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The Effect of L-Carnitine, Green Tea Extract and Lotus Leaf Extract on the Body Fat Percentage in High Energy Diet-Induced Obese Rats

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ABSTRACT

Background
Obesity has become a public health issue of global concern. Obesity is often associated with the occurrence of many diseases, and will also increase mortality; it not only affects personal health, but also increases healthcare costs, thus reducing social productivity and causing negative social and economic impacts. Therefore, ameliorating obesity is an issue worth attention and effort. The development of a natural and safe anti-obesity combination is worthy of further research. It is known that L-carnitine, green tea and lotus leaves have anti-obesity potential, but there is no research and discussion on this novel combination to improve body fat.

Objective
This study explored how the dietary supplement formula containing L-carnitine, green tea extract and lotus leaf extract (CGL) lowered the body fat accumulation in rats induced by high-energy diet.

Design
The test used 60 male Sprague Dawley® white rats aged 6 weeks, which were first divided into the control group (12 rats were given normal feed) and the experimental group (48 rats were given high energy diet; HE). The HE group was further divided into H_2O and CGL groups (296, 593 and 1186 mg/kg, to be designated as CGL-L, CGL-M, CGL-H respectively). The rats were first fed with feed for five-weeks, and then fed with different doses of CGL by gavage starting from the sixth-week. After nine-weeks of feeding, the rats were sacrificed to obtain their body weight, feed intake, body fat, serum biochemical indices and liver lipid measurements.

Results
The results show that the final body weight of HE+CGL-L (578.8±41.6 g) was significantly lower than that of HE+H_2O (634.9±42.2 g), and the body fat amount of HE+CGL-L (36.6±9.8 g) was significantly lower than that of HE+H_2O (49.4±13.8 g). Feed efficiency and calorie efficiency of HE+CGL-L were also significantly lower than that of HE+H_2O (p<0.001). HE+CGL-M and HE+CGL-H were also able to significantly reduce the final body weight, body fat amount and serum-free fatty acid concentration (p<0.05).

Conclusion
CGL can significantly reduce the final body weight, body fat amount, body fat ratio, feed efficiency and calorie efficiency. CGL has the potential as a new dietary supplement for weight loss. However, the significance of these results on humans taking the supplement for prolonged periods of time is unknown and should be a focus for future investigations.

Keywords
Obesity; L-Carnitine; Green tea extract; Lotus leaf extract; Weight loss.

INTRODUCTION

Obesity is a global concern. According to the estimation of the World Obesity Federation, the global number of overweight or obese adults will increase from 2 billion in 2014 to 2.7 billion by 2025. Obesity control is a major challenge to global public health. Compared with healthy people, obese people are more likely to suffer from diabetes, metabolic syndrome, dyslipidemia,

hypertension, cardiovascular disease, knee arthritis, etc.[1-3] In order to prevent the occurrence of obesity, dietary ingredients can also help to improve lipid metabolism, control body weight, and reduce fat deposits.

L-carnitine can help transport fat into mitochondria for energy metabolism. Contradictory outcomes have been observed in previous studies: some studies found that non-obese healthy people who took L-carnitine did not lose weight[4,5]; however, the results of the meta-analysis of the database show that it did affect weight loss, with the mean weight reduction being 1.33 kg and body mass index (BMI) reduction 0.47 kg/m^2. [6]

Green tea contains lots of antioxidant substances, such as catechins and tannins. Green tea can suppress oxidation in a dose-dependent manner, and its resistance to oxidation is six-fold than that of black tea.[7] Previous studies confirmed that green tea is rich in catechins, which can regulate blood lipids, promote metabolism and reduce weight.[8] An adult who drinks green tea containing 583 mg of catechins every day can reduce BMI, body fat, waist circumference and hip circumference.[9] In a 12-week experiment, drinking 625 mg of catechins daily in combination with moderate intensity exercise, three times a week could eliminate abdominal fat and reduce the blood concentrations of fatty acids and triglycerides,[10] which confirms the effect of catechins in green tea on weight loss.

Leaves of lotus (*Nelumbo nucifera*) have many medicinal uses in traditional culture. Various pharmacologically active substances can be extracted from different parts of lotus plants, such as nuciferine, flavonoids, polyphenols and glycosides,[11] and they have numerous effects, such as anti-obesity[12] and cholesterol reduction.[13]

The purpose of this study was to explore how a new formula, a dietary supplement containing L-carnitine, green tea extract and lotus leaf extract (CGL), helped reduce body fat accumulation by using the model of Sprague Dawley® rats fed with high energy diet to induce body fat accumulation.

MATERIALS AND METHODS

Supplement Composition

Each tablet (717.06 mg/tablet) of compound plant-based supplements provided by HealthTake Corporation contains L-carnitine, green tea extract (80 to 120 mg of catechin per tablet) and lotus leaf extract.

Study Design

The animal experiment project was reviewed and approved by the Institutional Animal Care and Use Committee of China Medical University (approval ID: CMUIACUC-2018-241) and was carried out according to the institutional animal ethics guidelines of the China Medical University. 60 male Sprague Dawley® rats aged 6 weeks were purchased from BioLASCO Taiwan Co., Ltd., which were raised at the Animal Laboratory of China Medical University. The temperature of the Animal Laboratory was set at 22±2 °C, and the light was on for 12 h and off for 12 h (light on at 8 AM, and off at 8 PM). After a week of acclimation, the experimental group began to be given high energy diet. The high energy diet (5.24 Kcal/g; 20% protein, 60% fat, 20% carbohydrates) was imported feed (D12492, Research Diet Inc., New Brunswick, NJ, USA). The control group was given normal (2.85 Kcal/g; 24% protein, 11% fat, 65% carbohydrates) imported feed (maintenance diet/Altromin 1320, Altromin Spezialfutter GmbH & Co. KG, Im Seelenkamp, Germany), and the drinking water was treated with high-pressure sterilization.

The experimental animals were divided into the control group with 12 rats and the high energy diet (HE) group with 48 rats.[14] Control group was fed with normal feed, while HE group was fed with high energy diet. In the first 3-weeks, three rats were housed in one cage, and from the fourth-week to the ninth-week, every rat had its own cage to facilitate the measurement of food intake and feed efficiency. After having been fed with high energy diet for five-weeks, control group continued to be fed with normal feed; HE group was divided into four groups, each with 12 rats: with and without CGL. One group was given sterilized water (HE+H_2O). The oral dose of rats was calculated based on the recommended daily intake of CGL for humans (4 tablets, each 717.06 mg) and the metabolic conversion ratio of 6.2 between humans and rats. The daily dose of rats was 2868.24 mg/60 kg (human body weight)×6.2=296 mg/kg; 296 mg/kg is equivalent to one time the human body dose. The daily doses used in this experiment were 296, 593 and 1186 mg/kg, which are one, two and four times the human doses, to be designated as HE+CGL-L, HE+CGL-M and HE+CGL-H, respectively. The samples were prepared as suspensions of 29.6, 59.3 and 118.6 mg/mL by adding deionized water. The rats were fed by gavage once a day, and the volume given was 1 mL/100 g body weight. After 4-weeks' feeding, the experimental animals were sacrificed after the feeding was stopped for one night. Blood was collected from the celiac artery for blood biochemical analysis when the rats were under anesthesia, and epididymal, perirenal and mesenteric adipose tissue in the peritoneal cavity was removed for measurement of its precise weight and calculation of body fat ratio (%).

Body Weight and Food Intake

The rats were weighed once a week during the experiment as the basis for administering the weekly amount of test substances. The weights of the rats at the beginning and end of the experiment were compared. The daily feed intake was recorded from the fourth-week to the ninth-week to calculate each rat's average daily feed intake. At the end of the experiment, the feed efficiency during the four-weeks of administering the test substances was calculated as follows: Feed efficiency (%) defined as (weight gain/food intake)×100.

Calorie Intake

Food intake was converted into calorie intake. The normal feed was calculated at 2.85 Kcal/g, HE was calculated at 5.24 Kcal/g, and CGL was calculated at 3.9 Kcal/g. At the end of the experiment, the calorie efficiency was calculated; Calorie efficiency (%)

defined as (weight gain / calorie intake) × 100.

Measurement of Liver Lipid Concentration

After the blood was collected from the celiac artery, the liver was washed with saline solution, and then stored at -80 °C until further analysis. After lipid extraction was done, the concentrations of liver triglyceride and cholesterol were measured.[15] We took 0.1 g of liver tissue, added 2 mL of extracting solvent (chloroform : methanol=2:1), and then homogenized it with a homogenizer. We let it stand at room temperature for 60 min before centrifuging it at 2200 xg for 5 min. We took the supernatant and placed it into a clean 1.5 mL centrifuge tube, added 0.2 mL 0.9% NaCl and mixed it well; at this moment the solution appeared white and turbid, centrifuged it at 300 xg for 5 min to make it separate to two layers; kept the bottom layer in an oven to be blown-dry with nitrogen at 55 °C; after drying, added 100 μL solvent (tert-butyl alcohol: triton X-100: methanol=2:1:1), heated to dissolve it at 65 °C for 15 min, and then tested with commercially available cholesterol and triglyceride reagents (Roche, Switzerland).

Measurement of Serum Biochemical Properties

After the blood was collected, it was centrifuged at 2000 xg for 15 min to acquire the blood plasma for biochemical analysis. The concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride, uric acid, creatinine were analyzed by using commercial kits (Roche, Germany) and evaluated by biochemical analyzer (COBAS MIRA PLUS, Roche, Switzerland). Free fatty acid was determined by using a non-esterified fatty acid kit (RANDOX, County Antrim, UK). Total cholesterol, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), sodium and potassium were determined by using commercial kits (Fortress Diagnostics Limited, Antrim, United Kingdom). Ketone body was determined by β-Hydroxybutyrate (ketone body) colorimetric assay kit (Cayman Chemical, MI, USA). Blood glucose levels was measured using a CareSens blood glucose meter (i-SEN, Korea).

Statistical Analysis

Results are expressed as the mean±SD. All experimental data were analyzed by one-way analysis of variance using the Dunnett's test, provided the data passed a normality test. A value of $p<0.05$ was used to indicate statistical significance between groups.

RESULTS

Body Weight Changes

As shown in Table 1, the initial weight of rats in the control group was not different from that of the rats in the four HE groups. The weight of rats in the HE+H_2O group was significantly higher than that in control group from week-1 to week-9. When the rats in the HE feed group had been fed for 5-weeks, there was no difference in the average weight of the rats in the four HE groups. When the rats in the CGL groups had been fed with high energy diet for 8-weeks to 9-weeks, the average weight of rats was significantly lower than that in the HE+H_2O group ($p<0.05$).

Feed Efficiency and Calorie Efficiency

High energy diet was administered for 9-weeks in total. After having been fed with high energy diet for 5-weeks, the rats started receiving sterilized water or supplements for a total of 4-weeks until they had been fed with high energy diet for 9-weeks. Weight gain of each rat was the weight at week-9 minus the weight at week-5, and the total food intake was the summation of food intake from week-6 to week-9. As shown in Table 2, the total weight gain in the HE+H_2O group was significantly higher than that in the control group ($p<0.05$). The total weight gain in the CGL groups were significantly lower than that in the HE+H_2O group. From week-6 to week-9, compared with the control group, the HE+H_2O group had significantly lower total food intake, yet significantly higher total calorie intake. In terms of total food intake and total calorie intake, there was no difference between the CGL groups and the HE+H_2O group. Feed efficiency and calorie efficiency were calculated from the total weight gain, total food intake and total

Table 1. *Effect of Supplements on the Weight of Rats Induced by High Energy Diet*

Weeks	Body Weight (g)				
	Control	HE+H_2O	HE+CGL-L	HE+CGL-M	HE+CGL-H
Week 0	225.1±7.2	226.8±19.7	228.8±5.0	224.3±17.6	225.8±6.8
Week 1	275.8±9.8	294.6±11.1$^{\#\#}$	289.4±14.4	291.3±17.9	293.4±15.1
Week 2	326.8±10.6	351.9±13.4$^{\#\#}$	347.3±15.1	349.9±20.1	351.2±17.9
Week 3	359.8±12.2	406.9±17.7$^{\#\#\#\#}$	405.3±20.5	403.4±23.8	405.1±21.9
Week 4	391.3±9.4	452.5±26.9$^{\#\#\#\#}$	450.3±21.0	450.1±19.3	443.8±31.6
Week 5	412.0±13.5	499.8±30.3$^{\#\#\#\#}$	504.8±29.9	507.5±21.8	498.8±37.4
Week 6	439.7±14.6	534.2±35.5$^{\#\#\#\#}$	521.8±35.0	523.9±22.6	516.3±41.5
Week 7	465.3±17.4	569.2±39.9$^{\#\#\#\#}$	535.3±37.0	531.4±25.1*	524.5±44.3**
Week 8	480.5±19.4	592.3±45.5$^{\#\#\#\#}$	542.8±39.4**	539.6±25.4**	530.6±34.2***
Week 9	495.0±20.1	634.9±42.2$^{\#\#\#\#}$	578.8±41.6**	574.7±24.8**	560.3±53.4***

Control, normal diet group ; HE+H_2O, high energy diet with sterilized water; HE+CGL-L, high energy diet with 296 mg/kg/day of CGL ; HE+CGL-M, high energy diet with 593 mg/kg/day of CGL; HE+CGL-H, high energy diet with 1186 mg/kg/day of CGL. Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#}p<0.01$, $^{\#\#\#\#}p<0.001$ as compared with the control group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with the HE+H_2O group.

Table 2. *Effect of Supplements on Feed Efficiency and Calorie Efficiency of Rats Induced by High Energy Diet*

Treatments	Weight Gain (g)	Food Intake (g)	Feed Efficiency (%)	Calorie Intake (Kcal)	Calorie Efficiency (%)
Control	83.0±10.5	810.1±68.1	10.3±1.2	2308.1±193.6	3.6±0.4
HE+H_2O	135.2±14.5$^{\#\#\#}$	614.0±47.2$^{\#\#\#}$	22.0±1.7$^{\#\#\#}$	3216.6±247.6$^{\#\#\#}$	4.2±0.3$^{\#\#\#}$
HE+CGL-L	73.9±17.4***	603.6±21.2	12.2±2.9***	3180.9±110.4	2.3±0.5***
HE+CGL-M	67.2±11.6***	591.4±15.8	11.4±1.9***	3132.9±83.2	2.1±0.4***
HE+CGL-H	61.5±22.1***	580.1±46.6	10.5±3.1***	3106.5±244.7	2.0±0.6***

Control, normal diet group ; HE+H_2O, high energy diet with sterilized water ; HE+CGL-L, high energy diet with 296 mg/kg/day of CGL ; HE+CGL-M, high energy diet with 593 mg/kg/day of CGL; HE+CGL-H, high energy diet with 1186 mg/kg/day of CGL.Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared with the control group. $^{***}p<0.001$ as compared with the HE+H_2O group.

Table 3. *Effect of Supplements on Body Fat Amount and Body Fat Percentage of Rats Induced by High Energy Diet*

Treatments	Weight of Adipose Tissue			Body Fat (g)	Body Fat Ratio (%)
	Epididymal Adipose Tissue (g)	Perirenal Adipose Tissue (g)	Mesenteric Adipose Tissue (g)		
Control	6.2±1.1	6.4±1.0	2.9±0.5	15.5±1.9	3.1±0.4
HE+H_2O	16.0±3.7$^{\#\#\#}$	24.1±7.1$^{\#\#\#}$	9.3±3.4$^{\#\#\#}$	49.4±13.8$^{\#\#\#}$	7.7±1.7$^{\#\#\#}$
HE+CGL-L	12.1±2.4*	18.3±5.6*	6.2±2.7**	36.6±9.7***	6.3±1.4*
HE+CGL-M	11.7±2.0**	14.7±2.4***	6.5±1.7*	32.9±5.3***	5.7±0.8**
HE+CGL-H	11.6±3.6**	14.8±5.0***	5.2±1.9***	31.7±9.8***	5.6±1.3***

Control, normal diet group ; HE+H_2O, high energy diet with sterilized water ; HE+CGL-L, high energy diet with 296 mg/kg/day of CGL ; HE+CGL-M, high energy diet with 593 mg/kg/day of CGL; HE+CGL-H, high energy diet with 1186 mg/kg/day of CGL. Body fat defined as the sum of epididymal adipose tissue, perirenal adipose tissue, mesenteric adipose tissue. Body fat ratio (%) defined as (Body fat (g) / Body weight (g))×100.Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared with the control group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with the HE+H_2O group.

Table 4. *Effect of Supplements on Liver Triglyceride and Cholesterol Concentrations in Rats Induced by High Energy Diet*

Parameters	Control	HE+H_2O	HE+CGL-L	HE+CGL-M	HE+CGL-H
Liver (g)	11.2±0.6	16.4±0.9$^{\#\#\#}$	14.5±1.8**	14.4±1.7**	13.9±2.1***
Liver (%)	2.3±0.1	2.6±0.1$^{\#\#}$	2.5±0.3	2.5±0.3	2.5±0.3
Cholesterol (mg/g tissue)	3.0±0.6	6.1±3.0$^{\#\#}$	5.5±1.5	6.0±2.6	5.9±2.2
Triglyceride (mg/g tissue)	12.4±4.6	44.7±11.5$^{\#\#\#}$	39.0±13.0	37.9±13.5	33.1±10.6*

Control, normal diet group ; HE+H_2O, high energy diet with sterilized water; HE+CGL-L, high energy diet with 296 mg/kg/day of CGL ; HE+CGL-M, high energy diet with 593 mg/kg/day of CGL; HE+CGL-H, high energy diet with 1186 mg/kg/day of CGL.Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared with the control group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with the HE+H_2O group.

calories from week-5 to week-9. The feed efficiency and calorie efficiency of the HE+H_2O group were significantly higher than those in the control group ($p<0.05$). The feed efficiency and calorie efficiency of the CGL groups were significantly lower than those in the HE+H_2O group.

Changes of Body Fat and Liver Indicators

As shown in Table 3, epididymal, perirenal mesenteric adipose tissue, body fat weight and body fat ratio (%) in the CGL groups were all significantly lower than those in the HE+H_2O group ($p<0.05$). As shown in Table 4, the absolute and relative liver weights, and concentrations of liver cholesterol and liver triglyceride in the HE+H_2O group were all significantly higher than those in the control group; the absolute liver weight in the three CGL groups were significantly lower than that in the HE+H_2O group ($p<0.05$), but there was no significant difference in the relative liver weight and the concentration of liver cholesterol; the concentration of liver triglyceride in the HE+CGL-H group was lower than that in the HE+H_2O group ($p<0.05$).

Serum Biochemical Test

As shown in Table 5, among the 13 biochemical indicators tested in serum, the concentrations of triglyceride, free fatty acid, ketone body and glucose in the HE+H_2O group were significantly higher than those in the control group, and the remaining 9 indicators did not differ significantly. All three doses of the CGL groups were able to reduce the triglyceride concentration in serum; HE+CGL-M and HE+CGL-H reduced the free fatty acid, ketone body and glucose concentrations in serum, but they had no significant effect on the other 9 indicators, no abnormal was observed.

Table 5. *Effect of Supplements on Plasma Biochemical Properties of Rats Induced by High Energy Diet*

Parameters	Control	HE+H_2O	HE+CGL-L	HE+CGL-M	HE+CGL-H
AST(U/L)	153.3±16.6	172.2±28.4	190.1±32.9	176.9±20.7	165.2±44.7
ALT(U/L)	48.9±7.8	48.7±11.9	51.8±16.2	45.5±5.9	47.7±9.2
TG (mg/dL)	36.3±11.1	47.4±6.1$^{\#}$	39.8±5.6	39.0±9.0	36.9±11.2*
TC (mg/dL)	61.0±11.2	69.4±16.3	63.5±16.8	58.1±13.8	60.9±13.5
LDL-C (mg/dL)	40.1±9.3	37.9±11.4	37.4±13.3	36.6±11.6	39.0±11.2
HDL-C (mg/dL)	18.6±5.1	23.0±9.8	23.4±3.3	18.3±3.4	21.1±4.9
FFA (mmol/L)	0.85±0.09	1.01±0.09$^{\#}$	0.93±0.26	0.80±0.16**	0.78±0.11**
KB (nmol/L)	199.9±49.5	362.8±65.6$^{\#\#\#}$	313.6±82.4	294.3±60.2	267.1±94.4**
Crea (mg/dL)	0.48±0.21	0.46±0.10	0.44±0.21	0.42±0.14	0.50±0.27
UA (mg/dL)	1.54±0.35	1.82±0.30	1.60±0.32	1.55±0.22	1.80±0.42
Na (mEq/L)	144.0±0.9	144.8±1.0	144.8±0.8	144.3±0.9	144.1±1.0
K (mEq/L)	4.7±0.4	4.7±0.6	4.4±0.4	4.6±0.1	4.7±0.3
Glu (mg/dL)	112.1±23.8	153.8±17.3$^{\#\#\#}$	138.0±19.6	112.6±10.3***	129.3±29.4*

Control, normal diet group ; HE+H_2O, high energy diet with sterilized water ; HE+CGL-L, high energy diet with 296 mg/kg/day of CGL; HE+CGL-M, high energy diet with 593 mg/kg/day of CGL; HE+CGL-H, high energy diet with 1186 mg/kg/day of CGL.Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ as compared with the control group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with the HE+H_2O group.AST, aspartate aminotransferase;ALT, alanine aminotransferase;TG, triglyceride;TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; FFA, free fatty acid; KB, ketone body; Crea, creatinine; UA, uric acid; Na, sodium; K, potassium; Glu, glucose.

DISCUSSION

Feeding one, two and four-fold the doses recommended for humans had a significant inhibitory effect on the weight, feed efficiency, calorie efficiency, body fat amount and body fat ratio of the rats increased by high energy diet. The initial weight of Sprague Dawley® male rats used in this study was approximately 225 g, and they were fed with high energy diet (5.24 Kcal/g) for 9-weeks. The results in Tables 1 and 3 show that compared with the control group the HE+H_2O group had 28.2% higher body weight, and 218% higher body fat (epididymal, perirenal, mesenteric adipose tissues). High energy diet significantly increased the accumulation of body fat in the rats. After being fed with high energy diet for 5 weeks, CGL supplements were administered daily for 4-weeks. Calculation was made on the basis of the data of those having been fed with high energy diet for 9-weeks. HE+CGL-L reduced body weight by 8.8% and body fat by 25.7%; HE+CGL- M reduced body weight by 9.4% and body fat by 33.2%; and HE+CGL-L reduced body weight by 11.8% and body fat by 35.9%. These results show that the CGL significantly reduced body fat accumulation in the rats.

Feed efficiency refers to the degree of digestion, absorption, and utilization of food after being ingested. The higher the percentage, the more fully the food can be utilized by the body. During the period of administering CGL supplements, feed efficiency and calorie efficiency can be calculated from the total weight gain, total feed intake and total calorie intake from week-5 to week-8. The feed efficiency and calorie efficiency of the CGL groups were significantly lower than those in the HE+H_2O group. These results show that the CGL reduced body weight and body fat accumulation by lowering feed efficiency and calorie efficiency. The caloric intake converted from feed intake gave the same results, that is, the calorie efficiency of CGL groups were significantly lower than that of the HE+H_2O group. In the safety test of 13 items in blood plasma, the CGL reduced the concentrations of free fatty acid, triglyceride, ketone body and glucose, but had no effect on the other 9 parameters, including AST, indicating that liver and kidney function indices were unaffected. As there is higher oil and fat content in high energy diet, besides the increase in blood free fatty acid and triglyceride concentrations, the concentrations of liver triglycerides and cholesterol in the rats also increased significantly; HE+CGL-H was able to significantly improve the concentration of liver triglycerides.

Previous study found that feeding mice with a high-fat diet and containing 0.5% (w/w) catechins helps to inhibit obesity caused by high-fat diet. After 12-weeks of feeding, the mice's weight can be significantly decreased. Tea catechins may prevent or improve obesity by modulating lipid metabolism.[16] The report of the meta-analysis on green tea and weight loss shows that taking catechins for 12-weeks on average could reduce the mean weight of the treatment subjects by 1.31 kg.[17] Daily intake of 270 to 1200 mg of catechin products could clearly affect human weight and cause physical changes. The possible mechanisms of catechins in green tea to help weight loss include energy consumption, changes in lipid metabolism, appetite suppression, and reduction in the absorption of glucose, probably by inhibiting the activities of α-amylase and α-glucosidase to reduce nutrient absorption.[8] Based on another meta-analysis on the results of 15 studies, compared with decaffeinated catechin, the combination of catechin and caffeine was able to significantly reduce the weight by 0.44 kg, BMI and waist circumference.[18] Supplements used in this study contain 80 to 120 mg of catechin per tablet, and the green tea extract contains approximately 0.7% caffeine. Taking 4 tablets per day would result in a dose of 320 to 480 mg catechin, which is consistent with the trend in the literature on the meta-analysis.

The lotus leaf extract used in this study contains 2% nuciferine and 5% flavonoids. In the *in vitro* tests, lotus leaf ex-

tract had a stronger inhibiting effect on lipase than on α-amylase. After phenolic compounds were eliminated from the lotus leaf extract, the inhibitory activity on lipase and α-amylase also disappeared.[12] The inhibiting effect on enzymes is known to lower the carbohydrate digestion rate, reduce the digestion and absorption of glucose, and help ameliorate postprandial hyperglycemia, thus preventing glucose from being transported to fat tissues, as well as suppressing the synthesis and accumulation of triglyceride.[19] In a study, it was pointed out that epigallocatechin (EGC), which is rich in lotus seedpod extract (LSE), has a hepatoprotective effect. It uses oleic acid to induce Hepa G2 cells to conduct experiments. By measuring oxidative stress and apoptosis pathways, it is confirmed that LSE has the effect of reducing lipid accumulation and lipid toxicity.[20] In addition, the part of nuciferine also has the effect of improving lipid metabolism and anti-oxidation. Studies have shown that *in vitro* experiments, using oleic acid to induce Hepa G2 cells for experiments, nuciferine can reduce triglyceride accumulation and effectively reduce fatty acid content.[21] Previous study indicated that nuciferine can reduce the weight gain and fat accumulation of high fat diet-fed rats, affect the intestinal microbial composition, increase short-chain fatty acids. The anti-obesity effect may be related to the composition of the intestinal flora and the regulation of potential functions.[22] It was also observed in this study that the supplement containing nuciferine tended to lower total triglyceride concentration in liver, and all three doses of the supplements could significantly reduce serum total triglyceride concentration ($p<0.05$).

Previous study have pointed out that feeding rats with a high-fat diet and then giving 250 mg/kg L-carnitine, the results show that the rats' blood lipids can be normalized, and decrease serum triglycerides, cholesterol, and peripheral adipose tissue.[23] On the other hand, it has also been observed that L-carnitine can change the lipid metabolism of high fat diet-fed rats with inhibition of stearoyl-CoA desaturase-1 (SCD-1) activity inducing an increase in fatty acid oxidation and a decrease in body fat.[24] When obese women aged 20 to 40-years-old took 1000 mg of L-carnitine daily for 12-weeks, its effect was better than raspberry ketone; the former could significantly improve cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL).[25] The supplements used in this study contain 300 mg of L-carnitine per tablet; if an adult takes 4 tablets daily, he or she will be getting 1200 mg of L-carnitine, and the long-term use is expected to reduce obesity-related parameters.

LIMITATION

Although the results of animal experiments are remarkable, the effects and variation in humans are still unknown. The prevention and maintenance of obesity after weight loss is still to be discussed.

CONCLUSION

CGL supplements can significantly reduce the weight, body fat (epididymal, perirenal, mesenteric adipose tissue), body fat ratio, feed efficiency and calorie efficiency of the rats. These results clearly show that CGL can alleviate fat accumulation in rats induced by high energy diet. In conclusion, the results of the animal experiments clearly show that the fat accumulation and high body fat percentage induced by high energy diet can be significantly reduced in rats by using supplement of the same dose recommended for humans. It has the potential as a new dietary supplement for weight loss. Therefore, human experiments should be tested and thorough the study of the effects in the future, investigate how to achieve the maintenance of human body effects and the improvement of variables.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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