



Role of Omega 3 Fatty Acids Against Ehrlich Ascites Carcinoma-Induced Hepatic and Brain Dysfunctions in Gamma Irradiated Mice

A Thesis

Submitted in Partial Fulfillment of the Requirements for the
Master Degree of Science
in Biochemistry

By

Mustafa Mohamed Mustafa El-Gharib
B.Sc. Biochemistry (2009)

Faculty of Science
Biochemistry Department
2014



Role of Omega 3 Fatty Acids Against Ehrlich Ascites Carcinoma-Induced Hepatic and Brain Dysfunctions in Gamma Irradiated Mice

A Thesis

Submitted in Partial Fulfillment of the Requirements for the
Master Degree of Science in Biochemistry

By

Mustafa Mohamed Mustafa El-Gharib
B.Sc. Biochemistry (2009)

Under Supervision of

Prof. Dr. Amina M. Medhat

Professor of Biochemistry
Faculty of Science
Ain Shams University

Prof. Dr. Ussama Z. Said

Professor of Physiological Chemistry
National Center for Radiation
Research and Technology
Atomic Energy Authority

Prof. Dr. Neamat H. Ahmed

Professor of Cell Biology and Histology
National Center for Radiation Research and Technology
Atomic Energy Authority

Faculty of Science
Biochemistry Department
2014

I declare that this thesis has been composed by myself and that the work which is recorded herein after has been done by myself. It has not been submitted for a degree at this or any other university.

Mustafa Gharib

Dedication

I dedicate this work with all my love to my family and for all my friends and those from whom I have learned, whenever and wherever they are.

Mustafa Gharib

ACKNOWLEDGMENTS

I am greatly honored to express my deep gratitude to **Prof. Dr. Amina M. Medhat**, Professor of Biochemistry, Faculty of Science, Ain Shams University, Egypt, for her kind supervision, moral support, instructive guidance and kind advice throughout this work.

I am sincerely grateful to **Prof. Dr. Ussama Z. Said**, Professor of physiological chemistry, Radiation Biology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Egypt, for suggesting the plane of the work and providing all the facilities for the accomplishment of this work as well as his continuous valuable guidance and helpful discussion throughout all stages of the study and also for handwriting of this manuscript.

Grateful thanks are expressed to **Prof. Dr. Neamat H. Ahmed**, Professor of cell biology and histology, National Centre for Radiation Research and Technology, Atomic Energy Authority, Egypt, for her unlimited efforts, for giving me the opportunity to perform this work under excellent working atmosphere, her encouragement, patience and interest that she showed in my work during the study period.

Acknowledgments

I am grateful to all my colleagues at Radiation Biology Department, NCRRT, especially my colleagues at the units of Physiological Chemistry and Cell Biology, for providing a good working environment, working assistance whenever necessary, and for sharing their scientific knowledge.

CONTENTS

	Page
Abstract	I
List of Abbreviations	III
List of Figures	VII
List of Tables	1
Introduction	7
Aim of the work	
1. Review of Literature	
1.1. Cancer.....	9
1.1.1. Factors involved in cancer development.....	10
1.1.2. Role of free radicals in cancer development.....	15
1.1.3. Cancer Metabolism	15
1.1.4. Overview of cancer therapeutic agents	19
1.2. Reactive Oxygen Species	25
1.2.1. Reactive Oxygen Species and Cancer	26
1.2.2. Reactive Oxygen Species and Antioxidant Systems	27
1.2.3. Reactive Oxygen Species and Ionizing Radiation	29
1.2.4. Impact of Oxidative Stress on Brain and Liver Tissues	31
1.3. Cancer and Brain-Liver interaction	36
1.4. Chemoprotection	37
1.5. Fatty Acids	43
1.6. Omega 3 Fatty Acids	45
1.6.1. Structure of Omega 3 Fatty Acids	46
1.6.2. Lipid mediators generated from ω -3 versus ω -6 PUFAs	47
1.6.3. Role of Omega 3 fatty acids in health and disease	48
1.6.4. Omega 3, Brain and Vision	49
1.6.5. Omega 3 and depression	51
1.6.6. Omega 3 and inflammation	52
1.6.7. Omega 3 and liver dysfunctions	52
1.6.8. Antioxidant activity of omega 3	53
1.6.9. Omega 3 and Cancer	53
1.6.10. Synergism of ω -3 FAs with antineoplastic radio- and chemo-therapy	57
1.6.11. Omega 3 and Radiation	60
1.6.12. Toxicity of Omega 3 Fatty Acids	64

Contents

2.	Materials and Methods	
2.1.	Materials	
2.1.1.	Experimental Animals	65
2.1.2.	Radiation Facility	65
2.1.3.	Tumor Transplantation	66
2.1.4.	Omega 3 Fatty Acids Treatment	66
2.1.5.	Experimental Design	67
2.1.6.	Biological Samples Preparation	69
2.2.	Methods	
2.2.1.	Measurement of Tumor Size	70
2.2.2.	Assessment of systemic inflammation	70
2.2.2.1.	Determination of Serum Tumor Necrosis Factor- Alpha Level	70
2.2.2.2.	Determination of Serum C-Reactive Protein Level	75
2.2.2.3.	Determination of Total Leukocytic Count	77
2.2.3.	Assessment of oxidative stress and antioxidant enzymes activities	78
2.2.3.1.	Determination of Lipid Peroxidation Level	78
2.2.3.2.	Determination of Reduced Glutathione Content ...	80
2.2.3.3.	Determination of Glutathione Peroxidase Activity	82
2.2.3.4.	Determination of Catalase Activity	85
2.2.3.5.	Determination of Superoxide Dismutase Activity	88
2.2.4.	Assessment of metabolic alterations in liver tissue ...	90
2.2.4.1.	Determination of Serum Alanine Aminotransferase Activities	90
2.2.4.2.	Determination of Serum Aspartate Aminotransferase Activity	92
2.2.4.3.	Determination of Serum Alkaline Phosphatase Activity	94
2.2.4.4.	Determination of Serum Lactate Dehydrogenase Activity	96
2.2.5.	Assessment of metabolic alterations in brain tissue ...	98
2.2.5.1.	Determination of Brain Dopamine, Epinephrine and Norepinephrine Levels	98
2.2.5.2.	Determination of Brain Serotonin Level	101
2.2.6.	Histopathological Examination	104
2.3.	Statistical analysis	104

3.	Results	
3.1.	Ehrlich Carcinoma (EC) tissue	107
3.1.1.	Monitoring of Ehrlich Tumor size	107
3.1.2.	Inflammatory Responses	110
3.1.2.1.	Total Leukocytic Count	110
3.1.2.2.	Serum Tumor Necrosis Factor-Alpha Levels	111
3.1.2.3.	Serum C - reactive protein Levels	112
3.1.3.	Tumor TBARS levels and Antioxidant Status	117
3.1.4.	Histopathological Examination of EC Tissue	122
3.2.	Liver tissue	124
3.2.1.	Liver Function Tests	124
3.2.2.	Liver TBARS Levels and Antioxidant status	128
3.2.3.	Histopathological Examination of Liver Tissue ...	132
3.3.	Brain tissue	138
3.3.1.	Brain Monoamines Levels	138
3.3.2.	Brain TBARS Levels and Antioxidant Status	142
3.3.3.	Histopathological Examination of Brain Tissue	146
4.	Discussion	153
5.	Summary and Conclusion	189
6.	Recommendations	193
7.	References	195
8.	Arabic Summary	
9.	Arabic Abstract	

LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AGEs	Advanced Glycation End Products
ALA	α -Linolenic Acid
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATI	After Tumor Inoculation
ATP	Adenosine Triphosphate
CAT	Catalase
CK	Creatine Kinase
COX	Cyclooxygenase
CRP	C-Reactive Protein
DA	Dopamine
DHA	Docosahexaenoic Acid
DNA	Deoxyribonucleic Acid
EAC	Ehrlich Ascites Carcinoma
EC	Ehrlich Carcinoma
EDTA	Ethylene Diamine Tetra Acetic Acid
EP	Epinephrine
EPA	Eicosapentaenoic Acid
FADH₂	Reduced Flavin Adenine Dinucleotide
FAs	Fatty Acids
GSH-Px	Glutathione Peroxidase
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
Gy	Gray