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Preventive Effects of *Ophiocordyceps sinensis* Mycelium on High-Fat Diet Induced Lipid Dysregulation and Hepatic Inflammation of Mice

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Abstract Dyslipidemia is regarded as one of risk factors related to cardiovascular disease and hepatosteatosis widely. Due to westernized diet habits and lifestyle changes, there is a high prevalence of those lipid-dysregulated diseases, i.e. fatty liver, hyperlipidemia, diabetes etc. However, the liver holds the lipid homeostasis so hepatoprotective nutraceuticals against high-fat diet (HFD) induced dyslipidemia may be potential for a public demand. This study demonstrated that OSM containing 10% polysaccharides and 0.25% adenosine can decrease ($p<0.05$) serum and liver triglyceride (TG) contents, and meanwhile, increased ($p<0.05$) fecal cholesterol (TC) levels in HFD fed mice. Moreover, *Ophiocordyceps sinensis* mycelium (OSM) also decreased ($p<0.05$) serum low-density lipoprotein cholesterol (LDLC) levels and the atherosclerosis index (LDLC/high-density lipoprotein cholesterol (HDLC)) in HFD fed mice. Regarding the liver damage, OSM supplementation attenuated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values, and liver tumor necrosis factor-α (TNF-α) levels in HFD fed mice. Taken together, OSM showed an ameliorative effect of the hepatosteatosis development and lipid-dysregulated-related diseases in a HFD habit.

Keywords: *anti-inflammation, high-fat diet, lipid-lowering effect, liver, Ophiocordyceps sinensis mycelium*

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1. Introduction

Recently, pathological changes that threat the public wellness in the modern society may relate to the westernized lifestyle, nutritional imbalance and so on. Consequently, a high prevalence of metabolic syndromes, i.e. hyperlipidemia, fatty liver, diabetes, cardiovascular diseases, results in a major problem globally. According to a statistical data from World Health Organization [1], more than 10% of the adults are obese in the world. Meanwhile, lipid dysregulation and obesity has been regarded as major factors enhancing the mortality on the human clinical research. It also occurs in Taiwan, where there are more than 51.5% male and 32.6% female obese or overweight, respectively [2].

Ophiocordyceps sinensis (OS, renamed from *Cordyceps sinensis* in 2007), regarded as a traditional tonic food supplementation, is usually found in elevation of 3000 to 5000 meters of mountains in southwest of China. It is rare and precious due to its slow generation time and its bioacive benefits in the oriental society. An ancient Chinese medical book, Compendium of Materia and Medic, revealed its therapeutic functions, which inspire scientists' studies. Due to the rareness of OS, *Ophiocordyceps sinensis* mycelium (OSM) has become another major subject in following investigations. Based on previous studies, the bioactivities of OS and OSM were found as immunomodulation [3], anti-inflammation [4], anti-tumor [5], antioxidation [6], cardiovascular-protection [6], anti-fibrosis [7], and regulating male reproductive function [8]. Besides, the evidences for exercise-endurance promotion and anti-fatigue activities of OS were demonstrated, and it meditated upregulation of some metabolic regulators, such as AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1), and peroxisome

proliferator activated receptor (PPAR), in the skeletal muscle [9]. Recently, it was reported that cordycepin, one nucleotide derivatives in OS, could activate AMPK in HepG2 cells and then decrease intracellular lipid accumulation [10]. These evidences showed that OS or OSM would affect lipid metabolism *in vitro*. Briefly, OS-origin materials have shown its potential of medical and nutraceutical candidates; hence, the aim of the present study was to investigate the alleviative effects of OSM on the development of dyslipidemic diseases by a high-fat diet (HFD) induced mouse model.

2. Materials and Methods

2.1. *Ophiocordyceps sinensis* Mycelium

OSM powder (TCM-606F®) which contains 10% polysaccharides and 0.25% adenosine was obtained from the TCM Biotech International Corp. (Taipei, Taiwan). According to the composition analysis, the dry matter mass of OSM powder is 72.82%.

2.2. Animal, Treatment, and Diet

C57BL/6 mouse is the most widely used strain in HFD inducing fatty liver model, and it was proven to highly similarity with clinical symptoms [11]. Totally 60 male C57BL/6 mice (4 weeks old) were used in this study and obtained from Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei, Taiwan). The animal use and protocol was reviewed and approved by the National Taiwan University Animal Care Committee (IACUC No.: 101-032). Two animals with an ear tag (No. 1 or 2) were housed in one cage in an animal room at 22±2°C and approximately 60 to 80% relative humidity with a 12 h light/dark cycle. A chow diet (Laboratory Rodent Diet 5001, PMI® Nutrition International/Purina Mills LLC, USA) and water were freely accessible for mice. After an acclimatization for 4 weeks, mice were randomly divided into 5 groups as followings: 1) Control: control diet with 0.2 mL ddH_2O; 2) HFD: HFD with 0.2 mL ddH_2O; 3) OSML (low dosage of OSM supplemented group): HFD with 405.6 mg TCM-606F®/Kg BW/day in 0.2 mL ddH_2O; 4) OSMM (medium dosage of OSM supplemented group): HFD with 1216.8 mg TCM-606F®/Kg BW/day in 0.2 mL ddH_2O; 5) OSMH (high dosage of OSM supplemented group): HFD with 2433.6 mg TCM-606F®/Kg BW/day in 0.2 mL ddH_2O. Control diet and HFD were manufactured according to the AIN-93G formulation where control diet and HFD contain 7.0 and 25.8% fat, respectively, and their energy contents are 4.00 and 4.88 Kcal/g, respectively (Table 1). The OSM powder was re-dissolved in sterile saline without heat, and given to animal via an oral administration.

2.3. Sample Collection and Preparation of Liver Homogenate

During the experimental period, food and water intakes, and body weights were recorded weekly. At the end of the experimental period, all mice fasted overnight before sacrificed, and blood and tissue samples were harvested for further analyses. The heart, liver, and kidney from each mouse were removed and weighted individually. Livers were cut into one cubic millimeter for histopathology section, and others were placed into liquid N_2. A 0.5 g of liver was homogenized on ice in 4.5 mL of phosphate-buffered saline (PBS, pH 7.0, containing 0.25 M sucrose) and centrifuged at 10,000 x g for 15 min at 4°C (Model 3740; KUBOTA, Tokyo, Japan) to obtain clear supernatant for further analyses. The protein content in the supernatant was measured based on the procedures of a Bio-Rad protein assay kit (catalog #500-0006; Bio-Rad Laboratories, Inc., Hercules, CA, USA) using bovine serine albumin (Sigma-Aldrich Co., Ltd.) as a standard.

2.4. Analyses of Serum Biochemical Values, as well as Liver and Fecal Lipids

Blood samples were collected and placed for one h at room temperature for clotting. Then, the sera were collected by a centrifugation at 3,000 × g for 10 min (Model 3740; KUBOTA, Tokyo, Japan) and then stored at -80°C for subsequent analyses. Serum ALT, AST, ALP, TG, TC, HDLC, and LDLC levels were assayed using commercial enzymatic kits with the SPOTCHEM™ EZ SP-4430 automated analyzer (ARKRAY, Inc., Kyoto, Japan). An atherosclerosis index was calculated by a formulation of LDLC level/HDLC level. Liver and fecal lipid concentrations were measured based on the methods of a previous report [12]. Briefly, liver and fecal lipids were extracted by a chloroform/methanol solution (2:1, v/v). For an efficient dissolution, the extracts were dried under N_2 and resuspended in isopropanol via an ultrasonic cleaner (model: DC150H, Taiwan Delta New Instrument Co. Ltd., Taiwan). Liver and fecal cholesterol and triglyceride concentrations were measured using commercial kits (Randox Laboratories Ltd., Antrim, U.K.).

2.5. Antioxidative Capabilities and Cytokine Analyses

The liver TBARS was used to represent the level of liver lipid peroxidation while reduced glutathione (reduced GSH), trolox equivalent antioxidant capacity (TEAC), and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assayed as indices for liver antioxidant capacities. They all were performed according to procedures described by Lin et al. (2017) [12]. Hepatic TNF-α (R&D System DuoSet® ELISA mouse TNFα, Cat.: DY410) and IL-1β (R&D System DuoSet® ELISA mouse IL-1β/ IL-1F2, Cat.: DY401) levels were measured by using ELISA kits and then performed in an ELISA reader (Bio Tek Inc. Synergy H1 Hybrid Multi-Mode Microplate Reader, Winooski, Vermont, USA) according to the commercial manufacturer's instructions (R&D system Inc., Minneapolis, Minnesota, USA). Hepatic TNF-α and interleukin-1β (IL-1β) levels were both expressed by pg/mg protein.

2.6. Histological Sections and Stainings

For a histopathological study, liver tissues from No. 1 mouse of each cage were placed in formalin for more than

24 h and were fixed in new neutral-buffered formalin solution, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. These blockers were later sectioned using a microtome, dehydrated in graded alcohol, embedded in paraffin section, and stained with hematoxylin and eosin (H&E). For Oil-red staining, the liver tissues from No. 2 mouse of each cage were immersed in graded sucrose buffers directly and embedded in optimal cutting temperature (OCT) compound (Sakura Finetek Inc., Torrance, CA, USA) and then frozen under -80°C. The frozen block was removed from the -80°C freezer and allowed to equilibrate in the cryostat chamber. The molten tissue-freezing medium was added to the block in order to fix it onto the cryostat holder. The block on the holder was then trimmed by a razor blade. Then the trimmed tissue was carefully transferred from the cryostat blade onto a slide. The slides were then treated as the same protocol as H&E staining and then stained with Sudan red and haematoxylin or Sudan red only. Finally, the results of microscopic examination were demonstrated as a prevalence table. Photomicrographs under H&E and Oil red staining were taken under a Zeiss Axioskop microscope AxioCam ERc 5s camera system with AxioVision Release 4.8.2 (06-2010) software (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). The quantitative results of Oil red stain were based on former studies [13,14]. All photos for H&E and Oil red stainings, respectively, were taken under the same multiple factor, and the area calculation was conducted according to the scale bar on ImageJ. The total area indicated the total stained area in red color.

Table 1. Formula of Control and High-fat diets

	Control diet		High-fat diet	
	(gm%)	(kcal%)	(gm%)	(kcal%)
Protein	20.3	20.2	23.9	19.5
Carbohydrate	67.9	64.0	44.7	31.8
Fat	7.0	15.8	25.8	47.7
Other		1.0		1.0
Total		100		100
Kcal/gm	4.00		4.88	
		Ingredients		
Casein	200.000	800.000	234.990	939.960
L-cystine	3.000	12.000	3.520	14.080
Corn starch	397.500	1590.000	234.990	939.960
Maltodexdrin	132.000	528.000	88.150	352.600
Sucrose	100.000	400.000	65.320	261.280
Cellulose	50.000	0.000	58.740	0.000
Soybean oil	70.000	630.000	82.240	740.160
Lard	0.000	0.000	176.240	1586.160
AIN mineral mix	35.000	0.000	41.120	0.000
AIN Vitamin mix	10.000	40.000	11.750	47.000
Choline bitartrate	2.50	0.000	2.930	0.000
TBHQ	0.014	0.000	0.014	0.000
Dye	0.001	0.000	-	-
Distilled H_2O	500.000	0.000	500.000	0.000
Total	1000.000	4000.000	1000.003	4881.200

*Base on the AIN-93G formula

2.7. Statistical Analysis

The experiment was conducted using a completely random design. Data were analyzed using analysis of variance. When a significant difference was determined at 0.05 probability level, the differences among treatments were distinguished byusing the Duncan's multiple range test. All statistical analyses of data were performed using SAS 9.0 (SAS Institute, Inc., NC, USA, 2002).

3. Results

3.1. Effects of OSM on Growth Performance, Organ Sizes, and Liver Injury Indices

Prospective greater increases in final body weight of HFD-fed mice (HFD, OSML, OSMM, and OSMH groups) were showed after 20 weeks of feeding while significantly increased ($p<0.05$) body weight gain in HFD fed mice compared to Control mice were observed (Table 2). There were no ($p>0.05$) differences in average daily water and energy intakes among all experimental groups (Table 2) although the average daily food intake in HFD group was lower ($p<0.05$) than that of Control group. Regarding organ (heart, liver, and kidney) weights, although there was a higher tendency of liver weights toward HFD feedings, no ($p>0.05$) differences among groups were observed (Table 2). Regarding serum liver damage indices (AST, ALT, and ALP values) and hepatic cytokines (TNFα and IL-1β), serum ALP values and hepatic IL-1β levels (except OSMM group) were not ($p>0.05$) altered among groups, but higher ($p<0.05$) ALT, AST values, and hepatic TNFα levels in HFD group than those in Control group were detected, but those increases were decreased ($p<0.05$) by OSM supplementation (Table 2). According to the H&E stainings, the liver tissue morphology among all HFD fed groups were determined as hepatic hypertrophy without severe monocyte infiltration (Figure 1).

3.2. Effects of OSM on Serum, Liver, and Fecal Lipids

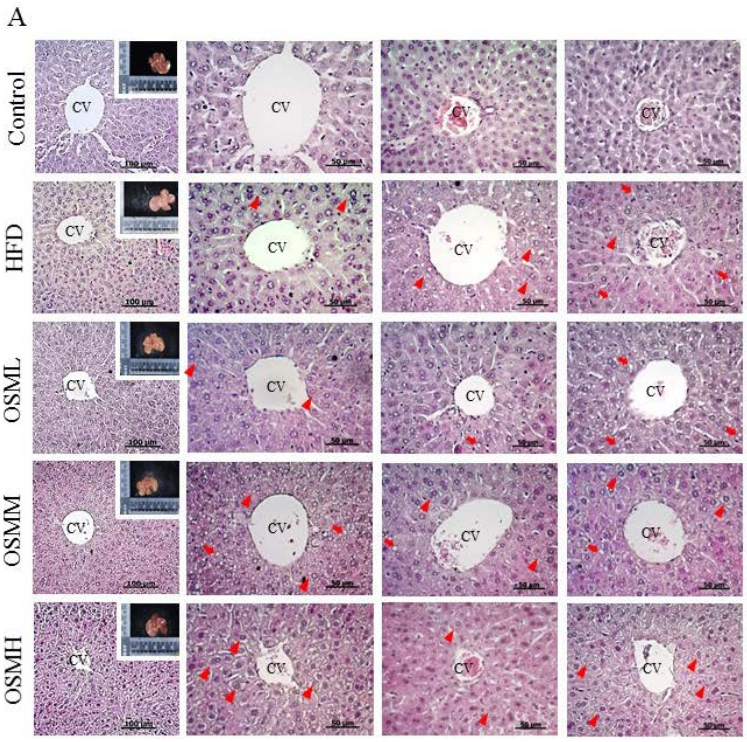
According to serum lipid profile (Table 3), higher ($p<0.05$) both serum TG and TC levels of HFD group were assayed compared to those of Control group. Meanwhile, only serum TG levels were normalized ($p<0.05$) by OSM supplementation. Regarding the serum cholesterol profile, although HFD fed groups (HFD, OSML, OSMM, and OSMH) had higher ($p<0.05$) HDLC levels than Control group, OSM supplementation alleviated ($p<0.05$) the increased LDLC levels in HFD fed mice. Hence, OSML, OSMM, and OSMH groups had lower ($p<0.05$) atherosclerosis indices (LDLC/HDLC) compared to that of HFD group while those calculated values were even similar ($p>0.05$) to that of Control group (Table 3). Similarly, the higher ($p<0.05$) liver TG level in HFD group was assayed compared to that of Control group, and also was alleviated ($p<0.05$) by OSM supplementation. Liver TC levels were not ($p>0.05$) different among groups. Although fecal TG levels were not ($p>0.05$) different among all groups, the significantly increased ($p<0.05$) fecal TC levels in HFD fed mice was observed in medium dosage of OSM supplemented group. Meanwhile, there was a higher tendency in those of OSML and OSMH. Based on results of H&E staining, the OSM supplementation attenuated the lipid accumulation (lipid vacuoles) form medium to light (Figure 1B).

Similarly, the more red-color spots which are Oil-red-stained lipid droplets were illustrated in livers of HFD fed mice compared to Control mice, but OSM supplementation decreased ($p<0.05$) the spot aggregation under microscope observation in an amplification factor of 400X (Figure 2A & Figure 2B). Figure 2C also showed that OSMsupplementation ameliorates the status of lipid accumulation from severe to medium in prevalence table.

Table 2. Growth parameters, organ sizes, hepatic injured indices, lipid peroxidation value, and antioxidant capacities in experimental mice

Parameter	Treatment				
	Control	HFD	OSML	OSMM	OSMH
			Growth parameters		
Initial body weight (g)	22.34±0.39a	21.75±0.22a	22.27±0.19a	22.68±0.06a	22.43±0.16a
Final body weight (g)	26.41±1.15b	29.39±1.34ab	30.18±0.66a	31.16±1.00a	29.71±0.74a
Body weight gain (g)	4.07±1.22b	7.64±1.31a	7.83±1.03a	8.39±0.99a	7.29±0.76a
Food intake (g/mouse/day)	2.55±0.13a	2.18±0.05b	2.24±.008ab	2.29±0.12ab	2.34±0.10ab
Water intake (mL/mouse/day)	3.75±0.16a	3.70±0.07a	3.76±0.26a	3.87±0.51a	4.15±0.43a
Energy intake (kcal/mouse/day)	10.22±0.53a	10.36±0.23a	10.64±0.39a	10.89±0.55a	11.14±0.46a
Food efficiency (g weight gain/g diet)	0.011±0.001c	0.020±0.000ab	0.020±0.001a	0.021±0.001a	0.018±0.001b
			Organ weights		
Heart (g)	0.14±0.01a	0.14±0.01a	0.14±0.00a	0.13±0.01a	0.15±0.01a
Liver (g)	0.95±0.04a	1.09±0.06a	1.06±0.03a	1.04±0.03a	0.97±0.04a
Kidney (g)	0.34±0.01a	0.36±0.02a	0.38±0.01a	0.39±0.01a	0.37±0.02a
			Serum biochemical values and inflammatory cytokines		
Serum AST (IU/L)	102.33±4.94c	153.00±6.06a	134.67±5.92ab	117.75±7.23bc	136.67±6.82a
Serum ALT (IU/L)	38.33±1.83b	53.50±3.64a	39.92±4.74b	37.75±4.11b	40.25±4.61b
Serum ALP (IU/L)	55.83±4.39a	49.83±3.15a	52.83±4.49a	51.08±2.00a	52.67±3.07a
Serum glucose (mg/dL)	94.08±19.60b	202.58±35.35a	222.92±29.57a	238.50±26.87a	238.75±25.17a
Liver TNF-α (pg/mg protein)	38.03±1.77b	46.70±2.63a	35.77±1.52b	32.83±2.18b	31.67±2.01b
Liver IL-1β (pg/mg protein)	7.98±0.63ab	9.57±0.90a	9.32±0.56a	7.16±0.52b	7.65±0.80ab
			Liver lipid peroxidation level and antioxidative capacities		
TBARS (nmol MDA/mg protein)	140.65±10.21ab	163.38±9.79a	136.10±4.16b	134.10±9.00b	147.34±8.76ab
Reduced GSH (nmol/mg protein)	107.20±7.70b	97.80±5.13b	141.16±10.27a	120.32±11.90ab	111.42±5.77b
TEAC (nmol/mg protein)	194.18±19.24a	183.44±17.49a	231.76±8.19a	228.42±9.35a	208.12±19.16a
SOD (unit/mg protein)	23.60±1.04a	22.22±0.72a	22.57±0.42a	22.53±0.76a	19.51±1.00b
CAT (unit/mg protein)	173.55±8.00a	156.05±6.00ab	176.36±4.40a	148.85±6.83b	155.01±7.68ab
GPx (nmol NADPH oxidized/min/mg protein)	1096.44±80.33a	747.39±102.12b	915.74±125.90ab	669.40±26.19b	712.34±75.23b

*The data are given as means ± SEM (n=12). Mean values without the common letters [(a-c)] in each test parameter were significantly different ($p< 0.05$).



B

	Control	HFD	OSML	OSMM	OSMH
Normal (0%)	4/6	0/6	2/6	1/6	1/6
Light (<10%)	2/6	3/6	3/6	2/6	2/6
Medium (10-33%)	0/6	2/6	1/6	3/6	3/6
Severe (33-66%)	0/6	1/6	0/6	0/6	0/6
Highly severe (66-100%)	0/6	0/6	0/6	0/6	0/6
Comment	Normal to light	Medium	Light	Light to medium	Light to medium

Figure 1. Pathohistological analysis and morphology of hepatocytes on H&E stained liver sections of experimental mice. (A) The central of hepatic classic lobules in different amplification factors were showed from left (100X, the scale is 100 μm) to right (200X, the scale bar is 50 μm), back-side appearances of livers were shown in right-upper part of first column with the scale. Each cavity that locates in central of the view was hepatic central vein that as abbreviated as CV. The nuclei were stained by hematoxylin, whereas the cytoplasms were stained by Eosin. (B) The prevalence table indicated the quantitative results of microscopic examination, the scores were given according to the density of micro- (as where red triangle indicates) or macro-steatosis (as where red arrow indicates)

Table 3. Liver, serum, and fecal lipid profiles of experimental mice

Parameter	Treatment				
	Control	HFD	OSML	OSMM	OSMH
			Serum lipid profile		
TG (mg/dL)	46.00±5.25b	78.00±5.49a	46.75±1.88b	48.25±2.25b	46.17±2.51b
TC (mg/dL)	73.59±7.49b	118.64±10.09a	117.06±6.46a	109.63±7.44a	104.69±4.71a
HDLC (mg/dL)	58.04±6.49b	77.53±8.60a	94.07±5.86a	86.15±5.98a	82.02±4.17a
LDLC (mg/dL)	11.46±1.06b	28.53±3.40a	16.52±0.84b	15.08±1.58b	13.42±1.08b
Atherosclerosis index (LDLC/HDLC)	0.22±0.02b	0.40±0.04a	0.18±0.01b	0.18±0.02b	0.17±0.02b
			Liver lipid profile		
TG (mg/g liver)	10.27±0.89b	14.26±1.39a	8.54±0.87b	10.54±1.26b	9.62±0.97b
TC (mg/g liver)	1.03±0.07a	1.13±0.13a	1.04±0.07a	1.03±0.14a	1.06±0.08a
			Fecal lipid profile		
TG (mg/g dried feces)	11.28±0.64a	13.70±1.71a	14.56±0.97a	13.67±2.76a	14.55±1.09a
TC (mg/g dried feces)	1.70±0.15c	2.50±0.27bc	3.25±0.17b	4.67±0.58a	3.24±0.48b

*The data are given as means ± SEM (n=12). Mean values without the common letters [a-c] in each test parameter were significantly different ($p < 0.05$).

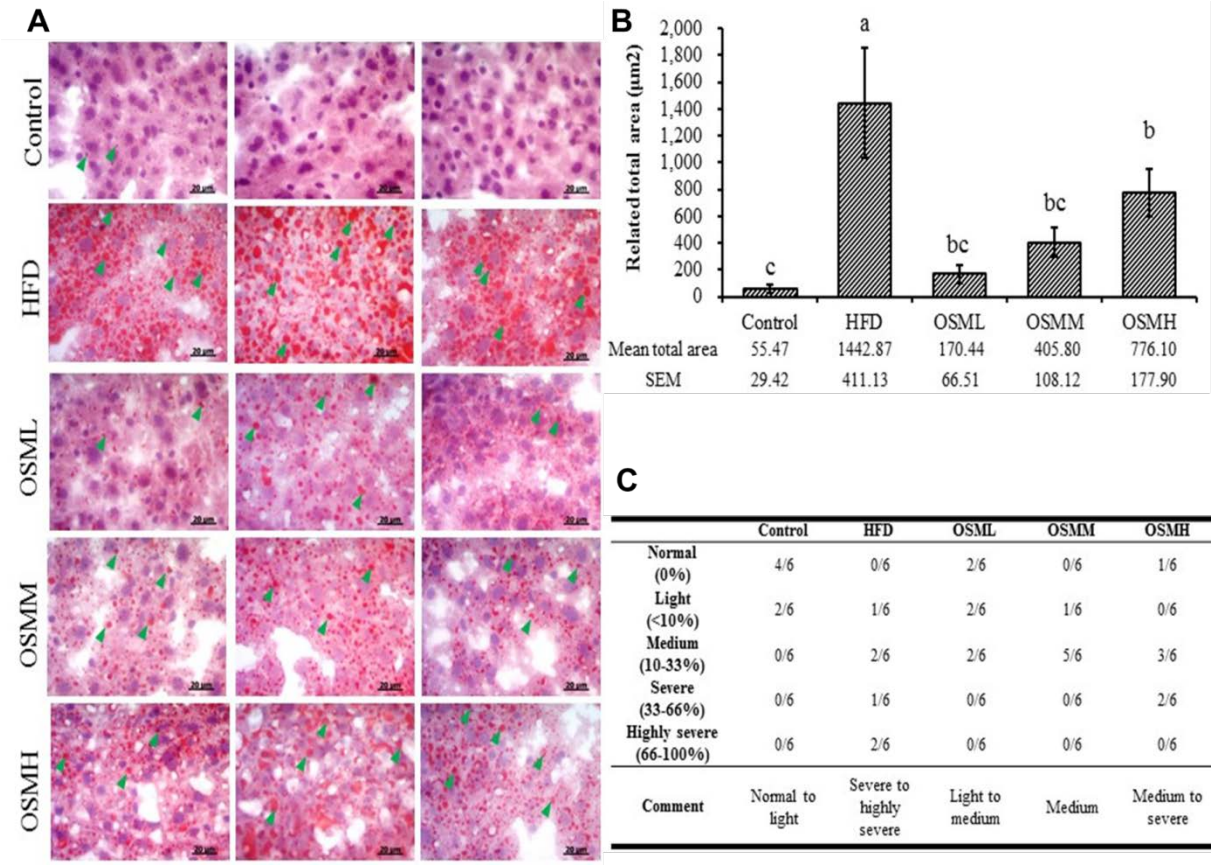


Figure 2. Pathohistological analysis and morphology of hepatocytes based on Oil red stained liver sections of experimental mice. (A) The results of Oil red O staining were showed. The lipid droplets were shown as light red in Oil red stainings (green triangle), and all tissues were also stained by hematoxylin in order to remark the cell nucleus. All the pictures of Oil red stainings were shown in an amplification factor of 400X (the scale is 20 μm). (B) The quantitative results of lipid-droplet densities via ImageJ. The means of total area and SEM were showed below the bar chart. The bars are given as means±SEM (n=6). Data points without the common letter [a-c] indicate significant differences ($p<0.05$). (C) The prevalence table indicated the quantitative results of microscopic examination, the scores were given according to the density of lipid droplets

3.3. Effects of OSM on Anti-oxidative Capacities

Under HFD feedings, only a higher tendency toward liver TBARS values were assayed in HFD group compared to Control group, but OSM supplementation indeed reduced TBARS values (OSML and OSMM groups vs. vs. HFD group, $p<0.05$) (Table 2). OSMsupplementation also increased ($p<0.05$) liver reduced GSH levels (OSML group vs. HFD group, $p<0.05$), but in comparison with HFD group, OSM supplementation only performed a higher tendency of TEAC levels. Regarding antioxidative enzymatic activities, liver SOD and CAT activities were not ($p<0.05$) influenced among groups, except the lower ($p<0.05$) SOD activity in OSMH group, while the lower ($p<0.05$) liver GPx activity in HFD fed mice compared to Control mice was amended in OSML group where the GPx activity of OSML group was similar as that of Control group (Table 2).

4. Discussion

In our results, the major pathological outcomes were consistent to what mentioned by Hebbard and George

(2011) [15]. They proclaimed that remarkable pathological outcomes of high fat diet induced mouse model are obesity and increased level of visceral fat, but the degree of liver injury is not as severe as other non-alcoholic fatty liver disease (NAFLD) models. It showed increased weight gains (Table 2) in this study, and the inflammatory levels in livers was not severe according to H&E stainings (Figure 1) although AST, ALT, and hepatic TNF-α levels showed statistical differences (Table 2). The liver weight showed no significant differences ($p>0.05$) but a heavy tendency in HFD fed mice (Table 2) while the lipid accumulation was obvious according to liver TG contents by HFD feedings (Table 3). Moreover, the lipid droplets were observed in results of H&E (Figure 1) and Oil red stainings (Figure 2A) as well, while the quantification of stained areas showed the significant increase ($p<0.05$) between Control and HFD groups (Figure 2B). Several previous studies also indicated the similar phenotypes under a HFD induced mouse model [16,17]. In addition, it was proclaimed that chronic inflammatory responses in livers or adipose tissues might be the driving force for NAFLD pathological progressing [15]. It may involve intrinsic and extrinsic factors at the same time where lipotoxicity [18,19], hepatocytes necrosis/apoptosis [20,21], oxidative stress [22,23] etc. are possible intrinsic factors, and a constant exposure to microbiota from gut may also trigger innate immune reaction [24,25]. As matters stand, our results demonstrated that the HFD enhances the secretion ($p>0.05$) of pro-inflammatory cytokine, TNF-α, in liver tissues (Table 2), but monocyte infiltration and necrotic cells were not clearly observed under a microscopic examination in the H&E stainings. According to histological activity index (HAI) score analysis which followed by Brunt's method [26], liver tissues of HFD fed mice were considered as slight inflammation in portal, intra-lobular, and periportal area (data not shown). Hence, it can be demonstrated that the HFD induction manner performed the diagnostic NAFLD phenotype of hepatosteatosis.

As we know, a high-fat dietary habit always results in an increased serum or liver lipids that stimulates an oxidative stress and further leads to liver damage. Higher serum AST, ALT, and free fatty acids, as well as liver TC, TG, TBARS, and cytokine levels were easily observed in a HFD fed rodent models [22,27,28]. It has been reported that the hepatoprotection of OSM against CCl_4 resulted in decreased serum AST and ALT values. Concurrently, increased antioxidative capabilities by OSM supplementation were also observed [29]. Based on our results, there are not ($p>0.05$) differences on antioxidant enzymatic activities in livers among groups, except GPx activity; and meanwhile, OSM supplementation did not powerfully enhance those enzymatic activities in our study. That phenomenon may be explained that the experimental period in this study is quite longer (20 weeks), and meanwhile, the neutralization ability of OSM on reactive oxygen species (ROS) has reached the maximum effect in this study. Apparently, it demonstrated a tendency of higher CAT, GPx activities, and TEAC values in OSML group compared with those of HFD group because the severity of hepatic lipid accumulation was the least among other groups (Figure 1 & Figure 2 and Table 3). However, the overall antioxidant levels (reduced GSH and TEAC levels) and lipid peroxidation status (TBARS values) in livers of HFD fed mice were still improved by OSM supplementation which could highly result from the lower serum and liver TG levels (Table 3 and Figure 2). Besides, the lower liver TBARS in HFD fed mice supplemented with OSM were also corresponding the lower liver damages (Table 2). Many phytochemicals, i.e. polysaccharide, glycopeptide, showed powerful immunomodulatory, anti-inflammatory, and antioxidative activities [30,31,32,33]. Furthermore, *Ophiocordyceps sinensis* revealed the improvement of an exercise endurance capacity of rats by activating not only the skeletal muscle metabolic regulators but also a coordinated antioxidative response [9].

Obesity, accompanying with hypertriglyceridemia and hepatic lipid accumulation, is the outcome of abnormal lipid homeostasis. Previous studies showed that fatty-liver development is associated with the metabolic syndrome [34]. In this investigation, the fat in the HFD offers a majority of caloric intake (47.7%) which is from lard (high proportion of saturated fatty acids and oleic acids) which shown to be a strong stimulator of triglyceride synthesis and secretion by hepatocytes [35]. Some previous reports indicated that polysaccharides can prevent obesity and liver lipid accumulation via decreasing food consumption [36,37], but our results are not the same as theirs (Table 1). It is reasonable to infer that the increasing tendency of hepatic lipid accumulation among OSM supplemented groups (Figure 1 & Figure 2 and Table 3) was resulted from consumption manner (food and energy intakes) (Table 2) while antioxidative capacities and enzymatic activities of OSMH were also influenced (Table 2). Based on our data, OSM regulated the lipid homeostasis via advancing excretion of cholesterol into feces, thus decreasing the serum LDLC levels and atherosclerosis index (Table 3). A previous investigation of red algae which contains 45.9 and 15.4% water soluble dietary fiber and insoluble dietary fiber, respectively, showed similar results of serum and fecal lipid profile as ours [38]. In addition, Kim et al. found two new anti-obesity compounds from *Cordyceps*, cordyrroles A and B, which are effectively inhibited pancreatic lipase activity [39]. Surprisingly, the OSM use in this study also decreased the liver TG levels, but it did not enhance ($p>0.05$) the TG output in feces, so it may not be related to lipase activities. Probably, the decreased liver TG levels by OSM treatment may be related to the energy expenditure in hepatic or other tissues like skeletal muscle [9]. It has been proclaimed the Cordymin, a compound in OS, could enhance the mitochondria biogenesis in livers [40,41]. On the other hand, OS supplementation on HFD-fed rats can enhance the protein expression of uncoupling protein 2 (UCP-2), further increasing energy expenditure in electronic transport chain, and then attenuate the steatosis [42]. The UCP-2 was considered as an important therapeutic target in NAFLD [43], and many pharmacological researches had focused on related mechanisms to restore the lipid homeostasis [27,42,43,44]. Overall, the OSM or its metabolites may modulate the whole body lipid regulation.

5. Conclusion

Based on the results of this investigation (Figure 3), the HFD clearly induced features of steatosis in livers of mice. This OSM supplementation could modulate the lipid contents in sera and livers of HFD fed mice where decreased serum TG and LDLC levels, and atherosclerosis indices, as well as the liver TG accumulation were assayed. Besides, increased serum AST and ALT, and liver TNF-α levels by HFD feedings were also ameliorated by supplementing our food-grade OSM. Hence, the ameliorative effects of OSM on the NAFLD development induced by HFD were expounded in this study.

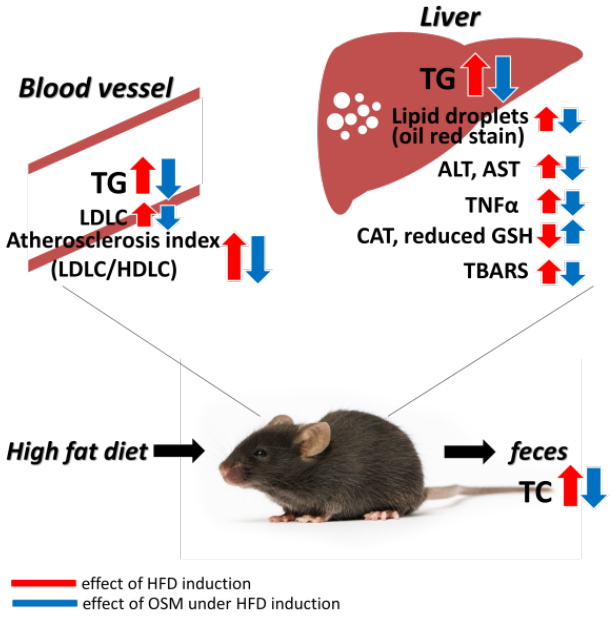


Figure 3. The graphic illustration of this study. The red arrow shows effects of high-fat diet, and the protective effects of OSM were indicated with the blue arrow

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