



Cairo University
Faculty of Veterinary Medicine
Department of Pharmacology

Comparative studies on hepatoprotective agents in rats

Thesis presented by

Zeinab Adel Mahmoud El- Gendy

(B.V.Sc. South Valley University, 2011)

For

Master Degree

(Veterinary Pharmacology)

Prof. Dr. Salah El-Din Abdel Hamid Mohamed Youssef

Prof. of Pharmacology

Faculty of Veterinary Medicine

Cairo University

Prof. Dr. Amer Ramadan Ali

Ayad

Prof. and Head of Pharmacology

Department

Faculty of Veterinary Medicine

Cairo University

Prof. Dr. Seham Abdel Satar

El-Batran

Prof. and Head of Pharmacology

Department

Medical Division

National Research Center

-2016-



Cairo University
Faculty of Veterinary Medicine
Department of Pharmacology

Supervision sheet

Supervisors

Dr. Salah El-Din Abdel Hamid Mohamed Youssef.

Professor of Pharmacology.
Faculty of Veterinary Medicine.
Cairo University.

Dr. Amer Ramadan Ali Ayad.

Prof. and Head of Pharmacology Department.
Faculty of Veterinary Medicine.
Cairo University.

Dr. Seham Abdel Satar El-Batran.

Prof. and Head of Pharmacology Department.
Medical Division.
National Research Centre.
Cairo city.



Cairo University
Faculty of Veterinary Medicine
Department of Pharmacology

Name :-Zeinab Adel Mahmoud El-Gendy.

Date of birth :-6/12/1988

Nationality :-Egyptian

Degree :-M.V.Sc.

Specification :-Pharmacology

Thesis title :-Comparative studies on hepatoprotective agents in rats.

Supervisors :-Prof. Dr. Salah El-Din Abdel Hamid Mohammed Youssef.

Prof. Dr. Amer Ramadan Ali Ayad, Prof. Dr. Seham Abdel Satar El Batrran.

ABSTRACT

This study was designed to investigate the hepatoprotective and antioxidant activities of *omega*-3, *l*-carnitine, *vitamin c* and their combination against acetaminophen-induced hepatotoxicity in rats. Rats were pretreated with *omega*-3, *l*-carnitine, *vitamin c* and their combination for twenty-one days prior to oral ingestion of acetaminophen. Then rats received a single toxic dose of acetaminophen. The obtained results showed that pretreatment with *omega*-3, *l*-carnitine, *vitamin c* and their combination limited the toxic effects of acetaminophen. This study concluded that animals treated with the combination (25 OLV) [25 mg/kg b.wt. p.o. Omega-3 plus 50 mg/kg b.wt. p.o. L-Carnitine plus 100 mg/kg b.wt. p.o. Vitamin-C] showed the highest hepatoprotective activity against experimentally induced hepatotoxicity in rats. That appeared in most performed tests ALT, AST, Cholesterol, HDL-cholesterol, LDL- cholesterol, Il-6, TNF- α , MDA, NO_x, GSH, FTIR, Histopathology and Immunohistochemistry (Caspase-3 and Bax).

Key words: Acetaminophen, Omega-3, L-carnitine, Vitamin c, Liver injury, Interleukin-6, FTIR.

I would to dedicate this achievement to my dear loving parents, my father Adel Mahmoud El-Gendy and my mother Mhassen El-Sayed Mohamed.

ACKNOWLEDGEMENTS

In the name of ALLAH (SWT) the Almighty who taught man about matter that he does not know and prayers and peace be upon our Holy Prophet Muhammad (SAW) and his good followers till the Day of Judgment.

First of all I would like to extend my thanks to Prof. DR. Salah El-din AbdelHamid Mohamed Youssef, Professor of Pharmacology Department - Faculty of Veterinary Medicine - Cairo University, for his excellent supervision, encouragement, guidance and valuable instruction that he offered to me while performing this work. His intensive and creative comments have helped me step by step throughout this study and his great help in revising and finishing this thesis. Indeed, I am very honored and lucky to work under his supervision.

I express my deep gratitude, sincere and heartfelt thanks to my supervisor Prof. DR. Amer Ramadan Ali Aiad, Professor and head of Pharmacology Department - Faculty of Veterinary Medicine - Cairo University, for his kind co-operation, constant support, untiring patience, stimulating suggestions and encouragement helped me in both, all the time of research work and the writing of this thesis. This thesis would never have to be completed without his help.

I fondly thank with deepest gratitude to Prof. DR. Seham Abdel satar El-Batran, Professor and Head of Pharmacology Department- National Research Center, Egypt, for her constant guidance, knowledge on the subject, regular observation, support and encouragement in every step of my thesis work and her great help in revising and finishing this thesis.

My deep appreciation and gratitude are as well to Dr. Rania Fouad Abdel-reheem Ahmed, Pharmacology Researcher - National Research Center, Egypt, for

the long discussions and suggestions that helped me to sort out scientific and technical details of some of my thesis work, I would extend my special thanks to her supervision, positive criticism, and fruitful help and for her great help in writing and revising this thesis.

Also special thanks to Dr. Rofanda Bakeer, Pathology Researcher-National Research Center, Egypt, for great help in performing and interpreting the histopathological investigations.

Last, but not the least, I would like to thank my Parents for providing me with support and confidence and for their continuous prayers. Special thanks go to them for their moral support and encouragement has been the driving force behind my efforts and responsible for whatever I am today.

N	Subject	Page
o		
	Introduction.....	1
	Review of literature.....	4
	2.1. Liver Anatomy.....	6
	2.2. Liver microenvironment.....	8
	2.2.1. Kupffer cells.....	8
	2.2.2. Liver Dendritic Cells (DCs)	9
	2.2.3. Liver sinusoidal endothelial cells (LSECs)	10
	2.2.4. Hepatocytes.....	10
	2.2.5. Hepatic stellate cells.....	10
	2.3. Drug-induced liver injury (DILI)	11
3.	Acetaminophen toxicity.....	15
	3.1. Hepatocyte cell death: amplification and injury phase.....	20
	3.2. Sterile inflammation and cytokine formation	22
	3.3. Apoptosis and necrosis.....	23
	3.4. Inter leukin-6.....	24
	3.5. Acetaminophen-induced responses at low doses.....	25
4.	Nutraceuticals.....	26
	4.1. Omega-3 fatty acids.....	27
	4.2. Carnitine.....	34

	4.2.1.	Carnitine function.....	36
	4.2.2.	Fatty acid activation inside the cells.....	37
	4.2.3.	Carnitine in fatty acid oxidation.....	38
	4.3.	Vitamin C (Ascorbic Acid)	42
	4.3.1.	Vitamin C hepatoprotective potential.....	43
	4.3.2.	Vitamin C hepatoprotective synergism with other agents.....	44
5.		Silymarine	45
6.		Aime of the work	45
7.		Fourier Transform Infrared (FTIR) micro-spectroscopy	46
8.		Material and methods	47
	8.1.	Material.....	47
	8.1.1.	Experimental animals	47
	8.1.2.	Plant extracts	47
	8.1.3.	Drugs, chemicals and solutions.....	41
	8.1.4.	Other chemicals and solutions.....	51
	8.1.5.	Instruments.....	52
	8.2.	Methods.....	55
	8.2.1.	Experimental Design.....	55
	8.2.2.	Determination of liver function parameters.....	57
	8.2.2.1.	Determination of transaminases activity (AST and ALT)	57
	8.2.3.	Determination of lipid profile parameters.....	60
	8.2.3.1.	Determination of total cholesterol.....	60

	Determination of high density lipoproteins.....	62
8.2.3.2.		
	Determination of low density lipoproteins.....	63
8.2.3.3.		
	Determination of anti-inflammatory activity...	64
8.2.4.		
	Determination of Interleukin 6.....	64
8.2.4.1.		
	Determination of serum tumor necrosis factor-	68
8.2.4.2.	alpha (TNF- α).....	
	Determination of oxidative stress, anti-oxidant	70
8.2.5.	activity and nitrosative stress.....	
	Determination of oxidative stress	70
8.2.5.1.	malondialdehyde.....	
	Determination of anti-oxidant activity	71
8.2.5.2.	glutathione reduced.....	
	Determination of hepatic nitrosative stress	73
8.2.5.3.	(nitrite plus nitrate) (NO _x) level	
	Histopathological examination.....	75
8.2.6.1.		
	Immunohistochemistry.....	76
8.2.6.2.		
	Tissue Preparation Methods in FTIR Micro-	76
8.2.7.	Spectroscopical Analysis of Biological	
	Tissues.....	
8.2.8.	Statistical Analysis.....	79