Carbohydrate Polymers 146 (2016) 388–395

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antioxidative and renoprotective effects of residue polysaccharides from *Flammulina velutipes*

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ARTICLE INFO

Article history:
Received 17 January 2016
Received in revised form 10 March 2016
Accepted 23 March 2016
Available online 26 March 2016

Keywords:
Antioxidative effects
Diabetic nephropathy
Flammulina velutipes
Renoprotective effects
Residue polysaccharides

ABSTRACT

Three extractable polysaccharides including Ac-RPS, Al-RPS and En-RPS were extracted from the residue of *Flammulina velutipes* and their antioxidative and renoprotective effects on STZ-induced mice were investigated. Biochemical and antioxidant analysis showed that the En-RPS had potential effects in decreasing the serum levels of CRE, BUN, ALB and GLU significantly, increasing the renal activities of SOD, CAT and GSH-Px remarkably, and reducing the renal contents of MDA prominently. Furthermore, the histopathological observations also displayed that En-RPS could alleviate kidney damage. These results demonstrated that En-RPS extracted from the residue of *F. velutipes* possessed potent antioxidant activities, and could be used as a promising therapeutic agent for inhibiting the progression of diabetic nephropathy. In addition, the monosaccharide compositions of these three RPS were also analyzed.

1. Introduction

It has been reported that over five million tons of mushroom residues were produced in China annually (Zhu, Sheng, Yan, Qiao, & Lv, 2012). However, serious problems including waste of resources and environmental pollution have been exposed owing to the low-efficiency utilization of mushroom residues. The mushroom residue, a kind of byproduct of mushroom cultivation containing mushroom mycelium and high levels of substance such as trace elements of Fe, Ca, Zn and Mg, cellulose, hemicellulose, lignin, carbohydrate, crude protein and fat, has been received more and more attentions in recent years (Zhu et al., 2012; Paredes et al., 2009). Strategically, there has been a desired interest in the exploitation of value-added products based on mushroom residue.

Mushroom polysaccharides, one of the most important components isolated from fungus, have been aroused much attentions due to the abundant pharmacological activities including antioxidant, immunomodulatory, anti-inflammatory, antihyperglycemic, and antihyperlipidemic (Gao, Wang, Wang, & Wang, 2013; Li, Lu, Zhang, Lu, & Liu, 2008; Liu, Sun, Rao, Su, & Yang, 2013). Besides, the natural mushroom polysaccharides were superior to the synthetic antioxidants owing to their non-toxicity, local accessibility and environment friendly (Argo et al., 2014). Hence, there is a considerable desiderating to process further research and development of natural polysaccharides.

Flammulina velutipes, one of the most popular edible fungi, has been widely cultivated in the world (Johansen, Harris, Rychly, & Ergul, 2014). Researchers have conducted on anti-proliferation, hepatoprotective and immunomodulatory activities of polysaccharides isolated from the fruiting bodies or mycelia of *F. velutipes* (Ma et al., 2015; Yang et al., 2012; Pang et al., 2007; Wu et al., 2014). Thus far, none of the above applications have been investigated about polysaccharides isolated from the residue of *F. velutipes*.

It has been reported that the DN is one of the major complications in diabetes patients characterized by hyperglycemia and pathological alterations of morphology and function of kidney (Reeves & Andreoli, 2000). Many evidence have demonstrated that ROS can react with membrane lipids and produce cell damage (Bhatia, Madhu, Gambhir, & Prabhu, 2003; Fridlyand & Philipson,

Abbreviation: Ac-RPS, acidic residue polysaccharides; ALB, albumin; Al-RPS, alkalic-RPS; Ara, arabinose; GLU, blood glucose; CAT, catalase; CRE, creatinine; DN, diabetic nephropathy; En-RPS, enzymatic-RPS; GC, gas chromatography; Glu, glucose; Gal, galactose; GSH-Px, GSH peroxide; MDA, malondialdehyde; Man, mannose; MC, model control group; NC, normal control group; ROS, reactive oxygen species; RPS, residue polysaccharides; Rha, rhamnose; Rib, ribose; STZ, streptozotocin; SOD, superoxide dismutase; BUN, urea nitrogen; Xyl, xylose.

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http://dx.doi.org/10.1016/j.carbpol.2016.03.071
0144-8617/

2005), and lots of evidences in both experimental and clinical studies have indicated that the excessive production of ROS can be regarded as a widely accepted participant in the development, progression and pathogenesis of kidney damage (Fridlyand & Philipson, 2005). Besides, the antioxidant and pre-oxidant properties of polysaccharides have been demonstrated to be involved in the hpyerglycemic-related activities, suggesting that mushroom polysaccharides have potential activity for the prevention of kidney damage (Johansen, Harris, Rychly, & Ergul, 2005; Young, Tate, Lightbody, McMaster, & Trimble, 1995). Therefore, it is quite necessary and significative to explore potential oral anti-hyperglycaemic agents for preventing and treating DN.

The objective of present work was conducted to evaluate the antioxidative and renoprotective effects of the three RPS of *F. velutipes* including Ac-RPS, Al-RPS and En-RPS on diabetic mice induced by STZ-injection, aiming to get the better understanding of the possible renoprotective mechanisms and their health benefits. In addition, the monosaccharide compositions of these three polysaccharides were also analyzed.

2. Materials and methods

2.1. Materials and chemicals

Dried residue of the *F. velutipes* was collected from Shandong Ronfun Mushroom Co., Ltd. (Dongying, China). Standard monosaccharides and STZ were purchased from Sigma Chemicals Company (St. Louis, USA). The diagnostic kits for antioxidant indicators were purchased from Nanjing Jiancheng Bioengineering Insititute (Nanjing, China). All other chemicals used in present work were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.2. Preparation of three RPS

The dried residue of the *F. velutipes* was crushed into powder using a disintegrator (Shanghai, China). The three kinds of Ac-, Al-, and En-RPS were extracted with HCl (0.5 M, 1:10, w/v), NaOH (0.5 M, 1:10, w/v) at 80 °C and snailase solution (4%, 1:4, w/v) at 37 °C for 6 h, respectively. After centrifugation (3600 r/min, 15 min), the supernatant was mixed with 3 volumes of ethanol (95%, v/v) at 4 °C overnight. The precipitate was considered as RPS and the carbohydrates contents were determined by the phenol-sulfuric acid colorimetric method, using glucose as standard (Chaplin & Kennedy, 1994). After removing the protein by the method of Sevag (Staub, 1965), the precipitate was collected and lyophilized to yield Ac-, Al-, and En-RPS, which were used for further experiments.

2.3. Animal experiments

The male Kunming strain mice (20 ± 2 g) were purchased from Taibang Biologic Products Inc. (Taian, China) and used in present study. All mice were acclimated for 5 days and they had free access to standard chow and tap water *ad libitum* under conditions of temperature (22 ± 2 °C), humidity (55 ± 5%) and a 12 h light-dark cycle. The experiment was approved by animal ethics guidelines of the Institutional Animal Ethics Committee.

After 5 days of accommodation, the diabetes was induced by a triple-successive intraperitoneal injection with STZ (80 mg/kg, freshly prepared in citrate buffer solution, 0.1 M, pH 4.5) except NC. After 12 h fasting, all STZ-injected mice were assessed by measuring glucose levels in tail vein. The mice with glucose levels over 13.3 mM were considered as diabetic (Aksoy, Vural, Sabuncu, & Aksoy, 2003). Then, NC mice received citrate buffer alone and diabetic mice were randomly divided into MC which received distilled water only, and nine dosage groups (five mice per group) which were treated with Ac-, Al-, and En-RPS, each polysaccharide included three dosages of 800, 400 and 200 mg/kg, respectively. After a 15-consecutive gavage, all mice were weighed and sacrificed under anesthetic treatment on an empty stomach. Blood samples from retrobulbar vein were centrifuged at 10,000 r/min for 10 min and the serums were obtained. The serum levels of CRE, BUN and ALB were measured by using automatic biochemical analyzer (ACE, USA). The kidneys were rapidly excised, weighed (kidney index was calculated by kidney weight/body weight × 100) and homogenized immediately in phosphate buffer solutions (PBS, 0.2 M, pH 7.4) at a proportion of 1:9 (w/v). The homogenates were centrifuged (4000 r/min) at 4 °C for 10 min and the supernatants were collected for further biochemical analysis. The renal SOD, CAT and GSH-Px activities and MDA contents were analyzed by commercial kits according to the instructions. Moreover, the pathological kidney cortex was preserved at 4% formalin, which was observed under a light microscope at magnifications of 400×.

2.4. Acute toxicity study

Another twelve male Kunming strain mice were collected for the acute toxicity study. Totally three groups with four in each group were processed and the mice were gavaged with Ac-, Al- and En-RPS at increasing dosages of 300, 600, 900 and 1500 mg/kg, respectively. The mice were observed continuously for gross behavioral changes, toxic symptoms and mortality during the whole feeding period.

2.5. Monosaccharides composition analysis

The monosaccharide compositions of RPS was determined by GC on a GC-2010 (Shimadzu, Japan) using the previous method (Zhu et al., 2012) with some slight modifications. The dried RPS (0.5 g) was hydrolyzed with 1.8 mL trifluoroacetic acid (TFA, 2 M) at 110 °C for 4 h. Then, the hydrolyzed product was cooled to room temperature and methanol was added to remove TFA. The sample (1 mL), including 0.3 mL ammonium hydroxide (12 M) and 0.3 mL $NaBH_4$ (dissolved in ammonium hydroxide) were incubated at 45 °C for 1 h, and the reaction was terminated at pH 6–7 adjusting by glacial acetic acid (0.4 mL). After being acetylated by mixing with 1-methylimidazole (1 mL) and acetic anhydride (4.5 mL), the sample (1 mL) was extracted by dichloromethane (3 mL). Monosaccharide compositions were identified by comparison with seven standard sugars including Glu, Gal, Rib, Ara, Man, Rha and Xyl. According to the chromatogram, the relative molar ratios of monosaccharides were analyzed by the area normalization method (Deng, Liu, Li, & Yu, 2000).

2.6. Statistical analysis

Results were reported as the mean ± standard deviation (SD). The differences between multiple groups were evaluated by one-way ANOVA and paired sample T test (SPSS 16.0 software package, USA). $P < 0.05$ was considered as significant difference.

3. Results

3.1. Effects of RPS on GLU levels, body weights and kidney index

The date concerning GLU levels and body weights were shown in Table 1. The GLU levels of all groups were initially similar. However, after being injected with STZ, MC group mice showed diabetic as evidenced by significantly ($P < 0.01$) increased in the GLU levels compared to NC group mice. The treatment of three RPS at different dosages exhibited significant decrease in GLU levels in the diabetic mice when compared to that in MC group mice. The GLU levels of