

**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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• {قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ }

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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.*

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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-κB	Nuclear factor κ B
P53	protein 53-kilo Daltons
PBS	Phosphate buffer saline
PKA	Protein kinase A
PKCδ	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 $2^{-\Delta\Delta CT}$. 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).