Universal Library of Medical and Health Sciences Research Article

ISSN: 3064-6545 | Volume 3, Issue 2

Open Access | PP: 09-16

DOI: https://doi.org/10.70315/uloap.ulmhs.2025.0302002

Influence of the Anti-Hyperlipidemia Effect of Fish Oil Supplement in High-Cholesterol Diet Rats

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Abstract

Complex fish oil capsule have been widely reported as a useful supplement to reduce fasting blood triglyceride levels in individuals with hyperlipidemia in high cholesterol diet (HCD) mice. The mice difference five groups (control, HCD, complex fish oil capsule dose 1X-flod, 2X-flod and 5X-flod) for six weeks. The evaluate complex fish oil use blood biochemical values, liver and feces assay. Thus feed a high-cholesterol diet resulted in significant dyslipidemia and fatty liver formation in experimental animals. Supplementation with 1, 2, or 5X-flod the dose of FO for 6-weeks resulted in a significant reduced 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 ($p<0.05$). By the experimental data can help reduce serum TG, TC and LDL-C concentrations, leading to a healthy effect on cardiovascular disease prevention.

Keywords: *Fish Oil; Lipid; Cardiovascular Diseases; Hyperlipidemia; High Cholesterol Diet.*

INTRODUCTION

Lipids are essential nutrients that provide critical energy and fatty acids, playing a significant role in various physiological and metabolic functions[1].Recent year hyperlipidemia effects many diseases such as Diabetes mellitus (DM), Cardiovascular diseases (CAD), and Hypertension. High concentrations of serum cholesterol, especially LDL-cholesterol, have been implicated as a high-risk factor for cardiovascular diseases as the deposition of oxidized low density lipoproproteins (LDL) leads to plaque formation and thickening of the arteries, resulting in cardiovascular complications[2-5]. Recently, reports correlating improvement in cardiovascular health with the administration of botanical dietary supplements have been on a continual increase[6-8]. According to data from the Global Burden Disease, Injuries, and Risk Factors Study (GBD) 2019, the total number of CVD cases (272 million to 523 million), death (12.1 million to 18.6 million), and disability-adjusted life years (DALY, 279.8 million to 393.1 million) increased significantly in the overall population from 1990 to 2019[9].

Over the past 20 years, there has been a dramatic increase in the scientific scrutiny of and public interest in omega-3 and omega-6 fatty acids and their impact on personal health. Omega-3 fatty acids possess anti-inflammatory, anti-arrhythmic, and anti-thrombotic properties; omega-6 fatty acids are proinflammatory and prothrombotic[10-13].

Omega-3 fatty acids to reduce blood lipids and prevent events of atherosclerotic cardiovascular diseases[14-16]. Also the fish oils have been widely reported as a useful supplement to reduce fasting blood triglyceride levels in individuals with hyperlipidemia[17].

Astaxanthin is a xanthophyll carotenoid pigment found in marine animals, and in addition to inhibition of lipid peroxidation and LDL-oxidation, astaxanthin has been reported to decrease serum TG and increase HDL-cholesterol and adiponectin in insulin resistant rats and in obese mice fed a high fat diet[18]. The other hand supplementation of nature astaxanthin increases serum HDL-cholesterol and increase in serum adiponectin levels in humans[19-21]. Even now astaxanthin is a natural antioxidant can help cardiovascular risk in individuals with prediabetes and dyslipidemia[22,23].

Sesame seeds (Sesamum indicum) a member of the Pedaliaceae family is regarded as the oldest oil-yielding seed crop known to humanity. The oil seed is due to its wide spectrum of pharmacological activities, therapeutic and nutritional importance which includes cholesterol-lowering effect and prevention of high blood pressure. Dietary sesame seeds reduce serum total cholesterol and LDL cholesterol concentrations in hypercholesterolemic patients[24-27].

Vitamin E is transported within lipoproteins while circulating in the blood. As a consequence, vitamin E concentration

Citation: Wan-Li Chu, Yi-Ju Hsu, Chi-Chang Huang, Yu-Chin Lin, Yu-Jou Chien, "Influence of the Anti-Hyperlipidemia Effect of Fish Oil Supplement in High-Cholesterol Diet Rats", Universal Library of Medical and Health Sciences, 2025; 3(2): 09-16. DOI: https://doi.org/10.70315/uloap.ulmhs.2025.0302002.

is closely correlated with that of cholesterol and total lipid. Vitamin E distribution is related to the kinetics of lipoprotein metabolism, abnormalities of the plasma lipid status associated with hyperlipidemia may affect functional vitamin E status[28]. Tocotrienols have been shown to regulate cholesterol metabolism by reducing the oxidation of LDL-C and by inhibiting the expression of HMG-CoA reductase[29]. Animal studies have also supported the preventive role of tocotrienols in the progression of atherosclerosis[30,31].

Although the individual components of FO 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 FO on lipid regulation in a hamster model of hyperlipidemia induced by a high-cholesterol diet (HCD).

MATERIAL & METHODS

Sample Treatment

The Fish oil complex (FO) supplement was provided by HealthTake Co., Ltd. (500 mg per capsule). The formula contained sesame extract, astaxanthin extract and vitamin E.

Animal Design

Forty-five male hamsters, aged 8-weeks, were purchased from the National Applied Research Laboratories and randomly assigned to five groups of 9 hamsters each. 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-11107) 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 FO tested: low (FO-1X), medium (FO-2X) and high (FO-5X). Since this is a hamster animal model test, to ensure that each hamster receives the recommended amount of FO 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 FO had significantly lower lipid levels than the control group fed a HCD ($p<0.05$) Figure 1.

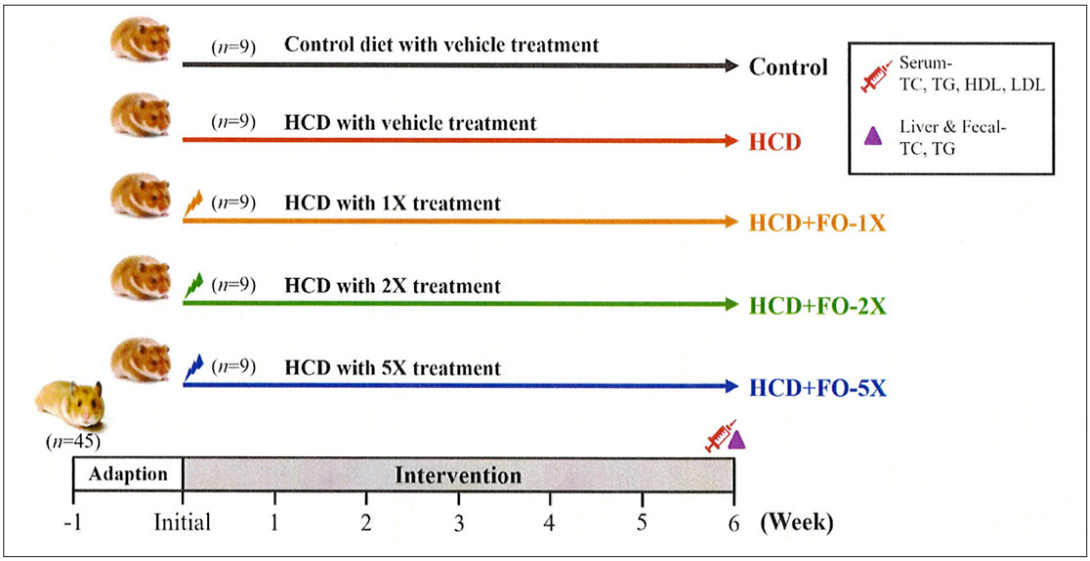

Figure 1. Schematic Representation of the Experimental Design Time line

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.{ Alpha-linolenic acid but not conjugated linolenic acid is hypocholesterolaemic in hamsters; Trans-8, cis-10+ cis-9, trans-11-conjugated linoleic acid mixture alters body composition in Syrian golden hamsters fed a hypercholesterolaemic diet.; Hypoilpidemic effects and safety of Lactobacillus reuteri 263 in a hamster model of hyperlipidemia. }

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

The liver of 10 mg was taken and 1 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 400 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. TG was then analyzed with the RANDOX assay kit (GPO-PAR kit, TR1697). Total Cholesterol RANDOX assay Kit (CHOD-PAP, CH3810).

Determination of TC and TG Concentrations in Feces

The feces were 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. TG was then analyzed with the RANDOX assay kit (GPO-PAR kit, TR1697). Total Cholesterol RANDOX assay Kit (CHOD-PAP, CH3810).

Pathological Examination

The liver tissue was fixed by formalin, embedded in paraffin, and sliced. Two staining methods were used: a general Hematoxylin and Eosin stain (H.E. stain). The liver injuries were divided into four classes and graded according to their pathology.

Statistical Analysis

All values are expressed as mean ± SD and the number of hamsters in each group was 9. 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 FO on Body Weight and Diet Intake

As shown in Table 1, before the intervention, there was no significant difference in body weight in all groups, but after 5-weeks of intervention, the body weight of all hamsters in the HCD intake group was significantly higher than the normal diet group ($p<0.05$). However, compared with the HCD group, the FO-1X, FO-2X and FO-5X groups had significant enhance of 1.09-flod, 1.08-flod, 1.07-flod and 1.06-flod(P<0,0001), but supplementation of FO-5X the body weight compared to the HCD group had reduce 2.61%(p=0.0193).

Table 1. Effect of FO supplement on growth parameters in high-fat diet-induced obese rats

Body weight (g)	ND	HCD			
		Control	FO-1X	FO-2X	FO-5X
Week 0	120.2±4.1^{a}	120.1±3.9^{a}	120.1±3.1^{a}	120.1±2.0^{a}	120.4±1.3^{a}
Week 1	122.0±4.1^{a}	123.6±3.8^{a}	123.1±3.0^{a}	123.4±2.1^{a}	124.0±1.2^{a}
Week 2	124.4±4.0^{a}	127.8±3.7^{a}	127.2±2.9^{b}	127.3±2.3^{a}	127.7±1.3^{a}
Week 3	126.9±3.9^{a}	132.8±3.4^{b}	131.5±2.9^{b}	131.5±2.5^{b}	131.4.±1.6^{b}
Week 4	129.3±4.0^{a}	138.1±3.1^{b}	136.5±2.8^{b}	136.1±3.0^{b}	135.7±1.8^{b}
Week 5	131.9±4.0^{a}	143.0±3.1^{c}	141.7±2.9^{abc}	140.7 ±3.4^{abc}	140.0±2.0^{b}
Week 6	134.6±4.1^{a}	147.7±3.0^{c}	146.0±3.2^{abc}	144.8±3.9^{abc}	143.8±2.3^{b}
Final	133.8±4.1^{a}	145.6±3.1^{c}	144.3±3.2^{abc}	142.8±3.8^{abc}	141.8±2.3^{b}

The reported values are the mean ± SD (n=9). Different superscript letters (a, b, and c) indicate significant differences between groups ($p < 0.05$). BW change = final BW - induced BW. Feed efficiency (%) = [Body weight change (g) ÷ total feed intake (g)] × 100%. BW, body weight.

Effect of 6-Week Supplementation of FO on Lipid Concentrations in HCD-fed Hamsters

As shown in Figure 2A, after 6-weeks of the experiment, serum TG concentrations were significantly improved in the HCD, FO-1X, FO-2X and FO-5X groups by 2.62-flod, 2.24-flod, 2.12-flod and 2.0-flod ($p<0.0001$), respectively, compared to the

control group. Thus, feeding HCD for 6-weeks significantly increased serum TG concentrations in animals. In contrast supplementation with FO-1X, FO-2X and FO-5X for 6-weeks effectively reduced by 14.55%,19.09% and 23.64% ($p<0.0001$), respectively, compared to the HCD group. The effect of FO supplementation on blood TG was dose-dependent ($p<0.0001$).

As shown in Figure 2B, after 6-weeks of the experiment, serum TC concentrations were significantly raised in the HCD, FO-1X, FO-2X and FO-5X groups by 3.76-flod, 3.39-flod, 3.27-flod and 3.16-flod ($p<0.0001$), respectively, compared to the control group. Thus, feeding HCD for 6-weeks significantly increased serum TC concentrations in animals. The other hand supplementation with FO-1X, FO-2X and FO-5X for 6-weeks effectively reduced by 9.86%,13.15% and 15.89% ($p<0.0001$), respectively, compared to the HCD group. The effect of FO supplementation on blood TC was dose-dependent ($p<0.0001$).

Figure 2C shows the changes in serum HDL-C concentrations in each group of animals after 6-weeks of the experiment. There were no significant differences in mean serum HDL-C concentrations between the four groups fed the HCD, FO-1X, FO-2X and FO-5X groups. Feeding HCD for 6-weeks caused an increase in serum HDL-C concentrations, whereas supplementation with FO for 6-weeks had no significant facilitative or inhibitory effect on the HCD-induced increase in HDL-C concentrations ($p<0.0001$).

Figure 2D shows the changes in serum LDL-C concentrations in each group of animals after 6-weeks of the experiment. Supplementation with FO-1X, FO-2X and FO-5X for 6-weeks is effective in raising the effect of elevated LDL-C concentrations in the blood caused by HCD. The effect of FO supplementation on blood LDL-C was dose-dependent($p<0.0001$).

As shown in Figure 2E, in the LDL-C/HDL-C ratio fraction of serum in all groups of animals after 6-weeks of the experiment, feeding HCD for 6-weeks was the main cause of the enhance in serum LDL-C/HDL-C ratio, while supplementation with FO-1X, FO-2X and FO-5X for 6-weeks was effective in preventing the HCD-induced increase in blood LDL-C/HDL-C ratio. Supplementation with FO-1X, FO-2X and FO-5X for 6-weeks was effective in preventing the improve in LDL-C/HDL-C ratio caused by HCD. The effect of FO supplementation on the LDL-C/HDL-C ratio was dose-dependent ($p<0.0001$).

As shown in Figure 2F, in the LDL-C/TC ratio fraction of serum in all groups of animals after 6-weeks of the experiment, feeding HCD for 6-weeks was the main cause of the decreased in serum LDL-C/TC ratio, while supplementation with FO-1X, FO-2X and FO-5X for 6-weeks was effective in preventing the HCD-induced increase in blood LDL-C/TC ratio. The other hand supplementation with HCD, FO-1X, FO-2X and FO-5X for 6-weeks effectively reduced by 53.57%, 50.0%, 48.21% and 44.64% ($p<0.0001$), respectively, compared to the HCD group. The effect of FO supplementation on the LDL-C/HDL-C ratio was dose-dependent ($p<0.0001$).

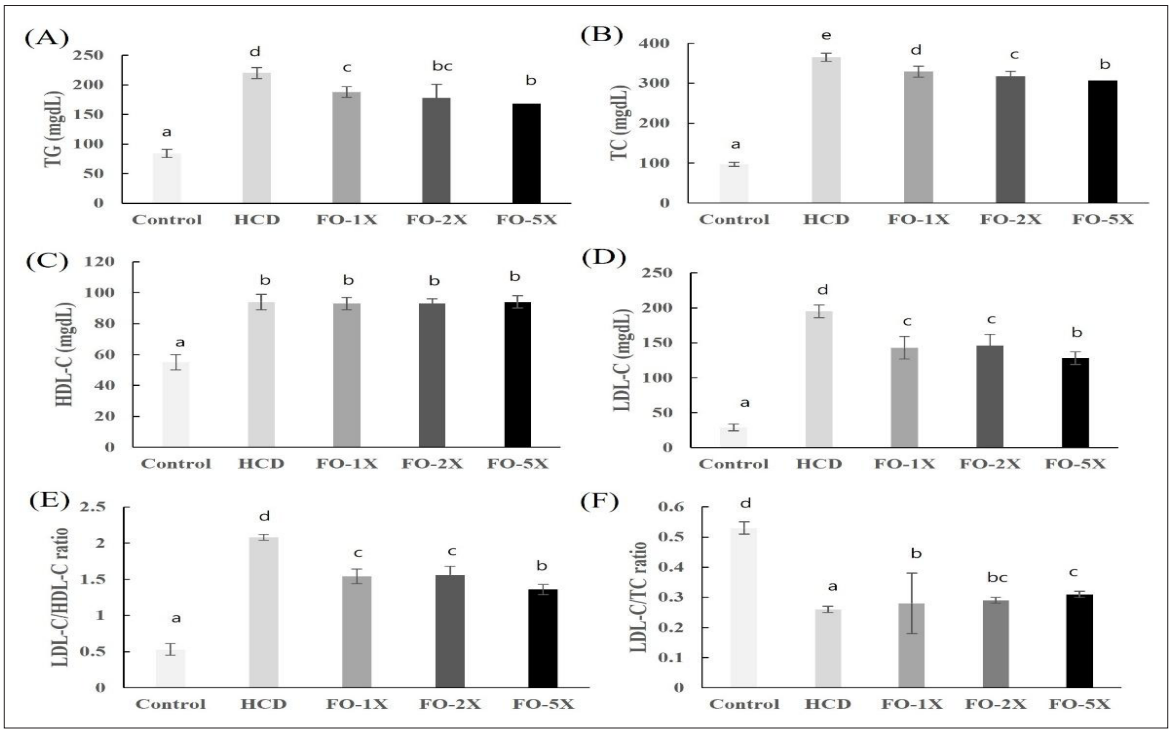

Figure 2. Effect of FO supplement on serum biochemical parameters in high-fat diet-induced obese rats.

The reported values are the mean ± SD (n=9). Different superscript letters (a, b, c and d) indicate significant differences between groups ($p < 0.05$). TC, total cholesterol. TG, triglyceride. LDL-C, low density lipoprotein-cholesterol. HDL-C, high density lipoprotein-cholesterol. LDL-C/HDL-C ratio, low density lipoprotein-cholesterol/ high density lipoprotein-cholesterol ratio. LDL-C/TC ratio, low density lipoprotein-cholesterol/ total cholesterol ratio.

Effect of 6-week FO Supplementation on Liver Lipid Content in HCD-fed Rats

Figure 3A shows the changes in liver TG content in each group after 6-weeks of the experiment. The liver TG content was significantly raised in the HCD, FO-1X, FO-2X and FO-5X groups by 4.47-flod, 4.01-flod, 3.78-flod and 3.22-flod ($p<0.0001$), respectively, compared to the control group. Thus, feeding HCD for 6-weeks significantly increased liver TG content in animals. The liver TG content in the FO-2X and FO-5X supplemented groups was significantly lower than that in the HCD group by 15.57% ($p=0.0072$) and 27.90% ($p<0.0001$).

Figure 3B shows the changes in liver TC content in each group after 6-weeks of the experiment. The liver TC content in the FO-1X, FO-2X and FO-5X supplementation groups was significantly lower than that in the HCD group by 21.17%, 33.06% and 35.09% ($p<0.0001$). Therefore, FO-1X, FO-2X and FO-5X supplementation for 6-weeks were all effective in reducing the effect of HCD on elevated liver TC-levels. The effect of FO supplementation on liver TC was dose-dependent ($p<0.0001$).

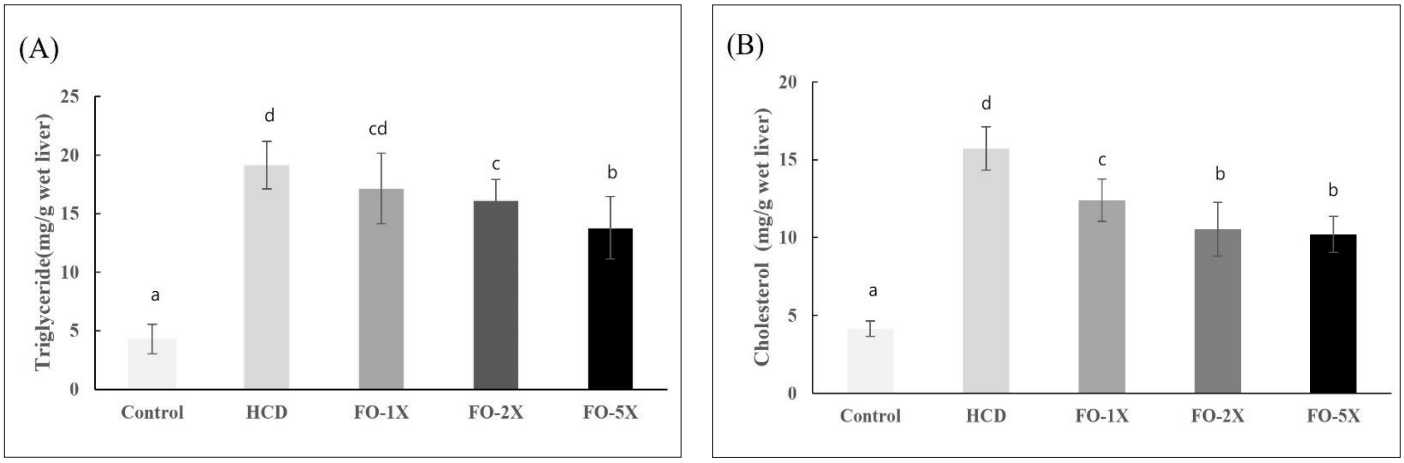

Figure 3. Effect of FO supplement on liver weight (A) triglyceride (B)cholesterol level in high-fat diet-induced obese rats.

The reported values are the mean ± SD (n=9). Different superscript letters (a, b, c, and d) indicate significant differences between groups ($p < 0.05$).

Effect of Supplemental 6-week FO on Fecal Lipid Content in HCD-fed Hamsters

Figure 4A shows the changes in TG content in the feces of each group at the end of 6-weeks of the experiment. The TG content in the feces of the HCD, FO-1X, FO-2X and FO-5X supplemented groups enhance significantly by 3.22-fold, 3.51-flod, 3.71-fold and 3.91-flod ($p<0.0001$) compared with that of the control group. Therefore, supplementation with FO-5X for 6-weeks was effective in increasing TG excretion in feces. The effect of FO supplementation on fecal TG was dose-dependent.

Figure 4B shows the changes in TC content in the feces of each group after 6-weeks of the experiment and the TC content in the feces of FO-1X, FO-2X and FO-5X supplemented groups promote significantly by 1.24-fold ($p=0.0004$),1.31-flod ($p<0.0001$) and 1.28-flod ($p<0.0001$) compared with the HCD group. These results supplemented FO-1X, FO-2X and FO-5X were effective raise TC content in feces.

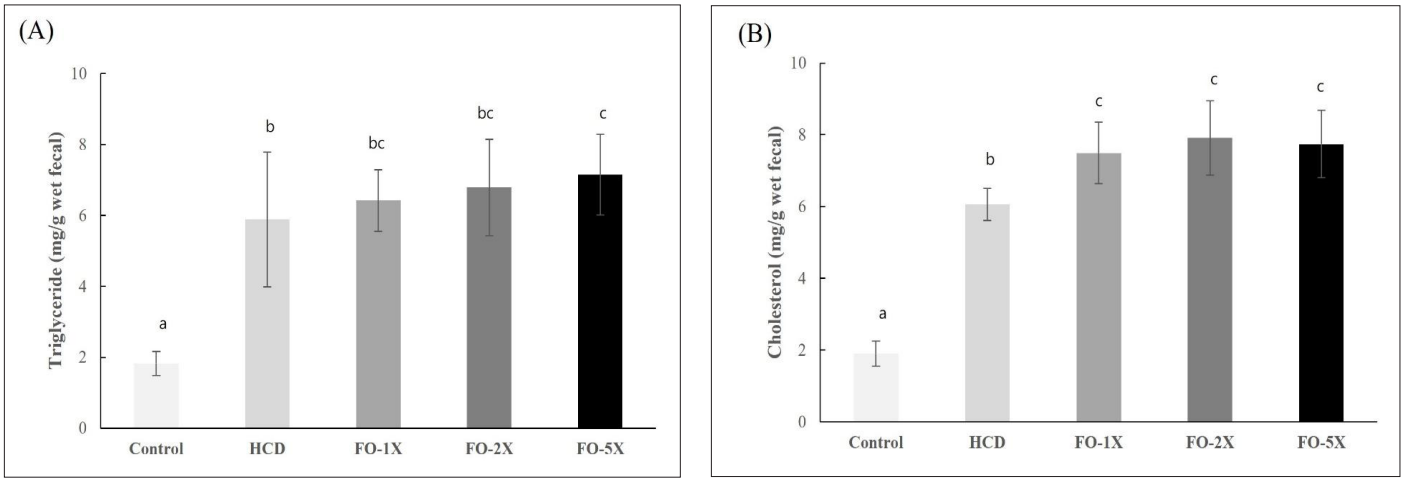

Figure 4. Effect of FO supplement on fecal weight (A) triglyceride (B)cholesterol level in high-fat diet-induced obese rats.

The reported values are the mean ± SD (n=9). Different superscript letters (a, b, c, and d) indicate significant differences between groups ($p < 0.05$).

Pathological Changes

As shown in Figure 5, the HCD-induced in the rats stained by H.E. showed apparent tissue vacuolization and necrosis (Figure 5B). The liver tissue vacuolization and necrosis degrees of the three dose groups of FO-1X, FO-2X and FO-5X. These results supplemented FO-1X, FO-2X and FO-5X were effective reduce vacuolization in liver.

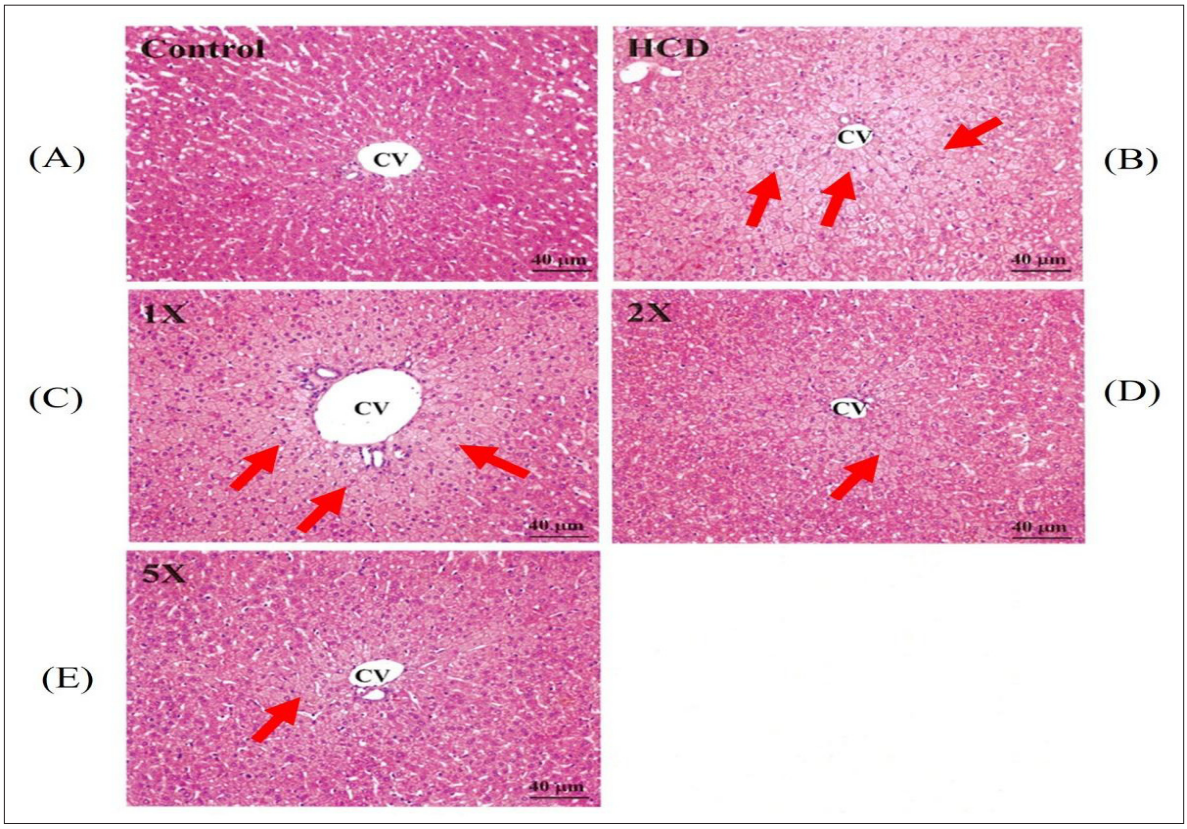

Figure 5. Rat Liver Tissue Structure (H.E. staining)

(A) Normal liver tissue structure of the normal control group .

(B) Liver vacuolization and necrosis of the HCD group.

(C) Liver fibrosis of the low dose group.

(D) Liver fibrosis of the medium dose group.

(E) Liver fibrosis of the high dose group.

CV: central veins

DISCUSSION

Hyperlipidemia is one of the major risk factors for atherosclerosis and ischemic diseases such as myocardial infarction and cerebral infarction. Elevated concentrations of LDL-C, TC and TG, known as hyperlipidemia, are a form of dyslipidemia. Dyslipidemia is a recognized modifiable cardiovascular risk factor.

In this study in order to understand the effect FO supplementation on the regulation of blood lipids. We found that serum LDL-C, TC and TG concentrations were significantly reduced after 6-weeks of FO supplementation, indicating that FO had a significant inhibitory effect on the enhanced in LDL-C, TC and TG concentrations induced by HCD.

Over the 20 years many people profession knowledge fish oil supplements appropriate to dyslipidemia. Then this study fish oil can reduce the LDL-C, TG and TC concentrations induce by HCD animals. In human for clinical trial demonstrate fish oil of omega-3 polyunsaturated fatty acids on blood were reduce triacylglycerol levels in normolipidemic and hyperlipidemic. Other studies verify supplements 2g/day of fish oil was maintenance of cardiovascular health and disease prevention[32-34]. Another study in human clinical trial demonstrate fish oil EPA/DHA ratios verify supplements 12 g/day of EPA-rich (EPA/DHA: 2.3) or DHA-rich (EPA/DHA: 0.3) fish oil for 8-weeks.The results fish oil EPA/DHA ratios effects on total lipids/lipoproteins, but differences were observed in lipoprotein subfraction composition and assignation, which could influence on the use of EPA versus DHA for ameliorating cardiovascular health[33].

Lipoproteins mainly function in maintaining lipid homeostasis by transporting lipids (including cholesterol and TGs) through the vascular and extravascular fluids in TC, phosphoilpids

and LDL- cholesterol levels are correlated with reduced vulnerability to atherosclerosis and other degenerative cardiovascular diseases[35]. In our study FO supplementation including fish oil, astaxanthin extract, sesame extract, and vitamin E related regulation lipids through the vascular and extravascular fluids in the body[4,5,35].

Astaxanthin has been reported to improve dyslipidemia and metabolic syndrome in animal models. The other hand the astaxanthin supplementation for eight weeks on cardiometabolic risk factors can reduce TC and LDL-C concentrations in human clinical trial. The present study suggests that astaxanthin can improve the serum lipid profile in humans, including an increase in HDL-cholesterol, for atherosclerotic cardiovascular disease through a variety of mechanisms, including reverse cholesterol transport from peripheral tissues to liver[36,37]. Our study pathological changes show FO supplementation can significantly decrease vacuolization in liver tissue.

Sesame seeds in general and its major lignan sesamin in particular, have been associated with various biochemical actions, mainly related to lipid metabolism, including a hypocholesterolemic effect in both animal model and human trials[26,38]. The sesame seeds can effect on serum cholesterol and triglycerides levels in animal rat. The present study demonstrates the hypoilpidemic potency of sesamin in ameliorating hyperlipidemia and its associated complications, facilitated by the inhibition of HMG-CoA reductase activity in animal model[39,40].

CONCLUSION

The results of the study showed that FO-1X, FO-2X and FO-5X supplementation for 6-weeks had a significant effect on preventing HCD-induced weight gain. In addition, a number of clinical lipid biochemical values showed that supplementation with FO-1X, FO-2X and FO-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) FO 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.

Acknowledgment

This research was founded by HealthTake Co., Ltd.

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