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Effect of Shilianhua extract and its fractions on body weight of obese mice

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Abstract

Five commercial botanical products (Shilianhua [SLH] tablets, Shiu Huo pills, Fenulyn, Bitter Melon, and Glucose Metabolic Support), available in the US market, with reported claims for regulation of metabolism were screened for their effect on body weight gain in high-fat diet–induced obese mice. Pilot results suggested that SLH tablets attenuated body weight gain, whereas Shiu Huo pills and Fenulyn tended to promote weight gain in the mice on the high-fat diet. To investigate the bioactive components in the SLH tablet, the wild SLH plant (*Sinocrassula indica Berge)* was collected from China and used to make a variety of extracts including aqueous extract, ethanol extract (SLH-E), and subfraction F100. In the study of metabolic activities, the extracts were administrated through food intake by incorporating them into the diet. A rigorous evaluation of the extracts on body weight was conducted in 2 animal models. The aqueous extract and SLH-E were tested in dietary obese mice, while F100 together with SLH-E was tested in KK-Ay mice, a genetic diabetic model. In the 12- to 16-week study, body weight was not significantly altered by the SLH extracts in the 2 animal models. The results suggest that neither the total extract nor the purified components from the SLH plant have a clear effect in the regulation of body weight. The weight reduction observed with the over-the-counter SLH tablet in the pilot studies may be secondary to other components in the tablet, but not from the SLH extract.

1. Introduction

Many over-the-counter (OTC) supplements have been marketed as being effective to promote glucose metabolism in humans, and consumption of dietary supplements by the general public has increased in the United States to facilitate the management of blood glucose [1]. Among these dietary supplements, botanical extracts are extremely popular components and have been promoted to enhance the therapeutic activities and reduce the adverse effects of synthetic drugs. However, the clinical efficacy and mechanism of action of many botanicals have not been well characterized. In this study, we evaluated the weight regulation activities of several botanical products that are marketed in the United States and make claims for metabolic regulation. Reduction of body weight is known to decrease blood glucose through improvement of insulin sensitivity. Five commercial products, based on reported claims, were chosen for evaluation in this study. Specifically, they were Shilianhua (SLH) tablets, Shiu Huo pills (SHP) (Chinese Medicine United Pharmaceutical Factory, Guangzhou City, China), Fenulyn (Fen) (Princess Lifestyle LLC, Baldwin Park, CA), Bitter Melon (BM) (Nature's Herbs, American Fork, UT), and Glucose Metabolic Support (GMS) (Now Foods, Bloomingdale, IL). The efficacies of these products in the management of body weight were tested in the dietary obese mice by oral administration.

The commercial SLH tablet contains several botanical products including SLH (*Sinocrassula Berger*), spirulina, *Lycium* berries, soy, fiber, guar gum, et al. The SLH (*Sinocrassula indica*) is also patented to reduce blood glucose in the United States, Japan, and China. *Shilianhua* is the Chinese name for houseleek, which is widely distributed in the world. In addition, it is consumed as tea in Taiwan and Japan. To study bioactivities of the SLH plant, we purified extracts from the wild *S indica* and examined their efficacy in the regulation of body weight in obese mice. The SLH extract was also divided into 4 subfractions, and one subfraction (F100) was tested in mice for weight regulation. The result suggests that the SLH extracts have no activities in the regulation of body weight.

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2. Materials and methods

2.1. Mouse models and treatment

Dietary obesity was generated in the male C57BL/6J mice with a high-fat diet (HFD) as described elsewhere [2]. Male C57BL/6J mice and KK-Ay (KK.Cg-Ay mutant) mice were purchased from Jackson Laboratory (Bar Harbor, ME) and housed singly in the study. The C57BL/6J mice were fed on an HFD (58% calories as fat, D12331; Research Diets, New Brunswick, NJ) at 5 weeks of age to induce obesity. The KK-Ay mice were fed a defined low-fat diet (D12329, Research Diets) throughout the experiment. The control group was fed the defined diet, and the treatment group was fed the same diet containing one of following products: SLH tablets (5.2 mg/[kg d]), SHP (100 mg/[kg d]), Fen (150 mg/[kg d]), BM (150 mg/[kg d]), and GMS (200 mg/[kg d]). In addition, animals had diets supplemented with SLH fractions (see preparation and purification below) consisting of SLH aqueous extract (SLH-A; 0.4% wt/wt), SLH ethanol extract (SLH-E; 1% wt/wt), or F100 at 0.05% (wt/wt). Body weight was measured weekly. Food intake was measured twice for 1-week intervals during weeks 3 and 6.

2.2. Preparation of the SLH-A

The photographs of fresh and dried SLH plants (*S indica*) are shown in Fig. 1A. The extract of SLH used in this study was prepared from the aerial parts of wild SLH plant. The plant was collected from the vast arid and mountainous areas in Guizhou province, southwestern China, where SLH has been used as a medicinal herb by the local residents for hundreds of years. The SLH sample was certified by a taxonomist at the Institute of Medicinal Plant Development, a Chinese authority in the identification and authentication of traditional Chinese herbs and medicinal plants. The fresh aerial part of SLH was air-dried under shade to reduce moisture content to approximately 8% wt/wt. The dry material was ground into 6-mm or smaller pieces. The grounded material was first soaked in deionized water at 1:8 wt/vol ratio for 60 minutes at room temperature and then extracted twice at 50°C in a rotary extractor for 6 hours. The water-soluble extract was separated from the solids (structural components of fibers, cellulose, semicellulose, debris of cells) by first centrifuging at 3500 rpm with an Allegra 6KR Centrifuge (Beckman Coulter, Palo Alto, CA) and then filtering with Whatman 4 paper. The liquid product was then concentrated in a rotary evaporator of 20-L capacity (Rotavapor R-220; Buchi, Flawi, Switzerland) and followed by freeze drying (Labconco, Kansas City, MO) into an aqueous extract powder (SLH-A) that accounted for 29.8% wt/wt of the raw herb.

2.3. Preparation of the SLH-E

The aqueous extract (about 1.3 kg) was further fractionated to yield a concentrated SLH extract. It was dissolved

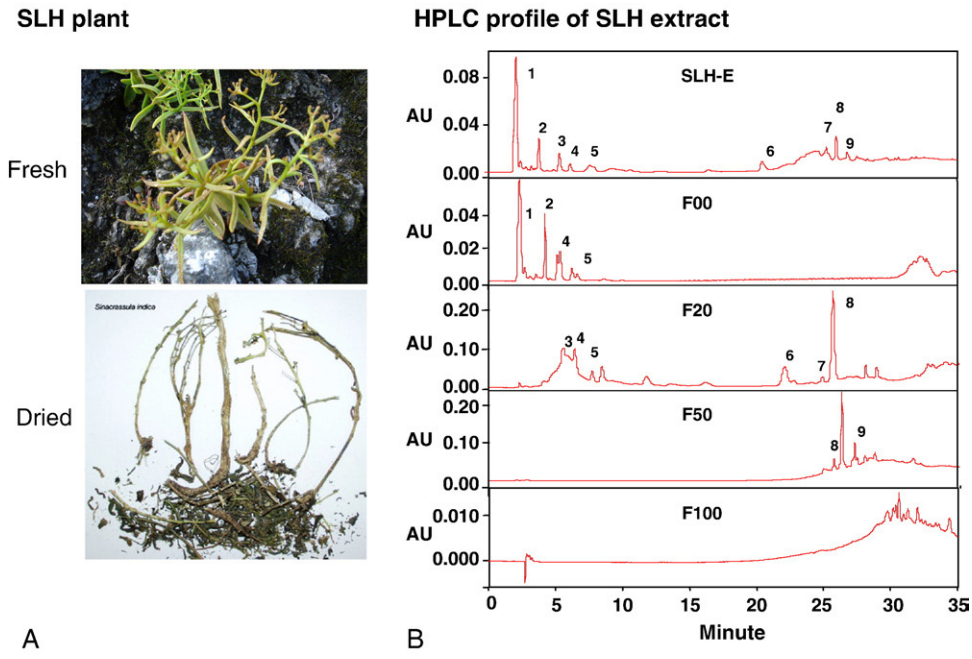

Fig. 1. Shilianhua extracts. A, Picture of wild SLH plant (*S indica*) used in this study. The plant was collected in the Guizhou province, southwestern China. B, Chromatographic fingerprints of SLH and its fractions. Nine major components were observed in the chromatographic fingerprinting profile of SLH-E. To enrich the metabolic activity, SLH-E was fractionated into 4 fractions in HPLC on the basis of polarity of components. These were F00, F20, F50, and F100. The fingerprint profile shows that components 1, 2, 4, and 5 were located in the F00 fraction; components 3, 5, 6, and 8 were included in the fraction F20; components 8 and 9 were contained in the fraction F50; and the least polar components were retained in the fraction F100.

into 15 L of deionized water, loaded into a 15-kg macroporous adsorbent polymer resin column (L493; Sigma Chemical, St Louis, MO), washed with 5 L of water, and then eluted with 20 L 95% ethanol, which was evaporated to obtain SLH-E.

2.4. Preparation of subfractions of SLH

The ethanol in 95% ethanol elutes was removed by evaporation under reduced pressure. The SLH was fractionated using a high-performance liquid chromatography (HPLC) system with C18 column (Sigma Chemical) that was eluted with water, 20% MeOH, 50% MeOH, and 100% MeOH in a sequence to obtain 4 subfractions, that is, F00, F20, F50, and F100. F100 was found to be the most active subfraction in the inhibition of inflammatory responses (data not shown) and was therefore selected as the subfraction for in vivo assessment on body weight gain along with the SLH-E and SLH-A extracts.

2.5. Characterization of SLH-E and subfractions

Chemical fingerprint of the SLH extracts was developed using a Waters (Milford, MA) HPLC system consisting of a Waters Delta 600 pump, a Waters 717 plus autosampler, and a Waters 2996 Photodiode Array Detector (190-800 nm) (Fig. 1B). The system is controlled by computer, and the data were analyzed with the Empower software system (Waters). The mobile phase consisted of HPLC-grade methanol and water run through a gradient elution from 3:97 ($MeOH/H_2O$) to 10:90 ($MeOH/H_2O$) for the first 6 minutes, followed by a gradient elution to 80:20 ($MeOH/H_2O$) for 14 minutes and gradient elution to 100:0 ($MeOH/H_2O$) for 5 minutes; kept for 5 minutes; and then equilibrated to 3:97 for 15 minutes before the next sample was injected.

2.6. Statistical analysis

In the bar figures, mean value and standard error of multiple data points or samples were used to represent the final result. Student *t* test or 1-way analysis of variance was used in the statistical analysis of the data, with a significance level of $P < .05$.

3. Results

3.1. Commercial SLH tablet attenuates diet-induced weight gain

The effects of 5 commercial products on body weight were investigated in dietary obese mice. The products were administrated through food intake by incorporating them into the HFD. Among the 5 products, the SLH tablets reduced gain in body weight on HFD (Fig. 2A, B). At the end of 12 weeks, the body weight was 38 g in the chow diet group (lean control) and 43 g in the HFD group (13% increase, $P < .05$). With SLH supplementation, the body

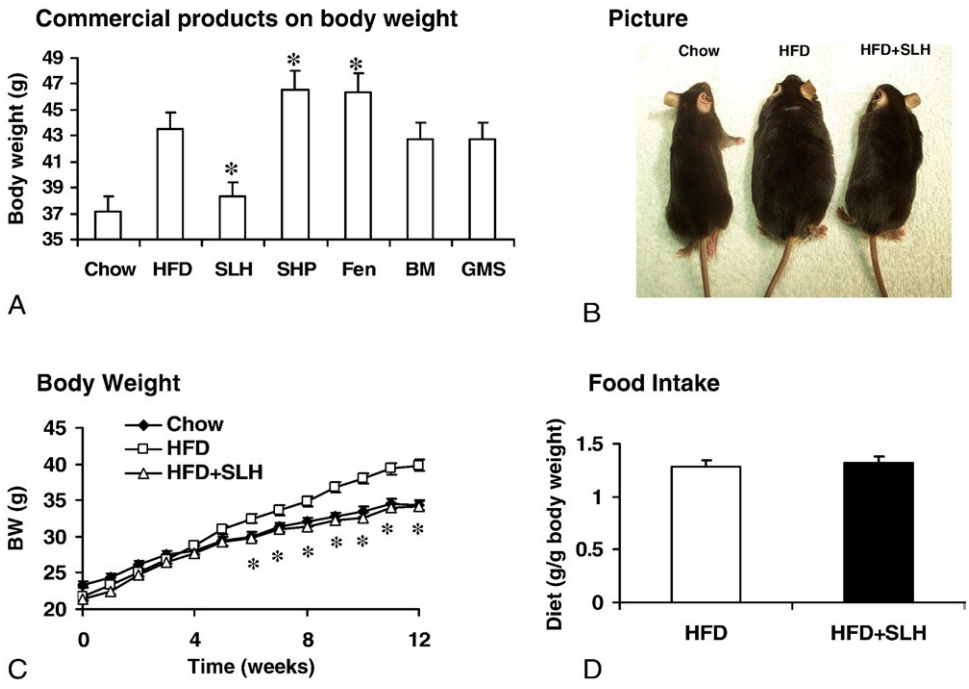

Fig. 2. Regulation of body weight by commercial botanical products. Five commercial botanical products including SLH, SHP, Fen, BM, and GMS were tested in the prevention of dietary obesity in mice fed an HFD. A, The SLH tablets reduced gain in body weight significantly when compared with the other commercial products. The body weight was examined at the end of 12 weeks on HFD. B, Picture of mice at 12 weeks on HFD and SLH tablets. C, Time course of body weight gain on HFD and SLH tablets. D, Food intake (grams) per body weight (grams) over 4 weeks from 12 to 16 weeks on HFD. Each point presents mean ± SE (n = 11). Compared with HFD control: *$P < .01$.

weight was 38 g in mice on HFD, which was identical to that of the chow diet group (Fig. 2C). With SHP or Fen supplementation, the body weight was 5% ($P < .05$) higher than that of the HFD group. These data suggest that SLH tablet attenuated body weight gain, whereas the SHP and Fen tablets promoted body weight gain in mice on HFD. This antiobesity effect of SLH was not a result of alteration in food intake because there was no significant difference in the food intake in the control (HFD) and experimental (SLH) groups (Fig. 2D). This pilot study suggested that the commercial SLH tablet may contain bioactive compounds that attenuates HFD-induced obesity in mice.

3.2. Chemical fingerprints of SLH extracts

Because the major component of the commercial SLH tablet is the extract of the Shilianhua plant, we made an effort to study the bioactivity in the plant *S indica.* A variety of extracts was isolated from the plant, and their chemical fingerprints were analyzed with HPLC. Nine major components were observed in the chromatographic fingerprinting profile of SLH-E (Fig. 1B). The SLH-E was fractionated into 4 fractions in HPLC on the basis of polarity of components. These were identified as F00, F20, F50, and F100. The fingerprint profile suggests that components 1, 2, 4, and 5 were located in the F00 fraction; components 3, 5, 6, and 8 were included in the fraction F20; components 8 and 9 were contained in the fraction F50; and the least polar components were retained in the fraction F100. F100 was used as an organic extract of SLH because it was collected through elution with 100% MeOH.

3.3. Purified SLH extracts had no effects on body weight of mice

The purified SLH extracts were studied in the mouse model of obesity in identical experiments as assessed with the commercial products. First, SLH-A was tested in the dietary obese mouse model through oral administration (dietary supplementation). The dosage of extract was 0.4% (wt/wt) in the diet, which is equivalent to 500 to 700 mg/kg body weight per day. Both in the control and in the SLH-A–treated mice, body weight was increased in the mice in a time-dependent manner on HFD. No difference was observed between the SLH group and control group in body weight (Fig. 3A). Because SLH-A had no obvious actions in the dietary obese mouse model, we tested SLH-E in the HFD mice. The dose of SLH-E was increased to 1% (wt/wt) in the diet. No change in body weight was observed for SLH-E (Fig. 3B). Finally, SLH-E was also tested in KK-Ay diabetic mice that develop hyperglycemia and insulin resistance on low-fat diet. F100, a subfraction of SLH-E, was also tested in the study. The SLH-E and F100 were administrated orally through diet supplementation at 2% and 0.05% wt/wt in the low-fat diet, respectively. In the study, body weight was not significantly changed by SLH-E

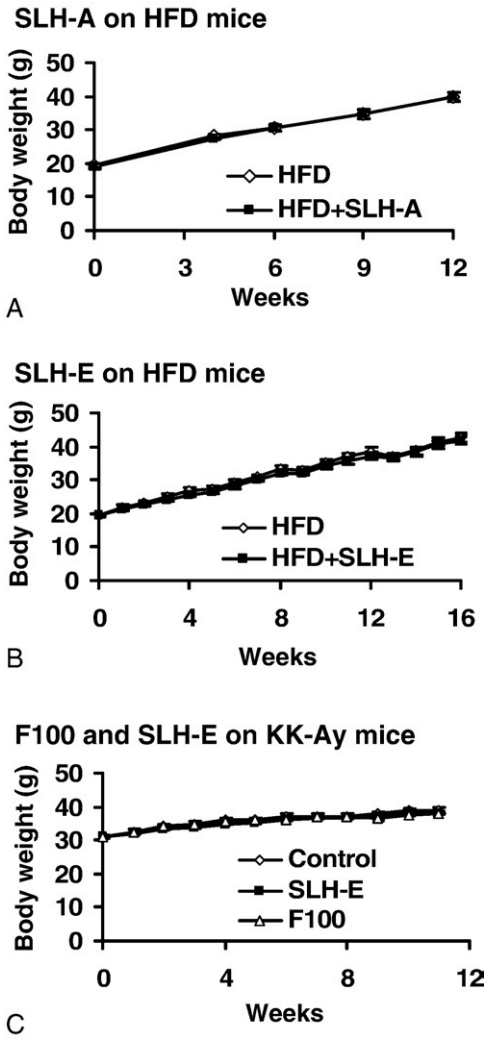

Fig. 3. Shilianhua had no effects on body weight of mice. A, Aqueous extract of SLH had no effects on HFD-induced obese mice (n = 9). B, Ethanol extract of SLH had no effects on HFD-induced obese mice (n = 11). C, F100 and SLH-E had no effects on KK-Ay mice (n = 9).

or F100 compared with the control mice (Fig. 3C), suggesting that the extracts of SLH were unable to regulate body weight in the diabetic KK-Ay mice.

4. Discussion

This study represents a very rigorous assessment of OTC supplements in the regulation of body weight. In this study, the commercial SLH tablet prevented body weight gain, whereas SHP and Fen promoted body weight gain in the dietary obese mice. However, when the major component of the SLH tablet was tested in the same animal model, no significant activity was observed in the regulation of body weight. Thus, this work strongly suggests that the SLH extract does not have an effect to regulate body weight.

The plant SLH is a shrub in the Crassulaceae family that grows in the southwestern part of China including Yunnan,

Guangxi, and Guizhou provinces. It had been used as an herb for hundreds of years in southwest China, and SLH is also patented to reduce blood glucose in the United States, Japan, and China. As we have described, when the 5 commercial products we tested were compared, only the SLH tablet was found to have a potent effect on regulation of metabolism. Thus, to identify the bioactive components for the activity for this commercial product, the SLH plant was obtained and used to make a variety of extracts including SLH-A, SLH-E, F00, F20, F50, and F100. Among them, the aqueous extract, that is, SLH-A, was the crude extract that contained all bioactive components except the fibers, cellulose, and debris of the cells that are felt not to be bioactive. The ethanol extract, that is, SLH-E, is a 95% ethanol extract of the plant and much more concentrated than SLH-A when assessing activity of inhibition of inflammatory response (data not shown). Compared with SLH-A, SLH-E contains more small-molecule compounds and fewer components with large molecules like polysaccharides and peptides, which are suggested to be inactivated in the gut after oral administration. In the subfractions of SLH, F100 was the organic extract with the lowest polarity in the SLH-E. F100 contains small molecules, such as saponin and alkaloid, which are water insoluble and with bioactivity in general. F100 is the most active subfraction in the inhibition of inflammation response (data not shown). In this study, SLH-A, SLH-E, and F100 were tested in dietary obese mice and KK-Ay mice, which are models for obesity and type 2 diabetes mellitus. However, no antiobesity effect was observed for these SLH extracts in the mice. The results indicate that SLH has no bioactivities in the regulation of body weight.

If the SLH plant does not have an activity in the regulation of body weight, what was responsible for the weight control activity of the SLH tablet in the pilot studies? First, the bioactivity of SLH tablet may be due to components other than SLH extracts. In addition to the SLH plant extract, the SLH tablet also contains products from 4 other plants, such as *Spirulina maxima*, *Lycium* berries, soy fiber, and guar gum. Spirulina was reported to reduce blood glucose and alleviate dyslipidemia and fatty liver [3-5]. Berry of *Lyceum barbarum* is a popular traditional Chinese herb that is used to treat aging-related disease. Berry of *Lyceum barbarum* was reported to have hypoglycemic, hypolipidemic, and antioxidant effects in both type 1 and type 2 diabetes mellitus in animal models [6-9] Soy fiber is able to decrease postprandial blood glucose by regulation of glucagon, pancreatic polypeptide, and somatostatin secretion [10,11]. Guar gum, a well-established water-soluble fiber, is known to reduce hypercholesterolemia, hyperglycemia, and obesity [12-14]. The combination of these botanical products in the SLH tablet may have constituted the antiobesity activity. However, to definitively state that the effect is from these extracts, the individual extracts also will have to be rigorously tested. In addition, it could also be argued that other components, for example, pharmacological agents, may have been involved. Over-the-counter agents are not regulated in the same fashion and do not have the same quality control standards as demanded by the Food and Drug Administration for prescription drugs. Thus, the claims made for products rarely are ever validated in placebo-controlled trials.

In summary, we conclude that the SLH plant as a total extract, or when separated into specific bioactive fractions, has no effects on regulation of body weight. The effect of weight reduction, if any, in the SLH tablet is clearly not due to SLH extract. As such, the effect of the commercial tablet on body weight with repeated testing was inconsistent. Thus, no scientific support exists for the role of SLH in the regulation of weight gain in our studies.

Acknowledgment/Conflict of Interest

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