Pleurotus sajor-caju and *Pleurotus florida* Mushrooms Improve Some Extent of the Antioxidant Systems in the Liver of Hypercholesterolemic Rats

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Abstract: *Objectives:* There is evidence that the antioxidant systems are imbalanced in hypercholesterolemic rats due to oxidative stress. Dietary supplementation of *Pleurotus sajor-caju* and *P. florida* are anti-hypercholesterolemic in rats. In this study, it was investigated whether these mushrooms have any effects on the antioxidant systems in hypercholesterolemic rat liver, which are altered.

Methods: Twenty young Long-Evans rats were randomly divided into 4 groups: control (fed basal diet), HC (fed 1% cholesterol with basal diet), HC+PS (fed 5% *P. sajor-caju* powder with 1% cholesterol and basal diet) and HC+PF (fed 5% *P. florida* powder with 1% cholesterol and basal diet). Rats were fed for 40 days, and then after sacrifice livers were collected, washed and homogenized. The content of reduced glutathione (GSH), thiobarbituric acid reactive substance (TBARS), and the activity of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) in homogenate were measured.

Results: All the studied parameters were altered in hypercholesterolemic rats significantly in comparison with control group, except catalase (non-significant). Feeding of *P. florida* protected liver lipid peroxidation in hypercholesterolemic rat, as TBARS was significantly lower (by 34.5%) in HC+PF group than in HC group. Feeding of both mushrooms showed significant improvement in the activity of GPx in liver of hypercholesterolemic rats (in HC+PS and HC+PF, the activity was higher by 50% and 80%, respectively than in HC group). However, mushroom feeding showed no significant effect on GSH level, SOD and catalase activity in hypercholesterolemic rats.

Conclusion: This investigation confirms the antioxidant activities of *P. sajor-caju* and *P. florida* to some extent in hypercholesterolemic rats in addition to their anti-hypercholesterolemic activity.

Keywords: *Pleurotus sajor-caju*, *Pleurotus florida*, Hypercholesterolemic rats, Lipid peroxidation, Glutathione peroxidase, Supeoxide dismutase, Catalase, Glutathione.

INTRODUCTION

Hypercholesterolemia is one of the major complications in health of the people all over the world, which mainly contribute to cardiac diseases and hypertension. Hypercholesterolemia is also related to diabetes and it has role in inducing oxidative stress [1, 2]. Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, neurodegeneration, aging etc. and most importantly, cancers [3-5]. Since the ancient times, mushrooms have been regarded as important medicinal food items, which have preventing and protective effects against many disorders, including hypercholesterolemia. Several studies have proven the anti-hypercholesterolemic effects of some edible mushroom species in experimental animals, and even in human [6-8]. One of the previous works of our related research group [9] also showed that dietary mushrooms of *Pleurotus* spp. have hypocholesterolemic effect on experimentally induced hypercholesterolemic rats.

Many species of mushrooms like *Ganoderma lucidum*, *Lentinus edodes*, *Grifola frondosa*, *Cordyceps sinensis*, and even some *Pleurotus* spp. like *P. ostreatus* have scientifically proved antioxidant activities [10-12]. This property of mushrooms is supposed to be effective also against the hypercholesterolemia induced oxidative alterations. Present study investigated the effect of dietary mushrooms *Pleurotus sajor-caju* and *Pleurotus florida* on the antioxidant systems (lipid peroxidation, reduced glutathione level, and antioxidant enzyme activities) of liver of hypercholesterolemic rats, as a continuation of the previous work of our laboratory [9]. However, *P. ostreatus* mushroom was excluded from this study, because its effects on the antioxidant system of

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1876-3960/11

hypercholesterolemic rats have already been well established [13]

MATERIALS AND METHODS

Mushroom Sample

The fruit bodies of *P. sajor-caju* and *P. florida* were collected from the production laboratory of National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh. Mushrooms were then dried and crushed into powder, and mixed with basal diet to feed rats.

Animal

Twenty young Long-Evans rats (*Rattus rattus*) were taken for this study (of 95± 5 g of weight), and were randomly divided into four groups: control group (fed basal diet), HC group (fed 1% cholesterol with basal diet), HC+PS (fed 5% *P. sajor-caju* powder with 1% cholesterol and basal diet) and HC+PF (fed 5% *P. florida* powder with 1% cholesterol and basal diet). Rats were housed in an animal room at 23± 2^0C, under 12 h dark-light cycles. The composition of the orally fed basal diet was as follows (g/100 g): wheat flour 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 the vitamin mixture in the diet was as follows (g/100 g vitamin): 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 the mineral mixture added to diet was as follows (g/100 g minerals): calcium gluconate 28.5, K_2HPO_4 17.3, $CaCO_3$ 26, $MgSO_4$ 12.6, KCl 12.6, $CuSO_4$ 0.06, FeSO4 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} and $CrSO_2$ 1.5×10^{-3}. Rats were fed for 40 days and then sacrificed. Liver was collected and washed with phosphate buffer saline (PBS) and homogenized in PBS.

Lipid Peroxidation Assay

The level of the liver lipid peroxidation (LPO) was estimated according to the thiobarbituric acid reactive substrates (TBARS) test of Ohkawa *et al.* [14] with slight modification. The liver homogenate (10%) was mixed with 0.02% butylhydroxytoluene to inhibit spontaneous oxidation. To each 50 µl of homogenate sample, 300 µl of 8.1% sodium dodecyl sulphate, 2.0 ml of 0.04% thiobarbituric acid in 20% acetic acid (pH 3.5) and 500 µl distilled water were added. The mixture was then incubated at 95º C for 1 h. After cooling with tap water, 1.0 ml distilled water and 2.5 ml of 15:1 (v/v) n-butanol-pyridine were added, and the mixture was shaken vigorously for about 20 min. After centrifugation at 1000 × g for 10 min, the absorption of the upper organic layer was determined at 530 nm by spectrophotometer. TBARS of liver was determined as nmol/ mg protein.

Protein Assay

Protein was determined according to the method of Lowry *et al*. [15]. The rat liver homogenate was heated at 95 ^{0}C for about 60 min in 0.1N NaOH for complete dissolution of cellular proteins. After cooling, the lysate was reacted with alkaline copper reagent to produce protein-Cu^{++} complex, which in turn reduced Folin reagent and produced blue colored complex. The absorption of the complex was measured against standard protein solution at 660 nm. Bovine serum albumin (BSA) was used as positive control and distilled water as negative control in protein estimation.

Glutathione Assay

Reduced glutathione (GSH) was assayed according to the slight modified method of Moron *et al.* [16]. Briefly, 1 ml of tissue homogenate was precipitated with 10% TCA (trichloro acetate), and centrifuged at 1000 × g. To the aliquot of supernatant, 2 ml of PBS and 0.5 ml of DTNB (5,5'-dithio 2-nitro benzoic acid) were added and final volume was made 5 ml with distilled water. The yellow colored product's optical density was measured at 412 nm by spectrophotometer. Commercial glutathione was used as standard. The level of GSH was expressed as µg/ mg protein.

Superoxide Dismutase Assay

Superoxide dismutase (SOD) activity was measured according to the slight modified method previously described by Marklund and Marklund [17]. Briefly, 500 µl of tissue homogenate was added into the assay mixture containing 0.25 ml absolute ethanol and 0.15 ml chloroform. After 15 min of shaking the suspension was centrifuged (at 8000 × g for 2 min) and supernatant was collected. The reaction mixture for auto-oxidation consisted of 2 ml Tris-HCl buffer (pH 8.2), 0.5 ml 2 mM pyrogallol and 2 ml of distilled water. The rate of auto-oxidation was measured in every 3 min. This was considered as 100% auto-oxidation. The assay mixture for enzyme contained 2 ml Tris-HCl buffer (pH 8.2), 0.5 ml 2 mM pyrogallol, 1.5 ml of distilled water and 0.5 ml of supernatant of tissue homogenate. This was immediately read at 470 nm every 3 min by a spectrophotometer. The activity of SOD was expressed as unit/ mg protein (one unit was the amount of enzyme that was utilized to inhibit 50% of auto-oxidation of pyrrogallol/ min).

Catalase Assay

Catalase activity was measured according to slight modified method of Sinha [18]. Briefly, 100 µl of tissue homogenate was added into the assay mixture containing 0.5 ml 0.2 M H_2O_2, 1 ml sodium phosphate buffer (0.01 M, pH 7.0) and 0.4 ml distilled water. Then 2 ml of dichromate-acetic acid was added after 15, 30, 45 and 60 s to stop the reaction. The tubes were then heated for 10 min and allowed to cool; the green colored product's color intensity was measured at 570 nm by spectrophotometer. The activity of catalase was expressed as unit/ mg protein (one unit was the amount of enzyme that utilized 1 µmol of H_2O_2/ min).

Glutathione Peroxidase Assay

The activity of Glutathione peroxidase (GPx) was determined according to slight modified method by Rotruck *et al.* [19]. Briefly, an assay mixture containing 0.5 ml sodium phosphate buffer, 0.1 ml 10 mM sodium azide, 0.2 ml 4 mM GSH and 0.1 ml 2.5 mM H_2O_2, was first prepared. To this, 100 µl of homogenate sample were added, and the total volume was made 2.0 ml with distilled water. This was incubated at 37 ^{0}C for 3 min and the reaction was finally terminated by adding 0.5 ml of 10% TCA. After centrifugation (1000 × g for 10 min), the supernatant was obtained and

4.0 ml of disodium hydrogen phosphate (0.3 M) solution and 1.0 ml of DTNB were added. The color intensity of the product was measured at 412 nm by spectrophotometer. Suitable aliquots of a standard (GSH) were also treated similarly. The enzyme activity was expressed as units/ mg protein (one unit was the amount of enzyme that converted 1 μmol of GSH to the oxidized form of glutathione (GSSH) in the presence of H_2O_2/ min).

Statistical Analysis

Data were analyzed and graphs were constructed by statistical program, SPSS-12.0 and Microsoft Excel. One way ANOVA and then post hoc tests were performed. $P \leq 0.05$ was regarded statistically significant.

RESULTS

The effect of mushroom feeding on the liver GSH level of hypercholesterolemic rats was non-significant at $P \leq 0.05$. The GSH was significantly lower in hypercholesterolemic group (HC) in comparison with the control group. Feeding of any of the *P. sajor-caju* and *P. florida* did not show any significant improvement in liver GSH level of hypercholesterolemic rats (Table **1**).

The lipid peroxidation in the liver of hypercholesterolemic rats was significantly protected by *P. florida* feeding. The TBARS (product of LPO) content in the HC group was significantly higher than the control group. Feeding of *P. florida* controlled the LPO, as the TBARS was significantly lower in HC+PF group than in HC group (by 34.5%). However, the effect of *P. sajor-caju* was not significant at $P \leq 0.05$ (Table **1**).

Fig. (**1**) presents the effect of mushroom feeding on the antioxidant enzyme activities in the liver of hypercholesterolemic rats. Liver SOD activity was significantly decreased in HC group rats in comparison with control group. Mushroom feeding had no significant improvement on this enzyme, i.e, the SOD activity varied statistically non-significantly among HC, HC+PS and HC+PF groups. Also, the liver catalase activity varied non-significantly among the control and experimental groups. However, both mushroom feeding showed significant improvement in the activity of GPx in hypercholesterolemic rats. Cholesterol diet decreased the liver GPx activity in HC group than in control group. But in HC+PS and HC+PF group, the activity was found higher (by 50% and 80%, respectively) which were statistically significant in comparison with HC group.

DISCUSSION

This study was performed to monitor the change of antioxidant activities in hypercholesterolemic rats and the effect

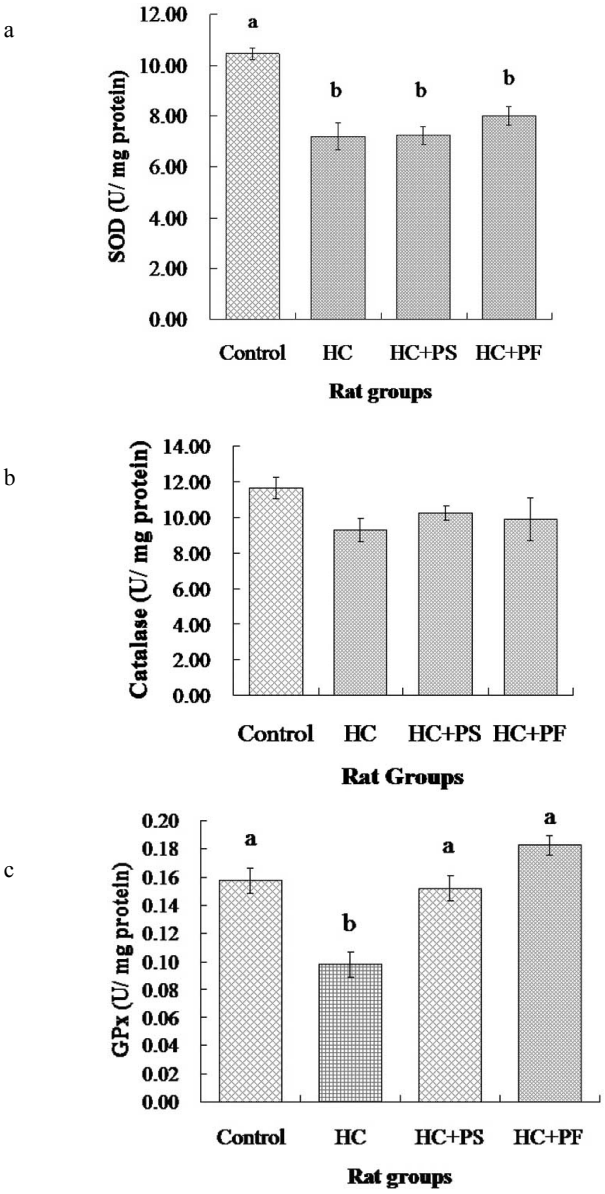

Fig. (1). Effect of Mushroom feeding on the antioxidant enzyme activities of hypercholesterolemic rats. Results (bars) are expressed as mean±SEM (n=5). Bars in each figure with different letters are significantly different at $P \leq 0.05$. Liver SOD (superoxide dismutase) activity was decreased in hypercholesterolemic rats in comparison with control group. Mushroom feeding had no significant effect on this enzyme. The liver catalase activity varied non-significantly among the groups. However, both mushroom feeding significantly increased the activity of GPx (glutathione peroxidase) in hypercholesterolemic rats, which was decreased.

Table 1. Effect of Mushroom Feeding on the Glutathione Level and Lipid Peroxidation in Liver of Hypercholesterolemic Rats

	Control	HC	HC+PS	HC+PF
GSH (μg/ mg protein)	9.53 ± 0.48^{a}	7.16 ± 0.76^{b}	7.56 ± 0.69^{b}	7.44 ± 0.64^{b}
TBARS (nmol/ mg protein)	1.04 ± 0.1^{a}	1.74 ± 0.21^{c}	$1.58 \pm 0.08^{b,c}$	$1.14 \pm 0.18^{a,b}$

Results are expressed as mean±SEM (n=5). Values in a single row with different superscript are significantly different at $P \leq 0.05$. HC- hypercholesterolemic rat group; HC+PS- *P. sajor-caju* fed hypercholesterolemic rat group; HC+PF- *P. florida* fed hypercholesterolemic rat group.

of the mushrooms with hypercholesterolemic potential on the antioxidant systems. As, hypercholesterolemia induces cellular oxidative stress, to protect cells or tissues antioxidant systems have to fight against this. Due to hypercholesterolemia, the antioxidant systems are supposed to be altered usually [1, 2, 8]. In this study, it has been found that all the studied parameters (GSH, TBARS, SOD, GPx and catalase) were altered in the liver of hypercholesterolemic rats significantly, except catalase activity (catalase activity was lower in HC group than in control, but the difference was non-significant). These findings just support some previous studies, which showed the decrease in GSH level, and antioxidant enzyme activities [13] and increase in TBARS level [8] in hypercholesterolemic rat's liver in comparison with control normocholesterolemic rats. Feeding of *P. sajor-caju* and *P. florida* recovered the alterations in some degrees. It has been found that the TBARS was significantly lower in *P. florida* fed HC+PF group than in HC group. And most importantly, both *P. florida* and *P. sajor-caju* fed groups (HC+PF and HC+PS, respectively) had significantly higher GPx activity in liver. These findings suggest that these mushrooms are potential to recover and improve the alteration in antioxidant systems by hypercholesterolemia. The antioxidant activities of these mushrooms in lowering lipid peroxidation and increasing GPx activities may be due to their nutritional or chemical composition. Vitamin E is regarded as an important natural antioxidant, which has capability to protect lipid peroxidation. *Pleurotus* mushrooms contain a considerable amount of vitamin E [10]. Also, the enzyme GPx is involved in the protection of lipid peroxidation, of which cofactor is selenium (Se) [19]. Selenium is one of the important trace elements found in the fruiting bodies of *P. florida* and *P. sajor-caju* [20].

In this study, for the first time the antioxidant activity of these two mushrooms in hypercholesterolemic rats is being reported, at least to some extent. The antioxidant activities of *P. ostreatus* and some other mushrooms have been reported earlier [10-12, 21]. Also a few reports exist about the antioxidant and antitumor role of *P. florida* [22]. But the report about the antioxidant potential of *P. sajor-caju* is rare. *P. sajor-caju* is nutritionally rich with higher protein [20, 23] than other *Pleurotus* mushrooms, and it has significant hypocholesterolemic effects [9]. So, this mushroom is recommended for the common people as a nutritious and health beneficial food supplement. The findings of this study suggest that in addition to nutritional and hypocholesterolemic importance, both *P. florida* and *P. sajor-caju* have roles in recovering and improvement of antioxidant systems of hypercholesterolemic rat liver at least in some degrees.

CONCLUSION

It is well established that *P. florida* and *P. sajor-caju* have significant health benefits through the modulation of physiological functions that include various atherogenic lipids in hypercholesterolemia. Also, these mushrooms are nutritionally rich food items. The findings of this study confirm the antioxidant activities of these mushrooms in hypercholesterolemic rats in addition to their hypocholesterolemic activity, at least to some extent. It is suggested to regular intake of these mushrooms to protect hypercholesterolemic condition and oxidative stress. More research are recommended on the investigation of antioxidant activities of the extracts or powder of the fruiting bodies of *P. florida* and *P. sajor-caju* on different human cell lines, animal model and human subjects.

ACKNOWLEDGMENT

This study was supported by the Strengthening Mushroom Development project of Ministry of Agriculture, People's Republic of Bangladesh.

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Received: January 05, 2011 Revised: January 27, 2011 Accepted: January 28, 2011