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THE HYPOCHOLESTEROLEMIC EFFECT OF EXTRACELLULAR POLYSACCHARIDE FROM THE SUBMERGED FERMENTATION OF MUSHROOM

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ABSTRACT

Male Sprague-Dawley rats were fed two semisynthetic diets supplemented with 2% cholesterol and 1% β-glucan type extracellular polysaccharide isolated from two liquid cultures of straw mushroom (*Volvariella volvacea*) mycelium containing different carbon sources. The experimental diets containing the two mycelial extracellular polysaccharides had induced a significant reduction in the levels of serum total cholesterol, LDL-cholesterol, and liver total cholesterol in the animals. There was no significant changes in the concentrations of serum triacylglycerol, HDL-cholesterol and liver total lipids. The fecal neutral steroid level was significantly increased while the fecal bile acid excretion was not significantly affected. Both mycelial extracellular polysaccharides exhibited hypocholesterolemic activity in rats with alimentary-induced hypercholesterolemia.
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KEYWORDS: Mushroom mycelium, *Volvariella volvacea*, Extracellular polysaccharide, β-glucans, Cholesterol

INTRODUCTION

Volvariella volvacea, "straw mushroom", is a widely cultivated tropical mushroom in Southeast Asia. Straw mushroom is not only a popular ingredient in traditional Chinese cuisine but also has a folk history concerning its medicinal applications in China (1). Our laboratory has demonstrated that both the fruiting bodies and the mycelia of straw mushroom exerted hypolipidemic effect in rats (2,3). The fruiting bodies and the mycelia of straw mushroom contain a large amount of cell-wall polysaccharides (25% and 50% dry weight, respectively) of which about two-thirds are the β-glucan type (4). Recently there have been extensive studies on fungal polysaccharides, especially β-glucans as biological response modifiers (5). The hypocholesterolemic effect of cereal and fungal β-glucans has also been reported (6,7,8). Most of these studies concentrated on the intracellular β-glucans isolated from the plant cell rather than the extracellular ones. During the submerged fermentation of the straw mushroom mycelium (9), it was found that a soluble β-glucan type extracellular polysaccharide was present in the liquid medium and could be recovered by ethanol precipitation (unpublished data). The following study investigated the potential cholesterol-lowering effect of this mycelial extracellular polysaccharide in rats with alimentary-induced hypercholesterolemia.

MATERIAL AND METHODS

Mycelium of the straw mushroom was cultivated by submerged fermentation using either a complete medium (CM) or an extract made from soy milk waste (SMW) as described previously (9). After 7 days of growth period, the liquid medium was separated from the mycelium and mixed with four volumes of 95% ethanol to precipitate the extracellular polysaccharide. The crude products (1.5 g and 1.9 g per litre of liquid medium for CM and SMW, respectively) were reprecipitated by ethanol and dried in a vacuum oven at 50°C for 6 hours. The final precipitate had a yield (per litre of liquid medium) of about 1 g for the CM and 1.4 g for the SMW. The carbohydrate content and the sugar composition of the final precipitate was determined by the gas chromatographic method described elsewhere (9).

The basal diet used was a modification of the AIN-76™ semi-purified diet with the following ingredients (g/100g): sucrose, 52.0; casein, 20; cornstarch, 15; vegetable oil, 5; mineral mix, 3.5; cholesterol, 2; vitamin mix, 1.0; DL-methionine, 0.3; choline chloride, 0.2. While the two experimental diets (EP-CM and EP-SMW), contained 1% by weight of the extracellular polysaccharide from the complete medium and the soy milk waste extract, respectively, the control diet had similar amount of cellulose instead. Cholesterol at 2% by weight was added to the diet groups to induce alimentary hypercholesterolemia. Male Sprague-Dawley rats of initial body weight of about 100 g were randomly assigned to three groups of ten. Each animal was housed individually in quarter maintained at 25°C with 12-h dark-light cycle. The animals were fed the three diets *ad libitum* for two weeks. Daily food intakes and body weights were recorded during the entire feeding period. Feces were collected over a 3-d period during the last week of the experiment and were kept at -70°C until analysis. Blood samples were obtained from the fasting animals (18-h) after exsanguination under anesthesia (sodium pentobarbital 50mg/kg). Total cholesterol, HDL-cholesterol, LDL-cholesterol, and triacylglycerol levels in the serum were measured by enzymatic methods (Sigma diagnostic procedure no. 352, 352-4, 353, and 336, respectively). The total cholesterol and total lipids of the liver were also determined by a colorimetric method and the Folch gravimetric method, respectively (10,11). Fecal neutral steroids and bile acids were analyzed by gas chromatography as trimethylsilylesters and methylesters, respectively (12). The results were analyzed for any significant differences by analyses of variance (ANOVA) and by Student's two-tailed *t*-test.

RESULTS

Both partially purified precipitates from the liquid medium contained mainly carbohydrate (90 and 92% for CM and SMW, respectively) with the following sugar composition (normalized %): glucose 95.4, galactose 3.8, arabinose 0.8 for CM; glucose 94.8, galactose 4.2, arabinose 1.0 for SMW. These results suggested that the precipitates from the liquid medium were most probably β glucan-type polysaccharide with minor arabinogalactan content.

There was no significant difference in the final body weight (mean ± SEM) between the control (283 ± 7 g) and the two experimental groups (278 ± 9 g for EP-CM and 281 ± 7 g for EP-SMW). The levels of daily food intake (mean ± SEM) of the EP-CM and EP-SMW diet groups (21.3 ± 1.9 g and 20.9 ± 1.5 g, respectively) were not significantly different from the control diet group (22.1 ± 1.8 g). As compared to the control diet, both experimental diets significantly lowered the serum total cholesterol by 17% (EP-CM) and 20 % (EP-SMW), respectively (Table 1). The serum LDL-cholesterol levels in the EP-CM and EP-SMW were also significantly decreased by 13 and 17%, respectively as shown in Table 1. There was also a significant reduction in the liver total cholesterol contents in the EP-CM and EP-SMW diet groups by 22 and 27%, respectively (Table 1). As shown in Table 2, the fecal excretion of neutral steroids in the EP-CM and EP-SMW diet groups was significantly increased (40 and 44%, respectively). The serum HDL cholesterol and triacylglycerol levels, the amounts of total liver lipids (Table 1) and fecal bile acids (Table 2) in the two experimental groups were not significantly different from the control group.

TABLE 1

Serum and Hepatic Cholesterol and Lipid Content in Rats Fed Basal Diet Supplemented with Mycelial Extracellular Polysaccharide

Diet Groups	Control	EP-CM *	EP-SMW #
Serum ($mmol.l^{-1}$)			
Total cholesterol	5.57±0.21 [a]	4.62±0.14 [b]	4.45±0.17 [b]
LDL cholesterol	4.55±0.11 [a]	3.96±0.09 [b]	3.77±0.13 [b]
HDL cholesterol	0.73±0.06 [a]	0.65±0.04 [a]	0.64±0.03 [a]
Triacylglycerol	1.33±0.16 [a]	1.31±0.12 [a]	1.20±0.09 [a]
Liver			
Total cholesterol($mmol.kg^{-1}$)	243±18 [a]	189±15 [b]	177±14 [b]
Total lipids ($mg\ kg^{-1}$)	110±7 [a]	103±5 [a]	111±5 [a]

* EP-CM Experimental diet with mycelial extracellular polysaccharide from complete medium
# EP-SMW Experimental diet with mycelial extracellular polysaccharide from soy milk waste extract
Values are means ± SEM for 10 animals per group. Within a horizontal row, figures with unlike superscript letters were significantly different ($p < 0.05$).

TABLE 2

Fecal Neutral Steroid and Bile Acid Levels of Rats Fed Basal Diet Supplemented with Mycelial Extracellular Polysaccharide

Diet Groups	Control	EP-CM *	EP-SMW #
Feces dry weight (g/day/rat)	1.31±0.27 [a]	1.37±0.29 [a]	1.24±0.23 [a]
Total neutral steroids (mg/day/rat)	20.23±1.33 [a]	28.33±1.70 [b]	29.13±1.65 [b]
Total bile acids (mg/day/rat)	7.89±0.76 [a]	8.33±0.65 [a]	7.93±0.86 [a]

* EP-CM Experimental diet with mycelial extracellular polysaccharide from complete medium
# EP-SMW Experimental diet with mycelial extracellular polysaccharide from soy milk waste extract
Values are means ± SEM for 10 animals per group. Within a horizontal row, figures with unlike superscript letters were significantly different ($p < 0.05$).

DISCUSSION

The two β-glucan type extracellular polysaccharides isolated from the straw mushroom mycelia cultivated in the complete medium (CM) and the soy milk waste extract (SMW) were both effective in lowering the serum total- and LDL-cholesterol levels, and the amount of liver total cholesterol. Although there was no significant difference in the hypocholesterolemic effect between the two mycelial β-glucans, the one from SMW seemed to consistently show lower levels of cholesterol values than the one from CM. It has been reported that mycelial extracellular polysaccharide production depended on the carbon source supplied to the cultures (13). Since the major carbon source of the CM (glucose) and SMW (arabinogalactan) are very different, it was likely that the two extracellular polysaccharides produced from the these two media might have different structures that affected their hypocholesterolemic properties. Further work is needed to optimize the production and to characterize the structure of these extracellular polysaccharides.

The hypocholesterolemic effect observed in the mycelial extracellular polysaccharide could be mainly due to the β glucans present in it. The viscous properties of the soluble β-glucan type polysaccharides could probably lower the cholesterol absorption by inhibiting the formation of micelles in the small intestine and altering the physical characteristics of the intestinal mucosa of rats (14). This is consistent with the result of an increase in fecal neutral steroid excretion. In contrast to other findings (15), the levels of bile acid excretion in the experimental diets did not increase significantly compared to the control diet. This indicated that the binding of bile acids to the β-glucan type extracellular polysaccharide and subsequent conversion of liver cholesterol to bile acids was not an essential mechanism in lowering the cholesterol levels. However, the liver total cholesterol levels were significantly lower in the experimental diets, showing that hepatic biosynthesis of cholesterol was suppressed. This could be due to a reduction in the activity of the liver enzyme, 3-hydroxy-3-methylglutaryl coenyme A (HMG-CoA) reductase (16). It is therefore suggested that the hypocholesterolemic effect of mycelial extracellular polysaccharide might be mediated by several mechanisms. Further investigation on the dose-response relationship of the mycelial extracellular polysaccharide and its cholesterol-lowering effect is required to establish the potential use of such material as a natural hypocholesterolemic agent. The physiological effect of this extracellular polysaccharide on rats will also be further studied for a longer feeding period.

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