Published in final edited form as:

J Food Sci. 2007 October ; 72(8): S590–S594. doi:10.1111/j.1750-3841.2007.00481.x.

American Ginseng Berry Juice Intake Reduces Blood Glucose and Body Weight in *ob/ob* Mice

J.T. Xie, C.Z. Wang, M. Ni, J.A. Wu, S.R. Mehendale, H.H. Aung, A. Foo, and C.S. Yuan

Tang Center for Herbal Medicine Research, Committee on Clinical Pharmacology and Pharmacogenomics, and Dept. of Anesthesia & Critical Care, The Pritzker School of Medicine, Univ. of Chicago, Chicago, IL 60637, U.S.A

Abstract

Diabetes is a serious chronic metabolic disease and has a significant impact on patients' lives and the health care system. We previously observed that the organic solvent extract of American ginseng berry possessed significant antidiabetic effects in obese diabetic *ob/ob* mice after intraperitoneal injection. If American ginseng berry is useful as a dietary supplement, simple preparation and oral intake would be a convenient, safe, and practical means for consumers. In this study, the simply prepared berry juice was first analyzed using high-performance liquid chromatography, and then administered orally in the *ob/ob* mice. The animals received daily berry juice 0.6 mL/kg or vehicle for 10 consecutive days. The results indicated that oral juice administration significantly lowered fasting blood glucose levels, and this effect continued for at least 10 d after cessation of the treatment. Data from intraperitoneal glucose tolerance test demonstrated that there was a notable improvement in glucose tolerance in the juice treated group. In addition, the berry juice significantly reduced body weight. Our data suggest that ginseng berry juice, as a dietary supplement, may have functional efficacy in consumers with diabetes.

Keywords

American ginseng; berry juice; hyperglycemia; obesity; *ob/ob* mice; type 2 diabetes

Introduction

Diabetes mellitus is a major and serious chronic metabolic disorder throughout the world. Diabetes has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. In the United States, the overall prevalence of diabetes is almost 8% of the population, of which 90% is type 2 diabetes (American Diabetes Assn. 1996; Skyler 2004). Treatment and care of diabetes represent a substantial portion of national health care expenditure. In 1992, for example, diabetes care cost approximately 15% of every dollar spent on U.S. healthcare (Rubin and others 1994).

Type 2 diabetes represents a syndrome with disordered metabolism of carbohydrate and fat. The most prominent clinical feature is hyperglycemia (Abaira and others 1995). The ability of insulin to mediate tissue glucose uptake is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load (Rang and Dale 1991; Kruszynska and Olefsky 1996). Patients with type 2 diabetes exhibit a marked reduction in insulin-mediated glucose disposal (Reaven 1983). Insulin resistance is independently associated with obesity, and with

the 80% of type 2 diabetic patients in the West who are obese (Kruszynska and Olefsky 1996; Ajjan and Grant 2006), and it is noted that insulin resistance is more severe in these patients (Seely and Olefsky 1993).

In addition to currently used pharmacological agents (oral hypoglycemic agents/insulin), diabetic patients and healthcare professionals are increasingly considering complementary and alternative approaches, such as using botanicals with antihyperglycemic activities (Lee 1999; Cicero and others 2004). We previously reported that the berry of American ginseng (*Panax quinquefolium* L.) possesses significant antidiabetic effects in C57BL/6J *ob/ob* and C57BL/Ks *db/db* mice (Attele and others 2002; Xie and others 2002, 2005). In these studies, an organic solvent was used to prepare the berry extract, and the extract was subsequently administered intraperitoneally in the experimental animals. If American ginseng berry can be useful as a dietary supplement, simple preparation and oral intake would be a convenient, safe, and practical means for consumers. In this article, we simplified the preparation of ginseng berry, and the prepared juice was administered orally in obese diabetic *ob/ob* mice. The constituents of the juice were also analyzed using high-performance liquid chromatography.

Materials and Methods

Chemicals

All solvents were of high-performance liquid chromatography (HPLC) grade from Fisher Scientific (Norcross, Ga., U.S.A.). Milli Q water was supplied by a water purification system (US Filter, Palm Desert, Calif., U.S.A.). Standards for ginsenosides Rb₁, Rb₂, Rc, Rd, Re, and Rg₁ were obtained from Indofine Chemical Co. (Somerville, N.J., U.S.A.); ginsenosides Rb₃, Rg₂, 20(R)-Rg₂, Rg₃, Rh₁, and Rh₂ were obtained from the Delta Information Center for Natural Organic Compounds (Xuancheng, Anhui, China). All standards were of biochemical-reagent grade and at least 95% pure as confirmed by HPLC.

Preparation of American ginseng berry juice (AGBJ)

Field-grown American ginseng berry was collected by our group from a ginseng farm in Wausau, Wis., U.S.A. The berry was first mixed in distilled water. The seeds were then removed, and the pulp was collected. The pulp was blended using a commercial food blender for 1 min, and filtered by medical cotton. The solution was heat-treated, condensed by an evaporator, and then refrigerated. Three hundred fifty grams of fresh berry yielded approximately 200 mL of juice for oral administration.

AGBJ analysis using high-performance liquid chromatography (HPLC)

The HPLC system was a Waters 2960 instrument (Milford, Mass., U.S.A.) with a quaternary pump, an automatic injector, a photodiode array detector (Model 996), and Waters Millennium 32 software for peak identification and integration. The separation was carried out on a Prodigy™ ODS(2) column (150 × 4.6 mm I.D.) (Phenomenex, Torrance, Calif., U.S.A.) with a guard column (Phenomenex Prodigy, 3.0 × 4.0 mm I.D.) (Wang and others 2006). For HPLC analysis, a 20-μL sample was injected into the columns and eluted at room temperature with a constant flow rate of 1.0 mL/min. Acetonitrile (solvent A) and water (solvent B) were used. Gradient elution started with 20% solvent A and 80% solvent B, changed to 20.3% A for 25 min, then changed to 26.8% A for 3 min and held for 26 min; changed to 35.6% A for 20 min; changed to 50% A for 11 min; changed to 68% A for 10 min; changed to 95% A for 1 min and held for 3 min; changed to 20% A for 3 min and held for 8 min. The detection wavelength was set to 202 nm. All tested solutions were filtered through Millex 0.2-μm nylon membrane syringe filters (Millipore Co., Bedford, Mass., U.S.A.) before use. The linearity of this method was assayed by analyzing standard solutions in the range of 2 to 400 μg/mL for the 12 ginsenosides. Calibration curves were constructed from the measured peak areas and the related amount of

ginsenosides. Ginsenosides Rb₁, Rb₂, Rb₃, Rc, Rd, Re, Rg₁, Rg₂, 20(R)-Rg₂, Rg₃, Rh₁, and Rh₂ in extract samples were identified by comparison of their retention times with those obtained from the chromatograms of mixed ginsenoside standards. The content of saponins in each sample was calculated using standard curves of ginsenosides.

Animals

The study protocol was approved by the Institutional Animal Care and Use Committee of the Univ. of Chicago. Adult male C57BL/6J *ob/ob* mice, that are known to be hyperglycemic, glucose intolerant, and obese, phenotypically resembling human type 2 diabetes (Zhang and others 1994), were purchased from Jackson Laboratory (Bar Harbor, Maine, U.S.A.). Mice were housed in environmentally controlled conditions with a 12-h light/dark cycle. Animals had free access to food (standard rodent pellet, Zeigler Bros., Gardners, Pa., U.S.A.) and water *ad libitum*, except when they were fasted before experiments. After 3 wk of acclimation under these conditions, animals were grouped for experiments. Ten animals, approximately 20 wk old, weighting 76.6 ± 2.6 g, were randomly divided into 2 groups used for the experiments: vehicle group and AGBJ group.

AGBJ was administrated orally (oral gavage) once a day at a dose of 0.6 mL/kg. Vehicle group animals received saline solution. No adverse events or irritation was observed following daily oral gavage of the berry juice or vehicle.

Blood glucose level measurements and intraperitoneal glucose tolerance test (IPGTT)

Fasting blood glucose levels were measured after animals fasted for 4 h (starting at 9:00 a.m.) on day 0 (before treatment) and day 10 (last day of treatment). Blood glucose levels were determined in blood samples from the tail vein at 1:00 p.m. using a Glucose Analyzer (Hemocue AB, Angelholm, Sweden). Animal body weight was also obtained at 1:00 p.m.

IPGTT was performed on day 0 and day 10. Animals were fasted for 4 h followed by an intraperitoneal (IP) administration of glucose (2 g/kg). Blood glucose levels were measured at 0 (baseline), 30, 60, and 120 min after glucose administration.

Statistical analysis

Data are expressed as mean $\pm$ SE. Statistical significance between the vehicle-treated group and juice-treated group was determined by paired Student's *t*-test. Differences were considered significant if $P < 0.05$.

Results

HPLC analysis of AGBJ

As shown in Figure 1, a good separation of 12 ginsenosides was obtained. The contents of 12 ginsenosides in AGBJ are shown in Table 1. The total ginsenoside content in AGBJ was 5.46 mg/mL. The main constituents in AGBJ were ginsenosides Re (0.88 mg/g), Rb₂ (0.77 mg/g), Rb₃ (2.90 mg/g), and Rd (0.39 mg/g) (Table 1).

Effects of AGBJ on fasting blood glucose levels

Four-hour fasting blood glucose levels were measured on day 0 and day 10 after daily oral administration of AGBJ 0.6 mL/kg or vehicle. As shown in Table 2, the baseline or day 0 fasting blood glucose levels were very high in both the vehicle group ($n = 5$) and AGBJ group ($n = 5$), that is, 230.0 ± 9.7 and 228.0 ± 14.5 mg/dL, respectively. After daily administration of AGBJ 0.6 mL/kg for 10 d, blood glucose concentrations of *ob/ob* mice decreased

significantly to 157.8 ± 13.9 mg/dL ($P < 0.01$, compared to the vehicle group of 223.0 ± 18.0 mg/dL). The glucose level did not change significantly over 10 d in the vehicle group.

Interestingly, after cessation of the AGBJ administration, its hypoglycemic effect continued. Figure 2 shows a prolongation of the antihyperglycemic activity of AGBJ. On day 20, *ob/ob* mice still had a significantly reduced blood glucose level (146.6 ± 17.6 mg/dL; $P < 0.01$ compared to vehicle). The blood glucose level returned to that of the control group as measured on day 30.

Effects of AGBJ on glucose tolerance test (IPGTT)

Glucose disposal was evaluated by IPGTT, prior to and 10 d post-treatment with vehicle or the AGBJ. As shown in Figure 3, on day 0, all *ob/ob* mice demonstrated basal hyperglycemia, and this hyperglycemia was exacerbated by the IP glucose load, and did not return to baseline after 120 min indicating glucose intolerance and impaired disposal. Figure 3A shows the IPGTT data after treatment with vehicle, and no significant changes were seen in any time points. As shown in Figure 3B, after 10 d of treatment with AGBJ, there was a significantly higher rate of glucose disposal at 30, 60, and 120 min ($P < 0.01$ compared to vehicle group). To evaluate the overall glucose exposure, the area under the concentration curve (AUC) was calculated. A significant improvement in glucose exposure was noted in the AGBJ-treated mice (day 10), in which the AUC decreased 38.9% compared to day 0 ($P < 0.01$). In contrast, there was no significant change in the AUC of animals who received vehicle.

Effects of AGBJ on body weight changes

Table 3 shows the effect of AGBJ on body weight changes in *ob/ob* mice. After 10 d of AGBJ administration, body weight in *ob/ob* mice reduced from 76.6 ± 2.6 g on day 0 to 74.1 ± 2.0 g on day 10 ($P < 0.05$). However, the body weight of animals in the vehicle group did not change significantly. After cessation of AGBJ administration, the body weight returned to that of the control group on day 20.

Discussion

Diabetes is a serious metabolic disease. The chronic nature of the disease and its complications are a significant medical problem. Diabetes can cause blindness, kidney failure, and nerve damage, and confers an increased risk of ischemic heart disease, stroke, and peripheral vascular disease (Chang and others 2005; Liu and others 2006). There are over 100 million people in the world with diabetes, and this number is projected to grow to over 200 million in the next 40 to 50 y (Proietto and others 1999). However, currently available drugs for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (DeFronzo 1999; Ajjan and Grant 2006). This situation has led to the search for alternative therapies that have a similar degree of efficacy without the troublesome side effects associated with conventional treatments.

Based on our previous observation of the antidiabetic effect of American ginseng berry extract (Attele and others 2002; Xie and others 2002), we simplified the preparation of ginseng berry, and the prepared juice was administered orally in *ob/ob* mice in this study. Unlike our previous published studies using organic solvent, we used water extraction in this study. With water extraction, 2 different types of antidiabetic components, ginsenosides and polysaccharides, were obtained. Additionally, water extraction does not have organic solvent residue problems in the extracts.

In *ob/ob* mice, mutation of the obese gene leads to morbid obesity and metabolic abnormalities (Zhang and others 1994; Yuan and others 2000). We observed that fasting blood glucose levels

decreased significantly after 10 d of oral administration of the berry juice, and this hypoglycemic effect continued for at least another 10 d after cessation of the treatment. Our IPGTT data indicated that, after the juice treatment, there was a markedly increased glucose disposal, suggesting an improvement in glucose tolerance. In addition, we observed that the berry juice reduced the body weight in the obese mice. Different from previously used intraperitoneal extract injection to our experimental animals, oral administration is a convenient and safe way to deliver medication and dietary supplements. In this study, oral berry juice administration was used and efficacy was observed. It appears that as a dietary supplement, the berry juice may have a functional efficacy in people with diabetes.

The active compounds in American ginseng are dammarane saponins, commonly referred to as ginsenosides, and the responsible constituents in the berry juice are also likely to be ginsenosides (Attele and others 2002). In this study, we measured the major ginsenosides using HPLC analysis, and the levels of the compounds can be used to develop quality controls for AGBJ preparations. Our analytical data showed that the ginsenoside proportions in AGBJ were much different from that in American ginseng root. In contrast to the main constituent of American ginseng root, which is ginsenoside Rb₁, the main constituent of AGBJ is ginsenoside Rb₃ (2.90 mg/g; see Table 1). This is the 1st report showing the high content of Rb₃ in the American ginseng berry water extract. Ginsenoside Rb₁ was a trace compound in AGBJ (0.05 mg/g), since the total ginsenoside content was 5.46 mg/mL. The actions of the ginsenosides may be multifaceted, but the exact mechanisms of action remain unclear. Based on previous reports, there are several plausible hypotheses that may work independently or concurrently, such as inhibiting digestion, increasing energy expenditure, improving sensitivity to insulin and changing blood insulin level, and promoting antioxidant activities (Lillioja and others 1993; Taylor and others 1994; Vuksan and others 2000; Attele and others 2002; Mehendale and others 2004; Cabioglu and Ergene 2006).

Conclusions

Data from this study demonstrated that oral administration of AGBJ significantly reduced high blood glucose levels and body weight in *ob/ob* mice. Our results suggest that the hypoglycemic activity of the berry juice may prove to be beneficial in the prevention and management of type 2 diabetes.

Acknowledgements

This work was supported in part by the NIH/NCCAM grant AT002445 and AT003255.

References

- Abraira C, Colwell JA, Nuttall FQ, Sawin CT, Nagel NJ, Comstock JP, Emanuele NV, Levin SR, Henderson W, Lee HS. Veterans Affairs Cooperative Study on glycemic control and complications in type II diabetes (VA CSDM). Results of the feasibility trial. Veterans Affairs Cooperative Study in Type II Diabetes. *Diabetes Care* 1995;18:1113–23. [PubMed: 7587846]
- Ajjan RA, Grant PJ. Cardiovascular disease prevention in patients with type 2 diabetes: the role of oral anti-diabetic agents. *Diab Vasc Dis Res* 2006;3:147–58. [PubMed: 17160909]
- American Diabetes Assn. Vital statistics. *Diabetes*. 1996
- Attele AS, Zhou YP, Xie JT, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan CS. Antidiabetic effects of Panax ginseng berry extract and the identification of an effective component. *Diabetes* 2002;51:1851–8. [PubMed: 12031973]
- Cabioglu MT, Ergene N. Changes in levels of serum insulin, C-peptide and glucose after electroacupuncture and diet therapy in obese women. *Am J Chin Med* 2006;34:367–76. [PubMed: 16710886]

- Chang WT, Dao J, Shao ZH. Hawthorn: potential roles in cardiovascular disease. *Am J Chin Med* 2005;33:1–10. [PubMed: 15844828]
- Cicero AF, Derosa G, Gaddi A. What do herbalists suggest to diabetic patients in order to improve glycemic control? Evaluation of scientific evidence and potential risks. *Acta Diabetol* 2004;41:91–8. [PubMed: 15666575]
- DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. *Ann Intern Med* 1999;131:281–303. [PubMed: 10454950]
- Kruszynska YT, Olefsky JM. Cellular and molecular mechanisms of non-insulin dependent diabetes mellitus. *J Investig Med* 1996;44:413–28.
- Lee L. Introducing herbal medicine into conventional health care settings. *J Nurse Midwifery* 1999;44:253–66. [PubMed: 10380444]
- Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 1993;329:1988–92. [PubMed: 8247074]
- Liu SQ, Yu JP, Chen HL, Luo HS, Chen SM, Yu HG. Therapeutic effects and molecular mechanisms of *Ginkgo biloba* extract on liver fibrosis in rats. *Am J Chin Med* 2006;34:99–114. [PubMed: 16437743]
- Mehendale SR, Aung HH, Yin JJ, Lin E, Fishbein A, Wang CZ, Xie JT, Yuan CS. Effects of antioxidant herbs on chemotherapy-induced nausea and vomiting in a rat-pica model. *Am J Chin Med* 2004;32:897–905. [PubMed: 15673195]
- Proietto J, Filippis A, Nakhla C, Clark S. Nutrient-induced insulin resistance. *Mol Cell Endocrinol* 1999;151:143–9. [PubMed: 10411329]
- Rang, HP.; Dale, MM. The endocrine system pharmacology. Harlow, U.K.: Longman Group Ltd; 1991. p. 504-8.
- Reaven GM. Insulin resistance in noninsulin-dependent diabetes mellitus. Does it exist and can it be measured? *Am J Med* 1983;74:3–17. [PubMed: 6337484]
- Rubin RJ, Altman WM, Mendelson DN. Health care expenditures for people with diabetes mellitus, 1992. *J Clin Endocrinol Metab* 1994;78:809A–F.
- Seely, BL.; Olefsky, JM. Potential cellular and genetic mechanisms for insulin resistance in common disorders of obesity and diabetes. In: Moller, D., editor. *Insulin resistance and its clinical disorders*. Chichester, U.K.: John Wiley & Sons, Ltd.; 1993. p. 187-252.
- Skyler JS. Diabetes mellitus: pathogenesis and treatment strategies. *J Med Chem* 2004;47:4113–7. [PubMed: 15293979]
- Taylor SI, Accili D, Imai Y. Insulin resistance or insulin deficiency. Which is the primary cause of NIDDM? *Diabetes* 1994;43:735–40. [PubMed: 8194657]
- Vuksan V, Sievenpiper JL, Koo VY, Francis T, Beljan-Zdravkovic U, Xu Z, Vidgen E. American ginseng (*Panax quinquefolius* L) reduces postprandial glycemia in nondiabetic subjects and subjects with type 2 diabetes mellitus. *Arch Intern Med* 2000;160:1009–13. [PubMed: 10761967]
- Wang CZ, Wu JA, McEntee E, Yuan CS. Saponins composition in American ginseng leaf and berry assayed by high-performance liquid chromatography. *J Agric Food Chem* 2006;54:2261–6. [PubMed: 16536605]
- Xie JT, Zhou YP, Dey L, Attele AS, Wu JA, Gu M, Polonsky KS, Yuan CS. Ginseng berry reduces blood glucose and body weight in db/db mice. *Phytomedicine* 2002;9:254–8. [PubMed: 12046868]
- Xie JT, McHendale S, Yuan CS. Ginseng and diabetes. *Am J Chin Med* 2005;33:397–404. [PubMed: 16047557]
- Yuan CS, Attele AS, Zhang L, Lynch JP, Xie JT, Shi ZQ. Leptin reduces body weight gain in neonatal rats. *Pediatr Res* 2000;48:380–3. [PubMed: 10960507]
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425–32. [PubMed: 7984236]

Figure 1. HPLC analysis of ginsenosides in American ginseng berry juice (AGBJ). Chemical structures of 3 main constituents in AGBJ are shown in (A). HPLC chromatogram of AGBJ recorded at 202 nm is shown in (B). HPLC conditions are described in the Materials and Methods section.

Figure 2. Prolonged hypoglycemic effect of American ginseng berry juice (AGBJ) on fasting blood glucose levels in *ob/ob* mice. Vehicle treated group ($n = 5$). AGBJ 0.6 mL/kg treated group ($n = 5$).

Figure 3. Effect of American ginseng berry juice on intraperitoneal glucose tolerance test (IGPTT) in *ob/ob* mice. (A) Vehicle treated group ($n = 5$). (B) AGBJ 0.6 mL/kg treated group ($n = 5$).

Table 1
Ginsenoside content in American ginseng berry juice

Ginsenoside	Rg ₁	Re	Rh ₁	Rg ₂	20R-Rg ₂	Rb ₁	Rc	Rb ₂	Rb ₃	Rd	Rg ₃	Rh ₂	Total
Content (mg/mL)	0.04	0.88	0.003	0.06	0.02	0.05	0.30	0.77	2.90	0.39	0.03	0.002	5.46

Table 2
Effects of American ginseng berry juice (AGBJ) on blood glucose levels in *ob/ob* mice

Group	<i>n</i>	Blood glucose (mg/dL)	
		Day 0	Day 10
Vehicle	5	230.0 ± 9.7	228.0 ± 14.5
AGBJ (0.6 mL/kg)	5	228.0 ± 14.5	157.8 ± 13.9 ^a

^a $P < 0.01$.

Table 3
Effects of American ginseng berry juice (AGBJ) on body weight changes in *ob/ob* mice

Group	<i>n</i>	Body weight (g)	
		Day 0	Day 10
Vehicle	5	77.9 ± 2.1	76.9 ± 3.7
AGBJ (0.6 mL/kg)	5	76.6 ± 2.1	74.1 ± 2.0 ^a

^a*P* < 0.05.