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Effects of the crude and the *n*–hexane extract of *Nigella sativa* Linn. (kalajira) upon diabetic rats

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Abstract

The study was carried out to investigate into the effect of concomitant administration of the crude and the *n*-hexane extract of *Nigella sativa* (kalajira) upon streptozotocin-induced diabetic adult male rats. Diabetes was induced by a single intraperitoneal injection of streptozotocin on day 1 while crude nigella powder in deionized water and the *n*-hexane extract of nigella were administered orally concomitantly from day 1 to 21. Rats were sacrificed on day 22. The serum glucose and cholesterol concentrations that were elevated in diabetic rats were normalized or near normalized by the crude nigella or the *n*-hexane extract administration; while the elevated serum triglyceride concentrations of the diabetic rats were brought down to lower than control values. The pancreatic GSH was closer to control value, and pancreatic histology suggested reappearance of β cells. The crude nigella concomitant to streptozotocin (STZ+Nc) administration appeared to provide better alleviation compared to the *n*-hexane extract of nigella concomitant to streptozotocin

Introduction

The increasing prevalence of diabetes mellitus in the present day world is a cause of concern to the mankind. Diabetes mellitus, whether of type I or II category, is primarily characterized by either lack of insulin or its action (due to any cause whatsoever) which starts with derangement of carbohydrate metabolism to eventually entangle derangement of protein and lipid metabolism as well. However, the preliminary diagnosis of diabetes mellitus is carried out by estimation of the blood sugar concentrations which in the normal individual rarely exceeds 7 mmol/L while in the fasting diabetic person, this is >7.8 mmol/L and is more than 10 mmol/L 2 hours after a meal in the diabetic.

A whole range of metabolic disorders (of carbohydrate, protein and lipid) create a disastrous condition into the person suffering from diabetes mellitus. The disease is not curable, and treatment is a must for the survival of the person. The present day available treatment starts with controlling or restriction of the diet along with exercise, insulin replacement in type I and oral hypoglycemic drugs ultimately being replaced by insulin in type II diabetes mellitus. Both oral hypoglycemic drugs and insulin have their drawbacks which have lead to the quest for new drugs which would be cheap, effective and would produce lesser adverse effects. Drugs of herbal origin may fulfill many of these criteria although doubt about their innocuous nature still persists and is subject for research. South Asia and Bangladesh harvests a reach source of herbs, shrubs and trees of medicinal value. *Nigella sativa* Linn. (kalajira) is one such herbal product which has been in use as a spice from ancient times. Its medicinal value to treat various ailments is also well-known. Until now its antihypertensive (Rashid et al., 1987), antibacterial (Ara, 1999), antidiabetic (Uddin et al., 2002; Uddin et al., 2005), lipid lowering (Shaha et al., 2004) and renoprotective (Begum et al., 2006) potentialities have been obtained through research.

Table I

Schedule of treatment

Group	Treatment, dose and duration		Day of sacrifice
	Day 1	Day 1-21	
I = (C)		Rat diet and water	22
II = (STZ)	Strepto-zotocin	Rat diet and water	22
III = (Nc)		Crude nigella pow-der along with rat diet and water	22
IV = (STZ+Nc)	Strepto-zotocin	Crude nigella pow-der along with rat diet and water	22
V = (H)		*n*-hexane extracts of nigella with rat diet and water	22
VI = (STZ+H)	Strepto-zotocin	*n*-hexane extracts of nigella with rat diet and water	22

Number of rats in each group is six; dose of streptozotocin was 50 mg/kg body weight; dose of crude negella powder or hexane extract was 10 g/kg/day

We were interested in observing the antidiabetic effect of crude nigella sativa and its *n*-hexane extract. An expectation to make an overall comparison of the antidiabetic effect of the crude nigella with that of the *n*-hexane extract of nigella has led the present researchers to initiate this study. The present study has taken into account estimation of lipid parameters and the anti-oxidant status (GSH) of the pancreas of the treated groups.

Materials and Methods

Animals

A total number of 60 adult male rats of the Long Evans strain (weighting about 200-250 g) were taken, divided into 6 groups, each group containing 10 rats. The rats were housed in the animal house and were kept in standard sized metallic cages (3 rats/cage) in an well ventilated room, and at a temperature of about 26-28°C. They were fed normal rat diet and water.

Group I (C = control group) was allowed normal rat diet and water *ad libitum*. Diabetes was induced in rats of Group II (STZ) by a single i.p. injection of streptozotocin (50 mg/kg, freshly dissolved in citrate buffer, pH 4.5) on day 1. Rats of Group III (Nc) were given crude nigella powder (10 g/kg body weight/day) orally mixed in deionised water through ryles tube from day 1 to day 21. Rats of Group IV (STZ + Nc) were administered crude nigella for 21 days concomitant with streptozotocin i.p. (50 mg/kg body wt) (single injection). Rats of Group V (H) were orally administered the *n*-hexane extract of nigella (10 g/kg body wt) for 21 days and rats of group VI (STZ+H) were administered the *n*-hexane extract of nigella (orally at a dose of 10 g/kg body wt/day for 21 days) concomitant with streptozotocin treatment (50 mg i.p. single dose on the first day). Rats of all groups were sacrificed on day 22.

Biochemical procedure

Rats were fasted for 18 hours before sacrifice. They were given only water *ad libitum* during the fasting period, and were sacrificed under light chloroform anesthesia. Blood was collected in test tubes and serum was separated after centrifugation. Serum glucose concentration was estimated by using an oxidase and peroxidase method (Trinder, 1969); pancreatic reduced glutathione (GSH) concentrations was estimated by Ellman's method (Ellman GL, 1959); serum triglyceride was measured by triglycerides liquicolor and cholesterol level was determined by Chod–pap method.

Histological procedure

Pancreas was dissected out, cut into small pieces and were fixed in 10% formalin. They were embedded in paraffin and were cut into 5 µm thickness in microtome. These sections were collected in slides and then stained with Hematoxylin and Eosin (Steward, 1960). The stained sections were examined under low and high power by using an Olympus microscope.

Statistical analysis

The data were expressed as mean ± SE. Students unpaired t-test was used to compare between the control and the treatment groups. $p<0.001$ was acknowledged as levels of significant difference between the groups.

Results

Significantly ($p<0.001$) high levels of serum glucose, cholesterol and triglyceride were produced as a result of streptozotocin administration in diabetic rats. The pancreatic GSH concentrations were significantly ($p<0.001$) reduced in diabetic rats compared to those of the control values (Table II). The blood sugar concentrations of Group I, III, IV, V and VI remained almost at similar levels. Treatment by crude (Group III) and *n*-hexane extract of nigella (Group V) did not change the blood sugar concentrations from those of the control values, while serum triglyceride and cholesterol concentrations were significantly ($p<0.001$) lowered by crude nigella (Group III) and *n*-hexane extract of nigella treatment (Group V) (Table II). The pancreatic reduced glutathione concentration were significantly ($p<0.001$) elevated by both crude nigella (Group III) and *n*-hexane extract of nigella treatment (Group V) (Table II), and

Table II

Biochemical observations

Groups	Number of rats	Serum glucose (mg/dL)	Pancreatic GSH (mg/g of protein)	Serum triglyceride (mg/dL)	Serum cholesterol (mg/dL)
I = (C)	10	5.3 ± 0.5	1.9 ± 0.01	160.3 ± 1.5	143.0 ± 0.9
II = (STZ)	10	9.7 ± 0.5[a]	0.9 ± 0.01[a]	208.2 ± 2.6[a]	169.3 ± 1.7[a]
III = (Nc)	10	5.3 ± 0.1[NS]	3.2± 0.1[a]	121.4 ± 1.7[a]	119.3 ± 4.9[a]
IV = (STZ+Nc)	10	5.9 ± 0.02[a]	1.2 ± 0.04[b]	97.9 ± 1.7[a]	139.7 ± 0.8[b]
V = (H)	10	5.6 ± 0.1 [NS]	3.3 ± 0.1[a]	118.0 ± 2.1[a]	114.3 ± 3.4[a]
VI = (STZ+H)	10	5.1 ± 0.02[a]	1.1 ± 0.03[b]	78.4 ± 2.9[a]	142.7 ± 1.7[b]

Group I (C) = Control; Group II (STZ) streptozotocin induced diabetic rat group (50 mg/kg body wt. administered i.p.); Group III (Nc)– crude nigella administration at 10 g/kg/day for 21 days; Group IV (STZ + Nc) - streptozotocin (50 mg/kg) single i.p. injection on day 1 and crude nigella powder administration at 10 g/kg/day for 21 days; Group V (H) – *n*-hexane extract of nigella orally at 10 g/kg/day for 21 days; Group VI (STZ + H) - streptozotocin (50 mg/kg) single i.p. injection on day 1 and n-hexane extract of nigella orally at 10 g/kg/day for 21 days; NS indicates no significant difference; [a]p<0.001; [b]p<0.01

were at much higher levels compared to those of the control group. The blood sugar concentrations in the STZ + Nc (Group IV) and STZ+H (Group VI) groups did not vary from those of the control values (Table II). The pancreatic GSH concentration of the STZ + Nc (Group IV) and STZ + H (Group VI) groups were significantly (p<0.001) increased compared to those of the streptozotocin-treated groups although did not attain the control values and remained at much lower concentrations compared to those of the Nc and H treated groups (Table II). The serum cholesterol and triglyceride concentrations of STZ + Nc (Group IV) and STZ + H (Group VI) groups had a similar pattern, serum triglyceride being lower (p<0.001) than control while serum cholesterol concentrations demonstrated values similar to those of the control group (Table II).

Histologically sections from streptozotocin-treated group (Group II) demonstrated shrunken islets of Langerhans within which there appeared degenerations and necrotic cells. β cell numbers appeared decreased (p<0.001), and 72% of β cells appeared damaged. In the Nc (Group III) and H (Group V) groups, no damaged cells apparently appeared. The STZ + Nc (Group IV) and STZ + H (Group VI) groups demonstrated lesser (p<0.001) number of β cells compared to sections from those of the control group (Group I). The islets of Langerhans appeared less shrunken compared to those from the streptozotocin-treated group. The percentage of damaged β cells in the STZ + Nc (Group IV) group was 24.9 and in the STZ + H (Group VI) group were 46.4.

Discussion

Treatment with crude nigella for 21 days in adult male rats did not change the blood sugar concentrations from those of the control value while serum cholesterol and triglyceride concentrations were significantly (p<0.001) reduced. The ameliorating effects of nigella are better exerted in presence of damage and necrosis (Begum et al., 2006), probably due to this reason the blood sugar concentrations of rats of Group III were not changed. The pancreatic GSH concentrations were about 2-fold elevated, and histology suggested increased number of β cells in the islets of Langerhans. Nigella administration has endowed with either stimulating the enzymes which enhance GSH synthesis or inhibiting the enzymes which destroy or metabolize GSH (Khan et al., 2003). Probably it was this property of nigella due to which the GSH concentrations were increased and apparently more β cells appeared in histology.

Treatment with only the *n*-hexane extract of nigella (Group V) has produced observations quite similar to those produced by crude nigella administration.

Concurrent administration of crude nigella along with streptozotocin (Group IV) in adult male rats and concomitant administration of the *n*-hexane extract of nigella along with streptozotocin (Group VI) in adult male rats has demonstrated that the blood sugar concentrations of these two groups remained closer to control values while serum triglyceride concentrations were significantly (p<0.001) lowered compared to those in the control and serum cholesterol concentration did not differ from those of the control group. The pancreatic GSH concentrations of these two groups appeared recovering from the streptozotocin-induced damage yet could not attain the control levels. Histology of the pancreas suggested that about 24.9% and about 46.4% β cells were damaged following STZ administration in Group IV (STZ + Nc) and in Group VI (STZ + H) respectively. This observation in the STZ + H group was contrary to our expectations that the *n*-hexane extract would provide better amelioration

Table III

After RAA treatment

Groups	Average number of β cell in each islet	Number of intact β cell	Number of damaged β cell	%damage β cell
I = (C)	720	720	-	
II = (STZ)	196	124	72	72.8
III = (Nc)	531	531	-	-
IV = (STZ + Nc)	541	456	85	24.9
III = (H)	666	666	-	-
IV (STZ + H)	386	355	31	46.4

compared to those of crude nigella concurrent administration (Group IV). This observation suggests that probably crude nigella contain better component than the *n*-hexane extract for the alleviation of the β cell damage or for the replenishment of pancreatic GSH, and differs from that reported by Begum et al., 2006; that the *n*-hexane extract significantly ameliorated signs of gentamicin–induced nephrotoxicity.

It may be expected that higher doses of crude nigella (or the alleviating component contained within it) would produce better recovery from the streptozotocin-insult of the pancreatic β cells of adult male rats. It is concluded that both the crude nigella and the *n*-hexane extract of *N. sativa* were alleviating to streptozotocin-induced diabetes mellitus and its consequences in adult male rats upon concurrent administration and that crude nigella appeared more effective in this regard.

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