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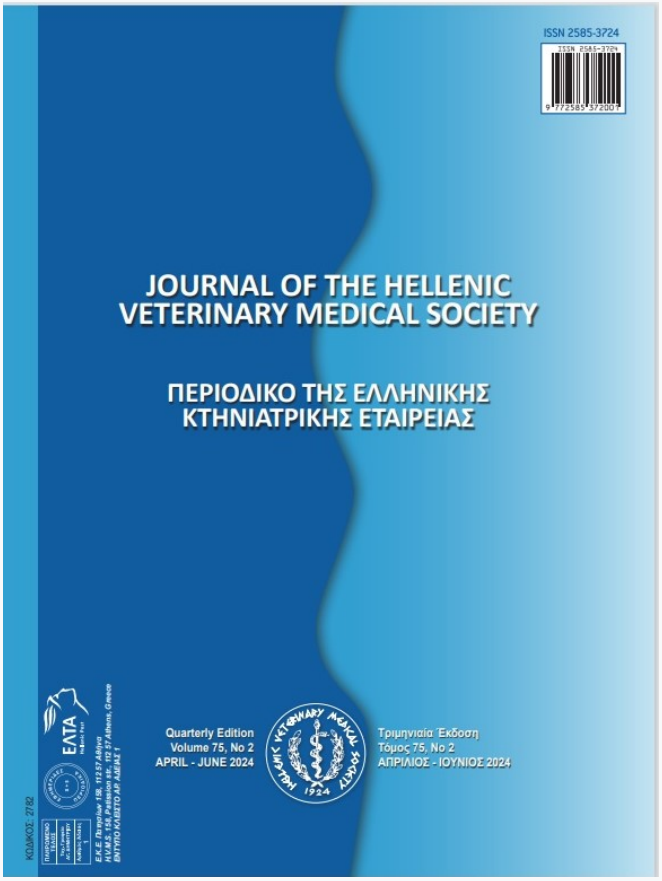

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Research article
Ερευνητικό άρθρο

Effect of black cumin (*Nigella sativa)* and high fat supplemented diet on lipid profile, C-reactive protein and cardiac histopathology in rabbits

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ABSTRACT: Present study determined effects of *Nigella sativa* on risk factors associated with cardiovascular disease. A typical marker for predicting cardiovascular risk is lipid profile. C-reactive protein, an independent predictor of coronary heart disease, has recently been found to be the inflammatory marker. People who consume a high fat diet have higher amounts of the CRP (C-reactive protein) levels and lipid profile. Current study was designed to evaluate the effects of *Nigella sativa* and high fat diet and their co-administration in 7 months old rabbits (*Oryctolagus cuniculus*) for 30 days. Effects of *Nigella sativa* and high fat diet were analyzed on the structure of heart, lipid profile and C-reactive protein in rabbits. For this purpose, rabbits (n=15) were kept in animal house under standard laboratory conditions. 15 rabbits were divided into three groups, one control and two experimental. Control group was treated with standard diet. Experimental group 1 was treated with high fat diet and experimental group 2 was treated with 5% *Nigella sativa* and high fat diet. Body weight showed significant (P>0.05) increase in group 1 fed with high fat diet and significant (P>0.05) decrease in body weight was observed in group 2 (5% *Nigella sativa)*. Cholesterol, triglycerides, LDL, VLDL and C-reactive protein showed significant (P>0.05) increased in group1 and in contrast significant decreased level of HDL was found in group1. Group 2 (5% *Nigella sativa)* showed significant (P>0.05) decrease in C-reactive protein levels, cholesterol, triglycerides, LDL, VLDL as compared to the high fat diet group. Histo-morphological study of heart showed moderate congestion, loss of sarcoplasm and fragmentation in many cardiac muscle fibers in group 1. However, group 2 (5% *Nigella sativa)* heart tissues showed no morphometric changes in the heart structure which indicate that 5% *Nigella sativa* might rescue or protect the heart structure from damage. It was concluded from present study that 5% *Nigella sativa* had anti-atherogenic cardioprotective properties. Using this plant with fat-rich diets simultaneously may reduce their adverse health effects.

Keywords**:** *Nigella sativa;* C-reactive protein levels; high fat diet; lipid profil

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INTRODUCTION

Nigella sativa, locally called as black cumin, has been utilized traditionally for hundreds of years around the world. It has been noted that among all the plants employed in medicine, *Nigella sativa* has the longest and most mystical history. Currently it is believed that black cumin seeds are an essential part of complementary medicine for cure as well as avoiding a variety of illnesses (Ermumcu and Şanlıer, 2007). Natural products are employed as complementary therapies for many disorders since their good medical effectiveness and less harmfulness. Natural products provide a sustainable source with a considerable efficacy to treat and overcome several disorders and fatal diseases, including cancer (Asma et al., 2022). One of them is the plant *Nigella sativa*. *Thymoquinone* could become more effective medications that treat a variety of additional ailments as a result of chemical improvements (Ijaz et al., 2017).

Increased consumption of high fat diets is known to be associated with major clinical problems and it can be considered a problem for public health in both industrialized and developing nations (Hariri and Thibault, 2010). The majority of earlier studies focused on harmful eating habits, particularly those that included excessive fat consumption, which leads to an unbalanced energy supply. For instance, a higher intake of meat-instant foods which are heavy in animal protein, saturated fat, sugar, sodium and food additives were found to be strongly linked to elevated CRP and metabolic disorders (Ren et al., 2018; Kucukkurt et al., 2023).

In addition to being one of the leading global causes of illness and death, cardiovascular disease also places a heavy financial load on the current health care systems. The impact of various dietary compositions on lipid profiles and risk-markers for cardiovascular diseases have recently been the focus of researchers (Daoud et al., 2014). Some of the contributing causes to cardiovascular diseases include hypertension, atherosclerosis, excessive cholesterol and other metabolic illnesses. In order to prevent this condition, controlling your weight and improving your lipid profile are crucial. Although *Nigella sativa* is said to lessen the danger of cardiac ailment as well as improve lipid profiles (Ermumcu and Sanlıer, 2017).

A typical lipid profile for predicting cardiovascular risk contains TG, TC, LDL-C and HDL-C. Several studies have demonstrated that lowering both can lower the risk of cardiovascular disease and mortality because it is widely known cardiac related disease is associated with cholesterol level and low-density lipoprotein cholesterol (Langsted and Nordestgaard, 2019).

An independent predictor of future coronary heart disease has recently been found to be the inflammatory marker C-reactive protein. People who consume a high fat diet have a higher amounts of the CRP levels (Sproston and Ashworth, 2018). The potential usefulness of black seeds in lowering cholesterol levels has also been investigated. Heart disease is linked to high cholesterol levels (Farhangi et al., 2018). In this study we investigated that that either the intake of 5 % black cumin reduced the CRP levels and Lipid profile in the rabbits.

MATERIALS AND METHODS

The research was conducted at Animal house, Zoology Department, Minhaj University Lahore. 12 rabbits of 4-5 month age were used and kept in wooden cages under regulated environment e.g. 12 hour cycle of day and night. After 5 days of adaptation to the new environment, three groups were formed. One group served as the control group and labelled as G_o while two groups were used as experimental groups and labelled as G_1 and G_2. The seeds of *Nigella sativa* were purchased from local market and grounded in grinder machine. Beef fat was purchased from butcher's shop locally. After cleaning the fat, it was heated at 55°C to make the oil.

After completing four weeks of experiment rabbits were sacrificed. Blood was collected in yellow capped tubes contained acid citrate dextrose (ACD) for serum collection. After collection, the sample was centrifuged for 10 minutes at 3000 rpm. For blood testing (Lipid Profile and CRP) serum was isolated and stored in Eppendorf tubes. An incision made

Table 1: Rabbits Feeding and Treatment Groups under Experiment

Treatment groups	No. of Animals	Diet	Supplementation
Control Group (G_o)	4	Pellets No. 44	None
Experimental Group (G_1)	4	Pellets No. 44	8% corn oil and 10 % beef fat
Experimental Group (G_2)	4	Pellets No. 44	8% corn oil and 10% beef fat + 5 % black cumin

through skin from sternum to the pelvis, heart was exposed and weighted. Lipid profile were measured by automatic analyzer and CRP have been measured by ELISA kit (Sandwich ELISA - Mouse C Reactive Protein ELISA Kit, Sigma Aldrich, USA) method.

Heart tissues were preserved in 10% formalin. Alcohol was used to first dehydrate the sample, followed by xylol to clean it. Before being sectioned with a microtome, tissues were imbedded in 10% melted paraffin wax. In order to expose tissues, wax was scraped from block surfaces. Before sectioning, blocks were refrigerated for ten minutes in an ice tray. Using a rotating microtome, sections were made. Five µm tissue slices were made. Hematoxylin and eosin were used to stain the clear cells. Coverslips were placed over the tissues and examined under low and high resolution of microscope.

Statistical Analysis: Data was analyzed by using Graph Pad Prism version 9 and unpaired t-test statistics were applied for finding mean, standard error mean and p-value for each parameter.

RESULTS

The Mean and SEM values of heart weight were 32.96±1.44, 36.80±0.19 and 30.30±2.09 in control group (G_o), experimental group (G_1) and experimental group (G_2) respectively. High fat diet along with simple diet was given to the experimental group (G_1) and it was observed that their herat weight was higher as compare to the other groups due to the deposition of fat. Experimental group (G_2) was given 5% black cumin along with high fat diet and simple diet and it was observed that that their heart weight was less as compared to the control group (G_0) and experimental group (G_1).

Simple feed was given to control group (G_o) rabbits and they had normal CRP levels. Where as experimental group (G_1) rabbbits was given high fat diet along witth simple diet and it was observed that their CRP levels were extremely high due to consumption of high fat diet. Experimental group (G_2) was given 5% black cumin along with high fat diet and simple diet and it was observed that that their CRP levels was less as compared to the experimental group ($G_{1)}$ as shown in figure below. Asterisks shoed the significant difference among all groups.

The overall Mean and SEM of TC, TG, VLDL, LDL, HDL of rabbits fed simple, high fat diet and 5% black cumin are shown in the table below respectively. Experimental group ($G_{1)}$ had increased TC, TG, VLDL, LDL and low HDL levels as compared to the control group (Go) due to high fat consumption. TC, TG, VLDL, LDL in experimental group ($G_{2)}$ was low and HDL was high as compared to the experimental group (G_1) because black cumin had curing effects and did not develop heart disease.

Table 2: Heart weight (Mean and SEM values) of control and experimental groups

Groups	Control group (G_o)	Experimental group ($G_{1)}$	Experimental group ($G_{2)}$
Heart weight	32.96±0.44	36.80±0.19^{a}*	30.30±2.09^{a}*

**Letter indicated with superscript are significantly different.*

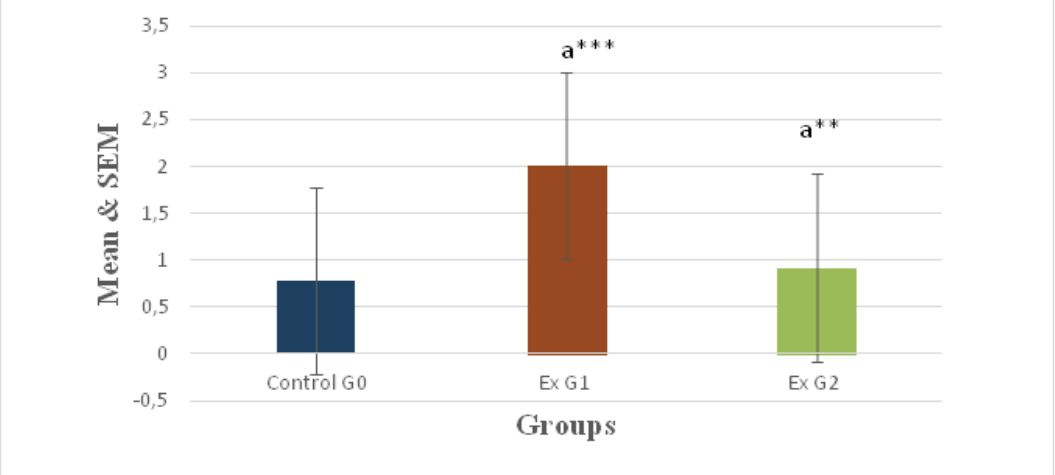

Figure 1: Morphometric analysis of CRP levels between rabbits of control group (G_0) and experimental groups (G_1 and G_2)

Table 3: Lipid profile values (Mean and SEM) of control and experimental groups

Groups	Control group (G_0)	Experimental group (G_1)	Experimental group (G_2)
Total cholesterol	67.25±4.44	90.50±3.30^{a}**	71.75±4.60
Triglycerides	105.50±2.72	192.25±3.56^{a}****	123.00±3.41a** b****
HDL	52.61±0.63	26.97±0.66^{a}****	50.33± 0.82^{a}****
VLDL	28.50±2.95	45.75±2.68^{a}**	32.50±2.06^{a}**
LDL	31.60±0.54	122.50±1.04^{a}****	44.00±2.34^{a}** ^{b}****

Values are expressed as Mean ± SEM
p>0.05, *p*<0.05*, *p*<0.01**, *p*<0.001***, *p*<0.0001****

Histopathological Examination of Heart Tissues

Histopathological results are shown in figures 2, 3 and 4.

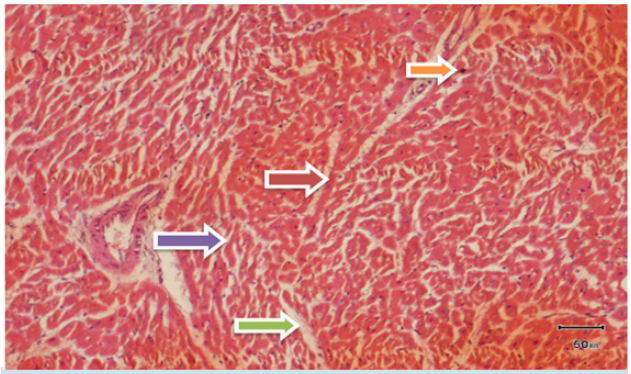

Figure 2: Rabbit heart almost normal, no tissue changes were seen. Red arrow: Sarcolemma. Orange arrow: Single central nucleus for muscle cell. Green arrow: Connective tissue containing purkings fibers and blood vessels among muscle cells. Purple arrow: Branched cardiac muscles cell.

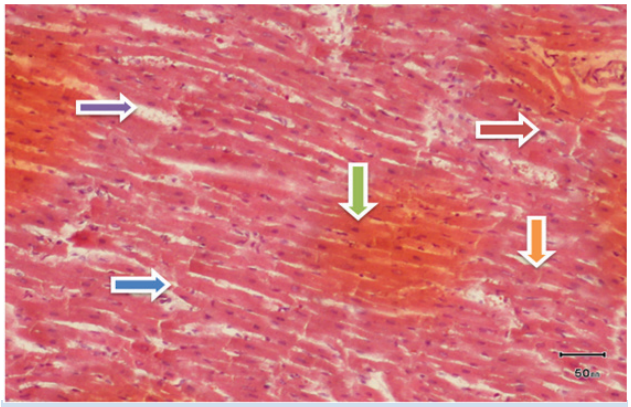

Figure 3: Moderate congestion is seen. Loss of sarcoplasm and fragmentation in many cardiac muscle fibers is seen. Blue Arrow: Intra muscular capillary. Purple Arrow: Connective tissue containing purkings fibers and blood vessels among muscle cells. Orange Arrow: Sarcolemma. Red Arrow: Single central nucleus for muscle cell. Green Arrow: Myocardium congestion.

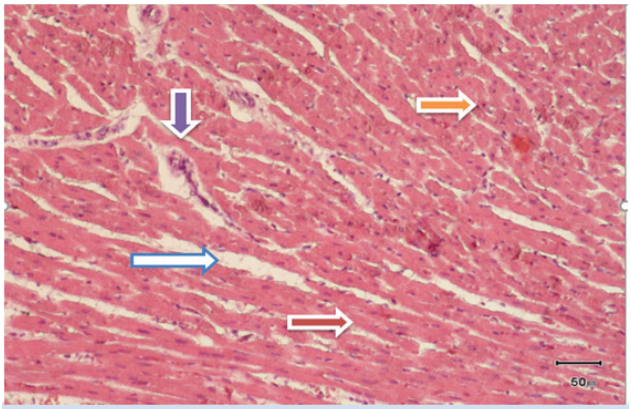

Figure 4: Almost normal no tissue changes were seen. Orange Arrow: Single central nucleus for muscle cell. White Arrow: Connective tissue containing purkings fibers and blood vessels among muscle cells. Purple Arrow: Branched cardiac muscles cell Red Arrow: Sarcolemma.

DISCUSSION

Present study was based on *Nigella sativa* with high fat diet to observe CRP levels, lipid profile and cardiac histopathology of 4-5 months male and female rabbits. Current study showed that rabbits fed with high fat diet (HFD) considerably show more fat deposition in their bodies due to oil supplementation than control group. After the dissection of rabbit, heart weight of all the rabbits were measured. It was observed that weight of experimental group (G_1) were higher than control group (G_o) and group (G_2) due to consumption of high fat diet. Increase in heart weight indicated that muscles of heart became thick and their thickness showed heart disease. It is concluded that HFD caused the heart disease. Many epidemiological studies of past few decades stated that intake of HFD is directly proportional to the heart disease (Wali et al., 2020).

Current study revealed that black cumin increased the heart weight by not causing disease in it. Experimental group (G_2) not showed any heart disease due to the consumption of 5% black cumin. Previously, no

such work was conducted that demonstrates the effect of black cumin on heart weight. Asterisks represented the significant difference between control group (G_o) and experimental groups ($G_{1,}$ $G_{2)}$ $p<0.05$ and $p<0.05$ treated with 5% black cumin.

Previous studies has shown that intake of high fat diet are positively correlated with CRP. The plasma CRP levels in the rabbits who were taking high fat diet were greater than in rabbits with healthy metabolisms (Hammed and Amao, 2022). Increased adipose tissue caused by HFD had been studied and are hazardous to numerous organ systems of the body (Kaki et al., 2018) After the centrifugation serum CRP was separated and CRP levels were measured and it was observed that CRP levels of experimental group (G_1) were higher than control group (G_o) due to the consumption of high fat diet and in the second experimental group (G_2) CRP levels were less as compared to the experimental group (G_1) due to the intake of 5 % black cumin. Asterisks showed the significant difference between the groups. A significant difference was examined in the parameters of experimental group (G_1) $p<0.001$ when compared with experimental groups (G_2) $p<0.01$ treated with 5% black cumin.

Previous research on the lipid profile revealed that *Nigella sativa* raised HDL and decreased total cholesterol, triglycerides and LDL (Laudadio et al., 2022). *Nigella sativa* is highly effective in the detection and cure of cardiovascular disorders as well as in lowering levels of TC and LDL which in turn reduce arterial wall lipid accumulation and contribute to atherogenesis (Seidavi et al., 2020) Current study showed that the Mean and SEM values of total cholesterol, triglycerides, very low density lipoprotein, low density lipoprotein was normal in (G_o) group, high in experimental group (G_1) and less in experimental group (G_2) respectively. While Mean and SEM values of HDL showed it was normal in (G_0) group, low in experimental group (G_1) and high in experimental group (G_2) respectively.

CONCLUSION

The result of current study showed that rabbits which were exposed to high fat diet showed increased fat. The inclusion of high fat diet caused the metabolic disturbance which may be evolved from inflammation and non-significant values of parameters that negatively affect the cardiac histopathology. The supplementation of 5% *Nigella sativa* (black cumin) seeds had balanced the CRP levels and lipid profile, also prevented the incidence of heart diseases that showed significant results. On the basis of present study, it is recommended that the utilization of herbal products is cheaper source and easily accessible that could be used for improving the incidence of heart diseases.

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