Protective effects of the herbal medicine goshajinkigan in a rat model of non-alcoholic fatty liver disease

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ABSTRACT
This study was designed to investigate the effect of an herbal medicine—goshajinkigan (GJ)—on the regulation of total body weight, as well as liver and adipose tissue weights in rats fed a high-fat diet (HFD) and drinking of 30% sucrose (HFDS) (HFD; the rats received 19.6% energy from carbohydrates, 18.2% from proteins, and 62.2% from lipids; total energy, 506 kcal/100 g). Control rats were fed a standard diet (the rats received 60.5% energy from carbohydrates, 26.2% from proteins, and 13.3% from lipids; total energy, 360 kcal/100 g). Over a period of 12 weeks, rats were allowed free access to either the standard diet or HFDS containing 0, 1, or 3% GJ. In comparison with the control group, the HFDS rats showed a significant decrease in overall body weight and adipose tissue weight, and an increase in liver weight at 12 weeks. GJ treatment significantly reversed the HFDS-induced decrease in body and adipose tissue weight and reduced the elevated liver weight dose-dependently. Similarly, GJ reduced the elevated serum aspartate aminotransferase levels observed in HFDS rats. These results suggest that GJ may have the potential to alleviate damage to the liver in subjects with long-term consumption of HFDS.

Increased hepatic production and accumulation of triacylglycerols are directly related to metabolic syndromes (8). Accumulation of triacylglycerols, termed as hepatic steatosis, is proposed to be an indication of more severe liver diseases including non-alcoholic steatohepatitis, which is characterized by fibrosis and necroinflammation and can progress to cirrhosis and terminal liver failure (1). Sugatani et al. found that intake of a high-fat and high-sucrose diet for 8 weeks resulted in marked accumulation of hepatic and serum triacylglycerols in rats, leading to hepatic steatosis but not hepatic necroinflammatory lesions (8).

Goshajinkigan (GJ), an herbal medicine, has been widely used to treat patients with melosalgia, pain in the lower back, and numbness (7, 11). Recently, GJ was reported to be effective in alleviating subjective symptoms of diabetic neuropathy (9, 10). Furthermore, GJ exhibited a significantly better antinociceptive effect in diabetic mice than in nondiabetic mice, as determined on the basis of nitric oxide (NO) production (6, 7). Furthermore, administration of GJ to rats with streptozotocin (STZ)-induced diabetes reduced hyperinsulinemia; GJ mediated its effects via the NO pathway (7). Further, the homeostasis model assessment-insulin resistance (HOMA-R) index in patients with type 2 diabetes significantly decreased after GJ treatment (8). Moreover, we observed that STZ-induced diabetic rats which were fed chow containing 1% GJ exhibited more stable levels of serum triglyceride, cholesterol, and free fatty acid (unpublished). We also found that GJ has the potential to alleviate hypertriglyceridemia and hyperinsulinemia in rats fed a high-sucrose diet (2, 3). However, GJ’s effects on
hitherto hepatic steatosis due to intake of a high-fat diet and sucrose solution (HFDS) have been described in few reports. In the present study, rats fed HFDS for up to 12 weeks were used as a model to investigate GJ’s protective effects against progressive non-alcoholic hepatic steatosis due to long-term HFDS consumption.

Male Wistar rats (Japan SLC Inc., Shizuoka, Japan) weighing 180–190 g were used for this study. The rats were maintained on a standard powder diet (MF® diet; Oriental Yeast, Tokyo, Japan) for 1 week. They had free access to rat chow and water, and were kept in a room maintained at 22°C ± 2°C with a 12-h light/dark cycle (diurnal time, 8 AM to 8 PM). All experimental procedures were conducted according to Osaka Ohtani University Guidelines for the Care and Use of Laboratory Animals. GJ extract was manufactured and supplied by Tsumura & Co. Ltd. (Tokyo, Japan). The composition of GJ was as follows: 5 g of Rehmanniae Radix (Rehmannia glutinosa Liboschitz); 3 g each of 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 rats were randomly divided into 4 groups with 8 rats per group. Rats in the control group were maintained on standard chow. The HFDS group was maintained on a high-fat diet (HFD-60® diet; Oriental Yeast, Tokyo, Japan). The composition of the standard chow was 7.7% water, 23.6% protein, 5.3% fat, 2.9% fiber, and 54.4% nitrogen-free extracts; total energy content was 360 kcal/100 g. The high-fat diet (HFD) consisted of 23.0% protein, 35.0% fat, 6.6% fiber, and 25.3% carbohydrate; total energy content was 506.2 kcal/100 g. The HFDS diets containing 1% or 3% GJ (HFDS + 1% GJ and HFDS + 3% GJ, respectively) were prepared by adding GJ extract at a concentration of 1.0 g or 3.0 g per 100 g of chow including the HFD. The dose of GJ used in this study was about 10-fold higher than that used in humans. The rats had access to chow and tap water or chow and 30% sucrose solution ad libitum. Body weights of rats and food intake per cage were measured on a weekly basis. At the end of 12 weeks, the rats were anesthetized and blood samples were collected from the inferior vena cava; we immediately separated these blood by centrifugation. The epididymal adipose tissue (EAT) and liver tissue were removed from each rat and rinsed with cold saline; the tissues were then weighed and stored at −80°C. Serum aspartate aminotransferase (AST) and glucose levels were determined using commercial kits (Transaminase CII-Test Wako and Glucose CII-Test Wako, respectively; Wako Pure Chemical Industries Ltd., Osaka, Japan). Serum triglyceride and total cholesterol levels were determined using the commercial kits Triglyceride E-Test Wako and Cholesterol E-Test Wako, respectively, which were purchased from Wako Pure Chemical Industries Ltd. Experimental data are expressed as the mean ± standard deviation (SD). Statistical analysis of the differences between the mean values was performed using Tukey’s multiple comparison test and an unpaired Student’s t-test with a significance level of \( P < 0.05 \).

The data we obtained in this study are shown below. Changes in the body weights of the rats are shown in Fig. 1. Compared to the rats in the control group, the rats in the HFDS, HFDS + 1% GJ, and HFDS + 3% GJ groups showed significantly decreased body weights. However, the body weights of the rats in the HFDS + 3% GJ group increased significantly compared to the rats in the HFDS group week 9 onward. At week 12, the body weights of the rats in the HFDS group significantly differed from those of the rats in the HFD + 1% GJ group. The food intake of rats in the HFDS and HFDS + GJ groups was almost similar; however, the intake of these groups was lower than that of the
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cies by feeding rats with high fat diet and sucrose solution with high caloric intake in order to produce non-alcoholic steatohepatitis without inducing obesity associated with intra-abdominal fat deposition. This model rats have decreased intake amount of HFD and their serum glucose has glucose levels lower than rats fed a standard diet. So it is necessary to determine serum insulin and leptin levels in future studies. We demonstrated that intake of HFDS for 12 weeks results in a suitable rat model of hepatic steatosis. This condition has been associated with hepatic injury, although we did not confirm the development of inflammation and fibrosis in the livers of HFDS-fed rats. The observed decrease in body weights most likely occurred as a consequence of lipid metabolic dysregulation because of the high fat in the chow and sucrose solution. As a result, a significant increase in the liver deposition and reduction in the intra-abdominal fat

control group (data not shown).

The EAT and liver weights of rats are summarized in Fig. 2. The EAT weights (g/100 g body weight) were significantly lower in rats belonging to the HFDS group than in those belonging to the control group (P<0.01). The EAT weights of the HFDS + 3% GJ group rats were significantly higher than those of the HFDS group rats (P<0.01). There were no significant differences in adipose weights between the control group rats and the HFDS + 3% GJ group rats. Liver weights (g/100 g body weight) of rats belonging to the HFDS group were significantly higher than those of the rats belonging to the control group (P<0.01). Compared to the rats of the HFDS group, the HFDS + 3% GJ group rats had significantly decreased liver weights (P<0.01).

The AST levels were significantly higher in rats belonging to the HFDS group, HFDS + 1% GJ, and HFDS + 3% GJ groups than in those belonging to the control group (P<0.01). Compared to the HFDS group rats, the HFDS + 3% GJ group rats had significantly decreased AST levels (P<0.05) (Fig. 3). The serum glucose, triglyceride and total cholesterol levels were significantly lower in the HFDS, HFDS + 1% GJ, and HFDS + 3% GJ groups than in the control group (P<0.01)(data not shown). Similarly, triglyceride and total cholesterol levels in the livers of the rats in the HFDS, HFDS + 1% GJ, and HFDS + 3% GJ groups were significantly higher than the corresponding levels in the rats in the control group (data not shown). However, HFDS + 1% and 3% GJ groups did not differ significantly from the HFDS group in terms of serum and liver levels (data not shown).

In this study, we attempted to mimic human spe-

Fig. 2 Effects of GJ on epididymal adipose tissue and liver weight in rats fed HFDS. **P < 0.01 compared with the control group. *P < 0.01 compared with the HFDS group. Data are mean ± SD (n = 8).

Fig. 3 Effects of GJ on AST in rats fed HFDS. **P < 0.01 compared with the control group. *P < 0.05 compared with the HFDS group. Data are mean ± SD (n = 8).
tissue, as well as decreases in serum glucose, triglyceride, and total cholesterol levels was observed in the HFDS group rats. GJ administration attenuated HFDS-induced hepatotoxic injury, evidenced by the changes in body weight, liver and EAT weight, and serum AST levels. Therefore, rats fed HFDS up to 12 weeks were used as a model of lipid metabolic dysregulation to evaluate the protective effects of GJ on HFDS-induced hepatotoxic injury. These results suggest that the potential protective effects of GJ may lead to the prevention of liver injury produced by long-term consumption of highly caloric diets. Nevertheless, total cholesterol and triglyceride concentrations in the liver did not decrease on GJ administration. The specific effects of high lipid content in the liver and adipose tissue weight on lipid and glucose metabolism dysfunction remain to be clarified. Additional studies are required to elucidate the links between impaired hepatic fat deposition and oxidative stress and associated pathological changes in liver. Oxidative stress has been associated with metabolic syndrome and is related to non-alcoholic fatty liver disease (5). The HFD-induced metabolic syndrome in animal models increases oxidative stress (6). Consequently, long-term administration of GJ in non-diabetic individuals may have potential use in the mild (not drastic) prevention of hepatic steatosis as an adjunctive therapy.

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REFERENCES


