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Long-term effects of *Nigella sativa* L. oil on some physiological parameters in normal and streptozotocin-induced diabetic rats

Ayed Al-Logmani[1], Talal Zari[2*]

[1]Northern Border University, Arrar, Saudi Arabia;
[2]King Abdulaziz University, Jeddah, Saudi Arabia; [*]Corresponding Author: talzari@yahoo.com

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ABSTRACT

The long-term effects of *Nigella sativa* L. oil on some physiological parameters were investigated in normal and streptozotocin (STZ)-induced diabetic male Wistar rats. STZ-induced diabetic rats showed significant increases in the levels of blood glucose, triglycerides, cholesterol, low density lipoprotein (LDL-cholesterol), uric acid, urea, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while high density lipoprotein (HDL-cholesterol) and total protein levels were significantly decreased compared to normal rats. Administration of black seed oil to diabetic rats resulted in a significant decrease in blood glucose, triglycerides, cholesterol, LDL–cholesterol ALT, AST and uric acid while HDL–cholesterol level was markedly increased compared to untreated diabetic rats after seven weeks of treatment. The results of this study indicate that the diet containing the oil of N. sativa improves the examined physiological parameters in STZ- induced diabetic rats especially when it is used for a longer period.

Keywords: Streptozotocin; Diabetes; Black Seed Oil; Rats

1. INTRODUCTION

Diabetes mellitus is probably the fastest growing metabolic disorder in the world and it is a major source of morbidity in developed countries. Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. Many studies have confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities [1,2]. Moreover, during the past few years some of the new bioactive drugs isolated from hypoglycaemic plants showed antidiabetic activity with more efficacy than oral hypoglycaemic agents used in clinical therapy [3]. Presently, there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents [4-6]. More than 400 plants with glucose lowering effect are known [7]. Also a number of plants have a hypolipidemic effect [8]. However, there is little information about plants with both hypoglycemic and hypolipidemic effects [9].

The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides [10]. Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents have been successful in maintaining euglycaemia and controlling long-term microvascular and macrovascular complications [10-12]. Insulin therapy is used for management of diabetes mellitus but there are several drawbacks, which include insulin allergy, insulin antibodies, lipodystrophy, autoimmunity and other delayed complications like morphological changes in kidney and severe vascular complications [13-15]. Thus, new, relatively non-toxic, therapeutic agents are needed to treat hyperglycemia, which also would correct dyslipidemia to reduce the risk of cardiovascular complications of diabetes [16].

Nigella sativa L. (*N. sativa*) is a spice plant belonging to the family Ranunculaceae. It is cultivated in several countries in the Mediterranean region and Asia, known in vernacular as "sannouj, habbat el Baraka or habbah saouda" [17]. The seeds were used in the orient as condiments or flavourings and also in traditional medicine

applications [18]. It has been shown that *N. sativa* has bronchodilatory [19,20], anti bacterial [21], hypotensive [22], immunopotentiating [23], antioxidant [24], antitumoral [25] and antidiabetic properties [26-28]. The oil of *N. sativa* was potent analgesic and anti-inflammatory drug in rats [29,30] and had *in vitro* and *in vivo* cytotoxic and immunosuppressive properties [31]. The petroleum ether extract exerted lipid-lowering and insulin-sensitizing actions in rats [32].

Induction of diabetes in laboratory animals is a convenient and useful strategy in the understanding and treatment of the disease. An appropriate dose of streptozotocin was used to induce experimental diabetes. Streptozotocin selectively destroyed pancreatic β-cells, resulting in hypoinsulinemia [33]. Streptozocin-treated rats are often used as diabetic animals with insulin-deficiency resulting from damage of beta-cells caused by the drug. These rats are hyperglycemic and have reduced uptake of glucose in skeletal muscles [34-36]. It is generally considered that hyperglycemia is the major factor in the pathogenesis of diabetic complications [37]. In diabetes there is inability to store fat and protein along with breakdown of existing fat and protein stores. Streptozotocin induced diabetic rats showed significant increases in the levels of cholesterol, phospholipids, triglycerides, and free fatty acids [38,39]. These changes remain important in terms of explaining the accelerated atherosclerosis. In addition, there is a loss of body weight [40,41]. Impairment of kidney function is a prominent feature of diabetes. Elevated levels of urea and decreased concentrations of uric acid and creatinine were shown in diabetes [42,43]. Overtime diabetic nephropathy will develop, characterized by proteinuria, a loss of renal function, and a rapid progression to end stage renal failure [44].

Little information exists concerning the effects of *N. sativa* oil on physiological parameters in normal and STZ-induced diabetic rats. Therefore, the aim of this study is to find if the administration of the oil of *N. sativa* could have beneficial effects on some physiological parameters in normal and STZ-induced diabetic rats after seven weeks. The physiological parameters include blood glucose, triglycerides, cholesterol, high density lipoprotein HDL-cholesterol, low density lipoprotein LDL-cholesterol, total protein, creatinine, urea, uric acid, alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

2. MATERIALS AND METHODS

2.1. Materials

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Al-Saggaf Est. (Jeddah, Saudi Arabia). The oil of *N. sativa* was obtained from Dreams Essential Oils Est. (Jeddah, Saudi Arabia). *N. sativa* oil was extracted by steam. The major compounds of this oil were thymoquinone (29.7%), p-cymene (23%), carvacrol (11.5%), α-pinene (8.6%), 4-terpineol (3.7%), longifoline (2.8%), carvone (1.8%) and t-anethole (0.8%).

2.2. Experimental Animals

Male Wistar rats weighing (180 - 230 g) were obtained from the Animal Experimental Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. The rats were housed in well- aerated cages in an animal room and maintained in a temperature-controlled room (24˚C ± 1˚C) with a 12 h light/12 h dark cycle, 55% ± 10% humidity. They were fed with normal commercial chow and water *ad libitum*. Throughout the experiments, animals were processed according to the suggested international ethical guidelines for the care of laboratory animals and all experimental procedures were approved by the Animal Care and Use Committee of King Abdul Aziz University.

2.3. Induction of Diabetes

The experimental animals were fasted for 12 h and then diabetes was induced by a single intraperitonial injection of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA), dissolved in a freshly prepared physiological saline solution (0.9% NaCl) at a dose of 65 mg/kg body weight. While normal rats received only the saline solution (0.9% NaCl) in the same volume and through the same route. After injection, all animals were returned to their cages and given free access to food and water. After 3 days, the fasting blood glucose levels were measured from tail blood samples by using an OneTouch Ultra® glucometer (Lifescan; Johnson & Johnson, Milpitas, CA, USA). Animals with blood glucose levels more than 277 mg/dL were considered diabetic and used for the experiment.

2.4. Experimental Design

A total of 40 rats were used in the experiment. The rats were divided into 4 groups of 10 animals each as follows: Group 1: Normal control (non-diabetic normal rats) received normal commercial chow and water *ad libitum*. Group 2: STZ-Control (diabetic control rats) received the same diet given in group 1. Group 3: STZ + *N. sativa* oil received diet containing 5% *N. sativa* oil. Group 4: Normal control +*N. sativa* oil received diet containing 5% *N. sativa* oil. All of the experimental groups received the treatments for a period of 7 weeks.

2.5. Blood Collection

After 7 weeks, the rats were fasted for 8 h before blood sampling, water was not restricted. Blood samples were collected from the orbital venous plexus of the rat under mild ether anaesthesia by heparinized capillary tube and into non-heparinized tubes [45] indicated that brief exposure and little amount of anesthetic used do not influence the activity of hepatic cytochrome P450 2E1 and P450 reductases in the rat. Clear serum samples were separated by centrifugation at 3000 rpm for 20 min and then collected and stored at –20˚C for different biochemical analyses, prior immediate determination of glucose, triglycerides, cholesterol, high density lipoprotein HDL-cholesterol (HDL-C), low density lipoprotein LDL-cholesterol (LDL-C), total protein, creatinine, urea, uric acid, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). All of these parameters were measured using an automatic analyzer (Architect c8000 Clinical Chemistry System, USA).

2.6. Statistical Analysis

Statistical analyses were performed using SPSS package for Windows version 13.0. Data are expressed as mean ± SE. One-way ANOVA and t-tests were used to analyze differences among groups. Differences between groups were considered statistically significant at $p < 0.05$.

3. RESULTS

The mean values of blood glucose of both control and experimental groups are presented in **Table 1**. STZ-induced diabetic rats showed a highly significant ($p < 0.001$) increase in the levels of blood glucose, registering increases of 246.2% after 7 weeks compared to the controls. Administration of *N. sativa* oil to diabetic rats resulted in a significant ($p < 0.001$) decrease in blood glucose levels of 64.9% after 7 weeks, compared to untreated diabetic rats. In comparison with control, administration of black seed oil in non-diabetic rats showed no significant differences in the level of blood glucose after 7 weeks.

The changes in the levels of serum lipids in control and experimental groups are illustrated in **Table 1**. There was a significant ($p < 0.001$) decrease in the level of HDL-cholesterol (24.2%) and significant ($p < 0.001$) increases in the levels of cholesterol, LDL-cholesterol and triglycerides in STZ-induced diabetic rats, with percentages of 64.7%, 70.9% and 176.1% respectively, compared to the controls. However, treatment of STZ-induced diabetic rats with *N. sativa* oil resulted in a significant ($p < 0.001$) decrease in the levels of triglycerides, cholesterol and LDL-cholesterol compared to untreated diabetic rats. While HDL-cholesterol level was significantly ($p < 0.001$) increased after 7 weeks. On other hand, normal rats fed on *N. sativa* oil showed no significant differences in the levels of blood triglycerides, cholesterol, HDL-cholesterol and LDL-cholesterol after 7 weeks compared to control.

The mean values of blood total protein, urea, uric acid and creatinine concentrations of both control and experimental groups are presented in **Table 2**. STZ-induced diabetic rats showed a significant ($p < 0.05$) decrease in blood total protein with percentage of 7% compared to the control. In contrast, STZ-induced diabetic rats showed a significant ($p < 0.001$) increase in blood uric acid, urea and creatinine by 23.7%, 155.4% and 29.7% respectively, compared to the control. Administration of *N. sativa* oil to diabetic rats resulted in a significant ($p < 0.01$) decrease in the levels of uric acid by 30% while there were no significant differences in the levels of total protein, urea and creatinine after 7 weeks, compared to untreated diabetic rats. In comparison with control, administration of black seed oil in non-diabetic rats showed no significant differences in the level of blood total protein, urea, uric acid and creatinine after 7 weeks.

Table 2 shows the mean values of AST and ALT activities of both control and experimental groups after 7 weeks. In STZ-induced diabetic rats the activities of blood AST and ALT were significantly ($p < 0.001$) increased by 38.9% and 136%, respectively, compared to their normal levels. On the other hand, treatment of the STZ-induced diabetic rats with *N. sativa* oil caused reduction in the activity of these enzymes in blood by 36.1% for AST and by 55.9% for ALT, compared to the

Table 1. Effects of *N. sativa* oil supplementation on blood glucose, triglyceride, cholesterol, LDL-C and HDL-C in rats.

Parameters	Treatment			
	Normal control	STZ	STZ + Black seed oil	Black seed oil
Glucose (mg/dl)	126.72 ± 2.17***	438.70 ± 4.48###	153.84 ± 1.85***	125.56 ± 2.84***
Triglyceride(mg/dl)	55.55 ± 5.27***	153.40 ± 6.52###	89.69 ± 1.39***	50.68 ± 8.75***
Cholesterol (mg/dl)	62.39 ± 3.11***	102.77 ± 2.14###	78.53 ± 2.62***	61.84 ± 2.74***
HDL-C (mg/dl)	20.37 ± 0.46***	15.45 ± 0.50###	19.01 ± 0.19***	18.96 ± 0.47***
LDL-C (mg/dl)	31.63 ± 0.55***	54.06 ± 1.37###	40.87 ± 2.42***	31.84 ± 2.39***

The number of animals was 5 for each treatment except for the control and STZ, in which it was 10;All values are expressed as means ± SE; Significantly different from untreated STZ-induced diabetic rats (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$); Significantly different from control (# $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$).

Table 2. Effects of *N. sativa* oil supplementation on blood total protein, urea, uric acid, creatinine, AST and ALT in rats.

Parameters	Treatment			
	Normal control	STZ	STZ + Black seed oil	Black seed oil
Total protein (g/L)	6.53 ± 0.08**	6.07 ± 0.09$^{\#\#}$	6.10 ± 0.09	6.32 ± 0.08
Creatinine (mg/dl)	0.37 ± 0.02**	0.48 ± 0.03$^{\#\#}$	0.43 ± 0.02	0.29 ± 0.06***
Uric acid (mg/dl)	0.97 ± 0.04*	1.20 ± 0.07$^{\#}$	0.84 ± 0.11**	1.01 ± 0.0
Urea (mg/dl)	16.87 ± 0.50***	43.08 ± 4.28$^{\#\#\#}$	33.11 ± 4.44	15.35 ± 1.12***
AST (U/L)	96.50 ± 2.90***	134.00 ± 8.20$^{\#\#\#}$	85.60 ± 4.63***	89.60 ± 2.38***
ALT (U/L)	46.10 ± 2.06***	108.80 ± 9.92$^{\#\#\#}$	48.00 ± 3.78***	43.40 ± 2.27***

The number of animals was 5 for each treatment except for the control and STZ, in which it was 10; All values are expressed as means ± SE; Significantly different from untreated STZ-induced diabetic rats (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$); Significantly different from control ($^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ and $^{\#\#\#}$ $p < 0.001$).

mean values of untreated diabetic group. In comparison with control, administration of black seed oil in non-diabetic rats showed no significant differences in activities of blood AST and ALT after 7 weeks.

4. DISCUSSION

Results of the present study showed that diabetic rats exhibited a significant increase in blood glucose level. This result is in consistent with other studies in rats [1,46,47]. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in STZ-induced diabetes mellitus rats [48-50]. In the present study, the oil of *N. sativa* significantly reduces blood glucose levels in STZ-induced diabetic rats after 7 weeks of treatment, which also demonstrates that there is significantly higher rate of glucose disposal. In a previous study, the oil of *N. sativa* significantly reduces blood glucose levels in STZ-induced diabetic rats after 3 weeks of treatment [2]. Similar observations were also obtained by Al-Awadi *et al.* [26], who reported that plant mixture extract comprising of *N. sativa*, Myrrh, Gum olibanum, Gum asafetida and Aloe to have a blood glucose lowering effect. Also, the intraperitoneal administration of volatile oil of *N. sativa* to fasting normal and alloxon-diabetic rabbits produced significant hypoglycemic effects [51]. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effects of the diabetogenic agent streptozotocin [33]. Previous studies demonstrated that the essential oils of black seed and their active constituents have proven free radical scavenging and antioxidant activities [24,52,53]. Based on above mentioned reports, it is suggested that the possible mechanism of action by the oil of *N. sativa* could be related to antioxidants that aid to recover from impaired metabolism of glucose [2]. The diabetes caused by streptozotocin administration increases fat mobilization in skeletal muscle [54] inducing significant weight loss [55]. Lipids play a vital role in the pathogenesis of diabetes mellitus. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [56]. In our study, we have noticed significantly increased levels of serum total cholesterol, triglycerides and LDL-cholesterol but markedly decreased level of serum HDL-cholesterol in STZ-induced diabetic rats. These results are in agreement with those obtained by [39,40,57,58]. The abnormal high concentrations of serum lipids in diabetic animals are due mainly to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase [59]. Excess fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins [60].

The present study showed that *N. sativa* oil had favourably modified serum lipid profile in rats with significant decreases in total cholesterol, LDL-cholesterol, triglycerides and increased HDL. Moreover, the effects of *N. sativa* oil on the tested physiological parameters in streptozotocin-diabetic rats are more beneficial after 7 weeks than after 3 weeks [2]. In this study, the STZ + *N. sativa* oil group showed significant decreases in the levels of glucose (15.97%), triglyceride (6.21%) and cholesterol (7.1%) after 7 weeks when compared with those after 3 weeks [2]. Zaoui *et al.* [61] reported that serum cholesterol, triglycerides and glucose levels were significantly decreased in *N. sativa* oil (1 ml/kg/day) for 12

weeks treated rats. The antilipidemic action of *N. sativa* oil may reside in their ability to stimulate insulin secretion and action. Fararh *et al.* [62] investigated the possible insulinotropic properties of *N. sativa* oil in streptozotocin plus nicotinamide-induced diabetes mellitus in hamsters. The results of their study indicate that there is a significant decrease in blood glucose level together with significant increase in serum insulin level after treatment with *N. sativa* oil for 4 weeks. In addition, there are big areas with positive immuno-reactivity for the presence of insulin in the pancreases from *N. sativa* oil-treated group compared to non-treated one using immunohistochemical staining. These data show that the hypoglycemic effect of *N. sativa* oil in streptozotocin plus nicotinamide diabetic hamsters resulted, at least partly, from a stimulatory effect on β cell function with consequent increase in serum insulin level. Therefore, these results indicate that *N. sativa* oil has insulinotropic properties in type 2-like model.

Cardiovascular disease (CVD) is directly related to plasma concentration of low density lipoprotein cholesterol (LDL-C) and inversely related to high density lipoprotein cholesterol (HDL-C) [63]. Increased levels of LDL are linked with cardiovascular disease; more specifically, it has been reported that oxidation of LDL particles is likely a key step in the development of atherosclerotic plaques [64]. Recent evidence suggests that lipid-lowering therapy reduces cardiovascular morbidity and mortality and causes regression of coronary atherosclerosis [65]. Zahida *et al.* [66] reported that *N. sativa* has ability to reduce lipid profile which is a major risk factor for coronary artery disease in cardiac patients. The exact mechanism of action of *N. sativa* is not known, however, it has been proved that volatile oil of *N. sativa* has two main constituents *i.e.* nigellone and thymoquinone which play a key role in heart disease prevention [67-69]. According to Feldman [70] antioxidants (e.g., vitamins E and C) may lessen the risk CVD by decreasing oxidized LDL, which is more atherogenic. Thymoquinone (TQ) and *ter*-butylhydroquinone (TBHQ) of *N. sativa* have strong antioxidant potentials through scavenging ability of different free radicals [71].

The data revealed significant elevations in blood urea, uric acid and creatinine concentrations in STZ-induced diabetic rats. A similar effect was recorded previously [42]. A decrease in body weight of diabetic rats is possible due to catabolism of fats and protein, even though the food intake is more in diabetic rats than control. Due to insulin deficiency protein content is decreased in muscular tissue by proteolysis [72]. The highly signifycant increase in serum urea concentrations of diabetic rats may be due to depletion of serum protein, increase in the rate of circulating amino acids and deamination takes place that consequently leads to the formation of large amount of ammonia which is eventually converted to urea. The breakdown of amino acids during gluconeogenesis in the liver results in increased production of urea, fostering negative nitrogen balance [73]. In contrast, serum total protein was decreased in diabetic animals. The decrease in blood total protein observed in diabetic rats is coinciding with the findings of [74] and [75]. This decline may be due to the inhibited oxidative phosphorylation processes which lead to decrease of protein synthesis, increase in the catabolic processes and reduction of protein absorption [76,77]. Previous changes in serum urea, uric acid and creatinine concentrations strongly suggested impairment of kidney function in diabetes. The treatment with *N. sativa* oil lead to significant decreases in the levels of uric acid in STZ-induced diabetic rats compared to untreated STZ-induced diabetic rats after 7 weeks. The main effect of the *N. sativa* oil is presumably due to its ability to increase insulin secretion [2].

In STZ-induced diabetic rats the activities of blood AST and ALT were significantly increased compared to their normal levels. These results indicated that diabetes may be induced due to liver dysfunction [78] also found that liver was necrotized in STZ-induced diabetic rats. Therefore, increase in the activities of AST and ALT in blood may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [79], which gives an indication on the hepatotoxic effect of STZ. On the other hand, treatment of the diabetic rats with *N. sativa* oil caused reduction in the activity of these enzymes in blood compared to the mean values of diabetic group and consequently may alleviate liver damage caused by STZ-induced diabetes. These results are in agreement with those obtained by [9] who reported that oral administration of cinnamaldehyde for 45 days significantly restores the enzyme levels to near normal in diabetic rats. A possible explanation for the differential effects of *N. sativa* oil on the activities of AST and ALT in blood is that these treatments may inhibit the liver damage induced by streptozotocin [2].

5. CONCLUSIONS

The results of this study indicate that black seed oil possesses hypoglycemic, hypolipidemic and antioxidant effects in STZ-induced diabetic rats and suggest that this oil may be an excellent adjuvant support in the therapy of diabetes and its complications especially when it is used for a longer period.

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