Study of Mustard Oil (*Brassica nigra* L.) As A Hypolipidemic

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ABSTRACT

Background: The present study investigated the effect of mustard oil on body weight and lipid profile in normal and hyperlipidimic mice was studied. The work involved the following studies.

Materials and methods: Twenty-four female mice are divided into four groups each group contain six animals. The normal control group (A) treated with (0.1 mL) distilled water (D.W) for 30 days, group (B) treated with daily high cholesterol diet (5% cholesterol) for 30 days, and the group C treated with (0.1 mL/ 25 kg) for mustard oil besides high cholesterol diet (5% cholesterol) for 30 days. The group D treated with (0.1 mL/ 25 kg) for mustard oil besides standard diet.

Results: Group (B) showed a significant increase ($P < 0.01$) in the body weight compared with normal control group(A). Mustard oil is significantly decreased the body weight in groups (C & D) compared with group(B). Lipid profile measured: Group (B) rats showed a significant increase ($P < 0.01$) in serum TC, TG, LDL and VLDL levels compared with normal control group(A). Serum HDL levels are a significant decrease in group (B) ($P < 0.01$) compared with normal control group(A). Mustard oil is significantly decreased the levels of TC and LDL in groups (C & D) compared with group(B) ($P < 0.01$). While, there was non-significant reduction in TG and VLDL of group (C) compared with group (B) ($P < 0.01$).

Conclusions: Mustard oil has effect on reduce body weight and serum lipid profile and atherogenic index. **Keywords:** Mustard oil, hypolipidemic

INTRODUCTION

The major lipids are fatty acids, triglycerides (TG), cholesterol (free and esterified cholesterol) and phospholipids (PL). They are important in maintaining the structure of cell membrane (cholesterol and phospholipids), steroid hormone synthesis (cholesterol), and energy metabolism (TG and fatty acid) (Liu ,2002). Plasma lipoproteins are typically classified into five major subclasses on the basis of their densities (Betteridge,2000).

1. High density lipoproteins (HDL) collect cholesterol from the body's tissues, and bring it back to the liver.
2. Low density lipoproteins (LDL) carry cholesterol from the liver to cells of the body.
3. Intermediate density lipoproteins (IDL) are intermediate between VLDL and LDL. They are not usually detectable in the blood.
4. Very low density lipoproteins (VLDL) carry (newly synthesized) triacylglycerol from the liver to adipose tissue.
5. Chylomicrons (CM) transport exogenous lipids to liver, adipose, cardiac, and skeletal muscle tissue.

Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is a major risk factor for the development of cardiovascular diseases. The search for new drugs able to reduce and/ or to regulate serum cholesterol and triacylglycerol levels has gained importance over the years, resulting in numerous reports on significant activities of natural agents (Jahromi *et al*., 1993 and Makni *et al*., 2008).

The World Health Organization (WHO) estimates that every year 12 million people worldwide die from cardiovascular diseases, with most of them being from the developing world (Kmietowicz, 2002).

Cardiovascular diseases have been implicated as leading cause of death across the world (Davey *et al.*,1990).

Several factors, such as a high caloric diet, age, lack of exercise, smoking, alcohol consumption, and genetic predisposition have been linked with cardiovascular disease (Asaolu *et al.*,2010). Intervention trials and prospective studies have shown that hypercholesterolemia, especially increased concentrations of LDL cholesterol, leads to the development of atherosclerosis (Stamler *et al.*,1986; Shepherd *et al.*,1995). In contrast, prospective studies have demonstrated a negative correlation between plasma HDL cholesterol and cardiovascular disease (Gordon *et al.*,1989). There is also evidence that oxidized LDL has a pathogenic role in the development of atherosclerosis (Steinberg *et al.*,1989). Atherosclerosis is degeneration, hardening and loss of elasticity (Stephens,2008), it includes accumulation of lipid, inflammatory cells, and fibrous tissue in the intima, which causes intimal thickening of large and mid-sized arteries. The clinical manifestations differ depending on the circulatory bed affected. The coronary arteries are particularly susceptible to atherogenesis; atherosclerosis of the coronary arteries may lead to angina pectoris and myocardial infarction (Ginsberg and Goldberg,1998 ; Falk , 2005) .

Atherosclerosis is a disease of large and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow, and diminished oxygen supply to target organs (Anderson,1999). A number of risk factors are associated with cardiovascular disease and these may be classified into two categories: fixed and modifiable. Fixed risk factors include genetic composition, age, menopausal status, and gender. The modifiable factors are a series of environmental cues and lifestyle choices including (but not limited to) diet, smoking, status of concurrent diseases (e.g. diabetes), exercise and ethanol consumption. When considering these risk factors, it is noteworthy that many, if not all, contribute to disease progression at least in part via oxidative stress **(**Fearon and Faux, 2009). Mustard oil is extracted from *Brassica nigra*. Mustard oil has been used internally and externally since ancient times. Mustard and its oil have been used as a topical treatment for rheumatism and arthritis, as a foot bath for aching feet, and in the form of plasters over the back and chest to treat bronchitis and pneumonia (Felter and Lloyd, 1983). Internally, mustard seeds have been used as appetite stimulants, emetics, and diuretics (Leung, 1980). Mustard oil is characterized by the presence of higher level of erucic acid and it has the lowest saturated fatty acids content among all the edible vegetable oils. Mustard oil contains fatty acids like erucic, oleic, linoleic and α-linolenic acid. Rapeseed-mustard consists of saturated fatty acid such as palmitic (C16:0), stearic (C18:0) and monounsaturated fatty acids such as oleic (18:1) eicosenoic (C 20:1) and erucic acid (C 22:1) and polyunsaturated fatty acids such as linoleic (C 18:2) and linolenic acid (C 18:3), known as essential fatty acids .Of the total fatty acids, erucic acid (C 22:1) (monounsaturated omega-9 fatty acid) is predominant in mustard oil (about 50 per cent) (Tanvir *et al.*, 2014) .

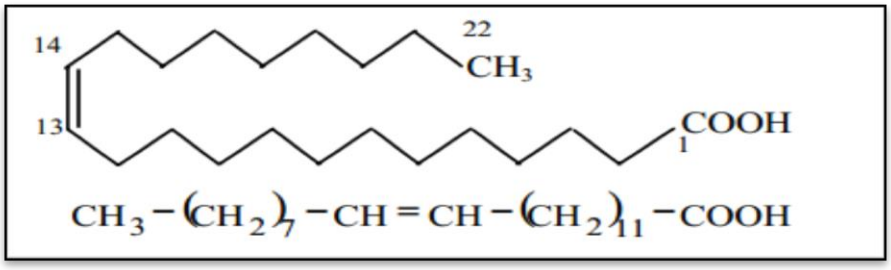

Figure 1. The structure of erucic acid (Tao and He, 2005).

Dietary saturated fatty acid increase blood cholesterol level, while polyunsaturated fatty acids (PUFAs) reduce blood cholesterol. It has been documented that fish and vegetable oils containing polyunsaturated fatty acids (PUFAs) reduce blood lipid profile. Volatile mustard oil is derived from steam distillation or by expression. There have been numerous phytochemical investigations on mustard seed; however, few clinical trials exist to support a clinical application of mustard oil.

MATERIALS AND METHODS

A. Mustard oil :

Mustard oil used in the present was collected from local market in nasiriyah city , Iraq.

B. Experimental animals :

Twenty four healthy female mice weighing (22-30g) for 4 weeks age were used in the present study. Animals were housed in the animal house of biology department / college of science / university of Thi-Qar / Iraq. Animals were housed in iron boxes bedded with wooden chips. During the experimental period six animals were kept in each box and they were housed under standard laboratory conditions (12h light: 12h dark photoperiod (LD) at 22 ± 2 C^{o} and relative humidity 45-55%. Animals were fed on standard rabbit pellet and tap water *ad libitum*. The standard pellet contains wheat 66.6%, soya 25.6%, and sun flower oil 4.4%, lime stone 1.5%, salt 0.63%, methionine 0.158%, choline chloride 0.062% and trace elements 0.05%.

C. Effects of Mustard Oil on Body Weight :

- Method of Food Preparing (High Cholesterol Diet):

5% of high cholesterol diet prepared from 50 g of cholesterol dissolved in 200 g of olive oil and heated in a water bath, and after soluble cholesterol in the oil were added to 1 kg of feed, then was cut into small pieces fit with the size of the holes in the lid iron to boxes, to facilitate the process taken up by rats (Cook *et al.,* 1950).

-Laboratory Animals :

Experimental animals were divided into four groups (6 mice in each group) upon the following designed:

- **Group A**: control (normal) that were treated daily with (0.1 mL D.W)
- **Group B**: Mice were treated with daily high cholesterol diet for 30 days (Cook *et al.,* 1950) .
- **Group C**: Mice were daily treated with (0.1 mL/ 25 kg). of mustard oil besides high cholesterol diet for 30 days.
- **Group D**: Mice were daily treated with (0.1 mL/ 25 kg). of mustard oil besides standard diet for 30 days.

Measured the weights of animals in the first and last day of the experiment using animals balance_stanton461, then calculate the difference was the weight of the animals.

D. Biochemical Parameters :

-Laboratory Animals :

Experimental animals were divided into four groups (6 mice in each group) as in (C).

-Blood collection :

1mL of blood were drawn from each animal of experimental groups, by heart puncture method after 12 hours fast. Using 60 gauge syringes, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at 2-8ºC until the time of assay. Several considerable methods were used to measure the studied parameters. It is notable that all measurements were duplicated for each sample.

E. Measurement of serum lipid profile :

The used reagents were supplied by Biolabo (France), and Serum total cholesterol was measured according to(Allan and Dawson,1979) and Serum TG was measured according to (Tietz *et al*., 1994 and Tietz *et al*., 1999). while serum HDL was measured according to (Lopes-Virella,1977), and measurement of LDL and VLDL according to (Friedwald *et al*.,1972). LDL , VLDL and atherogenic index concentration was measured as follows :

LDL (mg/dL) = total cholesterol – (HDL + VLDL)

VLDL(mg/dL) = serum TG /5

Atherogenic Index = LDL / HDL

Statistical Analysis :

Statistical analysis was done using the software **SPSS** version 17.0; the results were expressed as mean ± standard deviations (mean ± SD). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P < 0.01$) were considered statistically significant.

RESULTS and DISCUSSION

Vegetable oils in human diet constitute an important source of energy and have considerable importance human health.

Effects of Mustard Oil on Body Weight :

The body weight changes were shown in table (1) and figure (2). During 30 days, there was a significant increase in the body weight in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in body weight in groups (C and D) as

compared with group (B) ($P < 0.01$), On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

These results are similar to the result of Rahman, *et al*. (2012) who reported that the mustard oil treated group was found lower the body weight than that of the control group. The monounsaturated fatty acids and proper ratio of polyunsaturated fatty acids in mustard oil which improve heart health and keeps the balance of cholesterol levels in the body, which also lowers triglycerides and prevent obesity.

Effects of Mustard Oil on Serum Lipid Profile and Atherogenic Index Levels

Serum TC, LDL and Atherogenic index levels concentration was changed as shown in tables (2,5 and 7) and figures (3,6 and 8) , at 30 day there was significant increase in the serum concentration of TC, LDL and Atherogenic index levels in group (B) as compared with normal control group(A)($P < 0.01$) . At these times, there was significant reduction in the serum concentration of TC, LDL and Atherogenic index in groups (C and D) as compared with group (B) ($P < 0.01$). While, there was a significant increase in TC of groups (C and D) compared with group (A) ($P < 0.01$). On the other hand, a significant increase can be observed in LDL and Atherogenic index levels between group(C) compared to control group (A) ($P < 0.01$). While, there was non significant increase in LDL and Atherogenic index levels of group (D) compared with group (A) ($P < 0.01$). At 30 day there is a significant increase in the serum concentration of TG and VLDL in group (B) as compared with normal control group (A) ($P < 0.01$). Tables (3,6) and figure (4,7), illustrate a significant decrease ($P < 0.01$) in concentration TG and VLDL in groups (D) after having been treated for (30) days with (0.1 mL / 25 kg B.W) of mustard oil when compared to group (B). While, there was non significant reduction in TG and VLDL of group (C) compared with group (B) ($P < 0.01$). On the other hand, a significant increase can be observed in TG and VLDL between groups (C and D) compared to control group (A) ($P < 0.01$). Changes in the serum concentration of HDL is shown in table (4) and figure (5), at 30 day, HDL concentration decreased significantly in group (B) as compared with normal control group (A) ($p < 0.01$). Groups (C and D) showed significant increase in the serum HDL concentration as compared with group (B) ($p < 0.01$). While, there was non significant increase in HDL of groups (C and D) compared with group (A) ($P < 0.01$). These results are similar to the result of Mustafizur *et al*. (2014) who reported that the mustard oil and fish oil reduce the serum TC, LDL and TG, but had

increase the good cholesterol HDL level in the hypercholesterolemic rats. The stronger anticholesterol activity of mustard oil is because of ω-6 PUFAs. In giving the effect on good cholesterol HDL level, mustard oil showed the strongest increasing effect, which was followed by black seed oil, flax seed oil, sesame oil, and soybean oil (Mustafizur *et al*., 2014). Furthermore, the atherogenic index markedly decreased due to significant reduction in LDL/HDL ratio.Epidemiological studies show that eating fish or vegetable oil (mustard oil, black seed oil, flax seed oil, sesame oil, and soybean oil) concurrently decreases blood cholesterol and LDL levels and increases HDL, and thus reduces the risk of coronary death (Yamori *et al*., 1985; Dyeberg *et al*., 1975).

It is also shown that high concentrations of HDL(over 60 mg/dl) have protective effect against cardiovascular diseases such as ischemic stroke and myocardial infarction but higher LDL promotes cardiovascular disease. Very low LDL increases the risk of cardiovascular disease, if their HDL level is not high (Barteret *et al*., 2007). It is important to have a balance of omega-3 and omega-6 in the diet. The typical American diet tends to contain 14-25 times more omega-6 fatty acids than omega-3 fatty acids. The Mediterranean diet, on the other hand, has a healthier balance between omega-3 and omega-6 fatty acids. Recent studies have demonstrated that ingestion of polyunsaturated fatty acids (ω-3 and ω-6) including alpha linolenic acid (ALA), present in vegetable oils, is inversely related to the incidence of heart disease by decreasing cholesterol and triacylglycerol plasmatic levels (Vijaimohan *et al*., 2006). The major total fatty acids present in seed mixture are unsaturated fatty acids such as oleic acid,linolenic acid and linolenic acid , which play a crucial role in reducing blood cholesterol in human and rats (Movahedian *et al*,.2007). In a previous study, a 12-month, randomized, placebo-controlled trial examined the effects of fish oil versus mustard oil in 360 patients with suspected acute myocardial infarction (MI). Patients in group A (n = 122) received fish oil 1.08 g/day orally, group B (n = 120) received mustard oil 2.9 g/day orally, and 118 patients received placebo. Results indicated a reduction in total cardiac events in patients treated with fish oil or mustard oil compared with placebo (24.5% and 28% vs 34.7%; $P < 0.01$) (Singh *et al*,.1997). Mustard oil is rich in monounsaturated fats and polyunsaturated fats as well as omega-3 and omega-6 fatty acids. These acids to balance the levels of cholesterol in the blood by the levels of HDL, and low levels of LDL, which reduces the risk of cardiovascular disease. The ingestion of polyunsaturated fatty acids present in vegetable oils is inversely related to the incidence of heart diseases by decreasing cholesterol and

triacylglycerol . It leads to the reduction of cholesterol levels, which is mainly Low Density Lipoprotein (LDL) (Coffin *et al*,. 1955). The mustard oil showed cholesterol-lowering benefits in a study published in the May 2011 issue of the journal "Nutrition." Levels of low LDL , cholesterol, and triglycerides decreased in both groups. Both groups showed an increase in high density lipoprotein, or HDL, the good form of cholesterol. The study was conducted at the Department of Chemical Technology, University of Calcutta, India. increased intake of α-Linolenic Acid (ALA) can lower the risk of fatal coronary heart disease (Ingeborg *et al.*, 2004).

CONCLUSIONS

At the end of this study points below can be concluded:

- Mustard oil has effect on reduce body weight and serum lipid profile and atherogenic index .

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Table (1): Changes in the body weight in the all studied groups

Groups	N	ΔWeight (gm) Mean ± S.D
A	6	4.50 ± 1.37 [b]
B	6	7.33 ± 1.03 [a]
C	6	2.00 ± 0.33 [b]
D	6	3.83 ± 2.04 [b]
L.S.D		2.23

Note: Each value represents (mean ± SD) values with non identical superscript (a, b or c …etc.) were considered significantly different (P ≤ 0.01). n= no. of animals

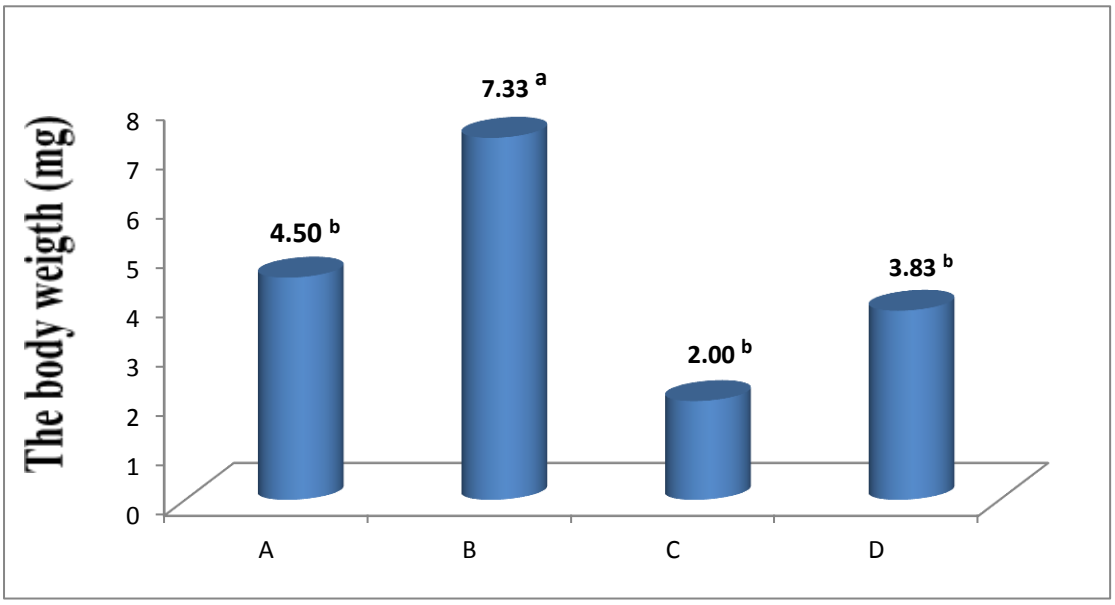

Table (2): Serum TC concentration in the all studied groups

Groups	n	TC (mg/dL) Mean ± S.D
A	6	120.50 ± 3.27 [d]
B	6	285.00 ± 10.37 [a]
C	6	180.50 ± 9.35 [b]
D	6	135.33 ± 4.58 [c]
L.S.D		12.34

Legend as in table (1)

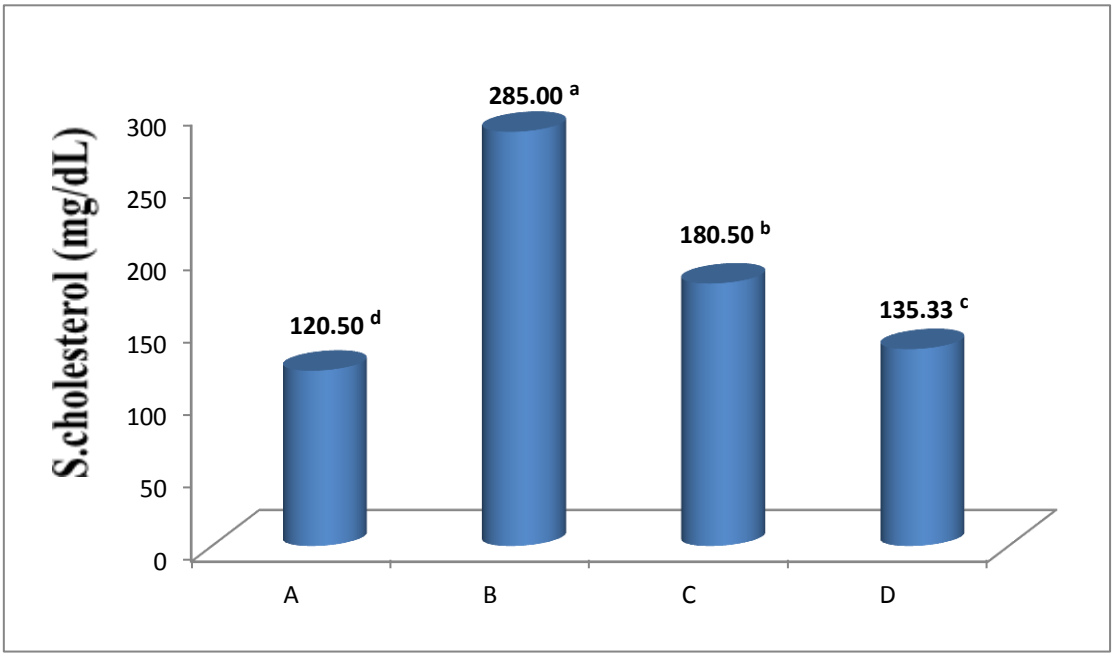

Table (3): Serum TG concentration in the all studied groups

Groups	n	TG (mg/dL) Mean ± S.D
A	6	109.16 ± 3.81 [c]
B	6	146.83 ± 5.87 [a]
C	6	139.66 ± 5.92 [a]
D	6	123.00 ± 4.38 [b]
L.S.D		8.33

Legend as in table (1)

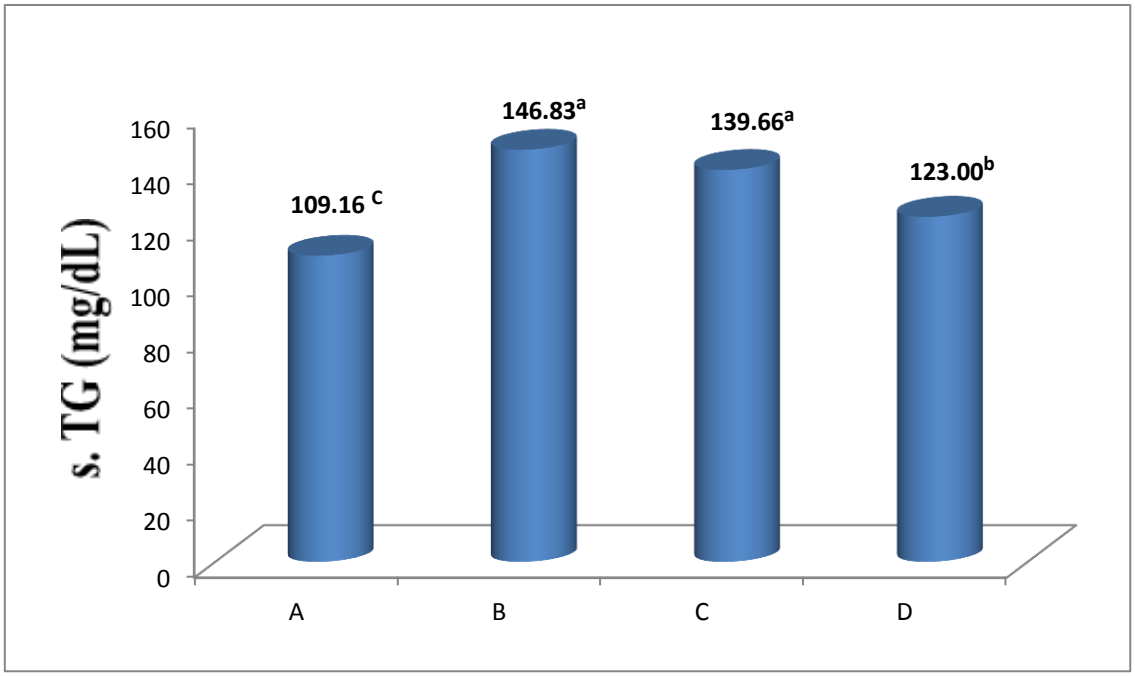

Figure (4): Serum TG concentration in the all studied groups

Table (4): Serum HDL concentration in the all studied groups

Groups	n	HDL (mg/dL) Mean ± S.D
A	6	78.33 ± 4.15 [a]
B	6	72.31 ± 3.20 [b]
C	6	84.20 ± 3.59 [a]
D	6	87.20 ± 3.54 [a]
L.S.D		7.99

Legend as in table (1)

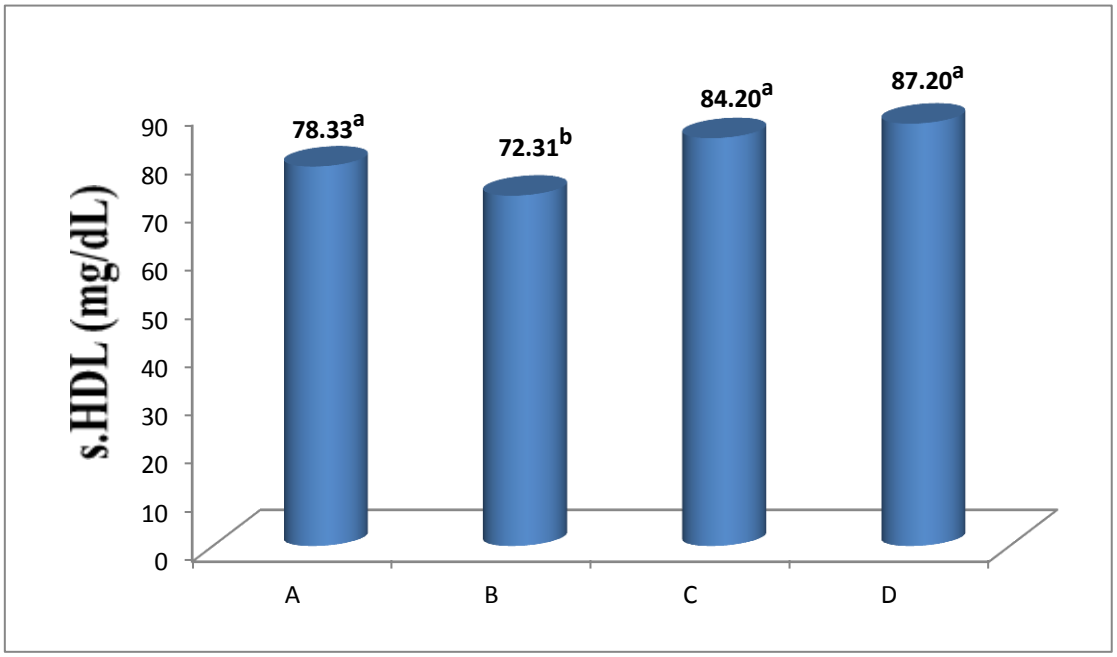

Figure (5): Serum HDL concentration in the all studied groups

Table (5): Serum LDL concentration in the all studied groups

Groups	n	LDL (mg/dL) Mean ± S.D
A	6	20.33 ± 1.19 [c]
B	6	183.31 ± 7.45 [a]
C	6	68.36± 3.14 [b]
D	6	23.53 ± 1.46 [c]
L.S.D		13.89

Legend as in table (1)

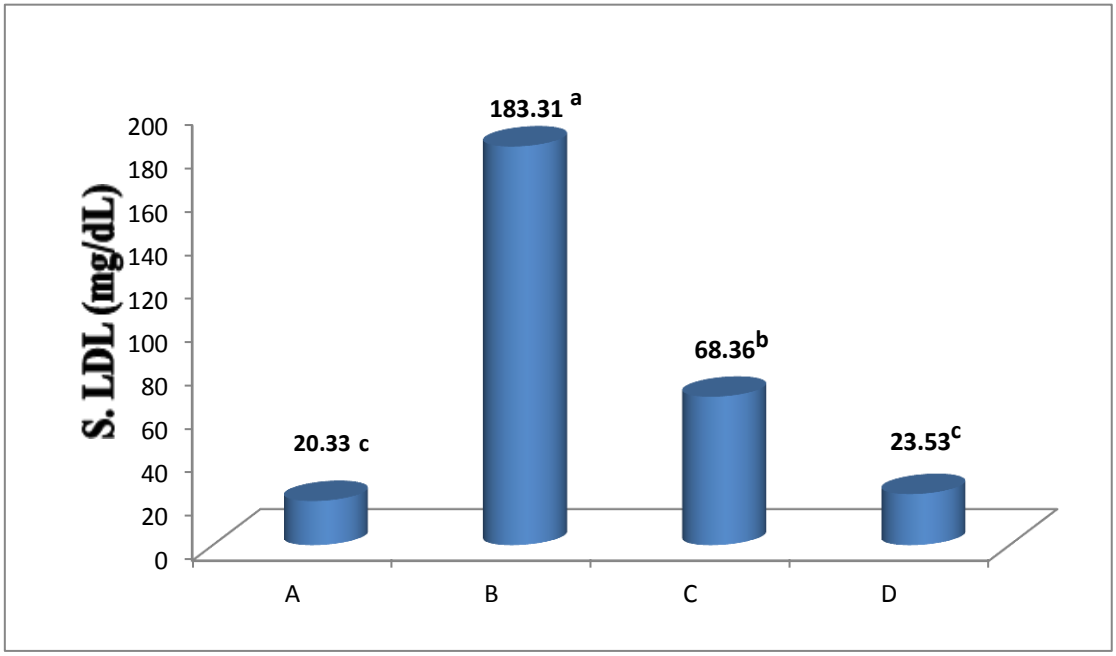

Figure (6): Serum LDL concentration in the all studied groups

Table (6): Serum VLDL concentration in the all studied groups

Groups	n	VLDL (mg/dL) Mean ± S.D
A	6	21.83 ± 0.76 [c]
B	6	29.36 ± 1.17 [a]
C	6	27.93 ± 1.18 [a]
D	6	24.60 ± 0.87 [b]
L.S.D		1.66

Legend as in table (1)

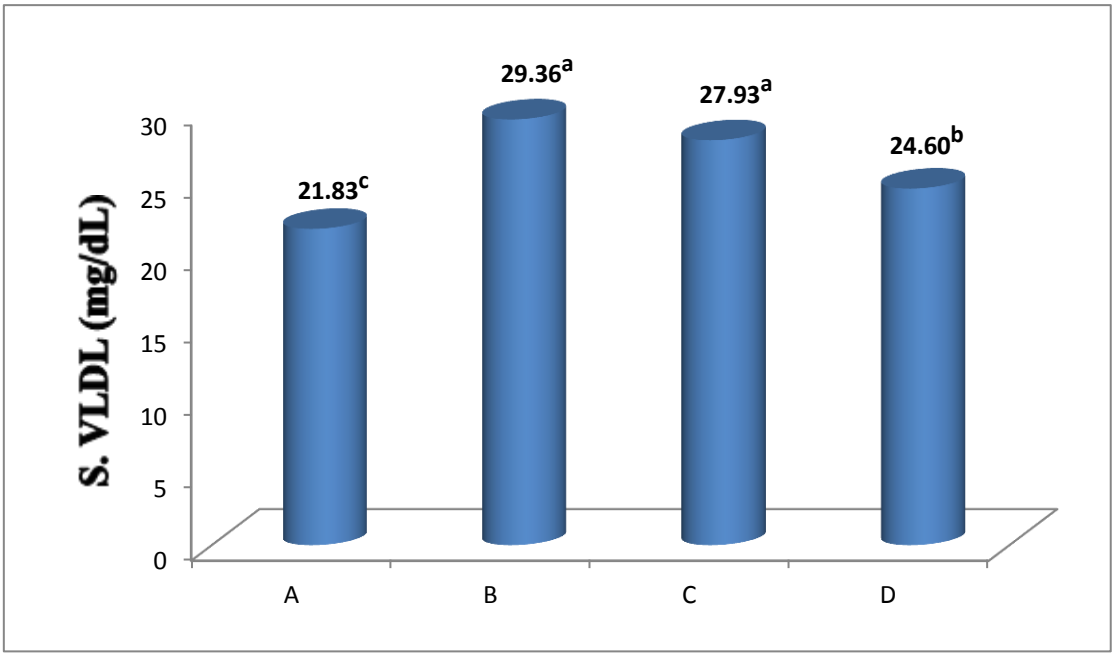

Figure (7): Serum VLDL concentration in the all studied groups

Table (7): The Atherogenic Index levels in the all studied groups

Groups	n	Atherogenic Index Mean ± S.D
A	6	0.26 ± 0.05 [c]
B	6	2.53 ± 0.14 [a]
C	6	0.82 ± 0.21 [b]
D	6	0.27 ± 0.07 [c]
L.S.D		0.22

Legend as in table (1)

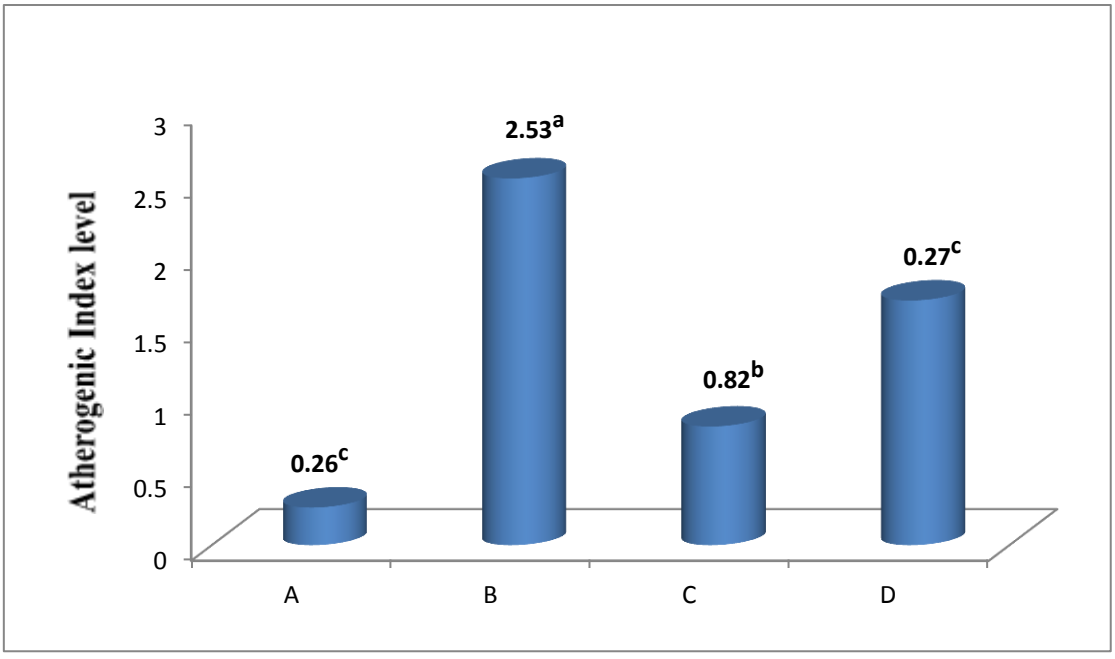

Figure (8): The Atherogenic Index levels in the all studied groups

المستخلص:-

هدفت الدراسة الحالية للبحث في تأثير زيت الخردل على وزن الجسم و مستوى دهون الدم في إناث فئران مرتفعة الدهون.

المواد وطرائق العمل: استعمل (24) من إناث فئران والتي قسمت إلى أربع مجاميع كل مجموعة تحتوي على ستة حيوانات. مجموعة السيطرة A جرعت بـــ 0.1 مل ماء مقطر لمدة 30 يوم. مجموعة عالية الدهون B تلقت عليقة مضاف اليها الكولسترول لمدة 30 يوم، مجموعة C تلقت عليقة مضاف اليها الكولسترول وتجريع 0.1 مل / 25 كيلو غرام من زيت الخردل لمدة 30 يوم إما مجموعة D تلقت عليقة اعتيادية وتجريع 0.1 مل / 25 كيلو غرام من زيت الخردل لمدة 30 يوم. النتائج : المجموعة B أظهرت ارتفاع معنوي في وزن الجسم بالمقارنة مع مجموعة السيطرة A. زيت الخردل أظهرا انخفاض معنوي في وزن الجسم مجموعتين C و D بالمقارنة مع مجموعة B. المجموعة B أظهرت ارتفاع معنوي في مستويات الكولسترول الكلي , الكليسريدات الثلاثية، البروتينات الدهنية منخفضة الكثافة و البروتينات الدهنية منخفضة الكثافة جداً بالمقارنة مع مجموعة السيطرة A. وانخفاض معنوي لمستوى البروتينات الدهنية عالية الكثافة بالمقارنة مع مجموعة السيطرة A. زيت الخردل أظهرا انخفاض معنوي في مستويات الكولسترول الكلي والبروتينات الدهنية منخفضة الكثافة في مجموعتين C و D بالمقارنة مع مجموعة B و ارتفاع معنوي لمستوى البروتينات الدهنية عالية الكثافة في مجموعتين C و D بالمقارنة مع مجموعة B.