Ain Shams University
Faculty of Science
Zoology Department

Molecular Characterization of the Potential Antitumor Mechanisms of Certain Polyphenols and γ-irradiation in Hepatocellular Carcinoma HepG2 Cell Line.

A THESIS

Submitted for the Award of Ph.D. Degree of Science
In Zoology "Molecular Biology"

By

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2018

{قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ }

سورة البقرة آية "32"

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Approval Sheet

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Date of examination: / /2018

Declaration

This thesis has not been previously submitted for any degree at this or any other University.

Azza El-Sayed Mohamed Kaed

Dedication

I dedicate this work with all my love
To the spirit of my father and mother
To my husband
To my sister, brothers
To my son, daughters

"Praise to Allah for choosing you to be my family"

ACKNOWLEDGEMENT

*With the name of **ALLAH** and thanks to **ALLAH** who gave me chance to present this work hoping the benefit of the patients.*

*I would like to express my deepest respect and sincere gratitude to **Prof. Dr. Nagwa Hassan Ali Hassan,** professor of Cytogenetics, Zoology Department, Faculty of Science, Ain Shams University; for her generous and precious supervision, valuable advices and constant guidance, to her precious reading and valuable comments. Without her generous and valuable assistance, this work would lose its value. It is an honor working under her supervision.*

*I would like to express my sincere gratitude and deep thanks to **Prof. Dr. Tarek Khaled Abdellah El Maghraby,** Professor of Molecular Biology in Radiobiology Department, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority for suggesting and planning for this work, enrichment of the research point and valuable effort in supervising such research work.*

*Thanks and gratitude also to **Prof. Dr. Mohammed Ahmed Ali, and Ahmed Elsayed Mohammed** researcher assistant at Center of Scientific Excellence for Influenza Viruses, National Research Centre, Egypt.*

I will not forget to thank all my real friends for their encouragement to exceed all barriers in my way and during the hard times.

CONTENTS

List of Abbreviations

AP-1	Activator protein 1
ANOVA	Analysis of variance
cDNA	Complementary Deoxyribonucleic Acid
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid
ERK	Extracellular signal-regulated kinase
G_0	Resting phase of cell cycle
G_1	Gap between mitosis and DNA synthesis
G_2	Gap between DNA synthesis and mitosis
Gy	Gray (measure of dose of irradiation)
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HepG2	Human hepatocellular carcinoma cell line
Her-2/neu	Human epidermal growth factor receptor 2
IC_{50}	Inhibitory concentration 50%
MDM2	Mouse double minute 2 homolog
MMP9	Matrix metalloproteases 9
MTT Assay	Methylthiazol tetrazolium assay
NF-kB	Nuclear factor κ B
P53	protein 53-kilo Daltons
PBS	Phosphate buffer saline
PKA	Protein kinase A
PKCd	Protein kinase C
WHO	World Health Organization
MAPK	Mitogen-activated protein kinase
µg	Microgram
M.W.	Molecular weight
µM	Micromolar
RNA	Ribonucleic acid
r-RNA	Ribosomal Ribonucleic acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
ROS	Reactive Oxygen species
SOD	Superoxide dismutase
TCA	Total antioxidant capacity
Sv	Sievert
VEGF	Vascular endothelial growth factor

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ABSTRACT

BACKGROUND: Hepatocellular carcinoma (HCC) is the common type of liver malignancy and the high incidence. HCC is the cause of high level of mortality due to the resistance to radiotherapy and the harmful effect of chemotherapy.

Aim of the study:

1) To evaluate the antitumor effect of different doses of polyphenol compounds "Curcumin, hesperidin, and quercetin"
2) To study the ability of polyphenol compounds to decrease the unwanted side effects of radiotherapy and to increase cells radiosensitivity to respond to low doses of radiotherapy.

METHODS: HCC cells treated with different concentrations of curcumin, hesperidin, and quercetin alone or combined with different doses of γ-radiation. The cell viability examined by MTT assay to all groups in different times, and gene expressions evaluated by Real-time PCR for *P53*, *Her2/neu*, and *MMP9* genes, also total anti-oxidant capacity (TAC) had been measured.

RESULTS: The cytotoxicity mean values show significant increase, so the cell proliferation were decreased in all groups in a time and dose-dependent manner. Real-time PCR results show enhancement in gene expression levels, that *p53* gene increased in all groups, but *Her2/neu*, and *MMP9* decreased in all groups when compared with reference gene *GAPDH* calculated by 2-ΔΔCT. total antioxidant capacity (TAC) were increased only by quercetin treatment at high concentrations..

CONCLUSION: Curcumin, hesperidin, and quercetin with or without γ-radiation can decrease cancer cell viability, and enhance some important gene expressions.

KEY WORDS: Hepatocellular carcinoma, γ-radiation, curcumin, hesperidin, and quercetin, real-time PCR, total antioxidant capacity (TAC).