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Role of Black Seed Oil (Nigella sativa) and Olive Oil Combination on Serum Rabbit Treated with Cholesterol

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Abstract: ***This study concluded the identification of the role of black seed oil and olive oil on the cholesterol and total protein and triglycerides: experiment on twenty-five rabbit divided into five totals: total control of outdoor graduation was drenched just water. And the first treatment series: a dose of black seed oil 25% and 75% of olive oil. And the second group outdoor graduation was drenched black bean oil 50% and 50% olive oil. And third treatment group outdoor graduation was drenched black seed oil 75% and 25% of olive oil. And group fourth treatment 100% outdoor graduation was drenched black caraway seeds only. results: low cholesterol in the group treated first, second and third, depending on the concentration of black seed oil and olive oil, while increases when there are black cumin oil only in the fourth set. The level of total protein in group treatment first, second and third while dipping in the fourth treatment group outdoor graduation was drenched black caraway seeds only. Low triglycerides in group treatment first, second and third, depending on the concentration of black seed oil and olive oil, while increases when there are black cumin oil only in Group IV. Conclusion: from this we and the impact of LDL, LDH so black bean t. ether doing black seed oil on blood depends on the mix concentration of black seed oil and olive oil***.

Keywords: Niglla sativa oil, Black seed oil, olive oil , Rabbits

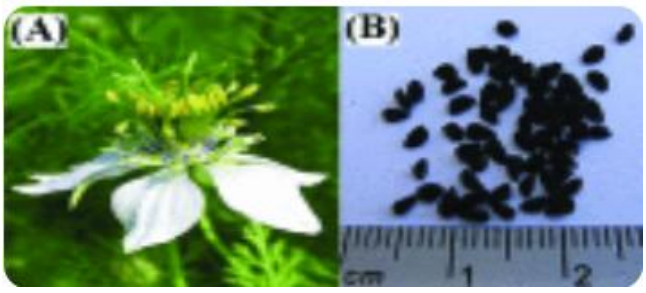
1. Introduction

Nigella sativa L. (Family: Ranunculaceae; commonly known as Black Cumin) is an annual herb possessing a wide range of medicinal uses(**1,2**) not withstanding its commercial significance as a spice yielding plant(**3)** Black cumin seeds are most revered (Holy herb of the Middle East – Yarn ell and Abascal**(4**). Effective utilization of *N. sativa* for therapeutic purposes as well as for trade will vastly depend upon yield (raw plant product- seeds; bioactive compounds- essential oil) and its quality. Existing germplasms may not substantiate the need for future, if not, at present. Therefore, it is of utmost essentiality to raise desirable plant type(s) in *N. sativa* through induced genetic variations and efficient breeding Endeavour. Considering nearly all essential aspects of *N. sativa*, a monograph is conducted with the laid formulation of WHO as well as with other significant parameters which will provide unabridged repository of references for present and future researchers who are looking to eugenize the species as a „potential medicinal herb" for human benefits.

2. Common Names

English: fennel flower, nutmeg flower, Roman coriander, black seed or black caraway, black sesame; India: Assamese - kaljeera or kolajeera, Bengali - kalo jeeray, Kannada – Krishna Jeerige, Tamil - karum jeerakam, Hindi/Urdu - kalaunji/man grail; Russian: Chernushka; Hebrew: Ketzakh; Turkish: çörek out; Arabic: habbat al-barakah; Persian: siyâh Dane; Indonesian: jintan hit am; Bosnian: čurekot**(5)** French: nigelle de Crète, toute épice; Germany: Schwarzkümmel; Portuguese: cominho-negro; Spanish: ajenuz, arañuel; Swedish: svartkummin(**6).**

The seed oil contains cholesterol, camp sterol, stigma sterol, β-sit sterol, α-spinasterol, (+)-citronellol, (+)-limonene, p-cymene, citronellyl acetate, carvone(**7),**nigellone, arachidic, linoleniclinolenic, myristic, oleic, palm tic, palmitoleic and stearic acids. Fixed oil: linolenic acid (55.6%), oleic acid (23.4%) and palm tic acid (12.5%). Volatile oil: trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%) **(8**), methoxypropyl)-5-methyl-1, 4-benzenediol, thymol and carvacrol **(9**). Root and shoot are reported to contain vanillicacid(**10)**.



Therapeutic Uses

Traditional Uses: In traditional system of medicine black cumin seeds are effective against cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrheal, obesity, diabetes, paralysis, hemiplegic, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea**(11).** It has also been used as a stimulant, diuretic, emmenagogue, lactagogue, anthelmintic and carminative(**12)** as well as it is applied to abscesses, nasal ulcers, orchitis, eczema and swollen joints**(11)**. Seed oil is considered to be local anesthetic **(13,14).**

3. Pharmacological Significance

Antibacterial Effect
The isolated saponin compounds from *N. sativa* (seeds) showed significant inhibiting effect on the growth of some bacteria, which include: *Staphylococcus aurous*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Pseudomonas aeruginosa***(15)**.

Antifungal Effect
It was found that methanolic extract of black seeds exhibits potent inhibition of fungus growth against *Candida Parapsilosis*, and *Issatchenkia Orientale'*swith IC50 Value 4.846 µ g/ml, and 6.795µg/ml, respectively and ethanolic extract also shows significant anti-fungal activity against fungus strain *Issatchenkia Oriental*with IC50 value 5.805 µg/ml **(16)**.

Ant parasitic Effect
It was revealed that the water extract of *N. sativa L*. seeds effect against trophozoites isolated from chronic and acute cases of *Entamoeba histolytic*in Baquba General Hospital, Diyala**(17).**

Anticancer Effect
N. sativa seed, its oil and extracts and some of its active principles, particularly thymoquinone and alpha-hederin, possess remarkable in vitro and *in vivo* activities against a large variety of cancers **(18).**

Anti- hepatotoxicity
The role of *N. sativa* was investigated in the prevention of carbon tetrachloride (CCl4)-induced liver toxicity, their results indicated that its' oil decreased significantly the elevated serum levels of liver enzymes and improve the state of oxidative stress induced by CCl4 **(19)**. Similar study confirm the protective role of vitamin E and flavonoids of *N. sativa* seed against hepatic dysfunction caused by sodium nitrate manifested by structural and functional change [20]. Another study confirmed that the black seeds have protective effect of against AlCl3 induced toxicity in rabbits**(21).**

Anti-Diabetic
N. sativa seeds were used as an adjuvant therapy in patients with diabetes mellitus type two added to their anti-diabetic medications. A dose of 2 gm/day of *N. sativa* might be a beneficial adjuvant to oral hypoglycemic agents (reductions in fasting blood glucose [FBG], , blood glucose level two hours postprandial [2hPG], and glcosylated hemoglobin [HbA1]) in type 2 diabetic patients (**22**).

Hypocholesterolemic and antiatherogeniccardio protective properties

N. sativa produces antiatherogenic effect by decreasing low density lipoprotein cholesterol level significantly**(23,24).** Serum triglycerides, total and LDL cholesterol decreased significantly after treatment with 750 mg of powdered grains of *N. sativa* enclosed in a capsule twice daily for 28 days, While HDL cholesterol increased significantly**(25)** .Similar results revealed that *N. sativa* oil decreased he levels of total cholesterol , triglycerides, phospholipids , LDL cholesterol and uric acid **(26)**. *N. sativa* either in powder or oil forms was shown to significantly reduce total cholesterol (TC) and low-density lipoprotein cholesterol (LDL) levels and enhance high-density lipoprotein cholesterol (HDL) levels after treatment for 2, 4, 6 and 8 weeks compared to the positive control group**(27)**.

Effects on Reproduction
The administration of 1ml/kg/day of *N.sativa* oil stimulated the secretion of sexual hormones that led to improve protein synthesis of hepatic enzymes, white blood cells count and decrease the serum cholesterol concentration in blood**.(28)**

Effect on immunity
Treatment of typhoid-antigen-challenged rat with the volatile oil revealed an immunosuppressant action as evidenced by the significant decreases in the antibody titer and the splenocytes and neutrophils counts **(29).**

Impact on the gastrointestinal system
Black cumin seed has been widely used as gastrointestinal disorders. The aqueous extract of the seeds was reported to exhibit anti-ulcer activity by decreasing the volume of acid in gastric juice in acetylsalicylic acidtreatedrats treated rat**(30)**.
The volatile oil and ethanolic extract inhibited spontaneous movements of the rabbit jejunum as well as agonist induced contractions and the spasmolytic effect involved calcium channel blockade **(31).**

Others
Black seeds act as analgesic, anti-inflammatory action, anti-asthmatic, antihistaminic, anti-allergic, antihypertensive, antihypertensive and anti-oxidant **(,29,32,33,34).**

4. Material and Method

Animals: a total number of 25 local mature rabbits were used in this investigation .Animals were two weeks for adaptation, they were fed ordinary pellet diet and green herb, the temperature was 22-29c.Their weight was recorded in the beginning of experimental the determine the dose of olive oil and black seed oil .their weight range from 1250-1500 gm and their age ranged from 8-12 month. Animals were kept individually in meshed stainless steel cage (100)cm each cage contain 5 rabbits. The light and dark cycle was (12:12) .The animals had free access food and water,care was taken to avoid unnecessary stress and noise cage crowding

Table 1: Instrument used in this study with their suppliers and sources

Instrument	*Supplier*	*Sources*
Centrifuge	Hettich	Germany
Spectrophotometer	Optima sp-300	Japan
Micropipette	SLAMED	Germany

Table 2: Chemicals used in this study with their suppliers and sources

Chemicals	**supplier**	**source**
Diethyl ether	BDH	England
Cholesterol kit	BIOLABO	France
Total protein	BIOLABO	France
Triglyceride	BIOLABO	France

5. Method

Use 500 ml of Diethyl ether and 5g of cholesterol, each 100 ml mixed with 1gm of cholesterol this process continuous about 1 month (orally).

Experimental design:
Control: which give water only**.**
Treated 1: give 25% nigella seed oil and 75% from olive oil (mix 1cc of nigella seed oil and 1cc of olive oil) in the water of rabbit.

Treated 2 : give 50% nigella seed oil and 50% from olive oil (mix 1cc of nigella seed oil and 1cc of olive oil) in the water of rabbit.

Treated 3 :give 75% nigella seed oil and25% olive oil (mix 1.5cc of nigella seed oil and 0.5cc of olive oil)in the water of rabbit**.**

Treated 4: give 100% nigella seed oil.(give 2cc in the water of rabbit) after 1 month collect of blood sample from each group of rabbit that make of cholesterol and triglyceride and total protein ratio of rabbit (that give nigella seed oil and olive oil)and ratio comparative with ratio of cholesterol and triglyceride and total protein of control rabbit.

Blood sample Collection : two ml were obtained via cardiac puncture from each animal by using disposable syringe washed with heparin before administration and the sample used for :

Serum Biochemical parameter two ml used for isolation of plasma by centrifugation for 30 min in 3000 PPM /min. Used plasma for measurement cholesterol ,total protein and triglycerol by spectrophotometer

Statistical analysis :used F test one way **(35).**

Determination of triglyceride
Principle the triglycerides are enzymatic ally hydrolyzed to glycerol according to the following:-

Triglycerides →glycerol +fatty acid
Glycerol+ATP→glycerol -3-Phosphate +ADP
Glycerid -3-P→Dihydroxyacetone –P +H2O2
H2O2 +4 –aminophenazone +p -chlorophenol→H2O +Quinonimine

Procedure
Wave length ……505nm(490 -550)
Temperature……37C (25C)
Cuvette ……1cm light path

	Blank	Stander	Sample
Stander	…….	10ul	……..
Sample	…….	……	10ul
Working reagent	1Ml	1Ml	1Ml

Mix and incubation 5 min. at 37C or 10 min. at 25C. The colure is stable for 30 min.

Calculation

$$\text{Triglyceridescon} = \frac{\text{O.D.Sample}}{\text{O.D.Stander}} \times n$$

Determination of total protein

Protein give an intensive violate- blue complex with copper in an alkaline medium. Iodide is including as antioxidant. The intensity of the color formed is proportional to the total protein concentration in the sample

Procedure:
1) Assay condition:
Wavelength……540 (530-550) nm
Cuvette ……..1cm .light path.
Temperature ……37C /15-25 C
2) Adjust the instrument to zero with distilled water.
3) Pipette into a cuvette:
4) Mix and incubate 5 min at 37c or 10 min at room temperature.
5) Read the absorbance (A) of the sample and standard, against the blank

The colour is stable for at least 30 minute.
Calculations

$$\frac{(A)\text{Sample}}{(A)\text{standard}} \times 7\ (\text{standard conc.}) = \frac{g}{dl}\ \text{of total protein in the sample}$$

Determination of cholesterol

Principle of method
The cholesterol present in the sample originate a colored complex according the following
Cholesterol +H2O → cholesterol +fatty acid
Cholesterol +O2 → cholesterol +H2O2
2H2O2 +Phenol +minophazone → Quinonimine +4H2O

The intensity of the color is proportional to the cholesterol concentration in the sample

Procedure:
1) Assay Condon
Wavelength……..505 nm (500-550)
Cuvette……1 cm light path
Temperature…….37C \15-25 C
2) Adjust the instrument to zero with distal water
3) Pipette in to a Cuvette
4) Mix and incubate for 5 min at 37C or 10 min at room temperature
5) Read the absorbance (A) of sample and stander .Against the Blank .

The colour is stable for at least 60 min

	Blank	Stander	sample
WR(ML)	1	1	1
Standard(ul)	.....	.....	10
Sample(ul)	.....	.....	10

Calculation:
(A) sample/(A) stander ×200 (standard conc.)= Mg\dl cholesterol in sample
Conversion factor : Mg/dl×mmol \L

6. Result

The result in table explain that black seeds administration in combination with oliveoil.
C**holesterol**:the result obtain in **(Figure 1)** significantly decrease p<0.05 (T3) as compared with control,(T1) and (T2). While (T4) result reverse increase..

Total protein :- the effect of black seeds on total protein conc. Of (T3) is significantly increase p<0.05 as a compared with control, (T1) and (T2) .

While (T4) result reverse decrease (**Figure 2).**

Triglycerol: -the data pertaining to triglyceride concentration of control and treated groups are depicted in table .triglyceride concentration shown a significant decrease p< 0.05 (T3) as a compared with control, (T1), (T2) While (T4) result reveres increase (**Figure 3).**

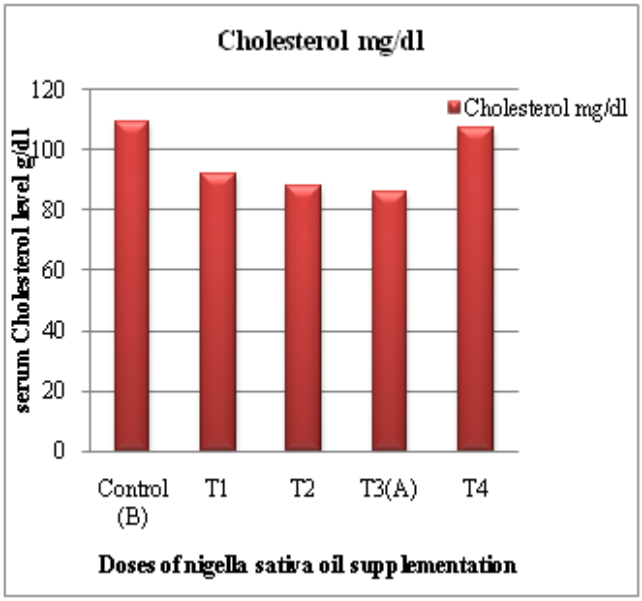


Figure 1: Change in cholesterol level of rabbit supplement with various doses of nigella sativa oil for one month

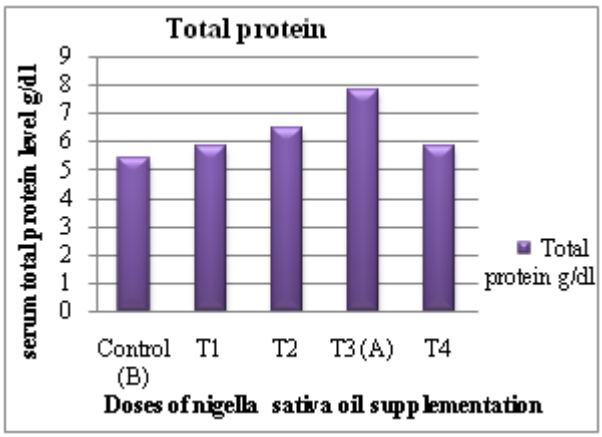


Figure 2: change in Total protein level of rabbit supplement with various doses of nigella sativa oil for one month

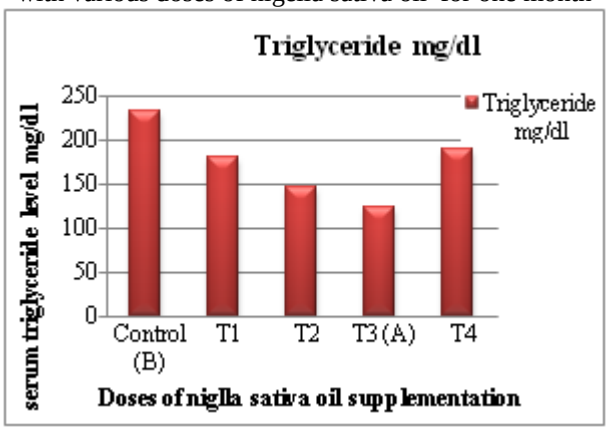


Figure 3: change in Triglyceride level of rabbit supplement with various doses of nigella sativa oil for one month

Table 3: The effect of black seed oil and olive oil combination on serum total protein, cholesterol and Triglycerol of rabbits administration for 30 day

Group \ Parameter	Cholesterol	Total protein	Triglyceride
	Control	Mean ± SE 109.32±23. 427 (B)	Mean ± SE 5. 4 ± 1.256 (B)	Mean ± SE 233.1 ± 25.717 (B)
T1	25%	91.86± 29.142	5.8 ± 0.607	179.08 ± 67. 086
T2	50%	88.1 ± 56.66	6.5 ± 1.985	146.6 ± 34.585
T3	75%	86.02 ± 20.173 (A)	7.8 ± 1.531 (A)	122.8 ± 28.362 (A)
T4	100%	106.9 ± 11.760	5.8 ± 426	190.26 ± 18.330

Control gives distal water only
(T1) combination 25% black seed oil and 75% olive oil
(T2) combination 50% black seed oil and 50% olive oil
(T3) combination 75% black seed oil and 25% olive oil
T4)100 % black seed oil only)
Values are expressed as mean± SE n=5 animal/group

Capital letters denote significant differences p<0.05 between group

LSD for total protein =2.35

7. Discussion

It is well that a successful for treatment of dyslipidemia is primary prevention of postprandial hyper lipidemia by aggressive delaying fat digestion and absorption (4,2). Previously , it has shown that oligomeric procyanidians containing .it was found that degree of polymerization of oligomeric procyanidians was an important factor to increase potency on pancreatic lipase inhibition (11). In the study the effect of black seed oil combination with olive oil at 75% on cholesterol, total protein and triglyceride (12, 13. 14) compartment with control, 25%, 50%.

Finding showed that acute administration of black seed oil is markedly suppressed the elevation of serum cholesterol and triglyceride in high concentration of black seed oil of 75% (14, 23, and 24).The black seed oil reduced plasma lipid profile and prevent high .while in total protein is increased 75%. Fat diet mauced obesity in hamster and related metabolic (26). The supplemented with proanthocyanidin-rich extract from black seed oil inhibits progression of Atherosderosis in cholesterol in rabbit (27). The mechanism of action in related to prevention of low. Density lipoprotein (LDL) oxidation in the arterial well diet (30).

We suggest that large term and high concentration supplementation of black seed oil reduced plasma liquid cholesterol and triglyceride while total protein increased from these point of view , an intake of black seed oil combination with olive oil feasible therapeutic strategy for prevention and treatment of patient with hyperlipidemia and obesity.

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