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Effects of Geniposide isolated from *Gardenia jasminoides* on Metabolic Alterations in High Sugar Diet-fed Rats

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The effects of geniposide isolated from Gardeniae Fructus ("San-shi-shi" in Japanese) on metabolic alterations in high sugar diet-fed rats were investigated. It was found that the oral administration of the high sugar diet caused hyperlipemia, liver injury with elevation of glutamic pyruvic transaminase (GPT) in the serum, and the accumulation of lipid peroxide in the liver.

Geniposide reduced the serum triglyceride, lipid peroxide, phospholipid, glucose, insulin and GPT in the high sugar diet-fed rats. It was found that the administration of geniposide reduced the sucrase activity in the small intestine and the deposition of lipid peroxide in the liver.

Keywords——*Gardenia jasminoides*; iridoid; geniposide; lipid metabolism; hyperlipemia; high sugar diet; liver injury; lipid peroxide

Gardeniae Fructus ("San-shi-shi," in Japanese), the fruits of *Gardenia jasminoides* Ellis f. *grandifloria* (Lour.) Makino (Rubiaceae), has been used in Chinese medicine as a remedy for inflammation and hepatic disorder. Harada *et al.*[1] reported that geniposide and genipine increased bile secretion, inhibited both the spontaneous contraction and the pilocarpine-induced contraction of rat stomach, and also inhibited the writhing behavior in mice induced by acetic acid. It has also been reported that genipine shows a weak anti-acetylcholine action and a weak anti-histamine action on the isolated mouse or guinea pig ileum. Yamauchi *et al.*[2] reported that genipine and geniposide showed cathartic action.

We reported that the oral administration of a high fat diet for 8 weeks causes a fatty liver, and liver injury with elevation of glutamic pyruvic transaminase (GPT) in rats.[3] The present report describes the effects of geniposide isolated from Gardeniae Fructus on metabolic alterations in rats induced by a high sugar diet for 7 weeks. In addition to *in vivo* experiments, the effects of geniposide on lipid peroxidation induced by ADP and NADPH in microsomes of liver were studied.

Materials and Methods

Materials——Geniposide isolated from the commercial drug by the method described by Inoue *et al.*[4] was used in the present investigations.

Animals——Young male Wistar-King strain rats, weighing 150—180 g and housed in a room maintained at 25±1°C and 60% relative humidity, were allowed free access to food and water. The room was illuminated for 12 h a day, starting at 7: 00 a.m.

Estimation of Serum and Liver Lipids, Serum Glucose, Insulin, Transaminases, and Sucrase Activity in Mucous Membrane of Small Intestine——The rats of high sugar diet-fed groups received the high sugar diet [casein 20%, sugar 60%, corn oil 15%, vitamin mixture 1% (Oriental Yeast Co., Ltd.), chocola A 200 I.U. (Eisai Co., Ltd.) per 100 g of diet, and 50% choline HCl 0.4 ml per 100 g of diet] and water for 7 weeks *ad libitum*. Rats of the experimental groups received the high sugar diet with geniposide (300 mg/kg or 100 mg/kg body weight) for 7 weeks. They were killed 8 h after the last oral intake of the high sugar diet and geniposide. Blood was taken by venous puncture and centrifuged at 1630×*g* for 10 min to separate the serum. Total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-ch), free

fatty acids (FFA), phospholipids (PL), glucose, insulin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lipid peroxides (LPO) in the sera were determined by using Total Cholesterol A-Test (Wako Co.), Triglyceride G-Test (Wako Co.), HDL-Cholesterol-Test (Wako Co.), NEFA-Test (Wako Co.), Phospholipids Test (Wako Co.), Glucose B-Test (Wako Co.), EIA Insulin-Test (Medical and Biological Lab. Co., Ltd.) and the methods of Reitman-Frankel[5] and Yagi *et al.*,[6] respectively. A portion (0.5 g) of the liver tissue was homogenized in Krebs–Ringer phosphate buffer (pH 7.4) (4.5 ml). The homogenate (0.2 ml) was extracted with $CHCl_3$–MeOH (2: 1) (4 ml), and the extract was dried and concentrated. The residue was analyzed for TC and TG by the methods described above. Liver homogenate was directly subjected to estimations of PL, FFA and LPO. Sucrase activity in the small intestine was determined by the methods of Saito *et al.*[7]

Preparation of Microsomes of Liver Tissue——Rats were killed by decapitation, and their liver tissue was quickly removed. Microsomes were isolated from the liver tissue by the method of Oda *et al.*[8] The estimation of protein in isolated microsomes were carried out by the method of Lowry *et al.*[9] with bovine serum albumin as a standard. Microsomes were suspended in Krebs–Ringer phosphate buffer (pH 7.4) for use.

Estimation of ADP and NADPH-induced Lipid Peroxidation in Microsomes of Liver Tissue——A mixture of 0.5 ml of microsomal suspension (equivalent to 12 mg of protein), 0.2 ml of Krebs–Ringer phosphate buffer (pH 7.4), 0.1 ml of Krebs–Ringer phosphate buffer containing 40 mM ADP solution, 0.1 ml of 4 mM NADPH solution and the indicated amount of geniposide was incubated at 37°C for 1 h in a final volume of 1 ml. Then the reaction was stopped by cooling the mixture to 4°C, and the lipid peroxides of microsomes were estimated by the method of Yagi *et al.*[6] with malondialdehyde as a standard.

Results

Effects of Geniposide on Serum Lipids, Glucose, Insulin and Transaminases (GOT and GPT)

As shown in Table I, it was found that the administration of the high sugar diet for 7 weeks caused hyperlipemia as compared to control rats. Serum TG, PL, LPO, glucose, insulin and GPT levels were found to be reduced in the rats orally given geniposide (100 mg/kg or 300 mg/kg) as compared to the high sugar diet-fed groups.

Table I. Effects of Geniposide on Serum Lipids, Glucose, Insulin and Transaminases in Rats fed a High Sugar Diet for 7 Weeks

	Control groups	High sugar diet-fed groups	Geniposide	
			100 mg/kg	300 mg/kg
TC (mg/dl)	$119.7 \pm 3.81^{N.S.}$	149.0 ± 19.0	$115.6 \pm 12.1^{N.S.}$	$140.9 \pm 15.8^{N.S.}$
HDL-ch (mg/dl)	$32.4 \pm 4.42^{N.S.}$	36.7 ± 3.58	$34.1 \pm 4.18^{N.S.}$	$38.0 \pm 4.09^{N.S.}$
TG (mg/dl)	$121.9 \pm 8.62^{d)}$	211.4 ± 18.1	$149.0 \pm 17.8^{b)}$	$129.4 \pm 8.85^{d)}$
FFA (meq/l)	$0.24 \pm 0.05^{N.S.}$	0.44 ± 0.08	$0.47 \pm 0.05^{N.S.}$	$0.50 \pm 0.08^{N.S.}$
PL (mg/dl)	$157.9 \pm 15.3^{d)}$	273.0 ± 17.9	$228.1 \pm 14.7^{a)}$	$243.9 \pm 9.72^{N.S.}$
LPO (nmol/ml)	$2.78 \pm 0.03^{a)}$	4.58 ± 0.97	$3.20 \pm 0.33^{N.S.}$	$2.69 \pm 0.09^{a)}$
Glucose (mg/dl)	$94.7 \pm 3.71^{d)}$	131.6 ± 6.39	$134.9 \pm 8.01^{N.S.}$	$98.7 \pm 9.93^{b)}$
Insulin (μU/ml)	$33.3 \pm 3.53^{N.S.}$	31.3 ± 6.36	$23.2 \pm 3.91^{N.S.}$	$10.6 \pm 3.91^{c)}$
GOT (Karmen unit)	$74.5 \pm 4.95^{N.S.}$	76.1 ± 3.75	$75.4 \pm 6.86^{N.S.}$	$76.0 \pm 4.54^{N.S.}$
GPT (Karmen unit)	$26.5 \pm 2.12^{d)}$	55.0 ± 1.57	$41.1 \pm 2.56^{d)}$	$42.3 \pm 5.14^{d)}$

The results are expressed as means ± standard errors of 6 rats. Significantly different from the high sugar diet-fed groups: *a)* $p < 0.05$, *b)* $p < 0.02$, *c)* $p < 0.01$, *d)* $p < 0.001$, N.S.: not significant. TC, total cholesterol; HDL-ch, high density lipoprotein-cholesterol; TG, triglyceride; FFA, free fatty acids; PL, phospholipids; LPO, lipid peroxides.

Effects of Geniposide on Body Weight, Liver Weight and Liver Lipids

As shown in Table II, the body weight and liver weight were reduced in rats given the high sugar diet as compared to the control rats. In the high sugar diet-fed rats, simultaneous oral administration of geniposide did not change the body weight or liver weight as compared to the high sugar diet-fed groups. It was found that the administration of the high sugar diet caused elevation of LPO in the liver as compared to the control groups. In the high

Table II. Effects of Geniposide on Body Weight, Liver Weight and Liver Lipids in Rats fed a High Sugar Diet for 7 Weeks

	Control groups	High sugar diet-fed groups	Geniposide	
			100 mg/kg	300 mg/kg
Body weight				
Initial (g)	$150 \pm 2.1^{N.S.}$	150 ± 2.3	$150 \pm 2.0^{N.S.}$	$150 \pm 1.8^{N.S.}$
Final (g)	$349 \pm 8.5^{c)}$	118 ± 2.7	$127 \pm 3.8^{N.S.}$	$110 \pm 6.1^{N.S.}$
Liver weight (g)	$14.0 \pm 0.70^{c)}$	5.53 ± 0.25	$5.55 \pm 0.31^{N.S.}$	$5.16 \pm 0.48^{N.S.}$
Liver				
TC (mg/g)	$4.23 \pm 0.61^{N.S.}$	4.72 ± 0.65	$4.51 \pm 0.66^{N.S.}$	$4.01 \pm 0.57^{N.S.}$
TG (mg/g)	$10.5 \pm 1.89^{N.S.}$	11.1 ± 1.25	$7.29 \pm 0.78^{a)}$	$5.21 \pm 0.93^{c)}$
FFA (μeq/g)	$4.65 \pm 0.42^{b)}$	3.32 ± 0.18	$2.84 \pm 0.25^{N.S.}$	$2.18 \pm 0.16^{c)}$
PL (mg/g)	$38.2 \pm 2.55^{N.S.}$	37.9 ± 0.96	$38.1 \pm 0.82^{N.S.}$	$37.9 \pm 0.96^{N.S.}$
LPO (nmol/g)	$205.8 \pm 10.8^{a)}$	283.7 ± 29.7	$194.8 \pm 9.05^{b)}$	$229.8 \pm 31.8^{N.S.}$

The results are expressed as means ± standard errors of 6 rats. Significantly different from the high sugar diet-fed groups: *a*) $p < 0.02$, *b*) $p < 0.01$, *c*) $p < 0.001$, N.S.: not significant. TC, total cholesterol; TG, triglyceride, FFA, free fatty acid; PL, phospholipids; LPO, lipid peroxides.

sugar diet-fed rats, oral administration of geniposide partly inhibited the elevation of liver LPO. Liver TG and FFA were significantly lowered by oral administration of geniposide as compared to the control and the high sugar diet-fed groups.

Effects of Geniposide on Sucrase Activity in Mucous Membrane of Small Intestine

Table III showed that sucrase activity in the mucous membrane of the small intestine was significantly reduced by oral administration of geniposide (100 mg/kg or 300 mg/kg) as compared to that of the control and the high sugar diet-fed rats.

Table III. Effects of Geniposide on Sucrase Activity of the Small Intestine in Rats fed a High Sugar Diet for 7 Weeks

	Sucrase activity (nmol/min/mg protein)
Control groups	$43.9 \pm 4.73^{N.S.}$
High sugar diet-fed groups	37.0 ± 2.87
+Geniposide (100 mg/kg)	$25.2 \pm 2.12^{b)}$
+Geniposide (300 mg/kg)	$29.4 \pm 1.53^{a)}$

The results are expressed as means ± standard errors of 6 rats. Significantly different from the high sugar diet-fed groups: *a*) $p < 0.05$, *b*) $p < 0.01$, N.S.: not significant.

Effects of Geniposide on ADP and NADPH-induced Lipid Peroxidation in Rat Liver Microsomes (*in Vitro*)

The formation of lipid peroxide in microsomes was found to be increased 4.67-fold by the addition of ADP (4 mM) and NADPH (0.4 mM). Geniposide had no effect on the ADP and NADPH-induced lipid peroxidation in rat liver microsomes.

Discussion

The present investigation demonstrated that geniposide of Gardeniae Fructus (San-shi-shi in Japanese) affects lipid and carbohydrate metabolism in high sugar diet-fed rats.

It is known that a high fat diet causes a fatty liver and a high carbohydrate diet induces hyperlipemia in rats.[10] In the present experiments, it was found that administration of

the high sugar diet for 7 weeks induced both hyperlipemia and the elevation of lipid peroxide in the liver as compared to the control rats. We have reported that the oral administration of lipid peroxide to rats induced liver injury with the elevation of serum GPT levels and accumulation of lipid peroxides in the liver.[3] In the present investigation, it was found that the oral administration of the high sugar diet for 7 weeks induced liver injury with the elevation of GPT in the serum and of lipid peroxides in the serum and liver. It was postulated that functional disorder of the liver of rats fed the high sugar diet might be induced by lipid peroxide accumulated in the livers. Geniposide did not affect lipid peroxidation induced by ADP and NADPH in rat liver microsomes. Therefore, the protection from liver injury provided by geniposide can't be explain in terms of the inhibition of the formation of lipid peroxides in the liver cells. The administration of geniposide in the high sugar diet-fed rats was found to reduce the levels of triglyceride, phospholipid, glucose and insulin, in addition to GPT and lipid peroxide, in the serum. The sucrase activity in the small intestine was also reduced by the administration of geniposide. Therefore, it is suggested that the effect of geniposide on metabolic alterations in the high sugar diet-fed rats might be at least partly a result of the inhibition of absorption of the high sugar diet in the small intestine, possibly by the inhibition of sucrase activity.

Experiments are now in progress to clarify the actions of geniposide in more detail.

References

1) *a*) M. Harada, N. Tenmyo, M. Aburada and T. Endo, *Yakugaku Zasshi*, **94**, 157 (1974); *b*) M. Aburada, H. Sasaki and M. Harada, *ibid.*, **96**, 147 (1976).
2) *a*) K. Yamauchi, R. Sakuragi, S. Kuwano and H. Inoue, *Planta Medica*, **25**, 219 (1974); *b*) K. Yamauchi, N. Fujimoto, S. Kuwano, H. Inoue and K. Inoue, *ibid.*, **30**, 39 (1976).
3) *a*) H. Okuda, *Igaku to Yakugaku*, **2**, 411 (1979); *b*) T. Mizunuma, Y. Takahashi, Y. Kishino, F. Sato and H. Okuda, *Lipid Peroxide Research*, **2**, 88 (1978).
4) *a*) H. Inoue, S. Saito, H. Taguchi and T. Endo, *Tetrahedron Lett.*, **1969**, 2349; *b*) H. Inoue, Y. Takeda, S. Saito, H. Nishimura and R. Sakuragi, *Yakugaku Zasshi*, **72**, 577 (1974).
5) S. Reitman and S. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 (1957).
6) H. Ohkawa, N. Ohishi and K. Yagi, *Anal. Biochem.*, **95**, 351 (1979).
7) *a*) M. Saito, E. Murakami, T. Nishida, Y. Fujisawa and M. Suda, *J. Biochem.* (Tokyo), **78**, 475 (1975); *b*) E. Murakami, M. Saito and M. Suda, *Eiyo to Shokuryo*, **30**, 1 (1977).
8) T. Oda and S. Seki, *J. Electron Microscopy*, **14**, 210 (1965).
9) O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
10) T. Mizunuma, F. Sato, T. Tezuka and H. Okuda, *Eiyo to Shokuryo*, **29**, 213 (1976).