Original Article

Comparative Pre- and Post-treatment Effects of *Nigella Sativa* Oil on Lipid Profile and Antioxidant Enzymes in a Rat Model of Diabetes Mellitus

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Abstract

Background: Lipid profile dysregulation and oxidative stress are important risk factors for cardiovascular disease in diabetic individuals. *Nigella Sativa* (NS) oil has been reported to have a favorable effect on triglycerides (TG) in rat models of diabetes mellitus. There is a dearth of information available about preventive or corrective use to manage and ameliorate diabetes. **Aim:** This study sought to ascertain the comparative pre and post-treatment effects of the oil on TG, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, and key antioxidant enzymes levels in diabetic rats. **Methods:** Thirty (30) Wistar rats were divided into 6 groups of 5 rats each as follows: Group I rats took normal chow *ad libitum* and served as Control. Group II rats were induced with diabetes using streptozocin (50 mg/Kg BW). Group III and IV rats were pre-administered with 0.5 and 1 ml of the oil, respectively, before induction, whereas Group V and VI rats were treated with 0.5 and 1 ml of the oil after induction. The listed parameters were assessed in the plasma at the end of the study. **Results:** Diabetes induction caused a significant increase in the TG level. There was no significant change in the oxidative stress parameters. Only post-administration caused a significant reduction in TG level, whereas both pre and post-administrations caused a significant improvement in HDL levels. Both pre- and post-administrations caused an increase in superoxide dismutase and catalase levels when causing a significant reduction in malondialdehyde level. **Conclusion:** Post-induction treatment may be more effective in the correction of lipid dysregulation and oxidative stress in diabetes.

Keywords: Antioxidant enzymes, atherogenic index, diabetes, lipid profile, *Nigella sativa*

Introduction

Diabetes mellitus (DM) remains the most common metabolic disorder worldwide.[1] The hallmark of this disorder includes the abnormally high level of triglycerides (TGs), reduced high-density lipoprotein (HDL), and reduced low-density lipoprotein (LDL) level.[1] Apart from these, hyperglycemia is known to play a contributory role in the development of oxidative stress in diabetes.[2] The oxidative stress if unchecked has been shown to continuously lead to damage to the biological system which eventually leads to increased production of markers of lipid peroxidation like malondialdehyde (MDA)[3] contributing to the development of diabetic complications.[4] The abnormalities in circulating lipids and lipoproteins are thus considered to be important risk factors for cardiovascular disease in diabetic individuals.[5] Reversal of these abnormalities in lipid profile may reduce accelerated atherosclerosis and the related macrovascular complications in patients with DM.[6]

The use of natural oil and herbal products for disease control is the new order, especially in the management of diabetic complications and correcting the associated metabolic abnormalities.[7] *Nigella sativa* (NS) (black cumin or black seed) is among the natural sources described to have therapeutic effects in the management of many diseases.[8]

Access this article online

Quick Response Code:

Website: www.njmonline.org

DOI: 10.4103/NJM.NJM_42_20

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How to cite this article: Busari AA, Adejare AA, Kelani SO, Imam KO, Awesu AA, Adefila-Sanni I. Comparative pre- and post-treatment effects of *Nigella sativa* oil on lipid profile and antioxidant enzymes in a rat model of diabetes mellitus. Niger J Med 2020;29:415-21.

Submitted: 29-Apr-2020 **Revised:** 02-Jul-2020
Accepted: 08-Jul-2020 **Published:** 18-Sep-2020

The oil has been widely reported to have anti-inflammatory, immunomodulatory, anticancer, antiparasitic, antiasthmatic, and antihypertensive effects.[9,10] It has also been reported to have antidiabetic, analgesic, antimicrobial, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastroprotective, and antioxidant properties.[11] Moreover, the seeds of *NS* are commonly used in the management of various diseases such as bronchitis, diarrhoea, rheumatism, and skin disorders.[10] Recently, our team was able to establish the therapeutic but non-synergistic effect it has when used with Vitamin E (α-tocopherol) in the management of cisplatin-induced renal toxicities.[12] Interestingly, thymoquinone which had earlier been identified as the active component in the oil is not only an effective superoxide radical scavenger but also protects the liver from toxins.[13]

The hypolipidemic and antioxidative effects of *NS* oil have been demonstrated in experimental animals where it was reported to have a favourable effect on TG and lipoprotein the pattern in normal rats.[14] Similar findings were encountered by the administration of thymoquinone, the active ingredient of NS, to rabbits fed on cholesterol-enriched diet[15] and to hypercholesterolemic rats.[16] Even though the majority of authors are of the opinion that NS oil administration improves hyperglycemia,[17] oxidative stress, and lipid peroxidation,[18] there is a paucity of information on whether this oil should be given before the development of diabetes or after, especially in individuals with a family history of diabetes. This study, therefore, sought to determine the comparative therapeutic effects of pre- and post-supplementation of the oil on lipid profile and antioxidant levels in a rat model of diabetes.

Animals and Methods

Ethical approval

Ethical approval for this study was granted by the College of Medicine of the University of Lagos Animal Care and Use Research Ethics Committee with reference number (CMUL/HREC/02/20/715).

Animals

The study was carried out in adult male Wistar rats weighing 140–160 g. For acclimatization, the animals were kept in the faculty animal house for 2 weeks before the start of the experiment. The Wistar rats were kept in wire mesh cages under room conditions. The animals were allowed free access to water and food pellets at a room temperature of about 29°C ± 2°C throughout the period of this experiment. They had a 12-hour light and 12-hour dark cycle. Experimental protocols complied with the Guide for the Care and Use of Laboratory Animals.[19]

Grouping and treatments per group

A total of thirty (30) Wistar rats were divided into 6 groups of 5 rats each as follows: Group I rats were fed normal rat chow *ad libitum* without any induction and served as Control. Group II rats were induced with diabetes without treatment (DB group). Group III rats were pretreated with 0.5 ml/kg NS oil before diabetes induction (NS0.5 + DB group) intraperitoneally. Group IV rats were pretreated with 1 ml/kg NS oil before diabetes induction (NS1 + DB group). Group V rats were treated with 0.5 ml/kg NS oil intraperitoneally after diabetes induction (DB + NS0.5) and finally Group VI rats were treated with 1 ml/kg NS oil after diabetes induction (DB + NS1) intraperitoneally. Group III and IV rats were sacrificed 72 h after induction, whereas Groups V and VI rats were sacrificed after completion of treatment.

This arrangement is illustrated in Table 1.

Induction of diabetes

Diabetes was induced in Groups II, III, IV, V, and VI by a single intraperitoneal injection of a freshly buffered (0.1 M citrate, pH 4.5) solution of streptozotocin (STZ) at a dosage of 50 mg/kg body weight. After 72 h of STZ administration, the tail vein blood was collected to determine blood glucose level with the aid of a very sensitive glucometer. Only rats with blood glucose over 250 mg/dl were considered diabetic and included in the experiments.[20]

Administration of *Nigella sativa* oil

Groups III and IV rats were preadministered with 0.5 and 1 ml of the pure oil, respectively, for 2 weeks intraperitoneally before the induction of diabetes. Groups V and VI rats were administered with 0.5 and 1 ml of the pure oil, respectively, for 2 weeks intraperitoneally after the induction of diabetes.

Blood sample collection

Each group of animals was sacrificed on different days after completion of the expected exposure and treatment periods. After overnight fasting, rats were sacrificed following euthanization by cervical dislocation.[21] Blood was collected via cardiac puncture into EDTA bottles and centrifuged immediately at 3000 rpm for 10 min to obtain serum for estimation of lipid profile and antioxidant enzymes.

Assessment of lipid profile

TG, total cholesterol, HDL, and LDL in the serum were analyzed using a kit meant for animals obtained from Randox Laboratories Limited, Crumlin, UK. This was carried out using the method of Morakinyo *et al*.[21] with some minor modifications. To 5 µL of serum, 0.5 mL of enzyme reagent was added and kept at 37°C for 5 min. Then, 5 µL of cholesterol standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510 nm. To 5 µL of serum, 0.5 mL of enzyme reagent was added, mixed well, and incubated at room temperature for 10 min. Then, 5 µL of TG standard and distilled water (blank) were also processed similarly. The absorbance was measured at 510 nm. LDLs, very LDL, and chylomicron fractions were precipitated quantitatively in 0.5 mL of serum by the addition of 50 µL of HDL precipitant (phosphotungstic acid in the presence of magnesium ions). After centrifugation, the cholesterol concentration in the HDL (supernatant) fraction was also determined. HDL and VLDL fractions were eliminated by

Table 1: Animal groups

Group	Group name	Induction	Pre-treatment	Post-treatment
I	Control	Distilled water	No treatment	No treatment
II	Diabetic (DB)	STZ-induced diabetes	No treatment	No treatment
III	NS0.5 + DB	STZ-induced diabetes	*N. sativa* 0.5 ml ip	No treatment
IV	NS1 + DB	STZ-induced diabetes	*N. sativa* 1 ml ip	No treatment
V	DB + NS0.5	STZ-induced diabetes	No treatment	*N. sativa* 0.5 ml ip
VI	DB + NS1	STZ-induced diabetes	No treatment	*N. sativa* 1 ml ip

STZ: Streptozotocin, NS: *Nigella sativa*, ip: Intraperitoneal, *N. sativa: Nigella sativa*

cholesterol esterase, cholesterol oxidase, and, subsequently, catalase (CAT). Thereafter, the specific measurement of LDL cholesterol marked by the intensity of the quinine imine dye produced was measured at 600 nm.

Assessment of antioxidant enzymes level and lipid peroxidation

The previously described standard methods[21] were used to carry out oxidative analyses. Briefly, the method described by Sun and Zigman[22] was used to determine the activity of superoxide dismutase (SOD) enzyme. The reduced glutathione (GSH) content of the serum was measured using the method previously described by van Doorn *et al*.[23] CAT activity was determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed in units per milligram of protein as previously described by Aebi.[24] Lipid peroxidation was estimated with the method of Mihara and Uchiyama[25] by measuring the level of MDA. Absorbance was recorded using a Shimadzu recording spectrophotometer (UV 160, Kyoto, Japan) in all measurements.

Statistical analysis

The data were expressed as mean ± standard error of the mean and analyzed using one-way analysis of variance followed by Neumann–Keul *post hoc* test. $P < 0.05$ was considered statistically significant. Data analysis was carried out using the GraphPad Version 5.05 for Windows Vista (GraphPad Software, San Diego, CA, USA).

Results

Effect of pre- and post-administration of *Nigella sativa* oil on cholesterol level

Induction of diabetes did not cause a significant ($P > 0.05$) change in the cholesterol level in the DB group compared to control. Both pre- and post-administration of the oil did not show any significant change ($P > 0.05$) in the level of cholesterol across all the groups. This is illustrated in Figure 1.

Effect of pre- and post-administration of *Nigella sativa* oil on triglyceride level

As illustrated in Figure 2, the induction of diabetes caused a significant ($P < 0.05$) increase in the TG level in the DB group compared to control. Pre-administration of the oil in the NS0.5 + DB and NS1 + DB groups did not cause a significant ($P > 0.05$) change in the level of TG. There was,

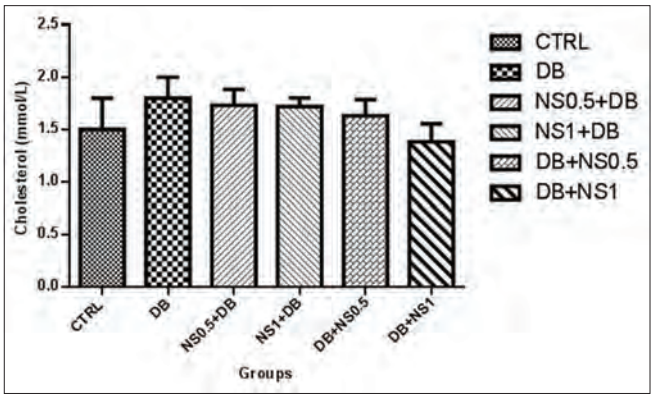

Figure 1: Cholesterol level across the groups. Values are represented as mean ± standard error of the mean. Group I: Control (CTRL), Group II: Diabetic (DB), Group III: *Nigella sativa* group pretreated with 0.5 ml (NS0.5 + DB), Group IV: *Nigella sativa* group pretreated with 1ml (NS1 + DB), Group V: *Nigella sativa* group post-treated with 0.5 ml (DB + NS0.5), Group VI: *Nigella sativa* group post-treated with 1 ml (DB + NS1)

however, a slight reduction in the level. Post-administration of the oil caused a significant ($P < 0.01$) reduction in the level of TG in the DB + NS1 group compared to the DB group. Post-administration in the DB + NS1 group was also more effective to cause reduction than the DB + NS0.5 group ($P < 0.01$). Post-administration of NS oil, however, did not significantly ($P > 0.05$) change the level of TG in the DB + NS0.5 group compared to the DB group.

Effect of pre- and post-administration of *Nigella sativa* oil on high-density lipoprotein level

There was a slight reduction in the level of HDL, but this reduction was statistically not significant ($P > 0.05$) in the DB group compared to the control group. Pre-administration with NS oil in the NS0.5 + DB group caused a significant increase ($P < 0.05$) in the HDL level compared to the DB group. Post-administration with *NS* oil in the DB + NS0.5 and DB + NS1 groups also caused a significant increase ($P < 0.05$) in the HDL level compared to the DB group. This is illustrated in Figure 3.

Effect of pre- and post-administration of *Nigella sativa* oil on low-density lipoprotein level

Induction of diabetes did not cause a significant ($P > 0.05$) change in the LDL level in the DB group compared to control. Both pre- and post-administration of NS oil did not show any significant

Table 2: Antioxidant levels across the groups

	CTRL	DB	NS0.5±DB	NS1±DB	DB±NS0.5	DB±NS1
GSH (µmol/ml)	13.65±0.82	9.65±1.58	20.57±1.73*	15.19±2.75	15.85±1.11	18.6±2.03
SOD (µmol/ml)	60.46±5.43	60.93±2.64	81.19±2.74**	78.91±4.29**	84.62±5.00**	82.10±4.35**
CAT (µmol/ml)	439.60±59.69	372.10±27.44	615.90±58.95**	455.60±45.50	475.80±36.40**	687.81±38.47***$^{\alpha,\beta,\mu}$
MDA (µmol/ml)	3.64±0.68	5.23±0.46$^{\delta}$	4.52±0.6*	3.52±0.41**	3.46±0.49**	3.42±0.69***

Values are represented as mean±SEM. Significant, *P<0.05, **P<0.01, ***P<0.001 versus DB group, $^{\beta}$Significant increase (P<0.01) versus NS1 + DB, $^{\mu}$Significant increase (P<0.01) versus DB + NS0.5, $^{\delta}P$<0.05 versus control group. Group I: Control (CTRL), Group II: Diabetic (DB), Group III: *N. sativa* group pretreated with 0.5 ml (NS0.5 + DB), Group IV: *N. sativa* group pretreated with 1 ml (NS1 + DB), Group V: *N. sativa* group posttreated with 0.5 ml (DB + NS0.5), Group VI: *Nigella sativa* group posttreated with 1 ml (DB + NS1). GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, *N. sativa*: *Nigella sativa*, SEM: Standard error of mean

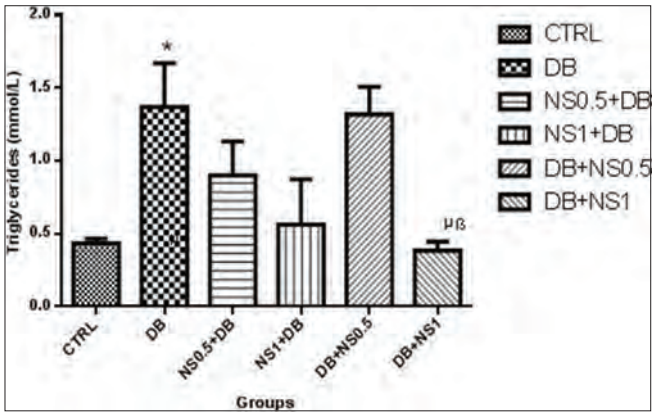

Figure 2: Triglyceride level across the groups. Values are represented as mean ± standard error of the mean. *: Significant increase ($p < 0.05$) versus Control group. µ: Significant reduction ($P < 0.01$) versus DB group, β: Significant reduction ($P < 0.01$) versus DB + NS0.5 group. Group I: Control (CTRL), Group II: Diabetic (DB), Group III: *Nigella sativa* group pretreated with 0.5 ml (NS0.5 + DB), Group IV: *Nigella sativa* group pretreated with 1 ml (NS1 + DB), Group V: *Nigella sativa* group post-treated with 0.5 ml (DB + NS0.5), Group VI: *Nigella sativa* group post-treated with 1 ml (DB + NS1)

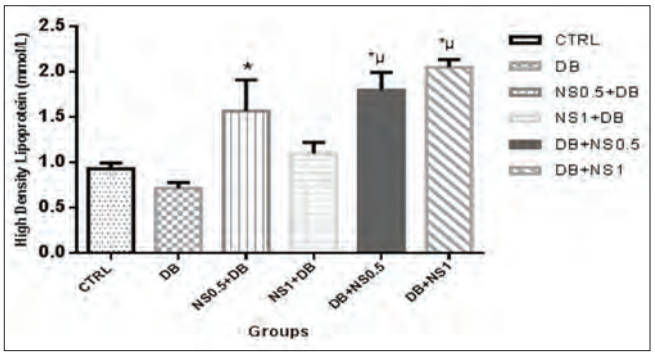

Figure 3: High-density lipoprotein level across the groups. Values are represented as mean ± standard error of the mean. *: Significant increase ($P < 0.05$) versus DB group. µ: Significant increase ($P < 0.01$) versus Control group. Group I: Control (CTRL), Group II: Diabetic (DB), Group III: *Nigella sativa* group pretreated with 0.5ml (NS0.5 + DB), Group IV: *Nigella sativa* group pretreated with 1 ml (NS1 + DB), Group V: *Nigella sativa* group post-treated with 0.5 ml (DB + NS0.5), Group VI: *Nigella sativa* group post-treated with 1 ml (DB + NS1)

change ($P > 0.05$) in the level of LDL across all the groups even though there appears to be a general reduction in the pre- and post-treatment groups. This is illustrated in Figure 4.

Effect of pre- and post-administration of *Nigella sativa* oil on atherogenic index

Induction of diabetes did not cause a significant ($P > 0.05$) change in the atherogenic index in the DB group compared to control. Both pre-and post-administration of the oil did not show any significant change ($P > 0.05$) in the atherogenic index across all the groups. This is illustrated in Figure 5.

Effect of pre- and post-administration of *Nigella sativa* oil on reduced glutathione level

As illustrated in Table 2 for the antioxidant enzymes, the level of GSH appears to be reduced, but this reduction was found not to be statistically significant ($P > 0.05$) when the DB group is compared with control. Pre-administration of the oil in the NS0.5+DB group caused a statistically significant ($P < 0.05$) increase in GSH level when compared with the DB group. Pre-administration of the oil in the NS1+DB group, however, did not cause a statistically significant ($P > 0.05$) difference in GSH level when compared with DB group. post-administration of *NS* in the DB + NS0.5 and the DB + NS1 groups also did not cause a statistically significant ($P > 0.05$) difference in GSH level when compared with DB group.

Effect of pre-and post-administration of *Nigella sativa* oil on superoxide dismutase level

The level of SOD was not statistically different ($P > 0.05$) in the DB group compared to control. Pre-administration of the oil in the NS0.5 + DB and NS1 + DB groups caused a statistically significant ($P < 0.01$) increase in SOD level when compared with the DB group. In a similar manner, post-administration of the oil in the DB + NS0.5 and DB + NS1 groups caused a statistically significant ($P < 0.01$) increase in SOD level when compared with the DB group.

Effect of pre- and post-administration of *Nigella sativa* oil on catalase level

It appears that CAT level was reduced in the DB group compared to control. However, this reduction was found not to be statistically significant ($P > 0.05$). Pre-administration in the NS0.5 + DB group and post-administration in the DB + NS0.5 and DB + NS1 groups caused a significant increase ($P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively) in the CAT level. CAT level was also found not to be significantly different ($P > 0.05$) in the NS1 + DB group compared to the DB group.

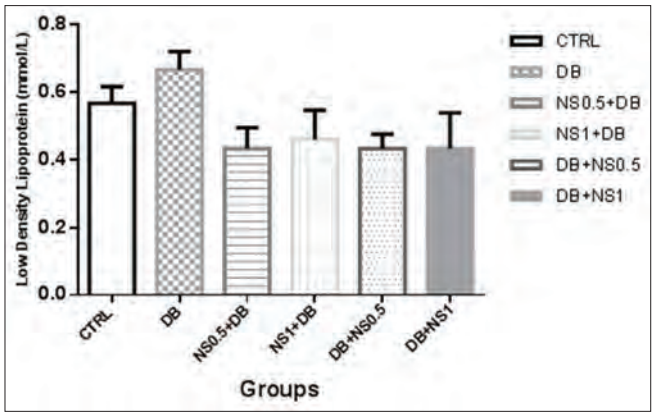

Figure 4: Low-density lipoprotein level across the groups. Values are represented as mean ± standard error of the mean. Group I: Control (CTRL), Group II: Diabetic (DB), Group III: *Nigella sativa* group pretreated with 0.5ml (NS0.5+DB), Group IV: *Nigella sativa* group pretreated with 1ml (NS1+DB), Group V: *Nigella sativa* group post-treated with 0.5 ml (DB+NS0.5), Group VI: *Nigella sativa* group post-treated with 1 ml (DB+NS1)

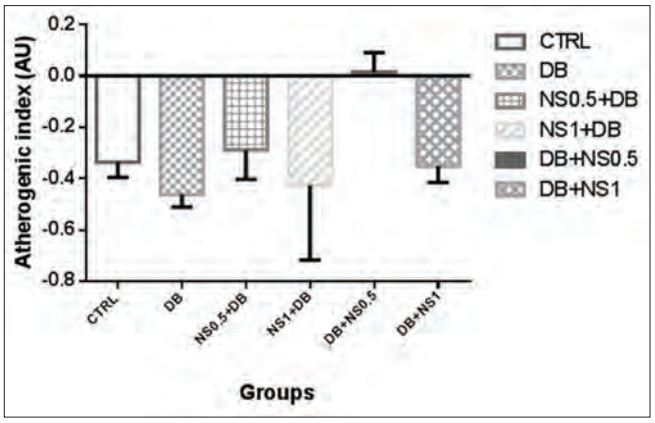

Figure 5: Atherogenic index across the groups. Values are represented as mean ± standard error of the mean. Group I: Control (CTRL), Group II: Diabetic (DB), Group III: *Nigella sativa* group pretreated with 0.5 ml (NS0.5+DB), Group IV: *Nigella sativa* group pretreated with 1ml (NS1+DB), Group V: *Nigella sativa* group post-treated with 0.5 ml (DB+NS0.5), Group VI: *Nigella sativa* group post-treated with 1 ml (DB+NS1)

Effect of pre- and post-administration of *Nigella sativa* oil on malondialdehyde level

The level of MDA was significantly higher ($P < 0.05$) in the DB group compared to control. MDA level was significantly ($P < 0.05$) lower in the NS0.5 + DB and NS1 + DB groups ($P < 0.01$) compared to DB group. MDA level was also found to be markedly reduced in the DB + NS0.5 ($P < 0.01$) and DB + NS1 groups when compared with the DB group.

Discussion

Even with the advancement in the management of DM, exploration for innovative agents continues since the existing synthetic agents have numerous limitations.[26] Even though NS oil has been shown to have a lot of beneficial effects; it is crucial to decide whether it should be used as a protective or corrective agent. This study is, thus, the first to compare the pre- and post-treatment effects in an animal model of DM.

Cholesterol level appeared to be high in the diabetes group, while neither pre-treatment nor post-treatment with the oil had a significant effect on the level. Increased serum total cholesterol concentrations are directly associated with an increased risk of coronary heart disease.[27] Both pre- and post-treatment with the oil slightly reduced the cholesterol level, and this effect may be due to *de novo* cholesterol synthesis inhibition or stimulation of excretion of bile acid. It is well known that both effects would lead to a decrease in serum cholesterol.[28,29] The slight reduction could also be due to the presence of phytosterols such as beta-sitosterol, polyunsaturated fatty acids, and their antioxidant activity.[30,31] These results further confirmed earlier reports that *NS* oil could be used as an adjunct therapy for glycemic control[28] either for prevention or correction of high glucose level.

The level of TG was observed to be significantly high in the DB group in this study. Elevated TG level is no doubt a risk factor[32] for atherosclerosis and this could be due to decreased clearance rate of TG.[33] It is worthy of note that post-treatment with the oil caused a significant reduction in the TG level, while the effect appears to be minimal when given before the induction. Kocyigit *et al*.[14] had demonstrated the hypolipidemic potential of the oil when used in diabetic rats, and our result further confirmed this earlier result and others.[15,16]

Our results also showed the ability of the oil to cause a marked improvement in the level of HDL when used either before or after diabetes induction. This is a pointer to the ability of the oil to either prevent or correct the HDL level in diabetes. This observation is in line with the result of Kaatabi *et al*.[33] HDL is a key lipoprotein that helps remove lipids from macrophage foam cells in the arterial wall and thereby offer useful protection from LDL oxidation.[34] Surprisingly, the obvious reductions observed in the LDL in pre- and post-treated rats were found not to be statistically significant. In essence, there is no obvious mechanism to explain the antiatherogenic pattern of lipoprotein profile found in the present study following oil pre- or post-treatment. This could, however, mean the non-preference of pre-treatment over post-treatment and vice-versa. Our study also showed the ability of post-treatment to ameliorate the atherogenic index compared to the pre-treatment slightly. Based on the results of this study, neither pre-treatment nor post-treatment of the oil can, thus, be guaranteed as a treatment option usable to correct this cardiovascular risk factor.

Diabetes induction in this study caused a slight reduction in GSH and CAT levels. It also caused a slight increase in MDA level. Only pre-treatment caused a significant improvement in GSH level. Both pre-treatment and post-treatment with the oil caused a marked improvement in the level of SOD and CAT.

It is interesting to know that both pre-treatment and post-treatment with the oil also caused a marked reduction in the level of MDA. In agreement with some of these observations, Omidi *et al*.[35] reported a significant improvement in antioxidant parameters in diabetic rats following NS oil administration. Also, Pencina *et al*.[36] reported a reduction in MDA following NS oil administration, and this was also the observation made by El Rabey *et al*.[37] It does mean that both pre- and post-treatment with NS oil have the potential to improve the antioxidant system and reduce lipid peroxidation, but the post-treatment appears to be more effective. Contrary to these observations, however, the dietary black seed had been shown not to significantly affect TAS, SOD, and GPX levels in hyperlipidemic rabbits.[38] This finding could, however, be attributed to the difference in the route of administration of the oil or species-specific cum genetic differences in the animals used.

Acknowledgment

The authors wish to thank Prof. I. A. Oreagba, Head of the Department of Pharmacology, Therapeutics and Toxicology of the College of Medicine of the University of Lagos, for his unwavering support. We gratefully thank Prof. S. O. Olayemi, former Head of the Department of Pharmacology, Therapeutics and Toxicology of the College of Medicine of the University of Lagos, for his useful comment and encouragements.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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