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The anti-obesity effect of green tea polysaccharide, polyphenols and caffeine in rats fed with high-fat diet

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Abstract

Beneficial effects of green tea (*Camellia sinensis*, Theaceae) extract against obesity have reported, however, the anti-obesity ability of the major components of green tea, polysaccharide, polyphenols and caffeine is not clear. Therefore, total green tea extract, polyphenols, polysaccharide, caffeine, polysaccharide and polyphenol at a dose of 400 or 800 mg/kg were conducted with high-fat diet fed rats for 6 weeks to investigate their anti-obesity effects. The results indicated that polyphenols and polysaccharide were responsible for the suppressive effect of green tea extract on body weight increase and fat accumulation. Moreover, polyphenols, polysaccharide, or caffeine can improve blood lipid and antioxidant levels, and effectively reduce rat serum leptin levels, inhibit the absorption of fatty acids, markedly reduce the expression levels of IL-6, TNF-α gene. Furthermore, it was shown that polysaccharide and polyphenols were synergistic in reduction serum leptin level and in anti-inflammatory activity. These results suggest that polysaccharide combination with polyphenols might be a potential therapy against obesity.

**Key words:** green tea, obesity, polysaccharide, polyphenols, caffeine
1. Introduction

Obesity is a serious health medical problem in the world, and the prevalence of obesity has increased dramatically for several decades \(^1\text{-}^3\). It is associated with increased health-care costs, reduced quality of life, and increase risk of various chronic diseases such as type II diabetes, hypertension, coronary artery disease, and various forms of cancer \(^4\text{-}^5\). Obesity is a complex metabolic disorder which caused by a positive energy balance, where energy intake exceeds energy expenditure \(^6\text{-}^7\).

Currently, available therapeutic approaches for treating obesity have a number of side effects \(^8\text{-}^9\). Therefore, growing attention has been given to natural products that are characterized as the anti-obesity agents \(^10\text{-}^11\).

As a beverage, green tea is well consumed in the world, especially in East Asian countries \(^12\). It contains abundant bioactive substances, including polysaccharide, caffeine and catechins. The catechin found in green tea mainly comprised epigallocatechingallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG), and epicatechin (EC) \(^13\), which has been suggested to be responsible for health effects \(^14\). Due to the ever-growing obesity pandemic, the anti-obesity effects of green tea are being increasingly investigated \(^15\). In 1999, Dulloo et al. \(^16\) found that administration of a green tea extract significantly increased energy expenditure and fat oxidation in a group of young males. Since then, several clinical trials have reported
the effects of tea preparations on increasing energy expenditure, fat oxidation, weight loss, fat mass, and weight maintenance after weight loss\textsuperscript{17-19}. Nevertheless, whether these effects are due to catechins or caffeine has yet to be resolved. Several studies in rodent models showed that green tea extract decreased weight gain and body fat gain\textsuperscript{20, 21}. In 2005, it was reported that treatment with TEAVIGO, a green tea extract containing 94% EGCG and 0.1% caffeine, significantly reduced body weight (BW) and body fat in different strains of mice fed a high-fat diet\textsuperscript{22, 23}. Choo reported that consumption with the water extract of green tea for 2 weeks decreased the body fat accumulation in high fat diet rats\textsuperscript{24}.

There are many reports about anti-obesity of green tea\textsuperscript{19, 25-28}, however, little is known about the underlying mechanism of action, in the regulation of body weight, lipolytic action and its relationship with inflammatory status. Recently, scientists showed that green tea polyphenols had anti-obesity effect by up-regulating adiponectin level in rats. Some studies suggested that the involved mechanisms were the inhibition of erk activation, alleviation of peroxisome proliferator-activated receptor $\gamma$ (PPAR-$\gamma$) phosphorylation, and increases in the PPAR-$\gamma$ expression\textsuperscript{29}. In addition, Lu et.al\textsuperscript{30} found that the gene expression of interleukin 6 receptor alpha (IL-6ra) was significantly increased in the rats fed high-fat diet compared to normal control.
In fact, different tea or the same tea from different area their function of anti-obesity is probably different and the different compositions of the same tea may also have different effects. Analytically, it is difficult to determine a particular composition in green tea singly responsible for the anti-obesity effect and the studies on the relationship between bioactive substances and anti-obesity ability have not been conduct yet. Therefore, the aim of this study was to isolate polyphenols, polysaccharide and caffeine from green tea and then investigate their influence on the development of obesity then we can comprehensive conclude that how the green tea extract reduce body fat of rats.

2. Materials and methods

2.1 Chemicals and animal

The coarse old green tea produced in Fujian China. Experimental animals were Sprague-Dawley (SD) male rats, 100 ~ 130 g which were purchased from Peking University Health Science Laboratory Animal Science, license number SCXK (Beijing) 2009-0017.

2.2 Separation and extraction from green tea

The total water extract of green tea was got under conditions which was that hot water heated it to 85°C by solid-liquid ratio 1:15 for 3 times. Tea polysaccharide was extracted from tea water by a method of water extracting-alcohol precipitating. Then
ethyl acetate was used to extracted tea polyphenols and trichloromethane was used to extracted caffeine from tea infusion. The purity of tea polyphenols and caffeine was analyzed by high performance liquid chromatography (HPLC), and Tea polysaccharide by phonel-sulfate method. The purity of the polyphenols which main contain EGC(45.43%), EGCG(28.64%), EC(11.27%), ECG(14.66%) was 71%, caffeine was 73.5% and tea polysaccharide (neutral sugar) was 27.5%.

2.3 Experimental design and dietary treatment

All the experimental procedures were approved by the Committee on the Ethics of Animal Experiments of Tianjin University of Science & Technology and according to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

One hundred and ten male SD rats were purchased from the Peking University Health Science Laboratory Animal Science and kept in a specific pathogen-free facility. The rats were housed at 23±3 °C, provided free access to water and food, and subjected to a 12 h/12 h light/dark cycle. The animals were acclimatized for 7 days and then randomly divided into normal control group, positive control group (orlistat), model control group, the total water extract, tea polyphenols, tea polysaccharide, the complex of tea polysaccharide and polyphenols and caffeine test group and each treatment group in addition to caffeine was divided into two doses of high and low. These groups were referred to as NC, MC, OC, TWH, TWL, TPPH,
TPPL, TPSH, TPSL, TPSM and CF groups, respectively. Since the start of the experiment, the positive control group, model control group, treatment group received high-fat diet (79% of the basal feed, 10% lard, 10% egg yolk powder, 0.5% cholesterol, 0.5% cholate). Normal control group was given the basal diet. Specific design was shown in Table 1. Weigh and measure body length daily, intragastric administration in regular. Record each animal’s food intake and leftover food daily. Collect rat droppings once a week and freeze-drying for later use.

2.4 Biochemical analyses of serum parameters

After six weeks experiment, all rats fasted for 12 hours and then were dissected, meanwhile fat accumulation in liver and internal of rats was examined with the naked eye. Take blood from the rat femoral artery and separate serum. An automatic biochemical apparatus was used to estimate the levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total superoxide dismutase (T-SOD), malondialdehyde (MDA), in serum. In addition, the level of leptin was determined by ELISA.

2.5 Adipose and liver histopathology

Suitable epididymal adipose tissue and liver of rats were selected and fixed in 10% formalin for 16 hours. Then all tissue were dehydrated in graded ethanol (70% ethanol
10min; 80% ethanol 10min; 95% ethanol 10min × 3 times, 100% ethanol 15min × 3 times). Using xylene to make tissue more clear (15min × 2 times). Then the tissue were dipped in wax two times at 60 °C, each time 1-2 hours and paraffin embedded at the same temperature. Fat and liver tissue blocks were cut into 5-micro sections and stained with hematoxylin and eosin.

2.6 Analysis of fecal fatty acids

The feces of rats were collected weekly and lyophilized. Extract fat in feces. Then after methyl esterification, the kind and content of fatty acids were detected by gas chromatography (GC). Accurately 0.5g lyophilized and pulverized feces and join 9 mL chloroform-methanol mixed solution. Then it was stored in refrigerator overnight at -80°C and filtered into a test tube with stopper. Crude extract was added to 0.2 times the volume of chloroform-methanol-0.29% NaCl solution and mixed well. Wait a moment to stratification, aspirate the top and clean the interface with a small amount of chloroform-methanol mixed solution. Then the appropriate amount of methanol was added and homogenate. In the end adding an appropriate amount of anhydrous Na₂SO₄, filtering again, evaporating with a stream of nitrogen. Adding 4% sulfuric acid-methanol solution in the test tube, then it was placed in a pre-heated to 75 °C water bath, heated reaction 1h. After completion of the reaction 2mL hexane and 5mL distilled water were added in that system. When it appeared stratification
then moved supernatant into a small beaker with anhydrous sodium sulfate. At last
mount the organic syringe filters (0.22\,\mu m) on syringe, filter into a centrifuge tube, for
GC measurement.

2.7 Research methods of detecting mRNA expression
Rats were anesthetized and euthanized, and adipose samples were collected at
dead of the experiment. The total mRNA was extracted by trizol (Takara). Then they
were reverse transcribed into cDNA. At last the gene expression of these related genes
were detected by RT-PCR (Bio-Rad). The method of operation was according to
instructions, the dye used SYBR Green. Gene specific primers used are given in Table
5. Relative quantification of gene expression with real-time PCR data was calculated
relative to GAPDH.

2.8 Statistical analysis
Results are presented as means with their standard errors. Statistical analysis was
performed using the SPSS program. Data were analyzed by one-way ANOVA.
Differences between the groups were established using the least significant difference
(LSD) test and the criterion for statistical significance was set at $p < 0.05$.

3 Results

3.1 Changes of rat body weight, food utilization and Lee's index
The changes in body weight, food utilization and Lee’s index were shown in
Table 1. The change of body weight in 6 weeks were shown in Figure 1. The final body weight of animals in the MC group was significantly higher compared with NC group, while other group except OC, TWL, TPSL had a significant reduction in body weight compared with MC group. The food utilization of rats in the MC was significantly higher compared with NC group and other group except TPSL had a significant reduction in body weight compared with MC group. Lee’s index could reflect the degree of obesity. The result showed that the lee’s index of MC group was significantly higher compared with NC group, while all medicated group had a significant reduction compared with MC group.

3.2 Body fat weight and fat index

After 6 weeks experiment, the epididymal and perirenal adipose tissues were collected and measured. Table 2 indicated that MC group had a significantly higher weight of two part fat when compared with the NC group, the similar results also were found in fat index. While the body fat weight and fat index of all medicated group except OC and TPSL had a significant reduction compared with MC group.

3.3 Serum lipids and antioxidant

Serum lipids and antioxidant profiles are shown in Table 3. The triglyceride and cholesterol levels of rats in MC increased significantly compared with the NC group. The serum triglyceride levels of TWL group had not a significantly reduction
compared with the MC group. And serum cholesterol levels of TWH and TPPH group
had not yet a significantly reduction compared with the MC group. However, the
serum LDL-C levels in all the groups were significantly reduced except that the TWH,
TWL and TPPH groups. It was interesting that the serum HDL-C levels of only TPSL
and TPSM groups were significantly higher compared with MC group but the serum
HDL-C level of CF group was lower than MC group. In order to investigate the
relationship of serum antioxidant and obesity, the levels of MDA and T-SOD were
analyzed. The levels of serum MDA of MC group had a significantly higher compared
with the NC group, but only OC, TPPH, TPSH, TPSL and TPSM group attenuated the
level of MDA. On the other hand the levels of serum T-SOD of MC group had a
significantly lower compared with the NC group, and the levels of T-SOD of TWH,
TPPL, TPSH and TPSM were significantly higher compared with MC group. Serum
leptin and insulin level were elevated in rats fed high fat diet, OC, TWL, TPPL, TPSH
and TPSM significantly lowered serum leptin levels compared to the MC group.

3.4 Histological analysis of liver and epididymal white adipose tissue

The histology of ratepididymal white adiposetissue was shown in Figure 2 and
the numbers of adipocyte within the same field were expressed in Table 2. The
adipocyte size of MC group was significantly bigger than NC group, and the numbers
of fat cells were significantly less than NC group. All medicated group, their
adipocyte size were significantly smaller than MC group, in another word their numbers of fat cells were significantly less than MC group. Changes in the degree of infiltration of lipid droplets in the liver were exhibited in Figure 3. The representative liver section of the MC group showed increased infiltration of lipid droplets, leading to hepatic steatosis condition. While the lipid droplet infiltration in the representative liver section of the other group except CF was markedly reduced, even the liver histology section of TPSH and TPSL groups were free from lipid droplets.

3.5 Rat feces fatty acids analysis

The rat feces fatty acids were determined by GC, and the result was expressed in table 4. According the result we found that the total fatty acids were mainly composed with palmitic acid, stearic acid, oleic acid and linoleic acid. Both the total amount of fatty acids and specific one in rat feces were significantly increased except TPSL group while compared to MC group, which indicated that green tea extract, polyphenols, polysaccharide, and caffeine can inhibit the absorption of fatty acids in the body.

3.6 The result of detecting mRNA expression

The mRNA expression levels of LEP, IL-6 and TNF-α were determined in white adipose tissue (Figure 4). As compared to the NC group, rat fed with high fat diet caused the up-regulation of LEP, IL-6 and TNF-α genes. OC, TWH, TWL, TPPH,
TPPL, TPSL, TPSM and CF markedly reduced the expression levels of LEP compared to MC group (Figure 4A). However, only OC, TPPH, TPSH, TPSL and TPSM significantly attenuated the expression of IL-6. In addition, Figure 4C indicated that all the medicated group except OC and CF group showed a lower level of TNF-α expression compared to the MC group.

4 Discussion

Several studies have evaluated the effects of green tea extracts on the development of obesity. However, there is extremely limited research focused on the relationship between bioactive substances of green tea and their anti-obesity. Therefore, in the present study we isolate polyphenols, polysaccharide and caffeine from green tea and then investigate their anti-obesity capabilities. According to the present study, we found that TWH, TPPH, TPPL, TPSH, TPSM and CF could significantly prevent the rat body weight gain compared with the model group, but TWL and TPSL did not change the rat body weight. There might be dose-dependent effects of tea water extract and polysaccharide, high dose green tea water extract or polysaccharide is more efficacious in prevent obesity.

Sayama et al. reported green tea could reduce weight of mice by suppression of food intake. Kao et al. also observed reduction in food intake after the administration of EGCG which was contained in tea polyphenol. We also found a
decrease in weight gain induced by polyphenols, polysaccharide, caffeine or the complex of tea polysaccharide and polyphenol respectively, is relevant with food utilization.

Zheng et al.\textsuperscript{33} found that caffeine and theanine could suppress body weight and body fat, while catechins did not change these parameters but reduced serum triglycerides and free fatty acids in female ICR mice. Our results suggested that polyphenols, polysaccharide, caffeine or the complex of tea polysaccharide and polyphenol can significantly reduce serum triglyceride levels. Furthermore, tea polysaccharides could effectively reduce serum LDL-C levels.

In this study, we demonstrate polyphenols, polysaccharide, caffeine or the complex of tea polysaccharide and polyphenol can inhibit the absorption of fatty acids in the body respectively. This suggests that, in agreement with previous reports studying green tea extracts presents anti-obesity properties\textsuperscript{36}.

Accumulated evidence indicates that obesity induced by high fat food can increases production of obesity related inflammation cytokines, such as leptin, IL-6, TNF-\textalpha, etc. Matsubara et al. reported that high-fat diet resulted in an increase in the content of saturated fatty acid could lead to an upward trend of fat cytokine mRNA such as leptin and adipsin\textsuperscript{37}. Our studies revealed that polysaccharide, caffeine or the complex of tea polysaccharide and polyphenol markedly reduced the expression
levels of leptin in white adipose tissue except high dose of polyphenol. Some studies have reported that serum leptin levels in obesity patients were significantly higher than the control group while several studies have shown that serum leptin levels of obese patients was significantly lower. Our results showed that suitable dose of green tea extract, polysaccharide, or polyphenols significantly lowered serum leptin levels. Moreover, it was shown that polysaccharide and polyphenols were synergistic in reduction serum leptin levels. Wu et al. suggested fruit polyphenols exerted potentially anti-inflammatory effect. In the present study we found both of green tea extract, polysaccharide, and polyphenols revealed anti-inflammatory. In addition, polysaccharide and polyphenols were synergistic in anti-inflammatory activity which means that polysaccharide combination with polyphenol could strengthen the function of anti-obesity than their individual effects.

In conclusion, coarse old green tea extract prevent body weight gain in male SD rat, polyphenols, caffeine, especially polysaccharide may play important part. The present experiments demonstrated that multiple factors in green tea contributed to anti-obesity effects. Each main ingredient in green tea all contributed to anti-obesity function and every ingredient in green tea may played many beneficial roles to achieve weight loss effects such as reducing food utilization, lowing serum triglyceride levels, inhibiting the absorption of fatty acids, regulating some relevant
genes’ expression and so on. In addition, tea polyphenols, polysaccharide, or caffeine effectively inhibit the absorption of fatty acids and markedly reduce the expression levels of inflammatory gene. Furthermore, polysaccharide and polyphenols were synergistic in reduction serum leptin level and in anti-inflammatory activity. Therefore polysaccharide combination with polyphenols might be a potential therapy to treat obesity, and further clinical studies are needed.

**Conflicts of interest**

The authors declare no conflict of interest.

**Acknowledgements**

The research was supported by Project of the Ministry of Science and Technology of the People’s Republic of China (No. 2012BAD33B08).
References


365  physiology (Bethesda, Md. : 1985), 2012, 112, 1637-1643.
367  2013, 4, 1654-1661.
Figure Caption

Figure 1. Changes in body weights of the SD rats when fed suitable feed for 42 days. Data are presented as the mean from twelve rats per group. NC was fed the standard chow plus saline; MC was fed high-fat diet with 79% of the standard chow, 10% lard, 10% egg yolk powder, 0.5% cholesterol, 0.5% cholate content plus saline; OC was fed high-fat diet feed plus orlistat capsule 40mg/kg BW; TWH was fed high-fat diet feed plus total water extract 800mg/ kg BW; TWL was fed high-fat diet feed plus total water extract 400mg/ kg BW; TPPH was fed high-fat diet feed plus tea polyphenols 800mg/ kg BW; TPPL was fed high-fat diet feed plus tea polyphenols 400mg/ kg BW; TPSH was fed high-fat diet feed plus tea polysaccharide 800mg/ kg BW; TPSL was fed high-fat diet feed plus tea polysaccharide 400mg/ kg BW; TPSM was fed high-fat diet feed plus the complex of tea polysaccharide and polyphenols (1:1) 800mg/ kg BW; CF was fed high-fat diet feed plus caffeine 400g/ kg BW.

Figure 2. Histopathological analysis of adipose tissues. All pictures were taken at 200 times. The letters A-K among the figure represent in proper order following groups: NC, MC, OC, TWH, TWL, TPPH, TPPL, TPSH, TPSL, TPSM and CF groups.

Figure 3. Histopathological analysis of liver. All pictures were taken at 200 times. The letters A-K among the figure represent in proper order following groups. NC, MC, OC, TWH, TWL, TPPH, TPPL, TPSH, TPSL, TPSM and CF groups.
Figure 4. Effect of green tea extract on the mRNA expression level of LEP (a), IL-6 (b), TNF-α (c) in the adipose tissues. NC, MC, OC, TWH, TWL, TPPH, TPPL, TPSH, TPSL, TPSM and CF groups. Compare with NC group MC group \(^*\)P < 0.05. Compare with MC group drug group \(^*\)P < 0.05.
Table 1

Table 1 Body weight and other characters of rats in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial BW(g)</th>
<th>Final BW(g)</th>
<th>Lee’s index</th>
<th>Food utilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>176.54±7.54</td>
<td>349.06±35.70</td>
<td>316.57±10.30</td>
<td>11.13±0.55</td>
</tr>
<tr>
<td>MC</td>
<td>182.98±13.93</td>
<td>384.96±32.59</td>
<td>327.63±8.36</td>
<td>15.06±1.07</td>
</tr>
<tr>
<td>OC</td>
<td>175.80±9.02</td>
<td>363.23±46.27</td>
<td>315.06±5.67</td>
<td>12.12±0.49</td>
</tr>
<tr>
<td>TWH</td>
<td>179.91±10.65</td>
<td>337.05±37.38</td>
<td>315.11±9.48</td>
<td>12.59±0.72</td>
</tr>
<tr>
<td>TWL</td>
<td>175.40±11.28</td>
<td>350.68±39.05</td>
<td>314.36±10.58</td>
<td>11.36±0.80</td>
</tr>
<tr>
<td>TPPH</td>
<td>175.39±5.07</td>
<td>341.44±19.55</td>
<td>312.68±12.53</td>
<td>12.93±0.61</td>
</tr>
<tr>
<td>TPPL</td>
<td>181.18±12.53</td>
<td>319.87±36.19</td>
<td>318.89±15.69</td>
<td>12.98±0.53</td>
</tr>
<tr>
<td>TPSH</td>
<td>182.09±16.42</td>
<td>338.76±33.31</td>
<td>312.83±11.59</td>
<td>12.91±0.81</td>
</tr>
<tr>
<td>TPSL</td>
<td>174.39±5.26</td>
<td>368.56±47.46</td>
<td>316.99±11.03</td>
<td>15.04±0.77</td>
</tr>
<tr>
<td>TPSM</td>
<td>177.11±12.90</td>
<td>316.07±32.60</td>
<td>309.58±10.66</td>
<td>13.04±0.89</td>
</tr>
<tr>
<td>CF</td>
<td>172.02±14.42</td>
<td>311.76±46.26</td>
<td>307.31±14.04</td>
<td>12.62±1.07</td>
</tr>
</tbody>
</table>

All values are means ± SD (n = 12). Compare with NC group MC group *P < 0.05. Compare with MC group drug group †P < 0.05. NC, standard chow plus saline; MC, high-fat diet with 79% of the standard chow, 10% lard, 10% egg yolk powder, 0.5% cholesterol, 0.5% cholate content plus saline; OC, high-fat diet feed plus orlistat capsule 40mg/kg BW; TWH, high-fat diet feed plus total water extract 800mg/kg BW; TWL, high-fat diet feed plus total water extract 400mg/kg BW; TPPH, high-fat diet feed plus tea polyphenols 800mg/kg BW; TPPL, high-fat diet feed plus tea polyphenols 400mg/kg BW; TPSH, high-fat diet feed plus tea polysaccharide 800mg/kg BW; TPSL, high-fat diet feed plus tea polysaccharide 400mg/kg BW; TPSM, high-fat diet feed plus the complex of tea polysaccharide and polyphenols (1:1) 800mg/kg BW; CF, high-fat diet feed plus caffeine 400mg/kg BW.
### Table 2

Table 2 Some body fat figures of rats in each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fat weight(g)</th>
<th>Fat index (%)</th>
<th>Number of cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>6.24±2.51</td>
<td>1.96±0.47</td>
<td>128.45±15.33</td>
</tr>
<tr>
<td>MC</td>
<td>10.99±2.24*</td>
<td>2.85±0.45#</td>
<td>56.67±5.05##</td>
</tr>
<tr>
<td>OC</td>
<td>8.61±3.11b</td>
<td>2.56±0.68</td>
<td>110.82±14.68&quot;</td>
</tr>
<tr>
<td>TWH</td>
<td>6.88±2.54&quot;</td>
<td>2.02±0.63&quot;</td>
<td>116.83±31.29&quot;</td>
</tr>
<tr>
<td>TWL</td>
<td>7.21±2.21&quot;</td>
<td>2.01±0.61&quot;</td>
<td>86.08±21.90&quot;</td>
</tr>
<tr>
<td>TPPH</td>
<td>7.31±2.70&quot;</td>
<td>2.33±0.39&quot;</td>
<td>110.83±18.88&quot;</td>
</tr>
<tr>
<td>TPPL</td>
<td>4.77±1.32&quot;</td>
<td>1.80±0.26&quot;</td>
<td>116.9±12.35&quot;</td>
</tr>
<tr>
<td>TPSH</td>
<td>7.29±2.95&quot;</td>
<td>2.35±0.63&quot;</td>
<td>99.55±32.90&quot;</td>
</tr>
<tr>
<td>TPSL</td>
<td>9.32±1.63</td>
<td>2.53±0.32</td>
<td>104.16±32.92&quot;</td>
</tr>
<tr>
<td>TPSM</td>
<td>6.57±1.08&quot;</td>
<td>2.24±0.62&quot;</td>
<td>143.18±30.15&quot;</td>
</tr>
<tr>
<td>CF</td>
<td>5.22±1.81&quot;</td>
<td>1.81±0.60&quot;</td>
<td>118.36±23.50&quot;</td>
</tr>
</tbody>
</table>

All values are means ± SD (n = 12). Compare with NC group MC group *P < 0.05. Compare with MC group drug group °P < 0.05.
### Table 3

**Table 3 Some serum figures of rats in each group**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mmol/(L))</th>
<th>TG (mmol/(L))</th>
<th>HDLC (mmol/(L))</th>
<th>LDLC (mmol/(L))</th>
<th>MDA (mmol/(L))</th>
<th>T-SOD (U/ml)</th>
<th>LEP (µg/(L))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>2.85±0.60</td>
<td>0.64±0.14</td>
<td>1.02±0.14</td>
<td>1.69±0.65</td>
<td>3.25±1.10</td>
<td>271.85±53.22</td>
<td>6.13±0.77</td>
</tr>
<tr>
<td>MC</td>
<td>4.32±0.88*</td>
<td>1.53±0.22#</td>
<td>1.04±0.11</td>
<td>3.04±0.93#</td>
<td>3.93±1.08#</td>
<td>193.04±48.02#</td>
<td>7.43±0.95#</td>
</tr>
<tr>
<td>OC</td>
<td>3.12±0.77*</td>
<td>1.01±0.27*</td>
<td>1.13±0.45</td>
<td>1.60±0.51*</td>
<td>2.79±0.98*</td>
<td>243.82±63.52</td>
<td>6.19±0.68</td>
</tr>
<tr>
<td>TWH</td>
<td>3.76±0.79*</td>
<td>0.86±0.08*</td>
<td>1.23±0.34</td>
<td>2.25±0.77</td>
<td>4.13±0.93</td>
<td>292.23±50.02</td>
<td>6.88±0.80</td>
</tr>
<tr>
<td>TWL</td>
<td>3.62±0.59*</td>
<td>1.33±0.16</td>
<td>0.99±0.49</td>
<td>2.05±0.62</td>
<td>3.77±0.63</td>
<td>189.55±66.88</td>
<td>5.99±0.90</td>
</tr>
<tr>
<td>TPH</td>
<td>4.23±1.08</td>
<td>0.89±0.19*</td>
<td>1.23±0.21</td>
<td>2.57±0.54</td>
<td>3.14±0.66*</td>
<td>225.81±68.62</td>
<td>7.00±0.58</td>
</tr>
<tr>
<td>TPPL</td>
<td>2.81±0.39*</td>
<td>0.90±0.12*</td>
<td>1.14±0.64</td>
<td>1.51±0.36*</td>
<td>3.96±0.77*</td>
<td>269.57±82.01</td>
<td>5.95±0.84</td>
</tr>
<tr>
<td>TPSH</td>
<td>2.95±0.99*</td>
<td>0.93±0.21*</td>
<td>1.05±0.29</td>
<td>1.93±0.19*</td>
<td>3.38±0.39*</td>
<td>276.44±57.45</td>
<td>6.32±0.74</td>
</tr>
<tr>
<td>TPSL</td>
<td>2.78±0.36*</td>
<td>0.94±0.20*</td>
<td>1.75±0.56*</td>
<td>1.51±0.40*</td>
<td>3.59±0.76*</td>
<td>254.22±69.10</td>
<td>6.94±0.27</td>
</tr>
<tr>
<td>TPSM</td>
<td>2.64±0.50*</td>
<td>0.73±0.19*</td>
<td>1.79±0.24*</td>
<td>1.52±0.43*</td>
<td>3.39±0.91*</td>
<td>289.87±57.4*</td>
<td>6.15±0.76</td>
</tr>
<tr>
<td>CF</td>
<td>2.78±0.36*</td>
<td>0.86±0.16*</td>
<td>0.89±0.31</td>
<td>0.94±0.18*</td>
<td>4.66±1.65</td>
<td>238.74±61.95</td>
<td>6.75±0.61</td>
</tr>
</tbody>
</table>

All values are means ± SD (n = 12). Compare with NC group MC group  *P < 0.05. Compare with MC group drug group  ^P < 0.05.
Table 4

Table 4 Fatty acids in rat feces

<table>
<thead>
<tr>
<th>Groups</th>
<th>Palmitic acid (mg/g)</th>
<th>Stearic acid (mg/g)</th>
<th>Oleic acid (mg/g)</th>
<th>Linoleic acid (mg/g)</th>
<th>Total acid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.12±0.01</td>
<td>0.11±0.01</td>
<td>0.06±0.01</td>
<td>0.06±0.00</td>
<td>0.3±0.04</td>
</tr>
<tr>
<td>MC</td>
<td>0.43±0.01</td>
<td>0.28±0.02</td>
<td>0.34±0.01</td>
<td>0.22±0.03</td>
<td>1.4±0.22</td>
</tr>
<tr>
<td>OC</td>
<td>0.60±0.04*</td>
<td>0.30±0.01*</td>
<td>0.44±0.01*</td>
<td>0.28±0.01*</td>
<td>1.8±0.22*</td>
</tr>
<tr>
<td>TWH</td>
<td>0.93±0.05*</td>
<td>0.60±0.04*</td>
<td>0.73±0.02*</td>
<td>0.38±0.03*</td>
<td>2.81±0.22*</td>
</tr>
<tr>
<td>TWL</td>
<td>0.92±0.05*</td>
<td>0.60±0.04*</td>
<td>0.75±0.01*</td>
<td>0.42±0.03*</td>
<td>2.91±0.25*</td>
</tr>
<tr>
<td>TPPH</td>
<td>0.56±0.04*</td>
<td>0.30±0.02*</td>
<td>0.47±0.01*</td>
<td>0.29±0.04*</td>
<td>1.99±0.29*</td>
</tr>
<tr>
<td>TPPL</td>
<td>0.63±0.03*</td>
<td>0.40±0.02*</td>
<td>0.48±0.02*</td>
<td>0.31±0.03*</td>
<td>1.87±0.26*</td>
</tr>
<tr>
<td>TPSH</td>
<td>0.71±0.02*</td>
<td>0.40±0.02*</td>
<td>0.56±0.01*</td>
<td>0.38±0.02*</td>
<td>2.25±0.23*</td>
</tr>
<tr>
<td>TPSL</td>
<td>0.48±0.03</td>
<td>0.29±0.01</td>
<td>0.37±0.02</td>
<td>0.25±0.02</td>
<td>1.51±0.20</td>
</tr>
<tr>
<td>TPSM</td>
<td>0.92±0.04*</td>
<td>0.60±0.02*</td>
<td>0.79±0.02*</td>
<td>0.51±0.03*</td>
<td>2.95±0.02*</td>
</tr>
<tr>
<td>CF</td>
<td>0.73±0.03*</td>
<td>0.50±0.01*</td>
<td>0.60±0.02*</td>
<td>0.39±0.01*</td>
<td>2.37±0.24*</td>
</tr>
</tbody>
</table>

All values are means ± SD (n = 12). Compare with MC group drug group *P < 0.05.

Table 5

Table 5 Primer sequences used in the study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 LEP</td>
<td>F: 5'-AAAAAGACGGGACAGAACA-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-TGACCAAGTGACATAGCG-3'</td>
</tr>
<tr>
<td>2 IL-6</td>
<td>F: 5'-TGCCCTTCTGGGACTGATG-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-ATACTGGTCTTTGTGGGTG-3'</td>
</tr>
<tr>
<td>3 TNF-α</td>
<td>F: 5'-CCACGCTTCTTCTGTCTACTG-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-GCTACCGGGCTTGTCACTC-3'</td>
</tr>
<tr>
<td>4 GAPDH</td>
<td>F:5'-GCAAGTTCACGGCAGCAG-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-GCCAGTAGACTCCAGCAGAT-3'</td>
</tr>
</tbody>
</table>
Figure 1
Figure 2
Figure 4

Relative gene expression of LEP

Relative gene expression of IL-6

Relative gene expression of TNF-α