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Full Length Research Paper

Effect of *Allium sativa* L. on pancreatic β. cells in comparison to *Nigella sativa* L. in streptozotocin induced diabetic rats

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The present study was carried out to determine the effects of *Allium sativa* and *Nigella sativa* on glucose level, insulin level, and histological appearance of beta cells of pancreas in streptozotocin induced diabetic rats. Aqueous *A. sativa* extract and *N. sativa* oil were prepared and stored at -20°C till use. Forty eight male albino rats were divided into four groups (n = 12 in each); normal control group (NC), diabetic control group (DC), diabetic *A. sativum* treated group (D-AS), and diabetic *N. sativa* treated group (D-NS). After induction of diabetes mellitus, the rats of D-AS and D-NS groups were treated with 100 mg/kg/day *A. sativa* extract and 0.2 ml/kg/day *N. sativa* oil, respectively for thirty days. Three animals from each group were sacrificed after induction of diabetes mellitus (DM) and then on 10, 20, and 30th days after *A. sativa* and *N. sativa* treatment for blood and pancreatic tissue sample collection. Results showed significant (*p = 0.001*) decrease in elevated serum glucose, rise in lowered serum insulin and partial improvement in histological appearance of pancreatic β cells of DM induced groups after 30 days of treatment with individual herbs. No significant difference noted for *A. sativa* (*p* = 0.42) and *N. sativa* (*p* = 0.79) activity which explains similarities in their effects.

Key words: Diabetes, rats, *Allium sativa, Nigella sativa*.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of carbohydrate, fat, and protein metabolism resulting in hyperglycemia due to defects in insulin secretion, insulin action, or both. Insulin is the main hormone responsible for the utilization of glucose and it is produced solely by the pancreatic β cells (Alberti and Zimmet, 1998). Streptozotocin (STZ) is a monofunctional nitrosourea derivative isolated from Streptomyces achromogenes. It is often used to induce diabetes in experimental animals through its toxic effects on pancreatic β cells (Bolzan and Bianchi, 2002). STZ has been used to induce DM in rats by giving an I/V dose of 60 mg/kg body weight (Ganda et al., 1976). Insulin is the main stay for the management and control of virtually all patients of type I DM and number of patients of type II DM. Many oral hypoglycemic agents are in use (AACE, 2007). On the other aspect, a lot of herbs are being used which have anti-hyperglycemic effects; are in the attention towards the natural therapy is going to increase. However there is a need for controlled studies on the effectiveness and potential risks of such products (Guinan, 2004). *Allium sativa* is one of the natural plants originated in Central Asia and belongs to Liliaceae family. It is regarded as one of the earliest documented examples of plants employed for treatment of diseases and maintenance of health (Omar et al., 2007). It is proven to have anti-hyperlipidaemic effects, alleviate coronary heart disease, decrease high blood pressure, cures fungal infections in addition to its anti-neoplastic, anti-bacterial (Rivlin, 2001) and anti-mycobacterial properties (Hannan et al., 2011). Anti-diabetic effect of *A. sativa* has proven its ability to

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Abbreviations: NC, Normal control group; **DC,** diabetic control group; **STZ,** streptozotocin; **SACS,** s-allyl cysteine sulfoxide; **DM,** diabetes mellitus.

reduce blood glucose level and improve insulin level in diabetic rats. In one of experimental studies, *A. sativa* treatment showed an increase in serum insulin level and decrease in serum glucose level in STZ induced diabetic rats (Eidi et al., 2006).

In another study, an anti-oxidant component of *A. sativa*; s-allyl cysteine sulfoxide (SACS) used for treatment of alloxan diabetic rats and the diabetes was improved comparatively as effects of glibenclamide and insulin. SACS; active ingredient also increased the insulin secretion from the β cells of normal rats (Augusti and Sheela, 1996). *Nigella sativa* is a spice plant belongs to the family Ranunculaceae L and it has been used in for centuries as a food flavour and remedy of many ailments in the Middle East, South Asia, and Far East (Anwar, 2005). Many studies have been reported for its anti-microbial, anti-hyperlipidaemic, anti-hyperglycaemic, diuretic and anti-oxidant effects. The beneficial effects of *N. sativa* are related to the main active components that is, thymoquinone and nigellone (Nickavar et al., 2003). Recent investigations have been conducted about anti-hyperglycaemic effects of *N. sativa* and showed to decrease the serum glucose levels and promotes certain hepatic enzymes activity such as SGPT enzyme (El-Daly, 1994). Scientific investigations have proven that the *N. sativa* causes direct stimulation of insulin release from isolated pancreatic Islets of langerhans *in- vitro* (Rchid et al., 2004). In another study it showed proliferation/ partial regeneration effect in STZ damaged pancreatic β cells (Kanter et al., 2003). The objective of present study was to observe the comparative effects of *A. sativa* and *N. sativa* on serum insulin levels, serum glucose levels and morphological changes of pancreatic β cells in STZ induced diabetic albino rats.

MATERIALS AND METHODS

Prior to the start of the study, approval was obtained from the Ethical Committee, University of Health Sciences, Lahore, Pakistan.

Preparation of *A. sativum* aqueous extract

Fresh bulbs of *A. sativa* (garlic) were purchased from local market. The cloves were separated and peeled to obtain the edible portion. Fifty grams of the edible portion was chopped and homogenized in 100 ml of autoclaved water in Warring blender. The homogenate was then filtered by using a 25 mm pore-sized filter paper (Millipore, St. Quentin, France) to give a crude aqueous extract of 500 mg/ml. This was collected in sterile container kept at 20°C till use (Iwalokun et al., 2004).

Preparation of *N. sativa* oil

The *N. sativa* seeds were washed and air-dried. Twenty grams of the powdered seeds were added to 400 ml of distilled water, and the extraction was carried out by steam distillation. The process continued until about 200 ml of distillate was collected. The distillate was then extracted 3 times with chloroform. After removing moisture by use of anhydrous sodium sulphate, the extract was evaporated on a water bath (40°C) to give the volatile oil. The yields from various extractions were pooled together, giving an average yield of 0.2% (Aqel and Shaheen, 1996).

Experimental animals

A total of 48 male albino rats weighing 200 to 250 g were procured and were randomly divided into four groups (n = 12 in each). These were labeled as NC and DC, *A. sativa* treated group (D-AS) and *N. sativa* treated rats (D-NS). Each group of rats was kept in a separate cage, in the same room with standard temperature and light period 07:00 a.m to 07:00 p.m. at 21±1°C. They were fed on standard rat diet and water in the experimental research laboratory of University of Health Sciences, Lahore, Pakistan. The rats were allowed to acclimatize for one week before the start of experiments.

Experimental procedure

The animals (albino rats) were divided into four groups including NC and DC diabetic *A. sativa* treated group (D-AS), diabetic *N. sativa* treated group (D-NS). The normal control group was kept normal without treatment while the diabetes mellitus was induced by intra-peritoneal administration of a single dose (50 mg/kg body weight) of STZ into rest of three groups. The diabetic group was kept as diabetic without treatment. The *A. sativa* and *N. sativa* treated groups were treated with 100 mg/kg/day *A. sativum* extract and 0.2 ml *N. sativa* oil, respectively for thirty days.

Sample collection

Three animals from each group were taken for blood samples and pancreatic tissue after induction of DM. While after the start of treatment with *A. sativa* and *N. sativa*, three rats from each group were again taken on the days 10, 20 and 30th for sample collection. Rats were anaesthetized using chloroform vapour and blood samples were collected by cardiac puncture taken into vaccutainers with gel and centrifuged for serum separation. Serum samples were separated into sterile eppendorff's tubes and stored at -20°C until used for glucose and insulin analysis. The pancreatic tissue samples were taken from rats and kept in 10% formalin solution for fixation.

Laboratory investigation

The glucose levels were estimated by a Glucose Oxidase method (Sigma) while the insulin levels were analysed by ELISA method (Bioversion, Germany).

Histological examination

The pancreatic tissue was incised from rat groups and samples were fixed in 10% formalin solution for 24 h. The average diameters of pancreatic beta cells were measured using Culling CF technique. Pancreatic tissue samples were processed for dehydration and clearing to embed the tissue in paraffin wax by automated tissue processor (Histotech III, Germany) and then tissue blocks were made. Sections of 4 to 5 µm thickness were cut by Rotary microtome (Leica, Germany). Sections were then stained by standard haematoxylin and eosin procedure. After the staining, microscopy was performed to visualize the morphology of pancreas.

Table 1. Effect of *Allium sativum* and *Nigella sativa* on serum glucose and insulin levels in different groups of rats.

Time vs group	Biochemical parameters	NC group (Mean ±S.E.M)	DC group (Mean ±S.E.M)	D-AS group (Mean ±S.E.M)	D-NS group (Mean ±S.E.M)
After induction of diabetes	Serum glucose (mg/dl)	96.67±7.3	360.26.46	386.67±24	340±23.09
	Serum insulin (µU/l)	8.38±0.57	2.55±0.28	2.85±0.47	2±0.48
10th day of treatment	Serum glucose (mg/dl)	96.67±8.82	320±15.28	210±20.82	260±30.56
	Serum insulin (µU/l)	8.07±0.61	2.28±0.23	4.01±0.07	3.41±0.54
20th day of treatment	Serum glucose (mg/dl)	96±2.89	330±15.28	151.67±10.14	210±20.82
	Serum insulin (µU/l)	7.71±0.47	2.39±0.29	4.47±0.24	4.37±0.15
30th day of treatment	Serum glucose (mg/dl)	95±2.89	333.33±14.53	115±5*	133.33±3.33*
	Serum insulin (µU/l)	9.95±0.41972	2.22±0.3267	5.12±0.07007*	5.19±0.1456*

* indicates significant effect = $p<0.05$.

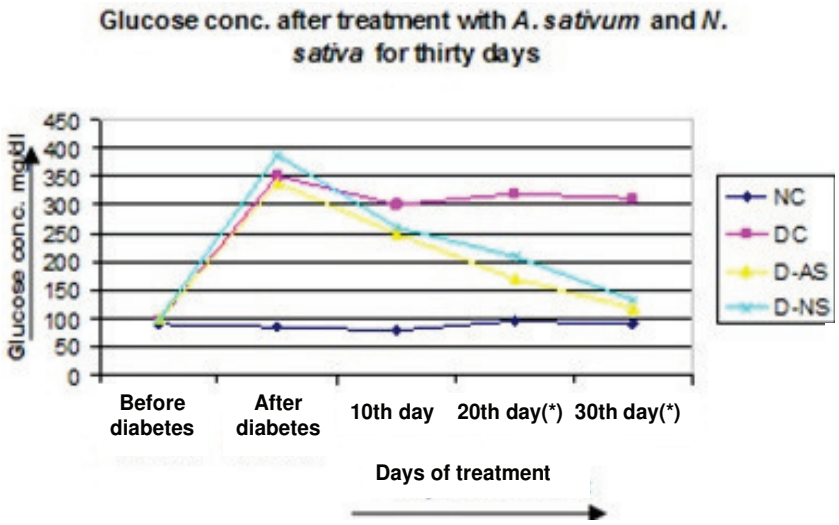

Figure 1. Glucose concentration (mg/dl) of normal control, diabetic control, *A. sativum* treated, and *N. sativa* treated rats before and after the STZ induction and during treatment. *Denotes a significance different ($p = 0.001$) between diabetic control and *A. sativum* and *N. sativa* separately. It is seen in the chart that the effects of *A. sativum* and *N. sativa* gave similar results in lowering the glucose levels in diabetic rats.

Statistical analysis

The data was collected and analysed using SPSS version16.0. Mean ± S.E.M is given for quantitative variables. Frequencies, percentages, and graphs are given for qualitative variables. One-way analysis of variation (ANOVA) was applied to observe group mean differences. Post Hoc Turkey test was applied to observe which groups mean differs. Fisher exact test was applied to observe association between qualitative variables. A p-value of < 0.05 was considered as statistically significant.

RESULT

After induction of DM by STZ, the serum glucose level raised above 300 mg/dl ($p = 0.91$) and serum insulin level fallen below 5 µU/L ($p = 0.96$) in all diabetes induced groups. The level of glucose in Group-NC and Group-DC rats with Mean ± S.E.M was 96.67±8.82 and 320±15.28, respectively. After treatment, the results of glucose con-centrations (mg/dl) for D-AS and D-NS rats (at 10th day of treatment) with Mean ± S.E.M were 210±20.82 and 260±30.56, respectively as given in Table 1. These results were continuously improved in treated rats with significant value ($p = 0.001$) at day 20th with Mean ± S.E.M for D-AS was 151±10.14 and for D-NS was 210±20.82 and level of glucose further decreased at day 30th with Mean ± S.E.M was 115±5 and 133±3.33,

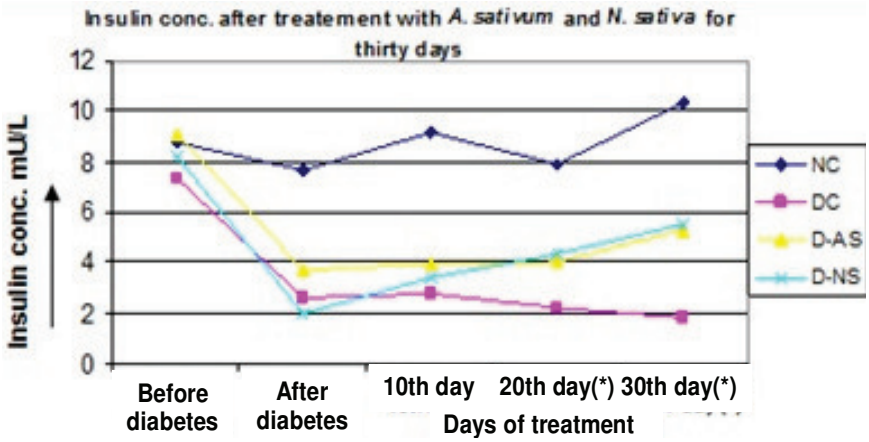

Figure 2. Insulin concentration of normal control, diabetic control, *A. sativum* treated, and *N. sativa* treated rats before and after the STZ induction. The data is experssed as mean ± S.E.M. *Denotes a significance different (p = 0.001) between diabetic control and *A. sativum* and *N. sativa* separately. It is seen in the chart that the effects of *A. sativum* and *N. sativa* gave similar results in elevating the lowered insulin concentration in diabetic rats.

respectively (Figure 1).

The results of insulin (µU/l) for D-AS and D-NS rats (at 10th day of treatment) with Mean ± S.E.M were 4.01±0.07 and 3.41±0.54, respectively (Table 1). These results were noted to increase the insulin level in these treated rats (p = 0.001) at day 20th with Mean ± S.E.M for D-AS was 4.47±0.24 and for D-NS 4.37±0.15 and at day 30th with Mean ± S.E.M was 5.12±0.07007 and 0.49±0.1456 respectively (Figure 2). The result showed a significant (p = 0.001) decrease in elevated serum glucose level and raise in lowered serum insulin level after thirty days of treatment both with *A. sativa* and *N. sativa*. Histological appearance of pancreas in NC group of rats was normal with an average diameter of 5 µm in size (Figure 3a). The morphology of pancreatic islets in DC group showed shrunken langerhans and the islet cells appeared small and oval shaped. The size of pancreatic cell was reduced with an average diameter of 3.5 µm (Figure 3b). In D-AS and D-NS groups treated with *A. Sativa* and *N. sativa*, the severity of necrotic and hyaline changes was less on the 20th day than those in the DC group. On day 30th, the necrotic and hyaline changes were improved to normal as compared to DC group. The size was increased to normal with average diameter of 5 µm of pancreatic beta cells in both treated groups (Figure 3c and 3d).

DISCUSSION

DM is a metabolic disorder characterized by a high level of glucose. *A. sativa* also known as garlic is being used for centuries for the remedies of diseases (Omar et al., 2007). *N. sativa* has been employed as food flavour and as herbal medicine. It has been used in the Middle East, south Asia, and Far East for a long time to treat many ailments and also as an additive to food (Anwar, 2005). In this study we induced DM by single intra-peritoneal injection of 50 mg/kg body weight of STZ. Second day after induction of diabetes using STZ injection, we observed that the rats became diabetic and the level of glucose ranged from 300 to 450 mg/dl. There was decrease in the levels of insulin in DC group as compared to NC group. In D-AS group, there was a decrease in the elevated glucose levels and improvement in the lowered insulin level towards normal. D-AS treated group showed maximum effects after 30 days of treatment. Similarly, D-NS group demonstrated the same activity on biochemical and histological changes of pancreas. The histological features of the β cells appeared spherical shaped and with an average diameter of 5 µm in NC group. In rats of DC group the islets of langerhans showed atrophic and hyaline changes. The cells appeared oval shaped with an average diameter of 3.5 µm. The histological abnormalities caused by DM induction were observed and showed improvement after different days of treatment with both the herbs. In previous studies, it showed that the STZ causes an increase in serum glucose level and decrease in serum insulin level.

These changes in blood glucose and insulin concentrations reflect abnormalities in pancreatic beta cell function. STZ impairs glucose oxidation and insulin biosynthesis and secretion. First of its action was seen to abolish the beta cell response to glucose which later followed a permanent loss and cells are damaged. The STZ is taken up by beta cells via glucose transporter GLUT2. The main reason for the STZ-induced beta cell death is alkylation of DNA which is related to its nitrosourea moiety. However, the synergistic action of

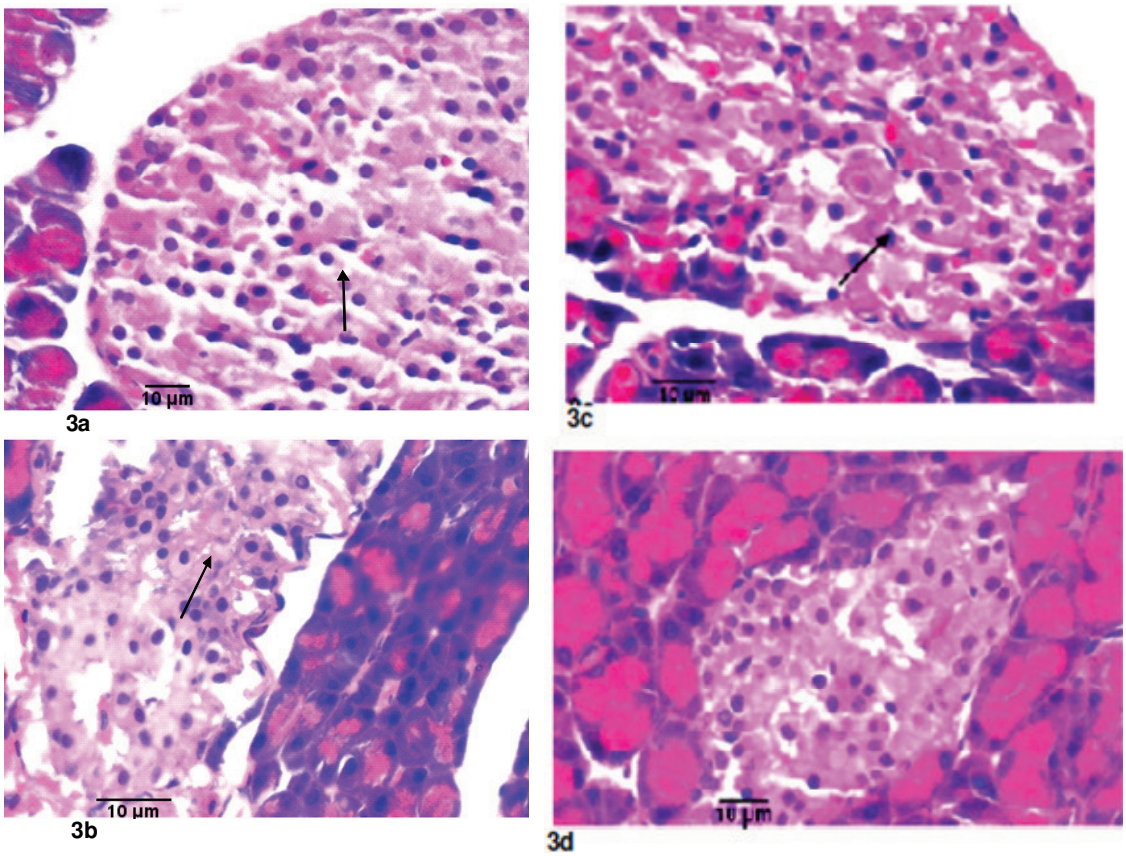

Figure 3. Photomicrograph of rat's pancreatic Islet of langerhans (H and E. ×80), (3a). NC rats with normal islets and no hyaline and necrotic changes with average diameter of 5 µm diameter. (3b). DC rats with shrunken islets of langerhans and showing hyaline and necrotic changes. The islet cells are small and oval in shape with an average diameter of 3.5 µm. (3c). In D-AS rats, the islet cells appeared in spherical shaped and larger in size and displayed increase in size and light hyaline changes in the majority of cells after 30 days treatment. (3d). In D-NS rats, the islet cells appeared in spherical shaped and larger size. The islets of langerhans in both treated groups are showing slight increase in size with an average of 5 µm diameter and hyaline changes in the majority of cells treated for 30 days with *N. sativa*.

both nitrous oxide (NO) and reactive oxygen species (ROS) that released from STZ may also contribute to DNA fragmentations and other deleterious changes caused by STZ. NO and reactive oxygen species can act separately or form the highly toxic peroxynitrate and therefore the intracellular antioxidants or NO scavengers substantially attenuate STZ toxicity (Szkudelski, 2001). In one of another study, the glucose levels were decreased with Mean ± S.E.M from 287±7.20 to 91±4.86 and 85±6.10 in Alloxan diabetic rats after treatment one group with 1 ml *A. sativum* juice/100 kg/day and in another group with 1ml *A. cepa* juices by gastric lavage for four weeks, respectively El-Demerdash et al., 2005). In another study the intra-gastric tube administration of *A. sativum* extracts at doses 0.25 g and 0.5 g/kg in wister rats tended to bring serum glucose significantly towards normal (Eidi et al., 2006). In previous study carried out on the antidiabetic and hypolipidaemic properties of *A. sativum* that showed that the glucose levels decreased by 29% after two weeks of oral treatment as compared to diabetic control (Thomson et al., 2007). The mean insulin levels in D-AS group reached near normal range after thirty days but still significantly lower (p = 0.001) than that of NC group. In present study, insulin levels increased from 2.85 µU/l to 5.12 µU/l as compared to a previous study which showed an increase in the insulin level from 2 IU/l to around 13 IU/l after oral treatment with *A. sativum* 500 mg/kg/day for fourteen days (Eidi et al., 2006). The insulin level in D-NS group reached near normal range but was significantly lower than NC group which increased from 2.00 to 5.49 µU/l as compared to a previous study which showed the increase insulin level from 10 µU/l to 16 µU/l after daily intra-peritoneal treatment with 0.2 ml of 0.2% *N. sativa* oil (Kanter et al., 2003).

In one of our earlier study, *N. sativa* caused a sharp decrease in the elevated serum glucose and slight increase in serum insulin concentration in STZ diabetic rats and in addition, it also protected the majority of the langerhans islet cells (Kanter et al., 2004). In different

study where the *N. sativa* oil was given intra-peritoneally to an STZ diabetic albino rats caused a decreased ($p = 0.001$) in the elevated serum glucose and an increase ($p<0.05$) in the lowered serum insulin concentrations as well as an improvement in histological appearances of islets of langerhans of diabetic rats. The hypoglycaemic effect of *N. sativa* could be partly due to amelioration in beta cells of pancreatic islets causing an increase in insulin secretion (Kanter et al., 2009).

Conclusion

It is concluded that there is no difference in effect of *A. sativum* and *Nigella sativa but A. sativum* is slightly more effective on biochemical parameters in comparison to *N. sativa*. Therefore, *A. sativum* and *N. sativa* have similar results in lowering glucose levels, elevating insulin levels and improving histological features in STZ induced diabetic rats. It is also recommended that more studies are needed to demonstrate pharmacokinetics and pharmaco-dynamics properties of *A. sativum* and *N. sativa* so that these natural plants could be utilized for the management of DM.

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