Hypolipemic Effect of *Cyclocarya paliurus* (Batal) Iljinskaja in Lipid-Loaded Mice

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We investigated the inhibitory effect of *Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*) extract on postprandial hyperlipemia in mice. A single oral administration of *C. paliurus* extract (250 mg/kg) suppressed an increase in plasma triacylgycerol (TG) levels when fed with 5 ml/kg of lard and olive oil. The inhibition rates were 28.6% and 24.1%, respectively, but free fatty acid (FFA) levels in plasma were not significantly affected as compared with control group mice. In addition, *C. paliurus* extract showed inhibitory activity toward pancreatic lipase, a key enzyme of dietary TG absorption, with an IC_{50} of 9.1 μg/ml *in vitro*. Our results suggested that the hypolipemic action of *C. paliurus* extract was probably interrelated with suppression of the activity of digestive lipase, and as a result, the blood lipid level was reduced.

Key words *Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*); triacylgycerol (TG); pancreatic lipase

Cyclocarya paliurus (Batal.) Iljinskaja (*C. paliurus*) is a Juglandaceae plant, an endemic tree growing on cloudy and foggy highlands in the Xiushui area of southern China.[1] The leaves of *C. paliurus* have been a food source for maritime people for a long time, and are known to have beneficial effects on health and used as a traditional remedy for ailments, the enhancement of mental efficiency, and recovery from mental fatigue. Recently, epidemiological research revealed that there are few patients with hyperglycemia and diabetes mellitus in the Xiushui area and it was recognized that drinking *C. paliurus* is beneficial to the prevention of these diseases.[2] In China, the health benefits of *C. paliurus* have been reviewed.[3] These studies suggested that *C. paliurus* protects insulin-producing cells under stress, improves insulin secretion and promotes the utilization of blood glucose as energy.[4] Yang *et al.*[5] isolated a sweet dammarane triterpenoid saponin, cyclocarioside A from the leaves of *C. paliurus*. Shu *et al.* also found two dammarane triterpenoid saponins in *C. paliurus*, cyclocarioside II and cyclocarioside III.[6] It has been demonstrated that triterpenoid saponins exhibit an insulin-like activity in adipocytes, *in vivo* and *in vitro*.[7,8] Shimizu *et al.* reported that triterpenoid saponins promote glucose tolerance in rats.[9] Therefore, these compounds of *C. paliurus* probably have beneficial effects on health and prevention of diseases. However, to our knowledge, no other research has been conducted on the hypolipemic effect of this plant.

It is well known that excessive amounts of triacylgycerol (TG) in a diet induce obesity and hypercholesterolemia, which are cardiovascular risk factors. Lipid is usually absorbed as fatty acid and 2-monoglyceride generated by pancreatic lipase. Pancreatic lipase is the most important enzyme in TG digestion. Its inhibitors have been demonstrated to promote weight loss through suppression of fat absorption.[10] Therefore, in the present study, we examined whether *C. paliurus* reduces the plasma TG level by inhibiting digestive lipase.

MATERIALS AND METHODS

Materials and Preparation of *C. paliurus* Extract *C. paliurus* was purchased from the Xiushui Tea Import & Export Co., Ltd. (Jiangxi province, China). Dry leaves of *C. paliurus* were treated with 20 parts of hot water for 1 h at 100 °C. After filtration and evaporation of the water, the recovered residue was powderized under frozen-decompression conditions. The recovery rate was 13%, and the extract residue was dissolved in water immediately before experiments. Olive oil and lard oil were purchased from Nacalai Tesque Inc. (Kyoto, Japan) and Sigma Chemical Company, respectively. (−)-Gallocatechin gallate (GCG; Sigma Chemical Co.) was used as a positive control in the present experiment.

Animals Seven-week old male ICR mice were purchased from CLEA Japan Inc. (Shimizu, Japan). The animals were kept in a specific pathogen-free animal room at 23±1 °C with a 12 h light and dark cycle (lights on from 600 to 1800 h), and were provided standard laboratory chow (CE-2; CLEA Japan, Inc.) and tap water. The animals were kept for 1 week before the experiment.

Lipid Absorption Test and Measurement of Plasma Lipid Levels For the lipid absorption test, a lard oil and olive oil solution was orally administered at 5 ml/kg body weight, 30 min after 250 mg/kg of *C. paliurus* extract was orally administered to ICR mice that had been starved overnight for 20 h. An equal volume of distilled water was administered to the control mice. The mice were analyzed for chronological changes in plasma TG and free fatty acid (FFA) levels, with five animals studied at each time point. Blood samples were taken from the heart under anesthesia with diethyl ether, and were put in a tube containing 2% sodium heparin. Then, the tubes were centrifuged at 5000 rpm for 5 min to obtain supernatant as a sample. All samples were stored at −20 °C until the assay. Plasma TG was measured using a Hitachi 7070 Automatic Serum Analyzer (Hitachi Co., Ltd., Japan) by the glycerol kinase/glycerol-3-phosphate oxidase (GK-GPO) method,[11] plasma FFA was determined as previously described,[12] and the assay kit was purchased from International Reagents Corp. Kobe, Japan.

Measurement of Pancreatic Lipase Activity The pancreatic lipase activity was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate. Pancreatic lipase

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(Type VI-S, from porcine pancreas) and 4-MU oleate were purchased from Sigma Chemical Co., respectively. Twenty five microliters of sample solution dissolved in water and 50 μl of 0.1 mM 4-MU solution dissolved in a buffer consisting of 13 mM Tris–HCl, 150 mM NaCl, and 1.3 mM $CaCl_2$ (pH 8.0) were mixed in the well of a microtiter plate, and then 25 μl of the lipase solution (50 U/ml) in the above buffer was added to start the enzyme reaction. After incubation at 25 °C for 30 min, 0.1 ml of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. The amount of 4-methylumbelliferone released by the lipase was measured with a fluorometrical microplate reader (Fluoroskan Ascent LabSystems Inc.) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm.[13] The IC_{50} of the test sample was obtained from the least-squares regression line of the plots of the logarithm of the sample concentration (log) *versus* the pancreatic lipase activity (%).[14]

Statistical Analyses Statistical analyses were performed with Student's *t*-test. Differences were considered to be significant when the probability value was less than <0.01.

RESULTS

As shown in Fig. 1A, plasma TG levels of the control mice significantly increased after oral administration of 5 ml/kg of lard oil. The plasma TG reached a maximum level at 3 h after administration, and decreased after that. The plasma FFA level showed the same pattern of chronological change (Fig. 1B). Administration of *C. paliurus* extract (250 mg/kg) significantly reduced plasma TG levels as compared with the control at 3 h after administration of lard oil ($p<0.005$). The mean value of the *C. paliurus* group was 523.3±29.4, 27.7% lower than that of the control group (723.7±41.9). On the other hand, the *C. paliurus* extract had no effect on plasma FFA levels.

The hypolipemic effect was also observed using olive oil (5 ml/kg)-loaded mice as shown in Fig. 2A. *C. paliurus* (250 mg/kg) extract lowered the plasma TG level to 70.6% at 3 h which is the time the maximum level wasattained, as compared with the control group after oral administration of olive oil ($p<0.01$). However, the extract did not cause a significant decrease in the plasma FFA level when compared to the control after oral administration of olive oil.

The inhibitory effect of *C. paliurus* extract on pancreatic lipase activity was investigated together with GCG as a positive control. *C. paliurus* extract inhibited pancreatic lipase activity in a dose-dependent manner as shown in Fig. 3 and the IC_{50} of the extract was 9.1 μg/ml as calculated from inhibition curves. However, the inhibitory effect of the *C. paliurus* extract was weaker than that of the GCG (IC_{50} value: 0.9 μg/ml).

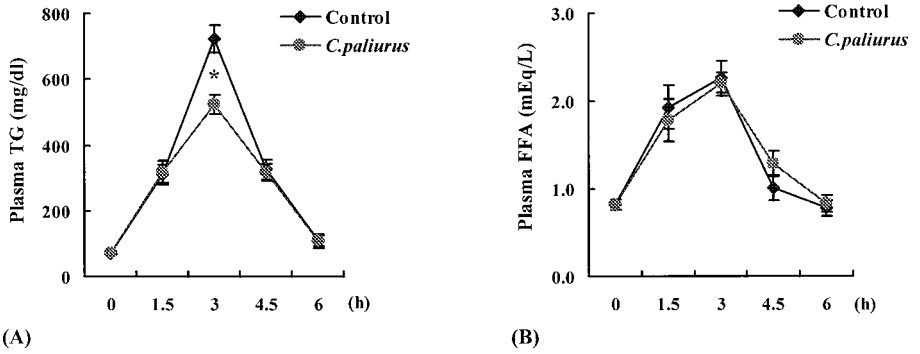

Fig. 1. Inhibitory Effect of a Single Oral Administration of *C. paliurus* Extract on Plasma TG Levels in Lard Oil-Loaded ICR Mice

Seven-week old male ICR mice were used in the lipid absorption test. Lard oil was orally administered at 5 ml/kg body weight, 30 min after the oral administration of 250 mg/kg of *C. paliurus* extract to mice that had been deprived of food for 20 h prior. Blood TG and FFA levels were analyzed chronologically, and the results represent the mean±S.E. of the values obtained from 5 animals at each time point. *Significantly different from the control mice ($p<0.005$) as determined by Student's *t*-test.

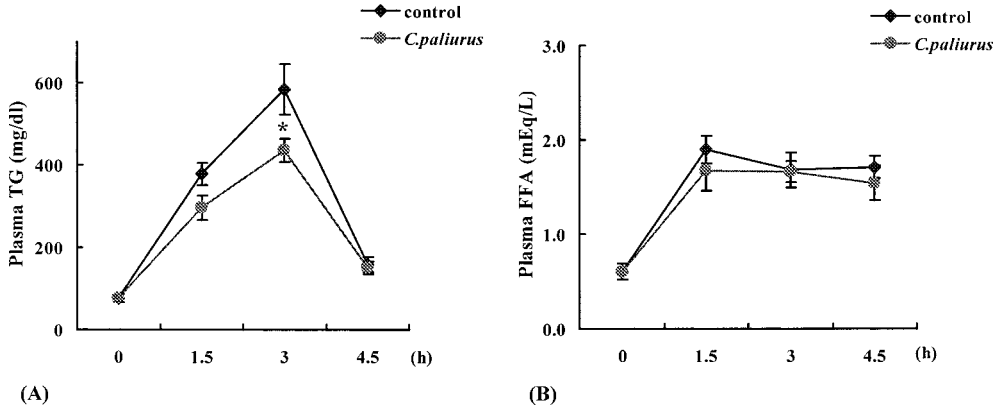

Fig. 2. Inhibitory Effect of a Single Oral Administration of *C. paliurus* Extract on Plasma TG Levels in Olive Oil-Loaded ICR Mice

Seven-week old male ICR mice were used in the lipid absorption test. Olive oil was orally administered at 5 ml/kg body weight, 30 min after the oral administration of 250 mg/kg of *C. paliurus* extract to mice that had been deprived of food for 20 h prior. Blood TG and FFA levels were analyzed chronologically, and the results represent the mean±S.E. of values obtained from 5 animals at each time point. *Significantly different from the control mice ($p<0.01$) as determined by Student's *t*-test.

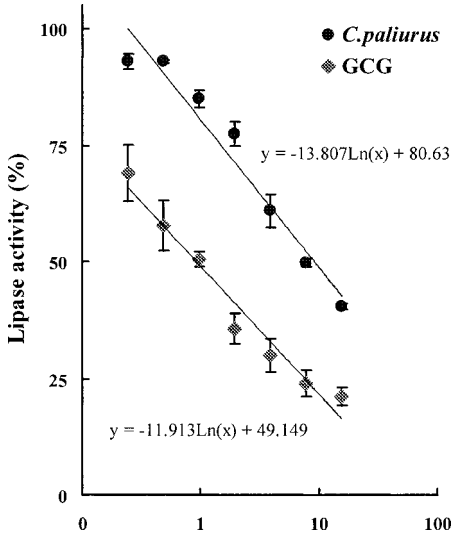

Fig. 3. Dose–Response Curves for Pancreatic Lipase Inhibition by Water Extract of *C. paliurus* Extract

The activity of pancreatic lipase was studied following the addition of *C. paliurus* extract and GCG to the enzymatic reaction solution, respectively, according to reported methods. The values are presented as the mean±S.E. of four samples.

DISCUSSION

In the present study, we investigated whether *C. paliurus* extract reduced plasma lipid levels in ICR mice loaded with lard oil or olive oil. The results suggested that *C. paliurus* extract significantly suppressed the postprandial hyperlipemia as compared with the control. Although we are unable to clearly describe the mechanism behind the hypolipemic effect of *C. paliurus* extract, administration of the extract did not significantly affect plasma levels of FFA, which may be derived from cytoplasmic TG, de-novo lipogenesis and TGs derived from incoming lipoproteins. As a result, we suggest that this hypolipemic effect is due to the suppression of lipid absorption. Alternatively, the hypolipemic effect increased the utilization of TG as an energy source by hormone-sensitive TG lipase, but this would result in higher levels of plasma FFA as hydrophilic product.

It is well known that the hypolipemic effect is useful in reducing the intestinal absorption of dietary lipid by inhibiting one or more of the metabolic processes implicated in lipid digestion and absorption.[15] It is genrally recognized that ingested lipid is an insoluble oil at body temperature. Therefore, the basic process of lipid absorption is the conversion of this oil into water-soluble compounds that can be efficiently absorbed. The key to this process is the pancreatic enzyme lipase, which is the principal lipolytic enzyme localized in the digestive tract,[16] and split lipids in a meal are degraded into fatty acids and monoglycerides before intestinal absorption.[17] Though the inhibitory activity of *C. paliurus* extract in pancreatic lipase is weaker than that of GCG which is known to have hypolipemic activity[18] as a positive control, the limited intestinal absorption of lipid may have been partly due to the reduction in blood lipid levels by the extract. It was suggested that the hypolipemic effect of *C. paliurus* extract was interrelated with suppression of the activity of enzymes such as pancreatic lipase.

Lipids are known to be an important energy source, but excess intake may induce obesity and hyperlipidemia. Obesity is directly associated with various chronic diseases such as coronary heart disease and diabetes, and because of its epidemic nature in developed societies, is a very important public health problem.[19,20] Some clinical studies have indicated a correlation between hyperlipidemia and life style-related diseases, and hyperlipidemia is a risk factor for arteriosclerosis.[21,22] Keeping the blood TG level within the normal range is important for the prevention of diseases such as arteriosclerosis, cerebral apoplexy and myocardial infarction. In China, *C. paliurus* is used as a beverage with beneficial effects on health. But the effect of *C. paliurus* on blood TG levels has never been reported. Our results suggest that the hypolipemic activity exhibited by *C. paliurus* extract probably involves control of the absorption of lipid through suppression of the activity of pancreatic lipase. Therefore, we demonstrated that *C. paliurus* might be useful for preventing disease by reducing blood lipid levels, improving lipid metabolism, and preventing many life-style related diseases that are caused by hyperglycemia.

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