

## Effect of Ethanol Extract of *Sesamum indicum* Seeds on Lipid Profile *in vivo*

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**Abstract:** The present study was conducted to investigate the effect of active compound (sesamin) found in defatted ethanol extract of *Sesamum indicum* seeds on the serum levels of lipid in mice. The qualitative and quantitative determination of sesamin bioactive compound in defatted ethanol extract using High Performance Liquid Chromatography (HPLC) analysis was carried out and compared with standard sesamin. It was found that the concentration of sesamin was 79.9% of ethanolic extract according to total peak area. The effect of active compound (sesamin) in defatted ethanol sesame extract was evaluated in mice blood serum (*in vivo*) after feeding them high and low fat diet for one month. Defatted Sesame extract at concentration 500 mg/kg of body weight (B.W) showed significant reduction ( $p \leq 0.05$ ) in the level of total cholesterol (62.26 , 56.14) mg/dl, triglycerides (61.54 , 61.12) mg/dl , and low density lipoprotein LDL (29.97 , 23.21) mg/dl and significant increase in the level of high density lipoprotein HDL (19.39 , 20.70) mg/dl in comparison with both high and low fat diet groups which recorded (80.43 , 68.24) mg/dl, (77.50 , 74.16) mg/dl, (16.80 , 19.32) mg/dl, and (48.09 , 34.08) mg/dL, respectively.

**Key words:** HDL, HPLC, LDL, Lipid profile, *Sesamum indicum*

### INTRODUCTION

There is considerable interest in the potential health benefits of oil seeds, such as sesamin, soy and flax seed especially regarding cardiovascular disease and cancer. this interest in oil seeds relates to their high content of polyunsaturated fatty acids ,vegetable protein ,soluble fiber , and flavonoids and related compounds ,which may possess cholesterol lowering and antioxidant activities (Jenkins *et al.*, 1999).

*Sesamum indicum* Linn. (Pedaliaceae) has long been used extensively as a traditional food in the orient for various purposes and has varieties of medicinal properties. The seed powder is useful in amenorrhea, dysmenorrhea, ulcers and bleeding piles (Visavadiya and Narasimhacharya, 2008).

Sesamin is a major lignan constituent of sesame and sesame oil, many studies have revealed that sesamin is effective in preventing hypertension, thrombogenesis (Noguchi *et al.*, 2001), and hypercholesteremia by increasing hepatic fatty acid oxidation (Ide *et al.*, 2001). Several modern drugs are being used as hypocholesteremic agents, but its cost and potential side effects have led many people to search for natural resources to reduce cholesterol levels (Singh *et al.*, 2003).

The relationship between dyslipidaemia and atherosclerosis has been well documented, the major risk factors for the development of atherosclerosis are hypercholesteremia and elevated LDL-cholesterol

concentration (Grundy, 1997). Further more, free radical mediated peroxidative modification of polyunsaturated fatty acids of LDL and VLDL is an important event in the development of atherosclerotic lesions. According to the oxidative-modification hypothesis, LDL accumulates in the extracellular sub-endothelial space of arteries and is oxidized, which is highly atherogenic and cytotoxic to vascular system (Spiteller, 2005).

Therefore, this study was aimed to assess the effect of defatted ethanol extract of sesamin seeds on lipid profile in mice blood serum.

### MATERIALS AND METHODS

This study was conducted in 2010 at Department of Biotechnology, College of Science, Baghdad University.

#### Seeds collection and preparation of crude extract:

*Sesamum indicum* seeds were collected from local market, grinded into coarse powder 100 g of grinded seeds was defatted with n-hexane (500 mL), the resulting slurry was filtered and air dried. the defatted residue extracted with 80% ethanol for 6 h (Shahidi *et al.*, 2006) .

#### Detection of sesamin using (HPLC):

HPLC analysis was done using C-18 column, 50×4.6 mm I.D column, the mobile phase used was 1% phosphate buffer(pH = 4.5): acetonitrile:water (60:40), and the flow rate was 1ml/min at 264 nm. The volume of injected extract and standard sesamin were 20 µL.

**Experimental animals:** Fourty three adult of albino female mice were purchased from the Institute of Embryo Research and Fertility/Iraq were used, their age were ranged between (8-12) weeks and weighted about (21-26) g. They were housed in plastic cages containing hard wood chips for bedding in controlled animal house at  $25\pm 2^{\circ}\text{C}$ , 4/10 h light/dark cycle. The animals were fed with a suitable quantity of water and complete diet. The animals were divided into three groups as follows:

- **Group I:** Consisted of 20 animals and were fed with high fat diet for 30 days. The diet composed of: Casein 305 g, Corn starch 150 g, Wheat flour 245 g, egg yolk 180 g, Fat 120 g, Vitamin 100 mg, mineral 30 mg and Cholesterol 3 mg (Neves *et al.*, 2006)
- **Group II:** Consisted of 20 animals and were fed with low fat diet for 30 days. The diet composed of: Casein 305 g, Corn starch 150 g, wheat flour 305 g, egg yolk 180 g, Fat 60 g, Vitamin 100 g, mineral 30 mg and Cholesterol 1.5 mg.  
Each group was divided into four subgroups, each subgroup contained five animals as follows:
  - **Subgroup 1:** Animals were fed with high or low fat diet for another 7 days only.
  - **Subgroup 2:** Animals injected intraperitoneally with *S. indicum* seed extract at a concentration 250 mg/kg of body weight for 7 doses daily for 1 week.
  - **Subgroup 3:** Animals injected intraperitoneally with *S. indicum* seed extract at concentration 500 mg/kg of body weight for 7 doses daily for 1 week.
  - **Subgroup 4:** Animals were fed special diet contained defatted *S.indicum* seed orally for 14 days. The diet was composed of: Casein 150 g, Wheat flour 447 g, corn starch 150 g, egg yolk 180 g, vitamin 10 mg, minerals 37 mg, and 346 g defatted sesame (Gorinstein *et al.*, 2003).
- **Group III:** Consisted of three animals and were fed with basal diet which composed of: casein 150 g, corn starch 150 g, wheat flour 793 g, egg yolk 180 g, vitamin 10 mg and minerals 37 mg (Gorinstein *et al.*, 2003).

When treatment period was finished, animals were weighted then obtained the blood from heart to study the level of lipid profile.

**Serum lipid profile assay:** From each animal, 0.7-1 mL of blood sample was obtained by cardiac puncture method using disposable insuline syringes (1 mL), put in eppendorff tube and left for about one hour to clot at room temperature, then separated by centrifugation at 4000 rpm for 10 min to collect serum. The separated serum was used to assay lipid profile.

Total Cholesterol (TC) and Triglyceride (T.G) were determined using commercially available kit (Human, Germany at 500nm (Schettler and Nüssel, 1975).

HDL cholesterol was assayed using liquicolor test kit after precipitation at VLDL and LDL with phosphotungstic acid and magnesium chloride (Gordon *et al.*, 1977).

LDL cholesterol concentration was calculated according to Friedewald equation as follows :

$$\text{LDL-C (mg/dl)} = \text{TC} - \text{HDL-C} - (\text{TG} / 5)$$

**Statistical analysis:** The data were analysed using completely randomized design (CRD) and a  $p > 0.05$  was established as the criterion for Least Significant Differences (LSD).

## RESULTS AND DISCUSSION

**Quantitative and qualitative analysis by HPLC technique:** HPLC analysis was accomplished to determine the most important bioactive phenolic compound (sesamin) in seeds extract using sesamin standard as a reference.

Results showed that the Retention Time (RT) of standard sesamin was 2.905 min comparing with (RT) of partially purified sesamin (2.848 min). The concentration of isolated sesamin was 79.93% of ethanolic extract according to Total Peak Area (TPA) and it was approximately close to standard peak Fig. 1.

Yasumoto *et al.*, (2003) demonstrated that retention time of sesamin was approximately 2.5 min by using 90% methanol as mobile phase in HPLC analysis, while it was approximately 4 min by using 80% methanol as mobile phase, Sesamin was separated by waters Radial-Pak 80×100 mm Cartridge C18 column, Flow rate 2 mL/min at 280 nm. The differences in retention time may due to the difference in mobile phase, on the other hand the different dielectric constant of solvents and polarity may affect the quantity of the extracted active compound.

**Effect of sesame extract on Total Cholesterol (TC):** Feeding mice with high or low fat diet increased the total cholesterol significantly (80.43, 68.24) mg/dl, respectively in comparison with control (60.64 mg/dl).

Results in Table 1 displayed that sesame extract reduced significantly ( $p \leq 0.05$ ) total cholesterol level in serum (74.66 and 62.26) mg/dl in mice fed high fat diet for both concentrations (250 and 500) mg/kg, respectively in comparison with mice fed for 1 month by high fat diet (80.43 mg/dl).

Also administration sesame extract reduced the serum cholesterol level significantly (63.14 and 56.14) mg/dl in mice fed low fat diet for both concentrations respectively in comparison with mice fed for 1 month by low fat diet (68.24 mg/dl).

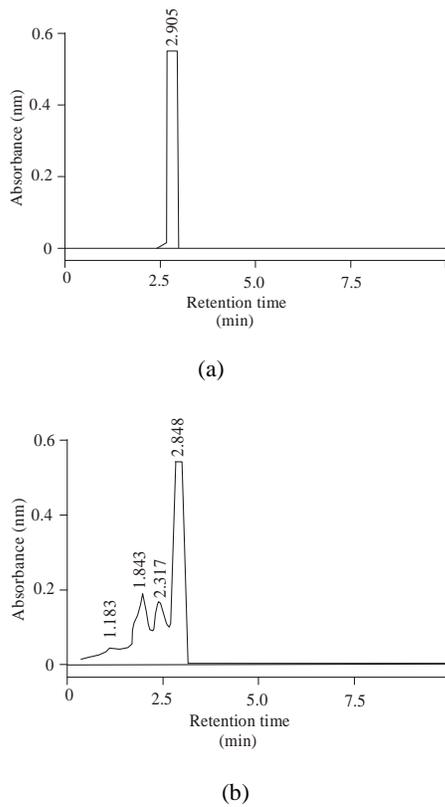


Fig. 1: Chromatographic resolution by HPLC for standard sesamin (a) and fraction components of sesamin extracted from seeds (b) in reverse phase on column C-18 , mobile phase 1% phosphate buffer (pH = 4.5): acetonitrile (60:40) at flow rate 1 mL/min, at 264 nm.

Table 1: Effect of administration sesame extract(IP) and defatted sesame diet in level of total cholesterol in mice fed for 30 day with high and low fat diet

Treatments	Animals groups	
	(Total cholesterol mg/dL)	Mean ± SE
Control	60.64±0.25 C	60.64±0.25 B
After feeding for 1 month by high and low fat diet	High fat diet (mg/dL)	Low fat diet (mg/dL)
	80.43±1.92 a	68.24±1.02 A
Feeding additionally 2 weeks by defatted Sesame	A	B
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Sesame extract concentrations (mg /kg)	250 74.66±1.41B	500 63.14±1.13B
	500 62.26±1.05C	56.14±1.36C

\*(p≤0.05); Different letters refer to a significant differences between treatments

Feeding mice with defatted sesame diet for 2 weeks showed no significant differences in the level of serum cholesterol (81.52 mg/dl) compared with mice fed high fat diet (80.43 mg/dl), while defatted sesame diet treatment

showed a significant reduction in serum cholesterol level (63.91 mg/dl) compared with mice fed low fat diet (68.24 mg/dl).

The hypocholesterolemic effect of sesamin, refers to component lignan in sesame seeds, which inhibits the absorption of cholesterol in the intestinal tract, increase the excretion of cholesterol in bile, and decrease the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase in rats (Hirose *et al.*, 1991).

Results obtained were in agreement with (Koh, 1987) who found that sesame oil was moderately but significantly more hypocholesterolemic than corn oil in serum of rat at dietary level of 15%.

The lipid lowering effects of sesame seed in hypercholestaemic rats is related to an increased excretion of cholesterol, neutral sterol, bile acid and an increase in hepatic bile acid content (Visavadiya and Narasimhacharya, 2008).

**Effect of sesame extract on triglyceride (TG):** After feeding mice with high and low fat diet, the serum triglyceride significantly increased to (77.5 and 74.16) mg/dl, respectively in comparison with control (68.00 mg/dl)(Table 2).

Treated animals with 250 and 500 mg/kg sesame extract recorded a significant decrease in triglyceride level depending on sesame extract concentration for both types of feeding high and low fat diet (68.63, 67.08) mg/dl, While there were no significant effects in serum triglyceride of mice fed additionally with defatted diet in comparison with previously feeding high and low fat. In contrast this diet showed a significant increase in triglyceride level compared with control. The present results showed that both sesame extract concentrations lowered triglyceride to the normal level.

The hypotriglyceridemic effect of partially purified sesamin on reducing triglyceride level might be due to

Table 2: Effect of administration sesame extract (IP) and defatted sesame diet in level of triglyceride in mice fed for 30 day with high and low fat diet

Treatments	Animals groups	
	(Triglyceride mg/dL)	Mean±SE
Control	68.00±1.09 B	68.00±1.09 B
After feeding for 1 month by high and low fat diet	High fat diet (mg/dL)	Low fat diet (mg/dL)
	77.50±1.08 A	74.16±1.04 A
Feeding additionally 2 weeks by defatted Sesame	A	A
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Sesame extract concentrations (mg /kg)	250 68.36±0.86 B	500 67.08±1.28 B
	500 61.54±1.00	61.12±0.86
	C	C

\*(p≤0.05); Different letters refer to a significant differences between treatments

Table 3: Effect of administration sesame extract (IP) and defatted sesame diet in level of HDL -cholesterol in mice fed for 30 day with high and low fat diet

Treatments	Animals groups	
	(HDL-C mg/dL)	Mean±SE
Control	21.66±0.79 A	21.66±0.79 a
After feeding for 1 month by high and low fat diet	High fat diet (mg/dL)	Low fat diet (mg/dL)
	16.80±0.73 C	19.32±0.52 B
Feeding additionally 2 weeks by defatted sesame	22.20±0.79 A	15.10±0.47 C
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Sesame extract concentrations (mg /kg)	250	18.00±0.87 B
	500	19.39±0.53 B
		21.36±0.49 A
		20.70±0.36 Ab

\*: (p<0.05); ns: non-significant; Different letters refer to a significant differences between treatments

inhibition of hepatic lipogenesis (Venkatesan *et al.*, 2003), and enhancing metabolism of exogenous-free fatty acid to oxidation by promote ketogenesis at the expense of esterification into TG in rat liver (Jeng and Hou, 2005).

**Effect of sesame extract on high density lipoprotein (HDL-C):** The normal level of HDL-C (21.66 mg/dl) in control group decreased significantly after feeding mice with high and low fat diet for 1 month reached to 16.8 and 19.32 mg/dl, respectively.

Treating mice fed with high fat diet with sesame extract at concentrations 250 and 500 mg/kg showed significant increase in HDL-C level (18.00 and 19.39 mg/d), respectively in comparison with mice fed for 1 month by high fat diet, while in mice fed with low fat diet, sesame extract showed a significant increase in HDL level (21.36 mg/dl) for 250 mg/kg but no significant differences in HDL level (20.70 mg/dl) were found for 500 mg/kg in comparison with mice fed for 1 month by low fat diet (19.32 mg/dl) and control (21.66 mg/dl).

Animal group which fed additionally with defatted sesame diet showed significant increase in the level of HDL-C (22.20 mg/dl) in comparison with animals fed with high fat diet for 1 month (16.80 mg/dl), but no significant differences appeared comparing with control (21.66 mg/dl), while mice fed with low fat diet, treatment with defatted sesame diet showed significant reduce in the level of HDL(15.10 mg/dl) compared with control (21.66 mg/dl) and mice fed with low fat diet for 1 month (19.32 mg/dl). (Table 3)

The present in vivo results demonstrate that the sesame seeds defatted extract has the potential for increasing HDL-C concentration (Daniel *et al.*, 2003). Administration of sesame seed powder orally raised HDL-C levels in hypercholesteraemic animals, while dietary fibers are not known to elevate HDL-C levels (Romero *et al.*, 2002).

Table 4: Effect of administration sesame extract (IP) and defatted sesame diet in level of LDL -cholesterol in mice fed for 30 day with high and low fat diet

Treatments	Animals groups	
	(LDL-C mg/dL)	Mean±SE
Control	25.38±0.82 D	25.38±0.82 Bc
After feeding for 1 month by high and low fat diet	High fat diet (mg/dL)	Low fat diet (mg/dL)
	48.09±2.34 A	34.08±1.24 A
Feeding additionally 2 weeks by defatted sesame	43.54±1.10 B	34.39±1.65 A
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Sesame extract concentrations (mg /kg)	250	42.97±1.90 B
	500	29.97±0.89 C
		28.36±1.25B
		23.21±1.74 C

\*: (p<0.05); Different letters refer to a significant differences between treatments

**Effect of sesame extract on low density lipoprotein (LDL- C):** Treating mice with concentrations 250 and 500 mg/kg of sesame extract after consumption high fat diet caused a significant decrease in the level of LDL (42.97 and 29.97) mg/dl, respectively in comparison with mice fed for 1 month by high fat diet (48.09 mg/dl) and significantly increased as compared with control (25.38 mg/dl).

While the mice fed with low fat diet, showed a significant reduce for both concentrations of sesame extract (250,500) mg/kg in LDL-C level (28.36 and 23.21) mg/dl in comparison with mice fed for 1 month by low fat diet (34.08 mg/dl) and no significant differences observed in comparison to control (25.38mg/dl).(Table. 4).

On the other hand, feeding animals with defatted sesame diet after feeding with high fat diet for one month (48.09mg/dl) caused a significant reduction in the level of LDL (43.54 mg/dl) and a significant increase compared with control(25.38 mg/dl),while no significant differences recorded with defatted sesame diet treatment in mice fed for 1 month by low fat diet (34.08 mg/dl) but significantly increased in comparison to control (25.38 mg/dl).

A significant decline in plasma LDL-Cholesterol occur due to the fiber content of sesame which reported to lower plasma LDL-Cholesterol by interrupting the cholesterol and bile acid absorption and increasing LDL receptor activity, the LDL-Cholesterol is then subsequently catabolized to bile acid (Venkatesan *et al.*, 2003; Romero *et al.*, 2002; Everson *et al.*, 1992). a twenty one cases of hypercholesterolemic individuals consumed 40 g of roasted sesame seeds for 4 weeks, significantly decreased the levels of serum TC (6.4%) and LDL-C (9.5%). However, the effect of sesame seeds on cholesterol disappeared when patients stopped the consumption of sesame diet (Chen *et al.*, 2005).

## CONCLUSION

Defatted Sesame seeds extract showed reducing ability in the level of total cholesterol, Triglyceride, HDL, and LDL in comparison with control at concentration 500 mg/kg of extract in mice blood serum. Further study on the efficacy of purified sesamin will be conducted to get better understanding of the mechanism of reducing cholesterol levels .

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