



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



Ain Shams University
Faculty of Women for Arts, Science and Education,
Biochemistry and Nutrition Department

A Comparative Study of Antidiabetic Effect of Zinc, Chromium and Selenium Nanoparticles in Rats

Thesis

Submitted for Faculty of Women for arts, Science and Education, Ain Shams
University, In Partial Fulfillment For the Requirement of Ph.D. Degree of
Biochemistry and Nutrition

By

Rasha Mustafa Hassan Amin

M.Sc., in Biochemistry and Nutrition, Biochemistry and Nutrition Department,
Faculty of Women for arts, Science and Education, Ain Shams University

Under Supervision of

Prof.Dr. Tahany El Sayed Kholeif

Professor of Biochemistry
Biochemistry and Nutrition Department
Faculty of Women for Arts, Science and Education
Ain Shams University.

Prof.Dr. Naglaa Hassanen Mohamed

Professor of Food science
Special Food and Nutrition Department
Agricultural Research center

Prof.Dr. Jehan Abdallah Gafer

Professor of Biotechnology
Animal Reproduction Research Institute

Dr. Mai Elsayed Abd Elkawi

Lecturer of Biochemistry and Nutrition
Biochemistry and Nutrition Department
Faculty of Women for Arts, Science and Education
Ain Shams University

2021



Ain Shams University

Faculty of Women for Arts, Science and Education,

A Comparative Study of Antidiabetic Effect of Zinc, Chromium and Selenium Nanoparticles in Rats

Thesis

THESIS ADVISORS

APPROVED

Prof. Dr. Tahany El Sayed Kholeif

Professor of Biochemistry
Biochemistry and Nutrition Department
Faculty of Women for Arts, Science and Education
Ain Shams University.

Prof. Dr. Naglaa Hassanen Mohamed

Professor of Food science
Special Food and Nutrition Department
Agricultural Research center

Prof. Dr. Jehan Abdallah Gafer

Professor of Biotechnology
Animal Reproduction Research Institute

Dr. Mai Elsayed Abd Elkawi

Lecturer of Biochemistry and Nutrition
Biochemistry and Nutrition Department
Faculty of Women for Arts, Science and Education

Approval of Chemistry Department Council / / 2021

Approval of Faculty Council / / 2021

Approval of University Council / / 2021

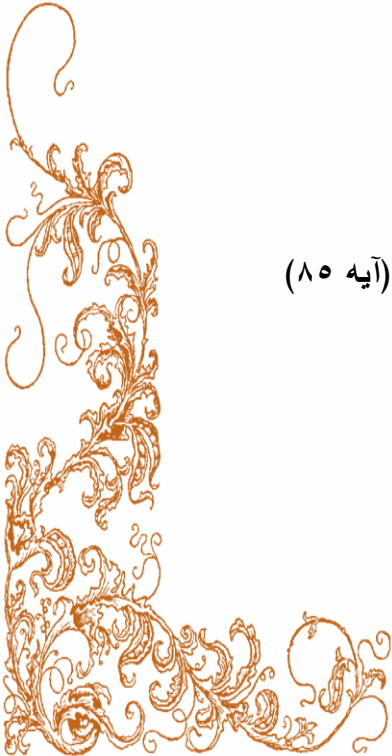


بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

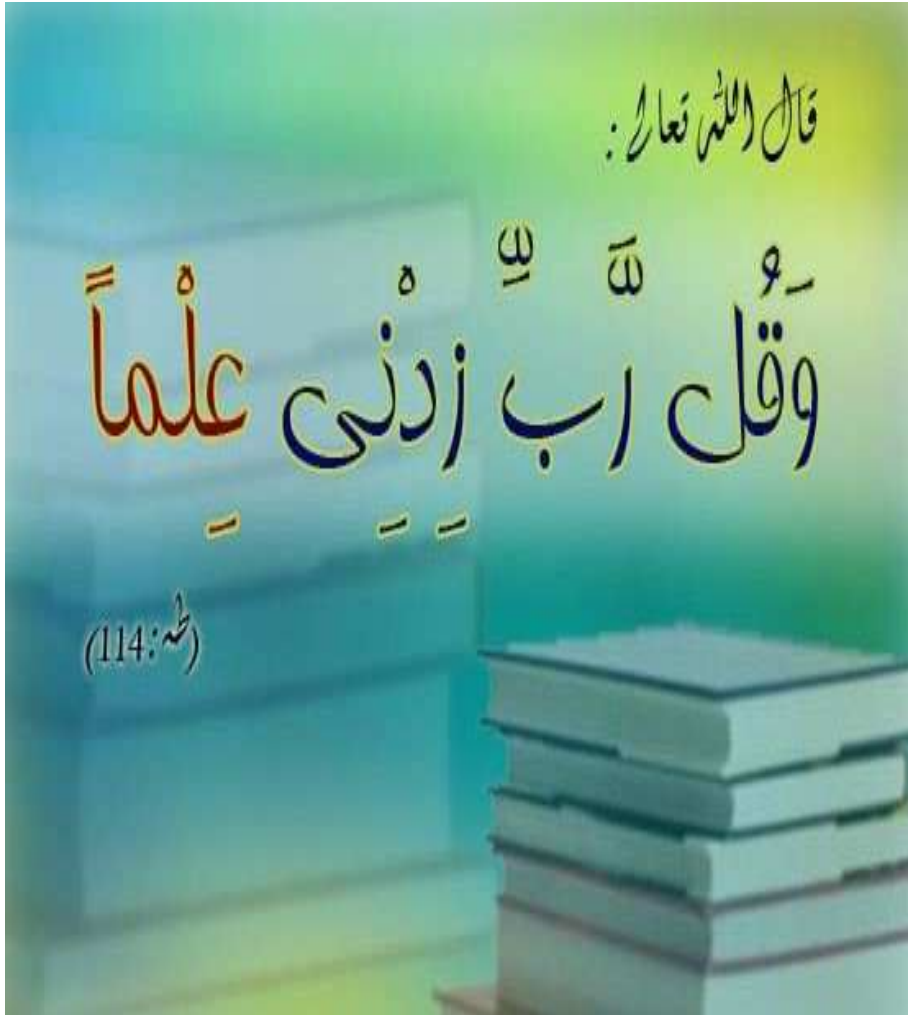
وَمَا أَوْزِنُنَا مِنَ الْعِلْمِ إِلَّا قَلِيلًا ﴿٨٥﴾

الْحَقِّيقُ
الْعَظِيمُ

الإسراء (آيه ٨٥)



“Allah”
The most Gracious
The most Merciful



Acknowledgment

In the name of God, the Most Gracious, the Most Merciful. All praise and gratitude to God Almighty for giving me the strength, ability and approval to complete this work.

First and foremost, I offer my sincerest gratitude to my esteemed supervisors, **prof. Dr. Tahany El Sayed Kholeif**, Prof. of Biochemistry, Department of Biochemistry and Nutrition, Faculty of Women for Art, Science and Education, Ain Shams University, who has supported me throughout my thesis with patience, knowledge, encouragement and effort and without her this thesis, would not have been completed. she helped me all the time of research. I don't find words to express my deepest thanks for her kindness, support and valuable hints

I would like to express my gratitude and appreciation for **Prof. Dr. Naglaa Hassanen Mohamed**, Prof. of Food science, Special Food and Nutrition Department, Agricultural Research Center and **Prof. Dr. Jehan Abdallah Gafer**, Prof. of Biotechnology, Animal Reproduction Research Institute, whose guidance, and encouragement has been invaluable throughout this study.

From the bottom of my heart I would like to express my gratitude and appreciation for **Dr. Mai Elsayed Abd Elkawi**, Lecturer of Biochemistry and Nutrition department of Biochemistry and Nutrition, Faculty of Women for Art, science and Education, Ain Shams University, whose guidance, support and encouragement has been invaluable throughout this study. Her overall insights in this field have made this an inspiring experience for me.

I am thankful to **Dr. Yasser Attia**. Professor of Physical Chemistry. National Institute of Laser Enhanced Sciences. Cairo University. For the help that he offered in nanoparticles synthesis carried out in this study.

I am thankful to **Dr. Kawkab Ahmed**. Professor of pathology. Faculty of veterinary medicine, Cairo University for the help that she offered in the histopathological examination carried out in this study

I am particularly grateful for the staff members of Biochemistry & Nutrition Department, Faculty of Women for Art, Science and Education, Ain Shams University, for their help and support.

X

Rasha Mustafa

Dedication

This thesis is dedicated to my “Father” who passed away but his soul is present by his advices and support, giving with no limits ,what gives me patience is that i believe that Allah willing he will be in a better place due to his good deeds and actions. I wished that he would have been with us today to reep the fruits of his efforts and suffering with us to be eminent and successful characters in society. I assume that he is now proud of me in this special moment. May Allah have mercy on him and bless him with heaven.

I would extend my gratitude and thanks to my dear “Mother” a source of motivation and strength during all the difficult moments who devoted het time and life to make us happy and successful.may god bless her life with good health and happiness

Also ,I dedicate this work to my sisters “Asmaa & Ayat” and my brothers, “Mohamed & Ahmed” who helped me and support me through the work.

Last but not least, this thesis is dedicated to my little heart, my twin sons, “Mohamed and Yasmine”, who were my great source of motivation and inspirationfor me.

Abstract

A Comparative Study of Antidiabetic effect of Zinc, Chromium and Selenium Nanoparticles in Rats. Rasha Mustafa Hassan Amin, Ph.D. degree, Biochemistry and Nutrition Department, Faculty of Women for Art, Science and Education, Ain Shams University.

This study was intended to evaluate the antidiabetic effect of single or combined administration of zinc oxide nanoparticles (ZnONPs), chromium oxide nanoparticles (Cr₂O₃NPs), and selenium nanoparticles (SeNPs), on genetic and metabolic insult in fructose/streptozotocin diabetic rat model. Type 2 diabetes mellitus was induced by feeding adult male albino rats with a high fructose diet accompanied by a single *i.p.* injection of streptozotocin (STZ). The rats were divided into 6 groups (10 rats/each): healthy (G1), diabetic control (G2), ZnONPs treated (G3, 10 mg/kg b.wt), for Cr₂O₃NPs treated (G4, 1 mg/kg b.wt), SeNPs treated (G5, 0.4 mg/kg b.wt) and nanoparticles mixture treated (G6). The results displayed that diabetes significantly decreased body weight, serum insulin, C-peptide, adiponectin levels, erythrocyte glutathione peroxidase and serum superoxide dismutase activities, as well as high-density lipoprotein cholesterol, and total antioxidant capacity levels. While, it caused a substantial increase in serum glucose, glycosylated Hb, C-reactive protein, atherogenic index, HOMA-IR, malondialdehyde, lipid profile, interleukin-6 levels, liver and kidney functions parameters. Furthermore, the findings showed a decrease in hepatic insulin receptor substrate-1 (IRS-1) mRNA expression level and adipocyte peroxisome proliferator-activated receptor (PPAR- γ) mRNA expression level in type 2 diabetic rats. DNA damage was confirmed by performing the comet assay. Moreover, histological observation and measuring the lesion score of pancreatic and hepatic tissues were performed, which were consistent with the biochemical results. The present study confirmed that oral administration of ZnONPs, Cr₂O₃NPs, SeNPs, and their mixture improved all the biochemical and genetic parameters toward normal levels and ameliorated the diabetic consequences that were manifested by restricting cellular DNA damage which maintaining pancreatic and hepatic tissues from oxidative damage. These nanoparticles showed antidiabetic, anti-inflammatory, antioxidant, hypolipidemic, and antiapoptotic agents. The best mitigated antidiabetic effect was observed in the mixture administered group as it combined all previous synergetic mechanisms of each single compound.

List of Contents

Title	Page
• Introduction	1
• <u>Aim of the work</u>	4
• <u>Review of literature</u>	6
1. Diabetes Mellitus	7
1.1. Classification of diabetes mellitus.	9
1.1.1. Type 1 diabetes.	10
1.1.2. Type 2 diabetes.	13
1.2. Correlations with and influencing factors on T2DM	13
1.2.1. Heritable genetic correlation	14
1.2.2. Susceptibility loci	20
1.2.3. Lifestyle factor correlation	22
1.2.4. Gut metagenome correlation	23
1.2.5. Micronutrients Biochemistry	27
1.3. Complications of T2DM	28
1.3.1. Cardiovascular diseases	29
1.3.2. Diabetic neuropathy	29
1.3.3. Diabetic nephropathy	29
1.3.4. Diabetic retinopathy	29
1.3.5. Cancers	30
1.4. The crosstalk between oxidative stress and inflammation in the progression of T2DM complications.	36
1.5. Risk assessment of T2DM.	37
1.5.1. Prediction models with noninvasive measures	37
1.5.2. Prediction models including biochemical measures	37
1.6. Treatment of T2DM	37
1.6.1. Non-pharmacologic treatment	37
1.6.2. Anti-diabetes pharmacotherapy	38
2. Nanotechnology	39
3. Nanoparticles	40
3.1. Zinc oxide nanoparticles (ZnONPs).	44
3.1.1. Biological function of zinc	44
3.1.2. Biological importance of zinc oxide nanoparticles	46
3.1.2.1. Anticancer therapy.	47
3.1.2.2. Antidiabetic agent	49
3.1.2.3. Antimicrobial activity	51

Title	Page
3.1.2.4. Anti-inflammatory and antioxidant activities.	52
3.2. Chromium oxide nanoparicles (Cr₂O₃NPs)	52
3.2.1. Biological function of chromium	52
3.2.2. Biological importance of Cr ₂ O ₃ NPs	57
3.3. Selenium nanoparicles (SeNPs)	60
3.3.1. Biological function of selenium.	60
<u>3.3.2. Biological importance of SeNPs.</u>	62
3.3.2.1. The role of Selenium in Antimicrobial applications	64
3.3.2.2. The role of Selenium in Anticancer Applications	66
3.3.2.3. The role of SeNPs in antidiabetic application	67
3.3.2.4. The role of SeNPs in antioxidant and anti-inflammatory application	69
Materials and Methods	
1. Materials	72
1.1. Chemicals	72
1.2. Animals	72
1.3. Diet	72
1.3.1. Standard diet	72
1.3.2. High fructose diet.	73
2. Methods	77
2.1. Synthesis of nanoparticles	77
2.1.1. Synthesis of zinc oxide nanoparticles (ZnONPs).	77
2.1.2. Synthesis of chromium oxide nanoparicles (Cr ₂ O ₃ NPs)	77
2.2.3. Synthesis of selenium nanoparicles (SeNPs)	77
2.2. Nanoparticles characterization	78
2.3. Experimental design	80
2.4. Sample preparation	82
2.5. Biological measurements	82
2.5.1. Food intake	82
2.5.2. Body weight change	83
2.5.3. Feed efficiency ratio	83
2.5.4. Relative organs weight	83
2.6. Biochemical Analysis	83
2.6.1. Glucose homeostasis measurements	83
2.6.1.1. Determination of serum glucose level	83
2.6.1.2. Determination of serum insulin level.	85
2.6.1.3. Calculation of Homeostatic Model Assessment of Insulin Resistance (HOMA -IR) level	87
2.6.1.4. Determination of serum proinsulin C-peptide level.	87
2.6.1.5. Determination of serum adiponectin level.	89

Title	Page
2.6.1.6.Determination of blood Glycosylated Hemoglobin (HbA1c)level.	90
2.6.2.Determination of gene expression of Insulin Receptor Substrate-1 and Peroxisome Proliferator Activated Receptors Gamma.	93
2.6.3.Determination of DNA damage in pancreas by comet assay	101
2.6.4.Determination of serum antioxidant and oxidative biomarkers	103
2.6.4.1.Determination of serum Glutathione peroxidase (GPx) activity.	103
2.6.4.2.Determination of serum Superoxide dismutase (SOD) activity.	105
2.6.4.3.Determination of serum total antioxidant capacity (TAC) level.	107
2.6.4.4.Determination of lipid peroxides as malondialdehyde (MDA) level.	108
2.6.4.5.Determination of nitric oxide (NO) level.	110
2.6.5.Determination of serum Inflammatory markers.	112
2.6.5.1.Determination of serum C-reactive protein (CRP).	112
2.6.5.2.Determination of serum Interleukin 6 (IL-6).	113
2.6.6- Determination of serum lipids profile.	115
2.6.6.1.Determination of serum total cholesterol (TC)	115
2.6.6.2.Determination of serum triacylglycerols (TAGs)	117
2.6.6.3.Determination of serum high density lipoprotein cholesterol	119
2.6.6.4.Calculation of serum low density lipoprotein Cholesterol	121
2.6.6.5.Calculation of serum very low density lipoprotein cholesterol	121
2.6.6.6.Calculation of serum atherogenic index (AI) level	121
2.6.6.7.Calculation of serum atherogenic coefficient	121
2.6.7-Determination of liver function measurements	122
2.6.7.1.Determination of serum aspartate amino transferase	122
2.6.7.2.Determination of serum alanine amino transferase.	124
2.6.7.3.Determination of serum albumin	126
2.6.8.Determination of kidney function measurements	127
2.6.8.1.Determination of serum urea	127
2.6.8.2.Determination of serum creatinine	128
2.7.Microscopic examination of liver and pancreas	130
Results and Discussion	
1.Nanoparticles characterization	131
2.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on body weight change, food intakes, and feed efficiency ratio, and relative organs weight in experimental group.	137
3.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on glucose level , HbA1c % , insulin, C-peptide, HOMA- IR, HOMA- β, and adiponectin levels in experimental groups	148
4.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on liver function tests (ALT and AST) activities,	165

Title	Page
as well as albumin level) in experimental groups	
5.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on of treatment on kidney functions tests (urea, and creatine levels) in experimental groups	170
6.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on of treatment on antioxidant/ oxidative stress): Erythrocyte GPx and serum SOD activities, serum TAC, MDA, and NO levels in experimental groups	175
7.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on treatment of lipid profile analysis in experimental groups	187
8.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on inflamatory markers (Serum interlukin-6 and C-reactive protein) levels in experimental	193
9.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on adipocyte gamma peroxisome proliferator activated receptor (γ - PPAR) gene and hepatic insulin receptor substrate - 1(IRS-1) gene in experimental groups	201
10.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs, SeNPs ,and their mixture on DNA damage in pancreatic cells by comet assay in experimental groups	206
11.Effect of single or combined oral administration of ZnONPs, Cr ₂ O ₃ NPs and SeNPs on lesion scores and microscopic examination of pancreatic and hepatic tissues in experimental groups	214
11.1.Histopathological lesion scores of the pancreas and liver in experimental groups:	214
11.2.Microscopic examination for pancreatic tissues in experimental groups	216
11.3.Microscopic examination for liver tissue in experimental groups	222
Summary	227
Conclusion	238
Recommendation	240
References	241
Arabic summary	

List of Tables

Table No.	Items	Page
1	Composition of standard diet	73
2	Composition of high fructose diet	74
3	Composition of vitamin mixture that supplies the recommended concentration of vitamin for AIN-93M diet.	75
4	Composition of mineral mixture that supplies the recommended concentration of elements for AIN-93 M diet	76
5	Body weight change, food intake and feed efficiency ratio in experimental groups.	139
6	Relative organs weight of (liver, kidney, heart, spleen, pancreas, adipose tissue, and testis) in experimental groups.	142
7	Serum glucose, HbA1c %, insulin, C-peptide, HOMA- IR, and adiponectin levels in experimental groups.	150
8	Serum AST and ALT activities, as well as serum albumin level in experimental groups.	166
9	Serum urea and creatinine levels in experimental groups.	171
10	Erythrocyte GPx and serum SOD activities, serum TAC, MDA, and NO levels in experimental groups	177
11	Serum lipid profile levels in experimental groups.	188
12	Serum interleukin-6 and C-reactive protein levels in experimental groups.	194
13	Adipocyte gamma peroxisome proliferator activated receptor (PPAR- γ) gene and hepatic insulin receptor substrate -1(IRS-1) gene expression in experimental groups.	202
14	Pancreatic DNA damage determined by comet assay in experimental group.	207
15	Histopathological lesion scores of the pancreas and liver in experimental groups	214