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Comparison of Hypoglycemic Activity of Trace Elements Absorbed in Fermented Mushroom of *Coprinus comatus*

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Abstract The effect of fermented mushroom of *Coprinus comatus* rich in trace elements, including vanadium, chromium, zinc, magnesium, copper, iron, and nickel, on glycemic metabolism was studied in this paper. Alloxan-induced hyperglycemic mice were used in the study. The blood glucose, glycohemoglobin, and glycogen synthesis of the mice were analyzed, respectively. At the same time, the gluconeogenesis of the normal mice was also determined. After the mice were administered (ig) with *C. comatus* rich in vanadium (CCRV), the blood glucose and the glycohemoglobin of alloxan-induced hyperglycemic mice decreased ($p<0.05$, $p<0.01$), glycogen synthesis of alloxan-induced hyperglycemic mice elevated ($p<0.01$), the gluconeogenesis of the normal mice was inhibited ($p<0.01$), and the sugar tolerance of the normal mice was improved. However, the same result did not occur in other groups. Vanadium at lower doses in combination with *C. comatus* induced significant effect on glycemic metabolism in mice.

Keywords Trace elements · Blood glucose · HbA1c · Glycogen · Gluconeogenesis · Sugar tolerance

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Introduction

Many trace elements have been studied and found effective in reducing blood glucose in diabetes. It is well known that trace elements serve as structural components of tissues and as constituents of the body fluids and vital enzymes in major metabolic pathways and are essential for the function of all cells [1]. As diabetes is a disease of metabolic abnormality, elements as such or as a component of enzymes may play a significant role in the development and control of diabetes.

The elements chromium, magnesium, and zinc are responsible for the secretion of insulin from beta cells of the islets of Langerhans and are involved in potentiating insulin action [2–5]. Some studies revealed the beneficial role of adequate supplementation of copper, iron, and nickel for the control and management of diabetes [6, 7]. Vanadium compounds have the ability to imitate action of insulin [8–9]. Oral administration of inorganic vanadium (IV, V) salts, have shown antidiabetic activity in patients [10].

However, the toxicity associated with trace elements limits their therapeutic efficacy [11–14]. By using them at lower doses, in combination with herbs or edible mushrooms that have been ascribed antidiabetic properties, is one potent way to reduce trace element-associated toxicity and maintain their effect.

Edible fungi have a long history of use in traditional Chinese medicine [15]. Various edible mushrooms have been ascribed to have antidiabetic properties. [16–18]. 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 [19–20]. *Coprinus comatus* is a mushroom claimed to benefit glycemic control in diabetes.

The purpose of this study was to investigate the effect of fermented mushroom of *C. comatus* rich in various trace elements on glycemic metabolism. Therefore, the effect of them on blood glucose and the HbA1c, glycogen synthesis, gluconeogenesis, and the sugar tolerance were studied, respectively.

Materials and Methods

Chemicals

Alloxan was of analytical grade, was purchased from Sigma. L-alanine was of analytical grade, was purchased from Betapharma, Shanghai, China. Xiaoke pills were purchased from Jilin Liuhe Pharmaceutic Factory, China. Xiaoke pill is a kind of Chinese medicine used for 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 of *C. comatus* Rich in Various Trace Elements

The fermented mushroom of *C. comatus* rich in various trace elements were produced by the way it was introduced by Chunchao Han [21, 22]. Briefly, the seed of *C. comatus* was grown at 28°C for 5 days on PDA slants (1,000 mL 20% potato extract liquid + 20.0 g dextrose + 20.0 g agar). 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% $MgSO_2$). 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 72-h-old fermented liquid culture was fermented mushroom of *C. comatus* rich in vanadium (CCRV).

Fermented mushroom of *C. comatus* rich in chromium (CCRC), fermented mushroom of *C. comatus* rich in zinc (CCRZ), fermented mushroom of *C. comatus* rich in magnesium (CCRM), fermented mushroom of *C. comatus* rich in copper (CCRP), fermented mushroom of *C. comatus* rich in iron (CCRI), and fermented mushroom of *C. comatus* rich in nickel (CCRN) were produced using the same method to produce CCRV except that there were other trace elements, namely. $CrCl_3$, $ZnSO_4$, $MgSO_4$, $CuSO_4$, $FeSO_4$, and Ni $(CH3COO)_2$ in the fermented culture medium instead of $NaVO_3$.

Fermented Mushroom of *C. comatus*

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

Sodium Vanadate Solution

Sodium vanadate (SV; 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.

Animals

Female Kunming strain mice weighing 20–22 g, grade II, 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.

Induction of Diabetes

Animals were fasted for 12 h and were then injected (i.v.) with alloxan (75 mg/kg) solution that was made with saline [23]. 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). The blood glucose level of mice greater than 11.1 mmol/L was selected as hyperglycemic mice.

Experimental Design

One hundred and thirty-two hyperglycemic mice were selected and allocated equally into 11 groups: alloxan-induced hyperglycemic group, alloxan and Xiaoke Pill-treated group, alloxan and CCRV-treated group, alloxan and CCRC-treated group, alloxan and CCRZ-treated group, alloxan and CCRM-treated group, alloxan and CCRP-treated group, alloxan and CCRI-treated group, alloxan and CCRN-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 12 groups of mice were administered (ig) saline, Xiaoke Pill (0.028 mg/kg/day), CCRV (0.18 mg/kg/day

vanadium), CCRC (0.18 mg/kg/day chromium), CCRZ (0.18 mg/kg/day zinc), CCRM (0.18 mg/kg/day magnesium), CCRP (0.18 mg/kg/day copper), CCRI (0.18 mg/kg/day iron), CCRN (0.18 mg/kg/day nickel), FMCC, SV (0.18 mg/kg/day vanadium), and saline, respectively. Twenty days later, 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 II, Bio-Rad Laboratories). The animals were sacrificed by decapitation, and the liver was dissected out for the measurement of hepatic glycogen.

Estimation of Hepatic Glycogen

The liver was homogenized in ice-cold 0.6 M $HClO_4$. The mixture was immediately centrifuged at 3,000 × *g* and subjected to determination of free glucose in the tissue by the glucose oxidase method. Amyloglucosidase solution (10 U/ml) in 0.2 M sodium acetate buffer (pH 4.8) was then mixed and incubated in the mixture at 40°C for 2 h. After incubation, pH of the mixture was adjusted to 7 and subjected to determination of total glucose. Free glucose was subtracted from total glucose to obtain glycogen content. The glycogen was expressed as milligram per gram wet tissue [24].

Estimation of Gluconeogenesis

One hundred and eight normal mice were selected and allocated equally into nine groups: Xiaoke Pill-treated group, CCRV-treated group, CCRC-treated group, CCRZ-treated group, CCRM-treated group, CCRP-treated group, CCRI-treated group, CCRN-treated group, saline group used as the control group. From then on, the three groups of mice were administered (ig) with Xiaoke Pill (0.028 mg/kg/day), CCRV (0.18 mg/kg/day vanadium), CCRC (0.18 mg/kg/day chromium), CCRZ (0.18 mg/kg/day zinc), CCRM (0.18 mg/kg/day magnesium), CCRP (0.18 mg/kg/day copper), CCRI (0.18 mg/kg/day iron), CCRN (0.18 mg/kg/day nickel), and saline respectively. At the end of the experimental period (15 days later), they were fasted for 12 h. After administration 1 h later, the mice were injected (s.c.) with L-alanine. The blood samples from the tail vein of the mice were collected at the 0th minute and 60th minute to determine blood glucose level.

Blood Samples to Determine Sugar Tolerance

One hundred and twenty normal mice were selected and allocated equally into ten groups: Xiaoke Pill-treated group, CCRV-treated group, CCRC-treated group, CCRZ-treated group, CCRM-treated group, CCRP-treated group, CCRI-treated group, CCRN-treated group, two groups of saline-treated mice used as the control group. The former eight groups of mice were administrated (ig) Xiaoke Pill (0.028 mg/kg/day), CCRV (0.18 mg/kg/day vanadium), CCRC (0.18 mg/kg/day chromium), CCRZ (0.18 mg/kg/day zinc), CCRM (0.18 mg/kg/day magnesium), CCRP (0.18 mg/kg/day copper), CCRI (0.18 mg/kg/day iron), and CCRN (0.18 mg/kg/day nickel); the others were administrated (ig) saline. On the eighth day, after the last administration, the former nine groups of mice were injected (ip) with glucose (2 g/kg); the last group was injected (ip) with saline. 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).

Statistical Analysis

All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test [25]. The data are shown as the mean ± SEM. The significant level of 5% ($p<0.05$) was used as the minimum acceptable probability for the difference between the means.

Results

Results on Blood Glucose and HbA1c

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 other groups.

Results on Hepatic Glycogen

CCRV produced the increase in the level of hepatic glycogen. The glycogen levels were 28.0±5.2 mg/g tissue in CCRV-treated mice ($p<0.01$). Treatment of diabetic mice with Xiaoke Pill also produced a significant increase in the levels of hepatic glycogen to 19.1± 0.5 mg/g tissue ($p<0.05$). Concentrations of hepatic glycogen were lower in diabetic mice (14.1±3.8 mg/g) than those in normal mice (24.1±4.3 mg/g) and CCRV-treated mice (Table. 3). Although the concentrations of hepatic glycogen were low in other groups, there were no significant difference between them and diabetic mice.

Results on Gluconeogenesis

At the 60th minute, the level of blood glucose of the mice in the CCRV group was not increased significantly (from 95.9±17.9 to 96.3±9.0 mg/mL) after the mice were injected (s.c.) with L-alanine. On the contrary, the level of blood glucose of the mice in the other

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

Different groups	Blood glucose (mmol/L)
Alloxan-treated	22.0±1.9[a]
Alloxan and Xiaoke pill-treated	12.9±3.2[b]
Alloxan and CCRV-treated	11.1±2.5[b]
Alloxan and CCRC-treated	18.7±3.8[a]
Alloxan and CCRZ-treated	17.9±2.0[a]
Alloxan and CCRM-treated	16.0±4.0[a]
Alloxan and CCRP-treated	16.1±3.0[a]
Alloxan and CCRI-treated	15.9±2.8[a]
Alloxan and CCRN-treated	15.3±4.1[a]
Alloxan and FMCC-treated	18.9±2.9[a]
Alloxan and SV-treated	18.5±2.8[a]
Control group	5.7±2.2

The different letters in the same column indicate a statistical difference, b was compared with a ($p<0.05$)

Table 2 Effect of CCRV and Other Treatments on HbA1c from Hyperglycemic Mice Induced By Alloxan (%)

Different groups	Results of HbA1c
Alloxan-treated	11.8±0.33[a]
Alloxan and Xiaoke pill-treated	8.3±0.33[b]
Alloxan and CCRV-treated	7.8±0.18[b]
Alloxan and CCRC-treated	8.9±0.36[a]
Alloxan and CCRZ-treated	10.1±0.19[a]
Alloxan and CCRM-treated	10.0±0.26[a]
Alloxan and CCRP-treated	11.0±0.40[a]
Alloxan and CCRI-treated	9.0±0.46[a]
Alloxan and CCRN-treated	9.3±0.31[a]
Alloxan and FMCC-treated	10.0±0.26[a]
Alloxan and SV-treated	9.8±0.33[a]
Control group	4.9±0.19

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

groups increased significantly after the mice were injected (s.c) with L-alanine. The result was shown in Table 4 ($p<0.01$).

Results on Sugar Tolerance

The ascension of blood sugar induced by glucose was inhibited 30 min later in all groups (Fig. 1). 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 other groups did not decrease to that of control group 120 min later. From Fig. 1, we can see that the sugar tolerance of normal mice was improved after administration (ig) of CCRV.

Discussion

Alloxan produces selective cytotoxicity in pancreatic β cells through the generation of reactive oxygen species resulting in reduced synthesis and release of insulin [26]. In our study, diabetic mice were induced with alloxan.

Many trace elements have been studied and found effective in reducing blood glucose in diabetes. However, there were few findings about them in the control of diabetes mellitus.

Table 3 Effect of CCRV and Other Treatments on Hepatic Glycogen

Different groups	Hepatic glycogen (mg/g tissue)
Alloxan-treated	14.1±3.8
Alloxan and Xiaoke pill-treated	19.1±0.7[b]
Alloxan and CCRV-treated	28.0±4.2[a]
Alloxan and CCRC-treated	16.1±2.8
Alloxan and CCRZ-treated	16.6±4.0
Alloxan and CCRM-treated	17.1±1.8
Alloxan and CCRP-treated	16.5±2.6
Alloxan and CCRI-treated	17.0±3.5
Alloxan and CCRN-treated	15.9±3.4
Saline-treated	24.1±4.3

The different letters in the same column indicate a statistical difference. CCRV-treated groups were compared with diabetic group, $p<0.01$

Table 4 Effect of CCRV and Other Treatments on Gluconeogenesis

Different groups	Blood glucose (mg/mL) at 0th minute	Blood glucose (m/mL) at 60th minute
Xiaoke pill-treated	92.5±15.5	98.8±11.9
CCRV-treated	95.9±17.9	96.3±9.0[a]
CCRC-treated	92.0±15.1	98.7±12.9
CCRZ-treated	91.9±14.5	97.8±10.9
CCRM-treated	92.4±10.1	97.0±9.9
CCRP-treated	91.6±9.5	98.1±12.0
CCRI-treated	92.0±14.9	97.8±10.0
CCRN-treated	92.7±10.5	96.9±9.8
Saline-treated	91.6±14.7	105.0±12.2[b]

The different letters in the same column indicate a statistical difference. CCRV-treated groups were compared with diabetic group, $p<0.01$

The toxicities associated with them limit their roles as therapeutic agents for diabetic treatment. One way to reduce their toxicities is to reduce their 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. By using trace elements at lower doses, in combination with herbs or edible mushrooms that have been ascribed antidiabetic properties, is one potent way to reduce trace element-associated toxicity and maintain their effect. *C. comatus* is one mushroom claimed to benefit glycemic control in diabetes. [27, 28]. More importantly, *C. comatus* has the ability to take up and accumulate trace metals [10]. In this study, we investigated the effect of fermented mushroom of *C. comatus* rich in various trace elements (Cr, Mg, Zn, Cu, Fe, Ni, and V) on glycemic metabolism at the same dose. Only the hypoglycemic effects of CCRV on hyperglycemic animals are significant.

Conclusions

Blood glucose, HbA1c, hepatic glycogen gluconeogenesis, and sugar tolerance are important parameters in diabetes. The hypoglycemic effects of CCRV on blood glucose are significant ($p<0.05$), though the dose of vanadium is only 0.18 mg/kg/day. Meanwhile,

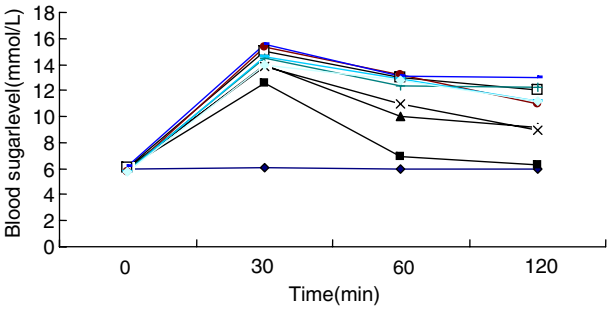

Fig. 1 Effects of CCRV and other treatments on sugar tolerance of normal mice (*filled diamonds* control group, *filled squares* CCRV-glucose group, *empty squares* saline-glucose group, *filled triangles* Xiaoke pill-glucose group). The level of blood sugar in CCRV-glucose group was very close to that of the control group 120 min later. However, the level of blood in other groups did not decrease than that of the control group

CCRV could reduce the concentration of the HbA1c in plasma of hyperglycemic animals ($p<0.01$). As the same time, the sugar tolerance of healthy mice was improved. Our data suggests that normalization of blood glucose in animal models could be due, at least in part, to the reduction in gluconeogenesis through acute and chronic effects and to the restoration of the depressed hepatic glycogen levels by CCRV. The action of CCRV on glycemic metabolism is the co-effect of *C. comatus* and vanadium. It indicates that by using vanadium at lower doses, in combination with edible mushrooms that have been ascribed antidiabetic properties, is one potent way to reduce vanadium-associated toxicity and maintain its effect.

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