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Research Article

Isolation and Characterization of Carbohydrate Fractions of the Fruiting Body of the *Basidiomycete Ganoderma Applanatum* and the Study of Their Hepatoprotective Activity

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Abstract

Polysaccharides isolated from basidiomycete raw materials *G. applanatum* have been studied, their physicochemical properties and hepatoprotective activity have been studied. Using the method of sequential water extraction, water-soluble polysaccharides were isolated with a yield of 5.45%. The carbohydrate content in the purified polysaccharide sample was determined by phenol-sulfuric acid methods and amounted to 71.52%. Polysaccharides were separated into neutral and anionic fractions using ion exchange chromatography and purified from proteins and peptides. The carbohydrate composition was determined. The MW of the resulting polysaccharide sample was 18.7kDa, the polydispersity index was 1.3. The results of IR spectroscopic studies showed that the polysaccharide, according to its structural characteristics, belongs to the β-glucan type polysaccharide. Using a model of acute toxic hepatitis in mice caused by the administration of a 50% carbon tetrachloride solution, their hepatoprotective activity was assessed. Polysaccharides isolated from the mushroom *G. Applanatum* at a dose of 50mg/kg, when administered orally, have high hepatoprotective activity. Polysaccharides (50mg/kg) in mice with toxic hepatitis cause a decrease in the activity of liver enzymes - ALT by 1.86±3.0, AST by 2.12±0.85 and ALP by 2.31±0.87 times, compared with the control group.

Keywords: Water-soluble Polysaccharides, Glucose, Galactose, β-glucan, Basidiomycete raw materials, Hepatoprotective activity

Introduction

To date, numerous studies have shown that polysaccharides isolated from basidiomycetes have a variety of biological activities, hepatoprotective [1,2], antioxidant [3,4] anti-inflammatory [5,6] hypoglycemic [7,8] antiviral [9,10] antiproliferative effect [11,12] liver protection [13] enhancing immunity [14] and other properties. The biological activity of polysaccharides depends on the physicochemical properties, molecular parameters, monosaccharide composition and the nature of the branching links in the polysaccharide macromolecule. Most polysaccharides isolated from basidiomycetes are branched β-glucans with (1,3) -(1,6) glycosidic linkages [15]. Studying the composition of mushroom polysaccharides makes it possible to determine their biological activity and structural relationships and expands the areas of application of these mushrooms. The purpose of this work is to isolate and characterize carbohydrate fractions of the fruiting body of the basidiomycete *G. applanatum* and study their hepatoprotective activity.

For preliminary purification of the starting material, the crushed fruiting body of the mushroom, *G. applanatum,* was extracted with a degumming reagent and the yield was 3.2%. After degumming the raw materials, to isolate water-soluble polysaccharides, sequential extraction was carried out and the yield of polysaccharides was 5.45%. The isolated polysaccharides are a cream-colored powder that is highly soluble in water, forms an opalescent solution at low concentrations, and forms a viscous solution at high concentrations.

Polysaccharides were purified from proteins by the Savage method and UV spectroscopic studies showed that no peaks corresponding to proteins were observed at 280nm and 260nm. Next, the total amount of carbohydrates was determined by the phenol-sulfate method and the yield was 71.52%.

At the next stage of the study, the polysaccharides were purified by ion exchange and gel chromatography. As a result of chromatography, polysaccharides were divided into two types - neutral and acidic fractions of polysaccharides. Neutral polysaccharides (yield 27.18%) were extracted with purified water, and anionic polysaccharides (yield 5.67%) were extracted with highly concentrated saline solution. Further purification of water-soluble polysaccharides was carried out by fractionation with purified water on a column with Sephadex G-75. The total carbohydrate content in purified polysaccharide samples when tested by the phenol-sulfuric acid method was 94.65%.

The results of studying the molecular weight of water-soluble polysaccharides using gel chromatography showed that the average molecular weight of the extracted samples of the aqueous fraction was Mw=50830 Da, and the polydispersity index value was 2.5. When studying the monosaccharide composition of isolated water-soluble polysaccharides, it was found that 75.9% glucose, 9.3% galactose, 3.4% fructose, 7.7% mannose and 0.8% xylose are monosaccharides. These results indicate that the isolated polysaccharides are glucan-type polysaccharides. The IR spectrum of the resulting polysaccharide sample revealed absorptions specific to polysaccharides at 3430, 2920, 1630, 1420-1380, 1200-750cm^{-1}. In the region of 1460-1650cm^{-1} of the IR spectrum, absorption intensity is observed, corresponding to the stretching vibrations of the C=C bonds of the aromatic ring of melanin and the C=O bonds of the carboxyl group.

Absorption in the region of 1200-800cm^{-1} is characteristic of vibrations of the C-C, C-O, C-N bonds in the pyranose ring. Low-intensity absorption in the region of 898 and 940cm^{-1} of the spectrum is characteristic of β-glycosidic bonds of polysaccharides [16]. This means that these polysaccharides contain β-type glycosidic linkages. In vivo studies of hepatoprotective activity were carried out on a model of toxic hepatitis caused by CCl4 in mice. The results of the study are presented in Table 1. According to the data obtained, against the background of the administration of β-glucan polysaccharide, isolated from the basidiomycete fungus *Ganoderma applanatum,* a high level of detoxification was observed in animals that reached 100% survival, while in the control this figure was 20% (Table 1).

Table 1: Effect of *Ganoderma applanatum* polysaccharide on survival, body and liver weight of mice with toxic hepatitis (M±m, n=6).

Groups	survival Rate of Mice (%)	Life Expectancy of Dead Mice (Days)	Liver Weight Coefficient	Coefficient of Change in Body Weight	Total CHA
Intact animals	100	-	66.79±1.04	(+) 2.17±0.31	1
Control group	20	2.8±0.2	92.15±3.05	(-) 2.0±0.24	0
Polysaccharide(50mg/kg)	100	-	59.29±0.59*	(+) 0.40±0.4	0.829

Note*: CHA is the coefficient of hepatoprotective activity. *- p<0.05 relative to the control group.

The best shielding of destructive events in the liver of mice, characterized by a decrease in inflammatory processes, is observed with the introduction of a polysaccharide sample, the liver weight coefficients of which were respectively 59.29±0.59, and this indicator is statistically significant (p<0.05) lower than the control-92.15±3.05. There are practically no differences with the intact group. According to the data presented in Table 1, the total CHA for all indicators showed the greatest effectiveness of the polysaccharide (0.829), which can be used as components of a drug with a high degree of hepatoprotective activity (Table 2).

Table 2: At the next stage, we studied the functional state of the liver of experimental animals.

Groups	ALT(U/l)	AST(U/l)	Alkaline Phosphatase (U/l)
Intact group	51±1	111±3	115±6
Control group	122.5±5.83*	109.4±4.38*	82.9±4.2*
Polysaccharide (50mg/kg)	65.8±5.5**	51.53± 4.24**	35.9±6.1**

Note*: *- p<0.05 relative to the control group.

The administration of carbon tetrachloride caused acute toxic liver damage in mice. The activity of liver enzymes (ALT, AST and alkaline phosphatase) significantly increased compared to control animals by 2.1 and 2.4 times. In the experimental groups of polysaccharides, these indicators were close to normal. The data obtained indicate that the isolated polysaccharides are promising biologically active components, on the basis of which it is possible to create drugs with hepatoprotective activity.

Conclusion

Water-soluble polysaccharides were isolated from the basidiomycete *Ganoderma applanatum*, a mushroom native to Uzbekistan, and their composition and physicochemical properties were studied. The monosaccharide composition of the polysaccharide was found to include 75.9% glucose, 9.3% galactose, 3.4% fructose, 7.7% mannose, and 0.8% xylose. IR spectroscopy revealed a band at 898-900cm-1 in the polysaccharide spectrum,

indicating the presence of a β-type glycosidic bond. Polysaccharides isolated from the *G. applanatum* mushroom at a dose of 50mg/kg, when administered orally, exhibited high hepatoprotective activity.

Conflicting Interests

None.

Acknowledgments

None.

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