Effect of Atorvastatin and Black Seed (Nigella Sativa) in Experimentally Induced Hypercholesterolemia in Rabbits

Suhrita Paul[1]
Kalyan Kumar Paul[2]
Sobhan Palodhi[3]
Sikha Dutta[4]

[1] Associate Professor, Department of Pharmacology, Medical College, Kolkata, India
[2]Chief Medical Officer, ESIC Hospital & ODC(EZ), Joka, Kolkata, India
[3]Demonstrator, Department of Pharmacology, Medical College, Kolkata, India
[4]Professor & Head, Department of Pharmacology, Medical College, Kolkata, India

Summary

Cholesterol lowering ability of finely powdered seeds of Nigella sativa(NS) was evaluated in comparison to atorvastatin, the most frequently used lipid lowering agent. Hypercholesterolemia was induced to the animals with cholesterol powder (50mg/kg/day) for the study duration (16 weeks). At the end of 4 weeks, they were randomly selected and divided into 3 groups (n=6). Group II received Cholesterol + atorvastatin (10 mg/day); Group III received Cholesterol + NS(5G/kg/day) while Group I continued with cholesterol powder (to serve as control) for the rest study period. Serum cholesterol, LDL, HDL and Triglycerides (TG) were estimated using the enzymatic method at 0, 4, 8, 12, 16 weeks in all the groups. The results were tabulated and analyzed statistically using one way ANOVA test. The results indicate that both atorvastatin and Nigella sativa have a definite role in retarding the rate of weight gain as a consequence to high cholesterol diet in rabbits. Also, there is fewer rises in all the lipid parameters in both the treatment groups when compared to the control group. Though atorvastatin is definitely more effective in reducing the lipid parameters, it also significantly lowers HDL where as NS shows promising results when compared to placebo and also has a favourable effect on HDL. NS is commonly used as food additive and has a wide range of medicinal properties. It can be recommended as a dietary supplement for long term use without toxic effects in the primary prevention of hypercholesterolemia and atherosclerosis and also has therapeutic potential in patients with coronary artery disease.

***Key Word*:** Hypercholesterolemia, Nigella sativa, atorvastatin

Introduction

The consistent observation that human atherosclerosis is prominent in clinical states accompanied by hypercholesterolemia have strongly implicated cholesterol as an important etiological agent in the development of atherosclerosis[1] and associated conditions like coronary artery disease, ischemic cerebrovascular diseases etc. Many drugs are used in the treatment of hypercholesterolemia of which the statins are most frequently prescribed. Statin-mediated lowering of low-density lipoprotein cholesterol (LDL -C) is regarded as the foundation of lipid-modifying therapy as among the lipoproteins, LDL cholesterol has been found to be directly associated with CHD.[2] However, this has failed to reduce cardiovascular event rates by more than about 20%–40% relative to placebo[3] indicating the need for therapeutic intervention against other lipid targets as well as non-lipid risk factors. Moreover, the potential side effects limit long term use of these drugs. Lifestyle modification and pharmacological measures must be taken together to achieve the target. In recent years there is ongoing search for natural substances that can combat hypercholesterolemia.

Nigella sativa L., commonly known as black seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world.[4] Studies on *N. sativa* seed and its oil have provided scientific support for the traditional use of the seed and its oil for treatment of rheumatism, immune stimulation, diabetes, cancer and related inflammatory diseases.[5] *Nigella sativa* seeds oil and thymoquinone have been shown to have a hypocholesterolemic activity in rats.[6] Although much work has been done on crude extract of NS and its constituents, NS seeds are customarily consumed either in their intact or powdered form. Therefore, the present study was undertaken to evaluate the cholesterol lowering ability of *N. sativa* powder in aqueous suspension in comparison to atorvastatin, the most frequently used lipid lowering agent in experimentally induced hypercholesterolemia in rabbits.

Material and Methods:

Animals:

Rabbit has been used as experimental animal in studies related to hypercholesterolemia and subsequent atherosclerosis since 1913[7]. Healthy male rabbits weighing between 1- 1.25 kg, 6 months to 1 year of age were used for this experiment. The rabbits were kept in individual polysulfone cage with wire mesh bottom. They received normal diet containing Bengal gram, green grass, one tea spoonful maize flower and drinking water at libitum. The temperature was maintained at 22 - 26^{o}C while the relative humidity was 50-60%. The experiment was conducted in the Department of Pharmacology, MGM Medical College & Hospitals, Jamshedpur in collaboration with Department of Pharmacology, Medical College, Kolkata. The experiments were performed following approval by the Institutional Animal Ethics Committee.

Drugs and Chemicals:

Cholesterol powder
Olive oil
Atorvastatin-10mg (Lupin)
Finely ground powder of Nigella sativa(NS)
Gum acacia
Distilled water

Plant material and preparation of aqueous suspension

Nigella Sativa seeds were purchased from a local herb dealer, identified under expert guidance and preserved for future reference. The seeds were dried and ground to a very fine powder and suspended in 1% gum acacia in distilled water and preserved for use in different experimental procedures.

Procedure:

All the rabbits (n=18) received normal diet for ***pre experimental period*** of 4 weeks for adaptation. At the end of this period, animals were weighed, and fasting blood samples were taken from the marginal ear vein for estimation of serum lipids.

Total ***experimental period*** of study was 16 weeks. Hypercholesterolemia was induced to the animals by feeding them with cholesterol powder 50 mg/kg dissolved in 5 ml of olive oil daily for the whole duration of study (16 weeks). At the end of 4 weeks, they were randomly selected and divided into 3 groups (n=6). Group II received Cholesterol + atorvastatin 10 mg/day (10 mg tablets were powdered and mixed with 5ml of glycerine and fed with the help of a canula to the animals); Group III received Cholesterol + 5G NS/kg/day (suspended in 5 ml distilled water with 1% gum acacia and fed with a canula) and Group I continued with cholesterol powder (to serve as control) for the rest study period of 12 weeks. . After the end of study, all the drugs were withdrawn, and the rabbits were kept on normal diet for a convalescent period of six months before considering them for any other study.

Serum cholesterol, LDL, HDL and Triglycerides were estimated using the enzymatic method at 0, 4, 8, 12, 16 weeks in all the groups. The results were tabulated and analyzed statistically using one way ANOVA test.

Results

There was a steady weight gain in the rabbits of all the groups as they were fed with cholesterol diet. The increase was less in Group II (At) and Group III(NS) in comparison to Group I, although Group II & III were comparable. (Table I). There was a steady rise in all the lipid parameters in Group I, II and III but the rise was significantly less in treatment groups II & III. Comparing Group II and III, atorvastatin more effectively reduced the cholesterol, LDL, TG levels than NS while the HDL level was more in Group III(chart 1-5)

Table I:

Mean body wt (0, 4, 8, 12 & 16 weeks) of the groups I, II, III (n=6)

	weight in grams (mean± SD)				
	0	4	8	12	16
Group I	1077 ± 60.36	1138 ± 64.13	1219 ± 53.97	1254± 45.44	1289 ± 56.46
Group II	1082± 69.49	1115 ± 70.31	1149± 73.93	1172 ± 74.11	1202 ± 73.27
Group III	1069 ± 47.33	1123 ± 59.6	1166± 57.9	1202± 58.8	1232± 71.1

Cholesterol Powder	Cholesterol	LDL	TG	HDL
Baseline	48.33± 4.13	26.08± 4.13	32.7 ± 4.8	12.4 ±1.3
4weeks	129.5± 6.1	78± 6.33	87.7±4.1	27.9 ±12.9
8weeks	165.4± 5.3	107.12± 8.5	125.8±5.1	33.9
12 weeks	193.6± 7.2	119.8± 7.5	139.9±6.2	41.2±4.1
16 weeks	213.3± 6.6	131.6± 4.9	147.9± 4.33	43.6±6.3

Table II:

Lipid profile for 16 weeks in experimentally produced hypercholesterolemia in rabbits

Table III:
Effects on the lipid profile of Atorvastatin 2 mg for 16 weeks in experimentally produced hypercholesterolemia in rabbits (n=6)

Ator 2mg + Chol powder	Cholesterol	LDL	TG	HDL
Baseline	49.1± 4.8	25.08± 5.8	33.7 ± 4.8	12.6 ±1.3
4weeks	93.1± 5.8	48± 3.53	57.7±4.1	24.9 ±12.9
8weeks	108.4± 6.3	57.12± 4.5	65.88±5.4	30.3±3.8
12 weeks	128.2± 4.18	63.7± 6.8	76.9±6.2	33.2±4.1
16 weeks	137.6± 3.48	68.6± 4.56	83.4± 4.33	37.9±6.3
P value	<0.0001	<0.0001	<0.0001	0.93

Table IV
Effects on the lipid profile of Nigella sativa (NS) in experimentally produced hypercholesterolemia in rabbits (n=6)

Chol+ NS	Cholesterol	LDL	TG	HDL
Baseline	47.2± 5.8	26.01± 2.7	37.7 ± 4.8	11.6 ±1.3
4weeks	89.8± 4.2	53.84± 4.22	67.7 ± 4.1	23.9 ± 2.9
8weeks	119.4± 3.5	65.93± 6.3	77.2±3.9	32.1±4.8
12 weeks	143.5± 6.2	78.4± 5.7	89.22±5.19	39.3±5.1
16 weeks	156.9± 4.23	83.8± 4.8	98.67±6.2	41.4±3.2
P value	<0.0001	<0.0001	<0.0001	0.61

Chart I showing the mean increase in body wt. in the Three Groups

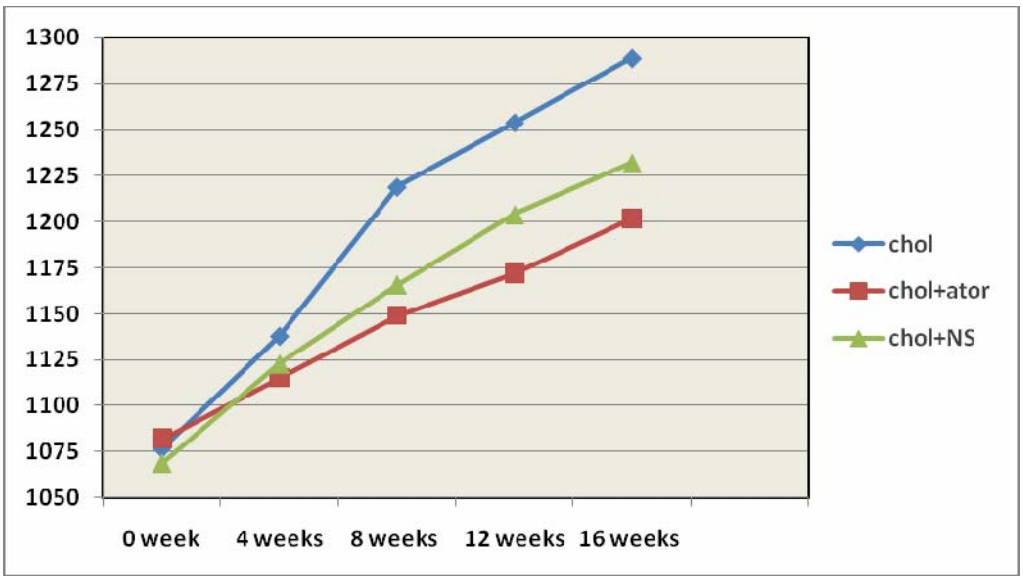


Chart II showing the cholesterol level in the three groups for 16 weeks

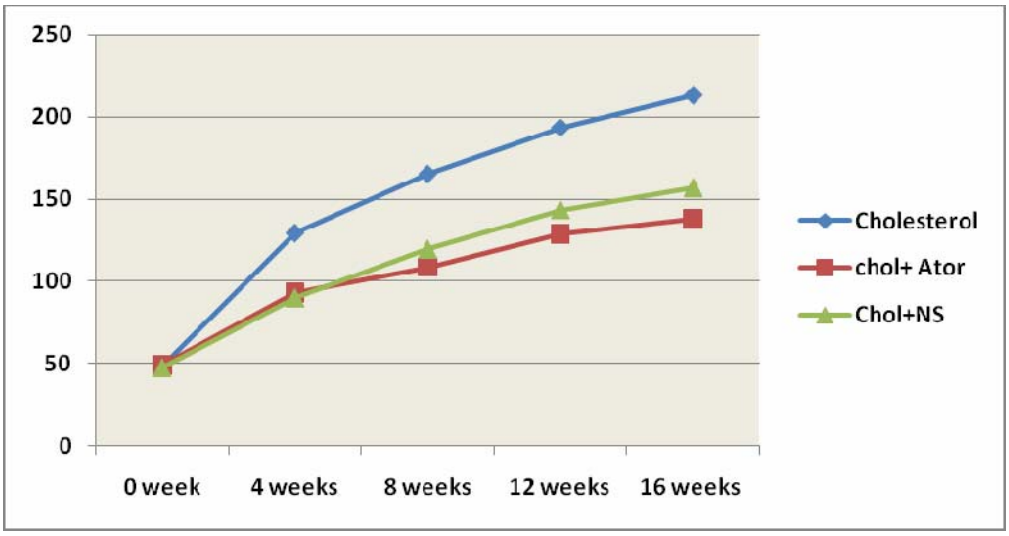


Chart III showing the LDL value in the three groups for 16 weeks

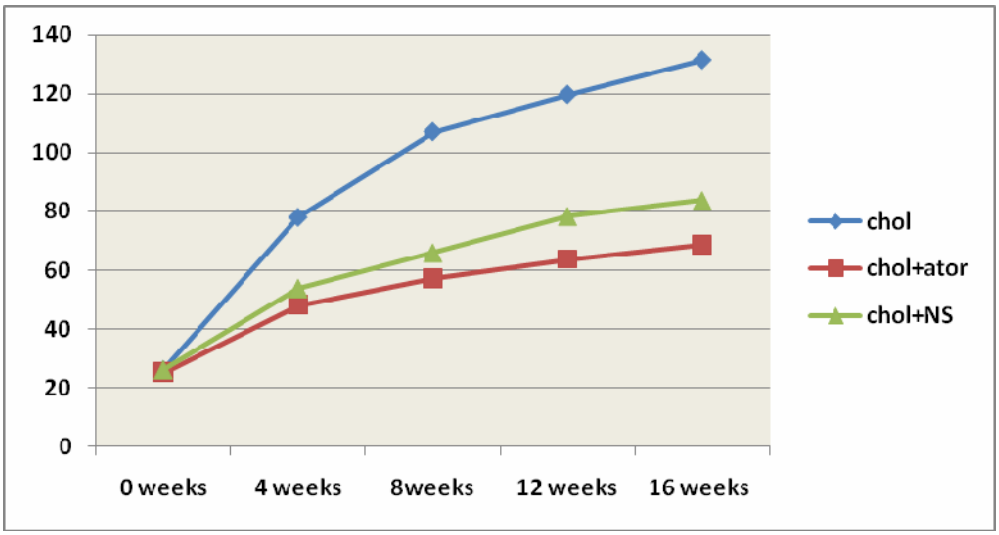


Chart IV showing the TG value in the three groups for 16 weeks

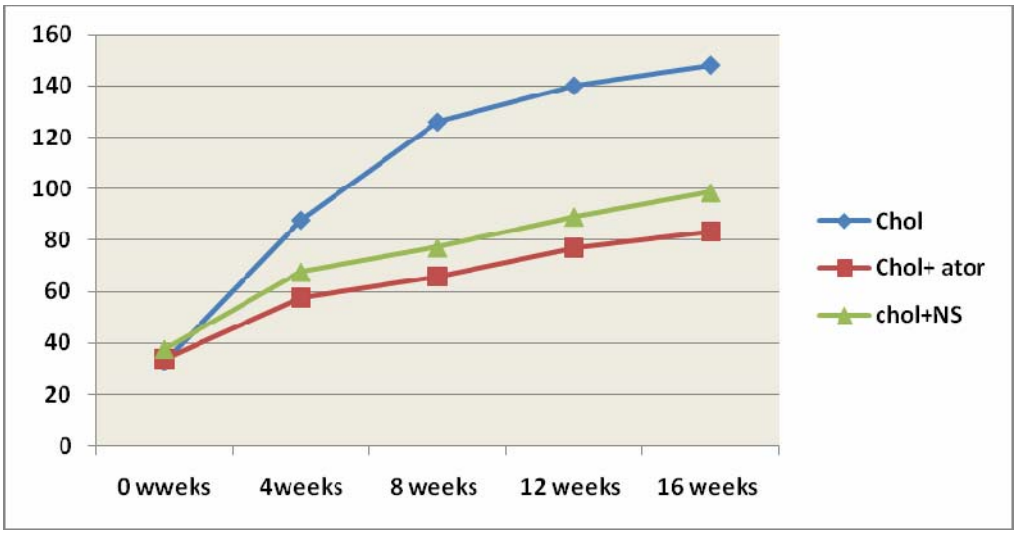
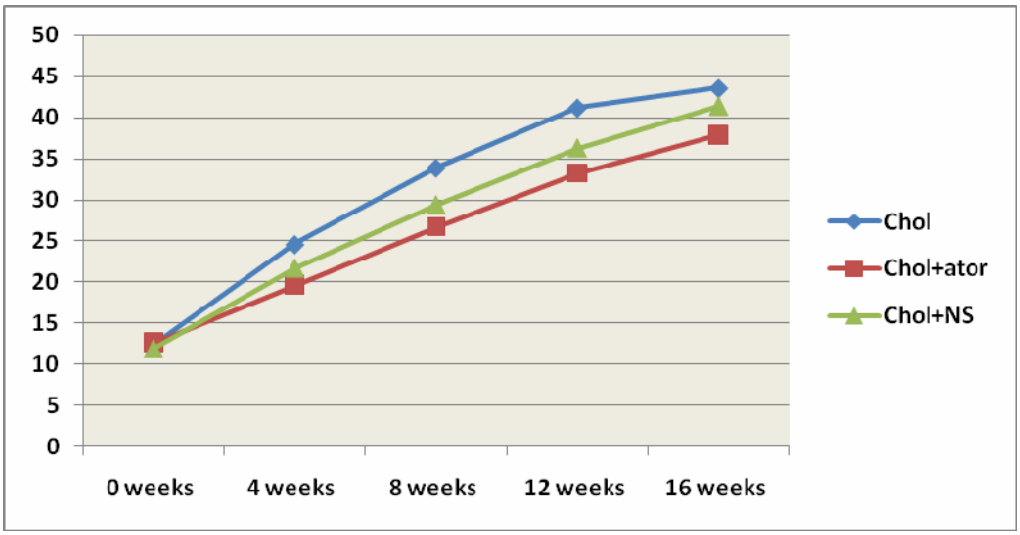


Chart V showing the HDL value in the three groups for 16 weeks



Discussion

Sedentary life style, urbanization, industrialization, alcohol, cigarette smoking etc increases the incidence of hypercholesterolemia and consequently atherosclerosis and CHD. So attempts are being made to find safe and effective drugs and dietary factors that would lower blood cholesterol levels. All the currently available hypolipidaemic drugs have significant side effects and potential toxicities without defined benefits. They should be reserved for patients with familial hyperlipidemia or others at elevated risk of CHD. Even in these patients the drug should be discontinued after one to two months if the serum cholesterol is not reduced by at least an additional 10% of the value achieved by dietary modification alone.[8]

The statins (atorvastatin, rosuvastatin, lovastatin, simvastatin etc) are a class of hypolipidaemic drugs that act by inhibiting HMG Co-A reductase, the rate limiting enzyme in the mevalonate pathway of cholesterol biosynthesis. Inhibition of this enzyme in the liver stimulates LDL receptor resulting in an increased clearance of LDL from blood and decrease in cholesterol level by 30-50%. They also reduce the TG level but the rise of HDL level is less than fibrates and nicotinic acid (other hypolipidaemic drugs). The NCEP Guidelines state that diet and lifestyle modification should be followed before staring statins or any other hypolipidaemic agents.[9] Adverse effects of statins are myalgia, muscle cramps and G.I. Symptoms, myositis and rarely rhabdomyolysis (pathological breakdown of skeletal muscles) which leads to acute renal failure. The risk of myopathy and rhabdomyolysis increases in proportion to the plasma Statin concentration. [10]

Nigella sativa or Black seed is an annual herbaceous plant that belongs to the Ranuculacea family. The seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world.[11] The seeds are considered as a natural food additive and a condiment typically consumed mixed with honey, and in baking products or pastries. Many active ingredients have been isolated from *N. sativa*, including: thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine and alpha-hedrin.[12] In addition, many pharmacological effects of *N. sativa* and its active principles have been identified, such as immune stimulation, anti-inflammatory, anti-cancer and antimicrobial activity. [11]

The results show that there was a steady weight gain in the rabbits of all the groups as they were fed with cholesterol diet. The increase was less in Group II(At) and Group III(NS) in comparison to Group I, although Group II & III were comparable. (Table I). This is similar to the study of Le et al (2004)[13] where *Nigella sativa* treatment of rats (2 g kg^{-1} day^{-1} of the original seed for 1 week) reported to cause a reduction in the body weight accompanied by significant and sustained reduction in food intake. Significant elevation in plasma TC and LDL levels and slight decrease in plasma HDL levels were used as indicators of hypercholesterolemia resulted from feeding rabbits cholesterol supplemented diet. These findings were in the same line as with those results reported by Prasad.[14] In addition, the significant reduction of TC and LDL levels and enhancement of HDL levels due to *N. sativa* treatment are in agreement with the previous studies as reported by El-Dakhakhani *et al* who found that feeding rats with *N. sativa* oil (800 g kg^{-1} day^{-1}) orally for 4 weeks caused significant decreases in the serum LDL and TG levels, and an elevation of serum HDL levels. [4] Oil extracted from *N. sativa* seeds is rich in unsaturated fatty acids, which could be responsible for the decrease of TC and LDL cholesterol levels as reported by other researchers.[14] The hypocholesterolemic effect of *N. sativa* seeds and their oil could be attributed to the seeds contents of total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) as observed by Al-Nageeb *et al.*[15] In addition, it was found that several dietary fibers significantly decrease plasma cholesterol levels in human subjects and thereby may reduce the risk of coronary heart diseases.[16] The present study demonstrated that finely powdered seeds of Nigella sativa (5 g kg^{-1} day^{-1}) significantly decreased TC and LDL levels when compared to control group over the treatment period and these findings are in agreement with those results obtained by other researchers[17]. When compared to NS atorvastatin is definitely more effective in reducing the lipid parameters but it also significantly lowers the good cholesterol, i.e. HDL. Though NS is less efficacious than atorvastatin, it shows promising results when compared to placebo and also has a favourable effect on HDL. NS is commonly used as food additive and has a wide range of medicinal properties. It can be recommended as a dietary supplement for long term use without toxic effects in the primary prevention of hypercholesterolemia and atherosclerosis and also has therapeutic potential in patients with coronary artery disease. However further studies need to be done including clinical trials for further exploration of the medicinal prospects of NS.

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