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Sweet tea leaves extract improves leptin resistance in diet-induced obese rats

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ABSTRACT

Aim of the study: Dietary obesity is usually characterized by leptin resistance and abnormal lipid metabolism. *Lithocarpus polystachyus* Rehd.(Sweet Tea) leaf is a kind of Chinese folkloric medicine, and it has been widely used for obesity, diabetes, and hypertension in South China. The present study is aimed at investigating the pharmacological mechanism of the anti-hyperleptinaemia effects of Sweet Tea leaves extract in high fat diet-induced obese rats.

Materials and methods: We induced high fat diet obesity for 14weeks to test the corrective effects of three ST doses (75, 150 and 300 mg/kg per day) for 8 weeks. At the end of the experiment, body weight, fasting blood glucose and serum lipids, superoxide dismutase (SOD), malondialdehyde (MDA), fasting serum insulin and leptin, C-reactive protein, adiponectin and resistin levels were measured, Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was also calculated. mRNA gene expression of PPARγ (peroxisome proliferator-activated receptor γ) and C/EBPα(CCAAT/enhancer-binding protein α) in epididymal adipose tissue of DIO control and experimental groups were evaluated.

Results: Sweet Tea leaves extract could significantly decrease the levels of serum lipids, attenuate body weight gain and lower circulatingleptin and insulin levels, ameliorate the state of oxidative stress, raise serum adiponectin, reduce circulating CRP and resistin levels, and depress the expression of PPARγ and C/EBPα in epididymal adipose tissue of obese rats.

Conclusion: The present findings suggest that ST can effectively attenuate the leptin resistance at least through anti-hyperlipidemic activity and thus has the therapeutic potential in treating hyperlipidemia and hyperleptinaemia related to dietary obesity.

1. Introduction

Prevalence of obesity is increasing worldwide annually, which seriously threatens people's health. Since obesity is closely associated with coronary heart disease, cerebrovascular disorder, hypertension, hyperlipidemia, type 2 diabetes and certain cancers (Barsh et al., 2000; Kopelman, 2000; Luchsinger, 2006; Lavie et al., 2009), it has become a major global public health issue. However, currently available drugs for obesity have a number of limitations, such as severely adverse effects and high rates of secondary complications.

Leptin, which is almost completely secreted by adipose tissue, acts upon the brain to regulate energy balance (Bjørbaek and Kahn, 2004). Circulating leptin levels can thus reflect the state of body energy storage. Leptin modulates energy balance all over the body by controlling processes related to energy intake and

Abbreviations: ST, Sweet Tea (*Lithocarpus polystachyus* Rehd.); HFD, high fat diet; DIO, diet-induced obesity; ST(L), ST(M) and ST(H), low, medium and high dose of ST leaves extract; HPLC, high performance liquid chromatography; FBG, fasting blood glucose; SOD, superoxide dismutase; MDA, malondialdehyde; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FFA, free fatty acids; FINS, fasting insulin; CRP, C-reactive protein; APN, adiponectin; PCR, polymerase chain reaction; PPARγ, peroxisome proliferators-activated receptor gamma; C/EBPα, CCAAT/ enhancer-binding protein alpha; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; S.E.M., standard error of mean; WAT, white adipose tissue

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expenditure (Myers, 2004). Previous studies also found central administration of recombinant leptin to normal rodents reduced food intake and raised energy consumption, resulting in loss of fat mass, so leptin originally was regarded as a potential remedy for obesity (Campfield et al., 1995; Halaas et al., 1997; Friedman and Halaas, 1998; Inui, 1999). Nevertheless, later evidences indicated that most obese patients and animals have elevated circulating leptin levels but this sufficient leptin fails to suppress feeding and decrease body weight, showing that most obesity exists a phenomenon of leptin resistance, and peripherally administration of leptin cannot develop its proper physiological effects (Fujioka et al., 1999; Myers et al., 2008; Morris and Rui, 2009). So that more and more scientists are seeking to exploit anti-obesity medicines that can ameliorate leptin resistance.

Lithocarpus polystachyus Rehd. (Fagaceae family), which is popularly named "Sweet Tea" (ST) in folk China, distributes mainly as a wild plant in the mountainous areas in South China with abundant natural resources. Its leaves have been used as a traditional herbal medicine and healthcare plant drink. Previous experiments revealed that it contains plentiful bioactive flavonoids and polyphenolic compounds, e.g. phlorizin, trilobatin, 3-hydroxy-phlorizin, etc (Zhou et al.,1992). These components have extensive pharmacological activities, such as anti-diabetes (Gaisano et al., 2002; McCrimmon et al., 2002; Zhao et al., 2004; Zhang et al., 2011), memory improvement (Hall et al., 1992; Boccia et al., 1999), anti-aging (Xiang et al., 2011), inhibition of lipid peroxidation (Ridgway et al., 1997) and the growth of human colon cancer cells (Ugocsai et al., 2005; Veeriah et al., 2006), and so on.

Even so, there were no pharmacological reports on anti-obesity effects of ST extract to date. Some promising preliminary results obtained in our laboratory revealed that ST has hypolipdemic effects in diabetic rats (Hou et al., 2011). Therefore, we hypothesized that the long-term administration of ST water extracts would have beneficial effects on leptin resistance by decreasing lipid accumulation in male rats with diet-induced obesity. The current studies were undertaken to evaluate its hypoleptinaemia effects in diet-induced obese rats and to elucidate its potential pharmacological mechanisms.

2. Materials and methods

2.1. Reagents

The kits of triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C), were obtained from Biosino Bio-Technology & Science Incorporation (Beijing, China). The kits of superoxide dismutase (SOD), malondialdehyde (MDA), were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Radioimmunoassay kit of insulin were purchased from Beijing Chemclin Biotech Co., Ltd. (Beijing, China). Rat ELISA kits of leptin and C-Reactive Protein (CRP) were obtained from Millipore Corporation (St. Charles, Missouri, USA), rat adiponectin ELISA kit from Invitrogen Corporation (Grand Island, NY, USA), rat resistin ELISA kit from BioVendor Laboratory Medicine, Inc. (Evropska, Czech); FFA hypersensitivity assay kit from Applygen Technologies Inc. (Beijing, China), portable blood glucose meter and paper from Roche Pharmaceutical Ltd. (Basel, Switzerland) were used in the study.

2.2. Animals and diet

Male Sprague-Dawley (SD) rats, weighing between 80 and 120 g, were obtained from the Laboratory Animal Services Center, Guangzhou University of Chinese Medicine (Guang zhou, China). The rats were housed in stainless steel cages with uniform temperature between 20 and 25 °C, humidity of 55 ± 5%, 12 h/12 h light/dark cycle and maintained with free access to food and water. Normal rats were fed with uniform laboratory chow. Obesity was induced by feeding with high fat diet (46% calories from lard) for14 weeks. All animals were weighed weekly throughout the experimental period. Body weights in high fat diet-fed rats were 20% higher than those of normal control littermates were considered adiposity. All procedures and studies were conducted in accordance with the Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation and approved by the Animal Care and Use Committee of Guangzhou University of Chinese Medicine. Twelve hours before blood collection, food was withdrawn but water remained available ad libitum.

2.3. Plant

The fresh leaves of *Lithocarpus polystachyus* Rehd. were collected from Jiangxi Province in summer, and identified by Prof. Lai Xiao-Ping in the School of Chinese Materia Medica, Guangzhou University of Chinese Medicine. The leaves were dried in the shade. A voucher specimen is deposited in the herbarium of Guangzhou University of Chinese Medicine.

2.4. Preparation of ST leaves extract

The dry leaves (1 kg) were pulverized and extracted twice by Soxhlet's extractor with 5 L water for 2 h each time. The aqueous extract solution was filtered and concentrated by using a rotary evaporator. Then the extract was dried to powder in a vacuum dryer at 105 °C to get the flavonoid-rich extract. And the flavonoid-rich fraction of Sweet Tea leaves was prepared by low temperature equipment with different extracting pressure (ZLUPD-S-350A, Guangzhou Zeli Pharmtech Co., Ltd., Guangzhou, China). Then the aqueous extract solution was processed by ultrafilter membrane and purified by HPD-826 macroporous resin column chromatography. Finally, the extract was dried to a powder on an atomizing drier to yield a yellow powder extract. The concentration of ST and total flavonoids were determined and calculated by spectrophotometer (UV-2550, SHIMADZU) at 284 nm, using phlorizin as a standard (ST, yield 12.0%; total flavonoids content is about 95%). The extracts were freshly dissolved with distilled water just before administration to animals during experiment.

2.5. HPLC analysis

The HPLC analysis was performed on a Summit HPLC system (Dionex, Germany), equipped with a Dikma Platisil-TM-ODS column (250 mm × 4.6 mm I.D., 5 μm particle size). The mobile phase consisted of a 40:60 (v/v) mixture of acetonitrile (A)/water (B) at a flow rate of 0.8 mL/min. A gradient program was used as follows: 0 min, 78% B; 10 min, 78% B; 30 min, 60% B; 40 min, 50% B; 50 min, 78% B. The column effluent was scanned from 200 nm to 600 nm with a UV diode-array detector, the monitor wave length was set at 285 nm as described by Dong et al. (2007), using phlorizin as a standard. The column was maintained at room temperature. Fig. 1 shows the HPLC fingerprint of ST extract. The two main peaks stand for phlorizin and 3-hydroxy-phlorizin, which structures were identified by ultraviolet, infrared, mass spectra, nuclear magnetic resonance (data was not shown), as described by Yang et al. (1991).