Effect of Sesame Oil, *Nigella sativa* L Oil and their Mixtures on Lipid Profile and Liver Enzymes in Hypercholesterolemic Rats

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Abstract: Objective: The present study aimed to investigate the effect of sesame oil and *Nigella sativa* L oil and their mixture in a dose of (5 mg/kg b.wt.) on lipid profile and liver enzymes in hypercholesterolemic rats for 6 weeks on body weight gain %, feed efficiency ratio,serum levels of total lipid, total cholesterol (TC), triglycerides (TG), lipoprotein fractions and liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed. Histopathological examination of liver andheart were also carried out. **Methods:** Thirty five male Wistar rats were distributed into five equal groups as follows: negative (normal rats), positive (hypercholesterolemic rats) control groups and positive groups orally given sesame oil and *Nigella sativa* L oil and their mixturein a dose of 5 mg/kg b.wt., respectively. **Results:** The results showed that oral administration of sesame oil and *Nigella sativa* L oil and their mixture in a dose of 5 mg/kg b.wt. to hypercholesterolemic rats for 6 weeks significantly decreased serum levels of TL,TC, TG, low density lipoproteins cholesterol (LDL-c), very low density lipoproteins cholesterol (VLDL-c) and liver enzymes when compared to the control positive group. Level of high density lipoprotein cholesterol (HDL-c) was significantly increased as compared to the control positive group. Histopathological examination of liver and heart of sesame oil and *Nigella sativa* L oil and their mixture in a dose of 5 mg/kg b.wt. showed amelioration of histological changes caused by high level of cholesterol in the positive control group. **Conclusion:** Results indicated that sesame oil and *Nigella sativa*L oil and their mixture in a dose of 5 mg/kg b.wt., have potent antiatherogenic and antioxidant effects in hypercholesterolemic rats. This study recommends that consuming sesame oil and *Nigella sativa*L oil and their mixture in a dose of 5 mg/kg b.wt. may be beneficial for patients who suffer from hypercholesterolemia and/orarteriosclerosis.

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Keywords: sesame oil; *Nigella sativa* L oil; lipid; liver; enzyme; hypercholesterolemic rat

1. Introduction

Hypercholesterolemia in combination with raised LDL cholesterol concentration represent major risk factors for the development and progression of atherosclerosis and consequently of cardiovascular disease. Based on this evidence, scientific research is targeting the discovery of new drugs with hypocholesterolemic effects. Plant based dietary therapies and natural food components are being proposed nowadays for the prevention of dyslipidemia. (**Laskarina *et al*., 2010**). Sesame oil has been evaluated as one of the familiar health foods of ancient time. However, compared with other vegetable oils, sesame oil contains a relatively high percentage of unsaponifiable matter (1%-3%) which includes sterols, sterol esters, mainly α-tocopherol and unique compounds called sesame lignans **(Frank, 2002)**. The two major oil-soluble lignans, sesam in and sesamolin are considered responsible for the unique properties of sesame seed oil. Sesamin is known to reduce the absorption and biosynthesis of cholesterol in rats and plasma cholesterol in humans, sesamin also elevates α-tocopherol levels in humans. **(Ali and Afaf, 2006),** The seed of *Nigella sativa* L (NS), an annual Ranunculaceae herbaceous plant, has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of asthma. NS contains more than 30 of a fixed oil and 0.40-0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4-24% thymoquinone and 46% many monoterpenes such as p-cymene, and α-pinene **(El Tahir *etal*., 1993).** Recently conducted clinical and experimental researches have shown many therapeutic effects of NS extracts such as immunomodulator, anti-inflammatory and anti-tumour, antibacteria agents **(Alam *et al*., 2010; Rogozhin *et al*., 2011).** Therefore, the present study was designed to investigate the effect of sesame oil and *Nigella sativa* oil on hypercholesterolemic rats.

2. Materials and Methods

Chemicals and kits

Cholesterol was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Kits for biochemical analysis of serum total lipids, triglycerides, total cholesterol, HDL-C, Serum aspartate aminotransferase, alanine aminotransferase

andalkaline phosphatase were purchased from Sigma-Aldrich Company.

Rats

Forty adult male Sprague Dawley rats weighing 180-190 g body weight and 10-11 weeks old were used in this study. Animals were obtained from Faculty of Pharmacy, King Abdul-Aziz University, Jeddah, Saudi Arabia.Rats were housed in a well ventilated laboratory room under standard conditions of 24 °C temperature, 50-52% relative humidity and 12 hr light/12 hr dark cycles. Experiment was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee (IAEC).

Sesame oil and *Nigella sativa* oil:

Sesame oil and *Nigella sativa*oil were purchased from a local market, Jeddah, Saudi Arabia.

Preparation of basal and cholesterol containing-diets:

The basal diet (AIN-93M) was prepared according to **Reeves *et al.* (1993)**. Diet was formulated to meet the recommended nutrients levels for rats.

Cholesterol containing diet was prepared by formulated basal diet with 1% cholesterol and 0.25% bile salts to induced hypercholesteremic in rats as described by **(Cara *et al.*, 1991).**

Experimental design:

Fourty rats weighing 195±3 were housed in healthy condition at temperature rooms (21-23^{o}C), with 40-60% humidity, exposed to a 12:12-hlight-dark cycle and fed on the basal diet and water was provided ad libitum for one week before starting the experimental for acclimatization. After acclimatization period rats were divided into five groups of eight rats each as follows:

Group (1): Served as a control negative group (normal rats) and fed on cholesterol free-diet for 6weeks.

Group (2): Kept as a control positive group and fed on cholesterol containing-diet for 6 weeks.

Group (3): Fed on cholesterol containing-diet and orally given sesame oil in a dose of 5 ml/kg b.wt.

Group (4): Fed on cholesterol containing-diet and orally given *Nigella sativa* oil in a dose of 5 ml/kg b.wt.

Group (5): Fed on cholesterol containing-diet and orally given Sesame oil and *Nigella sativa* oil in a dose of 5 ml/kg b.wt.

At the end of the experimental period (6 weeks), diets were withheld from experimental rats for 12-h and then rats were sacrificed. Blood samples were collected from the portal vein into dry clean centrifuge tubes for serum separation. Serum samples were frozen at -10^{o}C until chemical analysis. Heart and liver of sacrificed rats were kept in 10% formalin solution till processed for histopathological examination.

Determination of feed 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 using the following formulas:

BWG = Final body weight - Initial body weight

FER = BWG (g)/Food consumed (g)

Lipid profile and lipoprotein cholesterol assay:

Total Lipid (TL) concentrations were determined colorimetricusing spectrophotometer apparatus adjust at 520 nm as described by kit instructions (Randox Co., Ireland). Triglycerides (TG), Total Cholesterol (TC) and High Density Lipoprotein Cholesterol (HDL-C) concentrations were determined using enzymatic methods as described in the instructions provided with the kits (Analyticon® Biotechnologies AG, Germany). The absorbance of the testes samples were read using spectrophotometer adjusted at 546 nm for TG, TC and500 nm for HDL-C. Low Density Lipoprotein Cholesterol (LDL-C) concentration was calculated by using formula of **Friedwald *et al.* (1972)** and Very Low Density Lipoprotein Cholesterol (VLDL-C) was calculated using the following equation:

LDL-Cholesterol = Total cholesterol - (HDL-C + TG/5)

Liver functions assay:

Serum aspartateaminotransferase (AST) and alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) activities were determined using colorimetric methods as described in the kits instruction (Sigma-Aldrich Chemical Company). The absorption of the test samples were read at 505nmfor GOT and GPT and at 510 nm for ALP.

Histopathological examination:

Heart and liver of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 micronsthickness and stained with Heamtoxylin and Eosin stainfor examination of the liver as described by **Carleton (1979).**

Statistical analysis:

Results were expressed as a (mean ±SD). Data were analyzed statistically by analysis of variance, for statistical significance using L.S.D. test, one way ANOVA, post hoc multiple comparisons according to **Snedecor and Cochron (1989).** SPSS version 20 was used for these calculations.

3. Results

The effect of cholesterol-containing diet and the effect of oral administration of Sesame oil, Nigella sativa oil and their mixture on Feed Intake (FI), Body, Feed Efficiency Ratio (FER) and Weight Gain (BWG) were recorded in Table 1.

Feed intake, feed efficiency ratio and Body weight gain% were significantly (P< 0.05) increased in hypercholesterolemic rats (positive control group), compared to normal rats. Oral administration of Sesame oil, Nigella *sativa* oil and their mixture had significant (P<0.05) decreases in Feed intake, feed efficiency ratio and Body weight gain% when compared with the positive control group (model group).

Table 4.1 Effect of oral administration of Sesame oil, Nigella *sativa* oil and their mixture on feed intake, feed efficiency ratio and Body weight gain% in hypercholesterolemicrats.

Paramete / Groups	Mean of daily feed intake(g/d)	Feed efficiency ratio (FER)	Body weight gain (%)
Negative control	**23.43b**	**0.061 ± 0.005c**	**14.35 ± 1.22 c**
Positive control	**29.23 a**	**0.083 ± 0.001 a**	**29.16 ± 1.30 a**
Sesame oil **5 ml/kg**	**22.44b**	**0.076 ± 0.003b**	**23.82 ± 1.58 b**
Nigella sativa oil **5 ml/kg**	**21.59b**	**0.072± 0.001 b, c**	**22.91 ± 2.95 b**
Mixture of Sesame oil **and***Nigella sativa*oil **5 ml/kg**	**20.89b**	**0.072 ± 0.004 b, c**	**15.86 ± 1.33 c**

It is clear from **Table 4.2** that hypercholesterolemic rats had significant increases in total lipid (TL), triglycerides (TG) and total cholesterol (TC) compared to control negative group by 24.28, 47.57 and 24.34% respectively. Oral administration of Sesame oil, *Nigella sativa* oil and their mixture had significant decreases (p<0.05) in serum concentrations of TL by 12.3, 13.28, 18.3 and TG by 20.33, 21.24, 31.02 as well as TC by 12.15,14.82 and 18.71% compared to the positive controlrats.

Table 4.2 Effect of oral administration of Sesame oil, *Nigella sativa* oil and their mixture on total lipid, triglycerides and total cholesterol in hypercholesterolemicrats.

Paramete / Groups	Total lipid (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)
Negative control	320.56 ± 11.47 c	94.56 ± 5.42 c	74.98 ± 1.56b
Positive control	398.39 ± 12.62 a	139.54 ± 6.54 a	93.95 ± 3.95a
Sesame oil **5 ml/kg**	349.37 ± 12.84 b	111.17 ± 7.67 b	81.32± 4.43 b
Nigella sativa oil **5 ml/kg**	345.48 ± 14.24 b	109.9 ± 6.75 b	79.19 ± 5.45b
Mixture of Sesame oil **and** *Nigella sativa* oil **5 ml/kg**	325.46 ± 12.59 c	96.25 ± 6.55 c	76.83 ± 4.34 b

Results in **Table 4.3** revealed that positive control group had significant increase in the serum level of LDL-C as compared to the negative control group by 53.98%. Oraladministration of Sesame oil, *Nigella sativa* oil and their mixture caused significant reduction in the serum level of LDL-C at p<0.05 by 24.63, 27.26 and 33.14 respectively as compared to the positive control group.

Data also showed that rats fed cholesterol-diet had significant decrease in the serum level of HDL-C at p<0.05 by 31.54% as compared to negative control group. Oral administration of Sesame oil, Nigella *sativa* oil and their mixture caused significant increase in the serum level of HDL-C at p<0.05 by 26.46,27.22 and 41.41% respectively as compared to the positive control group.

With regard to the serum level of VLDL-C, results revealed that positive control group had significant increase in the serum level of VLDL-C at p<0.05 by 98.3% as compared to the negative control group. Groups orally given Sesame oil, *Nigella sativa* oil and their mixture had significant decrease in the serum level of VLDL-C at p<0.05 by21.55,26.53 and 38.05% respectively, compared to the positive control group.

Results in Table **4.4** revealed that positive control group had significant increases in serum levels of AST, ALT and ALP at p<0.05 by70.1, 68.2 and 36.58% respectively ascompared to the normal control group. Whereas, Oral administration of Sesame oil, Nigella *sativa* oil and their mixture had significant decreases in serum levels of AST, ALT and ALP at p<0.05 as compared to positive control group.

Table 4.3 Effect of oral administration of Sesame oil, Nigella *sativa* oil and their mixture on serum levels of lipoprotein fractions (HDL-c, LDL-c and VLDL-c)

Paramete / Groups	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
Negative control	28.82 ± 1.8 a	39.07 ± 1.6 c	7.09± 1.9 c
Positive control	19.73 ± 1.1 b	60.16 ± 1.4 a	14.06 ± 3.7 a
Sesame oil **5 ml/kg**	24.95 ± 1.3 a	45.34 ± 1.8 b	11.03± 1.5 b
Nigella sativa oil **5 ml/kg**	25.10 ± 2.1 a	43.76 ± 1.2 b	10.33 ± 2.8 b
Mixture of Sesame oil **and** *Nigella sativa* oil **5 ml/kg**	27.90 ± 2.4 a	40.22 ± 1.6 c	8.71 ± 1.2 c

Table 4.4 Effect of oral administration of Sesame oil, Nigella *sativa* oil and their mixture on serum levels of liver enzymes (AST, ALT and ALP) in hypercholesterolemicrats.

Paramete / Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative control	65.6 ± 1.8 c	37.21 ± 1.6 c	86.57 ± 1.9 c
Positive control	111.6 ± 2.1 a	62.59 ± 2.4 a	118.24 ± 1.2 a
Sesame oil **5 ml/kg**	88.8 ± 2.1 b	51.65 ± 2.8 b	96.53 ± 2.8 b
*Nigella sativa*oil **5 ml/kg**	80.6 ± 2.3 b	45.25 ± 2.6 b	95.27 ± 2.5 b
Mixture of Sesame oil **and** *Nigella sativa* oil **5 ml/kg**	71.3 ± 2.4 c	40.73 ± 2.2 a	87.45 ± 2.2 c

4. Histopathological study:

Examination of liver of normal rats fed on basal diet showed normal histological structure of hepatic lobule (Fig.1). Livers of rats fed on high cholesterol diet revealed congestion of hepatic central vein, granularity of cytoplasm of the hepatocytes and pyknosis of hepatic nuclei (Fig.2). Oral administrations of Sesame oil (5 mg/kg b.wt.) or *Nigella sativa* (5 mg/kg b.wt.) for 4 weeks to hypercholesterolemic rats showed slight congestion of central vein and hepatic sinusoids. Mixture of Sesame oil and Nigella *sativa* oil 5 ml/kg b.wt. when given to hypercholesterolemic rats alleviated the histopathological changes which seen in the liver of hypercholesterolemic rats (Fig.4). The histological examination of the heart tissue of normal healthy rats showed normal histological architecture manifested by normal cardiac vessels wall thickness, normal size and appearance of cardiac muscles and blood capillaries as illustrated in Figure 5. In rats fed on high - cholesterol diet, the examination of the heart revealed some degenerative changes with inflammatory cell infiltration and marked congestion of blood capillaries as demonstrated in Figure 6.Treatment with Sesame oil (5 mg/kg b.wt.) or *Nigella sativa* (5 mg/kg b.wt.) showed a moderate improvement except cardiac vessels still had focalthickening and some cardiac muscles looked dark Figure 7. Oral administration of Mixture of Sesame oil and Nigella *sativa* oil 5 ml/kg b.wt. revealed a marked improvement in histological architecture of the heart tissue except presence of few apoptotic dark cells in the cardiac muscle as shown in Figure 8.

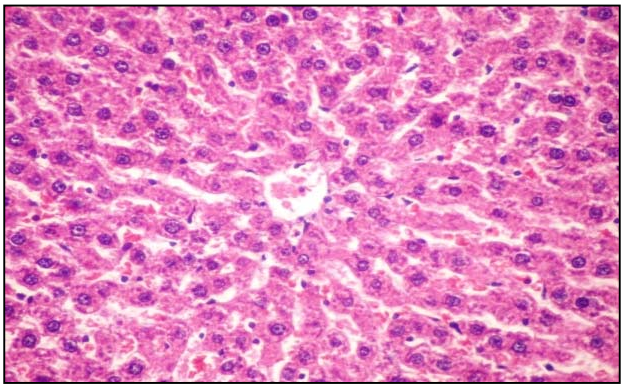

Fig (1) Liver of a control negative rat showing normal histological structure of hepatic cells.

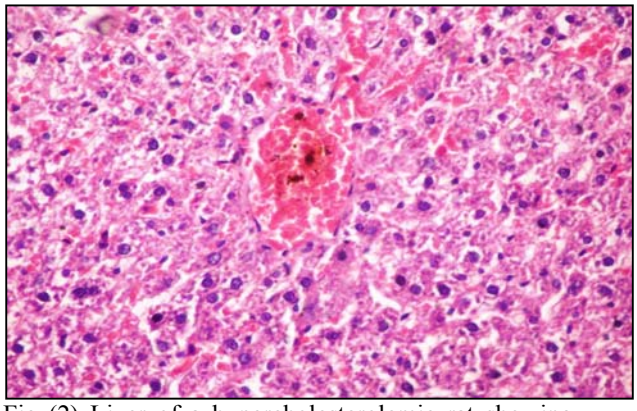

Fig (2) Liver of a hypercholesterolemic rat showing congestion of hepatic central vein (Arrow), granularity of cytoplasm of the hepatocytes (Arrow) and pyknosisof hepatic nuclei.

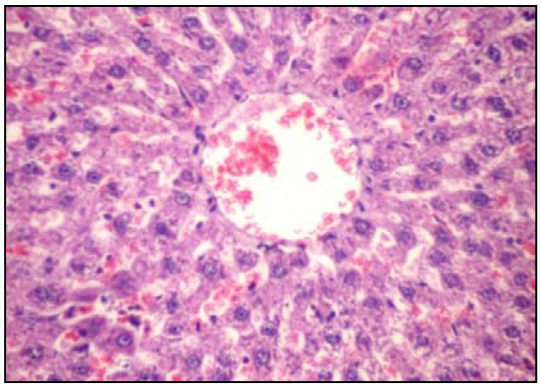

Fig (3) Liver of a hypercholesterolemic rat given Sesame oil (5 mg/kg b.wt.) or *Nigella sativa* oil (5 mg/kg b.wt.) for 4 weeks showed slight congestion of central vein and hepatic sinusoids.

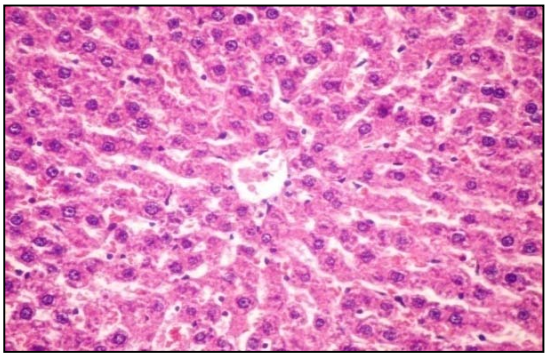

Fig (4) Liver of a hypercholesterolemic given Mixture of Sesame oil and *Nigella sativa* oil 5 ml/kg b.wt. for 4 weeks showing slight normal histological structure of hepatic lobules

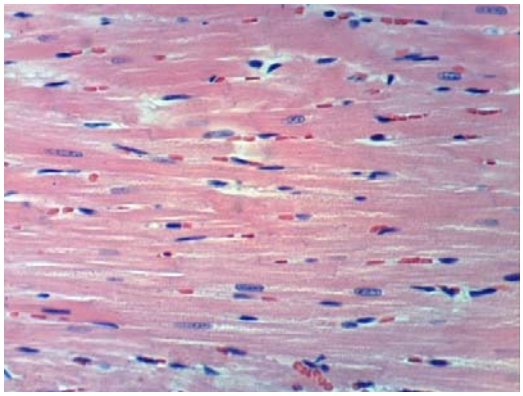

Fig. (5): Section from rat heart of normal rats (negative control) showing cardiac vessels with normal wall thickness.

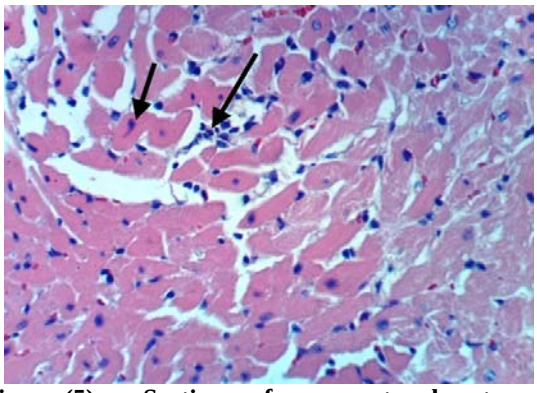

Fig. (5): Section from rat heart of hypercholesterolemic rat (positive control) showing degenerative changes of some cardiac muscles with inflammatory cell infiltrates (black arrows).

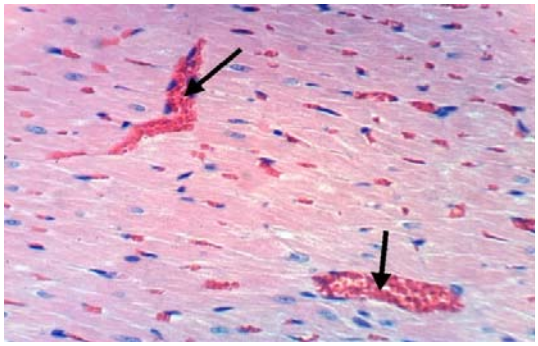

Fig (7) Heart of a hypercholesterolemic rat given Sesame oil (5 mg/kg b.wt.) or *Nigella sativa* oil (5 mg/kg b.wt.) for 4 weeks showing relative improvement Cardiac vessel still showed focal thickening.

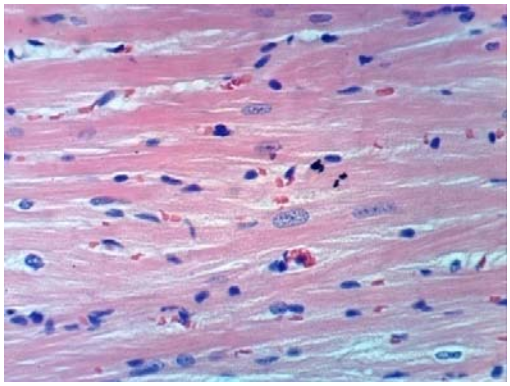

Fig (8) Heart of a hypercholesterolemic given Mixture of Sesame oil and Nigella *sativa* oil 5 ml/kg b.wt. for 4 weeks showing partial improvement of cardiac valve changes with some residual thickening and blood stasis.

4. Discussion

The present study aimed to investigate the effectiveness of oral administration of Sesame oil, *Nigella sativa* oil and their mixture on hypercholesterolemic rats The present data revealed that change in feed intake, feed efficiency ratio and body weight gain were significantly ($P< 0.05$) increased in the hypercholesterolemic rats(positive control group), compared to normal rats. These findings were in agreement with those obtained by **Nwozo *et al.* (2011)** who confirmed our results. The increase in body weight of hypercholesterolemic rats might be due to the increase of feed and caloric intake by rats. However, the change in body weight gain in treatment groups might beattributed to lower feed intake. Results of the present study revealed that feeding of rats on high - cholesterol diet resulted in significant increases in serum levels of TL, TG, TC, LDL-c and VLDL-c accompanied with a significant decrease in HDL-c level as compared to the negative control group. The increases in serum concentrations of the above mentioned parameters and the reduction in serum HDL-c as a result of feeding high - cholesterol diet have been pointed out as risk factors for the development of atherosclerosis and related cardiovascular diseases. These results were confirmed by histopathological examination of heart which showed degenerative changes of some cardiac muscles with inflammatory cell infiltration associated with a marked congestion of blood capillaries, compared to the negative control group. The present findings were in the same line as with those reported by **Frantz *et al.* (2012)** who demonstrated that lipid metabolism in rats fed high fat - diet (HFD) presented disorders and levels of serum TC and TG increased significantly, compared with the negative control group.

Regarding to serum LDL-c and HDL-c levels in rats fed with high cholesterol diet, the current results were in agreement with **Kumar *et al.* (2010)**. The previous authors concluded that oxidation of LDL-c resulted in formation of a wide range of biologically active products, including peroxides and malondialdehyde **Tebib*et al.* (1994)** found that activity of the lipoprotein lipase enzyme augmented in hypercholesterolemic rats. Lipase transforms VLDL-c into LDL-c that would lead to an increase in serum concentration of LDL-c.

Sesame seeds contain two unique substances, sesamin and sesamolin, during refinement the two phenolic antioxidants, sesamol and sesaminol are formed. Both of these substances belong to lignans and have been shown to possess cholesterollowering effect in humans (**Ogawa *et al.*, 1995).** Presumably, the oil contains chemical agents which help in maintaining the blood cholesterol at low level. The oils might be having antilipolytic effects in the body and prevent LDL-C from being oxidized (**Penalvo *et al.*, 2006)**. In this study, the observed cholesterol-lowering effects of combinations of sesame oil and Nigella sativa oil administered to hypercholesterolemic rats could be related to an increased excretion of cholesterol, neutral sterols and bile acid.

In our study, the significant reduction of TC and LDL levels and enhancement of HDL levels due to *N. sativa* oil, in agreement with the previous studies reported by **El-Dakhakhani *et al.*, (2000**) found that feeding rats with N. sativa oil (800 g kg−1 day−1) orally for 4 weeks caused significant decreases in the serum LDL and TG levels, and an elevation of serum HDL levels. Recently, it was reported that the petroleum ether extract of *N. sativa* significantly reduced plasma TG and increased HDL cholesterol **(Le *et al.*, 2004).** The volatile oil of *N. sa*tiva was observed to be as efficient as the cholesterol-reducing drug ST **(Settaf *et al.*, 2000).** Furthermore, a study in hypercholesterolemic rats showed that feeding rats with N. sativa oil decreased serum TC, TG and LDL levels **(Zaoui*et al.*, 2002).** On the other hand, previous results reported by **Al-Naqeeb *et al.*, (2009**) showed that *N. sativa* seeds oil is rich in vitamin E and total antioxidant activity, which may explain the significant reduction in plasma TC, LDL levels. As shown by **Jorge *et al.*, (1998)** (vitamin E administered to hypercholesterolemic rabbits significantly reduced the plasma LDL and vessel wall oxidation after 2 and 4 days of treatment, respectively, which was associated with a decrease in vessel and plasma TC levels and an improvement in endothelial cell functioning after 6 days.It was also found that oil extracted from N. sativa seeds is rich in unsaturated fatty acids, which could be responsible for the decrease of TC and LDL cholesterol levels. Yet these combinations significantly reduced the lipid profiles and improved the body antioxidant capacity of hypercholesterolemic rats.

On the other hand, the elevation in liver enzymes may be attributed to their release from the cytoplasm into the blood circulation after rupture of the plasma membrane **(Lu *et al.*, 2007).** In this concern, studies of Prasad (2010) and Saki e*t al*. (2011) showed that a high cholesterol diet moderately elevated serum levels of ALT, AST and ALP in rats. The discrepancy in the serum levels of these enzymes could be attributed to the levels and duration of hypercholesterolemia **(Lu *et al.*, 2007)**. Our results revealed that feeding rats on cholesterol-enriched diet produced liver injury as indicated by marked elevation in serum levels of AST and ALT enzymes associated with markedly histopathological changes. These changes consisted of diffuse vacuolar degeneration; fat vacuoles and necrosis of hepatocytes and markedly focal fibrosis

Results of the present study showed that there were significant decreases in serum levels of AST, ALT and ALP enzymes in hypercholesterolemic rats orally given N. sativa oil in a dose of 5 mg/kg b.wt., compared to the positive control group. The present results agreed with the results obtained by **Abdal-Wahhab *et al* (2005)** whoreported that N. sativa oil decrease oxidative stress and thus preventing liver damage. The present study showed that there were significant decreases in serum levels of AST, ALT and ALP enzymes in hypercholesterolemic rats orally given Sesame oil in a dose of 5 mg/kg b.wt., compared to the positive control group. These results may be due to its antioxidant effect which found to protect against oxidative stress and hepatic injury **(Chavali *et al*., 2001).** The best results in our study for liver enzymes were found in mixture group this may be due to the power antioxidant activity for both N. sativa oil and Sesame oil. The biochemical results of our study were confirmed by histopathological findings, which seen in liver sections. The histological findings of liver of the treated rats showed almost completely normal structure with regular arrangement of hepatocyte cell cords and exhibited reduction in fat accumulation.

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