Obesity is a state in which body fat is accumulated to an excessive extent and may be the cause of the onset of diabetes, dyslipidemia, hypertension, gout, cholelithiasis, osteoporosis, and other diseases. As obesity progresses, other severe, diseases appear that are difficult to treat or manage, including angina, ischemic heart disease, stroke, and malignant tumors. It has been also reported that the hypertrophy of adipocytes and inflammation can cause insulin resistance and obesity, resulting in increased leptin and decreased adiponectin secretions [1-3]. Therefore, as obesity is closely related to various diseases, its prevention has a very important impact in alleviating these diet-related diseases.

Kaempferia parviflora (KP) is a plant belonging to the family Zingiberaceae and is grown specifically in Thailand and Laos. It has historically been well known as Kurachai Dum in Thailand and was used as a traditional remedy. Unlike ginger, the rhizomes of KP are black. The rhizome was used to improve obesity, inflammation, allergy, and gastrointestinal disorders [4-6]. In addition, it has been reported that KP extract (KPE) has various properties, including anti-obesity effects, anti-oxidant and anti-inflammatory activity, and the capacity to reinforce skeletal muscles [7-11].

Dietary supplementation with KPE in obese mice reportedly suppresses body weight increases, body fat accumulation, and glucose intolerance [12-14]. Other reports suggest that oral intake of KPE increases energy expenditure and fat utilization in humans [15, 16]. Moreover, dietary intake of KPE reportedly decreases body fat (visceral and subcutaneous fat) in overweight and pre-obese subjects [17]. Collectively, these findings suggest that KPE could be used to reduce body fat in humans. However, its precise mechanism of action has not been elucidated yet.

In this study, we tried to explore the visceral fat reduction mechanism of KP in vivo.

Materials and Methods

Animals

We obtained 5-week-old male C57BL/6j mice from SLC (Shizuoka, Japan) to the animal experimental facility in Yokohama University of Pharmacy and bred them under controlled conditions of temperature (22 ± 3°C), humidity (55 ± 15%) and light (0700-1900 h) for 8 weeks. Dietary intake and body weight were measured daily. The visceral fat and subcutaneous fat were

Abstract

*Kaempferia parviflora* (KP) is a plant in Thailand known historically as Kurachai Dum. It belongs to the ginger family and was used as a remedy medicine. Its rhizomes were used to improve obesity, blood flow, inflammation, allergy, and gastrointestinal disorders. However, the mechanism of its anti-obesity effect has not been elucidated. In this study, our purpose was to explore the visceral fat reduction mechanism of KP in vivo. Five weeks old C57BL/6j male mice were used. The mice were fed for 8 weeks with a test food limited to 3 g/day/mouse. We divided the mice into 4 groups as follows: ① normal diet group (controls), ② high fat diet group (HFD), ③ high fat diet + 0.5% black ginger extract group (HFD + KP 0.5%), and ④ high fat diet + black ginger extract 1.0% group (HFD + KP 1.0%). At the end of the 8th week, the visceral fat of the mice was collected and weighed and the expression levels of adiponectin, leptin, IL-6, and IL-1β in adipose tissues were measured by RT-PCR. Leptin and IL-6 expressions were decreased with a significant difference between group 4 and group 2. Adiponectin expression was significantly higher in group 4 than in group 2. The present study indicated that the anti-obesity effect of KP in vivo normalizes the function of leptin by suppressing its resistance upon ingestion of high-fat meals and inhibits fat accumulation by thermogenesis in brown adipocytes.

Keywords: *Kaempferia parviflora*, anti-obesity, leptin, adiponectin, adipose tissue, C57BL/6j mice

Introduction

In recent years, obesity has been regarded as a serious health problem worldwide, including in Japan. Many Japanese develop dietary problems, although Japanese people genetically do not have a tendency for obesity. However, due to the wide spread of European and American food culture, Japanese food habits have changed from "Japanese style food," containing considerable dietary fibers, to "Western style food," mainly consisting of animal fat, resulting in an increase in obesity rate among the Japanese population.

Obesity is a state in which body fat is accumulated to an excessive extent and may be the cause of the onset of diabetes, dyslipidemia, hypertension, gout, cholelithiasis, osteoporosis, and other diseases. As obesity progresses, other severe, diseases appear that are difficult to treat or manage, including angina,
measured by X-ray CT scan (La Theta, Aloka, Tokyo) once per week. On the last day of the 8-week period, we removed the liver and the visceral adipose tissue (testicle, mesenteric, perirenal) after 24 h fasting. This study was conducted with the approval of the Animal Experiment Committee of Yokohama University of Pharmacy.

**Test Food**

The rhizomes of KP from Thailand were washed thoroughly in water, dried and powdered (Maruzen pharmaceuticals co. Ltd). The rhizome powder was extracted with 50% ethanol, evaporated in vacuo and freeze-dried to obtain a dry extract. All mice were fed normal diets (CE-2, CLEA Japan, Inc. Tokyo) for one week to permit acclimation to the environment, and then they were divided into 4 groups (n = 5-8 in each group) according to their diet as follows: ① control feed group fed diet containing 10 Kcal% fat (Research Diet, Inc. D12450B), ② high fat feed group (HFD) fed diet containing 60 Kcal% fat (Research Diet, Inc. D12492), ③ HF+ KP 0.5% group (KP 0.5%) fed high fat diet plus 0.5% KP extract and ④ HF+ KP 1.0% group fed high fat diet plus 1.0% KP extract. Food was administered at a rate of 3 g daily by a gauge individually for each animal.

**Biochemical Analysis**

The blood was collected in heparinized tubes and centrifuged at 3,000 rpm at 4 °C for 15 minutes, after which plasma was collected. Plasma concentrations of glucose, cholesterol, NEFA, and insulin were determined using an E-Test Wako system (Wako Pure Chemical Industries, Ltd., Osaka).

**X-Ray Computed Tomography (CT) Analysis**

Mice were injected with 10 mL/kg pentobarbital diluted 10x at the peritoneal cavity. The mice were anesthetized, and after confirmation of loss of reflexes, visceral and subcutaneous fat were observed by CT scanning once a week. A series of CT images of tissue fat was scanned at slices of 1.0 mm thickness.

**Histopathological Study**

Liver and tissue epididymal fat was obtained from all animals and fixed with 10% neutral buffered formalin solution, embedded in paraffin, and sectioned at 3 μm width slices. Hematoxylin-Eosin (HE) solution was used for tissue staining. The stained tissues were observed under a light microscope (magnification: ×400).

**Real-Time PCR**

For total RNA extraction, Isogen (Nippon Gene Co., Ltd., Tokyo) was added to the extracted fat from each mouse and homogenized with POLYTRON-PT 1300 D (Central Scientific Trade Co., Ltd., Tokyo). Chloroform (Nacalai Tesque, Inc., Kyoto) was added to the homogenate; after centrifugation, the supernatant was recovered in a new tube. Isopropanol (Nacalai Tesque Co., Ltd., Kyoto) was added to the supernatant and after another centrifugation, the precipitate was recovered and dissolved in sterilized water and RNA concentration was measured. cDNA was obtained according to the protocol of Super Script VIRO cDNA Synthesis Kit (Invitrogen, Thermo Fisher Scientific Inc.). After completion of the reverse transcription PCR, we proceeded to Real Time PCR for leptin, adiponectin, IL-6, and IL-1β, with GAPDH as an internal standard using the Light Cycler 480 system (Roche, Basel, Switzerland) [18, 19] Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Universal Probe Library</th>
<th>Forward (Left)</th>
<th>Reverse (Right)</th>
</tr>
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<tr>
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<td>TTTGATGTTGGGTCCTCG</td>
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<tr>
<td>ADIPONECTIN</td>
<td>#95</td>
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<tr>
<td>IL-6</td>
<td>#60</td>
<td>TCTTCTAAAGTATGGGCTGGGA</td>
<td>AAAGGGAGCTCCTTAACATGC</td>
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</table>

**Statistical Analysis**

Each result was represented as mean ± standard error (SE). Statistical analyses among the control, HF, and KP groups were carried out using Tukey-Kramer test and t test with a significance level of 0.05 (P<0.05) or 0.01 (P<0.001). These data were analyzed using software Excel statistics.

**Results**

**Effects of KP on Body Weight Variation**

In the HFD group, body weight gain was significantly higher than that in the control group 1 week after the start of the experiment, whereas body weight in the KP 1.0% group was significantly lower at the 3rd week than that in the HFD group, and this effect continued until the final day of the study. The body weight on the final day was 22.1 ± 0.29 g in the control group, 28.4 ± 0.26 g in the HFD group, 27.7 ± 0.21 in the KP 0.5% group, and 26.3 ± 0.3 g in the KP 1.0% group Figure 1. There were no significant differences in feeding rate among the groups Figure 2.
The Anti-Obesity Effect of Kaempferia Parviflora (KP) is Attributed to Leptin in Adipose Tissue

Figure 1: Effects of KP on variations in body weight. In the HFD group the body weight increased compared to the control group with a significant difference the first week of the study, and KP 1.0% inhibited the body weight gain in C57BL/6J mice 3-weeks after the initiation of the study #p<0.05 vs control, *p<0.05 vs HFD

Figure 2: Effects of KP on feeding rates. There was no difference in the feeding rates in all groups

Weight of Total Visceral Fat

The weight of visceral fat was 0.32±0.07 g in the control group, 1.75±0.14 g in the HFD group, 1.54±0.18 g in the KP 0.5% group, and 1.22±0.10 g in the KP 1.0% group. The weight of total visceral fat in the KP 1.0% group was significantly less than that in the HFD group Figure 3.
The Anti-Obesity Effect of Kaempferia Parviflora (KP) is Attributed to Leptin in Adipose Tissue

Figure 3: Effect of KP on the weight of the total visceral fat (testis, renal, and mesenteric) the 8th week after the initiation of the study. There was a significant increase in visceral fat in the HFD group compared to the control group, and KP 1.0% significantly inhibited the increase of visceral fat. Each value the mean ± SE #p<0.05 vs control, *p<0.05 vs HFD

Image analysis of visceral and subcutaneous fat by X-ray CT

After the first 2 weeks an increase in visceral fat was observed in the HFD group with significant difference compared to the control group, and this result remained the same until the end of the study. The KP 1% group showed significantly lower visceral fat at the 4th week than the HFD group, and this result remained unaltered until the end of the study Figure 4. The subcutaneous fat was increased in the HFD group at the 3rd week with a significant difference compared to the control group. In the KP 1.0% group, a decrease in the subcutaneous fat was observed with significant difference compared to the HFD group at the 6th week Figure 5. This is an image of visceral fat and subcutaneous fat obtained from X-ray CT analysis at 0-week, 5-week and 8-week.

At the beginning of the study, there was no difference between the 4 groups. In the CT image of the 5th week, a decrease was apparent in both visceral and subcutaneous fat in the KP 1.0% group compared to that in the HFD group. In the CT image at the 8th week, the decreases in visceral and subcutaneous fat were obviously confirmed in both KP 0.5% and KP 1.0% groups compared to the HFD group Figure 6.

Figure 4: Time course of the effect of KP on visceral fat by CT measurements. Significant increase in visceral fat was observed in the HFD group the 2nd week after the initiation of the study, and significant decrease in visceral fat in the KP 1.0% group compared to the HFD group the 4th week after the initiation of the study #p<0.05 vs control *p<0.05 vs HFD

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**Figure 5:** Time course of the effect of KP on subcutaneous fat by CT measurements. Significant increase in subcutaneous fat was observed in the HFD group 3 weeks after the initiation of the study and significant decrease in subcutaneous fat in the KP group compared to that in the HFD group 6 weeks after the initiation of the study. \#p<0.05 vs control. *p<0.05 vs HFD

**Figure 6:** X-ray CT image of visceral fat and subcutaneous fat at 8 weeks after intake of fat meal. In the CT images, the viscera fat appears red and the subcutaneous fat yellow.
Histopathological Study

KP significantly decreased the adipocyte size Figure 7A. In histological analysis, the adipocyte’s size of the HFD group was larger than that of the normal diet group. However, the KP 1.0% group showed significant decrease in adipocyte size compared to the HFD group. To observe the effect of KP on fat volume, we used micro-CT analysis to quantify it. The results showed that the HFD group exhibited larger fat volume than the normal diet group, suggesting that the KP considerably lowered the fat volume. The HFD group had significantly higher adipose tissue weight than the normal diet group; however, the KP 1.0% group had also decreased adipose tissue weight. These results indicate that the oral administration of KP reduces the adipocyte size and fat volume, leading to a significant reduction in fat accumulation. There were no differences in the liver weight in all groups. In histopathological analysis, the KP 1.0% group had significantly lower incidence of fat accumulation in the liver than the HFD group Figure 7B.

The Expression Level of Visceral Adipose Tissue Markers

The mRNA expression levels of visceral fat markers were measured by RT-PCR at the end of the study. Gene expression levels were normalized to the expression level of the GAPDH gene and were compared to the expression level of HFD group.

The adiponectin gene expression level was 2.72±0.13 in the control group, 2.60±2.02 in the KP 0.5% group, and 8.58±1.64 in the KP 1.0% group. The expression level of adiponectin was significantly lower in the HFD group than in the control group, and significantly higher in the KP 1.0% group Figure 8A.

The leptin gene expression level was 0.0058±0.0049 in the control group, 0.57±0.11 in the KP 0.5% group, and 0.20±0.0064 in the KP 1.0% group. The expression level of leptin was significantly higher in the HFD group than in the control group, and significantly lower in the HFD group than in the KP 1.0% group Figure 8B. The IL-6 gene expression level was 0.19±0.043 in the control group, 0.037±0.031 in the KP 0.5% group, and 0.080±0.040 in the KP 1.0% group. The expression level of IL-6 was significantly increased in the HFD group compared to the control group, and the KP groups, in both the 0.5% group and the 1.0% group, were significantly decreased compared to the HFD group Figure 8C. IL-1β gene expression level was 0.16±0.071 in the control group, 0.71±0.26 in the HFD KP 0.5% group, and 0.64±0.10 in the KP 1.0% group. In the HFD group, the expression level of IL-6 was significantly higher than that in the control group, but there was no significant difference from that in the KP group; only a decreasing trend was observed Figure 8D.
The Anti-Obesity Effect of Kaempferia Parviflora (KP) is Attributed to Leptin in Adipose Tissue

Discussion

The purpose of this study was to explore the mechanism of anti-obesity effect of KP. This study was breed individual gage with mice and them freely ingested. Approximately 70% of 4.5 g, the usual amount of mice daily food intake, was normally fed to the mice.

In the obesity mouse model, the suppression of obesity increase was shown in the KP 1.0% group. Although the feed intake of the KP group was the same as that of the target group, the KP group significantly prevented body weight gain and visceral fat and liver lipid accumulation. In addition, with respect to adipocytokines, a significant decrease in leptin and a significant increase in adiponectin were observed in the KP 1.0% group, as well as a significant decrease in levels of the inflammatory marker IL-6.

Previous reports have shown that KP effectively attenuated obesity and insulin resistance by suppression of fat accumulation, hyperinsulinemia, glucose intolerance, hypertension, and insulin resistance in type 2 diabetic mice [12]. These results indicate that KP can improve the obesity related to metabolic dysfunctions and insulin sensitivity in obese mice.

Serum levels of hepatotoxic biomarkers were not measured in this study; however, liver weight was not significantly altered among groups, which indicates a probable protective effect of KP. Hepatic fat accumulation induced by high fat diet leads to increase of hepatotoxic biomarkers, which may cause fatty liver disease and hepatic inflammation resulting in imbalance in lipid metabolism [18]. KP reduces hepatic fat accumulation thus preventing the obesity induced hepatic injury.

The present study shows that KP exerts an anti-obesity effect. KP effectively prevents body weight gain, reduce fat size and mass, and attenuates hepatic fat accumulation without any liver damage in obese mice.

Adiponectin is a hormone secreted from adipose tissue that regulates glucose and lipid metabolism by stimulating fatty acid oxidation in almost all major target tissues, including skeletal muscle, liver and adipocytes [19-21].

Blood levels of leptin are positively correlated with increase in obesity and weight in humans and rodents [22, 23]. It has been reported that with a high fat diet, blood leptin concentration augments and leptin resistance can be developed [24, 25].

It has been also reported that there is a decrease in the secretion of adiponectin in high fat diets [26]. In this study, the expression level of adiponectin was significantly higher in the KP 1.0% group than in the HFD group, and the expression level of leptin was significantly lower in the KP 1.0% group than in the HFD group.

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The 4-week administration of KP had no effect on adiponectin; however, significantly higher leptin levels were observed in the KP group than in the HFD group (unpublished data).

Previous reports have stated that KP is involved in thermogenesis in brown adipocytes [14, 27]. It has also been reported that leptin activates Uncoupling protein 1 (UCP-1) of BAT by activating sympathetic nerves that secrete noradrenaline, enhancing the capacity for thermogenesis and the energy consumption of individuals [28-30].

From the above data, it is suggested that KP enhances lipolysis by the β receptor (β-AR) system and consumption by the UCP by improving leptin resistance and is involved in fat reduction.

Conclusion

This study has shown that KP exerts an anti-obesity action by improving leptin resistance preventing effectively body weight gain, decreasing fat size and body weight, and hepatic fat accumulation without liver damage. The present study indicates that the anti-obesity effect of KP in vivo normalizes the function of leptin by suppressing the induction of leptin resistance upon ingestion of high-fat meals and inhibits fat accumulation by thermogenesis in brown adipocytes.
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Authors’ Contributions

MM, KY, TM and YM performed most of the experiments. NI gave many advices during the research. KH gave many helpful suggestions on the statistical analysis. YW designed the experiments. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

NA

Clinical Trial Registration

NA

Acknowledgement

The authors are thankful to dear friends, Mr. Yu Kuwahara, Mr. Jun Sakurai and Mr. Kazuto Homma, students of Functional Food, Yokohama University of Pharmacy, for their helpful assistances to prepare for this experiment.

Funding

Annual Research Grant from Yokohama University of Pharmacy

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