Effect of *Nigella Sativa* on the integrity of parotid salivary gland of albino rats and its activity for insulin and glucagon

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Abstract: This study investigated the effect of *Nigella Sativa* (*N.sativa*) on the histological features of parotid salivary glands of aging albino rats and its role on the activity of the glands for secretion of insulin- and glucagon-like peptides. Forty five male albino rats aged ten months were kept on the laboratory diet over a period of two months. Then, they were divided into three equal groups; young control (sacrificed at the end of the two months), old control (sacrificed three months later) and the experimental group (supplemented with *N. sativa* in a daily dose of 300mg/200gm body weight over a period of three months and then sacrificed). The parotid glands were then dissected out and subjected to histological and immunohistochemical investigations. The results showed only minimal amount of fibrosis and inflammatory cell infiltration in the *N. sativa* supplemented group. There were no distinctive changes in the architecture of the glands compared to that of young control. They did not show the prominent extensive features of aging manifested in the old aged control group. Moreover, the *N. sativa* supplemented group showed obvious increase in immunohistochemical reactivities for insulin and glucagon in the glandular tissue when compared to the rats of old control. Finally it could be concluded that *N.sativa* has got a cytoprotective effect against the degenerative changes of age and a beneficial role on the integrity of parotid salivary glands of aged rat. Also, *N. sativa* has been shown to increase the activity of parenchymal cells of rat parotid gland for insulin and glucagon that was markedly diminished with advance of age.

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1. Introduction:

The seeds of *Nigella sativa* commonly known as black seed or Habbat al-Barakah are used in folk (herbal) medicine all over the world. They are commonly used for the treatment and prevention of a number of diseases.According to **EL Bokhari, 2001** it is most famous for the tradition of **the holy prophet Muhammad (sws)** "*Hold on to the use of the black seed because it has a remedy for every illness except death".*

The seeds contain both fixed and essential oils, proteins and alkaloids. The pharmacological action of the crude extracts gives protection against nephrotoxicity and hepatotoxicity induced by either diseases or chemicals. The seeds are also characterized by a very low degree of toxicity. Moreover, the seeds/oil have anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activities. Also, the oil has been found to decrease blood pressure and improve respiration. **(Ali and Blunden, 2003)Turkdogan *et al.,* 2001** compared the role of different antioxidants such as Vitamin C (Vit C), Vitamin E (Vit E), Selenium and *N. sativa* on the prevention of liver fibrosis in rabbits. They found that *N. sativa* might partly be successful in the prevention of liver fibrosis, while Vit E plus Selenium and Vit C had little therapeutic effect or seemed to be ineffective. *Nigella sativa* has been found to have antidiabetic effect, **Farah *et al.,* 2002** and **Kanter *et al.,* 2003** reported that the hypoglycemic effect of *N. sativa* in diabetic rats resulted from a stimulatory effect on pancreatic β-cell function with consequent increase in serum insulin level. These results indicated that *N. sativa* has insulinotropic properties.*Nigella sativa* oil and thymoquinone (active ingredient) both possess potent anti-inflammatory effects on several inflammation-based models including experimental encephalomyelitis, colitis, peritonitis, edema, and arthritis. They resulted in suppression of the inflammatory mediators prostaglandins and leukotriens. **(Salem, 2005).** Moreover**, El-Dakhakhny *et al.,* 2000** and **Al Ghamdi, 2001** supported the use of *N. sativa* in medicine as an analgesic and anti-inflammatory agent.*Nigella sativa* has been shown to have antineoplastic effect as the volatile oil of *N. sativa* has been found to inhibit colon carcinogenesis of rats as well as inactivating human breast adenocarcinoma, unveiling opportunities for promising results in the field of

prevention and treatment of cancer. **(Salim and Fukushima, 2003; Farah and Begum, 2003)Smith and Toms, 1986** reported that the salivary glands are a known source of several biologically active peptides and hormones. Various reports indicated that these glands contain and secrete peptides with immunological similarity to such pancreatic hormones as insulin, glucagon and somatostatin. They described an Avidin-Biotin immune-cytochemical technique to localize cells containing insulin- or glucagon-like peptides in the major salivary glands of rats. Cells with insulin-like staining were observed in the intercalated ducts of both parotid and submandibular glands. A discrete population of cells with intense glucagon-like immunostaining was associated with the acini of all three major salivary glands. The biosynthesis of insulin-like material in rat and human parotid glands was confirmed in vitro by a specific separation method using anti-insulin antibody. These findings suggested that the parotid gland may be a further extra-pancreatic source of insulin, and that insulin biosynthesis does occur in extra-pancreatic tissues**(Murakami *et al.*,1982)**.Therefore, the aim of the current research was to study the effect of N. Sativa on the histological structure of parotid salivary glands of aging albino rats and to detect the effect of the black seeds on the activity of the glands for secretion of insulin- and glucagon- like peptides.

2. Materials and Methods

The study was carried out on forty five male albino rats aged ten months (adult). The animals were obtained from the "Animal research Institute ''of Faculty of Medicine, Cairo University, where they were properly observed since the day of birth till they reached the adequate age. They were kept under strict supervision by veterinarians. The rats were housed under controlled laboratory conditions (room temperature 23 ±2^{o}C and humidity (60± 5%).They were fed on a standardized laboratory balanced diet over a period of two months. The rats aged one year are considered to be at the end of the adult period**. (Vongvatcharanon *et al.,* 2006).** At this age, the animals were divided equally into three groups as follow:

Group 1: It comprised the young rats aged one year which were sacrificed by cervical dislocation. The parotid glands were dissected out and prepared for examination.

Group 2: The rats were kept on the laboratory diet over a period of three months to reach old age.

Group 3: The rats were fed the laboratory diet supplemented with *Nigella sativa* in a pulverized form. The supplementation was continued over a period of three months in a daily dose of 300mg/200gm body weight. This dose has been considered to give the optimal satisfactory healing properties for many diseases. **(Khanam and Dewan, 2008).**

At the end of the experimental period, the rats of groups 2 and 3 were sacrificed by cervical dislocation and the parotid glands were dissected out and prepared for examination as group1**.** Fixation was performed in neutral formol. Dehydration was carried out in ascending grades of ethyl alcohol, and then the specimens were cleared in xylene and mounted in paraffin wax. The prepared sections were 3-4 microns in thickness and were subjected to the following investigations:

I) Histological examination:

1- Haematoxylin & eosin (H&E) staining:

The routine H&E staining was performed to examine the histological features of the parotid salivary gland and to detect any structural change.

2-Masson's Trichrome staining:

The technique was carried out for examination of collagen and fibrosis. **(Lillie, 1954)**

II) Immunohistochemical investigation:

Avidin-biotin technique was used for localization of insulin- and glucagon- like peptides in the parotid salivary gland tissues. **(Smith and Toms, 1986)**

3. Results

In old rats(group 2), there was loss of the normal architecture of the rat parotid salivary gland. The acinar cells were hypertrophied and exhibited cytoplasmic vacuolation. At several sites, groups of acini were seen compacted together and showing ill defined cell boundaries. The cytoplasm of the acinar cells showed extensive decrease in basophilic stain by age, so that it appeared with similar stain to the ductal cells. The nuclei appeared with gross alteration in size, shape and chromatin density. Irregular, thick and dense bands of fibrous tissue densely infiltrated with chronic inflammatory cells surrounded the duct system and particularly the excretory ducts. They appeared formed of numerous heavy and thick concentric layers. Fibrosis was also manifested in the blood vessels wall, so that they appeared as thick, regular and fibrous rings enclosing RBC's. With respect to the ducts, they also showed cytoplasmic vacuolations and discontinuity of their lining cells.

However, in old supplemented rats(group 3) the parotid gland did not show the prominent extensive features of aging manifested in the previous group. The acini were also hypertrophied and obliterating the spaces in between, but no evidence of fibrosis was detected. Delicate connective tissue intervened between the acini simulating the young age group. No apparent decrease in the number of nuclei was noticed in the parenchymal elements. They displayed regular shape, size and chromatin density. Also, the ductal cell lining was intact and no signs of

vacuolations appeared in the cytoplasm. No noticeable change appeared in vascularity by age. Only minimal amount of fibrous tissue and inflammatory cell infiltration were detected surrounding the duct system when compared to the old unsupplemented group (plates 1 and 2).

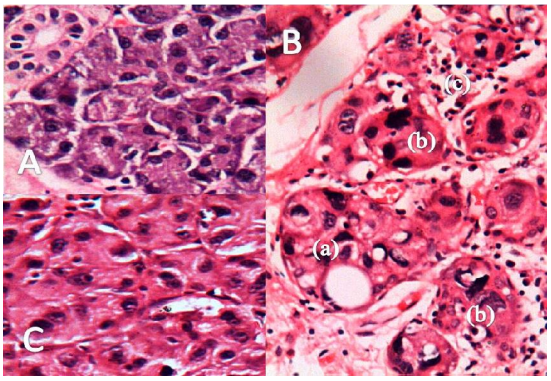

Plate 1: photomicrograph of the histological structure of parotid glands. A: normal architecture of parotid gland appeared in young rats B: In old age,the acini showed cytoplasmic vacuolation(a),gross alteration in shape, size and chromatin density apperared in the nuclei(b) and fibrous tissue infilterated with chronic inflammatory cells separated the parenchymal elements(c). C:the acini in group 3 were hypertrophied and showed regular cell lining with absence of vacuolations,fibrosis and dense inflammatory cell infilteration. H&E, Orig. Mag. 200.

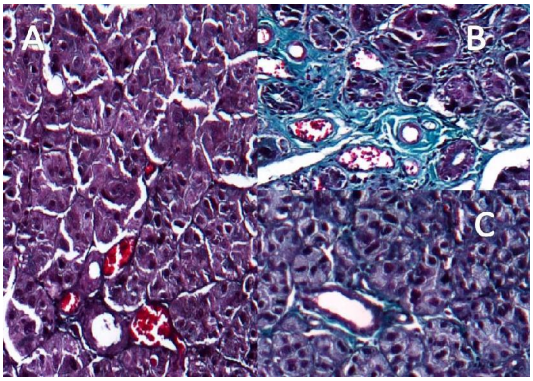

Plate 2: photomicrographs showing the fibrous tissue of parotid glands. A:the young age group showed absence of fibrosis. B: extensive fibrosis appeared around duct system and blood vessels and separated acini in old age group. C: minimal amount of fibrous tissue surrounded the ducts in old rats receiving N.sativa. Masson's Trichrome, Orig. Mag. 100.

Regarding the immunohistochemical results, the parotid salivary gland of adult rats (group 1) showed weak to mild reactivities for insulin and glucagon.

However, the old aged rats (group 2) showed marked decrease in immunoreactivity for insulin so that the parenchymal elements of the salivary gland showed negative reaction for the peptide. Whereas, very weak cytoplasmic immunoreactivity was expressed to glucagon by age. On the other hand, the old supplemented rats (group 3**)** showed obvious increase in immunohistochemical reactivities to both insulin and glucagon in the parotid salivary gland tissue (plates 3 and 4).

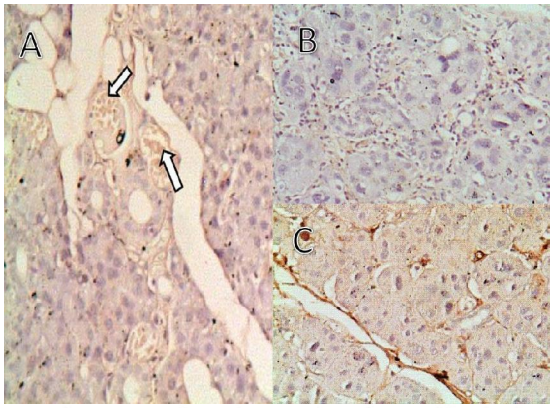

Plate 3 : photomicrographs of parotid glands showing insulin reactivity. A: the parenchymal cells of young rats showed weak to mild immunoreactivity and moderate reaction appeared within the B.Vs. B: negative reaction for insulin appeared in parenchymal tissue of old rats. C: obvious increase in insulin immunoreactivity appeared in parenchymal tissue of group 3. Insulin(DAB),Orig. Mag. 100

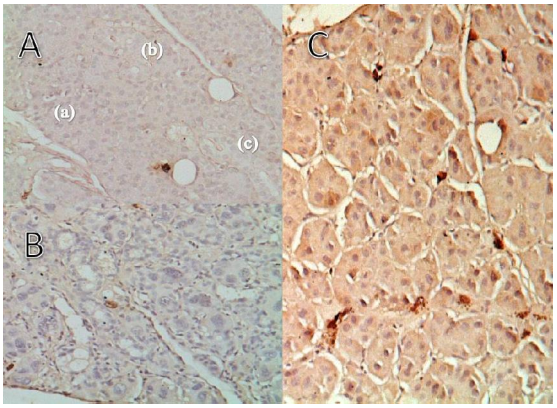

Plate 4 : photomicrographs of parotid glands showing glucagon reactivity. A: the parenchymal cells of young rats showed weak to mild cytoplasmic reaction(a) and weak reaction in intercalated duct(b) & striated duct (c). B: decreased reactivity for glucagon appeared in parenchymal tissue of old rats. C: noticeable increase in reaction for glucagon appeared in parenchymal tissue of group 3. Glucagon(DAB),Orig. Mag. 100

4. Discussion

In the current research, the parotid salivary glands of old non-supplemented rats revealed massive increase in fibrous tissue with the advance of age. Multiple heavy layers of fibrous tissue, densely infiltrated with chronic inflammatory cells surrounded the duct system and blood vessels in concentric manner. However the absence of fibrosis together with the minimal amount of inflammatory cells observed in old supplemented group might be correlated to the antioxidant effect of *N. sativa* and its role on the integrity of the fibroblasts. This assumption is based on the fact that high number of unstable free radicals produces chromosomal changes, collagen alteration and fibrosis **(Willingham, 1999)**.

Moreover**, Turkdogan *et al.,* 2001** and **Kanter *et al.*, 2005** found that *N. sativa* might be successful in the prevention of liver fibrosis possibly through immunomodulator and antioxidant activities. *N. sativa* was also found to have anti-inflammatory properties as reported by **El-Dakhakhny *et al.,* 2000** and **Al Ghamdi, 2001.** Furthermore, valuable amount of unsaturated fatty acid as lonolenic acid which stabilizes the cell membrane and inhibits inflammation has been identified in the black seeds **(Charkravarty, 1993)**.

In the present investigation, the acini by aging appeared hypertrophied with decreased number of lining cells. Their cytoplasm revealed decreased basophilia so that they were not easily distinguished from the ductal cells. Moreover, numerous areas of vacuolation were detected. The nuclei revealed reduction in number as well as gross alterations in size, shape and chromatin density. On the other hand, no sign of cytoplasmic vacuolations were detected in the salivary gland of old rats supplemented with *N. sativa*. The parenchymal cells were intact and the lining cells were continuous. Similar findings were reported by **Musa *et al.,* 2004** as they found that treatment with *N. sativa* extract resulted in improvements of the morphological features of stressed cells, along with a reduction in cytoplasmic vacuoles and cell membrane blebbing because of its antioxidants properties.

In the current study, the parotid salivary gland in *N. sativa* supplemented group presented normal structural morphological features for the nuclei in parenchymal tissue. The size, shape and chromatin density appeared almost similar to the young age group. The nuclear shape changes were considered by **Robertson *et al.*,2000** as a stage in the apoptotic morphological events. *N. sativa* has been found to have a protective role against progressive apoptosis as demonstrated by **Worthen *et al.,* 1998.** It decreased the expression of growth inhibitory factors or apoptotic factors. This antiapoptotic effect might be related to its antioxidant properties since **Willingham,1999** concluded that the cumulative effect of free radicals on the cell make it unable to resist stress and lead to death.

In the present study, the wall of the blood vessels appeared with normal thickness in the N.Sativa supplemented group. *N. sativa* has been shown in a previous study to prevent atherosclerosis in rabbit **(Asgari *et al.,* 2007)** The antioxidant properties of the seeds might protect the blood vessels from the destructive effect of free radicals. A decreased incidence of atherosclerosis has been demonstrated by **Khattab and Nagi, 2007.** They found that oral supplementation of *N. sativa* protected rats from induced hypertension by a mechanism related to its ability to scavenge superoxides.

In the present study, the rat parotid salivary gland showed immunoreactivity to insulin and glucagon among the acini and ducts,but their expression in parenchymal tissue decreased with the advance of age. Insulin reactivity in particular, was markedly diminished so that the acini and duct system presented negative staining. This investigation substantiates the results of **Chang and Halter, 2003** who reported lower levels for insulin in older people suggesting β-cells dysfunction. They reported a high prevalence of type 2- diabetes and post challenge hyperglycemia in the older population.

Increased immunoreactivity among the acini and duct system to insulin and glucagon was detected in the glands of old supplemented rats of the present investigation. This finding supports the antidiabetic role of N. Sativa as well as maintaining the integrity of the tissues and decreasing the expression of growth inhibitory factors or apoptotic factors. **Farah *et al.,* 2002** and **Kanter *et al.,* 2003** assumed that the hypoglycemic action of *N. sativa* was partly due to amelioration in the beta-cells of pancreatic islets causing an increase in insulin secretion which indicated that *N. sativa* oil has insulinotropic properties.

Kaleem *et al.,* 2006 confirmed that N.Sativa has an antidiabetic activity through its antioxidant properties. Furthermore, **Maritin *et al.,* 2003** concluded that insulin reactivity is ameliorated by antioxidants. However, **Houcher *et al.,* 2007** stated that the antidiabetic action of *N. sativa* was partly dependent on decreasing liver gluconeogenesis and was neither mediated through elevating plasma insulin levels nor by suppression of the intestinal absorption of glucose.

In agreement with **Donati *et al.,* 2008** glucagon reactivity in the present study was markedly reduced by age. However the increased perinuclear reactivity to glucagon demonstrated in the parenchymal cells of old supplemented group might

indicate a potentiating effect of *N. sativa* for biosynthesis of glucagon material which was impaired in old non-supplemented group. **In 2005, Salem** attributed the medicinal properties of the seed oil and its active ingredient, thymoquinone, to the antioxidant properties scavenging free radicals, neutralizing toxic products induced by insults and maintaining cell integrity

5. Conclusions:

N. sativa has got a beneficial role on the integrity of parotid gland in aging rats.It. exerted a cytoprotective effect against the degenerative changes of age. It increased the activity of rat parotid gland to insulin & glucagon which were markedly diminished with the advance of age. Therefore we suggest that *N. sativa* might have an analogous protective role on the integrity of alpha & beta pancreatic cells, thereby decreasing the incidence of diabetes by age. Further attention should be paid to herbs in folk medicine particularly if they have a prophetic reference. They might be beneficial in the treatment of a lot of incurable diseases.

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