



## The role of different edible vegetable oils as hypolipidemic agents on experimental hyperlipidemic rat model



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### Abstract

Hyperlipidemia is a common disorder that includes genetic and functional disorders represented by elevated lipid levels in the body. The objective of this study was to characterize the physicochemical parameters of the edible seed oils separated from linseed, soybean, olive seed and blends from different proportion, then identify the optimum blend in controlling serum lipid profile in experimental hyperlipidemic rat model. Histopathological examinations of the liver section were carried out to support the laboratory investigation. Sixty male albino rats were used for the study. The animals were divided into ten groups, where group 1 represents negative control, while rats in group 2 received high fat diet and served as positive control. Groups from 3 to 10 were fed high-fat diet and then supplemented with different vegetable oils. At the termination of the 6 weeks trial, blood samples and organs were collected for estimation of lipid profile. Results showed that rats administered different vegetable oils significantly decrease levels of serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) cholesterol as well as increase levels of high-density lipoprotein (HDL) cholesterol in comparison with rats fed high-fat diet. Histopathological examination of liver tissue in positive control showed severe dilatation and congestion in the central vein with fatty change in the surrounding adjacent hepatocytes. While administration of different vegetable oils showed marked improvement in the histological feature of liver tissues. In conclusion, the findings suggest that balanced blend from linseed, soybean and olive oils help in controlling hyperlipidemia and restoring the lipid profile.

**Key words:** Hyperlipidemia, Vegetable oils, Total cholesterol, Triglycerides, Histopathological examination.

### 1. Introduction

Hyperlipidemia is defined as abnormal blood lipid metabolism, characterized by increasing in total cholesterol, low density lipoprotein levels as well as the reduction in high density lipoprotein (HDL-C) levels [1, 2]. In recent years, much interest has been aroused in the anti-hyperlipidemia effect of dietary supplementation with certain edible oils [3, 4]. Vegetable oils consist of triacylglycerols (TAG) that are composed of three fatty acids linked to a glycerol backbone. TAG are major sources of essential fatty acids in the human diet and provide the essential omega-6 fatty acid linoleic acid and the omega-3 fatty acid  $\alpha$ -linolenic acid [5, 6]. The fatty acid composition of TAG is a primary determinant of the nutritional quality and health-promoting properties of vegetable oils. Vegetable oils are commercially obtained from seeds of plants such as soybean and linseed or from fruit mesocarps that surround seeds of plants such as olive and palm oil [7]. TAGs are one of the major

constituents of vegetable oils such as palm, soybean, canola, sunflower, and peanut oils. The relative amount of these fatty acids varies among the different vegetable oils, which affects their nutritional quality and functionality [8-10]. Fatty acids (FA) one of the main components of the living cell. FA could be divided into several groups with different functions, including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) [11-13]. Current research focuses on increasing attention on polyunsaturated fatty acids (PUFA) as essential components in the human diet. PUFA is one of the major components of biomembranes, including the plasma membrane, endoplasmic reticulum, and mitochondrial membrane. They are particularly considered as scavenger free radicals in living systems and in foods upon oxidation of the lipids [14]. It has been stated that edible vegetable oils rich in PUFA lower total cholesterol (TC), triacylglycerol (TG), LDL cholesterol as well as

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increase the beneficial HDL cholesterol [15]. The PUFA presented in flaxseed oil, olive oil, sunflower oil, and soybean oil could be used as hypolipidemic agents [16]. Flaxseed oil is a one of the richest sources of the plant-based  $\omega$ -3 fatty acid. Soybean oil is rich in essential fatty acids linoleic and  $\alpha$ -linolenic acids [17]. Rodriguez *et al.* [18] recommended that daily intake of 1.5-3.0g  $\alpha$ -linolenic acid in human diet tend to be helpful in the treatment and prevention of cardiovascular diseases. Olive oil is distinguished by high MUFA constituents with low SFA and low  $\omega$ -6:  $\omega$ -3 ratio with enhanced oxidative stability [19]. Previous studies have indicated that MUFA-rich oils such as olive, rapeseed and safflower and PUFA-rich oils such as soybean, sunflower oil are hypocholesterolemia agents [20]. Studies indicate that human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids (EFA) of approximately 1: 1, whereas in Western diets the ratio is 15/1 to 16.7/1 [21]. The research study aims to assess the chemical composition of three edible oils such as soybean oil, olive oil and linseed oil, also evaluate the effect of these oils on hyperlipidemic rats.

## 2. Materials and Methods

### 2.1. Materials

Extra virgin olive and linseed oil was kindly supplied from National Research Centre, Cairo, Egypt. Refined, bleached and deodorized (RBD) soybean oil (free from additives) was kindly supplied from Arma Company for Oils, Cairo, Egypt. The oils were stored in a dark bottle at 4°C until laboratory investigation. All solvents and chemicals were used during this study were in HPLC grade and purchased from Sigma-Aldrich, Darmstadt, Germany.

### 2.2. Methods

#### 2.2.1. Physicochemical analysis of oils under investigation

Physical properties such as refractive index, specific gravity and melting point were determined according to the method described by AOAC [22]. Chemical characteristics such as free fatty acids content (Acidity %) was determine according to the method adapted from IUPAC (1987) [23]. The Iodine value of the tested samples was determined using Wijs method according to the method described in AOAC [22]. Peroxide value, Saponification value and unsaponifiable matter content of the tested samples were determined according to the standard methods described in AOAC [22]. Ester value was calculated according to AOAC [22] by subtracting the acid value of oil from the saponification value according to the equation:

$$\text{Ester value} = \text{Saponification value} - \text{Acid value.}$$

#### 2.2.2. Fatty acid composition

The fatty acid profile was detected by the conversion of seed oil to a fatty acid methyl ester (FAMES) according to the modified method by Zahran and Tawfeuk [24]. The FAMES were prepared by adding 1.0 mL of n-hexane to 15 mg of oil followed by 1.0 mL of sodium methoxide (0.4 mol), then the mixture was vortexed for 30 seconds and allowed to settle for 15 min. The upper phase containing the FAMES was recovered and analyzed by gas chromatography (GC-FID). The FAMES were separated with an HP 6890 plus gas chromatography (Hewlett Packard, USA), using a capillary column Supelco™ SP-2380 (30 m×0.25 mm×0.20  $\mu$ m), (Sigma-Aldrich, USA), Detector (FID) and the injector and detector temperature was 250°C. The column temperature was 100°C (hold for 3 min) and raised to 130°C, at rate of 10°C/min, and held for 1 min. Then, the temperature raised to 170°C at 8°C/min, and raised to 215°C at 3°C/min (hold for 7 min), finally the temperature raised to 230°C at 40°C/min, and held for 3 min. The carrier gas was helium at flow rate 1.2 mL min<sup>-1</sup>, and split ratio at 20:1. FAMES were identified by comparing their relative and absolute retention times to those authentic standards of FAMES (Supelco™ 37component FAME mix). The fatty acid composition was reported as a relative percentage of the total peak area.

#### 2.2.3. Antioxidant activity

Antioxidant activity of oil samples were determined by the utilization of DPPH (2, 2diphenyl-1-picrylhydrazyl) radical scavenging activity according to modified method described by Blois [25]. Where oil samples (0.1 mL) were diluted and mixed with 3.9 mL methanolic solution of DPPH (0.1 mM), after incubation in darkness at room temperature for 30 min, the absorbance at 517 nm decreased. A sample free DPPH solution was used as a control. The following equation was used to determine the DPPH radical scavenging activity (%):

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A) / A_0] * 100$$

Where  $A_0$  is the absorbance at 515 nm of the control sample at time  $t=0$  min and  $A$  is the final absorbance of the test sample at 515 nm.

## 2.3. In vivo biological study

### 2.3.1. Composition of basal diet

The Basal diet is consisting of corn starch 46.5%, casein 14%, Soybean oil 4%, fiber 5%, mineral mixture 3.5% and vitamin mixture 1% as described in AIN-93M [26].

### 2.3.2. Animals

Sixty male albino rats weighing (120-140g) were used for the study. Animals were purchased from National Organization for Drug Control and Research, Giza, Egypt. All the animals were acclimatized for a week under standard husbandry conditions. The animals had free access to a standard pellet diet and water ad libitum was available to the animals. Animal handling and experimental procedures were approved by the Research Ethical Committee at National Research Centre, NRC.

### 2.3.3. Induction of hyperlipidaemia in rats

Hyperlipidemia was induced in rats according to the method of Gone *et al.* [27] by feeding the animal a high-fat diet contained 20% coconut oil and cholesterol (1%), the high-fat diet contained cholic acid (0.5%) to enhance the central absorption of lipid.

### 2.3.4. Design of study

The rats consumed basal diet throughout the 6 weeks of the trial, and they were randomly divided into 10 equal groups (6 rats per group):

G1: Negative control; G2: Positive control (high fat diet); G3: Olive oil / Linseed oil (90:10); G4: Olive oil / Linseed oil (50:50) G5: Olive oil / Soybean oil (90:10); G6: Olive oil / Soybean oil (50:50); G7: Olive oil / Linseed oil/ Soybean oil (50: 25: 25); G8: Olive oil; G9: Linseed oil and G10: Soybean oil.

Vegetable oil blends were given orally for the rats daily in a dose of (1 ml/kg body weight) throughout the feeding period (6 weeks). At the end of the feeding trial, the diet was withdrawn, and all animals were fasted overnight (12 h). The rats were anesthetized by intramuscular injection by ketamine chloride (24 mg/kg body weight). Blood was sampled from the orbital sinus of the eye and collected in clean dry test tubes and the serum was separated by centrifugation (3000 g, 10 min, 4°C). Serum obtained was used for various biochemical estimations.

### 2.3.5. Biochemical analysis

Serum total cholesterol (TC), triglycerides (TAG), low density lipoprotein (LDL) and high-density lipoprotein cholesterol (HDL) were determined using commercial kits (Randox Laboratories Ltd., Antrim, UK, BT294QY). The assays followed the manufacturer's protocol based on published techniques according to Tzang *et al.* [28].

### 2.3.6. Histopathological examination

The liver organs were separated, fixed in 10% formalin in saline for twenty-four hours. The organs were then washed with tap water, followed by sequential soaking in diluted methanol, diluted ethanol, and finally absolute ethyl alcohol for dehydration. The specimens were cleared in xylene and embedded in paraffin and left in hot air oven at 56°C for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness at room temperature by slide microtome (SLEE medical, Germany). The sections were embedded on glass slides, deparaffinized, stained by hematoxylin & eosin stain for examination through the light electric microscope [29].

### 2.4. Statistical analysis

Data obtained from the experiments were analyzed statistically on SPSS 17.0 computer software package. Differences between the treatment and control groups were compared using one-way analysis of variance (ANOVA). Differences between the various groups were considered statistically significant at  $P < 0.05$ . Graphs were plotted using GraphPad Prism software, version 5, for Windows (San Diego, CA, USA).

## 3. Results and Discussion

### 3.1. Physicochemical characteristics of the studied seed oils and their blends

Table (1) presents the results of the three studied physical parameters, the specific gravity, refractive index, and the melting points. The results of the five chemical parameters including free fatty acids, peroxide value, saponification value, unsaponifiable matter, ester value and the iodine value are also presented in the same table. The three studied oils differed significantly ( $P < 0.05$ ) in their melting points, being highest for soybean oil and lowest for linseed oil. The acidity of olive oil is significantly higher ( $P < 0.05$ ) compared with the two other seed oils. The iodine value of linseed oil was significantly higher than the other two seed oils ( $P < 0.05$ ). No significant differences could be found between all different studied oils in their refractive index ( $P < 0.05$ ). The Refractive index is considered one of the most important physical characteristics of oils, because it assesses the degree of their saturation, as well as their purity and monitoring the progress in reaction such as catalytic hydrogenation, oxidation and isomerization [30-32].

**Table 1.** Physicochemical properties of individual oils and their blends

Oil samples	Specific gravity (at 25 °C)	Refractive index (at 25 °C)	Melting point (°C)	Acidity (%)	Peroxide value (mEq./kg)	Saponification number (mg/g)	Unsaponifiable matter (%)	Ester value	Iodine number (g/100g)
Olive oil	0.912 <sup>a</sup>	1.4650 <sup>a</sup>	22.0 <sup>e</sup>	0.84 <sup>a</sup>	19.5 <sup>a</sup>	192.0 <sup>b</sup>	1.50 <sup>a</sup>	190.3 <sup>b</sup>	90 <sup>g</sup>
Linseed oil	0.915 <sup>a</sup>	1.4700 <sup>a</sup>	24.0 <sup>a</sup>	0.11 <sup>d</sup>	2.0 <sup>d</sup>	190.0 <sup>b</sup>	1.30 <sup>e</sup>	189.8 <sup>b</sup>	138 <sup>c</sup>
Soybean oil	0.932 <sup>a</sup>	1.4780 <sup>a</sup>	19.0 <sup>f</sup>	0.37 <sup>c</sup>	4.0 <sup>d</sup>	195.0 <sup>a</sup>	1.60 <sup>a</sup>	194.3 <sup>a</sup>	198 <sup>a</sup>
OO:LO, 90:10	0.914 <sup>a</sup>	1.4660 <sup>a</sup>	21.5 <sup>d</sup>	0.80 <sup>a</sup>	19.0 <sup>a</sup>	192.3 <sup>b</sup>	1.51 <sup>ab</sup>	190.7 <sup>b</sup>	100 <sup>f</sup>
OO:LO, 80:20	0.916 <sup>a</sup>	1.4670 <sup>a</sup>	21.0 <sup>d</sup>	0.74 <sup>a</sup>	18.0 <sup>a</sup>	192.6 <sup>b</sup>	1.52 <sup>ab</sup>	191.1 <sup>b</sup>	111 <sup>e</sup>
OO:LO, 70:30	0.918 <sup>a</sup>	1.4680 <sup>a</sup>	20.5 <sup>e</sup>	0.65 <sup>b</sup>	16.0 <sup>ab</sup>	192.9 <sup>b</sup>	1.53 <sup>a</sup>	191.6 <sup>b</sup>	122 <sup>d</sup>
OO:LO, 60:40	0.920 <sup>a</sup>	1.4690 <sup>a</sup>	20.0 <sup>e</sup>	0.56 <sup>b</sup>	14.0 <sup>b</sup>	193.0 <sup>ab</sup>	1.54 <sup>a</sup>	192.1 <sup>ab</sup>	134 <sup>c</sup>
OO:LO, 50:50	0.922 <sup>a</sup>	1.4700 <sup>a</sup>	19.5 <sup>ef</sup>	0.46 <sup>c</sup>	12.0 <sup>b</sup>	193.5 <sup>a</sup>	1.55 <sup>a</sup>	192.6 <sup>a</sup>	146 <sup>b</sup>
OO:SO, 90:10	0.912 <sup>a</sup>	1.4655 <sup>a</sup>	22.0 <sup>e</sup>	0.56 <sup>b</sup>	16.0 <sup>ab</sup>	191.6 <sup>b</sup>	1.46 <sup>b</sup>	190.5 <sup>b</sup>	99 <sup>f</sup>
OO:SO, 80:20	0.913 <sup>a</sup>	1.4660 <sup>a</sup>	22.5 <sup>e</sup>	0.46 <sup>c</sup>	14.2 <sup>b</sup>	191.4 <sup>b</sup>	1.44 <sup>b</sup>	190.5 <sup>b</sup>	104 <sup>e</sup>
OO:SO, 70:30	0.913 <sup>a</sup>	1.4665 <sup>a</sup>	23.0 <sup>bc</sup>	0.37 <sup>c</sup>	12.5 <sup>b</sup>	191.2 <sup>b</sup>	1.42 <sup>b</sup>	190.5 <sup>b</sup>	109 <sup>e</sup>
OO:SO, 60:40	0.913 <sup>a</sup>	1.4670 <sup>a</sup>	23.2 <sup>b</sup>	0.33 <sup>c</sup>	10.8 <sup>c</sup>	191.0 <sup>b</sup>	1.40 <sup>b</sup>	190.3 <sup>b</sup>	113 <sup>e</sup>
OO:SO, 50:50	0.914 <sup>a</sup>	1.4675 <sup>a</sup>	23.5 <sup>b</sup>	0.78 <sup>a</sup>	17.9 <sup>a</sup>	192.1 <sup>b</sup>	1.49 <sup>b</sup>	190.5 <sup>b</sup>	97 <sup>f</sup>
OO:LO:SO, 90:5:5	0.913 <sup>a</sup>	1.4650 <sup>a</sup>	22.0 <sup>e</sup>	0.72 <sup>a</sup>	16.2 <sup>ab</sup>	192.1 <sup>b</sup>	1.49 <sup>b</sup>	190.7 <sup>b</sup>	105 <sup>e</sup>
OO:LO:SO, 80:10:10	0.914 <sup>a</sup>	1.4660 <sup>a</sup>	21.9 <sup>e</sup>	0.66 <sup>b</sup>	14.6 <sup>b</sup>	192.2 <sup>b</sup>	1.48 <sup>b</sup>	190.8 <sup>b</sup>	113 <sup>e</sup>
OO:LO:SO, 70:15:15	0.915 <sup>a</sup>	1.4670 <sup>a</sup>	21.4 <sup>d</sup>	0.60 <sup>b</sup>	12.9 <sup>b</sup>	192.2 <sup>b</sup>	1.48 <sup>b</sup>	191.0 <sup>b</sup>	121 <sup>d</sup>
OO:LO:SO, 60:20:20	0.916 <sup>a</sup>	1.4680 <sup>a</sup>	21.0 <sup>d</sup>	0.54 <sup>b</sup>	11.3 <sup>bc</sup>	192.3 <sup>b</sup>	1.47 <sup>b</sup>	191.2 <sup>b</sup>	129 <sup>d</sup>
OO:LO:SO, 50:25:25	0.917 <sup>a</sup>	1.4690 <sup>a</sup>	20.5 <sup>e</sup>	0.84 <sup>a</sup>	19.5 <sup>a</sup>	192.0 <sup>b</sup>	1.50 <sup>a</sup>	190.3 <sup>b</sup>	90 <sup>g</sup>

Where: OO= Olive oil, LO= linseed oil and SO= Soybean oil; Data expressed as (mean values) of 3 replicates, within the same columns, values with different letters are significantly different ( $p < 0.05$ ).

### 3.2. Fatty acid composition of oil samples under investigation

Table (2) demonstrated that samples of olive oil are major unsaturated fatty acids (82.40%), while saturated fatty acids were at a rate of 17.60%. Predominantly in the composition of unsaturated fatty acids is oleic acid (65.34%). Moreover, the predominantly saturated fatty acids composition is palmitic acid (13.54%), and stearic acid observed (4.06%). The experimental values obtained are comparable to those in the literature, and that the composition of oleic acid obtained in this work dose not differ significantly from literature data, [33]. The samples of linseed oil are major unsaturated fatty acids (87.61%), while saturated fatty acids were at a rate of 12.39%. Predominantly in the composition of unsaturated fatty acids is linolenic acid (53.11%), also the category of unsaturated fatty acids was oleic acid (20.53%) and linoleic acid observed (13.97%). On the other hand, the predominantly saturated fatty acids composition is palmitic acid (6.04%), and stearic acid observed (5.05%). The obtained values are

comparable to those in the literature, and it notes that the composition of oleic acid obtained in this work do not differ significantly from literature data, namely 20.9-24.4% and 15.81-27.99% [34], also the composition of linoleic acid, values obtained not differ much from the literature [35]. The samples of soybean oil are major unsaturated fatty acids (82.54%), while saturated fatty acids were at a rate of 17.45%. Predominantly in the composition of unsaturated fatty acids is linoleic acid (61.78%), also the category of unsaturated fatty acids was oleic acid (20.16%). However, the predominantly saturated fatty acids composition is palmitic acid (11.07%), and stearic acid observed (4.97%). The illustrated values are comparable to those in the literature, and it noted that the composition of oleic acid obtained in this work dose not differ significantly from literature data [36].

Table (2) showed that samples of olive and linseed oil blends had unsaturated fatty acids which ranged between 81.47 to 88.04%, while saturated fatty acids were ranged between 12.07 to 18.54%. Predominantly in the composition of unsaturated fatty acids as oleic acid (41.34 - 63.28%), also the category of unsaturated fatty acids was linoleic acid observed

(12.51-15.36%) and linolenic acid (5.35-32.11%). Moreover, the composition of saturated fatty acids was palmitic acid (8.64-15.30%), and stearic acid observed (3.20-3.60%). The obtained values of olive oil blended with soybean oil were major unsaturated fatty acids which ranged between 82.09 to 83.74%, while saturated fatty acids were ranged between 16.26 to 17.91%. Predominantly in the composition of unsaturated fatty acids is oleic acid (36.49 - 67.64%), followed by linoleic acid observed (15.06-45.82%). Moreover, the predominantly in the composition of saturated fatty acids were palmitic acid (12.57-14.39%), and stearic acid observed (2.89-3.77%). However, the samples of olive oil which blended with linseed and soybean oil were major unsaturated fatty

acids that ranged between 82.51 to 84.17%, while saturated fatty acids were ranged between 15.74 to 17.50%. Predominantly in the composition of unsaturated fatty acids were oleic acid (27.75 - 64.51%), followed by linoleic acid observed (14.88-35.73%) and linolenic acid (3.12-20.69%). However, in the main composition of saturated fatty acids were palmitic acid (11.94-14.01%), and stearic acid observed (2.86-3.74%).

From previous results, five blends of vegetable oils were selected and compared with individual oils (olive, linseed and soybean oils). These blends were: OO:LO, 90:10, OO:LO, 50:50, OO:SO, 50:50, OO:LO:SO, 90:5:5 and OO:LO:SO, 50:25:25.

**Table 2.** Fatty acid composition of oil samples and their blends

Oil samples	Fatty acids%									TSFA	TUFA
	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0	C18:1	C18:2	C18:3		
Olive oil	Nd	Nd	13.54	Nd	4.06	Nd	65.34	17.06	Nd	17.6	82.4
Linseed oil	Nd	Nd	6.04	1.3	5.05	Nd	20.53	13.97	53.11	12.39	87.61
Soybean oil	0.11	0.08	11.07	0.9	4.97	0.32	20.16	61.78	0.6	17.45	82.54
OO:LO, 90:10	Nd	0.04	15.3	Nd	3.2	Nd	63.28	12.84	5.35	18.54	81.47
OO:LO, 80:20	Nd	0.16	14.76	Nd	3.23	Nd	58.89	12.51	10.19	18.15	81.59
OO:LO, 70:30	Nd	Nd	14.07	Nd	3.42	Nd	53.46	15.36	13.68	17.49	82.5
OO:LO, 60:40	Nd	Nd	10.36	Nd	3.6	Nd	51.29	13.74	21.22	13.96	86.25
OO:LO, 50:50	Nd	Nd	8.64	Nd	3.43	Nd	41.34	14.59	32.11	12.07	88.04
OO:SO, 90:10	0.11	Nd	13.96	Nd	2.89	0.33	67.64	15.06	Nd	17.29	82.7
OO:SO, 80:20	Nd	Nd	12.57	Nd	3.69	Nd	61.48	22.26	Nd	16.26	83.74
OO:SO, 70:30	Nd	0.05	14.39	0.05	3.42	Nd	37.66	44.43	Nd	17.91	82.09
OO:SO, 60:40	Nd	0.04	14.05	0.04	3.54	Nd	36.49	45.82	Nd	17.67	82.31
OO:SO, 50:50	Nd	0.06	13.29	Nd	3.77	Nd	37.53	45.02	0.33	17.12	82.88
OO:LO:SO, 90:5:5	Nd	Nd	13.73	0.24	3.07	0.46	64.51	14.88	3.12	17.5	82.51
OO:LO:SO, 80:10:10	Nd	Nd	14.01	Nd	2.86	Nd	58.01	17.79	7.33	16.87	83.13
OO:LO:SO, 70:15:15	Nd	Nd	13.68	0.11	3.38	Nd	52.1	20.5	10.18	17.17	82.78
OO:LO:SO, 60:20:20	Nd	0.04	12.28	Nd	3.72	Nd	42.58	25.65	15.28	16.04	83.51
OO:LO:SO, 50:25:25	Nd	0.06	11.94	Nd	3.74	Nd	27.75	35.73	20.69	15.74	84.17

Where: OO= Olive oil, LO= linseed oil and SO= Soybean oil; Nd, not detectable.

### 3.3. Antioxidant activity of selected vegetable oil blends

The antioxidant activity of the oil samples under investigation is mainly related to their bioactive compounds, such as phenolics, flavanols, and

flavonoids. In this study, the antioxidant capacity of oil samples and their blends was systematically evaluated. The DPPH inhibition of different oil samples and their blends were summarized in Table (3). Significant differences in DPPH scavenging

activity were found between the pure oil samples investigated, ranging from 14.56 to 36.79% and between their blends investigated ranging from 19.60 to 32.51%. This wide range of antioxidant activity may be attributable to the wide variety of bioactive compounds, such as phenolics, flavanols, carotenoids, and tannins, present in the selected oils.

Soybean oil clearly showed the highest activity being 36.79% followed by olive oil (17.91%) and linseed oil (14.56%). On the other hand, the samples of blended oils showed less significant differences, which the antiradical activity (%) were ranged between 19.60 to 32.51%. It could be noticed that the soybean oil was the playmaker in all blends effect on antioxidant activity in the oil blends.

The high vitamin E content in soybean oil acts as a powerful antioxidant while similarly protecting the skin from the damage of free radicals. Vitamin E is directly associated with improving the appearance of blemishes, reducing acne scarring, protecting the skin against sunburn, and stimulating the regrowth of new skin cells to promote healing. However, vitamin E enhanced activities of antioxidant more significantly and the possible role of vitamin E in reducing the risk of chronic diseases related to oxidative stress [37].

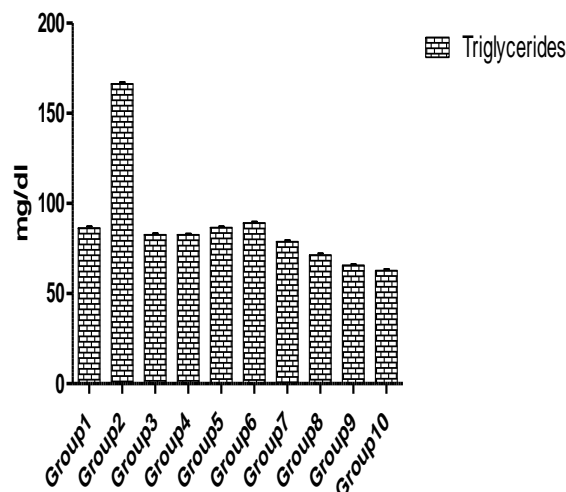
**Table 3.** Total antioxidant activity of oil samples and their blends

Oil samples	Antioxidant activity (%) by DPPH
Olive oil	17.91 <sup>b</sup>
Linseed oil	14.56 <sup>a</sup>
Soybean oil	36.79 <sup>c</sup>
OO:LO, 90:10	21.61 <sup>b</sup>
OO:LO, 50:50	19.60 <sup>b</sup>
OO:SO, 50:50	31.23 <sup>c</sup>
OO:LO:SO, 90:5:5	22.44 <sup>b</sup>
OO:LO:SO, 50:25:25	32.51 <sup>c</sup>

Where: OO= Olive oil, LO= linseed oil and SO= Soybean oil; Data expressed as (mean values) of 3 replicates, within the same columns, values with different letters are significantly different ( $p < 0.05$ ).

### 3.4. Impact of oil samples and their blends on the lipid profile

Data in Figure (1) showed the effect of vegetable oils blends on the serum triglycerides levels. A significant increase was observed in serum triglycerides level in positive control as compared to the negative control. Administration of different vegetable oils blends showed significant decrease in serum triglycerides as compared to positive control. Administration of soybean oil blend (G10) possessed the lowest level of TG followed by linseed oil blend (G9) in comparison with the other groups.



**Fig 1.** Level of serum triglycerides (mg/dl) in the different experimental groups

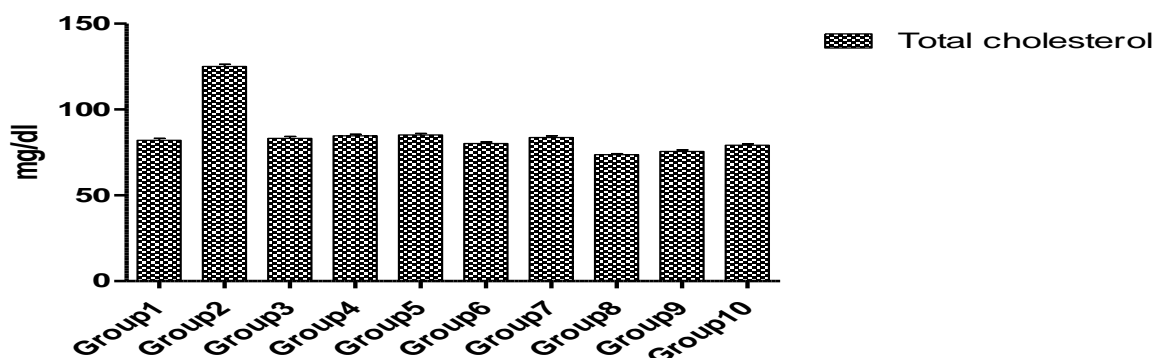
G1: Negative control; G2: Positive control (high fat diet); G3: Olive oil / Linseed oil (90:10); G4: Olive oil / Linseed oil (50:50) G5: Olive oil / Soybean oil (90:10); G6: Olive oil / Soybean oil (50:50); G7: Olive oil / Linseed oil/ Soybean oil (50: 25: 25); G8: Olive oil; G9: Linseed oil and G10: Soybean oil.

Furthermore, the data presented in Figure (2) showed the effect of vegetable oils blends on serum total cholesterol levels of the studied groups. Data showed a significant increase in total cholesterol levels in positive control as compared to negative control. Administration of different vegetable oils blends caused a significant decrease in total cholesterol as compared to positive control. After 6 weeks of feeding period, data showed that blend containing olive oil had the lowest serum TC followed by blend containing linseed oil in comparison with the other groups (Figure 2).

Data in Figure (3) illustrated the levels of serum high density lipoprotein-cholesterol (HDL-c) of hyperlipidemic rats fed different vegetable oils. Data showed a significant decrease in serum HDL-c in positive control as compared to the negative control. Administration of different vegetable oils significantly increases serum HDL-c as compared to positive control. After 6 weeks period, administration with a blend containing linseed oil (G9) possessed the highest level of HDL-c followed by blend containing olive oil/soybean oil (50:50) (G6) in comparison with the other groups. Moreover, the data of Figure (4) showed the effect of vegetable oils blends on LDL-c level in different groups.

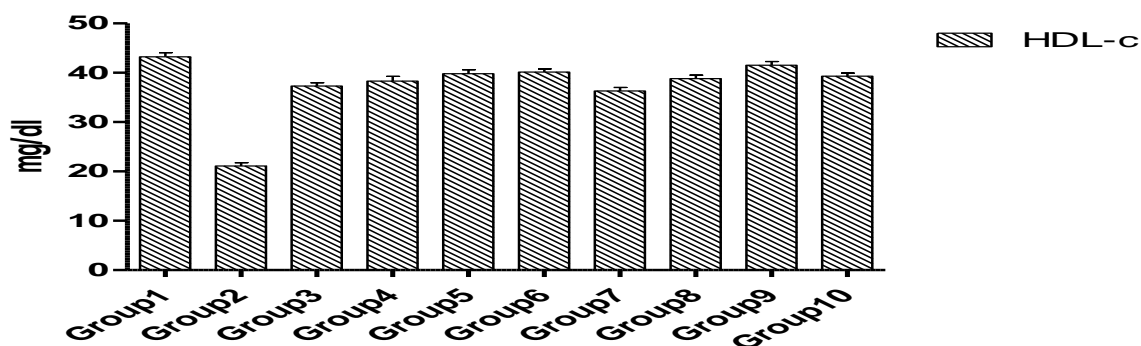
Data revealed a significant increase in serum LDL-c levels in positive control as compared to the negative control. Administration of different vegetable oils revealed that all groups significantly decrease the

LDL-c levels in comparison with positive control. Administration of olive oil blend (G8) possessed the lowest level of LDL-c followed by blend containing linseed oil (G9) in comparison with the other groups.



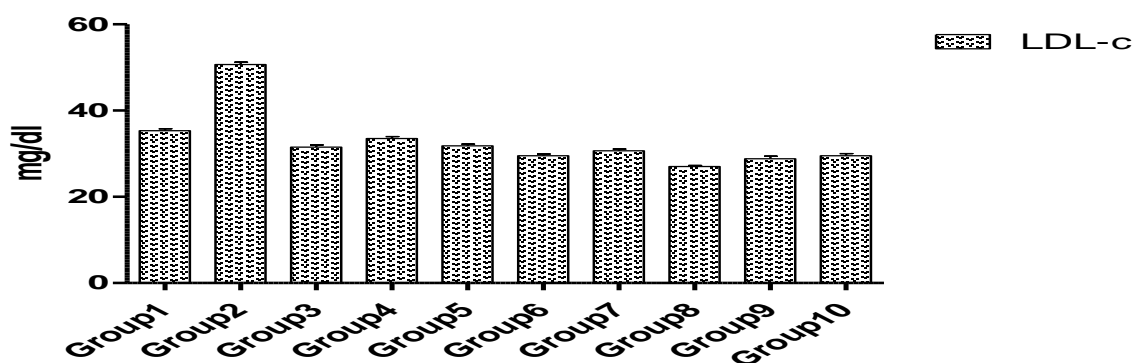
**Fig 2. Level of total cholesterol (mg/dl) in the different experimental groups**

G1: Negative control; G2: Positive control (high fat diet); G3: Olive oil / Linseed oil (90:10); G4: Olive oil / Linseed oil (50:50) G5: Olive oil / Soybean oil (90:10); G6: Olive oil / Soybean oil (50:50); G7: Olive oil / Linseed oil/ Soybean oil (50: 25: 25); G8: Olive oil; G9: Linseed oil and G10: Soybean oil.



**Fig 3. Level of HDL-c (mg/dl) in the different experimental groups**

G1: Negative control; G2: Positive control (high fat diet); G3: Olive oil / Linseed oil (90:10); G4: Olive oil / Linseed oil (50:50) G5: Olive oil / Soybean oil (90:10); G6: Olive oil / Soybean oil (50:50); G7: Olive oil / Linseed oil/ Soybean oil (50: 25: 25); G8: Olive oil; G9: Linseed oil and G10: Soybean oil.



**Fig 4. Level of LDL-c (mg/dl) in the different experimental groups**

G1: Negative control; G2: Positive control (high fat diet); G3: Olive oil / Linseed oil (90:10); G4: Olive oil / Linseed oil (50:50) G5: Olive oil / Soybean oil (90:10); G6: Olive oil / Soybean oil (50:50); G7: Olive oil / Linseed oil/ Soybean oil (50: 25: 25); G8: Olive oil; G9: Linseed oil and G10: Soybean oil.



Consumption of linseed, soybean and olive oils is beneficial for human health. These oils contain highest source of polyunsaturated fatty acids (PUFA) that are essential in the human diet. The results showed that all groups administered with different oils blends significantly decrease serum TG levels as compared to positive control group. Generally, high fat diet creates an increase in levels of TG due to lipoprotein lipase TG hydrolysis, so that the accumulation in the liver becomes more evident [38]. In contrast, the impact of PUFA can be attributed to a lowering in the hepatic synthesis of fatty acid, which lessens the level of TAG in the liver.

These results are in harmony with study of Ramadan *et al.*, [39] who reported that vegetable oils containing soy oil, linseed oil and sunflower oil could be useful formulations for the treatment of hypercholesterolemia and lowering total cholesterol, LDL and increase HDL. Similar results were obtained by Ahmed, [40] who found that soy oil, sunflower oil with linseed oil caused enhancement the lipid profile. The current study shows that administration of different oils blends caused a significant decrease in TC as compared to positive control group. These results are in line with Riediger *et al.*, [41] who found that feeding mice with diet containing flaxseed oil decrease serum total cholesterol.

Additionally, Tzang *et al.* [28] showed a significant decrease in total cholesterol and triacylglycerol in hamsters fed with flaxseed oil as compared to coconut oil. Moreover, the study of Sandstrom *et al.*, [42] revealed that oils with a low content of saturated fatty acids and high content of PUFA significantly decrease serum total cholesterol level. The results also indicated that administration of different oils significantly increase serum HDL-c as compared to positive control. HDL-C has been exhibited as a marker in the development of atherosclerosis [43].

Moreover, Amr *et al.*, [44] reported that dietary intake of olive oil and soybean oil improved lipid profile in mice. These findings also revealed that administration of different vegetable oils significantly decrease the LDL-c levels in comparison with positive control. These results were compatible with data obtained by Abdu Rahim and Jamal, [45] who stated that the consumption of olive oil significantly decrease triglycerides, total cholesterol, and LDL in the tested groups. Furthermore, Lichtenstein, [46, 47] reported that saturated fatty acids elevate LDL levels, whereas monounsaturated fatty acids and PUFA lower LDL levels.

### 3.5. Histopathological examination

The histopathological examination of liver tissues of the control group showing a normal

histological structure of the central vein and surrounding hepatocytes in the parenchyma (Figure 5). The liver tissue of the positive control group illustrated in Figure (6) showed severe dilatation and congestion in the central vein with fatty change in the surrounding adjacent hepatocytes. Liver tissue of rats administered with olive oil / linseed oil (90:10) illustrated in Figure (7) and showed mild dilatation in the central vein associated with a mild fatty change in the surrounding few hepatocytes. The liver section from rats orally administered with olive oil/linseed oil (50:50) had almost congestion in the portal vein as well as few inflammatory cells infiltration with mild fatty change in few surrounding adjacent hepatocytes (Figure 8). Liver tissue of rats administered with olive oil / soybean oil (90:10) showed dilatation and congestion in the central vein (Figure 9). The liver tissue of rats administered with olive oil/soybean oil (50:50) illustrated sever congestion was detected in the portal vein associated with periductal inflammatory cells infiltration surrounding the bile ducts (Figure 10). Liver tissue of rats administered with olive oil / linseed oil/ soybean oil (50: 25: 25) illustrated in Figure (11) and showed congestion in the central vein. The liver tissue of rats administered with olive oil showed congestion in the central vein and sinusoids (Figure 12). Liver tissue of rats administered with linseed oil showed focal hemorrhage in the hepatic parenchyma and congestion in the portal vein with few inflammatory cells' infiltration surrounding the bile ducts (Figure 13). Liver tissue of rats administered with soybean oil showed focal hemorrhage associated with congestion in the central vein (Figure 14).

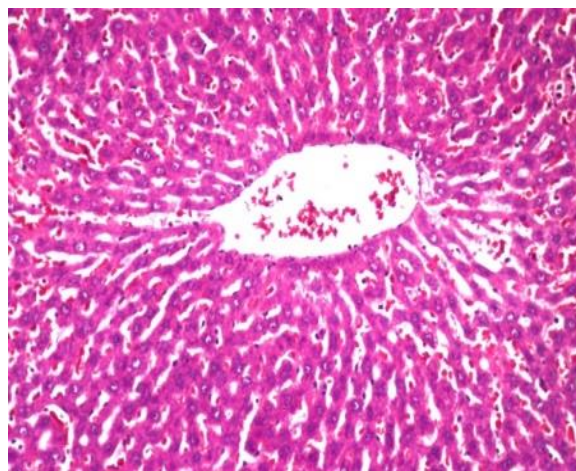


Figure 5. Liver section from group1 showed normal histological structure of the central vein and surrounding hepatocytes in the parenchyma were recorded (H&E, x200, x400).



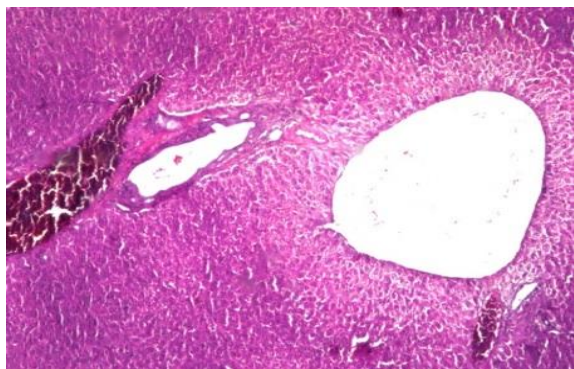


Figure 6. Liver section from group 2 (positive control) showed sever dilatation and congestion in the central vein with fatty change in the surrounding adjacent hepatocytes (H&E, x200, x400)

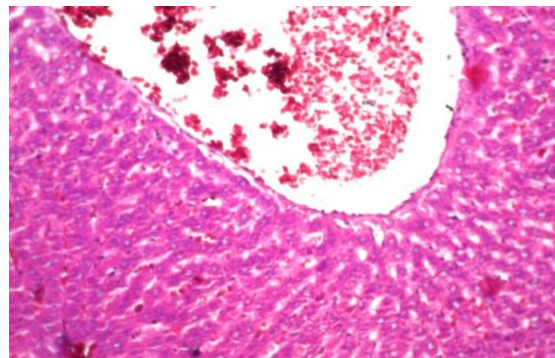


Figure 9. Liver section from group 5 showed there was dilatation and congestion in the central vein (H&E, x200, x400).

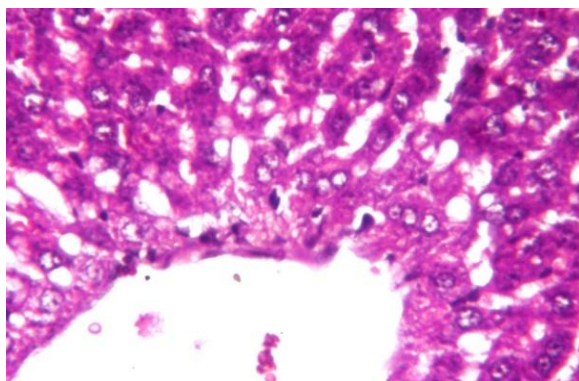


Figure 7. Liver section from group 3 showed mild dilatation was noticed in the central vein associated with mild fatty change in the surrounding few hepatocytes (H&E, x200, x400).

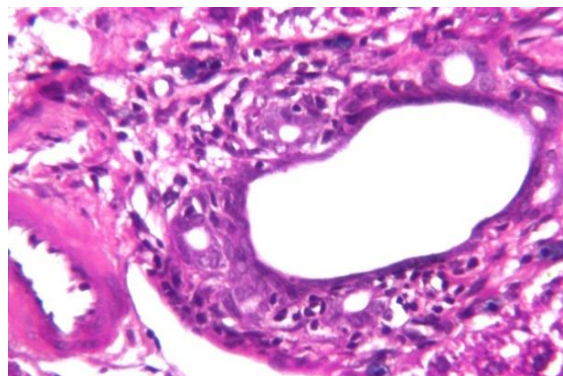


Figure 10. Liver section from group 6 showed sever congestion was detected in the portal vein associated with periductal inflammatory cells infiltration surrounding the bile ducts (H&E, x200, x400).

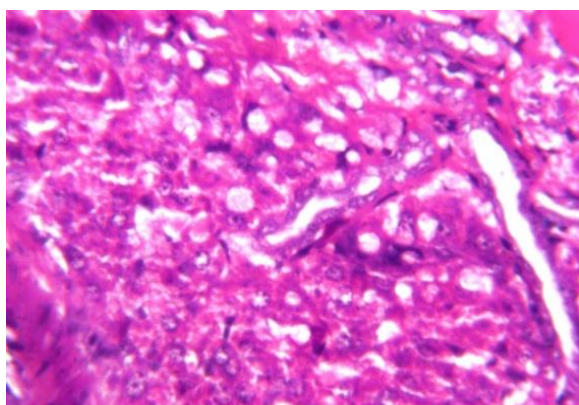


Figure 8. Liver section from group 4 showed the portal area showed congestion in the portal vein as well as few inflammatory cells infiltration with mild fatty change in few surrounding adjacent hepatocytes (H&E, x200, x400).

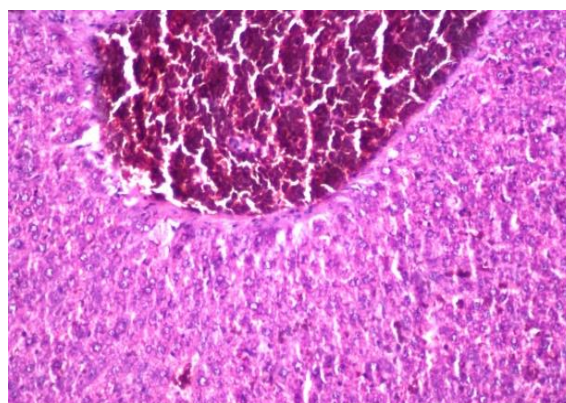


Figure 11. Liver section from group 7 showed congestion in the central vein (H&E, x200, x400).



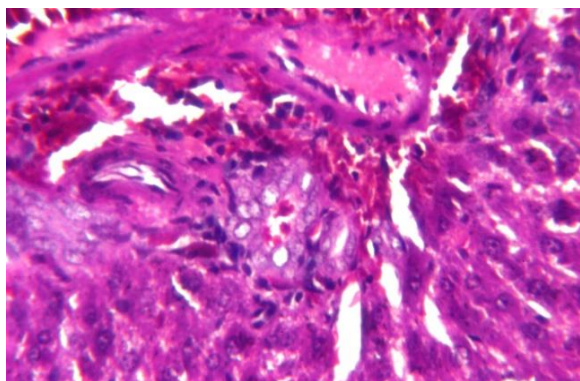


Figure 12. Liver section from group 8 showed congestion was observed in the central vein and sinusoids (H&E, x200, x400).

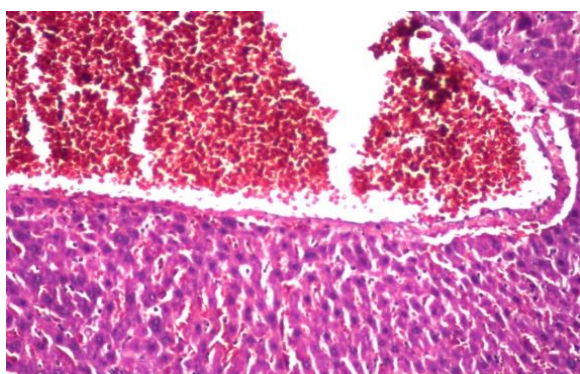


Figure 13. Liver section from group 9 showed focal haemorrhage was noticed in the hepatic parenchyma, while the portal area showed congestion in the portal vein with few inflammatory cells' infiltration surrounding the bile ducts (H&E, x200, x400).

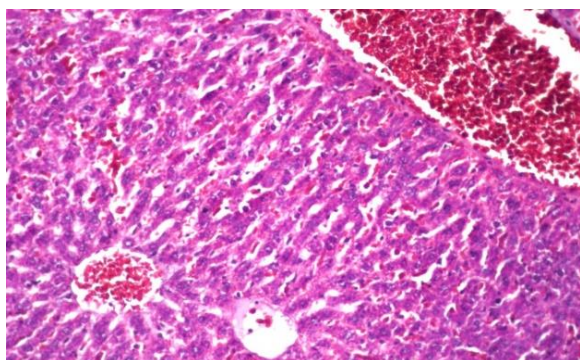


Figure 14. Liver section from group 10 showed the hepatic parenchyma showed focal haemorrhage associated with congestion in the central vein (H&E, x200, x400).

#### 4. Conclusion

The present study demonstrated that the balanced ratios of linseed oil rich in Omega-3 fatty acids with soybean oil (Omega-6) and olive oil (Omega-9) could be considered as a useful therapy for hyperlipidemia through ameliorating lipid profile. These findings also revealed that administration of different vegetable oils significantly lower levels of serum total cholesterol (TC), triglycerides (TG), and density lipoprotein (LDL) cholesterol as well as increase levels of high-density lipoprotein (HDL) cholesterol in comparison with positive control. Administration of different vegetable oils showed improvement in the histological examinations of liver tissues.

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#### Conflicts of interest

The authors declare no conflicts of interests.

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