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AN IN VIVO ASSESSMENT OF ANTIHYPERLIPIDEMIC EFFECT OF *NIGELLA SATIVA* ON HIGH FAT INDUCED HYPERLIPIDEMIC RAT MODEL

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ABSTRACT

Seeds of *Nigella sativa* is one of the well-known seed that is used for treatment of hyperlipidemia traditionally. In our study, high fat induced hyperlipidemia in rats were treated with *Nigella sativa* extract with a dose of 500 mg/kg in one group of rats and atorvastatin with a dose of 50 mg/kg in another group. We measured total cholesterol, Triglyceride, HDL and LDL level of rat. It was found that the hypolipidemic efficacy of *Nigella sativa* was comparable to that of atorvastatin ($p > 0.05$). It was seen that both atorvastatin and seed of Nigella sativa improved the pathological condition induced by high fat. Furthermore, in healthy individual rats, both atorvastatin and seed extract of *Nigella sativa* did not significantly alter normal physiological state. It can, therefore, be inferred that seed oil of Nigella sativa could be utilized as a good alternative therapy to treat hyperlipidemia.

KEYWORD: *Nigella sativa*, atorvastatin, hyperlipidemia, total cholesterol, HDL, LDL, triglyceride.

INTRODUCTION

Hyperlipidemia is believed to be one of the substantial risk factors causing cardiovascular diseases (CVDs). CVDs is responsible for one third of total deaths around the globe**.** It is believed that CVDs will turn out to be the main cause of death and disability worldwide by

the year 2020.[1,2] Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters phospholipids and plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels.[3,4]

Hypercholesterolemia and hypertriglyceridemia are the main causes of atherosclerosis which is vigorously related to ischemic heart disease (IHD).[5] There is a persistent relation between IHD and the high mortality rate. Furthermore, elevated plasma cholesterol levels cause more than four million deaths in a year.[6]

Atherosclerosis is a procedure of arteries hardening because of deposition of cholesterol in the arterial wall which causes narrowing of the arteries. Atherosclerosis and atherosclerosis-associated diseases like coronary, cerebrovascular and peripheral vascular diseases are triggered by the presence of hyperlipidemia.[7]

Efficient control of hyperglycemia in diabetic patients is crucial for reducing the risk of micro and macrovascular complications.[8] Natural sources play a significant role in the management of diabetes mellitus, especially in developing countries, delaying the development of diabetic complexities and correcting the metabolic abnormalities.[9]

Nigella sativa (black seed) is among the natural sources examined to have beneficial effects in the medicament of many diseases.[10] N. sativa has many advantages such as an anticancer, cardiovascular, anti-inflammatory, renal, immunomodulatory, and antidiabetic effects as well as many other effects like antiasthmatic, antimicrobial and antihypertensive effects. Furthermore, the seeds of N. sativa are broadly used in the medicament of various diseases like bronchitis, diarrhea, rheumatism, and skin disorders.[11] The efficacy of N. sativa is linked to various active components which have been isolated from seeds and its oil containing thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine, and alpha-hederin[12], as well as flavonoids.[13]

Various studies clearly showed that *Nigella sativa* significantly reduced the elevated blood glucose levels of different animals with experimentally-induced diabetes mellitus.[14] The apparent antidiabetic effect of *Nigella sativa* was attributed to its insulinotropic action[15,16], and the antioxidant properties which reduce the oxidative stress and preserve pancreatic β-cell integrity.[17-19] The glycemic control obtained by *Nigella sativa* was also ascribed to its

extrapancreatic actions, primarily the inhibition of hepatic gluconeogenesis.[20,21]

MATERIALS AND METHODS

Chemicals

Active Pharmaceutical Ingredient of Atorvastatin, was bought from Incepta Pharmaceuticals limited, Dhaka, Bangladesh.

Nigella sativa was collected from Mirpur Botanical Garden, Dhaka, Bangladesh. High Fat diet was purchased from Sigma Aldrich, Germany. Humalyzer 3000 a Semi-Automated Clinical Chemistry Analyzer, originated from USA was used to measure Total Cholesterol, HDL, LDL and triglyceride. Total cholesterol, HDL, LDL and triglyceride measuring kits were brought from Plasmatic Laboratory Product Ltd, UK. Glucometer Alere GI of Alere Inc, USA was purchased from Farmgate, Dhaka, Bangladesh.

Extraction procedure

At first seeds were collected and washed properly with water after that it was dried under sunlight for 7 days. Afterwards, the dried seeds were crushed into powder and then powdered leaves were soaked in ethanol for 21 days with shaking using a metabolic shaker. Next, the extract was filtered and the filtered liquid was collected. Then the rotary evaporator machine was used to make the collected filtered concentrate. Then, extract was collected carefully.[22]

Experimental design and Animal Handling

30 adult healthy male Wistar rats with body weight from 130-160 gram were collected from the Department of Pharmacy of Jahangirnagar University, Savar, Dhaka, Bangladesh. Then the rats were kept under controlled 12±1h light/dark cycle and 25◦ C temperature. In the Institute of Nutrition & Food Science, University of Dhaka. Initially for acclimatization, rats were kept there for 7 days. Then, the body weights of all rats were measured the rats were divided into 6 groups where each group contained 5 rats.

Group 1: Normal control (C)
Group 2: High fat diet induced control (H.F.D)
Group 3: High fat diet induced rat receiving Atorvastatin 50mg/kg of body weight (H.F.D + A.V).
Group 4: High fat diet induced rat receiving the seed extract of *Nigella sativa* 500 mg/kg body weight (H.F.D + N.S).

Group 5: Normal healthy rat receiving atorvastatin 50 mg/kg body weight (A.V)

Group 6: Normal healthy rat receiving seed extract of *Nigella sativa* 500 mg/kg body weight (N.S).

High fat diet

High fat diet was composed of 3% cholesterol (Sigma Aldrich Co., USA), 81.8% normal chow, 15% beef tallow, 0.2% cholic acid (Sigma Aldrich Co., USA). The component that was added were taken as the percentage of total diet.

Statistical Analysis

The data that we received from rats belonged different groups were expressed in mean±SD. For statistical analysis, SPSS used 16 software to analyze the data. Here we go through "One Way Anova T Test" to find out whether the intra-group differences are statistically significant ($p>0.05$) or not. Here, the level of significance was set at $p<0.05$, when p value was observed smaller than 0.05, the intra group difference was considered statistically significant.

RESULTS

Change in body weights

Body weights of rats were measured initially and again prior to sacrifice. The differences between the changes in weight were taken into consideration. The results are shown in Table 1 and Figure 1.

Body Weight (gram)	C	H.F.D	H.F.D + A.V	H.F.D+N.S	A.V	N.S
Initial Body weight	144.38±7.599	142.76±5.43	142.12±4.86	146.08±6.86	144.4±6.57	143.84±7.16
Final Body weight	161.42±7.79	208.76±9.95	175.92±4.87	176.88±6.25	163.48±5.29	158.82±8.17

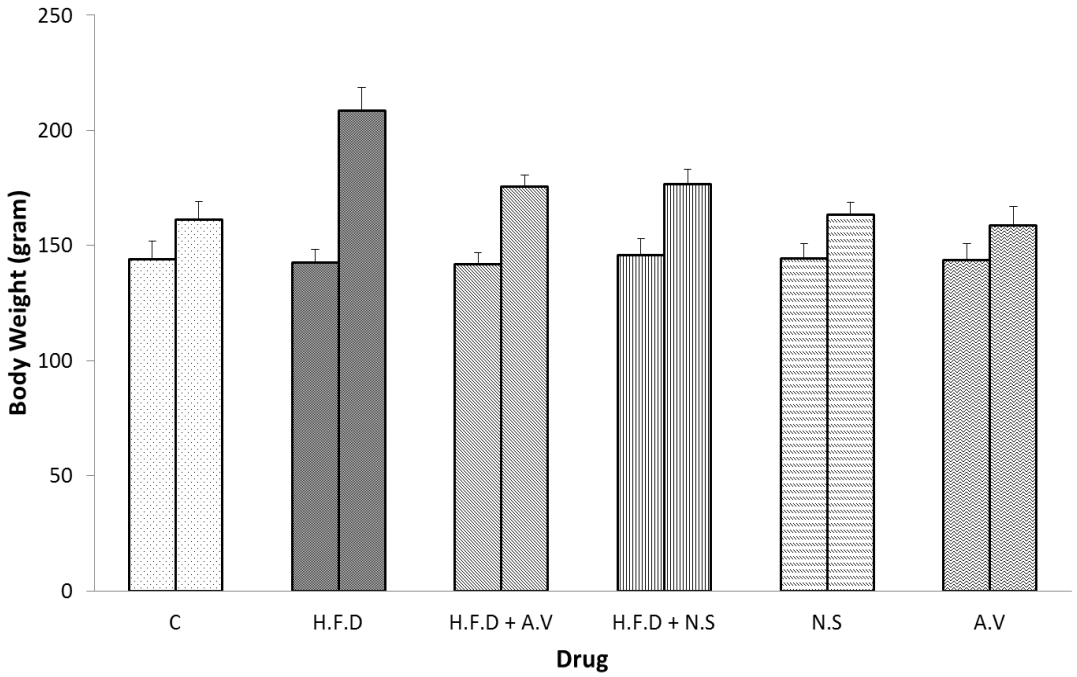

Figure 1: Comparision between the average body weight (mean±standard deviation) of rats belong to 6 groups.

Level of Total Cholesterol, HDL, LDL and triglyceride

The assessment of Total Cholesterol, HDL, LDL and triglyceride levels were measured after sacrificing.

The results are shown in Table 2 and in Figure 2,3,4 and 5.

Parameter(mg/dl)	C	H.F.D	H.F.D + A.V	H.F.D+N.S	A.V	N.S
Total Cholesterol	94.48±6.86	251.72±20.47	173.56±9.56	184.74±7.86	94.92±8.93	96.48±4.71
HDL	42.7±3.15	110.36±8.18	71.76±9.037	82.02±2.76	43.06±4.33	42.14±2.16
LDL	36.47±2.88	98.47±8.99	71.88±10.34	71.12±3.58	44.81±20.35	37.58±1.99
Triglyceride	81.53±5.15	214.46±18.66	149.6±8.64	157.71±7.76	81.77±8.36	83.75±5.32

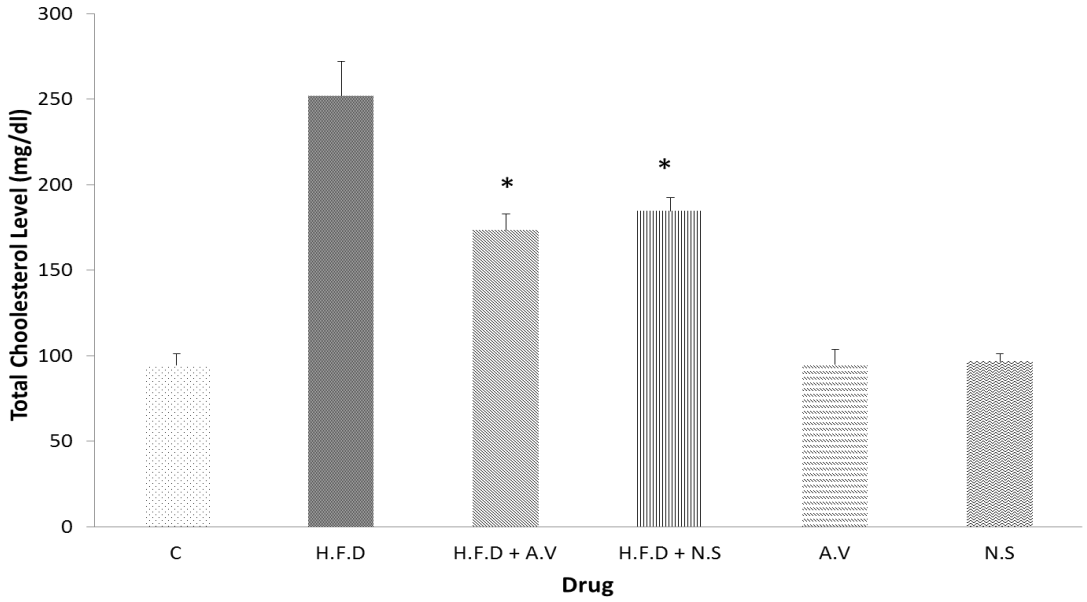

Figure 2: Total Cholesterol Level of Rats belonged six groups. The data were expressed as mean± standard deviation. * Expresses the significant change.

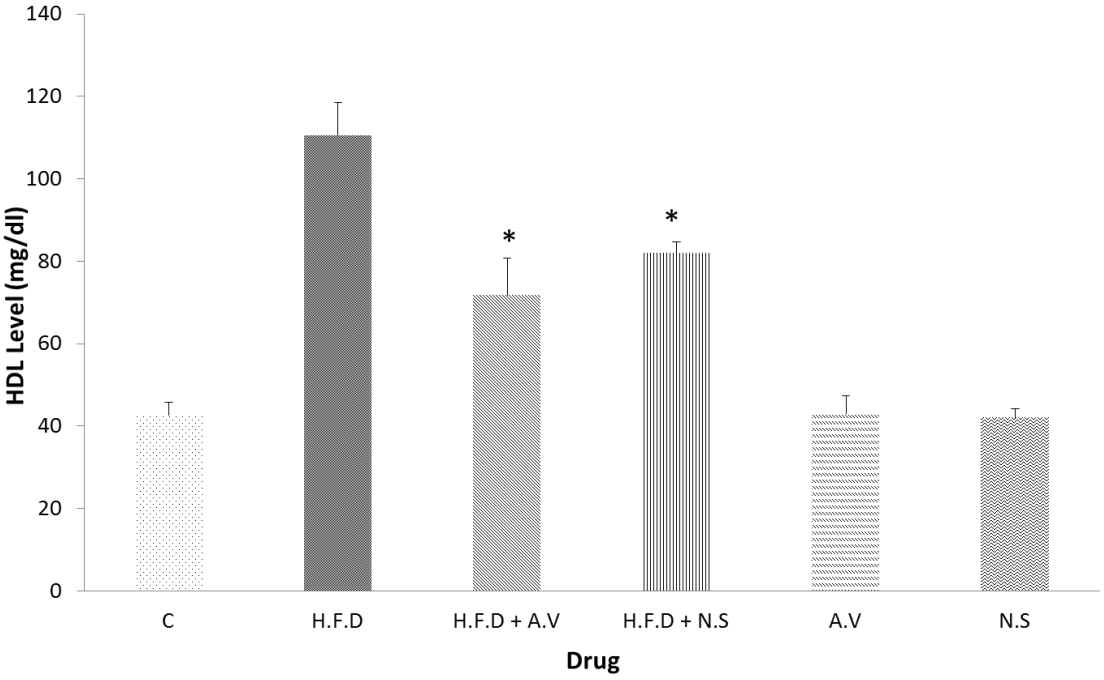

Figure 3: HDL Level of Rats belonged six groups. The data were expressed as mean± standard dviation. * Expresses the significant change.

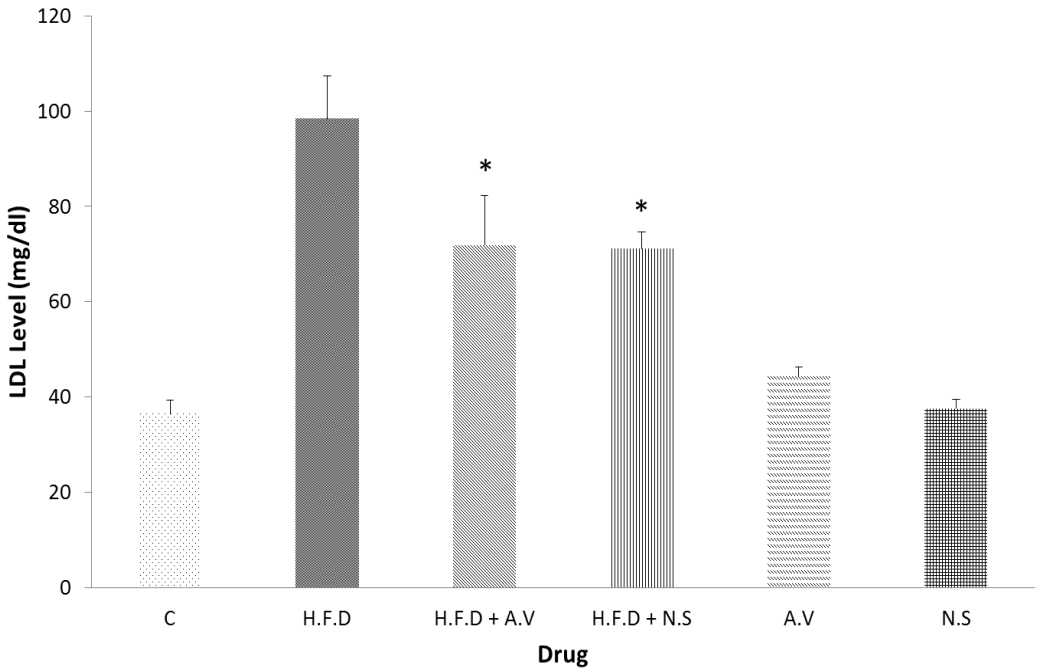

Figure 4: LDL Level of Rats belonged six groups. The data were expressed as mean± standard dviation. * Expresses the significant change.

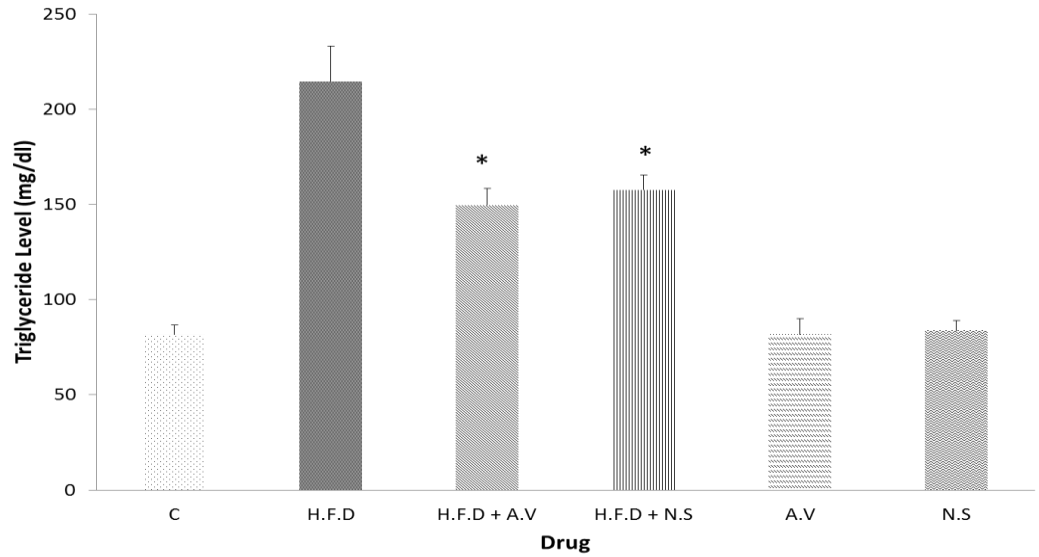

Figure 5: Triglyceride Level of Rats belonged six groups. The data were expressed as mean± standard dviation. * Expresses the significant change.

DISCUSSION

In our study, it has been observed that body weight of rats were increased in all groups and the maximum enhancement was observed in case of group 2 as rats were treated with high fat and no medicaments were given to them given to counteract the effect. In group 3 and 4 due

to high fat diet induction, weight enhancement rate was higher than group 1, 5 and 6 but lower than group 2 as rats belonged to group 3 and 4 are treated with Atorvastatin and *Nigella sativa.*

The rats belonged to group 2,3 and 4 were fed with high fat diet for 8 weeks. Then, treatment was begun and treatment period was 10 weeks.

After 10 weeks of treatment rats were sacrificed and blood was collected, as well as, centrifugation was done. Afterward plasma was collected. Then Total cholesterol, HDL, LDL and triglyceride was measured.

Here, group rats belonged to group 1 showed normal total cholesterol HDL, LDL, triglyceride level in contrast in group 2 total cholesterol HDL, LDL, triglyceride level was higher than that of all other group. It has been observed that both atorvastatin and *Nigella sativa* can significantly decrease the total cholesterol HDL, LDL, triglyceride level when compared with rats belonged to group 2 ($p<0.05$). Between atorvastatin and *Nigella* sativa atorvastatin showed better effect but with statistical significance in case of HDL. But in case of total cholesterol, LDL, triglyceride level, the difference between group 2 and group 3 was found statistically non-significant ($p>0.05$).

When the total cholesterol, HDL, LDL, triglyceride level of rat belonged to group 1, 5 and 6 were compared, no statistical significance was found ($p>0.05$).

CONCLUSION

From the above results, it may be concluded that the *N.sativa* seed oil provide similar but slightly lower effect than atorvastatin with null statistical significance (except HDL) level. Furthermore, in high fat diet induced rats, it ameliorated the conditions. Consequently, these parameters are found unchanged when normal rats were fed with *N.sativa* seed oil and atorvastatin with identical dose. We, thus, conclude that these herbal remedy can be incorporated for disease management of Hyperlipidemia.

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Authors' contribution: This work was carried out in collaboration among all authors. Authors AAC and MSA designed and wrote the research protocol. The authors MMHK, FN and LRMDC performed the tests equally and analyzed the data with the active co-operation of KH, AM,. Authors NA and ZI helped to trim the data of the work and performed the statistical interpretation.

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