



"Protective effect of marine animal extracts on carbon tetrachloride induced hepatotoxicity in mice"

"A Thesis Submitted for the degree of Master of Science As a partial fulfillment for requirements of the Master of Science"

By

Eman Abdel Aal Abdel Aati Khalil

(B.Sc.,2010)

Under the Supervision of

Prof.Dr. Hamdy Hamed Swelim

Prof. of Cell Biology and Histology
Department of Zoology
Faculty of Science
Ain Shams University

Dr. Mohamed Ahmed Zaki

Lecturer of Marine toxins
National Institute of Oceanography
and Fisheries
Suez Branch



Faculty of Science

Department of Zoology

Date of examination:19/11/2018

Approval Sheet

Protective effect of marine animal extracts on carbon tetrachloride induced hepatotoxicity in mice

By

Eman Abdel Aal Abdel Aati Khalil B.Sc. Zoology (2010)

This thesis for M.Sc. degree has been approved by:

Prof.Dr. Saied Abdel Halem
Professor of Fish Biology, National Institute of Oceanography and Fisheries, Cairo Branch.
Prof.Dr. Hassan Ibrahim Hassan
Professor of Embryology and Cell Biology, Zoology Department, Faculty of Science, Mansoura University.
Prof.Dr. Hamdy Hamed Swelim
Professor of Cell Biology and Histology, Zoology Department, Faculty of Science, Ain Shams University.

بسم الله الرحمن الرحيم

" وَقُل رَّبِ أَدْخِلْنِي مُدْخَلَ صِدْقِ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا"

صدق الله العظيم سورة: الإسراء

Acknowledgement

Firstly, Ultimate thanks to "Allah" (Almightly)

I wish to express my deepest thanks, respect, appreciation and profound gratitude to **Prof. Dr. Hamdy Hamed Swelim,** Professor of Cell Biology and histology, Department of Zoology, Faculty of Science, Ain Shams University for suggesting the problem of this study, direct supervision, and planning the whole work. I wish also to thank him for kind help, constructive criticism, support in overcoming all the problems that faced me during this work, and for critical reading of all the manuscript.

I would like to express my deep thank and gratitude to **Dr. Mohamed Ahmed Zaki,** Lecturer of Marine toxins, National Institute of Oceanography and Fisheries, Suez for his kind supervision, facilities he offered, generous hospitality during my presence in his lab.

I would like to thank all the staff members in Zoology Department, Faculty of Science, Ain Shams University for their kind help and invaluable co-operation throughout this research.

CONTENTS

	Page
LIST OF ABBREVIATIONS	i
LIST OF FIGURES	v
LIST OF TABLES	X
ABSTRACT	xii
INTRODUCTION	1
AIM OF THE WORK	6
REVIEW OF LITERATURE	
Liver functional anatomy and histology	7
Liver Fibrosis	10
Drug-induced liver injury	17
Carbon tetrachloride (CCl4) Model	21
Mechanism of Carbon tetrachloride (CCl4) induced liver injury	22
Liver Injury Amelioration	24
Natural products from marine sources	25
Chitons	30
Toxins produced by fishes	32
Tetrodotoxin	32
Puffer fish	34
MATERIALS AND METHODS	
1) Sample Collection	44

	1.1 Chiton	44
	1.2 Puffer fish	45
2) 1	Preparation of Extracts	
2	.1 Chiton	46
2	.2 Puffer fish	46
3) l	Experimental Animals	47
4) 7	Toxicological studies	
4	.1 Chiton	47
4	.2 Puffer fish	48
5) 1	Experimental Groups	49
6) 1	Blood collection	50
7)	Chemical Studies	
	1. Evaluation of antioxidant capacity by phosphomolybdenum method	51
	2. Determination of Free Radical Scavenging Activity	52
	3. GC/MS determination of active components of Chiton Extract	54
8) I	Biochemical assays	
	4. Determination of serum Alanine aminotransferases (ALT) and aspartate aminotransferases (AST) activities	56
	5. Determination of serum Albumin	56
9) 1	Histopathological examination	57
10) \$	Statistical analysis	60

RESULTS			
Chiton (Acanthopleura vaillantii)			
1) Chemical Studies of Chiton extract			
1.1 Evaluation of antioxidant capacity by phosphomolybdenum method	61		
1.2 Determination of Free Radical Scavenging Activity	61		
1.3 GC/MS determination of active components	63-65		
2) Toxicity Studies of Chiton extract	65-67		
3) Biochemical studies of Chiton extract	68-72		
Puffer fish (Lagocephalus sceleratus)			
1) Toxicity Studies of Puffer fish extract	73-75		
2) Biochemical studies of Puffer fish extract			
2) Biochemical studies of Puffer fish extract 75-80 Histological studies			
Group I and II: Histoarchitecture of the control	81		
groups	01		
Group III : CCl ₄ treated group	98		
Group IV : Mice treated with 10 mg Chiton extract only	118		
Group V : Mice treated with 10 mg Chiton extract + CCl ₄	130		
Group VI : Mice treated with 20 mg Chiton extract only	143		
Group VII : Mice treated with 20 mg Chiton extract + CCl ₄	154		

Mice treated with 1 µg Tetrodotoxin Group VIII: extract only 165 Mice treated with 1 µg Tetrodotoxin Group IX: 176 extract + CCl₄ 189-219 **DISCUSSION** Chiton (Acanthopleura vaillantii) 1) Chemical Studies of Chiton extract 2) Biochemical studies of Chiton extract 3) Histological studies Puffer fish (Lagocephalus sceleratus) 1) Biochemical studies of Puffer fish extract 2) Histological studies SUMMARY AND CONCLUSION 220-227 REFERENCES 228-270 **ARABIC SUMMARY**

LIST OF ABBREVIATIONS

Alb : Albumin.

ALT : Alanine aminotransferase.

A : Artery

AST : Aspartate aminotransferase.

B.W: Body weight.

BD : Bile duct.

CCl₄ : Carbon tetrachloride.

CCl₃* : Trichloromethyl free radical.

CCl₃OO*: Trichloromethyl peroxyl radical.

CAM : Chick embryo chorioallantoic membrane.

Conc. : Concentration.

CV : Central Vein.

CYP : Cytochrome P-450

DILI : Drug-induced liver injury.

DMBA : 7,12-di methyl benz(a)anthracene.

DNA : Deoxyribonucleic acid.

DOX : Doxorubicin

DPPH : diphenyl-β-picrylhydrazyl

EA : Ethyl acetate

EAC : Ehlrich ascites carcinoma

EC : Endothelial cell

ECM: Extracellular matrix.

Fig(s). : Figure(s).

GC/MS : Gas chromatography mass spectrometry.

GMA : Glycol methacrylate

HepG2 : Liver hepatocellular carcinoma cells.

HSCs : Hepatic stellate cells

Hx & E: Haematoxylin and Eosin.

IC₅₀: Inhibition concentration.

IFN: interferon

IL : Interleukin.

I.P. : Intraperitoneal(ly).

K : Kupffer cell(s).

LD : Lethal dose.

LD₅₀ : Median lethal dose.

MMPs : Matrix metalloproteinases.

NASH : Non-alcoholic steatohepatitis.

N : Two Nuclei

NK : Natural killer cell.

NKT : Natural killer T cell.

NO : Nitrous oxide

PAS : Periodic acid-Schiff

PV : Portal vein

RER : Rough endoplasmic reticulum

ROS : Reactive oxygen species.

RT : Retention times.

S : Sinusoids

SD : Standard deviation.

SPSS : Statistical Package for Social Science.

TBRI: Theodor Bilharz Research Institute.

TGF-α,-β : Transforming growth factor $-\alpha$,-β.

TIMPs : Tissue inhibitors of matrix metalloproteinases.

TTX : Tetrodotoxin.

TNF: Tumor necrosis factor.