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Original Article

Determination of Proximate Composition of Nigella sativa L. Seeds and its Effective Role in Improving Lipid Profile Among Hyperlipidemic Women

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ABSTRACT

Hyperlipidemia is characterized by abnormally elevated levels of body's plasma cholesterol and triacylglycerol. **Objective:** To find out the proximate composition of *Nigella sativa L.* and its hypolipidemic effect on blood lipid profile among hyperlipidemic women. **Methods:** To determine the proximate composition of *Nigella sativa* seed the kjeldhah method, soxhlet extraction using petroleum ether, and AOAC procedure were used. For the identification of hypolipidemic impact, a total of 64 women with mild to moderate hyperlipidemia, aged between 25-35 years were selected for the 8 weeks of study. The sample was selected from the University of Lahore Teaching Hospital, Lahore. After removing physical contaminants like dirt, dust, and other foreign grains black seeds were air dried. After drying, seeds were grounded into fine powder by using commercial blender. Each capsule was prepared with 500mg powder of *N. sativa*, and was transferred in air tight jars. After screening the participants of experimental group, they were advised to use 2 capsules of *N. sativa* supplement before breakfast and two capsules in the afternoon prior to their meal for 8 weeks. Participants were also advised to follow the given 7-day diet plan for 8 weeks. The anthropometric measurements, biochemical evaluation(lipid profile), and dietary intake data were collected. **Results:** The mean age group of Hyperlipidemic patients enrolled in study was 32.5±0.34 years. There was a significant reduction in Low Density Lipoprotein(LDL), total cholesterol level, and triglyceride levels with p-value less than 0.05. High density lipoprotein levels were also improved from 49.5±8.38mg/dl to 51.81±10.21mg/dl. **Conclusion:** The study concluded that *Nigella sativa L.* seed powder supplementation showed a significant improvement in lipid profile of hyperlipidemic patients. The study found an increase in high density lipoprotein among patients.

INTRODUCTION

Hyperlipidemia is a condition in which the body's plasma cholesterol (hypercholesterolemia) and triacylglycerol (hypertriglyceridemia) levels are abnormally high. Hyperlipidemia is also known as hyperlipoproteinemia since the lipoprotein must coexist with elevated levels of cholesterol and triacylglycerol in the plasma. As a result, high triglyceride levels represent increased levels of triglyceride-rich lipoproteins such as chylomicrons and VLDL, as well as their remains. A rise in cholesterol levels typically indicates an increase in LDL, which may or may not be accompanied by an increase in VLDL [1]. Hyperlipidemia, a significant systemic illness, is a modifiable risk factor for coronary heart disease and extracoronary atherosclerosis, as well as a higher risk of Cardiovascular Disease (CVD), which is the leading cause of death globally [2]. Several prospective studies have found that high-density lipoprotein cholesterol (HDL-C) content is inversely related to coronary heart disease. As a result, a

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low HDL-C level is a well-known risk factor for coronary heart disease [3]. BMI is now the most extensively used anthropometric test for predicting health risk connected to weight status, and several research have found a link between BMI and hyperlipidemia [2]. Hyperlipidemia is inherited polygenically in the majority of patients, and the disorder's expression is heavily impacted by factors such as (central) obesity, saturated fat intake, and the cholesterol content of a person's diet. Another process includes high levels of "apo B-100" lipoproteins in the blood, which can lead to atherosclerotic disease even if the patient does not have a family history of the condition. It is common for a person's risk of developing hyperlipidemia and cardiovascular disease to be influenced by a combination of hereditary and environmental variables [4]. Hyperlipidemia is linked to a number of causes, including hereditary, environmental, and lifestyle factors. Excessive alcohol use, smoking, high blood pressure, and other risk factors may all be managed. Secondary hyperlipidemia, also known as acquired hyperlipidemia, is associated to diabetes, renal failure, and alcoholism [5]. Vascular disease, which can be fatal if left untreated, is one of the most common complications of untreated hyperlipidemia. Coronary artery disease, peripheral artery disease, cerebrovascular accidents, aneurysms, type II diabetes, excessive blood pressure, and even mortality are examples of these complications [6]. Nigella sativa was used in traditional medicine for millennia, and a wide spectrum of chemical components discovered in N. sativa reflect its broad therapeutic properties. The seeds produce various alkaloids (the isoquinoline alkaloids like nigellicin and pyrazole alkaloids) [7]. Unsaturated fatty acids, including eicosadienoic acid (3%), oleic acid (20%), the dihomolinoleic acid (10%), saturated fatty acids palmitic acid linoleic acid, and the saturated fatty acids, for example, stearic acid (3%) are found in black cumin seeds. Crude fibre and vitamins, involving ascorbic acid, pyridoxine, thiamine, folic acid, and minerals like Fe, P, Ca, Zn, Na, and Cu have also been found in the seeds [8]. Furthermore, the seed oil has been used to separate free sterols, steryl glucosides, steryl esters, and acylated steryl glucosides [9]. Black cumin seed oil contains B-carotene (Pro-vitamin A) and tocopherol compounds, as well as phytosterols, such as the beta-sitosterol and, in lower levels, campesterol, stigmasterol, and lanosterol [10]. There are a total of four different reported phospholipid classes, including phosphatidylserine, therphophatidylinositol, phosphatidycholine, and phosphatidylethanolamine, among others [11].

METHODS

Two main procedures were used for the assessment, a) Kjeldhal method and soxhlet extraction procedure using petroleum ether AOAC processes and b) Interventional Non-randomized (Quasi Experiment) with pre-, post-testing. The proximate composition determined by using procedures at labs of University Institute of Diet and Nutrition Science, University of Lahore, Lahore. A total of 32 women, aged between 25-35 years with mild to moderate hyperlipidemia were selected for the 8 weeks of study. The sample was selected from the University of Lahore Teaching Hospital, Lahore. To determine the proximate composition and preparation of N. sativa supplementation black cumin seeds were purchased from local market. After removing physical contaminants like dirt, dust, and foreign grains, black seeds were air dried. The protein content of seeds was evaluated by kjeldhal method, fat content was analysed by soxhlet extraction of seed for 24 hrs using petroleum ether, and moisture fibre and ash content was assessed by AOAC procedures [18]. To prepare capsule, the seeds were ground into fine powder by using commercial blender and each capsule was prepared containing 500mg powder of N. sativa followed by transferring the product into air tight jars [12]. The participants who meet the study inclusion criteria were enrolled in the study. The baseline data was comprised of blood lipid profile (HDL, LDL, TC, and TG), anthropometric measurements (weight, height, & BMI) and 24-hour dietary recall. After screening the participants of experimental group, they were advised to use two capsules of N. sativa supplement before breakfast and two capsules in the afternoon prior to their meal for as long as 8 weeks [12]. Participants were also advised to follow the given 7-day diet plan for 8 weeks. The follow up for patients was conducted twice in a month. The anthropometric measurements of experimental group were collected in each follow up. Participants were asked for facing any constraints and barrier to follow the study procedure. After 8 weeks of study the same protocol of baseline, visitations were concluded for experimental groups. The baseline and post-test study data were compared to test the study hypothesis.

RESULTS

N. sativa seeds contain protein, fat, moisture, ash and carbohydrates with approximate percentage of 22.8%, 36.24%, 5, 41%, 3.30%, and 30.95% respectively. The percentage composition of the seeds is demonstrated in Table 1.

Macronutrien	%per 2g
Protein	22.8%
Fat	36.24%
Moisture	5.41%
Ash	3.30%
Carbohydrates	30.95%

Table 1: Proximate composition of Nigella sativa L. seed

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Table 2 exhibits the average age distribution trends in multiple hyperlipidemic patients that were enrolled to partook in the trial.

Age in Mean± SD	Minimum	Maximum
32.5±0.34	26	35

Table 2: Average age distribution of hyperlipidemic patients enrolled in study

Graphical demonstration was formulated for the demonstration of average socioeconomic status among the patients of hyperlipidemia that were selected for the trial. This graphical representation showed that 46.7% of participants belonged to upper middle class and 26.7% belonged to lower middle class, making the remaining patients belong to upper class.

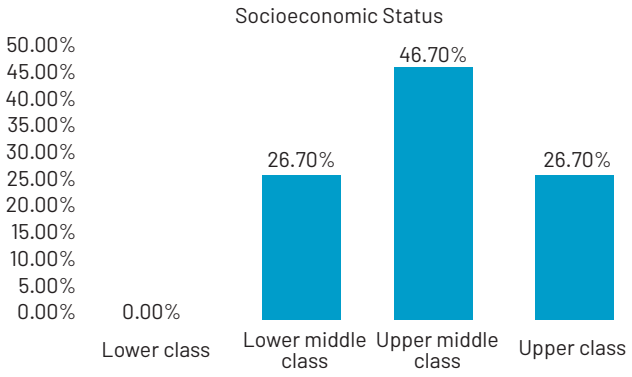

Figure 1: Average socioeconomic status among hyperlipidemic patients

The mean HDL of patients was 49.5±8.38mg/dl before the treatment, whereas the mean HDL after treatment was 51.81±10.21mg/dl. Findings showed statistically significant difference in pre- and post- treatment with p-values 0.001. The mean LDL of patients was 162.84±30.20mg/dl before the treatment, whereas the mean LDL after treatment was 158.84±22.56mg/dl. Findings showed statistically significant difference in pre and post treatment with p-values 0.001. The mean TC of patients was 227.81±11.95 mg/dl before the treatment, whereas the mean TC after treatment was 224.93±18.75mg/dl. Findings showed statistically significant difference in pre and post treatment with p-values 0.001. The mean TG of patients was 201.78±27.55mg/dl before the treatment, whereas the mean TG after treatment was 197±27.84mg/dl. Findings showed statistically significant difference in pre and post treatment with p-values 0.001, Table 3.

Macronutrien		Mean ± SD	Paired sample t-test (p-value)
High-Density Lipoprotein (HDL)	Pre	49.5±8.38	0.000
	Post	51.81±10.21	
Low-Density Lipoprotein (LDL)	Pre	162.84±30.20	0.001
	Post	158.84±22.56	
Total cholesterol (TC)	Pre	227.81±11.95	0.001
	Post	224.93±18.75	
Triglycerides (TG)	Pre	201.78±27.55	0.001
	Post	197±27.84	

Table 3: Comparison of average HDL, LDL, TC and TG pre- and post- treatment

DISCUSSION

It is estimated that more than half of all individuals in the United States have high LDL levels, with only about a third of those patients control their high LDL levels well, implying that the condition is undertreated [4]. In both the developed and developing countries, hyperlipidemia is the major cause of mortality, accounting for 16.7 million deaths each year [13]. Hyperlipidemia affects 63 percent of the Pakistani population. At least one major lipid-fraction, such as Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), or Triglycerides (TG), was abnormal in the study population. Low HDL-C was the most prevalent kind of isolated dyslipidemia (17.3 percent) [14]. There are many interventions that have done to improve hyperlipidemia and major influence have been given to the dietary interventions. Different plants have been approved to improve lipid ameliorating effects, and N. sativa is one of them. Existing analysis shows that active components in N. sativa improve many health complications, especially thymoquinone helps in prevention of lipid per oxidation and niacin improve weight management results. In another study Razamposh and his colleagues determine that. N. sativa capsules raised serum HDL cholesterol, decreased serum LDL cholesterol, and decreased the TC/HDL-C ratio [15]. In the present study the N. sativa seed powder capsule was prepared for the evaluation of proximate composition and its effective role in improvement of lipid profile among hyperlipidemic women visiting University of Lahore Teaching Hospital. The proximate analysis of N. sativa shows the presence of protein, fat, moisture, ash, and carbohydrates in percentage of 22.8%, 36.24%, 5, 41%, 3.30%, and 30.95%, respectively determined by extraction method used by Khoddami and his colleagues [18]. The formulation of seed powder capsule was developed by following the similar work performed by Ibrahim RM and his colleagues, on analysing the potential of therapeutic impact of N. sativa on different disease it has been evaluated that N. sativa helps to improve lipid profile [12]. In current study it has been evaluated that after consuming N. sativa seed powder capsules the lipid profile and antioxidants levels improved as compare to initial results. LDL- cholesterol level was reduced from 162.84±30.20 to 158.84±22.56mg/dl with p-value less than 0.05. Total cholesterol levels were also reduced (227.81±11.95 to 224.93±18.75) with p-value less than 0.005. In a randomised, double-blind, controlled study performed in Kerman, Iran, 20 physically inactive overweight females were categorized into two groups and given either 2g N. sativa supplementation (N. sativa capsules) and a placebo

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for eight weeks. During that time, both groups enrolled in an aerobic training programme (3 times per week). At the start of the study and at end of the eight weeks, blood lipids as well as VO2 max were measured. Supplementing with N. sativa reduced Total Cholesterol (TC), triglycerides, Low-Density Lipoprotein (LDL), and the body mass index while increasing HDL and VO2 max. A high-intensity aerobic training programme reduced TC and LDL while increasing VO2 max [19]. In another study the impact of crushed N. sativa on blood lipids, anthropometric factors and glucose homeostasis, in individuals with Hashimoto's thyroiditis were investigated by MA Farhangi and colleagues. After 8-week administration of N. sativa seed powder, blood concentrations of LDL cholesterol and triglyceride (TG) reduced with p value less than 0.05, whereas serum HDL cholesterol rise considerably [20]. In the present study the LDL cholesterol, Total cholesterol and Triglycerides were mitigated with p value less than 0.05 as well as the HDL cholesterol of patient considerably increase from 49.5±8.38 to 51.81±10.21 with p value less than 0.05.

CONCLUSION

The study concluded that N. sativa L. seed powder supplementation showed a significant improvement in hyperlipidemic condition. The study found an increase in high density lipoprotein among patients.

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