**Elaeagnus mollis** Oil Attenuates Non-alcoholic Fatty Disease in High-fat Diet Induced Obese Mice via Modifying the Expression of Lipid Metabolism-related Genes

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Abstract: *Elaeagnus mollis* oil (EMO), which is a type of plant oil, was extracted from the nuts of *Elaeagnus mollis* Diels that is known as a precious woodyoilcrop in China. The present study investigated the ameliorative effects of EMO on high-fat diet-induced non-alcoholic fatty liver disease (NAFLD) and explored relative regulation mechanism. The analysis of EMO fatty acids showed that EMO rich in unsaturated fatty acids (92.07%), such as linoleic acid (48.24%), oleic acid (34.20%) and linolenic acid (7.57%). In addition, supplementation of EMO could ameliorate the increase in body weight, fat weight, and abnormal serum lipids induced by high-fat diet. A further important implication is that the levels of serum ALT, serum AST, hepatic TG, TC, SOD, GSH/GSSG ration and MDA were improved after supplementing with EMO. All these changes may be due to the ability of EMO to inhibit fatty acid synthesis via reducing the mRNA expression of SREBP-1c, PPARγ and FAS, and elevate fatty acid oxidation by increasing the mRNA expression of PPARα and CPT-1. Meanwhile, our results also showed that endogenously synthesized n-3 PUFAs could significantly increase after treating with EMO. In conclusion, the results suggested that EMO could be regarded as a healthy food for preventing NAFLD.

Key words: *Elaeagnus mollis* oil, non-alcoholic fatty liver disease, high-fat diet, obesity, lipid metabolism

1 Introduction

Nowadays, the incidence of obesity is on the rise in global development because of a global shift in diet towards increased intake of energy-dense foods. According to the report of World Health Organization (WHO), the number of obesity reached 650 million in 2016, which is increased about three times compared with the year of 1975. Meanwhile, obesity vastly increased the risk of metabolic disease, especially NAFLD. NAFLD is a common chronic disease, which is characterized by lipids accumulation in the hepatocytes in absence of alcohol consumption. In general, the formation of NAFLD is defined when liver fat exceeds 5% of liver weight. If it is not treated in time, steatohepatitis, hepatic fibrosis, cirrhosis, and even hepatic cancer might be subsequently progressed. Recently, a literature reported that NAFLD occupied 10-24 percent of the population in the clinic all over the world. Thus, NAFLD has become a public health problem and the management of NAFLD is gaining a global concern. Plenty of studies have shown the methods of preventing NAFLD, mainly including medication, exercise and lifestyle modification at present. Medication therapeutic intervention is an effective strategy for improvement of NAFLD, whereas that might generate a series of side effects or contraindications under the condition of drug therapy. Tragically, it’s hard for the majority of people to change lifestyle and insist on long-term exercise. Therefore, dietary supplement as a potential approach has gained interest in interposing NAFLD.

*Elaeagnus mollis* Diels, which belongs to Elaeagnaceae

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family, mainly distributes at altitudes of 800-1500 m in west China, and it has obtained serious attention regarded as a precious woody oil crop. Accordingly, *Elaeagnus mollis* oil (EMO) is extracted from the nuts of *Elaeagnus mollis* Diels, and the EMO that we selected was extracted by the method of supercritical carbon dioxide extraction, and this method can better reserve effective constituents. Generally, oils, rich in unsaturated fatty acid, always possessed wonderful hepatoprotective effect on people. For example, several studies have reported that fish oil, which rich in n-3 polyunsaturated fatty acid, can ameliorate reductive dysfunction, lipid homeostasis and NAFLD. Krill oil, contained highly contents of eicosapentaenoic acid and docosahexaenoic acid, can suppress hepatic steatosis and obesity induced by high-fat diet. Also, *Starfish* oil, which abounding polyunsaturated fatty acid, has been reported that the improvement effect on hyperlipidemia and hepatic steatosis using C57BL/6N mice treated with high-fat diet. Recently, it has been reported that 6 weeks old mice were divided into control group, peanuts oil group and EMO group, and the levels of serum TC, TG, LDL-C, aspartate aminotransferase, and alanine aminotransferase were examined. The results indicated that EMO could protect against hyperlipidemia and hepatic oxidant stress. However, the alleviative effects of EMO on NAFLD induced by a high-fat diet have not been explored. The purpose of this study was to investigate the protective effects of EMO against NAFLD induced by high-fat diet. We analyzed the fatty acid profiles of EMO, then investigated the potential protective effect of EMO and further explored lipid synthesis and metabolism in the high-fat diet-induced NAFLD mice models.

2 Experimental

2.1 Chemicals, reagents, and animals

*Elaeagnus mollis* oil (EMO) was extracted and purified by using supercritical carbon dioxide extraction method from *Elaeagnus mollis* Diels seed, and was obtained from Shanxi qierkang samara biological products co. ltd (Taiyuan, China). Assay kits for total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA), glutathione (GSH), oxidized glutathione (GSSG) and superoxide dismutase (SOD) were purchased from Nanjing jiancheng bioengineering institute (Nanjing, China). Hematoxylin, eosin, absolute ethyl alcohol and xylene were purchased from local chemical reagent company (Taiyuan, China). The high-fat diet, consisting of 45% kcal fat, 35% kcal carbohydrate, and 20% kcal protein, was provided by Beijing keao cooperation feed co., ltd (Beijing, China) and the production license number was SCXK (SU)2016-0010. A low-fat diet, consisting of 10% kcal fat, 70% kcal carbohydrate, and 20% kcal protein, was purchased from Shanxi medical university (Taiyuan, China). Four-week-old male C57BL/6 mice (14.0 ± 2.0 g) were obtained from Nanjing junke bioengineering co., ltd (Nanjing, China) and the license number was SCXK (SU)2016-0010.

2.2 Fatty acid profile in EMO

The methylation of the fatty acids was carried out using a method of our laboratory. Briefly, 0.2 g EMO was mixed with 6 mL hexyl hydride and 1.4 mL of 2 mol/L KOH solution (dissolved in methanol), and the mixture was shaking for 2 min. After that the liquid was standing for 20 min at room temperature. Then, 2 mL distilled water was added, standing, and the upper solution dehydrated with anhydrous sodium sulfate. The residual solution was collected as a sample for analysing of fatty acids by gas chromatography-mass spectrometer (GC-MS). The chromatographic separation was performed on an RTX-WAX capillary column using helium (1.0 mL/min) as a carrier gas. 0.2 μL sample solution was injected with the 10:1 of split ratio and the injector temperature was 250°C. The temperature procedure as follow: initial temperature of 50°C, held for 2 min; elevated at 5°C/min to 140°C, held for 10 min; elevated at 1°C/min to 160, 180, 200 and 220°C in sequence and held for 10 min in each temperature point; then ramped at 2°C/min to 230°C, held for 30 min. The condition of the mass spectrum was set up as follows: the mass range (30-500 m/z) in a full-scan mode for electron ionization (70 eV), the temperature of an ion source, transmission line, and quadrupole were 230, 280 and 150°C, respectively. Identification of fatty acid was carried out by contrasting mass spectra to standard (NIST 11 library) and the relative content was detected by the normalization method of peak-area.

2.3 Animal protocol

The four-week-old male C57BL/6 mice used in this study were approved by the Center for Disease Control and Prevention of Shanxi Province (license number: SYXK (JING) 2015-0002). The C57BL/6 mice (n = 30) were housed in an environmentally controlled room at 20-26°C with the humanity of 40-60% and a 12 h light/dark cycle. There were two mice in a plastic cage and all mice were free to access to food and water during the experiment. After one week of acclimatization, the abnormal mice were removed, and the rest were randomly divided to three groups (n = 8): low-fat diet feed group (LFD), high-fat diet feed group (HFD) and the high-fat diet supplemented with a dose of 3.0 g/kg·d EMO group (EMO). The dose of EMO was selected based on a daily dose for adults, in which presented dietary guidelines for Chinese residents, and then it switched into the equivalent dosage while feeding the mice as described by previous report. Meanwhile, the LFD
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The liver and adipose tissues that fixed in the 10% formaldehyde solution for hepatic pathology; the second liver was stored at -80°C for biochemical analyses, antioxidant capacity analysis and fatty acid profiles; and the rest of the liver was put into centrifuge tube without ribonuclease and stored at -80°C for relative gene expression.

2.4 Serum biochemical analyses

The levels serum TC, TG, HDL-C, LDL-C, ALT and AST were measured according to the instruction of the commercial kits.

2.5 Measurement of hepatic profiles

The preparation of homogenized liver was conducted to the manufacturer’s instruction of commercial assay kits. Briefly, 1 g of liver tissue was added to a tube with 9 mL saline and then homogenized using a pulp refiner (FK-A, Youlian instruments institute, Jingtan, China). After that, the liver sample was centrifuged under the condition of 4°C, 2500 rpm, 10 min, and the supernatant was collected for testing hepatic profiles.

2.6 Analyses of antioxidant capacity

The method of homogenized liver tissue as indicated above. Hepatic SOD, MDA GSH and GSSG were measured in line accordance with manufacturer’s instruction, and the ration between GSH and GSSG was calculated.

2.7 Histopathology

The liver and adipose tissues that fixed in the 10% formaldehyde solution were embedded in paraffin and then were cut by a histotomy (Leica RM2235, Shanghai baihe instrument technology co. ltd, Shanghai, China), the thickness of section was approximate 6 μm. Sections were stained with hematoxylin and eosin (H&E), and pathology was observed using light microscopes (Olympus CX31, Japan Olympus corporation ltd, Tokyo, Japan). Besides, the histological scoring system was established based on a ratio of the area of hepatocyte lipid degeneration to total areas in accordance with previous method\textsuperscript{10}, and introduced briefly as follow: 0 grade (<5%), 1 grade (5% -33%), 2 grades (34%-66%), 3 grades (>66%).

2.8 Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from liver tissues using RNA Simple Total RNA Kit (TIANGEN biochemical technology co. ltd, Beijing, China)\textsuperscript{11}, and then cDNA was synthesized by using First-Strand cDNA Synthesis kit offered by US Everbright Inc (Suzhou, China). qRT-PCR was performed using SYBR Green qPCR Master Mix (US Everbright Inc, Suzhou, China) with the real-time fluorescent quantitative PCR instrument (ABI Fast7500, Shanghai aiyan Biotechnology limited co. ltd, Shanghai, China). The primer was designed in strict accordance with the principle of quantitative real-time PCR and the primer sequences were presented in Table 1. β-actin was regarded as the housekeeping gene.

2.9 Preparation and analysis of fatty acid in the livers

The extraction of liver and methylation of the fatty acids were carried out according to the previous report with minor modification\textsuperscript{12}. In brief, 300 mg liver homogenate was mixed with 2 mL methanol, and shaking for 30 min. Then 4 mL chloroform was added into the mixture for standing 24 h. After that, the mixture was filtered and the filtrate was added into 1.2 mL 0.9% of sodium chloride solution, and chloroform phase was collected as sample. Methylation of the fatty acids as follows: 10 mg sample was added to the flask with 1 mL 5% of the concentrated sulfuric acid-methanol solution, placed for 1 h at 70°C, and 1 mL of normal hexane was added, then standing until layered. The supernatant was used for GC-MS analysis, the chromatographic separation was performed on a FFAP capillary column using helium (1.0 mL/min) as a carrier gas. 1 μL sample solution was injected with the 15:1 of split ratio and the injector temperature was 250°C. The temperature procedure as follow: initial temperature 120°C, held for 5 min; elevated at 10°C/min to 190°C, held for 1 min; elevated at 2°C/min to 230°C and held for 12 min. The condition of mass spectrum is consistent with the method mentioned above.

2.10 Statistical analyses

All experimental data were expressed as the means ± standard deviation (SD) and were analyzed by one-way ANOVA followed by Duncan’s multiple range test using SPSS 17.0 (IBM, Armonk, NY, USA). Correlation analysis (R) was performed using Pearson coefficient or Spearman coefficient in accordance with normal distribution and outlier. \( P<0.05 \) was considered a significant difference.

3 Results

3.1 Fatty acid profile in EMO

The results of fatty acids profiles in EMO were presented

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in Fig. 1, and eighteen types of fatty acid were detected (shown in Table 2). It could be seen that the relative content of saturated fatty acid was only 7.93%, mainly including 4.13% of palmitic acid and 2.44% of stearic acid. While the relative content of unsaturated fatty acid took up 92.07%, which included linoleic acid (48.24%), oleic acid (34.20%), linolenic acid (7.57%), and n-6/n-3 polyunsaturated fatty acid ratio was 6.32:1. Above all, the results preliminarily indicated that EMO might be a kind of beneficial oil for people’s healthy because of higher contents of unsaturated fatty acid.

### 3.2 Effect of EMO on body weight, food intake, energy intake, food efficiency ratio, liver, and fat mass

The effect of EMO on body weight, food intake, food efficiency ratio, liver, and adipose tissue mass was shown in Table 3. There was no significant difference ($p > 0.05$) in initial body weight among the three groups. The final body weight of mice in the HFD group was significantly higher than mice in the LFD group ($34.54 - 37.19, 95\%$ Confidence interval(95\% CI) vs $27.38 - 29.42, 95\%$ CI; $p < 0.05$), supplementation of EMO decreased body weight ($30.71 - 35.05, 95\%$ CI vs $34.54 - 37.19, 95\%$ CI; $p < 0.05$). Besides, mice

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**Fig. 1** Fatty acid chromatogram of EMO obtained from GC-MS analysis.

**Table 1** Primer sequences used in this study.

| Gene       | Primer sequences         | PCR product (bp) | Sequence number |
|------------|--------------------------|------------------|-----------------|
| SREBP-1c   | F: CACAGCGGTTTTGGAACGACA R: CTCTCAGGAGATGTCGGCACC | 147              | NM_011480.3     |
|            | F: TTTTCAAGGGTGCCAGTTCGATCC R: AATCCCTTGCCCTCTGAGAT | 198              | NM_001127330.1  |
| PPARγ      | F: GGCCAGTGCTATGCTGAGAT R: AGGTCAAGTCTGCTCCTCA | 108              | NM_133360.2     |
| ACC        | F: GCTGCGGAACCTCAGGAAAT R: AGAGACGTGTCACTCCTGGAAT | 84               | NM_007988.3     |
| FAS        | F: CAGGCGCTAGGGTGATACACT R: TTGCGAGCTCCGATCACATT | 111              | NM_00113418.1   |
| PPARα      | F: CTCAGTGGGAGGGCTCTTCACCA | 105              | NM_013495.2     |
| CPT-1      | F: GCCCTGTCCTTGGACGACAA | 189              | NM_001271898.1  |
| ACOX       | F: CAGGGATTCGACGAGGTG | 156              | NM_007393       |
| β-actin    | F: CAGGGATTGCTGACAGGATG R: TGCTGATCCACATCTGCTGG | 156              | NM_007393       |

SREBP-1c: sterol regulatory element-binding protein, PPARγ: peroxisome proliferator-activated receptor, ACC: Acetyl-CoA carboxylase, FAS: fatty acid synthase, PPARα: peroxisome proliferator-activated receptor α, CPT-1: carnitine palmitoyl transterase-1, ACOX: acyl coenzyme a oxidase.
Table 2 Fatty acid profile of EMO.

| Retention time | Fatty acid                      | relative contents (%) |
|----------------|---------------------------------|-----------------------|
| 1              | Myristic acid (C14:0)           | 0.031                 |
| 2              | Pentadecanoic acid (C15:0)      | 0.024                 |
| 3              | Palmitic acid (C16:0)           | 5.131                 |
| 4              | 7-Hexadecenoic acid (C16:1)     | 0.032                 |
| 5              | 9-Hexadecenoic acid (C16:1)     | 0.087                 |
| 6              | Heptadecanoic acid (C17:0)      | 0.020                 |
| 7              | 10-Heptadecanoic acid (C17:1)   | 0.036                 |
| 8              | Stearic acid (C18:0)            | 2.435                 |
| 9              | Oleic acid (C18:1)              | 34.197                |
| 10             | Oleic acid (Z)- (C18:1)         | 1.061                 |
| 11             | Linoleic acid (C18:2)           | 48.244                |
| 12             | 10-Nonadecenoic acid (C19:1)    | 0.036                 |
| 13             | Linolenic acid (C18:3)          | 7.574                 |
| 14             | Eicosanoic acid (C20:0)         | 0.248                 |
| 15             | 11-Eicosenoic acid (C20:1)      | 0.742                 |
| 16             | Eicosadienoic acid (C20:2)      | 0.063                 |
| 17             | Docosanoic acid (C22:0)         | 0.013                 |
| 18             | Tetracosanoic acid (C24:0)      | 0.024                 |

Table 3 Effect of EMO on body weight, food intake and tissue mass.

| Groups       | LFD                  | HFD                  | EMO                  |
|--------------|----------------------|----------------------|----------------------|
| Body weight  |                      |                      |                      |
| Initial body weight (g) | 20.84 ± 1.10\textsuperscript{a} | 21.43 ± 0.82\textsuperscript{a} | 21.09 ± 0.88\textsuperscript{a} |
| Final body weight (g)   | 28.12 ± 0.89\textsuperscript{b} | 35.87 ± 1.26\textsuperscript{c} | 30.57 ± 1.74\textsuperscript{b} |
| Body weight gain (g)   | 7.25 ± 1.28\textsuperscript{a} | 12.70 ± 2.64\textsuperscript{b} | 9.41 ± 2.07\textsuperscript{b} |
| Food intake           |                      |                      |                      |
| Feed intake (g/d/mouse)| 2.68 ± 0.37\textsuperscript{b} | 2.47 ± 0.39\textsuperscript{c} | 2.04 ± 0.20\textsuperscript{b} |
| Energy intake (kcal/d/mouse)| 10.28 ± 0.81\textsuperscript{a} | 11.29 ± 0.91\textsuperscript{a} | 10.33 ± 0.68\textsuperscript{a} |
| Food efficiency ratio (%) | 4.86 ± 0.36\textsuperscript{a} | 9.29 ± 0.94\textsuperscript{b} | 8.26 ± 0.56\textsuperscript{b} |
| Tissue mass           |                      |                      |                      |
| Liver weight (g)      | 1.06 ± 0.53\textsuperscript{a} | 1.35 ± 0.09\textsuperscript{b} | 1.20 ± 0.12\textsuperscript{b} |
| Liver coefficient (%) | 3.65 ± 0.15\textsuperscript{a} | 4.01 ± 0.39\textsuperscript{b} | 3.90 ± 0.30\textsuperscript{b} |
| Fat weight (g)        | 1.15 ± 0.25\textsuperscript{a} | 3.83 ± 0.52\textsuperscript{b} | 2.63 ± 0.67\textsuperscript{b} |
| Fat coefficient (%)   | 3.45 ± 0.30\textsuperscript{a} | 10.61 ± 1.35\textsuperscript{b} | 8.13 ± 1.24\textsuperscript{b} |

All values are means ± SD (n = 8). Different letters indicate a significant difference (p < 0.05). Energy from low-fat diet was 3.85 kcal/g, energy from high-fat diet was 4.73 kcal/g, energy from EMO was 9 kcal/g.

Food efficiency ratio (%) = 100 × (weight gain/food intake for whole experimental period); Liver coefficient (%) = 100 × (liver weight /final body weight); Fat coefficient (%) = 100 × (fat weight /final body weight).
in EMO group had lower food intake than HFD group (1.89 – 2.19, 95% CI vs 2.19 – 2.73, 95% CI; p < 0.05), whereas significant difference of energy intake was not observed (9.62 – 11.05, 95% CI vs 10.15 – 12.64, 95% CI; p > 0.05). Additionally, the data revealed that the food efficiency ratio of mice in the EMO group was not significantly reduced (7.68 – 8.85, 95% CI vs 8.30 – 10.27, 95% CI; p > 0.05) compared with the mice in the HFD group. Liver weight and fat weight of mice in the HFD group were significantly increased compared with the LFD group (liver weight: 1.27 – 1.43, 95% CI vs 1.01 – 1.11, 95% CI; fat weight: 9.48 – 11.75, 95% CI vs 3.06 – 3.83, 95% CI; p < 0.05). While the liver weight and fat weight were significantly decreased in EMO group compared with HFD group (liver weight: 1.09 – 1.30, 95% CI vs 1.27 – 1.43, 95% CI; fat weight: 5.36 – 10.90, 95% CI vs 9.48 – 11.75, 95% CI; p < 0.05). Additionally, the fat coefficient of mice in EMO group was also significantly decreased compared with HFD group (1.71 – 3.54, 95% CI vs 3.30 – 4.36, 95% CI; p < 0.05). Accordant to previous report, the changing of adipocyte cell number and cell size was the major factor in the fat accumulation, thus adipose tissue pathological sections were analyzed and a clear adipocyte hypertrophy was observed in the HFD group, whereas treated with EMO significantly decreased the areas of adipocyte, and this result was presented in Fig. 2A, B.

3.3 Effect of EMO on serum lipids

To evaluate the effect of EMO on metabolic disorders, the levels of serum TG, TC, HDL-C, and LDL-C were detected, and the results were given in Fig. 3. The levels of TG, TC and LDL-C were increased markedly in HFD group relative to the LFD group (TG: 1.19 – 1.72, 95% CI vs 0.71 – 1.02, 95% CI; TC: 4.48 – 5.64, 95% CI vs 1.73 – 2.72, 95% CI; LDL-C: 2.58 – 4.19, 95% CI vs 0.32 – 1.51, 95% CI; p < 0.05). Whereas treatment with EMO was effective in preventing TG, TC and LDL-C levels (increased by 30.34%, 47.82%, and 69.23%, respectively) (TG: 0.90 – 1.18, 95% CI vs 1.19 – 1.72, 95% CI; TC: 2.03 – 3.33, 95% CI vs 4.48 – 5.64, 95% CI; LDL-C: 1.48 – 2.78, 95% CI vs 2.58 – 4.19, 95% CI; p < 0.05). Besides, serum HDL-C level of mice in HFD group was decreased compared with LFD group (1.27 – 1.73, 95% CI vs 1.64 – 2.29, 95% CI; p < 0.05), while the supplement of EMO increased the level of HDL-C, but no significant change was observed (1.37 – 1.87, 95% CI vs 1.27 – 1.73, 95% CI; p > 0.05). Compared with LFD group, the values of LDL-C/HDL-C and atherosclerosis index value (AI) of mice in HFD group were significantly increased (AI: 0.68 – 0.99, 95% CI vs 0.33 – 0.60, 95% CI; LDL-C/HDL-C: 0.38 – 0.61, 95% CI vs 0.88 – 2.26, 95% CI; p < 0.05), and a lower LDL-C/HDL-C level was observed in EMO group compared with HFD group (0.87 – 1.71, 95% CI vs 0.38 – 0.61, 95% CI; p < 0.05).

3.4 Effect of EMO on NAFLD-related indicators

3.4.1 Effect of EMO on liver injury

Long-term high-fat diet consumption induced the first sign of NAFLD such as liver injury. Serum ALT and AST...
levels, the common indicators of liver injury, were measured and the results were shown in Fig. 4A. The levels of ALT and AST of HFD group were significantly higher than that of the LFD group (ALT: 50.39–86.19, 95% CI vs 23.82–34.97, 95% CI; AST: 50.26–85.91, 95% CI vs 11.12–25.16, 95% CI; p < 0.05), EMO treatment could significantly decrease those values at the end of the study (ALT: 29.94–41.86, 95% CI vs 50.39–86.19, 95% CI; AST: 26.58–40.90, 95% CI vs 50.26–85.91, 95% CI; p < 0.05), which could reduce to 50.45% and 43.33%, respectively, and there was no significant change between LFD group and EMO group (p > 0.05).

3.4.2 Effect of EMO on hepatic lipids

Excessive lipid accumulation in the liver is another risk factor for NAFLD. In this study, we investigated the lipid accumulation status of three groups by evaluating hepatic histopathological sections. Based on visual observation, the livers of mice in the HFD group were pale yellow and enlarged compared with the LFD group (Fig. 5A). Meanwhile, normal hepatocytes and order hepatic cord were observed in LFD group, while the hepatic sections in HFD group were occupied by large areas of microvesicular steatosis and hepatic cord arranged mussily, indicating that fatty liver degeneration was induced by feeding with a high-fat diet. However, supplementing EMO effectively blocked excessive hepatic lipid accumulation (Fig. 5B). And this finding was corroborated by directly assessing hepatic lipids. Hepatic TG and TC contents of the HFD group were

**Fig. 3** Effect of EMO on serum lipids of high-fat diet induced mice. A: Serum triglyceride levels; B: Serum cholesterol levels; C: Atherosclerosis index = (cholesterol - high-density lipoprotein cholesterol)/cholesterol; D: Serum high-density lipoprotein cholesterol levels; E: Serum low-density lipoprotein cholesterol levels; F: The ratio between high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. All values are means ± SD (n = 8). Different letters indicate a significant difference (p < 0.05).
increased significantly in comparison to the LFD group (Hepatic TG: 0.40 – 0.47, 95% CI vs 0.18 – 0.22, 95% CI; Hepatic TC: 0.27 – 0.57, 95% CI vs 0.09 – 0.14, 95% CI; p <0.05) (Fig. 4B, C). And hepatic TG of mice in EMO group showed a significant reduction and decreased by 39.53% compared with HFD group (0.18 – 0.36, 95% CI vs 0.40 – 0.47, 95% CI; p <0.05). Additionally, the level of hepatic TC was reduced by supplementing EMO, while the significant change was not observed (0.19 – 0.42, 95% CI vs 0.27 – 0.57, 95% CI; p >0.05).

3.4.3 Effect of EMO on hepatic oxidative stress

To explore whether EMO ameliorates oxidative stress in the liver, hepatic SOD, GSH/GSSG ration and MDA were determined in three groups and the results were shown in Fig. 4D-F). A tendency toward lower of hepatic SOD activity was observed in HFD group relative to LFD group (143.30 – 209.91, 95% CI vs 258.44 – 296.14, 95% CI; p <0.05), while it was markedly enhanced after treating with EMO (increased by 29.10%). The GSH/GSSG ration of mice in HFD group was decreased compared to LFD group (0.97 – 2.40, 95% CI vs 1.93 – 3.32, 95% CI; p <0.05), while a light alteration of GSH/GSSG ration was shown in EMO group (1.84 – 2.73, 95% CI vs 0.97 – 2.40, 95% CI; p <0.05). Higher MDA levels in the liver of HFD group compared with LFD group were reversed by supplementing with EMO (0.87 – 1.04, 95% CI vs 1.08 – 1.56, 95% CI; p <0.05), and there was no significant difference (0.87 – 1.04, 95% CI vs 0.66 – 1.39, 95% CI; p >0.05) between EMO group and LFD group.

3.5 Correlation analysis

In order to further understand how EMO alleviates the hepatic steatosis, the relationship between BW, serum lipids and NAFLD-related indicators including liver injury
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With the mRNA expression level of FAS, this fact indicated that EMO might alleviate hepatic steatosis by reducing body weight or preventing lipids metabolic disorder while further investigation is needed to define the molecular mechanisms.

3.6 Effect of EMO on hepatic mRNA expressions of lipid metabolizing gene

Disorder of lipids metabolism is regarded as a major factor for the development of NAFLD, and the mRNA expressions of lipid metabolizing gene in liver were examined. It has been observed that high-fat diet significantly elevated the mRNA expressions level of SREBP-1c, PPARα, and FAS about 1.7, 1.8 and 1.3 folds, respectively. The mRNA expression level of SREBP-1c was more closely correlated with the mRNA expression level of FAS, this fact indicated that down-regulation of SREBP-1c could regulate the expression of FAS thereby depressing lipidsynthesis. Meanwhile, there was a strong correlation between mRNA expressions level of SREBP-1c and PPARα, suggesting that PPARα might also participate in preventing lipidsynthesis. Additionally, the mRNA expression level of ACC was markedly decreased in HFD group compared with LFD group (0.67 – 0.94, 95% CI vs 1.00 – 1.01, 95% CI; p < 0.05), but there was no significant difference (0.77 – 1.09, 95% CI vs 0.67 – 0.94, 95% CI; p > 0.05) between HFD group and EMO group.

Furthermore, there was no difference of the PPARα mRNA expression level between LFD group and HFD group (0.91 – 1.13, 95% CI vs 1.00 – 1.02, 95% CI; p < 0.05), while the mice treated with EMO significantly increased this level relative to the HFD group (1.56 – 2.71, 95% CI vs 0.91 – 1.13, 95% CI; p < 0.05). The mRNA expression of CPT-1 in HFD group was significantly elevated about 1.3 folds in comparison with LFD group (1.17 – 1.49, 95% CI vs 1.00 – 1.01, 95% CI; p < 0.05), and there was a further heightened in EMO group, which was about 1.4-fold higher compared with HFD group (1.54 – 2.28, 95% CI vs 1.17 – 1.49, 95% CI; p < 0.05). The mRNA expression of ACOX in HFD group was down-regulated relative to LFD group, and no affect with EMO treatment (0.40 – 0.70, 95% CI vs 0.50 – 0.70, 95% CI; p > 0.05). Meanwhile, there was a high correlation of mRNA expressions between PPARα and CPT-1, suggesting that EMO might affect lipids metabolism by alleviating the PPARα gene expressions and further regulated the expression of CPT-1.

3.7 Effect of EMO on the compositions and contents of fatty acids in the liver

The result of fatty acid composition in the livers was shown in Fig. 8. It showed that C14:0, C16:0, C16:1, C16:2, C17:0, C18:0, C18:1, C18:2, α-C18:3, γ-C18:3, C20:1, C20:2, C20:3, C20:4 and C22:6 were detected in the livers among three groups. And it can be observed preliminarily that the relative contents of the fatty acids C16:1, C16:2, C17:0,
C18:0, C18:1, C18:2, γ-C18:3, C20:1, C20:4 and C22:6 showed a significant difference ($p < 0.05$) between LFD group and the HFD group. After administration with EMO, the relative contents of C16:2, C18:1, C18:2, γ-C18:3, C20:4, C22:6 were changed toward the normal levels ($p < 0.05$), indicating that C16:2, C18:1, C18:2, γ-C18:3, C20:4, C22:6 might have effects on EMO preventing NAFLD. Based on the above results, the potential biomarkers mainly focused on unsaturated fatty acid, especially polyunsaturated fatty acid, thus the unsaturated degree was summarized (shown in Table 4). It can be found that the mice in the HFD group had the higher levels of MUFA, MUFA/UFA and n-6/n-3 PUFA ($p < 0.05$), EMO treatment could significantly decrease ($p < 0.05$) those values at the end of the study. Compared with the LFD group, lower levels of PUFA, PUFA/UFA, n-6 PUFA, and n-3 PUFA were observed of mice in the HFD group ($p < 0.05$). However, it could be seen that supplementing EMO effectively blocked the decrease in these values, especially n-3 PUFAs ($p < 0.05$), suggested administration of EMO to mice might result in

Fig. 7  Effect of EMO on mRNA expression of liver lipid metabolism. A: mRNA expression levels of SREBP-1c, PPARγ, ACC and FAS in the liver; B: Correlation between SREBP-1c, PPARγ, ACC and FAS; C: mRNA expression levels of PPARα, CPT-1, and ACOX in the liver; D: Correlation between PPARα, CPT-1, and ACOX. The correlation analysis was performed by using Pearson coefficient, and the correlation was presented from strong to weak in the order of the red, blue and yellow. All values are means ± SD (n = 8). Different letters indicate a significant difference ($p < 0.05$).

Fig. 8  The chromatogram of fatty acids of mice in LFD, HFD and EMO group by GC-MS.
n-3 PUFA participate in the metabolism of fatty acid in the liver to alleviate NAFLD.

### 4 Discussion

Different types of oils have different amounts and compositions of fatty acids, and both are important in regulating hepatic lipid metabolism\(^1\). The saturated fatty acid could promote the progression of lipids accumulation in the liver, while the unsaturated fatty acids are essential for lipid metabolism\(^2\). Plenty of studies have reported that EMO rich in unsaturated fatty acid, especially linoleic acid, oleic acid and linolenic acid\(^3, 20, 25\), and our data showed that unsaturated fatty acid in EMO reached to 91.28%. According to a report of a joint FAO/WHO consultation about preparation and use of food-based dietary guidelines, the balance between n-6 and n-3 polyunsaturated fatty acid plays an important role in determining the biological effects of dietary polyunsaturated fatty acid, and the recommended ratio of n-6 to n-3 polyunsaturated fatty acid should be between 5:1 and 10:1\(^25\). In this study, we found that the ratio of n-6 to n-3 polyunsaturated fatty acid reached 6.32:1 in EMO, which was closer to the FAO/WHO recommended values. Besides, several studies have evidenced the hepatoprotective effect of n-3 polyunsaturated fatty acids. All values are means ± SD (n = 8). Different letters indicate a significant difference (p < 0.05).
fatty acid\textsuperscript{2, 5, 23, 39}, and in this study we found that linolenic acid was main n-3 polyunsaturated fatty acid in EMO, which was reached 7.57\%. Therefore, we assumed that EMO might have a beneficial effect on attenuating NAFLD and related metabolic diseases.

It is generally known that long-term high-fat diet consumption directly induced obesity, which accompanied with NAFLD\textsuperscript{14, 40}. NAFLD is a pervasive chronic disease and is characterized to hepatic lipids accumulation and lipids metabolic disorders\textsuperscript{11, 17}. According to the report of Marchesini, the lifestyle modification and body weight change play an important role in the pathogenesis of NAFLD\textsuperscript{20}. Another study has evidenced that reducing body weight was a great strategy for preventing NAFLD\textsuperscript{8}. Generally, the formation of obesity is attributed to fat accumulation, thus decreasing fat weight may be a good way to achieve the reduction of body weight thereby alleviating NAFLD. Peng et al found that the reduction of total body fat could efficiently block liver steatosis\textsuperscript{34}. Moreover, increasing adipocyte cell number and cell size was the primary reason why the adipose tissue expansion\textsuperscript{27}, thus the beneficial effect on protecting against NAFLD was reducing adipocyte cell number and cell size to a normal level. In the present study, the body weight and fat weight were reduced significantly after supplementation with EMO (Table 3), and the improvement effect of adipocyte cell size was clearly observed in Fig. 2A, B. Besides, the strong correlation between the body weight (BW) and serum lipids parameters as well as NAFLD-related indicators (Fig. 6), suggesting the weight gain might be a potent signal for the formation of lipids metabolic disorders and pathogenesis of NAFLD.

In addition to, it has been established that a high-fat diet is one of the major reasons not only for obesity but also for lipids metabolic disorder. High-fat diet could lead to abnormal serum lipids by increasing serum TG, serum TC, serum LDL-C levels and decreasing serum HDL-C levels, which caused the formation of hyperlipemia\textsuperscript{13, 19}. At present, the effective method in the treatment of the hyperlipemia was taking hypolipidemic, such as lovastatin\textsuperscript{7}. Meanwhile, there have been many reports that oil containing unsaturated fatty acid, such as Silkworm pupa oil, Lemongrass oil, Starfish oil and so on\textsuperscript{3, 17, 42}, were beneficial for attenuating hyperlipidemia thereby achieving the same effect as hypolipidemic. And similar results, which EMO could improve the serum lipids disorders, were presented in our data (Fig. 3). Thus hypolipidemic property of EMO might be a support to our result of a beneficial effect on decreasing hepatic lipids accumulation, and this point was further confirmed through strong correlation between serum lipids and NAFLD-related indicators (Fig. 6).

As mentioned above, the high-fat diet directly caused lipid metabolism disorder, and simultaneously lead to the imbalance of lipids synthesis and catabolism in the liver. And mild NAFLD was formatted accompany with excessive hepatic lipids accumulation as a result of higher calories food\textsuperscript{22}. Similar results were observed by liver pathology changes in this study (Fig. 5B). Meanwhile, the levels of ALT and AST basically reflected the mild stage of hepatic steatosis, and ALT is usually regarded as a monitor that mirroring early or mild liver damage\textsuperscript{6}. Besides, accumulation of hepatic TG would also enhance the production of fatty acid oxidation. And MDA is usually regarded as an indicator to evaluate the oxidative damage of the liver. Additionally, the formation of hepatic oxidation stress would cause a reduction of GSH, which is one of the key antioxidants involved in protecting cells from damages by reactive oxygen species. GSH reduces disulfide bonds in cytoplasmic proteins to cysteines, in which it is converted to its oxidized form GSSG\textsuperscript{30}. Meanwhile, SOD, GSH-Px and CAT, which are antioxidant enzymes, can prevent against hepatic oxidative stress as a primary defense system\textsuperscript{43}. Correspondingly, the various risk factors, containing hepatic TG, hepatic TC, AST, ALT, hepatic SOD, hepatic GSH/GSSG ration and hepatic MDA, which are known to be involved in the progression of NAFLD, were detected in this study. And the results showed that the risk factors were improved by supplement with EMO (Fig. 4), indicating that EMO exerted a critical effect on protecting against high-fat diet induced hepatic lipids accumulation and liver damage. Interestingly, we also found that body weight and serum TG were all strongly correlated with NAFLD-related factors, indirectly demonstrated that it was beneficial for the improvement of NAFLD via alleviating obesity and hyperlipidemia.

In order to further clearly explore the relative mechanisms of EMO on high-fat diet induced NAFLD, we carried out real-time qPCR to analysis the mRNA expression of genes and performed GC-MS to analyze the fatty acids of livers. Indeed, pathogenesis of NAFLD is complex so that it is difficult to totally unveil the molecular mechanisms responsible for beneficial effects of EMO. In general, the imbalance between lipogenesis and lipids catabolism in the liver was the major factor that caused NAFLD. For this reason, we have mainly paid attention to the specific genes for lipid-related pathways combined with synthesis and catabolism of fatty acids in the liver. According to previous reports, lipid-associated pathways including SREBP1c-, PPARγ- and PPARα-dependent pathways. What’s more, literature reported that the over-expressed of lipogenic genes such as SREBP-1c, PPARγ, FAS and so on in the liver increased the risk of inflammation and hepatic steatosis\textsuperscript{21}. SREBP-1c is regarded as a critical transcription factor for TG synthesis\textsuperscript{35}, which could decrease the expression of lipogenic enzymes, including FAS, ACC, and SCD1\textsuperscript{25, 24}. PPARγ, which regarded as an important transcription factor in adipogenesis\textsuperscript{38}, involving in the micro vesicular accumulation of lipids droplets and glucose homeostasis\textsuperscript{30},
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and its downstream gene mainly contained FAS, AP2, and LPL. In this study, EMO dramatically reduced the mRNA expressions of SREBP-1c, PPARγ, and FAS (Fig. 7A) and suggested that the ameliorative action of EMO was attained by influencing SREBP1c-, PPARγ- and FAS-dependent pathway. Additionally, the strong correlation between SREBP-1c and PPARγ with FAS were presented in Fig. 7B, suggested that EMO could decrease lipogenesis by a pathway that SREBP-1c down-regulated the expression of FAS and PPARγ, and the similar result was observed that SREBP-1c-regulated the mRNA levels of PPARγ and FAS. In addition to, PPARγ also be able to induce the C/EBP-α which is a transcription factor can directly control adipocyte differentiation, while it needs to be further confirmed whether EMO could improve NAFLD via this mechanism. In contrast, the expressions of the genes related with β-oxidation of fatty acid were also analyzed. PPARα, which is a ligand-induced transcription factor, could regulate the process of lipolysis. Higher expression of PPARα could upregulate the expression of CPT-1, ACOX, and UCP, which is related to energy expenditure. Some studies have found that PPARα agonists could prevent the NAFLD induced by high-fat diet. As expected, our study also demonstrated that EMO significantly elevated the expression of PPARα. Meanwhile, the mRNA expression of CPT-1 was higher in MEO group when compared with the HFD group, and the strong correlation between PPARα and CPT-1 was presented in this study, indicated that EMO possessed a good ability to prevent NAFLD via enhancing β-oxidation of fatty acid.

What’s more, there were studies reported that endogenously synthesized n-3 PUFAs can ameliorate high-fat diet induced fatty liver and hyperlipidemia in fat-1 mice, leading to significant attenuation of NAFLD and hepatic injury. In this study, we found that the compositions of PUFAs, which mainly n-3 PUFAs, significantly increased in the livers after treatment with EMO, indicated that endogenously synthesized n-3 PUFAs might can ameliorate high-fat diet induced NAFLD by administration of EMO. Besides, the n-3 PUFAs are ligands for PPARα, and PPARα activation increases the fatty acid oxidation. Thus, when PUFAs were increased, fatty acid oxidation is elevated and thereby reducing lipid accumulation in the liver. Our data showed that the expression of lipogenic genes (SREBP-1c and FAS) was decreased, whereas the expression of lipolytic genes (PPARα and CPT1) was increased after EMO administration, and these results may be related to an increase in the amounts of endogenously synthesized n-3 PUFAs in the liver. Meanwhile, Guo et al has found that endogenously synthesized n-3 PUFAs participate in inhibiting TG synthesis and accelerating fatty acid oxidation in the liver, which is consist with our results. Above all, we preliminarily thought that EMO could prevent NAFLD via endogenously synthesized n-3 PUFAs participate in lipid synthesis and lipidolysis in the liver.
5 Conclusion

In conclusion, this study suggested that EMO could significantly alleviate high-fat diet induced NAFLD via improving body weight, fat weight, serum lipids, hepatic lipids accumulation, and hepatic damage. Meanwhile, EMO could also regulate hepatic lipids metabolism by decreasing the mRNA expressions of SREBP-1c, PPARγ and FAS, and increasing the mRNA expressions of PPARα and CPT-1. And the TG synthesis and fatty acid oxidation were associated with endogenous n-3 PUFAs. Based on the above results (Fig. 9), it preliminarily showed that EMO could be regarded as a dietary supplement for preventing the development of NAFLD. Furthermore, it is important to explore the expression of the proteins of related hepatic lipids metabolism and prove the effect of EMO on inflammation and insulin resistance, thus these researches need to further investigate in the future.

Practical Application

We concluded three highlights in this research as follow: (1) the novelty of materials, EMO is extracted from Elaeagnus mollis Diels, which is a precious woody oil crop in China. (2) It provided the effective evidence that EMO as a functional food could efficiently improve obesity, abnormal serum lipids, hepatic lipids accumulation, and liver injury. (3) The ameliorated mechanism of EMO on NAFLD was preliminarily explored from the aspect of lipids metabolism by gene expression and fatty acids profiles in the liver, which provided a theoretical basis for further research.

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Author Contributions

C.X. Guo guided the whole study, interpreted the results and took part in drafting manuscript; J.P. Qiao collected test data and drafted the manuscript; S.W. Zhang and M.P. Li participated in the guidance of the study and reviewed the manuscript; J. Li provided the materials and reviewed the manuscript; H. Shaimaa revised the typewriting and grammar errors which were appeared in the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Barbosasasilva, S.; Souzamello, V.; Magliano, D.C.; Marinho, T.S.; Aguilera, M.B.; andarim-de-Lacerda, C.A. Singular effects of PPAR agonists on nonalcoholic fatty liver disease of diet-induced obese mice. Life Sci. 127, 73-81 (2015).
2) Beppu, F.; Li, H.; Yoshinaga, K.; Nagai, T.; Yoshinda, A.; Kubo, A.; Kanda, J.; Gotoh, N. Dietary starfish oil prevents hepatic steatosis and hyperlipidemia in C57BL/6N mice fed high-fat diet. J. Oleo Sci. 66, 761-769 (2017).
3) Bae, J.S.; Park, J.M.; Lee, J.; Oh, B.C.; Jang, S.H.; Lee, Y.B.; Han, Y.M.; Ock, C.Y.; Cha, J.Y.; Hahn, K.B. Fat-1 mice prevent high-fat plus high-sugar diet-induced non-alcoholic fatty liver disease. Metabolism 66, 32-44 (2017).
4) Chen, Y.N. Effects of Elaeagnus mollis Diels. seed oil on antioxidant capacity and lipid metabolism of mice. China Oils and Fats 42, 77-80 (2017).
5) Chiu, C.Y.; Wang, L.P.; Liu, S.H.; Chiang, M.T. Fish oil supplementation alleviates the altered lipid homeostasis in blood, liver, and adipose tissues in high-fat diet-fed rats. J. Agric. Food Chem. 66, 4118-4128 (2018).
6) Esteghamati, A.; Noshad, S.; Khalilzadeh, O.; Khalili, M.; Zandieh, A.; Nakhjavan, M. Insulin resistance is independently associated with liver aminotransferases in diabetic patients without ultrasound signs of nonalcoholic fatty liver disease. Metab. Syndr. Relat. D. 9, 111-117 (2011).
7) Feng, W.W.; Kuang, S.Y.; Tu, C.; Ma, Z.J.; Pang, J.Y.; Wang, Y.H.; Zang, Q.C.; Liu, T.S.; Zhao, Y.L.; Xiao, X.H.; Wang, J.B. Natural products berberine and curcumin exhibited better ameliorative effects on rats with non-alcoholic fatty liver disease than lovastatin. Biomed. Pharmacother. 99, 325-333 (2018).
8) Ghaemi, A.; Taleban, F.A.; Hekmatdoost, A.; Rafiei, A.; Hosseini, V.; Amiri, Z.; Homayounfar, R.; Fakheri, H. How much weight loss is effective on nonalcoholic fatty liver disease? Hepat. Mon. 13(12), e15227 (2013).
9) Guo, X.F.; Gao, J.L.; Li, J.M.; Li, D. Fat-1 mice protect against high-fat plus high-sugar diets induced non-alcoholic fatty liver disease. Food Funct. 8, 4053-4061 (2017).
10) Huang, J.H.; Huang, X.H.; Chen, Z.Y.; Zheng, Q.S.; Sun, R.Y. Dose conversion among different animals and healthy volunteers in pharmacological study. Chinese Journal of Clinical Pharmacology & Therapeutics 9, 1069-1072 (2004).
11) Hwang, J.T.; Shin, E.J.; Chung, M.Y.; Park, J.H.; Chung,
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J. Oleo Sci.

S.; Choi, H.K. Ethanol extract of Allium fistulosum inhibits development of non-alcoholic fatty liver disease. *Nutr Res Pract.* **12**, 110-117 (2018).

12) Im, A.R.; Yang, W.K.; Park, Y.C.; Kim, S.H.; Chae, S. Hepatoprotective effects of insect extracts in an animal model of nonalcoholic fatty liver disease. *Nutrients* **10**, 735 (2018).

13) Ji, G.; Zhao, X.; Liang, L.; Jiang, Z. Comparison of dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. *Lipids Health Dis.* **10**, 23 (2011).

14) Jung, H.Y.; Ji, Y.; Kim, N.R.; Kim, D.Y.; Kim, K.T.; Choi, B.H. A *Foimotopsis pinicola* Jeseng formulation has an anti-obesity effect and protects against hepatic steatosis in mice with high-fat diet-Induced obesity. *Evid. Based Complement. Alternat. Med.* **2016**, 1-10 (2016).

15) Kim, J.B.; Wright, H.M.; Wright, M.; Spiegelman, B.M. AADD1/SREBP1 activates PPARγ through the production of endogenous ligand. *Proc. Natl. Acad. Sci. USA* **95**, 4333-4337 (1998).

16) Kleiner, D.E.; Brunt, E.M.; Natta, M.V.; Behling, C.; Contos, M.J.; Cummings, O.W.; Ferrell, L.D.; Liu, Y.C.; Torbenson, M.S.; Unalp-Arida, A.; Yeh, M.; McCullough, A.J.; Sanyal, A.J. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* **41**, 1313-1321 (2005).

17) Kumar, V.R.; Inamdar, M.N.; Nayeemunnisa; Viswanatha, G.L. Protective effect of lemongrass oil against dexamethasone induced hyperlipidemia in rats: possible role of decreased lecithin cholesterol acetyl transferase activity. *Asian Pac. J. Trop. Med.* **4**, 658-660 (2011).

18) Kim, E.H.; Bae, J.S.; Hahn, K.B.; Cha, J.Y. Endogenously synthesized n-3 polyunsaturated fatty acids in fat-1 mice ameliorate high-fat diet-induced non-alcoholic fatty liver disease. *Biochem. Pharmacol.* **84**, 1359-1365 (2012).

19) Kuo, Y.H.; Lin, C.H.; Shih, C.C. Ergostatrien-3β-ol from *Antrodia camphorata* inhibits diabetes and hyperlipidemia in high-fat-diet treated mice via regulation of hepatic related genes, glucose transporter 4, and AMP-activated protein kinase phosphorylation. *J. Agric. Food Chem.* **63**, 2479-2489 (2015).

20) Kan, L.; Wang, L.; Ding, Q.; Wu, Y.; Ouyang, J. Flash eExtraction and physicochemical characterization of oil from *Elaeagnus mollis* Diels seeds. *J. Oleo Sci.* **66**, 345-352 (2017).

21) Lima-Cabello, E.; García-Mediavilla, M.V.; Miquelena-Colina, M.E.; Vargas-castrillon, J.; Lozano-Rodrigues, T.; Fernandez-Bermejo, M.; Olcoz, J.L.; Gonzalez-Galego, J.; García-Monzon, C.; Sanchez-Campos, S. Enhanced expression of pro-inflammatory mediators and liver x-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C. *Clin. Sci. (Lond.)* **120**, 239-250 (2011).

22) Lee, H.S.; Nam, Y.; Chung, Y.H.; Kim, H.R.; Park, E.S.; Chung, S.J.; Kim, J.H.; Sohn, U.D.; Kim, H.C.; Oh, K.W.; Jeong, J.H. Beneficial effects of phosphatidylcholine on high-fat diet-induced obesity, hyperlipidemia and fatty liver in mice. *Life Sci.* **118**, 7-14 (2014).

23) Lee, M.F.; Lai, C.S.; Cheng, A.C.; Hou, J.S.; Badmaev, V.; Ho, C.T.; Pan, M.H. Krill oil and xanthigen separately inhibit high fat diet induced obesity and hepatic triglyceride accumulation in mice. *J. Funct. Foods* **19**, 913-921 (2015).

24) Li, L.; Yun, J.H.; Ryoo, J.E.; Lee, K.J.; Choi, B.C.; Baek, K.H. 54G/C polymorphism of SREBF-1 gene is associated with polycystic ovary syndrome. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **188**, 95-99 (2015).

25) Liang, S.; Yang, R.; Dong, C.; Yang, Q. Physicochemical properties and fatty acid profiles of *Elaeagnus mollis* Diels nut oils. *J. Oleo Sci.* **64**, 1267-1272 (2015).

26) Liu, F.J.; Liang, D.; Miao, L.Y.; Li, P.; Li, H.J. Liver-specific metabolomics characterizes the hepatoprotective effect of saponin-enriched *Celosia semen* extract on mice with nonalcoholic fatty liver disease. *J. Funct. Foods* **42**, 185-194 (2018).

27) Makni, M.; Fetoui, H.; Gargouri, N.K.; Garoui, E.M.; Jaber, H.; Makni, J.; Boudawara, T.; Zegha, N. Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in omega-3 and omega-6 fatty acids in hypercholesterolemic rats. *Food Chem. Toxicol.* **46**, 3714-3720 (2008).

28) Matsumoto, T.; Tera, S.; Oishi, T.; Kuwashiro, S.; Fujisawa, K.; Yamamoto, N.; Fujita, Y.; Hamamoto, Y.; Furutani-Seiki, M.; Nishina, H.; Sakaida, I. Medaka as a model for human nonalcoholic steatohepatitis. *Dis. Model. Mech.* **3**, 431-440 (2010).

29) Marchesini, G.; Petta, S.; Grave, R.D. Diet, weight loss, and liver health in NAFLD: pathophysiology, evidence and practice. *Hepatology* **63**, 2032-2043 (2015).

30) Mortezaee, K.; Khanlarkhani, N. Melatonin application in targeting oxidative-induced liver injuries: a review. *J. Cell. Physiol.* **233**, 4015-4032 (2017).

31) Organization, W.H. Obesity and overweight, 2018. URL http://www.who.int/zh/newsroom/fact-sheets/detail/obesity-and-overweight. Accessed Sep 25, 2018.

32) World Health Organization, Food and Agriculture Organization of the United Nations. In *Preparation and use of food-based dietary guidelines: report of a joint FAO/WHO Consultation* (WHO Technical Report Series 880). World Health Organization, pp. 52-55 (1998).

33) Park, S.; Shin, S.; Lim, Y.; Shin, J.H.; Seong, J.K. Korean pine nut oil attenuated hepatic triglyceride accumulation in high-fat diet-induced obese mice. *Nutrients* **8**, 59 (2016).

*J. Oleo Sci.*
34) Peng, C.H.; Lin, H.T.; Chung, D.J.; Huang, C.N.; Wang, C.J. Mulberry leaf extracts prevent obesity-induced NAFLD with regulating adipocytokines, inflammation and oxidative stress. *J. Food Drug Anal.* **26**, 778-787 (2018).

35) Rosen, E.D.; Walkey, C.J.; Puigserver, P.; Spiegelman, B.M. Transcriptional regulation of adipogenesis. *Genes Dev.* **14**, 1293-1307 (2000).

36) Rosen, E.D.; Hsu, C.; Wang, X.; Sakai, S.; Freeman, M.W.; Gonzalez, F.J.; Spiegelman, B.M. C/EBPa induces adipogenesis through PPARg: a unified pathway. *Genes Dev.* **16**, 22-26 (2002).

37) Suk, S.; Kwon, G.T.; Lee, E.; Jang, W.J.; Yang, H.; Kim, J.H.; Thimmegowda, N.R.; Chung, M.Y.; Kwon, J.Y.; Yang, S.; Kim, J.K.; Park, J.H.Y.; Lee, K.W. Gingerenone A, a polyphenol present in ginger, suppresses obesity and adipose tissue inflammation in high-fat diet-fed mice. *Mol. Nutr. Food Res.* **61**, 1700139 (2017).

38) Villarroya, F.; Iglesias, R.; Giralt, M. PPARs in the control of uncoupling proteins gene expression. *PPAR Res.* **2007**, 74364 (2007).

39) Wang, H.; Cai, Y.Z.; Shao, Y.; Zhang, X.F.; Li, N.; Zhang, H.Y. Fish oil ameliorates high-Fat diet induced male mouse reproductive dysfunction via modifying the rhythmic expression of testosterone synthesis related genes. *Int. J. Mol. Sci.* **19**, 1325 (2018).

40) Wang, T., Cui, V., Xie, L., Xing, R., You, P., Zhao, Y.L., Yang, Y.Q., Xu, Y.Q., Zeng, L., Chen H.Q., Liu, M.Y. Kisspeptin receptor GPR54 promotes adipocyte differentiation and fat accumulation in mice. *Front. Physiol.* **9**, 209 (2018).

41) Xu, T.Y.; Dong, Y.F.; Yang, W.; Xu, D.; Zhang, J.J.; Lou, Q.M. Analysis of lipids and fatty acids compositions of squid livers by NMR and GC-MS. *Journal of the Chinese Cereals and Oils Association* **31**(11), 140-144 (2016).

42) Zou, Y.; Shi, Y.; Liu, J.; Mu, L.X.; Liao, S.T. *Silkworm pupa* oil exerts hypolipidemic and antioxidative effects on rat model of high-fat diet induced hyperlipidemia. *J. Dent. Res.* **88**, 345-350 (2009).

43) Zhu, S.Y.; Jiang, N.; Yang, J.; Tu, J.; Zhou, Y.; Xiao, X.; Dong, Y. *Silybum marianum* oil attenuates hepatic steatosis and oxidative stress in high fat diet-fed mice. *Biomed. Pharmacother.* **100**, 191-197 (2018).