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ANTIDYSLIPIDEMIC AND ANTI-OXIDANT ACTIVITIES OF *NIGELLA SATIVA* SEEDS EXTRACT IN HYPERLIPIDEMIC RATS

Saumya Saxena [1], Pooja Tripathi Pandey [2], Vishnu Kumar [* 3] and Jitendra Kumar Saxena [4]

Department of Chemistry [1], Dayanand Anglo-Vedic College, Kanpur - 208001, Uttar Pradesh, India.
Department of Physiology [2], Autonomous State Medical College, Dharnipur, Jignera, Shahjahanpur - 242001, Uttar Pradesh, India.
Department of Biochemistry [3], SRMS Institute of Medical Sciences Bareilly - 243202, Uttar Pradesh, India.
Biochemistry Division [4], Central Drug Research Institute, Lucknow - 226031, Uttar Pradesh, India.

Keywords:

Antidyslipidemic activity, *Nigella Sativa*, triton WR 1339, Anti-oxidant activity, reactive oxygen species, High Fat Diet

Correspondence to Author:
Dr. Vishnu Kumar

Professor,
Department of Biochemistry,
SRMS Institute of Medical Sciences,
Bareilly - 243202, Uttar Pradesh,
India.

E-mail: madhwapur1976@gmail.com

ABSTRACT: This experimental study had approved by animal ethics of Central Drug Research Institute, Lucknow, and had undertaken to evaluate the antidyslipidemic and anti-oxidant activities of *Nigella Sativa* (*N. Sativa*), Hindi name Kalonji seeds extract in two models of hyperlipidemia. 1- triton and 2- cholesterol-rich high-fat diet (HFD) induced hyperlipidemia. *N. Sativa* and Gemfibrogil were macerated with 2% aqueous gum acacia, and the suspension had fed orally to rats at a dose of 500 mg/Kg (b.w.p.o.) respectively. Serum lipids were found to be lowered by *N. Sativa* in triton induced hyperlipidemia. On the other hand chronic feeding of *N. Sativa* extracts to rats in cholesterol-rich high fat diet-induced hyperlipidemia for 30 days caused lowering in lipid and apoprotein levels of β-lipoproteins followed by an increase in lipid and apoprotein levels of α lipoproteins. The results of the present study also demonstrate that *N. Sativa* seeds extract repaired hepatic lipid synthesis, increased fecal bile acid excretion, and increased plasma LCAT activity in rats. Furthermore, *N. Sativa* seeds extract (100 to 400 µg/ml) inhibited the *in vitro* generation of superoxide anions and hydroxyl radicals in both enzymatic and non-enzymatic systems in a concentration-dependent manner.

INTRODUCTION: *Nigella sativa* (*N. sativa*) black caraway, also known as black cumin, nigella, kalojeere, and kalonji) is an annual flowering plant in the family Ranunculaceae, native to south and southwest Asia. *N. sativa* grows to 20–30 cm (7.9–11.8 in) tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate and usually colored pale blue and white, with five to ten petals.

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The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds which are used as a spice, sometimes as a replacement for black cumin (*Bunium bulbocastanum)*.

The seeds of *N. Sativa* are used as a spice in Indian and Middle Eastern cuisines, and also in Polish cuisine. The black seeds taste like a combination of onions, black pepper, and oregano. They have a pungent, bitter taste and smell. In Palestine, the seeds are ground to make bitter qizha paste. The dry-roasted seeds flavor curries, vegetables, and pulses. They can be used as a seasoning in recipes with pod fruit, vegetables, salads, and poultry. In some cultures, the black seeds are used to flavor bread products and are used as part of the spice

mixture panch phoron (meaning a mixture of five spices) and alone in many recipes in Bengali cuisine and most recognizably in naan. Nigella is also used in Armenian string cheese; a braided string cheese called majdouleh or majdouli in the Middle East [1-5]. The seeds of *N. sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. And it is an important drug in the Indian traditional system of medicine like Unani and Ayurveda. Among Muslims, it is considered as one of the greatest forms of healing medicine available due to it was mentioned that black seed is the remedy for all diseases except death in one of the Prophetic hadith. It is also recommended for use on a regular basis in Tibbe-Nabwi (Prophetic Medicine) [6-10].

Dyslipipoproteinemia is an independent risk factor for the development of coronary artery diseases, myocardial infarction, and hypertension in hyperlipidemic patients [1]. Clinically diabetic patients are characterized by a marked increase in blood glucose level followed by normal or mild hyperlipidemia. Elevated level in low-density lipoprotein (LDL) along with triglyceride, especially in very-low-density lipoprotein (VLDL) and cholesterol in low-density lipoprotein with free radicals and oxidative stress-mediated formation of modified LDL is recognized as a leading cause of development of Atherosclerosis and coronary heart disease in diabetes mellitus [2]. Furthermore, hyperlipidemia may also induce abnormalities like oxidation of lipids, the formation of ketone bodies as well as resistance to insulin in muscle and liver cells in diabetic patients. Treatment of hyperlipidemia with available lipid-lowering drugs: fibrates and bile acid sequestrants are not free from many side effects such as myositis, gastrointestinal upset along with elevated hepatic, renal function tests [11-17].

MATERIALS AND METHODS:

Plant Material: *N. sativa* seeds were purchased from the local market of Lucknow and identified taxonomically by the division of Botany, Central Drug Research Institute, Lucknow India. 200 gm seeds powdered and extracted with absolute ethyl alcohol, the yield was 10% w/w. The alcohol content was evaporated to dryness. The final yield of 20.0 gm of crude extract (concentrate) was added with 50 ml of triple distilled water and was used for *in-vivo* and *in-vitro* studies. A dose of 500 mg/Kg was administered to rats orally, daily for 30 days [18].

Preparation of the Cholesterol-Rich High-Fat Diet (HFD): Deoxycholic acid (5g) was mixed thoroughly with 700 g of powdered rat chow diet supplied by Ashirvad Industries, Chandigarh, India. Simultaneously cholesterol (5g) was dissolved in 300g warm coconut oil. This oil solution of cholesterol was added slowly into the powdered mixture to obtain a soft homogenous cake. This cholesterol-rich high-fat diet (HFD) was molded into pellets of about 3 g each [19].

Animals: *In-vivo* experiments were conducted as per guidelines provided by the Animal Ethics Committee of Central Drug Research Institute, Lucknow, India. Male adult rats of Charles Foster strain (100-150g) bred in the animal house of the Institute were used. IAEC approval number Ref No. ELMC/R_Cell/EC/2016/124. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26 °C, relative humidity 50-60%, and 12/12 h light/dark cycle (light from 8:00 am to 8:00 pm) and provided with standard rat pellet diet and water ad libitum[20].

Lipid-Lowering Activity in Hyperlipidemic Rats: The rats were divided into four groups consisting of six rats in each group for triton model and the second set of groups in the same pattern for the HFD model separately. For Triton model: Group I (normal control) group II (triton treated hyper-lipidemic), Group III (Triton ± *N. sativa)* and Group IV (Triton ± gemfibrozil). In the acute experiments hyperlipidemia in rats of group III and IV was induced by administration of triton WR - 1339 (Sigma Chemical Company, St. Lovis, MO USA) at a dose of 400mg/Kg b.w. by intraperitoneal injection. *N. sativa* and Gemfibrogil were macerated with 2% aqueous gum acacia, and the suspension was fed orally to rats of group III and IV, respectively, at a dose of 500 mg/Kg (b.w.p.o.) with triton.

For HFD model Group I (normal control) group II (HFD treated hyperlipidemic), Group III (HFD ± *N. sativa*), and Group IV (HFD ± gemfibrozil).

HFD induced set of experimental rats; hyperlipidemia was induced in group III and IV by

feeding with HFD to animals (for 30 days). *N. sativa* extracts (500mg/Kg), and gemfibrozil (500 mg/Kg b.w.) were administered orally once daily for 30 days. Control animals received the same amount of normal saline. At the end of the experiments, rats were fasted overnight. On the next day, animals were anesthetized with thiopentone (50mg/kg). Blood was withdrawn from retro-orbital plexus, kept at 20 °C for 15 min and centrifuged at 2500x g for 20 min. The animals were sacrificed, and their livers were excised promptly. Feces were collected throughout the experimental period from all the groups. The cholic acid and deoxycholic acid content in the feces was estimated [20].

Biochemical Analysis: Plasma lecithin cholesterol acetyltransferase (LCAT) activity was measured [21] and post heparin lipolytic activity (PHLA) was assayed [22]. Serum was fractionated into very-low-density lipoprotein (VLDL), low-density lipo-protein (LDL) and high-density lipoprotein (HDL) by polyanionic precipitation [23]. Serum lipids were analyzed for their total cholesterol (TC) phospholipids (PL), triglyceride (TG), protein, and Apoprotein by standard procedures reported earlier [24]. Liver homogenate (10%, w/v) prepared in 0.1 M Tris HCl buffer (pH 8.1) was centrifuged at 2500 rpm for 10 min. The supernatant was used for lipoprotein lipase activity [25].

Assessment of Free Radical Scavenging Activity: Superoxide anions (O^{2-}) were generated enzy-matically by xanthine oxidase (0.04 units) and nitroblue tetrazolium (320µM) in absence or presence of test compounds in 100 mM phosphate buffer (pH 8.2). Fractions were well sonicated in phosphate buffer before use. The reaction mixture was incubated at 37 °C. After 30 min the reaction was stopped by adding 0.5 ml glacial acetic acid and the amount of formazone formed was measured at 560 nm on a spectrophotometer [26].

Percentage inhibition was calculated taking absorption coefficient of formazone as 7.2×10^3 M^{-1} cm^{-1}. In another set of experiments, the effect of test compounds on the generation of hydroxyl radicals (OH) was also studied by nonenzymatic reactants. Briefly, OH was generated in a non-enzymic system comprised of deoxyribose (2.8 mM), $FeSO_4.7H_2O$ (2 mM), Sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO_4 buffer, pH 7.4 to a final volume of 2.5 ml. The above reaction mixtures in the absence or presence of the test extracts were incubated at 37 ºC for 90 min. Reference tubes and reagent blanks were also run simultaneously [27]. Malondialdehyde (MDA) content in both experimental and reference tubes was estimated spectrophotometrically by thiobarbituric acid method [28].

Statistical Analysis: The statistical analysis of variance (ANOVA-New men's student's' test) was performed by comparison of values for the control, hyperlipidemia, and hyperlipidemic with drug-treated (*N. sativa* and gemfibrogyl) groups. Similarly, Streptozotocin-induced diabetic group was compared with the control and diabetic group with drug treatment. All hypothesis testing was two-tailed $p<0.05$ was considered statistically significant, and the results were expressed as mean ± SD. The statistical analysis was carried out by 14 graph pad JN STAT 3.0 software. Similarly, the generation of oxygen free radicals with four concentrations of *N. sativa* was compared with that of their formation without the addition of extracts in the reaction mixture. The values were tested for significance at a $p< 0.05$ [29].

RESULTS:

Effect of *N. sativa* Seeds Extract in Triton Induced Hyperlipidemia: The acute administration of triton WR-1339 induced a marked increase in serum level of TC (±3.18F), PL (±2.97F), TG (±2.87F) and protein (±1.59F). Treatment with *N. Sativa* seeds extracts caused reversal in the levels of TC (22&26%), PL (24&28%), TG (22&27%), and protein (19&24%), respectively **Table 1**. The lipid-lowering activity of these extracts in the hyperlipidemic rats was comparatively less to that of gemfibrozil.

Effect of *N. Sativa* Seeds Extract and Gemfibrozil on Lipid Composition in Serum Lipoproteins and Hepatic Lipids: The data in **Table 2** shows that administration of HFD in rats increased their serum levels of TC (±2.35 F), PL (±2.46F), TG (±2.47F) and protein (±1.38F) respectively. Feeding with the extract of *N. Sativa* decreased the levels of TC (23%), PL (22%), TG (25%), and protein (19%), respectively, in HFD treated animals. The analysis of hyperlipidemic

serum showed a marked increase in the level of lipids and apoproteins constituting b-lipoproteins and these effects were pronounced for VLDL-TG (2.24F) and LDL-TC (±4.84F). Treatment with *N. sativa* significantly reduced these levels of VLDL TG (23%) as well as LDL-TC (22%), PL (17%), TG (27%), and apo LDL (21%) respectively in hyperlipidemic rats. At the same time, the decreased levels of HDL and apo-HDL in these animals were partially recovered in **Table 2**. The increased level of TC (±1.5 F), PL (±1.55 F), TG (±1.52 F), protein (±1.44 F) in livers of HFD fed rats were observed to be lowered by their treatment with the *N. Sativa* seeds extracts **Table 2**.

TABLE 1: EFFECT OF *NIGELLA SATIVA* SEED EXTRACT AND GEMFIBROZIL ON SERUM LIPIDS IN TRITON INDUCED HYPERLIPIDEMIA

Parameters/Groups	Total cholesterol[a]	Phospholipids[a]	Triglyceride[a]	Protein[b]
Control (GrI)	88.74±5.91	84.12±6.29	81.30±5.30	5.64±0.23
Triton treated (GrII)	282.37±26.54*** (±3.18F)	250.40±20.17*** (±2.97F)	231.62±18.17*** (±2.87F)	8.97±0.31 (±1.59F)
Triton ± N. sativa (GrIII)	220.44±16.37** (–22)	190.10±12.88*** (–24)	180.20±14.10** (–22)	7.23±0.44* (–19)
Triton ± Gemfibrozil (standard drug) (Gr IV)	185.53±12.14*** (–34)	165.33±11.77*** (–33)	150.28±14.00*** (–35)	6.00±0.20*** (–33)

Units a: mg/dl; b: g/dL. Values are mean ± SD of six animals Triton treated group had compared with control, and triton plus *N. sativa* and gemfibrozil treated groups were compared with triton treated. Values in the parentheses are % change. ***p<0.001; **p<0.01; *p<0.05

TABLE 2: EFFECT OF *NIGELLA SATIVA* SEED EXTRACT AND GEMFIBROZIL ON HFD INDUCED HYPERLIPEMIC RATS

Groups Parameters	Control (GrI)	HFD (GrII)	HFD ± *N. sativa* (GrIII)	HFD ± Gemfibrozil (Gr IV)
A. Serum				
Total cholesterol[a]	82.77±6.38	194.69±18.65 *** (±2.35F)	151.22±13.12 ** (–22)	127.33±18.88 *** (–34)
Phospholipid[a]	81.24±10.08	200.33±14.00 *** (±2.46F)	158.27±12.14 ** (–21)	132.26±10.14 *** (–33)
Triglyceride[a]	80.20±6.23	198.23±13.77 *** (±2.47F)	150.12±12.10 *** (–24)	132.66±10.20 *** (–33)
Protein[b]	5.99±0.61	8.28±0.57*** (±1.38F)	6.80±0.32* (–18)	6.40±0.30 *** (–23)
B. VLDL				
Total cholesterol[a]	8.32±0.41	32.43±2.12 *** (±3.89F)	25.09±1.62**(–22)	20.80±1.00 *** (–35)
Phospholipid[a]	14.87±0.31	30.18±1.24*** (±2.02F)	26.12±1.80** (–20)	22.17±0.82***(–31)
Triglyceride[a]	38.69±1.27	86.77±5.12 *** (±2.24F)	65.01±2.82*** (–25)	60.66±4.00 *** (–30)
Apoprotein[a]	6.30±0.50	12.12±1.90*** (±1.92F)	9.75±0.64** (–20)	9.00±0.62 *** (–25)
C. LDL				
Total cholesterol[a]	13.23±0.88	64.16±5.72 *** (±4.84F)	50.29±3.67**(–21)	46.10±2.14 *** (–28)
Phospholipid[a]	12.14±0.47	43.36±3.36 *** (±3.57F)	36.41±2.73* (–16)	30.83±2.70 *** (–28)
Triglyceride[a]	15.12±0.17	36.62±2.68 *** (±1.58F)	27.12±2.12*** (–26)	25.78±1.66***(–30)
Apoprotein[a]	17.56±1.00	28.62±1.88 (±1.62F)	22.50±1.33** (–21)	20.37±1.00*** (–28)
D. HDL				
Total cholesterol[a]	45.38±2.71	38.14±2.80* (–16)	44.28±4.00*(±14)	45.00±4.10* (±15)
Phospholipid[a]	37.41±2.61	28.61±2.14 *** (–23)	32.83±2.66* (±13)	35.66±3.12** (±20)
Triglyceride[a]	15.14±1.10	12.13±0.94 ** (–20)	14.09±1.14* (±14)	14.27±1.18* (±15)
Apoprotein[b]	168.20±13.50	120.35±14.40 ***(–28)	140.80±7.50* (±15)	144.22±13.00 * (±17)
E. Plasma				
LCAT activity[c]	67.59±3.94	37.77±2.66 *** (–44)	48.39±2.42** (±22)	52.88±5.11 *** (±29)
PHLA[d]	17.66±1.06	10.38±0.70 ***(–41)	13.72±0.64*** (±24)	14.77±1.10*** (±30)

Units a: mg/dl; b: g/dL; c : nmol cholesterol released/hr /L plasma; d : n mol free fatty acid formed /hr/mL plasma. Values are mean±SD from 6 animals. HFD group had compared with control and HFD plus *N. sativa* and gemfibrozil treated groups had compared with HFD. Values in the parentheses are % change. ***p<0.001; **p<0.01; *p<0.05

Effect of *N. Sativa* on Lipolytic Enzymes and Faecal Excretion of Bile Acids: HFD feeding to rats caused the inhibition of plasma LCAT (–44%) and PHLA (–41%) respectively **Table 3** and total lipolytic activity (–45%) in liver **Table 3**. However, treatment with *N. sativa* and gemfibrozil partially reactivated these lipolytic activities in plasma and livers of hyperlipidemic rats. HFD feeding to rats caused a significant decrease in the fecal excretion of cholic acid (–41%) and deoxycholic acid (–56%), and these levels were shown to be recovered by the treatment with *N. sativa* (±18%), (±38%) and gemifibrozil (23&45%) respectively in HFD fed animals **Table 3**.

TABLE 3: EFFECT OF *NIGELLA SATIVA* SEED EXTRACT AND GEMFIBROZIL ON HEPATIC BIOCHEMICAL PARAMETERS AND FAECAL BILE ACID EXCRETION IN HFD INDUCED HYPERLIPIDEMIC RATS

Groups / Parameters	Control (GrI)	HFD (GrII)	HFD± N. sativa (GrIII)	HFD± Gemfibrozil (GrIV)
A. Liver				
LPL activity[a]	130.37±8.84	71.23±3.42 *** (–45)	81.81±6.12* (±13)	88.95±5.02 ** (±20)
Total cholesterol[b]	6.62±0.14	10.04±0.32 *** (±1.51F)	8.32±0.10* (–16)	7.32±0.27 *** (–27)
Phospholipid[b]	23.33±2.00	36.12±1.87 *** (±1.55F)	28.78±2.00** (–20)	25.00±1.88 *** (–30)
Triglyceride[b]	10.34±0.70	15.72±0.88 *** (±1.52F)	12.22±1.10** (–22)	11.00±0.77 *** (–30)
Protein[b]	150.30±12.50	217.50±15.0 *** (±1.44F)	180.01±10.39 * (–17)	160.12±13.19*** (–26)
B. Faecal bile acid				
Cholic acid[c]	81.47±4.87	47.63±3.12 *** (–41)	58.12±3.10 ** (±18)	61.99±3.77 *** (±23)
Deoxycholic acid[c]	53.66±3.12	23.41±1.77 *** (–56)	38.29±3.00 *** (±38)	43.27±4.00 *** (46)

Units: a: n mol free fatty acid formed/h/mg protein, b: mg/g; c/ µg/g. Values are mean±SD of six animals. HFD group had compared with control and HFD plus *N. Sativa*, and gemfibrozil treated groups were compared with HFD. ***p<0.001; **p<0.01; *p<0.05

Effect of *N. Sativa* Seed Extract on Generation of Super Oxide Anions: The data in **Table 4** showed that enzymic oxidation of xanthine to uric acid (A) as well as the generation of O_2^- anions in xanthine–xanthine oxidase system, as measured by reduction of NBT to Formazone (B) were inhibited to varying extents by *N. Sativa* extract in a concentration-dependent manner and this effect was maximum by 32 and 46% respectively at 400 µg/ml of *N. sativa* extract. The extract also trapped the O_2^- anions generated by non-enzymic system of NADH – Phenozine–Methosulphate and was responsible for the reduction of NBT in the reaction mixture. The effect was dose-dependent and was highest by 35%, 50% at 400 µg/ml of *N. Sativa* seeds extract, respectively.

Effect of *N. Sativa* Seeds Extract on Generation of Hydroxyl Radicals: The data in **Table 4** also showed that *N. sativa* extract inhibited the formation of OH^- by an enzymic system of hypoxanthine–xanthine oxidase and Fe^{++}. Addition of N. Sativa seeds extracts (100-400 µg) inhibited the OH^- mediated formation of 2, 3 dihydroxy-benzoate in concentration dependant manner, which was 50% at 500 µg/ml of test extract. Furthermore, this preparation, when added with reaction mixture containing Fe^{2+} –Sodium ascorbate- H_2O_2 employed for nonenzymic generation of OH^- inhibited fragmentation of deoxyribose into MDA and this effect was maximum by 38 and 51% at peak concentration 400 µg/ml of *N. Sativa* extract respectively.

TABLE 4: EFFECT OF CONCENTRATION OF *N. SATIVA* SEEDS EXTRACT ON GENERATION OF OXYGEN FREE RADICALS *IN-VITRO*

Concentration of *N. sativa* seeds extract	Generation of O_2- anions			Generation of OH· Radicals	
	Enzymic System		Non enzymic System	Enzymic System	Non enzymic System
	(Xn-XnOD-System)[a]	(Xn-XnOD-NBT-System)[b]	(NADH-PMS-NBT-System)[b]	(Sodium Salicylate-FeSO4 HypoXn-XnOD-System)[c]	(FeSO4– EDTA-H2O2-Sodium ascorbate-Deoxy ribose-System)[d]
None	45.42± 1.47	112.87 ± 23.70	323.98 ± 17.93	543.89 ± 43.86	28.12 ± 2.19
100µg/ml	37.21 * ± 1.12 (-18)	90.69 * ± 8.47 (-12)	229.91 ± 7.49** (-29)	500.69 ± 14.93* (-11)	21.00 ± 1.97** (-25)
200µg/ml	33.34 ** ± 0.78 (-27)	77.11 ** ± 3.86 (-32)	210.80** ± 12.83 (-35)	410.98** ± 24.67 (-24)	17.44** ± 0.78 (-38)
300µg/ml	29.78** ± 0.76 (-34)	65.65** ± 4.79 (-42)	180.94** ± 4.97 (-44)	359.99** ± 18.32 (-34)	14.98** ± 1.34 (-47)
400µg/ml	23.24** ± 0.36 (-49)	60.44** ± 2.64 (-46)	160.74** ± 11.81 (-50)	318.69** ± 18.53 (-41)	13.77** ± 2.21 (-51)

Values are mean ± SD of four separate observations. The systems added with the Concentration of *N. sativa* seeds extract had compared with those without adding concentration of *N. sativa* seeds extract separately. *p<0.05, ** p<0.001. Units: a; n mol uric acid formed/min, b; n mol formazon formed/min, c; n mol 2, 3 dihydroxy benzoate formed/hr, d; n mol Malondialdehyde formed/hr.

DISCUSSION: Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissues, resulting in increased blood lipid concentration [20].

The present study shows that *N. sativa* seeds extract possess antidyslipidemic and anti-oxidant activities altogether.

In the present study, *N. sativa* seeds were tested for their anti-dyslipidemic and anti-oxidant activities in two models of hyperlipidemia, triton, and cholesterol-rich high fat diet-induced hyperlipidemia. Lipases played a significant role in lipoprotein metabolism and decreased lipoprotein lipase activities are the main cause of atherosclerosis [30]. However, treatment with *N. Sativa* seeds extracts reversed these effects. *N. Sativa* seeds extract could increase the level of HDL by increasing the activity of LCAT, which might contribute to the regulation of blood lipids. LCAT plays a key role in lipoprotein metabolism and most of the lipoprotein changes are the outcome of primary abnormality owing to the diseases related with lipid metabolism [31]. The stimulation of lipolytic activity in the liver and the increase in the level of blood HDL-TC followed by the decrease of β-lipoprotein-lipids and the decrease in hepatic lipid levels by these extracts are of great utility for regressing atherosclerosis. The *N. sativa* seeds extract, and gemfibrozil caused significant decrease in the plasma levels of serum lipids in hyperlipidemic rats. *N. Sativa* seeds extract enhanced the excretion of bile acids through feces and this contributed to regress the cholestesrolosis in liver damage.

Dyslipidemia and oxidative stress are important etiologic factors implicated in the development of a variety of complications. To overcome these ailments, as drug having multifold properties such as lipid-lowering and anti-oxidant activities together is in great demand. *N. Sativa* seeds extract, and gemfibrozil caused a significant decrease in the serum level of lipids in triton induced hyperlipidemic rats.

In general oxidative damage takes place in LDL of plasma by the hydroxyl radicals (OH) generated by the metal ions present in the serum due to alterations in their oxidation states. It has been observed that oxidative damaged LDL is relatively more atherogenic than the native LDL. Currently, several drugs being used for dyslipidemia intervene by lowering cholesterol (LDL and total cholesterol) or by lowering triglyceride in plasma. These extracts may also enhance the synthesis of LDL apoprotein (ApoB) as well as receptor protein to accelerate protein decreased the rate of hepatic lipid synthesis and inhibition of oxidative modifications of LDL may regulate the cholesterol level in the body. It is increasing evidence that it involves the regeneration of islet β-cells by neutralization of cytotoxic free radicals. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin deficiency promotes the hormone sensitive lipases [32, 33].

N. sativa seeds extract might be due to inhibition of hepatic cholesterol biosynthesis, activation of tissue lipases and these beneficial effects may be due the bioactive compounds presents in *N. sativa* seeds like typical alkaloids linoleic acid, oleic acid, palmitic acid, and trans-anethole, and other minor constituents. Aromatics include thymoquinone, dihydrothymoquinone, p-cymene, carvacrol, α thujene, thymol, α-pinene, β-pinene and trans-anethole.

CONCLUSION: It is concluded that *N. sativa* seeds extract has the regulatory effect on the lipolytic activities of plasma and liver in hyperlipidemic conditions and they also possess the power of regulating the faecal excretion of bile acids. Treatment with *N. sativa* caused reversal in the levels of total cholesterol, phospholipids, triglycerides and free fatty acids in dyslipo-proteinemia. The outcomes of the present study suggest that the *N. sativa* seeds extracts can contribute their potential as antidyslipidemic and anti-oxidant drugs to the world of natural products in the field of dyslipoproteinemia. It should be pointed out here that plant-derived natural compounds have established a proven platform for developing new drug synthesis with fewer side effects. Our study validates a strong anti-oxidant and hypolipidemic activity of *N. Sativa* seeds extract in hyperlipidemic rats.

ETHICAL APPROVAL: This article does not contain any studies with human participants performed by any of the authors. The study was approved by the Institutional Animal Ethics Committee of Central Drug Research Institute and was carried out in accordance with the current

guidelines set by Organization for Economic Co-operation and Development (OECD), received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India for the care of laboratory animals

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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