

Ain Shams University
Faculty of Pharmacy
Pharmacology and Toxicology Department

Study of the Protective Effect of Blueberry Leaves Extract on Isoprenaline-Induced Myocardial Hypertrophy in Rats

A thesis submitted for the partial fulfillment of the requirements of M.Sc. in pharmaceutical sciences (Pharmacology and Toxicology).

By:

Radwa Ahmed Mohamed Eladwy

BPharm (Hons)

Demonstrator of Pharmacology and Toxicology Faculty of Pharmacy - Egyptian Russian University Under Supervision of:

Dr. Laila Ahmed A. Ramadan

Professor of Pharmacology and Toxicology Faculty of Pharmacy - Egyptian Russian University

Dr. Samar Saad-Eldeen Azab

Assistant Professor of Pharmacology and Toxicology Faculty of Pharmacy - Ain Shams University

Dr. Eman Mohamed Mantawy

Lecturer of Pharmacology and Toxicology Faculty of Pharmacy - Ain Shams University

بِسُ مِ اللَّهِ الرَّحْمِ الرَّحِيمِ

Acknowledgements

Acknowledgements

I would like to express my gratitude anyone who helped me in any way throughout my MSc.

I would like to thank **Prof. Dr. Laila Ramadan**, Professor of Pharmacology and Toxicology, Faculty of Pharmacy, Egyptian Russian University; for suggesting work, competent supervision and kind advices. My gratefulness to **Dr. Samar Azab**, Assistant Professor of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University for her helpful advices, sincere efforts and guidance throughout the entire work. I would like to declare that, this research work was greatly enhanced by her valuable contributions, a step-by-step guidance and precious remarks.

I would like to extend my appreciation to **Dr. Eman Mantawy**, Lecturer of Pharmacology and Toxicology, Faculty of Pharmacy, Ain-Shams University, for her support and competent guidance throughout the practical experiments. I owe my deepest gratitude to **Dr. Wesam El-bakly**, Lecturer of Pharmacology and Toxicology, Faculty of Medicine, Ain-Shams University, for her help in conducting the ECG and her generous help in generating ECG and immunohistochemistry figures.

I wish to express my great thanks and appreciation to Dr Adel Bekier, Dr Mohamed Fares, Dr Mustafa Fayed, Dr Hassan Afify, Dr Ahmed Mostafa for their assistance and collaboration during the time of my practical work and throughout my postgraduate study.

I would like to explicit my sincere thanks and gratitude to all members of the Pharmacology and Toxicology Department, Faculty of Pharmacy, Egyptian Russian University and all members of the Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University; for their cooperation and encouragement throughout this work.

Finally, I would like to express my gratitude to my mother, father, sisters, sons and my husband for their support and encouragement through my degree.

Radwa Eladwy

Table of Contents

Acknov	vledgement	I
List of A	Abbreviations:	I
List of	Γables	IV
List of l	Figures	V
Abstrac	rt	VIII
Introd	luction	1
A- D	efinition of cardiac hypertrophy:	1
	isk factors and prevalence of cardiac hypertrophy:	
	evelopment and classification of cardiac hypertrophy:	
	lechanism of cardiac hypertrophy	
	reatment of cardiac hypertrophy	
	utraceuticals	
	f the work	
	rials and Methods	
A.	Materials	
Dri	ugs and extracts	45
An	imals:	45
Ch	emicals and solutions:	46
Rea	adymade kits	48
An	tibodies:	49
В.	Methods:	50
1.	Electrocardiography (ECG):	50
2.	Assessment of heart indices:	50
3.	Serum preparation and preparation of tissue homogenate:	51
4.	Determination of serum aspartate amino transferase (AST):	52
5.	Determination of serum creatine kinase MB (CK-MB):	55
6.	Determination of serum lactate dehydrogenase:	57
7.	Determination of lipid peroxidation:	59

	8.	Determination of non-Protein Sulfhydryl Compounds	61
	9.	Determination of catalase (CAT) activity:	63
	10.	Determination of total proteins:	67
	11.	Determination of Troponin I activity	69
	12.	Determination of Interleukin-6 (IL-6)	73
	13.	Determination of Tumor necrosis factor-α (TNF-α):	79
	14.	Determination of Transforming growth factor beta 1 (TGF-β):	84
	15.	Histopathological examination:	89
	16.	Immunohistochemical examination	90
	17.	Statistical analysis:	92
Res	ults	5	93
A)) Ca	rdiotoxicity indices:	93
	1.	Electrocardiography (ECG)	93
	2.	Heart index and heart weight/tail length ratio	99
	3.	Biochemical cardiotoxicity markers:	103
B)	His	stopathological examination	109
C)) Me	echanistic study	112
	1. O	xidative stress markers:	112
	2. A	ssessment of Inflammatory mediators:	117
	3. In	nmunohistochemical detection of NF-κB (p65) and COX-2:	121
	4. A	ssessment of fibrosis:	126
Disc	cuss	sion	132
Sun	nma	ary and conclusion	142
		nces	
1761		IIVU	170

List of Abbreviations:

AAP	4-Aminophenazone
ABC	Avidin-Biotin-Peroxidase Complex
ACE	Angiotensin converting enzyme
AMP	Adenosine monophosphate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BB	Blueberry leaves extract
BSA	Bovine serum albumin
CaMKII	Calmodulin-dependent kinase II
c-AMP	Cyclic adenosine monophosphate
CAT	Catalase
CGA	Chlorogenic acid
СН	Cardiac hypertrophy
CK-MB	Creatine kinase MB
COX-2	Cyclooxygenase- 2
CVDs	Cardiovascular diseases
DCHBS	3,5-Dichloro-2-hydroxybenzene sulfonic
	acid
DMSO	Dimethyl sulfoxide
DTNB	5,5-dithio-bis (2-nitrobenzoic acid
ECG	Electrocardiogram

ĺ

ET-1	Endothelin-1
FAs	Fatty acids
G ₆ PDH	Glucose-6-phosphate dehydrogenase
GPx	Glutathione peroxidase
GSH	Glutathione
HK	Hexokinase
H ₂ O ₂	Hydrogen peroxide
HRP	Horseradish peroxidase
Ht index%	Heart index %
IL-6	Interleukin 6
iNOS	Inducible nitric oxide synthase
IP-3	Inositol-1,4,5-trisphosphate
ISO	Isoproterenol (Isoprenaline)
JAK	Janus kinase
LDH	Lactate dehydrogenase
LV	Left ventricle
LVH	Left ventricular hypertrophy
MDA	Malondialdehyde
MI	Myocardial infarction
mTOR	Mammalian target of rapamycin
NADPH	Nicotinamide adenine dinucleotide
	phosphate
NFAT	Nuclear factor of activated T cell

NF-κB	Nuclear factor kappa B
NO ⁻	Nitric oxide
\mathbf{O}^{2-}	Superoxide anion
O.D.	Optical density
OH.	Hydroxyl anion
PUFAs	Polyunsaturated fatty acids
RAAS	Renin-angiotensin-aldosterone system
REC-ASU	Research Ethics Committee of Ain Shams
	University
ROS	Reactive oxygen species
RV	Right ventricule
SOD	Superoxide dismutase
STAT	Signal transducer and activator of
	transcription
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBS	Tris buffered saline
TCA	Trichloroacetic acid
TGF-β	Transforming growth factor-beta
TMB	3,3',5,5'-tetramethylbenzidine
TNF-α	Tumor necrosis factor-alpha
TTE	Transthoracic echocardiography

List of Tables

Table 1: Description of the main electrocardiogram waves, their
representation and the linked disorder3
Table 2: Comparison of the pathological and physiological cardiac
hypertrophy6
Table 3: Effect of different doses of blueberry leaf extract (25, 50 and 100
mg/kg) on the cardiac hypertrophy induced by isoprenaline in rats 95
Table 4: Effect of different doses of blueberry leaf extract (25, 50 and 100
mg/kg) on the cardiac hypertrophy induced by isoprenaline 100
Table 5: Effect of different doses of blueberry leaf extract (25, 50 and 100
mg/kg) on AST, CKMB, LDH and troponin I levels in isoprenaline-
induced cardiotoxicity in rats
Table 6: Some oxidative stress markers of rats treated with 50 mg/kg
blueberry extract
Table 7: Effect of 50 mg/kg blueberry extract on inflammatory markers
IL-6 and TNF- α in isoprenaline-induced cardiotoxicity in rats 118
Table 8: Effect of the 50 mg/kg blueberry extract on the transforming
growth factor beta in isoprenaline-induced cardiac hypertrophy in rats.

List of Figures

Figure 1: Schematic representation of an ECG wave, which	ch is composed
mainly from P wave, QRS complex and ST segment	2
Figure 2: Schematic diagram of the RAAS pathway lead	ling to cardiac
hypertrophy and the possible intervention by some drug cl	lasses 12
Figure 3: Schematic diagram of the adrenergic β1-reco	eptor pathway
leading to cardiac hypertrophy	13
Figure 4: General chemical structure of anthocyanins, a	nthocyanidins,
chlorogenic acid and its precursors.	30
Figure 5: Timeline for the experimental work flow	40
Figure 6: Standard Calibration curve of AST	54
Figure 7: Standard calibration curve of MDA	60
Figure 8: Calibration curve of Troponin I	72
Figure 9: Calibration curve of IL-6	78
Figure 10 Calibration curve of TNF-α	83
Figure 11 Calibration curve of TGF-beta	88
Figure 12: Effect of different doses of blueberry (25, 50 a	nd 100 mg/kg)
on ECG pattern, in ISO-intoxicated rats.	94
Figure 13: Effect of different doses of blueberry (25, 50 a	nd 100 mg/kg)
on heart rate (beat/min) in rats subjected to isoprenaline	intoxication as
percentage of control group	96
Figure 14: Effect of different doses of blueberry (25, 50 a	nd 100 mg/kg)
on T wave amplitude (mv) in rats subjected to isoprenaling	ne intoxication
as percentage of control group	97
Figure 15: Effect of different doses of blueberry (25, 50 a	nd 100 mg/kg)
on R wave (mv) amplitude in rats subjected to isoprenali	ne intoxication
as percentage of control group.	98

Figure 16: Effect of different doses of blueberry (25, 50 and 100 mg/kg)
on heart index (%) in rats subjected to isoprenaline intoxication as
percentage of control group.
Figure 17: Effect of different doses of blueberry (25, 50 and 100 mg/kg)
on Heart weight/tail length ratio in rats subjected to isoprenaline
intoxication as percentage of control group
Figure 18: Effect of different doses of blueberry (25, 50 and 100 mg/kg)
on AST activity (IU/L) in rats subjected to isoprenaline intoxication as
percentage of control group.
Figure 19: Effect of different doses of blueberry (25, 50 and 100 mg/kg)
on CK-MB activity (IU/L) in rats subjected to isoprenaline intoxication
as percentage of control group.
Figure 20: Effect of different doses of blueberry (25, 50 and 100 mg/kg)
on LDH activity (IU/L) in rats subjected to isoprenaline intoxication as
percentage of control group.
Figure 21: Effect of different doses of blueberry (25, 50 and 100 mg/kg)
on Troponin I activity (ng/mL) in rats subjected to isoprenaline
intoxication as percentage of control group.
Figure 22: Effect of the blueberry leaf extract on ISO-induced histological
alterations of the heart tissue (x 100):
Figure 23: Effect of 50 mg/kg blueberry on GSH levels (mmol/g tissue) in
rats subjected to isoprenaline intoxication as percentage of control group.
Figure 24: Effect of 50 mg/kg blueberry on MDA levels (nmol/g tissue) in
rats subjected to isoprenaline intoxication as percentage of control group.