Improvement of Lipid Profile and Reduction of Body Weight by Shan He Jian Fei Granules in High Fat Diet-Induced Obese Rats

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* Supported by the National Natural Science Foundation of China (No.30271635), Natural Science Foundation of Guangdong Province (NO.7005956)
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SUMMARY

Background: The goal was to study lipid profiles (TG, TC, LDL, HDL), effects on serum leptin, and fat tissue adiponectin, and resistin as well as body weight effects of Shan He Jian Fei Granules (SHJFG) in rats on a high fat diet.

Methods: Rats were randomly divided into five groups: normal control group fed with normal fat diet, rats on high fat diet receiving low dosage, middle dosage, high dosage of Shan He Jian Fei Granules (SHJFG) as well as a high fat diet group receiving placebo. Rats were treated for 8 weeks. Body weight and naso-anal length of each rat were recorded and Lee’s index was calculated. Serum TG, TC, LDL, HDL and leptin concentrations were analyzed. The gene expressions of adiponectin and resistin in adipose tissues were tested by RT-PCR.

Results: Compared to the high-fat diet group, body weights, Lee’s indexes, weight of fat tissues and serum TG, TC, LDL and leptin of SHJFG groups significantly decreased (p<0.05), whereas mRNA expressions of adiponectin and resistin of SHJFG groups significantly increased (p<0.05).

Conclusions: SHJFG could significantly lower body weight and serum TG, TC, and LDL of obese rats. The effects of SHJFG in lowering leptin synthesis and raising mRNA expression of adiponectin and resistin in fat tissues may act as part of the mechanisms in lowering body weight of obese rats. Further studies are needed to demonstrate whether SHJFG may also reduce overall cardiovascular morbidity and mortality like other lipid lowering drugs.

KEY WORDS

obesity; high-fat diet; Shan He Jian Fei Granules (SHJFG); lipid; adiponectin; resistin; leptin

INTRODUCTION

Obesity is a medical condition in which excess body fat has accumulated. This condition is associated with an adverse effect on health, leading to reduced life expectancy and/or increased morbidity as well as associated costs for the health care systems [1,2]. Body mass index (BMI), a measurement which compares weight and height, defines people as overweight (pre-obese) when their BMI is between 25 kg/m² and 30 kg/m², and obese when it is greater than 30kg/m² [3]. Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, breathing difficulties during sleep,
certain types of cancer, and osteoarthritis [2]. Obesity is most commonly caused by a combination of excessive dietary calories, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness. Evidence to support the view that some obese people eat little yet gain weight due to a slow metabolism is limited; on average obese people have a greater energy expenditure than their thin counterparts due to the energy required to maintain an increased body mass [3,4,5].

The primary treatment for obesity is dieting and physical exercise. However, this strategy often fails due to non-compliance of the patients. Thus, there is a huge unmet medical need for drugs with a placebo-like safety profile and clear efficacy.

High fat diet induced obesity is frequently associated with an adverse lipid profile, in particular high high LDL. High LDL is a major risk factor for cardiovascular morbidity and mortality in the general population. Hence, we were likewise interested in potential effects of Shan He Jian Fei Granules on the lipid profile in rats on a high fat diet.

In the current study, we tested Shan He Jian Fei Granules (SHJFG) for the treatment of obesity associated with lipid disorders in an animal model of obesity. SHJFG is a mixture of haw, lotus leaf, cassia seed, alisma, and microcos paniculata. It exhibited good effects in weight loss of obese children in clinical use [6]. However, systematic preclinical or even clinical studies have not been performed so far. The current study thus aimed to test the hypothesis that SHJFG may reduce body fat content and to try to identify the potential pathways involved in weight-loss as well as analyses of the effects of this drug on the lipid profile.

MATERIALS AND METHODS

Materials

70 weaning Sprague-Dawley (SD) male rats, body weights ranging from 48 to 55 grams, were provided by the Laboratory Animal Center of Sun Yat-Sen University (certificate of conformity: No.00109004). They were kept in cages in a room at 19 - 24°C, 12h/12h of rhythmic light and dark and 45% humidity. Tap water and standard conventional feedstuff were freely available.

The drug was provided by China Resources Sanjiu Medical & Pharmaceutical CO., LTD. It consists of 20% haw, 20% lotus leaf, 20% cassia seed, 20% alisma, and 20% microcos paniculata linn. The granules were made as follows: The herbs were boiled separately for 30 min, then the suspension was dried carefully and small granules were produced. These granules were then mixed as described above and resuspended in hot water to obtain the drug consisting of the 5 different herbs.

All the herbs in the granules of SHJFG were approved drugs by the State Food and Drug Administration of China. The granules containing 20g of each herb were dissolved in water to form the liquid of 1.5 kg/L density. General feedstuff mainly contained corn powder, broomcorn powder, wheat powder, wheat bran, bean cake powder, bean powder, fish meal, bone meal and a little cod-liver oil, leaven, mineral composition, and other ingredients. High fat feedstuff consisted 76.5% general feedstuff, 3% cholesterol, 0.5% sodium cholate, 10% casein, and 10% lard oil.

Reverse transcriptase and RNase inhibitor were products of Invitrogen, Carlsbad, CA, USA. Random primers, buffer, dNTP and Taq enzyme were products of TaKaRa. Leptin ELISA kit was produced by Linco and provided by Shenzhen Jingmei BioTech Co., Ltd. Gene primer of adiponectin and resistin was synthesized by Dalian Biotechno Co., Ltd. GAPDH primer was synthesized by Shanghai Invitrogen Biotech Co., Ltd. Serum lipid test kit was provided by Zhejiang Dong’ou Bio-Engineering Co., Ltd. DY2W2 medium voltage electrophoresis was purchased from Beijing Liu Yi Instrument Factory. Other instruments used included an Olympus fully automated analyzer, a Biometra UNO PCR thermal cycler and Kodak electrophoresis data processing and analysis systems, and a gel analysis system.

Grouping and Treatment

The SD rats were randomly divided into a normal group (10 rats, group A) and a modeling group (60 rats) based on their basic weight after 3 days’ familiarity with the new environment. The modeling groups were fed with high fat feedstuff. After 8 weeks, the rats in the modeling group, whose body weights were 20% higher than the average body weight of the normal group, were defined as obese rat. Thus, 43 rats were obese by this standard. Then the 43 rats were randomly divided into an obesity control group (group B) and SHJFG low, middle, and high dosage groups (group C, D, E).

SHJFG groups were fed with high-fat-feedstuff and given SHJFG at a dose of 5 g/kg, 10 g/kg, and 20 g/kg to the corresponding group by gavage. Group A and group B were fed as before. Food and water intake, mental status, and feces of the rats were observed during the experiment. The experiment continued for another 8 weeks.

Sample Collection and Testing

The body weight (BW) of each rat was recorded before it was sacrificed. Anesthetizations of the rats were done by intraperitoneal injection of 1% pentobarbital sodium. The body length (BL), also called naso-anal length, was measured and Lee’s index

\[ \text{Lee’s index} = \frac{(BW^{10} \times 1000)}{BL (cm)} \]

was calculated.

The fat tissue around the epididymis was immediately taken out and weighed separately. Extracting 4 to 6 mL blood directly from the heart and heparin was added to anticoagulate to separate the serum. The serum and fat tissue were stored at -80°C for further use. A fully-automatic biochemical analyzer was used to test the amount
of serum TC, TG, LDL, and HDL of the rats. ELISA was used to test the amount of leptin.

Detecting mRNA Expressions of Adiponectin and Resistin in Fat Tissues
An RT-PCR method was adopted to test the mRNA expression of adiponectin in fat tissues. Referring to the steps of the Trizol reagent method, total RNA were extracted from 100 mg fat tissue. Then the rate of total RNA purity was detected by A260/A280 ratio. The first cDNA chains were synthesized by a 25 μL reaction system containing 2μL mRNA. The 25 μL PCR reaction system was prepared in an ice box with 1 μL RT reaction product as template. All forward and reverse PCR primers used were as follows; 5'-GGTGGCAAGGCCTCTGCTGTTCCCTCTCT-3' and 5'-GGTGGCCCTTCCGCTCTCTGTC TC-3' for adiponectin, and the amplified product was 266 bp. 5'-GGACAGGAGCTCATGCCCAGAAC CGTGTTTCCTCTC-3' and 5'-GGACAGGAGCTCATGCCCAGAAC C-3' for resistin, and the amplified product was 312 bp. 5'-GGTGTACAGGAGCTCATGCCCAGAAC CGTGTTTCCTCTC-3' and 5'-GGTGTACAGGAGCTCATGCCCAGAAC C-3' for GAPDH, and the amplified product was 504 bp. The PCR reaction was started at 94°C for 1 minute, followed by denaturation for 30 seconds at 94°C, 60°C for 40 seconds, and 72°C for 1 minute. All together there were 35 cycles. The last 7 minutes of the reaction were at 72°C. 10 μL PCR product was used for electrophoresis after the PCR reaction. An image scanner was then used for the grayscale scanning, and gel electrophoresis analysis software was used for the image analysis. Relative indicators of mRNA expression were reflected by the ratio of gray scale degree of adiponectin and resistin mRNA electrophoretic band compared with that of GAPDH’s.

Statistical Analysis
The statistical tests were performed with SPSS software version 12.0. All data was expressed as mean ± standard deviation (X ±s). Differences between two groups were analyzed with the Student’s t-test. Differences among three or more than three groups were analyzed with analysis of variance.

RESULTS

General Conditions after Experiment
Rats of group A had normal shape, reacted quickly, cibation and excretion were normal. Rats of group B almost had a normal appetite, but less activity and loose or dry stools. Rats of group C, D and E had normal appetite and excretion, mental states were normal, too. Some rats sacrificed because of inappropriate intragastric administration were confirmed by anatomy.

Effects of SHJFG on Body Weight, Lee’s index, and Fat Mass of Rats
Compared with group A, body weight, Lee’s index and fat mass of group B were obviously higher (p<0.05). There was no significant difference among all groups in body height of the rats (p>0.05). Compared with group B, body weight, Lee’s index, and fat mass of SHJFG groups (group C, D, E) decreased (p<0.05). Body weight and Lee’s index were not significantly different among the three SHJFG groups (p>0.05) (Table 1).

Effects of SHJFG on Serum Lipid Level of Obese Rats
Serum TC, TG, and LDL of group B were obviously higher, there were significant differences when compared with group A (p<0.05), and serum HDL of group B was significantly lower than group A (p<0.05). Compared with group B, serum TC, TG, and LDL of SHJFG groups decreased (p<0.05). But there were no significant differences among the three SHJFG groups (p>0.05). Serum HDL of SHJFG groups increased, but not statistically significant, when compared with group B. And there were no differences among the three SHJFG groups on serum HDL. Table 2 shows results with SHJFG at low, middle and high dosages.

Effects of SHJFG on Serum Leptin Level of Obese Rats
Serum leptin of group B was significantly higher when compared with group A (p<0.05). Compared with group B, serum leptin levels of group C, D, and E were significantly lower (p<0.05). But there were no statistical differences among the three SHJFG groups (p>0.05) (Table 3).

Effects of SHJFG on mRNA Expression of Adipose Tissue Adiponectin of Obese Rats
Compared with group A, mRNA expression of adipose tissue adiponectin of group B was obviously lower (p<0.05), while mRNA expression of adipose tissue adiponectin of SHJFG groups were significantly higher than group B (p<0.05). But there were no statistical differences among the three SHJFG groups (p>0.05) (Table 4 and Figure 1).

Effects of SHJFG on mRNA Expression of Resistin in Adipose Tissue of Rats
Compared with group B, mRNA expression of resistin expression of adipose tissues of SHJFG groups was significantly lower (p<0.05). There was a dose-dependent trend decreasing more obviously when dosage was higher. However, there were no significant differences among the three SHJFG groups. mRNA expression of group A was the lowest. mRNA expression of resistin of adipose tissues of group B was significantly higher than that of group A (p<0.05) (Figure 2 and Table 5).
Table 1. Body weight, Lee’s index, weight of fat tissues of rats in each group (±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Body length (cm)</th>
<th>Lee’s index</th>
<th>Weight of lipid tissues (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>285.3 ±25.4</td>
<td>19.7 ±0.7</td>
<td>334.2 ±2.4</td>
<td>2.56 ±0.21</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>351.2 ±221.2</td>
<td>19.8 ±0.9</td>
<td>356.3 ±4.2</td>
<td>4.14 ±0.42</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>321.6 ±23.2</td>
<td>20.1 ±0.5</td>
<td>340.9 ±3.4</td>
<td>3.13 ±0.25</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>319.3 ±14.4</td>
<td>19.6 ±1.1</td>
<td>348.7 ±3.1</td>
<td>3.11 ±0.39</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>314.6 ±19.3</td>
<td>19.9 ±0.8</td>
<td>341.8 ±4.6</td>
<td>3.03 ±0.15</td>
</tr>
</tbody>
</table>

Notes: *p < 0.05 vs. group B; #p < 0.05 vs. group A. A: normal rats; B: obese rats; C, D, and E: rats treated with SHJFG at low, middle and high dosage, respectively.

Table 2. Serum lipids of each group (±s, mmol/L).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TC</th>
<th>TG</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>1.65 ±0.15</td>
<td>1.18 ±0.27</td>
<td>0.78 ±0.13</td>
<td>1.15 ±0.16</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>1.92 ±0.23</td>
<td>1.62 ±0.32</td>
<td>1.06 ±0.26</td>
<td>0.92 ±0.11</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>1.69 ±0.35</td>
<td>1.32 ±0.16</td>
<td>0.77 ±0.21</td>
<td>0.92 ±0.13</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>1.69 ±0.31</td>
<td>1.31 ±0.17</td>
<td>0.76 ±0.12</td>
<td>0.94 ±0.23</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>1.67 ±0.24</td>
<td>1.30 ±0.22</td>
<td>0.78 ±0.15</td>
<td>0.94 ±0.07</td>
</tr>
</tbody>
</table>

Notes: *p < 0.05 vs. group B; #p < 0.05 vs. group A. A: normal rats; B: obese rats; C, D, E: rats treated with SHJFG at low, middle and high dosage, respectively.

Table 3. Serum leptin of each group (±s, ng/mL).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Leptin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>0.398 ±0.113</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>0.687 ±0.126</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>0.539 ±0.471</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>0.564 ±0.253</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>0.572 ±0.363</td>
</tr>
</tbody>
</table>

Notes: *p < 0.05 vs. group B; #p < 0.05 vs. group A. A: normal rats; B: obese rats; C, D, E: rats treated with SHJFG at low, middle and high dosage, respectively.

Table 4: Adiponectin mRNA expression in rats (±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gray ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>0.369 ±0.024</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>0.262 ±0.031</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>0.461 ±0.026</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>0.453 ±0.033</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>0.452 ±0.041</td>
</tr>
</tbody>
</table>

Notes: *p < 0.05 vs. group B; #p < 0.05 vs. group A. A: normal rats; B: obese rats; C, D, E: rats treated with SHJFG at low, middle and high dosage, respectively.
Table 5. mRNA expression of resistin in rats (X ±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gray ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>1.899 ±0.243</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>1.193 ±0.236</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>1.364 ±0.346*</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>1.386 ±0.267▲</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>1.442 ±0.171▲</td>
</tr>
</tbody>
</table>

Notes: *p<0.05 vs. group B; ▲p<0.05 vs. group A. A: normal rats; B: obese rats; C, D, E: rats treated with SHJFG at low, middle and high dosage, respectively.

Figure 1. Electrophoresis picture of adiponectin mRNA and GAPDH of each group
Notes: Lane1: group B; Lane2: group A; Lane3: group E; Lane4: group D; Lane5: group C; M: DNA marker, DL 2000.

Figure 2. Electrophoresis picture of resistin mRNA and GAPDH of each group
Notes: Lane1: group C; Lane2: group D; Lane3: group A; Lane4: group E; Lane 5: group B; M: DNA marker, DL 2000
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DISCUSSION

SHJFG has been clinically used for more than 20 years. It is used for the treatment of obesity and hyperlipoidemia based on long-term experience. However, systematic animal studies to test its properties have not yet been done. According to knowledge based on experience in the Traditional Chinese Medicine (TCM), the compound prescription of lotus leaf, alisma and microcos paniculata can remove dampness and turbid, haw can dissipate blood stasis and cassia seed can dispel masses. All herbs together remove dampness and dissolve blood stasis of the body, thus may have a good effect in treating simple obesity [6-11]. Here we used a systematique approach to test the properties of this drug. Rats were used to copy the simple obesity in human body by feeding them with high fat diet in this study.

SHJFG is a TCM drug. The active compound is not a small molecule like in typical Western drugs such as for example statins where the chemical structure and molecular targets are well known. The TCM drugs consists of herbs, usually the molecular pathway of the mode of action of the drug is unknown. It is believed that TCM drugs interfere simultaneously with multiple molecular pathways. There seems to be not just one active component, it is rather the mixture of the different herbs that causes the effects. One of the current research topics in TCM pharmacology is to identify these molecular pathways as it was done in our study.

Our data indicate that SHJFG significantly lowers serum TC, TG, and LDL-C. Although serum HDL-C did not significantly increase, there was an increasing trend. Statins, typically used for the treatment of hypercholesterolemia, only significantly reduce total cholesterol and LDL cholesterol with minor effects on HDL-C [12]. It is of particular note, that SHJFG significantly lowered body weight while western drugs, like statins, had little effect on body weight [13].

Adiponectin is a kind of plasma protein that interacts with the extracellular matrix. It is a negative regulation hormone in fat cells that has the functions of lowering blood glucose and lipids, reducing body weight, inhibiting angiogenesis and other functions, and is anti-inflammatory [14]. Arita [15] and other researchers found that serum adiponectin would decrease if body fat increased, although it was synthesized and secreted by adipocytes. In obese people, excess adipose tissue secretes a large amount of tumor necrosis factor. At the same time, the body restricts adiponectin’s synthesis and secretion through autocrine or paracrine signals. That may be the mechanism by which adiponectin decreases while body weight increases [16]. Some researchers believe that adiponectin reduction is the result of feedback inhibition regulation based on the excess adipose tissue accumulation of obese people. Whether adiponectin amount decreases or not depends on genetic susceptibility to obesity [17]. This experiment showed that, compared with group A, mRNA expression of group B significantly decreased ($p < 0.05$). It illuminated that the change of adiponectin is in inverse proportion to body weight. This result is in accordance with the reported feedback regulation mechanism. Resistin is mainly secreted by white adipose tissue. So its expression in white adipose tissue is higher than in yellow adipose tissue. Experiments confirmed that resistin expression in omental fat and subcutaneous fat was higher than in fat tissues of chest and thigh. Resistin played a role in obesity, type 2 diabetes, and cardiovascular diseases [18-21]. Compared with group B, adiponectin mRNA expression of SHJFG groups was significantly higher, and it increased as the dosage increased. This proved that adiponectin mRNA expression in obese rats could be increased by SHJFG. In rodents, acute injection of adiponectin can reduce the glucose output of the liver. In vivo, adiponectin can reduce the triglycerides in muscle and liver, increase fatty acid oxidation by raising the genes expression involved in the oxidation [22], thus lowering peripheral fatty acids to further ameliorate insulin resistance. This experiment also proved that SH JFG could increase the resistin gene expression. Therefore, SHJFG had good effects in losing weight and lowering blood lipid. Up-regulating gene expressions of adiponectin and resistin, thus reducing triglyceride and increasing fatty acid oxidation may be the mechanism of its therapeutic effects. Further study is needed to detect how SHJFG treats obesity and hyperlipoidemia by increasing gene expressions of adiponectin and resistin through signal transduction.

We have to acknowledge that the rats were not pair fed and that food intake was not monitored. Thus an effect of the drug on appetite cannot be excluded. In any case the effects were remarkable and may translate to cardiovascular risk reduction.

In conclusion SHJFG effectively lowers serum lipid concentrations (TG, TC, and LDL-C) and simultaneously body weight. The effects might be partially mediated by SHJFG induced modulation of adipokines.

Declaration of Interest:
There is no financial conflict of interest for any of the authors.

References:


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