

Hypolipidaemic Effect of Alpha-Tocopherol and *Nigella Sativa* Seed on Serum Lipid Profile of Albino-Rats Fed from Atherogenic Diet

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Abstract

The α -tocopherols (α -TP) or vitamin E are essential vitamin antioxidant. Its consumption is reported to reduce the incidence of cardiovascular disease. α -TP increases the biological activity of nitric oxide (NO); which inhibits the proliferation of vascular smooth muscle and moreover inhibits the platelet aggregation. The proposed under *Nigella sativa* (NS) seed, popularly known as “black seed”, had long been used as food condiment and flavoring agent. Its medicinal properties are known since historical times and have found their role to develop different remedies in Greek, Arabic and Chinese medicinal systems. *Nigella sativa* (NS) seed, popularly known as “black seed”, had long been used as food condiment and flavoring agent. Its medicinal properties are known since historical times and have found their role to develop different remedies in Greek, Arabic and Chinese medicinal systems. Various studies had reported different biological activities and effects as immune stimulatory, hypoglycemic effect, anti-inflammatory effects and anti-hypertensive [4] and anticancer activity Lipid lowering effects of has been reported by Kocyigit. Various studies had reported different biological activities and effects as immune stimulatory, hypoglycemic effect, anti-inflammatory effects and anti-hypertensive and anticancer activity [5]. Lipid lowering effects of has been reported by Kocyigit. Free fatty acids, cholesterols, triglycerides and phospholipids are considered the major blood lipids of human blood including some lipid soluble substances such as steroids, steroid hormones and fat soluble vitamins E, A, D, K. As the lipids are water insoluble hence, in the blood, they are present in form of emulsions as chylomicrons, or combined with proteins as lipoproteins [8]. Lipoproteins are of five types named chylomicrons remnants, VLDL, IDL, LDL and HDL [9]. Abnormally elevated blood lipoproteins are termed as hyperlipidemia and hypercholesterolemia. Dietary intake of polyunsaturated fatty acids and vitamin E reduced the risk of atherosclerosis and lipid peroxidation in smokers. Ingesting saturated fats raised the total serum cholesterol and ingestion of mono unsaturated fat increased HDL-c and polyunsaturated oil reduced cholesterol and increased HDL-c.

Aim of Study: First Insight Study of Hypolipidaemic Effect of Alpha-Tocopherol and *Nigella Sativa* Seed On Serum Lipid Profile of Albino-Rats Fed from Atherogenic Diet.

Study Design: Experimental Study.

Place and Duration of Study: Pathology Department, Post Graduate Medical Institute, Lahore August 2015 to January 2016.

Methodology: 50 male rats selected in 5 groups for 8 weeks given atherogenic diet with alpha-tocopherol and *Nigella saiva*, lipid profile determined and data analysed on SPSS 21.0 using one way ANOVA & post Hoc Tukey s test with P-value of significance at < 0.05.

Results: Present study evaluated the combined affects of alpha-tocopherol and *Nigella sativa* on lipid profile in experimental rats fed on atherogenic diet which shows significance differences at baseline and week 8. Serum HDL-c was elevated and serum cholesterol, serum triglyceride and serum LDL-c was significant reduction.

Conclusion: In present study, the *Nigella sativa* and vitamin E showed hypolipidemic effects. The present study concludes that the *Nigella sativa* combined with vitamin E (alpha-tocopherol) may prove effective in controlling blood lipids and preventing atherosclerosis.

Keywords: *Nigella sativa* and *Nigella saiva*; Alpha-Tocopherol; Atherogenic Die; Lipid; Rats

Introduction

Coronary artery disease (CAD) has already become a common type of heart disorders. CAD is the most frequent single cause of premature deaths in America, Russia, Europe, New Zealand, Australia and other countries. If estimated carefully, the CAD is proposed to be the major cause of mortality throughout the World by the year 2020 [1].

Pathophysiology of CAD involves atheroma formation (atherosclerosis) which is a lipid plaque. Atheroma may be deposited in any artery of human body with a high predilection of coronary arteries. Coronary artery atherosclerosis usually presents clinically as angina pectoris, ischemic heart disease, cardiac arrhythmias, and sudden deaths. Reported mortality of CAD disease is highly variable and is prevalent in European countries. In UK the mortality of CAD is reported as 1 of 3 males and 1 of 4 females dies because of ischemic cardiac events [1].

The α -tocopherols (α -TP) or vitamin E are essential vitamin antioxidant. Its consumption is reported to reduce the incidence of cardiovascular disease. α -TP increases the biological activity of nitric oxide (NO); which inhibits the proliferation of vascular smooth muscle and moreover inhibits the platelet aggregation. The proposed underlying mechanism of above mentioned effects is the inhibition of protein kinase C by the α -TP through increased biological activity of nitric oxide. This inhibition may prove beneficial in the setting of atherosclerosis. Previous study reported that the persons taking 100 IU of Vitamin E daily for > 2 years lowers the rates of coronary events and progressions of coronary atherosclerotic lesions and CVD and CAD [2].

Nigella sativa (NS) seed, popularly known as “black seed”, had long been used as food condiment and flavoring agent [3]. Its medicinal properties are known since historical times and have found their role to develop different remedies in Greek, Arabic and Chinese medicinal systems. Various studies had reported different biological activities and effects as immune stimulatory, hypoglycemic effect, anti-inflammatory effects and anti-hypertensive [4] and anticancer activity [5] Lipid lowering effects of has been reported by Kocyigit [6].

Free fatty acids, cholesterol, triglycerides and phospholipids are considered the major blood lipids of human blood including some lipid soluble substances such as steroids, steroid hormones and fat soluble vitamins E, A, D, K [7]. As the lipids are water insoluble hence, in the blood, they are present in form of emulsions as chylomicrons, or combined with proteins as lipoproteins [8]. Lipoproteins are of five types named chylomicrons remnants, VLDL, IDL, LDL and HDL [9]. Abnormally elevated blood lipoproteins are termed as hyperlipidemia and hypercholesterolemia. Dietary intake of polyunsaturated fatty acids and vitamin E reduced the risk of atherosclerosis and lipid peroxidation in smokers. Ingesting saturated fats raised the total serum cholesterol and ingestion of mono unsaturated fat increased HDL-c and polyunsaturated oil reduced cholesterol and increased HDL-c [10].

Aforementioned literature suggested that both tocopherols and *Nigella sativa* seed have been demonstrated to lower the lipids. The study may help clinicians and patients equally, to prevent or delay the development of atherosclerosis and coronary artery disease.

Aim of Study

First Study of Hypolipidaemic Effect of Alpha-Tocopherol and *Nigella Sativa* Seed on Serum Lipid Profile of Albino-Rats Fed from Atherogenic Diet.

Methodology

The present experimental study was conducted at the Pathology Department, PGMI, Lahore over 1 year duration. A sample of 50 male rats were selected through simple random sampling. In inclusion criteria rats of age 8 weeks and weight between 150 to 200 grams and in exclusion criteria those showing sign of any disease. Animals were divided into 5 groups, each group contain 10 rats. Group A (Negative Control Group) fed regular rat chow throughout study, Group B (Positive Control Group) fed atherogenic diet for 8 weeks, Group C fed atherogenic diet and alpha tocopherol for 8 weeks, Group D fed atherogenic and *Nigella sativa* seed and Group E fed atherogenic diet and alpha tocopherol and *Nigella sativa* by oral rout in morning All rats were fed on atherogenic diet for 08 weeks except negative control group. Atherogenic diet contained cholesterol 1.5 gm, sodium cholate 1 gm, and coconut oil 8 ml in 100 gram of ragular rat chow [11].

Rats were anaesthetized and sampling was done by cardiac puncture. Following lipid parameters were determined by enzymatic method using microlab chemistry analyzer. Total cholesterol, Triglycerides, LDLc, HDLc.

Statistical Analysis

The data after collection was processed by using Statistical Package for Social Sciences (SPSS 21.0). Application of one way ANOVA to determine the mean difference in SC, TAG, LDL-c, HDL-c among different groups. Application of post Hoc Tukey’s test for multiple comparisons. A P value ≤ 0.05 was considered significant.

Results

The present study evaluated the individual and combined effects of α -tocopherol (α -TP) and *Nigella sativa* seed on the blood lipid profile in experimental rats fed on atherogenic diet. SC, TAG, LDL-c and HDL-c were checked in beginning termed as baseline values, and on experimental week 8 respectively.

Baseline findings SC, TAG, LDL-c and HDL-c at baseline are shown in table I and graphs 1 - 5 respectively. Animals in 5 groups; A to E showed no differences for the SC, TAG and LDL-c as shown by the statistical analysis. However, HDL-c showed significant differences by ANOVA & post Hoc Tukey’s test showed p-value of 0.024. This reveals that our experimental animals under study were SC, TAG, LDL-c matched, except for the HDL-c. The HDL-c ranged from 40.40 to 46.70 among 5 groups ($p = 0.024$).

Week 8 findings SC, TAG, LDL-c and HDL-c at week 8 are shown in following table. Serum cholesterol was raised in Groups B, C, D and E. Serum cholesterol at week 8 showed statistically significant differences compared to baseline findings as below.

Serum cholesterol (mg/dl)	Baseline vs. Week 8	
Group A. Negative controls	121.10	115.50
Group B. Positive controls	121.30	284.60
Group C. α -tocopherol	124.90	242.70
Group D. <i>N sativa</i>	119.40	207.10
Group E. Combined (α -tocopherol+ <i>N. sativa</i>)	123.40	185.90

Most severe rise in serum cholesterol was noted in Positive control group B (serum cholesterol 284.60mg/dl). 8th week inter group showed much decrease in *N. sativa* group D and combined α -tocopherol+ *N.sativa* group E experimental rats.

Serum TAG showed a rise at week 8 compared to baseline. Groups B, C and D showed a rise in TAG compared to group E. Serum TAG shows significant difference at week 8 compared to baseline are as under.

Serum Triglycerides (mg/dl)	Baseline vs. Week 8	
Group A. Negative controls		
Group B. Positive controls	79.20	431.60
Group C. α -tocopherol		339.70
Group D. <i>N sativa</i>		302.70
Group E. Combined (α -tocopherol+ <i>N. sativa</i>)		236.90

Inter group comparison of TAG at week 8 shows significant lipid lowering of α -tocopherol + *N. sativa* (Group E). Also the *N. sativa*-group D showed a reduction in serum TAG.

Serum LDL-c showed most significant rise in group B- positive control rats. Serum LDL-c at week 8 showed significant rise compared to baseline shown as below in table 3.

Serum LDL-c (mg/dl)	Baseline vs. Week 8	
Group A. Negative controls	94.30	106.70
Group B. Positive controls	90.00	205.80
Group C. α -tocopherol	91.00	168.80
Group D. <i>N sativa</i>	92.30	150.10
Group E. Combined (α -tocopherol + <i>N. sativa</i>)	93.30	115.00

Inter group comparison of LDL-c at week 8 shows lipid lowering effect of *N. sativa*. However, combined α -tocopherol + *N. sativa* (Group E) significant decrease compared to other groups.

Serum HDL-c showed a severe reduction in Groups B, C and D. Group E showed a rise in HDL-c. Serum HDL-c at week 8 showed significant differences compared to baseline, shown as under in table 4.

Serum HDL-c (mg/dl)	Baseline vs. Week 8	
Group A. Negative controls	43.00	45.11
Group B. Positive controls	46.70	24.40
Group C. α -tocopherol	40.90	31.50
Group D. <i>N sativa</i>	40.90	38.50
Group E. Combined (α -tocopherol + <i>N.sativa</i>)	40.40	47.40

Inter group comparison of HDL-c at week 8 shows much elevation in Group E. Group E showed a boosting effect on HDL-c at baseline - 40.40 and week 8 - 47.40 mg/dl. Hence combined α -tocopherol + *N. sativa* showed better effects on serum HDL-c.

Discussion

The present experimental study evaluated the effects of α -tocopherol (α -TP) and *Nigella sativa* seed on the blood lipids rats fed on atherogenic diet. SC, TAG, LDL-c and HDL-c were checked in beginning termed as baseline values, and on experimental weeks 8 respectively. *Nigella sativa* (NS) seed, popularly known as "black seed", had long been used as food condiment and flavoring agent [3]. Its medicinal properties are known since historical times and have found their role to develop different remedies in Greek, Arabic and Chinese medicinal systems [4]. Lipid lowering effects of has been reported by Kocyigit [6]. Vitamin E as tocopherols is a complex family of diverse molecules collectively termed as Tocopherols. Another family within Vitamin E includes that of the tocotrienols. Each having 4 isomers labeled as alpha, beta, gamma and delta [12]. Of the tocopherol and tocotrienols analogues, the α -tocopherols show the highest biological activity [13], possible because of its maximum blood levels. Other tocopherols are absorbed either less or not at all [14]. A previous study demonstrated and reported protective effects of α -TP against the hypercholesterolemia which is a predisposing factor for atherosclerosis [15].

Atherosclerosis is a chronic inflammatory process that is characterized by the formation of plaques consisting of foam cells, immune cells, vascular endothelial cells, smooth muscle cells, platelets, extracellular matrix, and a lipid-rich core with extensive necrosis and fibrosis of surrounding tissues [16]. Hyperlipidemia is an important risk factor for cardiovascular disease. Its major role in the pathogenesis of atherosclerosis has been implicated by several clinical and epidemiological studies [17].

It is evident that most of the hypocholesterolemic drugs must be used for several weeks to be effective. This may expose patients to several side effects, especially liver injury [18]. *Nigella sativa* seeds has been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world [16]. It is a widely investigated herb for use as medicine. It has been traditionally used as medicine in Mediterranean region, Asia and Africa. The usefulness of *Nigella sativa* has been mentioned in various Ahadees-e-Nabvi. The prophet (PBUH) said, "Hold onto the use of the black seed for it has a remedy for every illness except death". Different scholars reported *Nigella sativa* as a beneficial medicinal herb like Al- Biruni, Ibne-Sina and Greek physician Dioscoredes. *Nigella sativa* and α -tocopherol showed significant reductions in the blood lipids- the cholesterol, triglycerides, LDL-c and HDL-c [19]. Major rise in serum cholesterol was noted in Positive control group B (serum cholesterol 410.40 mg/dl). Cholesterol showed non-significant increase in *N. sativa* group D and combined α -tocopherol + *N. sativa* group E experimental animals at week 8th.

Serum TAG showed a rise at week 8 compared to baseline ($p = 0.0001$). Serum TAG at week 8 compared to baseline showed significant differences (shown in table I and II) ($p = 0.0001$).

Serum LDL-c showed most significant rise in group D (*N. sativa*) animals. Serum LDL-c at week 8 showed significant rises compared to baseline values as shown in table I and II ($p = 0.0001$).

Serum HDL-c showed a severe reduction in Group A- negative control animals. Groups B, C, D and E showed a rise in HDL-c. Serum HDL-c at week 8 showed significant rises compared to baseline and values shown in table I and II ($p = 0.0001$).

The findings of present study are comparable to previous studies cited in the medical literature [16].

El-Morsy has reported a recent study that Black cumin (*Nigella sativa*) was used to study the effect on induced atherosclerosis in cholesterol-enriched diet fed rabbits. 30 male rabbits were fed atherogenic diet. Black cumin powder was given orally for 20 weeks at dose of 150 mg/kg body weight /day. End points of study were reported that the black cumin inhibited the atherosclerosis in aorta. A reduction in triglycerides, total-cholesterol (TC), LDL-c was observed, the findings are comparable to our present study. HDL-c was reported to rise which is also a supporting finding to present study. El-Morsy concluded that the black cumin has anti atherosclerosis and hypolipidemic potential. We are of opinion that the *N. sativa* reduces bad cholesterol (LDL-c) and increases HDL-c as is noted in present study also and is a consistent finding to El-Morsy [20].

Ahmed [21] has reported an experimental study on the effects of vitamin A, vitamin C and Vitamin E on the lipid profile and cardiovascular pathophysiology in salt-loaded albino rats. The results indicated a significant increase ($p < 0.05$) in pulse rate, total cholesterol (TC) and LDL cholesterol and a significant decrease in HDL-cholesterol and no change in Angiotensin converting enzyme (ACE) activity in negative control animals. But the animals which were treated with vitamins including vitamin E showed a significant reduction in pulse rate, TC and LDL-cholesterol and a significant rise in HDL-c. It was concluded that the anti oxidants like vitamin E have good effects on the blood lipids. The finding of Ahmed is a comparable finding to present study as we have noted a significant reduction in triglycerides, serum cholesterol and LDL-c in α -tocopherol treated rats.

Therefore, the present study concludes the *Nigella sativa* along with vitamin E (α -tocopherol) may be very good combination in controlling blood lipids and preventing atherosclerosis. The present study reports *Nigella sativa* and vitamin E show hypolipidemic effects. Further studies are warranted to confirm the findings of present study. *Nigella sativa* may be good treatment option of herbal origin for treating hyperlipidemia; however this needs further exploration of the underlying mechanisms at molecular level to reach to a proper conclusion.

Conclusion

In present study, the *Nigella sativa* and vitamin E (α -tocopherol) showed hypolipidemic effects. The present study concludes the *Nigella sativa* combined with vitamin E (α -tocopherol) may prove effective in controlling blood lipids and preventing atherosclerosis.

Outcome and Utilization

This α -tocopherol and *Nigella sativa* is easily available and cheap material. They can be a good substitute to the costly and conventional treatment of hyperlipidemia.

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