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Investigating the Lipid-Regulating Effects of Bergamot, Red Yeast Rice, Pine Bark, Vitamin E and Sesame Seed Extracts in Hamsters

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ABSTRACT

Background

With the rapid development of society, lifestyles and dietary habits are gradually changing. Due to the rich variety of food, high-fat and high-sugar diets are becoming more common. Humans who consume high-fat diets for a long time are prone to dyslipidemia, which is one of the main risk factors for the development of cardiovascular diseases (CVDs) such as coronary heart disease, myocardial infarction, atherosclerosis and hypertension. It is becoming a major health problem facing the world. Based on many studies, it is known that bergamot (*Citrus bergamia*) extract, red yeast rice (*Monascus anka*) powder, pine bark (*Pinus radiata*) extract, vitamin E and sesame (*Sesamum indicum*) extract have the potential to regulate blood lipids. However, there are no actual investigations or studies on this novel combination to control blood lipids.

Objective

This trial investigated whether lipid-clearing capsules (LCC) containing bergamot extract, red yeast rice powder, pine bark extract, vitamin E and sesame extract had an improved effect on regulating blood lipids.

Design

In this experiment, a high-cholesterol diet (HCD) containing 0.2% cholesterol was used to induce hyperlipidemia in experimental animals. Experimental animals (hamsters, male, n=50, 5-6-weeks old, mean weight about 90 g) were purchased from the National Center for Experimental Animal Reproduction and Research (NCEAR) and randomly divided into 5 groups (n=10) after a one-week adaptation period: (1) normal control group (Control), (2) high cholesterol diet group (HCD, fed high cholesterol diet), (3) 1-time dose of LCC group (LCC-1X, fed HCD+1-fold daily dose of LCC), (4) 2-times dose of LCC group (LCC-2X, fed HCD+2-fold daily dose of LCC), (5) 5-times dose of LCC group (LCC-5X, fed HCD+5-fold daily dose of LCC). Blood biochemistry, liver and stool analyses were performed after 8-weeks to assess the lipid-regulating effect of the capsules.

Results

Eight weeks of HCD feeding resulted in significant increases in serum triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations, as well as significant increases in hepatic TG and TC-levels and fecal TG and TC-levels. Thus, feeding a high-cholesterol diet resulted in significant dyslipidemia and fatty liver formation in experimental animals. Supplementation with 1, 2, or 5 times the dose of LCC for 8-weeks resulted in a significant decrease in serum TG, TC, LDL-C and LDL-C/high-density lipoprotein cholesterol (HDL-C) ratio ($p<0.05$) and a significant decrease in TG and TC in the liver ($p<0.05$). Supplementation with LCC also significantly increased TC excretion in the feces.

Conclusion

The results of this study confirm that LCC has lipid-regulating effects. By translating the experimental data into a daily supplement for adults, it is recommended that a daily intake of 2 LCC (1000 mg internal volume) can help reduce serum TG, TC and LDL-C concentrations, leading to a healthy effect on cardiovascular disease prevention.

Keywords

Bergamot extract; Red yeast rice powder; Pine bark extract; Vitamin E; Sesame seed extract; Cardiovascular diseases; Blood lipids.

INTRODUCTION

According to global epidemiological data, non-communicable diseases (NCDs) are now a major factor affecting human health and socio-economic development.[1] The draft action plan for the 66th World Health Assembly (WHA), held in Geneva, Switzerland, from May 20 to 28, 2013, points out that in 2008, 57 million people died worldwide, with 63% of those deaths attributed to NCDs. A major public health challenge, the action plan focuses on the four types of NCDs with the highest morbidity and mortality rates: cardiovascular disease (CVD), cancer, chronic respiratory disease and diabetes. In addition, the World Economic Forum (WEF) and the World Health Organization (WHO) estimate that the economic cost of NCDs in low- and middle-income countries will be approximately $7.28 trillion between 2011 and 2025, with CVD accounting for more than 50% of that cost.[1]

Cardiovascular diseases (CVDs) are a group of diseases related to blood vessel and heart function, such as myocardial infarction, heart failure and atherosclerosis. They have the highest mortality and morbidity rates and are the leading cause of death worldwide. According to the World Health Organization, an estimated 17.5 million people died from CVD in 2012, accounting for 31% of all deaths worldwide.[2] In European countries, nearly 4 million people die from CVD each year and the number of CVD-related deaths worldwide is expected to exceed 23.6 million by 2030 due to increasing disease incidence.[1] Hyperlipidemia, or hypercholesterolemia, is an important risk factor for the development of atherosclerosis and CVD.[3,4] The main pathogenic blood parameters are elevated low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC) and triglyceride (TG) concentrations. Several natural plant polyphenolic components have shown good hypolipidemic activity in animal and human studies, with optimal ability to prevent hyperlipidemia and cardiovascular complications.[5-7]

Research on lipids has not diminished in the last 20-years and continues to receive attention, as the concept of synergistic effects and multifunctionality has been an important direction in the development of natural or health foods internationally.[8,9] In addition, in recent years, agricultural products, food factors, or phytochemicals have been considered important complementary sources with health-promoting properties or even as lipid modulators. Lipid clearing capsules (LCC) contains bergamot extract, red yeast rice powder, pine bark extract, vitamin E and sesame extract, which have the potential to modulate lipid bioactivity and were therefore evaluated for their lipid-modulating function. Bergamot produced hypolipidemic activity in rats, reducing TC, LDL-C and TG-levels and significantly lowering blood glucose. In addition, it inhibited 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity and enhanced reactive vasodilation, making it an effective phytotherapy against hyperlipidemia and hyperglycemia.[10] Pectin- and flavonoid-rich citrus peel extracts may lower cholesterol by modulating hepatic HMG-CoA, possibly by binding bile acids and increasing blood and liver cholesterol turnover.[11-13]

Many studies have shown that the red yeast rice metabolite monacolin K may act as an inhibitor of HMG-CoA reductase, the most important regulator of the cholesterol synthesis pathway, leading to reduced cholesterol synthesis and lower cholesterol concentrations *in vivo*.[14,15] In 2014, Li et al[16] conducted a meta-analysis of 13 studies in approximately 804 subjects and showed that an average daily intake of monacolin K in red yeast rice was effective in reducing serum LDL-C, TC and TG concentrations for an average of 2-24-weeks. In another crossover double-blind trial, subjects taking 10 mg of monacolin K for 4-weeks showed a decrease in blood concentrations of TC and LDL-C.[17]

Pine bark extract significantly prolonged the oxidation of LDL and enhanced the antioxidant defense of LDL and monocytes, which may have a preventive effect on the progression of atherosclerosis.[18] Pine bark extract is a strong antioxidant with ameliorating effects on cardiovascular, skin, cognitive and menstrual disorders, as well as other diseases and disease processes such as diabetes and inflammation. We also obtained evidence for activation of nuclear factor-kappa B (NF-κB) and subsequent expression of various NF-κB-induced genes, such as adhesion molecules and endothelin-1, in cultured vascular endothelial cells, as well as anti-hypertensive effects in hypertensive rats attributed to its antioxidant properties. The protective effects on endothelial dysfunction and endothelial vasodilation are related to the antioxidant properties in hypertensive rats.[19]

Vitamin E supplementation has been shown to significantly reduce lipid peroxidation and TG-levels, which may be beneficial in reducing the risk of CVD in diabetic patients.[20] Other forms of vitamin E, such as tocotrienols, have also been found to reduce the risk of CVD by lowering levels of TC and TG, which are major risk factors for CVD.[21] Tocotrienols have been shown to regulate cholesterol metabolism by reducing the oxidation of LDL-C and by inhibiting the expression of HMG-CoA reductase.[22] Animal studies have also supported the preventive role of tocotrienols in the progression of atherosclerosis.[23,24]

Sesame is a medicinal and edible plant with strong antioxidant activity, mainly due to the presence of lignans and vitamin E, which scavenge free radicals in the body and prevent the peroxidation of unsaturated fatty acids in cells. Black sesame oil is rich in unsaturated fatty acids and other antioxidant components that have hypolipidemic effects and can prevent and reduce the occurrence and development of atherosclerosis.[25] Black and white sesame and their kernels have significant hypolipidemic effects by improving hyperlipidemia and increasing the antioxidant capacity of the liver. Combined with pathological observations, it has been shown that black and white sesame and their kernels can improve hepatic steatosis and disorders of lipid metabolism.[26]

Although the individual components of LCC are known to have lipid-regulating effects, there is a lack of studies investigating the effects of this combination on lipid regulation. Therefore, the purpose of this experiment was to evaluate the effects of LCC on lipid regulation in a hamster model of hyperlipidemia induced by a high-cholesterol diet (HCD).

MATERIALS AND METHODS

Sample Treatment

LCC provided by Healthtake Corporation, each weighing 500 mg, is formulated with a blend of bergamot extract, red yeast rice powder, pine bark extract, vitamin E and sesame extract. In this study, we could not show the percentages of the contents of the capsules, as the manufacturer indicated that the development of this product is ongoing and different formulas are being tested.

Dose calculations: As metabolic rates differ between humans and experimental animals, the maximum safe dose for humans was converted to a dose administered to experimental animals, following the estimated maximum safe starting dose in initial clinical trials of therapeutic agents in adult healthy volunteers (US Food and Drug Administration (FDA), 2005). The conversion factor between humans and hamsters is 7.4, so the 1X, 2X and 5X dose groups for hamsters were 123, 247 and 617 (mg/kg/day) in order. For a hamster with a body weight of 100 g, each hamster should be fed 12.3, 24.7 and 61.7 mg per day in the 1X, 2X and 5X dose groups, respectively.

Feed Ingredients

The test was based on a powdered standard feed (Rodent Laboratory Chow 5001, Purina Co., USA) with 0.2% cholesterol added to the feed. To successfully induce hypertriglyceridemia simultaneously, we also added 5% lard to the diets according to the literature.[27-29] The feed composition and formulation for each group of animals are shown in Table 1.

Table 1. *The Feed Composition and Formulation for each Group of Animals*

Group / Composition	Control	HCD	LCC-1X	LCC-2X	LCC-5X
Powdered Chow 5001 (g)	1000	948	948	948	948
Lard (g)	-	50	50	50	50
Cholesterol (g)	-	2	2	2	2

Experimental Groups

Fifty male hamsters, aged 5-6-weeks, were purchased from the National Applied Research Laboratories and randomly assigned to five groups of 10 hamsters each. The groups were as follows: a blank control group (Control) fed a normal standard solid diet and given the formulated dosing solution directly; a HCD group with no dosing given the solvent used to prepare the LCC-1X, LCC-2X and LCC-5X capsules and fed with 1x, 2x and 5x doses of LCC, respectively. The LCC solutions were prepared at concentrations of 12.3 mg/mL, 24.7 mg/mL and 61.7 mg/mL, respectively.

Experimental Design

The animal experimental procedures in this program were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Taiwan Sport University (No. IACUC-10920) and were housed in the animal house of the National Taiwan Sport University. The animals were kept in the animal house of the National Sports University. The temperature of the animal room was 22±2 °C, the humidity was 65±5% and there was light for 12-hours and darkness for 12-hours (lights on at 6:00 and off at 18:00). The animals were pre-housed for 1-week and the experiments were started after they had acclimatized to their environment. The experiments were performed in an animal model of induced hyperlipidemia, with three doses of LCC tested: low (LCC-1X), medium (LCC-2X) and high (LCC-5X). Since this is a hamster animal model test, to ensure that each hamster receives the recommended amount of LCC based on its body weight, the feeding material is mixed with reverse osmosis water and then fed through an oral tube. In addition, a normal control group and a HCD control group were required for each experiment, so there were five groups in this experiment. The normal control group was fed with normal chow and given orally with reverse osmosis water, while the HCD control group was fed with a HCD and given orally with reverse osmosis water. The body weight of the hamsters was measured periodically during the experiment and the daily intake of fatty chow was recorded to compare the body weight at the beginning and at the end of the experiment. After the animals were fasted for 12-hours, blood was collected, centrifuged after anesthesia and analyzed for clinical blood biochemical concentrations. The efficacy of the product in regulating lipids was assessed based on blood biochemical values and liver and fecal measurements. The product was considered to have lipid-lowering properties if the experimental group fed LCC had significantly lower lipid levels than the control group fed a HCD ($p<0.05$) (Figure 1).

Serum Biochemical Tests

Biochemical values included serum TG, TC, high-density lipoprotein cholesterol (HDL-C) and LDL-C values. Serum samples were examined using a hematology analyzer (Hitachi 7060, Hitachi, Tokyo, Japan) to analyze various clinical blood biochemical concentrations in the blood.

Determination of TC and TG Concentrations in the Liver

Following the experimental method of our previous report Huang et al,[29] 10 mg of liver was taken and 200 mL of organic solvent (chloroform: isopropanol: NP40=7:11:0.1) was added. Samples are centrifuged at 15,000×g for 10-minutes and evacuated in a vacuum at 200 mL (50 °C, 30-minutes). After evacuation, the reagents were redissolved with the cholesterol assay buffer provided in the kit and mixed well by ultrasonication and vortex shaking. TC was then analyzed with the BioVision Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay Kit (Item No. K603-100), while TG was analyzed with another commercially available kit, the Cayman Colorimetric Assay Kit (Item No. 10010303).

Determination of TC and TG Concentrations in Feces

The stool was oven dried to a constant weight, 0.1 g was added to 1 ml of phosphate-buffered saline (PBS), ground in a homogenizer, extraction solvent (chloroform: methanol=2:1, v/v) was added and filtered through filter paper (Whatman No. 5). The filtrate was dried under vacuum and 1 ml of dimethyl sulfoxide (DMSO) was added to the filtrate and mixed well with ultrasonic and vortex shaking. The TC was then analyzed with the BioVision TC and Cholesteryl Ester Colorimetric/Fluorescence Analysis Kit (Item No. K603-100), while the TG was analyzed with another commercially available kit (Item No. 10010). The TG was analyzed with another commercially available kit, the Cayman Colorimetric

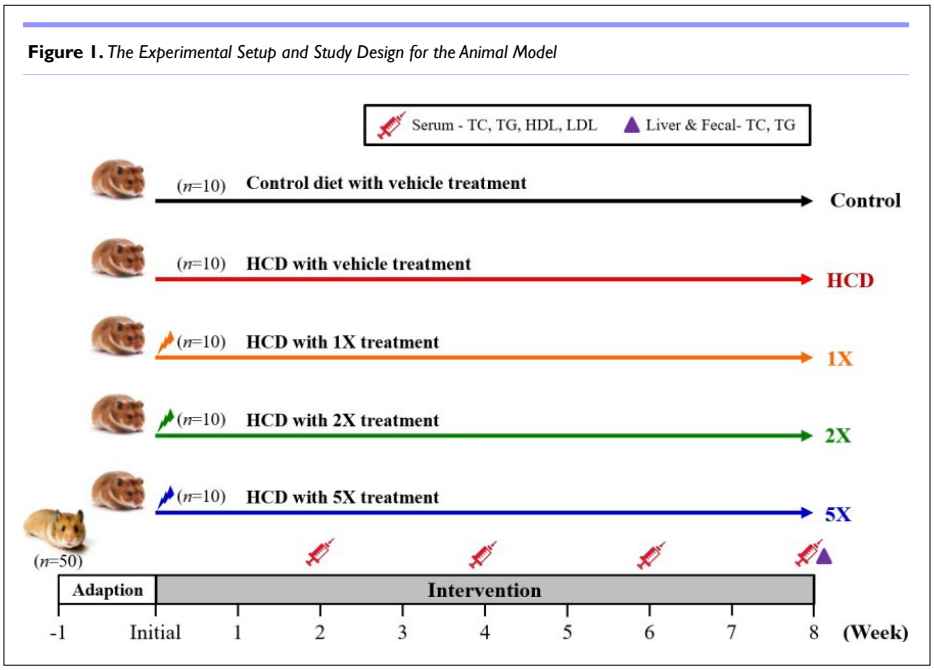


Figure 1. *The Experimental Setup and Study Design for the Animal Model*

Table 2. *Effect of LCC Supplementation on Body Weight, Body Composition and Diet Intake*

Characteristics	Control	HCD	LCC-1X	LCC-2X	LCC-5X
Initial BW (g)	85.7±4.2^{a}	85.5±5.5^{a}	85.4±8.1^{a}	85.9±4.7^{a}	85.4±5.8^{a}
Final BW (g)	101.1±6.7^{a}	118.2±9.5^{b}	114.4±5.0^{b}	116.2±7.0^{b}	111.5±9.1^{b}
Diet (g/mouse/day)	8.2±1.8^{a}	8.0±2.0^{a}	8.0±1.9^{a}	8.0±1.9^{a}	8.0±1.8^{a}
Calorie intake from diet (Kcal/mouse/day)	27.6±6.1^{a}	26.9±7.1^{a}	27.0±6.8^{a}	26.9±7.0^{a}	26.9±6.7^{a}
Water (ml/mouse/day)	14.7±2.9^{a}	14.5±3.0^{a}	14.5±3.0^{a}	14.6±2.4^{a}	14.9±2.6^{a}
Liver (g)	3.72±0.35^{a}	5.89±0.29^{d}	5.44±0.36^{c}	5.35±0.35^{bc}	5.13±0.34^{b}
Relative liver weight (%)	3.68±0.29^{a}	5.00±0.35^{c}	4.75±0.19^{bc}	4.62±0.42^{b}	4.62±0.45^{b}

Analysis Kit (Item No. 10010303).

Statistical Analysis

All values are expressed as Mean±SD and the number of hamsters in each group was 10. One-way analysis of variance (ANOVA) was performed using the statistical analysis software (SAS) computerized statistical package and Duncan's test was used to test for differences between treatments, with $p<0.05$ indicating statistical significance.

RESULTS

Effect of Supplementation of LCC on Body, Liver Weight and Diet Intake

As shown in Table 2, before the intervention, there was no significant difference in body weight in all groups, but after 8-weeks of intervention, the body weight of all hamsters in the HCD intake group was significantly higher than that in the normal diet group ($p<0.05$). However, all groups had no significant differences in diet, kcal, or water intake. In terms of liver weight, all groups ingesting HCD had significantly higher liver weight than the control group; however, compared with the HCD group, the LCC-1X, LCC-2X and LCC-5X groups had significant decreases of 7.61% ($p=0.0050$), 9.11% ($p=0.0010$) and 12.91% ($p<0.0001$), respectively. Similar results were found for the relative liver weight, LCC-2X and LCC-5X groups, which were significantly lower than the HCD group by 7.59% ($p=0.0232$) and 7.61% ($p=0.0199$), respectively.

Effect of 4-Week Supplementation of LCC on Lipid Concentrations in HCD-fed Hamsters

As shown in Figure 2A, after 4-weeks of the experiment, serum TG concentrations were significantly lower in the LCC-1X, LCC-2X and LCC-5X groups by 4.27% ($p=0.0048$), 4.74% ($p=0.0016$) and 4.25% ($p=0.0036$), respectively, compared to the HCD group. Thus, feeding HCD for 4 weeks significantly increased serum TG concentrations in animals, while supplementation with LCC-1X, LCC-2X and LCC-5X for 4-weeks effectively reduced the effect of HCD on elevated blood TG concentrations. The effect of LCC supplementation on blood TG was dose-dependent ($p<0.0001$).

Figure 2B shows the changes in serum TC concentrations in each group after 4-weeks of the experiment. The serum TC concentrations in the LCC-2X and LCC-5X supplementation groups were significantly reduced by 4.03% ($p=0.0001$) and 6.96% ($p<0.0001$), respectively, compared with those in the HCD

group. Therefore, supplementation with LCC-2X and LCC-5X for 4-weeks was effective in reducing the effect of elevated TC concentrations in the blood caused by HCD. The effect of LCC supplementation on blood TC was dose-dependent (p=0.0266).

Figure 2C shows the changes in serum HDL-C concentrations in each group of animals after 4-weeks of the experiment. There were no significant differences in mean serum HDL-C concentrations between the four groups fed the HCD, LCC-1X, LCC-2X and LCC-5X HCDs. Feeding HCD for 4-weeks caused an increase in serum HDL-C concentrations, whereas supplementation with LCC for 4-weeks had no significant facilitative or inhibitory effect on the HCD-induced increase in HDL-C concentrations.

Figure 2D shows the changes in serum LDL-C concentrations in each group of animals after 4-weeks of the experiment. Supplementation with LCC-1X, LCC-2X and LCC-5X for 4-weeks is effective in reducing the effect of elevated LDL-C concentrations in the blood caused by HCD. The effect of LCC supplementation on blood LDL-C was dose-dependent (p=0.0264).

As shown in Figure 2E, in the LDL-C/HDL-C ratio fraction of serum in all groups of animals after 4-weeks of the experiment, feeding HCD for 4-weeks was the main cause of the increase in serum LDL-C/HDL-C ratio, while supplementation with LCC-1X, LCC-2X and LCC-5X for 4-weeks was effective in preventing the HCD-induced increase in blood LDL-C/HDL-C ratio. Supplementation with LCC-1X, LCC-2X and LCC-5X for 4-weeks was effective in preventing the increase in LDL-C/HDL-C ratio caused by HCD. The effect of LCC supplementation on the LDL-C/HDL-C ratio was dose-dependent (p<0.0001).

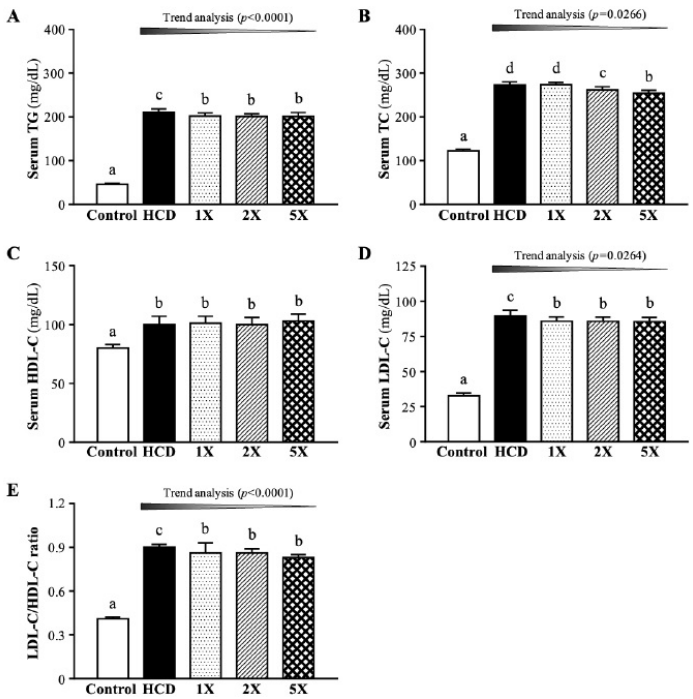
Effect of 6-week Supplementation of LCC on Lipid Concentrations in HCD-fed Hamsters

As shown in Figure 3A, after 6-weeks of the experiment, feeding HCD caused a significant increase in serum TG concentrations. Conversely, serum TG concentrations in the LCC-1X, LCC-2X and LCC-5X groups were significantly lower compared to the HCD group by 5.22% (p=0.012), 6.09% (p=0.003) and 5.22% (p=0.012), respectively. Thus, supplementation with LCC-1X, LCC-2X and LCC-5X for 6-weeks was effective in reducing the effects of elevated TG concentrations in the blood caused by HCD. On the other hand, Figure 3B shows serum TC concentrations were significantly reduced by 4.01% (p=0.0078), 16.04% (p<0.0001) and 15.24% (p<0.0001) in the LCC-1X, LCC-2X and LCC-5X supplemented groups, respectively, compared to the HCD group. Therefore, LCC-1X, LCC-2X and LCC-5X supplementation for 6-weeks were all effective in reducing the effects of elevated blood TC concentrations caused by HCD. The effect of LCC supplementation on blood TC was dose-dependent (p<0.0001).

Figure 3C shows the changes in serum HDL-C concentrations in each group of animals after 6-weeks of the experiment. Six-weeks of HCD feeding caused an increase in serum HDL-C concentrations in the animals, while six-weeks of LCC supplementation did not significantly promote or inhibit the HCD-induced increase in HDL-C concentrations.

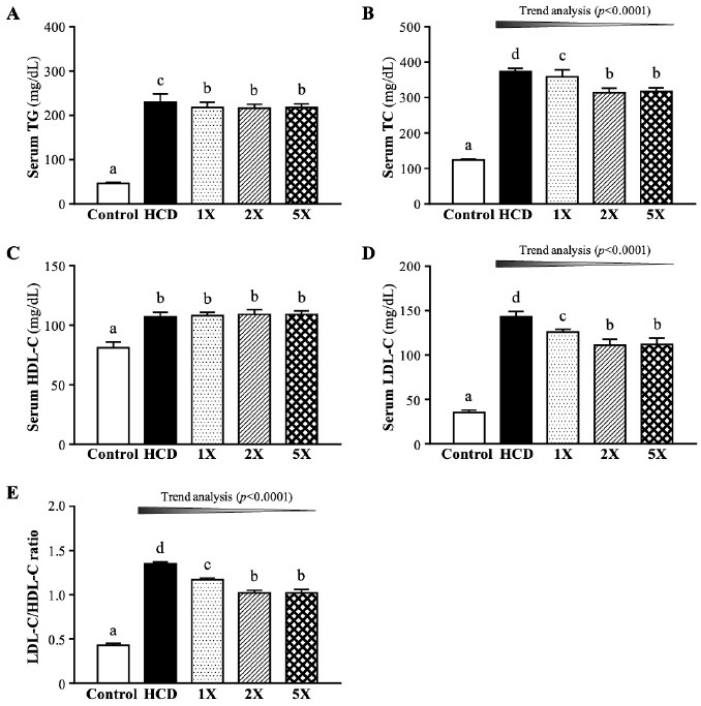
Figure 3D shows the changes in serum LDL-C concentration in each group of animals after 6-weeks of the experiment. Six-weeks of HCD feeding was the main reason for the increase in serum LDL-C concentration in animals, while six-weeks of

Figure 2. *Effect of 4-week Supplementation of LCC Capsules on Serum Triglyceride*



(A) Total cholesterol (B) HDL cholesterol (C) LDL cholesterol (D) Concentrations and LDL to HDL cholesterol ratio (E) High cholesterol-fed hamsters

Figure 3. *Effect of 6-week Supplementation of LCC Capsules on Serum Triglyceride*



(A) Total cholesterol (B) HDL cholesterol (C) LDL cholesterol (D) Concentrations, and LDL to HDL cholesterol ratio (E) High cholesterol-fed hamsters

LCC-1X, LCC-2X and LCC-5X supplementation were effective in reducing the effect of HCD on the increase in blood LDL-C concentration. The effect of LCC supplementation on blood LDL-C was dose-dependent (p<0.0001).

As shown in Figure 3E, the LDL-C/HDL-C ratio in the serum of each group of animals after 6-weeks of the experiment showed that feeding HCD for 6-weeks was the main cause of the increase in serum LDL-C/HDL-C ratio, while supplementation with LCC-1X, LCC-2X and LCC-5X for 6-weeks was effective in preventing the increase in LDL-C/HDL-C ratio induced by HCD. The effect of LCC supplementation on LDL-C and HDL-C was dose-dependent (p<0.0001).

Effect of 8-week Supplementation of LCC on Lipid Concentrations in HCD-fed Hamsters

As shown in Figure 4A, after 8-weeks of the experiment, serum TG concentrations were significantly reduced by 14.14%, 19.66% and 21.03% in the animals of each group supplemented with LCC-1X, LCC-2X and LCC-5X, respectively, compared with the HCD group (p<0.0001). Thus, LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks was effective in reducing the effects of HCD-induced elevated TG concentrations in the blood. The effect of LCC supplementation on blood TG was dose-dependent (p<0.0001).

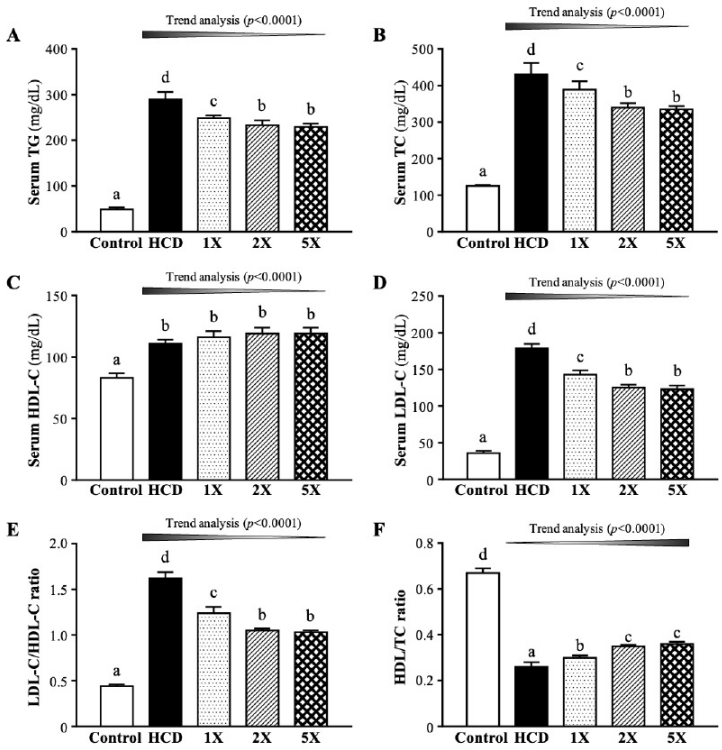
Figure 4B shows the changes in serum TC concentration in each group of animals after 8-weeks of the experiment. The serum TC concentrations in the LCC-1X, LCC-2X and LCC-5X supplementation groups were significantly reduced by 9.74%, 21.11% and 22.27%, respectively, compared with those in the HCD group (p<0.0001). Therefore, LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks can effectively reduce the effect of elevated TC concentrations in the blood caused by HCD. The effect of LCC supplementation on blood TC was dose-dependent (p<0.0001).

Figure 4C shows the changes in serum HDL-C concentrations in each group at the end of 8-weeks of the experiment. The HDL-C concentrations in the LCC-1X, LCC-2X and LCC-5X supplementation groups increased significantly by 4.66% (p=0.0038), 7.38% (p<0.0001) and 7.45% (p<0.0001), respectively, compared with the HCD group. Therefore, LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks was effective in enhancing the reduction of the HDL-C ratio in HCD induction. The effect of LCC supplementation on blood HDL-C was dose-dependent (p<0.0001).

Figure 4D shows the changes in serum LDL-C concentrations in each group after 8-weeks of the experiment. The serum LDL-C concentrations in the LCC-1X, LCC-2X and LCC-5X supplementation groups were significantly reduced by 20.01%, 30.07% and 31.53% (p<0.0001) compared with those in the HCD group. Therefore, LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks were all effective in reducing the effect of HCD on elevated LDL-C concentrations in the blood. The effect of LCC supplementation on blood LDL-C was dose-dependent (p<0.0001).

As shown in Figure 4E, at the end of 8-weeks of the experiment, the LDL-C/HDL-C ratios in the serum of animals in each group were significantly reduced by 23.46%, 35.19% and 36.42% in the LCC-1X, LCC-2X and LCC-5X supplemented

Figure 4. *Effect of 8-week Supplementation of LCC Capsules on Serum Triglyceride*



(A) Total Cholesterol (B) HDL Cholesterol (C) LDL Cholesterol (D) Concentrations and LDL to HDL Cholesterol Ratio (E)HDL Cholesterol to Total Cholesterol Concentration Ratio (F) High Cholesterol-fed Hamsters

groups, respectively, compared with the HCD group (p<0.0001). Thus, LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks were all effective in reducing the LDL-C/HDL-C ratio in the blood caused by HCD. The effect of LCC supplementation on the LDL-C/HDL-C ratio was dose-dependent (p<0.0001).

As shown in Figure 4F, after 8-weeks of the experiment, the HDL-C/TC ratios in the serum of animals in each group were significantly higher in the LCC-1X, LCC-2X and LCC-5X supplemented groups than in the HCD group by 1.15, 1.35 and 1.38-fold (p<0.0001). Thus, supplementation with LCC-1X, LCC-2X and LCC-5X for 8-weeks was effective in increasing the HDL-C/TC ratio in the blood caused by HCD. The effect of LCC supplementation on HDL-C/TC ratio was dose-dependent (p<0.0001).

Effect of 8-week LCC Supplementation on Liver Lipid Content in HCD-fed Rats

Figure 5A shows the changes in liver TG content in each group after 8-weeks of the experiment. The liver TG content in the LCC-1X, LCC-2X and LCC-5X supplemented groups was significantly lower than that in the HCD group by 20.86%, 25.67% and 35.29% (p<0.0001). Therefore, LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks were all effective in reducing the effect of HCD on elevated liver TG-levels. The effect of LCC supplementation on liver TG was dose-dependent (p<0.0001).

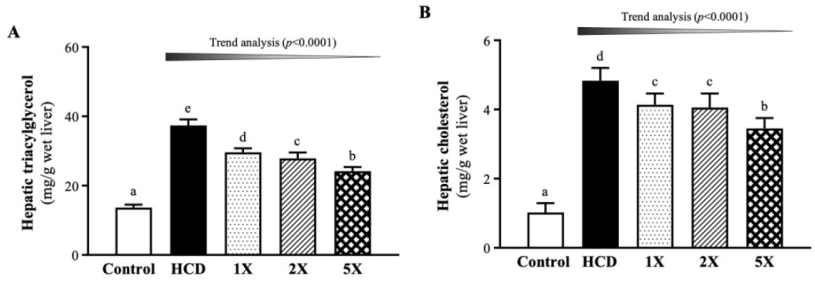
Figure 5B shows the changes in liver TC content in each group after 8-weeks of the experiment. The liver TC content in the LCC-1X, LCC-2X and LCC-5X supplementation groups was significantly lower than that in the HCD group by 14.18%, 15.67% and 28.61% (p<0.0001). Therefore, LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks were all effective in reducing the effect of HCD on elevated liver TC-levels. The effect of LCC supplementation on liver TC was dose-dependent (p<0.0001).

Effect of Supplemental 8-week LCC on Fecal Lipid Content in HCD-fed Hamsters

Figure 6A shows the changes in TG content in the feces of each group at the end of 8-weeks of the experiment. The TG content in the feces of the LCC-2X and LCC-5X supplemented groups increased significantly by 1.33-fold (p=0.0005) and 1.39-fold (p<0.0001) compared with that of the HCD group. Therefore, supplementation with LCC-2X and LCC-5X for 8-weeks was effective in increasing TG excretion in feces. The effect of LCC supplementation on fecal TG was dose-dependent (p<0.0001).

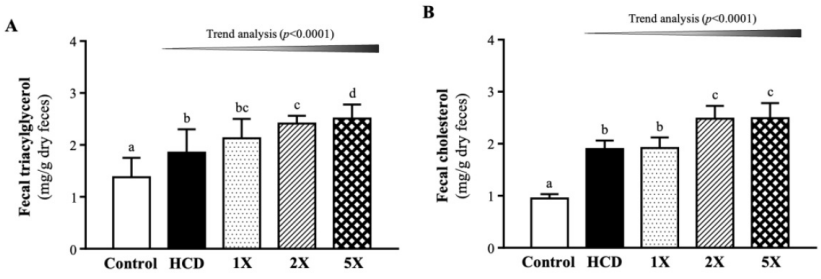
Figure 6B shows the changes in TC content in the feces of each group after 8-weeks of the experiment and the TC content in the feces of both LCC-2X and LCC-5X supplemented groups increased significantly by 1.31-fold compared with the HCD group (p<0.0001). Therefore, supplementation with LCC-2X and LCC-5X for 8-weeks could effectively improve TC excretion in feces. The effect of LCC supplementation on fecal TC was dose-dependent (p<0.0001).

Figure 5. *Effect of 8-week Supplementation with LCC Capsules on Triglyceride*



(A)Total cholesterol (B) Levels in the liver of high cholesterol-fed hamsters

Figure 6. *Effect of 8-week Supplementation with LCC Capsules on Triglyceride*



(A)Total cholesterol (B) Levels in the feces of high cholesterol-fed hamsters

DISCUSSION

Hypercholesterolemia is one of the major risk factors for atherosclerosis and ischemic diseases such as myocardial infarction and cerebral infarction. Dyslipidemia is an important risk factor for the development of atherosclerosis and eventual coronary artery disease. Elevated concentrations of LDL-C, TC and TG, known as hyperlipidemia, are a form of dyslipidemia. Dyslipidemia is a recognized modifiable cardiovascular risk factor.[30]

In this study, in order to understand the effect of LCC on the regulation of blood lipids, HCDs containing 0.2% cholesterol and 5% lard were used to induce hyperlipidemia in hamsters. Hamsters were fed a chow diet containing 0.5% or 1% cholesterol and a significant increase in serum TC concentrations was observed (Technical Sheet: Hamster Hypercholesterolemia Model). In this study, we found that serum LDL-C, TC and TG concentrations were significantly reduced after 4-weeks of LCC supplementation, indicating that LCC had a significant inhibitory effect on the increase in LDL-C, TC and TG concentrations induced by HCDs.

In the results of this trial, LCC supplementation was found to inhibit TG and TC-levels in the liver. Cholesterol in the body is derived from food and products of the cholesterol biosynthesis pathway in the liver. According to previous literature, the reason for the decrease in TC is that HDL-C transports cholesterol from peripheral cells, including blood vessel walls, to the liver, where it is synthesized into bile and then secreted into the intestine for excretion in the feces, resulting in a decrease in blood cholesterol and therefore an increase in fecal cholesterol.[31,32] Red yeast rice is a traditional Chinese fermented product that contains naturally occurring statins, which are known to have lipid-lowering properties.[33] Previous studies have also shown that red yeast rice can contribute to a decrease in TC in the blood, interfere with the absorption of cholesterol in the body and also bind with bile acids and bile salts to eliminate them from the body and promote the oxidation of cholesterol in the liver to produce bile acids in order to maintain a certain concentration of bile acids, increasing cholesterol consumption.[34] Another study also showed that feeding a 0.1% HCD induced hyperlipidemia in hamsters, while another group was fed a diet supplemented with red yeast rice powder, which showed lower plasma TC, TG and LDL-C concentrations than hyperlipidemic hamsters at weeks 4 and 8.[35] The mechanism behind the lipid-lowering effects of red yeast rice is attributed to the presence of monacolin K, which is structurally similar to the statin drug lovastatin.[36] Monacolin K inhibits the enzyme HMG-CoA reductase, which plays a key role in cholesterol synthesis in the liver. By inhibiting this enzyme, red yeast rice reduces cholesterol production and promotes its clearance from the bloodstream.[37] A past study showed that taking 3-10 mg of monacolin K daily had the lowest risk and reduced plasma LDL-C levels by 15-25% over 6-8-weeks.[36]

Other studies have systematically evaluated the effects of bergamot on lipid parameters in humans. According to the data, 75% of the studies showed significant reductions in TC, TG and LDL-C. The percentage reduction in TC ranged from 12.3 to 31.3%, in LDL-C from 7.6 to 40.8% and in TG from 11.5 to 39.5%. Eight trials reported an increase in HDL-C after the berga-

mot intervention.[38] The pine bark extract mainly contains proanthocyanidins, which have a variety of physiological effects, including antioxidant and anti-inflammatory ones. In other studies, pine bark extract was investigated for its anti-atherosclerotic effects in apoE-deficient mice and was found to reduce the area of heart valve lesions and serum and liver cholesterol levels in male mice.[39] Vitamin E is a fat-soluble antioxidant vitamin and alpha-tocopherol is its most naturally abundant and active form. Oxidation is a key step in the formation of atherosclerosis. Vitamin E has been shown to increase antioxidant properties *in vitro* and prevent the formation of atherosclerotic plaques in an experimental mouse model. Consumption of foods rich in vitamin E is associated with a lower risk of coronary heart disease in middle-aged and older men and women.[40] Preclinical studies have shown that sesame and its lignans induce beneficial changes in risk factors associated with CVD and the results suggest that sesame supplementation may reduce serum TC, LDL-C and lipid peroxidation and increase antioxidant status in hyperlipidemic patients.[41]

CONSTRAINTS

At present, we can see the initial significant results of animal experiments. In the future, we can observe the long-term effect of human consumption and whether it can regulate lipid-related problems is worth further investigation.

CONCLUSION

The results of the study showed that LCC-1X, LCC-2X and LCC-5X supplementation for 8-weeks had a significant effect on preventing HCD-induced weight gain and liver weight gain. In addition, a number of clinical lipid biochemical values showed that supplementation with LCC-1X, LCC-2X and LCC-5X could help reduce the increase in serum TG, TC, LDL-C, LDL-C/HDL-C ratio, liver TG and liver TC caused by a HCD and increase the amount of TC excreted in feces. Therefore, it can be concluded that the recommended daily intake of 2 (1000 mg internal volume) LCC for adults can help to (1) lower serum TG; (2) lower serum TG; and (3) lower LDL-C, which can further achieve the purpose of regulating blood lipid function.

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DISCLOSURE

I, the corresponding author, have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis

CONFLICTS OF INTEREST

We have no conflicts of interest to disclose. All authors declare that they have no conflicts of interest. The authors have not received personal financial gain from the sales of the LCC capsules. All findings and views expressed in this paper are those of the authors and do not necessarily reflect the view of HealthTake Corporation. All the authors consent to participate in the research project and the following has been explained to the authors: the research may not be of direct benefit to all the authors. All the authors' participation is completely voluntary. All the authors right to withdraw from the study at any time without any implications to the authors.

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