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ERUCA SATIVA MILL SEEDS OIL ALLEVIATES HYPERLIPIDEMIA AND NON-ALCOHOLIC FATTY LIVER DISEASE IN SYRIAN HAMSTER

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ABSTRACT

The impact of oils rich in long chain monounsaturated fatty acids (LCMUFA) against hyperlipidemia and non-alcoholic fatty liver disease (NAFLD) has been inadequately described. In addition, the chemical solvents and the high temperature used in vegetable oils extraction process from seeds cause severe loss of many vital compounds. So the goal of this paper was to examine the effect of cold pressed *Eruca sativa* Mill seeds oil (ESSO), as a source of LCMUFA, on hyperlipidemia and NAFLD in Syrian hamster. The ESSO content of fatty acids was analyzed using chromatographic methods. Fifty two (52) healthy male golden Syrian hamsters used in this experiment were randomly divided into 4 groups (Completely Randomized Design). Negative control group, CHD group, positive control group and ESSO group. This experiment was achieved in two periods. The first period continued 4 weeks, in which hyperlipidemia and NAFLD were induced in CHD, positive control and ESSO groups through feeding on a hyperlipidemic diet. The second period also lasted 4 weeks, in which ESSO was orally gavaged at 2 g/kg of the body weight daily to animals of ESSO group. The levels of total cholesterol (TC), triglycerides, HDL-C and glucose and the activities of ALT, AST, ALP, LDH and CK were analysed in the serum. One way analysis of variance (ANOVA) followed by Duncan's multiple range test was used for statistical analysis. The consumption of hyperlipidemic diet for 4 weeks caused a significant raise ($P<0.05$) of triglycerides, glucose, ALT, AST, LDH, CK and a significant reduction ($P<0.05$) of the HDL-C/TC ratio, at the same time created lipid accumulation in liver cells in CHD, positive control and ESSO groups in comparison with negative control group at the end of the first period. These negative influences were alleviated in ESSO group by administration of ESSO at the end of second period. In conclusion, The examined cold pressed ESSO has effective hypolipidemic and hepatoprotective effects in Syrian hamsters with hyperlipidemia and NAFLD.

Keywords: *Eruca sativa* seeds oil, Hamster, Hyperlipidemia, Non-alcoholic Fatty Liver Disease.

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INTRODUCTION

Recently, hyperlipidemia and nonalcoholic fatty liver disease (NAFLD) have been considered as life threatening diseases worldwide. Hyperlipidemia is described as a disorder of lipid metabolism that contains hypercholesterolemia and hypertriglyceridemia (Ma *et al*., 2012). Hyperlipidemia is one of the main causes of cardiovascular diseases (CVD) which have been reported to be the number 1 reason for death in the World (Berry *et al*., 2012). It was estimated that 17.9 million people died worldwide in 2016 due to the CVD, this number is expected to rise in the coming years (World Health Organization, 2017). NAFLD is the most common cause of chronic liver illness and the most well known reason for liver transplant in the world. It is characterized by abnormal accumulation of lipids into hepatocytes and increase of liver enzymes, especially alanineaminotransferase (ALT) and aspartateaminotransferase (AST). Hyperlipidemia, diabetes and obesity are the major causes of NAFLD. A close relationship has been reported between hyperlipidemia and NAFLD (Araújo *et al*., 2018; Chalasani *et al.*, 2018). NAFLD is present in roughly half of patients with hyperlipidemia (Assy *et al*., 2000). The cases of hyperlipidemia and NAFLD are closely related to diet composition. Foods rich in saturated fats and cholesterol are predisposing factors for hyperlipidemia which is presently accepted to be a fundamental factor in the development of NAFLD (Browning and Horton, 2004). The universal spread of NAFLD is 24–25% among general population (Bedogni *et al*., 2005). Therefore, the prevention and treatment of these two problems are strong public interest. Even though statins are effective in reducing the risk of CVD in patients with hyperlipidemia (Baigent *et al*., 2010; Stone *et al*., 2014), these drugs cause many harmful side effects such as, liver function disorders, high blood glucose level and musculoskeletal pain and inflammation (Shah and

Goldfine, 2012; Bruckert *et al*., 2005; Parker *et al*., 2013). Lately, there has been restored interest in studying beneficial seeds oils because of their healing properties. Multiple studies have shown that the use of conventional oils like flaxseed oil can diminish hyperlipidemia and improve fatty liver (Vijaimohan *et al*., 2006; Yang *et al*., 2009). These antihyperlipidemic and hepatoprotective effects have been attributed to the oil content of alfa linoleic acid (ALA), which has 3 double bonds and 18 carbon atoms (Vijaimohan *et al*., 2006). We have demonstrated that untraditional borage oil rich in gamma linolenic acid (GLA) with an 18 carbon atoms and 3 double bonds can improve fatty liver in male golden Syrian hamsters (Alhilal *et al*., 2019). Mert *et al*. (2020) showed that evening primrose oil rich in GLA regulates dyslipidemia and reduces steotosis in heptocytes of rats with metabolic syndrome. Studies about the impact of oils rich in long chain monounsaturated fatty acids (LCMUFA) with chain longer than 18 carbon atoms against hyperlipidemia and fatty liver have been inadequately described. *Eruca sativa* Mill seeds oil (ESSO) is an importanat source of LCMUFA (Uğur *et al*., 2010). Therapeutically, most uses of *Eruca sativa* were restricted to enhance the sexual ability of males. Mallah *et al*. (2017) observed that *Eruca sativa* leaves solution with sildenafil boosts the pharmacokinetics of sildenafil in rats. Although consumption of LCMUFA concentrate decreased plasma non-high density lipoprotein cholesterol (nonHDL-C) and improved hyperinsulinemia, it did not impact on plasma high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) and liver lipid content in mice (Yang *et al*., 2015). Alqasoumi (2010) showed that *Eruca sativa* leaves extract protects the liver from damage caused by carbon tetrachloride in rats. On the other hand, the oil quality is related with its content of unsaturated fatty acids (UFA). Generally, several solvents such as chloroform or ethanol are used to extract seeds oils. Pervious studies have shown that temperatures and diverse solvents used in extraction of oils affect the vital components of oils (Hou *et al.*, 2006; Jedidi *et al.*, 2020).

According to the available information, there is no report about the effect of ESSO, obtained by cold mechanical pressing and rich in LCMUFA, on hyperlipidemia and NAFLD in hamster. So, this study was designed to examine this ability in male golden Syrian hamster.

MATERIALS AND METHODS

Determination of fatty acids in ESSO: Ripe and dried *Eruca sativa* Mill seeds were gathered from private farms in Hama city, Syria and authenticated in Botany Department, Faculty of Science, University of Damascus. ESSO was obtained by cold mechanical pressing of *Eruca sativa* Mill seeds. For the analysis of ESSO content of fatty acids, Gas Chromatography GC3800 with Mass Spectrometry MS2000 was used. By comparison the mass spectra of fatty acids with those saved in the library of the GC-MS, the fatty acids content of ESSO was identificated.

Animals treatment and experimental protocol: This study was carried out in 2013, in the experimental animals unit, science research laboratory, faculty of veterinary medicine, Al-Baath University, Hama, Syria. During the experiment, all necessary measures were taken in accordance with international guidelines for the care and use of experimental animals. Al-Baath University Board (Homs, Syria) reviewed this protocol, approved all procedures and gave permission before applying the experiment (Approval Number 1011-12).

Coconut oil and sheep fat were obtained from local market in Hama city, Syria. Fifty two (52) male golden Syrian hamsters were procured from the animal health management (Damascus, Syria). The ages of animals ranged between 5-7 weeks and their weights between 45 to 50 grams at the start of the experiment. One hamster was placed in each cage. The animals were fed on standard animal feed for rodents with water *ad libitum* and kept under standard laboratory conditions (Temperature 22±2°C and Lighting 12/12 hour light-dark) in experimental animals unit, science research laboratory, faculty of veterinary medicine, Al-Baath University (Hama, Syria). For acclimation, the animals were left under these conditions for 15 days. To determine the health status of the animals used in this study, lipids and liver enzymes were measured in the blood serum before onset the experiment. The results showed that all animals were healthy. Fifty two (52) healthy male hamsters used in this experiment were randomly divided into 4 groups (13 hamsters in each group). Negative control group, CHD group, positive control group and ESSO group. The experiment was conducted in two periods. The first period lasted 4 weeks, in which hyperlipidemia and NAFLD were induced in CHD, positive control and ESSO groups through feeding on a hyperlipidemic diet that consists of 80% standard animal feed for rodents + 13.5% sheep fat + 6.5% coconut oil. The animals of negative control group were fed with standard animal feed for rodents. The second period also lasted 4 weeks, in which experiment groups were treated as follows: CHD group: Continued to be fed on hyperlipidemic diet. Positive control group: The hyperlipidemic diet was replaced by the standard animal feed for rodents. ESSO group: The hyperlipidemic diet was replaced by standard animal feed for rodents and the animals were orally gavaged with ESSO 2 g/kg of the body weight daily. Negative control, CHD and positive control groups were orally gavaged with distilled water 2 g/kg of the body weight daily to make the conditions of the experiment equal in all experimental groups.

Biochemical examination: For measuring the levels of total cholesterol (TC), HDL-C, TG and glucose and the activities of ALT, AST, alkalenfosfataz (ALP), lactate dehydrogenase (LDH) and creatine kinase (CK), two blood samples were collected from fasting animals (16 h), once at the end of first period and another at the end of second period via the retro orbital sinus into capillary tubes under anesthesia by diethyl ether (Wilson *et al*., 2006). Taken blood samples were left to coagulate and centrifuged at 3000 rpm for 30 minutes. The non hemolysed serum was collected and kept at -20°C until tests were conducted. BioSystems kits were used to measure all parameters enzymatically by using Spectrophotometer/BioSystems-Model BTS-310 EU/Spain.

Histopathological examination: For histopathological examination, samples were prepared with traditional techniques. After sacrifying hamsters under anesthesia, livers were promptly removed, fixed in 10% neutral formalin solution. Biopsies which were taken from various areas of specimens were implanted into paraffin. Histopathological evaluation was done by using light microscope after staining sections with hematoxylin and eosin (H&E).

Statistical analysis: One way analysis of variance (ANOVA/Duncan) was used to detect the significant differences among the four groups at the same period of time. The t-student's test was used at a confidence level of 95% to evaluate significant differences between the values obtained from 4 and 8 weeks of the same group. The results were expressed as mean ± standard deviation (SD). The data were analyzed by SPSS-17. $P<0.05$ was accepted to be the least limit of significance. All measurements were done in 3 replicates.

RESULTS AND DISCUSSION

ESSO fatty acid profile: The proportion of LCMUFA of ESSO in this study reached to 65.29% (Table 1), whilst ESSO obtained by using hexane solvent contained 63% of LCMUFA (Uğur *et al*., 2010) and ESSO extracted by using methanol solvent and elevated temperatures included 47.2% of LCMUFA (Chakrabarti and Ahmad, 2009). The percentage of linoleic acid in this study increased compared with (Chakrabarti and Ahmad, 2009; Uğur *et al*., 2010). This may perhaps be ascribed to the fact that no chemical substance was used in the ESSO extraction process in this study. Elevated percentage of linoleic acid is a positive factor, because this acid will be converted into GLA (Guil-Guerrero *et al*., 2017; Kapoor *et al*., 2015), which has antihyperlipidemic (Al-okbi *et al*., 2018) and hepatoprotective effects (Lukivskaya *et al*., 2012) in the body. In the present study, ESSO contained 4.91% saturated fatty acids (SFA) (Table 1), while this percentage increased to 14% and 11.8% according to (Uğur *et al*., 2010) and (Chakrabarti and Ahmad, 2009) respectively. Lowered level of SFA in current study indicates importance of the cold squeeze of seeds in suppressing the saturation of UFA, thus preserving UFA which give quality to oil.

Table 1. Fatty acids composition of *Eruca sativa* Mill seeds oil.

Fatty acids	% of total fatty acids
Palmitic(C16:0)	3.73
Palmitoleic(C16:1)	0.25
Stearic(C18:0)	1.18
Oleic(C18:1)(n9)	14.56
Linoleic(C18:2)(n6)	12.22
α-Linolenic(C18:3)(n3)	0.83
Eicosanoic (C20:1)	20.36
Erucic acid (C22:1)(n9)	44.93
Others	1.94
Total	100
LCMUFA	65.29
SFA	4.91

LCMUFA: long chain momounsaturated fatty acids, SFA: saturated fatty acids

Rodent model used: The golden Syrian hamster has been widely used in researches of lipid metabolism due to the similarity between the mechanisms of lipids metabolism in this animal and humans. The mechanisms of cholesterol synthesis, its transporting in the blood, uptake into the cell and undermining in humans and hamster are identical. Moreover, the concentration of cholesterol in hamster responds to dietary fats in the same way which happens in humans (Ohtani *et al.*, 1990; Spady *et al*., 1993). The golden Syrian hamster was used in this study. The model of this rodent was an important factor in inducimg hyperlipidemia within 4 weeks. In addition, the hamster was characterized by its small size, easy treatment, and great tolerance for daily fat doses.

Visceral fat and serum glucose: Visceral fats were collected at the end of the experiment. Hyperlipidemic diet created a significant increase ($P<0.05$) of visceral fat weight for CHD group in comparison with other groups (Fig. 1).

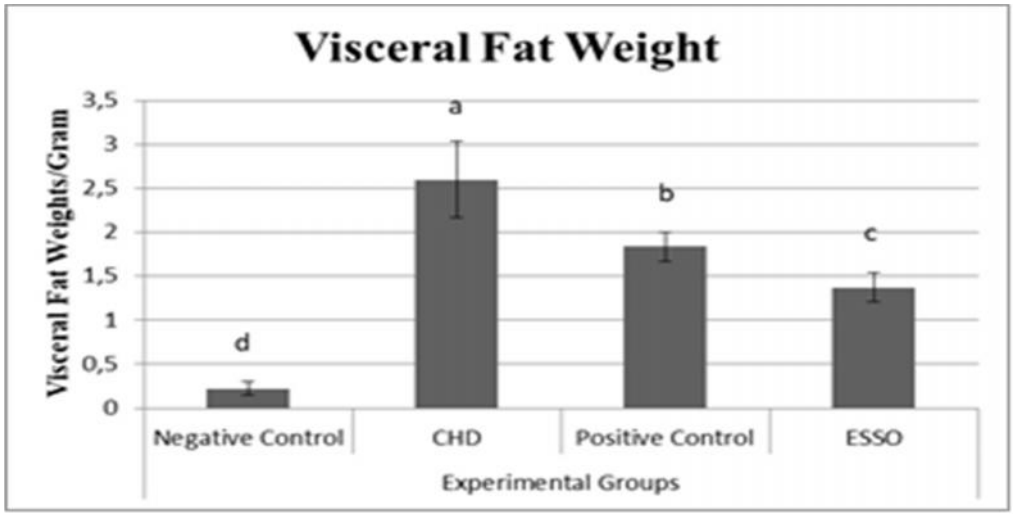

Fig. 1. Visceral fat weights in the end of second period.

Means followed by different small letters are significantly different (P<0.05), n=13. CHD: The group that treated with hyperlipidemic diet during the two periods of study. Positive control: The group that treated with hyperlipidemic diet only in the first period. ESSO: The group that treated with hyperlipidemic diet only in the first period and administered with *Eruca sativa* Mill seeds oil in the second period.

Tzang *et al*. (2009) observed an inclination towards bigger size of visceral fat in hamsters treated with coconut oil and butter. The increase in visceral fat in this study may be credited to the transmission of excess fat from the liver's need to peripheral tissues. A noteworthy increase in the weight of visceral fat was associated with elevated glucose level for CHD, positive control and ESSO groups compared to the negative control group at the end of first period (Table 2). High blood glucose concentration was observed in mice fed with hyperlipidemic diet (Sato *et al*., 2007). Several studies have shown that SFA are implicated in inducing diabetes as a result of their impact on insulin sensitivity and glucose/ lipid metabolism (Hulbert *et al.*, 2005; Abete *et al.*, 2011). In this study the increase of glucose level after treatment with hyperlipidemic diet may be attributed to the increased accumulation of visceral fat in the internal organs such as, liver and pancreas, which assume a fundamental function in the metabolism of carbohydrates. The livers of CHD, positive control groups seemed fragile, torn and missing their hardness, color, and natural gloss compared to the negative control group (Fig. 2).

At the end of second period, visceral fat weight decreased significantly (P<0.05) in ESSO group compared to the positive control group (Fig. 1). This reduction was associated with a significant decrease (P<0.05) in serum glucose (Table 2). Although plasma glucose level of mice were fed with saury oil rich LCMUFA was not different from those fed with lard, saury oil reduced adipocyte size (Yang *et al*., 2015). The decrease of visceral fat weight in ESSO group may be due to the role of LCMUFA in decreasing the TG synthesis. The liver returned normal appearance (Fig. 2). Thus serum glucose came back to normal level.

Serum lipid profile: Coconut oil was used in this study as a source rich in SFA, as well as sheep fat as a source rich in cholesterol. The aim of feeding hamsters with a diet rich in SFA and cholesterol for a period of 4 weeks was creating hyperlipidemia. For confirming the occurrence of hyperlipidemia, blood samples were taken from fasted (16h) hamsters. The use of hyperlipidemic diet showed significant increase (P<0.05) of the TC, HDL-C and TG concentrations almost (130%, 61%, 203%, respectively) and significant decrease (P<0.05) in HDL-C/TC ratio almost (30%) in each of CHD, positive control and ESSO groups at the end of first period in comparison with the negative control group (Table 2). High level of HDL-C was observed after using the hyperlipidemic diet in this study. HDL-C level increases when TC level raises. This increase is not seen as positive factor because the HDL-C/TC ratio decreased approximately by 30%. High TC concentration after feeding on hyperlipidemic diet may be construed by increased synthesis of cholesterol through using SFA with the aim of lowering their level in the blood. Raised TG level in this study can be attributed to high concentration of very low density lipoprotein cholesterol (VLDL-C) (Not referenced in the outcomes chapter), that is the primary transporter of TG produced by the liver. It is unclear whether the ESSO as one of the important sources of LCMUFA has a beneficial effect on hyperlipidemia. The administration of ESSO caused a significant decrease (P<0.05) of the TC, HDL-C and TG concentrations almost (32%, 24%, 16%, respectively) and significant increase (P<0.05) in HDL-C/TC ratio almost (11%) in ESSO group in comparison with the positive

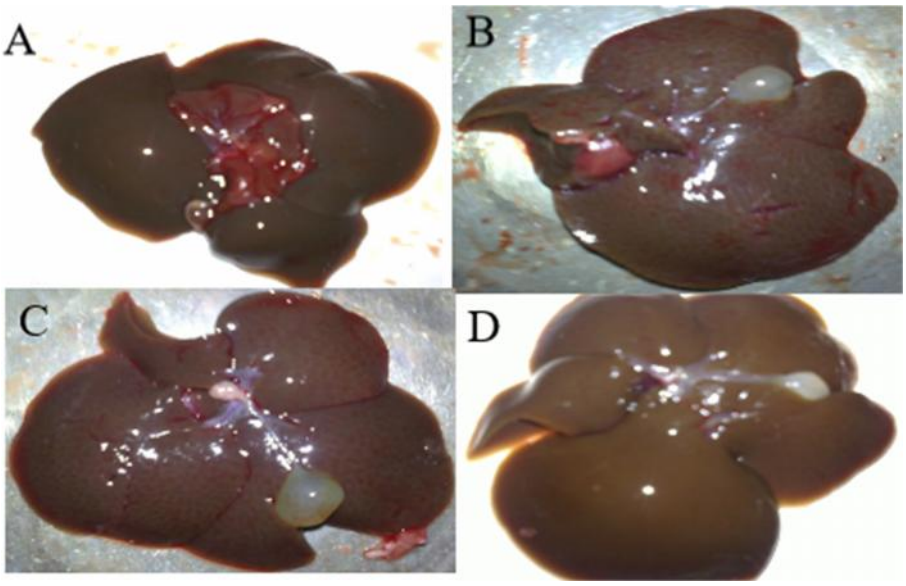

Fig. 2. Photographs of livers in the end of second period.

(A) Negative control group: Normal appearance of liver. (B) CHD group (The group that treated with hyperlipidemic diet during the two periods of study): Liver seems fragile, torn ve lacking its natural color and shine. (C) Positive control group (The group that treated with hyperlipidemic diet only in the first period): Liver seems fragile, torn ve lacking its natural color and shine. (D) ESSO group (The group that treated with hyperlipidemic diet only in the first period and administered with *Eruca sativa* Mill seeds oil in the second period): Normal appearance of liver.

Table 2. Effect of hyperlipidemic diet and *Eruca Sativa* Mill seeds oil on blood lipid profile and glucose.

Parameters	Experimental Groups			
	Negative Control	CHD	Positive Control	ESSO
TC(mg/dl)				
4 weeks(First Period)	^{c}116.53±5.23	^{ab}266.84±10.25^{B}	^{a}267.46±8.30	^{b}260.61±7.93^{A}
8 weeks(Second Period)	^{d}113.53±5.89	^{a}314.15±4.75^{A}	^{b}271.76±11.64	^{c}184.53±11.04^{B}
HDL-C(mg/dl)				
4 weeks(First Period)	^{c}42.69±3.70	^{a}67.92±2.49^{A}	^{a}68.76±3.19	^{b}64.15±3.82^{A}
8 weeks(Second Period)	^{d}42±3.93	^{b}64.69±3.30^{B}	^{a}68.07±3.14	^{c}51.23±4.39^{B}
HDL-C/TC				
4 weeks(First Period)	^{a}0.366±0.024	^{b}0.254±0.014^{A}	^{b}0.257±0.018	^{b}0.246±0.014^{B}
8 weeks(Second Period)	^{a}0.369±0.024	^{d}0.206±0.011^{B}	^{c}0.250±0.014	^{b}0.278±0.033^{A}
TG(mg/dl)				
4 weeks(First Period)	^{c}50.53±5.75	^{a}153.30±5.03^{B}	^{ab}148.53±7.98	^{b}144±7.46^{A}
8 weeks(Second Period)	^{d}54.61±7.82	^{a}190.46±8.01^{A}	^{b}153.38±8.89	^{c}128.53±7.52^{B}
Glucose(mg/dl)				
4 weeks(First Period)	^{c}93.76±10.19	^{a}127.30±9.54^{B}	^{b}119.30±9.76	^{b}118.30±9.23^{A}
8 weeks(Second Period)	^{c}96.30±9.40	^{a}163.46±10.52^{A}	^{b}123.61±11,13	^{c}97.46±8.61^{B}

Values are expressed as Mean±Standard Deviation; n=13. Different small letters indicate significant difference for each parameter in the same row (P<0.05), different large letters indicate significant difference for each parameter in the same column (P<0.05). TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride. CHD: The group that treated with hyperlipidemic diet during the two periods. Positive control: The group that treated with hyperlipidemic diet only in the first period. ESSO: The group that treated with hyperlipidemic diet only in the first period and administered with *Eruca sativa* Mill seeds oil in the second period.

control group at the end of second period (Table 2). Despite the observed decrease in HDL-C concentration, the HDL-C/TC ratio increased. This is a positive indication of the improvement in lipid metabolism thanks to ESSO. The experimental hyperlipidemia did not recede after the hyperlipidemic diet had been removed from the positive control group at the second period, where serum lipid levels were not different between the two trial periods of this group (Table 2). Yang *et al.* (2011) exhibited that pollock oil as a source of LCMUFA effectively improves hyperlipidemia in hyperlipidemic mouse model. The herring diet, rich in LCMUFA decreased TG and VLDL-C levels and increased HDL-C level in comparison with the beef diet without these fatty acids (Gabrielsson *et al*., 2012). The effect of ESSO in suppressing hyperlipidemia may be attributed to the role of LCMUFA in improving lipid metabolism, in particular fatty acids β-oxidation. LCMUFA have important role in improving lipid metabolism by increasing fatty acids β-oxidation and suppressing lipogenesis (Yang *et al*., 2016).**Liver enzymes:** Hyperlipidemia leads to deposition of excess fat from the metabolic capacity into liver cells, thus, hepatic steatosis called NAFLD occurs. In order to check the incidence and regression of NAFLD; ALT, AST, ALP and LDH enzymes activities were measured in serum. These enzymes perform their functions in the cells that made them and release to blood within the normal limits. Elevated plasma enzyme activity is a clinical indicator of tissue damage which synthesizes this enzyme. Although AST and LDH are present in the heart muscle and ALP in the bone, they are primarily localized in hepatic cells. The activity of ALT, AST, ALP and LDH increased approximately (157%, 183%, 23%, 206%, respectively) in each of CHD, positive control and ESSO groups in comparison with negative control group after treating with hyperlipidemic diet for 4 weeks (Table 3). These significant differences increased to reach approximately (214%, 247%, 36%, 237%, respectively) for CHD only in comparison with negative control group at the end of second period (Table 3). Raised ALT, AST, ALP and LDH activities in this work showed that hyperlipidemia was harmful to the liver and created NAFLD. This was reinforced by the histopathological examination of the liver as shown by H&E staining. The lipid accumulated in the liver cells and created small white vesicles in positive control group and big white gaps in CHD group (Fig. 3).

Table 3. Effect of hyperlipidemic diet and *Eruca sativa* Mill seeds oil on activities of some serum enzymes.

Parameters	Experimental Groups			
	Negative Control	CHD	Positive Control	ESSO
ALT(U/L)				
4 weeks(First Period)	^{b}62.84±7.51	^{a}162±12.51^{B}	^{a}159.69±10.01	^{a}162±12.98^{A}
8 weeks(Second Period)	^{d}65.76±7.70	^{a}206.69±9.05^{A}	^{b}163.15±13.23	^{c}87.38±7.80^{B}
AST(U/L)				
4 weeks(First Period)	^{c}77.38±13.54	^{ab}203.53±20.14^{B}	^{b}201.53±18.28	^{a}218.84±27.50^{A}
8 weeks(Second Period)	^{d}71.31±14.23	^{a}247.54±13.57^{A}	^{b}203.62±17.97	^{c}107.77±13.23^{B}
ALP(U/L)				
4 weeks(First Period)	^{b}227.61±24.52	^{a}278.76±22.78	^{a}279.92±22.91	^{a}260.61±27.58
8 weeks(Second Period)	^{d}220.92±7.06	^{a}299±24.96	^{b}283.38±22.10	^{c}257.84±14.72
LDH(U/L)				
4 weeks(First Period)	^{b}504.15±89.69	^{a}1545.6±205.4	^{a}1521.61±193.88	^{a}1472.10±243.83^{A}
8 weeks(Second Period)	^{c}477.92±68.50	^{a}1612.2±128.4	^{a}1550.30±159.93	^{b}793.84±78.57^{B}
CK(U/L)				
4 weeks(First Period)	^{b}170.38±11.66	^{a}202.38±11.89^{B}	^{a}198.23±7.56	^{a}201.76±7.49^{A}
8 weeks(Second Period)	^{c}171.92±10.73	^{a}241.53±12.19^{A}	^{b}202.23±7.04	^{c}176.84±7.90^{B}

Values are expressed as Mean±Standard Deviation; n=13. Different small letters indicate significant difference for each parameter in the same row (P<0.05), different large letters indicate significant difference for each parameter in the same column (P<0.05). ALT: alanineaminotransferase, AST: aspartateaminotransferase, ALP: alkalenfosfataz, LDH: lactate dehydrogenase and CK: creatine kinase. CHD: The group that treated with hyperlipidemic diet during the two periods. Positive control: The group that treated with hyperlipidemic diet only in the first period. ESSO: The group that treated with hyperlipidemic diet only in the first period and administered with *Eruca sativa* Mill seeds oil in the second period.

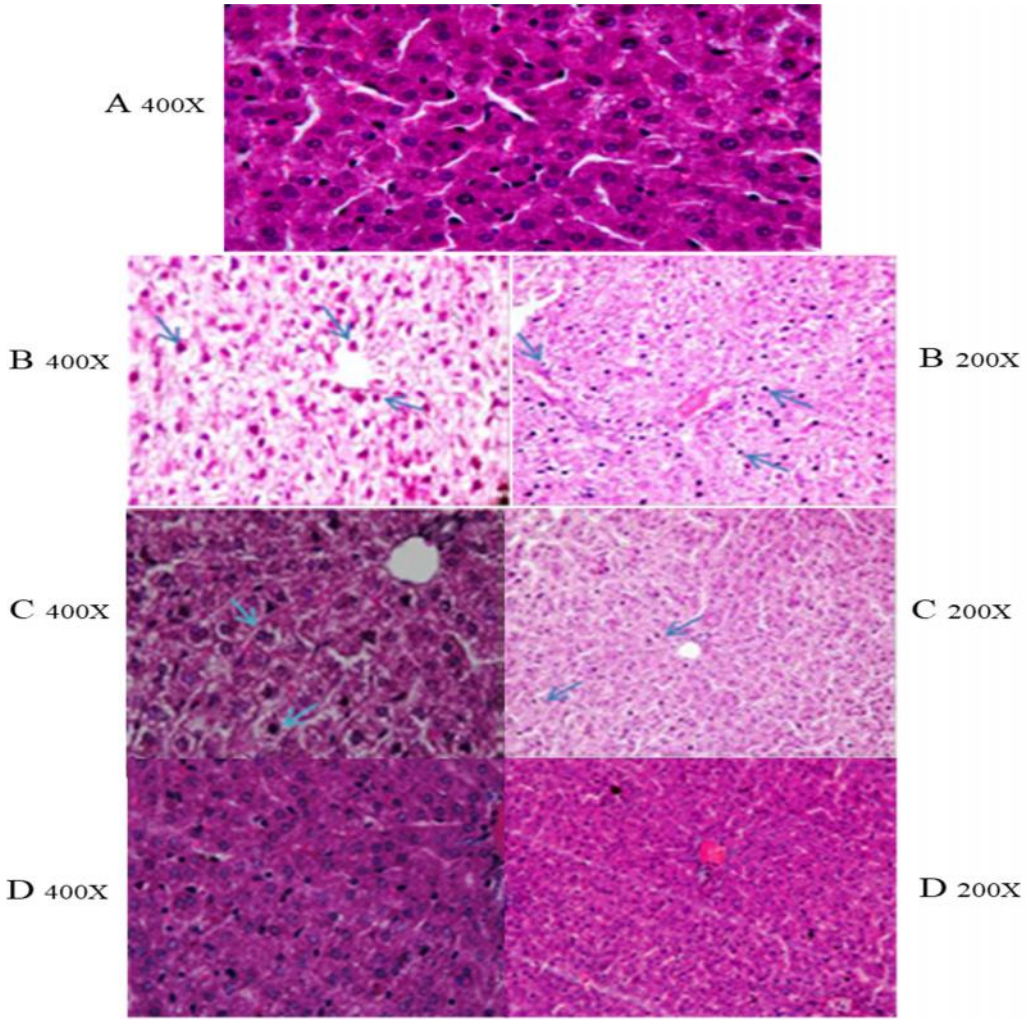

Fig. 3. Photomicrography of liver section in the end of second period, stained with H&E (400X, 200X).

(A) Negative control group: Normal histological structure, the cytoplasm seems dark red because it is rich in glycogen. (B) CHD group (The group that treated with hyperlipidemic diet during the two periods of study): Large gaps in the cells due to the accumulation of fat that pushed the nucleus towards the cell membrane. (C) Positive control group (The group that treated with hyperlipidemic diet only in the first period): Small gaps in the cells due to the accumulation of fat. (D) ESSO group (The group that treated with hyperlipidemic diet only in the first period and administered with *Eruca sativa* Mill seeds oil in the second period): Semi-normal histological structure.

The accumulation of fats in the liver cells or the occurrence of NAFLD may be due to the impaired ability of the liver to disposed of serum lipid. The oxidation of fatty acids in mitochondria is an important mechanism for eliminating lipid in the liver. The high activity of AST in this study demonstrated that the damage reached to the mitochondria of hepatocytes where the β-oxidation of fatty acids occurs. When the uptake of fatty acids exceeds their oxidation, NAFLD occurs (Ipsen *et al*., 2018). The increase of CK activity was approximately (18%) in each of CHD, positive control and ESSO groups in comparison with negative control group after treating with hyperlipidemic diet for 4 weeks. This is a clinical indicator of muscle damage which is likely to be cardiac in this study, because hyperlipidemia is risk factor for CVD. Although saury oil containing 21% LCMUFA decreased hepatic steatosis (Yang *et al*., 2015), it is unclear whether the ESSO as a source of LCMUFA can attenuate NAFLD. In addition to measuring the activities of serum enzymes like ALT, AST, ALP and LDH, histopathological examination was performed for evaluation the hepatoprotective effects of ESSO. The administration of ESSO caused a significant decrease ($P<0.05$) in the activities of ALT, AST, ALP and LDH approximately (46%, 47%, 9%, 49%, respectively) in ESSO group in comparison with positive control group at the end of second period (Table 3). This diminishing was associated with reduction in accumulated fat in liver cells

(Fig. 3) and the liver regained its texture and natural color (Fig. 2). The experimental NAFLD did not recede after hyperlipidemic diet had been excluded from the positive control group at the second period, where no significant differences (P>0.05) were observed in the liver enzymes of this group between the two trial periods (Table 3). Yang *et al*. (2015) indicated that saury oil decreases hepatic steatosis in mice fed a high-fat diet. Our results were in agreement with the previous study. Furthermore, CK enzyme activity diminished by 13% in ESSO group at the end of the second period. This is an indication of reduction in the potential heart damage. LCMUFA play a substantial rol in atherosclerosis decline (Yang *et al*., 2016). Beneficial effects of ESSO in this study may be attributed to the role of LCMUFA in rebalancing between lipid acquisition and disposal in the liver by promoting β-oxidation. Accordingly, accumulated lipids decreased, HDL-C/TC ratio increased and the enzymes activities improved by repairing the liver cells.

The ability of cold pressed ESSO to attenuate hyperlipidemia and NAFLD was examined by checking serum lipids, liver and heart enzymes and liver histopathology. In the present study, the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme which plays important role in regulation cholesterol synthesis was not measured. Notwithstanding, examined parameters gave promising results about the capability of this oil in attenuating these metabolic disorders. Undoubtedly, further studies are needed to clarify chemical mechanisms underlying the effect of ESSO.

Conclusion: In conclusion, the findings of this paper propose that cold pressed *Eruca sativa* mill seeds oil alleviates hyperlipidemia and non-alcoholic fatty liver disease in Syrian hamster.

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