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Full Length Research Paper

Effect of dietary supplementation with *Nigella sativa* L. on serum lipid profile, lipid peroxidation and antioxidant defense system in hyperlipidemic rabbits

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The seeds of *Nigella sativa* L. (black seed) have been used as a natural remedy to treat many diseases, including hyperlipidemia in some folk medicine. Therefore, the aim of the present study was to examine the hypolipidemic and antioxidant effects of dietary black seed in hyperlipidemic rabbits. Twenty four New Zealand male rabbits were fed a 0.5% cholesterol diet for 1 month and then were randomly assigned to two groups, the control group which received the previous hypercholesterolemic diet and the black seed group which was fed 7.5 g/kg b.w/day crushed black seed + 0.5% cholesterol diet, each for 2 months. Fasting blood samples were obtained at baseline, after hyperlipidemia, 1 month and 2 months of treatment to determine serum lipid profile, malondialdehyde (MDA), level, total antioxidant status (TAS), superoxide dismutase (SOD) and glutathione peroxidase (GPX). The concentrations of serum total cholesterol, triglyceride, low density lipoprotein cholesterol (LDL.C) and MDA were significantly lowered in black seed group ($P < 0.05$), as compared to control group at the end of study, but there were no significant differences in the levels of TAS, SOD and GPX between groups ($P > 0.05$). In conclusion, these findings demonstrate that dietary black seed can favorably decrease serum lipid profile and lipid peroxidation levels in hyperlipidemic rabbits, therefore, it may be considered as a useful therapy for hyperlipidemia.

Key words: *Nigella sativa*, lipid profile, malondialdehyde, antioxidant status, rabbits.

INTRODUCTION

Hyperlipidemia, being an important risk factor for cardiovascular disease, is a serious public health problem in the world. Its major role in the pathogenesis of atherosclerosis has been implicated by several clinical and epidemiological studies (Jaffar et al., 2004). Hyperlipidemia also has an indirect role by stimulating the production of oxygen free radicals (OFRs) from polymorphonuclear leukocytes (PMNLs) and monocytes (Prasad et al., 2005). Regarding its treatment, nowadays there is an increasing interest toward the potential health benefits of medicinal plants.

Nigella sativa Linn (*N. sativa*) commonly known as black seed or black cumin, is an annual herb from the botanical family of Ranunculaceae. The seeds of the plant have been used in the Southeast Asia, Middle and Far East as a natural remedy to treat many diseases, including asthma, hypertension, diabetes, hypercholesterolemia, inflammation, arthritis, tumor, gastrointestinal

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Abbreviations: (b.w); Body weight, **(GPX);** glutathione peroxidase, **(HDL.C);** high-density lipoprotein cholesterol, **(LDL.C);** low-density lipoprotein cholesterol, **(MDA);** malondialdehyde, **(NSO);** *Nigella sativa* volatile oil, **(OFRs);** oxygen free radicals, **(PMNLs);** polymorphonuclear leukocytes, **(PUFAs);** poly unsaturated fatty acids, **(SOD);** Superoxide dismutase; **(TQ);** thymoquinone, **(TAS);** total antioxidant status, **(TC);** total cholesterol, **(TG);** triglycerides and **(IM);** intramuscular.

disturbances and gynecological disorders for over 2000 years (Ali and Blunden, 2003; El-Din et al., 2006; Ramadan, 2007). They are extensively used as spice, condiment and aromatic which can be added to tea, coffee, casseroles or breads. The ground seed can be mixed with honey or sprinkled on salads (Ramadan, 2007).

The seed of *N. sativa* has over 100 different chemical components, including mucilage, crude fiber, reducing sugars, resins, alkaloids, flavonoids, organic acids, sterols, tannins and saponins, in addition to the high content of unsaturated fatty acids, especially linoleic acid (18:2 ω-6) and oleic acid (18:1 ω-9) and proteins. It also has yellowish volatile (essential) oil (Akram, 1999; Gilani et al., 2004). It is known that the biological activity of *N. sativa* seeds is attributed to its essential oil compo-nents (Hajhashemi et al., 2004). The main compounds contained are thymoquinone (30 - 48%), p-cymene (7 - 15%), carvacrol (6 - 12%), 4-terpineol (2 - 7%), t-anethole (1 - 4%) and a sesquiterpene longifolene (1 - 8%) (Burits and Bucar, 2000) in which thymoquinone (TQ) and its derivatives [dithymoquinone (DTQ), thymohydroquinone (THQ) and thymol (THY)] are the most putative pharmacologically active constituents of *N. sativa* (Padhye et al., 2008).

Regarding to the side effects of *N. sativa*, it has been shown that there were no toxic effects when its oil was given to mice via the stomach; it has also been reported that Nigella seed powder does not produce any toxic effect when given to rabbits by gastric incubation (Akhondian et al., 2007). Moreover, in another study the low toxicity of *N. sativa* fixed oil, evidenced by high LD50 values, key hepatic enzyme stability and organ integrity, suggested a wide margin of safety for therapeutic doses of its fixed oil (Zaoui et al., 2002a).

There is some scientific evidence for the hypolipidemic effect of black seed as its oil (El-Dakhakhny et al., 2000; Zaoui et al., 2002b; Kalus et al., 2003), extract (Le et al., 2004; Kaleem et al., 2006; Dehkordi and Kamkhah, 2008) or some pure compounds such as TQ (Badary et al., 2000; Bamosa et al., 2002). Moreover, the effect of *N. sativa* on serum or tissue lipid peroxidation and antioxi-dant status has been studied previously with inconsistent results (Meral et al., 2001; Kanter et al., 2005, 2006; Uz et al., 2008). However, to date, little attention has been paid to the effects of whole or crushed black seed on serum lipid profile and other biochemical parameters. Therefore, the present study was aimed to examine the effect of dietary crushed black seed on serum lipid profile, lipid peroxidation and antioxidant defense system in hyperlipidemic rabbits.

MATERIALS AND METHODS

The plant and chemicals

The *N. sativa* seeds were purchased from a local herb store and were authenticated at the Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran. A voucher specimen (Number: TbzMed-FPh-193) was deposited in the herbarium of the Faculty. The seeds were crushed in a miller before dietary supplementation.

Pure cholesterol powder (Sinopharm Chemical Reagent Co., Ltd, China) and Xylazine hydrochloride 2% (injectable solution, Alfasan, Poland) were obtained respectively from a laboratory equipment center and a veterinary pharmacy in Tabriz.

Animals

Twenty four New Zealand White male rabbits, weighing 1.8 - 2.4 kg were obtained from Razi Vaccine and Serum Research Institute (Karaj, Iran). Animals were housed in individual cages under standard laboratory conditions in a 12 h/12 h light/dark cycle and at a temperature of 21 - 23°C. Food and water were supplied *ad libitum*. The study was conducted in agreement with the Guides for Care and Use of Laboratory Animals of the National Institutes of Health and all efforts were made to minimize animal suffering and the number of animals used. The protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences.

Study design

All the rabbits were fed with rabbit laboratory chow diet (Nirou Sahand Company, Tabriz, Iran) during 2 weeks of adaptation and then received a hypercholesterolemic diet with 0.5% cholesterol for 1 month. Afterward, the rabbits were randomly assigned to two groups. The control group received the previous hypercholeste-rolemic diet and the black seed group received 7.5 g/kg b.w/day crushed black seed + 0.5% cholesterol diet, each for 2 months. The experimental diets were isocaloric and isonitrogenous and their compositions were analyzed by the Food and Drug Organization, Tabriz University of Medical Sciences (Table 1).

Fasting blood samples (5 cc) were obtained from central ear artery under slight anesthesia (Xylazine; 6 mg/kg IM) at baseline, after hyperlipidemia, and after 1 month and 2 months of treatment to determine serum lipid profile, including total cholesterol (TC), High density lipoprotein cholesterol (HDL.C), low density lipoprotein cholesterol (LDL.C), triglyceride (TG)], malondialdehyde (MDA), total antioxidant status (TAS), superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Biochemical analysis

Serum was obtained by high speed centrifugation and stored at -70°C until analysis. The concentrations of TC, HDL-C and TG were measured by enzymatic colorimetric methods with commercial kits (Cholesterol CHOD-PAP and Triglycerides GPO-PAP; Pars Azmone, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA) (Moghadasian et al., 2002). Serum LDL.C was calculated according to the Friedewald equation (Friedewald et al., 1972). Since the TC/HDL-C and LDL.C/HDL.C ratios determine the relative risk of coronary artery disease, they were also calculated.

Serum MDA level, as a marker of lipid peroxidation and oxidative stress was measured through reaction with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a pink colored complex. Then, its fluorescence intensity was measured at 547 nm with excitation at 525 nm by a spectrofluorimeter (Kontron, model SFM 25A, Italy) (Del Rio et al., 2003).

Measurement of TAS in serum and SOD and GPX in heparinated blood was performed by colorimetric method with commercial kits (TAS: RANDOX kits, SOD: RANSOD kits and GPX: RANSEL kits; RANDOX Laboratory, UK), on an automatic analyzer (Abbott model Alcyon 300, USA).

Table 1. Composition of experimental diets.

Variable	Energy (Kcal)	Lipid (g/100g)	Protein (g/100g)	Carbohydrate (g/100g)	Fiber (g/100g)	Ash (g/100g)	Moisture (%)
Hypercholesterolemic diet [a]	353.59	15.35	16.36	37.5	13.86	7.35	9.4
Black seed diet [b]	353.5	15.28	17.3	36.39	14.17	7.41	9.25

[a.] Rabbit laboratory chow diet (Nirou Sahand Company, Tabriz, Iran) + 0.5% cholesterol.
[b.] 7.5 g/kg b.w/day crushed black seed plus hypercholesterolemic diet.

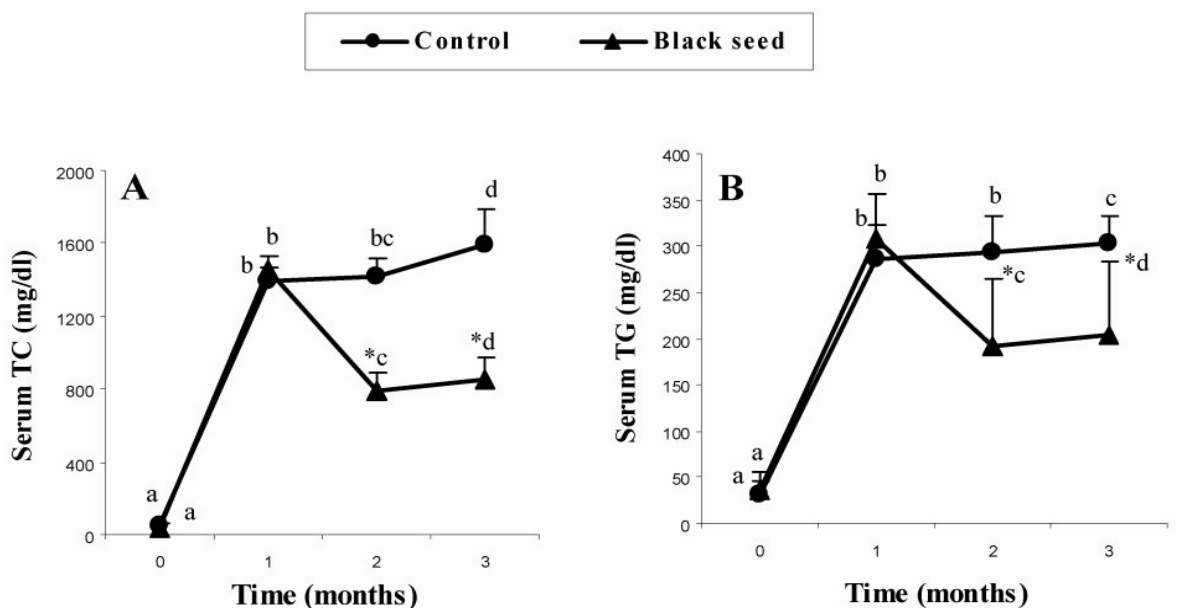

Figure 1. Sequential changes in serum total cholesterol (A) and triglyceride (B) concentrations of two groups. The time (months) refers to baseline (0), hyperlipidemic (1), first month of treatment (2) and second month of treatment (3). Results are expressed as mean ± SD. (*) $p < 0.05$, black seed group vs. control group at different intervals. (a, b, c …), statistically significant difference ($p < 0.05$) within each group at different intervals when there is not the same alphabet.

Statistical analysis

Results are expressed as mean ± SD. Normal distribution of collected data was examined with the Kolmogorov Smirnov test. Statistical analysis was performed on SPSS software (version 14) using unpaired Student's t-test, to compare results between two groups in different times. Moreover comparison within each group (before-after test) was conducted using paired Student's t test. P-values less than 0.05 were considered statistically significant.

RESULTS

Serum lipid profile

The sequential changes in serum TC, TG, LDL.C, HDL.C, TC/HDL.C and LDL.C/HDL.C are summarized in Figure 1 - 3. High cholesterol diet significantly increased the level of serum lipid profile compared to baseline ($p< 0.001$). There was no statistically significant difference in serum lipid profile between groups at baseline and after induction of hyperlipidemia ($p > 0.05$). After 1 month of treatment with black seed, the concentrations of serum TC, LDL.C, HDL.C, TG and TC/HDL.C were significantly lower in treatment group as compared to control group, by 43.7% ($p < 0.001$), 42.8% ($p < 0.001$), 22.3% ($p < 0.05$), 34.9% ($p < 0.01$) and 29.6% ($p < 0.05$), respectively. At the end of the study (after 2 months of treatment), black seed group also had lower levels of serum TC, LDL.C, TG, TC/HDL.C and LDL.C/HDL.C, compared to control group by 46.7% ($p < 0.001$), 48.2% ($p < 0.001$), 32.6% ($p < 0.05$), 38.5% ($p < 0.001$) and 40.19% ($p < 0.001$), respectively. Figure 2

In black seed group, the levels of TC, LDL.C, HDL.C, TG and TC/HDL.C significantly decreased after treatment at two months, compared to hyperlipidemic period. The reduction of TC, LDL.C, HDL.C, TG and TC/HDL.C were respectively by 45.2% ($p < 0.001$), 34.2% ($p < 0.001$), 30.95% ($p < 0.001$), 37.99% ($p < 0.01$) and 23% ($p < 0.05$) after one month of treatment and by 41.6% ($p <$

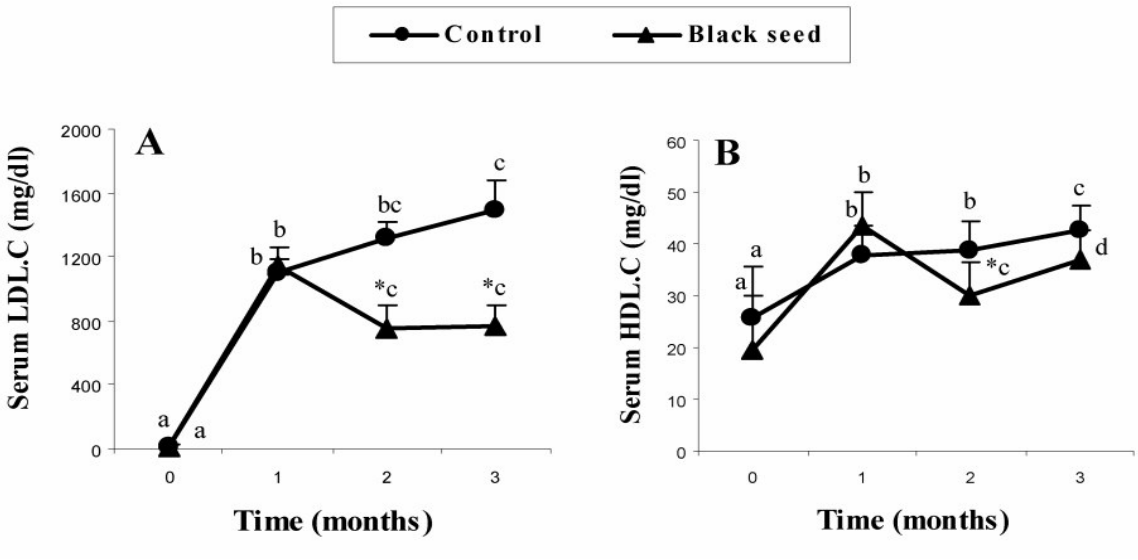

Figure 2. Sequential changes in serum LDL.C (A) and HDL.C (B) concentrations of two groups. The time (months) refers to baseline (0), hyperlipidemic (1), first month of treatment (2) and second month of treatment (3). Results are expressed as mean ± SD. (*) $p < 0.05$, black seed group vs. control group at different intervals. (a, b, c, …), statistically significant difference ($p < 0.05$) within each group at different intervals when there is not the same alphabet.

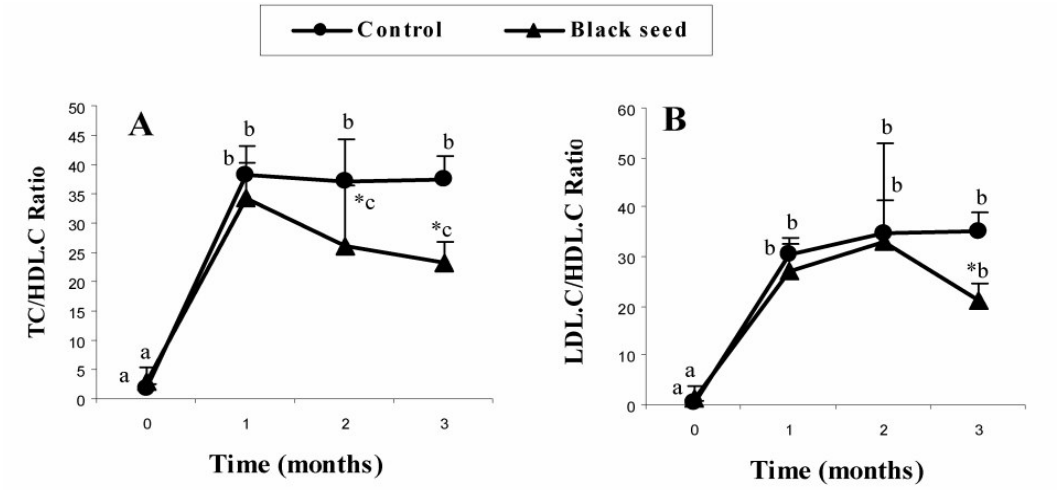

Figure 3. The Changes in serum TC/HDL.C (A) and LDL.C/HDL.C ratio (B) of two groups. The time (months) refers to baseline (0), hyperlipidemic (1), first month of treatment (2) and second month of treatment (3). Results are expressed as mean ± SD. (*) $p < 0.05$, black seed group vs. control group at different intervals. (a, b, c …), statistically significant difference ($p < 0.05$) within each group at different intervals when there is not the same alphabet.

0.001), 32.7% ($p < 0.01$), 14.76% ($p < 0.05$), 33.72% ($p < 0.05$) and 32.4% ($p < 0.01$) at the end of the study (Figure 3).

Serum malondialdehyde

The changes in the serum levels of MDA are summarized in Figure 4. High cholesterol diet had no significant effect on the levels of serum MDA ($P > 0.05$) and there was no statistically significant difference in serum MDA between groups at baseline and after induction of hyperlipidemic condition ($p > 0.05$) after 1 month and 2 months of treatment with black seed.

The level of MDA in black seed group were significantly lower by 51.1% ($p < 0.001$) and 49.2% ($p < 0.01$) during

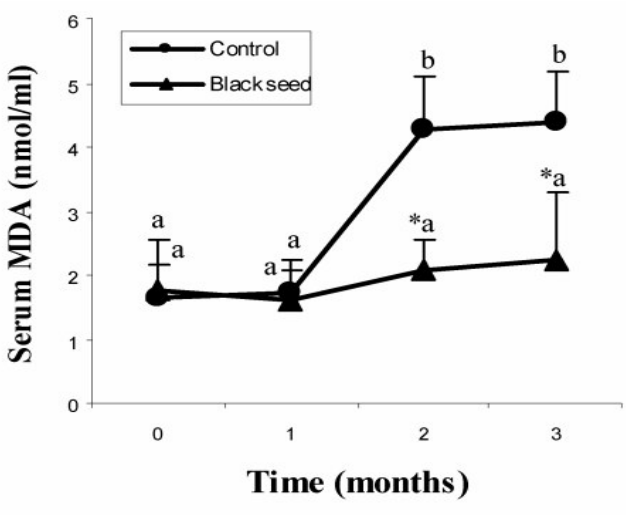

Figure 4. Sequential changes in serum MDA concentration of two groups. The time (months) refers to baseline (0), hyperlipidemic (1), first month of treatment (2) and second month of treatment (3). Results are expressed as mean ± SD. (*) $p < 0.05$, black seed group vs. control group at different intervals. (a, b, c…), statistically significant difference ($p < 0.05$) within each group at different intervals when there is not the same alphabet.

two months of treatment, as compared to control group. The changes within black seed group were not significant at different intervals ($p > 0.05$).

Antioxidant defense system

The sequential changes in serum TAS, blood SOD and GPX of the two groups are summarized in Figure 5. High cholesterol diet significantly decreased the levels of SOD and GPX in both groups ($p < 0.05$), but had no effect on total antioxidant status. Furthermore, no significant difference was observed in the levels of TAS, SOD and GPX between groups and within groups at different intervals in the present study ($p > 0.05$).

DISCUSSION

The results of the present study showed significant decrease in the development of hyperlipidemia in black seed group as serum lipid profile and malondialdehyde were significantly lower, compared to control group. Furthermore, dietary black seed had no significant effect on TAS, SOD and GPX levels in hyperlipidemic rabbits.

Although some studies have addressed different beneficial properties of *N. sativa* as its extract, oil or active compound(s) such as thymoquinone, to date few have examined the effects of whole or crushed seed, including its hypolipidemic and antioxidant properties. Only one study assessed the effect of crushed black seed on some blood parameters, including serum levels of cholesterol and triglyceride. The results showed significant decrease in the concentration of TC and TG; which was similar to our findings (Ibraheim, 2002).

Indeed, in consistent with our results, the study of Zaoui et al. (2002b) indicated that oral treatment with *N. sativa* oil, decreased serum cholesterol and TG levels by 15.5 and 22% in normal rats. In another study, *N. sativa* oil administration to rats significantly decreased serum TC, LDL.C and TG and increased HDL.C (El-Dakhakhny et al., 2000). Le et al. (2004) reported a significant decrease in plasma TG and an increase in HDL.C levels in black seed extract-oral treated rats, compared to the control group. Moreover, a significant reduction was observed in serum TC and LDL.C of patients with mild hypertension after 8 weeks of black seed extract oral administration (Dehkordi and Kamkhah, 2008). The results of Bamosa et al. (2002) demonstrated a decrease in serum TC, LDL.C, HDL.C and TG during intraperitonial injection of thymoquinone in rats. Furthermore, the hypotriglyceridemic effect of nigellamines (that is black seed diterpene alkaloids) was reported in an *in vitro* study, equivalent to the hypolipidemic agent, clofibrate (Morikawa et al., 2004).

The hypolipidemic effect of black seed does not seem to be due only to one component, but rather to the synergistic action of its different constituents, including TQ and nigellamine as mentioned above, soluble fiber (e.g. mucilage), sterols, flavonoids and high content of polyunsaturated fatty acids (PUFAs) (Ali and Blunden, 2003). Mechanism of hypolipidemic action of TQ is not fully understood; however, decreased cholesterol synthesis, and more importantly, its antioxidant role have been proposed. Lipid lowering effects of dietary soluble fibers (Brown et al., 1999; Talati et al., 2009) and sterols (Jones, 1999; Moruisi et al., 2006) are probably related to decreased dietary cholesterol absorption, increased primary bile acid synthesis and its fecal losses. Flavonoids may act by making liver cells more efficient to remove LDL.C from blood. To do this, flavonoids increase LDL receptor densities in liver and by binding to apolipoprotein B (El-Beshbishy et al., 2006; Weggemans and Trautwein, 2003). PUFAs are also well - known to decrease serum HDL and total cholesterol (Djousse et al., 2003).

Regarding the effect of black seed on lipid peroxidation and antioxidant defense system, Kanter et al. (2005) found that treatment with the volatile oil of *N. sativa* (NSO) decreased blood MDA levels and increased the antioxidant defense system activity in carbon tetrachloride treated rats. The same authors in another study showed that NSO treatment reduced the spinal cord tissue MDA and prevented from inhibition of SOD, GPX, and catalase (CAT) enzyme activities, following the experimental spinal cord injury in rats (Kanter et al., 2006). Furthermore, in a recent study oral administration of *N. sativa* oil to rats had no significant effect on the levels of

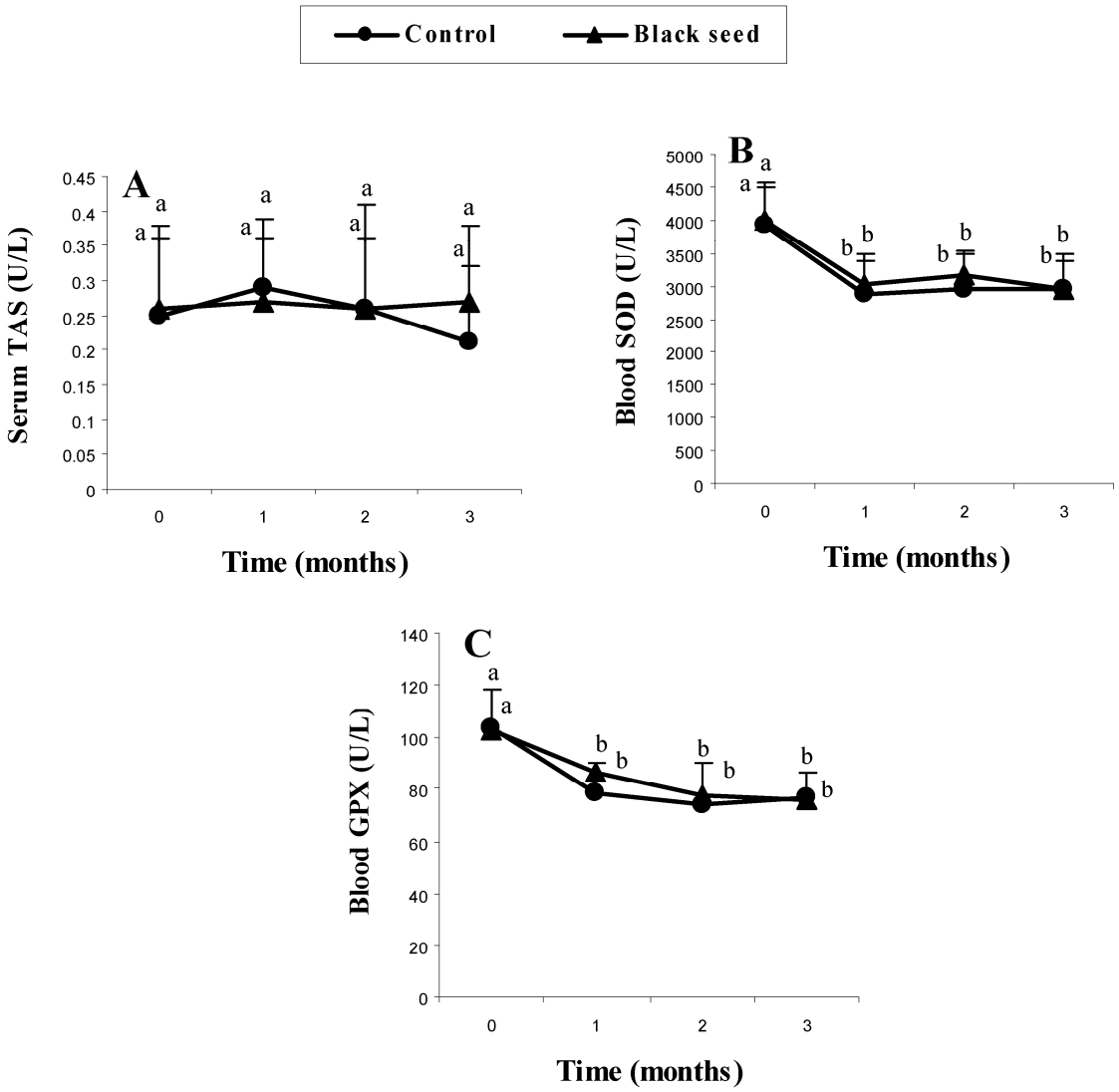

Figure 5. Sequential changes in serum TAS (A), RBC SOD (B) and GPX (C) levels of two groups. The time (months) refers to baseline (0), hyperlipidemic (1), first month of treatment (2) and second month of treatment (3). Results are expressed as mean±SD. (*) $p < 0.05$, black seed group vs. control group at different times. (a, b, c…), statistically significant difference ($p < 0.05$) within each group at different intervals when there is not the same alphabet.

heart tissue MDA and SOD, GPX, and CAT en-enzyme activities, compared to control group)Uz et al., 2008). The study of Meral et al. (2001) indicated that *N. sativa* extract decreased the elevated blood MDA concentration and increased the lowered glutathione and ceruloplasmin concentrations in diabetic rabbits.

In agreement with previous studies, we found a significant decrease in serum MDA levels, whereas in contrast with some of them our results did not show any significant differences in antioxidant status of the rabbits. It was probably attributable to the pharmacological form of black seed, kinds of antioxidant proteins, and enzymes or tissue or serum, which were studied. Moreover, most of the previous studies used a direct method before treatment with black seed to induce oxidative stress.

The antioxidant effect of black seed seems to be due to its oil, thymoquinone, flavonoids and also antioxidant vitamins like ascorbic acid. It has been shown that the *N. sativa* oil and TQ inhibit non-enzymatic lipid peroxidation in liposomes and both of them especially TQ, work as a scavenger of various reactive oxygen species, including superoxide anion and hydroxyl radicals (Padhye et al., 2008). In addition flavonoids are a class of polyphenolic compounds that seem to have antioxidant properties by suppressing reactive oxygen and nitrogen species formation, scavenging reactive oxygen and nitrogen species

and protecting the antioxidant defense system (Arts and Hollman, 2005; Moyers and Kumar, 2004)

In conclusion, dietary supplementation with crushed black seed favorably decreased serum lipid profile and lipid peroxidation levels in hyperlipidemic rabbits. Therefore, it may be regarded as a useful therapy for hyperlipidemia. However, further studies are required to study its effects on hyperlipidemic patients and compare it with lipid lowering drugs.

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