

Study on the biological effect of use flaxseed oil as a source of fat on the Biomarkers of experimental rats

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Abstract: Flaxseed oil comes from the seeds of the flax plant (*Linum usitatissimum*, L.). Flaxseed oil contains both omega-3 and omega-6 fatty acids, which are needed for health. Flaxseed oil contains the essential fatty acid alpha-linolenic acid (ALA), which the body converts into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the omega-3 fatty acids found in fish oil. Some researchers think that flaxseed oil might have some of the same benefits as fish oil, but the body is not very efficient at converting ALA into EPA and DHA. And the benefits of ALA, EPA, and DHA are not necessarily the same. Omega-3 fatty acids, usually from fish oil, have been shown to reduce inflammation and help prevent certain chronic diseases, such as heart disease and arthritis. Studies are mixed about whether flaxseed oil is useful for the same conditions. The present study was performed to evaluate the efficacy of flaxseed oil (FO) on the Biomarkers of experimental rat, The total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), alkaline phosphatase activity (ALP), and glucose, lipids profile and liver and kidney function, heart were measured Rats were divided into five groups; control groups (1&2) negative and positive were fed on basal diet without supplementation. All treated cirrhotic groups (3-5) were fed on experimental diets supplemented with by Flaxseed oil at different levels (20, 30 and 40 gm/kg). Results clearly revealed that the best treatment was flaxseed oil (FO) (40 gm/kg) which had lowest values of total lipid, triglycerides, total cholesterol, LDL, and had the highest values of HDL. While, all groups fed on basal diet with flaxseed oil (FO) by different levels (20,30,40gm/kg) showed significantly decrease of serum, triglycerides, total cholesterol LDL, HDL, ALK, BIL, ALB, TP, Urea, Creatinin and glucose had significant increase of serum HDL, respectively. It could be concluded that flaxseed oil (FO) by different levels ((20, 30, 40 gm. /kg) improve lipids profile and liver and kidney functions especially by flaxseed oil (FO) (40 gm/kg).

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Key Words: Flaxseed - serum lipid- liver- kidney functions- heart – Rats.

Introduction:

Flax oil is one of the best sources of omega-3 fatty acids. More than half of the oils found in the flaxseed come in the form of alpha-linolenic acid (ALA). Omega-3 fatty acids help to create flexible membranes in the cell walls and produce a substance that helps prevent blood clotting. Thus, flax oil can help reduce the risk of cardiovascular disease and strokes. Also, those who regularly take flax oil have lower blood pressure than those who do not. Several studies indicate that flaxseed oil, as well as ground flaxseeds, can lower cholesterol, thereby significantly reducing the risk of heart disease. Taking flaxseed oil may also protect against angina (chest pain) and high blood pressure. In addition, a five-year study done recently at Boston's Simmons College found that flaxseed oil may be useful in preventing a second heart attack. It may also help prevent elevated blood pressure by inhibiting inflammatory reactions that cause artery-hardening plaque and poor

circulation. In recent years, flaxseed (*Linum usitatissimum*) diets have shown to influence plasma lipid levels by changing lipid metabolism (Chena et al., 2005 Lucas et al., 2011) They are very rich source of lignans, α -linolenic acid (ALA), and soluble fiber (Beroza and Kinman 1955; Cunnane 2003), all that may positively affect women's coronary heart disease (CHD) risk. In few studies, the effect of FO on hypercholesterolemia and biomarkers of bone metabolism in ovariectomized Wistar rats was examined. Therefore, the aim of this study was to evaluate whether the incorporation of SO and FO to the diet can produce a beneficial effects on lipid profile and consequently on the prevention of atherosclerosis and bone markers using an ovariectomized rats as a suitable model of postmenopausal hypercholesterolemia. Postmenopausal coronary heart disease (CHD) has become a major cause of morbidity and mortality in women (Van der Schouw *et al.*, 1996). After the onset of menopause, the risk of

CHD in women increases dramatically because of hormone deficiency especially in estrogens (Rosenberg *et al.* 1981). Decreased ovarian function involved in increased plasma concentrations of total and LDL-cholesterol and in an increased LDL/HDL ratio are among the important risk factors for the development of CHD (Assmann, 1993) and (Martin *et al.*, 1986). Several lines of evidences indicate that estrogens are important regulators of lipid homeostasis (Basdevant, 1992) and (Mendelsohn and Karas, 1999). In patients with hypercholesterolemia, the consumption of sesame seed and flaxseed (FS) regimen reduces serum total cholesterol and LDL-cholesterol concentrations (Arjmandi *et al.*, 1998) and (Hirata *et al.*, 1996). Results are controversial regarding the hypocholesterolemic effects of flaxseed oil (FO).

This study aimed to evaluate the effect of different doses of flaxseed oil on lipids profile and liver, kidney functions, in rats.

2-Material and methods

Material

Flaxseed Oil (FO)

Flaxseed oil is derived from the hard, tiny seeds of the flax plant.

Rats and Diet:

A total of 25 Sprague–Dawley male rats were obtained from Farm of experimental animals in Helwan, Egypt. Rats weighing 120 ± 20 g and were housed in plastic cages and fed on basal diet and water for one week as an adaptation period. Animals were clinically healthy and they randomized and housed in stainless steel wire bottom cages (3 rats /cage) and maintained in air-conditioned room on a 12 h light/ dark cycle at 22 ± 2 °C. The basal diet composed of casein (12%), cellulose (5%), vitamins mixture (1%), salts mixture (4%), corn oil (5%) and corn starch (73%). The basal diet formulation was performed according to A.O.A.C (2006).

Experimental design:

A total of twenty five male healthy rats, weighing between (120 ± 20 g) were divided into five groups. Each group containing 5 rats. Control negative groups(1) fed on basal diet without supplementation contain 5% corn oil while control positive group(2) fed on basal diet without supplementation contain 5% flaxseed oil .

All groups (3, 4 &5) were fed on experimental diets as the following:

Group (3): Fed on basal diet containing 20 gm/kg diet flaxseed oil.

Group (4): Fed on basal diet containing 30 gm/kg diet flaxseed oil.

Group (5): Fed on basal diet containing 40 gm/kg diet flaxseed oil

Biological Determination:

Determination of food intake, body weight gain and feed efficiency ratio: Food Intake (FI) was calculated every other day, the biological value of the different diets was assessed by the determination of its effect on Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) at the end of the experimental period measured according to Chapman *et al.* (1959).

Biochemical Determination:

Determination of total Lipids, total Lipids (TL) were determined colorimetrically using sulfophosphovanillic mixture according to the method described by Schmitc (1964). Determination of total cholesterol, serum cholesterol was determined according to the enzymatic method described by Allain *et al.* (1974). Also, determination of triglycerides, the triglycerides in serum were colorimetrically determined according to Wahlefeld (1974). Determination of high density lipoprotein (HDL) cholesterol, the HDL-c was determined according to Albers *et al.* (1983) and determination of very low density lipoprotein (VLDL) cholesterol, the concentration of VLDL-c was estimated according to the Friedewald's equation, $VLDL-c = \text{triglycerides} / 5$. Determination of low density lipoprotein (LDL) cholesterol according to Friedewald *et al.* (1972), low density lipoprotein cholesterol can be calculated as follows: $LDL-c = \text{Total cholesterol} - (HDL-c) - (VLDL-c)$.

Determination of liver functions:

Serum activities of aspartate amino transferase AST, alanine amino transferase ALT Alkaline Phosphatase (ALP) activities were colorimetrically determined according to the method described by Reitman and Frankel (1957).

Determination of kidney functions:

Serum urea nitrogen, uric acid, creatinine were determined according to the methods described by (Patton and Crouch, 1977), (Fossati *et al.*, 1980) and (Husdan and , 1968) respectively.

Histopathological Examination

Specimens from the liver, kidney and spleen were taken immediately after sacrificing the rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, then cleared in xylene, embedded in paraffin, sectioned at 4-6 micron thickness and stained with Hematoxylen and

Eosin (Carleton, 1994) and examined microscopically.

Statistical Analysis:

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS, 1996).

3- Results and Discussions:

The effect of different levels of flaxseed oil on body weight gain (BWG %), Food intake and FER was illustrated in table (1). It could be observed that (control positive group) had significant decrease in body weight gain While the other groups from (3-5) which fed on basal diet with different levels of flaxseed oil (20, 30, 40gm/ to kg) indicate there were significantly increased. A diet high in saturated fats is considered to be one of main contributors to overweight and obesity. Fat is digested into monoglycerides and fatty acids by lipase and absorbed fat is accumulated in adipose tissue through excessive adipocyte differentiation (Yang et al., 2014). The significant increase of body weight with hyperlipidemic diet for eight weeks in the present study was in agreement with previous studies who attributed this increase to the high energy density of fat, since it provides ~9 kcal per gram in comparison to the carbohydrates and protein which provides only 4 kcal. Thus, increased fat intake can promote high energy consumption, increased energy density and palatability (Hill et al., 2000). The increase in body weight may also occur due to hyperphagia and consequently high energy intake induced by adipocyte-derived leptin hormone secretion (Dodd et al., 2014).

Our findings are similar to the results of (Vijaimohan et al 2006) and (Saman et al., 2011)

As shown in table (2), it could be observed weight of heart, liver and kidney had significantly increased compared by (control positive and negative groups

As shown in table (3), Effect of different levels of flaxseed oil on serum total cholesterol triglycerides, HDL-C, LDL-C AND VLDL it could be observed had significant decrease in Total cholesterol compare with control (+ve) in groups 4 and 5 and the same result record in Triglycerides with control (+ve) in groups 4 and 5. In case of LDL-C showed significant decrease compare with control (-ve). Our findings are similar to the results of Vijaimohan et al., (2006) who concluded that there was flaxseed oil (FO) supplementation significantly lowered the

increase in liver weight, plasma cholesterol, triglycerides, phospholipids, free fatty acids, high-density lipoprotein (HDL), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein (VLDL), LDL / HDL and TC / HDL ratio in HFD fed rats. FO significantly reduced the hepatic and plasma lipid levels indicating its hypolipidemic activity. Hepatic protection by FO is further substantiated by the normal liver histological findings in HFD fed rats. These data suggest that FO participate in the normal regulation of plasma lipid concentration and cholesterol metabolism in liver. No adverse effect of FO on growth parameters and plasma lipids in rats fed with fat-free diet. The results of the present study demonstrate that FO may be developed as a useful therapy for hyperlipidemia through reducing hepatic lipids, thereby proving its hypolipidemic.

Our results are in agreement with those of Shim et al. (2014) who have reported that the administration of 25 % flaxseeds lowers LDL cholesterol by 23 % in hypercholesterolemic rats. Makni et al. (2008) have reported that the administration of 33 % flax and pumpkin seeds mixture reduced plasma and liver TC and TG levels by 25 %; 15 % 39 % and 18 %, respectively in hypercholesterolemic rats. A meta-analysis of 28 studies demonstrated that consumption of 20 to 50 g/day of flaxseeds would decrease TC and LDL cholesterol particularly, in people with moderate hypercholesterolemia (Pan et al., 2009).

These results also corroborate with those of Prasad (2005) who have reported that the presence of lignans in flaxseeds is responsible for the cholesterol-lowering effects. Besides, secoisolariciresinol diglucoside (SDG), which is a phytoestrogen present in flaxseeds (Lamblin et al., 2008), is also known to reduce LDL and to increase HDL. Likewise. Otherwise, El-Waseif et al., (2014) have also shown that flaxseed oil might be used as functional therapeutical safe food for hyperlipidemia through decrease hepatic lipids. The increase in HDL-C is one of the most important criteria of anti-hypercholesterolemic agent. Moreover, numerous studies have demonstrated that high levels of HDL-C are associated with a lower incidence of CVD (Shali et al., 2001; Young, 2005). Moreover, epidemiological studies have shown that high HDL-C levels could potentially contribute to its

anti-atherogenic properties, including its capacity to inhibit LDL oxidation and protect endothelial cells from the cytotoxic effects of oxidized LDL (Assmann and Nofer, 2003).

In liver, the concentrations of TC and TG were lower in the group treated with flaxseeds compared with those of the untreated HC group. These results suggest that treatment with flaxseeds exert a cholesterol-lowering effect concomitant with the decrease in total hepatic cholesterol. This decrease is probably due to the presence of insoluble fiber in linseed. Fiber has been reported to reduce plasma levels of LDL-C by interrupting the absorption of cholesterol and bile acids and improving LDL receptor activity (Makni et al., 2008). In fact, it is known that the fibers interfere with the absorption of cholesterol and enterohepatic biliary flow and have caused a decrease in hepatic cholesterol pools (Romero et al., 2002; Lecumberri et al., 2007). In addition, diets rich in fibers are known to reduce TG levels by hindrance of hepatic lipogenesis (Venkatesan et al., 2003).

As shown in table (4) showed the result of GOT, GPT, ALK and BIL which indicate to significant increase in GOT and GPT these result not similar to the results of Shun *et al.*, (2009) whose found the liver damage indices [glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) values] in the flaxseed oil group were lower ($p < 0.05$) than those in the coconut oil (CO), and butter (BU), groups, which may result from higher ($p < 0.05$) glutathione (GSH) levels and a tendency toward lower malondialdehyde (MDA) levels in livers. Besides, lower ($p < 0.05$) gene expression and activity of hepatic matrix metalloproteinases-9 (MMP-9) in the FX group were lower ($p > 0.05$) compared to those in the CO and BU groups; however, no ($p > 0.05$) differences in gene expression activities of hepatic MMP-2 were observed among treatments. Those beneficial effects could explain the attenuation of FX on nonalcoholic fatty liver (NAFL) induced by a high-fat/cholesterol dietary habit. The result of ALK showed no significant all group record in the same limit. In the same table (4) the result of ALK indicate to no significant all group in the same range while the result of BIL showed significant decrease especially in group (4). As shown in table (5), it could be observed there was significantly decrease in, Urea and glucose in group (5) compare control negative and while both of creatinine, UA in the same rang in group (5) compare control negative. Another study showed Flaxseed oil slowed down early fibrosis

progression in renal injury of mice in a study of rats, flaxseed oil feeding produced hepatic and renal enrichments of n-3 PUFA and an increase in C18:>C18 PUFA ratios. The flaxseed oil-based diet was associated with lower mean cystic. Thus, flaxseed oil feeding moderates renal injury and the consumption of flaxseed oil-based products may provide health benefit (zhion@zhion.com).

Table (6) showed the results of TP, ALB, TG and the ratio of A/G these result indicate to TP all groups scored the same range with control (+ve) and (-ve) while results of ALB and TG showed the best result with group (5) which Fed on basal diet containing 40 gm/kg diet flaxseed oil.

Histopathological examination:

Microscopicall examination of Heart, liver and kidney of rat were investigated .the results of heart rats fed on basal diet without supplementation contain 5% corn oil control (-ve) showing no histopathological changes photo (1), while control (+ve) which feed on basal diet without supplementation contain 5% flaxseed oil showing intermyocardial photo (2). The experimental groups from 3 to 5 showed no histopathological changes as shown in photos 3, 4 and 5.

The result of kidney liver microscopicall examination showed control (+ve) photo (6) rats fed on basal diet contain 5% flaxseed oil showing atrophy of glomerular tuft. While Photo (7) rats fed on basal diet contain 5% flaxseed oil +20 gm/kg showing congestion of renal blood vessel. In case of rats fed on basal diet contain 5% flaxseed oil +30 gm/kg photo (8) showing no histopathological changes. Group NO (5) which fed on basal diet contain 5% flaxseed oil +40 gm/kg in photos No (9 and 10) showing focal tubular necrosis associated with inflammatory cells infiltration and atrophy of glomerular tuft and cystic dilatation of renal tubules.

The result of liver microscopicall examination showed control (-ve) group photo (11) which fed on basal diet contain 5% corn oil showing the normal histological structure of hepatic lobule while control (+ve) group photo (12) which fed on basal diet contain 5% flaxseed oil showing showing cytoplasmic vacuolization of hepatocytes . The experimental groups from 3 to 5 showed photo (13) rats fed on basal diet contain 5% flaxseed oil + 20 diet flaxseed oil showing cytoplasmic vacuolization of hepatocytes. Photo (14) rats fed on basal diet contain 5% flaxseed oil + 30 diet flaxseed oil showing Kupffer cells activation. photo (15) rats fed on basal diet contain 5% flaxseed oil + 40

diet flaxseed oil showing focal hepatic necrosis associated with inflammatory cells infiltration.

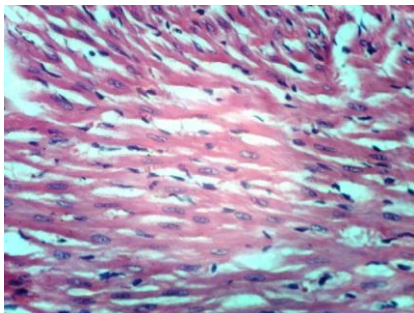


Fig (1): Microscopicall examination of Heart of rats fed on basal diet without supplementation contain 5% corn oil showing no histopathological changes (H & E X 400).

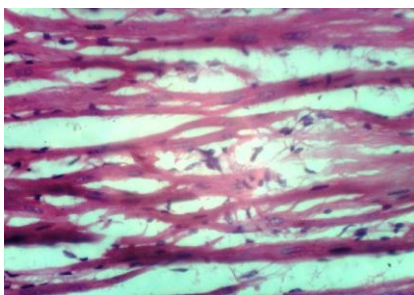


Fig (2): Microscopicall examination of Heart of rats fed on basal diet without supplementation contain 5% flaxseed oil showing intermyocardial oedema (H & E X 400).

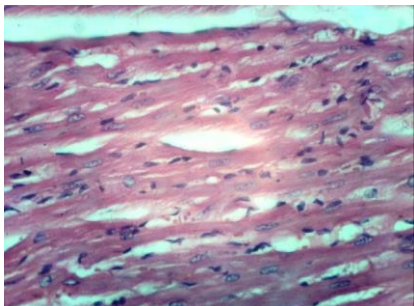


Fig (3): Microscopicall examination of Heart of rats fed on basal diet contain 5% flaxseed oil +20 gm/kg diet flaxseed oil showing no histopathological changes (H & E X 400).

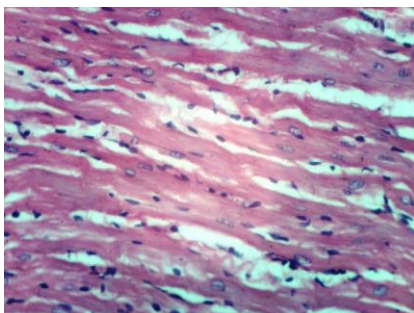


Fig (4): Microscopicall examination of Heart of rats fed on basal diet contain 5% flaxseed oil +30 gm/kg diet flaxseed oil showing no histopathological changes (H & E X 400).

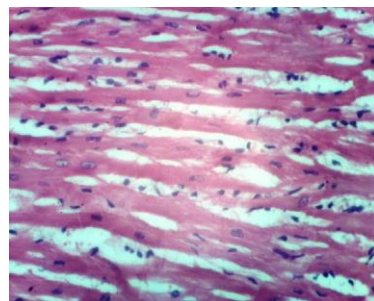


Fig (5): Microscopicall examination of Heart of rats fed on basal diet contain 5% flaxseed oil +40 gm/kg diet flaxseed oil showing no histopathological changes (H & E X 400).

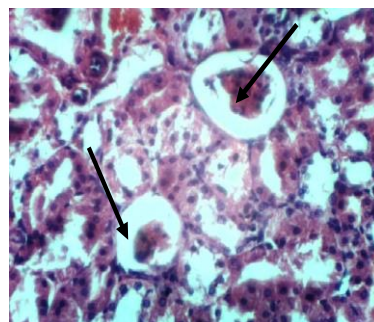


Fig (6): Microscopicall examination of Kidney of rats fed on basal diet contain 5% flaxseed oil showing atrophy of glomerular tuft (H & E X 400).

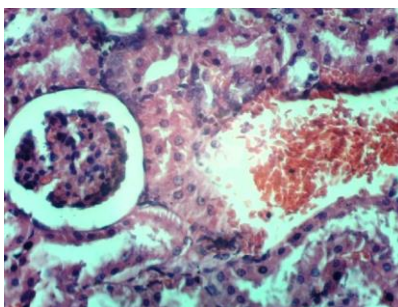


Fig (7): Microscopicall examination of Kidney f of rats fed on basal diet contain 5% flaxseed oil +20 gm/kg showing congestion of renal blood vessel (H & E X 4).

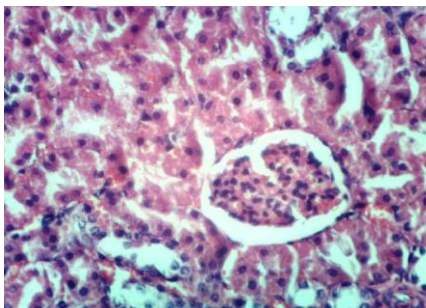


Fig (8): Microscopicall examination of Kidney f of rats fed on basal diet contain 5% flaxseed oil +30 gm/kg showing no histopathological changes (H & E X 400).

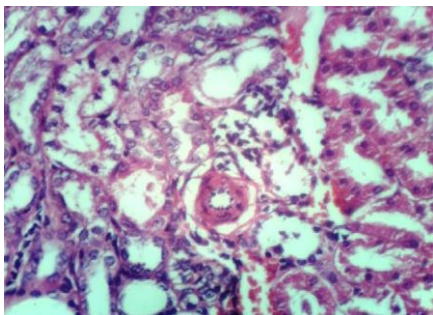


Fig (9): Microscopicall examination of Kidney f of rats fed on basal diet contain 5% flaxseed oil +40 gm/kg showing focal tubular necrosis associated with inflammatory cells infiltration (H & E X 400).

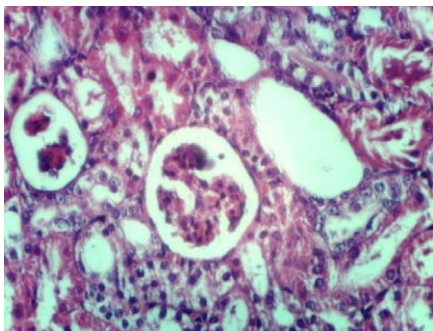


Fig (10): Microscopicall examination of Kidney of rats fed on basal diet contain 5% flaxseed oil +40 gm/kg showing atrophy of glomerular tuft and cystic dilatation of renal tubules (H & E X 400).

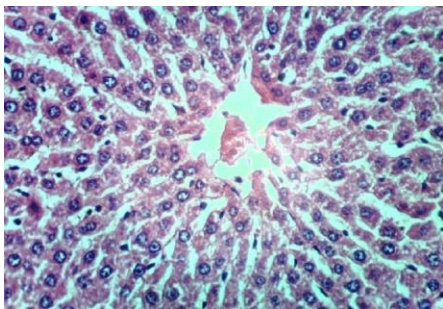


Fig (11): Microscopicall examination of Liver of rats fed on basal diet contain 5% corn oil showing the normal histological structure of hepatic lobule (H &

E X 400).

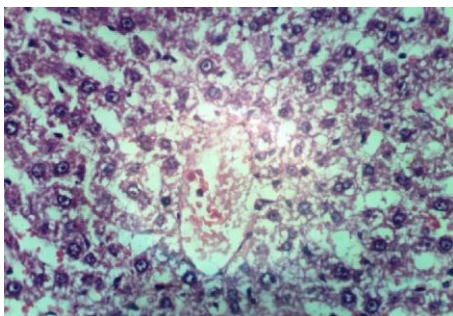


Fig (12): Microscopicall examination of Liver of rats fed on basal diet contain 5% flaxseed oil showing cytoplasmic vacuolization of hepatocytes (H & E X 400).

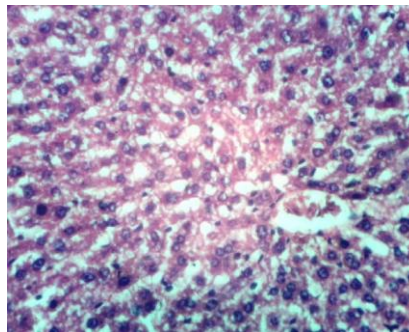


Fig (13): Microscopicall examination of Liver of rats fed on basal diet contain 5% flaxseed oil + 20 diet flaxseed oil showing cytoplasmic vacuolization of hepatocytes (H & E X 400).

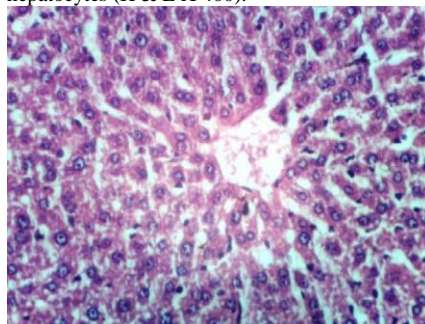


Fig (14): Microscopicall examination of Liver of rats fed on basal diet contain 5% flaxseed oil + 30 diet flaxseed oil showing Kupffer cells activation (H & E X 400).

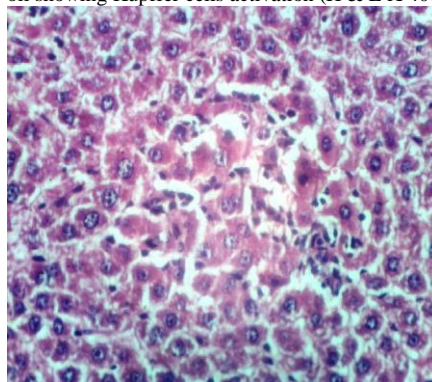


Fig (15): Microscopicall examination of Liver of rats fed on basal diet contain 5% flaxseed oil + 40 diet flaxseed oil showing focal hepatic necrosis associated with inflammatory cells infiltration (H & E X 400).

Table (1): Effect of different levels of flaxseed oil on B WG %, Food intake and FER of rats(mean+SD).

Groups	Week 1 g	Week 2 g	Week 3 g	week 4 g	BWG %	Food intake g/ day	FER
1-Control Negative	102.4±28.7 ^a	100.2±29.5 ^a	97.6±29.9 ^a	92.8±28.9 ^a	9.4±28 ^a	20.13±12.2 ^a	-4.67±11.4 ^a
2- Control Positive	101.8±21.8 ^a	99.4±21.4 ^a	94.8±21.1 ^a	89.6±16.5 ^a	10.8±12 ^a	20.24±23.1 ^a	-5.34±25.1 ^a
3- flaxseed oil 5% +20gm/km	102.2±12.1 ^a	110.2±12.3 ^b	118.1±22.4 ^{bc}	125.3±13.6 ^b	22.9±31 ^b	21.63±42.2 ^a	10.59±75.1 ^b
4 flaxseed oil 5% +30gm/km	112.0±22.4 ^b	120.3±15.7 ^{bc}	128.5±12.5 ^{bc}	135.7±10.2 ^{bc}	23.7±45 ^b	21.92±26.4 ^a	10.83±14.4 ^b
5- flaxseed oil 5% +40gm/km	124.2±24.4 ^b	133.7±12.2 ^{bc}	142.7±12.0 ^{bc}	149.1±12.2 ^{bc}	24.9±47 ^c	21.03±65.2 ^a	11.84±55.1 ^c

Data are presented as means ± SDM (n= 5) & Means within a column with different letters are significantly different at (P≤ 0.05).

Table (2): Effect of different levels of oil flaxseed on weight of rats organs (Heart, liver and kidney) (mean+SD).

Groups	Heart	Liver	Kidney
1-Control Negative	0.29±0.07 ^a	2.5±0.59 ^a	0.73±0.21 ^a
2- Control Positive	0.32±0.05 ^a	2.91±0.82 ^a	0.74±0.10 ^a
3- flaxseed oil 5% +20gm/km	0.41±0.05 ^b	3.53±0.40 ^b	0.78±0.03 ^a
4 flaxseed oil 5% +30gm/km	0.42±0.08 ^b	3.69±0.45 ^b	0.88±0.14 ^b
5- flaxseed oil 5% +40gm/km	0.45±0.02 ^b	3.87±0.31 ^b	0.85±0.14 ^b

Data are presented as means ± SDM (n= 5) & Means within a column with different letters are significantly different at (P ≤ 0.05).

Table (3): Effect of different levels of flaxseed oil on serum Cholesterol, Triglycerides, HDL, LDL-C, HDL-C and VLDL-c (mean+SD).

Groups	CH(mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL-c(mg/dl)
1-Control Negative	76.0±15.7 ^c	68.2±9.7 ^b	48.4±9.7 ^a	33.4±10.3 ^a	13.64±3.4 ^c
2- Control Positive	84.2±14.9 ^b	99.0±27.9 ^a	35.4±18.5 ^b	29.6±28.3 ^b	19.8±8.4 ^a
3- flaxseed oil 5% +20gm/km	95.8±7.2 ^a	85.0±9.9 ^b	46.4±7.3 ^a	31.3±5.3 ^a	17± 12 ^b
4 flaxseed oil 5% +30gm/km	82.8±9.4 ^b	67.0±16.5 ^b	45.0±2.8 ^a	20.4±7.1 ^{bc}	13.4±8.2 ^c
5- flaxseed oil 5% +40gm/km	83.2±9.6 ^b	70.8±33.2 ^b	44.2±3.7 ^a	22.0±14.5 ^{bc}	14.16±5.4 ^c

Data are presented as means ± SDM (n= 5) & Means within a column with different letters are significantly different at (P ≤ 0.05).

Table (4): Effect of different levels of flaxseed oil on serum AST,ALT, ALP and BIL (mean+SD).

Parameters as Mean ±SD				
Groups	COT (IU/L)	GPT (IU/L)	ALK (IU/L)	BIL
1-Control Negative	22.2±5.2 ^{bc}	7.2±1.7 ^{ab}	44.0±2.1 ^a	0.52±0.01 ^a
2- Control Positive	15.0±5.7 ^c	5.6±2.1 ^b	42.2±2.5 ^a	0.51±0.01 ^a
3- flaxseed oil 5% +20gm/km	33.2±4.0 ^{ab}	6.8±2.6 ^{ab}	43.0±3.8 ^a	0.45±0.13 ^b
4 flaxseed oil 5% +30gm/km	42.4±15.4 ^a	9.6±1.6 ^a	40.8±4.7 ^a	0.42±0.23 ^b
5- flaxseed oil 5% +40gm/km	38.2±8.0 ^a	9.2±3.3 ^a	43.6±6.5 ^a	0.49±0.06 ^{ab}

Data are presented as means ± SDM (n= 5) & Means within a column with different letters are significantly Different at (P ≤ 0.05).

Table (5): Effect of different levels of flaxseed oil on serum Creatinine, UREA, UA and GLU (mean+SD).

Groups	Creatinine (mg/dl)	UREA(mg/dl)	UA(mg/dl)	
1-Control Negative	0.62±0.08 ^{ab}	26.0±2.23 ^a	3.36±0.26 ^b	79.2±9.36 ^a
2- Control Positive	0.48±0.05 ^b	24.6±4.5 ^a	3.40±0.64 ^b	79.2±4.8 ^a
3- flaxseed oil 5% +20gm/km	0.69±0.31 ^{ab}	21.4±4.27 ^{ab}	2.48±0.87 ^c	74.6±7.6 ^a
4 flaxseed oil 5% +30gm/km	1.49±1.62 ^a	15.9±8.78 ^b	6.62±8.61 ^a	68.08±37.5 ^b
5- flaxseed oil 5% +40gm/km	0.63±0.10 ^{ab}	20.6±7.33 ^{ab}	3.52±1.00 ^b	60.80±6.68 ^{bc}

Data are presented as means ± SDM (n= 5) & Means within a column with different letters are significantly different at (P ≤ 0.05).

Table (6): Effect of different levels of flaxseed oil on serum TP, ALB, TG and A/G ratio (mean±SD).

Parameters as Mean ±SD				
Groups	TP	ALB	TG	A/G ratio
1-Control Negative	5.58±1.64 ^b	3.84±0.37 ^c	1.74±14.2 ^b	2.21±42.1 ^b
2- Control Positive	5.96±0.90 ^a	3.68±0.7 ^c	2.28±56.2 ^a	1.61±64.2 ^c
3- flaxseed oil 5% +20gm/km	5.50±0.62 ^b	4.0±0.15 ^c	1.5±21.3 ^c	2.67±23.4 ^b
4 flaxseed oil 5% +30gm/km	5.3±0.91 ^c	4.5±21.9 ^a	0.2±52.3 ^c	22.50±42.2 ^a
5- flaxseed oil 5% +40gm/km	5.26±0.65 ^c	3.38±0.69 ^c	1.88±32.5 ^b	1.80±26.4 ^c

Data are presented as means ± SDM ($n = 5$) & Means within a column with different letters are significantly different at ($P \leq 0.05$).

Conclusion:

It could be concluded that flaxseed oil by different levels (20,30,40gm/kg) showed improve lipids profile and liver functions

especially by flaxseed oil (40gm/kg) which has a best significant protective effect.

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