

Hypoglycemic and Hypolipidemic Potential of *Nigella sativa* L. Seed Extract in Streptozotocin (STZ)-Induced Diabetic Rats

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Abstract

Diabetes mellitus (DM) is the most common metabolic disease worldwide. Multiple medications and side effects, the most significant and prevalent themes in diabetes mellitus ultimately determine novel directions and avenues in drug discovery. The objective of this study was to determine the effects of NS (*Nigella sativa*) L. Seed hydroalcoholic Extract on hypoglycemic and hypolipidemic condition in Streptozotocin- induced diabetic rats. DM was induced by single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (65 mg/kg body weight) solution. Mice were divided into six groups. This study was conducted for 28 days and blood glucose level was measured at 7 days interval. Hyperlipidemic was induced by mixture of cholesterol (1.5%) and cholic acid (0.5%) with diet of rats. At the end of the treatment, the blood glucose level and lipid profile was measured by using commercial kits. It was observed that NS (*Nigella sativa*) has a potential hypoglycemic effect as it significantly ($p < 0.001$) decrease blood glucose level compared to diabetic group. The SGPT, SGOT and CRP were also decreased significantly ($p < 0.05$). Therefore NS (*Nigella sativa*) might be effective against liver malfunction. An indicative antilipidemic effect was also observed as TC, TG, LDL, VLDL showed significant ($p < 0.05$) decrease whereas HDL showed significant increase ($p < 0.001$) by NS (*Nigella sativa*) treatment compared to diabetic group. These results showed that hydroalcoholic extract of NS (*Nigella sativa*) at low doses has hypoglycemic effect and as well as lipid profile in diabetic subjects. *Nigella sativa* is a potential protective natural agent against atherosclerosis, hepatoprotective and cardiovascular complication in diabetes rats.

Keywords: *Nigella sativa*; Diabetes; CVD; Lipid profile; CRP

Introduction

Diabetes mellitus (DM) is the most common metabolic disease worldwide. Therapeutic compounds available to treat DM are either synthetic or formulated forms. Type 2 diabetes had global prevalence estimate of 2.8% in the year 2000 and is projected to be 4.4% in 2030 [1]. Prevention and control of DM is a major challenge and requires moulding lifestyle towards more physical activity and less calorie intake avoiding sedentary habits. However most people find it difficult to change their lifestyle and look for a less cumbersome alternative. A traditional component of food that can reduce appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, body weight, and can stimulate glucose induced secretion of insulin from beta-cells in pancreas, may prove to be useful for prevention and control of diabetes mellitus. Therefore, a variety of plants are used in the management and treatment of DM. Chemical and pharmacological studies on antidiabetic herbal remedies are in progress and might lead to inspiring elucidations. *Nigella sativa* Linn. (Ranunculaceae) seeds (NS) known as “black seed” or “black cumin” are commonly used in traditional medication systems [2-4]. Therefore a lot of patient nowadays use natural product to treat DM [5]. The seeds have been known by its contents, such as flavonoid, saponin, steroid/triterpenoid, quinon and alkaloid [6,7]. NS (*Nigella sativa*) can inhibit absorption of glucose in gut protect beta cell and increase AMPK pathway in muscle and hepatic cell [8]. There are lot of studies had been done regarding the antidiabetic activity of NS extract and/or fixed oil. It is not known whether the active substance was more abundant in fixed oil or in extract form. The components partially lyse cell membrane which increases glucose transport and lipid take up into adipose tissue. Based on the above properties, NS (*Nigella sativa*) could be considered as a therapeutic agent for diabetes and Cardiovascular Disease (CVD). However, very limited research has been done on this species of herbal medicine plant in broad and abroad to evaluate the complications between Diabetes and Cardiovascular diseases. There is no systematic

data available on the physicochemical and phytochemical properties of Bangladeshi NS (*Nigella sativa*). This study was undertaken to analyse Bangladeshi NS (*Nigella sativa*) for better understanding of its medicinal properties.

Materials and Methods

Plant material

The *N. sativa* seeds were collected from the survey of medicinal plant unit, Regional Research Institute of Biological science (IBSc), Rajshahi University, Bangladesh. Identification of the samples was further confirmed with the Department of Botany, Rajshahi University, Rajshahi, Bangladesh.

Preparation of plant extract

The *N. sativa* seeds were dried at room temperature. The seeds were powdered in an electrical grinder and stored at 5°C until further use. Seed powder (300 g) was extracted with petroleum ether (60-80°C) to remove lipids. Ethanol was evaporated in a rotary evaporator at 40-50°C under reduce pressure. The yield of extract was 15 g and substance was dissolved in water before use.

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Phytochemical screening of polyherbal formulation

The different extracts obtained were subjected to phytochemical screening for the presence of flavanoids, tannins, alkaloids, carbohydrates, phytosterols, triterpenoids, saponins according to standard procedures [9].

Experimental animals care

Experimental animals were collected from International Cholera and Dysentery Disease Research, Bangladesh (ICDDR). Ethical permission was provided by Institute of Biological science (IBSC), Rajshahi University. Male albino mice weighing ranged (25-27 g) were used for the study. They were individually housed in polypropylene cages in well-ventilated rooms under hygienic conditions, allowed free access to food (standard pellet diet) and water ad libitum and kept under maintained day night cycle. They were adapted for one week before the experiment.

Induction of diabetics

The overnight fasted animals were induced diabetes by a single administration of STZ (65 mg/kg b.w. i.p.) in ice-cold citrate buffer (pH 7.4). The threshold level of fasting serum glucose to diagnose diabetes was taken as >150 mg/dl (11.5 mmol/L) and only those animals were included in the study, rest are excluded from the study. The animals were divided into six groups of six in each group.

Blood collection

Blood samples from all groups were collected on days 1, 7, 14, 21 and 28 in a fasting state from rat's marginal ear vein by 26 G needle and syringe. Blood glucose levels, plasma cholesterol levels, triglyceride levels, LDL and HDL levels were determined by "Humylazer 2000" analyser (Human, Germany). The values were expressed as mean \pm SEM, Statistical analyses were performed by SPSS-16 one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test for multiple comparisons. $P < 0.05$ was considered as significant.

Experimental animals grouping and treatment

The animals were randomly divided into six groups. Each group contain six rats ($n=6$). The treatment of animals began on the initial day after STZ injection and this was considered as 1st day of treatment. The animals were treated for 4 weeks as follows:

Group-1: Control rats feed with standard pellet diet and water.

Group-2: The rats were made diabetic by an intra-peritoneal injection of single dose of 110 mg/kg body weight followed by 65 mg/kg body weight Streptozotocin. Animals whose blood glucose level exceeded 11.0 mmol/L at 72 h after treatment were considered diabetic. These animals served as untreated diabetic control.

Group-3: The diabetic rats treated with ethanol extract of NS (*Nigella sativa*) at a dose of 300 mg/kg body weight for 28 days.

Group-4: Hyper Cholesterol rats were given cholesterol (1.5%) and cholic acid (0.5%) mix with diet.

Group-5: Hyper cholesterol rats were treated with ethanol extract of NS (*Nigella sativa*) at a dose of 300 mg/kg body weight for 28 days.

Group-6: Diabetic rats were treated by Glibenclamide at a dose of 0.5 mg/kg b.wt.

Measurement of blood parameters

Plasma concentrations of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL, SGPT, SGOT and CRP were measured using a quantification kit (Linear chemicals, Barcelona, Spain) by automatic Bioanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

Statistical Analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). Results were analyzed by using Scientific Package of Social Science (SPSS) version 17.0. Two different set of statistics, which is descriptive and analytical statistics was applied. The descriptive statistic was used to analyse mean, standard deviation (SD) whereby analytical statistics, one-way ANOVA was used to determine statistical significance ($p < 0.05$, $p < 0.001$) among the groups.

Results

Comparing the blood sugar level in Streptozotocin induced diabetic rats, NS (*Nigella sativa*) administered subject showed significant reduction of blood glucose level which is as near as glibenclamide administered subject at ($P < 0.001$) (Table 1). Day 1, day 7, day 14, day 21 and day 28 NS (*Nigella sativa*) supplementation group's glucose levels maintained 32.58%-75.38% lower than the diabetic control group where as in case of glibenclamide it was 21.71%-87.63% lower than significantly diabetic control group ($P < 0.001$).

The Table 2 shows the serum levels of Total cholesterol (TC), Triglycerides (TG), LDL, VLDL, HDL and hypercholesterol of control and streptozotocin-induced diabetic rats. Reduction of Total Cholesterol (TC) level was 14.38%-23.05% observed by NS (*Nigella sativa*) treatment respectively in diabetic rats whereas hyper cholesterol reduces 19.04%-46.55%. The 3 to 28 days Total Cholesterol levels in the NS (*Nigella sativa*) treatment groups showed significant decrease compared with the diabetic control group and hyper cholesterol control group ($P < 0.05$). The effect of NS (*Nigella sativa*) treatment on serum triglyceride (TG) content in diabetic rats is illustrated in Table 2. The Levels of triglyceride in the diabetic and hyper cholesterol group on the 28 days increased. Compare with the diabetic and hyper cholesterol control group by the treatment of NS (*Nigella sativa*) serum triglyceride was considerably lower 6.30%-11.73% whereas reduction of hypercholesterol group was 52.30%-82.31%. During the course of the experiment the significantly decrease diabetic and hypercholesterol group ($P < 0.05$). The diabetic and hypercholesterol group shows an increase in LDL levels higher than the (normal) control group. LDL level was significantly reduced ($P < 0.05$) in diabetic group received NS (*Nigella sativa*) treatment 15.7%-27.30% and hypercholesterol 19.72%-38.03%. VLDL level was significantly reduced ($P < 0.05$) for due to received NS (*Nigella sativa*) treatment 9.2%-12.35% whereas hypercholesterol 63.00%-82.80%. The HDL level was increased significantly ($P < 0.001$) at diabetic 13.4%-30.43% whereas hypercholesterol increased 12.25%-37.40% (Table 2).

The Table 3 showed the phytochemical investigation revealed the fact that the hypoglycemic and hypolipidemic activity could be due to the presence of flavonoids, terpenoids or alkaloids.

Increasing of SGPT and SGOT level after diabetes induction which was compensated by NS (*Nigella sativa*) significantly ($P < 0.05$). The reduction of SGPT by NS (*Nigella sativa*) was 32.75% respectively whereas 25.23% for glibenclamide. The reduction of SGOT level was highly significant for NS (*Nigella sativa*) 29.84% than glibenclamide

Group	Blood sample drawing days				
	Day 1	Day 7	Day 14	Day 21	Day 28
Normal (control)	5.6 ± 0.1	5.8 ± 0.2	5.8 ± 0.1	5.8 ± 0.2	5.7 ± 0.1
Diabetic control	20.1 ± 0.5 ^{a*}	21.5 ± 0.4 ^{a*}	21.6 ± 0.7 ^{a*}	23.4 ± 0.5 ^{a*}	24.5 ± 0.5 ^{a*}
Diabetic+Glibenclamide	20.3 ± 0.9 ^{b*}	16.4 ± 0.4	13.5 ± 0.5 ^{b*}	11.5 ± 0.7 ^{b*}	9.3 ± 0.3 ^{b*}
Diabetic+NS (<i>Nigella sativa</i>)	21.3 ± 1.0 ^{b#}	18.2 ± 0.8 ^{b#}	16.3 ± 1.3	14.4 ± 1.1	12.4 ± 0.5 ^{b#}

Body weight decreases with injection of STZ. Body weights were measured with day 1 interval for 28 days. Each value is the mean ± SEM n=6. Blood glucose level in the treated rats were significantly different from normal and diabetic control groups at ^{a*}P<0.001, whereas ^{b*}P<0.001 and ^{b#}P<0.001 indicated the significant difference from diabetic control group.

Table 1: Effect of ethanol extract of NS (*Nigella sativa*) on the blood glucose level of experimental rats (mmol/L).

Groups	Total Cholesterol (mmol/L)	Triglycerides (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	HDL (mmol/L)
Normal (Control)	5.565 ± 0.119	1.423 ± 0.070	4.613 ± 0.143	0.646 ± 0.032	0.298 ± 0.011
STZ (Diabetic) control	6.556 ± 0.154 ^{a*}	1.965 ± 0.123 ^{a*}	5.427 ± 0.126 ^{a*}	0.892 ± 0.055 ^{a*}	0.231 ± 0.032 ^{a*}
Hypercholesterol	8.126 ± 0.580	3.458 ± 0.142	6.102 ± 0.537	1.571 ± 0.064	0.445 ± 0.056
Diabetic+Glibenclamide	5.603 ± 0.11 ^{b*}	1.725 ± 0.128 ^{b*}	4.610 ± 0.148 ^{b*}	0.783 ± 0.058 ^{b*}	0.205 ± 0.018 ^{b*}
Diabetic+NS (<i>Nigella sativa</i>)	5.135 ± 0.402 ^{b#}	1.765 ± 0.171 ^{b#}	3.825 ± 0.437 ^{b#}	0.813 ± 0.077 ^{b#}	0.398 ± 0.007 ^{b#}
Hyper Cholesterol+NS (<i>Nigella sativa</i>)	4.469 ± 0.05 ^{cc}	1.943 ± 0.163	2.953 ± 0.109 ^{cc}	1.368 ± 0.074 ^{cc}	0.49 ± 0.014 ^{cc}

Total Cholesterol, Triglycerides, LDL, VLDL and HDL in the treated rats were significantly different from normal and diabetic control groups at ^{a*}P<0.05, whereas Glibenclamide ^{b*}P<0.001 and *N. sativa* ^{b#}P<0.05 indicated the significant difference from Diabetic control group and ^{cc}P<0.05 indicated significant difference from hypercholesterol group.

Table 2: Effect of ethanol extract of NS (*Nigella sativa*) on Total cholesterol, Triglyceride, LDL, VLDL, HDL in experimental rats.

Tests	Chloroform	Petether	Ethylacetate	Ethanol	Aqueous extract
Alkaloids	-	+	-	+	+
Coumarines	+	+	+	+	+
Carbohydrate	+	+	+	+	+
Flavonoids	+	+	+	+	+
Glycosides	-	-	-	-	-
Saponins	+	+	+	+	+
Steroids and pyosteroids	-	-	-	+	-
Tannins	+	+	-	+	+
Terpenoids	+	-	+	-	-

Table 3: Phytochemical study for polyherbal formulation (powder of *Nigella sativa*).

61.23% (Figure 1).

The Figure 2 showed the C-reactive protein (CRP) levels were higher in diabetic and hypercholesterol control group than the normal group. By the treatment of NS (*Nigella sativa*) CRP significantly reduces 21.06% from 53.32% diabetic group whereas glibenclamide reduces 42.0% from 61.32%. By the treatment of NS (*Nigella sativa*) significantly reduction of CRP 32.51% from 73.14% in hypercholesterol group (p<0.05).

Discussion

Diabetes mellitus is a chronic, systemic, metabolic disease defined by hyperglycemia and characterized by alterations in the metabolism of carbohydrate, protein and lipid. Oxidative stress thought to be increased in a system where the rate of free radical production is increased and/or the antioxidant mechanisms are impaired [10,11]. In recent years, the oxidative stress-induced free radicals have been implicated in the pathology of insulin dependent diabetes mellitus [12-14]. The reduction in blood glucose levels, encountered herein, agree with the findings of previous studies conducted on streptozotocin induced diabetic rats treated with crude NS (*Nigella sativa*) [15], or with NS oil [16]. The possible beneficial effects of *Nigella sativa* and thymoquinone on histopathological changes of sciatic nerves in streptozotocin induced diabetic rats were evaluated. The treatment of both *Nigella sativa* and thymoquinone caused a sharp decrease in the elevated serum glucose and an increase in the lowered serum insulin concentrations in streptozotocin induced diabetic rats. *Nigella sativa*

and thymoquinone treatment resulted in increased area of insulin immuno reactive beta-cells significantly. Histological evaluation of the tissues in diabetic animals treated with thymoquinone and especially *Nigella sativa* showed fewer morphologic alterations [17]. As seen in the present studies also the level of serum lipids was usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease (CHD) [18]. Administration of *N. sativa* seeds extract improved considerably, serum lipids of diabetic rats which were however not completely normal. Oxidative stress plays a role in the causation of diabetes and Antioxidants have been shown to have a role in the alleviation of diabetes [19]. *Nigella sativa* treatment exerts a Therapeutic protective effect in diabetes by decreasing morphological changes and preserving pancreatic beta-cell integrity thus suggesting it can be clinically useful for protecting beta-cells against oxidative stress [20]. The plant mixture containing these seed revealed that blood glucose lowering effect was due to the inhibition of hepatic gluconeogenesis suggesting its use in non-insulin dependent diabetes mellitus [21]. *Nigella sativa* showed the maximum inhibition of α-amylase activity. It also showed a strong inhibition towards the flow of glucose across a dialysis membrane. It showed very strong anti-diabetic properties. In another study the extract of *Nigella sativa* was found to significantly lower blood pressure and lower level of cholesterol [22]. Supporting the traditional use of *Nigella sativa* as a treatment of dyslipidemia and hyperglycemia, the effects of the fixed oil of *Nigella* seeds in rats were investigated by monitoring blood homeostasis and body weight as well as toxicity. The serum cholesterol,

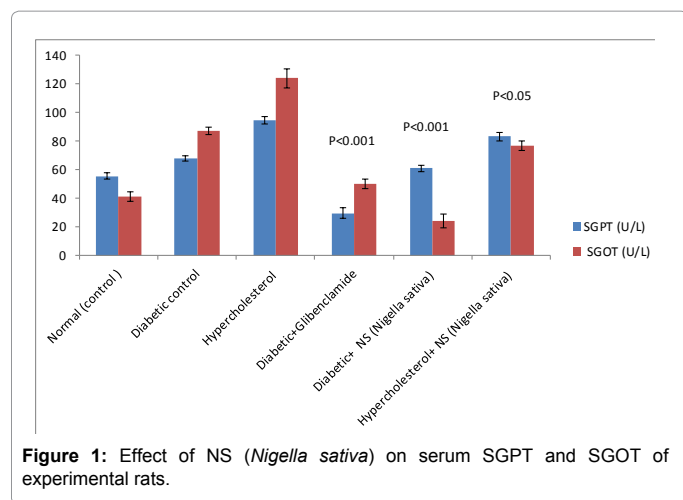


Figure 1: Effect of NS (*Nigella sativa*) on serum SGPT and SGOT of experimental rats.

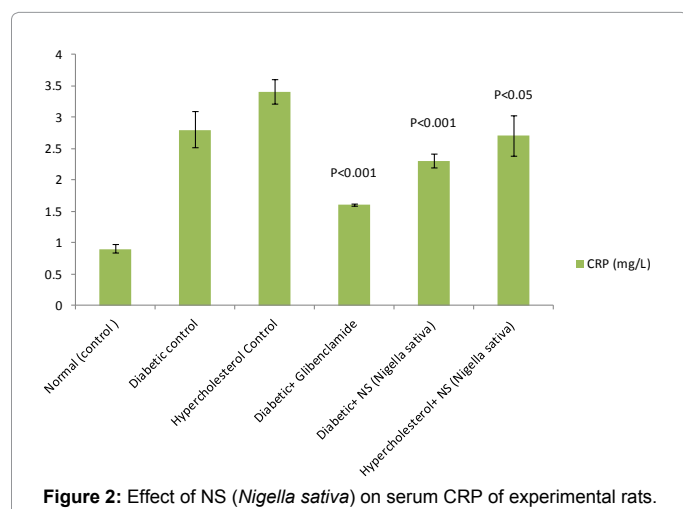


Figure 2: Effect of NS (*Nigella sativa*) on serum CRP of experimental rats.

triglyceride and glucose level and the count of leukocytes and platelets decreased while haematocrit haemoglobin levels increased significantly [23]. Recently, the effect of crushed seeds and total oil were studied on serum levels of glucose, cholesterol, triglycerides, creatine kinase, prolactin, red blood cells, white blood cells, platelets, haemoglobin, and some liver enzymes such as ALT, aspartate aminotransferase (AST), ALP and GGT [24]. The phytochemical study showed remarkable antidiabetic and hypolipidemic effect in streptozotocin induced diabetic rats. The extract showed no toxicity at significantly higher doses. Serum alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), lactate dehydrogenase (LDH) levels and total antioxidant capacity (TAC), catalase (CAT), total oxidative status (TOS), oxidative stress index (OSI) and myeloperoxidase (MPO) in hepatic tissue were determined. The results have shown that treatment with NS prevents hepatic ischemia-reperfusion injury to the liver [25]. It is reported that *N. sativa* (300 mg/kg) intraperitoneally relieves the deleterious effects of ischemia reperfusion injury on liver. Results suggested that *N. sativa* treatment protects the rat liver against hepatic ischemia reperfusion injury [26]. *N. sativa* administration protects hepatic tissue from deleterious effects of toxic metals such as lead, and attenuates hepatic lipid peroxidation following exposure to chemicals such as carbon tetrachloride [27]. C-reactive protein (CRP) is a simple cost effective test, which can predict the cardiovascular risk. The addition of CRP- testing to standard lipid screening appears to provide

an important method to determine Cardiovascular Disease (CVD) risk factor. The results showed the intrinsic cardiac contractile properties without evidence of an increased cardiac (CVD) work load or energy consumption *in vivo* which makes these seeds an isotropic agent with hemodynamic profile [28-30].

Conclusion

In conclusion, the experimental findings suggested that administration of *N. sativa*, at a safe dose level, suppresses STZ-induced diabetic in the rat. *N. sativa* seed extracts exhibited significant hypoglycaemic and hypolipidaemic effects. The most important action of *Nigella sativa* that may be responsible for its beneficial effect in metabolic syndrome is its insulin sensitizing action. *N. sativa* treatment may indicate its usefulness as a potential treatment in diabetic patients, our results suggested that hydroalcoholic extract of NS (*Nigella sativa*) at low doses has beneficial effect on FBG level and ameliorative effect on regeneration of pancreatic islets and may be used as a therapeutic agent in the management of diabetes mellitus.

Conflict of Interest

The Authors declare that they have no competing interests.

Acknowledgements

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