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The effect of different types of edible oils that contain omega-3 and omega-6 on blood lipids and the immune system in hypercholesterolemic rats

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Abstract

Macronutrients such as fatty acids and the effects of oils on human health and the immune system . Fish and flaxseed oil are great sources of omega-3 fatty acids, whereas plant oils, including chia seed oil, sunflower oil, and sesame oil, are excellent sources of omega-6 fatty acids. Randomization was used to divide the 42 adult male Sprague-Dawley rats into seven major groups (n = 7). As a negative control, Group 1 (healthy rats) was fed only a baseline diet. The other six groups received a The other six groups received a basic diet containing 2% cholesterol before to the experiment's start in order to elevate their cholesterol levels. . Six rats made up the second group, which was kept under positive control. For six weeks, the diets of groups (3), (4), (5), and (6) included 10% fish oil, flaxseed oil, chia seed oil, sunflower oil, and sesame oil, respectively. Following the trial, biochemical evaluations and histological studies were carried out to ascertain the effects of the examined oils. Results revealed that rats fed a supplemented diet with plant oils and fish oil, especially those with high contents of unsaturated and polyunsaturated fatty acids, reduced hypercholesterolemic levels in the blood, and the Contents of omega -3 and omega-6 reduced the risk of coronary heart disease and immune-regulatory properties. Omega- 6 fatty acids had an improved effect on the immune system than Omega- 3 fatty acids.

Keywords: omega-6, omega-3, blood lipids, immune system.

1. INTRODUCTION

Invading pathogens like viruses and bacteria are defended against by the immune system, which serves as a line of protection. Various cell types make up the mixture of immune cells, Immune cells can be generally categorized into two different groups based on their characteristics and defense mechanisms: cells of the innate immune system and cells of the adaptive immune system. Such cells as macrophages, neutrophils, eosinophils, basophils, mast cells, natural killer cells, and dendritic cells, are the first cellular line of defense. Although they usually move quickly, they rarely target a specific area. B cells and T cells have a higher level of specificity, but their activation is delayed. The secretion of cytokines and chemokines works as a coordination of the different immune cells, and regulation of their activity is crucial for mounting an effective immune defense (**Reyes-Caudillet al.,(2008)**. Dietary fats are a mixture of diverse fatty acids that are categorized as polyunsaturated (PUFA), saturated, and monounsaturated (MUFA) fatty acids. The unsaturated are classified into the omega series, being ω-9, which is nonessential for humans, ω-3 and ω-6., The polyunsaturated fatty acids (PUFA) omega-3 and omega-6 are regarded as necessary fatty acids, as they are not produced by the human body, and are supplied through food. Linoleic acid (LA), an omega-6 fatty acid, and alpha-linolenic acid (L.A.), among the essential fatty acids, are W-3 fatty acids **(Wasaki and Medzhitov ,2015).**

The cellular membrane's fluidity or the complex lipid raft assembly can both be controlled by omega-3 fatty acids. Research has been done on the effects of (PUFAs) on the immune system, with a special focus on the omega-3 PUFAs α-linolenic acid (ALA),eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is found in nuts and seeds whereas EPA and DHA are components of fish oil (**Calder, 2014)** . EPA and DHA can be synthesized from ALA , a process that involves several steps orchestrated by multiple elongases, desaturases, and β-oxidases . The same enzymes are involved in the metabolism of w-6 fatty acids **(Wasaki and Medzhitov, 2015).**

Immune regulation is vital and is mediated by metabolites produced from omega-3 and omega-6. These metabolites can be categorized into distinct groups and are commonly referred to as pro-resolving mediators (SPMs). prostaglandins, leukotrienes, thromboxanes, maresins, protectins, and resolvins. Their synthesis is orchestrated by cyclooxygenase, lipoxygenase, or cytochrome P450 enzymes **Rodrigues et al., (2016).**

Some studies have shown associations between higher intakes and/or blood levels of omega-3s and a decreased risk of certain cancers, including breast and colorectal cancers **Chiurchiu et al.,(2016) .** The present study was designed to evaluate the effects of different types of edible oils, such as flaxseed oil, fish oil, chia seed oil, sunflower oil, and sesame oil, that contain omega-3 and omega-6 fatty acids, on blood lipids and the immune system in rats.

2. MATERIALS AND METHODS

2.1- MATERIALS:

- Food items:

Oils (flaxseed oil, fish oil, chia seed oil, sunflower oil, and sesame oil) made up the study materials. All food items were obtained from the local market of agricultural herbs and medicinal plants in the Kingdom of Saudi Arabia. These oils were packed in dark brown bottles of 100 ml capacity each and prepared for chemical analysis by the **Bligh and Dyer (1959)** method.

- Cholesterol:

Elgomhoriya, a Cairo-based company for medical preparations, chemicals, and equipments, has supplied cholesterol in the form of a pure white crystalline powder.

- Rats:

Male Sprague Dawley albino rats, each weighing 110 ± 5g, were obtained from the Ministry of Health and Population's Laboratory of Animal Colony in Helwan, Cairo, Egypt.

2.2- METHODS:

- Experimental diets:

The dietary supply of protein, fat, carbohydrates, vitamins, and minerals was in accordance with the recommended dietary allowances for rats (American Institute of Nutrition, AIN) , according to **Reeves *et al.* (1993)**.

- Fatty-acid composition of seed oils

To determine fatty acid composition from flaxseed oil, fish oil , chia seed oil , sunflower oil and sesame seed, the extracted oils were saponified, esterified, and transferred to hexane, according to the **Hartman and Lago (1973)** method modified by **Maia and Rodriguez-Amaya (1993).** Fatty acid methyl esters were analyzed using a Shimadzu GC-2010 chromatograph with AOC-5000 autoinjector and flame ionization detector (FID). A Restek Stabilwax-DA fusedsilica bonded-phase column (30 m × 0.25 mm; 0.25 µm) was used, with both injector and FID operated at 250 °C. Initial oven temperature of 80 °C was maintained for 3 minutes and then raised to 140 °C at a rate of 10 °C/minutes and to 240 °C at 5 °C/minutes, which was kept for 11 minutes. Methyl ester peaks were identified by comparing their retention times on the column with those of standard fatty acid methyl esters (**Holland et al., 1994).**

- Experimental design

The Minufiya University's Shebin El-Kom Faculty of Home Economics was where the experiment was carried out. In a room that was kept at a constant temperature of 25 ± 2ºC, rats were housed in wire cages. Before the study began, all rats were given a weekly baseline diet to help them become used to it. The rats were sorted into two major groups after one week .The initial group (n = 6) of rats received the basal diet. The second main group (n = 36rats) was fed for six weeks on the diet containing cholesterol 2% to induce hypercholesterolemia before starting the experiment and were divided into seven groups (n=6) according to the following scheme. After the experiment, blood samples were taken for biochemical evaluation.

- **Group 1:** (control negative) was fed casein– basal diet (12% crude protein) for 45 days.
- **Group 2:**.(Control positive) was fed on a diet containing 2% cholesterol (non- treated rats).

- **Group 3**: Six hypercholesterolemic rats were fed a diet containing 10% flaxseed oil.
- **Group 4:** Six hypercholesterolemic rats were fed a diet containing 10% fish oil
- **Group 5:** Six hypercholesterolemic rats were fed a diet containing 10% chia seed oil.
- **Group 6**: Six hypercholesterolemic rats were fed a diet containing 10% sunflower oil.
- **Group 7**: Six hypercholesterolemic rats were fed a diet containing 10% sesame oil.

Each of the above groups was kept in a single wire cage. The diets were introduced to rats in special non-scattering feeding cups to avoid loss of food and contamination. Rats were given tap water via glass tubes that protruded through wire cages from upside-down bottles supported on one side of the cage. Rats were weighted at the beginning and at the end of the experiment.

2.3. Biochemical analyses

Blood samples were taken at the end of the experiment from all of the previously stated groups following a 12-hour fast. Blood was collected into a dry, clean centrifuge tube and allowed to coagulate in a water bath (37 °C) to separate the serum from the blood, it was centrifuged at 3000 rpm for 10 minutes enzymatic kit according to **Siest *et al*., (1981)** and a radioimmunoassay (RIA) assay was used to quantify insulin **(Yallow and Bauman, 1983).** Serum levels of total cholesterol and triglycerides were chemically determined according to **Ratliff and Hall (1973)** and **Jacob and Van-Denmark (1963)**, respectively.

2.4. Differential leucocytic count

Freshly collected blood samples of 20µl were spread on clean slides as a thin film. Each smear was left to air dry, and fixed with methanol for 2–3 minutes, and then labelled. Giemsa's stain (Aldrich) at 10% was used to stain blood smears, which were then inspected under a light microscope and had various blood leukocyte kinds quantified **(Schalm et al., 1975)**

2.5. Kidney function

Blood urea nitrogen (BUN) was determined using BioMérieux kits according to the method of **Patton and Crouch, (1977).** Serum uric acid (U.A.) was determined using the enzymatic colorimetric method as described by **Fossati *et al*., (1980)**. Serum creatinine (Cr) concentration was colorimetrically determined by the Jaffe reaction according to the method of **(Husdan and Rapoport, 1968).**

2.6 . The procedure is used to extract total lipids from the sample:

The extraction of total lipids is carried out by the method of **Bligh and Dyer (1959)**, using chloroform and methanol in a ratio of 1: 2. The methyl esters of the fatty acids were analyzed using a Shimadzu gas chromatograph-2010 (Kyoto, Japan). The analysis was done using a capillary column, model number CP7420 (Varian Inc., Palo Alto, CA), with a carrier gas of hydrogen and a make-up gas of nitrogen.The furnace's temperature is set in programmed mode for five steps. initial temperature of 51 °C which was maintained for 8 min, then increased at a 10°C /min to 170°C and maintained for 20 minutes, then increased to 4°C /min to 186°C and maintained for 19 minutes, again increasing to 4°C /min to 220°C and with 2°C /min to 240 °C to complete the process.

2.7- Histopathological study

At the conclusion of the trial, rats from each experimental group had their livers extracted and preserved in 10% neutral buffered formalin (pH=7.0):,dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 micron-thick sections were prepared and stained with hematoxylin and eosin for examining the liver using a light microscope at various magnifications **(Carleton , 1976).**

2.8. Statistical analysis

Statistical analysis was carried out with the computer program SPSS (Statistical Package for Social Science, 2008), and appropriate tests were used to compare values.

Table (1): Fatty acid composition of some common edible oils (g /100 g) in investigated oils

Variables	Oils				
	Flaxseed oil10%	fish oil 10%	chia seed oil 10%	**Sesame** oil **10%**	**Sunflower** oil **10%**
Saturated Fats (SFA)					
Capric Acid C10:0	-	-	-	3.22	-
Lauric Acid C12:0	.03	-	.03	14.59	-
Myristic Acid C14:0	06	.02	.o6	4.38	-
Palmitic Acid C16:0	5.34	.01	7.1	9	4
Stearic Acid C18:0	3.17	.4	3.24	4	2.8
Margaric acid (C17:0	.09	.17	.06	-	.02
Arachidic acid (C 20:0)	.18	.18	.24	.35	.21
Behenic acid (C 22:0)	.14	.07	.08	.08	-
Monounsaturated (MUFA)					
Oleic acid (C18:1 ω-9)	21	30.10	10.53	41	19
Palmitoleic acid (C16:1)	.05	.29	.2	.11	.12
Eicosenoic acid (20:1)	.2	4.4	.16	-	.18
Polyunsaturated Fats					
Linoleic Acid C18:2w6	16.13	8.43	20.37	45	68
Alpha Linolenic Acid C18:3w3	58.2	38	63.79	.29	1
Eicosadienoic acid (C20:2	-	46.0	0.08	.05	.02
n-3 PUFAs					
n-3 PUFAs	**62.7**	**21.19**	**58.2**	**.2**	**0.19**
n-6 PUFAs	**14.9**	**10.02**	**15.3**	**40.9**	**62.2**

3. RESULTS

3.1 Chemical composition of oils

The content of saturated fatty acids in the studied oils is presented in Table (1). The prominent representatives of this group of fatty acids are lauric acid (c12:0) and palmitic acid (C16:0) Sesame oil and flaxseed oil have a considerably higher content of lauric acid and palmitic acid compared to different investigated oils , however, the content of monounsaturated (MUFA) fatty acids is highest in sesame oil, fish oil, and flaxseed oil, followed by chia seed oils followed by the chia seed oils. Table 2 presents the content of polyunsaturated fatty acids (g/100 g fat) in the different oils. Prominent representatives of this group of fatty acids are Alpha Linolenic Acid (C18:3), Linoleic Acid (C18:2) and Eicosadienoic acid (20:2) . Our results indicated that flaxseed oil and chia seeds oil and fish oils have high lipid content and are a sources of PUFA (ω-3 and ω-6) when compared to the different investigated oils.

3.2 Food intake ,food efficiency ratio and body weight gain

Table (2) shows food the intake (F.I.) g , food efficiency ratio (FER) and body weight gain (BWG %) for different hypercholesterolemic groups fed on different oils. It is clear that food intake for control positive was lower than for control negative.It could be noticed that chia seed oil 10% and flaxeed oil 10%; treatments showed significantly ($p < 0.05$) higher food intake compared with the control positive 14.1 ±0.10 and 13.1 ±1. 01 mg/dl vs. 8.73 ± 0. 63 g respectively

Table (3) shows internal organs weight g , for control positive and different groups of hypercholesterolemic rats fed on different oils. It is clear that the liver for control positive was higher than control negative and all groups fed on different oils . Also data showed that groups fed on flaxseed oil 10% and chia seed oil 10% treatments showed significant differences compared with the control positive being (6.06±0.35 ,4.1 ± 0.55 and 5.2 ± 0.1 g , respectively).

Table (2) : Effect of different types of edible oils containing omega 3 and omega 6 on nutritional parameters of hyperchlesterolemic rats

Groups Variables	Control (+)	Control (-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame 10%	Sunflower 10%
FoodIntake: Mean±SD	8. 73 ± 0. 63	15.01±1.1	13.1± 1.01*	10.1± 1.01*	14.1± 0. 10	10.4±0. 52*	12 .2 ± 0.7
FER: Mean ±SD	1.012±0.013 *	3.053±0.005	1.721±0.059	2.496±0.045	1.98±0.136	1.311±0.02	1.521±0.0192
BWG : Mean ±SD	15. 8±2.2**	26. 66±3. 83	22.6 ± 5.62	25. 2 ±9.7	26. 83±32.2	13.68± 3.2**	18. 6 ±3.96*

* Differences are P < 0.05 significant
** Differences are P < 0. 001 highly significant

3.3 Lipid profile

Table 4 shows the fasting serum lipid fraction (mg/dl) for different groups of hypercholesterolemic rats fed on different oils that contain omega-3 and omega-6. It is clear that triglyceride for control (+) was higher than control negative. .It could be noticed that the lowest value of triglyceride (87.1±3.224 mg/dI) was observed for chia seed oil 10% treatment when compared with different groups of hypercholesterolemic and the highest value recorded for sesame oil 10% treatment 99±7. 607 mg/dI) when compared with control (+) (129.3±9.12 mg/dI) , The difference was significantly high (p<0.001).
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Regarding the HDL-c levels , the highest value was recorded for the fish oil 10% diet (36. 01± 0. 8 mg/dI) while the lowest was the sunflower oil 10 % diet (33. 6 ± 0.516 mg/dI) when compared with the control positive group (32. 2±367 mg/dl) , the difference was significantly high (p<0.001).

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Regarding VLDL-c was highest (16. 32±0.628 mg/dI) for the flaxseed oil 10% group and lowest (23 . 52 ± 1.80 mg/dI) for the sunflower oil 10% diet when compared with the positive control group (24.66±1. 83 mg/dl), and the difference was significantly high (p<0.001). Serum LDL-c was found to be highest (31.95 ± 5. 2 mg/dl) for fish oil 10% diet,being lowest in the sesame oil 10% diet rats group (56.448±3.76 mg/dI) in comparison with control positive group (55.47±9 23 mg/dl) ,difference were very high siginificant (p<0.05). from table (4) revealed that the lowest value of total cholesterol (75.33±4. 31 mg/dl) the chia seed oil 10% diet in comparison with the control positive group (113.33±10.59 mg/dl) differences are significant p< 0. 05. On one hand, the highest improvement in cholesterol was in the chia seed oil% and fish oil 10% groups, followed by flaxseed oil 10% .

3.4. Serum glucose

Results in Table (5) indicate levels of fasting serum glucose (mg/dl) for different Groups of hypercholesterolemic rats fed on different oils that contain (omega-3 and omega-6) The highest value was obtained for fish oil 10% treatment (102.04±1.78 mg/dl and the lowest for Sesame oil 10% diet (129.3±4.03 mg/dl) as compared with the positive control group (155.2 ±1. 78 mg/dl). The results agree with (Harcombe, 2019). They observed that rats' blood glucose levels were reduced when flaxseed and flaxseed oil were consumed.

3.5. Liver functions

Table (6) shows fasting serum AST, ALT and ALP (U /L) for different groups of hypercholesterolemic rats fed on different oils that contain (omega-3 and omega-6). It could be observed that the improvement of AST was highest for the fish oil 10% group while it was lowest for the sunflower 10% diet , The differences were very significant (p<0.001) and levels were (31. 6±2.06 and 36.6±2. 05 u/l) respectively.

Table (3) : Effect of different types of edible oils containing omega 3 and omega 6 on organs weight of hyperchlesterolemic rats

* Differences are P < 0.05 significant
** Differences are P < 0. 001 highly significant

Groups / *Variables*	*Control(+)*	*Control(-)*	*Oils*				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame 10%	Sunflower 10%
Liver Mean ±SD	6.06±0.35	5. 3±0.49	4.1 ± 0.55	6.02± 0.41	5.2±0.1	5.8±0.9	5.4±0.64
Heart Mean ±SD	0.53±0.05	0.8±0.001	0.5 ± 0.01	0.63±0.05	0.633±0.15	0.65±0.32	0.73 ± 0.05
Kidney Mean ±SD	1.04±0.20	1.18±.015	1.15 ± 0.05	1.11±0.11	1.761±0.05	1.462±0.15	1.7±0.73
Spleen Mean ±SD	0.58±0.15	0.57±0.20	0.62± .15*	0.53±0.15	0.8±0.17	0.76±0.20	0.73 ± 0.05
Lungs Mean ±SD	7.1±0.2	13.1±0.31	10.05±0.15	11.4±0.3	12.3±0.22*	13.86±0.38	9.13±0.25

Table (4): Effect of different types of edible oils containing omega 3 and omega 6 on lipid profile of hyperchlesterolemic rats

*   Differences are P < 0.05 significant
** Differences are P < 0. 001 highly significant

Groups / Variables	Control(+)	Control(-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame 10%	Sunflower 10%
Triglyceride Mean ±SD	129.3±9.12	85.9±7.155*	89.6 ± 3. 141*	95.2 ± 0. 894*	87.1±3.224*	99±7. 607*	97.5± .03*
HDL-c Mean ±SD	32. 2±367	43. 9±2.5**	35. 2 ±3. 57*	36. 01± 0. 89	35.01±0.894	35.1±1.78**	33. 6 ± 0.516*
VLDL-c Mean ±SD	24.66±1. 83	15.98±1.53* *	16. 32±0.628*	19.64 ± 0. 8*	18.42±0.644	20.06±1.52*	23. 52 ± 1.80
LDL-c Mean ±SD	55.47±9 23	21.42±4.76* *	40.08±1. 978*	31.95 ± 5. 2*	34.9± 4. 804	56.448±3.76	40.48 ± 3. 004
COHL Mean ±SD	113.33±10.5 9	83. 3±2. 35**	95. 6 ± 3. 38*	84. 6 ± 6.47	75.33±4. 31	100 6±3.14	101. 6 ± 1. 861
A.I	2.48	0.851	1.6	1.4	1.5	2.1	1.9

Table (5): Fasting serum glucose (mg/dl) for different Groups of hypercholesterolemic rats fed on different oils that contain (omega-3 and omega-6).

Table (6) : Effect of different types of edible oils containing omega 3 and omega 6 on liver enzymes of hyperchlesterolemic rats

Groups / Variables	Control(+)	Control(-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame 10%	Sunflower 10%
Glucose Mean ±SD	155.2 ±1. 78	98.8±0.884	120.2±5.36	102.04±1.78	116. 02±4.47*	129.3±4.03	125.3 ± 7. 17*

* Differences are P < 0.05 significant
** Differences are P < 0. 001 highly significant

Groups / Variables	Control(+)	Control(-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame 10%	Sunflower 10%
AST Mean ±SD	55.51±3.08	35.9±1.78***	32.1±1.788**	31. 6±2.06*	33.06±2.06**	35.66±0.516	36.6±2. 5**
ALT Mean ±SD	56.66±0.526	33.9±0.516**	34. 2±1.366	32.1±1.36	36. 2±1.54**	37. ±0.526	35.02±1.03**
ALP Mean ±SD	172.1±144.07	84.9±15.709	101.3 20.126	160.1±15.01 *	95.3±26.35	130.1±50.08	110. ±10.28

As regards the (ALT), the improvement was greatest for the fish oil10 % diet (32.1±1.36 u/l) being the lowest for the sesame oil

10 % diet group (37. 2±0.526). In the case of (ALP) activity , the highest improvement was also recorded for the chia seed oil 10% diet (95.3±26.35u /l) as compared with the control positive group (172.1±144.07 u/l) .

3.6 Kidney function

Data presented in table (7) illustrated serum uric acid , creatinine and urea (mg /dl) for control groups and different groups of hypercholesterolemic rats fed on different oils that contain (omega-3 and omega-6) . It could be noticed that uric acid for control positive was higher than for control negative. Feeding rats on different oils tested in this work reduced the uric acid level recorded for control (+) group . If sesame is possible, flaxseed oil (10%) reveals the highest improvement in uric acid. This was also found for the creatinine content of serum where olive oil 10 % was more potent in reducing the level of creatinine,although the values registered for different oils were evidently proximate, being however less than that of the control (+) rats group . Efficiency of olive oil 10 % was also much greater than in case of other tested oils in reducing urea of the control (+) rats group. Concerning kidney, groups fed on sunflower 10% and sesame oil 10% treatments showed no significant differences, with the control positive being 48.1±7.32 , 43.6± 3.38, and 45.3± 3.61 mg/dl, respectively.

3.7. Leukocyte function

Results in table (8) indicate the levels of effect of diets containing different oils (omega-3 and omega-6) on differential leukocyte count in hypercholesterolemic rats. It could be observed that the improvement of monocytes was highest for the sunflower oil 10% and sesame oil 10% groups, while it was lowest for the fish oil 10% diet. , differences were very high siginificant ($p<0.001$) and levels were (12.3 ± 0.7, 10.4 ± 2.4 and 0.2 ± 0.1u /l) respectively. As regards the neutrophils, the improvement was highest for the sesame oil 10 % diet (12.7 ± 0.3) being the lowest for the fish oil 10 % diet group (0.3 ± 0.1). In the case of eosinophil activity, the highest improvement was also recorded the sesame oil 10% and sunflower oil 10% diet (16.3± 0.5- 15.4 ± 2.4) being lowest for fish oil 10 % diet and Flaxseed oil10% group (2.4 ± 0.3, 2.5 ± 0.2).

Table (7) : Effect of different types of edible oils containing omega 3 and omega 6 on kidney functions of hyperchlesterolemic rats

* Differences are P < 0.05 significant
** Differences are P < 0. 001 highly significant

As regardes the Lymphocytes, the improvement was highest

Groups / Variables	Control(+)	Control(-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame 10%	Sunflower 10%
Uric Acid Mean ±SD	5. 5± 0. 267	3. 01±0. 57**	2.11±0. 36	3.2±0. 62**	3.45±0.68	3. 5±0. 373	4.2± 0.185*
Creatinine Mean ±SD	0. 98 ±. 050	0. 5±0.044***	0.58±0.157*	0.79±0. 08	0.57±0. 136	0.72± 0.089	0.89±0. 051*
Urea Mean ±SD	45. 3± 3. 61	36. 6± 4. 03	32.9±1.78**	42.6±2875*	37. 9±3. 14	43.6± 3.38	48.1±7. 32

for the sunflower oil 10% diet (1.63 ±. 12) being the lowest for the fish oil 10 % diet group (1.22 ±.02 %).in the case of macrophages activity , the highest improvement was also recorded for the sesame oil 10% diet (79 ± 10 %) being the lowest for the fish oil 10 % diet group (65 ± 18%). On the other hand, for phagocytosis activity, , the highest improvement was also recorded for the sunflower oil 10% diet (10.2 ± 0.5) with the lowest for the flaxseed oil 10 % diet group (0.2 ± 0.2). Regarding the cytokines levels, the highest value was recorded for sunflower oil 10% diet (9.2 ± 0.2) while the lowest was for fish oil 10 % diet (0.2 ± 0.3) when compared with the control positive group (0.3± 0.4) , the difference was very significant (p<0.001) for basophil levels, recorded for the Sunflower oil 10% diet (6.2 ± 0.3).

Table (8):Effect of different types of edible oils containing omega 3 and omega 6 on differential leukocyte count of hyperchlesterolemic rats

* Differences are P < 0.05 significant

Groups / Variables	Control(+)	Control(-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	**Sesame** oil **10%**	**Sunflower** oil **10%**
Monocytes (%)	**5.7 ± 0.3**	**6.2 ± 0.5**	**3.7 ± 0.2**	**0.2 ± 0.1**	**5.4 ± 0.7**	**10.4 ± 2.4***	**12.3 ± 0.7***
Neutrophils (%)	**0.2 ± 0.0**	**21.0 ± 1.0**	**0.3 ± 0.0**	**0.3 ± 0.1****	**10.2 ± 0.2**	**12.7 ± 0.3***	**10.2 ± 0.2**
Eosinophils (%)	**0.3 ± 0.0**	**12.7 ± 0.3**	**2.5 ± 0.2**	**2.4 ± 0.3**	**10.2 ± 0.2**	**16.3 ± 0.5****	**15.4 ± 2.4**
Lymphocytes (%)	**1.36±.19**	**1.50 ±.009**	**1.33 ±.015**	**1.22 ±.02**	**1.40 ± .32**	**1.51 ± .22***	**1.63 ±. 12***
Macrophages (%)	**75 ± 13**	**76 ± 20**	**70 ± 6**	**65 ± 18**	**75 ± 15**	**79 ± 10***	**78 ± 9.6**
Phagocytosis (%)	**6.4 ± 0.2**	**11.2 ± 0.2**	**0.2 ± 0.2**	**0.2 ± 0.1****	**7.2 ± 0.2**	**9.8 ± 0.1****	**10.2 ± 0.5***
Cytokines (%)	**0.3± 0.4**	**2.4 ± 0.3**	**0.3 ± 0.0**	**0.2 ± 0.3**	**5.2 ± 0.2**	**8.2 ± 0.2**	**9.2 ± 0.2***
Basophils (%)	**4.2 ± 0.2**	**3.2 ± 0.0**	**3.2 ± 0.1**	**2.2 ± 0.2***	**4.2 ± 0.3**	**5.2 ± 0.1**	**6.2 ± 0.3**
Cytokines (%)	**0.3± 0.4**	**2.4 ± 0.3**	**0.3 ± 0.0**	**0.2 ± 0.3**	**5.2 ± 0.2***	**8.2 ± 0.2***	**9.2 ± 0.2***
Basophils (%)	**4.2 ± 0.2**	**3.2 ± 0.0**	**3.2 ± 0.1**	**2.2 ± 0.2**	**4.2 ± 0.3**	**5.2 ± 0.1**	**6.2 ± 0.3***

** Differences are P < 0. 001 highly significant

Table 9 shows the effect of diets with different oils that contain omega-3 and omega-6 on total proteins (T.P.), albumin (Alb), and globulin (Glb) in differential hypercholesterolemic rats. It is clear that (T.P.), (Alb.), and (Glb.) for control positive were lower than control negative, and all groups fed on different oils. Also, data showed that groups fed on sesame oil 10% and sunflower oil 10% treatments showed significantly higher differences compared to the control positive (3.53±0.02, 7.30±0.04, and 6.92±0.03 g, respectively) for total protein. In the present study, (TP), (Alb), and (Glb) levels were higher in sesame oil and chia seed oil and lower in flaxseed oil and fish oil in hypercholesterolemic rats ($p < 0.05$)

Table (9): Effect of different types of edible oils containing omega 3 and omega 6 on protein status of hyperchlesterolemic rats

Groups / Variables	Control(+)	Control(-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame oil 10%	Sunflower oil 10%
TP(g/dL)	3.53±0.02	8.30±0.03	1.43±0.01	2.30±0.02	5.23±0.02	7.30±0.04*	6.92±0.03*
Alb (g/dL)	3.15±0.12	4.40±0.14	1.33±0.01	1.30±0.01	3.20±0.02	3.02±0.02*	3.32±0.02*
Glb (g/dL)	1.95±0.02	3.40±0.10	.53±0.04	2.31±0.01	2.21±0.01**	4.01±0.03	3.60±0.02

* Differences are P < 0.05 significant
** Differences are P < 0. 001 highly significant

Results in Table (10) showed the effect of diets with different oils that contain (omega-3 and omega-6) and their mediators on the various immune system cells. It could be observed that the improvement of NK cell Mast cells were highest for Sunflower oil 10% and chia seed oil 10%. sesame oil 10% groups , as well as T cells and B cells were highest for Sunflower oil 10% and sesame

oil 10% groups .while the was lowest for fish oil 10 % diet group when compared with the
control positive .

Table (10): Effect of different types of edible oils containing omega 3 and omega 6 on the various immune system cells of hyperchlesterolemic rats

groups Cell Types	Control(+)	Control(-)	Oils				
			Flaxseed oil10%	fish oil 10%	chia seed oil 10%	Sesame oil 10%	Sunflower oil 10%
Dendritic cells	↑ stimulation	↔ stimulation	↓ stimulation	↓ stimulation	↑ stimulation	↑ stimulation	↑ stimulation
NK cell	↓ stimulation	↔ stimulation	↓ stimulation	↓ stimulation	↑ stimulation	↑ stimulation	↑ stimulation
Mast cells	↓ stimulation	↔ stimulation	↓ stimulation	↓ stimulation	↑ stimulation	↑ stimulation	↑ stimulation
T cells	↓ stimulation	↔ stimulation	↓ stimulation	↓ stimulation	↑ stimulation	↑ stimulation	↑ stimulation
B cells	↓ stimulation	↔ stimulation	↓ stimulation	↓ stimulation	↑ stimulation	↑ stimulation	↑ stimulation

↓ shows a reduction, ↑ shows an increase, ↔ shows contradictive evidence
↓ indicates a decrease, ↑ indicates an increase, ↔ indicates contradictive evidence

4.Histopathology

The histopathological findings of rat liver were illustrated in Fig. 1. Examining the liver of a control, untreated rat revealed the normal histology of the hepatic lobule, which consists of centralvein and concentrically arranged hepatocytes around it (Photo.1). However, the liver of a hypercholesterolemic rat showed vacuolar degeneration of hepatocytes (Photo.2), necrosis of sporadic hepatocytes, and sinusoidal leucocytosis (**Photo**.2). Apparent normal hepatocytes with kupffer cells activation were noticed in the examined liver, which fed on flaxseed oil at 10% (Photo. 3), while sections of the liver of a rat from group 4 showed vacuolization of some hepatocytes (Photo. 6) and focal hepatic hemorrhage Photo. 6). Aparent normal hepatocytes were observed in the liver of rats fed flaxseed oil (10%), fish oil (10%), and chia oil (10%) (Photos). Moreover, some examined sections of rats fed sunflower oil 10% and sesame oil 10% revealed focal hepatic necrosis infiltrated with mononuclear inflammatory wells (Photos. 6, 7).

Fig.(1) Effects of different oils on The histopathological findings of rat liver

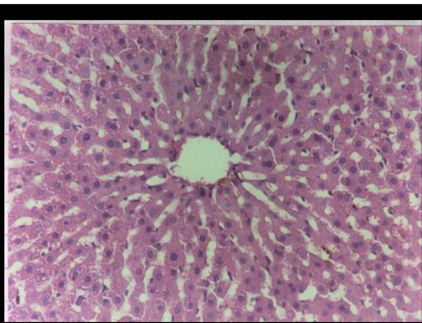


Photo (1): liver of control, untreated rat showing the normal histology of hepatic lobule (H and E X 200)

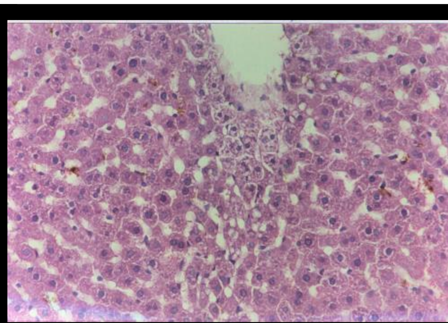


Photo (2): **)**:liver of hypercholesterolemic rat which feeding on (normal diet +) showing vacuolar degeneration of hypato sytes (H and EX 200

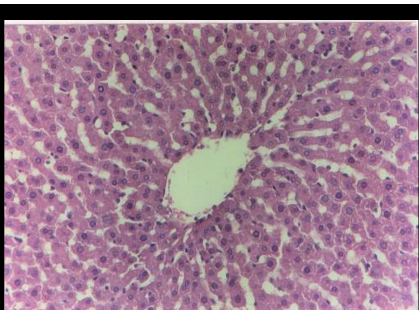


Photo (3): liver of rat which feeding on flaxseed oil 10% showing no histobathological alterations (H and E X 200).

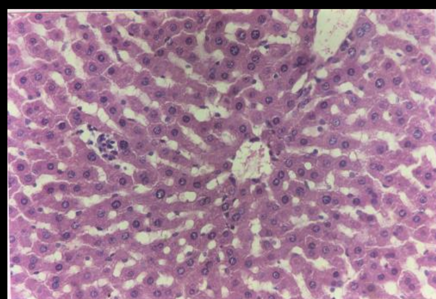
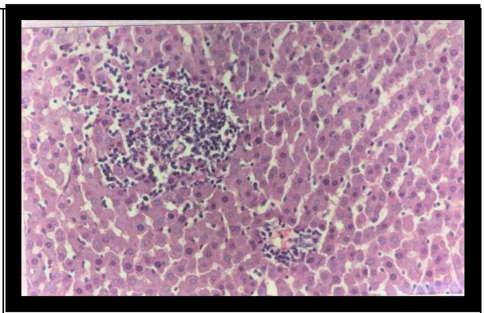


Photo (4):): liver of rat which feeding fish oil 10% showing minute apperant normal hepatocytes (H and E X 200).



Photo(5): liver of rat which feeding on chia oil (10%) showing Focal hepatic necrosis infiltrated with mononuclear inflammatory cells (H and E X 200).

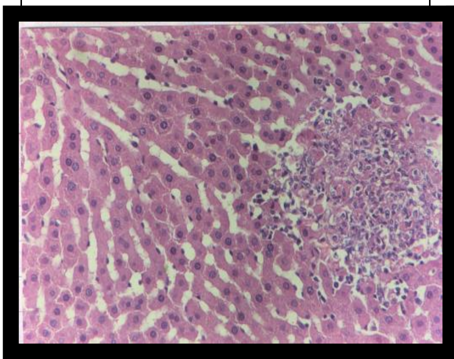


Photo (6): liver of rat which feeding on sunflower oil 10% showing local hepatic necrosis infiltrated with nonuclear cells inflammation (H and E X 200)

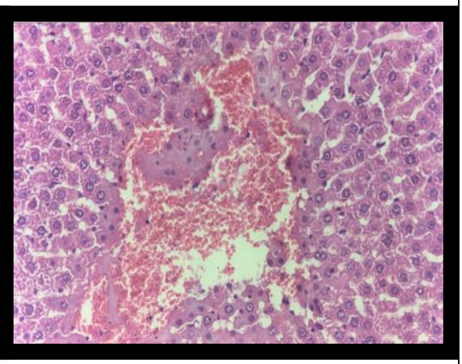


Photo (7):liver of rat which feeding on sesame oil 10% showing focal hepatic haemorrhage (H and E X 200).

5.DISCUSSION:

The present study aimed to evaluate the effect of different types of edible oils that contain omega-3 and omega-6 on blood lipids and the immune system in rats. Our results showed significant improvement in blood lipids in different groups of hypercholesterolemic , In the present study, rats fed flaxseed oil ($G._F$) had higher HDL and lower LDL and VLDL levels compared to the findings reported by **Morise et al. (2004),** who examined how adding flaxseed oil to rats' diets affected their health. esearchers found HDL of 32.8 ± 0.27 mg/dL $_{-1}$, LDL of 4.70 ± 0.05 mg/dL $_{-1}$ and VLDL of 3.00 ± 0.05 mg/dL $_{-1}$. This result is in agreement with many studies. Such as , **(Morise , 2004)** showed that omega-3 fatty acids found in flaxseed oil contribute to increasing cholesterol excretion via bile, thus depleting the liver cholesterol pool and increasing the synthesis of free cholesterol. Also **Halfen et al. (2016)** reported that fish oil reduces blood lipoproteins and mediates cellular inflammation. We speculated that fish oil and

flaxseed oil could reduce the level of nitrosative stress and oxidative stress . Also PUFA- rich, diets such as those containing flaxseed oil, might promote a cardioprotective effect and decrease total cholesterol and LDL-c to normal levels in patients with hypercholesterolemia **(Gupta et al., 2018). Yoon et al. (2021)** reported that nitrosative stress was shown to be closely related to cardiovascular and other diseases . The reaction between NO and superoxide during nitrosative stress results in the peroxynitrite anion (NO-), which produces 3-NT by nitrating proteins and other biomolecules. Other studies demonstrated that regular consumption of ω-3 PUFA is efficient in reducing total cholesterol, cholesterol fractions, and triglycerides **Cholewski et al. (2018).** In line with our results, the amount of PUFA in nut oil is significant (71%)., which accounts for its low oxidative stability. The linoleic acid content in the oil from the nuts varies from 49% to 55% **(Ozcan et al., 2010).** ALA is an important fatty acid that is converted to the long-chain omega-3 fatty acids such as DHA and, optionally EPA. DHA and EPA are found mainly in fish and have demonstrated cardioprotective properties **(Abdel-Moein et al., 2011 and Allam-Ndoul et al.,2017)** .

Also, Harcombe (2019) found that oils with low w-6/w-3 PUFA ratios controlled lipid metabolism, adipokines , improved metabolic disorders and hepatic steatosis . Elevated circulating n-6 fatty acids levels are associated with increased cardiovascular risk, which may be related to the increased oxidative stress in endothelial cells that is caused by FFAs **(Wang et al., 2019).** also found that ALA-rich Chia seed oil improved high-fat diet-induced hepatic steatosis and reduced hepatic lipid deposition in **mice (Han et al., 2020 and Wang et al., 2021).** Our study indicated that replacing of SFAs with PUFAs with different n-6/n-3 PUFA ratios might affect lipid metabolism because it reduces inflammation.

Our results showed significant improvement in serum blood glucose levels in different groups of hypercholesterolemic that were fed on different oils that contain (omega-3 and omega-6) by reducing blood glucose levels, The results agree with Other studies **(Marques et al., 2011)** reported that the combination of flaxseed and flaxseed oil in rat diets produces lower glucose levels (160.8 ± 33.6 mg.dL–1) than those of diets containing only roasted flaxseed probably because flaxseed provides a high content of soluble and insoluble fibers that contribute to decreased glucose.

Aspartate aminotransferase (AST) and alanine aminotransferase (ATL) blood levels were significantly higher in this study's rats after being fed cholesterol-induced hypercholesterolemia than in the rats used as the negative control. Aspartate aminotransferase (AST) and alanine aminotransferase (ATL) blood levels were significantly higher in this study's rats after being fed cholesterol-induced hypercholesterolemia than in the rats used as the negative control. Our data showed that the AST, ALT and ALP (U /L) for different groups of hypercholesterolemic rats fed on different oils that contain (omega-3 and omega-6). It could be observed that the improvement liver functions. The results agree with **(Han et al., 2018),** who noticed that balance in the dietary omega-6/3 ratio may be necessary for ensuring that an excessive and prolonged inflammatory response does not occur and omega-3 fatty acids from food encourage the liver's Treg population to grow and accumulate **Lian et al., (2015**). Oxidative stress and inflammation contribute to the progression of hepatic steatosis **(Li et al., 2022).**

It could be noticed that uric acid for control positive was higher than for control negative. Feeding rats with different oils tested in this work reduced the uric acid level recorded for the control (+) group . Results in Table (8) indicate the levels of effect of diets containing different oils (omega-3 and omega-6) on differential leukocyte count in hypercholesterolemic rats. Long-chain omega-3s can also reduce the amount of inflammatory cytokines produced by endothelial, macrophage, and monocyte cells. Numerous advantages of long-chain omega-3s are attributable to their capacity to reduce inflammatory cytokine gene expression, which is achieved in part by reducing NF-kB activation. It could be observed that the improvement of leukocyte count in hypercholesterolemic rats levels was higher in sesame oil , chia seed oil, sunflower oil and lower in flaxseed oil and fish oil of hypercholesterolemic rats ($p < 0.05$). The results agree with other studies **(Kumar et al., 2016**) that reported that DHA and EPA have been found to decrease gene expression of cytokines in macrophages in vitro. Also the results agree with Moustaka **et al. (2019)** that cytokines in macrophages produced by omega-3 fatty acids were low in production and secretion. Also the results were in agreement with **svahn et al. (2019)** noticed that it has been demonstrated that omega-3 fatty acids increase the ability of m**o**use neutrophils to phagocytose. In the present study, The effect of Omega- 3 fatty acids lower differential leukocyte count in hypercholesterolemic rats concentrations compared with negative control groups.

Neutrophil migration, phagocytic capability, generation of reactive oxygen species, and cytokine synthesis are just a few of the ways that omega-3 fatty acids and their metabolites regulate neutrophil function. The results agree with **Rees et al. (2006)** who found that EPA supplementation led to a decrease in reactive oxygen species production in older men and omega-3 fatty acids modulate T cell activation in vivo, and fish consumption had no significant association with the risk of colon cancer alone .The results agree with other studies **(Serhan, 2014)** They found that omega-3 fatty acids, modulate neutrophil function migration, phagocytic capacity, and the production of reactive oxygen species and cytokines. On the other hand, in various animal models and in human cells, omega-3 fatty acids reduce IgE-mediated activation of mast cells **(Farjadian et al., 2016).** Table (9) shows the effect of oils that contain (omega-3 and omega-6) on total proteins (T.P.), albumin (Alb) and globulin (Glb) in differential hypercholesterolemic rats . It is clear that (T.P), (Alb) and (Glb) for control positive were lower than control negative . Also data showed that groups fed sesame oil at 10% and sunflower oil at 10% treatments showed significantly higher compared to the control positive .In the present study (TP), (Alb) and (Glb) levels were higher in sesame oil , and chia seed oil and lower in flaxseed oil and fish oil in hypercholesterolemic rats ($p < 0.05$). The results disagree with **Amir et al., (2023)** who found that When hospitalized patients took omega-3 fatty acid supplements, their serum albumin and pre-albumin concentrations improved.

T cells are lymphocytes generated from the thymus that utilize the T cell receptor (TCR) to recognize antigens made available by antigen presentation cells (APCs). T cells are traditionally divided into two primary subsets, CD4+ T cells and CD8+ T cells, based on whether or not these cells express the CD4 or CD8 molecules on their surface. Both subsets have different immune characteristics and capabilities. While CD4+ T cells are important in the immune response to bacterial infections, CD8 T cells are important in the immune response to viral infections.cytotoxic T cells and Helper . Results in Table (10) showed the effect of diets with different oils that contain (omega-3 and omega-6) and their mediators on the various immune system cells. It could be observed that the improvement of NK cell Mast cells was highest for omega-6 fatty acids, as well. .while was lowest for fish oil 10 % and flaxseed oil 10% diet groups (omega-3 fatty acid) when compared with control positive. The results agree with **(Saray et al., 2019**) who they found that effects of omega-3 fatty acids on different cells from the immune system and their possible molecular mechanisms, The first method by which omega-3 fatty acids may affect T cell activation in vivo involves changes in the activation of APCs. Omega-3 fatty acid supplementation has been demonstrated to be advantageous in a number of T-mediated illnesses, including autoimmune hepatitis and cytotoxic T cell-mediated asthma. While cytotoxic cells kill virus-infected cells, Th cells control the activity of other immune cells.

our study were in the same line with those reported by **(Teague et al., 2013)** who they found that the two lymphocyte types that make up the majority of the adaptive branch of the immune response are B cells and T cells. A diet high in omega-3 fatty acids alters the percentages of the various B cell populations in various tissues in mice, with a decrease in the number of naive and mature B cells in the bone marrow but no changes in the percentages of pre-B cells or pro-B cells. This is despite the fact that the primary classical function of B cells is the production of antibodies.

Therefore, the present study was performed to research the impact of various n-6/n-3 PUFA ratios on immune system mediators in various cell types. As expected, our study indicated that n-6/n-3 PUFA ratios might affect different cells of the immune system where the values of the immune system cells increased with omega 6 more than with omega 3 **(Hashimoto and Hossain.,2018),** In the present study, rats fed flaxseed oil ($G._F$) had higher HDL and lower LDL and VLDL levels compared to the findings reported by **Morise et al., (2004),** who examined how adding flaxseed oil to rats' diets affected their health. Researchers found

6.Conclusion

The aim of the current study was to determine how various food oils containing omega-3 and omega-6, such as flaxseed oil, fish oil, chia seed oil, sunflower oil, and sesame oil, affected the immune system and blood lipids in rats. The current study did, however, also imply that increased dietary n-3 fatty acid intake enhances high-fat metabolic characteristics. Our findings showed that increased levels of circulating n-6 fatty acids are linked to an increased risk of cardiovascular disease because they may be related to the increased oxidative stress in endothelial cells that is brought on by FFAs. However, fish oil and flaxseed oil may be able to lower levels of oxidative stress and nitrosative stress. Omega-6 also increases and stimulates immune cells, whereas Omega-3 lessens this stimulation.

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تأثير أنواع مختلفة من الزيوت التي تحتوي على أوميجا ٣ وأوميجا ٦ على دهون الدم والجهاز المناعي في الفئران المصابة بأرتفاع الدهون

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الملخص العربي

تؤثر العناصر الكبرى مثل الأحماض الدهنية على صحة الإنسان والجهاز المناعي. تعتبر زيوت الأسماك وزيت بذور الكتان و زيت بذور الشيا مصادر جيدة لأوميجا ٣ الدهنية، في حين أن الزيوت النباتية بما في ذلك زيت عباد الشمس وزيت السمسم هي مصادر ممتازة لأحماض أوميجا ٦ الدهنية. تم أستخدام ٤٢ فار وتم تقسيمهم إلى سبع مجموعات رئيسية (ن = ٧). المجموعة الاولى (ضابطة سالبة) وتتغذى على الغذاء الاساسي . أما المجموعات الست الأخرى تم تغذيها على الغذاء الاساسي بالاضافة الى ٢٪ كوليسترول قبل بدء التجربة من أجل رفع مستويات الكولسترول لدى الفئران . وشكلت المجموعة الثانية ست فئران(ضابطة موجبة). أ ستمرت التجربة لمدة ستة أسابيع شملت الوجبات الغذائية بالاضافة الى الزيوت المضافة للمجموعات (٣)، (٤)، (٥)، و (٦) بنسبة ١٠٪ زيت السمك وزيت بذور الكتان وزيت بذور الشيا وزيت عباد الشمس وزيت السمسم، على التوالي. وفى بداية ونهاية فترة التغذية تم وزن الفئران وحساب معدل التغير فى وزن الجسم ، وفى نهاية فترة التجربة تم سحب عينات دم لقياس التقييم البيوكيميائي ومستويات دلالات الحالة المناعية للتأكد من آثار الزيوت التي تم فحصها. كشفت النتائج أن الفئران التي تم تغذيتها على نظام غذائي مدعم بالزيوت النباتية وزيت الاسماك وخاصة ذات المحتوى العالي من الأحماض الدهنية غير المشبعة و المحتوية على أوميجا ٣ وأوميجا ٦ خفضت مستوى الكوليستيرول في الدم و قللت من خطر الإصابة بأمراض القلب التاجية وكما اثرت الزيوت على كفاءة الجهاز المناعى حيث كان افضل تأثير على الجهاز المناعي فى مجموعة الزيوت المحتوية على اوميجا ٦ مثل زيت السمسم يلية زيت عباد الشمس مقارنة بالزيوت المحتوية على أحماض أوميغا ٣ الدهنية مثل زيت الاسماك وزيت الكتان وزيت بذور الشيا حيث خفضت من كفاءة الجهاز المناعى . وتوصي الدراسة بتناول الزيوت المحتوية على أوميجا ٣ وأوميجا ٦ حسب حالة المرضى الصحية .

الكلمات المفتاحية: أوميجا ٦ – أوميجا ٣– دهون الدم– الجهاز المناعي– بذور الشيا.