Note
Hypoglycemic Action of *Cyclocarya paliurus* (Batal.) Iljinskaja in Normal and Diabetic Mice

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This study examined the hypoglycaemic activity of *Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*) in ICR mice by oral glucose tolerance testing. The blood glucose level was significantly lower in the *C. paliurus* extract treatment group than in the control group after animals were given sucrose. This difference was not observed following the administration of glucose. We demonstrated that the chronological change in the level of blood glucose in genetically hyperglycemic obese KK-A^+^ mice is significantly lower when *C. paliurus* extract is administered daily for three weeks. An in vitro study showed that *C. paliurus* inhibits α-glucosidase, a disaccharide-degrading enzyme in the small intestinal mucosa, leading to a decrease in the absorption of glucose into the blood and a subsequent lowering of the blood glucose level.

Key words: *Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*); blood glucose; KK-A^+^ mice; α-glucosidase; glucose tolerance tests

*Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*) is a Juglandaceae plant, an endemic tree that grows on the cloudy and foggy high ground of the Xiushui area of southern China. The leaves of *C. paliurus* taste sweet and are consumed daily as a beverage in this region.  

C. *paliurus* has a beneficial effect on health and is used as a traditional remedy for ailments, the improvement of mental efficiency, and recovery from mental fatigue. Recently, epidemiological research showed that hyperglycemia and diabetes mellitus are very rare in the Xiushui area, demonstrating that *C. paliurus* is beneficial in the prevention of these diseases. In China, the health benefits of *C. paliurus* have been reviewed. These studies suggested that *C. paliurus* protects stressed insulin-producing cells, improves insulin secretion, and promotes the use of blood glucose as energy. However, the mechanism of this hypoglycemic activity of *C. paliurus* has not been investigated. Therefore, we studied the effect of *C. paliurus* on glucose tolerance in an animal model to clarify the mechanism involved.

*C. paliurus* was purchased from the Xiushui Tea Import & Export Co., Ltd., Jiangxi Province, China. Dried leaves of *C. paliurus* were extracted with 20 volumes of water with boiling for 1 h at 100°C. After filtration and evaporation of the water, the recovered residue was powdered under frozen-decompression conditions. The recovery rate was 12.9%, and the extract was used for this experiment. D-(+) glucose, sucrose, and starch were purchased from Nacalai Tesque (Kyoto, Japan). The samples were dissolved in water immediately before use. Seven-week-old male ICR and four-week-old male KK-A^+^ obese mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). The animals were kept in a pathogen-free animal room at 23 ± 1°C with a 12 hour light-dark cycle (lights on from 0600 to 1800), and were provided standard laboratory chow (CE-2; CLEA Japan, Inc.) and tap water. The animals were kept for 1 week before the experiment.

For the glucose tolerance test, a solution of 20% D-(+) glucose, 40% sucrose, and 40% starch was orally administered at 10 μl/g body weight, 5 min after 250 mg/kg *C. paliurus* extract was orally administered to ICR mice that had been starved overnight for 20 h before the test. An equal volume of distilled water was administered to the control mice. The mice were analyzed for chronological changes in blood glucose, with five animals studied at each time. To examine its chronic effects, *C. paliurus* extract, which had been dissolved in water, was orally administered to 11 KK-A^+^ obese mice for 21 days, at a dose of 2 g/kg per day. Water was orally administered to 10 animals in control group. Blood (20 μl) was taken from the tail vein, and blood glucose was measured with an ANTHENSE II Blood Glucose Analyzer (Sankyo Co., Ltd., Kyoto, Japan).
Fig. 1. Effects of C. paliurus on Glucose Tolerance Tests.

Seven-week old male ICR mice were tested for glucose tolerance. A solution of 20% D- (+)-glucose (A), 40% sucrose (B), and 40% starch (C) was orally administered at 10 ml/g body weight, 5 min after oral administration of 250 mg/kg C. paliurus extract to ICR mice that had been deprived of food for 20 h before administration. Blood glucose levels were analyzed chronologically, and the results represent the mean ± SD of the blood glucose levels obtained from 5 animals each time.*Significantly different from the control mice (p < 0.001) as measured by Student's t-test.

at 7, 14, and 21 days after C. paliurus extract administration. Body weight was also recorded during this period.

An α-glucosidase inhibitory assay was done by the chromogenic method. Yeast α-glucosidase (14 units/mg protein/ml, Sigma Chemical Co.) was dissolved in a 100-mM phosphate buffer (pH 7.0) containing 0.2% bovine serum albumin and 0.02% NaN₃, and was used as an enzyme solution. p-nitrophenyl-α-D-glucopyranoside (5 mM, Nacalai Tesque), which had been dissolved in this buffer (pH 7.0), was used as a substrate solution. Following this, 50 μl of enzyme solution and 10 μl of C. paliurus extract, which had been dissolved in distilled water, were mixed in the well of a microtiter plate. Epicatechin (Sigma) was previously dissolved in dimethyl sulfoxide and used for the enzyme inhibitory assay. The titer was measured (A₄₀₅) at zero time, using a microplate reader (Multiskan MS model 352, LabSystems Inc.). The substrate solution (50 μl) was incubated for 5 min, then added to the solution. The reaction mixture was incubated for 5 min at room temperature. The increase in absorbance from zero time was measured. Inhibitory activity was expressed as 100 min the absorbance difference (%) of the test samples relative to the change in absorbance of the control that used the carrier solvent instead of the test solution.

Care and treatment of the animals conformed to the guidelines established by the Japanese Society of Nutrition and Food Science (Law No. 105 and Notification No. 6 of the Japanese government).

The blood glucose levels of the ICR mice significantly increased (p < 0.0001) after oral administration of 2 g of glucose. The maximum level was attained in 30 min, and following this, the blood glucose level decreased with time (Fig.1 A). However, the C. paliurus extract did not produce a significant decrease in the blood glucose level when compared to the control group. As shown in Fig. 1 B, blood glucose levels peaked at 60 min after administration of an oral 4 g sucrose load in all of animals. Administration of C. paliurus extract reduced the blood glucose levels as compared with the control group (p < 0.001). The blood glucose level of the C. paliurus extract group was 290.2 ± 16.3 mg/dl (± SD), and was 19.3% lower than that of the control group (359.3 ± 17.9). This pattern was observed following the administration of starch (Fig. 1 C). Oral administration of 4 g of starch caused a 16.1% and a 26.8% decrease in the blood glucose level as compared with the control group, 60 and 90 min after administration, respectively.

Figure 2 A shows the chronological change in the level of blood glucose in the KK-A' obese mice. We found that the level remained lower in the C. paliurus extract group than in the control group throughout the 21-day experimental period. The increase in body weight of the extract-treated mice was slightly lower than that of the control mice, but this difference was not significant during the period of study Fig.2 B.

The inhibitory activity of C. paliurus extract and epicatechin against α-glucosidase were measured. C. paliurus extract inhibited α-glucosidase activity in a dose-dependent manner as shown in Fig.3. However, the inhibitory effect of C. paliurus extract was weaker than that of the epicatechin.

This study was done to evaluate the hypoglycemic activity of C. paliurus. Our results demonstrated that a single administration of C. paliurus extract reduced elevation of blood glucose levels induced by admin-
Fig. 2. Effects of *C. paliurus* on KK-A' Obese Mice.

Four-week old male KK-A' obese mice were used in this study. The results represent the mean ± SD of the blood glucose levels obtained from 10 or 11 animals of each group. The *C. paliurus* extract was dissolved in water and was orally administered to the KK-A' obese mice for 21 days at a dose of 2 g/kg/day. The same dose of water was administered to the control mice. Blood glucose levels were then measured. *Significantly different from the control mice (p < 0.01) as measured by Student's t-test.*

Fig. 3. Inhibitory Effects of *C. paliurus* on α-Glucosidase Activity.

The activity of α-glucosidase was studied following the addition of *C. paliurus* extract, and epicatechin to the enzymatic reaction solution, respectively, according to reported methods.

A hypoglycemic effect is useful to reduce the intestinal absorption of dietary carbohydrates by inhibiting the metabolic processes implicated in carbohydrate digestion and absorption. It is generally recognized that carbohydrates are digested into disaccharides by enzymes secreted from the digestive tract, and then converted into glucose by α-glucosi-
References