Wang BX *et al* / Acta Pharmacol Sin 2003 Jan; 24 (1): 50-54



# Hypoglycemic activity of ginseng glycopeptide[1]

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**KEY WORDS** hypoglycemia; ginseng; glycopeptides; blood glucose; liver glycogen

## ABSTRACT

**AIM:** To study the hypoglycemic activity of ginseng glycopeptide (GGP). **METHODS:** Normal mice or rabbits and alloxan or streptozotocin-induced hyperglycemic rats or mice were used in the study. Blood glucose and liver glycogen levels of the experimental animals during the trial period were analyzed by spectrophotometry with *O*-toluidine and iodine reagents, respectively. **RESULTS:** Significant decreases in blood glucose and liver glycogen levels were induced in a dose-dependent manner after administration of GGP 50, 100, or 200 mg/kg injected ip or sc to normal mice and injected im 30 or 60 mg/kg to normal rabbits. The hypoglycemic activity of GGP lasted for about 16 h, and were examined in both normal animals and hyperglycemic animals. **CONCLUSION:** GGP injection induced the pronounced decreases in blood glucose and liver glycogen levels in both normal and hyperglycemic animals.

## INTRODUCTION

Ginseng is one of valuable Chinese materia medica and plays an important role in the folk medicine in the East Asian countries, such as China, Japan, and Korea. The history of using ginseng for therapeutical purpose in these countries can be traced up to 5000 years. Ginseng has been recorded to treat "Xiaoke" (emaciation and thirst) symptom in many ancient Chinese medical literature[1]. "Xiaoke", in general, indicates diabetes mellitus. However, the history of using modern techniques to study this herb's pharmaceutical and pharmacological effects is no more than 90 years. In the last three decades, a large amount of pharmacological researches have revealed that ginseng can decrease blood glucose level in normal and experiment-induced hyperglycemic animals[2]. In the last decade, both our group and Japanese researchers have extended the previous results to the chemical and pharmacological effects of another kind of active component, ginseng glycopeptide (GGP)[3-6]. It has been reported that ginseng polysaccharides contain considerable amount of polypeptides ranging from 1.6 % to 27 %[7]. Since ginseng polysaccharides are not single component, we coined this GGP. GGP compound has following pharmacological effects: immunomodulatory, antitumor, antiulcer, and hypoglycemic activities. Among the abovementioned functions of ginseng, hypoglycemic activity is most outstanding.

[1] Project supported by the National Natural Science Foundation of China (No 38970901) and State Administration of Traditional Chinese Medicine, People's Republic of China (No 89234).
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Received 2001-11-26 Accepted 2002-09-27

## MATERIALS AND METHODS

**Animals** Male Wistar rats weighing 150-160 g, male Kunming strain mice weighing 20-22 g, male rabbits (CBWS strain) weighing 2-2.5 kg (Grade II, Certificate No 10-5110, Experimental Centre, Changchun College of Traditional Chinese Medicine) were used. They were maintained at room temperature under alternating 12-h light/dark photoperiod, and could access to standard laboratory food and fresh water *ad libitum*.

**Chemicals and reagents** Ginseng glycopeptide (GGP) was isolated from Jilin white ginseng (*Panax ginseng* CA Mayer), which was obtained by the Department of Phytochemistry, Academy of Traditional Chinese Medicine and Materia Medica of Jilin Province. Molecular weight of GGP was 6000. The glycon part of GGP consisted of rhamnose, arabinose, galactose, and glucose, and the ratio of these components is 0.46: 1.61:1:2.37. The peptide part of GGP consisted of 16 amino acids (Asp, Glu, Pro, and others). GGP was dissolved in physiological saline and made up into the predetermined concentrations. Insulin, alloxan, and streptozotocin were purchased from Sigma (St Louis, MO, USA).

**Experiment design** Four experiments were conducted in this study in order to investigate effects of GGP on glycometabolism of normal and hyperglycemic animals. All the experimental animals were randomly allocated into the predetermined groups (refer to tables). Physiological saline was used for all the animals in the control groups.

Experiment I, this experiment consisted of two trials: trial I, normal mice were used; and trial II normal rabbits were used. In trial I, animals in the treatment groups were daily injected with GGP (ip or sc, with regard to dosages, refer to tables) for 3 d; whereas, those in the control group were injected with saline at 10 mL/kg. Animals were killed at the end of the treatment. In trial II, animals were killed by decapitation 6 h after GGP injection (im). In the both trials, blood and liver tissue were rapidly collected from the animals for the measurement of blood glucose and glycogen levels.

Experiment II, normal mice were allocated into 6 groups. Groups 1 and 4 were the controls and injected with saline at 5 mL/kg. Groups 2 and 5 were injected with insulin at 5 U/kg, and Groups 3 and 6 were injected with GGP at 200 mg/kg. Animals were killed at different time intervals, and the blood and liver tissue were collected for the measurement of glucose and glycogen levels.

Experiment III, hyperglycemic rats were prepared by iv administration of 0.7 % alloxan (70 mg/kg) solution. The alloxan solution was made with saline. Before alloxan administration, the experimental animals were fasted over night. Rats having blood glucose level at 16.65- 24.98 mmol/L were selected and used at d 5 after alloxan intoxication. Forty-eight hyperglycemic rats were equally divided into 4 groups: alloxan-intoxicated, alloxan and insulin-treated, alloxan and two GGP-treated (different doses) groups. Twelve normal rats were injected with saline served as a control group. From d 6 to d 11 of intoxication, normal and intoxicated control groups were daily injected with saline ($2\ mL \cdot kg^{-1} \cdot d^{-1}$) sc and the 3 treated groups were injected with insulin at $1\ U \cdot kg^{-1} \cdot d^{-1}$, GGP 100 and 200 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. On d 8 and 11, blood samples were obtained through tail vein 1 h after administration of GGP for the measurement of blood glucose level, then treatment was stopped. On d 17 of intoxication, the blood glucose was measured again. From d 18 of intoxication, GGP was daily injected again. On d 21 of intoxication, all the rats were decapitated, blood glucose and liver glycogen contents were measured 1 h after final injection of GGP.

Experiment IV, 90 mice were injected ip with streptozotocin (STZ) prepared with 0.05 moL/L citric acid solution at 90 mg/kg, and the blood glucose level was measured on d 5 after injection. Sixty hyperglycemic mice having 13.88- 22.2 mmol/L blood glucose level were selected and divided equally into 5 groups. Extra 12 mice were taken as normal control group. From d 6 to d 11 after STZ injection , the normal and STZ-intoxicated groups were injected with saline at 10 mL/kg once a day sc. The other groups were injected sc with the medicines as shown in Tab 5. On d 8 and d 11 after STZ intoxication, blood sample was taken from orbital vein for the measurement of blood glucose level. Then treatment was stopped and blood glucose contents were measured again until d 14 after STZ intoxication. The treatment was resumed from d 15 to d 17 after STZ intoxication. Mice in all the groups were decapitated and blood was sampled for the measurement of blood glucose level 1 h after last injection of the medicines.

**Measurements of blood glucose and liver glycogen contents** Blood glucose concentration and liver glycogen contents were measured using *O*-toluidine[8], and iodine reagents[9], respectively.

**Data analysis** The values were expressed as mean±SD. The significance of difference *vs* the control group was determined by Student's *t* test.

## RESULTS

**Effects of GGP on the levels of blood glucose and liver glycogen in mice and rabbits** The levels of blood glucose and hepatic glycogen contents in both mice and rabbits were decreased after GGP injection either significantly ($P<0.05$) or highly significantly ($P<0.01$ or over). This decrease showed a dose-dependent manner (Tab 1).

**Tab 1. Effects of GGP on the levels of blood glucose and hepatic glycogen in mice and rabbits. *n*=8. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control group.**

| Group/ mg·kg$^{-1}$ | Blood glucose/mmol·L$^{-1}$ ip | sc | Hepatic glycogen/mg·g$^{-1}$ Wt ip | sc |
|---|---|---|---|---|
| Mice | | | | |
| Control | 6.7±1.3 | 5.9±1.6 | 43±17 | 38±24 |
| Ins 5 U·kg$^{-1}$ | 3.9±0.6[c] | 4.1±0.9[c] | 60±8[b] | 64±11[c] |
| GGP 50 | 5.0±1.0[a] | 4.9±0.6[a] | 31±11[a] | 19±16[a] |
| 100 | 4.8±1.0[b] | 4.7±0.8[a] | 27±11[b] | 19±15[a] |
| 200 | 4.7±1.0[b] | 4.5±0.9[b] | 22±10[c] | 14±11[b] |
| Rabbits | im | | im | |
| Control | 6.8±0.8 | | 10.0±2.0 | |
| Ins 5 U·kg$^{-1}$ | 6.6±0.7[a] | | 15.0±7.0[a] | |
| GGP 30 | 6.0±0.6[a] | | 7.4±1.7[a] | |
| 60 | 5.9±0.4[b] | | 6.7±1.7[b] | |

Wt: wet tissue; Ins: Insulin; U: Unit (same in below tables).

**Influences of singular GGP injection on the levels of blood glucose and liver glycogen in mice** All the results were summarized in Tab 2 (glucose level) and Tab 3 (glycogen level). These results showed that GGP significantly decreased blood glucose and liver glycogen levels during the period between 1 to 16 h after GGP injection. The blood glucose levels in insulin-treated groups were decreased 1 h after injection, but the liver glycogen levels of these groups were increased. However, the change in levels of blood glucose and liver glycogen was not statistically significant during the period between 4 to 24 h after injection of insulin.

**Tab 3. Effects of GGP on the levels of liver glycogen in mice. *n*=8. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control group. Wt:wet tissue.**

| Group/ mg·kg$^{-1}$ | Liver glycogen/mg·g$^{-1}$ Wt 1 h | 4 h | 8 h | 16 h | 24 h |
|---|---|---|---|---|---|
| (ip) | | | | | |
| Control | 42±16 | 48±19 | 29±14 | 52±10 | 47±12 |
| Ins 5 U·kg$^{-1}$ | 65±19[c] | 47±34[a] | 44±19[a] | 58±18[a] | 43±14[a] |
| GGP 200 | 30±12[a] | 26±15[b] | 19±8[a] | 38±15[b] | 52±13[a] |
| (sc) | | | | | |
| Control | 36±10 | 41±21 | 39±18 | 59±26 | 36±13 |
| Ins 5 U·kg$^{-1}$ | 55±16[c] | 61±30[a] | 57±24[a] | 63±21[a] | 39±17 |
| GGP 200 | 30±18[a] | 32±14[a] | 25±14[a] | 50±18[a] | 42±16[a] |

**Effects of GGP on alloxan-induced hyperglycemia in rats** The results from Experiment III showed

**Tab 2. Effects of GGP on the levels of blood glucose in mice. *n*=8. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control group.**

| Group/mg·kg$^{-1}$ | Blood glucose/mmol·L$^{-1}$ 1 h | 4 h | 8 h | 16 h | 24 h |
|---|---|---|---|---|---|
| (ip) | | | | | |
| Control | 6.0±0.7 | 6.4±0.7 | 6.6±0.9 | 5.9±1.2 | 6.3±1.4 |
| Ins 5 U·kg$^{-1}$ | 2.9±0.4[c] | 6.1±0.4[a] | 6.3±0.3[a] | 6.0±0.6[a] | 7.1±0.9[a] |
| GGP 200 | 4.5±0.9[c] | 4.2±0.6[c] | 5.6±0.6[b] | 4.2±1.2[c] | 7.3±1.1[a] |
| (sc) | | | | | |
| Control | 6.4±0.6 | 6.3±1.0 | 6.6±0.8 | 5.9±0.7 | 6.3±0.9 |
| Ins 5 U·kg$^{-1}$ | 3.1±0.5[c] | 6.3±0.4[a] | 6.3±0.3[a] | 6. 0±0.4 [a] | 6.0±1.0[a] |
| GGP 200 | 6.0±0.8[a] | 5.7±1.2[a] | 5.5±0.8[b] | 4.8±0.9[c] | 6.0±1.0[a] |

that repeated sc injection of GGP caused significant and dose-dependent decrease of alloxan-induced hyperglycemia (Tab 4). When administration of insulin or GGP was stopped, blood glucose level increased gradually and administration of GGP and insulin again induced decrease in blood glucose level. Although both GGP and insulin had hypoglycemic activities, the underlying mechanisms through which they function was different from each other. The former caused an increase, while the latter caused a decrease in hepatic glycogen. Consequently, the effects of GGP and insulin on carbohydrate metabolism could be through independent pathways.

**Effects of GGP on streptozotocin-induced hyperglycemia in mice** The repeated sc injection of GGP caused significant and dose-dependent decrease of streptozotocin-induced hyperglycemia. When treatment of insulin or GGP was stopped, blood glucose level increased gradually and administration of GGP and insulin again induced decrease in blood glucose level (Tab 5).

## DISCUSSION

The results from the present study revealed that GGP, irrespective of the way of injection (ip, sc, or im, but not oral administration) or the animal species (mouse, rat, or rabbit) we used, had significant effects on hyperglycemia and liver glycogen level. The hypoglycemic activity of GGP exhibited not only on the normal animals (mouse and rabbit), but also on the experiment-induced hyperglycemic animals (rat and mouse). The hypoglycemic action of GGP lasted for 16 h. GGP and insulin, although hypoglycemia can be induced by both drugs, exhibited differential effects on liver glycogen level. GGP decreased liver glycogen content, but insulin increased it. It was found that GGP decreased liver glycogen

**Tab 4. Effects of GGP on rat hyperglycemia induced by alloxan.** $n$**=12. Mean±SD.** $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ ***vs*** **saline.** $^{e}P<0.05$, $^{f}P<0.01$ ***vs*** **Ax.**

| Treatment | Blood glucose after alloxan intoxication/mmol· $L^{-1}$ | | | | | Glycogen |
|---|---|---|---|---|---|---|
| | 5 d | 8 d | 11 d | 17 d | 21 d | /mg· $g^{-1}$ Wt |
| Saline | 6±1 | 5±1 | 5±0.4 | 5±1 | 6±1 | 50±13 |
| Ax 70 mg· $kg^{-1}$ | 23±10$^{c}$ | 18±5$^{c}$ | 23±5$^{c}$ | 20±9$^{c}$ | 19±7$^{c}$ | 60±19$^{a}$ |
| Ax+Ins 1 U· $kg^{-1}$ | 22±11$^{c}$ | 6±1$^{f}$ | 9±3$^{cf}$ | 13±5$^{ce}$ | 10±6$^{bf}$ | 68±22$^{b}$ |
| Ax+GGP 200 mg· $kg^{-1}$ | 23±7$^{c}$ | 7±3$^{bf}$ | 11±4$^{cf}$ | 19±12$^{c}$ | 12±6$^{bf}$ | 23±18$^{cf}$ |
| Ax+GGP 100 mg· $kg^{-1}$ | 20±6$^{c}$ | 8±3$^{bf}$ | 13±5$^{cf}$ | 22±9$^{c}$ | 17±3$^{ce}$ | 26±19$^{cf}$ |
| | ←\| BSLBD →\| ←\| daily dosing →\| ←\| | | | no treatment →\| ←\| | daily dosing →\| | |

Ax=alloxan; BSLBD=blood sugar level before dosing; Wt: liver wet tissue.

**Tab 5. Effects of GGP on streptozotocin-induced hyperglycemia in mice.** $n$**=12. Mean±SD.** $^{c}P<0.01$ ***vs*** **control group;** $^{e}P<0.05$, $^{f}P<0.01$ ***vs*** **STZ group.**

| Treatment | Blood glucose level/mmol· $L^{-1}$ | | | | |
|---|---|---|---|---|---|
| | 5 d | 8 d | 11 d | 14 d | 17 d |
| Control | 6.0±0.2 | 6.3±0.9 | 6.3±0.8 | 6.3±1.3 | 6.3±1.3 |
| STZ 90 mg· $kg^{-1}$ | 16.9±2.3$^{c}$ | 17±3$^{c}$ | 15±3$^{c}$ | 16±5$^{c}$ | 16±6$^{c}$ |
| STZ+Ins 5 U· $kg^{-1}$ | 17.1±2.4$^{c}$ | 7.5±2.4$^{f}$ | 8.8±2.2$^{f}$ | 13±4$^{c}$ | 7±3$^{f}$ |
| STZ+GGP 200 mg· $kg^{-1}$ | 17.0±2.0$^{c}$ | 9.9±2.3$^{f}$ | 9.0±2.5$^{f}$ | 13.1±2.5$^{c}$ | 9.6±1.3$^{f}$ |
| STZ+GGP 100 mg· $kg^{-1}$ | 16.8±2.4$^{c}$ | 10.2±1.5$^{f}$ | 9±3$^{f}$ | 18±7$^{c}$ | 7.8±1.8$^{f}$ |
| STZ+GGP 50 mg· $kg^{-1}$ | 16.0±1.6$^{c}$ | 12±5$^{e}$ | 11±4$^{f}$ | 13±7$^{c}$ | 9.8±2.7$^{f}$ |
| | ←\| BSLBD →\| ←\| daily dosing | | →\| ←\| no treatment →\| ←\| | daily dosing →\| | |

STZ=streptozotocin; BSLBD=blood sugar level before dosing.

through the enhancement of phosphorylase activity[10].

Miyazaki (1989) reported that ginseng contained about 22 ginseng polysaccharides, all of which had the effectiveness in anti-hyperglycemia[7]. There exits a difference in the chemical structure of these polysaccharides, which include dextran, variety of acidic hetero-polysaccharides. The difference in the molecular weight among these polysaccharides is even greater than in chemical structure: from $M_r$ 1800 to $M_r$ 1 800 000. Every ginseng polysaccharide contains a certain amount of polypeptides (1.6 %–27 %). Despite of thse differences in chemical structure and molecular weight, all of these polysaccharides have the effectivness in anti-hyperglycemia, when they are administrated intraperitoneally (100 mg/kg).

In the present study, the polysaccharide we used was an acidic hetero-polysaccharide of ginseng, which contains 5.6 % polypeptides. We found that the polypeptides and the polysaccharides in our compound were linked via covalent bonds. Therefore, we suggested that this compound should be called ginseng glycopeptide (GGP). In the previous studies, only ginsenosides were recommended as the active compounds, but ginseng glycopeptides as wastes. In the present study, we convincingly demonstrated that ginseng glycopeptides had the effectiveness in anti-hyperglycemia. Therefore, the results from this study have laid the foundation to fully utilize ginseng chemically and pharmacologically.

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