



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY



“The Effect Of Titanium Dioxide Nanoparticles And Its Withdrawal On Submandibular Salivary Gland Of Albino Rats”

Histological and Immuno-Histochemical study

Thesis submitted in partial fulfillment for Master Degree in Oral Biology, Faculty of Dentistry, Ain Shams University

By

Mark Mikhles Fekry Tawadrous

B.D.S (2012)

Faculty of Dentistry, Cairo University

Demonstrator in Oral Biology Department, Faculty of Dentistry,
The British University in Egypt

Supervisor

Prof. Dr. Medhat Ahmed El-Zainy

Professor of Oral Biology

Former Vice Dean of Society and Environmental Affairs

Faculty of Dentistry, Ain-Shams University

Co-supervisor

Prof. Dr. Reham Magdy Amin

Professor of Oral Biology

Faculty of Dentistry, Ain-Shams University

**Faculty of Dentistry
Ain-Shams University
2020**

Acknowledgement

First, thanks to **GOD**, the most gracious and the most merciful. The completion of this thesis wouldn't have become possible without the help and support of several people to whom I'm greatly in debt. I believe it is my duty to register here my deep gratitude to them.

I would like to thank **Prof. Dr. Medhat Ahmed El-Zainy**, Professor of Oral Biology, Former Vice Dean of Society and Environmental Affairs, Faculty of Dentistry, Ain Shams University, for his support, continuous encouragement and appreciated suggestions that guided me to accomplish this work.

I want to express my deepest gratitude to **Prof. Dr. Reham Magdy Amin**, Professor of Oral Biology, Faculty of Dentistry, Ain Shams University, for her considerable support and valuable supervision. Her deep knowledge, sincere suggestions and contributions with her constant follow up have made this work fulfilled.

Many thanks to all the members of the Oral Biology Department, Faculty of Dentistry, Ain Shams University for offering lots of help.

Finally, to all my wonderful friends in the department and the BUE family each one of you was more like a brother or sister to me, your help is greatly appreciated.

Dedication

First of all, I am very grateful to God for giving me the strength to accomplish this work and for the endless blessings bestowed upon me.

To the most amazing wife anyone can wish for, without her outstanding help and support I wouldn't have finished my work.

To my lovely daughter Sophia, I love you so much you mean the world to me and cherish our life with your gorgeous spirit.

To my mother and father, words will never express my gratitude to both of you, I am who I am because of them, their sacrifices and belief in me. I wish I can always live up to their expectations and make them proud.

To my sisters and their families, having them around is a true blessing.

To my father, mother and sister in law, they are like second father and mothers to me and always by my side. Thank you can never be enough for what they have done.

To my big family, here is a chance to show you how proud I am to belong to such a loving, respectful and honorable family.

Table of Contents

| | |
|--|-----|
| List of Abbreviations | i |
| List of Figures..... | iii |
| List of Tables | v |
| Abstract | vi |
| Introduction..... | 1 |
| Review of literature | 3 |
| Anatomy of Submandibular salivary glands | 3 |
| Histology of Submandibular salivary glands..... | 4 |
| Nanotechnology | 6 |
| Nanoparticles..... | 7 |
| Applications of nanoparticles..... | 9 |
| Food Additives | 12 |
| Nanotechnology in food industry..... | 15 |
| Titanium Dioxide Nanoparticles | 17 |
| Side effects and toxicity of TiO ₂ NPs..... | 19 |
| Apoptosis..... | 22 |
| Aim of the study | 24 |
| Materials and Methods..... | 25 |
| Results | 33 |
| 1-Histological results:..... | 33 |
| 2-Immunohistochemical results:..... | 52 |
| Summary of the histological results:..... | 58 |
| Summary of the immunohistochemical results:..... | 60 |

| | |
|-------------------------------|-----|
| 3-Statistical Analysis: | 61 |
| Discussion | 64 |
| Summary | 73 |
| Conclusions..... | 78 |
| Recommendations..... | 79 |
| References..... | 80 |
| Arabic Summary | - - |

List of Abbreviations

| Abbreviation | Meaning |
|---|-----------------------------------|
| Ag | Silver |
| Al₂O₃ | Alumina |
| ATP | Adenosine Triphosphate |
| Au | Gold |
| Bax | Bcl-2-associated X protein |
| Bcl-2 | B-cell lymphoma 2 |
| BV | Blood vessel |
| BW | Body weight |
| CT | Connective tissue |
| DNA | Deoxyribonucleic acid |
| E530 | Magnesium oxide MgO food additive |
| ED | Excretory ducts |
| EM | Electron microscope |
| Fe₃O₄, Fe₂O₃ | Iron oxides |
| GCT | Granular convoluted tubules |
| gm | Gram |
| H&E | Hematoxylin and Eosin |
| H₂O₂ | Hydrogen peroxide |
| HCl | Hydrochloric acid |
| I.G | Intra-gastric |
| I.P | Intraperitoneal |
| ID | Intercalated ducts |
| IL-1b | Interleukin 1 beta |
| IL-6 | Interleukin 6 |
| kg | Kilogram |
| Lab | Laboratory |
| LM | Light microscope |
| m | meter |
| mag | Magnification |
| mg | Milligram |

| | |
|--------------------------------|----------------------------------|
| MgO | Magnesium oxide |
| MP | megapixel |
| NF-κB | nuclear factor-kappaB |
| nm | nanometer |
| NP | Nanoparticle |
| °C | Celcius |
| Orig. | Original |
| pH | Potential Hydrogen |
| R&D | Research and Development |
| RBCs | Red blood cells |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| SG | Salivary gland |
| SiO₂ | Silicon dioxide |
| SiO₂NP | silicon dioxide nanoparticle |
| SMG | Submandibular salivary gland |
| St.D | Striated ducts |
| TEM | Transmission electron microscope |
| TiO₂ | Titanium dioxide |
| TNF α | Tumor necrosis factor α |
| UK | United Kingdom |
| USA | United States of America |
| ZnO | Zinc Oxide |

List of Figures

| Figure | Description | Page |
|-----------|---|-----------|
| 1 | A photomicrograph of rat submandibular salivary gland of control group (H&E , orig. mag. X400). | 35 |
| 2 | A photomicrograph of rat submandibular salivary gland of control group (H&E , orig. mag. X400). | 36 |
| 3 | A photomicrograph of rat submandibular salivary gland of Group II (H&E , orig. mag. X400). | 39 |
| 4 | A photomicrograph of rat submandibular salivary gland of Group II (H&E , orig. mag. X400). | 40 |
| 5 | A photomicrograph of rat submandibular salivary gland of Group II (H&E , orig. mag. X400). | 41 |
| 6 | A photomicrograph of rat submandibular salivary gland of Group III (H&E , orig. mag. X400). | 43 |
| 7 | A photomicrograph of rat submandibular salivary gland of Group III (H&E , orig. mag. X400). | 44 |
| 8 | A photomicrograph of rat submandibular salivary gland of Group IV (H&E , orig. mag. X400). | 46 |
| 9 | A photomicrograph of rat submandibular salivary gland of Group IV (H&E , orig. mag. X400). | 47 |
| 10 | A photomicrograph of rat submandibular salivary gland of Group IV (H&E , orig. mag. X400). | 48 |
| 11 | A photomicrograph of rat submandibular salivary gland of Group V (H&E , orig. mag. X400). | 50 |
| 12 | A photomicrograph of rat submandibular salivary gland of Group V (H&E , orig. mag. X400). | 51 |
| 13 | An immunostained photomicrograph of control group (anti active caspase 3 orig. mag. X400) | 53 |
| 14 | An immunostained photomicrograph of Group II (anti active caspase 3 orig. mag. X400) | 54 |
| 15 | An immunostained photomicrograph of Group III (anti active caspase 3 orig. mag. X400) | 55 |
| 16 | An immunostained photomicrograph of Group IV (anti active caspase 3 orig. mag. X400) | 56 |

| | | |
|-----------|---|-----------|
| 17 | An immunostained photomicrograph of Group V (anti active caspase 3 orig. mag. X400) | 57 |
| 18 | Summary for the histological features of parenchymal elements of rats SMG in all groups | 58 |
| 19 | Summary for the histological features of excretory ducts of rats SMG in all groups | 59 |
| 20 | Summary for the immunohistochemical staining reaction of rats SMG to caspase 3 in all groups | 60 |
| 21 | Bar chart representing mean caspase 3 surface area in submandibular salivary gland cells of the five groups | 63 |

List of Tables

| Table | Title | Page |
|-------|---|------|
| 1 | Showing summary of the doses given to control subgroups | 27 |
| 2 | Showing summary of the doses given to experimental groups | 28 |
| 3 | Showing comparison of surface area ($\times 10^4$) among different studied groups | 62 |

Abstract

Background: Titanium dioxide (TiO₂) Nanoparticles is the most widely used white pigment in food industry, and despite its wide range of uses, it has many side effects on different body tissues.

Aim of the study: To investigate the toxic effect of titanium dioxide nanoparticles and the possible recovery after withdrawal on rat submandibular salivary gland (SMG) histologically and immunohistochemically.

Materials and methods: Forty-eight male albino rats of two months' age were used in this study and were divided into Group I (control) which were further subdivided into four sub-groups corresponding to experimental groups (Group IA, IB, IC and ID) and experimental groups (Group II, III, IV and V). Referring to the experimental groups, Group II received 50 mg/kg/day TiO₂NPs orally for 30 days while Group III, IV and V received same dose and duration as Group II but were left for recovery for two, four and six weeks respectively. The specimens of the right side of rats' SMG were stained by H&E and studied under light microscope for histological changes, while the left side were prepared and examined immunohistochemically by using caspase-3 apoptotic marker.

Results: Histological examination of the Group II showed that acinar cells demonstrated some histological alterations including pyknotic, hyperchromatic and crescent shaped nuclei with variable sized cytoplasmic vacuolations. The ducts showed some signs of degeneration with loss of their normal cellular outlines. However, there was some improvement in Group III and IV that escalated in Group V that was almost same histological picture as the control group.

Apoptotic changes expressed by anti-active caspase 3 were highly observed Group II and statistical results showed the highest statistical significance. Significant difference was observed between studied groups.

Conclusion: The use of TiO₂NPs leads to histological alterations in submandibular salivary glands of rats and induced apoptotic changes on their cells, which was confirmed statistically. These apoptotic changes were decreased gradually as the recovery periods increased and almost reversed to normal after 6 weeks.