

Protective Effect of Linseed Oil and Walnuts against Hypercholesterolemia Induced by Atherogenic Diet in Rats

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Abstract Hypercholesterolemia is the most common pathologic process underlying atherosclerosis and cardiovascular disease. Alpha-linolenic acid (ALA) is believed to benefit the cardiovascular system. The objective of this study was to determine the effect of different levels of dietary ALA from linseed oil and walnut on serum lipid profile of rats fed on atherogenic diet (AD). Sixty male Sprague-Dawley rats weighed 121.3 ± 10.6 g were divided into six groups. Rats were fed for 10 weeks on rodent diets contained 2% cholesterol, 3% corn oil and 20% fat whose source was from either palm stearin (saturated fat), corn oil, linseed oil or walnut at different levels of ALA that ranged from zero to 9.71%. Weight gain, liver weight, serum lipid profile and liver function parameters were determined. AD based on saturated fat resulted in significant alterations in serum lipids, increase in body weight gain and relative liver weight accompanied by negative effect on liver function parameters. Dietary ALA could counteract the detrimental effects brought about by the AD. The overall beneficial effects provided by high level of ALA were better than those given by low levels of ALA. Linseed oil exerted a hypolipidemic effect and could be considered as a promising functional food in cardiovascular disease.

Keywords: alpha-linolenic acid, linseed oil, walnut, hypercholesterolemia, lipid profile, liver function parameters

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1. Introduction

Hypercholesterolemia is a major risk factor for the cardiovascular diseases (CVD) [1]. High cholesterol raises risk of heart disease [2]. The biomarkers for elevated risk of CVD are elevated serum total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) [3].

Atherosclerosis is a progressive, pathologic process, and the primary contributing factor to CVD [4]. High fat diets can be used to generate a rodent model for the analysis of the path physiology of dyslipidemia [5]. National Heart Associations have recommended increased consumption of fatty fish or ω -3 polyunsaturated fatty acids (PUFA) supplements to prevent CVD. Non-fish sources of ω -3 PUFA vary in their capacity to regulate blood levels of eicosapentaenoic acid and docosahexaenoic acid (C20–22, ω -3 PUFA) and CVD risk factors [6].

Flax is an annual plant belonging to the genus *Linum* and the family *Linaceae*. Different varieties of *Linum* bred for fiber use are called flax, whereas the oilseed varieties are called linseed (*Linum usitatissimum* L.), oilseed flax or flax [7]. Linseeds (*Linum usitatissimum*) contain 32–45% of their mass, as oil. Linseed oil is the only oil of plant origin known to have the highest concentration of ALA. ALA serves as a precursor for long chain ω -3

polyunsaturated fatty acids such as C20–22, ω -3 PUFA. The ω -3 fatty acid found in linseed differ from those in fish. Linseed oil has been tested in clinical trials that have described its potential beneficial effect against disorders, such as dyslipidemia and cardiovascular disease [8,9].

Walnut, *Juglans regia* L. (*Juglandaceae*) is classified as a strategic species for human nutrition and is included in the Food and Agriculture Organization of United Nations (FAO) list of priority plants [10]. The high protein and oil contents of the kernels of walnut make this fruit essential for human nutrition [11]. Among nut oils, walnut oil contains the highest amounts of PUFAs [12]. The fatty acid composition of walnut oil is unique compared to other nuts oil because walnut oil contains predominantly linoleic acid (49 to 63%), and a considerable amount of ALA (8 to 15.5%) [13]. However, FDA [14] stated that, "there is no significant scientific evidence that consumption of walnuts may reduce the risk of coronary heart disease". Other researchers [15,16] reported that frequent consumption of walnuts and/or walnut oil may improve cardiovascular risk via mechanisms that extend beyond their established cholesterol-lowering action. Walnut consumption (42.5-85 g/day) reduced TC and LDL-C levels indicating cardio protective benefits. Beneficial effects of ALA on plasma lipid and lipoproteins are controversial [17,18,19]. The discrepancies between these results may be due to differences in study protocols,

diets, amounts of n-3 PUFA supplement and durations [20].

The present study was designed to investigate the hypocholesterolemic and biochemical effects of ALA levels derived from linseed oil and walnut in high fat diet fed rats.

2. Materials and Methods

2.1. Materials

Fresh crude hot screw pressed linseed oil; and refined palm stearin were obtained from Tanta Oil and Soap Company (Egypt), and Cairo Oil and Soap Company (Egypt), respectively. English walnut (*Juglans regia*) fruits and refined corn oil (Mazola brand) were purchased from local market, Cairo, Egypt. Walnut fruits were stored in their shells at -15°C. Cholesterol was purchased from Loba Chemie Pvt. Ltd. India. Cholic acid, choline chloride and standard fatty acid methyl esters were purchased from Sigma Chemical Company, St. Louis, Missouri, USA. Casein for nutrition was purchased from Nice Chemicals Pvt. Ltd. Kerala, India.

2.2. Extraction of Walnut Oil

The walnuts (about 100 g) were manually cracked and shelled. Walnut kernels were ground into a fine powder with a hand mortar. The oil was extracted from the kernels using cold ether [21]. The oil was stored at -20°C until analysis of its fatty acids and chemical characteristics.

2.3. Chemical Composition of Walnuts

Walnut kernels were ground and analyzed to determine, moisture (at 105°C to a constant weight), fat (as an ether extractable component using a Soxhlet apparatus), ash, crude fiber and protein (as crude nitrogen x 6.25) by the Kjeldahl method [22]. Analyses were performed in duplicate.

2.4. Chemical Properties of Oils

Acid value, saponification value, unsaponifiable matter and peroxide value in the investigated oils were determined according to the official methods [23].

2.5. Fatty Acid Composition of the Oils

Fatty acids of the investigated oils were separated by saponification and acidification [23]. The methyl esters of the fatty acids were prepared by using a mixture of methanol: concentrated sulfuric acid (99:1, v/v) and fatty acids: methylation mixture ratio of (1:13, v/v) at room temperature [24].

2.6. Identification of the Fatty Acid Methyl Esters by Gas Chromatography (GC)

The fatty acid methyl esters were analyzed by Agilent 6890 GC Gas Chromatograph coupled with an HP 5973 Mass Selective Detector. The chromatography apparatus was fitted with TR-FAME (Thermo 260 M142 P) (30 m, 0.25 mm ID, 0.25 µm film thickness) (70% Cyanopropyl – polysilphenylene-siloxane) capillary column. The column

oven temperature was programmed at 3°C/min from 80°C to 230°C, whereas, it was kept for 5 min. Injector and transferline were kept at 250°C, the MS source at 230°C, and the quadrupole at 150°C. The carrier gas of the sample was helium at 1.0 mL/min and the sample was injected in the split mode. The MS conditions were as follows: ionization energy 70 eV, with selected ion monitoring (SIM). For the identification of the compounds, the mass spectra of the samples were compared with those of the NIST/EPA/NIH Mass Spectral Library 2.0. Software adopted to handle mass spectra and chromatograms was a Chem Station.

2.7. Animals and Experimental Design

Sixty adult male albino rats weighing (121.3 ± 10.6 g) were obtained from the Holding Company for Biological Products and Vaccines, Egypt. Experimental animals were housed on a 12 h light-dark cycle at 25 ± 2°C and relative humidity of 30-60%. Rats were individually housed in rodent stainless steel mesh cages in the Animal House of Research Institute of Ophthalmology, Egypt. Animals were given free access to water and diets during the ten-week duration of the experiments.

Rats were fed on a basal diet [22] for 7 days prior to experimentation for acclimatization. Rats were randomly divided into a basal diet (control) group and five atherogenic diet (AD) groups of 10 animals each. All animals were fed purified experimental diets, which contained 4% salt mixture [22] and 1% vitamin mixture [22]. AD contained 2% cholesterol, 0.5% cholic acid, 0.2% choline chloride and 23% oil or fat (containing different levels of ALA). Diets were freshly prepared and the ingredients were separately stored at 4°C.

Table 1 shows the diets composition. The Ethics of Animal Use in Research Committee (EAURC) approved research protocol, by the Animal Care and Use Committee of University of Cairo (Giza, Egypt) under the number (CUFA/F/FS/2015/44).

The experiments were performed in compliance with the 'Guide for the Care and Use of Laboratory Animals' [25].

2.8. Biochemical Analyses

TC, HDL-C and TG concentrations of serum were determined using kits purchased from Stanbio, USA. Serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum lactate dehydrogenase (LDH) were determined using kits purchased from Chornlab, Barcelona, Spain. LDL-C was estimated using the Friedewald's formula as follows:

$$LDL-C = (TC - HDL-C) - \left(\frac{TG}{5} \right) \quad (1)$$

The Atherogenic indices were calculated as follows: Castelli's Risk Index [26]

$$(CRI) = \frac{TC}{HDL-C} \quad (2)$$

Atherogenic Coefficient [27]

$$(AC) = \frac{(TC - HDL-C)}{HDL-C} \quad (3)$$

2.9. Blood Sampling and Hepatosomatic Index

Rats were fasted for 12 h before blood sampling. Blood samples were taken at zero time and periodically until induction of hypercholesterolemia took place. Blood samples were collected from the retro orbital plexus vein, under light anesthesia with diethyl ether, using a fine capillary tube in dry clean centrifuge tubes. The tubes were kept at room temperature to allow blood to clot

before centrifugation at 3000 rpm for 15 min and the clear serum was separated and stored at -20°C for subsequent biochemical analyses. After blood sampling, at the end of 10 weeks animals were sacrificed by cerebral dislocation. The liver specimens were immediately excised, washed twice with cold physiologic saline (0.9%), blotted dry using filter paper and weighed. Hepatosomatic index (HI) was calculated as the percent of liver weight after dissection to body weight at the time of sacrifice [28].

Table 1. Composition of the Experimental Diets

Ingredient (g/100 g)	Basal diet			Atherogenic diets		
	G1	G2	G3	G4	G5	G6
Casein (80% protein)	15	15	15	11.43	7.86	15
Corn oil	8	3	13	3	3	3
Linseed oil	-	20	10	10	-	-
Palm stearin	-	-	-	-	-	20
Walnut	-	-	-	15.825	31.65	-
Cholesterol	-	2	2	2	2	2
Cholic acid	-	0.5	0.5	0.5	0.5	0.5
Choline chloride	-	0.2	0.2	0.2	0.2	0.2
Salt mixture	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1
Cellulose	1	1	1	0	0	1
Sucrose	35.5	26.65	26.65	26.025	24.895	26.65
Cornstarch	35.5	26.65	26.65	26.025	24.895	26.65
Total Calories (kilocalorie)	404	468.2	468.2	461.47	450.71	468.2
Fatty acid composition (g/100 g diet) ^a						
C16:0	0.90	1.71	2.15	2.10	2.5	11.4
C18:0	0.17	1.36	0.93	1.07	0.68	0.61
C18:1	2.67	5.04	6.36	4.95	4.86	7.45
C18:2(ω -6)	4.26	5.18	8.71	8.74	12.4	3.54
C18:3(ω -3, ALA)	0	9.71	4.85	6.14	2.56	0
ω -3/ ω -6 ratio ^b	0	1.87	0.55	0.70	0.21	0
P/S ratio ^c	4.01	4.87	4.48	4.79	4.70	0.27
%metabolic energy from oil	17.8	44.21	44.21	44.856	45.927	44.21

Note: ^a Calculated from the fatty acid composition of the oils; ω -3/ ω -6 ratio^b, The ratio of ω -3 to ω -6 polyunsaturated fatty acids; P/S ratio^c, The ratio of polyunsaturated to saturated fatty acids.

2.10. Statistical Analyses

The results are presented as mean \pm SD. The statistical analysis was performed using one-way analysis of variance; statistically significant effects were further analyzed and means were compared using Duncan's multiple range test. Statistical significance was determined at ($P < 0.05$). Statistical analyses were performed using Cost at Statistics Software, version 6.4 (Co Hort Software, California, USA).

3. Results

3.1. Fatty Acid Composition and Characteristics of the Investigated Oils

Linseed and walnut oils were rich (~66%) in the PUFA while palm stearin was poor (~10%) as illustrated in Table 2. ALA (ω -3) was the predominant fatty acid in linseed oil. It represented 73% and 19.1% of the polyunsaturated fatty acids in linseed and walnut oils, respectively. Walnut oil and corn oil were characterized by a higher content of linoleic acid (18:2, ω -6) compared to that of linseed oil. Palm stearin was rich in saturated fatty acids (palmitic and stearic acids) (~60%). Saturated fatty acids did not exceed 13% in the other investigated oils.

3.2. Chemical Composition of Walnut Kernels

The total oil, protein, fiber and ash contents of the walnut kernels were 63.2, 18.05, 5.33 and 1.7% on dry weight basis, respectively. These data were used to accommodate the protein and carbohydrate contents of the added walnuts in the formula of diets G4 and G5.

3.3. Feed Intake and Weight Gain of the Rats

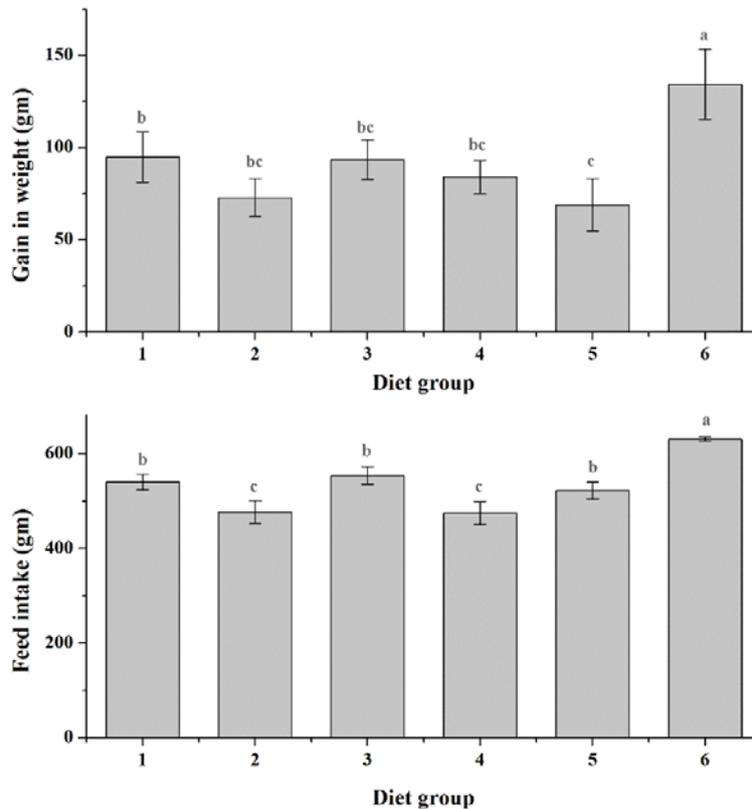
After 10 weeks of the experiment, the highest feed intake and weight gain were significantly ($P < 0.05$) observed in the group of rats that were fed on AD based on palm stearin (G6), when compared with the other investigated groups (G1 to G5), as shown in Figure 1. The highest increase in weight gain was associated with the decrease in the P/S ratio in the diet to 0.27 (G6) instead of > 4 in the other diets.

Feed intake of the rats fed on AD based on oils rich in PUFA, ALA $> 6\%$ (G2 and G4) for the same duration was significantly lower than that of rats fed on the basal diet or AD containing lower ALA (G3 and G5). Lowest weight gain was recorded for rats fed on AD containing high walnut kernel level (31.65%, G5) that its oil represented 39.9% of the total dietary energy.

Table 2. Fatty Acid Composition (%) and Characteristics of the Investigated Oils

Fatty acids	Linseed oil	Walnut oil	Corn oil	Palm stearin
Palmitic acid (C16:0)	6.83	10.8	9.11	55.33
Stearic acid (C18:0)	6.51	3.16	1.67	2.75
Oleic acid (C18:1)	20.21	19.26	27.01	32.24
Linoleic acid (C18:2, ω -6)	17.91	54	43.08	9.68
α -Linolenic acid (C18:3, ω -3)	48.55	12.78	ND	ND
Σ PUFA ^a	66.46	66.78	53.27	9.68
Σ SFA ^b	13.34	13.96	13.33	58.08
ω -3/ ω -6 ratio ^c	2.71	0.23	-	-
Chemical characteristics				
Acid value (mg KOH/g oil)	0.21	1.03	0.09	0.035
Peroxide value (meq. peroxide/ kg oil)	1.38	0	0.05	0.01
Saponification value (mg KOH/g oil)	190	197	189	200
Unsaponifiable matter %	1.786	1.827	1.287	0.743
Iodine value (g I ₂ /100g oil)	171.2	145.4	121.6	31.55

Note: ND, under the detection limit; Σ PUFA^a, Total polyunsaturated fatty acids; Σ SFA^b, Total saturated fatty acids; ω -3/ ω -6 ratio^c, The ratio of ω -3 polyunsaturated fatty acids to ω -6 polyunsaturated fatty acids.

**Figure 1.** Feed intake and weight gain of the experimental groups

Note: Data are shown as mean values \pm Standard Deviation. Values are for 6 animals in each group. Groups with the same superscript are not significantly different ($P < 0.05$) as assessed by 1-way ANOVA. Comparison between groups was made using Duncan's test. Group 1 (Basal diet, 8% corn oil), Group 2 (20% linseed oil+ 3% corn oil+ 2% cholesterol), Group 3 (10% linseed oil+ 13% corn oil+ 2% cholesterol), Group 4 (10% linseed oil+ 15.83% walnut+ 3% corn oil+ 2% cholesterol) and Group 5 (31.65% walnut+ 3% corn oil+ 2% cholesterol) and Group 6 (20% palm stearin+ 3% corn oil+ 2% cholesterol).

3.4. Induction of Hypercholesterolemia

Blood serum TC measurement was performed at the start (zero time) of the experiment and after 6 and 10 weeks in order to verify the development of hypercholesterolemia in rats, as shown in Figure 2.

TC level of rats at zero time was within the normal level (Figure 2) as reported by Go [29]. TC level of rats that were fed on a basal diet did not differ significantly ($P < 0.05$) over 6 weeks of the study. On the other hand, feeding rats on AD (G2-G6) for 6 weeks elevated their total serum TC level significantly ($P < 0.05$) regardless of the type of oil (or fat used) and the variations in ALA content and P/S ratio.

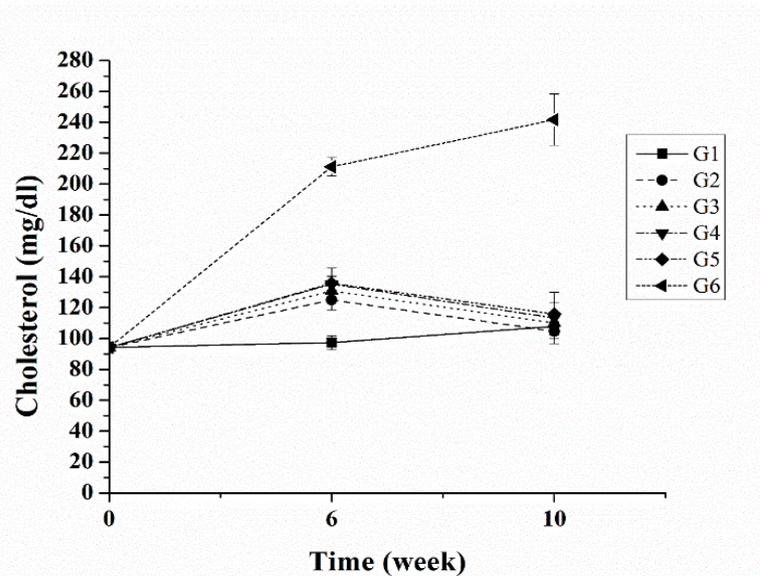


Figure 2. Serum cholesterol levels of the rats fed control and atherogenic diets for ten weeks

Note: Data are shown as mean values \pm Standard Deviation. Values are for 6 animals in each group.

Group 1 (Basal diet, 8% corn oil), Group 2 (20% linseed oil + 3% corn oil + 2% cholesterol), Group 3 (10% linseed oil + 13% corn oil + 2% cholesterol), Group 4 (10% linseed oil + 15.83% walnut + 3% corn oil + 2% cholesterol), Group 5 (31.65% walnut + 3% corn oil + 2% cholesterol) and Group 6 (20% palm stearin + 3% corn oil + 2% cholesterol)

TC level of rats that were fed on AD based on palm stearin (G6) was significantly ($P < 0.05$) higher than that of rats that were fed on the other investigated ADs without significant difference ($P \geq 0.05$) between groups 2 to 5. The rate of increase in TC level during the first 6 weeks of feeding rats on AD (Figure 2) could be related to saturated fat content in the diet. Continuous feeding rats on palm stearin diet (G6), four weeks thereafter, increased their serum TC level to >240 mg/dL.

Continuous feeding of rats on ADs based on oils rich in PUFA (G2 to G5), containing $\geq 2.56\%$ ALA, for another 4 weeks was associated with a significant ($P < 0.05$) reduction in their serum TC to the normal level.

3.5. Effect of Diet Composition on Blood Serum Parameters

Results in Table 3 show that rats fed on ADs for ten weeks exhibited significantly ($P < 0.05$) lower HDL-C when compared to rats in the control group (G1). However, no significant differences were observed among dietary groups (G2-G6) for HDL-C level. Feeding on ADs caused significant decrease in HDL-C.

LDL-C and TG levels of rats fed AD based on saturated fat (G6), for ten weeks, were significant ($P < 0.05$) higher by 2-3 fold than those of rats fed on the ADs based on oils rich in PUFA (G2-G5). In this group (G6), serum LDL-C and TG levels exceeded 180 mg/dL and 200 mg/dL, respectively. Atherogenic indices (CRI and AC) of the palm stearin group (G6) were significantly ($P < 0.05$) higher by 7 and 13 fold, respectively, than those of the control group. Feeding rats on ADs based on oils rich in PUFA (G2-G5) caused significant ($P < 0.05$) elevations in AC, CRI, LDL-C and TG levels by approximately 2-3 fold greater than those fed the control diet (G1). It could be noticed that the TG level of rats fed AD based on walnut as a source of ALA (G5) was significantly ($P < 0.05$) lower than that of rats fed on the other ADs based on oils rich in PUFA (G2-G4).

Hepatosomatic index (HI) of rats fed on a basal diet for 10 weeks was 2.34%. This result is in agreement with that reported by Sambu [28]. Meanwhile, HI of rats that received ADs (G2-G6) for 10 weeks increased approximately threefold over that of low fat fed rats (control group, G1). The livers of these animals (G2-G6) were enlarged and had a pale yellow granular appearance. However, feeding rats AD based on linseed oil containing 9.71% ALA alleviated significantly ($P < 0.05$) this increase. ALT, AST and ALP levels were significantly higher ($P < 0.05$) in rats fed the ADs compared to those of rats that were fed on the control diet. Feeding rats on AD based on palm stearin (G6), elevated significantly ($P < 0.05$) their ALT, AST and ALP levels higher than those of rats that were fed the ADs based on oils rich in PUFA (G2-G5). The rats fed on ADs (G3-G6) exhibited significantly ($P < 0.05$) higher AST/ALT ratio when compared to the control group (G1). On the other hand, the AST/ALT ratio of the rats fed on AD containing 9.71% ALA from linseed oil (G2) was insignificantly ($P < 0.05$) different from that of normal control. LDH level of rats fed AD based on palm stearin (G6) was (1574.83 U/L) threefold higher than that of rats fed the control diet (424 U/L). Feeding rats on AD based on linseed oil with 9.71% of ALA kept their LDH activity (557.01 U/L) slightly different from that of the control group.

4. Discussion

Much attention has been focused lately on the protective effects of ALA against hypercholesterolemia and atherosclerosis. The objective of the present study was to examine the protective effect level of ALA from linseed oil and walnut against atherogenic diet-induced hypercholesterolemia by studying the changes in serum lipid levels, and activities of liver enzymes.

Table 3. Effect of Dietary ALA Levels on Serum Lipid Profile and Liver Function of Rats Fed on Atherogenic Diets

Parameter	Diet groups					
	G1	G2	G3	G4	G5	G6
Cholesterol (TC, mg/dL)	107.8 ^b ± 5.57	104.7 ^b ± 4.61	110.0 ^b ± 4.77	113.3 ^b ± 10.91	115.9 ^b ± 7.59	241.7 ^a ± 16.66
HDL-C (mg/dL)	52.23 ^a ± 7.86	20.61 ^b ± 1.22	22.27 ^b ± 1.54	21.59 ^b ± 1.15	22.78 ^b ± 2.00	17.02 ^b ± 1.84
LDL-C (mg/dL)	41.73 ^c ± 4.61	65.23 ^b ± 4.54	66.57 ^b ± 2.80	74.00 ^b ± 10.93	77.59 ^b ± 5.83	183.57 ^a ± 14.48
Triglycerides(mg/dL)	68.98 ^e ± 5.58	94.29 ^c ± 7.83	105.88 ^b ± 3.34	88.48 ^c ± 4.61	77.90 ^d ± 2.02	205.37 ^a ± 2.84
Atherogenic Coefficient (AC)	1.08 ^c ± 0.22	4.08 ^b ± 0.09	3.95 ^b ± 0.19	4.27 ^b ± 0.75	4.10 ^b ± 0.22	13.24 ^a ± 0.72
Castelli's Risk Index (CRI)	2.08 ^c ± 0.22	5.08 ^b ± 0.09	4.95 ^b ± 0.19	5.27 ^b ± 0.75	5.10 ^b ± 0.22	14.24 ^a ± 0.72
Hepatosomatic index (HI)	2.34 ^c ± 0.04	5.74 ^b ± 0.31	6.36 ^{ab} ± 0.54	6.36 ^{ab} ± 0.55	7.42 ^a ± 0.24	7.24 ^a ± 1.30
ALT(U/L)	42.33 ^e ± 1.85	47.68 ^d ± 2.48	45.91 ^d ± 0.73	58.19 ^c ± 0.89	61.94 ^b ± 2.23	84.62 ^a ± 2.85
AST(U/L)	67.88 ^d ± 1.69	80.92 ^c ± 5.27	85.49 ^c ± 1.74	115.23 ^b ± 4.92	121.35 ^b ± 6.15	173.92 ^a ± 12.98
AST/ALT ratio	1.61 ^c ± 0.06	1.70 ^{bc} ± 0.20	1.86 ^{ab} ± 0.03	1.98 ^{ab} ± 0.11	1.96 ^{ab} ± 0.14	2.06 ^a ± 0.22
Alkaline phosphatase(U/L)	64.74 ^e ± 3.75	167.05 ^b ± 3.37	173.17 ^b ± 5.22	124.65 ^d ± 3.99	148.02 ^c ± 1.84	276.24 ^a ± 4.21
LDH(U/L)	424.00 ^e ± 26.23	557.01 ^d ± 74.60	979.43 ^b ± 23.06	939.57 ^b ± 72.54	789.65 ^c ± 39.40	1574.83 ^a ± 94.62

Note: Data are expressed as mean ± Standard Deviation. Data within columns with varying superscript letters are statistically different ($P < 0.05$). Values are for 6 animals in each group. Group 1 (Basal diet, 8% corn oil), Group 2 (20% linseed oil + 3% corn oil + 2% cholesterol), Group 3 (10% linseed oil + 13% corn oil + 2% cholesterol), Group 4 (10% linseed oil + 15.83% walnut + 3% corn oil + 2% cholesterol), Group 5 (31.65% walnut + 3% corn oil + 2% cholesterol) and Group 6 (20% palm stearin + 3% corn oil + 2% cholesterol)

Fatty acid composition of the investigated oils, besides chemical characteristics, is within the acceptable limits of the Codex standards of vegetable oils for edible purposes [30] and Egyptian Standard for edible linseed oil [31]. This indicates the freshness of the walnut and the crude linseed oil used and palm stearin and corn oil that were freshly refined. Data of chemical composition of walnut are in agreement with those reported by Ozkan and Koyuncu [32].

In this study, feed intake and weight gain were significantly higher in rats that were fed the AD based on saturated fat (palm stearin, 37.5% total caloric intake, polyunsaturated /saturated fatty acid ratio 0.27) compared to rats that were fed the AD based on oils rich in PUFA though diets had the same levels of energy and protein. These results are in agreement with those found by other authors [33,34,35]. They reported that the degree of saturation of the fatty acids in the diet might have a crucial role in the development of obesity-related metabolic complications. The current study indicates that weight gain of rats that were fed on AD (23% oil) containing ALA >4% (G2-G4) was not significantly different from that of the control group (8% corn oil) despite the difference in energy intake. The composition of fat in the diet is more important than the amount of fat [36]. On the other hand, weight gain of rats that were fed on AD containing 31.65% walnuts (ALA = 2.56%, G5) for 10 weeks was significantly lower than that of the control group. This could be attributed to different mechanisms such as increased satiety levels, increased resting energy expenditure or energy malabsorption as reported by Sabate [35].

Feeding rats on palm stearin diet for ten weeks, induced hypercholesterolemia since their serum cholesterol level exceeded 240 mg/dL, according to the American Heart Association [37]. The developed hypercholesterolemia may be attributed to the higher levels of dietary cholesterol (2%) in presence of saturated fat (20% palm stearin), besides long-term feeding (10 weeks). Consumption of dietary cholesterol increases blood cholesterol concentrations, but its effect is less than that of saturated fatty acids [38]. The low-saturated-fat diet would lower TC and LDL [39]. In the current study, TC increased during the first 6 weeks of feeding Ad rich in PUFA, and decreased progressively thereafter. Dupasquier

et al. [40] observed the same trend. It seems that inclusion of dietary ALA into AD ameliorated the increase of serum TC level in rats. Dietary ALA exhibited a hypocholesterolemic effect in a long feeding experiment. According to The National Cholesterol Education Program [3] LDL-C is the primary target of therapy in patients with hypercholesterolemia. A high-risk LDL-C level was defined as a serum level of more than 160 mg/dL [41]. In the current study, LDL-C in the AD groups based on oils rich in PUFA did not exceed 78 mg/dL after 10 weeks of feeding (Table 3). Triglyceride levels in Ads (G2-G5) and the control group were within the normal level [42].

n-3 PUFAs reduce TG and exert beneficial effects against CVD [20]. Among the groups studied, only in AD based on walnut, the TG level was significantly close to that of the control group though it contains low level of ALA (2.56%). This is a significant evidence that walnut has a protective effect as reported by Davis et al. [43]. Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of TG in the liver. NAFLD results from an imbalance among lipid intake, synthesis, degradation and secretion. It could be induced by high-fat diets that contain 30%-50% or more of kcal fat [44].

The development of atherosclerosis in rats was significantly related to an increase in the atherogenic coefficient (AC), and the serum TC level [46]. Atherogenic indices are powerful indicators of the risk of CVD, the higher the value, the higher the risk of developing the disease [47]. It has been stated that CRI could be considered desirable below 4.0, borderline 4.0-6.0, and high risk above 6.0 [48]. In the current study, dietary ALA ($\geq 2.56\%$) kept the atherogenic indices (AC and CRI) and LDL-c level in rats fed a hypercholesterolemic diet below borderline high, since low atherogenic indices and low levels of serum TC and serum TG are known to decrease appreciably the risk of CVD.

In the current study, HI of the hypercholesterolemic rats was significantly higher than that of the normal control group. This increase is primarily due to the accumulation of lipids in the cytoplasm of the hepatocytes.

It could be observed that the AD increased the activities of the investigated enzymes (ALT, AST, ALP and LDH) due to leakage into serum following tissue

damage. Effect of AD is more pronounced for AST rather than ALT. These activities were significantly lower in the rats given the ADs supplemented with dietary ALA. Most of AST in hepatocytes are located in mitochondria, whereas ALT is thought to be non-mitochondrial. ALT is released into serum with severe hepatic injury; mitochondrial membrane damage may result in the release of mitochondrial AST and elevating the AST/ALT ratio [49]. The present findings are consistent with those of similar studies [50,51].

In the present study, the elevated level of LDH enzyme (1574.83U/L) in the rats fed on palm stearin AD indicated that they are prone to hepatic and cardiac complications. However, feeding rats on AD containing 9.71% of ALA from linseed oil for 10 weeks kept their LDH activity at 557.01U/L close to that of the normal rats (424U/L).

5. Conclusion

These results suggest that prolonged feeding diet rich in ALA could suppress hypercholesterolemia. This effect was associated with a significant decrease in serum TC, LDL-C levels and atherogenic indices.

Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

ALA; alpha-linolenic acid, TC; total cholesterol, TG; triglycerides, LDL-C; low-density lipoprotein cholesterol, HDL-C; high density lipoprotein-cholesterol, ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase, LDH; lactate dehydrogenase, CVD; cardiovascular diseases, AD; atherogenic diet, PUFA; polyunsaturated fatty acids.

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