

Effect of medium -fat diets containing flaxseed, sesame seeds and their oils on non-alcoholic fatty liver disease in rats.

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Abstract

containing flaxseed, sesame seeds and their oils on non-alcoholic fatty liver disease (NAFLD) in rats, from some nutritional and biochemical parameters, in addition to estimate the chemical composition and fatty acids of the two types of the tested seeds. Fifty-four adult male Albino rats (Sprague Dawley strain), were used in this study and divided into two main groups. The first main group (6 rats) fed on basal diet, as a control negative group. The second main group (48 rats) fed on high fat diet (HFD) for 6 weeks to induce non-alcoholic fatty liver disease, after confirmation rats were randomly assigned to eight equal subgroups: Subgroup (1): fed on high fat diet (HFD) and used as a control positive group (+ve)1. Subgroup (2): fed on medium fat diet (MFD) and used as a control positive group (+ve)2. Subgroup (3): fed on (MFD), replace (10% sheep tallow with 10% flax seeds oil). Subgroup (4): fed on (MFD), replace (10% sheep tallow with flax seeds which provided the diet with 10% oil). Subgroup (5): fed on (MFD), replace (10% sheep tallow with 10% sesame oil). Subgroup (6): fed on (MFD), replace (10% sheep tallow with sesame seeds which provided the diet with 10% oil). Subgroup (7): fed on (MFD), replace (10% sheep tallow with 5% flaxseeds oil and 5% sesame oil). Subgroup (8): fed on (MFD), replace (10% sheep tallow with sesame seeds which provided the diet with 5% oil and flax seeds which provided the diet with 5% oil). The experiment lasted for 4 weeks. The results indicated that, treating NAFLD rats with MFD containing (10% flaxseeds oil, flaxseeds which provided the diet with 10% oil, 10% sesame oil, sesame seeds which provided the diet with 10% oil, 10% (flaxseeds oil and sesame oil) and (sesame and flaxseeds which

provided the diet with 10% oil) led to significant decrease in body weight gain %, organs weight%, peritoneal fat pad PFP /body weight % PFP, leptin, glucose, lipid profile including (cholesterol, triglycerides, LDL-c and VLDL-c), liver enzymes including (AST, ALT and ALP), while these treatments induced significant increase in feed intake, HDL-c and antioxidant enzymes including (GSH-GPx, SOD and CAT). The medium fat diet MFD alone or MFD containing the tested seeds and their oils improved the nutritional and biochemical parameters in non-alcoholic fatty liver disease, especially the group fed on MFD containing the combination of flaxseeds and sesame seeds which provided the diet with 10% oils, followed by the group fed on MFD containing 10% combination of these oils. The medium fat diet MFD which contain the combination of flaxseeds and sesame seeds, or their oils improved the health status of rats suffering from non-alcoholic fatty liver disease

Key words: Flaxseeds, Sesame seeds, Non-alcoholic fatty liver, High fat diet, Lipid profile, Liver enzymes, Antioxidant enzymes and rats

Introduction

Fat accumulation in the liver (hepatic steatosis) from non-alcoholic called Non-alcoholic fatty liver disease (NAFLD), this disease affects about 1.80 billion people (Younossi et al., 2016). In this respect, in 2009 Bellentani and Marino reported that, 10-35% of the adult population in the worldwide suffering from NAFLD. On the other hand, Alberti et al., (2005) decided that hypertension, insulin resistance, obesity and dyslipidemia occur during NAFLD. Aside from the gain of weight and also obesity, the dietary composition, types and the amount of fat induced the development of NAFLD, in this respect, Donnelly et al., (2005) reported that, feeding three day isoenergetic diet

containing 30% energy from fat increased the level of triglyceride by about 15% in the liver which derived from dietary fat.

High-fat diets (HFD) increased body weight, body fat and liver fat levels which associated with insulin resistance in rodent models (**Samuel et al., 2004**). On the other hand, **Anstee and Goldin (2006)** reported that, feeding mouse models on HFD does not produce liver fibrosis and only mild steatosis. While **Kien, (2009) and Tanaka et al., (2008)** reported that, high intake of dietary fat which contain an increase of the ratio of ω -6/ ω -3 and increase the intake of saturated fatty acids associated with liver inflammation and NAFLD. On the other side, **Araya et al., (2004)** reported also, Omega-3 polyunsaturated fatty acids PUFA levels decreased in the hepatic tissue of patients with NAFLD, while the ratio increased of ω -6/ ω -3 may contribute to the development of a fatty liver because of a decreased capacity to regulate liver lipid metabolism. **Cortez-Pinto et al., (2006)** reported that, lower dietary intake of ω -3 fatty acid led to NAFLD in individuals than that of healthy control. While **Kabisch et al., (2018)** found that, low-fat diets led to liver fat reduction.

Flaxseed (*Linum usitatissimum*) consists of 41% fat, 20% protein, and 28% fiber (by weight) (**Singh et al., 2011 and Katare et al., 2012**). Flaxseed contain high amount of ω -3 PUFA, which are reported in the above to have significant hepatoprotective properties and preventing oxidative stress as well as liver inflammation (**Parker et al., 2012 and Hussein et al., (2016)**). One cup of whole flaxseed

has approximately 23% α -linolenic acid (ALA) (by weight). Flaxseed contain several phenolic compounds including (35 to 70 mg/100 g flavonoids) and (1 to 26 mg/100 g lignans) (Martinchik et al., 2012 and Silva et al., 2013). Regarding flaxseed oil Ganorkar and Jain (2013) reported that, this oil contains a combination of fatty acid which is characterized by its high content of polyunsaturated fatty acid, especially (ALA) which constitutes 57% of the total fatty acids in flax.

Sesamum indicum (Pedaliaceae) is the traditional health food around the world, sesame meal is the residue after pressing the oil from the seed, and it is an excellent source of protein 47.1% to 52.9% (Kaneko et al., 2002). Sesame oil contains mono and polyunsaturated fatty acids, these fatty acids are good for heart healthy, because it lowers blood pressure and also cholesterol. In addition, sesame oil contains vitamin E and other antioxidant compounds known as lignans (Sankar et al., 2006). Therefore, the present study designed to evaluate the effect of medium -fat diets containing flaxseed, sesame seeds and their oils on non-alcoholic fatty liver disease in rats.

Material and Methods

Materials:

- 1- Casein, vitamins, minerals, cellulose and choline chloride obtained from El-Gomhorya Company, Cairo, Egypt.
- 2- Soy, sesame seeds (*Sesamum indicum* L.) and flaxseeds (*Linum usitatissimum*) obtained from Agricultural research center, Giza, Egypt.

3- Starch obtained from local market, Cairo, Egypt.

4- Kits for biochemical analysis obtained from Alkan for pharmaceutical and chemical , Dokki, Egypt.

Animals:

Male albino rat *Sprague Dawley* strain weighting 200 \pm 10g purchased from Helwan farm for experimental animals, Ministry of Health and Population, Helwan, Cairo, Egypt.

Methods:

Chemical analysis:

Moisture, total protein, total lipid, carbohydrate and fiber were analyzed and calculated in sesame and flax seeds chemically according to (*A.O.A.C. 2000*), while extraction of oils and fatty acids composition were determined in sesame oil and flaxseeds oil, according to (**Gunstone et al., 1994 and Yeshajahu, 1994**).

Experimental Animals:

Animals: Fifty-four (54) adult male Albino rats of (*Sprague Dawley strain*), weighting 200 \pm 10g were used in this study. All rats were fed the control (basal diet) diet for 4 consecutive days for adaptation. Each rat was housed in an individual stainless steel cage under hygienic controlled condition. Diets were introduced to rats in a special non – scattering feeding cup to avoid loss of food and contamination. Tap water was provided to rats by means of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage.

Basal Diet: the basal diet consists of casein (14%), corn oil (4%), choline chloride (0.25%), and vitamin mixture (1%), cellulose (5%) and the remainder is corn starch according to **Reeves et al, (1993)**, salt mixture which used in these study according to **Hegsted et al., (1941)** and vitamin mixture prepared according to **A.O.A.C. (1975)**.

After adaptation period, the rats divided into two main groups as follows: **The first main group:** (6 rats) fed on basal diet, as a control negative group. **The second main group** (48 rats) fed on high fat diet used to induce non-alcoholic fatty liver disease according to **Zarghani et al., (2016)** ; containing (carbohydrate 55.6% ; “starch 21.6%, sucrose 30% and fructose 4%”), (Fat 27.5% ; “soybean oil 2.5%, hydrogenated oil 5% and sheep tallow 20%’), protein 8.2%, cellulose 4.2%, salt mixture 3.5% and vitamin mixture 1%. After 6 weeks' body weight gain %, serum lipid, cholesterol and triglycerides were determined in the first and second main groups, to insure the induction of obesity and fatty liver, then the second main group divided into eight subgroups as the following:

Subgroup (1): fed on high fat diet (HFD) all over the experimental period, as a control positive group (+ve). **Subgroup (2):** fed on medium fat diet (MFD) containing (Fat 13.75% “soybean oil 1.25%, hydrogenated oil 2.5% and sheep tallow 10%’), protein 8.2%, cellulose 4.2%, salt mixture 3.5% and vitamin mixture 1% and the remainder was corn starch as position Cont.2. **Subgroup (3):** fed on (MFD), replace (10%sheep tallow with 10% flax seeds oil). **Subgroup (4):** fed on (MFD), replace (10%sheep tallow with flax seeds which provided the diet

with 10% oil). **Subgroup (5):** fed on (MFD), replace (10%sheep tallow with 10% sesame oil). **Subgroup (6):** fed on (MFD), replace (10%sheep tallow with sesame seeds which provided the diet with 10% oil). **Subgroup (7):** fed on (MFD), replace (10%sheep tallow with 5% flaxseeds oil and 5% sesame oil). **Subgroup (8):** fed on (MFD), replace (10%sheep tallow with sesame seeds which provided the diet with 5% oil and flax seeds which provided the diet with 5% oil).

During the experimental period (28 days), the diets consumed and body weights were recorded twice weekly according to **Chapman et al., (1959)**. At the end of the experiment, the animals were fasted overnight, and then the rats were anaesthetized and sacrificed. Blood samples were collected from the aorta. Blood samples were centrifuged and the serum was separated to estimate some biochemical parameters, i.e. serum leptin hormone according to **Guillaume and Bjorntorp (1996)**, glucose (**Trinder, 1959**), cholesterol (**Allain et al., 1974**), triglycerides (**Foster and Dumns, 1973**), high density lipoprotein HDL-c (**Lopes-Virella et al., 1977**), low density lipoprotein LDL-c and VLDL-c calculated according to (**FriedWald et al., 1972**), Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) by **Reitman and Frankel, (1957)**, alkaline phosphatase (ALP) by **Belfield and Goldberg, (1971)**. In the liver tissue homogenate catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activities were measured according to the methods described by **Aebi, (1984)** ; **Beauchamp and Fridovich (1971)** and **Ellman (1959)** respectively.

Liver, kidney and peritoneal fat pad were separated from each rat and weighted to calculate the liver, kidney and peritoneal fat pad / body weight %. After that, a specimen from each liver was stored in refrigerator until homogenate preparation. Results of biological evaluation of each group were statistically analyzed (mean \pm standard deviation and one-way ANOVA test) by using SPSS package Ver.25 and compared with each other using the suitable test (least significant differences at $P < 0.05$) according to (**Sendecor and Cochran, 1979**).

Results and Discussion:

Chemical composition of sesame and flaxseed.

Chemical analysis of flaxseeds and sesame seeds presented in Table (1). The data presented in this table showed that, the mean value of moisture and fiber were higher in flaxseeds than that of sesame seeds, while the amount of protein, oil, ash and carbohydrates were higher in sesame seeds than that of flaxseeds. Our results agree with **Morris, (2003)**, who found that, the averaged of fat, protein, dietary fiber, moisture and ash in brown Canadian flax 41%, 20%, 28%, 7.7% and 3.4%), respectively. On the other hand, **Daun and DeClercq, (1994)**, reported that, the protein content of the flaxseeds decreases as the oil content increases.

Nzikou et al., (2009) analyzed the chemical composition of sesame seeds and found that, the seed contained 5.7% moisture, 20% crude protein, 3.7% ash, 3.2% crude fiber, 54% fat and 13.4% carbohydrate. Other study by **Borchani., et al., (2010)** showed that, the chemical

composition of sesame seed is an important source of oil (44 - 58%), protein (18 - 25%), carbohydrate (~13.5%) and ash (~5%).

Table (1): Chemical composition of sesame seeds and flaxseed (g/100g Dry weight).

Nutrients	Flaxseed	Sesame seeds
Moisture*	6.75	5.1
Protein*	18.34	19.4
Oil*	42.00	51.33
Ash*	3.54	4.0
Fiber*	27.00	4.2
Carbohydrate	2.37	15.97

* The average of two estimates

Fatty acids composition of sesame seeds and flaxseed oils (g/100g)

The fatty acid composition of flaxseeds and sesame seeds oils presented in Table (2). Saturated fatty acid (SFA) of flaxseeds oil was 14.85%. Palmitic acid (C16:0) was the major SFA presented in this oil (8.35%), followed by stearic acid C18:0 (5.44%). On the other hand, SFA of sesame seeds oil was (37.72%), most of them short and medium chain fatty acids included (caprylic acid C8:0, capric acid C10:0 and lauric acid C12:0). The other major SFAs in sesame seeds oil were, palmitic (8%), stearic (5.11%) and myristic (4%) respectively.

Oleic acid (C18:1) were higher in sesame seeds oil, as compared to flaxseeds oil (30.77% vs. 22.25%), respectively. The total amounts of polyunsaturated fatty acid PUFA were higher in flaxseeds oil than that of sesame seeds oil (61.95% vs. 30.60%). Linoleic acid (C18:2) was the

major PUFA in sesame seeds oil (30.31%), while alpha-linolenic acid (C18:3) was the major PUFA in flaxseeds oil (46.08%).

In this respect, **Morris, (2003)** reported that, the averaged of saturated fat, monounsaturated fat, linoleic acid (omega-6 fattyacid) and α -Linolenic acid (omega-3 fatty acid) in flaxseed oil were (9%, 18%, 16% and 57%), respectively.

On the other hand, **Nzikou et al., (2009)** reported that, sesame seeds oil contain high levels of unsaturated fatty acids, especially oleic (up to 38.84%) and linoleic (up to 46.26%). *Sesamum indicum* L. oil can be classified in the oleic-linoleic acid group. The dominant saturated acids were palmitic (up to 8.58%) and stearic (up to 5.44%).

Table (2): Fatty acid composition of sesame oil and flaxseed oil (g/100g).

Fatty acids \ Samples	Flaxseeds	Sesame seeds
Caprylic acid (C8:0)	--	3.00
Capric acid (C10:0)	--	2.27
Lauric acid (C12:0)	--	14.50
Myristic acid (C14:0)	--	4.00
Palmitic acid (C16:0)	8.35	8.00
Heptadecanoic acid (C17:0)	--	0.05
Stearic acid (C18:0)	5.44	5.11
Arachidonic acid (C20:0)	0.22	0.55
Behenic acid (C22:0)	0.43	0.15
Lignoceric acid (C24:0)	0.41	0.09
Oleic acid (C18:1)	22.25	30.77
Palmitoleic acid (C16:1)	0.08	0.05
Linoleic acid (C18:2)	15.75	30.31
Alpha-linolenic acid (C18:3)	46.08	0.21
Eicosanoic acid (C20:2)	0.12	0.08
SFA	14.85	37.72
MUFA	22.33	30.82
PUFA	61.95	30.6
Unknown	0.87	0.86

The effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on feed intake and body weight gain% of non-alcoholic fatty liver disease in rats presented in Table (3). The mean value of feed intake (g/day/each rat) decreased in the control (+ve)¹ (Non-alcoholic fatty liver disease NAFLD group) which fed on high fat diet HFD, as compared to healthy rats fed on basal diet. On the other hand, fed intake of rats which suffer from non-alcoholic fatty liver disease and fed on medium fat diet (MFD)², increased significantly $p \leq 0.05$, as compared to control (+ve)¹.

Feeding non-alcoholic fatty liver disease rats on MFD containing 10% sesame seeds oil SSO, 10% flax seeds oil FSO, 5% SSO+5% FSO and their seeds which provided the diet with 10% oils led to significant increase ($P \leq 0.05$) in the mean values of feed intake, as compared to the positive control groups. The highest increase in the mean value of feed intake recorded for NAFLD group fed on MFD containing SS and FS, which provided the diet with 10% oils, this treatment showed non-significant changes in feed intake, as compared to the negative control group.

Table (3): Effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on feed intake and body weight gain% of non-alcoholic fatty liver disease in rats.

Parameters		Feed intake (g/day/each rat)	BWG%
Groups			
Control (-ve)		19.700 ^a ± 0.447	19.498 ^f ± 0.402
Control (+ve) ¹		16.188 ^f ± 0.483	29.162 ^a ± 0.738
Medium fat diet (MFD) Control (+ve) ²		17.770 ^e ± 0.674	26.574 ^b ± 1.013
MFD and replace 10% sheep tallow with	10% SS oil	18.138 ^{d,e} ± 0.352	25.846 ^b ± 0.793
	SS, which provided the diet with 10% oil).	18.854 ^{b,c} ± 0.511	25.973 ^b ± 0.832
	10% FS oil	18.746 ^{c,d} ± 0.578	22.538 ^c ± 1.019
	FS, which provided the diet with 10% oil.	18.500 ^{c,d} ± 0.353	20.294 ^{d,e} ± 0.701
	5% SSO and % FSO	18.934 ^{b,c} ± 0.427	20.569 ^d ± 0.859
	SS and FS, which provided the diet with 10% oils.	19.500 ^{a,b} ± 0.500	19.477 ^e ± 0.404

MFD: Medium-fat diets **SS:** sesame seeds **SSO:** sesame seeds oil

FS: Flax seeds **FSO:** Flax seeds oil

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

Body weight gain % (BWG%) of NAFLD group which was fed on HFD (control +ve)¹ increased significantly ($p \leq 0.05$), as compared to healthy rats fed on basal diet. On the other hand, when fed rats which suffer from NAFLD on MFD (control +ve)², the mean value of BWG% decreased significantly ($p \leq 0.05$), as compared to NAFLD group fed on HFD (control +ve)¹. Treating NAFLD rats with MFD containing 10% SSO, 10% FSO, 5% SSO+5% FSO and their seeds which provided the diet with 10% oils showed significant decrease in BWG%, as

compared to the positive control group which fed on HFD, on the other hand, all treated groups showed significant decrease ($p \leq 0.05$) in BWG%, except NAFLD groups which treated with 10%SS oil and sesame seeds which provided the diet with 10% oil), as compared to the positive control group fed on MFD.

In this respect, some researchers reported that, an excess of body fat tissue may be related not only to energy intake and energy expenditure in humans (**Flatt, 1995**) and (**Prentice, 1998**), but also to the type of diet, especially high-fat diets (HFD), which may lead to various metabolic alterations such as hyperphagia in humans (**Westerterp et al., 2008**), reduced lipolytic activity in fat tissue, reduction in leptin secretion and/or sensitivity, hypothalamic neuron apoptosis (**Moraes et al., 2009**), impairment of mitochondrial metabolism (**Pomplun et al., 2007**), insulin resistance, and obesity (**Wajchenberg, 2000**). On the other side, ecological studies between populations tend to show a positive relationship between fat and obesity, especially if populations with low fat intakes (**Seidell, 1998**). In addition to, extremely low fat, high carbohydrate diets are also very effective for weight loss (**Ornish et al., 1990**).

Dietary fibers play an important role in body weight regulation, through both hunger suppression and diminished nutrient absorption (**Hennes and Perry, 2006**). Flaxseeds contain ~30% dietary fibers of which one third are water-soluble and belonging to a group of heterogeneous polysaccharides (**Naran et al., 2008**). They can affect multiple aspects of the gastrointestinal function such as

gastric emptying rate and nutrient absorption rate in the small intestine (**Lairon et al., 2007**).

Brant et al., (2012) reported that, the development of metabolic syndrome was altered by flaxseed, because it has beneficial effects on lipids and glucose in addition to prevents the excess of body weight gain. **Baranowski et al., (2012)** reported that, alpha linolic acid (ALA-rich Flaxseed oil induced decrease in adipocyte hypertrophy, protein levels of inflammatory markers in adipose tissue in obese zucker rats. In addition to **Kristensen et al., (2011)** reported that, flaxseed fibers baked into bread rolls to significantly increase satiety in a dose dependent manner.

Raesi-Dehkordi et al., (2018) reported that, sesame products consumption did not significantly affect body weight and body mas index BMI ($P > 0.05$). Only sesame oil significantly decreased the body weight and BMI ($P < 0.05$). The body fat percent was significantly reduced after sesame consumption ($P < 0.05$). Sesame consumption did not affect other body composition indices ($P > 0.05$).

The effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on some organs weight/body weight% and peritoneal fat pad/body weight % of non-alcoholic fatty liver disease in rats presented in Table (4).

The data presented in this table revealed that, the mean values of liver, kidney and peritoneal fat pad **PF**/body weight % increased significantly in the control (+ve)¹ group (NAFLD group fed on HFD), as compared to the negative control group fed on basal diet. MFD induced significant decrease $p \leq 0.05$ in liver, kidney and peritoneal

fat pad/body weight %, as compared to the control (+ve)¹ group. All treated groups with 10% (SSO, FSO, combination of them) and the seeds which provided the diet with 10% oils showed significant decrease in the mean value of liver, kidney and peritoneal fat pad/body weight %, as compared to the positive control groups. The highest improvement in decreasing these organs and **PFP** recorded for the group treated with the combination of (SS and FS), which provided the diet with 10% oils, followed by the group treated with FS, which provided the diet with 10% oil.

Schumann et al., (2003) showed that the long-term use of 100gm/kg dietary ground flaxseed in laying hen diets reduced liver fat content and liver weight. The addition of the fatty liver supplement in the flaxseed diets also lowered liver malondialdehyde (MDA) content in hens.

Table (4): Effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on organs and PFP of non-alcoholic fatty liver disease in rats.

Parameters		Organs weight %		PFP%
		Liver	Kidney	
Control (-ve)		2.733 ^e ± 0.048	0.486 ^g ± 0.024	0.923 ^g ± 0.099
Control (+ve) ¹		3.365 ^a ± 0.048	0.768 ^a ± 0.014	2.124 ^a ± 0.066
Medium fat diet (MFD) Control (+ve) ²		3.241 ^b ± 0.035	0.690 ^b ± 0.033	1.933 ^b ± 0.065
MFD and replace 10%sheep tallow with	10% SS oil	3.068 ^c ±0.056	0.640 ^c ± 0.025	1.704 ^c ± 0.066
	SS, which provided the diet with 10% oil).	3.013 ^{c,d} ± 0.037	0.597 ^d ± 0.013	1.506 ^{e,f} ± 0.073
	10% FS oil	3.061 ^c ± 0.092	0.600 ^d ± 0.020	1.622 ^{c,d} ± 0.052
	FS, which provided the diet with 10% oil.	2.986 ^d ± 0.036	0.559 ^{e,f} ± 0.031	1.482 ^{e,f} ± 0.065
	5% SSO and % FSO	2.946 ^d ± 0.016	0.569 ^{d,e} ± 0.022	1.541 ^{d,e} ± 0.051

	SS and FS, which provided the diet with 10% oils.	2.950 ^d ± 0.030	0.530 ^f ± 0.033	1.425 ^f ± 0.059
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MFD: Medium-fat diets **SS:** sesame seeds **SSO:** sesame seeds oil
FS: Flax seeds **FSO:** Flax seeds oil **PPF:** peritoneal fat pad

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

The effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on serum leptin and glucose of non-alcoholic fatty liver disease in rats presented in Table (5).

Leptin and serum glucose increased in NALD group which fed on HFD, as compared with healthy rats fed on basal diet. Leptin and serum glucose increased by about (247.818% and 74.560%) in the NALD group (control +ve)¹ fed on HFD, than that of the negative control group. The increase of leptin and serum glucose decreased in NALD group which fed on MFD, as compared to the group fed on HFD.

Replacement 10% sheep tallow with 10% SSO or 10% FSO and their combination in the diet which used in feeding NAFLD rats showed significant decrease $p \leq 0.05$ in leptin hormone and serum glucose, as compared to the positive control groups^{1&2}, the same trend was observed when used SS, FS and their combination which provided the diet with 10% oil. The best results in leptin hormone and serum glucose recorded for NAFLD group which treated with MFD containing with the combination of SS and FS, which provided the diet with 10% oils, followed by the group treated with FS, which provided the diet with 10% oil, and the combination of 5% SSO and 5% FSO or the

group treated with SS, which provided the diet with 10% oil, respectively.

Our results are in agreement with **Fernandez-Riejos et al., (2010)** who reported that increased fat mass and larger adipocytes lead to elevated circulating leptin concentration. On the other hand, **Bluher and Mantozoros, (2004)** also reported that, human obesity is associated with increased level of leptin.

In this respect **Kratz et al., (2002)** demonstrated that omega 3 fatty acids are considered to be anti-inflammatory and thus may counteract some of the damaging effects of the obese condition. Higher intakes of dietary omega 3 fatty acids are associated with reduced lymphocyte proliferation and Th1 cell development, lower circulating levels of leptin. The dietary ALA-rich flaxseed oil decreased adipocyte size in epididymal and perirenal adipose tissue **Baranowski et al., 2012**).

Wang et al., (2013) reported that α -linolenic acid increases peripheral insulin sensitivity in obese patients and it may aid the prevention and treatment of type 2 diabetes mellitus and atherosclerotic vascular diseases. **Tharwat et al., (2017)** reported that, supplemented bakery with flaxseed or flaxseed oil for 12 weeks' decreased body mass index BMI, blood glucose and improved lipid profile in type 2 diabetics, thus incorporating flaxseed in bakery is recommended.

Ramesh et al., (2005) reported that, diabetic rats fed on a diet supplemented with sesame oil (6%) decreased the levels of blood glucose compared to the diabetic control.

Sesame oil contain high amount of monounsaturated fatty acid, the previous studies have suggested that a high monounsaturated fat diet improves glycemic control by exerting protective effect against β -cell death and augmenting insulin sensitivity (Brehm et al., 2009). On the other hand, Raesi-Dehkordi et al., (2018) reported that, sesame products consumption did not significantly affect body weight and body mass index BMI ($P > 0.05$). Only sesame oil significantly decreased the body weight and BMI ($P < 0.05$). The body fat percent was significantly reduced after sesame consumption ($P < 0.05$).

Table (5): Effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on serum leptin and glucose of non-alcoholic fatty liver disease in rats.

Groups		Parameters	
		leptin	Glucose
Control (-ve)		3.438 ^h ± 0.274	88.957 ^h ± 3.472
Control (+ve) ¹		11.958 ^a ± 0.461	155.284 ^a ± 3.700
Medium fat diet (MFD)		9.442 ^b	139.833 ^b
Control (+ve) ²		± 0.379	± 3.188
MFD and replace 10% sheep tallow with	10% SS oil	8.444 ^c ± 0.379	129.233 ^c ± 2.938
	SS, which provided the diet with 10% oil).	7.182 ^c ± 0.235	116.517 ^c ± 1.806
	10% FS oil	7.838 ^d ± 0.272	123.126 ^d ± 2.703
	FS, which provided the diet with 10% oil.	6.708 ^f ± 0.327	110.523 ^f ± 3.081
	5% SSO and % FSO	7.204 ^c ± 0.192	116.260 ^c ± 3.130
	SS and FS, which provided the diet with 10% oils.	6.192 ^g ± 0.254	100.141 ^g ± 3.416

MFD: Medium-fat diets **SS:** sesame seeds **SSO:** sesame seeds oil

FS: Flax seeds **FSO:** Flax seeds oil

Least significant differences at $P < 0.05$.

Means with the same letter are insignificantly difference.

The effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on serum lipid profile including, cholesterol, triglycerides, high density lipoprotein – cholesterol HDL-c, low density lipoprotein cholesterol LDL-c and VLDL-c (mg/dl) of non-alcoholic fatty liver disease in rats presented in Table (6).

Total cholesterol, triglycerides, LDL-c and VLDL-c increased significantly ($p \leq 0.05$), while HDL-c decreased in NAFLD rats fed on HFD (control +ve)¹, as compared to normal rats (control –ve group) fed on basal diet. On the other hand, feeding NAFLD rats on MFD (control +ve)² led to significant decreased in all parameters, except HDL-c which showed significant increase, as compared to NAFLD rats fed on HFD.

Treating NAFLD groups with 10% (SSO, FSO and their combination) or SS, FS and their combination (which provided the diets with 10% oils) caused significant decrease ($p \leq 0.05$) in serum cholesterol, triglycerides, LDL-c and VLDL-c, while HDL-c recorded significant increase, as compared to the positive control groups.

Table (6): Effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on organs and PFP of non-alcoholic fatty liver disease in rats.

Parameters		T.Ch.	Trig.	HDL-c	LDL-c	VLDL-c
		Groups				
Control (-ve)		84.166 ^g ± 4.241	51.802 ^g ± 2.634	52.767 ^a ± 1.901	21.038 ^h ± 1.996	10.360 ^g ± 0.526
Control (+ve) ¹		175.120 ^a ± 5.296	99.370 ^a ± 4.057	24.230 ^g ± 2.737	131.02 ^a ± 2.291	19.873 ^a ± 0.811
Medium fat diet (MFD) Control (+ve) ²		150.293 ^b ± 2.484	85.615 ^b ± 4.864	29.279 ^f ± 1.233	103.89 ^b ± 0.753	17.122 ^b ± 0.972
MFD and replace 10%sheep tallow with	10% SS oil	140.773 ^c ± 2.190	78.055 ^c ± 1.996	37.091 ^e ± 2.193	88.071 ^c ± 1.244	15.610 ^c ± 0.399
	SS, which provided the diet with 10% oil).	132.774 ^d ± 2.316	73.733 ^d ± 2.441	40.294 ^d ± 1.606	77.733 ^d ± 1.752	14.746 ^d ± 0.488
	10% FS oil	133.451 ^d ± 2.168	69.494 ^e ± 1.535	41.390 ^{cd} ± 1.708	78.163 ^d ± 1.649	13.898 ^e ± 0.306
	FS, which provided the diet with 10% oil.	124.124 ^e ± 1.487	67.665 ^e ± 1.984	42.397 ^{cd} ± 2.084	68.194 ^f ± 1.462	13.532 ^e ± 0.396
	5% SSO and % FSO	127.440 ^e ± 2.535	63.527 ^f ± 0.977	43.482 ^{bc} ± 1.855	71.259 ^e ± 2.380	12.705 ^f ± 0.195
	SS and FS, which provided the diet with 10% oils.	115.423 ^f ± 2.160	61.410 ^f ± 2.693	45.468 ^b ± 2.206	57.673 ^g ± 1.528	12.281 ^f ± 0.538

MFD: Medium-fat diets **SS:** sesame seeds **SSO:** sesame seeds oil

FS: Flax seeds **FSO:** Flax seeds oil

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

The results in this Table revealed that, lipid profile in the groups which treated with FS and FSO recorded more improvement, than that of SS and SSO groups. On the other hand, the best results in lipid profile recorded for NAFLD group fed on MFD containing SS and FS provided the diet with 10% oils, followed by the group treated with (5% SSO + 5% FSO) and the group which treated with FS which provided the diet with 10%, respectively.

Peters, (2003) reported that, dietary fat is considered an important factors contributing to the obesity. Flaxseed (*Linum usitatissimum* L.) classified as a functional food, flaxseed leading source of the dietary fiber, phytochemical (flavonoids, lignans, phenolic acids, phytic acid, and tocopherols) and high amount of alpha-linolenic acid (**Oomah and Mazza, 1998 and Oomah 2001**). Lignans in the flaxseed have antioxidant activity (**Xue et al, 1992**) and therefore may also be of benefit in the prevention of cardiovascular disease (**Hertog et al., 1993**). In this respect, **Park et al., (2012)** suggested that lignan - enriched flaxseed supplementation may provide beneficial effects such as the reduction of body weight and fat accumulation, and improving the lipid profiles of cholesterols.

Paulina et al., (2018) also documented Consumption of flaxseeds has been shown to improve dyslipidemia and reduce blood pressure. Flaxseeds also have some anti-inflammatory and anti-obesity actions. Interestingly, all the aforementioned beneficial effects of flaxseed have been attributed not only to PUFAs, mainly-linolenic acid, but also to fiber and lignans. **Thakur et al., (2009)** reported that, added flaxseed fiber to bread lowered the level of cholesterol in diabetics. Also, **Pan et al., (2009)** reported that, consumption of flaxseed decreased both total and LDL-cholesterol. On the other hand, **Mandasescu et al., (2005)** reported that flaxseed supplementation (as a source of linolenic acid, LNA) decreased total cholesterol by about (17.2%), LDL-C (3.9%), triglycerides (36.3%) and the ratio between TC/HDL-C (33.5%) in hyperlipidemic patients. Also **Bloodon and Szapary, (2004)**. suggested that

flaxseed and its omega-3 fatty acid, alpha-linolenic acid (ALA) induce 11% decreases in (TC).

Bhaskaran et al., (2006) reported that, diet containing sesame oil prevented atherosclerosis in male mice. The levels of plasma cholesterol, triglyceride, low and very low density lipoprotein-cholesterol were decreased in, as compared with atherosclerotic diet-fed animals, while plasma high density lipoprotein level was significantly increased in sesame diet-fed animals. In this respect, **(Satchithanandam et al., 1996)** reported that, sesame oil could lower the level of lipid in serum as well as in liver of rodents.

Ashakumary et al., (1999) reported that, sesame oil also contains lignans that are known to complex cholesterol from the gut and prevent cholesterol absorption. While **Akanya et al., (2015)** reported that, reduction in cholesterol levels observed in diabetic groups which were treated with sesame may be due to the presence of saponins. Saponins have been reported to form complexes with cholesterol and bile acids and prevent them from being absorbed through the small intestine hence lowers the cholesterol level in the blood and liver. On the other hand, **Sirato Yasumoto et al., (2001)** found that, sesame seed oil has been shown to maintain good cholesterol (HDL) and lowers bad cholesterol (LDL).

The effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on liver enzymes including Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and alkaline phosphatase (ALP) of non-alcoholic fatty liver disease in rats presented in Table (7).

Data in this table showed that, feeding NAFLD rats on HFD increased the mean values of serum AST, ALT and ALP significantly, as compared to healthy rats fed on basal diet. While feeding NAFLD rats on MFD induced significant decrease ($p \leq 0.05$) in all liver enzymes parameters, as compared to the rats which suffer from NAFLD and fed on HFD. Results in the present study indicated that, treating NAFLD groups with MFD containing SS, FS, combination of them and their oils improved the parameters of liver enzymes (AST, ALT and ALP), as compared to the group which treated with MFD only. The highest improvement in these parameters recorded for the group which treated with the combination of SS and FS which provided the diet with 10% oil, followed by the groups which treated with (5% SSO + 5% FSO) and FS which provided the diet with 10% oil.

Consumption of high caloric diet led to lipid accumulation (**Asai and Miyazawa, 2001**), increased the production of inflammatory cytokines, and the progression of liver disease (**Wei et al., 2007**). On the other hand, (**Cao et al., 2010**) showed that HFD could induce hyperlipidemia in rats, and hyperlipidemia could alter the related marker enzyme profiles in serum and liver tissue and progress to liver cirrhosis

Table (7): Effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on liver enzymes of non-alcoholic fatty liver disease in rats.

Parameters		AST	ALT	ALP
		U/l		
Control (-ve)		71.502 ^a ± 1.721	22.735 ^a ± 1.284	95.368 ^a ± 3.378
Control (+ve) ¹		126.894 ^a ± 4.294	79.394 ^a ± 3.196	268.319 ^a ± 6.867
Medium fat diet (MFD) Control (+ve) ²		108.768 ^b ± 4.532	65.519 ^b ± 3.248	249.818 ^b ± 7.178
MFD and replace 10%sheep tallow with	10% SS oil	90.622 ^c ± 2.225	59.780 ^c ± 2.598	226.001 ^c ± 1.611
	SS, which provided the diet with 10% oil).	85.866 ^d ± 2.145	55.479 ^d ± 2.978	215.447 ^d ± 3.119
	10% FS oil	87.187 ^{c,d} ± 2.161	54.323 ^d ± 2.438	216.482 ^d ± 2.502
	FS, which provided the diet with 10% oil.	81.370 ^e ± 2.531	49.627 ^e ± 3.536	204.236 ^e ± 2.987
	5% SSO and % FSO	81.742 ^e ± 2.341	49.056 ^e ± 2.518	207.313 ^e ± 3.408
	SS and FS, which provided the diet with 10% oils.	77.224 ^f ± 2.413	44.782 ^f ± 3.385	195.491 ^f ± 3.669

MFD: Medium-fat diets **SS:** sesame seeds **SSO:** sesame seeds oil

FS: Flax seeds **FSO:** Flax seeds oil

Least significant differences at P<0.05.

Means with the same letter are insignificantly difference.

Paulina et al., (2018) reported that, bacterial glycolytic activity increased in the distal intestine by flaxseeds and led to decrease hepatic fat " especially triglyceride accumulation" Flaxseed decrease lipid peroxidation in the kidneys and increase the blood HDL cholesterol concentration. Flaxseed which have high amount of omega-3 polyunsaturated fatty acid, have significant hepatoprotective properties (**Parker et al., 2012**). On the other hand, **Naqshbandi et al., (2012)** reported that, dietary supplementation of flaxseed oil improved the effects of hepatotoxic in rats which induced by Cisplatin. **Fasehuddin and Madhusudhan, (2007)** reported that,

flaxseed chutney supplemented diet could lower the serum cholesterol and as a potential source of antioxidants it could exert protection against hepatotoxic damage induced by carbon tetrachloride (CCl₄) in rats.

Sesame seed consumption appears to increase plasma gamma-tocopherol and enhanced vitamin E activities (**Baselt, 2008**) which are believed to prevent the fatty accumulation and vaculation at the cellular level (**Sanyal et al., 2004**). Sesame lignans have antioxidant property which can inhibit the absorption and the production of cholesterol in the liver (**Kato et al., 1998**). Also, the protective effect of flavonoid of sesamum seed was attributed to its antioxidant and free radical scavenging properties (**Takeoka and Dao (2003)**). Sesamin has been known to protect the liver from oxidative damage (**Sirato Yasumoto et al., 2001**).

The effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on antioxidant enzymes including Glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) of non-alcoholic fatty liver disease in rats presented in Table (8).

The mean values of GSH, SOD and CAT of NAFLD group which was fed on HFD (control +ve)¹ decreased significantly $p \leq 0.05$, as compared to the negative control group. Meanwhile, feeding NAFLD group on MFD (control -ve)² increased the antioxidant enzymes, as compared to those fed on HFD (control +ve)¹.

Treating NAFLD groups with MFD containing sesame oil, flax seeds oil, combination of them and their seeds, led to significant increase in the antioxidant enzymes

(GSH, SOD and CAT), as compared to the positive control groups (control -+ve)^{1&2}.

Flaxseeds and its oil showed more effect in increasing the antioxidant enzymes, than that of sesame seeds or sesame seeds oil. On the other hand, the best results in these enzymes was recorded for the group treated with combination of sesame seeds SS and Flaxseeds FS together, which provided the diet with 10% oils, followed by the groups fed on MFD containing FS, which provided the diet with 10% oil and the combination of sesame seeds oil and flaxseeds oil, respectively.

Safer and natural antioxidants are generally required for food, biological, and pharmaceutical systems. Plant constituents with antioxidant activity and free radical scavenging effects are regarded as the safe source for these antioxidants, as it was reported that synthetic antioxidants have shown adverse effects including mutagenic, carcinogenic, and toxic effects (**Kahn et al., 2015**). Phenolic compounds, which can be obtained from flaxseed (*Linum usitatissimum* L.) oil, are the most widely occurring chemicals, having strong antioxidant properties (**Gulçin et al., 2011**). Phenolic compounds exhibit their antioxidant activity owing to their redox property. p-hydroxybenzoic acid, ellagic acid, p-coumaric acid, ferulic acid, and ascorbic acid are the main phenolic acids in flaxseeds (**Han et al., 2018**).

Rajasha et al. (2006) indicated that, beneficial flaxseed antioxidant components help to restore the elevated activity of hepatic marker enzymes like catalase, SOD and peroxide dismutase at almost normal level in weanling

albino rats which treated with CCl₄. Further, in another study it was demonstrated that dietary flaxseed supplementation increases antioxidant defenses through both reduced ROS generation and increased ROS detoxification (Lee et al., 2008).

Mohamed et al., (2013) reported that, sesame oil offered significant protection against isoproterenol induced oxidative myocardial injury through augmentation of antioxidant enzyme, results of that study shows protective mechanism via decreasing thiobarbituric acid reactive substance (TBARS) and enhancing the endogenous antioxidant enzymes (reduced glutathione (GSH), superoxide dismutase (SOD) and Catalase) in male Wistar albino rats which treated orally with (5 and 10 ml/kgb.w.) for thirty days.

Table (8): Effect of medium-fat diets containing flaxseeds, sesame seeds and their oils on the antioxidant enzymes in liver tissue in non-alcoholic fatty liver disease rats.

Parameters		GSH mg/g Liver	SOD U/g liver	CAT mmol/g liver
Groups				
Control (-ve)		0.372 ^a ± 0.013	0.351 ^a ± 0.010	0.385 ^a ± 0.008
Control (+ve) ¹		0.202 ^g ± 0.014	0.251 ^f ± 0.007	0.144 ^g ± 0.008
Medium fat diet (MFD) Control (+ve) ²		0.236 ^f ± 0.008	0.276 ^e ± 0.010	0.248 ^f ± 0.006
MFD and replace 10%sheep tallow with	10% SS oil	0.256 ^e ± 0.012	0.295 ^d ± 0.009	0.299 ^e ± 0.008
	SS, which provided the diet with 10% oil).	0.286 ^d ± 0.007	0.319 ^c ± 0.003	0.310 ^{d,e} ± 0.009
	10% FS oil	0.306 ^c ± 0.008	0.320 ^c ± 0.007	0.315 ^{c,d} ± 0.009
	FS, which provided the diet with 10% oil.	0.327 ^b ± 0.006	0.331 ^b ± 0.010	0.328 ^b ± 0.009
	5% SSO and % FSO	0.329 ^b ± 0.009	0.327 ^{b,c} ± 0.006	0.324 ^{b,c} ± 0.008

SS and FS, which provided the diet with 10% oils.	0.360 ^a ± 0.008	0.337 ^b ± 0.007	0.332 ^b ± 0.009
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MFD: Medium-fat diets **SS:** sesame seeds **SSO:** sesame seeds oil

FS: Flax seeds **FSO:** Flax seeds oil

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

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