



Anti-Angiogenic Efficacy of Chitosan- Gallium Nanoparticles and Low Doses of γ -irradiation on Mice Bearing Ehrlich Carcinoma

A Thesis

Submitted for the degree of Master of Science as a Partial Fulfillment for requirements of the Master of Science in Biochemistry

By

Riham Mahmoud Mohammad Abdel Mawla
B.Sc. Biochemistry/Chemistry (2012)

Under Supervision of

Prof. Dr. Eman Ibrahim Kandil

Professor of Biochemistry, Biochemistry Department
Faculty of Science, Ain Shams University

Prof. Dr. Abdelfattah Mohsen Badawi

Professor Researcher of Applied chemistry
Egyptian Petroleum Research Institute

Prof. Dr. Neamat Hanafi Ahmed

Professor Researcher of Cell biology and Histology
National Center for Radiation
research and Technology
Atomic Authority

Faculty of Science
Ain Shams University

2019



Anti-Angiogenic Efficacy of Chitosan- Gallium Nanoparticles and Low Doses of γ -irradiation on Mice Bearing Ehrlich Carcinoma

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

By

**Riham Mahmoud Mohammad Abdel Mawla
B.Sc. Biochemistry/Chemistry (2012)**

Faculty of Science
Ain Shams University
2019

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.

Riham Mahmoud

Acknowledgment

First of all, Thanks first and last to **ALLAH**, to whom I relate any success in my life. Words stand short when they come to express my gratefulness to my supervisors.

I am really grateful to express my gratitude, appreciation and thanks to **Prof. Dr. Eman Ibrahim Kandil**, Professor of Biochemistry, Biochemistry Department, faculty of Science, Ain Shams University for her relentless support, splendid efforts, advice and her time, attention and guidance needed to let this thesis be completed.

I would like to express my profound gratitude and cordial appreciation to my eminent **Prof. Dr. Abdelfattah Mohsen Badawi**, Professor of Applied chemistry, Egyptian Petroleum Research Institute, for his moral support, valuable supervision, honest help, encouragement, keep interest and guidance throughout the performance of this work.

I am deeply grateful and deep appreciations are due to **Prof. Dr. Neamat Hanafi Ahmed**, Professor of Cell biology and Histology, National Center for Radiation research and Technology, Atomic Authority, for her supervision, encouragement, providing a lot of facilities, helpful discussions, directions, beneficial advice and continuous support and definitely, for her, no words of praise are sufficient.

Contents

Abstract.....	
List of Abbreviations.....	I
List of Figures.....	IV
List of Tables.....	VIII
Introduction.....	1
Aim of the work.....	6
 1.Review of Literature.....	 7
1.1.Cancer.....	7
1.1.1.Factors Involved in Cancer Development	8
A..Biological Factors	8
B..External Factors.....	10
1.1.2.Types of cancer:.....	13
1.1.3. Cancer treatment:.....	16
A..Surgery	17
B..Radiotherapy	17
C..Cancer chemotherapy	22
1.2..Angiogenesis	24
1.2.1..Angiogenic promoters	26
1.2.2..Angiogenic inhibitors	30
1.2.3..The mechanism of angiogenesis:.....	31
1.2.4..Angiogenesis in cancer	33
1.2.5..Antiangiogenic treatment of cancer	36
1.3..Reactive oxygen species (ROS)	37
1.3.1..Reactive Oxygen Species and Cancer	38
1.3.2..Role of Free Radicals in Cancer Development	40
1.3.3..Reactive Oxygen Species and Antioxidant Systems	41
1.3.4..Impact of Oxidative Stress on different tissues	43
1.4..Inflammation and cancer	46

1.4.1..Tumor Necrosis Factor- alpha (TNF- α)	48
15 ..Apoptosis.....	50
1.5.1..Caspase -3.....	51
1.6..Nanotechnology in cancer treatment.....	53
1.6.1..Tumor targeting	53
1.6.2..Cancer treatment.....	55
1.6.3..Drug delivery	56
1.7..Chitosan.....	56
1.7.1..Structure of chitosan.....	57
1.7.2..Antitumor effect of chitosan.....	59
1.7.3..Biodegradability and safety of chitosan	60
1.7.4..Sustained release provided by ChNPs	60
1.7.5..In vivo metabolic processing of ChNPs	61
1.8..Gallium nanoparticles	62
1.8.1..Anti-tumor effect of gallium.....	63
1.8.2..Transport and Cellular Uptake of Gallium	64
2..Materials and Methods	66
2.1..Materials	66
2.1.1..Experimental Animals	66
2.1.2..Radiation Facility	67
2.1.3..Tumor Transplantation	67
2.1.4..Preparation of Gallium-Chitosan nanoparticles	67
2.1.5..In vitro study cytotoxicity of Ch.GaNPs	68
2.1.6..Experimental Design	69
2.1.7..Biological Samples Preparation.....	70
2.2..Methods	71
2.2.1..Measurement of Tumor Size	71
2.2.2..Assessment of angiogenic response	71
A..Vascular endothelial cell growth factor (VEGF)	71

B..Platelet-derived growth factor (PDGF)	77
2.2.3..Assessment of inflammatory response	84
A..Tumor necrosis factor-alpha (TNF- α) level	84
2.2.4..Assessment of apoptotic response	89
A..Caspase-3(CASP-3) level	89
2.2.5..Assessment of Oxidative Stress and Antioxidant Enzymes Activities.....	95
A..Determination of Lipid Peroxidation Level	95
B..Determination of Reduced Glutathione Content	97
C..Determination of Glutathione peroxidase	98
2.2.6. Histopathological Examination.....	101
2.2.7. Characterization of cell death (apoptosis)	102
2.2.8. Statistical Analyses.....	103
3..Results.....	105
3.1..Characterization of Chitosan-Gallium nanoparticles (Ch.GaNPs).....	105
3.2..Chemosensitivity of Ch.GaNPs on Ehrlich ascite carcinoma (EACs) cells.....	106
3.3..Monitoring of Ehrlich tumor size.....	109
3.4..Angiogenesis regulators	112
3.4.1. Vascular Endothelial Growth Factor (VEGF) and Platelet- derived growth factor (PDGF) levels	112
3.5..Inflammatory response	116
3.5.1..Tumor Necrosis Factor Alpha (TNF- α) levels	116
3.6..Apoptosis regulator	119
3.6.1..Caspase-3 (Casp-3) levels	119
3.7..Antioxidant and histopathological status of different tissues in female mice bearing Ehrlich Carcinoma	122
3.7.1. Ehrlich carcinoma tumor tissue	122
I...Lipid peroxidation (LPO) levels and Antioxidant Status.....	122

II...Histopathological examination of EC tumor tissue in different animal group.....	126
III...Apoptotic and necrotic examination of Ehrlich carcinoma in different experimental group	128
3.7.2..Liver tissue	131
I...Lipid peroxidation (LPO) Levels and Antioxidant status.....	131
II...Histopathological Examination of Liver Tissue.....	135
III..Apoptotic and necrotic examinations in liver tissue of mice bearing Ehrlich carcinoma:	139
3.7.3. Kidney tissue	141
I...Lipid peroxidation (LPO) Levels and Antioxidant status.....	141
II..Histopathological examination of kidney tissue of mice bearing Ehrlich carcinoma	145
III...Apoptotic and necrotic examination in kidney tissue of the Ehrlich carcinoma.	148
3.7.4. Spleen tissue	150
I..Lipid peroxidation (LPO) Levels and Antioxidant status.....	150
II..Histopathological examination of spleen tissue of mice bearing EC.....	154
III...Apoptotic and necrotic examination of spleen tissue in female mice bearing EC:.....	158
 4..Discussion	 161
Summary.....	191
References.....	196
الملخص العربي.....	235.
المستخلص العربي.....	237..

List of Abbreviations

5-FU	5-Fluorouracil
Ab	antibody
Ang's	Angiopoietins
ATI	After Tumor Inoculation
ATP	Adenosine triphosphate
BAX	Bcl 2-associated X protein
BCAAs	Branched-Chain Amino Acids
Bcl.2	B-cell lymphoma-2
bFGF	basic Fibroblast Growth Factor
BSSP4	Brain-Specific Serine Protease 4
Ch.GaNPs	Chitosan Gallium nanoparticles
ChNPs	Chitosan nanoparticles
CRP	C-Reactive Protein
CSFs	Colony-Stimulating Factors
CYP450	Cytochromes P450
DLL4	Delta like ligand 4
DNA	Deoxyribonucleic Acid
dNTP	deoxyribonucleotide triphosphate
DSBs	double strand breaks
DTNB	5, 5'dithiobis-(2-nitro-benzoic acid)

E.IR	Ehrlich carcinoma irradiated group
EAC	Ehrlich ascites carcinoma
EC	Ehrlich carcinoma
ECF	extracellular fluid
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
ELISA	Enzyme Linked Immune Sorbent Assay
EPR	Enhanced Permeability and Retention
ETC	Electron Transport Chain
Ga	Gallium
GBM	glioblastoma multiforme
GSH	Reduced Glutathione
GSH-Px	Glutathione Peroxidase
GSSG	Glutathione in oxidized form
Gy	Gray
HIFs	Hypoxia-Inducible Factors
HO-1	Hemeoxygenase-1
HRP	Horseradish Peroxidase
i.p	intraperitoneal
IC₅₀	half maximal inhibitory concentration
IFN-γ	Interferon-gamma
I-kB	Inhibitor kappa B

IL-1b	Interleukin 1-beta
IR	Ionizing Radiation
LET	Linear Energy Transfer
MDA	Malondialdehyde
MMP	Matrix Metalloproteinases
MPS	Macrophage Phagocytic System
mROS	mitochondrial ROS
MT	Metallothionein
MTD	Maximum Tolerated Dose
N.C	Normal control
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide
NCRRT	National Center for Radiation Research and Technology
NF-κB	Nuclear Factor-κB
NOXs	NADPH oxidases
NPs	Nanoparticles
O.D	optical density
PBS	Phosphate buffer solution
PDGF	Platelet-derived growth factor
PIGF	Placental growth factor
r.p.m	Revolutions Per Minute

RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
S.C	subcutaneous
SPSS	Statistical Package for Social Science
TBA	Thiobarbituric acid
TCA	Trichloro acetic Acid
TEM	Transmission Electron Microscope
Tf	Transferrin
TFR	Transferrin receptor
TGF-α	Transforming growth factor- α
Th	T helper cell
TMB	Tetramethylbenzidine
TNF-α	Tumor Necrosis Factor-Alpha
TSTA	Tumor Specific Transplantation Antigen
VCAM-1	vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WHO	World Health Organization

List of Figures

Figure	Title	Page
Figure (1)	Angiogenesis and vasculogenesis in normal cell.	33
Figure (2)	Angiogenesis steps in tumor vascularization.	36
Figure (3)	Structure of Glutathione.	42
Figure (4)	Caspase-3 apoptotic pathways.	52
Figure (5)	Structure of chitosan.	58
Figure (6)	Standard curve of VEGF.	76
Figure (7)	Standard curve of TNF- α .	89
Figure (8)	Standard curve of Casp-3.	94
Figure (9)	Characterization of Chitosan-Gallium nanoparticles	105
Figure (10)	The effect of Ch.GaNPs on the viability of EAC cells.	108
Figure (11)	Effect of Ch.GaNPs and/or γ -radiation on tumor size of mice bearing EC.	111
Figure (12)	Percent change of VEGF and PDGF level in different animal groups, mice-bearing E.C treated with Ch.GaNPS and/ or γ -irradiation compared to E.C group.	115

Figure (13)	Percent change of TNF- α level in different animal groups, mice-bearing EC treated with Ch.GaNPS and γ -irradiation compared to E.C group.	118
Figure (14)	Percent change of CASP-3 level in different animal groups, mice-bearing EC treated with Ch.GaNPS and γ -irradiation compared to E.C group	121
Figure (15)	Percent change of tumor LPO, GSH and GSH-Px in mice-bearing EC treated with Ch.GaNPS and/or γ -irradiation compared to EC group.	125
Figure (16)	Photomicrograph of Ehrlich carcinoma bearing mice represents (A): control EC in mice. (B): treated with Ch.GaNPs. (C): exposed to low doses of γ - radiation. (D): treated with Ch.GaNPs and exposed to low doses of γ - radiation.	127
Figure (17)	Fluorescent imaging of sections in Ehrlich Carcinoma representing control EC in mice	129
Figure (18)	Fluorescent imaging of sections in EC represents (A): exposed to low doses of γ - radiation. (B): treated with Ch.GaNPs. (C): treated with Ch.GaNPs and exposed to low doses of γ - radiation.	130
Figure (19)	Percent change of liver LPO, GSH and GSH-Px in mice-bearing EC treated with Ch.GaNPS and/or γ -irradiation compared to NC group.	134

Figure (20)	Percent change of liver LPO, GSH and GSH-Px in mice-bearing EC treated with Ch.GaNPS and/or γ -irradiation compared to EC group	134
Figure (21)	Photographs of sections in liver of mice. A, B: Normal control sections. C&D: Liver sections of mice bearing EC	137
Figure (22)	Photographs of sections in liver of mice bearing EC. A, B: Liver sections of mice bearing EC exposed to γ - radiation. C, D: Ch.GaNPS treated group. E, F: treated with Ch.GaNPs and exposed to low doses of γ -radiation.	138
Figure (23)	Fluorescent imaging of liver sections represents (A): Section in normal liver. (B): bearing Ehrlich carcinoma. (C): exposed to low doses of γ - radiation. (D): treated with Ch.GaNPs. (E): treated with Ch.GaNPs and exposed to low doses of γ - radiation.	140
Figure (24)	Percent change of kidney LPO, GSH and GSH-Px in mice-bearing EC treated with Ch.GaNPS and/or γ -irradiation compared to NC group.	144
Figure (25)	Percent change of kidney LPO, GSH and GSH-Px in mice-bearing EC treated with Ch.GaNPS and/or γ -irradiation compared to EC group.	144
Figure (26)	Photomicrographs of sections in kidney of mice. A: Section in normal kidney. B&C: Section in kidney of mice bearing EC	146