

بسم الله الرحمن الرحيم

 $\infty\infty\infty$

تم رفع هذه الرسالة بواسطة / حسام الدين محمد مغربي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

AIN SHAMS UNIVERSITY

Since 1992

Propries 1992

ملاحظات: لا يوجد



Ain Shams University
Faculty of Science
Department of Biochemistry

Evaluation of the Therapeutic Impact of Mesenchymal Stem Cells in a Rat Model of Experimentally Induced Type 2 Diabetes

A Thesis for partial fulfillment of the requirements for Master Degree of Science in Biochemistry

Submitted By

Doaa Mahmoud Youssef

(B. Sc. in Biochemistry - Chemistry, 2010)

Under Supervision of

Prof. Dr. Azza Ahmed Atef

Professor of Biochemistry Faculty of Science Ain Shams University

Dr. Fatma Abd Elkarim Abozahra

Assistant Consultant of Molecular Biology and Tissue Culture Medical Ain Shams Research Institute Faculty of Medicine Ain Shams University

Dr. AlShaimaa Mohamed Taha

Lecturer of Biochemistry Faculty of Science Ain Shams University

2020



Ain Shams University
Faculty of Science
Department of Biochemistry

Biography

Name : Doaa Mahmoud Youssef Youssef Kereet

Date of Graduation: 2010

Degree awarded: B.Sc in Biochemistry - Chemistry, 2010

Grade : M.Sc in Biochemistry

Faculty name: Faculty of Science

University name: Ain Shams University

بسم الله الرحمن الرحيم

"قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم"

صدق الله العظيم (سورة البقرة آية: ٣٢)

Dedication

To my mother, my father, my brothers, my sister and my friends for their love, encouragement, help and prayers that made studies possible and to them I owe everything.

Doaa Mahmoud Youssef Youssef



Biochemistry Department

Approval sheet

Evaluation of the Therapeutic Impact of Mesenchymal Stem Cells in a Rat Model of Experimentally Induced Type 2 Diabetes

Thesis

Submitted for the degree of Master of Science as a partial fulfillment for requirements of M.Sc. degree in Biochemistry

Submitted by **Doaa Mahmoud Youssef**

(B. Sc. in Biochemistry - Chemistry, 2010)

Supervisors:

Prof. Dr. Azza Ahmed Atef Professor of Biochemistry, Faculty of

Science, Ain Shams University

Dr. Fatma Abd Elkarim Abozahra Assistant Consultant of Molecular,

Biology and Tissue Culture, Medical Ain Shams Research Institute, Faculty of Medicine, Ain Shams University

Dr. AlShaimaa Mohamed Taha Lecturer of Biochemistry, Faculty of

Science, Ain Shams University

Examiners committee:

Prof. Dr. Mohamed Hesham Mohamed Mahfouz BadranProfessor of Biochemistry, Department of Biochemistry, National Institute of

Diabetes and Endocrinology

Prof. Dr. Legaa Abd El Azim

Ahmed Moamen

Professor Researcher of Biochemistry and Molecular Biology and Chairwoman of Academic Sciences Department, Research Institute of

Ophthalmology, Ministry of Higher Education and Scientific Research

Prof. Dr. Azza Ahmed Atef Professor of Biochemistry, Faculty of

Mahmoud Science, Ain Shams University

Contents

Acknowledgement	I
Abstract	III
List of Abbreviations	V
List of Figures	XIII
List of Tables	XVI
Introduction	XIX
Aim of the Work	XXI
Chapter 1: Review of Literature	1
1. Glucose Homeostasis	1
1.1. Control of Glucose Homeostasis	1
1.1.1. Insulin: A Key Regulator for Glucose	
Homeostasis	2
1.1.1.1 Insulin Secretion	3
1.1.1.2. Insulin Functions	6
1.1.1.3. Normal Insulin Signaling	7
2. Diabetes mellitus	12
2.1. Definition	12
2.2. Classification	13
2.2.1. Type 1 Diabetes Mellitus	13
2.2.2. Gestational Diabetes Mellitus (<i>GDM</i>)	13
2.2.3. Type 2 Diabetes Mellitus	14

2.3. Impaired Insulin Signaling Pathway & Insulin	
Resistance	15
2.3.1. Dysfunctions of IRS1/2-PI3K Signaling	16
a. IRS1	16
b. IRS2	16
c. PI3K	17
2.3.2. Dysfunctions of Akt/PKB Signaling	18
2.3.3. Dysfunctions of aPKCs Signaling	18
2.3.4. Dysfunctions of MAPK Signaling— ERK,	
JNK, p38	19
2.4. Causes of Impaired Insulin Signaling Pathway	19
2.4.1. Obesity	19
2.4.1.1. Adipose Tissue and Adipokines	23
2.4.1.1.1 Leptin	24
• Leptin Signaling Pathway	25
• Leptin Functions	27
2.4.1.1.2. Adiponectin	27
Adiponectin Signaling Pathway	28
Adiponectin Functions	29
2.4.2. Oxidative Stress	30
2.4.3. β-Cell Dysfunction	38
2.4.3.1. Decline in β-Cell Function	39

2.4.3.2. Decline in β-Cell Mass	40
3. Pancreatic β cells Insulin like Growth Factor-1-	
Receptor (IGF-1R)	41
IGF Signaling Pathway	42
4. Bone Marrow-Mesenchymal Stem Cells in Treating	
Type 2 Diabetes	44
4.1. Stem Cell Sources	44
4.1.1. Embryonic Stem Cells (ESCs)	44
4.1.2. Adult Stem Cells (ASCs) or Mesenchymal	
Stem Cells (MSCs)	45
4.1.3. Induced Pluripotent Stem Cells (iPS)	45
4.2. Mesenchymal Stem Cells	45
4.3. Molecular Mechanism of Action of MSCs	47
4.3.1. Differentiation into Insulin Producing Cells	
(IPCs)	47
4.3.2. Promoting the Regeneration of Pancreatic Islet	
Beta Cells	48
4.3.3. Protection of Endogenous Pancreatic Islet Beta	
Cells	50
• Immune Regulatory Capacity	50
Protection against Oxidative Stress	51
Protection by Autophagy	51

4.2.3.4. Amelioration of Insulin Resistance	52
Chapter 2: Materials and Methods	55
I. Bone Marrow-Mesenchymal Stem Cells (BM-	
MSCs)	55
I.1. Isolation	55
I.2. Culturing	58
I.3. Counting and Trypsinization	59
I.4. Charecterization by Flow Cytometry	60
II. Experimental Model of Type 2 Diabetes	63
1. Animals	63
2. Induction of Type 2 Diabetes	64
a. High Fat Diet Preparation	64
b. Streptozotocin Preparation	65
III. Biochemical Analysis	67
1. Diabetic Biomarkers	67
-Determination of Fasting Plasma Glucose Level	67
-Determination of Fasting Serum Insulin Level	69
Calculation of the Homeostatic Model Assessment	
(HOMA)	72
2. Lipid Profile	73
-Determination of Serum Triacylglycerol	
Concentration	73
-Determination of Serum Total Cholesterol	

-Determination of Serum HDL-C Concentration	Concentration	75
-Calculation of Serum VLDL-C Concentration	-Determination of Serum HDL-C Concentration	77
-Calculation of Atherogenic Indices 1 & 2	-Calculation of Serum LDL-C Concentration	80
3. Insulin Sensitivity Markers	-Calculation of Serum VLDL-C Concentration	80
-Determination of Serum Adiponectin Concentration	-Calculation of Atherogenic Indices 1 & 2	80
-Determination of Serum Leptin Concentration	3. Insulin Sensitivity Markers	80
IV. Enzymes of Glucose Homeostasis Studies as well as IGF-1R	-Determination of Serum Adiponectin Concentration	80
as IGF-1R	-Determination of Serum Leptin Concentration	85
A. Determination of the Enzymes and IGF-1R at the Genetic Level	IV. Enzymes of Glucose Homeostasis Studies as well	
Genetic Level	as IGF-1R	90
A) RNA Extraction	A. Determination of the Enzymes and IGF-1R at the	
Step 1: Preparation of RNase-Free Tools and Bench	Genetic Level	90
Bench	A) RNA Extraction	91
Step 2: RNA Extraction	Step 1: Preparation of RNase-Free Tools and	
Calculation of RNA Concentration and Purity	Bench	91
B) Synthesis of Complementary DNA (cDNA) 96 C) Quantitative Real Time Polymerase Chain Reaction	Step 2: RNA Extraction	92
C) Quantitative Real Time Polymerase Chain Reaction	Calculation of RNA Concentration and Purity	96
Reaction	B) Synthesis of Complementary DNA (cDNA)	96
B. Determination of the Enzymes Content	C) Quantitative Real Time Polymerase Chain	
-Total Protein Measurement	Reaction	99
	B. Determination of the Enzymes Content	106
-Determination of Glucokinase Content	-Total Protein Measurement	106
	-Determination of Glucokinase Content	108

-Determination of Glycogen Synthase Content	111
VI. Histological Studies	115
VII. Statistical Analysis	117
Chapter 3: Results	118
Chapter 4: Discussion	149
English Summary	164
Conclusion	170
References	171
المستخلص	1
الملخص العربي	٣
الخلاصة	٨

Acknowledgment

First and foremost cordial thanks to Allah

No words could express my sincere appreciation and deepest thanks to **Prof. Dr. Azza Ahmed Atef Mahmoud,** Professor of Biochemistry, Faculty of Science, Ain Shams University, for her endless help, motherly attitude, close supervision, creative thinking, valuable suggestions and constant advice throughout this work.

I am deeply indebted to **Dr. Fatma Abd Elkarim Abo Zahra,** Assistant Consultant of Molecular Biology and
Tissue Culture, Medical Ain Shams Research Institute
(MASRI), Faculty of Medicine, Ain Shams University, for
her practical guidance, kind help, tremendous concern, care
and invaluable assistance.

I am so grateful to **Dr. AlShaimaa Mohamed Taha**, Lecturer of Biochemistry, Faculty of Science, Ain Shams University for her instructive and practical guidance, her enthusiastic encouragement and revision of every detail, as well as profound writing of the manuscript. I would like to thank **Prof. Dr. Adel Bakeer Kholoussy,** Professor of Pathology, Faculty of Veterinary Medicine, Cairo University, for the assistance with the histological study.

My deep thanks and regards to the Biochemistry Department, Faculty of Science, Ain Shams University and Medical Ain Shams Research Institute.

Abstract

Insulin resistance and defect in insulin-producing pancreatic beta cells are the key features of type 2 diabetes (T2D). Mesenchymal stem cells isolated from bone marrow (BM-MSCs) have differentiation, anti-inflammatory and immunosuppressive characteristics, making them suitable for treating T2D. Therefore, the present study aimed at investigating the possible therapeutic mechanisms of BM-MSCs infusion in high fat diet/streptozotocin (HFD/STZ)induced T2D rats. Thirty male Wistar rats were divided into 3 groups: Normal control, T2D, and T2D treated with BM-MSCs. The anti-diabetic effect of BM-MSCs was evidenced of hyperglycemia, by ameliorating the state hyperinsulinemia, and hyperlipidemia. BM-MSCs enhanced significantly insulin sensitivity in diabetic rats via decreasing levels in serum and consequently, increasing adiponectin/leptin T2D ratio, compared to group. Furthermore, BM-MSCs improved glucose homeostasis by up-regulating gene expressions of hepatic glucokinase and glycogen synthase in the liver and skeletal muscles as well as increasing their levels. Treatment of T2D with BM-MSCs stimulated pancreatic regeneration in diabetic rats by up-