Journal of Trace Elements in Medicine and Biology 20 (2006) 191–196

PHARMACOLOGY

Hypoglycemic activity of fermented mushroom of *Coprinus comatus* rich in vanadium

Chunchao Han[a,*], Junhua Yuan[b], Yingzi Wang[c], Lingjun Li[a]

[a]*School of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250014, PR China*
[b]*Department of Gastroenterology, QiLu Hospital of Shandong University, Jinan 250012, PR China*
[c]*Department of Pharmacy, Beijing University of Traditional Chinese Medicine, Beijing 100102, PR China*

Received 7 February 2006; accepted 25 June 2006

Abstract

The hypoglycemic activity of fermented mushroom of *Coprinus comatus* rich in vanadium (CCRV) was studied in this paper. Alloxan and adrenalin induced hyperglycemic mice were used in the study. The blood glucose and the HbA1c of the mice were analyzed, respectively. At the same time, the sugar tolerance of the normal mice was also determined. After the mice were administered (ig) with CCRV, the blood glucose and the HbA1c of alloxan-induced hyperglycemic mice decreased ($p<0.05$, $p<0.01$), ascension of blood glucose induced by adrenalin was inhibited ($p<0.01$) and the sugar tolerance of the normal mice was improved. Also, the body weight of the alloxan-induced hyperglycemic mice was increased gradually. In the fermented mushroom of *C. comatus*, vanadium at lower doses in combination with *C. comatus,* induced significant decreases of the blood glucose and HbA1c levels in hyperglycemic mice.

Keywords: *Coprinus comatus*; Vanadium; Blood glucose; HBA1c; Sugar tolerance

Introduction

Vanadium, element number 23, atomic weight 50.94, is normally present at very low concentrations ($<10^{-8}$ M) in virtually all cells in plants and animals. As a potential therapeutic agent, it is attracting increasing attention. Vanadium compounds have the ability to imitate action of insulin [1,2]. Oral administration of inorganic vanadium (IV, V) salts, have shown anti-diabetic activity in vitro [3], in vivo [4] and even in patients [5]. However, the toxicity associated with vanadium limits its role as a therapeutic agent for diabetic treatment [6]. Typical clinical manifestations are diarrhea, vomiting, abdominal cramps, green tongue, bronchospasm, and irreversible renal excretion damage [7]. In order to reduce the toxicity of vanadium, McNeill 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). In this paper, vanadium at lower doses (0.18 mg/kg/d) was absorbed by fermented mushroom of *Coprinus comatus,* which is one rare edible fungus that is able to absorb and accumulate trace elements. The co-effect of *C. comatus* and vanadium was studied.

Edible fungi have a long history of use in traditional Chinese medicine [10]. Various edible mushrooms have been ascribed to have anti-diabetic properties [11–13]. Another important property of the edible mushroom is

*Corresponding author. Tel./fax: +86 531 82613129.
E-mail address: biobook2@sohu.com (C. Han).

0946-672X/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2006.06.003

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 [14–16]. *C. comatus* is a mushroom claimed to benefit glycaemic control in diabetes. Many physiological functions of *C. comatus* have been reported, such as anti-cancer properties [17], immunomodulatory [18], and its hypoglycemic activity. It had been reported that there were hypoglycemic components in *C. comatus* [19]. An acute glucose-lowering effect was reported in normal rats and mice, and a chronic reduction in basal glycemia and improved glucose tolerance were also noted in normal mice consuming a diet containing 330 g dried fruit bodies of *C. comatus*/kg [20,21].

Materials and methods

CCRV

CCRV was produced in the Pharmaceutic Laboratory of Shandong University of Traditional Chinese Medicine, China.

The seed of *C. comatus* was purchased from the Agricultural Culture Collection of China.

First, the seed was grown at 28 °C for 5 days on PDA slants (1000 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 *C. comatus* 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 3 days.

A 72-h-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% $MgSO_2$ + 0.9% $NaVO_3$). The volume of inoculum was 15 mL, which was then cultivated under the same condition. The 72-h-old fermented liquid culture was CCRV. After stirred by a homogenizer, an ampule was filled with 0.4 mL of CCRV and then was sterilized in a microwave oven for 3 min.

Fermented mushroom of *C. comatus* (FMCC)

The fermented mushroom of *C. comatus* was produced using the same method to produce CCRV except that there was no $NaVO_3$ in the fermented culture medium.

Sodium vanadate solution (SV)

Sodium vanadate (0.9 g) was dissolved in 100 mL of normal saline. An ampule was filled with 0.4 mL of SV and then was sterilized in a microwave oven for 3 min.

Chemicals

Alloxan and adrenaline were analytical grade, were purchased, respectively, from Sigma Co. Ltd and Tianjin Amino acid Co. Ltd. Sodium vanadate (analytical grade) was purchased from Beijing Chemical Factory, China. 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.

Animals

Female Kunming strain mice weighing 20–22 g, Grade II, Certificate SCXK (Lu) 20040004, 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 [22]. 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 5 groups: Alloxan-induced hyperglycemic group, alloxan and Xiaoke Pill-treated group, alloxan and CCRV-treated group, alloxan and–FMCC treated group, and the alloxan and SV-treated group. The other 12 normal mice were injected (iv) with the normal saline and used as the control group. From then on, the 6 groups of mice were administered (ig) saline, Xiaoke Pill (0.028 mg/kg/d), CCRV (0.18 mg/kg/d vanadium), FMCC (0 mg/kg/d vanadium), SV (0.18 mg/kg/d vanadium), and saline, respectively. The body weights of the mice were measured on the 0th day, 5th day, 10th day, 15th day, and the 20th day. At the same time, after fasting the mice for 12 h on the 20th day, blood samples

were obtained from the tail veins to determine the blood glucose levels. On the 45th day, blood samples were collected from the orbital veins to measure the HbA1c with the HbA1c Apparatus (Variant α, Bio-Rad Laboratories).

Blood samples from adrenaline-induced hyperglycemic mice

Seventy-two healthy mice were allocated equally into 6 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-CCRV treated group, adrenaline-FMCC treated group, adrenaline-SV treated group and the control group. Blood samples from the tail vein of the mice were collected at the 0th minute and 60th minute to determine blood glucose level just as above.

Blood samples to determine sugar tolerance

Thirty-six healthy mice were divided equally into 3 groups. The first group was administered (ig) CCRV, the others were administered (ig) saline. On the 8th day, after the last administration, the first and second groups were injected (ip) with glucose (2 g/kg), the third group was injected (ip) with saline, namely, CCRV–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).

Results and discussion

All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test [23]. The data represents 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.

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 alloxan and CCRV-treated group and the body weights of mice in alloxan and FMCC treated group, increased gradually 10 days later. The body weights of mice in the alloxan and the Xiaoke Pill-treated group also increased. On the contrary, the body weights of mice in alloxan and SV-treated group did not increase significantly.

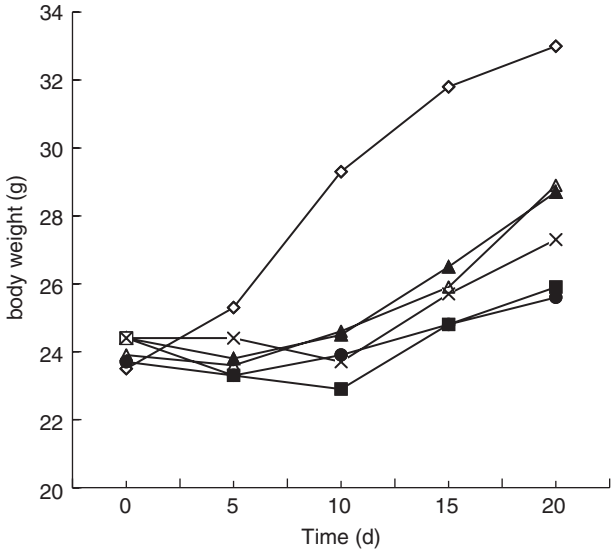

Fig. 1. Effects of CCRV on body weights of hyperglycemic mice (□ control group, × alloxan and Xiaoke Pill-treated group, ▯ alloxan and FMCC-treated group, ▲ alloxan and CCRV-treated group, ■ alloxan-intoxicated group, ◆ alloxan and SV-treated group) Contrasted with the alloxan-induced hyperglycemic group, the body weights of mice in alloxan and CCRV-treated group, alloxan and FMCC treated group, alloxan and the Xiaoke Pill-treated group increased gradually 10 days later. On the contrary, the body weights of mice in alloxan and SV-treated group, alloxan-induced hyperglycemic group did not increase significantly.

Table 1. Effect of CCRV and other treatments on blood glucose levels in alloxan-hyperglycemic mice

Different groups	Blood glucose (mmol/L)
Alloxan-treated	21.2 ± 2.1^{a}
Alloxan and Xiaoke Pill-treated	13.1 ± 3.2^{b}
Alloxan and CCRV-treated	10.5 ± 2.0^{b}
Alloxan and FMCC-treated	19.6 ± 3.0^{a}
Alloxan and SV-treated	18.9 ± 2.8^{a}
Control group	5.9 ± 1.2

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

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 CCRV and the Xiaoke Pill ($p<0.05$). Meanwhile, CCRV could decrease the concentration of HbA1c in plasma of alloxan-induced hyperglycemic group 45 days later ($p<0.01$), as shown in Table 2. However, the same result did not occur in the alloxan and FMCC treated group or the alloxan and SV-treated group.

Adrenaline activates glycogenolysis and glyconeogenesis to elevate serum glucose level [24] and its effect is relatively rapid [25]. The results of blood glucose from hyperglycemic mice induced by adrenaline are presented in Table 3. It showed that after administration (ig) CCRV 15 days, ascension of blood glucose induced by adrenaline was inhibited ($p<0.01$). However, the same results did not occur in the alloxan and FMCC treated group or the alloxan and SV-treated groups.

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

Bailey et al. [21] have studied the hypoglycemic functions of *C. comatus*. However, they did not study the hypoglycemic functions of *C. comatus* on the hyperglycemic mice. In our study, not only did the blood glucose and the HbA1c of alloxan-induced hyperglycemic mice decreased ($p<0.05$, $p<0.01$), but also the ascension of blood glucose induced by adrenalin was inhibited ($p<0.01$) after the hyperglycemic mice were fed (ig) CCRV.

The hypoglycemic functions of vanadium have been researched for many years. Meeks et al. [26] reported the effect of vanadium on metabolism of glucose in rats. Thompson et al. [27] studied the effect of vanadium (V) intake on blood glucose lowering using streptozotocin (STZ)-diabetic rats. All of these studies revealed the hypoglycemic functions of vanadium, but the toxicity of vanadium was not emphasized. One way to reduce the toxicity of vanadium is to reduce its dose.

The hypoglycemic effect of FMCC and SV was not significant. It is implied that the hypoglycemic effect on the hyperglycemic mice was caused by the co-effect of *C. comatus* and vanadium. Neither the *C. comatus* nor vanadium (0.18 mg/kg/d) could reduce the level of blood glucose when they were given to the hyperglycemic mice singly. The body weights of hyperglycemic mice induced by Alloxan increased as they were given *C. comatus*. It indicates that *C. comatus* could supplement nutrients to mice.

Table 2. Effect of CCRV and other treatments on HbA1c from hyperglycemic mice induced by alloxan (%)

Different groups	Results of HbA1c
Alloxan-treated	10.8 ± 0.23^{a}
Alloxan and Xiaoke Pill-treated	8.0 ± 0.30^{b}
Alloxan and CCRV-treated	7.9 ± 0.28^{b}
Alloxan and FMCC-treated	10.0 ± 0.26^{a}
Alloxan and SV-treated	9.8 ± 0.33^{a}
Control group	4.8 ± 0.22

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

Conclusion

Many transition elements have been studied and found effective in reducing blood glucose in diabetes. Shindea UA et al. have proved chromium picolinate significantly improves deranged carbohydrate and lipid metabolism of experimental chemically induced diabetes in rats [28]. Nomura et al. reported the effect of cobalt

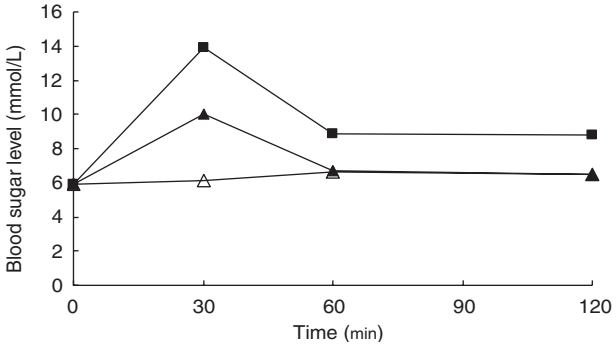

Fig. 2. Effects of CCRV on sugar tolerance of normal mice (△ Control group, ■ Saline-glucose group, ▲ CCRV-glucose group). The level of blood sugar in CCRV–glucose group was very close to that of the control group 120 min later. However, the level of blood in the saline–glucose group did not decrease to that of control group.

Table 3. Effect of CCRV and other treatments on blood glucose levels in adrenaline-hyperglycemic mice

Different groups	Blood glucose (mmol/L) at 0th min	Blood glucose (mmol/L) at 60th min
Adrenaline–treated	5.7 ± 2.6	15.1 ± 1.0^{a}
Adrenaline and Xiaoke Pill-treated	5.6 ± 2.2	9.3 ± 0.6^{b}
Adrenaline and CCRV-treated	5.8 ± 3.1	10.6 ± 1.5^{b}
Adrenaline and FMCC-treated	5.6 ± 2.6	14.0 ± 1.2^{a}
Adrenaline and SV-treated	5.7 ± 2.3	13.9 ± 1.6^{a}
Control group	5.7 ± 3.3	5.8 ± 3.2

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

on the liver glycogen content in the streptozotocin-induced diabetic rats [29]. When cobalt was administered to the rats, the glycogen returned to the level of healthy rats, concomitantly with the decrease in blood glucose. Vanadium is one such nutritional trace element that has been found to be very effective in reducing blood glucose. It is known to act as a potent insulin mimetic agent by increasing glucose transport and metabolism in skeletal muscle, liver and adipose tissue [2,29]. However, the toxicity associated with vanadium limits its therapeutic efficacy [6]. To reduce vanadium-associated toxicity and maintain its insulin-mimetic anabolic effects, vanadium is administered at lower doses in combination with *Trigonella foenum graecum*, an Indian herb. This combination has been found to show almost complete reversal of the raised blood glucose levels and altered metabolic changes in experimental diabetes in tissues such as the kidney [30]. By using vanadium at lower doses, in combination with herbs or edible mushrooms that have been ascribed anti-diabetic properties, is one potent way to reduce vanadium-associated toxicity and maintain its effect. *C. comatus* is one mushroom claimed to benefit glycemic control in diabetes [20,21]. *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 [31]. It indicates that *C. comatus* could supplement nutrients to the mice as well as lower blood glucose of hyperglycemic mice. More importantly, *C. comatus* has the ability to take up and accumulate trace metals. It has been reported that fermented mash of *C. comatus* is rich in chromium by liquid fermentation [32].

The hypoglycemic effects of CCRV on hyperglycemic animals are significant, irrespective of the hyperglycemic animals were induced by alloxan ($p<0.05$) or adrenaline ($p<0.01$), though the dose of vanadium is only 0.18 mg/kg/d. Meanwhile, CCRV could reduce the concentration of the HbA1c in plasma of hyperglycemic animals ($p<0.01$), which is a more useful parameter in diabetes. At the same time, the sugar tolerance of healthy mice was improved, which is another important parameter in diabetes. The hypoglycemic mechanism of CCRV had been studied, which included inhibiting gluconeogenesis, strengthening glycogan synthesis and insulin, C-peptide secretion as well as recovering β-cell injured by alloxan. It will be published in the following paper. From this study, we could conclude CCRV may be used as a hypoglycemic food or medicine for hyperglycemic people. The potential application of CCRV needs to be further studied.

References

[1] Gil J, Miralpeix M, Carreras J, Bartrons R. Insulin-like effects of vanadate on glucokinase activity and fructose 2,6-bisphosphate levels in the liver of diabetic rats. J Biol Chem 1988;263:1868–71.

[2] Shechter Y. Insulin-mimetic effects of vanadate. Possible implications for future treatment of diabetes. Diabetes 1990;39:1–5.

[3] Lu B, Ennis D, Lai R, Bogdanovic E. Enhanced sensitivity of insulin-resistant adipocytes to vanadate is associated with oxidative stress and decreased reduction of vanadate (+5) to vanadyl (+4). J Biol Chem 2001;276:35589–98.

[4] Semiz S, Orvig C, McNeill JH. Effects of diabetes, vanadium, and insulin on glycogen synthase activation in Wistar rats. Mol Cell Biochem 2002;231:23–35.

[5] Goldfine AB, Simonson DC, Folli F, Patti ME, Kahn CR. In vivo and in vitro studies of vanadate in human and rodent diabetes mellitus. Mol Cell Biochem 1995;153:217–31.

[6] Domingo JL. Vanadium and tungsten derivatives as antidiabetic agents: a review of their toxic effects. Biol Trace Elem Res 2002;88:97–112.

[7] Scior T, Guevara-García A, Bernard P, Do Q-T, Domeyer D, Laufer S. Are vanadium compounds drugable? Structures and effects of antidiabetic vanadium compounds: a critical review. Mini-Rev Med Chem 2005;5:995–1008.

[8] McNeill JH, Yuen VG, Hoveyda HR, Orvig C. Bis(maltolato)oxovanadium (IV) is a potent insulin mimic. J Med Chem 1992;35(8):1489–91.

[9] Sakurai H, Fujii K, Watanabe H, Tamura H. Orally active and long-term acting insulin-mimetic vanadyl complex: bis (picolinato) oxovanadium (IV). Biochem Biophys Res Commun 1995;214:1095–101.

[10] Demirbas A. Heavy metal bioaccumulation by mushrooms from artificially fortified soils. Food Chem 2001;74:293–301.

[11] Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Evaluation of traditional plant treatments for diabetes: studies in streptozotocin diabetic mice. Acra Diaberologiu Larinu 1989;26:51–5.

[12] Kiho T, Tsujimura Y, Sakushima M, Usui S, Ukai S. Polysaccharides in fungi. XXXIII. Hypoglycemic activity of an acidic polysaccharide (AC) from *Tremella fuciformis*. Yakugaku Zasshi 1994;114:308–15 [in Japanese].

[13] Kiho T, Sobue S, Ukai S. Structural features and hypoglycemic activities of two polysaccharides from a hot-water extract of *Agrocybe cylindracea*. Carbohydr Res 1994;251:81–7.

[14] Kalac P, Niznamska M, Bevilaqua D, Staskova I. Concentrations of mercury, copper, cadmium and lead in fruiting bodies of edible mushrooms in the vicinity of a mercury smelter and a copper smelter. Sci Total Environ 1996;177:251–8.

[15] Kalac P, Svoboda L. A review of trace element concentrations in edible mushrooms. Food Chem 2000;69:273–81.

[16] Malinowska E, Szefer P, Falandaysz J. Metals bioaccumulation by bay bolete, *Xerocomus badius*, from selected sites in Poland. Food Chem 2004;84:405–16.

[17] Cui M, Zhang H, An L. Tumor growth inhibition by polysaccharide from *C. comatus*. World Chinese J Digestol 2002;10:287–90.

[18] Xing F, Wang H, Han C, Wang Y. Study on the immunocompetence of polysaccharides from the *C. comatus*. J Food Sci 2003;24:139–41 [in Chinese].
[19] Gu Y, Ju Y. Food and officinal mushroom – *C. comatus*. Vegetable 1996;13:10 [in Chinese].
[20] Lelley J. Investigations on the culture of the ink cap, *C. coniurus* (Mull ex Fr) Gray. Mushroom J 1983;129:14.
[21] Bailey CJ, Turner SL, Jakeman K, Hayes WA. Effect of *C. comatus* on plasma glucose concentrations in mice. Planta Med 1984;50:525–6.
[22] You Y, Lin Z. Antioxidant effect of Ganoderma polysaccharide peptide. Acta Pharm Sinica 2003;38:85–8.
[23] Duncan D B. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957;13:164–76.
[24] Cherrington AD, Fuchs H, Stevenson RW, Williams PE, Alberti KG, Steiner KE. Effect of epinephrine on glycogenolysis and gluconeogenesis in conscious overnight-fasted dogs. Am J Physiol 1984;247(2 Part 1): E137–44.
[25] Issekutz Jr B, Allen M. Effect of catecholamines and methylprednisolone on carbohydrate metabolism of dogs. Metabolism 1972;21(1):48–59.
[26] Meeks MJ, Landolt RR, Kessler WV, Born GS. Effect of vanadium on metabolism of glucose in the rat. J Pharm Sci 1971;60:482–3.
[27] Thompson KH, Leichter J, McNeill JH. Studies of vanadyl sulfate as a glucose-lowering agent in STZ-diabetic rats. Biochem Biophys Res Commun 1993;197: 1549–55.
[28] Shindea UA, Sharma G, Xu YJ, Dhalla NS, Goyal RK. Insulin sensitising action of chromium picolinate in various experimental models of diabetes mellitus. J Trace Elem Med Biol 2004;18:23–32.
[29] Nomura Y, Okamoto S, Sakamoto M, Feng Z, Nakamura T. Effect of cobalt on the liver glycogen content in the streptozotocin-induced diabetic rats. Mol Cell Biochem 2005;277:127–30.
[30] Raju J, Gupta D, Rao AR, Yadava PK, Baquer NZ. *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol Cell Biochem 2001;224: 45–51.
[31] Liu Y, Zhang J. Recent advanced in the studies on the medicinal functions of *C. comatus*. Acta Edulis Fungi 2003;2:60–3 [in Chinese].
[32] Wang Y, Li X, Yin J, Gao C. Fermented mash of *C. comatus* rich in chromium (FCRC) can bring down high level of blood glucose. J Shandong Univ 2000;35: 17–20 [in Chinese].