Anti-obesity Effects of *Eucommia ulmoides* Leaves

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In this paper, we have examined the anti-obesity effects of *Eucommia ulmoides* leaves in rats and mice.

A high fat diet was orally administered to rats. In addition to this diet, a water solution of *E. ulmoides* leaf extract was administered. After 60 min, the levels of serum triglyceride decreased significantly in the 400 mg/kg *E. ulmoides* leaf extract group as compared with that in the high-fat diet group.

A diet of 40% beef tallow was fed to rats. These rats were divided into three groups; the *E. ulmoides* leaf extract powder (E-EP) group received 10% E-EP, the *E. ulmoides* leaf green powder (E-GP) group received 10% E-GP and the *E. ulmoides* leaf powder (E-LP) received 10% E-LP in addition to the beef tallow. Food and water were fed *ad libitum*. After 4 weeks, the body weight, visceral fat, and serum of triglyceride levels of the E-E and E-L groups were significantly lower than those of rats in the 40% beef tallow group.

Key words: *Eucommia ulmoides* leaf extract; obesity; anti-obesity effect; high-fat food; lipid

Introduction

Eucommia ulmoides (*Eucommia ulmoides* Oliver) is a deciduous tree and is the only species belonging to the genus *Eucommia* and family Eucommiaceae. Its bark has been known to have hypotensive, diuretic, and analgesic effects¹⁾ and has been used in China since the Han period. Further, it has been used in pharmaceutical products in Japan. Its leaves, on the other hand, have been used in food. Folklore studies conducted in western China have reported their use in tea and gruel since long. Currently, they are readily available as a health food product in Japan. Furthermore, recent pharmacological studies on the leaves have shown that they exhibit hypotensive effects in humans and spontaneously hypertensive rats (SHR)²⁾. The other effects demonstrated include anti-stress³⁾, analgesic^{4,5)}, and anti-obesity⁶⁻⁹⁾ effects. This study focused on the anti-obesity effect of *E. ulmoides* leaves and investigated the fat pad weight and levels of total serum triglyceride and cholesterol after the long-term and single administration of test materials.

Materials and Methods

Experiment 1: Effect of the long-term intake of *E. ulmoides* leaves on mice fed an obesity-inducing high-fat diet.

1. Preparation of materials and feeds for the experiment

The composition of the feed is shown in Table 1. It comprised 40% beef tallow, 10% corn starch, 9% granulated sugar, 4% mineral mixture, 1% vitamin mixture, 26% casein, and 10% test material. *E. ulmoides* leaf extract powder (E-EP), *E. ulmoides* leaf green powder (E-GP), and *E. ulmoides* leaf powder (E-LP) were used as the test materials.

Table 1. Components of the administration sample

Component	High Fat Diet (HFD)	Experiment group
Beef rallow	40	40
Corn starch	10	10
Granulated sugar	9	9
Mineral mix	4	4
Vitamin mix	1	1
Casein	36	26
Sample	-	10

(% of content)

The high-fat diet (HFD) group received the feed in which one of the test materials was replaced by 10% casein.

2. Animals

Female ICR mice, 4 weeks of age, were obtained from Japan SLC Inc. (Shizuoka, Japan). They were kept under a room temperature of 23°C ± 2°C, 50% ± 10% humidity, and a 12-h light-dark cycle (lights on at 8 AM and off at 8 PM). They were used in the experiment after a preliminary rearing period of 1 week.

3. Measurement of the levels of serum triglyceride and serum cholesterol at 4 weeks of administration.

The animals were allocated to either test group (E-EP, E-GP, and E-LP groups) or the HFD group according to body weight; each group comprised 7 animals. The animals of each group were allowed free intake of feed and water for 4 weeks, and their body weight and feed intake were measured every week. At 4 weeks after the onset of the administration, 12-h fasted mice were etherized, their visceral fat removed

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Fig. 1. Effect of the long-term intake of *E. ulmoides* on body weight.

Each point represents the mean $\pm$ SD of seven animals.

*: significantly different from the control ($p < 0.05$).

and weighed, and blood samples obtained by cardiac blood collection. The collected blood samples were set aside for 60 min at room temperature, and then centrifuged (5000 rpm, 25°C for 10 min) to separate the serum. The levels of serum triglyceride and serum cholesterol were measured using commercially available enzyme kits (triglyceride: E-Test Wako, cholesterol: E-Test Wako; Wako Pure Chemicals).

4. Statistical analysis

Data were analyzed using one-way ANOVA, and then differences among means were analyzed using Fisher's protected least significant differences (PLSD) multicomparison test. Differences at $p < 0.05$ were considered significant.

Experiment 2: Effects of the administration of a single dose of *E. ulmoides* leaves on the levels of serum triglyceride and serum cholesterol in HFD-rats

1. Preparation of materials and test feeds for the experiment

The fat was prepared in the form of an emulsion by sonicating a mixture of 3 ml corn oil, 40 mg cholic acid, 1 mg cholesterol oleate, and 3 ml distilled water. The sample solution for administration was prepared by adding the test material to the fat emulsion. The *E. ulmoides* leaf extract (E-E) to be used for administration was prepared to concentrations of either 200 mg/kg body weight (E-E200) or 400 mg/kg body weight (E-E400) using distilled water. Doses of 5 ml/kg of the fat emulsion and test material each were orally administered. The control (CONT) group received the mixture with distilled water, instead of the E-E.

2. Animals

Male Wistar rats, 8 weeks of age, were obtained from Nihon Dobutsu Inc. (Osaka, Japan). They were maintained under a room temperature of $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $50\% \pm 10\%$ humidity, and a 12-h light-dark cycle (lights on at 8 AM and off at 8 PM). They were used for the experiment after a preliminary rearing period of 1 week.

3. Measurement of serum triglyceride levels after a single administration

In order to divide the rats into groups based on the serum triglyceride level, blood was sampled from the orbital vein

Fig. 2. Effect of the long-term intake of *E. ulmoides* on visceral fat.

Each point represents the mean $\pm$ SD of seven animals.

*: significantly different from the control ($p < 0.05$).

of 12-h fasted rats. The collected blood samples were set aside for 60 min at room temperature, and then centrifuged (5000 rpm, 25°C for 10 min). Rats with comparable mean values of serum triglyceride, measured using the E-Test Wako, were allocated to the same group. On the day of administration, the sample solution was orally administered to 12-h fasted rats of the CONT group, who received only fat, and those of the test groups, namely, E-E200 and E-E400, by using a cannula. Blood sampling from the orbital vein was performed at 30, 60, 120, 180, and 300 min after administration. The collected blood samples were set aside for 60 min at room temperature and then centrifuged (5000 rpm, 25°C for 10 min) to separate the serum. Serum lipid levels were measured using the E-Test Wako.

4. Statistical analysis

Data were analyzed using one-way ANOVA, and then differences among means were analyzed using Fisher's protected least significant differences (PLSD) multicomparison test. Differences at $p < 0.05$ were considered significant.

Results

Experiment 1

Weight change in each group from the start of administration to 4 weeks later is shown in Fig. 1. Although the animals' body weights increased with rearing, the test groups (E-EP, E-GP, and E-LP groups) showed significantly lower body weight than the HFD group at the start of the first week of administration, and they showed no weight gain up to 4 weeks after the administration.

Figs. 2, 3, and 4 show the ratio of visceral fat to body weight, serum triglyceride levels, and total serum cholesterol levels, respectively, in each group at 4 weeks. The ratio of visceral fat to body weight of both test groups was significantly lower than that of the HFD group. The serum triglyceride levels of the E-EP and E-GP groups were significantly lower than that of the HFD group. Further, the total serum cholesterol levels of all the test groups were significantly lower than that of the HFD group.

Experiment 2

The effects of E-E on fat absorption were investigated using the blood fat level as an indicator by administering a

Fig. 3. Effect of the long-term intake of *E. ulmoides* on the serum triglyceride level

Each point represents the mean $\pm$ SD of seven animals.

*: significantly different from the control ($p < 0.05$).

Fig. 5. Effect of *E. ulmoides* on the serum triglyceride level after oral administration

200 mg/kg *Eucommia* leaf extract (E-E200; ●), 400 mg/kg *Eucommia* leaf extract (E-E400; ●), and control (CONT; ○)

Each point represents the mean $\pm$ SD of seven animals.

*: significantly different from the control ($p < 0.05$).

single oral dose of E-E200 or E-E400, fat emulsions containing *E. ulmoides* leaves, to rats. The serum triglyceride and total serum cholesterol levels were measured to determine the blood lipid level. In the CONT group, the serum triglyceride level (Fig. 5) started to increase at 60 min after the onset of fat administration and returned to the baseline level at 300 min. On the other hand, the E-E200 and E-E400 groups showed slower increases in the serum triglyceride level, and this level was significantly lower in these groups at 60 and 120 min after the onset of administration than in the CONT group. These levels tended to be slightly higher at 180 min than that of the CONT group, although there was no significant difference. No significant difference in total serum cholesterol levels (Fig. 6) was observed between the test and HFD groups.

Discussion

This research focused on the anti-obesity effect of *E. ulmoides* leaves and investigated the effects of long-term and single administration of *E. ulmoides* leaf products on fat pad weight and lipid metabolic parameters.

In Experiment 1, the effects of *E. ulmoides* leaves were examined by maintaining female ICR mice on a long-term high-fat diet. Compared to the HFD group, increase in body weight and visceral fat accumulation were significantly suppressed in all the test groups despite the fact that none

Fig. 4. Effect of the long-term intake *E. ulmoides* on the serum Cholesterol level

Each point represents the mean $\pm$ SD of seven animals.

*: significantly different from the control ($p < 0.05$).

Fig. 6. Effect of *E. ulmoides* on the serum cholesterol level after oral administration

Each point represents the mean $\pm$ SD of seven animals.

of the groups showed a variation in feed intake quantity throughout the 4-week administration period. With regard to serum lipid levels, the serum triglyceride levels of the E-EP and E-GP groups were significantly low, and the total serum cholesterol level of all the test groups was significantly low. Metori *et al.*⁹⁾ measured plasma lipid in rats after administering a high-fat diet for only 2 weeks followed by the administration of a combination of a high-fat diet and *E. ulmoides* leaves for an additional 2 weeks. This experiment revealed that *E. ulmoides* leaves significantly reduced plasma triglyceride levels. This experiment used serum lipid as an indicator and yielded comparable results. Thus, the long-term intake of *E. ulmoides* leaves reduced serum lipid and inhibited visceral fat accumulation and increase in body weight in mice on a high-fat diet.

Experiment 2 examined the effects of *E. ulmoides* leaves on male Wistar rats orally administered a single dose of fat. When only fat was administered, the serum triglyceride level started to increase at 60 min after the start of the experiment and returned to the baseline level at 300 min. On the other hand, when a combination of fat and E-E was administered, the serum triglyceride level tended to slowly increase until 180 min after the administration. However, these values tended to be lower than when only fat was administered. Lipid is absorbed through the intestinal walls and transported into the blood; this suggested that the E-E may play a role in the inhibition or delay of intestinal lipid absorption. Tanabe *et al.*¹⁰⁾ have shown one of the mechanisms of action of

E. ulmoides leaves on lipid metabolism, namely, *in vitro* inhibition of pancreatic lipase activity by E-EP and E-GP. This indicated that the anti-obesity effect of *E. ulmoides* leaves could be attributed to inhibition of lipid absorption. Future experiments are required in order to elucidate the mechanisms of lipid metabolism and inhibition of lipid absorption into the blood. This research has successfully demonstrated that *E. ulmoides* leaf products inhibit triglyceride absorption into the blood and prevent visceral fat accumulation. These obesity-reducing effects were suggested to be caused by the inhibition of lipid absorption from the intestine following ingestive behavior. Further studies are required in order to identify the component of *E. ulmoides* responsible for inhibiting lipid absorption to shed light on the role it plays in the regulation of fat accumulation in rats.

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