

**Effect Of Black Seeds (Nigella Sativa L. ) Oil On Low Density Lipoprotein In Albino Rats**  
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**Abstract**

In this study was to investigate the effect of black seed oil on the blood level of low density lipoprotein ( LDL ) in albino male rats .

This study was done for 60 days on 40 mature albino male rats , divided into two groups ( control and treated ) . Animals of both groups were fed on common rats diet for 60 days , 2% of cholesterol was added on fed of treated group.

At a day 30 of experiment , the animals were given daily oral dose of 500mg/kg body weight of oil extract of Black seeds by oral lavage.

The statistical analysis in treated group showed a significant increase (  $P < 0.05$  ) in LDL – cholesterol at days 15 and 30 and a significant decrease at 45 and 60 days of the experiment when compared with control group .

**Key word** : Black seed , Rats , low density lipoprotein

تأثير زيت الحبة السوداء على البروتينات الدهنية واطئة الكثافة في الارانب المقهاء

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**الخلاصة**

في هذه الدراسة هو لمعرفة تأثير زيت الحبة السوداء في البروتينات الدهنية واطئة الكثافة في ذكور الجرذان المقهاء . كانت فترة التجربة ستون يوما" استخدمت فيها اربعون جرذان قسمت الى مجموعتين ( مجموعة السيطرة ومجموعة العلاج ) ، غذيت الحيوانات في كلا المجموعتين على الغذاء الاعتيادي للجرذان لمدة ستون يوما" ، وتم اضافة

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2% كوليسترول للعلف لمجموعة العلاج ، وفي اليوم 30 من التجربة ولغاية نهاية التجربة اعطي لمجموعة العلاج عن طريق الفم جرعة 500 ملغم / كغم من وزن الحيوان المستخلص الزيتي للحبة السوداء التحليل الاحصائي في مجموعة العلاج اوضح ارتفاع معنوي بمستوى  $P < 0.05$  في قيم البروتين الدهني واطء الكثافة في اليوم 15 و 30 من التجربة ، وانخفاض معنوي في الايام 45 و 60 من التجربة مقارنة مع مجموعة السيطرة .  
الكلمات المفتاحية : الحبة السوداء ، الجرذان ، البروتينات الدهنية واطئة الكثافة

### Introduction

Hypercholesterolemia is the term used to describe high level of cholesterol in blood , its characterized by elevated level of LDL – cholesterol , normal or low level of HDL - cholesterol and normal or elevated of triglycerides <sup>[1,2]</sup> .

lipids mainly cholesterol , cholesterol esters and triglycerides enter the tunica intima probably from blood across a damaged endothelium . Much of the lipid is phagocytosed by foam cells probably macrophages and myointimal cells but some eventually become free and more lipid accumulates when bloated foam cells undergo cell death <sup>[3]</sup> .

Atherosclerosis is a chronic disease of large and medium size arteries with hardening and loss of elasticity of arterial walls which lead to narrowing of arterial lumen , the lesion starts initially as fatty streaks development into fatty plaques in smooth muscle cells migrate to the tunica intima and proliferate as foam cells arise from monocytes derived macrophages in the tunica intima and then necrosis with deposition of extracellular cell lipid and cholesterol crystals <sup>[4,5]</sup> .

The *nigella sativa* seed is known by various names, including <sup>[6]</sup>:

- Black cumin
- Black caraway
- Black onion seed
- Black seed
- Fennel flower
- Roman coriander
- Kalonji

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The seed contain a fatty oil rich with unsaturated fatty acids a smaller amounts of saturated fatty acids also contain nine essential amino acids which required for a healthy diet and a active compound called thymoquinone, by which it acquires an aromatic flavor. However, different brands contain varying chemical compositions , also the health enhancing potential of black cumin has been attributed to the active ingredients that are mainly concentrated in fixed and essential oil <sup>[6]</sup> .

Black cumin seed fixed oil contains appreciable quantities of unsaturated especially polyunsaturated fatty acids; constitute the bulk of oil ranging from 48-70%, while monounsaturated (18-29%) and saturated fatty acids (12-25%) are in lesser proportions <sup>[7, 8]</sup>. Besides better fatty acid profile, it also contains considerable quantities of tocopherols and allied bioactive compounds that are important in attenuating the overall antioxidant capabilities of the body , moreover, presence of phytosterols further strengthens its hypoglycemic and hypercholesterolemic perspectives <sup>[9, 10]</sup>. Likewise, pharmacological investigations explored the effectiveness of essential oil and its active ingredient i.e., thymoquinone against various maladies like oxidative stress, cancer, immune dysfunction and diabetic complications <sup>[11,12]</sup>.

### **Materials and Methods**

Healthy male albino rats were obtained at the age 2 month and the range weight ( 210 – 250 gm ) from drug control laboratory ( Baghdad ) and used in this study , they were reread in plastic cages at room temperature about 20-25 C and fed on pellets and tap water was offered ad libitum. Animals were left for 7 days for adaptation before beginning of the experiment.

The rats were divided into two groups , control animals ( 10 rats ) were fed on regular rats diet for 60 days , and the treated animals ( 30 rats ) were fed on regular common diet containing 2% cholesterol for 60 days . Thirty days after begin of the experiment , the animals were given daily oral dose of 500mg /kg B.W. of oil extract Black seeds by stomach tube for 30 days .

Cholesterol powder diet was dissolved by diethyl ether solution ( 99% ) , the solution was spilled upon the pellets and let to dry and then given to the rats <sup>[13]</sup> .

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Blood samples ( 3 ml ) was withdrawn by cardiac puncture process <sup>[ 14 ]</sup> for LDL - cholesterol tests , the blood was immediately transferred to a tube containing heparin ( 1000 U ) and centrifuged ( 3000 rpm ) for 10min. , plasma was separated immediately and kept at - 20C until assayed and the biochemical tests were carried out at days ( 0,15,30,45 and 60 ) of experiment . LDL- cholesterol test performed in this study by using kit ( linear com. Spain ) , the procedure was performed by attend the reagents and samples in room temperature , and the samples , blank and calibrator as represented in the following table :

**Table ( 1 )**

Tube	Blank	Sample	Calibrator
R1	300 ul	300 ul	300 ul
Sample		4 ul	
CAL			4 ul

The samples was mixed for 5 min. at 37C and added :

**Table ( 2 )**

R2	100 ul	100 ul	100 ul
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Then mixed and incubated further 5 min. at 37 C , and then reading the absorbance of the sample and calibrator at 600 nm against the reagent blank . LDL – cholesterol was calculated by the equation <sup>[15]</sup>

Calculation : LDL – cholesterol ( mg/dl ) = absorbance of sample/ absorbance of calibrator x concentration of calibrator

### **Preparation Of The Oil**

There is used local black seed , the seeds was washed , dried in open air , crushed and have been squeezed by hydraulic press and use pressure 400 bar , the uses dose 500mg / kg body weight from oil extract for each animal .

### **Statistical Analysis**

Statistical analysis of data of the present study was performed on the basis of two way analysis of variance ( ANOVA ) using significant level of  $P < 0.05$  <sup>[16]</sup> .

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**Results and Discussion**

In the table ( 3 ) shows the values of LDL – cholesterol showed significant increase  $P < 0.05$  at days 15 and 30 ( $32.86 \pm 2.58$  mg/dl and  $37.66 \pm 2.50$  mg/dl ) respectively with significant decrease at day 45 and 60 ( $35.45 \pm 2.27$  mg/dl and  $29.26 \pm 3.18$  mg/dl ) respectively compared with day zero and control group.

**Table ( 3 )**

Day	Control LDL-cholesterol( mg / dl )	Treated LDL-cholesterol( mg/dl )
0	$28.57 \pm 3.31$	$30.02 \pm 3.11$
15	$29.58 \pm 2.47$	$32.86 \pm 2.58$
30	$29.48 \pm 2.75$	$37.66 \pm 2.50$
45	$28.73 \pm 2.63$	$35.45 \pm 2.27$
60	$28.28 \pm 4.33$	$29.26 \pm 3.18$

The black seed oil contain thymoquinone it's consider strong antioxidant for lipids especially to LDL <sup>[17]</sup>.

Also the oil extract of black seed contain flavonoids which plays an important role as an antioxidant <sup>[18]</sup>.

Other study indicated that hypercholesterolemia is lead to elevation of LDL - cholesterol contrast to the uptake of native ( unoxidized ) LDL by the LDL ( Apolipoproteins B and E ) receptor on macrophage , the uptake of oxidized LDL by the scavenger receptor pathway is not subject to negative feed back regulation and thus results in massive uptake of cholesterol by the macrophages <sup>[19]</sup>.

The oxidative modification of LDL appears to have an important role in foam cell formation and atherogenesis , this link between the oxidation of LDL and atherogenesis provides a convenient and simple rationale for the beneficial effect of antioxidants on the incidence of coronary artery disease <sup>[20]</sup>

Also the black seed oil contain lipase enzyme which has an important role in the decomposition of fatty of triglycerides to monoglycerides & fatty acids <sup>[21]</sup>.

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Oxidized LDL is also cytotoxic to vascular cells thus promoting the release of lipids and lysosomal enzymes into the intimal extracellular space and enhancing the progression of atherosclerotic lesion <sup>[22]</sup>.

Several antioxidants have been shown to limit lipid accumulation in vessels of animals fed high cholesterol diets <sup>[23]</sup>.

Also the oil extract of black seed contains 58.1% of poly unsaturated fatty acids works to reduce the level of cholesterol and reduce blood pressure, which led to a counter-considered factor for atherosclerosis <sup>[24]</sup>.

The oil extract also contain the soluble fibers as it work to reduce the level of cholesterol by converting the cholesterol to bile acids <sup>[25]</sup>.

Sterols play an important role in the metabolism of lipids through inhibition of absorption of cholesterol, increased secretion of bile salts and also inhibition of synthesis of triglyceride in the liver leading to decrease level of LDL in blood and decrease of cholesterol in the liver, this could be considered as an antagonist to cholesterol of blood which helps in preventing atherosclerotic lesion <sup>[26]</sup>.

Epidemiologic studies suggested that low levels of antioxidants are associated with a high risk of cardiovascular diseases and their increased intake appears to be protective <sup>[27]</sup>.

LDL - cholesterol is one of the four major groups of lipoproteins which are important physiologically and in clinical diagnosis. The LDL cholesterol transports cholesterol from liver to the peripheral tissues, it is called (bad cholesterol) and its concentration in blood has positive correlation with incidence of cardiovascular diseases <sup>[28]</sup>.

In the current study, in the irradiated animals with cholesterol, there was an increase in the amount of oxidized LDL, circulating in plasma with a simultaneous increase in the tissue oxidized LDL levels, The increased oxidized LDL levels may have led to an increase in the plaque area of the animals that were fed with a cholesterol supplemented diet and irradiated.

The administration of seed oil to rats orally, caused significant lowering in serum LDL cholesterol <sup>[29]</sup>.

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**Conclusion**

The present study showed that the black seed oil and the presence of active compounds had significant effect in lowering the level of LDL – cholesterol in treated rats .

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