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EVALUATION OF ANTIOXIDANT EFFECTS OF *Nigella sativa* EXTRACT ON THE ULTRA STRUCTURE OF NEURAL TUBE DEFECTS IN DIABETIC RATS'S OFFSPRING

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Abstract

Maternal diabetes is responsible for many types of embryonic defects. Increased oxidative stress has been suggested to play a role in the pathogenesis of disturbed embryogenesis in diabetic pregnancies. The Nigella *sativa* seeds are used in folk medicine all over the world for treating many diseases. Regarding to antioxidant properties of Nigella Sativa, it was of interest to determine whether *Nigella sativa* extract has any effect on spinal cord neuroepithelium of diabetic rats' embryos. Rats were distributed to 4 groups, two diabetic and two control groups. Diabetes was induced by intrapritoneal injection of STZ. Rats were mated overnight and treatment with *Nigella sativa* extract or vehicle from 1 to day 17 of gestation at a dose of 1mg/kg body weight by gastric gavages. On the 17th day of gestation, rats were sacrificed. In offspring of vehicle-treated diabetic rats, a significantly decreased means (CRL) observed, in comparison with offspring of nondiabetic rats. Treatment with *Nigella sativa* slightly increased but did not normalize CRL compared with control group. No abnormal changes in histology of neural tube were seen in evaluation of neural tube in all groups. Micrograph of diabetic rats treated with vehicle showed some abnormal projection on the apical surface of neuroepithelial cells. These changes were not seen in other groups. It seems *Nigella sativa* has a protective effect against diabetic embryopathy and fetal loss.

Keyword:

Nigella sativa, Diabetic embryopathy, Oxidative stress, Neural tube defect.

Introduction

Offspring of women with diabetes have a high incidence (6% to10%) of congenital Anomalies, which represent a twofold to fivefold increase over rates observed in The non diabetic populations (1).

These malformations arise at the beginning of organogenesis, during the 8 weeks of gestation in human embryos and during the first 7-10 days in mouse and rat embryos (2). Diabetic embryopathy can affect any developing organ system, although defects of the neural tube and heart are among the most common defects (3). Similar anomalies were also found in animal with diabetes, rendering them a valid experimental model for studying diabetic embryopathy (4).

A recently proposed mechanism for diabetes related embryonic maldevelop-

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ment is the increased production of reactive oxygen species (5). Increased glucose metabolism in embryo cells increases oxidative stress through a complex network of altered biochemical pathways, which combine to increase reactive oxygen species production and to decrease availability of GSH(glutathione) for free radical scavenging. Oxidative stress inhibits expression of pax_3 in neuroepithelial cells (6). Expression of Pax-3 regulates neural tube closure (7). Nevertheless, with impaired pax_3 gene expression, there is insufficient pax_3 protein to prevent accumulation of P_{53} protein. P_{53} then induces expression of genes which cause apoptotic cell death. Apoptotic neuroepithelium fails to proliferate, migrate, and form a closed neural tube, thereby leading to a NTD (neural tube defect) (6).

There has been increasing interest regarding the role and use of natural antioxidants as a means of preventing oxidative damage in diabetes due to high oxidative stress (8). The seeds of *Nigella sativa* L., an annual Ranunculaceae herbaceous plant, have been used traditionally for centuries in the Middle East, Northern Africa and India for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, influenza, eczema, as a diuretic, lactagogue and vermifuge (9). The black seed, *Nigella sativa* is known to contain>30% of fixed oil and 0/4-0/45% wt. /wt. of volatile oil. The volatile oil is known to contain 18.4-24% thymoquinone (TQ) and 46% of monoterpenes such as p-cymene and α-piene (10). Recently, clinical and animal studies have shown that the extracts of the black seeds have many therapeutic effects such as bronchodilator, immunomodilative (11), antibacterial (12), hypotensive (13), hepatoprotective (14) and antidiabetic. It has been shown that both the fixed oil of *Nigella sativa* as well as thymoquinone (the main compound of the essential oil), inhibit non-enzymatic lipid per oxidation in liposome (15). Using thin-layer chromatography (TLC), it has also been shown that compounds isolated from *Nigella sativa* (including thymoquinone, carvacol, t-anethole and 4-terpinol) have appreciable free radical scavenging properties (9). Thymoquinone is reported to possess a strong antioxidant property (16). Thymoquinone protects organs against oxidative damage induced by a variety of free radical generating agents (17).

In regarding to antioxidant properties of Nigella sativa, it was of interest to determine whether *Nigella sativa* extract has any effect on spinal cord neuroepithelium of diabetic rats' embryos.

Materials and methods

The *Nigella sativa* seeds were powdered mechanically. The extract was obtained by cold extraction method forty gram portions of the seed powder were extracted with 200ml n-hexan with stiring at room temperature for 24hr to give hexan extract. These processes were repeated three times and were added. The solvent was then completely removed under reduced pressure. The obtained oily extract from *Nigella sativa* seeds has a chestnut color and agreeable perfume.

The 70 to 90 days of age Female Sprague-Dawley rats, weighing 200 to 250g were used in the study. They were subjected to a cycle of 12 hours of light followed by 12 hours of dark at an ambient temperature of 220c, and they had free access to tap water and laboratory standard commercial food. The rats were distributed to 4 groups, 2 control groups (group1 and 2) and 2 diabetic groups (group3 and 4). Each group contained 5 female animals and 10 embryos were observed.

Diabetes was induced in rats from group 3 and 4 by a single intraperitoneal injection of freshly prepared streptozocin (45mg/kg, dissolved in citrate buffer 0/1mol/l; pH 4.8). The induction of diabetes was

confirmed by blood glucose levels>300mg/dl measured 24 and 48h after STZ injection. Blood was obtained from the tail vein and analyzed for glucose levels with the Glucometer Elit. Diabetic female rats were mated overnight with non diabetic males of the same strain, and vaginal smears were examined the next day for the presence of coagulation plaque. The presence of spermatozoa was established as day zero of pregnancy. In control group non diabetic female rats were mated overnight with nondiabetic male rats. The *Nigella sativa* treated rat groups (groups 2 and 4) received daily administrations of 1ml/kg body weight of *Nigella sativa* fixed oil by oral gavages from day 1 to day 17 of gestation. Other groups of animal (groups 3 and 4) were treated in an identical fashion with 1mg/kg body weight of water. Blood glucose level was measured on the 17th day of gestation and rats were sacrificed with chloroform, fetuses were released from yolk sacs and surrounding deciduas. The offspring were transferred to a Petri dish. The CRL of fetus was measured. A fetus was defined as normal if examination revealed normal body flexure and both anterior and posterior neuropore closure. The spinal cord was separated and cut to small pieces. Some of these pieces were fixed in formaldehyde solution, and histological slides were prepared, followed by hematoxylin-eosin staining by standard methods. Other pieces were fixed in glutaraldehiyde and osmium tetroxide and were prepared for being considered by transmission electron microscope. Images were examined for histopathological changes by a clinical pathologist who was masked to the treatment group from which the neural tube was removed.

Results

In vehicle-treated diabetic group the mean blood glucose level during the pregnancy days (1 to 17) was significantly higher than nondiabetic animals. Daily gavages of *Nigella sativa* 1mg/kg significantly alter fed-state glucose levels, in diabetic rats. The administration of *Nigella sativa* to diabetic rats has made a significant reduction on plasma glucose level (Fig. 1). Pregnancy outcome: the number of implantation per litter was similar in all groups. However, the percentage of absorptions per litter was significantly elevated in vehicle-treated diabetic rats, in comparison with vehicle treated no diabetic rats and *Nigella sativa* treated diabetic rats. Treatment with *Nigella sativa* was significantly reduced absorption rates in diabetic rats (Fig. 2).

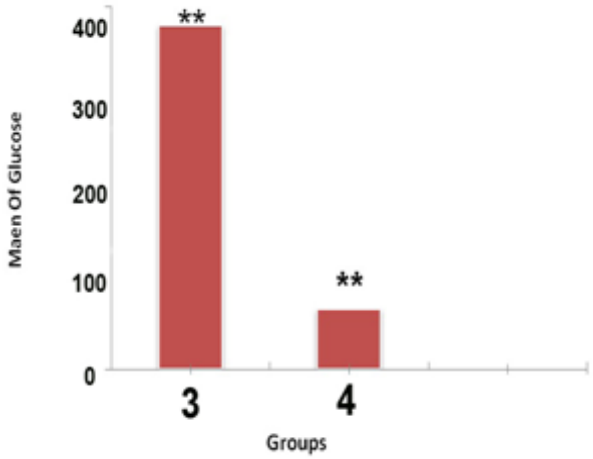

Fig. 1: Glucose amount between group 3 and 4. The administration of *Nigella sativa* to diabetic rats has been produced a significant reduction on plasma glucose level $p<0.05$.

To estimate embryonic development, we measured fetal crown-rump length (Fig. 3). In offspring of vehicle-treated diabetic rats (group 3); a significantly decreased mean crown-rump length was observed, in comparison with offspring of nondiabetic rats (group 1). Treatment with *Nigella sativa* slightly increased crown rump-length but did not normalize CRL compared with control group.

Neural tube defect: neural tube defect was significantly seen in embryos of vehicle treated diabetic rats. In control groups and *Nigella sativa* treated diabetic rats were not seen neural tube defects. In evaluation of neural tube in all groups with light microscope and hematoxylin-eosin staining, any abnormal changes in histology of neural tube weren't seen (Fig. 4).

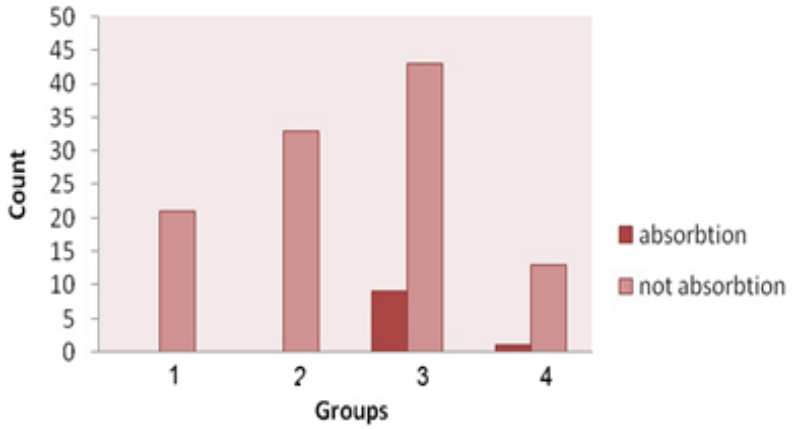

Fig. 2: Rate of absorption between different groups. The percentage of absorption was significantly reduced in *Nigella sativa* treated diabetic rats. P<0.05.

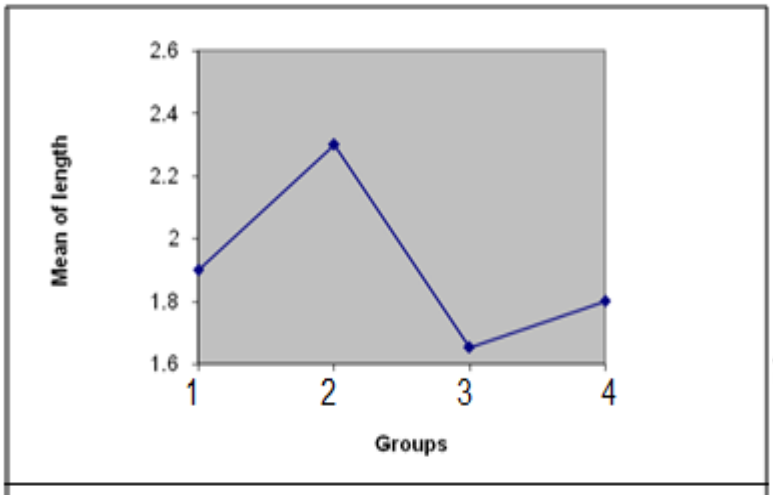

Fig. 3: comparison between CRL in different groups. Treatment with *Nigella sativa* slightly increased crown rump length. p<0.05.

Micrograph of electronic microscope of neural tube in diabetic rats treated with vehicle showed some abnormal projection on the apical surface of neuroepithelial cells (Fig 5, 6, 7). These changes weren't seen in the control groups and the diabetic group treated with *Nigella sativa* extract. Therefore it seems treatment with *Nigella sativa* extract has protective effects on neural tube defect and neuroepitelial cells and it can reduce neural tube defect in embryos of diabetic rats.

Discussion

In the present study we examined the therapeutic effect of *Nigella sativa* as an antioxidant agent against fetal malformations in diabetic pregnancy by using an animal model (streptozocin-induced diabetes). Streptozocin caused hyperglycemia in rats and also caused neural tube defect in embryos of diabetic rats. The mechanisms behind free radicals and the possible role of oxidative stress in the pathogenesis of diabetes and diabetic complications have been extensively studied for years in patients and animal models (14).

Numerous studies have found increased lipid peroxides or reactive oxygen species (ROS) and oxidative stress or both in different animal models of diabetes (18). Oxidative stress refers to an imbalance between the intracellular production of free radicals and the cellular defense mechanism. Protein, lipids, and DNA are sensitive target of ROS. An excess availability of free radicals accompanied by a reduction in the capacity of the natural antioxidant systems leads to cellular dysfunction and death (19). Free radical-induced DNA damage has been shown to be increased in embryos from diabetic rats (20). In this study *Nigella sativa* treatment was efficient in reducing blood glucose in diabetic rats. In *Nigella sativa* treated diabetic rats the blood glucose was significantly reduced. And this finding show *Nigella sativa* was affected on diabetes. etalutsop saw tI that the basis of the beneficial effect of *Nigella sativa* in diabetes might be its antioxidant property(21). Fetal size was significantly reduced in diabetic rats, in this study the CRL of embryos was reduced in diabetic rats but in *Nigella sativa* treated group reduction of embryo's size or CRL was lesser but effect of *Nigella sativa* on CRL in diabetic rats was small. *Nigella sativa* caused small increase in CRL in *Nigella sativa* treated group. Some antioxidant such as *Nigella sativa* and lipoic acid had effect on CRL in diabetic rats. Previous study had shown lipoic acid had efficient effect in increase embryo's CRL in diabetic rats (22).

Treatment with *Nigella sativa* slightly increased size of embryos in diabetic rats. Other most important findings in this study were positive effects of *Nigella sativa* on diabetes induced both embryonic malformation and absorption.

In previous study had shown antioxidant agents such as lipoic acid, vitamin E, folic acid and ergothionein cause reduction in embryo malformations and absorption. This study showed some abnormal projection on the surface of neuroepitelial cell in vehicle treated diabetic rats by TEM. The structure of neural tube in *Nigella sativa* treated group was similar to the control group. It seems *Nigella sativa* similar to other antioxidant agents causes reduction in neural tube defect. *Nigella sativa* oil has many antioxidant agents and it’s most important component is thymoquinone (15).

thymoquinone can reduce reactive oxygen species production indirectly and inhibit lipid peroxidation (23). *Nigella sativa* also had positive effect on reduction of embryo's absorption and causes increase in resorption in diabetic rats.

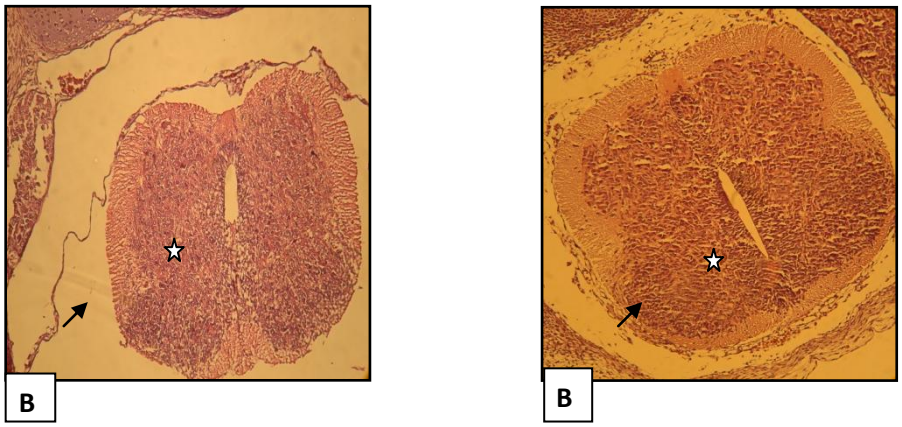

Fig. 4: light microscopic images of group3 (A) and 4(B). Wight matter (➔), gray matter (☆). (H&E x=100).

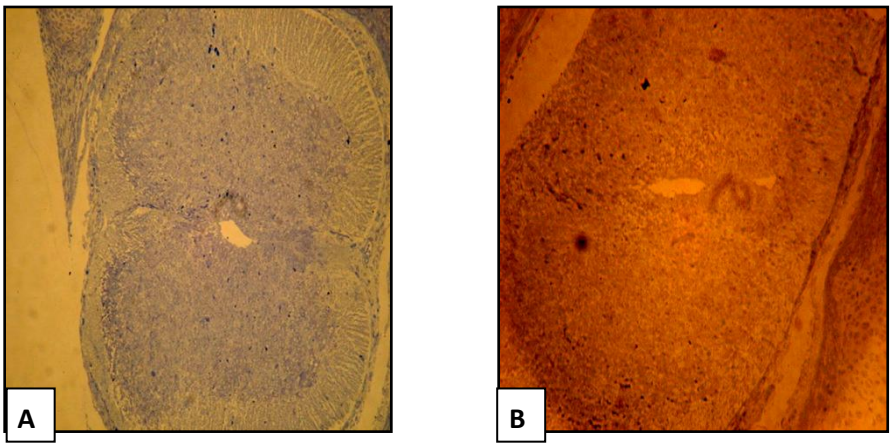

Fig. 5: semitin images of group3 (A) and 4(B). (tuloidiane blue x=100).

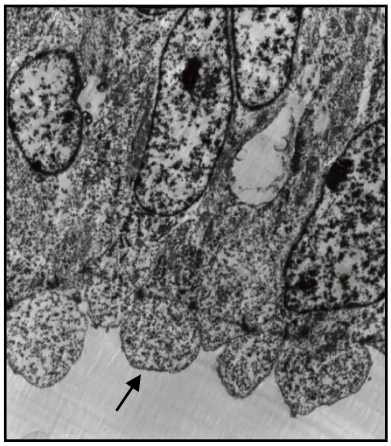

Fig. 6: Transmission electron micrograph of neural tube in group 3(vehicle treated diabetic rats). Abnormal projection (➔) on the apical surface of neuroepithelial cells (x=3900).

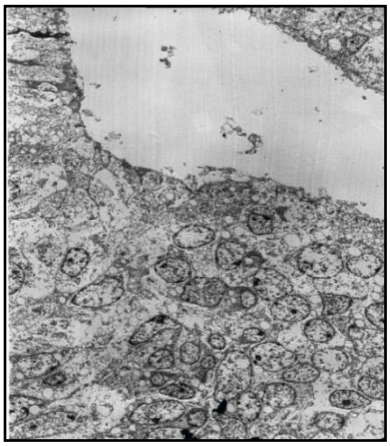

Fig. 7: Transmission electron micrograph of neural tube in group 4 (Nigella Sativa treated diabetic rats) (x=1200).

Conclusion

Our findings suggest that *Nigella sativa* extracts may have a protective effect against diabetes-related embryopathy and absorption.

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