3-O-β-D-Glucosyl-(1→6)-β-D-glucosyl-kaempferol Isolated from Sauropus androgynus Reduces Body Weight Gain in Wistar Rats

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Received March 31, 2006; accepted September 15, 2006

The young sticks and leaves of Sauropus androgynus (SA) that had been used as a health food for body weight reduction, led to an outbreak of obliterative bronchiolitis in Taiwan. This study tested the toxicity and anti-obesity features of the SA-isolated compound, 3-O-β-D-glucosyl-(1→6)-β-D-glucosyl-kaempferol (GGK), on male Wistar rats receiving 6 or 60 mg/kg of GGK orally as well as partial purified EtOAc and n-BuOH fractions of SA extract daily for 28 d. Sixty milligrams per kilogram GGK treatment significantly reduced food intake in rats by 15% (p<0.05). The reduced food intake corresponded to decreases in body weight in the high or low dose GGK groups, as compared to the control groups. The serum levels of free triglyceride significantly decreased in GGK-treated rats. GGK treatment led to successive reductions in daily food intake and body weight without obvious histopathological changes in Wistar rats. Thus, GGK may be potentially to be developed as a safe and novel compound for anti-obesity treatment.

Key words Sauropus androgynus; 3-O-β-D-glucosyl-(1→6)-β-D-glucosyl-kaempferol; body weight; weight reduction; anti-obesity.

Sauropus androgynus (SA), a member of the Euphorbiaceae family, is grown in high-temperature and humid regions of south Asia. It has been recorded as a tropical vegetable by the “United States Department of Agriculture,” and has a high-vitamin content earning it the name “multigreen” to provide a cheap source of dietary protein.1—3) Uncooked young sticks and leaves of SA have been used as a health food for body weight reduction in Taiwan till an outbreak of bronchiolitis obliterans.4—6) Lots of compounds such ligands, megastigamans, nucleosides, and flavonol glucosides have been found in SA extract. GGK is a flavonoid derivative isolated from SA, has dual effects in both decreasing body weight and food intake in Wistar rats. GGK is herein effective against obesity without obvious pathological changes.

MATERIALS AND METHODS

Animals Twenty-four male Wistar rats were randomly allocated into six groups. Each group consisted of 4 rats in a wire-bottomed cage in a room maintained at 22°C, on reversed phase lighting 12 h dark: light cycle. All rats were fed a diet for 4 weeks which consisted of 40.7% corn starch (S.C.G, Korea), 30% soybean oil (TSC, R.O.C), 19.3% casein (Fluka, Switzerland), 5% cellulose (ICN, U.S.A.), 3.5% mineral mixture (ICN, U.S.A.), 1% vitamin mixture (ICN, U.S.A.), 0.3% methionine (ICN, U.S.A.) and 0.2% choline (ICN, U.S.A.) by weight. This diet produces reliable weight gain, providing 31.8% of energy as carbohydrate, 52.7% as protein and 15.5% as fat.

SA Extract and GGK Preparation SA extract and GGK were prepared as described with a minor modification.7) Twenty-six kilograms of SA in 60 l was continuously methanol extracted for three times, followed by dissolution in 21 distilled water. SA in water was then partitioned with EtOAc and n-BuOH, resulting in the EtOAc extract and n-BuOH extract, sequentially. Forty grams of n-BuOH extract was further purified to yield 3—4 g, depending on the batches, of GGK which was then used in the animal study (Fig. 1).7)

Treatment Procedures Four rats in each group were given 720 mg/kg/d of the EtOAc extract of SA, the n-BuOH extract of SA at 82 mg/kg, GGK at 60 mg/kg or 6 mg/kg, pioglitazone at 2.5 mg/kg and vehicle in H2O (pH 5.9) for 4 weeks. Food intake and body weight of each individual rat was recorded daily for 4 weeks. At the end of the study, rats

![Fig. 1. Structure of 3-O-β-D-Glucosyl-(1→6)-β-D-glucosyl-kaempferol (GGK)](image)
were sacrificed by carbon dioxide inhalation and immediately underwent cardiac puncture for the blood collection. The lung, liver and kidney tissues were collected for histopathological examination.

**Determination of Serum Biochemical Parameters** All animals were sacrificed by decapitation at the end of the experiment. All serum samples were sterile, haemolysis-free, and were kept at 4 °C before determination of the biochemical parameters.

Serum Glutamate Oxaloacetate Transaminase (GOP), Glutamate Pyruvate Transaminase (GPT), Blood Urea Nitrogen (BUN), creatinine, triglyceride and cholesterol levels were measured with an AU 600 multiparametric analyzer (Olympus, Hamburg, Germany).

**Histology of the Organs** Immediately after removal, lung, liver and kidney tissues were fixed in 10% buffered formalin and processed for histopathological examination with conventional methods and stained with hematoxylin and eosin (H&E) or silver. All of the observed abnormal morphological findings were classified and registered.16)

**Statistical Analysis** Data were expressed as mean±S.D. unless otherwise specified. The Student’s t test was used to analyze individual differences. Rate comparison was analyzed by the T test. A value of p<0.05 was considered to be statistically significant.

RESULTS

**Effect of GGK on Food Intake in Wistar Rats** The rationale of the compound concentrations in this study is as details. GGK used for in vivo study is expected to be approximately equal to the effective concentration of GGK against adipogenesis in preadipocyte differentiation in vitro. Thus, GGK 6 mg/kg and tenfold concentration 60 mg/kg GGK were tested in this study. The content of GGK is about one fourteenth in n-BuOH fraction. Therefore, 82 mg/kg of n-BuOH fraction was used in the study. EtOAc fraction, the most abundant fraction in SA extract, has a 9 : 1 ratio to n-BuOH fraction. Therefore, 82 mg/kg of n-BuOH fraction was used in the study. EtOAc fraction, the most abundant fraction in SA extract, has a 9 : 1 ratio to n-BuOH fraction. Both GGK 60 mg/kg and GGK 6 mg/kg treatment groups reduced food intake, compared to vehicle-treated controls throughout the 28 d experimental period (Fig. 2). Food intake in the 60 mg/kg GGK group was decreased by 15% (p<0.05, n=4). The EtOAc fraction group had similar effect to the GGK 6 mg/kg group. On the other hand, there is less significance in reduction of total food consumption in rats treated with the n-BuOH fraction of SA extract.

**Effect of GGK on Body Weight** Reduction of the body weight gains were evident in groups treated with either 60 mg/kg or 6 mg/kg of GGK in comparison to negative (vehicle-treated) and positive (pioglitazone-treated) control groups; the 60 mg/kg GGK group was more significant throughout the 28 d experimental period (Fig. 3). Although the contents of GGK in the EtOAc and n-BuOH groups were supposed to be equivalent to that in the 6 mg/kg GGK group, the weight gains of the EtOAc group and n-BuOH group differed from that of vehicle-treated control slightly. Since the n-BuOH fraction of SA extract was partially purified, certain components which were unfavorable in reduction of weight gain were possibly contaminated.

**Blood Biochemical Analysis** All groups in our experiment fed with high-fat diet. Despite rats fed with high-fat diet exerted higher value of TG than that with normal diet; there is no difference in cholesterol level between high-fat and normal diet groups. High and low doses of GGK resulted in a significant reduction in total triglyceride levels by 35—47% in serum as compared to the high-fat fed control group (Table 1). In addition, statistically significant diminutions of serum GPT levels occurred after GGK treatment.

**Histopathological Changes in Lung and Liver Tissue** Tissue changes were examined in Wistar rats treated with n-BuOH or GGK 60 mg/kg under histopathological analysis of the lung tissue using H&E (Fig. 4A) or Silver (Fig. 4B) stain and the liver tissue using H&E stain (Fig. 4C). As a result, there is no significant difference of the lung tissues between the GGK group and control group. However, in the n-BuOH group, the bronchiolitis obliterans and organizing pneumonia (BOOP) changes were observed. Pericentral fatty changes with occasional apoptosis were also observed in liver tissue of the n-BuOH group. In fact, all groups showed mild inter-
stitial changes in lung and mild intrahepatocyte fatty changes in liver. Unlike the n-BuOH group, the GGK-treated group had no identifiable fat accumulation and no evident histopathological change in liver, such as inflammation or hepatocyte injury.

DISCUSSION

Regarding the amount of GGK used in animal study, we try to adapt in vitro effective concentration to achieve the considerable in vivo concentration. When the preadipocytes NIH3T3-L1 were treated with 6 μg/ml of GGK, there was an approximately 50% anti-adipogenesis effect in our previous study. We look 6 μg/ml of GGK as an effective concentration in vitro and suppose that as the corresponded concentration in blood. Thus, we treat the averaged body weight 500 g Wistar rats with 6 mg/kg GGK orally to yield an in vivo concentration 6—60 μg/ml approximately when we count the confounding factors such as absorption, metabolism and distribution. Tenfold concentration of GGK, 60 mg/kg, was also tested in the study.

In the present study, GGK, isolated from SA, significantly decreases food intake and reduces body weight gain in Wistar rats. In fact, food ingestion was apparently less in the GGK 6 mg/kg group than that in the n-BuOH fraction group although the amount of GGK treated was theoretically equivalent in these two groups. It may result from certain components contaminating the partially purified n-BuOH fraction of SA extract and interfering the reduction of food consumption. Similar episode occurred, regarding the reduction of body weight gain, in these two groups. Furthermore, GGK decreases the level of triglyceride at either 6 mg/kg or 60 mg/kg. It is still unclear the association of changes in blood serum biochemical levels with anti-obesity in the Wistar rat model. Histopathological examination of rats under GGK treatment shows no significant changes to lungs or liver, compared to the control group. The findings reported here therefore demonstrate that GGK administration can lead to reduction in daily food intake and body weight gain, without identifiable pathological change in Wistar rats.

Decreasing levels of triglyceride was noted in the serum of GGK-treated rats. However, GGK treatment also significantly reduced GPT value, with a negligible effect on GOT value. In human obesity, hepatic steatosis is often accompanied by steatohepatitis, which may progress to cirrhosis and

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<th>Table 1. Effect of GGK on Blood Serum Biochemical Levels in Wistar Rats</th>
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The rats were treated with 60 mg/kg or 6 mg/kg of GGK for 28 d. Values are expressed as mean±S.E.M. (n=4). *Difference statistically significant from control group: p<0.05.

Fig. 4. Pathological Changes in Lung and Liver Tissues

Lung specimens of Wistar rats fed with normal saline (left), n-BuOH fraction 82 mg/kg (middle) and GGK 60 mg/kg (right) for 28 d were stained with H&E (A) and Silver (B). Presence of bronchiolitis obliterans and organizing pneumonia (BOOP) changes was in the n-BuOH group. Nearly normal lung except for mild interstitial changes were observed in the GGK and control groups. Liver tissues were stained with H&E (C). Mild intrahepatocyte fatty changes were found in all groups. Pericentral fatty changes (arrow) with occasional apoptosis (arrow head) were observed in the n-BuOH group. ×200.
life-threatening liver disease. Resolution of hepatic steatosis without liver injury is an important adjunct to the loss of adipose tissue. Concurrent administration of medications with potential hepatoprotective effects may be a reasonable adjuvant for the treatment of GGK in obesity.

In summary, our results suggest that GGK could be an effective agent in the treatment of obesity, and does not exhibit detectable pathological changes in rats. Reductions in daily food intake and body weight in this study were dose dependent and time dependent. Even at the low dose of GGK (6 mg/kg) used in this study, loss in body weight was eventually observable. Furthermore, our data provide evidence that GGK can affect feeding behavior and, thus, may be of use in obesity therapy for the control of appetite and body weight.

Acknowledgement This work was supported by NSC Grant (NSC 89-2314-B-002-054).

REFERENCES


