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

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12 **Abstract**

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

27 **Key words:** green tea, obesity, polysaccharide, polyphenols, caffeine

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29 **1. Introduction**

30 Obesity is a serious health medical problem in the world, and the prevalence of
31 obesity has increased dramatically for several decades [1-3]. It is associated with
32 increased health-care costs, reduced quality of life, and increase risk of various
33 chronic diseases such as type II diabetes, hypertension, coronary artery disease, and
34 various forms of cancer [4, 5]. Obesity is a complex metabolic disorder which caused by
35 a positive energy balance, where energy intake exceeds energy expenditure[6, 7].
36 Currently, available therapeutic approaches for treating obesity have a number of side
37 effects [8, 9]. Therefore, growing attention has been given to natural products that are
38 characterized as the anti-obesity agents[10, 11].

39 As a beverage, green tea is well consumed in the world, especially in East Asian
40 countries[12]. It contains abundant bioactive substances, including polysaccharide,
41 caffeine and catechins. The catechin found in green tea mainly comprised
42 epigallocatechingallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG),
43 and epicatechin (EC)[13], which has been suggested to be responsible for health
44 effects[14]. Due to the ever-growing obesity pandemic, the anti-obesity effects of green
45 tea are being increasingly investigated[15]. In 1999, Dulloo *et al.* [16]found that
46 administration of a green tea extract significantly increased energy expenditure and fat
47 oxidation in a group of young males. Since then, several clinical trials have reported

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48 the effects of tea preparations on increasing energy expenditure, fat oxidation, weight
49 loss, fat mass, and weight maintenance after weight loss [17-19]. Nevertheless, whether
50 these effects are due to catechins or caffeine has yet to be resolved. Several studies in
51 rodent models showed that green tea extract decreased weight gain and body fat gain
52 [20, 21]. In 2005, it was reported that treatment with TEAVIGO, a green tea extract
53 containing 94% EGCG and 0.1% caffeine, significantly reduced body weight (BW)
54 and body fat in different strains of mice fed a high-fat diet [22, 23].Choo reported that
55 consumption with the water extract of green tea for 2 weeks decreased the body fat
56 accumulation in high fat diet rats[24].

57 There are many reports about anti-obesity of green tea[19, 25-28], however, little is
58 known about the underlying mechanism of action, in the regulation of body weight,
59 lipolytic action and its relationship with inflammatory status. Recently, scientists
60 showed that green tea polyphenols had anti-obesity effect by up-regulating
61 adiponection level in rats. Some studies suggested that the involved mechanisms were
62 the inhibition of erk activation, alleviation of peroxisome proliferator-activated
63 receptor γ (PPAR-γ) phosphorylation, and increases in the PPAR-γ expression [29]. In
64 addition, Lu et.al [30] found that the gene expression of interleukin 6 receptor alpha
65 (IL-6ra) was significantly increased in the rats fed high-fat diet compared to normal
66 control.

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67 In fact, different tea or the same tea from different area their function of
68 anti-obesity is probably different and the different compositions of the same tea may
69 also have different effects. Analytically, it is difficult to determine a particular
70 composition in green tea singly responsible for the anti-obesity effect and the studies
71 on the relationship between bioactive substances and anti-obesity ability have not
72 been conduct yet. Therefore, the aim of this study was to isolate polyphenols,
73 polysaccharide and caffeine from green tea and then investigate their influence on the
74 development of obesity then we can comprehensive conclude that how the green tea
75 extract reduce body fat of rats.

76 **2. Materials and methods**

77 *2.1 Chemicals and animal*

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

82 *2.2 Separation and extraction from green tea*

83 The total water extract of green tea was got under conditions which was that hot
84 water heated it to 85℃ by solid-liquid ratio 1:15 for 3 times. Tea polysaccharide was
85 extracted from tea water by a method of water extracting-alcohol precipitating. Then

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86 ethyl acetate was used to extracted tea polyphenols and trichloromethane was used to
87 extracted caffeine from tea infusion. The purity of tea polyphenols and caffeine was
88 analyzed by high performance liquid chromatography (HPLC), and Tea
89 polysaccharide by phonel-sulfate method [31]. The purity of the polyphenols which
90 main contain EGC(45.43%)，EGCG(28.64%)，EC(11.27%)，ECG(14.66%) was
91 71%, caffeine was 73.5% and tea polysaccharide (neutral sugar) was 27.5%.
92 *2.3 Experimental design and dietary treatment*
93 All the experimental procedures were approved by the Committee on the Ethics
94 of Animal Experiments of Tianjin University of Science & Technology and according
95 to the National Institutes of Health Guide for Care and Use of Laboratory Animals.
96 One hundred and ten male SD rats were purchased from thePeking University
97 Health Science Laboratory Animal Science and kept in a specific pathogen-free
98 facility. The rats were housed at 23±3 ℃， provided free access to water and food,
99 and subjected to a 12 h/12 h light/dark cycle. The animals were acclimatized for 7
100 days and then randomly divided into normal control group, positive control group
101 (orlistat), model control group, the total water extract, tea polyphenols, tea
102 polysaccharide, the complex of tea polysaccharide and polyphenols and caffeine test
103 group and each treatment group in addition to caffeine was divided into two doses of
104 high and low. These groups were referred to as NC, MC, OC, TWH, TWL, TPPH,

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105 TPPL, TPSH, TPSL, TPSM and CF groups, respectively. Since the start of the
106 experiment, the positive control group, model control group, treatment group received
107 high-fat diet (79% of the basal feed, 10% lard, 10% egg yolk powder, 0.5%
108 cholesterol, 0.5% cholate). Normal control group was given the basal diet. Specific
109 design was shown in Table 1. Weigh and measure body length daily, intragastric
110 administration in regular. Record each animal's food intake and leftover food daily.
111 Collect rat droppings once a week and freeze-drying for later use.

112 *2.4 Biochemical analyses of serum parameters*

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

121 *2.5 Adipose and liver histopathology*

122 Suitable epididymal adipose tissue and liver of rats were selected and fixed in 10%
123 formalin for 16 hours. Then all tissue were dehydrated in graded ethanol (70% ethanol

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124 10min; 80% ethanol 10min; 95% ethanol 10min × 3 times, 100% ethanol 15min × 3
125 times).Using xylene to make tissue more chear (15min × 2 times). Then the tissue
126 were dipped in wax two times at 60 ℃, each time 1-2 hours and paraffin embedded at
127 the same temperature. Fat and liver tissue blocks were cut into 5-micro sections and
128 stained with hematoxylin and eosin.

129 *2.6 Analysis of fecal fatty acids*

130 The feces of rats were collected weekly and lyophilized. Extract fat in feces.
131 Then after methyl esterification, the kind and content of fatty acids were detected by
132 gas chromatography (GC). Accurately 0.5g lyophilized and pulverized feces and join
133 9 mL chloroform-methanol mixed solution. Then it was stored in refrigerator
134 overnight at -80℃ and filtered into a test tube with stopper. Crude extract was added
135 to 0.2 times the volume of chloroform-methanol-0.29% NaCl solution and mixed well.
136 Wait a moment to stratification, aspirate the top and clean the interface with a small
137 amount of chloroform-methanol mixed solution. Then the appropriate amount of
138 methanol was added and homogenate. In the end adding an appropriate amount of
139 anhydrous Na_2SO_4, filtering again, evaporating with a stream of nitrogen. Adding 4%
140 sulfuric acid-methanol solution in the test tube, then it was placed in a pre-heated to
141 75 ℃ water bath, heated reaction 1h. After completion of the reaction 2mL hexane
142 and 5mL distilled water were added in that system. When it appeared stratification

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143 then moved supernatant into a small beaker with anhydrous sodium sulfate. At last
144 mount the organic syringe filters (0.22μm) on syringe, filter into a centrifuge tube, for
145 GC measurement.

146 *2.7 Research methods of detecting mRNA expression*

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

154 *2.8 Statistical analysis*

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

159 **3 Results**

160 *3.1 Changes of rat body weight, food utilization and Lee's index*

161 The changes in body weight, food utilization and Lee's index were shown in

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162 Table 1. The change of body weight in 6 weeks were shown in Figure 1.The final
163 body weight of animals in the MC group was significantly higher compared with NC
164 group, while other group except OC, TWL, TPSL had a significant reduction in body
165 weight compared with MC group. The food utilization of rats in the MC was
166 significantly higher compared with NC group and other group except TPSL had a
167 significant reduction in body weight compared with MC group. Lee's index could
168 reflect the degree of obesity. The result showed that the lee's index of MC group was
169 significantly higher compared with NC group, while all medicated group had a
170 significant reduction compared with MC group.

171 *3.2 Body fat weight and fat index*

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

177 *3.3 Serum lipids and antioxidant*

178 Serum lipids and antioxidant profiles are shown in Table 3. The triglyceride and
179 cholesterol levels of rats in MC increased significantly compared with the NC group.
180 The serum triglyceride levels of TWL group had not a significantly reduction

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181 compared with the MC group. And serum cholesterol levels of TWH and TPPH group
182 had not yet a significantly reduction compared with the MC group. However, the
183 serum LDL-C levels in all the groups were significantly reduced except that the TWH,
184 TWL and TPPH groups. It was interesting that the serum HDL-C levels of only TPSL
185 and TPSM groups were significantly higher compared with MC group but the serum
186 HDL-C level of CF group was lower than MC group. In order to investigate the
187 relationship of serum antioxidant and obesity, the levels of MDA and T-SOD were
188 analyzed. The levels of serum MDA of MC group had a significantly higher compared
189 with the NC group, but only OC,TPPH, TPSH,TPSL and TPSM group attenuated the
190 level of MDA. On the other hand the levels of serum T-SOD of MC group had a
191 significantly lower compared with the NC group, and the levels of T-SOD of TWH,
192 TPPL, TPSH and TPSM were significantly higher compared with MC group. Serum
193 leptin and insulin level were elevated in rats fed high fat diet, OC, TWL, TPPL, TPSH
194 and TPSM significantly lowered serum leptin levels compared to the MC group.

195 *3.4 Histological analysis of liver and epididymal white adipose tissue*

196 The histology of ratepididymal white adiposetissue was shown in Figure 2 and
197 the numbers ofadipocyte within the same field were expressed in Table 2. The
198 adipocyte size of MC group was significantly bigger than NC group, and the numbers
199 of fat cells were significantly less than NC group. All medicated group, their

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200 adipocyte size were significantly smaller than MC group, in another word their
201 numbers of fat cells were significantly less than MC group. Changes in the degree of
202 infiltration of lipid droplets in the liver were exhibited in Figure 3. The representative
203 liver section of the MC group showed increased infiltration of lipid droplets, leading
204 to hepatic steatosis condition. While the lipid droplet infiltration in the representative
205 liver section of the other group except CF was markedly reduced, even the liver
206 histology section of TPSH and TPSL groups were free from lipid droplets.
207 *3.5 Rat feces fatty acids analysis*
208 The rat feces fatty acids were determined by GC, and the result was expressed in
209 table 4. According the result we found that the total fatty acids were mainly composed
210 with palmitic acid, stearic acid, oleic acid and linoleic acid. Both the total amount of
211 fatty acids and specific one in rat feces were significantly increased except TPSL
212 group while compared to MC group, which indicated that green tea extract,
213 polyphenols, polysaccharide, and caffeinecan inhibit the absorption of fatty acids in
214 the body.
215 *3.6 The result of detecting mRNA expression*
216 The mRNA expression levels of LEP, IL-6 and TNF-α were determined in white
217 adipose tissue (Figure 4). As compared to the NC group, rat fed with high fat diet
218 caused the up-regulation of LEP, IL-6 and TNF-α genes. OC, TWH, TWL, TPPH,

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219 TPPL, TPSL, TPSM and CF markedly reduced the expression levels of LEP
220 compared to MC group (Figure 4A). However, only OC, TPPH, TPSH, TPSL and
221 TPSM significantly attenuated the expression of IL-6. In addition, Figure 4C
222 indicated that all the medicated group except OC and CF group showed a lower level
223 of TNF-αexpression compared to the MC group.

224 **4 Discussion**

225 Several studies have evaluated the effects of green tea extracts on the
226 development of obesity. However, there is extremely limited research focused on the
227 relationship between bioactive substances of green tea and their anti-obesity[19, 25-28].
228 Therefore, in the present study we isolate polyphenols, polysaccharide and caffeine
229 from green tea and then investigate their anti-obesity capabilities. According to the
230 present study, we found that TWH, TPPH, TPPL, TPSH, TPSM and CF could
231 significantly prevent the rat body weight gain compared with the model group, but
232 TWL and TPSL did not change the rat body weight. There might be dose-dependent
233 effects of tea water extract and polysaccharide, high dose green tea water extract or
234 polysaccharide is more efficacious in prevent obesity.

235 Sayama et al. [32-34] reported green tea could reduce weight of mice by suppression
236 of food intake. Kao et al.[35] also observed reduction in food intake after the
237 administration of EGCG which was contained in tea polyphenol. We also found a

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238 decrease in weight gain induced by polyphenols, polysaccharide, caffeine or the
239 complex of tea polysaccharide and polyphenol respectively, is relevant with food
240 utilization.

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

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

251 Accumulated evidence indicates that obesity induced by high fat food can
252 increases production of obesity related inflammation cytokines, such as leptin, IL-6,
253 TNF-α, etc. Matsubara *et al.* reported that high-fat diet resulted in an increase in the
254 content of saturated fatty acid could lead to an upward trend of fat cytokine mRNA
255 such as leptin and adipsin[37].Our studies revealed that polysaccharide, caffeine or the
256 complex of tea polysaccharide and polyphenol markedly reduced the expression

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257 levels of leptin in white adipose tissue except high dose of polyphenol. Some studies
258 have reported that serum leptin levels in obesity patients were significantly higher
259 than the control group while several studies have shown that serum leptin levels of
260 obese patients was significantly lower[38, 39].Our results showed that suitable dose of
261 green tea extract, polysaccharide, or polyphenols significantly lowered serum leptin
262 levels. Moreover, it was shown that polysaccharide and polyphenols were synergistic
263 in reduction serum leptin levels. Wu et al. suggested fruit polyphenols exerted
264 potentially anti-inflammatory effect[11, 40]. In the present study we found both of green
265 tea extract, polysaccharide, and polyphenols revealed anti-inflammatory. In addition,
266 polysaccharide and polyphenols were synergistic in anti-inflammatory activity which
267 means that polysaccharide combination with polyphenol could strengthen the function
268 of anti-obesity than their individual effects.

269 In conclusion, coarse old green tea extract prevent body weight gain in male SD
270 rat, polyphenols, caffeine, especially polysaccharide may play important part. The
271 present experiments demonstrated that multiple factors in green tea contributed to
272 anti-obesity effects. Each main ingredient in green tea all contributed to anti-obesity
273 function and every ingredient in green tea may played many beneficial roles to
274 achieve weight loss effects such as reducing food utilization, lowing serum
275 triglyceride levels, inhibiting the absorption of fatty acids, regulating some relevant

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276 genes' expression and so on. In addition, tea polyphenols, polysaccharide, or caffeine
277 effectively inhibit the absorption of fatty acids and markedly reduce the expression
278 levels of inflammatory gene. Furthermore, polysaccharide and polyphenols were
279 synergistic in reduction serum leptin level and in anti-inflammatory activity.
280 Therefore polysaccharide combination with polyphenols might be a potential therapy
281 to treat obesity, and further clinical studies are needed.

282 Conflicts of interest

283 The authors declare no conflict of interest.

284 Acknowledgements

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1 **Figure Caption**

2

3 Figure 1.Changes in body weights of the SD rats when fed suitable feed for 42 days.
4 Data are presented as the mean from twelve rats per group. NC was fed the standard
5 chow plus saline; MC was fed high-fat diet with 79% of the standard chow, 10% lard,
6 10% egg yolk powder, 0.5% cholesterol, 0.5% cholate content plus saline; OC was
7 fed high-fat diet feed plus orlistat capsule 40mg/kg BW; TWH was fed high-fat diet
8 feed plus total water extract 800mg/ kg BW; TWL was fed high-fat diet feed plus total
9 water extract 400mg/ kg BW; TPPH was fed high-fat diet feed plus tea polyphenols
10 800mg/ kg BW; TPPL was fed high-fat diet feed plus tea polyphenols 400mg/ kg BW;
11 TPSH was fed high-fat diet feed plus tea polysaccharide 800mg/ kg BW; TPSL was
12 fed high-fat diet feed plus tea polysaccharide 400mg/ kg BW; TPSM was fed high-fat
13 diet feed plus he complex of tea polysaccharide and polyphenols (1:1) 800mg/ kg BW;
14 CF was fed high-fat diet feed plus caffeine 400g/ kg BW.

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

20

21 Figure 3.Histopathologicalanalysis of liver. All pictures were taken at 200 times.
22 The letters A-K among the figure represent in proper order following groups. NC, MC,
23 OC, TWH, TWL, TPPH, TPPL, TPSH, TPSL, TPSM and CF groups,

24

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25 Figure 4.Effect of green tea extract on the mRNA expression level of LEP (a), IL-6 (b),
26 TNF-α (c) in the adipose tissues.NC, MC, OC, TWH, TWL, TPPH, TPPL, TPSH,
27 TPSL, TPSM and CF groups. Compare with NC group MC group $^{\#}P < 0.05$. Compare
28 with MC group drug group $^{*}P < 0.05$.

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Table 1

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

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

29 All values are means ± SD (n = 12). Compare with NC group MC group $^{\#}P < 0.05$. Compare with MC
30 group drug group $^{*}P < 0.05$. NC, standard chow plus saline; MC, high-fat diet with 79% of the standard
31 chow, 10% lard, 10% egg yolk powder, 0.5% cholesterol, 0.5% cholate content plus saline; OC,
32 high-fat diet feed plus orlistat capsule 40mg/kg BW; TWH, high-fat diet feed plus total water extract
33 800mg/ kg BW; TWL, high-fat diet feed plus total water extract 400mg/ kg BW;TPPH, high-fat diet
34 feed plus tea polyphenols 800mg/ kg BW; TPPL, high-fat diet feed plus tea polyphenols 400mg/ kg
35 BW; TPSH, high-fat diet feed plus tea polysaccharide 800mg/ kg BW;TPSL, high-fat diet feed plus tea
36 polysaccharide 400mg/ kg BW; TPSM, high-fat diet feed plus he complex of tea polysaccharide and
37 polyphenols (1:1) 800mg/ kg BW.CF, high-fat diet feed plus caffeine 400mg/ kg BW.

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39 **Table 2**

Table 2 Some body fat figures of rats in each group

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

40 All values are means ± SD (n = 12). Compare with NC group MC group $^{\#}P < 0.05$. Compare with MC

41 group drug group $^{*}P < 0.05$.

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Table 3

Table 3 Some serum figures of rats in each group

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

42 All values are means ± SD (n = 12). Compare with NC group MC group $^{\#}P < 0.05$. Compare with MC

43 group drug group $^{*}P < 0.05$.

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Table 4

Table 4 Fatty acids in rat feces

Groups	palmitic acid (mg/g)	stearic acid (mg/g)	oleic acid (mg/g)	linoleic acid (mg/g)	total acid (mg/g)
NC	0.12±0.01	0.11±0.01	0.06±0.01	0.06±0.00	0.3±0.04
MC	0.43±0.01	0.28±0.02	0.34±0.01	0.22±0.03	1.4±0.22
OC	0.60±0.04*	0.30±0.01*	0.44±0.01*	0.28±0.01*	1.8±0.22*
TWH	0.93±0.05*	0.60±0.04*	0.73±0.02*	0.38±0.03*	2.81±0.22*
TWL	0.92±0.05*	0.60±0.04*	0.75±0.01*	0.42±0.03*	2.91±0.25*
TPPH	0.56±0.04*	0.30±0.02*	0.47±0.01*	0.29±0.04*	1.99±0.29*
TPPL	0.63±0.03*	0.40±0.02*	0.48±0.02*	0.31±0.03*	1.87±0.26*
TPSH	0.71±0.02*	0.40±0.02*	0.56±0.01*	0.38±0.02*	2.25±0.23*
TPSL	0.48±0.03	0.29±0.01	0.37±0.02	0.25±0.02	1.51±0.20
TPSM	0.92±0.04*	0.60±0.02*	0.79±0.02*	0.51±0.03*	2.95±0.02*
CF	0.73±0.03*	0.50±0.01*	0.60±0.02*	0.39±0.01*	2.37±0.24*

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

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Table 5

Table 5 Primer sequences used in the study

	Gene	Primer sequence
1	LEP	F: 5’- AAAAGAACGGGACAGAACA -3’ R: 5’- TGACCAAGGTGACATAGCG -3’
2	IL-6	F: 5’- TGCCTTCTTGGGACTGATG -3’ R: 5’- ATACTGGTCTGTTGTGGGTG -3’
3	TNF-α	F: 5’- CCACGCTCTTCTGTCTACTG -3’ R: 5’- GCTACGGGCTTGTCACTC -3’
4	GAPDH	F:5’- GCAAGTTCAACGGCACAG -3’ R:5’- GCCAGTAGACTCCACGACAT -3’

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50 **Figure 1**

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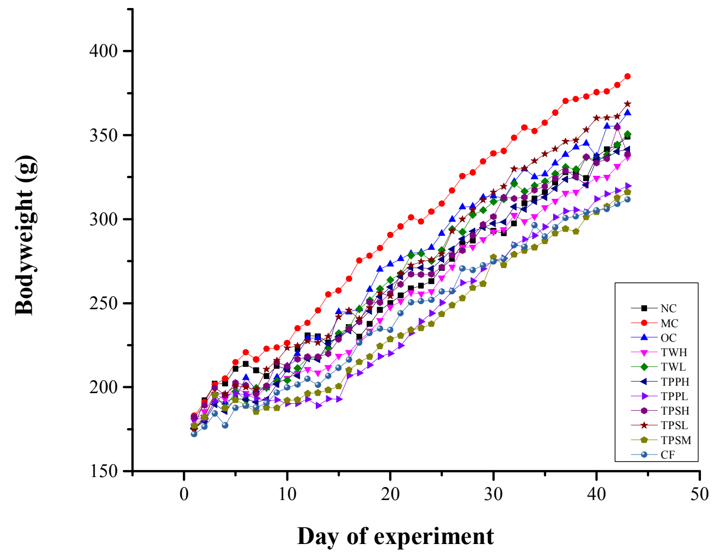

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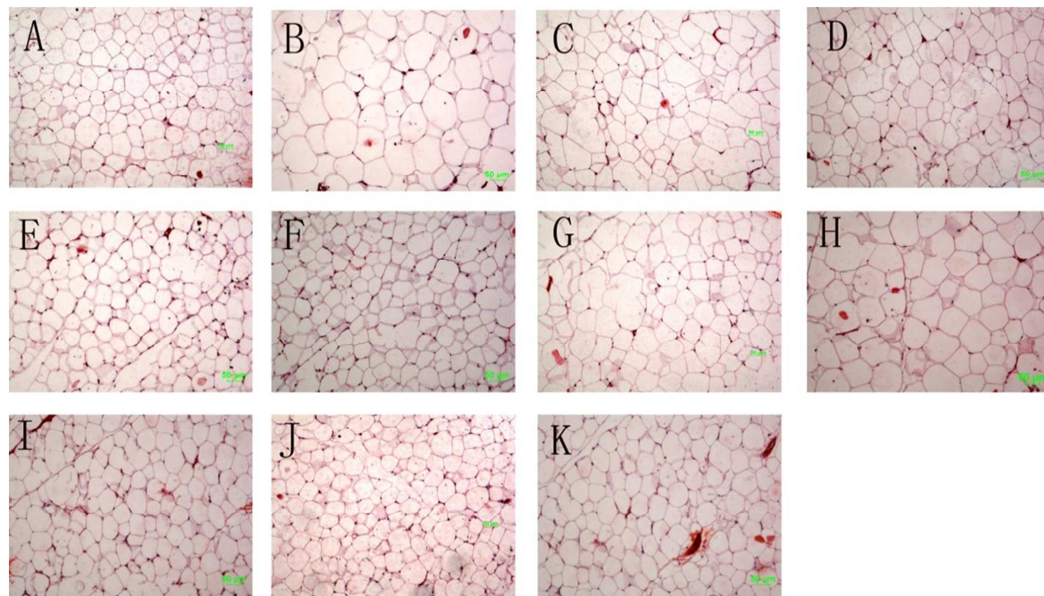
52 **Figure 2**



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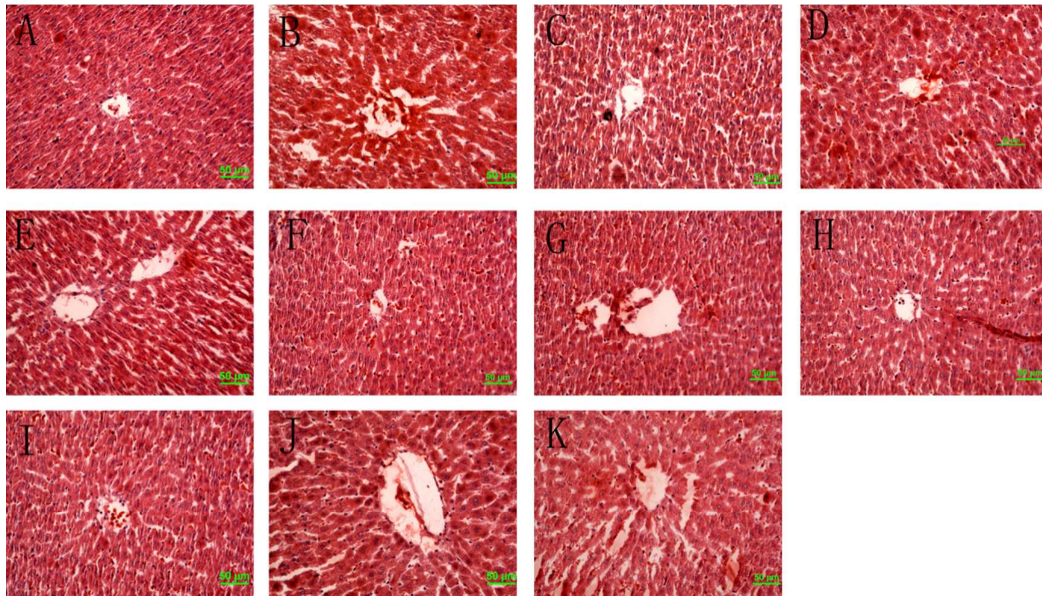
55 **Figure 3**



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58 **Figure 4**

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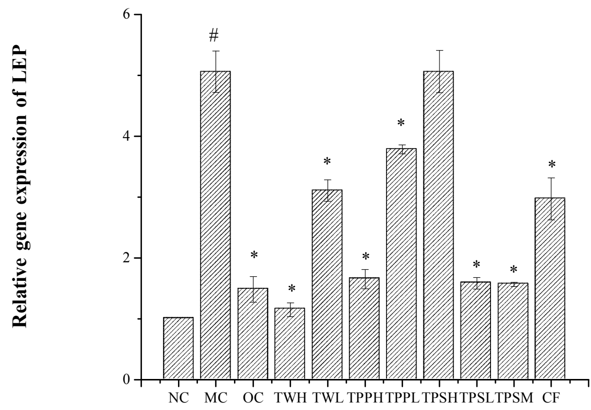

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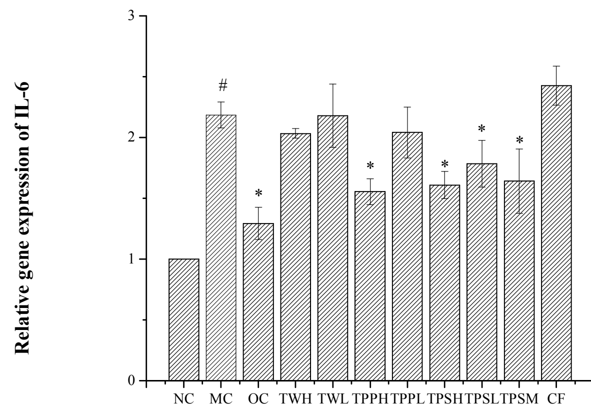

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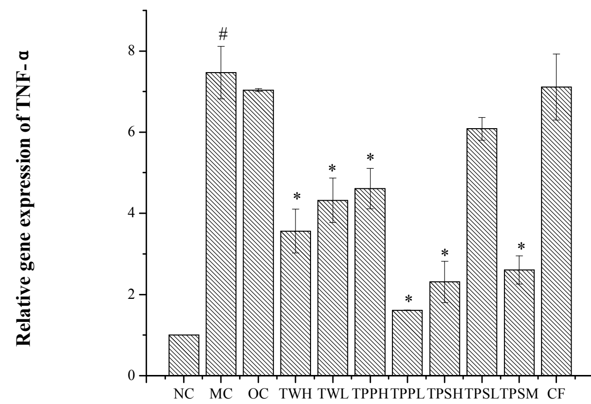

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