

**How to Cite:**

Khan, M. R., Khan, M. A., Singh, V. K., Saxena, A., Singh, T., Gangwar, A. K., & Shamim, S. (2022). Protective effect of aqueous extract of *Nigella sativa* on Oxidative Enzymes, Homocysteine, and Lipids in Methionine induced Hyperhomocysteinemic rats. *International Journal of Health Sciences*, 6(S1), 5764–5778. <https://doi.org/10.53730/ijhs.v6nS1.6144>

# **Protective effect of aqueous extract of *Nigella sativa* on Oxidative Enzymes, Homocysteine, and Lipids in Methionine induced Hyperhomocysteinemic rats**

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**Abstract**---Hyperhomocysteinemia has emerged as an independent risk factor for development of various diseases such as coronary, cerebrovascular and peripheral arterial occlusive diseases. Its association is found with atherosclerosis, cancer and some other aged-related illnesses including Alzheimer's disease. The present

study was designed to investigate the homocysteine lowering potential, effect on lipids and oxidative enzymes of standardized aqueous extract of *Nigella sativa* seeds (100 and 200 mg/kg body weight, *p.o.*) in hyperhomocysteinemia induced by L-methionine. Hyperhomocysteinemia was induced in wistar albino rats by methionine treatment (1 g/kg, *p.o.*) for 30 days. Folic acid (100 mg/kg, *p.o.*) given to rats as a standard drug treatment. Rats were fed with the aqueous extract of *Nigella sativa* (100 and 200 mg/kg, *p.o.*) for 30 days. The results of the present study after treatment with *Nigella sativa* aqueous extracts in two doses of 100 and 200 mg/kg body weight, showed lipid lowering, cardio- and neuro-protective potential of *Nigella sativa*. The results of test drug were compared with folic acid, a standard positive control. The present study results indicate that the aqueous extract of *Nigella sativa* seeds treatment protect the antioxidant defense against hyperhomocysteinemia, hyperlipidemia and oxidative stress in methionine-induced rat model.

**Keywords**---Homocystein, Methionin, Hyperhomocysteinemia, *Nigella sativa*.

## Introduction

Hyperhomocysteinemia has emerged as an independent risk factor for development of coronary, cerebrovascular and peripheral arterial occlusive diseases (Omenn *et al.*, 1998). It is one of the main factors that cause various diseases, such as atherosclerosis (Refsum *et al.*, 1998; Merkel 2004), diabetes (Luis *et al.*, 2004), cancer (Poirier *et al.*, 2001) and some other aged-related illnesses including Alzheimer's disease (Clarke *et al.*, 1998; Nilsson *et al.*, 2002). Although severe hyperhomocysteinemia is rare, mild elevations in homocysteine concentration have been found in nearly 7% of the general population and in 20 to 30% of patients with coronary and peripheral vascular disease (Clarke *et al.*, 1991; McCully 1996; Folsom *et al.*, 1998). Recent epidemiological studies support a positive association between plasma homocysteine concentration and risk for cardiovascular disorders (Arnesen *et al.*, 1995; Graham *et al.*, 1997). Hyperhomocysteinemia has been implicated as a risk factor for vascular disease as well as brain atrophy and therefore, may be related to the development of dementia and possibly Alzheimer's disease (den Heijer *et al.*, 2003).

During the past few years, elevated blood levels of homocysteine (a sulfur-containing amino acid) have been linked to increased risk of premature coronary artery disease, stroke, and thromboembolism (venous blood clots), also among people having normal cholesterol levels. The hypothesis that hyperhomocysteinemia (Hhcy) is a risk factor for cardiovascular disease was proposed over many decades ago by McCully, who observed advanced vascular lesions in children with severe hyperhomocysteinemia caused by inborn errors of methionine metabolism (McCully and Wilson 1969). It has been established that hyperhomocysteinemia is a pathological condition characterized by elevation of plasma total homocysteine (HCY).

A study compared 131 patients with severe blockages in two coronary arteries, 88 patients with moderate blockage of one coronary artery, and another group of healthy individuals without heart disease. The researchers found a linear relationship between blood homocysteine levels and severity of the coronary blockages: For every 10% elevation of homocysteine, there was nearly the same rise in the risk of developing severe coronary heart disease (Verhoef *et al.*, 1997). Another study has found that postmenopausal women with elevated homocysteine levels had a higher incidence of coronary heart disease (Ridker 1999). Another study found that homocysteine levels were much higher in people who developed vein clots than in similar people who did not (Loralie *et al.*, 2000). Yet another study found that elevated homocysteine levels may be associated with an increased risk of stroke in people who already have coronary heart disease (Tanne 2003).

Reducing homocysteine level by inhibiting its formation or promoting its transformation is becoming an attractive approach in disease prevention. Plant infusions and decoctions have been used as popular medicine in several under developed and developing countries as an alternative treatment for various pathophysiological conditions. The local communities residing in the biodiversity-rich areas of the North Eastern Region of India have traditionally used and relied on herbs for treating various ailments (Kayang *et al.*, 2005). Ramaswami *et al* 2004 have reported that curcumin blocks homocysteine-induced endothelial dysfunction in porcine coronary arteries. In another study investigator reported the protective effect of pre- treatment of *Nigella sativa* medicinal plant in Isoproterenol induced cardio toxicity in rats.

L-methionine administration is resulted in hyperhomocysteinemia as reported earlier and is commonly used in animal model to induce hyperhomocysteinemia (Ubbink *et al.*, 1996; Silberberg *et al.*, 1997; Bellamy *et al.*, 1998). Therefore, in the present study, L-methionine was used to induce hyperhomocysteinemia and it was thought worthwhile, to investigate the antihyperhomocysteinemic activity of standardized aqueous extract of *Nigella sativa* seed on L-methionine induced hyperhomocysteinemia in albino rats. The present study was designed to investigate the homocysteine lowering potential of standardized aqueous extract of *Nigella sativa* seeds (100 and 200 mg/kg body weight, p.o.) on hyperhomocysteinemia induced by L-methionine.

## **Material and Methods**

### **Extracts of *Nigella sativa* L. seeds**

The plant specimens for the study were collected from the local market and were positively identified and authenticated by the National Botanical Research Institute (Council of Scientific and Industrial Research), Rana Pratap Marg, Lucknow. A voucher specimen no. (NBRI-SOP-202), Reference no. (NBRI/CIF/296/2012), dated 10/04/2012.

### **Preparation of the aqueous extract of *Nigella sativa* seeds**

The dried and coarsely powdered drug (100 g) was packed in a Soxhlet apparatus and was subjected to extraction with water for 72 h. The filtrate was evaporated under vacuum drier and brown mass residue obtained was stored at 4°C for further use. The average yield of aqueous *Nigella sativa* extract was approximately 11.59 %. For experimental study, the weighed amount of aqueous *Nigella sativa* extracts (100 and 200 mg/kg) were suspended in 1% Tween 80 in distilled water and administered to adult male Wistar albino rats by oral route.

### **Experimental Design**

Healthy, male, adult, Wistar albino rats (200-250 g) procured from the School of pharmaceutical sciences, IFTM University, Moradabad and acclimatized under standard laboratory conditions at  $25 \pm 2^\circ\text{C}$ , relative humidity ( $50 \pm 15\%$ ) and normal photoperiod (12 h light dark cycle) for 7 days, were used for the experiment. Animals were fed with commercial rat pellet diet and water was provided *ad libitum*. Adequate measures were taken to minimize pain or discomfort, and that the experiments were conducted in accordance with international standards on animal welfare as well as being compliant with local and national regulations. After acclimatization for 7 days, Adult Wistar rats were divided into eleven groups (n=6 each) as shown below.

The protocol for animal experiment was approved by the Institutional Animal Ethics Committee (IAEC) of IFTM University, Moradabad, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration no. 837/ac/04/CPCSEA and dated 22/09/2004).

Group I. Vehicle Control: Rats were treated with 1% Tween 80 in distilled water (2 ml/kg body weight, *p.o.*) for 30 days

Group II. Pathogenic Control: Rats were treated with 1% Tween 80 in distilled water (2 ml/kg body weight, *p.o.*) + L-methionine (1 g/kg body weight, *p.o.*) daily in drinking water for 30 days.

Group III. Aqueous *Nigella sativa* extract (100 mg/kg) *per se*: Rats were treated with aqueous *Nigella sativa* extract (100 mg/kg body weight, *p.o.*) for 30 days.

Group IV. Aqueous *Nigella sativa* extract (200 mg/kg) *per se*: Rats were treated with aqueous *Nigella sativa* extract (200 mg/kg body weight, *p.o.*) for 30 days.

Group V. Aqueous *Nigella sativa* extract (100 mg/kg) treated: Rats were treated with aqueous *Nigella sativa* extract (100 mg/kg body weight, *p.o.*) + L-methionine (1 g/kg body weight, *p.o.*) daily in drinking water for 30 days.

Group VI. Aqueous *Nigella sativa* extract (200 mg/kg) treated: Rats were treated with aqueous *Nigella sativa* extract (200 mg/kg body weight, *p.o.*) + L-methionine (1 g/kg body weight, *p.o.*) daily in drinking water for 30 days

Group VII. Folic acid (100 mg/kg) treated: Rats were treated with folic acid (100 mg/kg body weight, *p.o.*) + L-methionine (1 g/kg body weight, *p.o.*) daily in drinking water for 30 days.

The blood samples of all overnight fasted animals groups were collected by the retro-orbital plexus using micro-capillary technique (Sorg and Buckner 1964). Serum was separated for biochemical estimations of various parameters.

In serum, homocysteine levels were estimated using the Fluorescence Polarization Immunoassay (FPIA) method described by Primus *et al* (1998). For Lactate Dehydrogenase Estimation Working reagent (1 ml) and serum (0.05 ml) was pipette into cuvette. Mixed and read the first absorbance of the test exactly at one minute and thereafter at 30, 60, and 90 seconds at 340nm. The mean change in absorbance per minute was determined using UV spectrophotometric method of analysis (Lum and Gambino 1974). Total Cholesterol in serum sample was determined by Measuring the OD of Sample and Test against Blank on a colorimeter with a yellow green filter or on a spectrophotometer at 560 nm (Demacher and Hijamaus 1980). High Density Lipoprotein and Cholesterol Estimation were also done by spectrophotometer at 560 nm (Burstein *et al.*, 1970). Estimation of Triglycerides was performed according to Foster and Dunn (Foster and Dunn 1973). Low Density Lipoprotein-Cholesterol, Very Low Density Lipoprotein-Cholesterol and atherogenic index were calculated according to Friedward *et al.* (1972) as following.

LDL-cholesterol = total cholesterol – HDL-cholesterol – Triglycerides/5

VLDL-cholesterol = triglycerides / 5

Atherogenic Index calculation

a. Total cholesterol / HDL-cholesterol

b. LDL-cholesterol / HDL-cholesterol

Lactate dehydrogenase (LDH) catalyses the oxidation of lactate to pyruvate, accompanied by a simultaneous reduction of NAD to NADH. Lactate dehydrogenase (LDH) activity in serum is proportional to the increase in absorbance, due to the reduction of NAD. Lactate dehydrogenase (LDH) and HDL-C levels were estimated using commercial diagnostic kits from Reckon Diagnostics Pvt. Ltd. Baroda, India.

At the end of experiment the animals were sacrificed by method of cervical dislocation. The hearts and brain tissues were removed quickly, rinsed in ice cold saline and dried for biochemical estimations. Homogenate (10 % w/v) was prepared for biochemical estimations (Bruce *et al.*, 1995). Homogenate (10%) of whole brain tissue in ice cold KCl (0.15 M) was used for assessment of the malondialdehyde (MDA), according to the method of Ohkawa *et al* (1979). In phosphate buffer (0.1 M, pH 7.0), it was used for the assay of glutathione (GSH) content (Sedlak and Lindsay 1968). Lipid peroxides (LPO) was measured by estimating thiobarbituric acid reactive substances (TBARS) i.e. malondialdehyde (MDA), and glutathione assay was based on the reaction with DTNB i.e. DTNB (5,5'-dithiobis- (2-nitrobenzoic acid)) is reduced by -SH group to form one mole of 2-nitro-5-mercaptobenzoic acid (yellow color).

### Statistical Analysis

Statistical analysis was carried out using Graphpad Prism 7.0 (Graphpad software; San Diego, CA). All results were expressed as Mean  $\pm$  S.E.M. Groups of

data were compared with an analysis of variance followed by Dunnett t-test. Values were considered statistically significant, when  $p < 0.01$ .

## Results

### **Biochemical studies**

#### **Effect on Serum Homocysteine Levels ( $\mu\text{g/mL}$ )**

A significant ( $p < 0.01$ ) increase in serum homocysteine levels was observed in the pathogenic control rats (i.e. group II), as compared to the vehicle control rats (i.e. group I rats). Aqueous *Nigella sativa* extracts (100 and 200 mg/kg body weight, *p.o.*) and folic acid treatment significantly ( $p < 0.01$ ) reduced the elevated levels of serum homocysteine. There was no significant ( $p > 0.05$ ) change observed in the serum homocysteine levels in aqueous *Nigella sativa* extract (100 and 200 mg/kg body weight, *p.o.*) *per se* treated rats (i.e. group III and IV), as compared to the group I rats (Table 1).

#### **Effect on Serum Lactate Dehydrogenase (LDH) Levels (IU/L)**

A significant ( $p < 0.01$ ) increase in serum LDH level was observed in L-methionine-treated group, as compared to vehicle control group rats. L-methionine- induced hyperhomocysteinemic rats, when treated with aqueous extracts of *Nigella sativa* (100 and 200 mg/kg body weight, *p.o.*) and folic acid (100 mg/kg body weight, *p.o.*), a significant ( $p < 0.01$ ) decrease in the serum LDH levels was observed as compared to pathogenic control (i.e. group II) rats. There was no significant ( $p > 0.05$ ) change in the serum LDH levels in aqueous *Nigella sativa* extracts (100 and 200 mg/kg body weight, *p.o.*) *per se* treated rats as compared to the group I rats (Table 1).

#### **Effect on Serum Lipid Profiles Levels (mg/dl)**

Serum total cholesterol, triglycerides and low density lipoproteins levels (LDL-C) were significantly ( $p < 0.01$ ) increased along with the significant ( $p < 0.01$ ) decrease in serum high density lipoproteins (HDL-C) levels in pathogenic control i.e. group II rats, as compared to vehicle control i.e. group I rats. With repeated administration of aqueous *Nigella sativa* extracts and folic acid treatment, the above mentioned parameters were reached to almost normal levels significantly ( $p < 0.01$ ), as compared to pathogenic control group. There was no significant ( $p > 0.05$ ) change in the lipid profiles levels were observed in aqueous *Nigella sativa* extracts (100 and 200 mg/kg body weight, *p.o.*) *per se* treated rats (i.e. group III and IV), as compared to group I rats (Table 2 and Table 3).

#### **Effect on Myocardial and Brain Lipid Peroxide Levels (nmoles of MDA/mg protein)**

The lipid peroxides levels in the myocardial and brain homogenates of L-methionine treated rats were found to be significantly higher ( $p < 0.01$ ), as compared to vehicle control rats. Aqueous *Nigella sativa* extracts (100 and 200 mg/kg body weight, *p.o.*) and folic acid (100 mg/kg body weight, *p.o.*) treatment significantly ( $p < 0.01$ ) reduced the elevated levels of myocardial and brain lipid

peroxides levels, as compared to the myocardial and brain lipid peroxides levels in pathogenic control rats (i.e. group II). However, there was no significant ( $p > 0.05$ ) change in the myocardial and brain lipid peroxides levels in aqueous *Nigella sativa* extracts (100 and 200 mg/kg body weight, *p.o.*) *per se* treated rats, as compared to the group I rats (Table 4 and Table 5).

#### **Effect on Myocardial and Brain Glutathione Levels ( $\mu$ moles of phosphorous liberated/min/ mg protein)**

In L-methionine treated rats (i.e. pathogenic control rats, group II), tissue glutathione levels decreased significantly ( $p < 0.01$ ) as compared to the vehicle control rats i.e. group I rats. Aqueous *Nigella sativa* extracts (100 and 200 mg/kg body weight, *p.o.*) and folic acid (100 mg/kg body weight, *p.o.*) treatment significantly ( $p < 0.01$ ) increased the levels of myocardial and brain glutathione levels, as compared to the pathogenic control rats (i.e. group II). However, there was no significant ( $p > 0.05$ ) change in the myocardial and brain glutathione levels in aqueous *Nigella sativa* extracts (100 and 200 mg/kg body weight, *p.o.*) *per se* treated rats, as compared to the vehicle control rats (Table 4 and Table 5).

#### **Discussion**

Oxidative stress is implicated in the pathogenesis of atherosclerosis, ischemia reperfusion injury (IR), cancer, inflammatory diseases, metabolic disease such as diabetes; and diseases of the central nervous system (CNS) such as Alzheimer's, Parkinson's, and stroke. Among the many health predictions the most alarming is that of cardiovascular diseases (CVD). Ischemic heart disease is a leading cause of death in India, with an estimated 3 million deaths per year accounting for 25% of all mortality (Mukherjee 1995). Epidemiological studies support a positive association between plasma homocysteine concentration and risk for cardiovascular disorders (Arnesen *et al.*, 1995; Graham *et al.*, 1997). A mere increase of 12% over the normal level of homocysteine has been associated with a 3-folds increase in risk for myocardial infarction (Naygard *et al.*, 1998).

It is interesting to note that different plants and plant extracts can also stimulate the synthesis of cellular antioxidants (Pathania *et al.*, 1998; Bhattacharya *et al.*, 1999; Banerjee *et al.*, 2001; Gauthaman *et al.*, 2001). Indeed, many antioxidative plants and their isolated active components have been reported to be cardioprotective in ischemia reperfusion-induced myocardial infarction (Banerjee *et al.*, 2002; Mohanty *et al.*, 2004; Rao *et al.*, 2005). *Nigella sativa* L. (Ranunculaceae) is a herbaceous plant which grows in Mediterranean countries and is cultivated in India. It is commonly known as black cumin seed and kalaungi in Hindi in India. It is used as a spice and for the treatment of various diseases. Recent pharmacological investigations of the seed extract revealed that a wide spectrum of biological activities including anti-inflammatory, analgesic, antibacterial, antifungal, anti-helminthic, bronchodilatory, hypertensive and immunoprotecting activities.

## Homocysteine and *Nigella sativa*

Several potential mechanisms underlying the deleterious effect of homocysteine in the brain have been proposed that include oxidative stress, alterations in DNA methylation and activation of the excitotoxic NMDA receptors (Mattson and Shea 2003). The mechanisms associated with homocysteine-induced endothelial dysfunction are mediated by increased oxidative stress (Kanani et al., 1999), leading to increased levels of oxidized LDL (Ventura *et al.*, 2005). Hyperhomocysteinemia may promote the generation of ROS such as H<sub>2</sub>O<sub>2</sub> and hydroxyl radicals via the autooxidation of sulfhydryl (-SH) group (Heinecke *et al.*, 1987) or by decreasing the intracellular levels of GSH that is involved in the elimination of free radicals. The results of the present study showed that by oral feeding L-methionine in a dose of 1 g/kg body weight, for 30 days to albino rats significantly ( $p < 0.01$ ) elevated the levels of homocysteine, LDH, total cholesterol, LDL-C, triglycerides in serum and LPO in heart and brain homogenates with a concomitant decrease in serum HDL-C levels and GSH content in heart and brain homogenates.

An increase in the levels of serum LDH indicates cardiac muscular damage and it could be due to the leakage of enzymes from the heart (Sheela et al., 2000). Free radicals generated by hyperhomocysteinemia, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of the membrane structural and functional integrity (Ajitha and Rajnarayana 2001). In the present findings, the levels of LPO were found to be significantly ( $p < 0.01$ ) increased in animals subjected to L-methionine treatment and data corroborates the findings of Ajitha and Rajnarayana (2001). Further, due to this increased lipid peroxidation, GSH levels were lowered. The same findings were also reported by Flohe (1989).

In the present study, elevated levels of homocysteine, LDH, total cholesterol, LDL-C and triglycerides in serum and LPO in heart and brain homogenates were reduced significantly ( $p < 0.01$ ) after treatment with aqueous *Nigella sativa* extract, suggesting, cardio- and neuro-protective potential of *Nigella sativa*. Further, the levels of HDL-C in serum and GSH in heart and brain homogenates were increased significantly ( $p < 0.01$ ), thereby, enhancing the endogenous antioxidant levels. Furthermore, the results of test drug were comparable to folic acid, a standard drug (Usui *et al.*, 1999). Folic acid administration significantly declines homocysteine levels and improves arterial endothelial function and has potential implications for the prevention of atherosclerosis in adults with 3 or more traditional risk factors of Coronary Artery Disease (CAD) and hyperhomocysteinemia (Guo *et al.*, 2004).

## Conclusion

Antioxidant treatment restores several toxic effects of homocysteine. In the present study, elevated levels of homocysteine, LDH, total cholesterol, LDL-C and triglycerides in serum and LPO in heart and brain homogenates were reduced significantly ( $P < 0.01$ ) after treatment with *Nigella sativa* aqueous extracts in two doses of 100 and 200 mg/kg body weight, suggesting lipid lowering, cardio- and neuro-protective potential of *Nigella sativa*. Further, the levels of HDL-C in serum

and GSH in heart and brain homogenates were increased significantly ( $P<0.01$ ), thereby, enhancing the endogenous antioxidant levels. Furthermore, the results of test drug were comparable to folic acid, a standard positive control. So it can be concluded that *Nigella sativa* decrease the levels of homocysteine. Therefore, the role of *Nigella sativa* in management of hyperhomocysteinemia is suggested.

Table 1  
Effect on homocysteine and lactate dehydrogenase (LDH) levels in serum in L-methionine-induced hyperhomocysteinemia in albino rats

Groups	Homocysteine ( $\mu\text{g/mL}$ )	LDH (IU/L)
I. Vehicle control	8.520 $\pm$ 0.189	29.101 $\pm$ 0.709
II. Pathogenic control	23.238 $\pm$ 0.339 <sup>#</sup>	58.864 $\pm$ 0.702 <sup>#</sup>
III. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (100 mg/kg, <i>p.o.</i> )	8.864 $\pm$ 0.987	29.748 $\pm$ 0.559
IV. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (200 mg/kg, <i>p.o.</i> )	9.104 $\pm$ 0.105	31.718 $\pm$ 1.107
V. Aqueous <i>Nigella sativa</i> extract treated (100 mg/kg, <i>p.o.</i> )	15.971 $\pm$ 0.029*	41.876 $\pm$ 0.432*
VI. Aqueous <i>Nigella sativa</i> extract treated (200 mg/kg, <i>p.o.</i> )	16.105 $\pm$ 0.051*	40.018 $\pm$ 0.426*
VII. Folic acid treated (100 mg/kg, <i>p.o.</i> )	15.048 $\pm$ 0.041*	35.124 $\pm$ 0.371*

All values expressed as Mean  $\pm$  SEM, n=6, control group (i.e., group I),

<sup>#</sup>p<0.01, vs vehicle

\*p<0.01, vs pathogenic control group (i.e., group II)

Table 2  
Effect on total cholesterol, triglycerides, LDL-C and HDL-C levels in serum in L-methionine-induced hyperhomocysteinemia in albino rats

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
I. Vehicle control	102.216 $\pm$ 0.794	86.952 $\pm$ 1.294	42.872 $\pm$ 0.602	40.486 $\pm$ 0.568
II. Pathogenic control	192.491 $\pm$ 1.714 <sup>#</sup>	180.628 $\pm$ 2.152 <sup>#</sup>	143.134 $\pm$ 1.624 <sup>#</sup>	15.142 $\pm$ 0.542 <sup>#</sup>
III. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (100 mg/kg, <i>p.o.</i> )	96.451 $\pm$ 0.514	86.874 $\pm$ 0.589	42.087 $\pm$ 0.282	38.984 $\pm$ 0.188
IV. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (200 mg/kg, <i>p.o.</i> )	98.142 $\pm$ 1.304	88.417 $\pm$ 1.288	40.527 $\pm$ 0.623	37.580 $\pm$ 0.430
V. Aqueous <i>Nigella sativa</i> extract treated (100 mg/kg, <i>p.o.</i> )	165.385 $\pm$ 1.024*	144.328 $\pm$ 1.603*	120.230 $\pm$ 0.728*	22.308 $\pm$ 0.389*

VI. Aqueous <i>Nigella sativa</i> extract treated (200 mg/kg, <i>p.o.</i> )	158.108 ± 1.869*	129.366 ± 2.210*	106.584 ± 0.728*	22.830 ± 0.602*
VII. Folic acid treated (100 mg/kg, <i>p.o.</i> )	149.305 ± 1.420*	121.720 ± 1.817*	96.587 ± 0.354*	28.324 ± 1.261*

All values expressed as Mean ± SEM, n=6,  
control group (i.e., group I),

#p<0.01, vs vehicle

\*p<0.01, vs pathogenic control  
group (i.e., group II)

Table 3: Effect on VLDL-C levels and atherosclerotic index in serum in L-methionine-induced hyperhomocysteinemia in albino rats

Groups	VLDL-C (mg/dl)	Atherosclerotic Index (Total Cholesterol/HDL-C)	Atherosclerotic Index (LDL-C/HDL-C)
I. Vehicle control	16.254 ± 0.268	2.139 ± 0.308	1.361 ± 0.032
II. Pathogenic control	36.204 ± 0.315#	14.198 ± 0.087#	11.987 ± 0.425#
III. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (100 mg/kg, <i>p.o.</i> )	16.358 ± 0.357	2.894 ± 0.031	1.108 ± 0.008
IV. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (200 mg/kg, <i>p.o.</i> )	16.875 ± 0.120	2.569 ± 0.009	1.075 ± 0.006
V. Aqueous <i>Nigella sativa</i> extract treated (100 mg/kg, <i>p.o.</i> )	27.938 ± 0.630*	7.896 ± 0.094*	5.512 ± 0.087*
VI. Aqueous <i>Nigella sativa</i> extract treated (200 mg/kg, <i>p.o.</i> )	26.750 ± 0.462*	6.798 ± 0.076*	4.687 ± 0.061*
VII. Folic acid treated (100 mg/kg, <i>p.o.</i> )	24.871 ± 0.492*	4.347 ± 0.129*	3.230 ± 0.112*

All values expressed as Mean ± SEM, n=6,  
control group (i.e., group I),

#p<0.01, vs vehicle

\*p<0.01, vs pathogenic control  
group (i.e., group II)

Table 4  
Effect on lipid peroxides (LPO) and glutathione (GSH) levels in myocardial homogenate in L-methionine-induced hyperhomocysteinemia in albino rats

Groups	LPO (nmoles of MDA/mg protein)	GSH ( $\mu$ moles of phosphorous liberated /min/mg protein)
I. Vehicle control	1.345 $\pm$ 0.004	11.798 $\pm$ 0.182
II. Pathogenic control	4.920 $\pm$ 0.038 <sup>#</sup>	6.098 $\pm$ 0.322 <sup>#</sup>
III. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (100 mg/kg, <i>p.o.</i> )	1.684 $\pm$ 0.031	12.265 $\pm$ 0.559
IV. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (200 mg/kg, <i>p.o.</i> )	1.687 $\pm$ 0.023	12.478 $\pm$ 0.357
V. Aqueous <i>Nigella sativa</i> extract treated (100 mg/kg, <i>p.o.</i> )	3.879 $\pm$ 0.025*	9.347 $\pm$ 0.314*
VI. Aqueous <i>Nigella sativa</i> extract treated (200 mg/kg, <i>p.o.</i> )	3.320 $\pm$ 0.017*	9.311 $\pm$ 0.187*
VII. Folic acid treated (100 mg/kg, <i>p.o.</i> )	2.782 $\pm$ 0.019*	10.977 $\pm$ 0.258*

All values expressed as Mean  $\pm$  SEM, n=6,  
control group (i.e., group I),

<sup>#</sup>p<0.01, vs vehicle

\*p<0.01, vs pathogenic control  
group (i.e., group II)

Table 5  
Effect on lipid peroxides (LPO) and glutathione (GSH) levels in brain homogenate in L-methionine-induced hyperhomocysteinemia in albino rats

Groups	LPO (nmoles of MDA/mg protein)	GSH ( $\mu$ moles of phosphorous liberated /min/mg protein)
I. Vehicle control	1.519 $\pm$ 0.007	12.988 $\pm$ 0.257
II. Pathogenic control	7.464 $\pm$ 0.221 <sup>#</sup>	7.699 $\pm$ 0.634 <sup>#</sup>
III. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (100 mg/kg, <i>p.o.</i> )	1.877 $\pm$ 0.066	14.117 $\pm$ 0.187
IV. Aqueous <i>Nigella sativa</i> extract <i>per se</i> (200 mg/kg, <i>p.o.</i> )	1.988 $\pm$ 0.061	14.494 $\pm$ 0.545
V. Aqueous <i>Nigella sativa</i> extract treated (100 mg/kg, <i>p.o.</i> )	4.163 $\pm$ 0.072*	10.464 $\pm$ 0.525*
VI. Aqueous <i>Nigella sativa</i> extract treated (200 mg/kg, <i>p.o.</i> )	3.496 $\pm$ 0.051*	11.493 $\pm$ 0.166*
VII. Folic acid treated (100 mg/kg, <i>p.o.</i> )	3.153 $\pm$ 0.064*	12.445 $\pm$ 0.749*

All values expressed as Mean  $\pm$  SEM, n=6,  
control group (i.e., group I),

<sup>#</sup>p<0.01, vs vehicle

\*p<0.01, vs pathogenic control group (i.e., group II)

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