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THE INFLUENCE OF FLAXSEED ON BODY WEIGHT, PLASMA LIPID AND HEPATIC ENZYMES LEVEL OF OVARICTOMIZED FEMALE ALBINO RATS FED ON HIGH FAT DIET

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ABSTRACT

Postmenopausal obesity sounds an alarm for women's health and is generally the major risk factor responsible for many chronic diseases such as metabolic syndrome. Non alcoholic fatty liver disease (NAFLD) is considered to be the hepatic manifestation of the metabolic syndrome. In recent years, flaxseed may play a role in the prevention and treatment of lipid disorders that may improve liver functions. The aim of the present study is to evaluate the influence flaxssed on plasma lipid profile and hepatic enzymes level of ovarictomized female albino rats fed on high fat diet. Animals were divided into five groups, 6 rats each, which were maintained on different diet formulas for 12 weeks. At the end of the experiment blood samples were collected for body weight and biochemical study. The highest mean value of final body weight, total cholesterol, triglyceride and low density lipoprotein cholesterol levels were recorded among group **(IV)** followed by those of group **(II)** and finally group **(III)**. The least mean value of aspartate transaminase and alanine transaminase level was recorded among the control group **(I)** followed by those of group **(III)** and **(V)** and finally those of group **(II)**. While, the highest mean value was recorded among group **(IV)**. One may recommend to avoid consuming high fat diet for long time especially in postmenopausal women. Flaxseed supplementation may provide a

new therapeutic strategy to reduce hyperlipidemia and nonalcoholic fatty liver especially in postmenopausal female.

Keywords: Liver, cholesterol, flaxseed, ovarictomized rats

INTRODUCTION

Onset of menopause is associated with declining estrogen levels, decreased energy expenditure, and decreased fat oxidation, all of which are accompanied by increases in total body fat, obesity, and visceral adipose tissue mass [1]. Obesity can be defined as a chronic disease that has severe consequences on physical and psychological health and not simply a cosmetic issue. Obesity is mainly due to the intensive lifestyle, high carbohydrate or fat dietary intake, and reduced energy consumption [2]. Excess body weight affects all social groups but is more common in lower socio-economic and socially disadvantaged groups, particularly among women [3]**.** Postmenopausal weight gain is one of stages that most women are afraid of. This is because weight gain during menopause does not only indicate that they are indeed aging but also means that they cannot really control the drastic changes that their body will go through. This extra weight generally tends to accumulate around the abdomen and women often notice the shape of their bodies slowly lose their hour-glass figure and begin to take on a rounder shape. This body transformation is a typical aspect of postmenopausal weight gain. As female enter the early stages of menopause, weight gain becomes more and more dangerous, and losing weight becomes almost impossible [4]. Postmenopausal obesity sounds an alarm for women's health and is generally the major risk factor responsible for many chronic diseases such as metabolic syndrome. Additionally, obesity is the most important risk factor for complex and chronic liver disorders and has been linked to the development of non-alcoholic fatty liver disease (NAFLD) that begins as steatosis and may progress to steatohepatitis, cirrhosis, liver failure, and hepatocellular carcinoma [5,6].

NAFLD in obese people is usually clinically silent in the absence of decompensated cirrhosis. Symptoms, if present, are minimal and nonspecific, such as fatigue and right upper quadrant discomfort. Most findings on physical examination are also normal, but should be suspected in patients with elevated aminotransferase levels at routine evaluation, hepatomegaly during physical examination

which has been reported in up to 50% of obese subjects or radiological evidence of a fatty liver that are usually made by ultrasonography allows detecting moderate and severe steatosis. More sensitive techniques, including Magnetic Resonance Image which is hindered by expense and lack of feasibility in large populations [7].

Until now, there is no specific treatment for NAFLD that is proven to be effective. However, an appropriate diet and exercise program are associated with a decrease in the incidence of metabolic syndrome and can also improve the histologic features of NASH in more than 80% of cases [8]. Previous studies have shown that gradual weight loss (1 kg/week) may lead to an improvement in liver biochemistries and liver histology [9]. However, in patients with a high degree of fatty infiltration, rapid weight loss may promote portal inflammation and fibrosis, bile stasis and focal necrosis [10]. Medical treatment of NAFLD is the second choice to decrease the incidence of metabolic syndrome, including lipid-lowering drug that decreases the content of hepatic triglyceride as Clofibrate [11] or Insulin sensitizers as Biguanides that act mainly by increasing hepatic insulin sensitivity [12]. Antioxidants vitamin E, being fat soluble, can stabilize mitochondrial function and is theorized to inhibit lipid peroxidation and subsequent free radical reaction [13, 14] . Ursodeoxycholic acid, hydrophilic bile acid, has an antiapoptotic and cytoprotective effect in NAFLD [15].

In recent years, plant seeds, particularly flaxseed may play a role in the prevention and treatment of several health conditions specially lipid disorders, heart disease and may improving liver functions [16]. The aim of the present study is to evaluate the influence flaxssed on plasma lipid profile and hepatic enzymes level of ovarictomized female albino rats fed on high fat diet. For simulating menopause, surgical bilateral ovariectomy was done.

MATERIAL & METHODS

-Thirty adult female ***Rattus norvogicus*** albino rats, ten weeks of age and weighed 145-150 g were used in the present study. All rats were freely provided with water and rat chow balanced diet one week before the starting of the experiment. One week after their arrival to the animal house, the animals were divided into five groups, 6 rats each. Animals were maintained on different diet formulas for 12 weeks. ***a. Rats fed on balanced diet*** **(GI):** Animals of this group were served as controls. They were daily fed on balanced diet, ***b. Rats fed on high fat diet*** **(GII):** Animals of this group were daily fed

on high fat diet. ***c. Ovarictomized rats fed on balanced diet*** **(GIII):** Animals of this group were bilaterally ovarictomized, and daily fed on balanced diet, ***d. Ovarictomized rats fed on high fat diet*** **(GIV):** Animals of this group were bilaterally ovarictomized, and daily fed on high fat diet till the end of this experiment and ***e. Ovarictomized rats fed on high fat diet containing ground flaxseed*** **(GV):**Rats were bilaterally ovarictomized, and daily fed on high fat diet mixed with ground flaxseed till the end of the experiment. Ovariectomy [17, 18] of adult female albino rat was performed to make these animals in a physiological condition simulating menopause**.**

-The animals of all groups were weighed individually at the beginning of the experiment and at the end just before collecting samples. The percentage of weight gain (**% wt**) is calculated by the following equation [19].

$$\text{Wt} = \frac{\text{(amount of increase weight in grams)}}{\text{Mean of intial weight}} \times 100$$

- Blood samples were collected at the end of the experiment. The rats were fasted over night (16 hour) before taking blood sample then they were lightly anaesthetized with ether after which blood samples were collected immediately in sterile tubes from the orbital sinus of each rat using a heparinized capillary tube. Samples were left to stand for at least 30 minutes at room temperature to coagulate before being centrifuged for 20 min at 2000 rpm to separate the serum[20]. The separated serum was collected to estimate the concentration of plasma lipids (serum total cholesterol (Tc), triglycerides (TGs) and serum high density lipoprotein cholesterol (HDLc). Low density lipoprotein cholesterol (LDLc) concentration was calculated by the following equation: TC– [TG/5+HDLc] [21]. Hepatic enzymes (Alanine aminotransferase (ALT), Aspartat-aminotransferase (AST) were also determined.

Statistical Analysis

Coding of data was carried out manually, and analysis was conducted through SPSS program version 12 statistics software. The different results were analyzed statistically using Student's **"T"** test for quantitative data of two independent samples.

RESULTS

I. Body weight

The higher mean value of final body was recorded among group **(II)** compared to group **(I);** group **(III)** compared to group **(I);** group **(IV)** compared to group **(I**) and finally, among group **(V)** compared to group **(I)**. These differences were of statistically significant values (**Table 1**). However, group

(IV) recorded a non-significant higher mean value than that of group **(II)** and group **(V)** showed a significant reduction in the final body weight comparing to group **(IV)** (**Table 2**).

II. Lipid profile

The higher mean values of serum total cholesterol level, triglyceride level and low density lipoprotein cholesterol were recorded among group **(II)** compared to group **(I)** and the lower mean value of serum high density lipoprotein cholesterol level was recorded among group **(II)** compared to group **(I)**. These findings were of statistically significant values (**Table 3).** it was observed also that group **(III)** had higher mean values of serum total cholesterol level, triglyceride level and low density lipoprotein cholesterol compared to group **(I)** and the lower mean value of serum high density lipoprotein cholesterol level was recorded among group **(III)** compared to group **(I)**. The higher mean values of serum total cholesterol level, triglyceride level and low density lipoprotein cholesterol were recorded also among group **(IV)** compared to group **(I)** and the lower mean value of serum high density lipoprotein cholesterol level was recorded among group **(IV)** compared to group **(I)**. It was noticed that the higher mean values in total cholesterol and low density lipoprotein cholesterol levels were recorded among group **(V)** compared to group **(I)**. All the previous findings were of statistically significant value (**Table 3**). On the contrary, the mean values of triglyceride level and high density lipoprotein cholesterol among group **(V)** were nearly equal to group **(I)**. This observed finding was statistically of a non-significant value (**Table 3**). As regarding the mean values of serum lipid profile among rats of group **(IV)** and rats of group **(V)**, it was observed that the lower mean value of total cholesterol, triglyceride and low density lipoprotein cholesterol levels were recorded among group **(V)** compared to group **(IV)**. However, the higher mean value of high density lipoprotein cholesterol level was recorded among group **(V)** compared to those of group **(IV)**. These observed findings were of statistically significant values (**Table 4**).

III. Serum Liver Enzymes Levels [Aspartate Transaminase (AST) and Alanine Transaminase (ALT)]

The higher mean value of AST level was recorded among group **(II)** compared to group **(I)** without a statistically significant difference. Also, the higher mean value of ALT level was recorded among group **(II)** versus group **(I)**, but this observed finding was of a statistically significant value**.** it was observed also that higher mean value of AST

level was among group **(III)** compared to group **(I)** without a statistically significant difference. On the other side, the higher mean value of ALT level was recorded among group **(III)** versus group **(I)** with a statistically significant value. The higher means values of both AST & ALT were recorded among group **(IV)** versus group**(I)** This observed finding was statistically of significant value. Furthermore, the higher means of both AST & ALT were recorded among group **(V)** versus group **(I)**, with a non- significant value **(Table 5).** When comparing the mean values of serum liver enzymes (AST & ALT) level among group **(IV)** and group **(V)**, it was found that the lower mean of AST was recorded among group **(V)** versus group **(IV)** without statistically significant value**.** Also, the lower mean of ALT level was recorded among group **(V)** versus group **(IV)** with a statistically significant value **(Table 6).**

Table (1): The mean values of final body weight among rats of all groups at the end of 12th week:

Items/Studied groups	Group(I) N = 6	Group(II)N = 6	Group(III) N = 6	Group(IV) N = 6	Group(V) N = 6
Final weight Mean(gm+SD)	189.2±14	240 ±10	228.3±12	247.5±5.2	220±11
Test of significance(T.test)		10	0.163	11	1.1
P. value		P1 (0.000*)	P2 (0.000*)	P3(0.000*)	P4 (0.000*)

N = Number of animals P1, P2, P3, p4 = P. value of each group versus control group * = P ≤ 0.05 = Significant

Table (2): The mean values of final body weight among rats of (II, IV, V)groups at the end of 12th week:

Items/Studied groups	Group(II) N = 6	Group(IV) N = 6	Group(IV) N = 6	Group(V) N = 6
Final weight mean(gm+SD)	189.2±14	247.5±5.2	247.5±5.2	220±11
Test of significance(T.test)		0.000		0.388
P. value		P1=1.0 NS		P2=0.001*

N = Number of animals P1= p. value in group IV versus group II
P2= p. value in group V versus group IV * = P ≤ 0.05 = Significan
NS = P > 0.05 = Non significant

Table (3): The mean values of serum lipid profile (mg/dl) among all rats of the experimental groups at the end of 12th week:

Studied groups/ Items	Group(I) N = 6	Group(II) N = 6	Group(III) N = 6	Group(IV) N = 6	Group(V) N = 6
Cholesterol mean (mg/dl+SD)	48.1±2.2	76.5±4	53.5±2	92±7.5	52±3
Test of significance(T.test)		17.2	0.241	0.56	0.003
P. value		P1 =0.000*	P2=0.001*	P3=0.001*	P4=0.03*
Triglyceride mean (mg/dl+SD)	31.2±6	93.2±6.2	42.1±2.5	103±10	34±7.3
Test of significance(T.test)		17.9	2.9	17.3	1
P. value		P1=0.000*	P2=0.002*	P3=0.002*	P4=0.5(NS)
HDLc mean (mg/dl+SD)	39±2.2	25±2	32±2	22±3	38.5±4
Test of significance(T.test)		10.2	0.013	1.2	2.4
P. value		P1=0.000*	P2=0.000*	P3=0.000*	P4=0.8(NS)
LDLc mean (mg/dl+SD)		33.6±2	13±1.2	48±5	6.3±2.2

Test of significance(T.test)	**2.9±0.64**	**37**	**4.2**	**6.7**	**21**
P. value		**P1=0.000***	**P2=0.000***	**P3=0.000***	**P4=0.004***

N = Number of animals * = P ≤ 0.05 = Significant
P1, P2, P3, p4 = P. value of each group versus control group NS = P > 0.05 = Non significant

Table (4): The mean values of serum lipid profile (mg/dl) among rats of (IV, V) groups at the end of 12th week:

Studied groups/ Items	Group(IV) N = 6	Group(V) N = 6
Cholesterol mean (mg/dl+SD)	**92±7.5**	**52±3**
Test of significance(T.test)		**9**
P. value		**P=0.000***
Triglyceride mean (mg/dl+SD)	**103±10**	**34±7.3**
Test of significance(T.test)		**0.81**
P. value		**P=0.000***
HDLc mean (mg/dl+SD)	**22±3**	**38.5±4**
Test of significance(T.test)		**1.2**
P. value		**P=0.000***
LDLc mean (mg/dl+SD)	**48±5**	**6.3±2.2**
Test of significance(T.test)		**31.6**
P. value		**P=0.000***

N = Number of animals * = P ≤ 0.05 = Significant
P= p. value in group V versus group IV

Table (5): The mean values of serum hepatic enzymes level (U/l) among rats of all experimental groups at the end of 12th week:

Studied groups/ Items	Group(I) N = 6	Group(II) N = 6	Group(III) N = 6	Group(IV) N = 6	Group(V) N = 6
AST mean (U/I +SD)	**35.4±6.4**	**47.2±7.3**	**44.5±11.4**	**53.4±11**	**46±18**
Test of significance(T.test)		**3**	**0.758**	**4**	**2**
P. value		**P1 =0.014 NS**	**P2=0.121 NS**	**P3=0.005***	**P4=0.22 NS**
ALT mean (U/I +SD)	**23.1±2.5**	**52±9**	**31±3**	**55.6±7**	**36.3±15**
Test of significance(T.test)		**7**	**0.010**	**10**	**4**
P. value		**P1=0.000***	**P2=0.000***	**P3=0.000***	**P4=0.06 NS**

N = Number of animals * = P ≤ 0.05 = Significant
P1, P2, P3, p4 = P. value of each group versus control group NS = P > 0.05 = Non significant

Table (6): The mean values of serum hepatic enzymes level (U/l) among rats of group (II) and (IV) at the end of 12th week:

Studied groups/ Items	Group(IV) N = 6	Group(V) N = 6
AST mean (U/I +SD)	**53.4±11**	**46±18**
Test of significance (T.test)		**0.769**
P. value		**P=0.386 =NS**
ALT mean (U/I +SD)	**55.6±7**	**36.3±15**
Test of significance (T.test)		**1**
P. value		**P=0.016***

N = Number of animals NS = P > 0.05 = Non- significant
P = p. value in group V versus group IV * = P ≤ * = P ≤ 0.05 = Significant

DISCUSSION

Bilateral ovariectomy was performed to adult female albino rats in order to make these animals in a physiological condition simulating menopause**.** Using an ovarictomized obese rat model combined with consuming fat enriched diet approximate the nonalcoholic fatty liver disease (NAFLD) [22]. Since flaxseed and its products have attracted a great deal of interest among consumers and health care professionals for their potential benefits on human health, this study tried to clarify its effect on reducing body weight, improving blood lipids and hepatic enzymes levels.

The increase in body weight in (**GIII**) is in consistence with the work of [23, 24] who stated that ovarian hormones play major role in the regulation of food intake and body mass. They found in their researches that withdrawal of ovarian hormones in rats by bilateral ovariectomy induces weight gain and explain the cause of increasing body weight in ovarictomized groups by two possibilities; **primarily** increases food consumption and hyperphagia, accelerating fat deposition and increasing body mass followed by decreases motor activity, **secondary**, is at least consistent with the recent discovery that adipose tissue secretes a substance into the blood stream, referred to as adipsin, that has a purported role in systemic energy balance directing body towards storage mood. The biological cause of the associating postmenopausal obesity is to elevate circulating endogenous estrogens from peripheral aromatization of androgens in adipose tissue in obese postmenopausal women [25, 26]. The increase body weights which was encountered in (**GII & GIV)** of the present work could be explained by the work of [27, 28, 29] who attributed the increase in body weight as a consequence of consuming high fat diet by the occurrence of leptin resistance across the central nervous system due to hyperlipidemia . They stated that there is a combination of both hormonal and neural changes that lead to obesity as leptin hormone under normal physiological condition has an important role in limiting further weight gain and preventing obesity. Leptin supplies a signal that is proportional to the level of fat to the hypothalamus and hindbrain which stimulates its receptor that sufficient energy stores exist. This signal decreases expression of neuropeptides which is orexigenic and increases expression of other neuropeptides that are anorexigenic such as neuropeptide-Y (NP-Y), reducing feeding behavior and in turn suppresses appetite. Furthermore, leptin stimulates thyroid function, accelerates metabolism and

increases energy expenditure by increasing sympathetic nervous system activity and thermogenic activity in brown adipose tissue with subsequent stimulatation of lipid oxidation. The reduction in body weight that was recorded in (**GV)** was in agreement with [30], who reported in his study on postmenopausal obese women that linseed is considered as part of a slimming diet, as it eases weight loss and explained this by: **lecithin** content in freshly crushed flaxseed dissolves the nasty fats from food in the digestive tract and taken them to be eliminated from body, rather than being stored as body fat. **Water soluble fibers** and **protein** contents of flaxseed with plenty of water, is slowly digested, delay gastric emptying and tend to increase intestinal transit time which gives feeling of fullness for longer time and contributes to suppress appetite. Also, **alpha linolenic acid** content in flaxseed increases the body's sensitivity to the leptin hormone, which contributes in reducing feeding behavior, stimulates thyroid function and thus speeds up metabolism and also possibly increases thermogenic activity in brown adipose tissue.

Marked increase in serum lipid parameters with the least value in high density lipoprotein cholesterol that was observed in both (**GII** & **GIV)** were in agreement with [32] who stated that dietary fatty acids are responsible for 35% of free fatty acid (FFA) supply to the liver and overfeeding increase both blood glucose and FFA. The uptake of hepatic glucose is subsequently increased and this excess glucose is diverted into the hepatic de novo lipogenesis and so contributes to increase intra hepato cellular pool of FFA and allowing greater synthesing of very low density lipoprotein cholesterol, triglyceride and cholesterol. In addition dietary fat favors the accumulation of triglycerides within hepatocytes resulting in the prominent hepatic accumulation of fat. Both authors concluded that about 26% of hepatic triacylglycerides accumulation are derived from increased de novo lipogenesis. The increase in serum lipid parameters with significant decrease in high density lipoprotein cholesterol levels which was recorded in (**GIII**) of the present study was in agreement with [31] who explained that estrogen plays an important role in the regulation of lipid metabolism and thus progressive withdrawal of estrogen during natural menopause or following bilateral ovariectomy is associated with many features of the metabolic syndrome, as well as the deteriorations of the blood lipid profile, this tend to have higher low density lipoprotein cholesterol and triacylglycerol levels and

lower high density lipoprotein cholesterol levels compared with premenopausal women. The improvement in serum lipid profile that was obtained in (**GV)** was in agreement with [33]. These authors showed in their studies on hypercholesterolemic rabbits, that dietary flaxseed prevented hypercholesterolemic atherosclerosis as it decreases blood levels of total cholesterol, low density lipoprotein cholesterol and triglycerides. On the contrary other studies on human subjects showed that flaxseed supplementation in the form of either a flaxseed containing bread or 50 gm of ground flaxseed /day for human subjects with hyperlipidemia for three months resulted in significant reductions in serum total cholesterol and low density lipoprotein cholesterol with increase in high density lipoprotein cholesterol level but with no effect on triglyceride level [34]. Different results obtained from studies on young female Sprague Dawley rats fed on isocaloric modified HFD diet supplemented with whole flaxseed for 56 days [20,35]. These authors found a decrease in the blood level of total and low density lipoprotein cholesterol without any significant effects on high density lipoprotein cholesterol, while the level of plasma triglyceride increases. The results of previous researchers' studies might suggest that the effects of flaxseed may vary according to the experimental species, amount and type of flaxseed preparation and the pre-existing level of serum lipids. Moreover, most authors in their studies have used the whole flaxseed form, not the crushed form, which is difficult to digest. The hypocholesterolemic effects of flaxseed is due to its water soluble fiber content that interact with cholesterol in the intestinal lumen to decrease its absorption [36]. The water soluble fiber content of flaxseed is almost fully fermented in the colon into short chain fatty acids that may inhibit liver cholesterol synthesis and increase the body clearance of low density lipoprotein cholesterol [37].

The increase in hepatic enzymes (AST and ALT) that were recorded in **GII, GIII** and **GIV** were in agreement with [38,39]. Elevated ALT more than AST level in both **GII** and **GIV** were in agreement with [40], who stated that with hepatocyte injury in NAFLD there is a two to three fold elevation in levels of both ALT and AST in plasma, they become 1.5-3 times the normal limit and ALT is usually more elevated to higher level than AST**.** He concluded that greater grades of hepatic steatosis and fibrosis are recorded in patients with marked elevation in ALT level. Improved liver enzymes (AST & ALT) levels that was encountered in **GV** was

in agreement with [16]. This author stated that the hepato protective effects of flaxseed meal is attributed to the effect of alpha linolenic acids on the hepatocyte itself as it is responsible for the proper functions and stability of hepatocytes cell membrane by maintaining its fluidity, produce flexible cell membranes and the proper activity of the membrane bound proteins.

Yet, until now the mode of action of flaxseed is unclear and further researches are still needed to study the effect of repeated use of different amounts and forms of flaxseed with different age and sex to avoid any possible undesirable effects if found. From the foregoing investigation, one may recommend to Avoid consuming diets contain excess amount of polysaturated fatty acids for long time especially in postmenopausal women. Flaxseed may be considered a part of a slimming diet, as it eases weight loss in people afflicted with obesity and it is advisable to use flaxseed products to improve general health. Also, flaxseed supplementation may provide a new therapeutic strategy to reduce hyperlipidemia and nonalcoholic fatty liver especially in postmenopausal female.

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