Biol Trace Elem Res (2009) 127:177–182
DOI 10.1007/s12011-008-8231-7

A Comparison of Hypoglycemic Activity of Three Species of Basidiomycetes Rich in Vanadium

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Received: 23 June 2008 / Accepted: 27 August 2008 /
Published online: 20 September 2008

Abstract The hypoglycemic activity of fermented mushroom of three fungi of basidiomycetes rich in vanadium was studied in this paper. Alloxan- and adrenalin-induced hyperglycemic mice were used in the study. The blood glucose and the sugar tolerance were determined. After the mice were administered (ig) with *Coprinus comatus* rich in vanadium, the blood glucose of alloxan-induced hyperglycemic mice decreased ($p<0.05$), ascension of blood glucose induced by adrenalin was inhibited ($p<0.01$) and the sugar tolerance of the normal mice was improved. However, the same result did not occur in *Ganoderma lucidum* and *Grifola frondosa* group. Compared with *Ganoderma* rich in vanadium and *Grifola frondosa* rich in vanadium, the hypoglycemic effects of *Coprinus comatus* rich in vanadium on hyperglycemic animals are significant; it may be used as a hypoglycemic food or medicine for hyperglycemic people.

Keywords Vanadium · *Ganoderma lucidum* · *Coprinus comatus* · *Grifola frondosa* · Basidiomycetes

Introduction

Vanadium is normally present at very low concentrations ($<10^{-8}$ M) in virtually all cells in plants and animals. Vanadium compounds have the ability to imitate the action of insulin [1, 2]. Oral administration of inorganic vanadium salts has shown antidiabetic activity [3–5]. However, the toxicity associated with vanadium limits its role as a therapeutic agent for diabetic treatment [6, 7].

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In order to reduce the toxicity of vanadium, McNeill JH et al. [8] studied the coordination complexes of vanadium compounds with maltol or kojic acid as ligands. Sakurai et al. [9] also studied the long-term-acting insulin-mimetic vanadyl complexes of oxovanadium (IV). *Ganoderma lucidum* (*GL*), *Coprinus comatus* (*CC*), and *Grifola frondosa* (*GF*) are three fungi of basidiomycetes that have a long history of use in traditional Chinese medicine, which has been popularly used for its health-promoting properties. Their beneficial effects on glycemic control in diabetes, hypercholesterolemia, and immunostimulating activities have been reported [10–14]. Another important property of the edible mushroom is the ability to take up and accumulate trace metals such as cadmium, lead, arsenic, copper, nickel, silver, chromium, and mercury in the body or mycelium of the mushroom [15–17]. In order to reduce the toxicity of vanadium, vanadium could be taken up and accumulated by fermented mushroom of *Ganoderma lucidum*, *Coprinus comatus*, and *Grifola frondosa.* The purpose of this study was to investigate the hypoglycemic activity of three fungi of basidiomycetes rich in vanadium. The coactivity of vanadium at lower doses and edible mushroom could reduce vanadium-associated toxicity and maintain its effect.

Materials and Methods

Chemicals

Sodium vanadate was of analytical grade and purchased from Beijing Chemical Factory, China. Alloxan and adrenaline were of analytical grade and were purchased, respectively, from Sigma Co., Ltd. and Tianjin Amino acid Co., Ltd. Xiaoke pills were purchased from Jilin Liuhe Pharmaceutic Factory, China. Xiaoke pill is a kind of Chinese medicine used in the treatment of diabetes. It is composed of glibenclamide and several traditional Chinese herbs, including Radix Puerariae, Radix Rehmannia, Radix Astragali, Radix trichosanthis, Corn Stigma, Fructus Schisandrae, and Rhizoma Dioscoreae.

Fermented Mushroom

GL, *CC*, and *GF* were offered by the Shandong Academy of Agricultural Sciences (Jinan, China). The seed was grown at 28°C for 5 days on potato dextrose and agar slants medium (1,000 mL 20% potato extract liquid + 20.0 g dextrose + 20.0 g agar) and then maintained at 4°C in a refrigerator. Five to six pieces of the mycelia of mushroom were transferred from a slant into 250-mL Erlenmeyer flasks containing 100-mL liquid medium (20% potato extract liquid + 2.0% dextrose + 0.1% KH_2PO_4 + 0.05% $MgSO_2$). The culture was incubated at 27°C on a rotary shaker at 180 rpm for 7 days.

A 7-day-old liquid culture was homogenized using a sterilized blender and then inoculated to 500-mL Erlenmeyer flasks containing 300 mL of fermented culture medium (20% potato extract liquid + 2.0% dextrose + 0.1% KH_2PO_4 + 0.05% $MgSO_2$ + 0.6%$NaVO_3$). The volume of inoculum was 15 mL, which was then cultivated under the same condition. The 72-h-old fermented liquid culture was stirred by a homogenizer; an ampule was filled with 0.4 mL of them (0.6%$NaVO_3$) and then was sterilized in microwave oven for 3 min.

Animal

Kunming strain mice weighing 20–22 g were purchased from the Experimental Animal Center, Shandong University. The mice were maintained at room temperature under

alternating natural light–dark photoperiod and had access to standard laboratory food and fresh water ad libitum.

Blood Samples from Alloxan-Induced Hyperglycemic Mice

One hundred mice were fasted for 12 h and were then injected (iv) with alloxan (75 mg/kg) solution that was made with saline [18]. Forty-eight hours later, blood samples were collected from the tail veins of the mice. The blood glucose was analyzed with a Glucometer-4 (Bayer). Sixty hyperglycemic mice (the blood glucose level greater than 11.1 mmol/L) were selected and allocated equally into five groups: alloxan-induced hyperglycemic group, alloxan- and Xiaoke-pill-treated group, alloxan- and GL-treated group, alloxan- and CC-treated group, and the alloxan- and GF-treated group. The other 12 normal mice were injected (iv) with the normal saline and used as the control group. From then on, the six groups of mice were administered (ig) saline, Xiaoke pill (0.028 mg/kg per day), GL (2.4 mg/day vanadium), CC (2.4 mg/day vanadium), GF (2.4 mg/day vanadium), and saline respectively. The body weights of the mice were measured on the zeroth day, fifth day, tenth day, 15th day, and the 20th day. At the same time, after fasting the mice for12 h on the 20th day, blood samples were obtained from the tail veins to determine the blood glucose levels.

Blood Samples from Adrenaline-Induced Hyperglycemic Mice

Seventy-two healthy mice were allocated equally into six groups. Each group was administered (ig) with different materials just as the above experiment. On the 14th day, they were fasted overnight. After administration 1 h later, the former five groups were injected (sc) with adrenaline and the last group with saline: namely, adrenaline-induced hyperglycemic group, adrenaline–Xiaoke-pill-treated group, adrenaline–GL-treated group, adrenaline–CC-treated group, adrenaline–GF-treated group, and the control group. Blood samples from the tail vein of the mice were collected at the zeroth minute and 60th minute to determine blood glucose level just as above.

Blood Samples to Determine Sugar Tolerance

Sixty healthy mice were divided equally into five groups. Three groups was administrated (ig) GL, CC, and GF; the others were administrated (ig) saline. On the eighth day, after the last administration, the former four groups were injected (ip) with glucose (2 g/kg); the last group was injected (ip) with saline: namely, GL–glucose group, CC–glucose group, GF–glucose group, saline–glucose group, and saline–saline group (control group). Blood samples were obtained from the tail veins of the mice at 0, 30, 60, and 120 min, respectively. Blood glucose values were determined with Glucometer-4 (Bayer).

All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test [19]. The data represent means and standard deviations. The significant level of 5% ($p<0.05$) was used as the minimum acceptable probability for the difference between the means.

Results and Discussion

The body weights of the hyperglycemic mice induced by alloxan are presented in Fig. 1. Contrasted with the alloxan-induced hyperglycemic group, the body weights of mice in GL-

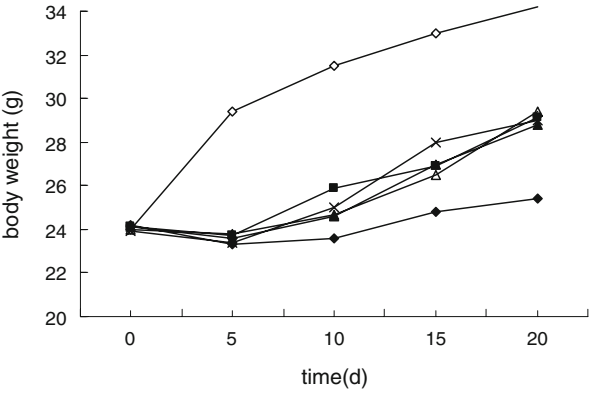

Fig. 1 Effects of basidiomycetes rich in vanadium on body weights of hyperglycemic mice (*empty diamonds* control group, *crosses* alloxan- and Xiaoke-pill-treated group, *empty triangles* alloxan- and GL-treated group, *filled triangles* alloxan- and CC-treated group, *filled squares* alloxan and GF-treated group, *filled diamonds* alloxan-induced hyperglycemic group)

treated group, CC-treated group, GF-treated group, and Xiaoke-pill-treated group increased gradually 10 days later. On the contrary, the body weights of mice in the alloxan-treated group did not increase significantly. All of the three species of fungi have a long history of use not only as traditional Chinese medicine but also as delicious food. *G. lucidum* has been popularly used for its health-promoting properties [10]. *G. frondosa* has recently attracted considerable attention for its various physiological activities [13]. *C. comatus*, on a dry weight basis, contains, on the average, 58.8% carbohydrate, 25.4% protein, and 3.3% fats, with the rest constituted of minerals [20]. It indicates that the three species of fungi could supplement nutrients to mice.

The results of blood glucose from hyperglycemic mice induced by alloxan are presented in Table 1. The levels of blood glucose decreased after administration of CC and the Xiaoke pill ($p<0.05$). However, the same result did not occur in GL and GF group or the alloxan group.

Adrenaline activates glycogenolysis and glyconeogenesis to elevate serum glucose level [21] and its effect is relatively rapid [22]. The results of blood glucose from hyperglycemic mice induced by adrenaline are presented in Table 2. It showed that, after administration (ig) of CC at 15 days, ascension of blood glucose induced by adrenaline was inhibited ($p<0.01$). However, the same results did not occur in the GL and GF group or the alloxan groups.

Table 1 Effect of Basidiomycetes Rich in Vanadium and Other Treatments on Blood Glucose Levels in Alloxan-Hyperglycemic Mice

Different groups	Blood glucose (mmol/L)
Alloxan-treated	20.2±3.1 a
Alloxan- and Xiaoke-pill-treated	11.1±3.2 b
Alloxan- and CC-treated	9.8±2.2 b
Alloxan- and GL-treated	17.6±3.0 a
Alloxan- and GF-treated	18.0±2.8 a
Control group	6.0±1.2

The different letters in the same column indicate a statistical difference ($p<0.05$)

Table 2 Effect of Basidiomycetes Rich in Vanadium and Other Treatments on Blood Glucose Levels in Adrenaline-Hyperglycemic Mice

Different groups	Blood glucose (mmol/L) at zeroth minute	Blood glucose (mmol/L) at 60th minute
Adrenaline-treated	5.7±2.6	16.8±1.8 a
Adrenaline- and Xiaoke-pill-treated	5.6±2.2	9.0±2.6 b
Adrenaline- and CC-treated	5.8±3.1	8.9±2.5 b
Adrenaline- and GL-treated	5.6±2.6	14.0±3.2 a
Adrenaline- and GF-treated	5.7±2.3	13.5±2.6 a
Control group	5.7±3.3	6.8±2.2

The different letters in the same column indicate a statistical difference ($p<0.01$)

From the Tables 1 and 2, we could see that the hypoglycemic effect of GL and GF was not significant. It is implied that the fermented mushroom of *C. comatus* rich in vanadium has the hypoglycemic effect, although the three mushrooms cultures have the same vanadium content. *Coprinus comatus* is a mushroom claimed to be beneficial in glycemic control in diabetes. It had been reported that there were hypoglycemic components in *C. comatus* [12].

The ascension of blood sugar induced by glucose was inhibited 30 min later in CC–glucose group (Fig. 2). However, the level of blood sugar of the mice in other groups did not decrease to that of the control group 120 min later. From Fig. 2, we can see that the sugar tolerance of normal mice was improved after administration (ig) of CC.

Conclusions

C. comatus, *G. lucidum*, and *G. frondosa* could take up and accumulate vanadium at lower doses. Compared with *G. lucidum* rich in vanadium and *G. frondosa* rich in vanadium, the hypoglycemic effects of *C. comatus* rich in vanadium on hyperglycemic animals are significant, irrespective of the hyperglycemic animals induced by alloxan or adrenaline. From this study, we could conclude that *C. comatus* rich in vanadium may be used as a hypoglycemic food or medicine for hyperglycemic people. Its potential application needs to be further studied.

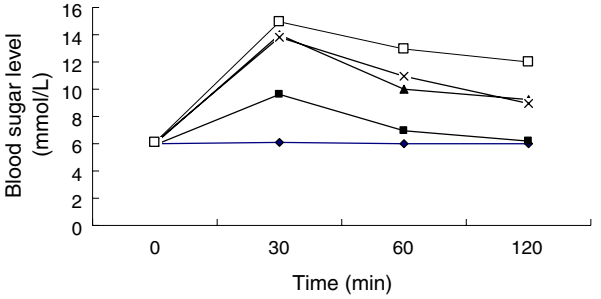

Fig. 2 Effects of basidiomycetes rich in vanadium on sugar tolerance of healthy mice (*filled diamonds* control group, *empty squares* saline–glucose group, *filled squares* CC–glucose group, *crosses* GL–glucose group, *filled triangles* GF–glucose group)

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