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Isulinotropic properties of *Nigella sativa* oil in Streptozotocin plus Nicotinamide diabetic hamster

K. M. FARARH[†], Y. ATOJI[‡], Y. SHIMIZU[†], T. TAKEWAKI[†,*]

[†]*Department of Physiology and Pathogenetic Veterinary Sciences, The United Graduate School, Gifu University, 1-1 Yanagido, 501-1112 Gifu, Japan,* [‡]*Department of Anatomy, The United Graduate School, Gifu University, 501-1112 Gifu, Japan*

SUMMARY

The present study was designed to investigate the possible insulinotropic properties of *Nigella sativa* L. (*N. sativa*) oil in Streptozotocin plus Nicotinamide-induced diabetes mellitus in hamsters. Nicotinamide was injected intraperitoneally 15 min before injection of Streptozotocin intravenously. Oral treatment with *N. sativa* oil began 4 weeks after induction of diabetes. Serum insulin was measured by enzymeimmunoassay. Islets insulin was stained using anti-insulin monoclonal antibody. Significant decrease in blood glucose level together with significant increase in serum insulin level were observed after treatment with *N. sativa* oil for 4 weeks. Big areas with positive immuno-reactivity for the presence of insulin were observed in the pancreases from *N. sativa* oil-treated group compared to non-treated one using immunohistochemical staining. Therefore, our data show that the hypoglycemic effect of *N. sativa* oil in Streptozotocin plus Nicotinamide diabetic hamsters resulted, at least partly, from a stimulatory effect on β cell function with consequent increase in serum insulin level. These results indicate that *N. sativa* oil has insulinotropic properties in type 2-like model.

β CELL defect and insulin resistance are essential features of non-insulin-dependent diabetes mellitus (NIDDM) (Cerasi and Luft 1967, Girard 1994) and both features are the focus of intensive investigations. In this context, plants are source of many biochemical substances that present interesting therapeutic properties (Balandrin et al 1985). Some plants with antidiabetic properties have been in use in many Middle Eastern countries as a natural remedy for diabetes in traditional medicine (Swamy and Tan 2000); *N. sativa* is one of these plants. It has a great potential in the treatment of diabetic animals because of its combined hypoglycemic (Al-Hader et al 1993) and immunopotentiating properties (Haq et al 1999). Also it is cheap and readily available.

Although there are some plants used in the treatment of diabetic dog (Ribes et al 1986) and its insulinotropic properties were investigated (Sauvaire et al 1998, Broca et al 1999) and considered as secretagogues with potential interest for the treatment of type 2 diabetes mellitus that characterized by defective insulin secretion associated with various degrees of insulin resistance (Cerasi et al 1972, De-Fronzo 1988, Kahn and Porte 1988), the insulinotropic effect of *N. sativa* has not been studied yet.

Therefore, the present study was designed to investigate the possible insulinotropic properties of *N. sativa* oil and whether it could correct the alterations observed in Nicotinamide plus Streptozotocin model of NIDDM. This model, which appears to be particularly suitable to test potential antidiabetic drugs as it shares some important features of type 2 diabetes.

MATERIAL AND METHODS

N. sativa *oil*

N. sativa oil was obtained from Kahira Pharm., (Egypt) that was extracted as follows. *N. sativa* seeds were authenticated by Botanical Department, Faculty of Agriculture, Zagazig University, Egypt. Then the seeds were washed, dried, and crushed to a powder with an electric microniser. 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 of distillation was continued until about 200 ml of the distillate were collected. The distillate was extracted three times with chloroform. Moisture was removed by anhydrous sodium sulphate and the resultant extract was evaporated using a water bath (40 °C); this led to the appearance of the volatile oil. The products of various extractions were pooled together giving an average yield of 0.3%. 500 mg of the volatile oil were dissolved by the initial addition of 1 ml of dimethyl sulphoxide (DMSO), followed by the addition of 9 ml of normal saline to yield a concentration of 50 mg volatile oil per 1 ml solution.

The oil was given once daily orally in a dose of 400 mg/kg body weight by using intra-gastric intubation.

*Corresponding author: T. Takewaki. Fax: +81-58-293-2992.
E-mail: tt@cc.gifu-u.ac.jp

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Animals

Forty male Syrian hamsters 8-weeks old (90–135 gm weight) were obtained from commercial company for laboratory animals, (Gifu, Japan) and placed in stainless steel cages and maintained under suitable lighting, temperature, and proper hygienic condition. Well-balanced ration and drinking water were available. The animals were observed for 14 days before the experimentation. Animals were anaesthetized with diethyl ether and killed via the carotid arteries. The surgical procedures and pre- and post-operative care of the animals conformed to the Gifu University, animal care and use committee in accordance with Japanese department of agriculture guide lines and all efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were divided into four groups, control normal group, diabetic-untreated group, diabetic-treated with DMSO, and diabetic-treated with *N. sativa* (10 animals in each group).

Streptozotocin plus Nicotinamide-induced diabetes mellitus

Thirty hamsters were injected Nicotinamide (NA) (Sigma) in a dose of 230 mg/kg body weight intraperitoneally 15 min before intravenous administration of Streptozotocin (STZ) (Sigma) in a dose of 65 mg/kg body weight according to the protocol described before (Masiello et al 1998). Control animals were injected with buffered solution alone. All animals were then maintained for 4 weeks with food and water with monitoring of blood glucose, body weight and food and water consumptions before beginning of treatment with *N. sativa* oil.

Blood sampling

Blood samples were collected from the medial canthus of the eye and by heart puncture in sterilized tubes for serum separation and in tubes containing heparin for separation of plasma according to standard method (Riley 1960).

Blood glucose

Blood glucose was measured according to the method adopted before (Miwa et al 1972) using glucose kit (enzymatic method) obtained from Wako (Japan).

Estimation of insulin

It was performed according to the method adopted before (Gerritsen and Blanks 1974, Barrington 1975) using enzymeimmunoassay (EIA) system obtained from Amersham Pharmacia Biotch (USA).

Immunohistochemical staining for insulin

It was performed according to the protocol described before (Miko et al 2001) briefly.

Pancreases from all groups of animals were collected and fixed in 10% buffered formalin and then embedded in paraffin. Thin sections (5 μm) were made. Deparaffinization with xylene and alcohol; washing with PBS three times for 5 min; immersion in 0.3% H_2O_2 in methanol; rinsing in PBS three times for 5 min; blocking with horse serum for 30 min; rinsing in PBS three times for 5 min; incubation with primary antibody (anti-mouse insulin) overnight at 4 °C; rinsing in PBS three times for 5 min; incubation with secondary antibody (horse anti-mouse IgG) for 30 min; rinsing in PBS three times for 5 min; incubation with ABC kit for 30 min; rinsing in PBS three times for 5 min; incubation with DAB (3,3-diamino benzidine tetrahydrochloride) in Tris–HCl for 5–10 min; rinsing in PBS three times for 5 min; washing with distilled water; rinsing in hematoxylin for few seconds; dehydration in alcohol and xyline; finally, mounting and covering with canada balsam were made.

Evaluation of immunohistochemical preparations was made by examination of 8–10 islets for each hamster.

Statistical analysis

Data are expressed as the mean ± SE. *N* represents number of hamsters used in each group. Differences between means were analyzed by using ANOVA for comparison between diabetic-untreated group and diabetic-treated with *N. sativa*. A *P* value of less than 0.05 was considered significant (Zar 1974).

RESULTS

Blood glucose level

There was a significant decrease ($p < 0.01$) in fasting plasma glucose level after first, second, third, and fourth weeks of treatment with *N. sativa* oil for 4 weeks in STZ–NA-diabetic hamsters (Fig 1).

Effect of N. sativa *oil on serum insulin level*

Data demonstrating the effect of *N. sativa* oil on insulin level in STZ–NA-diabetic hamsters are

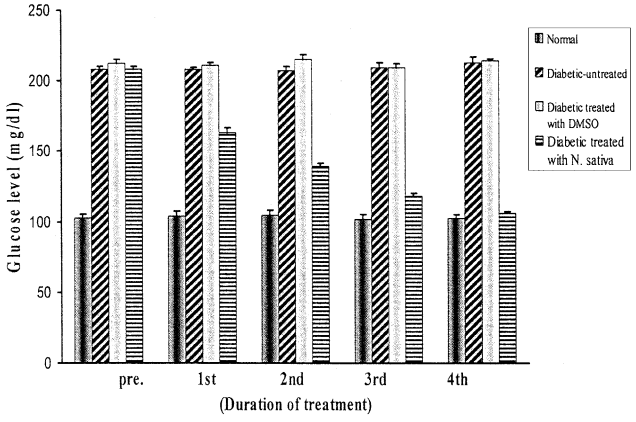

FIG 1: Changes in fasting plasma glucose level in STZ + NA-diabetic hamsters during 4 weeks treatment with *N. sativa* oil. Results are shown as mean ± SE $N = 10$.

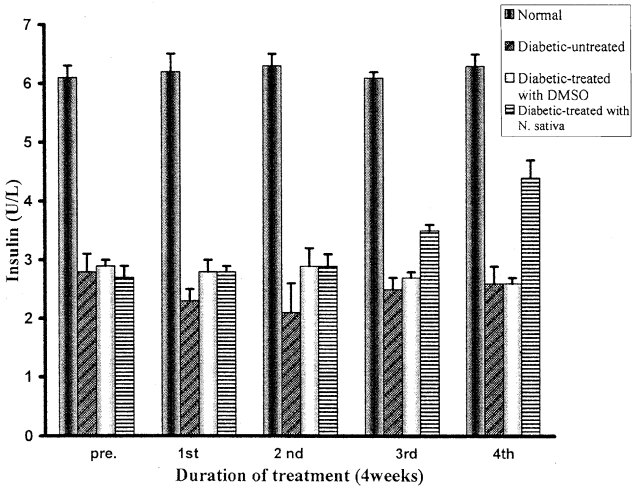

FIG 2: Changes in serum insulin level in STZ + NA-diabetic hamsters after 4 weeks treatment with *N. sativa* oil. Results are shown as mean ± SE $N = 10$.

summarized in Fig 2. Results showed that, there was a significant increase ($P < 0.05$) in serum insulin level after treatment with 400 mg/kg *N. sativa* oil for 4 weeks in STZ–NA-diabetic hamsters.

Immunohistochemical staining of the Pancreas

Fig 3B shows a small area (18% of islets size) with positive immuno-reactivity to the presence of insulin in pancreases from STZ–NA-diabetic hamsters.

After 4 weeks treatment with *N. sativa* oil, pancreas showed a big area (80% of islets size) with positive immuno-reactivity for the presence of insulin with many brown insulin granules distributed in the β cells in the islets of Langerhans (Fig 3D).

DISCUSSION

The results of the present study demonstrate that *N. sativa* oil has a significant hypoglycemic effect in STZ plus NA-induced diabetic hamsters. The decrease in the blood glucose was highly significant (reached to the normal level) after 4 weeks treatment with *N. sativa* oil. This result indicates that the hypoglycemic effect of the oil is time dependent. The administration period may be necessary for the induction of certain metabolic processes which might be essential to mediate the observed hypoglycemic activity (Al-Hader et al 1993, Al-Awadi et al 1985). Moreover, several other plants produced similar hypoglycemic effects in normal and hyperglycemic animals only after their administration for a period ranging from 3 to 12 days (Nobrega et al 1985, Ghannam et al 1986).

The volatile oil of *N. sativa* is known to have a hypoglycemic effect in STZ-induced diabetic rats and rabbits (Al-Hader et al 1993, Hedaya 1995, Al-Awadi et al 1991), but there was no increase in the level of insulin.

The failure to increase insulin may be related to the severe cytotoxicity of STZ on the β cells of the pancreas, which resulted in severe damage of islets cells (Terson 1951, Junod et al 1969), therefore there was no response to insulinotropic agents in this model of diabetes mellitus.

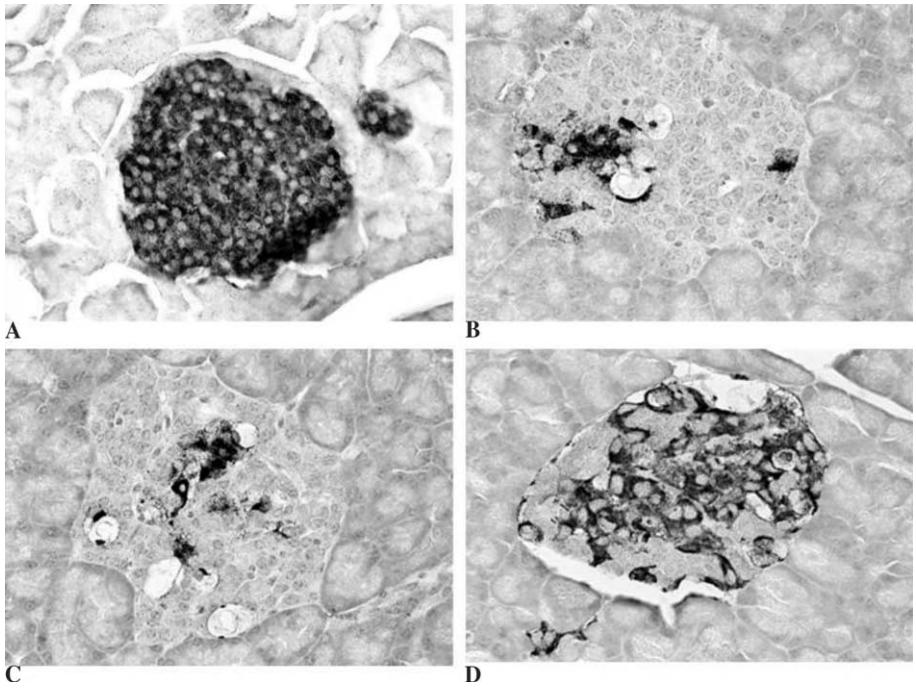

FIG 3: Insulin immuno-staining of β cells in islets of pancreas from normal (A), diabetic-untreated (B), diabetic-treated with DMSO (C), and diabetic-treated with *N. sativa* oil for 4 weeks (D). Original magnification 600×.

STZ plus NA-induced diabetes, constitutes a new experimental diabetic model that mimic some features of type 2 diabetes not shared by other established animal models of the disease, essentially a partial responsiveness to glucose and well-preserved sensitivity to insulinotropic agents (Masiello et al 1977, Masiello et al 1985).

The STZ plus NA diabetic animals are mildly diabetic without associated obesity, primarily characterized by reduced pancreatic insulin stores (40% of normal) and defective insulin secretion, which presented a good stability of its diabetic state during at least 9 weeks (Masiello et al 1998), therefore this model appeared particularly suitable to investigate the potential antidiabetic properties of *N. sativa* oil in vivo.

Our data demonstrated a significant increase in serum insulin level in STZ plus NA diabetic hamsters after 4 weeks oral treatment with *N. sativa* oil.

This increase in the insulin level resulted from activation of β cells of the pancreas from the *N. sativa* oil treated hamsters as demonstrated by immunohistochemical staining of pancreas using anti-insulin antibodies; where a high immuno-reactivity to anti-insulin antibodies was observed in the pancreases from *N. sativa* oil treated hamsters (80% of islets cells were positive) compared to that from non-treated ones, which contain small area with positive immuno-reactivity (18%). These results indicate activation of the β cells of the pancreas with *N. sativa* oil.

Al-Awadi et al 1991 found that a mixture of medicinal plants comprising *N. sativa* decrease hepatic gluconeogenesis through decreasing the activity of phosphenolpyruvate carboxykinase and pyruvate carboxylase enzymes in STZ-induced diabetic rats. So that the hypoglycemic action of *N. sativa* oil is mediated through a combined action of decreasing hepatic gluconeogenesis and activating pancreatic β cells with increase in serum insulin level. Therefore the observed decrease in glucose after first week of treatment may be due to decrease in hepatic gluconeogenesis, but after activating pancreatic β cells with increase in insulin level which became significant after the fourth week of treatment, a highly significant decrease in glucose level reaching to normal level was observed.

More investigations are needed to demonstrate the effect of *N. sativa* oil on insulin release from the isolated islets cells in vitro and its relation to glucose concentration in the medium.

In conclusion, our data suggest that the improvement of the diabetic state of STZ plus NA-induced diabetic hamsters resulted, at least partly, from stimulatory effect of *N. sativa* oil on β cell function and increase in insulin level.

Therefore, the new insulinotropic properties of *N. sativa* oil could now be considered of some interest for the improvement of NIDDM.

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