## بسم الله الرّحمن الرّحيم قالوا سُبْحَانَك لا عِلْمَ لَنَا إلاّ مَا عَلَمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ صدق الله العظيم

اية ( ٣٢) سورة البقرة

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

#### **Thesis**

Submitted to the Faculty of Oral and Dental Medicine
Cairo University
In partial fulfillment of the requirements
of the Master Degree in Oral Biology

#### PRESENTED BY

#### Radwa Taher Mostafa Elsharkawy

B.D.S
Faculty of Oral and Dental Medicine
Cairo University
2010

## Supervisors

Prof. Dr. Samía Mostafa Kamal
Professor Doctor
Oral Bíology Departement
Faculty of Oral and Dental Medicine
Cairo University

Dr.Rehab Alí Abdel Moneim

Lecturer

Oral Biology Departement

Faculty of Oral and Dental Medicine

Cairo University

## **Contents**

Introduction	1
Review of literature	3
Nigella Sativa	3
Chemical composition of N.Sativa	4
Protective role of N. sativa	5
Antioxidants properties of N.Sativa	6
Antidiabetic effect of N.Sativa	7
Effect of N.Sativa on liver and kidney	10
Anti-inflammatory and analgesic properties of N.Sativa	11
Antineoplastic effect of N.Sativa	13
Hematological effect of N.Sativa	15
Other activities of N.sativa	16
Adverse effects and toxicity of N.Sativa	17
Parotid gland	18
Metabolic upsets in Diabetes Mellitus	23
Aim of the study	26

Materials and Methods	27
Results	34
Histological results	34
Immunohistochemical results	55
Discussion	65
<b>Summary</b>	82
Conclusions	85
References	87
Arabic summary	

## <u>list of figures</u>

Figures	Page
Fig.1:Photomicrograph of the parotid gland of young rat (group 1 )showing the normal architecture of the gland. H&E,Orig. Mag.100	37
Fig.2:Photomicrograph of parotid gland of group 1 showing secretory terminal portion, striated duct, connective tissue septa ,loose connective tissue , blood vessels (B.Vs )& capillaries. H&E,Orig.Mag.200.	37
Fig.3:Photomicrograph of parotid gland of group 1 showing intercalated duct and B.Vs. H&E,Orig.Mag.200.	38
Fig.4: Photomicrograph of parotid gland of group 1 showing excretory duct & B.V engorged with RBCs . H&E,Orig.Mag.200.	38
Fig.5: Photomicrograph of the parotid gland of old rat (group 2) showing decreased basophilia in the cytoplasm, vacuolation in acini , dense fibrous tissue separating the parenchymal elements & thick B.Vs . H&E, Orig.Mag.100	42
Fig.6: Higher magnification of the previous figure showing vacuolation in acini & dense fibrous tissue separating the parenchymal elements .  H&E, Orig.Mag.200	42
Fig.7:Photomicrograph of parotid gland of group 2 showing alteration in shape, size and chromatin density of nuclei, areas of extravasation intralobularly and inflammatory cells infiltration.  H&E,Orig.Mag.200.	43

basophilia of the cytoplasm and hypertrophied acini. H&E,Orig.Mag.100	43			
Fig.9: Photomicrograph of parotid gland of group 2 showing thick and dense bands of fibrous tissue with chronic inflammatory cells infiltration surrounding the excretory ducts & thickened B.Vs .  H&E,Orig.Mag.100				
Fig.10:Photomicrograph of the parotid gland of group 2 showing extensive fibrosis and dense inflammatory cells infiltration, thickened B.Vs, extravasation and vacuolation in duct cells.  H&E,Orig.Mag.200	44			
Fig.11: Photomicrograph of the parotid gland of old rats of group 3 that had been receiving N.Sativa showing intact hypertrophied acini, secretory ducts with regular cell lining & B.Vs with normal thin wall. H&E,Orig.Mag.100.				
Fig.12: Higher magnification of parotid gland of group 3 showing the intact hypertrophied acini & minimal inflammatory cells infiltration .  H&E,Orig.Mag.200.				
Fig. 13:Photomicrograph of the parotid gland of group 3 showing decreased basophilia of the cytoplasm, mild inflammatory cells infiltration with minimal amount of fibrosis around the excretory ducts and B.Vs .  H&E,Orig.Mag.100.				
Fig.14:Photomicrograph of the parotid gland of group 3 showing normal intact secretory terminal portion ,minimal fibrosis, minimal amount of inflammatory cells infiltration & B.Vs wall with normal thickness.  H&E.Orig.Mag.100.				
Fig.15: Photomicrograph of the parotid salivary gland of group 1 showing absence of fibrosis in the young age group.  Masson's Trichrome.Orig.Mag.100.	51			
Fig.16: Higher magnification of the previous figure showing thin collagen fibers around the duct and B.Vs (arrows).  Masson's Trichrome.Orig.Mag.200.	51			

Fig.17: Photomicrograph of parotid salivary gland of group 2 showing dense & thick bands of collagen fibers extending throughout the whole glandular structure and form several layers of concentric rings around the ducts & B.Vs. Masson's Trichrome.Orig.Mag.100.	52
Fig.18: Higher magnification of the parotid salivary gland of group 2 showing the thick network of collagen separating & encircling the parenchymal elements of the gland.  Masson's Trichrome.Orig.Mag.200.	52
Fig.19: Photomicrograph of parotid salivary gland of group 2 showing a thick band of fibrous tissue running between the acini & encircling the blood capillaries .  Masson's Trichrome.Orig.Mag.200.	53
Fig.20: Photomicrograph of the parotid salivary gland of group 3 showing minimal amount of fibers around the parenchymal elements & B.Vs. Masson's Trichrome.Orig.Mag.60.	53
Fig.21: Photomicrograph of the parotid salivary gland of group 3 showing little amount of fibers surrounding the large ducts Masson's Trichrome.Orig.Mag.100.	54
Fig.22: Higher magnification of the parotid salivary gland of group 3 showing complete absence of fibrosis inbetween the parenchymal elements.  Masson's Trichrome.Orig.Mag.200.	54
Fig.23: Photomicrograph of the parotid salivary gland of group 1 showing weak to mild cytoplasmic immuno-reactivity for insulin in acinar cells, with stronger reaction in few scattered nuclei & weak cytoplasmic reaction in intercalated & striated ducts.  Insulin (DAB),Orig.Mag.100.	57
Fig.24: Photomicrograph of the parotid salivary gland of group 1 showing moderate immuno-reactivity to insulin within the blood vessels & mild to moderate reaction in connective tissue stroma.  Insulin (DAB),Orig.Mag.100.	57

Fig.25: Photomicrograph of the parotid salivary gland of group 2 showing negative reaction for insulin in the parenchymal elements & moderate to strong reaction in the connective tissue stroma & B.Vs. Insulin (DAB),Orig.Mag.100.	58
Fig 26: Photomicrograph of the parotid salivary gland of group 3 showing increased cytoplasmic reaction to insulin in the parenchymal elements and stronger perinuclear reactivity & moderate reaction in the B.Vs . Insulin (DAB),Orig.Mag.100.	58
Fig 27: Photomicrograph of the parotid salivary gland of group 3 showing increased cytoplasmic reaction to insulin in the acinar cells, stronger perinuclear reactivity & moderate to strong immuno-histochemical reaction in the connective tissue.  Insulin (DAB),Orig.Mag.100	59
Fig.28: Photomicrograph of the parotid salivary gland of group 1 showing weak to mild cytoplasmic reaction for glucagon in the acini, weak reaction in intercalated duct & striated duct & isolated areas of strong perinuclear reactivity.  Glucagon (DAB),Orig.Mag.100.	62
Fig.29: Photomicrograph of the parotid salivary gland of group 2 showing decreased reactivity for glucagon among the acini ,ducts & connective tissue stroma .  Glucagon (DAB),Orig.Mag.100.	62
Fig.30: Photomicrograph of the parotid salivary gland of group 3 showing noticeable increase in reaction for glucagon in the acini, more frequent & increased perinuclear reactivity.  Glucagon (DAB),Orig.Mag.100.	63
Fig.31: Photomicrograph of the parotid salivary gland of group 3 showing a slightly increased reaction for glucagon in the intercalated duct, striated duct & mild to moderate reaction within the B.Vs . Glucagon (DAB),Orig.Mag.100.	63
Fig.32: Photomicrograph of the parotid salivary gland of group 3 showing noticeable increase in glucagon reactivity in the excretory ducts . Glucagon (DAB),Orig.Mag.100.	64

## <u>Acknowledgement</u>

I would like to express my deepest appreciation and thanks to my supervisor professor Dr. Samia M.Kamal, professor of Oral Biology, Faculty of Oral and Dental medicine, Cairo University for her great effort, patience and support. Without her and her beneficial advices, this work would have never been accomplished in such manner. Actually I am very lucky to have such a wonderful person as my supervisor. I gained an experience and learned alot from working with her. I will always be thankful and grateful for her.

Also, I will never forget the valuable assistance and support of my supervisor Dr. Rehab A. Abdel Moneim, lecturer of Oral Biology, Faculty of Oral and Dental medicine, Cairo University.

## To my family

To my family for the love and support you have given me. They always have been there for me and my daughter caring and helping till I finished this thesis.

To my beloved husband who has been a great help to me. He was always there when I needed him and went through many difficulties in order to support and assist me in performing such work.

And finally to my beautiful daughter Salma. Her beautiful smile shine up my days and keeping me going on. And I think because of her "Allah" was always there for me.

## **Introduction**

The seeds of Nigella Sativa (N.Sativa) plant have been used to promote health and fight disease for centuries especially in the Middle East and Southeast Asia. In South Asia, it is called "Kalonji", its Arabic name is "Habat-ul-Sauda" and its English name is "Black cumin". It is also known in the Middle Eastern countries as "Habat-al-barakah". The plant is widely grown in different parts of the world and is an annual herb cultivated in India. As an oriental spice, N. Sativa has long been used as a natural remedy for the treatment of many acute diseases as fever, diarrhea, microbial diseases and inflammation as well as chronic diseases such as bronchial asthma, diabetes and hypertension. This plant has been a great focus of research and has several traditional uses and consequently has been extensively studied for its chemical constituents and biological activities. (Najmi et al., 2008)

Kaleem et al., 2006 reported that oral administration of N. Sativa seed to diabetic rats for 30 days significantly reduced the elevated levels of blood glucose, lipids and improved altered levels of lipid peroxidation products and antioxidant enzymes in liver and kidney. The results confirmed the antidiabetic activity of N. Sativa seeds extract and suggested that because of its antioxidant effects its

administration might be useful in controlling the diabetic complications in experimental diabetic rats.

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 extrapancreatic source of insulin, and that insulin biosynthesis does occur in extrapancreatic tissues.

(Murakami , Taniguchi & Baba ,1982)

Smith & Toms, 1986 reported that 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 immunocytochemical technique to localize cells containing an insulin- or glucagon-like peptide in the major salivary glands of rats. Cells with insulin-like staining were observed in the intercalated ducts of both the parotid and submandibular glands. A population of cells discrete with intense glucagon-like immunostaining was associated with the acini of all three major salivary glands.

## **Review of literature**

### Nigella sativa

Archeological evidence about the earliest cultivation of N. Sativa is still scanty. Seeds of this condiment had been found in several sites from ancient Egypt including Tutenkhamen's tomb. Although its exact role in Egyptian culture is unknown, we do know that items entombed with a pharaoh were carefully selected to assist him in the after life. N.Sativa was a traditional condiment of the Old World during classical times; and its black seeds were extensively used to flavor food. The seeds have been traditionally used in the Middle East and Southeast Asian countries to treat ailments including asthma, bronchitis, rheumatism and related inflammatory diseases. It was also used to increase milk production in nursing mothers, to promote digestion and to fight parasitic infections. Its oil has been prescribed to treat skin conditions such as eczema and boils and to treat cold symptoms. The various uses of nigella has earned for this ancient herb the Arabic approbation 'Habbat al barakah' meaning the seed of blessing.(Zohary and Hopf,2000)

**In Islam**, it is regarded as one of the greatest forms of healing medicine available. **The holy prophet Muhammed (sws)** once stated that the black seed can treat every disease except death as