

**HYPOLIPIDEMIC EFFECT OF
POLYSACCHARIDE EXTRACTED FROM
AURICULARIA NIGRICANS ON TRITON
WR-1339-INDUCED HYPERLIPIDEMIC MICE**

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Abstract

Auricularia nigricans is a medicinal mushroom - one of the most cultivated mushrooms due to its high nutritive and medicinal values. The objective of this study was to evaluate the effect of the polysaccharides extracted from *Auricularia nigricans* in Triton WR-1339- induced hyperlipidemic mice.

In order to meet the objective of the research, Triton WR-1339 was intraperitoneally injected at the dose of 400 mg/kg. At the same time, *Auricularia nigricans* polysaccharides (*Auricula PSP*) were orally administered at the doses of 0.87 g/kg and 1.74 g/kg according to the treatment or pretreatment protocol. Atorvastatin (75 mg/kg) was used as the reference drug. The plasma triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL) - cholesterol were determined by commercial kits.

Preliminary results showed that the oral administration of *Auricularia nigricans* polysaccharides (*Auricula PSP*) made reduce the plasma

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triglycerides, total cholesterol, and LDL-cholesterol and markedly increased the plasma HDL-cholesterol in mice injected Triton WR-1339. These results demonstrated that the polysaccharides extracted from *Auricularia nigricans* had the clearly benefit effect on hyperlipidemia and may be an ideal medicine material for the prevention of coronary heart diseases.

1. Introduction

Hyperlipidemia is a biochemical disorder that is characterized by abnormally high levels of lipids in the blood resulting in increasing the risk of coronary heart diseases (CHD). Two common lipid abnormalities are characterized either by high blood cholesterol levels (hypercholesterolemia) or high blood levels of triglycerides (hypertriglyceridemia). If blood cholesterol levels are elevated, large amounts of low density lipoprotein (LDL, so-called "bad") cholesterol can deposit in the arterial walls, representing the first stage in the narrowing of arteries, termed atherosclerosis. Hypercholesterolemia is especially dangerous when high density lipoprotein (HDL, "good") cholesterol levels are low [1].

Auricularia auricula has been known to have potent hypocholesterolemic effect [2]. The polymer of this mushroom also has various biological activities: such as anti-tumor [3], hypoglycemic [4], anticoagulant [5]. Hypolipidemic effect of biopolymers extracted from culture broth, mycelia, and fruiting bodies of *Auricularia auricula-judae* was investigated in dietary-induced hyperlipidemic rats. The results showed that the administration of the fruiting bodies reduced the plasma triglyceride, total cholesterol, low-density lipoprotein cholesterol, and atherogenic index while increasing the high-density lipoprotein cholesterol level, when compared to the control group [6]. However, the hypolipidemic effects of polysaccharide extracted from *Auricularia nigricans* cultivated in Vietnam in Triton WR-1339-induced hyperlipidemic mice remains unclear.

2. Research Methods and Contents

2.1. Test animals

Male *Swiss albino* mice, aged 5 weeks, were purchased from the Institute of Vaccines and Medical Bio-products in Nha Trang City, Vietnam and housed according to the principles stated in the Guide for Care and Use of Laboratory Animals [7]. The mice were fed with food provided by the Institute of Vaccines and Medical Bio-products and water ad libitum throughout the experimental period, and kept for at least 1 week prior to each experiment.

2.2. Chemical and reagents

Triton WR-1339 and atorvastatin were obtained from Sigma-Aldrich (USA). The commercial kits for analysis of plasma triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol were obtained from Human Ltd. Co. (Germany).

2.3. Polysaccharide extraction procedure

The extraction procedure of crude polysaccharide (PSP) was slightly modified in accordance with the method of Chen et al. [2] as follows:

200 mg of lyophilized powder of the *Auricularia nigricans* was extracted with 20 ml distilled water, and heated at 80°C for 3 hours at 100 rpm in the shaker. The extracted slurry was cooled and filtered with a 0.45 µm membrane filter (Millipore) and concentrated in a vacuum. Four volumes of 95 % ethanol was added stirred vigorously and then allow (add 'ed' to word 'allow') to precipitate overnight at 40°C. The precipitated PSP collected by centrifuging at 10,000 g for 20 minutes was rewashed with ethanol and centrifuged again described as above, then lyophilized and the weight of PSP was estimated.

2.4. Experimental protocols

The base of the experimentally induced hyperlipidemic animal model was the Triton WR-1339-induced model accordance with the method of Bertges et al. [8] with slight modification for mice [9].

2.5. Treatment protocol

To investigate the hypolipidemic effect of *Auricularia* PSP, the mice were divided into 4 groups of 10 mice per group: physiological control (saline), pathological control (Triton WR-1339 injection and saline treatment), Atorvastatin (75 mg/kg) treatment group, and two *Auricularia* PSP treatment groups at a dose of 0.87 g/kg or 1.74 g/kg. Triton WR-1339 was dissolved in saline and intraperitoneally injected at a dose of 400 mg/kg. The mice were treated 3 times with *Auricularia* PSP at 1 hour and 8 hours after the Triton WR-1339 injection. Atorvastatin was orally administered to the mice at 8 hours after the Triton WR-1339 injection (5:00 P.M.). Twenty-four hours after the Triton WR-1339 injection and one hour after the last administration of saline or *Auricularia* PSP, 1-ml blood samples were withdrawn from the mouse tail and centrifuged to collect the plasma for biochemical assays (analysis of plasma triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol) according to the protocols of commercial kits.

2.6. Pretreatment protocol

The mice were divided into 5 groups of 10 mice per group: physiological control (saline), physiological test group *Auricularia* PSP at a dose of 1.74 g/kg, pathological control (Triton WR-1339 injection and saline treatment), and two *Auricularia* PSP treatment groups at a dose of 0.87 g/kg or 1.74 g/kg. The mice in each group were administered saline or *Auricularia* PSP for 7 days. At day 7, Triton WR-1339 was intraperitoneally injected at a dose of 400 mg/kg. Twenty-four hours after the Triton WR-1339 injection and one hour after the last administration of saline or *Auricularia* PSP, biochemical assays were performed.

2.7. Statistical analysis

All the data were expressed as mean \pm SEM (Standard error of the mean) and analyzed using SigmaStat 3.5 software. One-way ANOVA followed by the Student-Newman-Keuls test was used to analyze with least significance difference $p < 0.05$ as the level of significance.

3. Results and Discussions

To investigate the effect of *Auricularia* PSP on the lipid levels in mouse plasma, the contents of triglycerides, total cholesterol, HDL - cholesterol, and LDL-cholesterol were measured. As shown in Fig. 1, when the mice were treated with Triton WR-1339 in the absence of the *Auricularia* PSP, the amounts of triglycerides after Triton WR-1339 injection were respectively increased by 459.93% (treatment protocol) and 458.98% (pretreatment protocol), compared with the physiological control.

However, either treatment or pretreatment with *Auricularia* PSP (0.87 or 1.74 g/kg) prior to Triton WR-1339 injection significantly reduced triglycerides in a dose-dependent manner. For instance, the amounts of triglycerides in treatment or pretreatment with *Auricularia* PSP (1.74 g/kg) were decreased by 28.47% and 33.93%, respectively, compared with the Triton WR-1339 control groups (pathological groups).

As shown in Fig. 2, the amounts of total cholesterol in the pathological groups were respectively increased by 324.42% (treatment protocol) and 375.14% (pretreatment protocol), compared with the physiological control. Either treatment or pretreatment with *Auricularia* PSP (0.87 or 1.74 g/kg) significantly reduced total cholesterol in a dose-dependent manner. The amounts of total cholesterol in *Auricularia* PSP (0.87 or 1.74 g/kg)-treated groups as well as Atorvastatin (75 mg/kg) group were decreased by 28.74%, 41.01%, and 45.17%, respectively, compared with the pathological groups. Pretreatment with *Auricularia* PSP (0.87 or 1.74 g/kg) for 7 days prior to Triton WR-1339

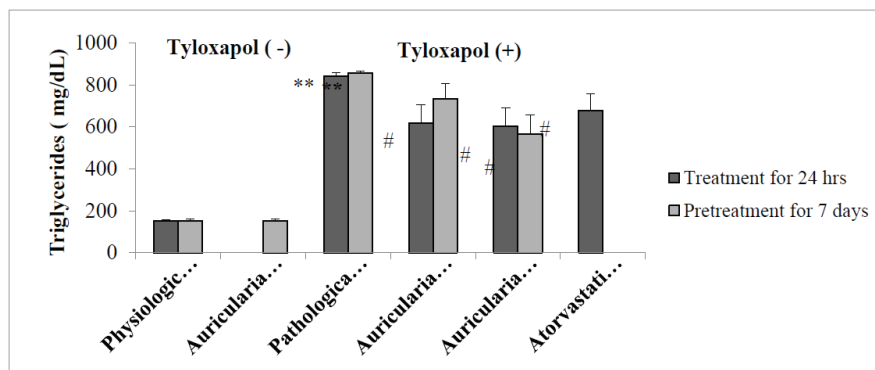


Fig. 1: The effect of *Auricularia* PSP on the plasma triglyceride levels. *Auricularia* PSP was administered according to treatment or pretreatment protocols. Atorvastatin was used as reference drug. Data were expressed as mean \pm SEM ($n = 10$). ** $p < 0.001$ as compared to the physiological control. # $p < 0.05$ and ## $p < 0.001$ as compared to the pathological controls.

injection showed the amounts of total cholesterol were decreased by 34.07% and 54.38%, respectively, compared with the pathological groups.

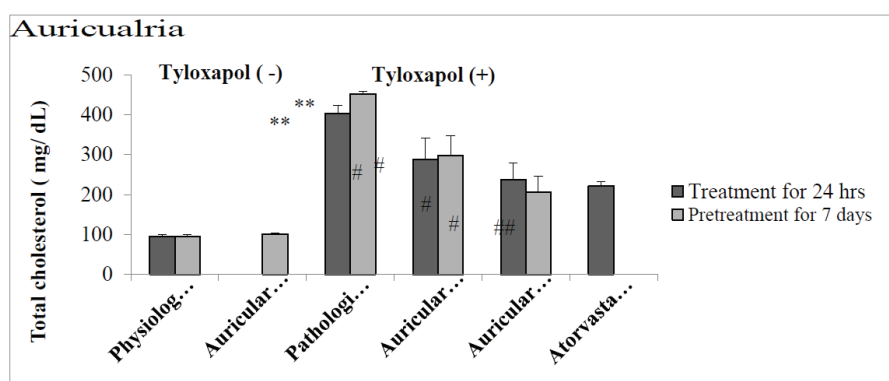


Fig. 2: The effect of *Auricularia* PSP on the plasma total cholesterol levels. *Auricularia* PSP was administered according to treatment or pretreatment protocols. Atorvastatin was used as reference drug. Data were expressed as mean \pm SEM ($n = 10$). ** $p < 0.001$ as compared to the physiological control. # $p < 0.05$ and ## $p < 0.001$ as compared to the pathological controls.

The amounts of LDL-cholesterol in the pathological groups were respectively increased by 111.63% (treatment protocol) and 93.28% (pretreatment protocol), compared with the physiological control (Fig. 3). Either treatment or pretreatment with *Auricularia* PSP (0.87 or 1.74 g/kg) significantly reduced LDL-cholesterol with the amounts of LDL-cholesterol in *Auricularia* PSP (0.87 or 1.74 g/kg)-treated groups as well as atorvastatin (75 mg/kg) group were

decreased by 42.61%, 40.17%, and 54.42%, respectively, compared with the pathological groups. Pretreatment with *Auricularia* PSP (0.87 or 1.74 g/kg) for 7 days prior to Triton WR-1339 injection showed the amounts of LDL-cholesterol were decreased by 39.13% and 44.35%, respectively, compared with the pathological groups.

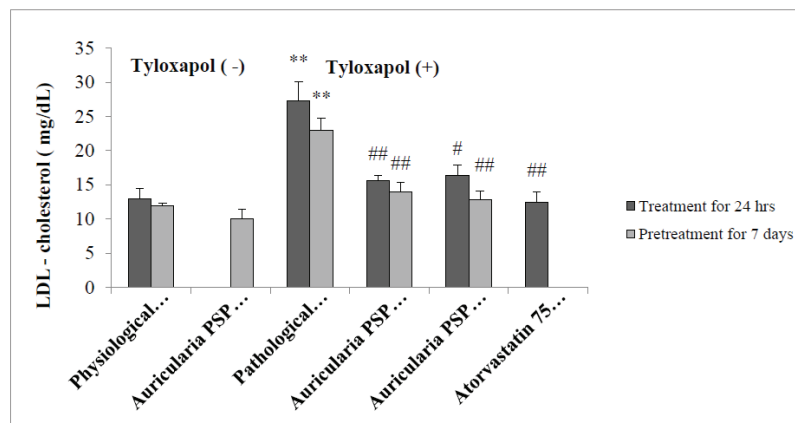


Fig. 3: The effect of *Auricularia* PSP on the plasma LDL-cholesterol levels. *Auricularia* PSP was administered according to treatment or pretreatment protocols. Atorvastatin was used as reference drug. Data were expressed as mean \pm SEM ($n = 10$). ** $p < 0.001$ as compared to the physiological control. # $p < 0.05$ and ## $p < 0.001$ as compared to the pathological controls.

As shown in Fig. 4, the levels of HDL-cholesterol in the Triton-treated control groups were diminished by 37.13% (treatment protocol) and 39.54% (pretreatment protocol), compared with the physiological control. Either treatment or pretreatment with *Auricularia* PSP (0.87 or 1.74 g/kg) significantly elevated HDL-cholesterol in a dose-dependent manner. The amounts of HDL-cholesterol in *Auricularia* PSP (0.87 or 1.74 g/kg)-treated groups as well as Atorvastatin (75 mg/kg) group were markedly increased by 42.59%, 77.81%, and 120.12%, respectively, compared with the pathological groups. Pretreatment with *Auricularia* PSP (0.87 or 1.74 g/kg) for 7 days prior to Triton WR-1339 injection showed the amounts of HDL-cholesterol were increased by 67.29% and 70.55%, respectively, compared with the Triton-treated control groups.

The nonionic detergent, Triton WR1339 (Tyloxapol or an oxyethylated tertiary octyl phenol formaldehyde polymer), has been used widely for screening natural or chemical hypolipidemic drugs. In a study by there was marked increase in the level of serum total cholesterol, triglycerides, phospholipids, LDL-cholesterol, and very low density lipoprotein (VLDL) cholesterol and a significant decrease in the level of HDL-cholesterol in the rats treated with Triton WR-1339 [8]. Triton WR-1339 blocks the uptake of triacylglycerol-rich lipoproteins from plasma in peripheral tissues to produce acute hyperlipidemia

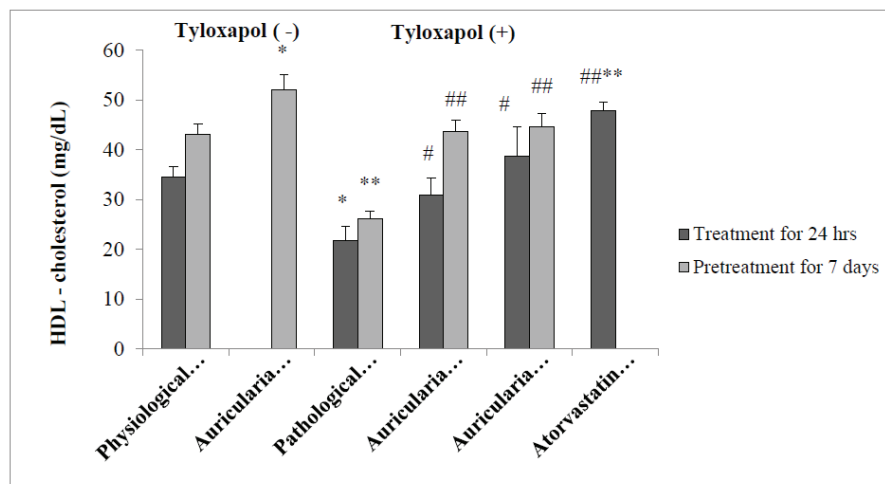


Fig. 4: The effect of *Auricularia* PSP on the plasma HDL-cholesterol levels. *Auricularia* PSP was administered according to treatment or pretreatment protocols. Atorvastatin was used as reference drug. Data were expressed as mean \pm SEM ($n = 10$). * $p < 0.05$ and ** $p < 0.001$ as compared to the physiological control. # $p < 0.05$ and ## $p < 0.001$ as compared to the pathological controls.

in animal models [10]. Its function is to inhibit the activity of the enzyme lipoprotein lipase, resulting in the accumulation of triglycerides and VLDL in plasma, which causes a significant increase in hepatic cholesterol biosynthesis by stimulating the activity of the enzyme HMG-CoA reductase [11]. Experimental evidence supports the concept that Triton WR-1339 physically alters VLDL-cholesterol, rendering it refractive to the action of lipolytic enzymes of blood and tissue [12].

The present study demonstrated the potential of *Auricularia* PSP in reducing the level of triglycerides, total cholesterol, and LDL-cholesterol in mice injected with Triton WR-1339. Such hypolipidemic effects of *Auricularia* PSP were the same as Atorvastatin, a selective and competitive inhibitor of HMG-CoA reductase (the rate-limiting enzyme in cholesterol synthesis). In animal models, Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL-cholesterol receptors on the cell surface to enhance uptake and catabolism of LDL-cholesterol [14]. Atorvastatin also reduces LDL-cholesterol production and the number of LDL-cholesterol particles. On the other hand, plasma LDL (which is quantitatively the most significant lipoprotein class in the control of serum cholesterol levels) values may be affected by modifications of very-low-density lipoprotein (VLDL) metabolism, including the rate of conversion of VLDL to LDL [15]. Therefore, the present

results suggest that polysaccharides from *Auricularia nigricans* may act on HMG-CoA reductase or lipoprotein metabolism.

Taken together, it is possible that the hypocholesterolemic effect of *Auricularia* polysaccharides in the present study appeared to be due to the reduced cholesterol synthesis in the liver. Also, the possibility of combined effects (the acceleration of enzyme lipoprotein lipase activity, the inhibition of cholesterol absorption, and/or the inhibition of biosynthesis of LDL and acceleration of their fractional turnover) cannot be ruled out. Therefore, further comprehensive chemical and pharmacological investigations may help in elucidating the exact mechanism of hypolipidemic

4. Conclusion

In conclusion, the results indicated that *Auricularia* polysaccharides had the hypolipidemic effect on the Triton WR-1339-induced hyperlipidemic model in mice. This finding supports the medicinal and therapeutic value of *Auricularia nigricans* cultivated in Vietnam in the prevention of coronary heart disease.

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