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Ultrasonic-assisted extraction process of crude polysaccharides from Yunzhi mushroom and its effect on hydroxyproline and glycosaminoglycan levels

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ABSTRACT

Yunzhi is a mushroom belonging to species of the Basidiomycetes class of fungi. Its medicinal value was recorded in China. The Yunzhi polysaccharide was extracted from Yunzhi mushroom in this work. The results of chemical composition indicated that Yunzhi polysaccharides comprised mainly of carbohydrate compound. Total carbohydrate content was determined to be 95%. The polysaccharides were composed of glucose and mannose as detected by GC in the ratio of 4.4:1. The absorption spectra of the polysaccharides showed that the polysaccharides-related absorption peaks at 1153, 1336, 1460, 1663, 1712 and 2927 cm^{-1}. Effect of Yunzhi polysaccharides on hydroxyproline, glycosaminoglycan, I collagens mRNA/β-actin and I collagens protein/GAPDH was investigated by employing animal model. Result showed that Yunzhi polysaccharides could enhance hydroxyproline, glycosaminoglycan, I collagens mRNA/β-actin and I collagens protein/GAPDH level in rat. These results indicated that Yunzhi polysaccharides could be beneficial for treatment of bone degenerative diseases.

1. Introduction

A variety of compounds including polysaccharide with antitumor activity have been reported to be present in mushrooms (Park, Lai, & Kim, 2004; Tao, Zhang, & Zhang, 2009; Ukawa, Ito, & Hisamatsu, 2000; Shahwar & Raza, 2009). Coriolus versicolor (CV), known as Yunzhi in China, is a mushroom belonging to species of the Basidiomycetes class of fungi. Its medicinal value was recorded in the Compendium of Chinese Materia Medica and Shen Non Compendium Medica thousands of years ago in China. Nowadays, its therapeutic potential has been gaining acceptance among patients worldwide (Kidd, 2000). Yunzhi polysaccharopeptide (PSP) and polysaccharide Kureha (PSK, Krestin) are a new type of Biological Response Modifier (BRM) extracted from the deep-layer cultivated mycelia of the Cov-1 and CM-101 strains of Yun Zhi (Coriolus versicolor), respectively.

Hydroxyproline differs from proline by the presence of a hydroxyl (OH) group attached to the C (gamma) atom. Other hydroxyprolines also exist in nature, the most notable ones being 2,3-cis-, 3,4-trans- and 3,4-dihydroxyproline, which occurs in diatom cell walls (Nakajima & Volcani, 1969) and is postulated to have a role in silica deposition. Hydroxyproline is also found in the walls of oomycetes, fungus-like protists related to diatoms (Alexopoulos, Mims, & Blackwell, 1996).

Glycosaminoglycans (GAGs) or mucopolysaccharides are long unbranched polysaccharides consisting of a repeating disaccharide unit. The repeating unit consists of a hexose (six-carbon sugar) or a hexuronic acid, linked to a hexosamine (six-carbon sugar containing nitrogen).

Collagen is a group of naturally occurring proteins. In nature, it is found exclusively in animals (Muller, 2003; Ali Shah, Hasan, Hameed, & Akhter, 2009). It is the main protein of connective tissue. It is the most abundant protein in mammals (Di LulloDagger, Sweeney, Körkkö, Ala-Kokko, & San Antonio, 2002), making up about 25–35% of the whole-body protein content. In muscle tissue it serves as a major component of endomysium. Collagen constitutes 1–2% of muscle tissue, and accounts for 6% of the weight of strong, tendinous muscles (Sikorski, 2001). Gelatin, which is used in food and industry, is derived from collagen.

In the present study, the Yunzhi polysaccharides were extracted using ultrasonic method. Its chemical structure was analysed by GC–MS and FT-IR. At last, pharmacological function of Yunzhi polysaccharides was evaluated.

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2. Materials and methods

2.1. Material

The Yunzhi mushroom, were collected from a market in Chongqing city, thoroughly washed with distilled water, freeze-dried and ground.

2.2. Extraction procedure

Ground Yunzhi mushroom was refluxed with 95% ethanol at 70 °C in a water bath for 3 h to deactivate the endogenous enzymes and remove some soluble materials, including free sugars, amino acids and some phenols. The ethanolic mixture extract was centrifuged (3000*g*, 10 min). The ethanol extraction was washed twice with 95% ethanol. The combined extract was vacuum-dried at 60 °C for 12 h, and it was suspended in the water and sonicated at the temperature of 40–80 °C and actual sonic power of 7.2–40.3 W for 10–30 min. After rapid cooling to room temperature using ice water, the supernatant was concentrated in a rotary evaporator under reduced pressure, and then mixed with four volumes of cold 95% ethanol (12 h, 4 °C) for isolation of the polysaccharides. All experiments were performed at least in duplicate.

2.3. GC–MS analysis

GC analyses were performed on an Agilent Technologies 6890N Network gas chromatograph coupled to an Agilent Technologies 5973 Network quadrupole mass selective spectrometer and provided with a split/splitless injection port. Helium was the carrier gas, at a linear velocity of 38 cm/s. Compounds were separated on a HP-5MS capillary column (Hewlett–Packard, Avondale, PA, USA) and successively on a SPB-1 capillary column (Supelco Ltd., Bellefonte, PA, USA), both 30 m × 0.25 mm ID, 0.25 μm film thickness. Column temperature was held at 40 °C for 5 min and increased to 75 °C at 4 °C/min, then at 8 °C/min to 250 °C holding 10 min. The injector temperature was 250 °C, and samples (1 μl) were injected in the splitless mode.

The temperatures of the ion source and the transfer line were 175 and 280 °C, respectively. Positive ion electron impact mass spectra were recorded at 70 eV ionisation energy, 2 scan/s.

2.4. FT-IR analysis

Polarized infrared external reflectance spectroscopy was used to obtain spectra in a single reflection mode using a nitrogen-purged thermo Nicolet Nexus Fourier transform infrared (FT-IR) spectrometer. The p-polarized light was incident at 80° from the surface normal. We used a narrow band mercury–cadmium–telluride detector for the reflected light and averaged 1024 scans, yielding the spectrum at a resolution of 4 cm^{-1}. The sample compartment was purged with dry CO_2-free air. Data manipulation was restricted to manually correcting the baseline for display purposes. Positions, band maxima and integral band areas were evaluated from the raw data.

2.5. Animals, diets and experimental design

Thirty-two Wistar rats seven weeks old and weighing ≈280 g were randomly divided into groups with approximately equal mean group body weights. The animals were housed in polycarbonate cages in a temperature controlled room (23 ± 1 °C) subjected to a 12-h light/dark cycle (lights on at 07:00 h) with free access to both food and water. After a 4-day adaptation period, the rats were randomly divided into four groups of eight animals each and fed an basic diet for 12 weeks (Control). The experimental groups were fed the same diet and orally given Yunzhi polysaccharides (100 mg/kg B.W., 150 mg/kg B.W. and 200 mg/kg B.W.). At the end of the experimental period, overnight-fasted animals were sacrificed under light ether anesthesia. Skeletal muscles were quickly excised, weighed and stored frozen immediately at −80 °C until analysis. The muscle tissue hydroxyproline (HYP) levels were determined by the method of Zamirul Hussain, Cross, Mustafa, and Bhatnagar (1976) after some pieces of samples were dried, weighed, digested in nitric acid/perchloric acid solution for 3 h. Muscle GAG content was quantified using the 1,9-dimethylmethylene blue assay (Suzuki, Suzuki, Nakamura, & Koizumi, 1976).

2.6. Real-time PCR and Western blot analysis

Specific primers were designed to selectively amplify β-actin and I collagens. The primer sequences and conditions for β-actin were as previously published (Ignatius et al., 2005).

Real-time PCR was performed in 96-well 0.2 ml thin-wall PCR plates using the iCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA) and carried out with QuantiTect SYBR Green PCR Master Mix (Qiagen), which contained HotStarTaq DNA Polymerase, QuantiTect SYBR Green PCR Buffer and SYBR Green I. The real-time PCR mixture contained 1× QuantiTect SYBR Green PCR Master Mix, 0.3 μM primer pairs and 1 μl cDNA in a total volume of 25 μl. The mixture was heated initially at 95 °C for 15 min in order to activate HotStarTaq DNA Polymerase and then followed by 40 cycles with denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 60 s.

I collagens protein level was performed using Western blot analysis.

2.7. Statistical analysis

Data are presented as means ± SD. One-way or two-way analysis of variance (ANOVA) was used for statistical analyses of data obtained within the same group of rats and between groups of rats, respectively. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. GC–MS and FT-IR analysis

The Yunzhi polysaccharides showed a single and symmetrically sharp peak. Total carbohydrate content was determined to be 95%. The polysaccharides were composed of glucose and mannose as detected by GC in the ratio of 4.4:1 (Fig. 1).

Yunzhi polysaccharides were measured using FT-IR spectroscopy. The absorption spectra of the polysaccharides are shown in Fig. 2. Among several peaks in the 3400–1000 cm^{-1} region, the polysaccharides-related absorption peaks at 1153, 1336, 1460, 1663, 1712 and 2927 cm^{-1}. In addition to assigning these peaks to vibrations of OH, we assign the peaks at 3400, 2900, 1712, 1336 and 1153 cm^{-1} to the vibrations of CH_2 asymmetric stretching, CH_2 symmetric stretching, C═O stretching, CH_2 deformation and CH_3 deformation, respectively (Kuo & Lai, 2009; Sun, Mark Lawther, & Banks, 1996). The bands in the region of 1000–500 cm^{-1} are due to the vibrations of C—N and other bands.

3.2. Pharmacological function of Yunzhi polysaccharides

Compared with normal rats (group I), treatment with Yunzhi polysaccharides (100, 150 or 200 mg/kg B.W.) produced a significant increase in hydroxyproline and glycosaminoglycan contents in rats of groups II, III and IV (Table 1). These results suggest that

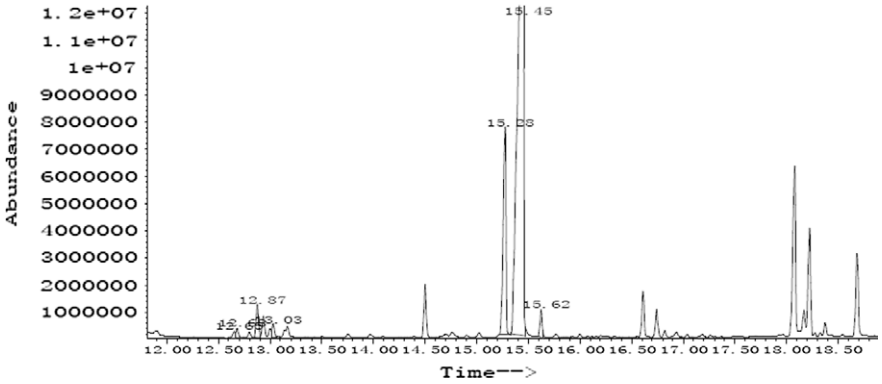

Fig. 1. GC–MS analysis of Yunzhi polysaccharides.

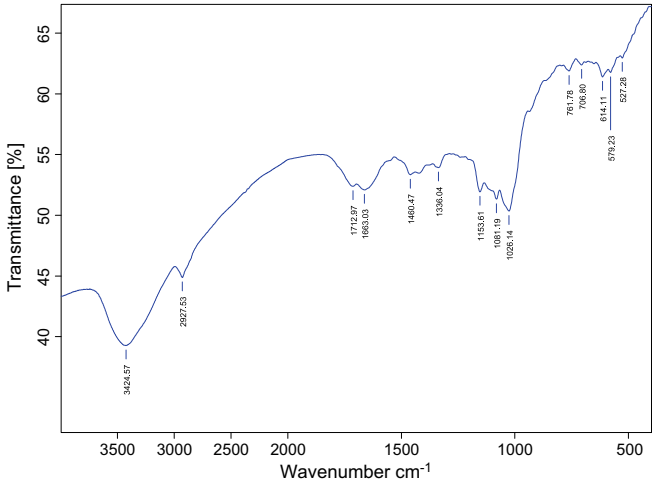

Fig. 2. FT-IR analysis of Yunzhi polysaccharides.

Table 1
Effect of Yunzhi polysaccharides on hydroxyproline and glycosaminoglycan levels.

Group	Hydroxyproline (HYP)	Glycosaminoglycan (GAG)
control	15.31 ± 0.31	24.28 ± 0.19
polysaccharides-treatment (100 mg/kg B.W.)	16.21 ± 0.15*	25.07 ± 0.35*
polysaccharides-treatment (150 mg/kg B.W.)	16.82 ± 0.22**	25.88 ± 0.24**
polysaccharides-treatment (200 mg/kg B.W.)	16.99 ± 1.86**	27.03 ± 0.20**

* $P < 0.05$, compared with control group rat.
** $P < 0.01$, compared with control group rat.

Table 2
Effect of Yunzhi polysaccharides on I collagens mRNA/β-actin and I collagens protein/GAPDH.

Group	I collagens mRNA/β-actin	I collagens protein/GAPDH
control	0.31 ± 0.02	0.68 ± 0.01
polysaccharides-treatment (100 mg/kg B.W.)	0.37 ± 0.01**	0.74 ± 0.02**
polysaccharides-treatment (150 mg/kg B.W.)	0.39 ± 0.01**	0.77 ± 0.02**
polysaccharides-treatment (200 mg/kg B.W.)	0.41 ± 0.02**	0.81 ± 0.02**

** $P < 0.01$, compared with control group rat.

Yunzhi polysaccharides can significantly ($P < 0.05$, $P < 0.01$) improve bone quality in a dose-dependent manner.

Compared with normal rats (group I), treatment with Yunzhi polysaccharides (100, 150 or 200 mg/kg B.W.) produced a significant increase in I collagens mRNA/β-actin expression and I collagens protein/GAPDH content in rats of groups II, III and IV (Table 2). These results suggest that Yunzhi polysaccharides can significantly ($P < 0.05$, $P < 0.01$) improve bone quality in a dose-dependent manner.

4. Conclusion

The polysaccharides were composed of glucose and mannose as detected by GC in the ratio of 4.4:1. Among several peaks in the 3400–1000 cm^{-1} region, the polysaccharides-related absorption peaks at 1153, 1336, 1460, 1663, 1712 and 2927 cm^{-1}. In our results, Yunzhi polysaccharides supplementation could significantly enhance hydroxyproline, glycosaminogly, I collagens mRNA/β-actin expression and I collagens protein/GAPDH contents in rats. This indicated that it could improve bone quality in a dose-dependent manner.

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