Original Article

Pre treatment by the crude and the n-hexane extract of *Nigella sativa Linn*. (Kalajira) alleviates diabetes mellitus

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Abstract

Methodology: Effect of pretreatment by crude and n-Hexane extract of *Nigella* (10mg/kg body wt./day for 21 days) on streptozotocin (STZ, 50 mg/kg body wt. single dose i.p.) administered rats were observed in the present study. Biochemical parameters (Serum glucose, serum TG, serum cholesterol, pancreatic GSH) were compiled together with pancreatic histology. ***Results:*** Both the crude and the n-Hexane extract of *Nigella* pre-treated diabetic rats had demonstrated significant ($P<0.001$) alleviation from the elevated serum glucose, lowered pancreatic reduced glutathione ($P<0.01$) and elevated serum TG concentrations ($P<0.01$). 72% β cells appeared to be damaged by STZ administration, while in the crude and the n-Hexane extract of *Nigella* pretreated diabetic groups this damage was 31% and 46% respectively, the crude *Nigella* pretreated group thus appeared to have better amelioration. ***Conclusions***: Further studies are suggested to obtain the protective ingredient from the crude *Nigella* and to observe its effect upon the above mentioned parameters of diabetic rats in higher doses for prolonged periods.

***Keywords*:** Diabetes, *Nigella sativa Linn*., Streptozotocin.

Introduction

From the time immemorial, varieties of herbals were used to alleviate different diseases of mankind. This study was an attempt to search for herbal remedies of diabetes mellitus, a disease which encircles disorders of carbohydrate, fat and protein metabolism in consequence of either absence of insulin or ineffective action of insulin; replacement of which may correct the blood sugar concentrations and reverse the metabolic disorders. *Nigella sativa Linn*. (Kalajira) is a widely used spice in Asia whose antimicrobial, antidiabetic, antitnephrotoxic and anti lipid properties have been reported[1,2,3,4]. Streptozotocin (STZ) was used to induce diabetes in adult male rats. Crude *Nigella* and its n-hexane extract were administered into these rats from day 1 to day 10 and were continued up to day 21. STZ was administered on day 11. The aim was to observe whether the crude *Nigella* or its n-Hexane extract pre-treatment would be able to produce beneficiary effects upon the elevated serum glucose and elevated serum lipid (TG, cholesterol) levels and upon the lowered reduced glutathione levels along with alleviation of pancreatic β cell damage in diabetic rats.

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Materials and methods

Animals: 52 adult male rats, Long Evans Norwegian strain, aged 8-12 weeks were obtained from the animal house of BSMMU and were kept in standard laboratory conditions. They were fed rat diet and given water *ad libitum* and rats were randomly divided into 6 groups.

Drug: Streptozotocin was obtained from Sigma Aldrich Cheme GmbH, Germany.

Test material: *Nigella sativa Linn*. (kalajira) was purchased from the local market.

Chemicals and reagents: Reagents for the estimation of serum glucose, serum TG and serum cholesterol were purchased from Human, Germany. Chemicals for the estimation of pancreatic reduced glutathione (GSH) were purchased from Sigma Aldrich Cheme GmbH, Germany and chemicals required for histopathology from E. Merck, Germany.

The crude and the n-hexane extract of Nigella: The seeds of *Nigella* were soaked in n-hexane for 48-72 hours. They were filtered and the filtrate was concentrated in a rotary vacuum evaporator. After evaporation a concentrated dark brown oily extract of *Nigella* was obtained. The left over seeds following filtration were obtained as the crude *Nigella.*

Experimental procedure: Normal rat diet and water *ad libitum* was allowed to control group of rats [group I(C)]. Rats of group II received single intraperitoneal injection of streptozotocin (STZ) 50mg/kg body weight in citrate buffer, pH 4.5 on day 11. Crude *Nigella* (10gm/kg body wt/day) dissolved in deionized water was administered through feeding tube to the rats of group III (Nc) for 21 days. Crude *Nigella* was administered from day 1 to day 21 while streptozotocin was administered on day 11 to rats of group IV (Nc+ STZ). The n-hexane extract of *Nigella* dissolved in deionized water was administered at the same dose as crude *Nigella* i.e. 10 gm/kg body wt. daily from day 1 to day 21 through Ryles tube to rats of group V (H). The n-hexane extract of *Nigella* was administered at 10 gm/kg body wt./day for 21 days while STZ was administered on day 11 intraperitoneally to rats of group VI (H+ STZ). All rats were sacrificed on Day 22. The rats were kept overnight fasting and only water was allowed to them and sacrifice was carried out under mild chloroform anesthesia and blood and the pancreas were collected from each rat. Serum was separated through centrifugation for biochemical analysis and the pancreatic tissue from each rat was processed separately for estimation of pancreatic GSH and histological study.

Table I - Schedule of treatment and days of sacrifice

Groups	n	Treatment	Dose	Duration	Day of sacrifice
I (C)	8	Rat diet and water	As needed	Day 1-Day21	22
II (STZ)	8	Streptozotocin	50 mg/kg body wt	Day 11 only	22
III (Nc)	8	Crude *Nigella*	10 gm/kg/day	Day 1-Day21	22
IV(Nc+STZ)	8	Crude *Nigella* + STZ	10 gm/kg/day + 50 mg/kg body wt	Nc= Day 1-21 STZ on day 11	22
V (H)	10	n-hexane extract of *Nigella*	10 gm/kg/day	Day 1-Day21	22
VI (H+STZ)	10	n-hexane extract of *Nigella*+ STZ	10 gm/kg/day + 50 mg/kg body wt	H= Day 1-21 STZ on day 11	22

The schedule of treatment and days of sacrifice has been presented on Table I. n= Number of rats in each group. C = Control, STZ = Streptozotocin, Nc = Crude *Nigella*, H = n-hexane extract of *Nigella.*

Biochemical measurements: Serum glucose was measured by oxidase-peroxidase method[5]; pancreatic GSH concentrations were estimated spectrophotometically by Ellman's method[6]. Serum TG and Cholesterol were measured by triglyceride liquicolor and Chod-pap method[7] respectively.

Histological procedure: Small portion of the dissected pancreas were fixed in 10% formalin, dehydrated in graded alcohol embedded in paraffin, cut at a thickness of 5 μm by microtome and then stained with haematoxylin and eosin[8] for light microscopic examination by an Olympus microscope.

Statistical analysis: The results obtained from the experiment are expressed as mean ± SE of the number of samples. Data were analyzed by Students unpaired t-test. $P<0.05$ were taken as significant.

Results

Biochemical observations: Administration of streptozotocin on day 11 has caused significantly elevated ($P<0.001$) serum glucose, serum TG ($P<0.01$) and serum cholesterol levels ($P<0.05$) on day 22 (Table II). The pancreatic reduced glutathione concentrations (GSH) were significantly ($P<0.001$) lowered compared to those of the control values (Table II). Treatment by crude *Nigella* alone (Nc) or by the n-hexane extract of *Nigella* (H) did not deviate the blood sugar concentrations from the control values, while serum TG and serum cholesterol concentrations were significantly ($P<0.001$) reduced; and the pancreatic reduced glutathione concentration was significantly ($P<0.001$) elevated. Blood sugar concentrations in the (Nc+ STZ) and (H+ STZ) groups were near to those of the control values. The serum TG in the (Nc+STZ) group and in the (H+STZ) group were lower ($P<0.05$ and $P<0.01$) compared to those of the STZ groups. Serum cholesterol concentration of the (Nc +STZ) and (H+STZ) groups remained at the level of the STZ group suggesting no alleviation in this parameter (Table II).

Table II - Serum glucose, pancreatic GSH and serum lipid levels

Groups	n	Serum glucose (mmol/L)	Pancreatic GSH (mg/gm of protein)	Serum TG (mg/dl)	Total Cholesterol (mg/dl)
I (C)	8	5.29±0.31	1.79±0.06	163.55±1.31	147.00±0.88
II (STZ)	8	9.88±0.39***	0.79±0.02***	211.11±2.59**	171.29±1.83*
III (Nc)	8	5.49±0.10^{NS}	3.39±0.03***	129.45±1.48**	123.22±3.99**
IV (Nc+STZ)	8	6.01±0.17***	1.07±0.02**	107.27±1.7*	164.43±1.11
V (H)	10	5.40±0.13^{NS}	3.19±0.66***	122.00±2.10**	121.21±3.2**
VI (H+STZ)	10	5.89 ±0.17***	1.69±0.03**	81.92±1.83**	166.67±1.40

n= Number of rats in each group. C = Control, STZ = Streptozotocin, Nc = Crude *Nigella*, H = n-hexane extract of *Nigella.* NS indicates no significant difference; P< 0.001= ***, P<0.01=**, P<0.05=*.

*Histological observations***:** In STZ-treated rats pancreatic sections stained with haematoxylin and eosin demonstrated degenerative and necrotic changes and shrunken islets of Langerhans. There was decrease ($P<0.001$) in the number of β cells (Fig-b). Pretreatment by crude *Nigella* and by the n-hexane extract of *Nigella*, i.e. in the (Nc+STZ) and in the (H+STZ) groups, the severity of degenerative and necrotic changes in the islet of Langerhans appeared to be less (Fig d and f). The number of the β cells were increased ($P<0.01$) and the islets appeared increased in size, compared to those of the STZ-treated group (Fig. b). Lymphoid cell infiltration in the peripheral parts of the islets of Langerhans in sections from the STZ-treated rats was apparent, which appeared to be less in the (NC+STZ) and (H+STZ) groups (Fig. d & f)

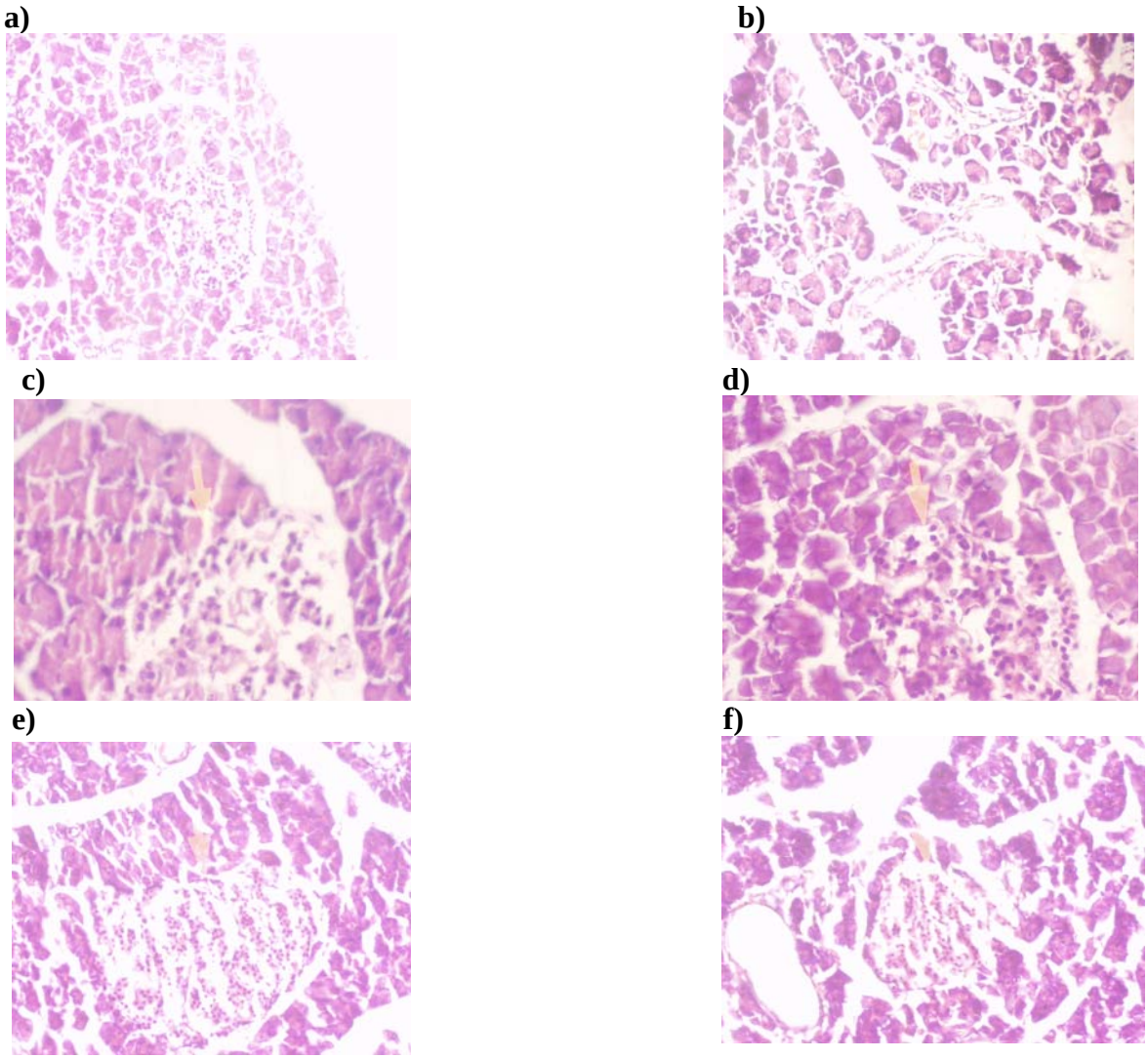

Fig. Histological appearance of pancreatic sections (×100) stained with H&E.
(a) control rat pancreas under light microscope showing an islet of Langerhans (Pale colonies of cells are surrounded by the darker exocrine tissue); (b) Sections of rat pancreas of STZ group showing reduced diameter and number of cell; (c) Sections of rat pancreas of Nc group demonstrating islets of Langerhans packed with cells; (d) Sections of rat pancreas (Nc+STZ), suggesting increased number and size of cells in the islet; (e) Sections of rat pancreas (H-group) under light microscope suggesting normal appearance of the islet of Langerhans packed with cells; (f) Sections of rat pancreas of the (H+STZ) suggesting increased size of the islet and number of cells in the islets had also increased.

Discussion

The high serum glucose concentrations of the diabetic rats appeared to lower down towards control levels by pretreatment with the crude *Nigella* and with the n-hexane extract of *Nigella* and was in agreement with those obtained by Uddin *et al.* (2002). The serum TG concentration was raised following STZ administration ($P<0.001$), however crude *Nigella* and n-hexane extract pretreatment brought them down to levels even lower compared to those in the control rats. This observation was similar to those obtained by Saha *et al.* (2004) when treatment with crude *Nigella* had lowered the serum TG concentrations of fat fed rats. The serum cholesterol concentrations of the crude and of the n-hexane extract of *Nigella* pretreated diabetic rats however, did not show alleviation and remained at levels similar to those of the STZ-group. Perhaps not enough β-cells had received protection by the pretreatment with the crude and the n-hexane extract of *Nigella*. The observations obtained from estimation of pancreatic GSH and pancreatic histology suggests partial alleviation of the pancreatic β-cells.

It can be concluded that pretreatment with *Nigella sativa Linn*. in STZ treated rats was beneficial in lowering the blood glucose concentrations, partial amelioration of the high lipids and partial elevation of the lowered pancreatic GSH concentrations of diabetic rats. Pancreatic histology has also suggested significant protection of pancreatic β cells following pretreatment of both crude and n-hexane extract of *Nigella sativa Linn.* However, the crude *Nigella* pretreated group appears to have better protection. Future studies may be designed to characterize the ameliorating factors from the crude extract of *Nigella* and to determine the dose and duration of treatment by the ameliorating factor.

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Reference

1. Ara N. Antimicrobial activity of the volatile oil of *Nigella sativa Linn*. Seeds [M.Phil thesis]. Dhaka: Bangabandhu Sheikh Mujib Medical University. 1999.

2. Uddin N, Dewan ZF, Zaman M, Saha RR, Sultana M. Effects of *Nigella sativa Linn.* (kalajira) on serum glucose concentration in streptozotocin-induced diabetic rats. *Bangladesh J Physol Pharmacol* 2002; **18** (1):6-9.

3. Begum NA, Dewan ZF, Nahar N, Mamun MR. Effect of n-Hexane extract of *Nigella sativa* on gentamicin-induced nephrotoxicity in rats. *Bangladesh J Pharmacol* 2006; **1** (1):16-20.

4. Saha RR, Dewan ZF, Uddin N. Effects of *nigella sativa* (kalajira) on serum lipid profile of hyperlipidemic rats. *Bangladesh J Physol Pharmacol* 2004; **20** (1):36-38.

5. Trinder P. Determination of the blood glucose using an oxidase peroxidase system with a non carcinogenic chromogen. *J Clin Path* 1969; **22** (2):158-61.

6. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82** (1):70-77.

7. Richmond W. Preparation and properties of acholesterol oxidase from *Nocardia* sp. And its application to the enzymatic assay of total cholesterol in serum. *Clin Chem* 1973; **19** (12):1350.

8. Steward HK. Manual of histologic and special staining technics. 2nd ed. New York, Mc Graw-Hill Book Company, Inc., 1960.

Narrated by Abu Hurairah (R) The Prophet (S) said: *"Use the Black Seed because, it contains the cure for every type of ailment, except for death."* [At-Tirmidhi, Ahmad and Ibn Hibban]