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Supplementation of Powdered Black Cumin (*Nigella sativa*) Seeds Reduces the Risk of Hypercholesterolemia

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ABSTRACT:

Background: Functional and nutraceutical foods are gaining immense popularity among the masses. Plants and their bioactive molecules are of prime importance. Although various plants from different geographical areas have been tested in the past, many horizons still need to be addressed. Black cumin (*Nigella sativa* L.) is one such example that is quite popular in South Asia and the Middle East.

Context and purpose: The present research study was designed to expedite the role of black cumin seed in reducing the risk of hypercholesterolemia. For the purpose, thirty Sprague dawley rats were procured from the National Institute of Health (NIH) in Islamabad, Pakistan, and further split up into three groups, (10 rats each). Experimental diets were prepared using powdered black cumin (PBC) at 1% and 2%, and compared with the placebo.

Results: The results revealed that PBC was effective in reducing the serum cholesterol, triglycerides, and low-density lipoproteins (LDL). Additionally, the experimental diets resulted in a non-significant increase in high-density lipoprotein (HDL). Overall, powdered black cumin at 1% and 2% reduced cholesterol level by 6.73, and 4.48%, LDL by 24.79, and 24.32% respectively. However, the supplementation of PBC at 2% resulted in marked variations as increasing tendency, which was recorded for cholesterol and triglycerides contents after 28 days of study.

Conclusion: Present research investigation brightened the prospects of using powdered black cumin seed in diet based therapies to improve the lipid profile. Further studies are still required to assess the phytochemistry of the plants and indeed the functional ingredients responsible for such health benefits. Such studies would bring meticulousness for utilization of black cumin seeds as a functional food.

Keywords: Functional foods, black cumin, lipid profile, cholesterol, triglycerides

BACKGROUND:

The diet-health linkage is a prime area of research in the domain of nutrition. The concepts of functional and nutraceutical foods flourished as a results of such research interventions. The consumer trends have also widened, and many have begun to look at food not only for its basic nutrition, but also for the allied health benefits [1-2]. Therefore, opportunities abound in functional foods to combat oxidative stress, hyperglycemia, and hypercholesterolemia. Now it's the responsibility of the nutritionists to pay attention towards their health claims and their safety issues [3-4].

Black cumin (*Nigella sativa* L.) contains several bioactive molecules that include thymoquinone, thymol, tocopherols, tocopherol, trans-retinols, and selenium. These functional ingredients are predominantly present in its fixed and essential oil [5]. *N. sativa* seeds also contain proteins, alkaloids (nigellicines and nigelledine), and saponins (α -hederin) in substantial amounts. Black cumin seeds have been used as herbal medicine by various cultures and civilizations to treat and prevent the number of diseases to ensure good health. Recent pharmacological investigations suggested its potential role, specifically for the amelioration of oxidative stress through free radical scavenging activity, the induction of apoptosis to cure various cancer lines, the reduction of blood glucose, and the prevention of complications from diabetes. It regulates hematological and serological aspects and can be effective in dyslipidemia and respiratory disorders. Moreover, its immunopotentiating and immunomodulating role brings balance to the immune system. Evidence is available supporting the utilization of *Nigella sativa* and its bioactive components in a daily diet for health improvement [6]. Like most herbs, the composition of black cumin varies with geographic distribution, time of harvest, and agronomic practices [7]. Black cumin seeds contain appreciable quantities of unsaturated especially polyunsaturated fatty acids (48 to 70%), while monounsaturated (18 to 29%), and saturated fatty acids (12 to 25%), are in lesser proportions. Besides a better fatty acid profile, it contains considerable quantities of tocopherols and allied bioactive compounds. Moreover, the presence of phytosterols in amounts of 0.33 to 0.36% further strengthens its hypoglycemic and hypcholesterolemic perspectives [8]. Several pharmacological investigations explored the effectiveness of thymoquinone against oxidative stress, cancer, immune dysfunction, and diabetic complications. Furthermore, it also regulates several hematological and serological functions, it maintains body homeostasis and bears a hypocholesterolemic effect [9].

Black cumin is a promising candidate in dietary modifications and could be utilized directly in the daily diet, or added as a functional ingredient in cereal based products [10]. Keeping in view the above information, the present research project was designed to expedite the role of black cumin (*Nigella sativa* L.) seed in reducing the risk of hypercholesterolemia. For the purpose, powdered black cumin seeds at 1% and 2% were added in the experimental diets of rodents and their influence was observed in balancing the lipid profile.

METHODS:

Black cumin seeds of the indigenous variety were obtained from Barani Agricultural Research Institute (BARI), Chakwal. Materials for experimental diets were procured from local markets, while the chemical and kits were purchased from Merck (Merck KGaA, Darmstadt, Germany).

Proximate and nutritional composition: The black cumin seeds were ground to 50mm mesh size, and further evaluated for their proximate composition as described in AACC [11]. The fixed and essential oils were also analyzed and the results are already published (See: Sultan et al. [5] Published in Pakistan Journal of Botany).

Efficacy study & housing of rats: In order to explore the hypocholesterolemic perspectives, experimental diets containing 1% and 2% powdered black cumin seeds were prepared, and the rest of the formulation remained the same as suggested by the National Institute of Health (NIH). Sprague dawley rats were maintained according to standard guidelines: they were housed in groups of ten in stainless steel top grill cages, with a controlled temperature ($23\pm 2^{\circ}\text{C}$), a relative humidity ($55\pm 5\%$), and a 12 hour light-dark cycle. Food and water was provided *ad libitum*, and their intake was monitored on a daily basis. Half of the overnight fasted rats were sacrificed after four weeks of the feeding trail, and the rest were sacrificed at the end of study. Blood samples were collected through cardiac puncture, and serum was collected following the method of Uchida et al. [12].

Serum lipid profile: The lipid profile includes cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides. For the purpose, cholesterol in the collected serum of individual rats of all groups was measured by the liquid cholesterol CHOD-PAP method, as described by Stockbridge et al. [13], to find out the effect of the individual diet on the cholesterol level of respective groups. Serum High Density Lipoprotein (HDL) was measured by the HDL Cholesterol Precipitant method as described by Assmann [14]. Low Density Lipoprotein (LDL) was measured by following the procedure of McNamara et al. [15]. Triglycerides in the collected serum of individual rats were measured by liquid triglycerides GPO - PAP method, as described by Annoni et al. [16].

Statistical analysis: Data obtained was analyzed statistically using the statistical package i.e. Cohort V-6.1 (Co-Stat-2003). The sample for each analysis was run quadruplet, and the values expressed are means \pm the standard deviation. Analysis of variance was applied to check the level of significance, and the means were compared through Tukey's HSD tests.

RESULTS:

Diet based therapies are gaining immense importance and usually rely on plant based remedies. Black cumin (*Nigella sativa*) is quite famous in Muslim communities. In the present research investigation, the efforts were diverted to utilize powdered black cumin seeds at 1% and 2% in the diets of Sprague dawley rats to assess the efficacy of black cumin as hypoglycemic agent.

The results obtained were subjected to statistical analysis using two factor-factorial design and mean squares (Table 1), which explicated that the lipid profile was affected significantly as a function of diets, except for high-density lipoprotein (HDL), while study intervals and their interaction remained non-significant with exception of their momentous impact on low-density lipoprotein (LDL).

Table 1. Effect of diets and study intervals on serum lipid profile in normal rats

SOV	Df	Cholesterol	HDL	LDL	Triglycerides
Diets (A)	2	0.4743ns	2.0657ns	2.5846*	4.1410*
Intervals (B)	2	2.5608ns	1.4493ns	0.3864ns	13.2879**
A x B	4	0.8087ns	0.3138ns	1.2035ns	3.3782*
Error	36	0.4743	2.0657	2.5846	4.1410
Total	44				

ns = Non-significant; * = Significant; ** = Highly significant

Diets containing powdered black cumin seeds reduced cholesterol contents significantly as compared to the control (Table 2). Maximum cholesterol 108.19±2.27mg/dL was recorded in the D₁ (control) group, while the D₂ (PBC 1%) and the D₃ (PBC 2%) groups had a lower mean cholesterol value, i.e. 105.30±2.43 and 107.17±2.80mg/dL, respectively. During the entire study, the placebo (control) group showed a non-significant increase in cholesterol contents from 107.85±4.27 to 112.27±2.68mg/dL. On the contrary, PBC 1% decreased cholesterol contents from 110.16±3.17 to 102.75±3.25mg/dL. However, higher concentration of powdered black cumin (PBC 2%) resulted in a varied response as cholesterol decreased in the initial four weeks (102.38±2.93mg/dL), but increased afterwards, and cholesterol contents were recorded 107.04±4.17mg/dL after 56 days of feeding trials.

Table 2: Level of cholesterol (mg/dL) in normal rats treated with powdered black cumin seeds

Diets	Study intervals (Days)			Means
	0	28	56	
D₁ (Control)	107.85±4.27	104.44±2.66	112.27±2.68	108.19±2.27
D₂ (PBC 1%)	110.16±3.17	102.99±2.86	102.75±3.25	105.30±2.43
D₃ (PBC 2%)	112.08±5.89	102.38±2.93	107.04±4.17	107.17±2.80
Means	110.03±1.22	103.27±0.61	107.35±2.75	

Means for HDL contents (Table 3) indicated non-significant variations due to the diets. However, it is observed that HDL contents slightly improved in D₂ (PBC 1%) and D₃ (PBC 2%). Data pertaining to serum triglycerides are presented in Table 4. The results indicated that triglycerides increased in control diets with an overall mean value of 94.51±2.05mg/dL. In comparison, D₂ (PBC 1%) containing diets decreased the triglycerides from the baseline values of 87.59±4.41 to 80.27±5.08mg/dL. However, the supplementation of powdered black cumin seeds at 2% resulted in a marked increase in triglycerides statistically at par with control diets.

Table 3: Level of HDL (mg/dL) in normal rats treated with powdered black cumin seeds

Diets	Study intervals (Days)			Means
	0	28	56	
D₁ (Control)	44.32±1.76	44.43±2.83	44.73±2.87	44.49±0.12
D₂ (PBC 1%)	45.61±2.29	48.77±2.57	51.32±3.25	48.56±1.65
D₃ (PBC 2%)	45.07±2.37	48.78±2.19	49.75±3.15	47.87±1.43
Means	45.00±0.38	47.33±1.45	48.60±1.99	

Table 4: Level of triglycerides (mg/dL) in normal rats treated with powdered black cumin seeds

Diets	Study intervals (Days)			Means
	0	28	56	
D₁ (Control)	91.55±3.63	93.53±5.95	98.45±6.32	94.51±2.05a
D₂ (PBC 1%)	87.59±4.41	89.07±4.69	80.27±5.08	85.64±2.72b
D₃ (PBC 2%)	88.57±4.65	90.26±4.06c	99.97±6.34	92.93±3.55a
Means	89.24±1.19	90.95±1.33	92.90±6.33	

Means sharing same letters in a column/row do not differ significantly at $P < 0.05$

It is obvious from the means (Table 5) that maximum LDL contents 44.79±1.90mg/dL were recorded in D₁ (control) whereas D₂ (PBC 1%) and D₃ (PBC 2%) exhibited lower LDL contents i.e. 39.61±3.72 and 40.71±4.32mg/dL, respectively. Furthermore, groups of rats fed on D₁ showed non-significant increase in LDL from 345.22±1.79 to 47.85±3.07mg/dL during the whole study, whilst, diets containing powdered black cumin at 1% and 2% decreased LDL level significantly from 47.03±2.37 to 35.37±2.24 and 49.29±2.59 and 37.30±2.37mg/dL, respectively.

Table 5: Level of LDL (mg/dL) in normal rats treated with powdered black cumin seeds

Diets	Study intervals (Days)			Means
	0	28	56	
D₁ (Control)	45.22±1.79b	41.31±2.63c	47.85±3.07a	44.79±1.90a
D₂ (PBC 1%)	47.03±2.37ab	36.42±1.92d	35.37±2.24d	39.61±3.72b
D₃ (PBC 2%)	49.29±2.59a	35.54±1.60d	37.30±2.37d	40.71±4.32b
Means	47.18±1.18a	37.76±1.80c	40.18±3.88b	

Means sharing same letters in a column/row do not differ significantly at $P < 0.05$

DISCUSSION:

The Sprague dawley strain of rats is frequently used in efficacy studies to achieve multifarious objectives; the present research investigation was conducted following the protocols already described by Morita et al. [17]. Rats fed on experimental diets containing powdered black cumin at 1% and 2% showed some promising results. It is obvious from the present research that diets containing powdered black cumin at 1% and 2% improved the lipid profile.

Overall, powdered black cumin at 1% and 2% reduced cholesterol level by 6.73 and 4.48%, LDL by 24.79 and 24.32%, respectively, whilst, HDL contents improved non-significantly (Figure 1). The improvement in the overall lipid profile can be interpreted due to several reasons. Black cumin is rich in polyunsaturated fatty acids [18] that mainly account for its lipid lowering potential [7]. Some other research interventions also supported the claims of the present research investigation. It produces a favorable effect on serum lipid profile like lowering total cholesterol, LDL, and triglycerides, while elevating the HDL level [19]. Moreover, it was hypothesized that the cholesterol lowering mechanism might be through PPAR α (Peroxisome Proliferator-Activated Receptor) activation [20-21].

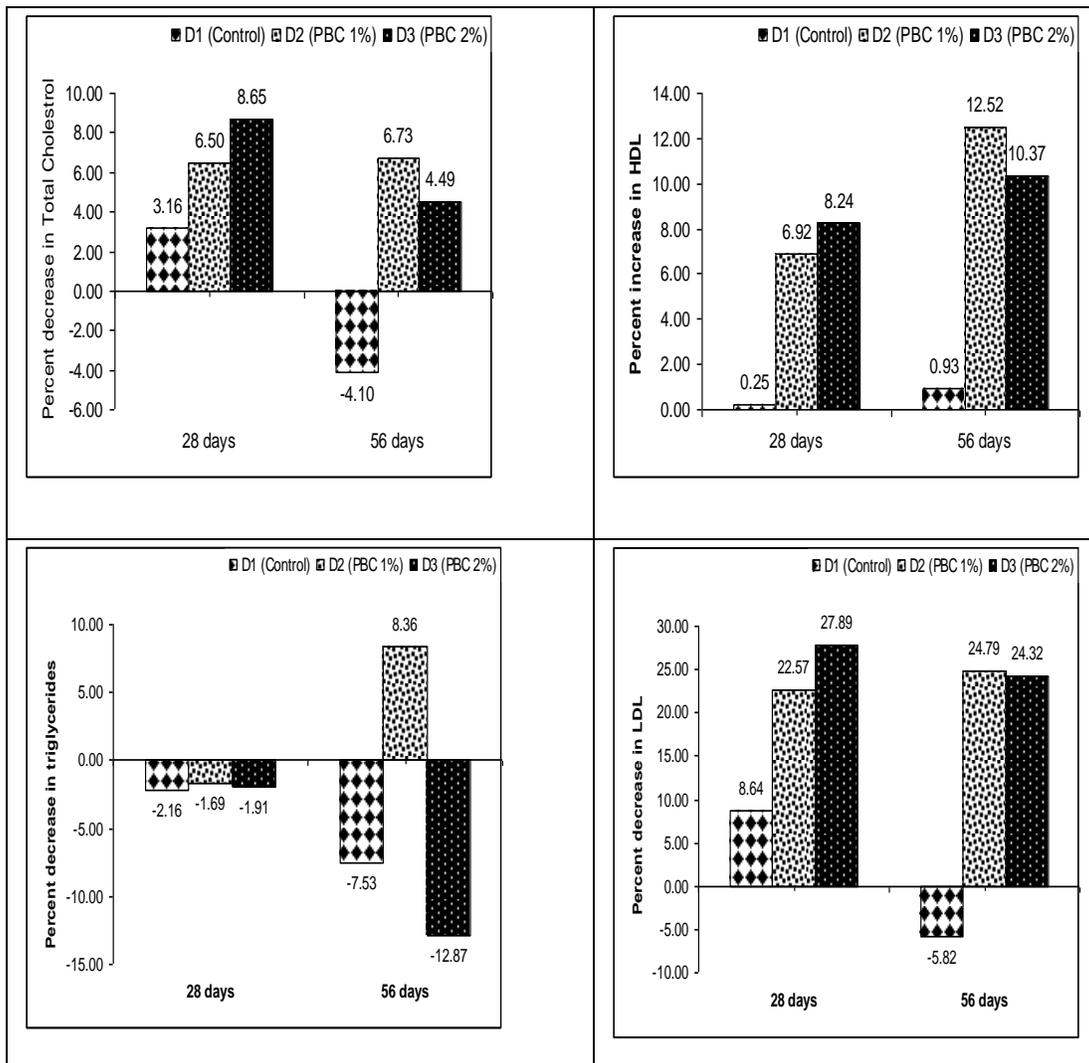


Figure 1: Percent increase/decrease in parameters related to hypercholesterolemia

The presence of substantial amounts of fat-soluble vitamins, especially tocopherols in black cumin seeds have been highlighted by Ramadan and Mörsel [22] and Al-Saleh et al. [23]. These bioactive compounds are responsible for reducing oxidative modification of LDL, resulting in their clearance from the blood stream. Moreover, the black cumin seeds also contain the appreciable amount of sterols, especially β -sitosterol that has been bestowed with the ability to inhibit absorption of dietary cholesterol. The active ingredients, i.e. thymoquinone, in few studies were reported to possess hypocholesterolemic potential [7, 24]. The other possible justification of cholesterol reduction is owed to the slight anorexic effect of black cumin, as it reduces the appetite [25].

It is obvious from the present research that diets containing black cumin fixed and essential oils improved the lipid profile, but the effect was more pronounced the in group of rats fed lower doses of powdered black cumin seeds.

CONCLUSION:

The conclusive approach drawn from this part of the efficacy study is black cumin holds potential to decrease total cholesterol and LDL, however, its dosage needs to be adjusted to bring in the desirable results. Nevertheless, there is need to conduct further trials in similar types of rats, modeling after inducing hypercholesterolemia to validate the findings. Moreover, the different fractions of black cumin seeds should also be tested for their therapeutic potential through animal modeling. The results from such studies would be important for meticulousness of the present findings.

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