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ARTICLE

Improvement of Obesity Phenotype by Chinese Sweet Leaf Tea (*Rubus suavissimus*) Components in High-Fat Diet-Induced Obese Rats

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Drinking an herbal tea to lose weight is a well-liked concept. This study was designed to examine the possible improvement of obesity phenotype by a new tea represented by its purified components, gallic acid, ellagic acid, and rubusoside (GER). Male obese-prone SD rats were given low-fat diet, high-fat diet, or high-fat diet plus GER at the dose of 0.22 g/kg of body weight for 9 weeks. GER significantly reduced body weight gain by 22% compared to the high-fat diet control group with 48% less abdominal fat gain. Food intake was not affected. Blood glucose was lowered in the GER-treated group, whereas serum triglycerides and cholesterol were significantly reduced by 50%. This improved obesity phenotype may be associated with the attenuated expression of vascular endothelial growth factor in preadipocyte 3T3-L1 cells. Although other underlying, possibly multiple, mechanisms behind the improved phenotype are largely unknown, the observed improvement of multiple obesity-related parameters by the new tea warrants further investigations.

KEYWORDS: Chinese sweet leaf tea; ellagic acid; gallic acid; obesity; *Rubus suavissimus*; rubusoside

INTRODUCTION

The prevalence of obesity is growing worldwide, overtaking the incidences of underweight, malnutrition, and some infectious diseases. In the United States, it is estimated that this prevalence has reached almost 30% (*1*). Searching for ways to manage a healthy body weight has been an active endeavor for individuals and biomedical researchers. Because of the lack and limitation of weight loss medicines, traditional herbal teas and functional food ingredients have once again become important tools in improving obesity-related parameters. There are numerous reports on the improvement of obesity phenotype and metabolic symptoms by plant-derived active ingredients (*2*−*4*). Green tea, for example, is one of the most extensively studied plants for the prevention of metabolic syndrome by stimulating fat oxidation and increasing energy expenditure (*5*, *6*).

The most effective herbal agents are perhaps ephedrine and caffeine, alone or in combination. For several years, ephedrine and caffeine were used as dietary supplements for weight loss until toxicity issues prompted the U.S. Food and Drug Administration (FDA) to halt their use for this purpose (*7*, *8*). Currently, there are no effective and safe herbal products for weight management despite numerous scientific investigations. Our search for effective weight management ingredients has focused on folk medicines with empirical indications of enhancing obesity or obesity-related parameters. One such folk medicine that came to our attention is the Chinese sweet leaf tea plant (*Rubus suavissimus*), one of the many *Rubus* species in the Rosaceae family.

The Chinese sweet leaf tea has been consumed as a beverage leaf tea in southwestern China. Due to its sweet flavor, it is better known as "sweet tea" by the local residents. The major sweet principle is due to the presence of diterpene glycosides, dominated by rubusoside (*9*). Rubusoside has a slightly bitter aftertaste, but the sweetness is approximately 115 times higher than that of sucrose, thus making it a natural sweetener (*10*, *11*). In addition to the common tea use, Chinese sweet leaf tea has been widely applied in China as folk remedies to alleviate type 2 diabetes, relieve cough, and maintain healthy kidneys (*12*). Studies also demonstrated that the sweet leaf tea extract was inhibitory of the activity of NF-κB (*13*) and α-amylase (*14*), which is closely related to glucose metabolism. Recent scientific investigations even found that the Chinese sweet leaf tea extract was highly potent against angiogenesis, with gallic acid being partially responsible for this activity (*15*). Ellagic acid, another major component in the sweet leaf tea extract, also possesses an antiangiogenic property by inhibiting both vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptors in endothelial and smooth muscle cells (*16*).

It was discovered that adipose tissues secrete several angiogenesis factors such as VEGF, fibroblast growth factor, and hepatocyte growth factor (*17*, *18*). Several studies have even linked excessive angiogenesis to diseases including cancer, obesity, and

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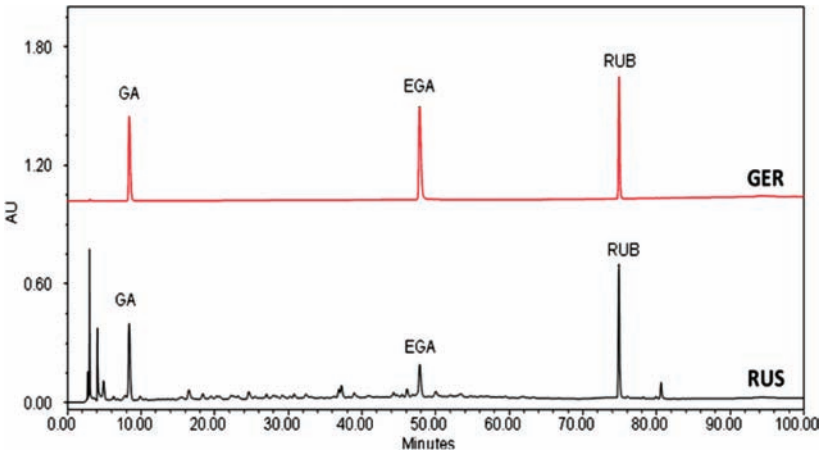

Figure 1. Chromatographic fingerprint of the purified Chinese sweet leaf tea extract (GER) composed of gallic acid (GA), ellagic acid (EGA), and rubusoside (RUB) in the similar ratio as the standardized extract (RUS). The chromatograms were generated using a Prevail C18 column (4.6 × 250 mm, 5 μm) and a gradient mobile phase of acetonitrile and 0.2% of phosphoric acid at a flow rate of 1.0 mL/min at 30 °C.

asthma (*18*, *19*). Even more supportive of the antiangiogenic approach to weight loss was the finding that, at cellular level, antiangiogenic therapy successfully inhibited the process of adipogenesis and the growth of pre-existing adipose tissues (*17*, *20*). Rupnick et al. (*20*) further showed that the use of an angiogenesis inhibitor caused weight loss, whereas treatment withdrawal restored body weight gain, suggesting obesity or fat accumulation is dependent on angiogenesis. Because the Chinese sweet leaf tea extract and its identified components were potent angiogenesis inhibitors (*13*, *15*, *16*, *21*), a hypothesis has emerged that it may work in the adipose tissues to slow or prevent the accretion of fat in obese subjects. Therefore, the objective of the present study was to determine if the three bioavailable components mimicking the Chinese sweet leaf tea extract could improve obesity phenotype and prevent fat accumulation at a nontoxic dose in obese animals.

MATERIALS AND METHODS

Materials. Gallic acid and ellagic acid were purchased from Sigma-Aldrich (St. Louis, MO). The reference standard of rubusoside was isolated in our own laboratory and identified by spectral data (UV, MS, ^{1}H NMR, ^{13}C NMR, and 2D-NMR). The procedures of isolation were reported previously (*22*). The rubusoside has purities of > 95% by HPLC-PDA analyses based on a peak area normalization method. The pre-adipocyte 3T3-L1 cells were purchased from ZenBio (Research Triangle Park, NC). The anti-VEGF antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-bFGF antibody was purchased from Upstate Biotechnology (Lake Placid, NY). β-Actin was purchased from Sigma-Aldrich (St. Louis, MO).

Extracts. The standardized sweet leaf tea extract (RUS) was produced in our laboratory, and the quality of the extract was determined by an HPLC fingerprint analysis with three major markers, gallic acid, ellagic acid, and rubusoside (*22*) (**Figure 1**). The purified Chinese sweet leaf tea extract (GER) was formulated on the basis of the abundant and bioavailable marker compounds in RUS and composed of only gallic acid (5%), ellagic acid (9%), and rubusoside (86%), accounting for 27% of RUS by weight as shown in **Figure 1**. Therefore, GER is a mixture of these commercially available products and represents a purified, reconstituted sweet leaf tea extract.

Animals. Male obese-prone SD rats (Crl:OP (CD); 4−6 weeks old) were purchased from Charles River Laboratories International Inc. (Wilmington, MA). Animals were housed individually in stainless steel cages in an air-conditioned room at 21 ± 2 °C and 50−60% relative humidity with a 12/12 h light/dark cycle. Prior to euthanization, urine was collected from each rat through an individual metabolic cage (Lab Products Inc., Seaford, DE), and blood or serum was collected via cardiac puncture. All procedures were performed under the protocols approved by the Institutional Animal Care and Use Committee of Louisiana State University (LSU-IACUC), Baton Rouge, LA.

Diets. The normal chow (Purina 5001 Lab Diet), with 13.5% calories from fat (ether extract), 58% calories from carbohydrate, and 28.5% calories from protein, was purchased from PMI Nutrition International (Brentwood, MO). The high-fat chow, with 60% calories from fat, 20% calories from carbohydrate, and 20% calories from protein, was obtained from Research Diets Inc. (New Brunswick, NJ). The major fat in the high-fat chow diet consisted of soybean oil (25 g/kg) and lard (245 g/kg).

Absorption of the Chinese Sweet Leaf Tea (RUS) Components in Orally Administered Normal SD Rats. Sixteen normal male SD rats were used in the experiment. Prior to treatment, all rats were fasted overnight. The treated group (n = 8) received extract at a dose of 1 g/kg of body weight by oral gavage, and the control group (n = 8) received the same volume of water via daily oral gavage for 3 consecutive days. All rats were given free access to food and water after the gavage. Urine was collected cumulatively for 24 h on the third day after treatment began. Later, the urine sample was extracted twice with ethyl acetate and subjected to HPLC fingerprint analysis as described in Chou et al. (*22*). The characteristic peaks (i.e., gallic acid, ellagic acid, and rubusoside) were identified on the basis of the retention times and UV spectra of the respective reference compounds.

Improvement of Obesity Phenotype by the Purified Chinese Sweet Leaf Tea Extract (GER) in Diet-Induced Obese SD Rats. *Experimental Design*. Forty-five obese-prone SD rats were divided into 3 groups of 15 rats per group. One group was fed normal chow and the other two groups, high-fat chow. Treatments were later assigned to each group. Group 1 received high-fat diet with purified Chinese sweet leaf tea (GER) at 0.22 g/kg of body weight. Group 2 was the high-fat control receiving a high-fat diet (HFD), and group 3 was the low-fat control receiving normal chow diet (LFD). The treated rats were first exposed to a small amount of relevant extract in liquid food at the beginning of the treatment to minimize extract aversion. GER was incorporated into the high-fat diet at 3% w/w. On the basis of the relative daily food intake as well as the acceptance of extract, the maximum achievable dose was 0.22 g/kg (equivalent to 0.8 g/kg RUS) for GER. All rats were provided ad libitum access to food and tap water throughout the 9 week treatment period. Body weight and food intake were measured weekly. Animals were also observed daily for any abnormal physical and behavioral changes or clinical signs of toxicity.

Blood Glucose Measurement. Fasting blood glucose was measured at week 0 (baseline), week 4, and week 8 after treatments began. This was done by drawing a small drop of blood from the tail vein, which was measured with a commercial glucometer (Abott Laboratories, North Chicago, IL) after a 6 h fast during the day.

Blood Sampling and Clinical Pathology. At the end of the treatment, all rats were anesthetized with isoflurane and euthanized. Blood samples were collected and examined for any possible toxicity or adverse effects resulting from the consumption of the extracts. For the hematology

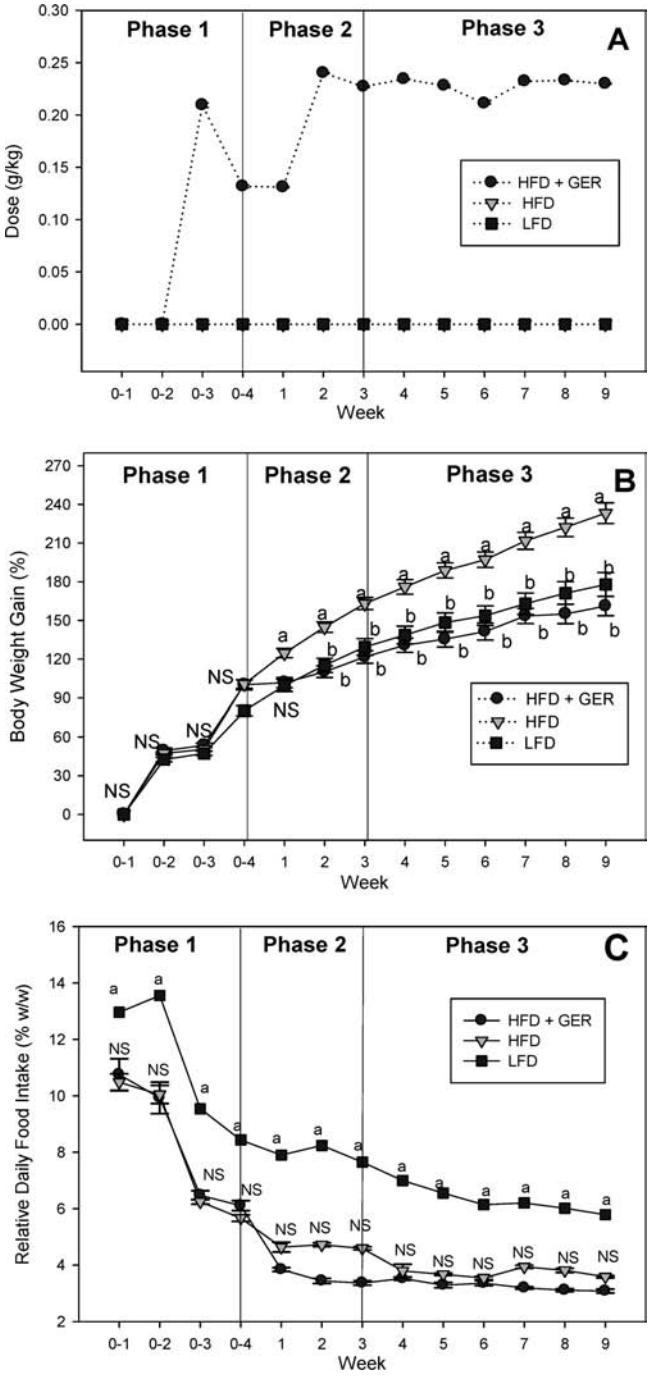

Figure 2. (**A**) Average dose (g/kg) of the purified Chinese sweet leaf tea extract (GER) received by the diet-induced obese SD rats. (**B**) Effects of GER on the body weight gain (%) of diet-induced male obese-prone SD rats compared to the control groups receiving high-fat (HFD) or low-fat (LFD) diet. (**C**) Effects of GER on the relative daily food intake (% w/w) of male obese-prone rats fed a high-fat diet for 9 weeks. All data are expressed as the mean ± standard error, where $n = 15$. Different letters at each week indicate a significant difference at $p \leq 0.05$. NS stands for not significant.

analysis, blood samples were collected in an EDTA vacutainer (Becton, Dickinson and Co., Franklin Lakes, NJ). The parameters tested included the erythrocyte, hemoglobin, hematocrit, red blood cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), and total WBCs. Blood chemistry samples were collected in a vacutainer and allowed to coagulate. Serum was separated via centrifugation at 2000*g* for 10 min. Serum chemistry test included glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (APH), creatine kinase (CK), total bilirubin, total protein, albumin, globulin, total cholesterol, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, sodium, potassium, chloride, bicarbonate, and anion gap. Both blood and serum samples were stored at −20 °C and immediately submitted to the Louisiana Animal Disease Diagnostic Laboratory (School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA) for analyses. Serum triglyceride level was analyzed via a serum triglyceride determination kit (Sigma-Aldrich, St. Louis, MO).

Cytotoxicity Determination. The preadipocyte 3T3-L1 cells were grown at a density of 1×10^4 cells/well in DMEM media. After a 24 h incubation period, cells were treated with various concentrations of GER (0.398−100 μg/mL). After incubation for an additional 72 h, inhibition of cellular proliferation was assessed by MTT assay (*23*). Absorbance was read at a wavelength of 570 nm and a reference wavelength of 650 nm using a V-Max microplate reader by Molecular Devices, Inc. (Sunnyvale, CA).

Western Blot Analysis. The preadipocyte 3T3-L1 cells were treated in serum-free conditions with 1−10 μg/mL of GER for 16 h. Cell lysates were collected and sonicated on ice for 3 min. Protein levels were quantified via the BioRad DC protein assay (Hercules, CA). Equal levels of protein (50 μg) were fractionated on precast gels (BioRad) and then transferred onto polyvinylidene difluoride membranes, according to the standard methods. Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween-20 for 1 h and then probed with primary antibodies diluted 1:2000 in blocking buffer overnight. Protein bands were visualized via chemiluminesence, using the ECL+ detection kit and hyperfilm (Amersham Biosciences, Piscataway, NJ). Equal loading of samples was illustrated by Western blotting for the presence of β-actin.

Statistical Analyses. All data were analyzed using the Statistical Analysis System (SAS, Cary, NC). Analysis of variance (ANOVA) with repeated measure was performed on the body weight changes, relative daily food intake, and blood glucose levels throughout the experimental period. Serum triglyceride levels, serum chemistry, and hematology data were analyzed using one-way ANOVA. Tukey's post hoc test was performed to compare group differences. The significance of all tests was set at $p \leq 0.05$. Outlier detection was performed to eliminate data with *R*-Student scores of ≥2.50. As a result, significant improvements were observed in the coefficient of variation, R^2, and test of normality. All results reported were expressed as mean ± SEM, unless otherwise stated.

RESULTS

Gastrointestinal Absorption of Chinese Sweet Leaf Tea Components. After a single oral administration of the Chinese sweet leaf tea extract at the dose of 1 g/kg of body weight, only gallic acid, ellagic acid, and rubusoside were detected in their original structures (unmetabolized) in the 24 h urine sample (data not shown). Gallic acid, ellagic acid, and rubusoside were regarded as being orally bioavailable. This finding plus known contents of each compound was the basis for reconstituting the three compounds into the GER formulation.

Body Weight Gain and Food Intake in Diet-Induced Obese SD Rats. Because of the feeding adaptation, the experimental results were presented in three phases based on the actual amount of extract consumed (**Figure 2A**). In phase 1, the rats consumed 0.2 g/kg of GER and the level of extract consumption affected neither weight gain nor food intake. After the successful initial adaptation of taste, the extract was incorporated into the high-fat chow diet, which was the only food source for these rats. In phase 2, a significant increase in the extract consumption was observed, which marked the beginning of the 9-week treatment period. At week 1 of the treatment period, the dose of GER reached 0.2 g/kg of body weight. Due to the increased extract consumption in phase 2, significant weight gain reduction in the HFD + GER group over the HFD control was observed, but the reduction was no different from the LFD group (low-fat control). The beginning of phase 3 was indicated at week 3 as constant extract consumption rate was observed for HFD + GER at 0.22 g/kg. The

Table 1. Effects of the Purified Chinese Sweet Leaf Tea (GER) on Body Weight and Tissue Weight[a]

	HFD	LFD	HFD + GER
body weight (g)			
initial	171.27 ± 5.28 a	172.00 ± 6.26 a	169.60 ± 5.43 a
final	683.47 ± 15.86 a	553.27 ± 12.42 b	530.20 ± 7.14 b
energy intake (kJ/day/g of body weight)	1.16 ± 0.03	1.15 ± 0.02	1.07 ± 0.04
tissue weight (% w/w)			
liver	2.95 ± 0.17 b	3.52 ± 0.23 a	2.87 ± 0.06 b
kidneys	0.54 ± 0.04 c	0.71 ± 0.03 a	0.62 ± 0.01 b
mesentery with fat	3.77 ± 0.71 a	1.95 ± 0.44 b	1.66 ± 0.07 b
total abdominal fat	10.07 ± 1.50 a	4.37 ± 0.88 b	5.28 ± 0.26 b
epididymal	3.90 ± 0.46 a	1.90 ± 0.40 b	2.18 ± 0.10 b
retroperitoneal	4.45 ± 0.93 a	1.66 ± 0.38 c	2.27 ± 0.13 b
perirenal	1.71 ± 0.30 a	0.81 ± 0.16 b	0.83 ± 0.04 b

[a] All values are expressed as mean ± standard error, where $n = 15$. Different letters in each row indicate a significant difference at $p \leq 0.05$. HFD, high-fat diet control group; LFD, low-fat diet control group; HFD + GER, high-fat diet group treated with purified Chinese sweet leaf tea.

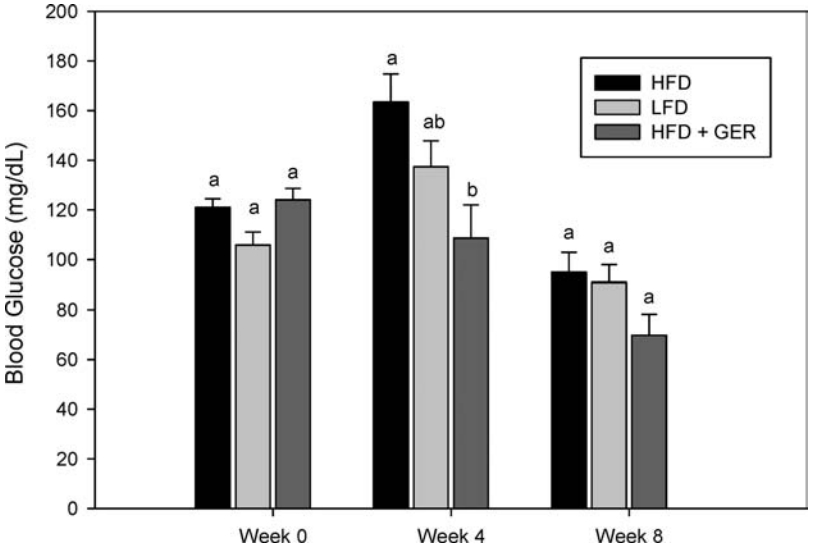

Figure 3. Effects of the purified Chinese sweet leaf tea extract (GER) on the blood glucose levels (mg/dL) of the diet-induced obese SD rats measured at week 0 (baseline), week 4, and week 8 of treatment compared to the control groups receiving high-fat (HFD) or low-fat (LFD) diet. All data are expressed as the mean ± standard error, where $n = 15$. Different letters on each week indicate a significant difference at $p \leq 0.05$.

reductive effect of HFD + GER on body weight continued after week 5, albeit at slower rates. At the end of the treatment period, body weight gain was reduced by 22% ($p \leq 0.001$) with the consumption of 0.22 g/kg of GER compared to the HFD control (**Figure 2B**). Body weight gain of the HFD + GER and LFD groups was reduced to the same 20% ($p = 0.2511$) compared to the HFD control. No difference in food intake (**Figure 2C**) or energy intake (**Table 1**) was observed between the two groups on high-fat diet. Overall, the group on the high-fat diet consumed about 4%, whereas that on normal diet consumed about 6%, of their body weight during the treatment period (**Figure 2C**). Although there was a significant difference in the amount of food intake between the food types (normal or high-fat diet), the total calorie intake was the same (**Table 1**).

Abdominal Fat Accumulation in Diet-Induced Obese SD Rats. Liver, kidneys, mesentery, epididymal, retroperitoneal, and perirenal adipose pads of rats were collected and weighed at euthanization. The weights (% w/w) of organs and tissues were calculated on the basis of the disemboweled body weight. Total abdominal fat (epididymal, retroperitoneal, and perirenal) was remarkably reduced in the LFD and HFD + GER groups by 57 and 48%, respectively, when compared to the HFD control (**Table 1**). The fat mass reduction was mainly due to the decrease of retroperitoneal and perirenal adipose pads. Mesentery fat in the LFD and HFD + GER groups was also significantly reduced by 48 and 56%, respectively, compared to the HFD control. Overall, the HFD + GER and LFD control groups produced the same effect on fat accumulation. The liver and kidney weights of the GER-treated groups were not significantly different from those of the HFD control. The livers and kidneys of the LFD group were slightly heavier than those of the groups on high-fat diet. This is possibly due to the relatively lower disemboweled body weight in that particular group as the absolute weight showed little or no difference.

Fasting Blood Glucose in Diet-Induced Obese SD Rats. Blood glucose was measured after a 6 h fasting period during the day. With a comparable baseline (week 0) to begin with, the blood glucose level was significantly lowered by 33% ($p = 0.001$) compared to the HFD control after 4 weeks of oral administration of GER (**Figure 3**). On week 8, HFD + GER tended to lower the blood glucose by approximately 26% over the HFD control, but it was statistically insignificant ($p = 0.6505$) due to interanimal variation.

Toxicology Related to Treatments. No clinical signs of toxicity or abnormal behavior were observed in the obese-prone rats after a 9 week administration of GER. There were no treatment-related adverse effects observed on the basis of the hematology (data not shown; see the Supporting Information) and blood

Table 2. Effects of the Purified Chinese Sweet Leaf Tea (GER) on Serum Chemistry Values

	groups[a]		
	HFD	LFD	HFD + GER
AST (IU/L)	142.78 ± 16.08[b]	194.89 ± 41.82[b]	206.44 ± 32.64
ALT (IU/L)	39.44 ± 2.14[b]	51.86 ± 3.84[c]	45.22 ± 4.98
AP (IU/L)	267.70 ± 15.35 a	227.90 ± 7.94 ab	205.40 ± 9.31 b
CK (IU/L)	3785.33 ± 1399	2847.50 ± 1295[b]	5792.44 ± 2149[b]
bilirubin (mg/dL)	0.19 ± 0.04	0.22 ± 0.04	0.36 ± 0.05
protein (g/dL)	5.91 ± 0.08	5.76 ± 0.12	5.98 ± 0.12
albumin (g/dL)	3.12 ± 0.07	3.12 ± 0.07	3.31 ± 0.07
globulin (g/dL)	2.79 ± 0.06	2.64 ± 0.06	2.67 ± 0.06
cholesterol (mg/dL)	103.50 ± 3.20 a[d]	61.80 ± 3.30 c	79.90 ± 4.72 b
BUN (mg/dL)	17.50 ± 0.48 ab	19.10 ± 0.43 a	16.40 ± 0.64 bc
creatinine (mg/dL)	0.30 ± 0.00 b	0.31 ± 0.01 b	0.33 ± 0.02 ab
calcium (mg/dL)	10.34 ± 0.15	10.26 ± 0.19	10.02 ± 0.24
phosphorus (mg/dL)	7.35 ± 0.22	8.00 ± 0.50	8.18 ± 0.60
sodium (mmol/L)	141.40 ± 0.48	141.30 ± 0.75	140.70 ± 0.96
potassium (mmol/L)	6.35 ± 0.24	6.51 ± 0.39	7.39 ± 0.63[b]
chloride (mmol/L)	100.30 ± 0.47	99.90 ± 0.64	101.00 ± 0.58
bicarbonate (mmol/L)	23.82 ± 0.45	24.28 ± 0.64	22.48 ± 0.86
anion gap (mmol/L)	23.63 ± 0.70	23.63 ± 0.91	25.13 ± 1.31
triglycerides (mg/dL)	168.00 ± 18.00 a	200.00 ± 30.00 a	89.00 ± 17.00 b

[a] All values are expressed as mean ± standard error, where $n = 10$, unless otherwise specified. Different letters in each row indicate a significant difference at $p \leq 0.05$. HFD, high-fat diet control group; LFD, low-fat diet control group; HFD + GER, high-fat diet group treated with purified Chinese sweet leaf tea. [b] $n = 9$. [c] $n = 7$. [d] $n = 8$.

chemistry (**Table 2**). Nevertheless, elevated values of CK were observed in all groups with excessively high variations. Although the reasons are unknown, we suspect systemic instrumental variation. In addition, the total cholesterol level was significantly lower in the LFD and HFD + GER groups than in the HFD control group, by approximately 40 and 20%, respectively. No other abnormal values were noted. Serum triglyceride levels are shown in **Table 2**. Compared to the HFD group, serum triglyceride levels, as a result of GER treatment, were reduced by almost 50% ($p = 0.002$). Surprisingly, the triglyceride level of the LFD control group, which was on a normal low-fat diet, was not significantly different from the HFD control group, even though weight gain was significantly blocked.

Cytotoxicity and Expression of Angiogenesis Growth Factors in Preadipocyte 3T3-L1 Cells. GER was minimally cytotoxic to 3T3-L1 cells. At the concentration of 10 μg/mL, GER inhibited the growth of 3T3-L1 cells by only approximately 20% (data not shown; see the Supporting Information). In contrast, at the same concentration of 10 μg/mL, GER significantly reduced the expression of VEGF, but not bFGF (**Figure 4**).

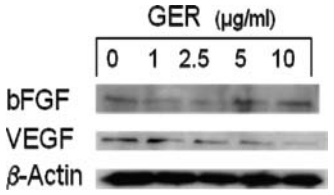

Figure 4. Effect of the purified Chinese sweet leaf tea extract (GER) on the expression of pro-angiogenic growth factors in preadipocyte 3T3-L1 cells. Cells (1×10^6) were plated overnight and treated with GER at the concentrations indicated above. After 24 h, cells were lysed in the lysis buffer as described under Materials and Methods. The expression of bFGF and VEGF was determined by immunoblotting. The data represent two repeated experiments.

DISCUSSION

Our study showed that the Chinese sweet leaf tea extract can slow body weight gain in subjects that are on a high-fat diet. The current paper demonstrates a significant 22% overall reduction in body weight gain and a 48% reduction in abdominal fat accumulation in obese animals that were orally administered 0.22 g of purified sweet leaf tea extract/kg of body weight.

Gallic acid, ellagic acid, and rubusoside are dominant compounds in the sweet leaf tea extract, accounting together for 27% by weight. This plus the data from the oral absorption study guided the successful composition of the purified extract referred as GER, with 86% of rubusoside, 9% of ellagic acid, and 5% of gallic acid. There were very limited reports on the bioactivity of rubusoside but plentiful information on its natural sweetening properties and toxicity (*10*, *11*).

Our preliminary study on normal rats demonstrated that GER had a robust representation of the standardized extract (RUS), producing 90% of the effect in body weight gain over the use of RUS without any changes in food intake (data not shown). Although either the whole extract (RUS) or only three compounds (GER) from the sweet leaf tea plant were effective in preventing body weight gain, GER has advantages over RUS as a candidate for improving obesity-related parameters. First, GER has a dose advantage. To prevent the same rate of weight gain requires 3.6-fold less of GER than RUS. This difference will be obvious when it is translated to human consumption. For example, at the beneficial dose of 0.22 g/kg in rats as found in this study, a 90 kg person would only need to drink the tea at 2.8 g GER (or take fewer than six 500 mg capsules) daily compared with 10 g RUS. Second, GER has a quality control advantage in manufacturing over the complex RUS extract. Because GER consists of only three compounds and RUS has tens of hundreds of compounds, it is obvious that GER can be formulated with minimal batch-to-batch variations, which translates to reproducible health effects in improving obesity-related parameters. In comparison, although the major components of the RUS are known, controlling batch-to-batch variations of a complex botanical extract in scale-up manufacturing remains a challenging task.

As abdominal fat accounts for the majority of the weight gain in men, it is reasonable to state that the pronounced weight gain prevention was in fact a prevention of fat accumulation rather than a result of reduced food intake or toxicity in GER. In addition, the finding that low-fat dieting was as effective as GER at preventing weight gain confirms the importance of a healthier diet alone. The fact that the rats on a high-fat diet (i.e., 60% of the total calories from fat) but supplemented with GER gained weight at the same rate as those on normal diet (14% of the total calories from fat) signified the extremely effective role of GER in preventing body weight gain and fat accumulation. Furthermore, the significant lowering of total cholesterol by GER indicates its ability to improve lipid profiles when a fatty diet is consumed. In addition to the reduced fat accumulation, the observed blood glucose level change (lowering) could be an indication of the involvement of carbohydrate metabolism. This represents a new piece of scientific evidence to support the use of Chinese sweet leaf tea as a potential therapy to treat diabetes (*12*). Because obesity and insulin resistance are interrelated, whether the anti-obesity effect of GER serves as a cause of the improvement of blood glucose levels remains unclear. Nevertheless, the reduced serum triglyceride level in the treated group but not the low-fat (LFD) or high-fat (HFD) diet control group could indicate increased insulin sensitivity in the obese model treated with GER (*24*).

The observed improvement of numerous obesity-related parameters by the purified tea compounds is strong, which raised the curious question on the underlying mechanisms of action. It is suspected that multiple mechanisms might have played roles and contributed to the overall phenotype. Exploring those mechanisms of action is beyond the scope of this study. However, because the tea has been reported to inhibit angiogenesis of human vascular endothelium (*15*), that revelation was followed by exploring possible underlying mechanisms behind the inhibition in preadipocytes. Angiogenesis is essential for the development of both healthy and pathological tissues. Recent studies have demonstrated that the growth and expansion of adipose tissues is associated with active angiogenesis (*17*−*20*). There was solid evidence that inhibition of angiogenesis prevented adipogenesis in obese animal models (*20*) and VEGF was one of the key targets (*25*, *26*). The present study provided additional evidence that attenuating VEGF in preadipocytes by the purified Chinese sweet leaf tea (GER) was a possible mechanism of preventing weight gain in the obese animals, rather than inhibiting the growth of preadipocytes. Other mechanisms might be involved as well. Gallic acid, for instance, has been shown to decrease body weight gain in high-fat diet-induced obese rats via the suppression of oxidative stress, hepatosteatosis, and dyslipidemia (*27*). In addition, gallic acid and ellagic acid have been reported to reduce α-amylase and α-glucosidase activity (*14*, *28*). Hence, the compounds in GER could have worked alone or in concert to alter the physiology of the gastrointestinal tract by reducing energy absorption or increasing energy expenditure.

The purified Chinese sweet leaf tea extract was shown to improve significantly the parameters related to obesity including the prevention of body weight gain and fat accretion and improvement of lipid profiles of the high-fat diet-induced obese rats without changing food intake. Our results also demonstrated that the inhibition of VEGF in the preadipocyte cells might be a possible mechanism and feasible approach toward improving obesity phenotype. More extensive mechanistic studies are clearly justified by the findings of this study.

ABBREVIATIONS USED

GER, gallic acid, ellagic acid, rubusoside; RUS, *Rubus suavissimus*; SD, Sprague−Dawley; LFD, low-fat diet; HFD, high-fat diet; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.

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Supporting Information Available: Table 1 and Figures 1−3. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Flegal, K. M.; Carroll, M. D.; Ogden, C. L.; Curtin, L. R. Prevalence and trends in obesity among US adults, 1999−2008. *JAMA, J. Am. Med. Assoc.* **2010**, *303*, 235−241.

(2) Choi, H.; Eo, H.; Park, K.; Jin, M.; Park, E.; Kim, S. H.; Park, J. E.; Kim, S. A water-soluble extract from *Cucurbita moschata* shows anti-obesity effects by controlling lipid metabolism in a high fat diet-induced obesity mouse model. *Biochem. Biophys. Res. Commun.* **2007**, *359*, 419−425.

(3) Lei, F.; Zhang, X. N.; Wang, W.; Xing, D. M.; Xie, W. D.; Su, H.; Du, I. J. Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. *Int. J. Obesity* **2007**, *31*, 1023−1029.

(4) Kamisoyama, H.; Honda, K.; Tominaga, Y.; Yokota, S.; Hasegawa, S. Investigation of the anti-obesity action of licorice flavonoid oil in diet-induced obese rats. *Biosci., Biotechnol.,Biochem.* **2008**, *72*, 3225−3231.

(5) Dulloo, A. G.; Duret, C.; Rohrer, D.; Girardier, L.; Mensi, N.; Fathi, M.; Chantre, P.; Vandermander, J. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am. J. Clin. Nutr.* **1999**, *70*, 1040−1045.

(6) Wolfram, S.; Wang, Y.; Thielecke, F. Anti-obesity effects of green tea: from bedside to bench. *Mol.Nutr.Food Res.* **2006**, *50*, 176−187.

(7) Haller, C. A.; Jacob, P.; Benowitz, N. L. Pharmacology of ephedra alkaloids and caffeine after single-dose dietary supplement use. *Clin. Pharmacol. Ther.* **2002**, *71*, 421−32.

(8) Dunnick, J. K.; Kissling, G.; Gerken, D. K.; Vallant, M. A.; Nyska, A. Cardiotoxicity of ma huang/caffeine or ephedrine/caffeine in a rodent model system. *Toxicol. Pathol.* **2007**, *35*, 657−664.

(9) Tanaka, T.; Kohda, H.; Tanaka, O.; Chen, F. H.; Chou, W. H.; Leu, J. L. Rubusoside (β-D-glucosyl ester of 13-*O*-β-D-glucosyl-steviol), a sweet principle of *Rubus chingii* Hu (Rosaceae). *Agric. Biol. Chem.* **1981**, *45*, 2165−2166.

(10) Ohtani, K.; Aikawa, Y.; Kasai, R.; Chou, W. H.; Yamasaki, K.; Tanaka, O. Minor diterpene glycosides from sweet leaves of *Rubus suavissimus*. *Phytochemistry* **1992**, *31*, 1553−1559.

(11) Sugimoto, N.; Sato, K.; Liu, H.; Kikuchi, H.; Yamazaki, T.; Maitani, T. Analysis of rubusoside and related compounds in tenryocha extract sweetener. *J. Food Hyg. Soc. Jpn.* **2002**, *43*, 250−253.

(12) Huang, P.; Jiang, S. Complex utilization of *Rubus suavissimus* S. Lee. *Guangxi Chem. Ind.* **2002**, *31*, 24−25.

(13) Liu, D.; Gao, Z.; Zhang, J.; Ye, J.; Liu, Z. Bioassay-guided fractionation of *Rubus suavissimus* leaf extracts possessing NF-κB inhibitory activities and a separable cytotoxicity. *Pharm. Biol.* **2005**, *43*, 713−717.

(14) Li, H.; Tanaka, T.; Zhang, Y. J.; Yang, C. R.; Kouno, I. Rubusiviis A−F, monomeric and oligomeric ellagitannins from Chinese sweet tea and their α-amylase inhibitory activity. *Chem. Pharm. Bull.* **2007**, *55*, 1325−1331.

(15) Liu, Z.; Schwimer, J.; Liu, D.; Lewis, J.; Greenway, F. L.; York, D. A.; Woltering, E. A. Gallic acid is partially responsible for the antiangiogenic activities of *Rubus* leaf extract. *Phytother. Res.* **2006**, *20*, 806−813.

(16) Labrecque, L.; Lamy, S.; Chapus, A.; Mihoubi, S.; Durocher, Y.; Cass, B.; Bojanowski, W.; Gingras, D.; Beliveau, R. Combined inhibition of PDGF and VEGF receptors by ellagic acid, a dietary-derived phenolic compound. *Carcinogenesis* **2005**, *26*, 821−826.

(17) Brakenhielm, E.; Cao, Y. Angiogenesis in adipose tissue. *Methods Mol. Biol.* **2008**, *456*, 65−81.

(18) Christiaens, V.; Lijnen, H. R. Angiogenesis and development of adipose tissue. *Mol. Cell. Endocrinol.* **2010**, *318*, 2−9.

(19) Fan, T. P.; Yeh, J. C.; Leung, K. W.; Yue, P. Y. K.; Wong, R. N. S. Angiogenesis: from plants to blood vessels. *Trends Pharmacol. Sci.* **2006**, *27*, 297−309.

(20) Rupnick, M. A.; Panigraphy, D.; Zhang, C.; Dallabrida, S.; Lowell, B. B.; Langer, R.; Folkman, M. J. Adipose tissue mass can be regulated through vasculature. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10730−10735.

(21) Oner, F. H.; Bezerra, Y.; Peyman, G. A.; Conway, M. D.; Lewis, J. M.; Liu, Z.; Greenway, F.; Woltering, E. A. Antiangiogenic effect of a Chinese sweet leaf tea extract in experimental corneal neovascularization. *Pharm. Biol.* **2007**, *45*, 45−47.

(22) Chou, G.; Xu, S. J.; Liu, D.; Koh, G. Y.; Zhang, J.; Liu, Z. Quantitative and fingerprint analyses of Chinese sweet tea plant (*Rubus suavissimus* S. Lee). *J. Agric. Food Chem.* **2009**, *57* (3), 1076−1083.

(23) Mosmann, T. Rapid coloricmetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. *J. Immunol. Methods* **1983**, *65*, 55−56.
(24) Nagle, C. A.; Klett, E. L.; Coleman, R. A. Hepatic triacylglycerol accumulation and insulin resistance. *J. Lipid Res.* **2009**, *50*, S74−S79.
(25) Nishimura, S.; Manabe, I.; Nagasaki, M.; Hosoya, Y.; Yamashita, H.; Fujita, H.; Ohsugi, M.; Tobe, K.; Kadowaki, T.; Nagai, R.; Sugiura, S. Adipogenesis in obesity requires close interplay between differentiating adipocytes, stromal cells, and blood vessels. *Diabetes* **2007**, *56*, 1517−1526.
(26) Ledoux, S.; Queguiner, I.; Msika, S.; Calderari, S.; Rufat, P.; Gasc, J.; Corvol, P.; Larger, E. Angiogenesis associated with visceral and subcutaneous adipose tissue in severe human obesity. *Diabetes* **2008**, *57*, 3247−3257.
(27) Hsu, C.; Yen, G. Effect of gallic acid on high fat diet-induced dyslipidaemia, hepatosteatosis and oxidative stress in rats. *Br. J. Nutr.* **2007**, *98*, 727−735.
(28) Li, J.; Lu, Y.; Su, X.; Li, F.; She, Z.; He, X.; Lin, Y. A norsesquiterpene lactone and a benzoic acid derivative from the leaves of *Cyclocarya paliurus* and their glucosidase and glycogen phosphorylase inhibiting activities. *Planta Med.* **2008**, *74*, 287−289.

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