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Effects of *Nigella sativa* Seed Extract on Insulin Resistant Non-insulin-Dependent Diabetic Guinea Pigs

Shamshun Nehar*, Hena Kauser, Prabha Rani and Imtiyaz Alam

Department of Zoology, Ranchi University, Ranchi-834008, India

***Corresponding author e-mail:** snehar1805@yahoo.co.in

ABSTRACT

In the present investigation, effects of ethanolic extract of *Nigella sativa* seed extract on insulin resistant non insulin dependent diabetic (NIDDM) guinea pigs were studied. Significant ($p<0.05$) decrease in blood glucose level and glycosylated haemoglobin were observed in animals treated with ethanolic extract of *N. sativa*. In addition, improvement in glucose tolerance, insulin level and insulin sensitivity was observed in treated diabetic animals. The results were more significant in animals treated with higher dose of *N. sativa* seed extract (500mg/kg BW) as compared to lower dose of extract (250 mg/kg BW). Protective effect of *N. sativa* seed extract was comparable to the standard drug (i.e., glimepride). Therefore it is concluded that *N. sativa* seed extract might prove beneficial in preventing hyperinsulinemia, impaired glucose tolerance, insulin resistance and eventually an effective means of improving hyperglycemia, without any toxic effect at the doses studied in the present study.

Keywords- *Nigella sativa,* Insulin resistance, Non-insulin dependent diabetes mellitus, STZ Guinea pigs.

INTRODUCTION

Type 2 diabetes mellitus (DM) is possibly the world's fastest growing metabolic disorder which is characterized by hyperglycemia due to impaired insulin secretion with or without insulin resistance[1]. Diabetes mellitus is a heterogeneous group of disorders in which there is a specific genetic pattern, which includes environmental factors and pathophysio-logical mechanisms, leading to impairment of glucose tolerance[2]. Long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels are associated with chronic diabetic hyperglycemia. The resistance to insulin in diabetic patient is a result of autoimmune destruction of β-cells of the pancreas with consequent insulin deficiency to abnormalities[3]. Symptoms of marked hyperglycemia include polyurea, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment

of growth and susceptibility to certain infections may also accompany chronic hyperglycemia[4].

In recent years, plant derived medicines have received great deal of attention compared to synthetic ones for the cure and prophylaxis of various diseases. Management of type 2 DM without any adverse effect is still a challenge to researchers. A large number of herbal medicines are known which is found to possess antihyperglycemic effect and they are used because of minimal side effects and low cost of preparation. *Nigella sativa* is one of such plants used as an alternative medicine for diabetes. *N. sativa* has been used for many years for its diuretic, anti-hypertensive[5], anti- diabetic[6], anticancer and immunodulatory, analgesic antimicrobial[7], anti-helminths and anti-inflammatory[8], spasmolytic, bronchiodilator[9], gastro-protective[10], hepato-protective[11-13], renal protective and antioxidant properties. The seeds of *N. sativa* are widely used in the treatment of various diseases like bronchitis, asthama, diarrhea, rheumatism and skin disorders. Additionally, used as liver tonic, digestive, anti-diarrhoeal, appetite stimulant, and to support immune system[14]. Nearly 32 compounds have been identified, of which thymoquinone, thymohydro-quinone, dithymoquinone, p-cymene, arvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene and á-pinene are some of the predominant compounds. Other derivatives found in trace amounts include carvone, limonene, citronellol. Many of these compounds are capable of inducing pharmacological effects in humans. Properties of whole seeds or their extracts are mainly attributed to quinine constituents, of which thymoquinone (TQ) is the most abundant as well as the potent pharmacologically active compound[15]. *N. sativa* oil as well as TQ have been shown to prevent oxidative injuries. It is reported that *N. sativa* and its derivative TQ inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation[16].

Keeping in view, the severity of disease and potential of *N. sativa*, it was thought valuable to explore the antidiabetic properties of ethanolic extract of *N. sativa* seed by investigating the effect of *N. sativa* seed extract on hyperinsulinemia, glucose intolerance and insulin sensitivity in non-insulin dependent diabetes mellitus (NIDDM) model of guinea pigs.

MATERIALS AND METHODS

Animals

Male guinea pig (*Cavia porcellus*) of age 6-8 weeks old (weight approx. 150gm/rat) were used in this study and housed in stainless steel cages (5 animals/ cage). They were acclimatized under laboratory conditions *viz.* 24 ± 1°C of ambient temperature and relative humidity 55±10%, with a 12:12 h light-dark cycle. Animals were fed standard chow and water *ad libitum* for whole period of the experiment.

Grouping of animals

Sixty animals were distributed into 6 groups (ten animals in each group) as follows- Animals of **Group-1:** normal non-diabetic, G**roup-2:** diabetic control, G**roup-3:** diabetic guinea pigs fed with ethanolic extract of *N. sativa* seed (250 mg/kg body wt.) in diet, G**roup-4:** diabetic guinea pigs fed with ethanolic extract of *N. sativa* seed (500 mg/kg body wt.) in diet, G**roup-5:** diabetic guinea pigs fed with Glimipiride (2 mg/kg body wt.) in diet and ethanolic extract of *N. sativa* seed was fed to guinea pigs of respective groups for eight weeks continuously. **Group-6:** Runner group (normal guinea pigs fed with ethanolic extract of *N. sativa* seed @ 500 mg/kg body wt.) for study of *N. sativa* seed toxicity. Guinea pigs were sacrificed at the 8th week

of experimental period and assessed for various parameters.

Preparation of ethanolic extract of *Nigella sativa* seed

The seeds of *Nigella sativa* were purchased from local market. Seeds of *N. sativa* were later shade dried for 5 days and powdered using mixer grinder. One thousand gram of shade dried *N. sativa* seed powder was soaked for 48 hours in a flask containing 1.5L of petroleum ether. The flask was kept at room temperature for overnight. Following day the mixture was filtered with Whatman's filter paper and supernatant powder was collected. Supernatant powder was dried for overnight. Next day extraction was done through soxhlet extractor by using ethanol. All the residue extract were collected and stored at room temperature.

Induction of type-2 diabetes (NIDDM) in rats

For induction of Type-2 diabetes (NIDDM), guinea pigs were fed with standard chow containing high carbohydrate for 15 days before STZ injection. Type-2 diabetes in guinea pigs was induced by a single dose of intra-peritoneal injection of STZ (freshly prepared in 0.1M citrate phosphate buffer) at a dose of 60mg/kg body weight[17] (Wang *et.al.,* 2007). All rats were fasted for 12 hours before STZ injection.

Blood glucose test

Glucose levels were determined by using one drop of blood samples, blood was drawn from tail vain. Bayer Contour TS Glucometer (Bayer Healthcare Ltd., Hong Kong) was used for glucose level analysis following manufacturer's protocol.

Oral glucose tolerance test (OGTT)

OGTT was performed between 0900-1400 h on 8th week as per the method described by Tran *et al*., (2003)[18]. The rats were deprived of food for 12-14 h before administration of oral glucose at concentration of 2 gm/kg BW. After glucose administration, blood samples were collected from the tail vein at 0 (before administration), 60 min and 120 min. Glucose levels were analysed by using one drop of blood samples in Bayer Contour TS Glucometer (Bayer Healthcare Ltd., Hong Kong).

Glycosylated hemoglobin (HbA1c) level

Bannon's (1982) method was used to estimate glycosylated hemoglobin level[19], using a commercial diagnostic kit from Monozyme India Ltd., Secunderabad, India.

Insulin level

Plasma insulin level was estimated quantitatively by ELISA method of Morgan and Lazarow (1963)[20]. For this purpose Insulin ELISA kit was used.

Insulin sensitivity

Insulin tolerance test (ITT) is used to assess peripheral insulin resistance[21]. This test measures insulin sensitivity using KITT as an index of insulin mediated glucose metabolism. Rats were kept fasted overnight before administering insulin challenge. Insulin (0.2 U/100 g BW human regular insulin; Eli Lilly, Indianapolis, IN) was administered by slow *i.v.* injection through tail vein. Later, blood samples were collected at 0, 30, 60 and 120 min after administration of insulin injection. Glucose was then estimated by glucose oxidase-peroxidase method. KITT was determined from the slope of a linear portion of regression line of natural logarithm of glucose *versus* time using the formula:

$$KITT = \frac{0.693 \times 100}{t1/2}$$

Where, t1/2 represents the half-life of plasma glucose decay. The half-life of plasma glucose was obtained by plotting

plasma glucose concentrations *versus* time on semi-logarithmic graph paper.

HOMA-R

HOMA-R was calculated by using fasting blood glucose (FBG) and fasting insulin (FI) level. FBG and FI levels were used for the determination of hepatic insulin resistance[22]. The insulin sensitivity level was calculated using the following formula:

HOMA-R = FI (µU/mL) x FBG (mg/dL)/22.5.

Statistical analysis

The data were analyzed by one way ANOVA (Analysis of Variance). Values expressed in Mean ± S.E.M. Differences in mean were considered significant at $P<0.05$.

RESULTS

Blood glucose test

Table 1 shows the effect of *N. sativa* seed extract on blood glucose level and glycosylated haemoglobin. After 8 weeks of treatment, diabetic guinea pigs fed with 500 mg/Kg BW *N. sativa* seed (group-IV) extract showed significantly ($P<0.05$) lower (103.66±3.17) blood glucose as compared to guinea pigs fed with 250 mg/Kg BW (group-III) *N. sativa* seed extract (189.33±2.60,). Comparable blood glucose concentration were recorded at 8th weeks of treatment in guinea pigs of 500 mg/Kg BW *N. sativa* (103.66±3.17), glimepiride (104±3), runner (79 ±2.5) and normal (78.33 ± 2.33) groups.

Oral glucose tolerance test (OGTT)

At the 8th weeks of treatment (Fig.-1), blood glucose concentration was restored back to normal in guinea pigs fed with 500 mg/Kg BW *N. sativa* (group-IV) or glimepiride (group-V). Guinea pigs fed with 500 mg/Kg BW *N. sativa* seed extract showed significantly ($P<0.05$) better oral glucose tolerance as compared to guinea pigs fed with 250 mg/Kg BW *N. sativa* seed extract. Effects of *N. sativa* seed extract at the concentration of 500 mg/Kg BW was found to be comparable with glimepiride fed (group-V) and guinea pigs of normal (group-I) and runner group (group-VI).

Glycosylated haemoglobin

A significant ($P<0.05$) decrease in glycosylated hemoglobin was observed, in diabetic guinea pigs fed with ethanolic extract of *N. sativa* seed, at the end of 8th weeks of treatment (Table 1). At this stage 4.79±0.81 percent of hemoglobin was glycosylated in guinea pigs of normal group (group-I) where it was 9.56±0.88 percent in guinea pigs of diabetic control group (group-II). Diabetic guinea pigs fed with 250 mg/Kg BW (group-III) or 500 mg/Kg BW (group-IV) *N. sativa* seed extract showed 7.95±0.89 and 6.82±1.60 percent glycosylated hemoglobin respectively. Feeding *N. sativa* seed extract at the concentration of 500 mg/Kg BW to diabetic guinea pigs showed significantly ($P<0.05$) better results as compared to feeding 250 mg/Kg BW and found to be comparable with that of glimepiride (group-V) fed (5.5±1.60) and guinea pigs of runner group (group-VI, 4.89± 0.81).

Insulin level

Table 2 shows the effect of ethanolic extract of *N. sativa* seed on insulin level. At 8th week of treatment, feeding of 500 mg/Kg BW *N. sativa* seed extract to diabetic guinea pigs resulted in significant ($P<0.05$) increase in plasma insulin (17.55±1.60). This increased plasma insulin was found to be non significant ($P<0.05$) with guinea pigs of normal (18.5±0.81), glimepiride (17.95±1.60) and runner group (18.0±0.81).

Insulin tolerance

Guinea pigs of diabetic control group (group-II) showed resistance towards insulin treatment. A non significant ($P>0.05$)

decrease in blood glucose (mg/dl) was observed in guinea pigs of diabetic control group (group-II) at 30 min (334±6.5) and 90 min (310±5.7) of insulin administration. Guinea pigs of non diabetic normal (group-I, 140±8.11 Vs. 86±5.6) and runner (group-VI, 141±6.8 Vs. 87±7.9) groups showed statistically significant ($P<0.05$) decrease in blood glucose at 30 min and 60 min. Feeding *N. sativa* ethanolic extract to guinea pigs of diabetic groups for 8 weeks improved the insulin tolerance and showed blood glucose back to normal. *N. sativa* ethanolic seed extract at the concentration of 500 mg/Kg BW (312±5.7 Vs 210±8.5) and glimepiride (117±6.9. Vs 212±7.7) showed significantly better insulin tolerance at 30 min and 60 min as compared to guinea pigs fed with that of 250 mg/Kg BW (228±8.6 Vs 214±8.5).

HOMA

At the end of 8th week, feeding guinea pigs with 250 mg/Kg BW (68.41±3.67) or 500 mg/Kg BW (63.05±4.42) *N. sativa* seed extract resulted in significant ($P<0.05$) decrease in insulin resistance index. This came back to normal at 8th week (Table 2) and recorded to be statistically non significant ($P>0.05$) with guinea pigs of normal (group-I, 62.8±3.54), glimepiride (group-V, 62.8±3.51) and runner (group-VI, 62.6±3.44) group.

DISCUSSION

Type-2 diabetes is characterized by abnormalities in carbohydrate and lipid metabolism[23], resulting from defects in insulin secretion or action or both[24-26] which lead to postprandial and fasting hyperglycemia, dyslipidemia and hyperinsulinemia[27]. Insulin resistance is considered to be the significant pathogenic factor in type 2 diabetes[28]. Insulin resistance also leads to other disorders such as obesity, dyslipidemia, hypertension and cardio-vascular disease, collectively termed as insulin resistance associated disorder (IRAD)[29]. One of the late complications of uncontrolled DM is the formation of advanced glycosylated end products (AGE)[30]. Some of these end products can react with other proteins and increased permeability and thickening of blood vessel walls with oxy radical damage[31]. Protein glycation and glucose auto oxidation may generate free radicals in the diabetic patient, which in turn catalyses lipid peroxidation, the antioxidant status of the diabetic is compromised and is unable to protect against harmful effect of free radicals[32].

There is an increased glycosylation of number of proteins, including haemoglobin and β-crystalline of lens in diabetic patients[33]. Glycosylated Haemoglobin (HbA1c) is an important tool of glycemic management[34]. Reason being that it provides an accurate measure to access the glycemic control and diagnosis of new diabetes mellitus. Samuel Rahbar *et al* (1969)[35] first described its relationship with diabetes. Individuals with inadequately controlled diabetes, the amount of these glycated haemoglobins are much higher than normal individual[36]. HbA1c level is directly proportional to the average blood glucose concentration. In the present study the HbA1c level was found to increase in NIDDM control animals. Treatment with two doses of *N. sativa* seed extract significantly decreased HbaA1c level in a dose dependent manner. Administration of *N. sativa* seed extract to NIDDM animals reduced the glycosylation of haemoglobin by virtue of its free radical scavenging property and thus decreased the level of HbA1c. A decrease in blood glucose level might also contribute to decreased level of HbA1c in *N. sativa* seed extract-treated NIDDM guinea pigs.

Feeding *Nigella* extract to diabetic guinea pigs at concentration of 250 mg/Kg BW or 500 mg/Kg BW or glimepiride,

resulted in significant ($P<0.05$) increase in plasma insulin as compared to non fed guinea pigs of control group. This plant extract act in a similar fashion like that of *Ocimum canum* plant extract and sulphonylureas which also promote insulin release by β-cells of the pancreas[37]. Our result is in agreement with those of Ali BH (1997)[38] who showed increase in plasma insulin level after treating diabetic rats with *Rhazya stricta* extract. Tolan *et al* (2001)[39] showed increase in plasma insulin leading to decrease in insulin binding on the insulin receptor in diabetic dog following the treatment with capsaicin, the active principles present in *Capsicum frutescens*. The hypoglycaemic effect of *N. sativa* reported here is in agreement with the report of Abdel *et al* (1998)[40]. The hypoglycaemic effect may be mediated through enhancement of peripheral metabolism of glucose and an increase in insulin release or may be due to intestinal reduction of absorption of glucose or partially due to amelioration in the β cells of pancreatic islets causing an increase in insulin secretion.

An insulin-resistance state is a key phase of metabolic syndrome, constituting the most important risk factor for the development of glucose intolerance and diabetes mellitus[41]. Therefore, interventions to decrease insulin resistance may delay the progress of diabetic complications. In this study, when animals were subjected to oral glucose tolerance test (OGTT), glucose lowering effects were found in groups administered with *N. sativa* seed extract. Hypothetically, the extracts may have the properties to stimulate or regenerate the ß-cell for the secretion of insulin and evidently effective in controlling diabetes[42]. Induction of diabetes with STZ can be associated with decrease in hepatic glycogen, which could be attributed to decrease in the availability of the active form of enzyme glycogen synthetase probably because of low levels of insulin[43]. Decreased activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase, which has been reported in diabetic animal, resulting in depletion of liver and muscle glycogen content[44]. Treatment with the *N. sativa* seed extract might increase the level of enzyme to the level which influence an over-all increase in glucose influx.

Evaluation of insulin sensitivity is an important mechanism for diagnosing the development and progression of diabetes and atherosclerotic disease, a common cause of mortality in diabetic individuals[29]. It is important to evaluate insulin resistance i.e., a decrease in the effect of insulin to stimulate glucose uptake at a given serum insulin concentration, for the prevention and treatment of NIDDM[45]. Thus Insulin tolerance test and HOMA levels were determined to check insulin sensitivity[46]. ITT was used to assess peripheral insulin resistance[21]. The results obtained here clearly showed that ITT was significantly improved by *N. sativa* seed extract treatment to STZ induced diabetic guinea pig. Additionally, *N. sativa* seed extract treatment significantly prevented the rise in HOMA in STZ treated animals. These findings suggest that *N. sativa* seed extract is pharmacologically effective in improving insulin sensitivity.

In the present study, significant decrease in blood glucose level, glycosylated haemoglobin, hyperinsulinemia and significant improvement in glucose tolerance and insulin sensitivity was observed in guinea pigs treated with *N. sativa* seed extract. Effects of higher doses were more significant than lower doses of extract. Protective effect of *N. sativa* seed extract was comparable to the standard drug i.e., glimepride. Therefore it is concluded that *N. sativa* seed extract might prove beneficial in preventing hyperinsulinemia, impaired glucose tolerance, insulin resistance and eventually an effective means

of improving hyperglycemia, without any toxic effect at the dose used in the present study.

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Table 1. Effect of ethanolic extract of *N.Sativa* seed on blood glucose and glycosylated haemoglobin (HbAlc) at 8th week of treatment indicating its anti-hyperglycemic effects

Groups	Blood Glucose (mg/dl)	Glycosylated Haemoglobin in %
Normal	78.33±2.33	4.79±0.81
Diabetic control	273.66±5.89*	9.56±0.88*
250mg/kg BW	189.33±2.60*	7.95±0.89
500mg/kg BW	103.66±3.17	6.82±1.60
Glimepride	104±3	5.5±1.60
Runner	79 ± 2.5	4.89 ±0.81

Value are Mean± S.E.M of three experiments, P<0.05.

Table 2. Effect of ethanolic extract of *N.Sativa* seed on Insulin resistance index (HOMA) at 8th week of treatment calculated by the formula, HOMA = insulin (µU/mL) X glucose (mg/dL)/22.5

Groups	Plasma Insulin(µu/ml)	HOMA
Normal	18.5±0.81	62.8±3.52
Diabetic control	7.8±0.88*	90.2±4.07*
250mg/kg BW	14.2±0.89	68.41±3.67
500mg/kgBW	17.55±1.60	63.05±4,42
Glimepride	17.95±1.60	62.8±3.51
Runner	18.0±0.81	62.6±3.44

Value are Mean ± S.E.M of three experiments, P<0.05.

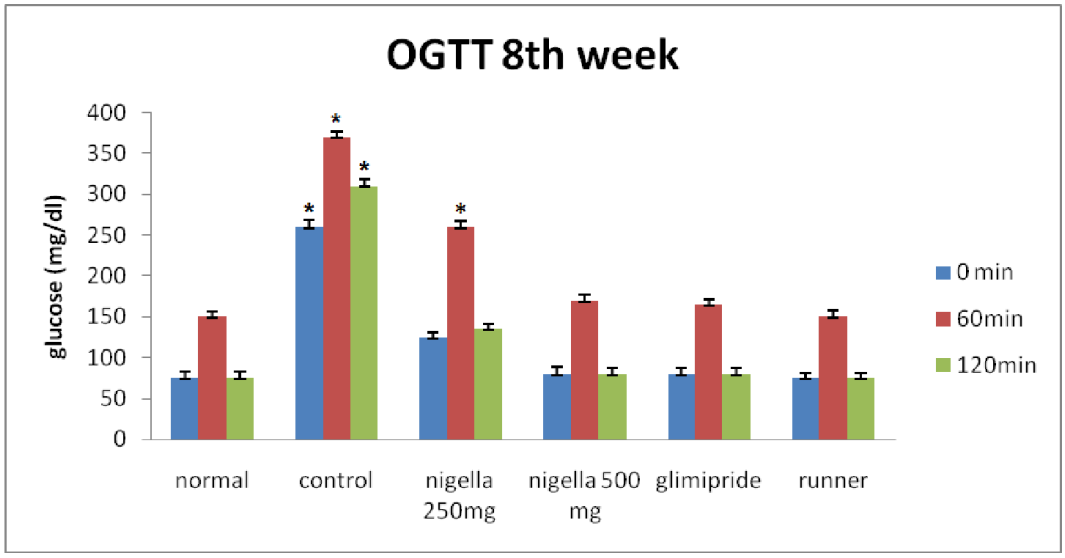

Figure 1. Effect of ethanolic extract of *Nigella sativa* and performance of all groups of guinea pigs for Oral Glucose Tolerance Test (OGTT) on 8th week of experiment

* P<0.05

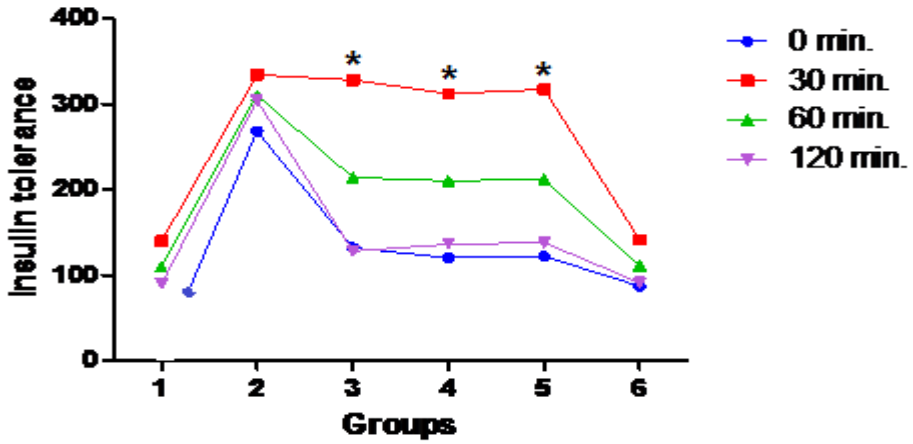

Figure 2. Effect of ethanolic extract of *N. sativa* seed on Insulin tolerance test at 8th week treatment indicates its anti-hyperglycemic effects

* P<0.05