Original Research Article

Antidiabetic potential of *Nigella sativa* L seed oil in alloxan-induced diabetic rabbits

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Abstract

Purpose: To evaluate the antidiabetic, hypolipidemic and antioxidant potential of seed oil of Nigella sativa L (NSO).
Methods: Nigella sativa seed oil (NSO) was extracted with Soxhlet apparatus using petroleum ether, and was given orally at a dose of 2.5 ml/kg body weight to alloxan-induced diabetic rabbits daily for 24 days. Biochemical parameters including total cholesterol (TC), triglycerides (TGs), low density lipoprotein cholesterol (LDL), very low lipoprotein cholesterol (VLDL), high density lipoprotein (HDL) and plasma glucose were determined in the treatment and control groups. Furthermore, bilirubin, vitamin C, catalase and mean body weight were assessed.
Results: NSO treatment significantly lowered serum blood glucose levels and lipid contents, but increased the mean body weight, HDL-C and vitamin C levels of diabetic rabbits (p < 0.001). Moreover, NSO significantly decreased catalase activity, TC, TGs, LDL-C and VLDL-C levels, but normalized bilirubin levels in diabetic rabbits.
Conclusion: These results indicate that NSO possesses significant antidiabetic potential. Thus, it may be useful as an adjunct with antidiabetic medication but further studies are required to ascertain this.

Keywords: Black cumin, Diabetes, Hypoglycemic, Hypolipidemic, Antioxidants

INTRODUCTION

Diabetes mellitus (DM) is a disease associated with severe complications. It is a prominent cause of illness and mortality throughout the world. Statistics have shown that 2.8 % of the world's population suffer from diabetes, with the likelihood of increasing to more than 5.4 % by 2025 [1]. Diabetes is also increasing alarmingly in the Indo-Pak region. According to Pakistan National Survey of diabetes for the period 2016-2017, one out of four Pakistanis is diabetic, which accounts for approximately 35.5 - 37.5 million people [2]. Hyperglycemia, and life style-related problems are major causative factors for diabetes mellitus and its complications [3]. It has

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been reviewed from the literature that diabetes mellitus is strongly associated with production of free radicals, resulting in oxidative stress, cardiac disorders, renal failure, neuro-degeneration and immune dysfunction [4]. Various plants rich in antioxidant components ameliorate hyperglycemia and hypercholesterolemia by quenching free radicals [5].

In Pakistan, *Nigella sativa* (black cumin or black seed) is locally called *kalonji* seed (Ranunculaceae). It is an annual herb cultivated in different parts of the world, including Indo-Pak. Phytochemically, it is comprised of many bioactive compounds such as saponins, tannins, fixed oil, protein, alkaloids, and essential oil [6]. *Nigella sativa* exhibits broad-spectrum biological activities including antioxidant [7], hepato-protective [8], and antidiabetic properties [9]. The volatile lipidic fraction of *Nigella sativa* possesses insulinotropic properties, as shown through its maintenance of β-cell integrity in the pancrease [10]. It has been reported that black cumin seed oil (thymoquinone) produced significant decrease in serum glucose, triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, when compared to the untreated diabetic rats [11].

EXPERIMENTAL

Collection and extraction of plant material

Nigella sativa L seeds (accession no. MP00023) were collected from Genbank of National Agricultural Research Center (NARC), Pakistan. The plant seeds were cleaned, desiccated and crushed to fine powder using mortar and pestle. The *Nigella sativa* seed powder (100 g) was extracted in petroleum ether (400 mL) for 4-6h using Soxhlet apparatus. The solvent was evaporated from the extract using rotary apparatus, resulting in *Nigella sativa* seed oil (*NSO*).

Animals

Fifteen male rabbits (mean weight = 1 kg) were purchased from University of Veterinary and Animal Sciences, Lahore (VUAS), Pakistan. The animals were kept in large spacious cages at the animal house of the Department of Pharmacy, University of Sargodha, and were maintained in line with "Principals of Laboratory Animal Care" (NIH publication 85-23, revised in 1985) [12]. The rabbits were acclimatized for fifteen days prior to start the experiment, and were provided standard diet and water *ad libitum*.

Study protocol

The animals were divided into three groups: control, diabetic control, and *NSO*-treated groups, with 5 rabbits per group. Following 14-h fast, diabetes was induced in the rabbits via intravenous injection of 10% alloxan (Sigma Chemical Co., St Louis, MO, USA) at a dose of 150 mg/kg (dissolved in isotonic NaCl) [13]. Three days after alloxan injection, diabetes was confirmed through increased levels of serum glucose (hyperglycemia). *Nigella sativa* oil was administered orally to the rabbits at a dose of 2.5 mL/kg body weight [14] daily for 24 days. The animals were not treated with insulin at any stage during the trial.

Body weights of all animals were measured on an electric balance on day 1, and at 6-day intervals for the 24 days of the study.

Blood sampling

On day 1 and at 6-day intervals, all rabbits were fasted for 12 h, and about 2.5 mL of blood was taken from the jugular vein using sterile syringes and needles. Blood glucose levels were determined with a glucometer (On Call EZ II, ACON® Laboratories, Inc.). The blood samples were collected in heparin vials to determine plasma lipid profile and antioxidant levels. Plasma was separated by centrifuging the heparinized blood at 4000 rpm for 9 min, and the plasma was kept at -80 ºC prior to analysis.

Determination of plasma lipid profile

The plasma samples were analyzed for TGs, cholesterol, HDL-Cholesterol, LDL-cholesterol, and VLDL-cholesterol levels using the respective assay kits (Analyticon® Biotechnologies AG, Germany).

Determination of plasma antioxidant levels

Plasma catalase was measured using the method by Goth [15]. Total bilirubin was determined according to the procedure of Jendrassik and Grof [16], while plasma vitamin C level was estimated with HPLC [17].

Statistical analysis

Results are presented as mean ± standard deviation. Statistical analysis was carried out with one-way ANOVA analysis and Tukey's post hoc test for multiple comparisons (differences among means), using Statistical Package for Social Sciences (SPSS) software, version 21.0.

Differences were considered significant *at p <* 0.05.

RESULTS

Effect of *NSO* administration on rabbit body weight

Mean body weight of animals from all study groups was recorded before and after oral administration of *Nigella sativa* oil. As shown in Figure 1, there was a significant reduction (2.43 %) in mean body weight of the diabetic rabbits ($p<0.001$). However, *NSO* treatment produced significant increase in mean body weight of the animals throughout the study period, when compared to the diabetic control animals at the various time points i.e. day 1 (1.25 %), day 6 (1.41 %), day 12 (1.49 %), day 18 (0.28 %), and day 24(1.14 %).
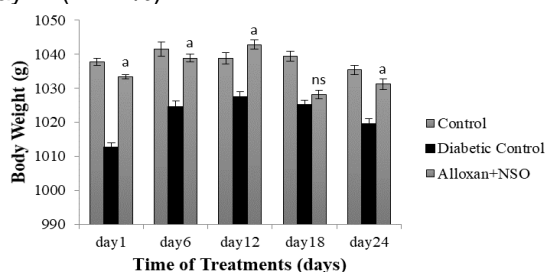

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Figure 1: Effect of *Nigella sativa* oil (*NSO*) treatment on body weight. A gradual rise in body weight was seen in alloxan + *NSO* treatment group, when compared to the diabetic control group. Values are expressed as mean $\pm$ SD (*n* = 5). [a]$p < 0.001$, compared to diabetic control; [ns] not significant, when compared with diabetic control. The data were analyzed using one-way ANOVA analysis and Tukey's post hoc test

Effect of *NSO* on blood glucose levels

There was a gradual increase in mean plasma glucose levels of diabetic rabbits (from +28.45% on day 1 to +92.10% on day 24). However, *NSO* treatment was very effective in lowering the blood glucose levels in the treatment group from -5.76% on day 12 to -18.06% on day 24, when compared to the diabetic group (Figure 2).

Effect of *NSO* on serum lipid profile

The results showed that *NSO* treatment produced significant effects on lipid profile of the diabetic rabbits. In the alloxan + *NSO* treatment group, *NSO* produced a highly significant (*p* < 0.001) but gradual decreases in plasma levels of cholesterol (from 4.4 % on day 1 to -33.23 % on day 24), and also decreased plasma TG from 0.21 % on day 1 to -14.5 % on day 24), when

compared to diabetic control group. An increase in plasma HDL content was found in alloxan + *NSO* treatment group from day 12 (24.48 %) to day 24 (66.66 %). Significant *(p < 0.001)* reductions in plasma LDL levels (-36.8 %), and VLDL levels (-24.43 %) were observed in alloxan + *NSO* treatment group on day 24, when compared to the diabetic control group. These results are shown in Table I.
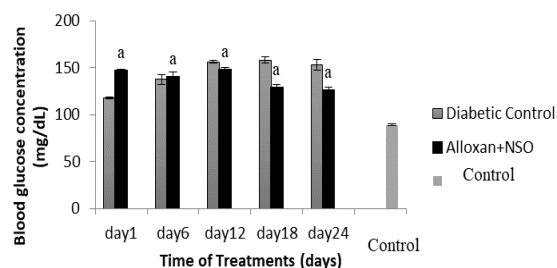

Figure 2: Effect of *Nigella sativa* oil (*NSO*) treatment on blood glucose levels. A gradual decrease in plasma glucose level was observed in alloxan + *NSO* treatment group, relative to the diabetic control group. Values are mean± SD (n=5), [a] *p* <0.001, compared to diabetic control; [ns] not significant, relative to diabetic control. The data were analyzed using one-way ANOVA analysis and Tukey's post hoc test

Effect of *NSO* on antioxidant contents

Alloxan treatment led to gradual decreases in plasma bilirubin levels (from -14.63 % on day 1 to -69.81 % on day 24). However, *NSO* treatment led to significant (*p* <0.001) rise in plasma bilirubin levels from day 12 (15.15 %) to day 24 (69.81 %). An almost similar trend was seen in vitamin C contents in all treatment groups from day 1 to day 24. A significant *(p<0.001)* rise in plasma vitamin C content was seen in alloxan + *NSO* treatment group, when compared to the diabetic control throughout the study. In contrast, there was a significant decrease in plasma catalase activity in the alloxan + *NSO* treatment group, when compared to diabetic control group (*p* <0.001; Table II).

DISCUSSION

Nigella sativa oil treatment has been found to be effective in lowering blood glucose levels of diabetic animals [18]. It was seen in the current study that the body weight of diabetic rabbits was decreased gradually till the start of treatment, and blood glucose level increased due to insufficient production of insulin. However, when the diabetic group was treated with *NSO*, the body weight of animals was increased while the glucose level was decreased.

Table 1: Effect of *NSO* on lipid profile of diabetic rabbits

Day	Treatment group	Lipid profile				
		Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/ dL)
1	Control	49.12 ± 0.82	92.00 ± 0.93	15.40 ± 0.56	16.00 ± 0.45	16.40 ± 0.3
	Diabetic Control	50.00 ± 0.98	91.80 ± 1.40	14.40 ± 0.66	16.20 ± 0.48	16.40 ± 0.3
	Alloxan + *NSO*	52.20 ± 1.08^{ns} (4.4%)	92.00 ± 0.88^{ns} (0.21%)	14.60 ± 0.33^{ns} (1.38%)	18.80 ± 0.29^{a} (16.04%)	19.40 ± 0.38^{a} (18.29%)
6	Control	48.40 ± 1.20	91.02 ± 1.74	13.20 ± 0.15	15.60 ± 0.41	17.20 ± 0.3
	Diabetic Control	57.80 ± 0.92	97.60 ± 1.04	12.00 ± 0.31	19.60 ± 0.31	19.00 ± 0.63
	Alloxan + *NSO*	55.60 ± 1.14^{ns} (-3.08%)	95.40 ± 1.12^{ns} (-2.25%)	11.80 ± 0.38^{ns} (-1.66%)	21.60 ± 0.15^{a} (10.20%)	22.40 ± 1.15^{a} (17.89%)
12	Control	49.20 ± 1.49	92.60 ± 1.08	13.60 ± 0.52	16.00 ± 0.43	17.00 ± 0.3
	Diabetic Control	60.20 ± 1.56	101.00 ± 1.03	9.80 ± 0.31	21.60 ± 0.15	20.38 ± 0.36
	Alloxan + *NSO*	53.16 ± 1.46^{a} (-11.69%)	93.38 ± 0.72^{a} (-7.54%)	12.20 ± 0.33^{a} (24.48%)	19.60 ± 0.15^{a} (-9.25%)	20.20 ± 0.42^{ns} (-0.88%)
18	Control	47.20 ± 1.49	89.00 ± 1.12	12.80 ± 0.22	15.80 ± 0.31	16.00 ± 0.2
	Diabetic Control	66.20 ± 1.47	102.20 ± 1.40	8.20 ± 0.29	23.60 ± 0.38	21.20 ± 1.0
	Alloxan + *NSO*	48.20 ± 1.49^{a} (-27.19%)	89.80 ± 0.92^{a} (-12.13%)	13.20 ± 0.12^{a} (60.97%)	17.60 ± 0.22^{a} (-25.42%)	18.40 ± 0.44^{a} (-13.2%)
24	Control	47.20 ± 1.47	89.20 ± 1.00	13.40 ± 0.15	15.60 ± 0.43	17.40 ± 0.5
	Diabetic Control	69.20 ± 1.44	103.40 ± 1.30	8.40 ± 0.29	25.00 ± 0.22	23.00 ± 0.31
	Alloxan + *NSO*	46.20 ± 1.47^{a} (-33.23%)	88.40 ± 0.69^{a} (-14.5%)	14.00 ± 0.25^{a} (66.66%)	15.80 ± 0.39^{a} (-36.8%)	17.38 ± 0.64^{a} (-24.43%)

Values are mean ± SD (n = 5); $^{a}p < 0.001$ comparison to diabetic control; ^{ns}not significant comparison to diabetic control. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test

Table 2: Effect of *NSO* on antioxidants levels

Day	Treatment group	Antioxidant		
		Bilirubin (mg/dL)	Vitamin C (mg/mL)	Catalase (KU/L)
1	Control	0.78 ± 0.02121	19.30 ± 1.2689	46.90 ± 1.2288
	Diabetic Control	0.82 ± 0.02915	16.50 ± 1.0559	52.00 ± 1.2748
	Alloxan + *NSO*	0.70 ± 0.01581^{a} (-14.63%)	20.10 ± 1.5843^{ns} (21.81%)	30.00 ± 1.1726^{a} (-42.3%)
6	Control	0.84 ± 0.05477	19.00 ± 1.2510	46.20 ± 1.5411
	Diabetic Control	0.76 ± 0.03808	15.70 ± 1.0536	55.00 ± 1.1113
	Alloxan + *NSO*	0.72 ± 0.02915^{ns} (-5.26%)	19.80 ± 1.0840^{a} (26.11%)	27.60 ± 1.3210^{a} (-49.81%)
12	Control	0.84 ± 0.05477	19.30 ± 1.2884	47.24 ± 1.4082
	Diabetic Control	0.66 ± 0.03808	15.78 ± 1.1367	55.30 ± 1.4107
	Alloxan + *NSO*	0.76 ± 0.03808^{ns} (15.15%)	19.80 ± 1.0794^{a} (25.47%)	25.50 ± 1.1489^{a} (-53.88%)
18	Control	0.84 ± 0.05477	19.00 ± 1.2865	45.50 ± 1.1225
	Diabetic Control	0.56 ± 0.03391	15.70 ± 1.0770	55.80 ± 1.0559
	Alloxan + *NSO*	0.86 ± 0.03162^{a} (53.57%)	20.00 ± 1.2510^{a} (27.38%)	26.60 ± 1.0124^{a} (-52.32%)
24	Control	0.86 ± 0.03162	18.70 ± 1.1000	46.50 ± 1.1023
	Diabetic Control	0.53 ± 0.02739	15.70 ± 1.1640	55.74 ± 1.0714
	Alloxan + *NSO*	0.90 ± 0.03674^{a} (69.81%)	20.08 ± 1.5353^{a} (27.89%)	27.04 ± 1.3353^{a} (-51.48%)

Values are mean± SD (n=5); $^{a}p < 0.001$, compared to diabetic control; ^{ns} not significant, compared to diabetic control. The data were analyzed using One-way ANOVA, followed Tukey's post hoc test

In a study, El-Bahr *et al* reported significant effects of turmeric and black cumin seed mixture on body weight of diabetic rats, when compared to untreated diabetic rats [18].

In the present study, increases in body weights of the treated rabbits were observed from day 1^{t} to day 12, with a slight decrease on day 18, followed by increases up to the 24th day of the study. *Nigella sativa* oil treatment also produced significant hypoglycemic potential in the diabetic rabbits. The high blood glucose levels of the diabetic animals were reduced gradually after the *NSO* treatment in alloxan + *NSO* group on day 24 (125.6 ± 3.5896). These results are comparable with those of Mohtashami *et al* who also reported significant decreases in serum glucose levels on different treatment days in STZ-induced diabetic rats treated with *Nigella sativa* seeds [19]. In addition, Asaduzzaman *et al*

reported a highly significant fall in serum glucose level of STZ-induced diabetic rat after *Nigella sativa* treatment [20]. Maintenance of animal body weight and blood glucose levels are indicators of normalized metabolism, especially carbohydrate metabolism which is affected primarily in diabetes.

The finding of present study revealed the ameliorating effect of *NSO* in diabetic rabbits. The *NSO* treatment resulted in increased levels of good cholesterol e.g. HDL-cholesterol, while it significantly reduced the levels of bad lipids i.e. cholesterol, TGs, LDL-cholesterol, and VLDL-cholesterol.

In a previous study, it was reported that the hypolipidemic effect of black cumin seed was due to the synergistic action of its different constituents i.e. nigellamine, thymoquinone (TQ), flavonoids, mucilage (soluble fiber), sterols, and polyunsaturated fatty acids (PUFAs) [21]. In the current study, there were significant reductions in the levels of cholesterol, TGs, LDL-C and VLDL-C in rabbits treated with alloxan + *NSO* on the 24th day. However, an increase in plasma HDL-C content was also observed in the alloxan + *NSO* group on the 24th day. These results are in agreement with those reported in a study by Al-Logmani and Zari [22] on long term beneficial effect of *black cumin* seeds on lipid profile of streptozotocin-induced diabetic rats. Asaduz-zaman *et al.* reported significant reductions in cholesterol, TGs, LDL and VLDL levels, and a significant rise in HDL levels in STZ-induced diabetic rats after *Nigella sativa* treatment [20]. In this study, it is likely that the increase in body weight and increase in HDL-cholesterol levels of diabetic rabbits after *NSO* treatment are indicators of the hypolipidemic effect of *NSO*.

Antioxidant molecules have the ability to scavenge free radicals, thereby suppressing cell membrane damage which may lead to diabetes. *Nigella sativa* seed oil possesses antioxidant phytochemical compounds such as thymo-quinone, carvacrol, anithole, and 4-terpineol. The determination of alterations in levels of endogenous antioxidants is a useful method for assessment of beneficial effects of antidiabetic drugs. Significant increases in bilirubin levels were seen in the treatment group on the 18th and 24th days of the treatment. Abdel-Daim and Ghazy have reported significant increases in total bilirubin in rabbits after oxytetracycline (OTC) + *NSO* treatment [23]. Bilirubin, a breakdown product of heme, is a lipid-soluble and cytotoxic compound, and a good antioxidant which suppresses lipid peroxidation [24]. El-khateeb *et al* reported significant increases in total serum bilirubin levels of tramadol-induced hepato- and nephrotoxic rats after *Nigella sativa* oil treatment [25].

Vitamin C acts as antioxidant due to its electron-donating nature. Decreased levels of vitamin C have been associated with various complications including high blood pressure and diabetes [26]. Kanter *et al* have reported increases in vitamin C levels in CCl_4-treated rats exposed to black seed (black cumin) L. and *stinging nettle* (*Urtica dioica*) L. [27]. Song *et al* have reported that *N. sativa* oil reduced liver damage in alloxan-induced diabetic animals by increasing blood levels vitamin C and decreasing catalase activity. In the present study, there were highly significant increases in vitamin C levels, and highly significant decreases in catalase activity in the treated animals. The decrease in catalase activity might be due to the decreased levels of oxidants in the cells [28]. Shahid *et al* have also reported decreases in catalase activity in cisplatin (CP)-induced hepatotoxic rat liver after CP+*NSO* treatment [7].

In the present study, it is proposed that the antioxidant potential of *NSO* augmented the levels of endogenous antioxidants in cells, thereby mitigating metabolic derangements under diabetic conditions, especially carbohydrate and lipid metabolisms.

CONCLUSION

The results obtained in the current study indicate that *Nigella sativa* seed oil (*NSO*) normalized key biochemical predictors of diabetes in alloxan-induced diabetic rabbits. The seed oil exerts its antidiabetic effects by boosting cellular antioxidant status. Thus, it may also limit the side effects of synthetic antidiabetic drugs.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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