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Biochemical effects of *Nigella sativa* L seeds in diabetic rats

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Oral administration of ethanol extract of *N. sativa* seeds (300 mg/kg body weight/day) to streptozotocin induced diabetic rats for 30 days significantly reduced the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation products (TBARS and hydroperoxides) and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase in liver and kidney. The results confirm the antidiabetic activity of *N. sativa* seeds extract and suggest that because of its antioxidant effects its administration may be useful in controlling the diabetic complications in experimental diabetic rats.

Keywords: Antioxidants, Diabetes mellitus, Free radicals, *Nigella sativa*, Oxidative stress

Diabetes mellitus (DM) affects a large number of people throughout the world and more so in India. Clinically, the disease is associated with a number of chronic complications including nephropathy, neuropathy, retinopathy and cardiovascular diseases[1]. In view of undesirable side effects of synthetic drugs[2], WHO has recommended evaluation of plants effective in different diseases. Many Indian medicinal plants have been found to be useful in successfully managing diabetes and from some of them active principles have been isolated[3]. Thus, it will be useful to look for new and if possible more efficacious drugs and the vast reserves of phytotherapy may be an ideal target. The beneficial effects of some synthetic and plant drugs have been shown to be due to their antioxidant properties. Plants often contain substantial amounts of antioxidants, flavonoids and tannins[4]. Implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free-radical generation but also due to non-enzymatic protein glycosylation[5] and alteration in antioxidant enzymes[6].

Among medicinal plants, *Nigella sativa* L (Ranunculaceae; called as Kalongi, in Hindi) seeds have bronchodilator[7], antibacterial[8], diuretic, hypotensive[9] and immunopotentiating properties[10]. El-Tahir *et al.*[11] have reported that *N. sativa* seeds contain several active constituents such as thymoquinone, many monoterpenes such as p-cymene and α-pinene. Kanter *et al.*[12] have reported antidiabetic activity of *N. sativa* on streptozotocin induced diabetic rats. The present study has been aimed to investigate the effect of ethanolic extract of *N. sativa* on lipid peroxides and enzymic antioxidants in liver and kidney tissues of streptozotocin (STZ) induced diabetic rats.

Materials and Methods

Chemicals—Bovine serum albumin (BSA), thiobarbituric acid (TBA) and streptozotocin were procured from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals used were of analytical grade and purchased locally.

Plant material—The *N. sativa* seeds were collected from The Survey of Medicinal Plant Unit, Regional Research Institute of Unani Medicine, Aligarh (U.P) India. Identification of the samples was further confirmed with the Department of Botany, Aligrah Muslim University (AMU), Aligarh and a voucher specimen (JK 021) was deposited in the department herbarium.

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. It was then filtered and the residue was extracted with 95% ethanol by soxhlet extraction. Ethanol was

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evaporated in a rotary evaporator at 40°-50°C under reduced pressure. The yield of extract was 15 g and the substance was dissolved in water before use.

Animals—Male albino Wistar rats (32), weighing about 150-180 g, obtained from Central Animal House, J.N. Medical College, AMU, Aligarh were used. The animals were maintained on standard rat feed supplied by Amruth, Pune. The experiments were conducted according to the ethical norms approved by Institutional Animal Ethics Committee (IAEC) Guidelines.

Experimental induction of diabetes—Diabetes was induced using streptozotocin (55 mg/kg body weight single *i.p.* injection) in 0.1 *M* citrate buffer *p*H 4.5(ref.13).

Experimental design—The rats were divided into following four groups of eight each:

Group 1: Healthy control rats receiving 0.1 *M* citrate buffer (*p*H 4.5).

Group 2: Diabetic controls.

Group 3: Diabetic rats treated with protamine-zinc insulin ip injection (6 units/kg/day) for 30 days.

Group 4: Diabetic rats given ethanol extract of *N. sativa* (300 mg/kg/day) in aqueous solution orally once daily for 30 days.

Rats in all the groups were provided with food and water *ad libitum*. The blood glucose and body weight were checked weekly. At the end of the experimental period, the rats were anaesthetized and sacrificed. Blood sample was collected in tubes containing potassium oxalate and sodium fluoride. The plasma was stored at -4°C until analysis was completed. The liver and kidney tissues were excised, rinsed in ice-cold saline, cut into small pieces and homogenized with Potter-Elvehjem glass-Teflon homogenizer in Tris-HCL buffer (*p*H 7.4). The homogenate was centrifuged at 10000 rpm for 10 min. Supernatant was used for various measurements. The following analyses were carried out: blood glucose, plasma total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were estimated using standard kits of Ranbaxy Laboratories, New Delhi. Plasma insulin level was assayed by enzyme linked immunosorbent assay (ELISA) kit (Boerhringer Mannheim, Germany). Low density lipoprotein (LDL-C)[14], thiobarbituric acid reactive substances (lipid peroxides)[15] and hydroperoxides[16], reduced glutathione (GSH)[17], superoxide dismutase (SOD)[18], glutathione peroxidase (GPx)[19], catalase (CAT)[20] and protein[21], were also estimated. All spectrophotometric measurements were carried out in a Camspec UV-Visible (Camspec M330B, UK) spectrophotometer.

Statistical analysis—All the data were statistically evaluated with SPSS/7.5 Software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant differences test. *P* values <0.05 were considered statistically significant.

Results

Body and organ weight—A significant ($P< 0.05$) decrease in body (27.3%) and liver weights (9.5%) and increase in kidney weight (25%) was observed in diabetic rats as compared to control rats. Administration of *N. sativa* extract to diabetic rats increased body (33.5%) and liver (41.3%) weight and decrease kidney (15.7%) weight to near normal level. In the group 3 animals treated with insulin, body and kidney weights returned to normal values, but liver weight returned to only 18.4% of normal value.

Blood parameters—The levels of blood glucose and plasma lipid profile (TC, LDL-C and TG) were significantly higher ($P< 0.05$) in diabetic rats as compared to control rats. Oral administration of *N. sativa* extract to diabetic rats, brought down blood glucose to near normal and showed significant decrease ($P< 0.05$) in the level of plasma lipid profile and an increase in plasma insulin level as compared to untreated diabetic rats (Table 1). The effect was however slightly less than that in animals treated with insulin.

Lipid peroxidation products—There was a significant elevation in the level of TBARS and hydroperoxides in both liver and kidney tissues of untreated diabetic rats. Administration of *N. sativa*

Table 1—Effect of treatment of diabetic rats with *N. sativa* seeds extract for 30 days on their blood glucose, plasma lipid profile and insulin levels

[Values are mean ± SD from 8 animals in each group]

Parameter	Control	Diabetic	Diabetic + Insulin	Diabetic+*N. sativa*
TC*	120.6±11.3	283.5±12.5	157.2±10.1[a]	171.2±6.0[a]
LDL-C*	62.4±5.54	186.18±7.1	71.4±8.8	85.0±11.1[a]
HDL-C*	40.6±4.1	59.8±3.4	68.1±5.4[a]	63.5±5.8[a]
LDL/HDL	1.53±0.12	3.11±0.34	1.04±0.45	1.33±0.22
TG*	89.3±6.7	185.1±12.1	102.4±10.7[a]	113.8±10.1[a]
Blood Glucose*	95.0±8.0	283±15.2	98±7.0[a]	102±7.3[a]
Plasma Insulin†	15.13±0.71	4.22±0.26	10.21±0.47	11.37±0.72[a]

[a]$P< 0.05$ when compared with diabetic rats

Units: *mg/100ml serum; †μUnits/ml serum

extract to diabetic rats significantly decreased ($P< 0.05$) the level of TBARS and hydroperoxides (Table 2). The effect of *N. sativa* extract was slightly more than that with insulin except in kidney in which the effect of both was equal.

Antioxidants—Significant decreases was observed in the activities of SOD, CAT, GPx and GSH in liver and kidney tissues of untreated diabetic rats. Administration of *N. sativa* seeds *extract* and insulin considerably improved the activities of these enzymes which were however not normal in treated diabetic rats (Table 2).

Discussion

Treatment of diabetic rats with *N. sativa* seeds extract showed improvement in many parameters. Their body, liver and kidney weights and blood glucose were near normal, indicating possible prevention of muscle wasting known to occur in diabetes. The studies confirm the earlier findings that *N. sativa* seeds extract has antidiabetic effect[12]. 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[22]. Lowering of serum lipid levels through diet or by drug therapy seems to be associated with a decrease in the risk of vascular disease[23]. 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[24]. In diabetes oxygen free radicals (OFRs) are generated by stimulating H_2O_2 *in vitro* and *in vivo* in pancreatic β-cells. OFRs scavenging enzymes normally respond to conditions of oxidative stress with a compensatory mechanism that increases the antioxidative enzyme activity in diabetic rats initially but reduced in chronic uncontrolled diabetes[25,26].

The results of the present study indicate significantly increased lipid peroxidation of rats exposed to STZ and its statistically significant attenuation by *N. sativa* seeds extract treatment. This suggests protective role of *N. sativa* seeds extract which could be due to the antioxidative effect of flavonoids present in the seeds which act as strong superoxide radicals and singlet oxygen quenchers[27]. The decreased activities of SOD, GPx and CAT in both liver and kidney during diabetes may be due to production of reactive oxygen free radicals. Treatment with *N. sativa* seeds extract increased the activity of these enzymes and thus may help to counteract the damage by the free radicals generated during diabetes. Reduced glutathione is a potent free radicals scavenger. Treatment of *N. sativa* seeds extract resulted in the elevation of the GSH levels, which is present in the islet β-cells and protects the membranes against oxidative damage by regulating the redox status of protein in the membrane[28].

The present results suggest that it is worthwhile carrying out further studies to find out whether *N. sativa* seeds extract could be a supplement, as an antioxidant therapy and may be beneficial for correcting the hyperglycemia and preventing diabetic complications due to lipid peroxidation and free radical oxidation. Longer duration studies of *N. sativa* seeds and its isolated active compounds on chronic models are necessary to develop a potent antidiabetic drug from *N. sativa* seeds.

Table 2—Effect of treatment of diabetic rats with *N. sativa* seeds extract for 30 days on superoxide dismutase, catalase, glutathione peroxide, reduced glutathione, TBARS and hydroperoxides in liver and kidney

[Values are mean ± SD from 8 animals in each group]

Groups	Control	Diabetic	Diabetic+Insulin	Diabetic+ *N. sativa*
Liver				
SOD[a]	20.16±1.60	14.26±1.30	18.72±2.00*	18.34±3.06*
CAT[b]	0.223±0.030	0.137±0.019	0.181±0.026*	0.210±0.017*
GPx[a]	0.187±0.020	0.131±0.014	0.168±0.038*	0.170±0.026*
GSH[c]	55.6±3.00	30.3±2.34	54.4±3.20*	52.7±2.30*
TBARS[d]	0.87±0.05	1.69±0.31	1.01±0.21*	0.93±0.06*
Hydro-peroxides[e]	62.5±2.5	88.3±4.1	67.1±3.5*	63.1±1.7*
Kidney				
SOD[a]	12.27±1.48	8.24±0.83	13.15±1.02*	12.18±2.25*
CAT[b]	0.116±0.014	0.072±0.009	0.104±0.018*	0.110±0.012*
GPx[a]	0.050±0.009	0.036±0.005	0.048±0.008*	0.046±0.004*
GSH[c]	31.4±1.50	19.2±1.16	26.5±1.95*	30.1±2.00*
TBARS[d]	1.25±0.16	2.08±0.22	1.66±0.15*	1.47±0.14*
Hydro-peroxides[e]	48.4±3.4	71.8±2.1	59.5±0.9*	60.2±1.4*

*$P< 0.05$ when compared with diabetic rats

Units: [a]U/mg protein, [b]U/mg protein×10^3, [c]mg/100g of tissue, [d]m*M* TBARS/100 g of wet tissue, [e]mM Hydroperoxides/100 g of wet tissue

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