Brief Report

DOI: 10.5582/ddt.2011.v5.4.181

Effects of Gosha-jinki-gan (Chinese herbal medicine: Niu-Che-Sen-Qi-Wan) on hyperinsulinemia induced in rats fed a sucrose-rich diet

Yoshihiko Hirotani[1,*], Ayaka Doi[1], Kenji Ikeda[1], Ryuji Kato[2], Yoshio Ijiri[2], Kazuhiko Tanaka[2], Michiaki Myotoku[1]

[1] *Laboratory of Clinical Pharmaceutics, Faculty of Pharmacy, Osaka Ohatani University, Osaka, Japan;*
[2] *Laboratory of Clinical Pharmacy and Clinical Pharmacokinetics, Osaka University of Pharmaceutical Sciences, Takatsuki, Japan.*

ABSTRACT: We investigated the effects of a Chinese herbal medicine, Gosha-jinki-gan (GJG), on the regulation of insulin levels in rats fed a sucrose-rich diet (SRD). Normal Wistar rats in the SRD group were fed an SRD for 4 weeks. Increased dietary sucrose did not alter plasma glucose levels but it increased plasma insulin levels at 2 and 4 weeks in the SRD-fed rats relative to control rats that were fed standard chow. GJG treatment significantly suppressed the SRD-induced elevation in plasma insulin levels. These results suggest that GJG improves hyperinsulinemia caused by an SRD.

Keywords: Herbal medicine, Gosha-jinki-gan, sucrose-rich diet, hyperinsulinemia, rats

1. Introduction

Insulin dysfunction, including hyperinsulinemia, is a major metabolic abnormality in populations with non-insulin-dependent diabetes mellitus (type 2 diabetes). Studies in rats have shown that a sucrose-rich diet (SRD) leads to abnormal insulin sensitivity in the liver and peripheral tissues, resulting in hypertriglyceridemia (*1*,*2*). Hypertriglyceridemia is an important risk factor for coronary heart disease, especially in populations with type 2 diabetes. In rats, hyperinsulinemia and subsequent dyslipidemia are known to occur after administration of an SRD (*2*,*3*). Hence, the relationship between hyperinsulinemia and hypertriglyceridemia is explained by the following correlations: *i*) insulin resistance and compensatory hyperinsulinemia, *ii*) hyperinsulinemia and hepatic synthesis and secretion of very low-density lipoprotein-triglyceride (VLDL-TG), and *iii*) the hepatic VLDL-TG secretion rate and plasma triglyceride concentrations (*4*).

Gosha-jinki-gan (GJG) (Niu-Che-Sen-Qi-Wan), a traditional Chinese herbal complex of 10 medical herbs, has been widely used to treat patients with melalgia, lower back pain, and numbness. Recently, GJG was reported to effectively attenuate the subjective symptoms of diabetic neuropathy (*5*,*6*). In addition, Suzuki *et al*. reported that the antinociceptive activity of GJG was significantly greater in diabetic mice than in non-diabetic mice as gauged by nitrous oxide (NO) production (*7*). Further, homeostasis model assessment of the insulin resistance (HOMA-R) index in patients with type 2 diabetes found that the index decreased significantly after GJG treatment (*8*). However, few reports have described the effects of GJG on hyperinsulinemia induced by sucrose in the diet.

The present study sought to investigate the effects of GJG on hyperinsulinemia in normal rats that were given an SRD for a period of 4 weeks.

2. Materials and Methods

2.1. *Animals*

Male Wistar rats (Japan SLC Inc., Shizuoka, Japan) weighing 180-190 g were used in this study. The rats were maintained on a standard powder diet (MF® diet; Oriental Yeast, Tokyo, Japan) for 1 week. They were allowed free access to rat chow and water and were kept in a room maintained at 22 ± 2°C with a 12-h/12-h light/dark cycle (light cycle begun at 8:00 AM). All experimental procedures were conducted according to the Osaka Ohtani University Guidelines for the Care and Use of Laboratory Animals, and the study protocol was approved by the local Animal Ethics Committee.

2.2. *Drugs*

Spray-dried GJG powder was manufactured and supplied by Tsumura & Co. Ltd. (Tokyo, Japan). The composition of GJG is as follows: 5 g of Rehmanniae Radix (*Rehmannia glutinosa* Liboschitz); 3 g each of

**Address correspondence to:*
Dr. Yoshihiko Hirotani, Laboratory of Clinical Pharmaceutics, Faculty of Pharmacy, Osaka Ohatani University, 3-11-1 Nishikiorikita Tondabayashi, Osaka 584-8540, Japan.
e-mail: hirotay@osaka-ohtani.ac.jp

Achyranthis Radix (*Achyranthes bidentata* Blume), Corni Fructus (*Cornus officinalis* Sieb. et Zucc), Dioscoreae Rhizoma (*Dioscorea batatas* Decaisne), Plantaginis Semen (*Plantago asiatica*), Alismatis Rhizoma (*Alisma orientale* Juzep), Hoelen (*Poria cocos* Wolf), and Moutan Cortex (*Paeonia suffruticosa* Andrews); and 1 g each of Cinnamomi Cortex (*Cinnamomum cassia* Blume) and Aconiti Tuber (*Aconitum carmichaelii* Debeaux). The three-dimensional high-performance liquid chromatography (HPLC) profile of the GJG extract powder provided by Tsumura Inc. is shown in Figure 1.

2.3. *Animal treatments and collection and preparation of blood samples*

The rats were randomly divided into 3 groups consisting of 5 rats each. Rats in the control group were maintained on standard chow. The rats in the SRD group were maintained on standard chow supplemented with 50% sucrose (370 kcal/100 g chow) without GJG, whereas those in the SRD + GJG group were fed chow containing 50% sucrose and 1% powdered GJG extract. The rats had access to the chow and tap water *ad libitum*. Body weights of the rats and the food and water intake per cage were measured on a weekly basis. For 4 weeks, non-fasting blood samples were collected daily from the jugular vein at 10:00 AM, and the samples were stored in chilled tubes with 30 mM EDTA (final concentration).

2.4. *Assays to determine plasma glucose, triglyceride, cholesterol, and insulin levels*

Plasma glucose levels were determined using a commercial assay kit (Glucose CII-Test Wako; Wako Pure Chemical Industries Ltd., Osaka, Japan). Plasma triglyceride and cholesterol levels were determined using the commercial lipid assay kits Triglyceride E-Test Wako and Cholesterol E-Test Wako, respectively (Wako Pure Chemical Industries Ltd.). Plasma immunoreactive insulin levels were measured using a commercial radioimmunoassay kit (Insulin Eiken RIA kit; Eiken Chemical Co. Ltd., Tokyo, Japan).

2.5. *Data analysis*

Experimental data are expressed as mean values with standard deviations (S.D.). Statistical analysis of the differences between the mean values obtained was performed using Tukey's multiple comparison test and an unpaired Student's *t* test with a significance level of $p < 0.05$.

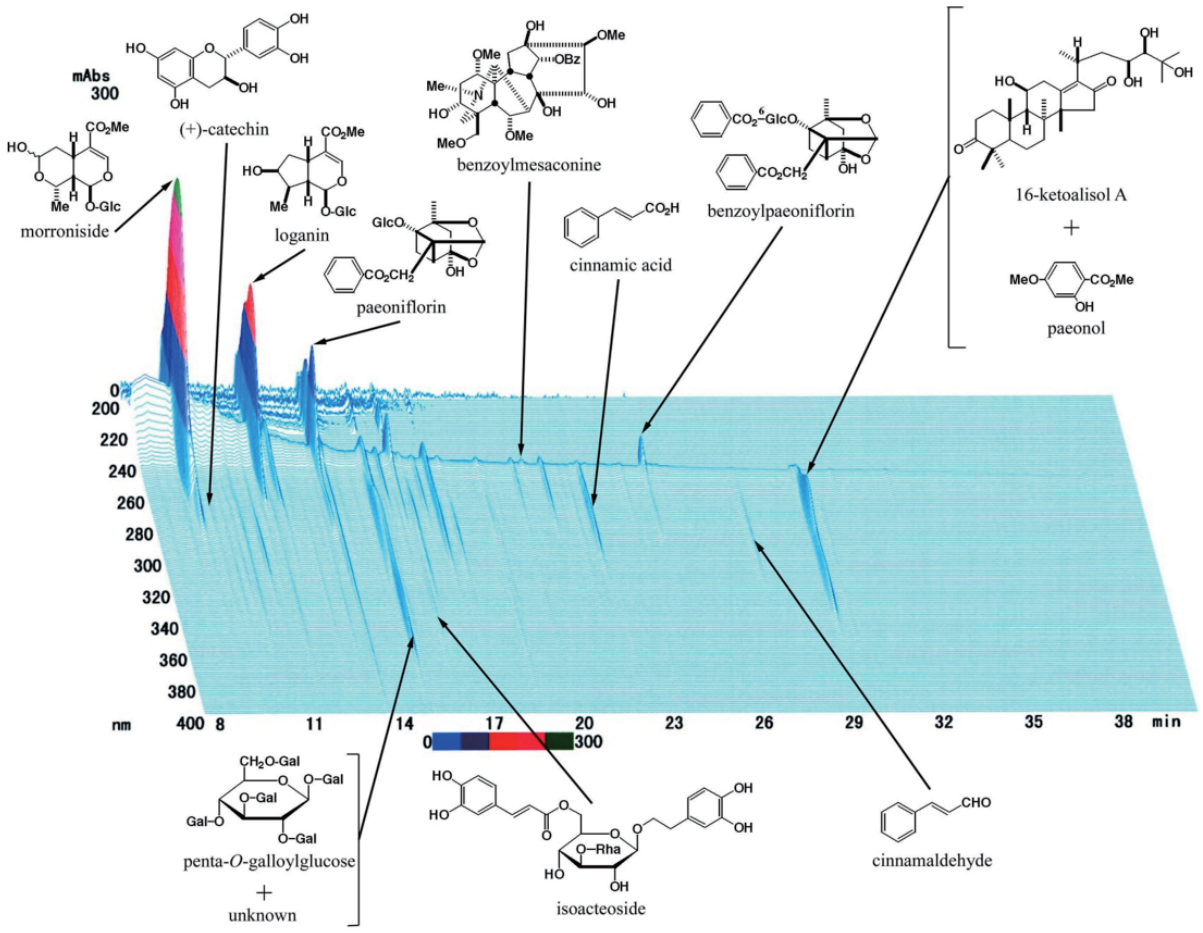

Figure 1. Three-dimensional HPLC profile of GJG.

3. Results and Discussion

Sucrose is a major ingredient of many processed foods and has been implicated in the development of obesity and dyslipidemia in humans (*9,10*). Further, diets rich in sucrose are known to induce hyperinsulinemia and hypertriglyceridemia in rodents (*11,12*). Rodent models of diet-induced hyperinsulinemia and hypertriglyceridemia are used to assess the therapeutic efficacy of drugs and nutrients that are likely to affect insulin sensitivity and lipid concentration in the blood (*12,13*). However, the effects of GJG in ameliorating the metabolic dysregulation induced by high sucrose intake have not been previously reported.

The three-dimensional HPLC profile of the GJG extract is shown in Figure 1. Standard compounds that have been isolated, purified, and identified (*via* mass spectroscopy, infrared spectroscopy, and nuclear magnetic resonance) from botanical raw materials in GJG are analyzed under the same conditions, and data from the UV spectra and column retention times are used to create a chromatogram library. Subjecting the library to a peak detector (an auxiliary function of HPLC) allows evaluation of the degree of similarity of peaks and peak purity. Morroniside, loganin, and paeoniflorin were the major components of GJG; (+)-catechin, penta-*O*-galloylglucose, isoacteoside, benzoylmesaconine, cinnamic acid, benzoylpaeoniflorin, cinnamaldehyde and 16-ketoalisol A were also detected.

The changes in the body weights of the rats are shown in Figure 2. These changes were significantly lower in the SRD-fed rats than in the control rats ($p < 0.05$ or $p < 0.01$), except in week 1. The changes in body weight in the rats administered SRD + GJG were similar to those in the control rats. The food and water intake by the SRD and SRD + GJG groups was similar to that by the control group (data not shown). An SRD alters energy partitioning in a way that is conducive to body-weight gain (*4,14*). However, the body weights of the SRD-fed rats significantly decreased in the current study (Figure 2). Sucrose has been reported to increase body weight when administered in solution form but not when added to chow (*4*). Addition of sucrose to the drinking water of rats induces obesity with intraabdominal fat deposition. Hence, the decrease in the body weights of rats in the present study was probably due to a decrease in food intake as a result of the high sucrose content in the chow. Notably, food and water intake did not vary among the 3 groups. These results indicate that GJG restored the body weights of the SRD-fed rats.

No significant changes were detected in the plasma glucose levels of any of the 3 groups throughout the study period, except in the SRD group in week 4 (Figure 3A). Figure 3B shows the changes in the non-fasting plasma insulin levels during the study period. In the SRD group, the non-fasting plasma insulin levels were significantly higher than those in the control group ($p < 0.05$). Further, the SRD + GJG group had significantly decreased plasma insulin levels in weeks 2 and 4 ($p < 0.05$) compared to the SRD group. In the present study, administration of SRD alone was followed by a significant increase in the plasma insulin levels; as a consequence, the plasma glucose levels tended to decrease. SRD + GJG administration significantly prevented the development of hyperinsulinemia in weeks 2 and 4 ($p < 0.05$) (Figure 3B); therefore, GJG normalized the plasma glucose and insulin levels of the rats fed SRD. An important point is that GJG alone does not reduce plasma glucose and insulin levels in normal rats (*15*). The nitric oxide pathway has been reported to potentially mediate the effects of GJG on insulin action in insulin-sensitive tissues (*15*). Cinnamomi cortex, a component of GJG, has been shown to improve insulin action by enhancing the insulin-signaling pathway in skeletal muscles (*16*). Qin *et al*. reported that GJG administration improved impaired insulin sensitivity in rats with streptozotocin (STZ)-induced diabetes (*17*). Since GJG is a complex medical preparation containing individual ingredients with different pharmacological actions, further studies are required to ascertain the molecular mechanism by which GJG affects insulin sensitivity.

No significant changes in the plasma triglyceride levels were detected in the SRD and SRD + GJG group

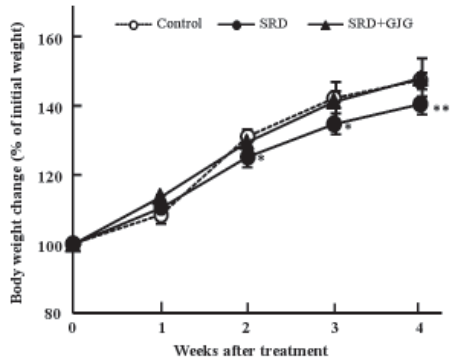

Figure 2. Effects of GJG on the post-treatment body weight of SRD-fed rats. * $p < 0.05$, ** $p < 0.01$ compared to the control group. Data are mean ± S.D. ($n = 5$).

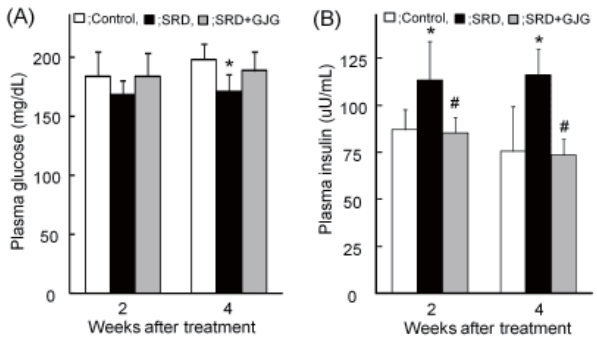

Figure 3. Effects of GJG on post-treatment plasma glucose (A) and insulin (B) levels in SRD-fed rats. * $p < 0.05$ compared to the control group. # $p < 0.05$ compared to the SRD group. Data are mean ± S.D. ($n = 5$).

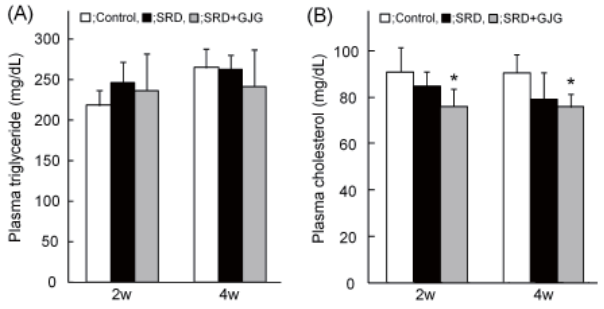

Figure 4. Effects of GJG on post-treatment plasma triglyceride (A) and cholesterol (B) levels in SRD-fed rats. * $p < 0.05$ compared to the control group. Data are mean ± S.D. ($n = 5$).

rats in weeks 2 and 4, compared to levels in the control group (Figure 4A). The non-fasting plasma cholesterol levels in the SRD group did not significantly differ from those in the control group, whereas those in the SRD + GJG group were significantly lower than the levels in the control group in weeks 2 and 4 ($p < 0.05$; Figure 4B). Chemical compounds such as alisol A and cinnamon in GJG components might decrease plasma cholesterol levels (*18,19*). That said, short-term administration of an SRD was not found to alter plasma triglyceride and cholesterol levels. However, long-term administration of GJG has been found to reduce elevated plasma triglyceride levels induced by SRD treatment (*20*).

In conclusion, the data in the present study suggest that GJG may prove useful in the treatment and/or prevention of hyperinsulinemia in non-diabetic subjects. However, further investigation is needed to elucidate the molecular mechanisms associated with the effects of GJG.

Acknowledgements

The authors wish to thank Tsumura Inc. for providing the GJG extract powder.

References

1. Soria A, D'Alessandro ME, Lombardo YB. Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocytes. J Appl Physiol. 2001; 91:2109-2116.
2. Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC, Lombardo YB. Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. J Nutr. 2003; 133:127-133.
3. Gutman RA, Basílico MZ, Bernal CA, Chicco A, Lombardo YB. Long-term hypertriglyceridemia and glucose intolerance in rats fed chronically an isocaloric sucrose-rich diet. Metabolism. 1987; 36:1013-1020.
4. Goodson S, Halford JC, Jackson HC, Blundell JE. Paradoxical effects of a high sucrose diet: High energy intake and reduced body weight gain. Appetite. 2001; 37:253-254.
5. Sakamoto N, Sato Y, Goto Y, Ikeda Y, Takahashi A, Yano S, Takeda K, Baba S, Kaneko T, Mimura G, Tanaka T. Treatment of diabetic neuropathy with traditional oriental medicine-comparison between Goshajinkigan and mecobalamin treatment. J Jpn Diab Soc. 1987; 30:729-737.
6. Tawata M, Kurihara A, Nitta K, Iwase E, Gan N, Onaya T. The effects of goshajinkigan, a herbal medicine, on subjective symptoms and vibratory threshold in patients with diabetic neuropathy. Diabetes Res Clin Pract. 1994; 26:121-128.
7. Suzuki Y, Goto K, Ishige A, Komatsu Y, Kamei J. Antinociceptive effect of Gosha-jinki-gan, a Kampo medicine, in streptozotocin-induced diabetic mice. Jpn J Pharmacol. 1999; 79:169-175.
8. Uno T, Kitamura Y, Sato Y. Diabetic complications and Kampo medicine. Aichi Gakuin University bulletin of the Faculty of Psychological & Physical Science. 2007; 2:69-74.
9. Fried SK, Rao SP. Sugars, hypertriglyceridemia, and cardiovascular disease. Am J Clin Nutr. 2003; 78:873S-880S.
10. Gross LS, Li L, Ford ES, Liu S. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: An ecologic assessment. Am J Clin Nutr. 2004; 79:774-779.
11. Lombardo YB, Drago S, Chicco A, Fainstein-Day P, Gutman R, Gagliardino JJ, Gomez Dumm CL. Long-term administration of a sucrose-rich diet to normal rats: Relationship between metabolic and hormonal profiles and morphological changes in the endocrine pancreas. Metabolism. 1996; 45:1527-1532.
12. Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA. Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. Am J Physiol. 1996; 271:R1319-R1326.
13. Chen C, Li TC, Li CI, Liu CS, Wang HJ, Lin CC. Serum resistin level among healthy subjects: Relationship to anthropometric and metabolic parameters. Metabolism. 2005; 54:471-475.
14. Diniz YS, Rocha KK, Souza GA, Galhardi CM, Ebaid GM. Effects of *N*-acetylcysteine on sucrose-rich diet-induced hyperglycaemia, dyslipidemia and oxidative stress in rats. Eur J Pharmacl. 2006; 14:151-157.
15. Hu X, Sato J, Bajotto G, Khookhor O, Ohsawa I, Oshida Y, Sato Y. Goshajinkigan (Chinese herbal medicine niu-che-sen-qi-wan) improves insulin resistance in diabetic rats *via* the nitric oxide pathway. Nagoya J Med Sci. 2010; 72:35-42.
16. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y. Cinnamon extract (traditional herb) potentiate *in vivo* insulin-regulated glucose utilization *via* enhancing insulin signaling in rats. Diabetes Res Clin Pract. 2003; 62:139-148.
17. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y. Gosha-jinki-gan (a Herbal Complex) corrects abnormal insulin signaling. Altern Med. 2004; 1:269-276.
18. Imai Y, Matsumura H, Aramaki Y. Hypocholestemic effects of alisol A-24-monoacetate and its related compounds in rats. Japan J Pharmacol. 1970; 20:222-228.
19. Kim SH, Hyun SH, Choung SY. Anti-diabeticeffect of cinnamon extract on bloodglucose in db/db mice. J Ethnopharmacol. 2006; 104:119-123.
20. Hirotani Y, Ikeda K, Myotoku M. Effects of the herbal medicine goshajinkigan on sucrose-rich diet-induced hypertriglyceridemia in rats. J Trad Med. 2009; 26:187-193.

(Received April 26, 2011; Revised May 27, 2011; Accepted July 06, 2011)