RESEARCH ARTICLE

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Effect of Nigella sativa extract on some physiological parameters and histological changes in alloxan-induced diabetic albino rats.

ABSTRACT

The present study aimed to clarify the antidiabetic activity of Nigella sativa (N.S.) aqueous extract on diabetic adult male albino rats. Rats were divided into three groups; the first served as control group, the second and third groups were injected with a single dose of alloxan (120 mg/kg body weight) and after 7 days of treatment the animals of the third group were treated with daily doses of water extract Nigella sativa (0.01mg/100 g b. wt). After 30 days of treatment half of each group was sacrificed and the other half was left for 15 days more without any additional treatment (as a recovery period).

The obtained results revealed significant decrease in body weight gain and significant increase in blood glucose level of diabetic rats with different histological changes in the cells of islets of Langerhans. These histological and physiological changes were ameliorated in rats treated with Nigella sativa (N.S.).

Water extract of Nigella sativa showed a significant hypoglycemic and hyperinsulinimic effect. On the other hand, a significant increase in body weight, β-cell number and liver glycogen content were achieved in comparison with diabetic rats.

The results of the present study clarify the role of Nigella sativa as an active antidiabetic plant and suggest a limit relationship between drenching Nigella sativa extract and insulin production.

Key word: Nigella sativa, diabetic, blood glucose, body weight, liver glycogen content, insulin, rats.

INTRODUCTION

Diabetes mellitus affects approximately 100 million persons worldwide (Amos et al., 1997). Five to ten percent have type 1 (formerly known as insulin-dependant) and 90% to 95% have type 2 (non-insulin-dependant) diabetes mellitus. It is likely that the incidence of type 2 diabetes will rise as a consequence of lifestyle patterns contributing to obesity (Meral et al., 2004). The pathogenesis of hyperglycemia in type 2 diabetes involves defects in both pancreatic secretory function and insulin sensitivity (Zaoui et al., 2002).

Diabetes is possibly the world's fastest growing metabolic disease, and as the knowledge of the heterogeneity of this disorder increases, so does the need for more appropriate therapies (Baily and Flott, 1986).

Traditional herbal remedies which are often free from side effects are still in use by diabetic patients, especially in third world countries and may therefore; present new avenues in the search for alternative hypoglycemic drugs (Ajagaokar, 1979) and such remedies are used in treatment of diabetes in developing countries. The actual medicinal action of plant drug lies in its active constituents such as alkaloids, cardiac glycosides saponins, flavonoids, volatile oils, terpenoids, resin and mucilage. Many investigators reported that folk medicine in Egypt had described 25 kinds of Egyptian herbs and plant prescriptions belonging to 13 families to be concerned with the treatment of diabetes mellitus (Mossa, 1985).

Nigella sativa is a medical plant used for treating many diseases such as rheumatism and cancer (Abd El-salam et al., 1992; Houghton et al., 1995). The essential oil of Nigella sativa exhibits hypoglycemic and bronchodilator properties (AL-Hader et al., 1993). In the latter work, water extract of a mixture composed of five plants was proved to have a good hypoglycemic effect on diabetic rats; Nigella sativa was one component of this mixture. So, this study was designed to follow up the action of Nigella sativa water extract treatment and to show whether it has an insulin-like action or it may accelerate insulin secretion from pancreatic cells. Also the study aims at revealing its effects on the pancreatic cell type and numbers.

MATERIALS AND METHODS

1- Experimental animals:

Thirty adult male albino rats (weighing 120 ± 20 g) were obtained from NAMRU, Cairo. Each rat was
weighed at the beginning and the end of the experiment. The percentages of body weight changes were calculated.

2- Plant extract:

50 g of *Nigella Sativa* seeds were finely ground then boiled with distilled water (200 ml) for 10 minutes. After cooling to room temperature, the extract was filtered and stored in refrigerator.

3- Experimental design:

Thirty male albino rats were given the regular laboratory stock diet and evaluated after overnight fast then divided into three groups (10 rats in each).

- **Group I:** Considered as control group and received no treatment.
- **Group II:** received a single subcutaneous dose of alloxan (120 mg/kg b. wt) and served as diabetic rats.
- **Group III:** This group received a single subcutaneous dose of alloxan (120 mg/kg b. wt.) and after 7 days the rats were treated daily with aqueous extract of *Nigella sativa* (0.01 g/100 g b. wt/day).

Five animals of each group were decapitated after 30 day of treatment and the other five animals were left without any additional treatment for 15 days as a recovery period.

4- Biochemical studies:

Serum glucose level, liver glycogen content and serum insulin level were determined according to the method described by Tietz (1986), Joseph (1955), and Reeves (1983), respectively.

5- Histological examinations:

Paraffin sections of the pancreas from each group were stained using 2 different stains:

- 1- Hematoxylin and Eosin (HE) stain.
- 2- Modified aldehyde fuchsin stain (Halami, 1952).

6- Quantitative studies:

Stained sections were used for morphological and cytophotometric analysis using a computerized imaging; Measurements were recorded by motic analyzing system for α, β & δ-cells of islet of Langerhans (number of cells, diameter of cells and nuclear diameter).

7- Statistics

Statistical analysis of the data was done by Student's t-test. P value was considered significant at level 0 p < 0.05.

Scanning Electron Microscopy:

The obtained larvae were put in glutraldehyde 2.5 % and processed for SEM according to Cowell and O’Connor (2000).

RESULTS

The present study recorded highly significant decrease (p<0.01) in body weight gain in diabetic group and *Nigella sativa*-treated group through the treatment period when compared with control rats, while, rats treated with *Nigella sativa* showed highly significant increase in percentage of body weight gain when compared with diabetic ones during the experimental period (Fig. 1).

![Graph showing percentage of body weight changes](image)

**Fig. 1.** Percentage of body weight change in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.

The present data showed severe hyperglycemia, hypoinsulinemia and highly significant decrease in liver glycogen content in diabetic rats when compared with control group throughout the experiment period. Otherwise, *Nigella sativa*-treated group showed insignificant changes when compared with the control rats in the previous parameters except serum insulin level which showed highly significant decrease when compared with the control rats, but recorded highly significant increases when compared with the diabetic group during the experimental period (Figs 2-4).

![Graph showing serum glucose level](image)

**Fig. 2.** Serum glucose level (mg/dl) in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.

![Graph showing liver glycogen content](image)

**Fig. 3.** Liver glycogen content (mg/100 g) in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.
In the current study, alpha cells recorded no significant change in their number, diameter and nuclear diameter in diabetic and *Nigella sativa*-treated groups as compared to control throughout the experiment period (Figs 7-9).

In sections of pancreas stained with HE in normal animals, the islets of Langerhans appeared as irregular spherical masses scattered throughout the pancreas with rich vascular supply. All cells display granular cytoplasm and central spherical nuclei (Fig. 5). With modified fuchsin stain, β-cells appeared as the most abundant cells and occupy the core of the islets. They contain numerous granules and are stained with violet colour. Alpha (yellow) and delta cells (green) occupy the periphery of the islets (Fig. 6).

![Fig. 4. Serum insulin level (μu/ml) in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.](image)

![Fig. 7. Means of changes in the number of α-cells in the islet of Langerhans in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.](image)

![Fig. 8. Means of changes in the diameter of α-cells in the islet of Langerhans in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.](image)

![Fig. 9. Means of changes in the nuclear diameter of α-cells in the islet of Langerhans in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period](image)
Concerning pancreatic β-cell number, the present study showed reduction in islets of diabetic rats, necrosis, intracellular vacuolation and degranulation in some surviving β-cells which recorded a significant decrease in their number and significant increase in cellular and nuclear diameter when compared with control group throughout the experimental period. While, treatment with *Nigella sativa* extract showed pronounced increase in β-cells number and less vacuolation. And also, there is no change in cellular and nuclear diameter of β-cells when compared with normal islets during the experimental period (Figs. 10-12 and 13-18).

**Fig. 10.** Means of changes in the number of β-cells in the islet of Langerhans in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.

**Fig. 11.** Means of changes in the diameter of β-cells in the islet of Langerhans in control, diabetic and *Nigella sativa* extract treated diabetic rats after 4 weeks of treatment and 2 weeks of recovery period.

**Fig. 13.** Photomicrograph of a section in pancreas of diabetic male rat, showing a small sized islet Note the pale disintegrated nuclei (n) and dark cells at the periphery of islet (d) and the normal structure of exocrine pancreas. (HE, X 400)

**Fig. 14.** Photomicrograph of a section in pancreas of diabetic male rat, showing a small sized islet Note the pale disintegrated nuclei (n), vacuolated deeply green d-cells (d), vacuolated β-cell (v) and faint stained α-cell (a). (modified aldehyde fuchsin X 1000).

**Fig. 15.** Photomicrograph of a section in pancreas of *Nigella sativa* treated rat, showing slightly vacuolation β-cell (→) with rounded nuclei and deeply stained basophilic nuclei cell (→). (HE, X 400)

**Fig. 16.** Photomicrograph of a section in pancreas of diabetic rat treated with *Nigella sativa*, showing slightly vacuolated β-cell (→) with rounded nuclei and acidophilic cytoplasm of a cell (→). (Modified aldehyde fuchsin X 1000).

**Fig. 17.** Photomicrograph of a section in pancreas of diabetic male rat after recovery period (45 days), showing destructed islet of Langerhans, which contain destructed nuclei (p), vacuolated cell and necrosis (s). (HE, X 400).
Fig. 18. Photomicrograph of a section in pancreas of diabetic male rat after recovery period (45 days), showing illustrated faintly stained nuclei (a), non-nucleated (n) and vacuolated β-cell (v). (Modified aldhyde fuchsin X 1000).

Otherwise, the present data recorded no significant changes in delta cells number, diameter and nuclear diameter in the diabetic group but these cells recorded significant increase after the recovery period. While, the rats treated with *Nigella sativa* extract showed a significant decrease throughout the experiment (Figs 19-21).

**DISCUSSION AND CONCLUSION**

Due to the high cost and poor availability of current therapies in developing countries, the diabetic patients need alternative therapies to control all the pathological aspects of diabetes and (Marles and Farnsworth, 1995). The traditional antidiabetic plants might provide this useful source of new oral hypoglycemic compounds. So, this study is a step to evaluate and follow up the effects of *Nigella sativa* water extract as a hypoglycemic agent.

The present results, revealed severe loss in body weight gain in diabetic rats when compared with the control rats. This loss may be explained by inhibition of synthesis of DNA and RNA in the diabetic animals and/or it is attributed to different side effects on the ability to use carbohydrates including lipolysis, glycogenolysis and acidosis. This result is mainly due to destructions of β-cells which lead to sudden drops of insulin secretion (Abdel-Moneim et al., 1999a; Ganong, 2003; Helal et al., 2003; Kechrid; Bouzerna, 2004; Rawi et al., 1996).

Severe hyperglycemia in diabetic rats recorded in the present work can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of alloxan on the β-cells of the pancreas which has a direct effect on their membrane permeability by causing failure of ionic pumps and increased cells size. It also inhibits intracellular energy generation, insulin secretion and causes sudden activation of quiescent cells for a high level of protein synthesis and produced rapid and massive beta cell death leading to a decrement in β-cells number (Majno and Joris, 1999). The destructive effect of alloxan on β-cells may be also attributed to its ability to inhibit enzymes of the tricarboxylic acid cycle and Ca²⁺- dependent dehydrogenases in β-cell mitochondria, causing ATP deficiency, cessation of insulin production and cell necrosis (Shafrir, 2003).

The results of the present study also showed β-cells with vacuolated cytoplasm in the diabetic group. Vacuolation of the islet is the most prominent lesion associated with functional islet abnormality and development of hyperglycemia (Bolaffi et al., 1986).
and Kessler et al., 1999). Also, the vacuolation may be due to the diabetogenic action of alloxan which induced highly reactive oxygen radicals, which are cytotoxic to β-cells (Fischer and Homburger, 1980). According to Yamamoto et al. (1981) and Ronald (1988) the fragmentation of nuclear DNA of pancreatic β-cells seems to be important for the development of diabetes and supposed to be resulted from the accumulation of superoxide or hydroxyl radicals in the β-cells.

El-Seifi et al. (1997) suggested that, the hypoglycemic effect of some medicinal plants could be attributed to factors other than stimulation of insulin release only, e.g. their effect on the number and/or affinity of insulin receptors on target cells and the post-receptors of these cells. Abdel-Moneim et al. (1999a) reported that the hypoglycemic effect of *Nigella sativa* may be attributed to an increase in the islet numbers and to its effect on the time-course of glucose resorption from the intestine.

On the other hand, the *Nigella sativa* treated group showed insignificant change in beta cells number and diameter as compared to the normal group. This plant may have a stimulatory effect on the division of β-cells or may contain non-metabolizable 2-deoxy and 3-O-methylglucoses, which share the entry site, block the diabetogenic action of alloxan and restore insulin production (Shafrir, 2003) and also resulted from their content of non-enzymatic radical scavengers such as NAD or chelators of metal ions which protect against alloxan injury (Malaisse, 1982).

The significant hypoglycemic and insulino tropic effect induced in diabetic rats by *Nigella sativa* treatment may result from its effect on the time-course of glucose resorption from the intestine. It also has an effect on peripheral tissues and regeneration of islet of Langerhans leading to lower blood glucose level. The treatment with *Nigella sativa* induced islet cells regeneration with increased number of β-cells (Abdel-Moneim et al., 1999b; EL-Daly, 1994). In agreement with the present results of AL-Hader et al. (1993) found that the administration of the volatile oil of *Nigella sativa* to fasting normal and alloxan diabetic rabbits produced a significant hypoglycemic effect.

On the other hand, the decrease in liver glycogen content of diabetic rats may be a result of increasing glucose output during insulin deficiency (Gold, 1970). Also, it may be due to the loss of glycogen synthetase-activating system (Annamala and Augusti, 1980) and/or increased activity of glucose-6-phosphatase (Abdel-Moniem et al., 2001). Increase in liver glycogen content after treatment with the extract of *Nigella sativa* could be attributed to increased insulin level which has a potent effect on glycogen synthesis activity as well as on hepatic hexokinase and glycogen 6-phosphatase activity (Sheela and Augusti, 1992). It also may be due to secretion of amylin from β-cells which increase the motility of the gastrointestinal tract and decreases the absorption in intestine. In the same time the increase of amylin leads to decreases of insulin secretion, but in this case all insulin secretion are active insulin rather the proinsulin in diabetic rats. Further investigations are needed to detect effects of different doses and time intervals of *Nigella sativa* needed in treatment of the diabetic animals.

It seems that supplementation of *Nigella sativa* immediately after diagnosis of diabetes may delay its complications. The benefit of this herb is not only due to its hypoglycemic effect, but also due to its protecting effect against injury and oxidation processes and due to its contents of many vitamins and enzymes. It contains 58% of essential fatty acids including omega 6 and omega 3. These are necessary for the formation of Prostaglandin E1 which balances and strengthens the immune system giving it the power to prevent infections and allergies and control chronic illnesses. Also, *Nigella sativa* oil contains about 0.5 - 1.5% volatile oils including nigellone and thymochinone which are responsible for its anti-oxidant activities.

The present study suggests that *Nigella sativa* is the one of choices that could be used either as a single medicine or in combination with other herbs in treatment of diabetes mellitus.

**REFERENCES**


التأثيرات الفسيولوجية والهسيولوجية للمستشفى المائي لنبات حبة البركة على الجردان

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تأتي هذه الدراسة لمساحة فاعلة المستضحل المائي لنبات حبة البركة عندما تستخدم كعلاج لمرض السكر. واستخدم لهذه الدراسة ثلاثين من ذكور الجردان البدائي بالرطوبة المتواضعة المائيةتعبير كجامع المجموعة الثانية، ثم حقنها بالأوكسان لإجراء مرض السكر والمجموعة الثالثة جردان مصابة بمرض السكر ثم تعالجها بالمستضحل المائي لنبات حبة البركة (0.01 ملجم/100 جم من وزن الجسم). وتمت المحاسنة لمدة ثلاثين يوماً وكذلك بعد فترة الاستشفاء (خمسة عشر يوماً) دون أي علاج إضافي.

وأظهرت الدراسة وجود تحسين حاد في حالة النسيج في نسبة زيادة وزن الجسم في الجردان المطابق بالسكر الدم، وكذلك في المجموعة المائدة السكر الدم. ولم يتم تفعيلها بالمستضحل المائي لنبات حبة البركة.

واختبرت النتيجة أيضًا وجود زيادة ملحوظة في حالة النسيج في معدل تجفيف لحم البدائي بالسكور الدم، وتراجع هذه النتيجة لانتظار النتائج لل bảngات الطبية في المجموعة المائية السكر الدم. لم يتم تفعيلها بالمستضحل المائي لنبات حبة البركة بالرطوبة المائدة خلال هذا الفحص.

وأظهرت النتيجة أيضًا وجود نقص في حالة النسيج في معدل التحفيز في النسيج السكري، وكذلك نسبة هرمون الأستروما في الجردان المطابق بالسكور الدم، بكلمها بالمستضحل المائي لنبات حبة البركة بالرطوبة المائدة خلال هذا الفحص.

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