Effect of Melatonin on High Salt-Induced Metabolic Changes in Ovariectomized Rats

Thesis

Submitted for Partial Fulfillment of Master Degree in Basic Medical Science (Medical Physiology)

By

Howida Ahmed El-sayed Saleh Khashab

(M.B.B.Ch-Ain Shams University)

Supervised by

Prof. Dr. Mona Ahmed Ahmed

Professor of Medical Physiology Faculty of Medicine - Ain Shams University

Dr. Dalia Abdel-Salam Saad

Lecturer of Medical Physiology
Faculty of Medicine - Ain shams University

Dr. Doaa Ahmed Abou-Bakr

Lecturer of Medical Physiology Faculty of Medicine - Ain shams University

Dr. Lobna Abdelrazek Elkhateb

Lecturer of Histology and Cell Biology Faculty of Medicine- Ain Shams University

Physiology Department
Faculty of Medicine, Ain Shams University
2020

Acknowledgement

First of all, I thank **ALLAH** for blessing this work as a part of his generous help throughout my life.

I would like to express my sincere gratitude and deepest thanks to **Prof. Dr. Mona Ahmed Ahmed,** Professor of Physiology, Faculty of Medicine, Ain Shams University, for suggesting and planning this work, her scientific support, judicious guidance, generous help and valuable supervision through the whole work. I am deeply indebted to her and I admit that I am so much privileged and honored to have her as my supervisor.

I would like to display my indebtedness to **Dr. Dalia Abdel-Salam Saad**, Lecturer of Physiology, Faculty of Medicine, Ain Shams University, for her careful guidance, keen supervision and valuable instructions.

I would like to display my indebtedness to **Dr. Doaa Ahmed Abou-Bakr**, Lecturer of Physiology, Faculty of Medicine, Ain Shams University, for her limitless help, kind encouragement and continuous support throughout the whole work.

I would, also, like to acknowledge **Dr. Lobna Abdelrazek Elkhateb,** Lecturer of Histology and Cell biology, Faculty of Medicine, Ain Shams University, for her useful and helpful contribution in the histological part of this work.

Last but not least, I would like to express my profound truthful love to my great husband, sincere regards and never-ending reverence to my mother, father, mother in law and father in law for the comfort and relief they provided me during their hectic schedules to accomplish this work.

Contents

Subject	Page
List of abbreviations	I
List of tables	III
List of figures	VIII
Introduction	1
Aim of the work	3
Review of literature	
- Estrogens	4
- Menopause	19
- High salt intake	27
- Melatonin	33
Materials and Methods	45
Results	90
Discussion	162
Summary and Conclusion	202
References	208
Arabic Summary	

List of Abbreviations

Abbreviation	Full Term
AMH	Anti-Mullerian Hormone
CRP	C-Reactive Protein
DAB	Di-amino-benzedene-tetra-hydro-chloride
E2	17β-Estradiol
ERα	Estrogen Receptor Alpha
ERβ	Estrogen Receptor Beta
GSH-Px	Glutathione Peroxidase
н&Е	Hematoxylin & Eosin
H_2O_2	Hydrogen Peroxide
HDL-C	High Density Lipoprotein Cholesterol
HFD	High Fat Diet
HOMA-IR	Homeostatic Model Assessment of Insulin
HRP	Horseradish Peroxidase
IL-1	Interleukin- 1
IL-12	Interleukin- 12
IL-18	Interleukin- 18
IL-1β	Interleukin- 1beta
IL-2	Interleukin- 2
IL-6	Interleukin- 6
IR	Insulin Receptor
IRS-2	Insulin Receptor Substrate-2
LDL-C	Low Density Lipoprotein -Cholesterol
LW	Liver Weight

Elist of Abbreviations

Abbreviation	Full Term
MDA	Malondialdehyde
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NAFLD	Nonalcoholic Fatty Liver Disease
PW	Pancreas Weight
TNF-α	Tumor Necrosis Factor- Alpha
VFW	Visceral Fat Weight
WC	Waist Circumference

List of Tables

Table No.	Title	Page
1	Initial and final body weight (BW, g) and body mass index (BMI, g/cm ²) and their percent change (% change) in sham-operated control group.	97
2	Initial and final body weight (BW, g) and body mass index (BMI, g/cm ²) and their percent change (% change) in ovariectomized group.	98
3	Initial and final body weight (BW, g) and body mass index (BMI, g/cm ²) and their percent change (% change) in high salt ovariectomized group.	99
4	Initial and final body weight (BW, g) and body mass index (BMI, g/cm ²) and their percent change (% change) in melatonin-treated high salt ovariectomized group.	100
5	Initial and final body weight (BW, g) and body mass index (BMI, g/cm ²) and their percent change (%change) in the different groups.	101
6	Initial and final waist circumference (WC, cm), WC percent change (% change), visceral fat weight (VFW, g) and visceral fat weight / body weight (VFW/BW, g/g) in sham-operated control group.	104
7	Initial and final waist circumference (WC, cm), WC percent change (% change), visceral fat weight (VFW, g) and visceral fat weight/ body weight (VFW/BW, g/g) in Ovariectomized group.	105
8	Initial and final waist circumference (WC, cm) and WC percent change (% change), visceral fat weight (VFW, g) and visceral fat weight/ body	106

Table No.	Title	Page
	weight (VFW/BW, g/g) in high salt	
	ovariectomized group.	
9	Initial and final waist circumference (WC, cm) and WC percent change (% change), visceral fat weight (VFW, g) and visceral fat weight / body weight (VFW/BW, g/g) in melatonin-treated high salt ovariectomized group.	107
10	Initial and final waist circumference (WC, cm) and WC percent change (%change), visceral fat weight (VFW, g) and visceral fat weight / body weight (FBW/BW, g/g) in the different groups.	108
11	Pancreas weight (PW, g), pancreas weight / body weight (PW/BW, g/g), liver weight (LW, g) and liver weight / body weight (LW/BW, g/g) in sham-operated control group.	111
12	Pancreas weight (PW, g), pancreas weight / body weight (PW/BW, g/g), liver weight (LW, g) and liver weight/body weight (LW/BW, g/g) in ovariectomized group.	112
13	Pancreas weight (PW, g), pancreas weight / body weight (PW/BW, g/g), liver weight (LW, g) and liver weight/body weight (LW/BW, g/g) in high salt ovariectomized group.	113
14	Pancreas weight (PW, g), pancreas weight / body weight (PW/BW, g/g), liver weight (LW, g) and liver weight/body weight (LW/BW, g/g) in melatonin-treated high salt ovariectomized group.	114
15	Pancreas weight (PW, g), pancreas weight / body weight (PW/BW, g/g), liver weight (LW, g) and liver weight /body weight (LW/BW, g/g) in the different groups.	115

Table No.	Title	Page
16	Plasma glucose (mg/dl), glucose uptake by diaphragm (mg/g/90 min), glucose output by kidneys (mg/g/60 min), serum insulin (ng/ml) and homeostatic model assessment of insulin resistance (HOMA-IR) in sham- operated control group.	117
17	Plasma glucose (mg/dl), glucose uptake by diaphragm (mg/g/90 min), glucose output by kidneys (mg/g/60 min), serum insulin (ng/ml) and homeostatic model assessment of insulin resistance (HOMA-IR) in ovariectomized group.	118
18	Plasma glucose (mg/dl), glucose uptake by diaphragm (mg/g/90 min), glucose output by kidneys (mg/g/60 min), serum insulin (ng/ml) and homeostatic model assessment of insulin resistance (HOMA-IR) in high salt ovariectomized group.	119
19	Plasma glucose (mg/dl), glucose uptake by diaphragm (mg/g/90 min), glucose output by kidneys (mg/g/60 min), serum insulin (ng/ml) and homeostatic model assessment of insulin resistance (HOMA-IR) in melatonin-treated high salt ovariectomized group.	120
20	Plasma glucose (mg/dl), glucose uptake by diaphragm (mg/g/90 min), glucose output by kidneys (mg/g/60 min), serum insulin (ng/ml) and homeostatic model assessment of insulin resistance (HOMA-IR) in the different groups.	121
21	Plasma triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl), high density lipoprotein- cholesterol (HDL-C, mg/dl) and low-density lipoprotein- cholesterol (LDL-C,	124

List of Tables

Table No.	Title	Page
	mg/dl) levels and atherogenic index (AI) in sham-operated control group.	
22	Plasma triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl), high density lipoprotein-cholesterol (HDL-C, mg/dl) and low density lipoprotein-cholesterol (LDL-C, mg/dl) levels and atherogenic index (AI) in ovariectomized group.	125
23	Plasma triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl), high density lipoprotein-cholesterol (HDL-C, mg/dl) and low density lipoprotein-cholesterol (LDL-C, mg/dl) levels and atherogenic index (AI) in high salt ovariectomized group.	126
24	Plasma triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl), high density lipoprotein-cholesterol (HDL-C, mg/dl) and low-density lipoprotein-cholesterol (LDL-C, mg/dl) levels and atherogenic index (AI) in melatonin-treated high salt ovariectomized group.	127
25	Plasma triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl), high density lipoprotein-cholesterol (HDL-C, mg/dl) and low-density lipoprotein-cholesterol (LDL-C, mg/dl) levels and atherogenic index (AI) in the different groups.	128
26	Serum estradiol (pg/ml), malondialdehyde (MDA, nmol/ml) and tumor necrosis factoralpha (TNF-α, pg/ml) and plasma catalase (U/L) in sham operated control group.	130
27	Serum estradiol (pg/ml), malondialdehyde (MDA, nmol/ml) and tumor necrosis factor-	131

List of Tables

Table No.	Title	Page
	alpha (TNF-α, pg/ml) and plasma catalase (U/L) in ovariectomized group.	
28	Serum estradiol (pg/ml), malondialdehyde (MDA, nmol/ml) and tumor necrosis factoralpha (TNF-α, pg/ml) and plasma catalase (U/L) in high salt ovariectomized group.	132
29	Serum estradiol (pg/ml), malondialdehyde (MDA, nmol/ml) and tumor necrosis factoralpha (TNF-α, pg/ml) and plasma catalase (U/L) in melatonin-treated high salt ovariectomized group.	133
30	Serum estradiol (pg/ml), malondialdehyde (MDA, nmol/ml) and tumor necrosis factoralpha (TNF-α, pg/ml) and plasma catalase (U/L) in the different groups.	134
31	Number of caspase-3 immune-positive cells/islet of pancreatic tissue in the studied groups	152
32	Histopathological scoring system showing number and frequency distribution (%) of each component examined in five different field sections in 4 rats (20 fields/ group).	161

List of Figures

Figure No.	Title	Page
Ι	Serial dilutions of TNF-α standard	70
II	Standard curve for TNF-α	72
III	Standard curve for insulin.	76
IV	Standard curve for estradiol.	79
1	Initial and final body weight (BW) and BW percent change (% change) in the different groups.	102
2	Initial and final body mass index (BMI) and BMI percent change (% change) in the different groups.	103
3	Initial and final waist circumference (WC) and WC percent change (% change) in different groups.	109
4	Visceral fat weight (VFW) and visceral fat weight / body weight (VFW/BW) in different groups.	110
5	Pancreas weight (PW, g), pancreas weight / body weight (PW/ BW, g/g), liver weight (LW, g) and liver weight / body weight (LW/ BW, g/g) in the different groups.	116
6	Plasma glucose, glucose uptake by diaphragm and glucose output by kidneys in the different groups.	122
7	Serum insulin and homeostatic model assessment of insulin resistance (HOMA-IR) in different groups.	123
8	Plasma triglyceride (TG, mg/dl), total cholesterol (TC, mg/dl), high density lipoprotein- cholesterol (HDL-C, mg/dl) and low density lipoprotein- cholesterol (LDL-C,	129

E List of Figures

Figure No.	Title	Page
	mg/dl) levels and atherogenic index (AI) in different groups.	
9	Serum estradiol (pg/ml), malondialdehyde (MDA, nmol / ml) and tumor necrosis factoralpha (TNF-α, pg/ml) and plasma catalase (U/L) in the different groups.	135
10	Correlations between serum level of estradiol and plasma glucose, glucose uptake by the diaphragm, serum insulin and HOMA-IR in the studied groups.	138
11	Correlations between serum level of estradiol and plasma triglycerides, total cholesterol, LDL-C, HDL-C and atherogenic index in the studied groups.	139
12	Correlations between serum level of estradiol and catalase, MDA, TNF- α in the studied groups.	140
13	Correlations between serum level of MDA and fasting plasma glucose, glucose uptake by the diaphragm, serum insulin and HOMA-IR in the studied groups.	141
14	Correlations between serum level of MDA and plasma triglycerides, total cholesterol, LDL-C, HDL-C and atherogenic index in the studied groups.	142
15	Correlations between serum MDA and plasma catalase and serum TNF- α in the studied groups.	143
16	Correlations between serum level of TNF-α and final body weight and final waist circumference in the studied groups.	144