

### Effect of Bone Marrow Derived Stem Cells versus Insulin on the Lingual Papillae of Diabetic Albino Rats (Light and Scanning Electron Microscopic Study)

Thesis submitted to
Faculty of Dentistry - Ain Shams University
In partial fulfillment of the requirements for
Doctorate Degree in Oral Biology
By

### Rania Osama Mohamed Mohsen Abd El Halim

B.D.S, 2010

MSc. Oral Biology, Ain Shams University, 2017 Assistant Lecturer of Oral Biology Faculty of Dentistry - Ain Shams University

### **Supervisors**

### Prof. Dr. Ahmed Mahmoud Halawa

Professor and Head of Oral Biology Department Faculty of Dentistry - Ain Shams University

### Dr. Rabab Hassan Ali

Lecturer of Oral Biology Faculty of Dentistry - Ain Shams University

> Faculty of Dentistry Ain Shams University 2019

# بسم الله الرحمن الرحيم

" قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ"

صدق الله العظيم (البقرة- الآية ٣٢)

### <u>ACKNOWLEDGEMENT</u>

All praise and all thanks to ALLAH who guided and enabled me by his mercy to fulfill this thesis.

I would like to express my deepest gratitude to prof. Dr. Ahmed M. Halawa, professor and head of Oral Biology, Faculty of Dentistry, Ain Shams University, for his kind support, advices, continuous encouragement and appreciated suggestions throughout this work that guided me to accomplish it.

I am also grateful to **Dr. Rabab Hassan**, lecturer of Oral Biology, Faculty of Dentistry, Ain Shams University, who freely gave me her time, effort and experience with continuous guidance throughout this work.

Thanks also for all the stuff of Oral Biology Department, Faculty of Dentistry, Ain Shams University, who gave me their time, help and support to finish this work.

## **DEDICATION**

To my great beloved parents and brother,

Thanks a lot for being there for me.

# **CONTENTS**

Title	Page
Introduction and Review of literature	1
Diabetes epidemiology and classification	1
General complications of diabetes mellitus	4
Oral manifestations of diabetes mellitus	8
The lingual papillae and diabetes mellitus	11
Streptozotocin	16
• Insulin	18
Alternative therapies for diabetes mellitus	21
Stem Cell based therapy	22
Bone marrow-mesenchymal stem cells	23
Therapeutic potential of Bone marrow- mesenchymal stem cells	25
<ul> <li>Bone marrow-mesenchymal stem cells therapy perspectives for type I diabetes mellitus</li> </ul>	26
Aim of The Study	31
Materials and Methods	32
Results	48

Light microscopic results for Haematoxylin and Eosin stained sections	48
Light microscopic results for Anti-Proliferating Cell Nuclear Antigen immunohistochemical stained sections	90
Fluorescent microscopic results	102
Scanning electron microscopic results	109
Statistical results	133
Discussion	153
<b>Conclusions and Recommendations</b>	174
Summary	176
References	186
Arabic Summary	-

# **LIST OF FIGURES**

Figure	Title	Page
No.		
1	A photograph showing (on call plus) glucometer.	37
2	A photograph showing (Leica) phase contrast fluorescent microscope used in the study.	42
3	An image of section to be analyzed viewed by image analysis software.	45
4	The image showed only the positive cells in the epithelium after adjustment of the brightness.	45
5	The image after convertion into binary black and white type with watershed.	46
6	The image showed ellipses to see where and which cells the program identified.	46
7	A photomicrograph of simple conical papillae of Gp I (H&E, original mag. x200).	50
8	A photomicrograph of simple conical papillae of Gp I (H&E, original mag. x400).	50
9	A photomicrograph of giant conical papillae of Gp I (H&E, original mag. x200).	51
10	A photomicrograph of true filiform papillae of Gp I (H&E, original mag. x200).	51
11	A photomicrograph of simple conical papillae of Gp II (H&E, original mag. x200).	54
12	A photomicrograph of simple conical papillae of Gp II (H&E, original mag. x400).	54
13	A photomicrograph of giant conical papillae of Gp II (H&E, original mag. x200).	55

14	A photomicrograph of true filiform papillae of Gp II (H&E, original mag. x200).	55
15	A photomicrograph of true filiform papillae of Gp II (H&E, original mag. x200).	56
16	A photomicrograph of simple conical papillae of Gp III (H&E, original mag. x200).	59
17	A photomicrograph of simple conical papillae of Gp III (H&E, original mag. x400).	59
18	A photomicrograph of giant conical papillae of Gp III (H&E, original mag. x200).	60
19	A photomicrograph of true filiform papillae of Gp III (H&E, original mag. x200).	60
20	A photomicrograph of simple conical papillae of Gp IV (H&E, original mag. x200).	63
21	A photomicrograph of simple conical papillae of Gp IV (H&E, original mag. x400).	63
22	A photomicrograph of giant conical papillae of Gp IV (H&E, original mag. x200).	64
23	A photomicrograph of true filiform papillae of Gp IV (H&E, original mag. x200).	64
24	A photomicrograph of fungiform papilla of Gp I (H&E, original mag. x400).	68
25	A photomicrograph of fungiform papilla of Gp II (H&E, original mag. x400).	68
26	A photomicrograph of fungiform papilla of Gp III (H&E, original mag. x400).	69
27	A photomicrograph of fungiform papilla of Gp IV (H&E, original mag. x400).	69

28	A photomicrograph of circumvallate papilla of Gp I (H&E, original mag. x100).	73
29	A photomicrograph of circumvallate papilla of Gp I (H&E, original mag. x400).	73
30	A photomicrograph of circumvallate papilla of Gp II (H&E, original mag. x100).	74
31	A photomicrograph of lower part of circumvallate papilla trough of Gp II (H&E, original mag. x400).	74
32	A photomicrograph of circumvallate papilla of Gp III (H&E, original mag. x100).	75
33	A photomicrograph of circumvallate papilla of Gp III (H&E, original mag. x400).	75
34	A photomicrograph of circumvallate papilla of Gp IV (H&E, original mag. x100).	76
35	A photomicrograph of circumvallate papilla of Gp IV (H&E, original mag. x400).	76
36	A photomicrograph of foliate papillae of Gp I (H&E, original mag. x200).	80
37	A photomicrograph of lower part of foliate papillae trough of Gp I (H&E, original mag. x400).	80
38	A photomicrograph of foliate papillae of Gp II (H&E, original mag. x200).	81
39	A photomicrograph of foliate papillae of Gp II (H&E, original mag. x200).	81
40	A photomicrograph of lower part of foliate papillae trough of Gp II (H&E, original mag. x400).	82
41	A photomicrograph of foliate papillae of Gp III (H&E, original mag. x200).	82

42	A photomicrograph of lower part of foliate papillae trough of Gp III (H&E, original mag. x400).	83
43	A photomicrograph of foliate papillae of Gp IV (H&E, original mag. x200).	83
44	A photomicrograph of foliate papillae of Gp IV (H&E, original mag. x400).	84
45	A photomicrograph of Von Ebner salivary glands of Gp I (H&E, original mag. x400).	88
46	A photomicrograph of Von Ebner salivary glands of Gp II (H&E, original mag. x400).	88
47	A photomicrograph of Von Ebner salivary glands of Gp III (H&E, original mag. x400).	89
48	A photomicrograph of Von Ebner salivary glands of Gp IV (H&E, original mag. x400).	89
49	A photomicrograph of simple conical papillae of Gp I (Anti-PCNA, original mag. x200).	92
50	A photomicrograph of giant conical papillae of Gp I (Anti-PCNA, original mag. x200).	92
51	A photomicrograph of true filiform papillae of Gp I (Anti-PCNA, original mag. x200).	93
52	A photomicrograph of simple conical papillae of Gp II (Anti-PCNA, original mag. x200).	93
53	A photomicrograph of giant conical papillae of Gp II (Anti-PCNA, original mag. x200).	94
54	A photomicrograph of true filiform papillae of Gp II (Anti-PCNA, original mag. x200).	94
55	A photomicrograph of simple conical papillae of Gp III (Anti-PCNA, original mag. x200).	95
56	A photomicrograph of giant conical papillae of Gp III (Anti-PCNA, original mag. x200).	95

57	A photomicrograph of true filiform papillae of Gp III (Anti-PCNA, original mag. x200).	96
58	A photomicrograph of simple conical papillae of Gp IV (Anti-PCNA, original mag. x200).	96
59	A photomicrograph of giant conical papillae of Gp IV (Anti-PCNA, original mag. x200).	97
60	A photomicrograph of true filiform papillae of Gp IV (Anti-PCNA, original mag. x200).	97
61	A photomicrograph of fungiform papillae of Gp I (Anti-PCNA, original mag. x400).	100
62	A photomicrograph of fungiform papillae of Gp II (Anti-PCNA, original mag. x400).	100
63	A photomicrograph of fungiform papillae of Gp III (Anti-PCNA, original mag. x400).	101
64	A photomicrograph of fungiform papillae of Gp IV (Anti-PCNA, original mag. x400).	101
65	A photomicrograph of simple conical papillae of Gp I (PKH26, original mag. x200).	102
66	A photomicrograph of giant conical papillae of Gp I (PKH26, original mag. x200).	103
67	A photomicrograph of true filiform papillae of Gp I (PKH26, original mag. x200).	104
68	A photomicrograph of fungiform papilla of Gp I (PKH26, original mag. x200).	104
69	A photomicrograph of lower part of circumvallate papilla trough of Gp I (PKH26, original mag. x200).	105
70	A photomicrograph of foliate papillae of Gp I (PKH26, original mag. x200).	105
71	A photomicrograph of simple conical papillae of Gp III (PKH26, original mag. x200).	106
72	A photomicrograph of giant conical papillae of Gp III (PKH26, original mag. x200).	106

73	A photomicrograph of true filiform papillae of Gp III (PKH26, original mag. x 200).	107
74	A photomicrograph of fungiform papilla of Gp III (PKH26, original mag. x200).	107
75	A photomicrograph of lower part of circumvallate papilla trough of Gp I (PKH26, original mag. x200).	108
76	A photomicrograph of foliate papillae of Gp III (PKH26, original mag. x200).	108
77	A scanning electron micrograph of simple conical papillae of Gp I (x300).	110
78	A scanning electron micrograph of giant conical papillae of Gp I (x350).	110
79	A scanning electron micrograph of true filiform papillae of Gp I (x250).	111
80	A scanning electron micrograph of simple conical papillae of Gp II (x300).	113
81	A scanning electron micrograph of giant conical papillae of Gp II (x350).	113
82	A scanning electron micrograph of true filiform papillae of Gp II (x250).	114
83	A scanning electron micrograph of simple conical papillae of Gp III (x300).	116
84	A scanning electron micrograph of giant conical papillae of Gp III (x350).	116
85	A scanning electron micrograph of true filiform papillae of Gp III (x250).	117
86	A scanning electron micrograph of simple conical papillae of Gp IV (x300).	119
87	A scanning electron micrograph of giant conical papillae of Gp IV (x350).	119

88	A scanning electron micrograph of true filiform papillae of Gp IV (x250).	120
89	A scanning electron micrograph of fungiform papilla of Gp I (x600).	123
90	A scanning electron micrograph of fungiform papilla of Gp II (x600).	123
91	A scanning electron micrograph of fungiform papilla of Gp II (x600).	124
92	A scanning electron micrograph of fungiform papilla of Gp III (x600).	124
93	A scanning electron micrograph of fungiform papilla of Gp IV (x600).	125
94	A scanning electron micrograph of circumvallate papilla of Gp I (x140).	128
95	A scanning electron micrograph of circumvallate papilla of Gp II (x140).	128
96	A scanning electron micrograph of circumvallate papilla of Gp III (x140).	129
97	A scanning electron micrograph of circumvallate papilla of Gp IV (x140).	129
98	A scanning electron micrograph of foliate papillae of Gp I (x130).	131
99	A scanning electron micrograph of foliate papillae of Gp II (x130).	131
100	A scanning electron micrograph of foliate papillae of Gp III (x130).	132
101	A scanning electron micrograph of foliate papillae of Gp IV (x130).	132
102	Line chart representing changes by time after diabetes confirmation in mean FBG level of the four groups.	136

103	Bar chart representing comparison of mean FBG level between the four groups.	139
104	Line chart representing changes by time after diabetes confirmation in mean BW of the four groups.	143
105	Bar chart representing comparison of mean BW between the four groups.	146
106	Bar chart representing comparison of mean number of Anti-PCNA positive cells at same papillae between the four groups.	150
107	Bar chart representing mean number of PKH26 labeled cells at different papillae within Gp III.	152

# **LIST OF TABLES**

Table	Title	Page
No.		
1	Descriptive statistics for FBG level (mg/dL) in the different groups after diabetes confirmation.	133
2	The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison of FBG level at different time periods after diabetes confirmation within the same group.	136
3	The mean, SD values and results of one-way ANOVA test for comparison of FBG level between the different groups.	139
4	Descriptive statistics for BW (gm) in the different groups after diabetes confirmation.	140
5	The mean, SD values and results of repeated measures ANOVA test for comparison of BW at different time periods after diabetes confirmation within the same group.	143
6	The mean, SD values and results of one-way ANOVA test for comparison of BW between the different groups.	146
7	Descriptive statistics for Anti-PCNA positive cells of epithelium of filiform and fungiform papillae in the different groups.	147
8	The mean, SD values and results of one-way ANOVA test for comparison of Anti-PCNA positive cells at same papillae between the different groups.	150
9	Descriptive statistics for PKH26 labeled cells in Gp III.	151