Journal of Food and Nutrition Research, 2014, Vol. 2, No. 7, 419-424
Available online at http://pubs.sciepub.com/jfnr/2/7/15

DOI:10.12691/jfnr-2-7-15

Antihyperglycemic Activity of Exopolysaccharide Produced by Mushroom *Pleurotus ferulae* with Submerged Liquid Culture on Streptozotocin-induced Diabetic Rats

Jinnchyi Wang[1], Chihchung Wu[2], Yukuei Chen[3], Chenyu Chen[4], Shuhui Hu[5,*], Suejoan Chang[6,*]

[1]Department of Food Science and Technology, Tajen University, 20, Weishin Rd., Shinerh Village, Yenpu Shiang, Pingtung, Taiwan
[2]Department of Nutrition and Health Sciences, Chang Jung Christian University, 396, Sec. 1, Changrong Rd., Gueiren Dist., Tainan, Taiwan
[3]Department of Food Science and Nutrition, Mei Ho University, 23, Pingguang Rd., Neipu Shiang, Pingtung, Taiwan
[4]Innovative Digi Tech-Enabled Applications & Services Institute, Institute for Information Industry, 3F-3, No 2, Fuxin 4th Rd., Kaohsiung, Taiwan
[5]Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University, 100, Shihchuan 1st Road, Kaohsiung, Taiwan
[6]Department of Life Sciences, National Cheng Kung University, No. 1, University Rd., Tainan, Taiwan
*Corresponding author: suhuhu@cc.kmu.edu.tw;sjchang@mail.ncku.edu.tw

Received June 07, 2014; Revised July 20, 2014; Accepted July 24, 2014

Abstract The production of many edible and medicinal mushrooms has been steadily increasing, because of several of their physiological effects. In the present study, we investigated the antihyperglycemic activity of large exopolysaccharide molecules (PSF), which are produced in the fermented broth of ferula mushroom *Pleurotus ferulae*, on streptozotocin-induced diabetic rats. All experimental rats were divided into 6 groups consist of 8 rats. The diabetic rats were fed a diet containing PSF for 6 weeks at a dose of 30 mg (PSFL-group), 90 mg (PSFM-group), or 250 mg/kg body weight (PSFH-group) daily, respectively. The fasting blood glucose level of the PSFH-group was the lowest among all 3 PSF-fed groups. Insulin levels increased and HbA1c levels decreased significantly for the three PSF-fed groups in comparison with negative control group during period of breeding. The PSFH-group's low-density lipoprotein cholesterol and triglyceride levels were lower than those of other groups. A dose-dependent effect revealed that the exopolysaccharide of *P. ferulae* might mitigate hyperglycemia at the highest dose of 250 mg/kg body weight.

Keywords: *exopolysaccharide, Pleurotus ferulae, antihyperglycemic activity, submerged culture, streptozotocin-induced diabetic rats*

Cite This Article: Jinnchyi Wang, Chihchung Wu, Yukuei Chen, Chenyu Chen, Shuhui Hu, and Suejoan Chang, "Antihyperglycemic Activity of Exopolysaccharide Produced by Mushroom Pleurotus ferulae with Submerged Liquid Culture on Streptozotocin-induced Diabetic Rats." *Journal of Food and Nutrition Research*, vol. 2, no. 7 (2014): 419-424. doi: 10.12691/jfnr-2-7-15.

1. Introduction

Mushrooms are a fat-free foodstuff that is rich in nutrients, and contain a variety of ingredients that have physiological effects and other prophylactic or therapeutic effects without significant toxicity [1,2,3]. Some studies have suggested that water-soluble polysaccharides (WSPs) should be extracted from mushrooms because they have physiological effects, such as inducing the proliferation of certain types of immune cells [4,5,6] and antihyperglycemic activity [7]. WSPs from *Hericium* spp., *Pleurotus citrinopileatus*, and other mushrooms have been demonstrated to possess antigenotoxic and immunoenhancing properties as well as inhibit artificially induced pulmonary metastasis both in vivo and in vitro [8,9]. Furthermore, it has been presumed that the immune-enhancing effect is associated with the antihyperglycemic activity. A few herbs or foodstuffs with hypoglycemic effects might independently enhance insulin secretion. Immune system activation caused by administering WSPs might repair a partially damaged pancreas or prevent its growing worse among diabetic rats with autoimmune damage, and cause a higher insulin secretion level. Jiang et al. treating STZ-induced diabetic rats with WSP, showed insulin secretion enhancement, and inhibition of increased the blood glucose level [10]. β-glucans from edible mushrooms, such as pleuran or lentinan, have been recognized since a very long time as anticarcinogens that also aid fat metabolism [11].

In the previous study, we also found the *Pleurotus ferulae* have the antigenotoxicity and antimutagenicity

effects [12]. Although treatment of diabetes with synthetic drugs takes effect rapidly, it occasionally induces unwanted side effects. Researchers seek natural products with no side effects that can effectively lower blood glucose level. Several mushrooms were shown to decrease blood glucose and increase blood insulin in streptozotocin-induced diabetes rats [13,14]. Lv et al. found that the *Coprinus comatus* mushroom elevated glycogen synthesis in hypoglycemic mice and inhibited gluconeogenesis in normal mice [15]. The *Grifola frondosa* mushroom, rich in vanadium, was found to decrease blood glucose level in alloxan-induced hyperglycemic mice [16]. Other studies have focused on the effects of lowering insulin resistance and improving glucose tolerance [17].

P. ferulae is a wild strain of mushroom with a delicious taste and several nutrients, nicknamed the "beef-liver mushroom of the prairie" [18]. It grows near or on the stem of the plant ferula and is also called the ferulae mushroom. In the present study, the large exopolysaccharide molecule was extracted from fermented *P. ferulae* broth to examine its antihyperglycemic activity in STZ-induced diabetic rats.

2. Materials and Methods

2.1. Spawn

Pleurotus ferulae (Pleurotaceae, Agaricomycetideae) was cultivated in potato dextrose broth (Difco, Detroit, USA) for 8 days at 23°C. This was used as the inoculum.

2.2. Preparation of Exopolysaccharide

P. ferulae was cultivated in GPY broth (glucose 30 g/L, peptone 10 g/L, yeast extract 2 g/L, pH 5.5) on a shaker at 100 rpm, at 25°C for 20 days. The fermented broth was filtered, and the water-soluble exopolysaccharide was extracted from the filtrate by ethyl alcohol precipitation. The exopolysaccharide was then deproteinated by the trichloroacetic acid method. After further isolation by ultrafiltration (Militan system, ELO 04; Millipore Co. USA), molecules of > 10^5 Da were obtained and lyophilized. This is the material refer to as PSF in this study.

2.3. Composition and Molecular Weight of PSF

The polysaccharide content of PSF was determined by the phenol-sulfuric acid method [19]. The composition of PSF was determined by thin-layer and gas-liquid chromatography (HP 5890C) as described by Wang et al. [9]. The molecular weight of PSF was determined by high-performance gel-permeation chromatography using the LC-10AT HPLC system (Algilent, USA) equipped with a TSK GMPW column (300 mm × 0.8 mm, TOSOH, Japan) and an RI detector (Algilent, USA). In each run, a sample solution (20 μL) was injected, and eluted with 0.1 M sodium nitrate solution at a flow rate of 0.8 mL/min. A standard curve was established with T-series dextrans (MW: 41,100–2,000,000 Da, Sigma Co.) [20].

2.4. Animals

Experiments with Sprague–Dawley rats (5 weeks old, 150 ± 10 g, male, purchased from the BioLasco Co., Ltd. Taiwan) were conducted in the qualified animal breeding room of the animal center at our institute. The Animal Care and Research Ethics Committee of Tajen University reviewed and approved the experimental protocol. Each rat was housed individually under standard environmental conditions (23 ± 1°C, humidity 55 ± 5%) with 12-hr light:12-hr dark cycles. The rats had free access to water and a semi-purified diet (AIN-76, ICN Biochemicals Inc. CA, USA) for 7 days.

2.5. Antihyperglycemic Activities of PSF

One group of 8 normal rats was used as the control group and received a regular AIN-76 diet. The other experimental rats were subjected to 18 hr of fasting, and then injected intraperitoneally with 65 mg/kg body weight STZ (Sigma, St. Louis, Mo, USA) dissolved in 0.1 M cold sodium citrate buffer (pH 4.5), to induce diabetes. In 8 days later after STZ injection, these diabetic rats, which had fasting blood glucose level above 220 mg/dL, were divided into 5 groups consist of 8 rats each. Two groups of STZ-induced diabetic rats were used as negative and positive controls. The former received a PSF-free regular diet and the latter were fed regular diet with insulin injected. The other three groups of STZ-induced diabetic rats were fed a regular AIN-76 diet with 1 mL of aqueous PSF administered by oral gavage, at a daily dose of 30 mg (the PSFL group), 90 mg (the PSFM group), or 250 mg (the PSFH group) per kg body weight. At the end of 42 days of PSF feeding, 4 rats from each group were killed. The remaining rats in the 3 PSF-fed groups were switched to either a regular PSF-free or an insulin-free diet until Day 49, at which point they were also killed. Blood samples were collected without anesthesia from the tail vein into heparinized fluoride oxalate-coated tubes, and analyzed immediately. Fasting blood glucose was determined every 7 days, following 18 h of food deprivation. Blood glucose was determined using kits (Randox Lab. Ltd. Co., UK). The glycosylated hemoglobin (HbA1c) was determined by Primus Ultra2 HPLC system (Primus Co., Kansas City, Kansas, USA) and the values were expressed as % of Hb [21]. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were determined using the Kodak Ektachem DT 60 Analyzer (Eastman Kodak Co., NY, USA) [2]. The blood insulin level was determined using the Elecsys Insulin kits (Roche Diagnostics Co., Indiana, USA) [22].

2.6. Statistical Analysis

Data are shown as the mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests were conducted using SPSS (version 10) software. Differences with $p<0.05$ were considered significant.

3. Results

The molecular weight of PSF was 6.3×10^5 Da, and its major compositions were galactose, mannose, and glucose at a ratio of about 5.2:2.2:1. PSF yield was 0.914% (w/v, dry weight).

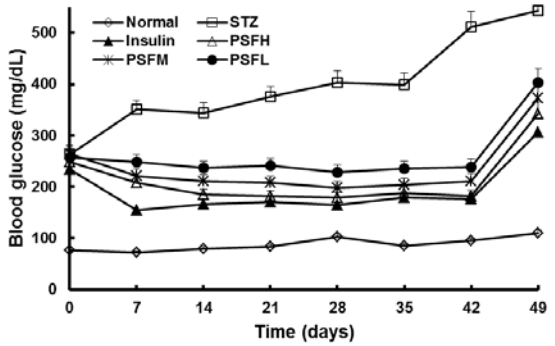

Figure 1. Effect of PSF feeding on blood glucose in induced-diabetic rats. The three groups of 8 STZ-induced diabetic rats were each fed with PSF at a daily dose of 30, 90, or 250 mg/kg, body weight (they were named as PSFL, PSFM, or PSFH, respectively) for 42 days, after which the PSF feeding stopped. Moreover, the rats were fed with a regular PSF-free or an insulin-free diet until Day 49. STZ and Insulin are the negative and positive control groups, respectively. Data are shown as the mean ± SD. Differences with $p<0.05$ were considered significant

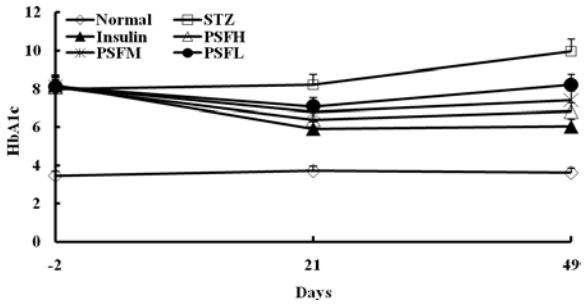

Figure 2. The changes of HbA1c while fed PSF or PSF-free diet. The experiment was described in the legend of Figure 1. The Day -2 was denoted as the 2 days before rats were fed PSF. The PSF-free regular diet was fed from Day 43 to Day 49

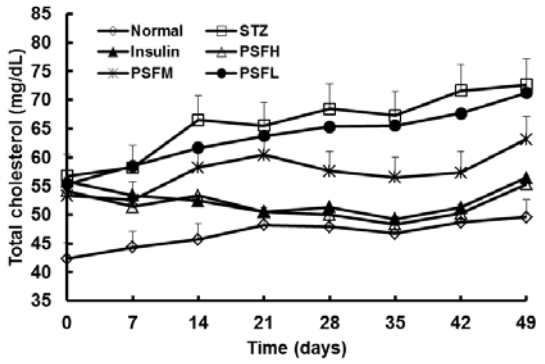

Figure 3. Effect of PSF feeding on total cholesterol in induced-diabetic rats. The experiment was described in the legend of Figure 1

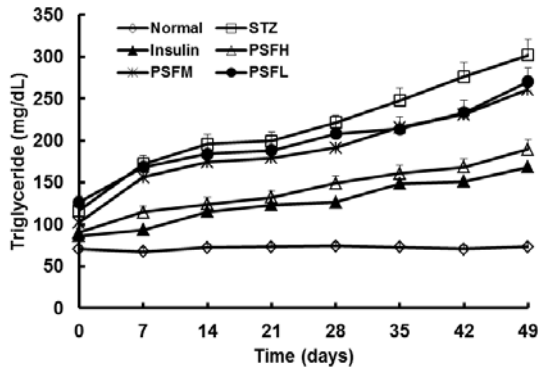

Figure 4. Effect of PSF feeding on triglyceride in induced-diabetic rats. The experiment was described in the legend of Figure 1

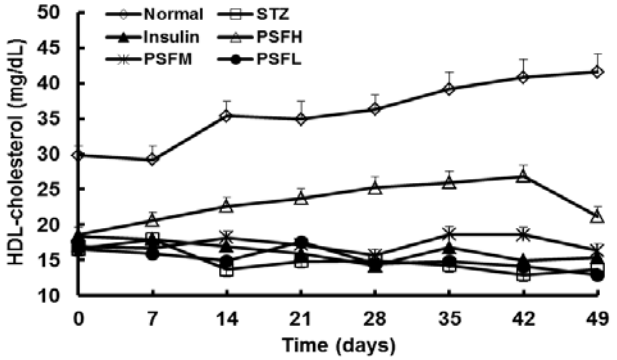

Figure 5. Effect of PSF feeding on HDL-cholesterol in induced-diabetic rats. The experiment was described in the legend of Figure 1

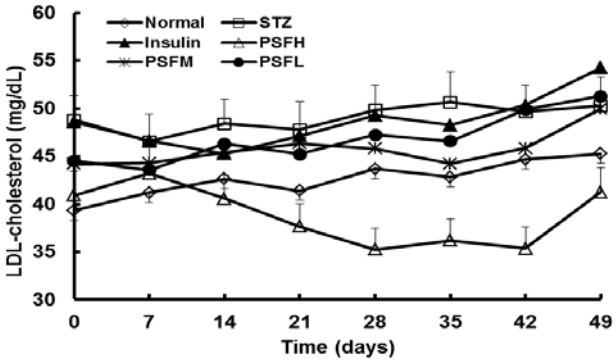

Figure 6. Effect of PSF feeding on LDL-cholesterol in induced-diabetic rats. The experiment was described in the legend of Figure 1

Compared with groups fed PSF-free diets beginning on Day 0 (negative control or normal group), the PSFH group showed a decrease in fasting blood glucose of 16.0%, 25.9%, and 27.0% on Days 7, 14, and 21, respectively (Figure 1). For the PSFL group, the fasting blood glucose decreased by 3.1%, 7.8%, and 5.9% on Days 7, 14, and 21, respectively. The fasting blood glucose in the PSF-fed groups showed a static trend of percentage decrease over 28 days, although the percentage decreases for Days 21 and 28 showed no significant difference ($p>0$ .05). The percentage decrease in fasting blood glucose was approximately 24%–28% for the PSFM and PSFH groups in comparison with Day 0 and 28. During days 28–42, the percentage decrease in fasting blood glucose in the PSFH group was approximately 2.6–3.8 times that in the PSFL group. The fasting blood glucose level in the PSFH and positive control groups was not significantly different ($p>0.05$) over 21 days. The fasting blood glucose level in the 3 PSF-fed groups were distinctly lower than that in the negative control group ($p<0.05$), and the level increased again in the 3 PSF-fed groups that were switched to a PSF-free diet. ΔHbA1c of the three PSF-fed groups were obviously lower than that of negative control group ($p<0.05$) on the 21th day (Figure 2). The ΔHbA1c of PSFL-group was about one time that in the PSFH-group.

Although the TC level in the PSFH and PSFM groups was significantly lower than that in the negative control group ($p<0.05$), the TC level in the PSFL and negative control groups was very similar (Figure 3). There was no significant difference between TC level in the PSFH and positive control groups ($p>0.05$). The change in serum TG level in the PSFH group was similar to that in the positive control group, but was higher than that in the PSFL group during feeding (Figure 4), as expected. When PSF was fed

for 28 days, there was no significant difference in the serum TG level between the PSFH and positive control groups ($p>0.05$). On Day 42, the serum TG level in the PSFH group was lower than that in the PSFL and negative control groups by 45.4% and 70.0%, respectively, and higher than that in the positive control group by 13.1%. The serum TG level in the 3 PSF-fed groups increased again significantly, once the rats were no longer fed PSF. In addition, the PSFL group had the highest percentage increase, which was approximately 1.82 times the percentage increase in the PSFH group. The serum HDL-C level in the PSFH group was distinctly higher than that in the PSFM and PSFL groups ($p<0.05$) during feeding (Figure 5), and there was no significant difference between the PSFM and PSFL groups ($p>0.05$). The HDL-C level in the PSFM, PSFL, and positive control groups was similar during days 7-42. The serum HDL-C level in the PSFH group was higher than that in the negative control group by an average of 40.0% for Days 14–42. The LDL-C level in the PSFH group decreased by approximately 3% after 28 days of feeding compared to the level after 7 days (Figure 6). When PSF feeding stopped on Day 42, PSF-free regular diet continued till to Day 49, which resulted in the LDL-C level decreased by 26.9% in the PSFH group. The serum LDL-C level in the other 2 PSF-fed and positive control groups was not significantly different ($p>0.05$). The insulin level of all 3 PSF-fed groups showed an increase (Figure 7). Among those fed PSF for 21 and 42 days, the insulin level of PSFH group had the highest percentage increase. Although the insulin level was not significantly different between the PSFH and PSFM groups, there was an obvious difference between these 2 groups and the PSFL group after feeding for 21 or 42 days ($p<0.05$). When the groups were fed a PSF-free regular diet after 42 days, the insulin level in the 3 PSF-fed groups decreased. The percentage decrease in the insulin level in PSFH group was lower than that in the other 2 PSF-fed groups ($p<0.05$). The average body weight change in the PSFH group was similar to that in the positive control group, which was fed a regular insulin diet, but was significantly greater than that in the PSFM or PSFL group ($p<0.05$) (Figure 8). Figure 9 shows that there was no significant difference in the food intake between the positive control group and the three PSF-fed groups during the experimental period ($p>0.05$). The food intake of the PSFM and PSFL groups was higher, however, than the normal group ($p<0.05$).

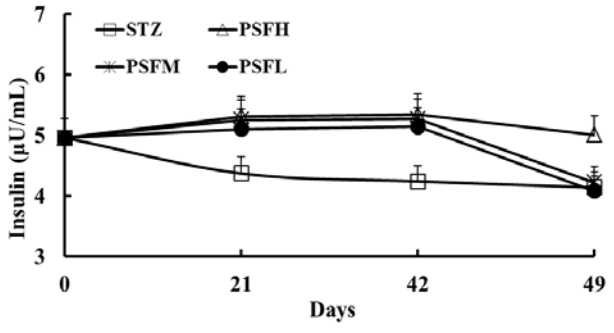

Figure 7. The percentage changes of insulin while fed PSF or PSF-free diet. The experiment was described in the legend of Figure 1. The PSF-free regular diet was fed from Day 43 to Day 49. The concentration unit of insulin was represented as μU/mL

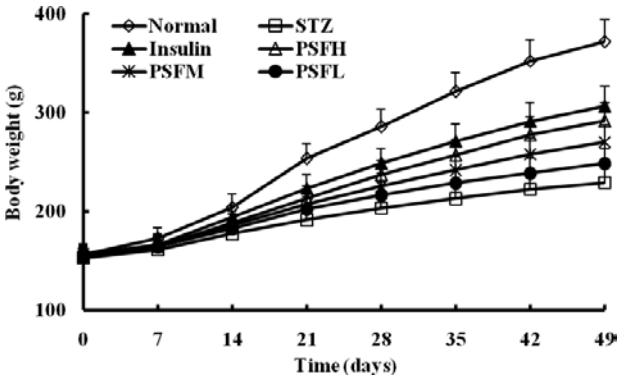

Figure 8. Effect of PSF feeding on body weight in induced diabetic rats. The experiment was described in the legend of Figure 1

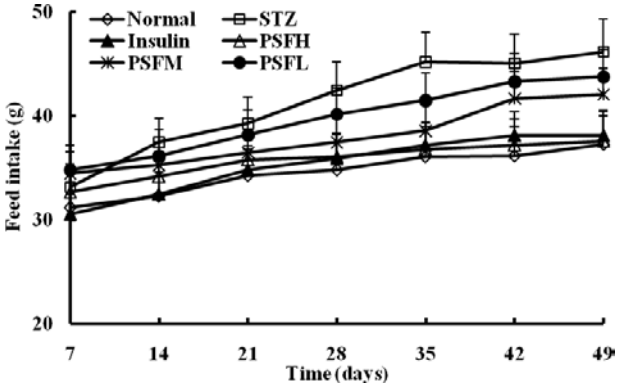

Figure 9. Effect of PSF feeding on feed intake in induced-diabetic rats. The experiment was described in the legend of Figure 1

4. Discussion

The composition or stereo-skeletal structures of exopolysaccharides are likely related to their physiological effects [23]. Many mushrooms with physiological effects contain heteropolysaccharides composed mainly of mannose or galactose. In this study, the PSF is a heteropolysaccharide. Fhernanda et al. found that the *Agaricus bisporus* produced high molecular weight glucan that contained glucose and galactose (2.4%) at a ratio of 4.1:1 [24]. Exopolysaccharide produced by *Agaricus brasiliensis* in submerged culture consists principally of mannose (58.7%), and galactose (21.4%) [25].

High glucose levels in diabetes cause complications to develop. Heart disease and stroke, kidney disease, eye disease, periodontal disease, foot problems, skin disorders, diabetic neuropathy and nerve problems are among serious complications of diabetes [26]. This study shows that the PSFH group experienced a stronger antihyperglycemic effect than the PSFL or PSFM groups. After PSF supplementation was stopped, the blood glucose and HbA1c levels in the 3 PSF-fed groups increased significantly. Monitoring blood glucose and HbA1c levels are critical measurements for controlling the onset of serious complications of diabetes. Increased HbA1c levels correlate with glucose intolerance in diabetes [27]. This serves as a marker for average blood glucose levels over prolonged periods of time [28]. The higher doses of PSF-fed could be help to alleviate the increasing HbA1c level. STZ-induced metabonomic alternations were related to promotion of gluconeogenesis,

disruption of lipid metabolism, and elevation of fatty acid [29]. Rats with STZ-induced diabetes fed the exopolysaccharide extracted from the medicinal mushroom *Phellinus baumii* had blood glucose level that was 52.3% lower than that in rats in the negative control group [30]. In a similar study, the WSP from *P. citrinopileatus*, 0.15 mg/kg body weight, displayed an obvious antihyperglycemic effect when administered to STZ-induced diabetic rats [7].

If mechanisms regulating blood glucose enhance the production of insulin or insulin receptors, then these mechanisms should also increase the penetration of blood glucose into identical cells. The abnormal secretion of insulin should hinder blood glucose from entering cells and make the blood glucose level rise. The present study shows that PSFH group had more effective insulin secretion than other experimental groups. These effects of PSF on insulin secretion are also consistent with the results obtained with other species of medicinal mushrooms. The exopolysaccharide of *Laetiporus sulphureus*, another edible mushroom, was also increase proliferation and insulin secretary function of rat insulinoma cells [31]. The basidiomata of *P. eryngii* were fed to db/db mice, and as a result, the serum glucose level decreased, and the insulin level rose. This promoted insulin secretion and caused the serum glucose level to drop [32]. This wealth of evidence suggests that exopolysaccharides derived from mushrooms have the ability to induce the insulin production, and help lower the glucose level in diabetic rats.

Some foodstuffs can regulate lipid metabolism, possibly activating lipase activity or suppressing the increase of triglycerides. Some compositions of polysaccharide might be able to enhance *de novo* hepatic lipogenesis as a result of reduced activity of lipogenic enzymes. Moreover, polysaccharide-enriched diet could down-regulated the genes of hepatic cholesterol synthesis [33]. *P. eryngii* has a clear inhibitory activity against pancreatic lipase in vitro. The water extract of the mushroom suppresses the plasma and chylomicron triacylglycerol level, but has no effect on lipoprotein lipase activity [34]. Although the TG level of the PSFH group had no significant improvement, the level was lower than that in the other 2 PSF-fed groups. This result suggests that PSF might suppress the increase of TG level at a dose over 90 mg/kg body weight. In general, insulin-dependent diabetic patients would have abnormal blood chylomicron and VLDL level. In this study, the highest dose (250 mg/kg body weight) of PSF caused the HDL-C level to rise obviously, but LDL-C level was lowered. Therefore, if PSF is fed at the highest dose it might be able to alleviate the increased level of harmful lipids. Cho et al. suggested the exopolysaccharide of *Tremella fuciformis* and *Phellinus baumii* might improve insulin sensitivity by regulating lipid metabolism [35]. In addition, lipids, proteins, and carbohydrates in a body might also exhibit abnormal metabolism in diabetes, while might further cause triglycerides and VLDL to rise excessively, and HDL level decrease abnormally. Jeong et al. found that the basidiomata of *Agaricus bisporus*, fed to STZ-induced diabetic rats, and significantly reduced the plasma glucose and TG level by 24.7% and 39.1%, respectively [36].

5. Conclusions

Large PSF (>10^5 Da) extracted from the fermented broth of *Pleurotus ferulae* showed an antihyperglycemic activity in the STZ-induced diabetic rats at a dose of 250 mg/kg daily. We suggest that PSF might be able to improve hyperglycemia and regulate blood lipid profiles in STZ-induced diabetic rats.

Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

WSP, water soluble polysaccharide; ESP, exopolysaccharide; PSF, exopolysaccharide extracted from fermented broth of *Pleurotus ferulae*; STZ, streptozotocin; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides

References

[1] Aidaa FMNA., Shuhaimia M., Yazidb M. and Maaruf AG., "Mushroom as a potential source of prebiotics: a review," *Trends in Food Science Technology*, 20. 567-575. 2009.

[2] Hu SH., Liang ZC., Chia YC., Lien JL., Chen KS., Lee MY. and Wang JC., "Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*," *Journal of Agricultural and Food Chemistry*, 54. 2103-2110. 2006.

[3] Jedinak A., Dudhgaonkar S., Jiang J., Sandusky G. and Sliva D., "*Pleurotus ostreatus* inhibits colitis-related colon carcinogenesis in mice," *Internatioal Journal of Molecular Medicine*, 26. 643-650. 2010.

[4] Barbara S. and Julita R., "Health-promoting potential of edible macromycetes under special consideration of polysaccharides: a review," *European Food Research and Technology*, 234. 369-380. 2012.

[5] Wong KH., Lai CKM. and Cheung PCK., "Immunomodulatory activities of mushroom sclerotial polysaccharides," *Food Hydrocolloids*, 25. 150-158. 2011.

[6] Inafuku M., Nagao K., Nomura S., Shirouch B., Inoue N. and Nagamori N., "Protective effects of fractional extracts from *Panellus serotinus* on non-alcoholic fatty liver disease in obese, diabetic db/db mice," *British Journal of Nutrition*, 107. 639-646. 2012.

[7] Hu SH., Wang JC., Liang ZC., Lien JL. and Liaw ET., "Antihyperglycemic effect of extract from fermented broth of *Pleurotus citrinopileatus*," *Applied Microbiology and Biotechnology*, 70. 107-113. 2006.

[8] Wang JC., Hu SH., Wang JT., Chen KS. and Chia YC., "Hypoglycemic effect of extract of *Hericium erinaceus*," *Journal of the Science of Food and Agriculture*, 85. 641-646. 2005.

[9] Wang JC., Hu SH., Liang ZC. and Yeh CJ., "Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* with submerged culture and its antitumor effect," *Applied Microbiology and Biotechnology*, 67. 759-766. 2005.

[10] Jiang S., Du P., An L., Yuan G. and Sun Z., "Anti-diabetic effect of *Coptis chinensis* polysaccharide in high-fat diet with STZ-induced diabetic mice," *International Journal of Biological Macromolecules*, 55. 118-122. 2013.

[11] Iris L., Lili N., Dana L., Irena P., Yitzhak H. and Betty S., "Glucans from the edible mushroom *Pleurotus pulmonarius* inhibit colitis-associated colon carcinogenesis in mice," *Journal of Gastroenterology*, 47. 504-518. 2012.

[12] Hu SH., Liang ZC., Lien JL., Wang JC. and Chang SJ., "Antioxidant and antigenotoxicity activities of extracts from liquid submerged-culture culinary-medicinal ferula mushroom

Pleurotus eryngii Lenzi (DC.) Quel. var. *ferulae* (Agaricomycetideae)," *International Journal of Medicinal Mushrooms*, 11. 395-408. 2009.

[13] Liu YT., Sun J., Luo ZY., Rao SQ., Su YJ. and Xu RR., "Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity," *Food and Chemical Toxicology*, 50. 1238-1244. 2012.

[14] Wasser SP., "Current findings, future trends, and unsolved problems in studies of medicinal mushrooms," *Applied Microbiology and Biotechnology*, 89. 1323-1332. 2011.

[15] Lv Y., Han L., Yuan C. and Guo J., "Comparison of hypoglycemic activity of trace elements absorbed in fermented mushroom of *Coprinus comatus*," *Biological Trace Element Research*, 131. 177-185. 2009.

[16] Cui B., Han L., Qu J., Lv Y., "Hypoglycemic activity of *Grifola frondosa* rich in vanadium," *Biological Trace Element Research*, 131. 186-191. 2009.

[17] Preuss HG., Echard B., Bagchi D., Perricone NV. and Zhuang C., "Enhanced insulin-hypoglycemic activity in rats consuming a specific glycoprotein extracted from maitake mushroom," *Molecular and Cellular Biochemistry*, 306.105-113. 2007.

[18] Akyuz M. and Kirbag S., "Effect of various agro-residues on nutritive value of *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi," *Journal of Agricultural Science*, 16. 83-88. 2010.

[19] Chaplin MF. and Kennedy JF., "Carbohydrate analysis. A practical approach," 2nd ed. IRL Press Ltd., USA, 1994.

[20] Wu X., Mao G., Fan Q., Zhao T., Zhao J. and Li F., "Isolation, purification, immunological and anti-tumor activities of polysaccharides from *Gymnema sylvestre*," *Food Research International*, 48. 935-939. 2012.

[21] Gosselin RC., Carlin AC. and Dwyre DM., "Comparison of the BioRad Variant and Primus Ultra2 high-pressure liquid chromatography (HPLC) instruments for the detection of variant hemoglobins." *International Journal of Laboratory Hematology*, 33. 159-167. 2011.

[22] Sapin R., Le Galudec V., Gasser F., Pinge M. and Grucker D., "Elecsys insulin assay: Free insulin determination and the absence of cross-reactivity with insulin Lispro," *Clinical Chemistry*, 47. 602-625. 2001.

[23] Li N., Fang JC., Wong JH., Ng TB. and Jiang Y., "Isolation and identification of a novel polysaccharide-peptide complex with antioxidant, anti-proliferative and hypoglycaemic activities from the abalone mushroom," *Bioscience Reports*, 32. 221-228. 2012.

[24] Fhernanda R., Smiderle GL., Sassaki J van A., Marcello I., Harry JW. and Leo JLD van G., "High molecular weight glucan of the culinary medicinal mushroom *Agaricus bisporus* is an α-glucan that forms complexes with low molecular weight galactan," *Molecules*, 15. 5818-5830. 2010.

[25] Lima LF., Habu S., Gern JC., Nascimento BM., Parada JL. and Noseda MD., "Production and characterization of the exopolysaccharides produced by *Agaricus brasiliensis* in submerged fermentation," *Applied Microbiology and Biotechnology*, 151. 283-294. 2008.

[26] Srinivasan S. and Pari L., "Antihyperlipidemic effect of diosmin: A citrus flavonoid on lipid metabolism in experimental diabetic rats," *Journal of Functional Foods*, 5. 484-492. 2013.

[27] Prabakaran D. and Ashokkumar N., "Antihyperglycemic effect of esculetin modulated carbohydrate metabolic enzymes activities in streptozotocin induced diabetic rats," *Journal of Functional Foods*, 4. 776-783. 2012.

[28] Abdulrazaqa NB., Cho MM., Win NN., Zaman R. and Rahman MT., "Beneficial effects of ginger (*Zingiber officinale*) on carbohydrate metabolism in streptozotocin-induced diabetic rats," *British Journal of Nutrition*, 108. 71194-71201. 2012.

[29] Xu WX., Wu JF., An YP., Xiao CN., Hao FH., Liu HB., Wang YL. and Tang HR., "Streptozotocin-induced dynamic metabonomic changes in rat biofluids," *Journal of Proteome Research*, 11. 3423-3435. 2012.

[30] Hwang HJ., Kim SW., Lim JM., Joo JH., Kim HO. and Kim HM., "Hypoglycemic effect of crude exopolysaccharides produced by a medicinal mushroom *Phellinus baumii* in streptozotocin-induced diabetic rats," *Life Science*, 76. 3069-3080. 2005

[31] Hwang HS., Lee SH., Baek YM., Kim SW., Jeong YK. and Yun JW., "Production of extracellular polysaccharides by submerged mycelial culture of *Laetiporus sulphureus* var. *miniatus* and their insulinotropic properties," *Applied Microbiology and Biotechnology*, 78. 419-429. 2008.

[32] Kim JI., Kang MJ., Im J., Seo YJ., Lee YM. and Song JH., "Effect of king oyster mushroom (*Pleurotus eryngii*) on insulin resistance and dyslipidemia in db/db mice," *Food Science and Biotechnology*, 19. 239-242. 2010.

[33] Kim H., Bartley GE., Rimando AM. and Yokoyama W., "Hepatic gene expression related to lower plasma cholesterol in hamsters fed high-fat diets supplemented with blueberry peels and peel extract," *Journal of Agricultural and Food Chemistry*, 58. 3984-3991. 2010.

[34] Mizutani T., Inatomi S., Inazu A. and Kawahara E., "Hypolipidemic effect of *Pleurotus eryngii* extract in fat-loaded mice," *Journal of Nutrition Science and Vitaminology*, 56. 48-53. 2010.

[35] Cho EJ., Hwang HJ., Kim SW., Oh JY., Baek YM. and Choi JW., "Hypoglycemic effects of exopolysaccharides produced by mycelial cultures of two different mushrooms *Tremella fuciformis* and *Phellinus baumii* in ob/ob mice," *Applied Microbiology and Biotechnology*, 75. 1257-1265. 2007.

[36] Jeong SC., Jeong YT., Yang BK., Islam R., Koyyalamudi SR. and Pang G., "White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats," *Nutrition Research*, 30. 49-56. 2010.