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NOTE

Camellia japonica Seeds Extract Suppresses Lipid-induced Hypertriglyceridemia and Fat Accumulation in Mice

Masaru Ochiai[1]* , Tsutomu Nozaki[2], Masaki Kato[2], and Ken-o Ishihara[2]

[1] *School of Veterinary Medicine, Kitasato University, 23-35-1 Higashi, Towada, Aomori 034-8628, JAPAN*
[2] *BHN Co., Ltd., 1-16, Kandanishiki, Chiyoda, Tokyo 101-0054, JAPAN*

Abstract: As the seed extract from *Camellia japonica* (CJ) contains saponins, inhibitory effects of pancreatic lipase activity and body fat accumulation are expected. To investigate the anti-obesity effect of CJ seed extract, ICR mice were fed with a high-fat diet that was either supplemented or not with 1% CJ seed extract for 53 days. Including CJ seed extract in the high-fat diets of mice increased fecal fat excretion and decreased the body weight gain and lipid parameters in plasma and in the liver. In addition, lipid-induced hypertriglyceridemia was delayed by a single administration of CJ in ddY mice. Small intestinal transit was increased in ddY mice that received the CJ seed extract, but gastric emptying remained unchanged. These data demonstrate that CJ seed extract can suppress excess fat absorption, which can lead to the prevention of diet-induced obesity.

Key words: seed, *Camellia japonica*, saponin, mice, fat absorption

1 INTRODUCTION

Hypertriglyceridemia is known to be an independent risk factor of insulin resistance and coronary artery disease[1]. Post-prandial hypertriglyceridemia is caused by an increase in chylomicron-triglyceride synthesis and lipid metabolic disorders. Natural food sources have been studied for use in suppressing post-prandial hypertriglyceridemia. Extensive investigations into the effect of natural food sources on inhibition of pancreatic lipase activity, micelle formation by bile acids, chylomicron synthesis and secretion in the small intestine have been conducted. To suppress lipid uptake into the blood, inhibition of pancreatic lipase activity and small intestinal lipid absorption is supposed to be more effective and safe. Pancreatic lipase inhibitors have been extensively reported in reviews[2-4]. In particular, saponins, which are hydrophilic glycosides that contain a lipophilic triterpene or steroid, have been reported as possible pancreatic lipase inhibitors.

The flowers and leaves of *Camellia* plants have been extensively studied, and many of its compounds, including saponins, have been reported to be useful for the prevention and treatment of several diseases such as hypertriglyceridemia, obesity, and diabetes[5-7]. The seeds of *Camellia japonica* L.(CJ; Japanese name "tsubaki")have been used as a stomachic and anti-inflammatory medicine in Japanese folk medicine and as Camellia oil. However, in the present, there are only a few reports on the anti-obesity effect of the CJ seed. In this study, the effects of CJ seed extracts on the development of obesity and hypertriglyceridemia in mice were investigated.

2 EXPERIMENTAL

2.1 Test materials

Powdered seed extract from CJ was produced by BHN Co., Ltd.(Tokyo, Japan). Oils in the CJ seeds were removed, and then, the residue was extracted with hot water, filtered, and then spray dried. The hot-water extract was used as the saponin-containing sample in this study. Regarding safety as a food material, CJ was considered to be safe since no obvious problems were exhibited by the Ames test, and acute- and 28 days chronic-toxicity tests (internal unpublished data). In short, the acute toxicity

*Correspondence to: Masaru Ochiai, School of Veterinary Medicine, Kitasato University, 23-35-1 Higashi, Towada, Aomori 034-8628, JAPAN
E-mail: mochiai@vmas.kitasato-u.ac.jp
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test was performed in male and female Wistar rats by oral administration at 2,000 mg/kg, and the 28 days oral subchronic toxicity test was performed in male and female ICR mice using 1% CJ diet (about 1,200-1,400 mg/kg) dose daily. On the basis of the phenol-sulfuric acid colorimetric analysis using a Sep-Pak C_{18} cartridge[8], at least 25% of the compounds in the hot-water extract were saponins. According to a previous report, several kinds of Camellia saponins are expected to be contained in hot-water extracts of CJ[9].

2.2 Animal treatment and diets

The animal experimental protocol was approved by the President of Kitasato University through the judgment by the Institutional Animal Care and Use Committee of Kitasato University (Approval No. 16-138).

2.2.1 Anti-obesity effect of *Camellia japonica* (experiment A)

Twenty-four ICR mice (male, 4 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). Mice were housed at 23 ± 2℃ with lights on between 07:00 and 19:00 and were given free access to water and a chow diet (CE-2; Japan Clea, Tokyo) for 2 weeks of acclimation. Mice were divided into three diet groups (eight mice per group): the AIN-93G [low fat (LF), 7 wt% fat, 10 wt% sucrose] diet group, AIN-93G-based high-fat and high-sucrose [high fat (HF), 30 wt% fat, 20 wt% sucrose] diet group, and HF diet supplemented with 1% saponin extract diet group. The diet composition was the same as our previous study[10].

Mice were fed *ad libitum* for 53 days. The body weight and dietary energy intake of each group were monitored twice a week. Feces were collected during the final 3 days and were freeze-dried and weighed. At the end of the feeding period, mice were fasted overnight and then euthanized under isoflurane anesthesia. Blood was collected from the abdominal vein and then centrifuged (6,200 × g, 4℃, 15 min) to obtain plasma. Immediately after euthanasia, liver and adipose tissues (perirenal, epididymal, and mesenteric) were removed and weighed. Plasma and tissues were stored at −80℃ prior to analysis. The plasma levels of triglycerides (TG), total cholesterol (TCHO), high-density-lipoprotein (HDL)-CHO, glucose, and insulin were measured using commercial kits (Wako Pure Chemicals, Osaka; Shibayagi, Gunma, Japan). The values of homeostasis model assessment (HOMA) of IR (HOMA-IR) and insulin secretion (HOMA-β) were calculated using following formulae[11]. Total lipids in the feces and liver were extracted using the Folch's method[12]. Total lipids in the feces were measured by volatilization of the extracted lipids. The contents of TG and CHO in the feces and liver were measured as described for the plasma samples.

2.2.2 Anti-hypertriglyceridemia effect of CJ (experiment B)

The ddY mouse strain has been found to be an appropriate model for lipid-induced hypertriglyceridemia[13], and it was used in this study. Nineteen ddY mice (male, 11 weeks old) were purchased from Japan SLC. Mice were housed at 23 ± 2℃ with lights on between 07:00 and 19:00 and were given free access to water and CE-2 for 1 week of acclimation. Before the lipid absorption test, mice were fasted for 10 h. Fasted mice were divided into two groups (9 or 10 mice per group). The CJ seed extract was dissolved in deionized water and orally administered (300 mg/kg), followed by oral administration of soybean oil (5 mL/kg; RIKEN Nosan-Kako, Fukuoka, Japan). The control group of mice was administered deionized water, followed with soybean oil. Before and 60, 120, 180, 240, and 360 min after the oil administration, blood (20 μL/mice) was collected from the tail vein and centrifuged (6,200 × g, 4℃, 5 min) to obtain plasma. Plasma TG levels were immediately measured as described in experiment A. The area under the curve of TG levels was calculated hourly by thetrapezoidal rule. After completion of experiment B, mice were maintained on CE-2 for 1 week and then used for experiment C.

2.2.3 Effect of CJ on small intestinal transit and gastric emptying (experiment C)

Small intestinal transit was investigated as previously described[14]. The ddY mice were fasted for 12 h and divided into two groups (nine mice per group): control (0) and CJ saponin extract (300 mg/kg). The treatment group mice were orally administered the CJ (300 mg/kg). The mice in the control group were orally administered water. Thirty minutes after the administrations, a test meal solution (0.5 mL) was orally administered. The test meal solution was prepared by adding 0.5% CMC and 5% (w/v) Evans blue to the LF diet (15%, w/v). Fifteen minutes after the test meal solution was given, the mice were euthanized by cervical dislocation. The small intestine from the pylorus to the ileocecal junction was removed. The total length of the small intestine and the length between the pylorus and the point to which the test meal solution had reached were measured. Small intestinal transit was calculated as the ratio of the distance reached by the test meal solution to the total length of the small intestine and was expressed as a percentage. The content in the stomach was weighed for an index of gastric emptying.

2.3 Statistical analyses

Energy intake and fecal parameters are expressed as the mean and their actual data without statistical analysis because mice were in group housing (four mice per cage). The other data are expressed as mean ± SE (n = 8–10). Statistical analysis of differences among the three groups (experiment A) was performed using one-way analysis of variance (ANOVA) and the Fischer-PLSD test. For experiments B and C which contained two groups, statistical analysis of differences was performed using the student's t-test. A difference with $p < 0.05$ was statistically significant. All statistical analyses were performed using Excel

Statistics 2015 (SSRI, Tokyo, Japan).

3 RESULTS

3.1 Experiment A

Body weight gain, energy intake, tissue weights, plasma biochemical parameters, and lipid content in the liver and feces are shown in Table 1. Mice on the HF diet that included CJ seed extract had significantly lower body weight

Table 1 Effect of CJ on body weight, food intake, tissues weight, serum biochemical parameter, and hepatic and fecal lipids content in mice fed the HF diet.

Group	LF	HF	CJ
Body weight and food intake			
Body weight (Initial) (g)	34.3 ± 0.70	35.0 ± 1.0	35.0 ± 0.4
Body weight gain (g/54 days)	14.2 ± 1.7**	26.9 ± 3.7	16.4 ± 2.4*
Energy intake (kcal/mouse/day)	23.0 (22.9, 23.1)	21.3 (21.0, 21.5)	19.5 (19.2, 19.8)
Energy efficiency (g BW/kcal)[1]	11.6 (9.3, 13.9)	23.9 (23.8, 24.0)	15.8 (15.0, 16.5)
Weight of tissues and organs, and fat weight			
Liver (g)	1.79 ± 0.06**	2.38 ± 0.18	1.88 ± 0.09**
(mg/g BW)	36.9 ± 1.4	40.1 ± 1.2	38.4 ± 1.4
Abdominal adipose tissues (g)	2.81 ± 0.40***	5.30 ± 0.45	3.85 ± 0.4*
(mg/g)	58.0 ± 8.0**	88.8 ± 3.8	77.5 ± 6.5
Perirenal (g)	0.64 ± 0.09**	1.06 ± 0.10	0.77 ± 0.09*
(mg/g BW)	13.2 ± 1.8*	17.9 ± 1.0	15.7 ± 1.6
Epididymal (g)	1.59 ± 0.21**	2.84 ± 0.23	2.27 ± 0.27
(mg/g BW)	32.6 ± 4.2**	48.0 ± 2.7	45.6 ± 4.0
Mesenteric (g)	0.59 ± 0.10***	1.40 ± 0.17	0.81 ± 0.10**
(mg/g BW)	12.2 ± 2.1***	22.9 ± 1.8	16.3 ± 1.4*
Serum biochemical component			
Triglyceride (mg/100 mL)	88.1 ± 7.5	83.4 ± 5.7	84.1 ± 10.6
Total cholesterol (mg/100 mL)	101.5 ± 5.6**	148.5 ± 12.2	121.7 ± 8.4*
HDL-cholesterol (mg/100 mL)	81.2 ± 8.2*	108.4 ± 8.7	99.9 ± 6.5
Glucose (mg/100 mL)	171.1 ± 15.6***	235.8 ± 8.1	212.6 ± 10.7
Insulin (ng/mL)	0.18 ± 0.04***	1.75 ± 0.34	0.60 ± 0.18**
HOMA-IR	2.1 ± 0.6***	27.0 ± 5.30	8.6 ± 2.8**
HOMA-β	15.6 ± 2.3***	93.4 ± 17.90	36.5 ± 9.3**
Liver lipids contents			
Triglyceride (mg/g tissue)	35.9 ± 2.1***	155.8 ± 24.9	61.2 ± 16.1***
Cholesterol (mg/g tissue)	5.6 ± 0.3	5.9 ± 0.4	4.9 ± 0.2*
Fecal lipids contents			
Total dry feces (g/3 days/mouse)	1.2 (1.2, 1.2)	1.1 (1.1, 1.2)	1.1 (1.1, 1.1)
Triglyceride (mg/3 days/mouse)	3.9 (3.6, 4.1)	5.9 (5.4, 6.4)	10.5 (9.8, 11.1)
Cholesterol (mg/3 days/mouse)	6.1 (6.1, 6.2)	9.8 (9.0, 10.6)	14.5 (14.4, 14.7)

Data present mean ± SE (n=8). Statistically significant differences were evaluated by one-way ANOVA and Fischer-PLSD test. A difference of $p < 0.05$ was considered to be statistically significant. *, **, ***$p < 0.05$, 0.01, 0.001 (*vs*. HF group). LF, low-fat; HF, high-fat; CJ, Camellia japonica. HOMA-IR, The values of homeostasis model assessment of IR; HOMA-β, The values of homeostasis model assessment of insulin secretion. [1]Energy efficiency (g/kcal) = Body weight gain (g) / Energy intake throughout the test period (kcal)

gain and abdominal adipose tissue weights as compared with mice on the HF diet. The relative weight (mg/g BW) of the mesenteric adipose tissue was also significantly lower in the CJ seed extract diet group. In plasma analyses, the increase in the levels of TCHO, insulin, and HOMA indices induced by the HF diet were significantly suppressed in the CJ seed extract diet group. The liver levels of TG and CHO were markedly suppressed in the CJ seed extract diet group. In fecal analyses, fecal TG and CHO excretions were markedly higher in the HF plus CJ seed extract diet group as compared with the HF diet group. Feces excretion was similar between the groups.

3.2 Experiment B

The results of the oral lipid absorption test are shown in Fig. 1. The plasma TG level at 30 min after the lipid administration was significantly lower in the group of mice administered 300 mg/kg of CJ seed extract than in the group of mice administered vehicle. The plasma TG levels were not significantly different between the CJ seed extract-treated and untreated groups at the other time-points as determined by comparing their AUC values.

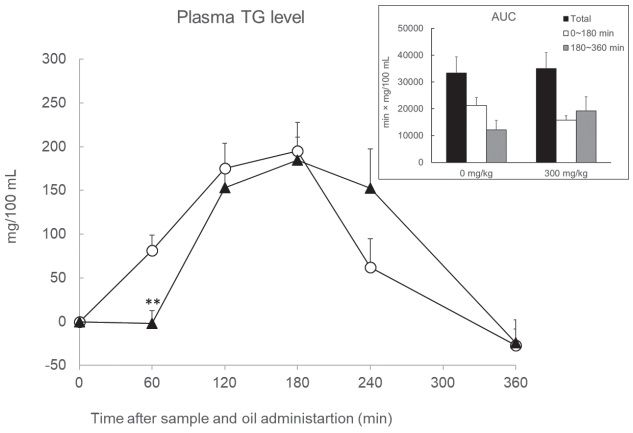

Fig. 1 Plasma triglyceride level and area under the curve (AUC) during the oral lipid absorption test (5 mL/kg) in ddY mice administered at the dose of 0 (vehicle) (○) or 300 mg/kg (▲). ACU was calculated by the trapezoidal rule (Experiment B). Results are mean ± SE (n = 9-10). Statistically significant differences were evaluated by student's t-test. A p value <0.05 was considered statistically different. **$p<0.01$ ($vs.$ 0 group).

3.3 Experiment C

The results of the small intestinal transit are shown in Fig. 2. The small intestinal transit time was significantly higher in the CJ seed extract group (300 mg/kg) as compared with the control group. However, there were no significant differences in the stomach contents between the two groups ($p = 0.10$).

4 DISCUSSION

The anti-obesity effects of CJ seed extract were investigated from the aspect of dietary TG absorption in the digestive tracts of mice. Mice on the HF diet that was supplemented with CJ seed extract have suppression of both body weight gain and fat accumulation in adipose tissues and in the liver. These changes may have resulted from the increases in fecal lipids excretion. The mechanisms that could explain the increase in fecal lipids excretion are the suppression of gastric emptying and lipid-induced hypertriglyceridemia.

Saponins are composed of sugars attached to a steroid or triterpene. Some reviews report that CJ contains many kinds of triterpene oligoglycoside-type saponins such as camelliasaponins. Some saponins that function as lipase inhibitors are reported to suppress dietary lipid-induced hypertriglyceridemia and adipose accumulation[4, 15]. Owing to their detergent property, saponins inhibit pancreatic lipase activity by affecting a surface property of the substrate

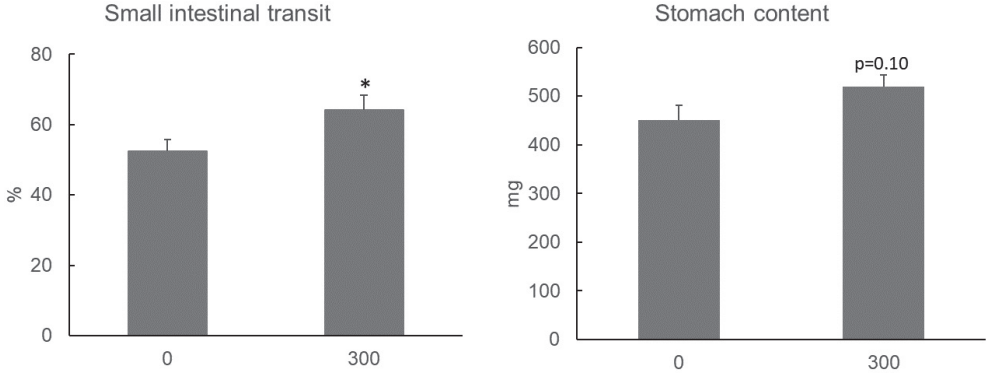

Fig. 2 Small intestinal transit and stomach content in ddY mice administered at the dose of 0 (vehicle) or 300 mg/kg (Experiment C). Results are mean ± SE (n = 9-10). Statistically significant differences were evaluated by student's t-test. A p value <0.05 was considered statistically different. *$p<0.05$ ($vs.$ 0 group).

rather than binding to the lipase as is the case for tannin[16]. Triterpene oligoglycoside-type saponins isolated from *Camellia sinensis* inhibit pancreatic lipase activity and lipid-induced hypertriglyceridemia in ddY mice[5]. Some saponins derived from *Camellia sinensis* are closely similar in structure to those from CJ[5, 9]. These previous findings may indicate that saponins derived from the seeds of CJ function in a similar manner to inhibit pancreatic lipase activity, micelle formation, and fat absorption, and that these effects could explain the increase in fecal fat (TG and CHO) excretion and the suppression of HF diet-induced obesity observed in this study. However, the suppression of body weight gain by chronic feeding of the HF diet plus CJ seed extract cannot be perfectly explained only by the suppression of abdominal adipose tissue accumulation. The substantial suppression of body weight by the CJ seed extract can indicate that subcutaneous adipose accumulation is suppressed as well as abdominal adipose accumulation and liver fat accumulation, although the mechanisms have not been clarified. Generally, excess lipids accumulation in the liver impairs fat metabolism and induces insulin resistance. Suppression of liver fat accumulation as well as intestinal fat absorption by the CJ seed extract can improve fat metabolic disorders and insulin resistance.

Gastrointestinal transit time and gastric emptying are considered to be important for metabolic health[17]. Matsuda *et al.* reported that tea flower saponins accelerate gastrointestinal transit and have anti-hyperlipidemia, anti-hyperglycemia, and anti-obesity effects in mice[6]. Tea saponins also accelerate gastrointestinal transit in mice as well as inhibit effects against pancreatic lipase activity, which contribute to the suppression of plasma TG elevation[5, 18–20]. It has been reported that triterpene saponins isolated from the seeds and leaves of *Camellia* plants are gastroprotective and can accelerate gastrointestinal transit[21]. The present data provide evidence that CJ saponins accelerate gastrointestinal transit. In this study, the small intestinal transit was significantly accelerated in mice treated with the CJ seed extract (300 mg/kg) as compared to the control mice. However, the gastric content was not significantly different between the two groups, which indicates that the gastric contents remained in the stomach, but the liquids passed through to the small intestine. Suppression of the lipids-induced acute hypertriglyceridemia can delay the digestion and absorption of lipids in small intestine and suppress the fat synthesis in tissues. In case of chronic feeding of the HF diet plus CJ seed extract, the fecal fat excretion is expected to be increased since these suppressive effects against fat absorption and fat synthesis continue for a long term. And it is also possible that a chronic feeding of the HF diet plus CJ seed extract influences the other fat metabolism as well as delays the intestinal fat absorption. On the other hand, in case of single administration of lipids plus CJ seed extract to the fasting mice, it is not expected that the total fat absorption and fecal fat excretion is changed as shown in the result of AUC value (Fig. 1) since the fasting mice do not meet the energy requirements. A contradiction of the results between in the acute and chronic administrations of the CJ seed extract to be resolved remains and the detailed mechanisms should be clarified in further experiments.

The chemical structures of camelliasaponins vary between *Camellia* species[4, 6, 9, 19, 20, 22], and their functions and metabolism differ in the gastrointestinal tract. For example, saponins from *Camellia sasanqua* inhibit gastric emptying, small intestinal mobility, and fecal excretion in mice[23]. On the other hand, Yoshikawa *et al.* reported that chakasaponins, which are saponins isolated from *Camellia sinensis*, accelerate gastrointestinal transit and inhibit pancreatic lipase activity. Camelliasaponins from the seed of CJ inhibit alcohol absorption in mice[9, 24]. Theasaponin E1, but not E2, isolated from the seeds of *Camellia sinen-*

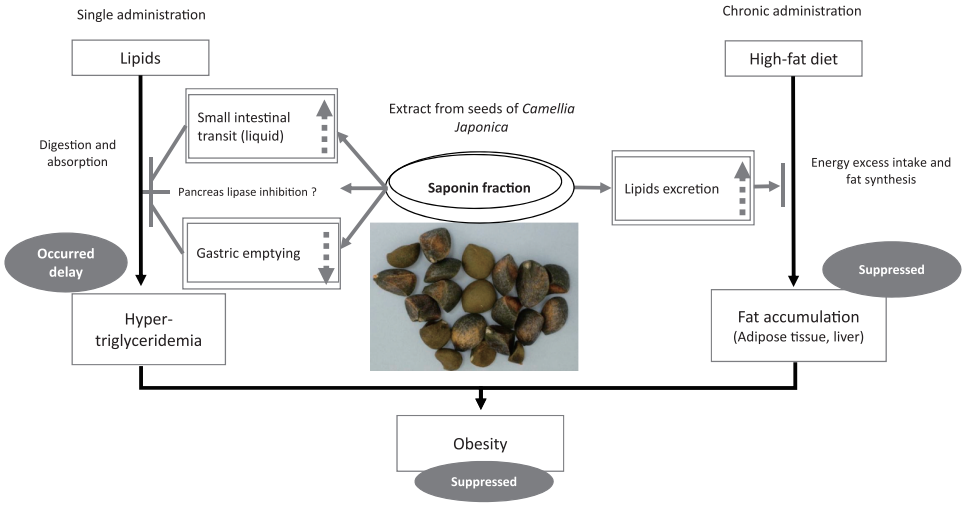

Fig. 3 Possible mechanism for the anti-obesity and anti-hypertriglyceridemia effects by the CJ in mice.

sis, was reported to inhibit gastric emptying and accelerate gastrointestinal transit[19].

Acceleration of gastrointestinal transit and suppression of gastric emptying have recently been considered to be related with the improvement of glucose homeostasis, but their detailed mechanism has not yet been determined[17]. In regard to glucose metabolism, the fasting plasma insulin level and HOMA indices were suppressed in the CJ seed extract diet group, which suggests that insulin resistance could be improved by treatment with CJ saponins. As plasma was collected at a fasted state, relationships among gastrointestinal transit, gastric emptying, and fasting plasma glucose metabolic parameters could not be determined in this study. However, the substantial suppression of plasma insulin level induced by the CJ seed extract diet can be explained by the improvement of digestive functions. These data suggest that CJ saponins may be useful for improving diabetes.

In conclusion, saponins extracted from the seeds of CJ show anti-obesity effects in mice. The possible mechanisms are the acceleration of the small intestinal transit coupled with a delay of food gastric emptying and fecal lipid excretion via pancreatic lipase inhibition (Fig. 3). These effects by the CJ seed extract may be beneficial for the prevention of lipid-induced hypertriglyceridemia in clinical situations.

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