



Faculty of Science
Zoology Department

Physiological and Molecular Studies on the Effect of Pomegranate (*Punica granatum* L.) Peel and Some of its Bioactive Components against Hyperlipidemia-Induced Atherosclerosis in Male Albino Rats

A Thesis

Submitted in Partial Fulfillment for the Degree of
Master of Science in Zoology

By

Randa Ratib Soliman Def-Allah

B. Sc. (Zoology/ Chemistry), 2012

Supervisors

Prof. Nadia Mohamed Abd El-Aziz El-Beih

Professor of Physiology,
Department of Zoology, Faculty of Science,
Ain Shams University

Dr. Enas Ali El-Husseny

Assistant professor of Physiology,
Department of Zoology, Faculty of Science,
Ain Shams University

(2018)



Faculty of Science
Zoology Department

Student Name: Randa Ratib Soliman Def-Allah

Thesis Title:

Physiological and Molecular Studies on the Effect of Pomegranate
(*Punica granatum* L.) Peel and Some of its Bioactive Components
against Hyperlipidemia-Induced Atherosclerosis
in Male Albino Rats

Degree: Master of Science in Zoology

This thesis has been supervised by:

- **Prof. Nadia Mohamed Abd El-Aziz El-Beih**

Professor of Physiology
Zoology Department - Faculty of Science
Ain Shams University

- **Dr. Enas Ali El-Husseney**

Assistant Professor of Physiology
Zoology Department - Faculty of Science
Ain Shams University



Faculty of Science
Zoology Department

Approval Sheet

Title of Thesis:

Physiological and Molecular Studies on the Effect of Pomegranate
(*Punica granatum* L.) Peel and Some of its Bioactive Components
against Hyperlipidemia-Induced Atherosclerosis
in Male Albino Rats

Name of Student: Randa Ratib Soliman Def-Allah

Degree: Master of Science in Zoology

This thesis has been approved by:

- **Prof. Hamdy Abd-El Hameed Ibrahim**
Professor of Physiology -Zoology Department
Faculty of Science - Mansoura University
- **Prof. Amr Abd-El Azeem Shalaby**
Professor of Physiology - Zoology Department
Faculty of Science - Zagazig University
- **Prof. Nadia Mohamed Abd-El Aziz El Beih**
Professor of Physiology - Zoology Department
Faculty of Science - Ain Shams University

Acknowledgements

First of all, I thanks immeasurably my God Almighty for blessing me with the completion of this work and to overcome the difficulties that I faced during its performance.

I am much honored to extend my thanks, respect and appreciation to my supervisor Prof. Nadia Mohamed Abd El-Aziz El-Beih, Professor of Physiology, Department of Zoology, Faculty of Science, Ain Shams University, whose name is always a sign of prestige and appreciation and also for her supervising and reviewing this work and its interest to be the best. She always guided me with her advice, knowledge and unlimited encouragement. I ask God Almighty to perpetuate her health and wellness.

I would like to extend my sincere thanks to Dr. Enas Ali El-Husseney, Assistant Professor of Physiology, Department of Zoology, Faculty of Science, Ain Shams University, for giving me a lot of her time to present and discuss practical and scientific results as well for her continuous effort to teach me the modern scientific methods. My great thanks are to her kindness, friendship and her constructive participation in my life. I do not forget her great support for me in all directions of life; mentally, psychologically and socially.

I am grateful to Prof. Khalid Gamal Al-Din, Professor of Medical Physiology, National Research Centre, for his

*scientific support in the part of the practical work of ELIZA.
All thanks and respect for him.*

I would like to express my gratitude to the head and all members of the Department of Zoology, Faculty of Science, Ain Shams University for their help and encouragement. Especially thanks to the Committees of Physiological Research Laboratory, Central Laboratory and the tissue culture laboratory in the department for providing me with facilities to complete my work.

I owe my lifetime thanks to my husband, Mohamed Farag Abo El-Hamd, teaching assistant, Department of Botany for his great moral support, scientific advice and his trials in solving the problems that faced me with his great wisdom. May Allah bless you and make you one of the famous scientists.

Finally, I would like to thank my family, my father, my mother and my brothers for their moral support and their prayers to Allah for my success.

Randa Ratib Soliman

List of Abbreviations

4AAP	4-amino-antipyrine
AC	Atherogenic coefficient
AIP	Atherogenic index of plasma
AMI	Acute Myocardial infarction
ANOVA	Analysis of variance
APA	Aminophenazone
BW	Body weight
CaCl₂	Calcium chloride
CAD	Coronary artery disease
CAT	Catalase
CD36	Cluster of differentiation 36
CD68	Cluster of differentiation 68
cDNA	Complementary deoxyribonucleic acid
CE	Cholesterol esterase
CO	Cholesterol oxidase
CPK-MB	Creatine phosphokinase-MB
CRI-I	Castelli's risk index I
CRI-II	Castelli's risk index II
Ct	Cycle threshold
CVDs	Cardiovascular diseases
DHBS	3,5-Dichloro-2-hydroxy benzene sulfonic acid
DHBS	3,5-dichloro-2-hydroxybenzene sulfonic acid
DTNB	5, 5`Dithiobis (2 nitrobenzoic acid)
EA	Ellagic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immune-sorbent assay
FFA	Free fatty acid
G-6-PD	Glucose-6- phosphate dehydrogenase
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
GK	Glycerol kinase
GPO	Glycerol phosphate oxidase

GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione reduced
GSSG	Oxidized glutathione
H₂O₂	Hydrogen peroxide
HD PPE	High dose pomegranate peel extract
HDL-C	High density lipoprotein-cholesterol
HFD	High fat diet
HK	Hexokinase
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
i.p.	Intraperitoneal
IL-1	Interleukin-1
IL-6	Interleukin-6
KCl	Potassium chloride
KH₂PO₄	Potassium phosphate monobasic
LD PPE	Low dose pomegranate peel extract
LDH	Lactate dehydrogenase
LDL	Low-density lipoproteins
LDL-C	Low density lipoprotein-cholesterol
LOX-1	Lectin-like oxidized low density lipoprotein receptor-1
LPL	Lipoprotein lipase
MDA	Malondialdehyde
MCP-1	Monocyte chemo-attractant protein-1
M-CSF	Macrophage colony stimulating factor
mRNA	Messenger ribonucleic acid
Na₂HPO₄	Sodium phosphate dibasic
NaCl	Sodium chloride
NAD⁺	Nicotinamide adenine dinucleotide
NADP⁺	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide 3-phosphate (reduced form)
NBT	Nitroblue tetrazolium
NOx	Nitric oxide

Ox-LDL	Oxidized lowdensity lipoproteins
OD	Optical density
PBS	Phosphate buffer solution
PC	Punicalagin
PCR	Polymerase chain reaction
PE	Pomegranate extract
PFE	Pomegranate flower Extract
PJ	Pomegranate juice
PLE	Pomegranate leaf extract
PMS	Phenazine methosulphate
POD	Peroxidase
PON1	Paraoxonase 1
PP	Pomegranate peel
PPE	Pomegranate peel extract
PPPs	Pomegranate peel polyphenols
PSO	Pomegranate seed oil
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT	Reverse transcription
RT-PCR	Reverse transcription polymerase chain reaction
SD	Sprague Dawley
SEM	Standard error of means
SOD	Superoxide dismutase
SR-B	Scavenger receptor class B
STZ	Streptozotocin
TAG	Triacylglycerol
TBA	Thiobarbituric acid
TC	Total cholesterol
TMB	3,3',5,5'-tetramethylbenzidine
TNF-α	Tumor necrosis factor alpha
VLDL-C	Very low density lipoprotein cholesterol
WFEP	Whole fruit extract of pomegranate
WHO	World health organization

XO	Xanthine oxidase
-----------	------------------

List of Tables

Table	Title	Page
Materials and Methods		
I	Reagents used for cDNA preparation.	59
II	Sequences of primers used for the Real-time PCR analysis.	60
III	Master mix used for real time PCR.	60
IV	Thermal cycling conditions of real time PCR program of GAPDH, CD-36 and LOX-1 genes.	60
Results		
1	Body weight change%, liver weight (g) and relative liver weight of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	66
2	Total Cholesterol (TC) and Triacylglycerol (TAG) concentrations (mg/dl) of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	70
3	Serum high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) concentration (mg/dl) of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	76
4	Atherogenic index and Atherogenic coefficient of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic Acid and	81

	punicalagin	
5	Castelli's risk index I and Castelli's risk index II of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	84
6	Liver glutathione reduced (GSH) level (mg/g tissue) and liver glutathione reductase (GR) activity (U/L) of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	88
7	Liver glutathione peroxidase (GPx) activity (U/g tissue) and liver superoxide dismutase (SOD) activity (U/g tissue)) of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	93
8	Catalase (CAT) activity (U/g tissue) and Liver malondialdehyde (MDA) level (nmol/g tissue) of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	99
9	Nitric oxide (NO _x) level (μmol/L) and serum paraoxonase 1 (PON1) activity (U/L) of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin	102
10	Serum Creatine phosphokinase (CPK-MB) activity (U/L) and serum lactate dehydrogenase (LDH) activity (U/L) of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	105

11	TNF- α level in serum of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	109
12	The expression of CD36 in dorsal aorta of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	111

List of Figures

Figure	Title	Page
Literature Review		
I	Initiation and development of atherosclerotic Lesion (Napoli, 2002).	10
II	Pomegranate fruit, flower, sundried pomegranate peel and peel powder (Ismail et al., 2012).	15
III	Principal therapeutic effects of pomegranate peel (Middha et al., 2013).	21
IV	Chemical structure of punicalagin and ellagic acid (Seeram et al., 2006).	23
Materials and Methods		
V	HPLC chromatographic profiles at 254 nm of ellagic acid (EA) standard (A), punicalagin (PC) standard (B) and pomegranate peel extract (C).	40
VI	Amplification curve of real time-PCR showing Ct.	61
VII	Dissociation curve of real time-PCR.	62
Results		
1	Body weight change % in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	67
2	Liver weight (g) in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	68

3	Relative liver weight in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	69
4	Total cholesterol concentration (mg/dl) in serum of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	71
5	Triacylglycerol concentration (mg/dl) in serum of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	72
6	High density lipoprotein cholesterol concentration (mg/dl) in serum of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	77
7	Low density lipoprotein cholesterol concentration (mg/dl) in serum of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	78
8	Very low density lipoprotein cholesterol concentration (mg/dl) in serum of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	80
9	Atherogenic index of plasma in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and	82

	punicalagin.	
10	Atherogenic coefficient in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	84
11	Castelli's risk index I in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	85
12	Castelli's risk index II in non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	86
13	Reduced glutathione level (mg/g tissue) in liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	89
14	Glutathione reductase activity (U/L) in liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	90
15	Glutathione peroxidase activity (U/g tissue) in liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	94
16	Superoxide dismutase activity (U/g tissue) in liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel extract (low and high doses), ellagic acid and punicalagin.	95
17	Catalase activity (U/g tissue) of liver tissue of non-hyperlipidemic and hyperlipidemic adult male albino rats treated with pomegranate peel	98