

Antidiabetic Efficacy of *Nigella sativa* Linn. in Alloxan-induced Diabetic Rabbits

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ABSTRACT

Introduction: 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 present study was conducted to investigate antidiabetic efficacy of extracts of *Nigella sativa* Linn. seeds (NS). **Methods:** Alloxan-induced diabetic rabbits (n = 28) were divided into four groups, including diabetic control. Two groups were treated with NS methanol (NSM) and ethanol (NSE) extracts, and one group were given glibenclamide. Serum glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were measured by kit methods. **Results:** NS demonstrated hypoglycaemic and hypolipidaemic activities. Although NS treatments decreased blood glucose, TC, TG and LDL-C and increased HDL-C in diabetic groups, it was unable to normalize all the parameters. NS extracts did not regenerate β -cells of islet of the pancreas. Glibenclamide showed significant ($P < 0.05$) decline in hyperglycaemia and hyperlipidaemia as well as prominent renewal of β -cells of islets of Langerhans. Few surviving beta cells of islet of the pancreas were apparent in NSM treated group. Standard drug glibenclamide showed a significant decline in hyperglycaemia and hyperlipidaemia as well as prominent renewal of beta cells. **Conclusion:** *N. sativa* L. extracts have curative effects in terms of diabetes-induced disturbances of glucose and lipids. Further investigations are suggested to ascertain the therapeutic role of NS on islet histopathology in diabetes mellitus.

KEYWORDS: Diabetes mellitus, glibenclamide, hypoglycaemia, *Nigella sativa* Linn.

INTRODUCTION

Diabetes mellitus (DM) is the most common metabolic disease worldwide. Therapeutic compounds available to treat DM are either synthetic or formulated forms. Multiple medications and side effects, the most significant and prevalent themes in DM ultimately determine novel directions and avenues in drug discovery. 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.¹ *Nigella sativa* Linn. (Ranunculaceae) seeds (NS) known as “black seed” or “black cumin” are commonly used in traditional medication systems.^{2,3,4} Antioxidant and anti-inflammatory actions of NS are already

acknowledged.^{5,6,7} Salutary characteristics of NS in the management of hyperglycemia, hyperlipidemia and bronchial asthma were cited previously.^{8,9,10,11} NS also possess chemo-preventive and insulinotropic effects.^{12,13,14,15,16}

In view of the established antidiabetic potentials, present study was performed to investigate antidiabetic efficacy of NS and to compare it with that of glibenclamide (glyburide), a reference antidiabetic drug. Glibenclamide enhances insulin secretion from islet cells and thus promotes hypoglycaemia and hypolipidaemia.

MATERIALS & METHODS

Nigella sativa seeds were collected from local retail market and authenticated at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. After washing and drying, seeds were ground into a fine powder state. NS (50 g) was homogenized with 150 ml methanol and ethanol separately and kept on an orbital shaker (120 rpm) for 48 hours at room temperature. Mixtures were filtered and concentrated at 50°C in water bath. The final yields were 5.7% and 3.6% for NS methanolic (NSM) and ethanol (NSE) extracts respectively. The suspensions of these extracts were then prepared in 0.5% w/v carboxymethylcellulose

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(CMC) in normal saline solution.

Healthy male rabbits (*Oryctolagus cuniculus*) weighing 1-1.5 kg were kept in metal cages with free access to standard feed and water ad libitum. The animals were acclimatized to the laboratory conditions prior to experimentation in the animal house of the Institute of Microbiology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad. Alloxan monohydrate (Sigma-Aldrich) was used (80 mg/kg body weight) through intravenous injection to ear vein. Rabbits having fasting blood glucose levels greater than 150 mg/dL were considered diabetics. Group 1 was the vehicle (seven healthy rabbits as negative control). The overnight fasted animals were randomly divided into four groups (seven animals per group). These groups were, Group 2: Diabetic control (diabetic rabbits as positive control), Group 3: Diabetic rabbits for oral administration of 100 mg/kg body weight NS methanol extract, Group 4: Diabetic rabbits for oral administration of 100 mg/kg body weight NS ethanol extract, Group 5: Diabetic rabbits for oral administration of glibenclamide (2.5mg/kg body weight).

All the groups were loaded with glucose solution (2.0 g/kg) intravenously after half an hour of extract/drug administration. Extract/drug treatment continued for two weeks. Fasting blood samples were collected at baseline and after treatment. Serum glucose, triglycerides (TG), total Cholesterol (TC), HDL-Cholesterol (HDL-C) was measured by kit methods (Ecoline Diasys GmbH Merck, Germany). LDL-C concentrations were calculated as: Total cholesterol - HDL.C- VLDL.C.¹⁷ Whereas, VLDL-Cholesterol was calculated as: Triglyceride/5.¹⁸

RESULTS

Hypoglycaemic Effects:

Alloxan monohydrate raised the blood-glucose levels up to 265.6 ± 30.26 mg/dL. Blood glucose was significantly higher (265.6 ± 30.26 mg/dL; P<0.05) in diabetic rabbits as compared to non-diabetic rabbits (99.5 ± 13.12 mg/dL). Glucose levels in NS treated groups (Diabetic + NSM and Diabetic + NSE) showed descending trends in the post-treatment analysis. However, lowered glucose levels were higher (Diabetic + NSM: 176.6 ± 26.75 mg/dL; Diabetic + NSE: 205.3 ± 31.12 mg/dL) than that of the vehicle (non-diabetic control group: 104.7 ± 13.23 mg/dL). NSM and NSE treatment made 32.64% and 22.87% change (P<0.05) in glucose as compared to the initial levels (Table 1). This reflected significant (P<0.05) hypoglycemic effect in NS treated rabbits as compared to the diabetic group. Nonetheless, final glucose levels were higher (Diabetic + NSM: 176.6 ± 26.75 mg/dL; Diabetic + NSE: 205.3 ± 31.12 mg/dL) than that of the vehicle (104.7 ± 13.23 mg/dL). Glibenclamide not only reduced the glucose level by 58% as compared to baseline concentrations, it also attained the normal range. NS managed to lower hyperglycemia, but it was less effective than glibenclamide.

Table 1. Effects of treatment with *N. sativa* seed extracts on serum glucose and lipids in alloxan diabetic rabbits

		Study Groups				
		Vehicle	Diabetic	Diabetic + NSM	Diabetic + NSE	Diabetic + G
Glucose	Initial	99.5 ± 13.12	265.6 ± 30.26	262.2 ± 29.45	266.1 ± 39.47	265.4 ± 24.39
	Final	104.7±13.23	296.3 ± 45.91	176.6 ± 26.75*	205.3 ± 31.12*	111.3 ± 19.18*
TG	Initial	88.19 ± 5.19	154.66 ± 7.6	158.44 ±9.01	152.22 ±8.93	159.74 ± 9.25
	Final	89.15 ± 6.37	166.81 ± 4.33	134.31 ± 10.0*	146.03 ± 10.5	78.32 ± 5.7*
TC	Initial	53.14±10.58	200.33 ± 5.01	194.21 ±7.26	197.09 ± 7.9	199.25 ± 6.38
	Final	54.67 ± 7.64	210.2 ± 4.77	177.15 ± 1.69*	186.42 ± 4.17	123.75 ± 2.80*
HDL-C	Initial	28.66 ± 3.7	45.33 ± 8.5	42.55 ± 2.95	46.41 ± 1.97	43.7 ± 2.85
	Final	27.99 ± 7.21	43.86 ± 3.65	43.11 ± 1.6	47.98 ± 3.70	51.54 ± 1.67*
LDL-C	Initial	6.85 ± 1.92	124.07 ± 2.2	119.98 ± 2.62	120.2 ± 3.64	123.61 ± 2.99
	Final	8.85 ± 5.61	132.98 ± 0.99	107.18 ± 1.91*	109.24 ± 1.82*	56.55 ± 2.97*

Data expressed as mean (mg/dL) ± SEM of triplicate measurements for groups of seven animals each.

*P < 0.05 as compared to diabetic control.

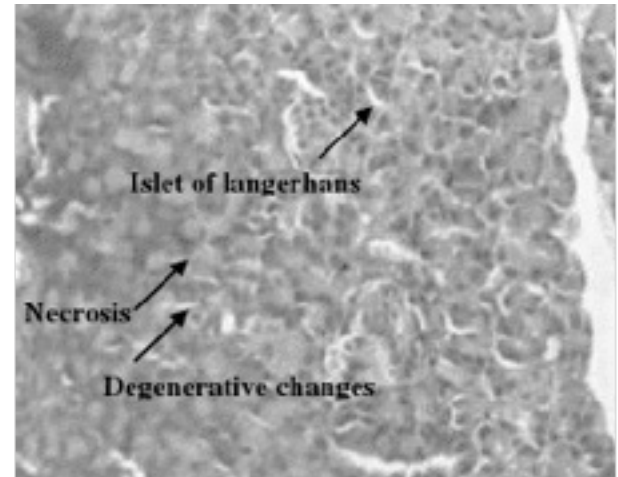
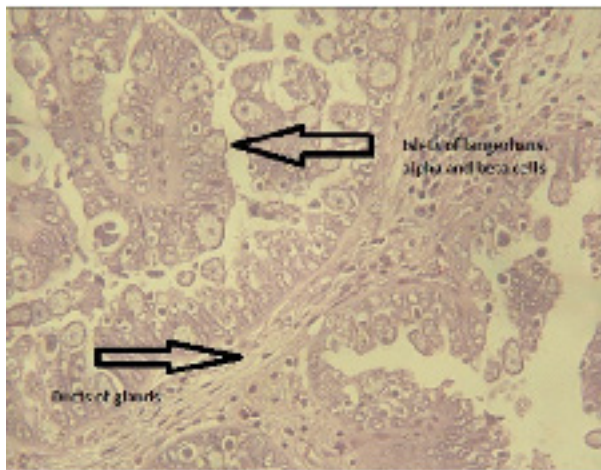
TG: triglyceride; TC: total cholesterol; Diabetic + NSM: Diabetic group given NS methanol extract; Diabetic + NSE: Diabetic group given NS ethanol extract; Diabetic + G: Diabetic group given glibenclamide.

Hypolipidaemic Effects:

Hypolipidaemic activity of NS is summarized in Table 1. Significantly higher ($P < 0.05$) levels of TC, TG, LDL-C and HDL-C in diabetic animals as that of vehicle control were observed at baseline. NS exhibited trivial antihypercholesterolemic and antihypertriglyceridemic activities in NS groups (Diabetic + NSM and Diabetic + NSE). NSM extracts accomplished 15% reduction ($P < 0.05$) in TG but was ineffective to reach normal range that was accomplished with glibenclamide (51%; $P < 0.05$). NSE extracts did not significantly affect TC. Glibenclamide significantly ($P < 0.05$) lowered TC by 38% than that of initial concentrations. Even though TC levels were significantly lowered (123.75 ± 2.80 mg/dL) by glibenclamide, these were higher than that of the vehicle group (54.67 ± 7.64 mg/dL). About 1.3-3.38% change in HDL-C as compared to the respective baseline measurements by NSM and NSE was quite insignificant. Contrary to that, a decline in initial LDL-C levels by NSM and NSE was noteworthy ($P < 0.05$). Nonetheless, more profound changes in high density lipoprotein cholesterol were

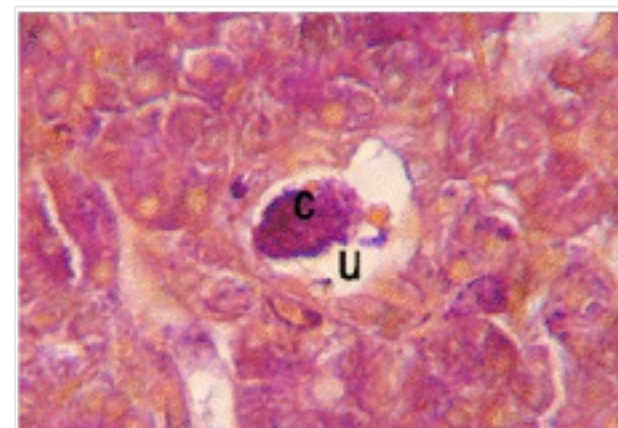
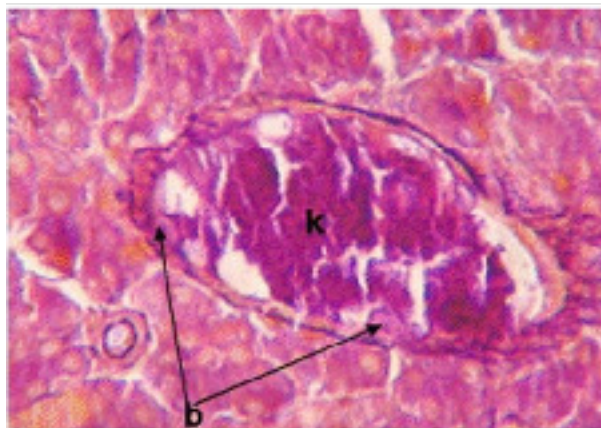
Pancreatic histopathology:

The microanatomical changes in the pancreatic tissues are presented in Figure 1 (A-E). Typical acini and normal cellular population were present in the islets of langerhans of the vehicle group (A). Alloxan monohydrate injection resulted in varying degree of damage to islet cells. Regular arrangement of cells was disturbed. Clumping, degranulation and hydropic degeneration were apparent in β -cells (B). Glibenclamide treatment caused partial regeneration of pancreatic beta cells (C) and NSM treated group had some survival β -cells (D) but not by NS ethanol extracts (E). Pancreas of glibenclamide treated group showed beta cells with normal granulations. Pancreatic anatomy of NSM and NSE groups did not match either with that of vehicle or diabetic group.



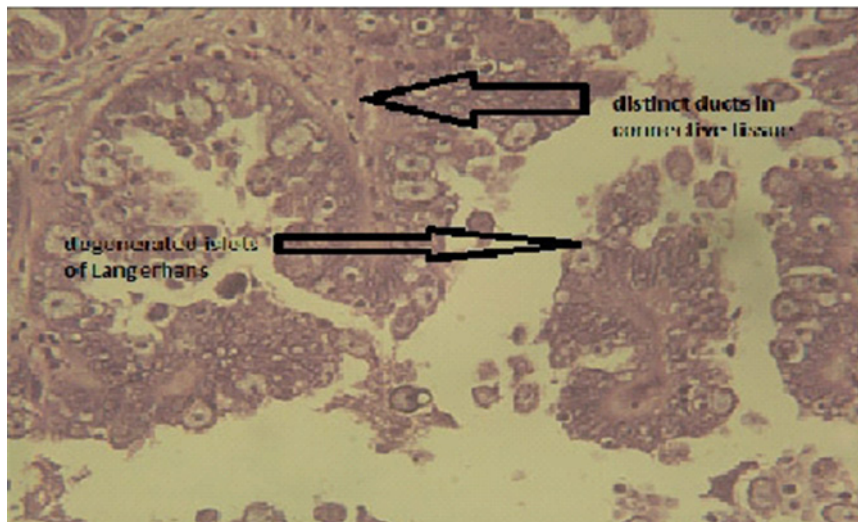
A

B



C

D



E

Figure 1. Histopathological sections of pancreas stained by chromium Haematoxyline-phloxine (A) pancreas of normal healthy rabbit with normal appearance of islets of langerhans; (B) pancreas of diabetic rabbit showing degenerative changes and necrosis; (C) pancreas of diabetic rabbit, treated with NS methanol extract with aggregates (shrunken mass) of cells (k) and few survivor beta cells (b) at the periphery; (D) pancreas of diabetic rabbit, treated with NS ethanol extract. The distorted beta (c) cells of islets and degeneration (u) are highlighted by deeply stained and white areas respectively; (E) pancreas of diabetic rabbit, treated with glibenclamide showing partial beta cells regeneration.

DISCUSSION

Hypoglycaemic Effects:

These observations were not supported by the findings of Al-Awadi and Gumaa.²⁰ Likewise; Meral et al. observed that despite a major decline in elevated glucose concentration by NS, these levels were still higher (194.41 ± 27.33 mg/dL) than those of the control animals (96.32 ± 8.37 mg/dL).²¹ Kaleem et al. and Bamosa et al. reported significant reductions in fasting blood glucose by NS in diabetic conditions.^{3,9} In a prospective study, Najmi et al. observed that NS oil (2.5 ml twice/day) rectified fasting glucose levels.²² The hypoglycaemic mechanism of NS, which is apparent in this study may be either through hepatic gluconeogenesis (Farrah et al.) or certain extrapancreatic changes (El-Dakhkhny et al.).^{23,24} An indirect hypoglycaemic nature of NS intervened by extrapancreatic actions was suggested by El-Dakhkhny et al.²⁴ Extrapancreatic mode of action can be explained by the presence of insulin-like peptides or glucose absorption inhibitory potentials of NS.^{24,25,26} Conversely, some studies pointed towards beta cells regeneration, enhancement of insulin secretion and hypoglycemia by NS administration.^{27,28}

Hypolipidaemic Effects:

Sabzghabae et al. reported reductions in TG, TC and LDL-C by NS capsules (2g/day) in Iranian subjects.¹¹ Conversely, no change in HDL-C was apparent. In another study (Al-Logmani and Zari), NS reduced TG, TC and LDL-C and increased HDL-C in experimental animals.²⁹ Similarly, when 800 mg/kg *Nigella sativa* oil was given orally to rats for four weeks, it significantly decreased TG, TC and LDL-C and increased serum

HDL-C levels.²⁴ Le et al. studied the effect of petroleum ether extract of NS (equivalent to 2g/kg/day) on blood lipids in the normal rat. NS-treated rats had lower fasting plasma levels of triglycerides and higher HDL-C as compared to controls.² Tasawar et al. found NS (500 mg/day) treatment lowered cholesterol, LDL, VLDL and triglycerides in coronary artery disease patients.³⁰ Administration of 5% NS oil to diabetic rats decreased lipid profile and elevated HDL-cholesterol level as compared to untreated diabetic rats.²⁹ In another prospective study (Najmi et al.), NS oil (2.5 ml twice/day) amended TC and LDL-C levels.²² Literature indicates the dose-dependent hypolipidaemic effect of *N. sativa*. Higher doses of NS, suitable solvent and longer treatment durations are most likely to induce pronounced hypolipidaemic effects. Results of the present study are partially in accordance with the previous data.^{3,4}

Pancreatic histopathology:

Several studies are available on restoration of beta cells of islets of the pancreas by NS.^{2,9,10,11,13,28,29} Some phytochemicals liable for pancreatic cells renewals were either deficient in our preparations or proper solvent and treatment times were required for conspicuous histopathological changes.

CONCLUSION

N. sativa seed extracts exhibited significant hypoglycaemic and hypolipidaemic potentials. However, extracts did not show substantial beta cells regeneration activities. Further investigations are suggested to ascertain the therapeutic role of *N. sativa* in diabetes mellitus.

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