Journal of Medicinal Plants Research Vol. 5(32), pp. 6963-6967, 30 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.644

Full Length Research Paper

Inhibitory effects on α-glucosidase and hypoglycemic effects of the crude polysaccharides isolated from 11 edible fungi

Chun Xiao[1,2,4], Qingpin Wu[2]*, Jianbin Tan[3], Wen Cai[3], Xiaobing Yang[2] and Jumei Zhang[2]

[1]South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China.
[2]Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, State Key Laboratory of Applied Microbiology (Ministry-Guangdong Province Jointly Breeding Base),South China, Guangdong Institute of Microbiology, Guangzhou 510070, China.
[3]Department of Toxicology, the Center for Disease Control and Prevention of Guangdong Province, Guangzhou 510020, China.
[4]Graduate School of the Chinese Academy of Sciences, Beijing 100049, China.

Accepted 18 October, 2011

This objective of this study was to investigate the inhibitory effect of polysaccharides extracted from 11 edible fungi on α-glucosidase *in vitro* and their hypoglycemic effect in type 2 diabetic mellitus (DM) mice. *In vitro,* α-glucosidase activity was determined by measuring the p-nitrophenol released from p-Nitrophenyl-α-D-Glucopyranoside at 400 nm. *In vivo,* Kunming mice with steptozotocin-induced combination high fat diet fed were used. At the end of the study, blood serum glucose and insulin of the mice were measured. *In vitro*, the crude polysaccharides of *Phellinus igniarius* and *Phellinus chrysoloma* showed strong α-glucosidase inhibitory activities of 79.72 and 89.26 %, respectively. *In vivo*, Serum glucose levels significantly differed (P < 0.01) between the polysaccharides dose-treated groups and the DM group. *Grifola frondosa* crude polysaccharides showed the greatest hypoglycemic effect in lowering the diabetic blood serum glucose level by 50.09%. The crude polysaccharides of *Ganaderma lucidum, Ganaderma sinense, G. frondosa, P. igniarius and P. chrysoloma* significantly increased the insulin sensitivity index (ISI) compared with the DM group (P < 0.05). The crude polysaccharides of *P. igniarius and P. chrysoloma* demonstrated high α-glucosidase inhibitory activities. All 11 edible fungi polysaccharides significantly decreased the fasting serum glucose level in type 2 DM mice. Furthermore, *G. lucidum, G. sinense, G. frondosa, P. igniarius and P. chrysoloma* crude polysaccharides were able to facilicate the utilization of insulin and improve insulin resistance.

Key words: Edible fungi, polysaccharides, hypoglycemic activity, type 2 diabetes mellitus mice, α-glucosidase inhibitory activity.

INTRODUCTION

Diabetes is a metabolic disorder (Mohler et al., 2009). The world prevalence of diabetes among adults (aged 20 to 79 years) was 6.4% (affecting 285 million adults) in 2010, and is expected to increase to 7.7% (439 million adults) by 2030 (Shaw et al., 2010). Diabetes mellitus (DM) is classified into two types of type 1 (insulin-dependent DM, IDDM) and type 2 (non-insulin-dependent DM, NIDDM) with type 2 DM represents above 90% of all known cases of DM.

Mushrooms have long been medicinally used to treat with diabetic in Asian countries. Previous clinical research has shown that the polysaccharide fractions of *G. frondosa* (SX fraction) and *G. lucidum* (Ganopoly) have hypoglycemic action in patients with type 2 DM (Lindequist et al., 2005). Type 2 DM animals experiments have identified several natural extracts from edible fungi, such as Fraction X (FXM) and MT-α-glucan obtained from *G. frondosa*, an acidic polysaccharide (TAP) was isolated from a hot water extract of the fruiting bodies of

*Corresponding author. E-mail: 13823320821@139.com, xiaochun960@hotmail.com. Tel: +86-20-8768-0942.

Tremella aurantia, water-soluble polysaccharide (FA) from that of *Auricularia auricular* and *G. lucidum* water-extract (Manohar et al., 2002; Lei et al., 2007; Kiho et al., 1995; Yuan et al., 1998; Seto et al., 2009).

One of the therapeutic approaches to decreasing of postprandial hyperglycemia is to retard glucose absorption by the inhibition of α-glucosidase in the brush border of enterocytes lining the intestinal villi. α-Glucosidase inhibitors such as acarbose were first used in the early 1990s and owing to high cost and limited efficacy, the use of this class remains low (Mohler et al., 2009). Safer natural agents that inhibit α-glucosidase are hence better alternatives for the treatment of type 2 DM. However, except for *G. frondosa* (Matsuura et al., 2002), literature on edible fungi showing α-glucosidase inhibitory activity are currently limited. This study investigated the inhibitory effects of polysaccharides extracted from 11 edible fungi on α-glucosidase *in vitro* and their hypoglycemic effect in type 2 DM mice.

MATERIALS AND METHODS

Edible fungi

G. lucidum, *G. sinense*, *Ganoderma applanatum*, *Lentinus edodes*, *G. frondosa*, *Agaricus blazei*, *Coriolus versicolor*, *Hericium erinaceus*, *A. auricular*, *P. chrysoloma*, *P. igniarius* were collected from Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application.

Isolation of polysaccharides

The dried fruiting bodies of edible fungi were homogenized to a fine powder. The powder was mixed with distilled water at a ratio of 1:20 (w/v) and extracted at about 80°C. The mixture was filtered and centrifuged (5000×g, 10 min, 4°C) The supernatant was concentrated under a reduced pressure, not exceeding 60°C, and treated with 4 volumes of absolute ethanol at 4°C overnight for precipitation. The resulting precipitate was dispersed in water, dialyzed to yield the crude polysaccharides. The carbohydrate content of the fraction was determined by the phenol–sulfuric acid method (Dubois et al., 1956). The concentrations of the crude polysaccharides were adjusted to 10 mg ml^{-1}.

Determination of α-glucosidase inhibitory activities

α-Glucosidase (EC_3.2.1.20) from yeast *Saccharomyces cerevisiae* and *p*-Nitrophenyl-α-D-Glucopyranoside (pNPG) as a synthetic substrate of α-glucosidase were purchased from Sigma Chemical Co. Acarbose was obtained from Bayer Healthcare Co., Ltd.

Inhibition *of α- glucosidase activity*: α-glucosidase (50 µl, 0.8 U) with 100 µl of the various edible fungi polysaccharides (100 mg ml^{-1}) was incubated in 67 mM of phosphate buffered saline (PBS) at 37°C for 10 min and then mixed with 100 µl of 116 mM pNPG as a substrate in PBS to start the reaction. The reaction was incubated at 37°C for 30 min and stopped by adding 5 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the *p*-nitrophenol release from pNPG at 400 nm. Acarbose was used as the positive control and the PBS as the negative. The following formula was used:

Inhibitory of α-glucosidase Activity Percent (%) = (1-A) / A_0 ×100 %

Where *A* is the optical density at 400 nm(OD_{400}) of the edible fungi polysacchariedes or acarbose, and A_0 is the OD_{400} of the negative control.

Animals and breeding conditions

All six-week-old male Kunming mice (body weight, 24 to 26 g) and standard pellet diet were provided by the Guangdong Province Experimental Animals Center [Production Certificate No. scxk (Yue) 2008-0011. Quality Certificate No. 0069870. Experimental Animals License No. syxk (Yue) 2008-0002]. All protocols complied with the guide for the care and use of laboratory animals and were approved by the Animal Care Committee of the Center for Disease Control and Prevention of Guangdong Province. The standard pellet diet and water were given ad libitum. The animals were maintained under a constant 12 h light / 12 h dark cycle and an environmental temperature of 21 to 23°C.

Hypoglycemic activity

The mice were adapted to the environment for 7 days and then fasted overnight before receiving an intraperitoneal (ip) injection of freshly prepared streptozotocin (STZ: 35 mg kg^{-1} body weight, dissolved in citrate buffer at pH 4.5). They were then administered a high-fat diet (Srinivasan et al., 2005). After 2 weeks, the mice were made to fast for 5 h, and their fasting blood serum glucose levels were determined. The mice with blood serum glucose level above 11.1 mM were considered to be diabetic and were used in the experiment. All the mice were randomly divided into four groups with 8 mice in each group namely: normal control group (NC group), diabetic control group (DM group), positive control and diabetic dose-treated group (100 mg kg^{-1} d^{-1}, ip). All mice were allowed free access to drinking water and pellet diet ad libitum for 7 days, and the control groups received the same dose of saline. At the end of the experiment, fasting blood was withdrawn from the orbital sinus of the mice, which were subsequently sacrificed.

Determination of blood serum glucose

Blood serum was separated by centrifugation (1000×g, 5 min, 4°C). Blood serum glucose was determined using a commercially available glucose kit (Jiancheng Bioengineering Institute, Nanjing, China) based on the glucose–oxidase method (Stevens, 1971).

Determination of blood serum insulin

Blood serum insulin was determined by ^{125}I-labled insulin radioimmunoassay kit**.** Insulin sensitivity index (ISI) was calculated as -(lnFINS+ lnFPG) (Li and Pan 1995), where FPG (mM) is fasting plasma glucose and FINS (mU l^{-1}) is fasting insulin.

Statistic analysis

All data were expressed as mean ± SD. Statistical significance was analyzed by one way analysis of variance (ANOVA) analysis in software SPSS 11.5, whereas intergroup comparisons were performed using the least significant difference (LSD) test. $P < 0.05$ was considered statistically significant.

Table 1. Effect of crude polysaccharides isolated from edible fungi on blood serum glucose in type 2 DM mice.

Group	Dose (mg kg^{-1} d^{-1})	Blood serum glucose (mM)	
		0d	7d
Normal	100	7.09±0.31	7.23±0.62**
DM Control	100	11.27±1.03	16.23±3.45**
Acarbose	100	11.64±1.29	12.26±2.39**
G. frondosa	100	11.51±1.29	8.10±1.58**αα
L. edodes	100	11.76±1.66	10.80±1.55**
A. blazei	100	11.50±1.10	12.15±1.95**
A. auricula	100	11.44±0.84	10.82±3.19**
H. erinaceus	100	11.23±0.93	10.69±1.32**
C. versicolor	100	11.29±1.00	9.54±1.30**αα
G. Lucidum	100	11.37±1.06	10.14±1.95**α
G. sinense	100	11.43±1.14	10.64±1.86**
P. igniarius	100	11.54±1.31	11.31±2.02**
P. chrysoloma	100	11.59±1.32	9.79±0.90**αα
G. applanatum	100	11.78±1.64	12.22±1.54**

(Values represent means ± SD (n=8). Significant difference from the DM control: * $P < 0.05$, ** $P < 0.01$. Significant from the positive acarbose: $^{\alpha}P < 0.01$).

Table 2. Effect of crude polysaccharides isolated from edible fungi on blood serum insulin in type 2 DM mice.

Group	Dose (mg kg^{-1} d^{-1})	FINS (mU l^{-1})	ISI
Normal	/	9.45±3.37**	–4.16±0.31**
DM Control	100	21.68±7.22	–5.74±0.43
Acarbose	100	21.09±8.98	–5.45±0.52
G. applanatum	100	20.18±6.70	–5.44±0.28
A. blazei	100	26.80±4.07	–5.80±0.16
P. igniarius	100	17.19±8.62	–5.10±0.68**
A. auricula	100	21.21±7.50	–5.33±0.64
L. edodes	100	20.15±5.34	–5.36±0.29
H. erinaceus	100	24.72±8.84	–5.48±0.46
G. sinense	100	15.82±6.67	–5.09±0.52**
G. lucidum	100	18.82±5.56	–5.19±0.18*
P. chrysoloma	100	13.84±6.01*	–4.81±0.44**
C. versicolor	100	25.70±6.86	–5.42±0.38
G. frondosa	100	12.13±2.07**	–4.53±0.30**

(FINS: fasting insulin ; ISI: insulin sensitivity index;Values represent means±SD (n=8);Significant from the DM control：*p< 0.05 , **p< 0.01)

RESULTS

Effects of edible fungi polysaccharides on blood serum glucose

As shown in Table 1, no significant difference was initially observed between the dose-treated groups and DM control group. After 7 days, the blood serum glucose of mice in all the dose-treated groups significantly decreased compared with those in the DM group ($P < 0.01$). This indicated that all the edible fungi crude polysaccharides were able to significantly decrease fasting serum glucose level. *G. frondosa* crude polysaccharides showed the greatest hypoglycemic effect in lowering the diabetic blood serum glucose level by 50.09%, close to the normal blood serum level.

Effects of edible fungi polysaccharides on blood serum insulin

As shown in Table 2, the blood serum insulin level of DM

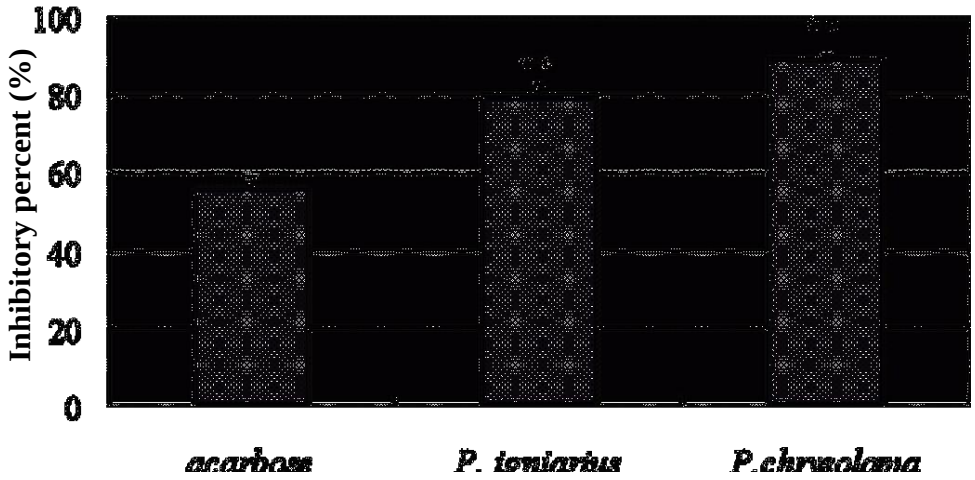

Figure 1. α-Glucosidase inhibitory activity of the edible fungi crude polysaccharides. (Values represent means±SD (n=3); Significant from the positive control acarbose：*p< 0.05 , **p< 0.01).

group was significantly higher compared with the NC group, *G. frondosa* and *P. chrysoloma* crude polyssaccharides significantly decreased the blood serum insulin level ($P < 0.05$). ISI was significantly higher in the *G. Lucidum*, *G. sinense*, *G. frondosa, P.igniarius* and *P. chrysoloma* polysaccharide-treated groups compared with the DM control group ($P < 0.05$).

Determination of α-glucosidase inhibitory activities

As shown in Figure 1, acarbose, *P. igniarius* and *P. chrysoloma* crude polysaccharides displayed 56.0, 79.72 and 89.26% of α-glucosidase inhibitory activities, respectively. Furthermore, *P. igniarius* and *P. chrysoloma* showed more prominent inhibitory activities than that of the positive control acarbose ($P < 0.01$).

DISCCUSSION

The α-glucosidase activity experiment is often used to screen the anti-diabetic substances *in vitro*. Based on the results of this study, *P. chrysoloma* was the most active α-glucosidase inhibitor, followed by *P. igniarius*. Furthermore, both fungi showed more prominent inhibitory activities than the positive control acarbose ($P < 0.01$). This is an important information, as *P. igniarius* and *P. chrysoloma* crude polysaccharides have not been previously evaluated for their α-glucosidase inhibitory activity. So *P. igniarius* and *P. chrysoloma* crude polysaccharides are promising antidiabetic α-glucosidase inhibitor candidate.

Type 2 DM is characterized by hyperglycemic and insulin resistance(Moller 2001). Animal models have been extensively used for the study of type 2 DM, such as Zucker diabetic fatty rat, OLETF rat as well as *db/db* mouse, *ob/ob* mouse, KK mouse, fat-fed STZ-treated rat, NSY mouse (Rees and Alcolado, 2005). The present study used mice receiving combination of high fat diet fed and low-dose STZ (Srinivasan et al., 2005). The results revealed that 11 varieties of edible fungi polysaccharides had hypoglycemic activity with different potencies, confirming their traditional indications. No study on the hypoglycemic activity in type 2 diabetic mice of *G. sinense*, *G. applanatum*, *L. edodes*, *A. blazei*, *C. versicolor*, *H. erinaceus*, *P. chrysoloma* and *P. igniarius* crude polysaccharides have been previously reported. In the present study, it is noteworthy that *G. frondosa* crude polysaccharide singnificantly decreased the diabetic blood serum glucose level by 50.09%, close to the normal blood serum level.

FXM obtained from *G. frondosa*, favourably influences glucose/insulin metabolism in KK mice (Manohar et al., 2002), and water-soluble polysaccharide (FA) from that of *A. auricula* has been reported to be effective in lowering plasma glucose level in KK mice. *G. lucidum* water extracts have exhibited hypoglycemic effects in *db/db* mice (Seto et al., 2009).

Insulin resistance is considered to be the significant pathogenic factor in type 2 DM and an obvious target for anti-diabetic medication (Olefsky and Nolan, 1995). Thus, more effective treatment of type 2 DM mainly relies on how to overcome insulin resistance. The crude polysaccharides of *G. lucidum*, *G. sinense*, *G. frondosa, P. igniarius* and *P. chrysoloma* can facilitate the utilization of insulin and improvement insulin resistance. MT-α-glucan from the fruit body of *G. frondosa* has an antidiabetic effect in KK mice, which might be related to its effect on insulin receptors (that is, increasing insulin sensitivity and ameliorating insulin resistance of peripheral target tissues) (Lei et al., 2007). FXM from the

fruit body of *G. frondosa* lowers circulating glucose and insulin concentrations, suggesting an effect on peripheral insulin sensitivity (Kubo et al., 1994; Manohar et al., 2002).

This study reveals that *G. frondosa* polysaccharide is potent anti-diabetic agent with considerably strong hypoglycemic activity and high potency of increasing insulin sensitivity, *P. igniarius* and *P. chrysoloma* crude polysaccharide are promising antidiabetic α-glucosidase inhibitor candidate in the light of their dual-action target of improvement insulin resistance and α-glucosidase inhibitory activity.

ACKNOWLEDGEMENTS

This work was supported by Guangdong Provincial Department of Science and Technology, Project No. 2009A080209002, 2009B090300298 and 2009A020700006.

REFERENCES

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem., 28: 350-356.

Kiho T, Morimoto H, Sakushima M, Usui S, Ukai S (1995). Polysaccharides in fungi 35. Anti diabetic activity of an acidic polysaccharide from the fruiting bodies of *Tremella aurantia*. Biol. Pharm. Bull., 18: 1627-1629.

Kubo K, Aoki H, Nanba H (1994). Antidiabetic Activity Present in the Fruit Body of Grifola-Frondosa (Maitake). Biol. Pharm. Bull., 17: 1106-1110.

Lei H, Ma X, Wu WT (2007). Anti-diabetic effect of an α-glucan from fruit body of maitake (*Grifola frondosa*) on KK-Ay mice. J. Pharm. Pharmacol., 59: 575-582.

Li G, Pan X (1995). A new insulin-sensitivity index for epidemiological study. Chin. Med. J. (Engl), 108: 55-56.

Lindequist U, Niedermeyer THJ, Julich WD (2005). The pharmacological potential of mushrooms. Evid-Based Compl. Alt., 2: 285-299.

Manohar V, Talpur NA, Echard BW, Lieberman S, Preuss HG (2002). Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice. Diabetes. Obes. Metab., 4: 43-48.

Matsuura H, Asakawa C, Kurimoto M, Mizutani J (2002). α-alucosidase inhibitor from the seeds of balsam pear (Momordica charantia) and the fruit bodies of Grifola frondosa. Biosci. Biotech. Bioch., 66: 1576-1578.

Mohler ML, He Y, Wu Z, Hwang DJ, Miller DD (2009). Recent and emerging antidiabetes targets. Med. Res. Rev., 29: 125-195.

Moller DE (2001). New drug targets for type 2 diabetes and the metabolic syndrome. Nature, 414: 821-827.

Olefsky JM, Nolan JJ (1995). Insulin-Resistance and Non-Insulin-Dependent Diabetes-Mellitus - Cellular and Molecular Mechanisms. Am. J. Clin. Nutr., 61: 980-986.

Rees DA, Alcolado JC (2005). Animal models of diabetes mellitus. Diabetic Med., 22: 359-370.

Seto SW, Lam TY, Tam HL, Au ALS, Chan SW, Wu JH, Yu PHF, Leung GPH, Ngai SM, Yeung JHK (2009). Novel hypoglycemic effects of Ganoderma lucidum water-extract in obese/diabetic (+db/+db) mice. Phytomedicine (Jena), 16: 426-436.

Shaw JE, Sicree RA, Zimmet PZ (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pr., 87: 4-14.

Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res., 52: 313-320.

Stevens JF (1971). Determination of Glucose by an Automatic Analyser. Clin. Chim. Acta., 32: 199-205.

Yuan Z, He P, Cui J, Takeuchi H (1998). Hypoglycemic effect of water-soluble polysaccharide from Auricularia auricula-judae Quel. on genetically diabetic KK-Ay mice. Biosci. Biotechnol. Biochem., 62: 1898-1903.