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Original Article

Comparison of Biochemical Parameters of Pre and Concomitant Treatment with Crude *Nigella sativa Linn.* (Kalajira) upon Diabetic Rats

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Abstract:
The study was carried out to compare the biochemical parameters of effect of pre and concomitant administration of the crude Nigella sativa (kalajira) (10 mg/kg body wt./day for 21 days) upon streptozotocin-induced diabetic adult male rats. Diabetes was induced by a single intraperitoneal injection of streptozotocin on day one. At first pretreatment by crude nigella powder in deionized water were administered before inducing diabetes then 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 both pre and concomitant treatment by crude nigella administration; while the elevated serum triglyceride concentrations of the diabetic rats were brought down to lower than control values. The pancreatic GSH (reduced glutathione) was closer to control value. The crude nigella concomitant to streptozotocin (STZ+Nc) administration appeared to provide better alleviation compared to the pretreatment.

Key words: Diabetes, Nigella sativa, Streptozotocin

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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 sativ*a (*N. sativa*) seeds, an annual Ranunculaceae herbaceous plant, have been used in folk medicine to treat diabetes[2]. The glucose lowering and antidiabetic effects of *N. sativa* has been reported in different diabetic animal models[3,4].

Antioxidant and anti-inflammatory actions of *N. sativa* are already acknowledged[5,6,7]. Salutary characteristics of *N. sativa* in the management of hyperglycemia, hyperlipidemia and bronchial asthma were cited previously [8,9,10,11]. *N. sativa* also possess chemo-preventive and insulin-tropic effects[12,13,14,15,16].

In view of the established antidiabetic potentials, present study was performed to investigate antidiabetic efficacy of *N. sativa*.

Materials and Methods:
Test material: *N. sativa* seeds were collected from local retail market. After washing and drying, seeds were ground into a fine powder state. Incubated at 45-50˚C temperature for 24-48 hours. Finally powdered by a blender to get a fine dried powder.

Animals: 50 adult male rats, Long evans Norwegian strain, aged 8-12 weeks were obtained from the animal house of Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka and were kept in standard laboratory conditions. They were fed rat diet and given water *ad libitum* and rats were randomly divided into 5 groups.

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

Chemicals and reagents: Reagents for the estimation of serum glucose, serum Triglyceride (TG) and serum cholesterol were purchased from Human, Germany. Chemicals for the estimation of

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pancreatic reduced glutathione (GSH) were purchased from Sigma Aldrich Cheme GmbH.

Experimental procedure: Group I (Control group) rats were fed with normal rat diet and water ad libitum. Rats of group II (STZ) received single intraperitoneal injection of streptozotocin (50 mg/kg, freshly dissolved in citrate buffer, pH 4.5) on day 1- day 21. Rats of Group III (Nc) were given crude *N. sativa* seeds powder (10 mg/kg/day) orally mixed in deionized water through ryles tube from day 1-day 21 while streptozotocin was administered on day 11 to rats of group IV (Nc+STZ) and group V (STZ+Nc) were administered crude nigella for twenty one days concomitant with streptozotocin intraperitoneal (50 mg/kg) (single dose).

All rats were sacrificed on Day 22. The rats were kept overnight fasting and only water was allowed to them and sacrificed under light chloroform anesthesia. Blood was collected in test tubes and serum was separated after centrifugation.

Table-I: Plan of Treatment (n=50)

Groups	Treatment			Dose
	Day 1	Day 11	Day 1-21	
I (Control) [n=10]			Rat diet + water	As needed
II (STZ) [n=10]	STZ		Rat diet + water	50 mg /kg
III (Nc) [n=10]			Crude *N. sativa* powder + rat diet + water	10 mg /kg
IV (Nc+STZ) [n=10]		STZ	Crude *N. sativa* powder + rat diet + water	10 mg /kg and 50 mg /kg
V (STZ+Nc) [n=10]	STZ		Crude *N. sativa* powder + rat diet + water	50 mg /kg and 10 mg /kg
All the rats were sacrificed at Day 22				

STZ = Streptozotocin; Nc = Crude *N. sativa* powder

Biochemical measurement: Serum glucose was estimated by oxidase peroxidase method[17]; pancreatic reduced glutathione (GSH) concentrations were estimated spectrophotometrically by Ellman's method[18], serum TG and Cholesterol were measured by triglyceride liquicolor and CHOD-PAP method[19] method respectively.

Statistical analysis: The results obtained from the experiment are expressed as mean ± SE of the number of samples. Student's unpaired t-test was used to compare between the pre and concomitantly treatment groups. $P<0.05$ were taken as significant.

Results:
In streptozotocin treated group (Group II) significantly elevated ($p<0.001$) serum glucose, serum cholesterol ($p<0.05$), serum TG ($p<0.01$) on day 22 were found. 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) showed no change in blood sugar concentration from the control values but serum TG and serum cholesterol were significantly ($p<0.001$) reduced; the pancreatic reduced glutathione concentration was significantly ($p<0.001$) elevated.

Table-II: Results of experiment (n=50)

Groups	Serum glucose (mg/dl)	Pancreatic GSH (mg/gm of protein)	Serum TG (mg/dl)	Total cholesterol (mg/dl)
I (Control) [n=10]	5.32 ± 0.45	1.85 ± 0.01	160.29 ± 1.45	143.03 ± 0.91
II (STZ) [n=10]	9.70 ± 0.47*	0.88 ± 0.01*	208.21 ± 2.62*	169.33 ± 1.70*
III (Nc) [n=10]	5.30 ± 0.09^{NS}	3.21 ± 0.06*	121.35 ± 1.68*	119.26 ± 4.93*
IV (Nc+STZ) [n=10]	6.01 ± 0.17*	1.07 ± 0.02*	107.27 ± 1.7*	164.43 ± 1.11
V (STZ+Nc) [n=10]	5.89 ± 0.02*	1.17 ± 0.04*	97.88 ± 1.65*	139.67 ± 0.77*

* = Significant Result; NS = Not Significant Result

Blood sugar and serum TG concentrations in the (Nc+STZ) and (STZ+Nc) groups were near to those of the control values. But (STZ+Nc) group showed more reduction in these parameters than the (Nc+STZ) group.

Serum cholesterol concentrations of the (Nc+STZ) treated group remained nearly at the level of the STZ group suggesting that no alleviation in this parameter whereas the (STZ+Nc) treated group demonstrated that the values similar to those of the control group (Table II).

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. In recent years, the oxidative stress-induced free radicals have been implicated in the pathology of insulin dependent diabetes mellitus[17,18].

The serum glucose concentration of the pre and concomitantly administered crude *N. sativa* treated diabetic rats were significantly lower than that of the STZ treated diabetic rats (Group II). The mechanism of the hypoglycemic effect of *N. sativa* has been suggested previously to be due to pancreatic actions via enhancing insulin secretion and inducing β-cell proliferation and regeneration[19,21]. Another group of researchers have proposed extra-pancreatic actions as a mechanism of *N. sativa* hypoglycemic effect through enhancing tissue sensitization to insulin, especially liver and muscles[20,22].

The Pancreatic glutathione concentrations were found significantly increased in both Group IV and V compared to STZ treated diabetic rats. *N. sativa* supplementation to patients with DM resulted in improved antioxidant status and reduction in oxidative stress, evident by significant increase in TAC and CAT activity associated with significant reduction in lipid peroxidation markers[22].

The serum TG and cholesterol concentrations were significantly lowered in comparison to the STZ treated diabetic rat group (Group I).

Among the pre and concomitantly crude *N. sativa* administered groups, the concomitantly crude *N. sativa* administered group was found comparatively more effective than pre-administration though statistically not significant but the changes in the parameters in percentages were more found in concomitantly treated groups.

Conclusion:

This study reveals that crude *N. Sativa* seeds have role in the management of Diabetes Mellitus as natural remedy. Further study is suggested in large number of subjects to confirm the beneficial effect of *N. Sativa* in Diabetes mellitus as well as in heperlipidaemia.

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