



A Cross Talk between Ursolic Acid and Possible Fertility Disorders Induced by Metabolic Syndrome in Experimental Male Rats

Thesis

*Submitted for Partial Fulfillment of the MD Degree
in Basic Medical Sciences (Physiology)*

Presented By

Ahmed Ezzat El-Sebaiee
(M.B., B.Ch. M.Sc. Physiology)

Under Supervision of

Professor. Dr. Bataa Mohamed El-Kafoury

*Professor of Physiology
Faculty of Medicine, Ain Shams University*

Dr. Enas Abd El-Aziz Abd El-Hady

*Assistant Professor of Physiology
Faculty of Medicine, Ain Shams University*

Dr. Wessam Ezzat Morsy

*Lecturer of Physiology
Faculty of Medicine, Ain Shams University*

Physiology Department
Faculty of Medicine - Ain Shams University
2018

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgments

First and foremost, I feel always indebted to Allah the Most Beneficent and Merciful.

In recognition of gratitude, I should express my appreciation to Prof. Dr. Amira Metwaly Abd El-Rahman, Professor of Physiology, faculty of medicine, Ain Shams University, for laying foundation rules of this work and her effort in drawing the major headlines and steps to start.

I would like to acknowledge my deepest and sincere gratitude, and appreciation to Prof. Dr. Bataa Mohamed El-Kafoury, Professor of Physiology, Head of Physiology Department, Faculty of Medicine, Ain Shams University, for planning and organization of this work, and for her meticulous supervision, scientific support and judicious guidance. Indeed, I'm very privileged and honored to have worked under her distinctive supervision.

I would like to express my thanks to Doctor. Enas Abd El-Aziz Abd El- Hady, assistant professor of Physiology, faculty of medicine, Ain Shams University, for her unique and featured support and psychological encouragement.

I would like to display my indebtedness to Doctor. Wessam Ezzat Morsy, lecturer of Physiology, faculty of medicine, Ain Shams University, for her real and fruitful aid throughout the theoretical and practical parts of this work.

Special thanks to Prof. Dr. Manal Hassan, professor of Histology, faculty of medicine, Ain Shams University, for her effort regarding histological examination of the samples.

Many thanks to Prof. Dr. Amany El-Shwarby, professor of Histology, faculty of medicine, Ain Shams University, for her kind share in providing me the electron microscope for semen analysis.

Also, I really appreciate the role of Mr. Mohamed El- Serafy, the animal house keeper at Physiology department, faculty of medicine, Ain Shams University for his help throughout the practical maneuvers concerning animal handling.

To all who shared me support and smile..



To:

Soul of my father

My Mother

&

My Sisters

***for their endless love, support,
and continuous care***



List of Contents

Title	Page No.
List of Tables	7
List of Figures	14
List of Photos.....	17
List of Abbreviations.....	18
Introduction.....	- 1 -
Aim of the Work	25
Review of Literature	26
Metabolic syndrome: a major health problem	26
Risk factors and pathophysiology of metabolic syndrome:-	27
Different models of metabolic syndrome induction:	42
The hypothalamic pituitary gonadal axis and metabolic syndrome	51
Possible fertility disorders induced by metabolic syndrome	55
Fructose: chemical structure, sources, uses, handling and its role in both metabolic syndrome and possible male fertility disorders.	70
Ursolic acid: chemical structure, sources, its different functions and impact on male fertility.	89
Materials and Methods	98
Results	141
Individual Tables	178
Discussion.....	215
Summary and Conclusion.....	237
References	245
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	Changes in final body weight (gm), % change in body weight, waist circumference (cm), body mass index, retroperitoneal fat weight (gm) and epididymal weight (gm) in different studied groups.....	143
Table (2):	Changes in fasting blood glucose (mg/dl), serum insulin (μ IU/mL) and calculated insulin resistance HOMA score in different studied groups.	148
Table (3):	Changes in final systolic blood pressures (mm.Hg), final diastolic blood pressures (mm.Hg) and heart rate (beat per minute) in different studied groups.	152
Table (4):	Changes in serum triglycerides (mg/dl), total cholesterol (mg/dl), high-density lipoproteins (HDL, mg/dl), low density lipoproteins (LDL, mg/dl), HDL/ LDL ratio and calculated atherogenic index in different studied groups.....	156
Table (5):	Changes in serum prolactin (ng/ml), follicular stimulating hormone (FSH, mIU/ml) and testosterone (ng/ml) levels in different studied groups.....	161
Table (6):	Changes in testicular tissue MDA (nmol /g tissue) and glutathione peroxidase activity (U/ g tissue) in different studied groups.	165
Table (7):	Changes in epididymal sperm count ($\times 10^6$) in different studied groups.	168
Table (8):	Correlation between epididymal sperm count ($\times 10^6$) and different parameters.	170

List of Tables cont...

Table No.	Title	Page No.
Table (9):	Correlation between epididymal weight (gm) and different parameters.	171
Table (10):	Changes in initial body weight (gm), final body weight (gm), % change in body weight, waist circumference (cm), body mass index, retroperitoneal fat weight (gm) and epididymal weight (gm) in control group.	178
Table (11):	Changes in initial body weight (gm), final body weight (gm), % change in body weight, waist circumference (cm), body mass index, retroperitoneal fat weight (gm) and epididymal weight (gm) in control group that received low dose of ursolic acid.	179
Table (12):	Changes in initial body weight (gm), final body weight (gm), % change in body weight, waist circumference (cm), body mass index, retroperitoneal fat weight (gm) and epididymal weight (gm) in control group that received high dose of ursolic acid.	180
Table (13):	Changes in initial body weight (gm), final body weight (gm), waist circumference (cm) % change in body weight, body mass index, retroperitoneal fat weight (gm) and epididymal weight (gm) in metabolic syndrome induced group.	181
Table (14):	Changes in initial body weight (gm), final body weight (gm), % change in body weight, waist circumference (cm), body mass index, retroperitoneal fat weight (gm) and epididymal weight (gm) in metabolic syndrome induced group that received low dose of ursolic acid.	182

List of Tables cont...

Table No.	Title	Page No.
Table (15):	Changes in initial body weight (gm), final body weight (gm), % change in body weight, waist circumference (cm), body mass index, retroperitoneal fat weight (gm) and epididymal weight (gm) in metabolic syndrome induced group that received high dose of ursolic acid.....	183
Table (16):	Changes in fasting blood glucose (mg/dl), serum insulin (μ IU/mL) and calculated insulin resistance HOMA score in control group.....	184
Table (17):	Changes in fasting blood glucose (mg/dl), serum insulin (μ IU/mL) and calculated insulin resistance HOMA score in control group that received low dose of ursolic acid.....	185
Table (18):	Changes in fasting blood glucose (mg/dl), serum insulin (μ IU/mL) and calculated insulin resistance HOMA score in control group that received high dose of ursolic acid.	186
Table (19):	Changes in fasting blood glucose (mg/dl), serum insulin (μ IU/mL) and calculated insulin resistance HOMA score in metabolic syndrome induced group.....	187
Table (20):	Changes in fasting blood glucose (mg/dl), serum insulin (μ IU/mL) and calculated insulin resistance HOMA score in metabolic syndrome Induced group that received low dose of ursolic acid.	188
Table (21):	Changes in fasting blood glucose (mg/dl), serum insulin (μ IU/mL) and calculated insulin resistance HOMA score in metabolic syndrome induced group that received high dose of ursolic acid.	189

List of Tables cont...

Table No.	Title	Page No.
Table (22):	Changes in the initial and final systolic blood pressure (mm.Hg), diastolic blood pressure (mm.Hg) and heart rate (beat per minute) in control group.	190
Table (23):	Changes in the initial and final systolic blood pressure (mm.Hg), diastolic blood pressure (mm.Hg) and heart rate (beat per minute) in control group that received low dose of ursolic acid.	191
Table (24):	Changes in the initial and final systolic blood pressure (mm.Hg), diastolic blood pressure (mm.Hg) and heart rate (beat per minute) in control group that received high dose of ursolic acid.	192
Table (25):	Changes in the initial and final systolic blood pressure (mm.Hg), diastolic blood pressure (mm.Hg) and heart rate (beat per minute) in metabolic syndrome induced group.....	193
Table (26):	Changes in the initial and final systolic blood pressure (mm.Hg), diastolic blood pressure (mm.Hg) and heart rate (beat per minute) in metabolic syndrome induced group that received low dose of ursolic acid.....	194
Table (27):	Changes in the initial and final systolic blood pressure (mm.Hg), diastolic blood pressure (mm.Hg) and heart rate (beat per minute) in metabolic syndrome induced group that received high dose of ursolic acid.	195

List of Tables cont...

Table No.	Title	Page No.
Table (28):	Changes in serum triglycerides (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low-density lipoproteins (LDL, mg/dl), HDL/ LDL ratio and the calculated atherogenic index in control group.....	196
Table (29):	Changes in serum triglycerides (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low-density lipoproteins (LDL, mg/dl), HDL/ LDL ratio and the calculated atherogenic index in control group that received low dose of ursolic acid.....	197
Table (30):	Changes in serum triglycerides (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low-density lipoproteins (LDL, mg/dl), HDL/ LDL ratio and the calculated atherogenic index in control group that received high dose of ursolic acid.....	198
Table (31):	Changes in serum triglycerides (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low-density lipoproteins (LDL, mg/dl), HDL/ LDL ratio and the calculated atherogenic index in metabolic syndrome induced group.....	199
Table (32):	Changes in serum triglycerides (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low-density lipoproteins (LDL, mg/dl), HDL/ LDL ratio and the calculated atherogenic index in metabolic syndrome induced group that received low dose of ursolic acid.	200

List of Tables cont...

Table No.	Title	Page No.
Table (33):	Changes in serum triglycerides (mg/dl), total cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), low-density lipoproteins (LDL, mg/dl), HDL/ LDL ratio and the calculated atherogenic index in metabolic syndrome induced group that received high dose of ursolic acid.....	201
Table (34):	Changes in serum prolactin (ng/ml), follicular stimulating hormone (FSH, mIU/ml) and testosterone (ng/ml) levels in control group.	202
Table (35):	Changes in serum prolactin (ng/ml), follicular stimulating hormone (FSH, mIU/ml) and testosterone (ng/ml) levels in control group that received low dose of ursolic acid.....	203
Table (36):	Changes in serum prolactin (ng/ml), follicular stimulating hormone (FSH, mIU/ml) and testosterone (ng/ml) levels in control group that received high dose of ursolic acid.....	204
Table (37):	Changes in serum prolactin (ng/ml), follicular stimulating hormone (FSH, mIU/ml) and testosterone (ng/ml) levels in metabolic syndrome induced group.....	205
Table (38):	Changes in serum prolactin (ng/ml), follicular stimulating hormone (FSH, mIU/ml) and testosterone (ng/ml) levels in metabolic syndrome induced group that received low dose of ursolic acid.	206

List of Tables cont...

Table No.	Title	Page No.
Table (39):	Changes in serum prolactin (ng/ml), follicular stimulating hormone (FSH, mIU/ml) and testosterone (ng/ml) levels in metabolic syndrome induced group that received high dose of ursolic acid.	207
Table (40):	Changes in testicular tissue MDA (nmol /g tissue) and glutathione peroxidase activity (U/ g tissue) in control group.	208
Table (41):	Changes in testicular tissue MDA (nmol /g tissue) and glutathione peroxidase activity (U/ g tissue) in control group that received low dose of ursolic acid.....	209
Table (42):	Changes in testicular tissue MDA (nmol /g tissue) and glutathione peroxidase activity (U/ g tissue) in control group that received high dose of ursolic acid.	210
Table (43):	Changes in testicular tissue MDA (nmol /g tissue) and glutathione peroxidase activity (U/ g tissue) in metabolic syndrome induced group.	211
Table (44):	Changes in testicular tissue MDA (nmol /g tissue) and glutathione peroxidase activity (U/ g tissue) in metabolic syndrome induced group that received low dose of ursolic acid.....	212
Table (45):	Changes in testicular tissue MDA (nmol /g tissue) and glutathione peroxidase activity (U/ g tissue) in metabolic syndrome induced group that received high dose of ursolic acid.	213
Table (46):	Epididymal sperm count ($\times 10^6$) in all studied groups.	214

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Mechanism of action of insulin on intracellular signaling pathways.....	29
Figure (2):	Role of inflammatory and oxidative mediators in the pathogenesis of atherosclerosis in the metabolic syndrome	38
Figure (3):	Excessive calorie intake leads to hypertrophy of adipose tissue and increased macrophage infiltration, a condition which favors inflammation and oxidative stress situations: a precursor of metabolic syndrome.....	39
Figure (4):	Normal male hypothalamic pituitary gonadal axis	54
Figure (5):	Role of adipocytes in male infertility	58
Figure (6):	Dysregulation of the normal male hypothalamic–pituitary–gonadal axis as a consequence of obesity.....	63
Figure (7):	Postulated central mechanisms underpinning etiology of decreased testosterone secretion in obese men	64
Figure (8):	Pathophysiology of male infertility in metabolic syndrome.....	69
Figure (9):	Structural formula of fructose	70
Figure (10):	Absorption of fructose in small intestinal cells and its metabolic end-products in the liver cells	74
Figure (11):	Fructose handling by the liver cell	74
Figure (12):	Metabolism and metabolic impact of fructose overconsumption.....	78

List of Figures cont...

Fig. No.	Title	Page No.
Figure (13):	(Left) & (Right): Role of the sympathetic nervous system and ET1 in fructose induced hypertension	81
Figure (14):	Potential deleterious effects of fructose-induced hyperuricemia and its role in induction of hypertension	83
Figure (15):	Proposed mechanisms of hypertension in fructose fed rats	85
Figure (16):	Structural formula of ursolic acid.....	89
Figure (17):	Small animal tail blood pressure system	108
Figure (18):	Recording of rat's tail blood pressure using the small animal tail blood pressure system	108
Figure (19):	Recording of rat's heart rate using the small animal tail blood pressure system	109
Figure (20):	Hemocytometer used in sperm counting.	124
Figure (21):	Changes in (A)-Final body weight (gm), (B)-% change in body weight in different studied groups.	144
Figure (21):	Changes in (C)-Waist circumference (cm) and (D)-Body mass index in different studied groups.	124
Figure (21):	Changes in (E) -Retroperitoneal fat weight (gm) and (F)- Epididymal weight (gm) in different studied groups.	125
Figure (22):	Changes in (A)- Fasting blood glucose (mg/dl) and (B)- Serum insulin (μ IU/mL) in different studied groups.....	149
Figure (22):	Changes in calculated insulin resistance HOMA score in different studied groups.....	129