



Faculty of Science
Department of Zoology

Evaluation of the ameliorative effects of safranal and selenium on the liver damage induced by thyrotoxicosis in adult male albino rats

A Thesis

Submitted for the award of the degree of
Ph.D. in Zoology (Physiology)

Submitted by

Rasha Ali Hussein Ali

Drug control specialist- National Organization
For Drug Control and Research
M.Sc. in physiology - Faculty of Science,
Helwan University
2012

Under supervision of

Dr. Wael Mohamed El-Sayed

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

Dr. Lobna Abd El-monem Mohamed Hassanin

Assistant Professor of Physiology,
National Organization for Drug Control and Research

Dr. Enas Ali El-Hussieny

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

2017

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَلِلَّهِ الْمُلْكُ الْأَمِينُ
إِذَا دُعِيَ إِلَى اللَّهِ وَالْيَوْمِ
الْآخِرِ أَفْوَاحًا
وَلِلَّهِ الْمُلْكُ الْأَمِينُ

صَدَقَ اللَّهُ الْعَظِيمُ

Dedication

To my beloved mother,

To my beloved father

To my second mother Zeinab Elfass

To my brothers,

Hussein, Tamer

To my sisters,

Rabab, Rania

To the new generation

My nephews,

Anas, Ahmed, Ali, yassein

And

To my nieces,

Mariam, Gana and Malak

Acknowledgments

ACKNOWLEDGMENTS

First of all, cordial thanks are due to Allah who enabled me to overcome all the problems which faced me throughout the work.

*I am greatly indebted to my supervisor **Dr. Wael M. El-Sayed**, Professor of Physiology, Department of Zoology, Faculty of Science, Ain Shams University for giving me the opportunity to perform this work under excellent working atmosphere, his encouragement, patience and interest that he showed in my work during the study period. I found him a true academician and I will always remember him with respect.*

*No words can express my sincere gratitude to **Dr. Lobna Abd El-monem**, Assistant Professor of Physiology, National Organization for Drug Control and Research for her encouragement, patience during the practical part, help during the preparation for this work and for her creative ideas and **Dr. Enas Ali El-Hussieny**, Lecturer of Physiology, Department of Zoology, Faculty of Science, Ain Shams University for her guidance and help during the gene expression part and her help and creative ideas.*

Acknowledgments

I would also like to express my thanks to all members of Hormone evaluation department, National Organization for Drug Control and Research.

Thanks are also extended to head and members of Zoology Department, Faculty of Science, Ain Shams University.

Rasha Ali Hussein Ali

List of Contents

Contents

Contents	Page
List of Abbreviations	I
List of Tables	III
List of Figures.....	IV
Abstract	1
1. Introduction	3
Aim of the work	4
2. Review of Literature	6
2.1 The Thyroid gland and Thyroid hormones	6
2.1.1 Thyroid hormones and liver.....	7
2.1.2 Thyrotoxicosis.....	8
2.1.3 Thyrotoxicosis and liver.....	8
2.2 Oxidative stress.....	9
2.2.1 Hyperthyroidism and oxidative stress.....	13
2.3 Apoptosis.....	14
2.4 Effect of thyrotoxicosis on different parameters.....	17
2.4.1 Effect of thyrotoxicosis on body weight, alanine aminotransferase (ALT), aspartate amino transferase (AST), oxidative parameters and liver histology.....	17
2.4.2 Effect of thyrotoxicosis on caspase-3, caspase-9, bax, bcl-2, DNAfragmentation.....	22
2.5 Safranal.....	23
2.5.1 Biological properties of saffron.....	23
2.5.2 Biological properties of safranal.....	25
2.6 Effect of safranal on various parameters.....	26
2.6.1 Effect of safranal on body weight, alanine aminotransferase (ALT), aspartate amino transferase (AST), oxidative parameters and liver histology.....	26

List of Contents

Contents (cont...)

Contents	Page
2.6.2 Effect of safranal on caspase-3, caspase-9, bax, bcl-2 and DNA fragmentation.....	28
2.7 Selenite.....	29
2.7.1 Role of selenium in the synthesis of thyroid hormones....	31
2.7.2 Biological properties of selenium.....	32
2.8 Effect of selenite on various parameters.....	32
2.8.1 Effect of selenite on body weight, ALT, AST, oxidative parameters and liver histology.....	32
2.8.2 Effect of selenite on caspase-3, caspase-9, bax, bcl-2 and DNA fragmentation.....	37
3. Materials and Methods	38
3.1. Materials	38
3.1.1 Agents	38
-Levothyroxine (L-T4).....	38
-Safranal.....	38
-Sodium selenite.....	38
3.1.2. Experimental animals.....	38
-Experimental design.....	39
-Animal groups.....	39
3.2 Blood collection and sample analysis.....	40
3.3 Tissue and sample analysis.....	41
3.4 Biochemical determinations.....	41
3.4.1 Determination of free triiodothyronine (fT3) and free thyroxine (fT4).....	41
3.4.2 Determination of thyroid stimulating hormone (TSH).....	45

List of Contents

Contents (cont...)

Contents	Page
3.4.3 Determination of alanine aminotransferase (ALT) activity.	48
3.4.4 Determination of aspartate aminotransferase (AST) activity.....	49
3.4.5 Determination of Malondialdehyde (MDA).....	51
3.4.6 Determination of Nitric oxide (NO).....	53
3.4.7 Determination of GSH.....	54
3.4.8 Determination of superoxide dismutase (SOD) activity.....	56
3.4.9 Determination of catalase (CAT) activity.....	58
3.4.10 Determination of total protein.....	59
3.5 Bcl-2, bax, caspase-3 and caspase-9 assay by RT-PCR.....	61
3.5.1 RNA Extraction.....	61
-Homogenization.....	61
-Phase Separation.....	61
-RNA Precipitation.....	62
-RNA Washing.....	62
3.5.2 RNA Quantification.....	62
3.5.3 RNA Electrophoresis.....	63
3.5.4 Reverse Transcription Polymerase Chain Reaction (RT-PCR).....	63
3.5.5 Real-time PCR analysis.....	64
3.6 DNA fragmentation% assay.....	65
3.7 Histopathological study.....	67
3.8 Statistical analysis.....	67
4. Results	68
5. Discussion	119

List of Contents

Contents (cont...)

Contents	Page
6. Summary	137
7. References	140
Arabic Summary	
Arabic Abstract	

List of Abbreviations

List of Abbreviations

Abbrev.	Full-term
AIF	Apoptosis-inducing factor
ALT	Alanine aminotransferase
ANOVA	analysis of variance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
Bcl-2	B cell lymphoma
Caspases	CysteinyI aspartate specific proteases
CAT	Catalase
DNA	Deoxyribonucleic acid
DEPC	Diethylpyrocarbonate
DPA	Diphenylamine
dNTP	Deoxyribonucleotide triphosphate
ELISA	Enzyme-linked immunosorbent assay
ft3	Free triiodothyronine
ft4	Free thyroxine
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
H ₂ O ₂	Hydrogen peroxide
IDD	Iodothyronine deiodinases
LPO	Lipid peroxidation
L-T4	Levothyroxine
MDA	Malondialdehyde

List of Abbreviations

List of Abbreviations (cont...)

Abbrev.	Full-term
NO	Nitric oxide
NOS	Nitric Oxide Synthase
NF- κ B	Hepatic nuclear factor Kappa B
OS	Oxidative stress
PTP	permeability transition pores
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RT- PCR	Reverse Transcription Polymerase Chain Reaction
Se	Selenium
Smac/Diablo	Second mitochondria-derived activator of caspases/direct IAP binding protein with low pI
SOD	Superoxide dismutase
T3	Triiodothyronine
T4	Thyroxine
THs	Thyroid hormones
TSH	Thyroid stimulating hormone
TAE	Tris-acetate-EDTA
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TE	Tris EDTA
TMB	Tetramethylbenzidine
TNF- α	Tumor necrosis factor

List of Tables

Table No	Title	Page
Table (3.1)	Sequences of primers used for the Real-time PCR analysis.....	65
Table (4.1)	Effect of safranal and selenite on body weight change, liver weight and relative liver weight in adult male albino rats.....	69
Table (4.2)	Effect of safranal and selenite on serum free triiodothyronine (fT3) and free thyroxine (fT4) and thyroid stimulating hormone (TSH) levels in adult male albino rats.....	74
Table (4.3)	Effect of safranal and selenite on serum alanine aminotransferase (ALT) and aspartate amino transferase(AST) activities in adult male albino rats.....	79
Table (4.4)	Effect of safranal and selenite on malondialdehyde (MDA) and nitric oxide (NO) and reduced glutathione (GSH) level in adult male albino rats.....	83
Table (4.5)	Effect of safranal and selenite on catalase and superoxide dismutase (SOD) activities in adult male albino rats.....	89
Table (4.6)	Effect of safranal and selenite on bax, bcl-2 mRNA expression ($2^{-\Delta\Delta CT}$), bax/bcl-2 ratio in adult male albino rats.....	93
Table (4.7)	Effect of safranal and selenite on caspase -9 and caspase- 3 mRNA expression ($2^{-\Delta\Delta CT}$) in adult male albino rats.....	100
Table (4.8)	Effect of safranal and selenite on DNA fragmentation (%) in adult male albino rats.	107
Table (4.9)	Effect of safranal and selenite on histological alteration in liver tissue in normal, L-T4- treated rats.....	118

List of Figures

List of Figures

Figure No	Title	Page
Figure 2.1	Death-receptor-mediated and mitochondrial pathways of cell apoptosis.....	17
Figure 2.2	Chemical structure of safranal	24
Figure 2.3	Structure of selenite.....	30
Figure 3.1	Standard curve of free triiodothyronine	44
Figure 3.2	Standard curve of free thyroxine.....	45
Figure 3.3	Standard curve of thyroid stimulating hormone....	47
Figure 3.4	Standard curve of ALT.....	49
Figure 3.5	Standard curve of AST.....	51
Figure 3.6	Standard curve of MDA.....	52
Figure 3.7	Standard curve of NO.....	54
Figure 3.8	Standard curve of GSH.....	56
Figure 3.9	Standard curve of protein.....	61
Figure 4.1	Effect of safranal and selenite on body weight change (%) in adult male albino rats.....	70
Figure 4.2	Effect of safranal and selenite on liver weight (g) in adult male albino rats.....	71
Figure 4.3	Effect of safranal and selenite on relative liver weight (g/g) in adult male albino rats.....	72
Figure 4.4	Effect of safranal and selenite on serum fT3 (pg/ml) level in adult male albino rats.....	75
Figure 4.5	Effect of safranal and selenite on serum fT4 (ng/dl) level in adult male albino rats.....	76
Figure 4.6	Effect of safranal and selenite on serum TSH (μ IU/ml) level in adult male albino rats.....	78
Figure 4.7	Effect of safranal and selenite on serum alanine amino transferase (ALT) activity (U/L) in adult male albino rats.....	80

List of Figures

List of Figures (cont...)

Figure No	Title	Page
Figure 4.8	Effect of safranal and selenite on serum aspartate amino transferase (AST) activity (U/L) in adult male albino rats.....	81
Figure 4.9	Effect of safranal and selenite on malondialdehyde (MDA) level (nmol/g tissue) in adult male albino rats.....	84
Figure 4.10	Effect of safranal and selenite on nitric oxide (NO) level ($\mu\text{mol/g}$ tissue) in adult male albino rats.....	86
Figure 4.11	Effect of safranal and selenite on reduced glutathione (GSH) level in (mmol/g tissue) adult male albino rats.....	87
Figure 4.12	Effect of safranal and selenite on catalase activity (U/mg protein) in adult male albino rats.....	90
Figure 4.13	Effect of safranal and selenite on superoxide dismutase (SOD) activity (U/mg protein) in adult male albino rats.....	91
Figure 4.14	Effect of safranal and selenite on the bax mRNA expression ($2^{-\Delta\Delta\text{CT}}$) in adult male albino rats.....	94
Figure 4.15	Amplification plot of bax.....	95
Figure 4.16	Amplification plot of GAPDH.....	95
Figure 4.17	Effect of safranal and selenite on the fold change of bcl-2 mRNA expression ($2^{-\Delta\Delta\text{CT}}$) in adult male albino rats.	96
Figure 4.18	Amplification plot of bcl-2.....	97
Figure 4.19	Effect of safranal and selenite on bax/ bcl-2 ratio in adult male albino rats.....	99
Figure 4.20	Effect of safranal and selenite on the fold change of caspase-9 mRNA expression ($2^{-\Delta\Delta\text{CT}}$) in adult male albino rats.....	101

List of Figures

List of Figures (cont...)

Figure No	Title	Page
Figure 4.21	Amplification plot of caspase-9.....	102
Figure 4.22	Effect of safranal and selenite on the fold change of caspase-3 mRNA expression ($2^{-\Delta\Delta CT}$) in adult male albino rats.....	104
Figure 4.23	Amplification plot of caspase-3.....	105
Figure 4.24	Effect of safranal and selenite on DNA fragmentation (%) in adult male albino rats....	108
Figure 4.25	A photomicrograph of a liver of rat (saline group) showing normal histological structure of the central vein (CV) and surrounding hepatocytes (h)(H&E) (X40).....	111
Figure 4.26	A photomicrograph a liver of rat (paraffin group) showing normal histological structure of the central vein (CV) and surrounding hepatocytes (h) (H&E) (X 40).....	111
Figure 4.27	A photomicrograph of a liver of rat (safranal group) showing normal histological structure of the portal area (pa) and surrounding hepatocytes (h) (H&E) (X40).....	112
Figure 4.28	A photomicrograph of a liver of rat (sodium selenite group) showing normal histological structure (H&E) (X40).....	112
Figure 4.29	A photomicrograph of a liver of rat (thyrotoxic animals) showing sever dialation and congestion in central vein (CV) (H&E) (X16).....	113
Figure 4.30	A photomicrograph of a liver of rat (thyrotoxic animals) showing sever dialation and congestion in central vein (CV) (H&E) (X40).....	113
Figure 4.31	A photomicrograph of a liver of rat (thyrotoxic animals) showing sever congestion in portal vein (PV) and dilation in bile duct (bd) and fibrosis (f) with oedema (o) in portal area (H&E) (X16).....	113