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HYPERLIPIDEMIC AND HYPOCHOLESTEROLIC ACTION OF SUBMERGE CULTURED MUSHROOMS

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Abstract

According to WHO, cardiovascular diseases are among the major causes of death on the planet. One of the main risk factors is elevated level of blood cholesterol. Therefore, development of new, effective and safe measures to reduce the level of blood cholesterol is an important and actual problem. Our study was devoted to hypolipidemic effect of mycelia of some basidiomycetes, which can be used as a food supplement, to reduce elevated levels of cholesterol. As it is known, fungi, being a good source of nutritive protein, also possess a wide range of different kinds of biological activities. Mushrooms which perform hypolipidemic activity can reduce cholesterol levels and could be a promising tool to control hyperlipidemia and hypercholesterolemia.

Submerged cultivation of basidiomycetes was carried out for 7 days on a medium containing glucose and peptone as carbon and nitrogen sources respectively. After culturing, the biomass was separated from the native liquid by filtration and dried at 50 ^{0}C, grinded and obtained powder preparation was used for the study of hypolipidemic and hypocholesterolic activity.

Experiment was performed during 21 days using white male rats received from Rappolovo station of Russian Academy of Medical Sciences (St. Petersburg). Animals have been divided into groups on seven animals in each. Animals in intact group received a standard dry fodder and water *ad libidum*. The control group received hyperlipidemic diet and water. Animals from experimental groups received hyperlipidemic diet with the addition of mushroom preparation.

The concentration of total cholesterol and cholesterol of high density lipoproteins in blood plasma was defined using a cholesterol determination kit of "Human" (Germany). The method is based on a combination of enzymatic reaction with a reaction of oxidation.

It has been shown, that addition of most of mycelia powders of investigated fungi to hyperlipidemic fodder of rats leads to a significant reduction in total cholesterol levels in the blood. However, in some cases, this decrease was related to decrease of alpha-cholesterol. The most decrease in atherogenic factor, and hence the risk of blockage of blood vessels was observed when mycelia of *Trichoderma ochracea* and *Panus conchatus* were added to hyperlipidemic fodder.

***Key words**: Hypocholesterolic action, Mushrooms.*

1. Introduction

According to WHO, cardiovascular diseases are among the major causes of death on the planet. One of the main risk factors for cardiovascular diseases are hypercholesterolemia and hyperlipidemia. Therefore, development of new, effective and safe measures to reduce the level of blood cholesterol is an important and actual problem.

Laboratory and clinical studies have shown beneficial effect of diets supplemented with fruits and vegetables on atherosclerosis [1, 2].

From ancient times mushrooms were attracting the interest of the people due to their nutritive properties. They are a good source of food proteins and biologically active substances. Promising results of submerge cultivation of higher basidiomycetes, are increasing the prospects for their wide utilization in food industry and for the health care [3 - 6].

One of the important and well known biological activities of mushroom compounds are immunomodulating and antitumor activities of beta-glucans derived from mushrooms [7 - 9].

During several recent decades, the problem of atherosclerosis and related diseases arises very strictly in the whole world.

Mushrooms possessing antisclerotic action, capable to decrease the level of cholesterol in blood, can be very perspective for medical industry and could become a valuable diet product [5, 6, 10 - 12]. Study of Slovakian scientists demonstrates, that the diet containing 5% of powdered oyster mushroom (*Pleurotus ostreatus*) or an equivalent amount of mushroom ethanolic extract feeded for 12 weeks significantly decreases cholesterol content in serum and liver [13].

The aim of our studies was to determine hypocholesterolic and hypolipidemic effects of supplements from submerge cultured mycelia of various mushrooms.

2. Materials and Methods

The objects of our study were submerge cultivated higher basidiomycetes: *Coriolus hirsutus, Cerrena unicolor, Trametes ochracea, Panus conchatus, Ganoderma lucidum, Flammulina velutipes, Coprinus lagopides* and *Pleurotus ostreatus.*

Submerge cultivation was performed on a culture media containing glucose as a carbon source and peptone as a source of nitrogen for 8 days. As a result of submerge cultivation necessary amount of biomass was collected, which was dried at 50 ^{0}C, powdered and used for the studies of hypocholesterolic activity.

2.1 Determination of hypocholesterolyc activity of basidiomycetes

The model of hyperlipidemia of rats has been chosen on the basis of experimental experience of Institute of experimental medicine of Russian Academy of Medical Sciences and other works [14, 15].

Experiment was performed during 21 days using white male rats received from Rappolovo station of Russian Academy of Medical Sciences (St. Petersburg). Animals have been divided into groups of seven animals in each. Animals from intact group received a standard dry fodder and water *ad libitum*. The control group received hyperlipidemic diet and water. Animals from experimental groups received hyperlipidemic diet with the addition of one of mushroom preparations.

The composition of Hyperlipidemic diet was as follows: dry crystalline cholesterol - 5%, pork fat - 25%, sunflower seed oil - 18%, vitamin D - 200 thousand units/day, the remaining - oat flakes. Hyperlipidemic fodder was prepared by mixing of the melted pork fat with crystalic cholesterol and with other above mentioned components. Animals received mushroom additives *per os*, mixed with a main bulk of a fodder in quantity of 2% from a daily diet. Amount of daily fodder for each animal was 20 grams. After 21 days the blood and a liver of rats was taken for analyses.

2.2 Determination of total cholesterol and cholesterol of high density lypoproteids

For a rough quantitative estimation of a degree of risk of an atherosclerosis in 1977 Klimov A.N. offered so-called cholesterol factor of atherogenity (Kcl), representing a ratio of cholesterol of atherogenic lipoproteins (cholesterol of low density lipoproteins - Cl LDL and cholesterol of very low density lipoproteins - Cl VLDL) and cholesterol of anti-atherogenic lipoproteins (cholesterol of high density lipoproteins or an alpha cholesterol - Cl HDL) [16].

$$Kcl = \frac{Cl\ LDL + Cl\ VLDL}{Cl\ HDL}$$

As total quantity of cholesterol of atherogenic lipoproteins can be presented as a difference between the total cholesterol and cholesterol of high density lipoproteins, the factor of atherogenity can be calculated on the basis of definition only of two parameters - the total cholesterol and cholesterol of lipoproteins of high density:

$$Kcl = \frac{Cl\ total - Cl\ HDL}{Cl\ HDL}$$

The concentration of total cholesterol and Cl HDL in blood plasma was determined using a cholesterol determination kit of "Human" (Germany). The method is based on a combination of enzymatic reaction with a reaction of oxidation. Hydrogen peroxide is formed as a result of these reactions due to the action of peroxidase reacts with 4-amynophenazon and phenol with formation of the colored product - chinonimin / Instruction of "Humana" cholesterol determination kit/.

Concentration of cholesterol was determined using following equation:

$$Ccl = 14{,}3 \times OD\ of\ sample\ (mol/L)$$

The concentration of total cholesterol in liver was determined using the following procedure: 200 mg of a liver tissue was dehydrated with 1,0 mL of methanol within 12 hours. Then 2 mL of a mixture of heptan and isopropanol in a ratio 2 : 1 was added. After 12 hours an extract was selected and evaporated. To a dry extract 0.1 mL of methanol and 2 mL of a standard reagent of "Human" company for definition of the total cholesterol were added. After 10 mines optical density at 500 nanometers was measured. Concentration of cholesterol was defined using above mentioned equation.

Coefficient of atherogenity was calculated based on obtained data.

3. Results and Discussion

It is known, that hypercholesterolemia increases the risk of heart disease. Elevated levels of circulating cholesterol cause deposits to form inside blood vessels. These deposits can result in a disease process called atherosclerosis, which can cause blood clots to form that will ultimately totally stop blood flow. If this happens in the arteries supplying the heart, a heart attack will occur. If it happens in the brain, the result is a stroke where a portion of brain tissue dies. Atherosclerosis causes more deaths from heart disease than any other single condition. The most common cause of elevated serum cholesterol is eating foods that are rich in saturated fats or contain high levels of cholesterol.

Cholesterol has been divided into two major categories: low-density lipoprotein (LDL), the so-called "bad" cholesterol, and high-density lipoprotein (HDL), the so-called "good" cholesterol.

We studied the influence of mushroom additives to hypercholesterolic diets on the levels of total cholesterol and high-density lipoproteins in rats. The obtained results are demonstrating that the addition of almost all of studied mushroom mycelia to hyperlipidemic diets of rats caused significant decrease in a level of total cholesterol (Fig. 1). Positive effect was not detected only for mycelia of *Coprinus lagopides*. In the cases of *Trametes ochracea* and *Panus conchatus* high levels of a-cholesterol in blood of experimental animals was almost normalizing the coefficient of atherogenity.

From the data presented in Figure 2 it could be concluded, that addition of the mycelia of *Trametes ochracea*, *Panus conchatus* and *Coprinus lagopides* to hyperlipidemic diets was significantly decreasing the risk of atherosclerosis. Addition of the first two mushrooms was even more efficient, decreasing the levels of cholesterol coefficient of atherogenity to the levels of normal (healthy) diet of intact group.

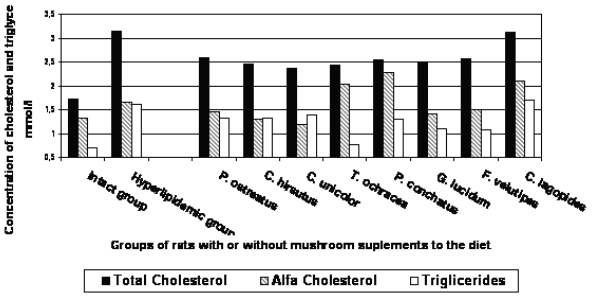

Figure 1. Concentrations of cholesterol and triglycerides in blood of different experimental groups

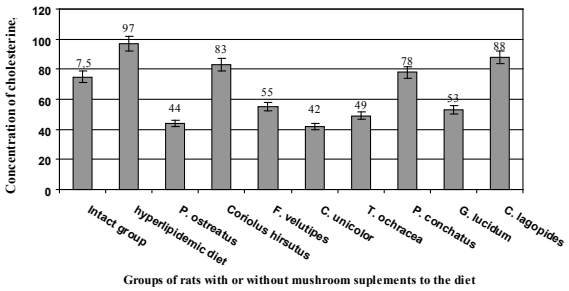

Figure 2. Levels of the factor of atherogenity for different diets

Studies of the cholesterol levels in rat livers, shows that addition of mushroom mycelia to diets was efficiently and significantly slowing the processes of synthesis of endogenous cholesterol in rat liver (Fig. 3).

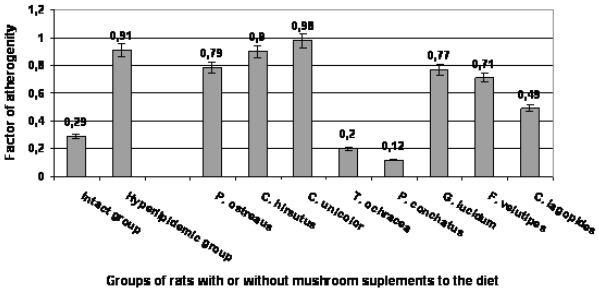

Figure 3. Contents of total cholesterine in the rat livers

For the groups receiving hyperlipidemic diets supplemented with mycelia from *Cerrena unicolor*, *Pleurotus ostreatus*, *Trametes ochracea*, *Flammulina velutipes* or *Ganoderma lucidum* the levels of cholesterol in rat livers were even lower, than for the group obtaining ordinary diet.

4. Conclusions

- As a result of conducted studies it is possible to draw a conclusion that the addition of a biomass of some mushrooms to hyperlipidemic diets leads to essential decrease in a risk of development of atherosclerosis.

- The highest hypocholesterolic activity was detected for the fruit bodies of *Pleurotus ostreatus* and mycelium of *Ganoderma lucidum*.

- Results of performed studies are demonstrating significant hypocholesterolic effect of uptake of supplements from various basidial mushrooms.

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