



*Ain Shams University
Faculty of Pharmacy
Pharmacology and Toxicology Department*

**A Study on the Potential
Chemomodulatory Effects of Biochanin-
A in Hepatocellular Carcinoma Cells**

A thesis submitted for the partial fulfillment of the requirements
of M.Sc. in pharmaceutical sciences (Pharmacology and
Toxicology).

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Acknowledgement

In the name of Allah, The Most Gracious and The Most Merciful. Peace and blessings be upon our Prophet Mohammad and his good followers till the Day of Judgment.

*At the beginning, I would like to explicit my sincere thanks and gratitude to my supervisor, Prof. Dr. **Ashraf B. Abdel-Naim**, Professor of Pharmacology and Toxicology, Faculty of Pharmacy, Ain-Shams University, for being so generous with his knowledge and being so supportive in all aspects. I am so grateful to him for his sincere efforts and guidance through the entire work. He is exemplary in his role as a mentor, a researcher, and a teacher, in the laboratory and in life.*

*I owe my sincerest thanks to my supervisor, Prof. Dr. **Amani Emam Khalifa**, Professor of Pharmacology and Toxicology, Faculty of Pharmacy, Ain-Shams University, for her kind help, constructive advice, and continuous wholehearted support. My work was greatly enhanced by her valuable contributions. I learned a lot from her step-by-step guidance and her precious remarks.*

*I owe my deepest gratitude to my supervisor, Prof. Dr. **Suher Zada**, Professor of Immunology, Biology department, American University in Egypt, for her constant, unwavering guidance and patience. She has not only provided me the opportunity to work in her lab, but her efforts to support me by all means will be fondly kept in my memory.*

*I would like to extend my appreciation and thankfulness to Dr. **Mai Fathy Tolba**, lecturer of Pharmacology and Toxicology, Faculty of Pharmacy, Ain-Shams University, for her persistent support, outstanding competent guidance and patience for all my concerns. Her genuine enthusiasm for scientific work and broad knowledge has greatly impressed me.*

*I am deeply grateful to Prof. Dr. **Eman El-Ahwany**, Professor of Immunology, Theodor-Bilharz Research Institute, for her generous support and feedback. Also, I am very thankful for her participation in funding my work.*

*I would like to thank Prof. Dr. **Mona Moussa**, Professor of Pathology, Theodor-Bilharz Research Institute, for her help with the immunocytochemical staining and analysis.*

Furthermore, I would like to thank my lab colleagues: Noha Nagdy, Mai Omar, Reem Tarek, Mohamed El-sayed and Mohamed Hussein, for their assistance and collaboration during the time of my practical work for my thesis.

*I am indebted to Prof. Dr. **Ihab Fettouh**, Dean of Faculty of Pharmacy, Egyptian-Russian University, my professors in the Pharmacology department: Prof. Dr. **Laila Ramadan** and Ass.Prof. Dr. **El-sayed Akool**, my colleagues: **Mohamed Edfawy**, **Mahmoud Nour**, **Noha Saied**, **Mostafa Fayed** and **Hassan Afify** for their help and support throughout my post-graduate study.*

I would also like to thank my parents and siblings for encouraging me throughout the years, helping me to pursue my dreams and also for being an integral part of it. Without their love and understanding, I could hardly endure the hard times throughout my study.

Finally, my deepest thanks go to my wife for always being there and believing anything is possible. She continuously inspires me to take on new challenges, both in career and life.

Mohi Youssef

Table of Contents

Introduction.....	1
Hepatocellular Carcinoma.....	1
Epidemiology:.....	1
Hepatocellular carcinoma in Egypt:	2
Etiology and risk factors:	4
Staging.....	11
Hepatocarcinogenesis:.....	12
Current trends in management of HCC:	16
Prognosis and Predictive factors	20
Prevention.....	21
Apoptosis.....	23
Sorafenib	28
Chemistry:.....	31
Pharmacodynamics:.....	32
Pharmacokinetics:	35
Pharmacogenetics:.....	37
Drug interactions:	39
Treatment plan and assessments:	40
Toxicity:.....	41
Isoflavones.....	44
Origin and Source:.....	44
Effects on diseases and disorders:	46
Pharmacokinetics:	50
Function in plants:.....	53
Factors affecting isoflavones' content:.....	53
Use in animals:.....	55
Toxicity:	55
Biochanin A.....	57

Chemistry:.....	57
Pharmacodynamics:	58
Pharmacokinetics:	62
Pharmacogenetics:	66
Drug interaction:.....	67
Toxicity:	71
<i>Aim of the work</i>	73
<i>Materials and Methods</i>	75
(A)- Design of the work:	75
(B)- Materials:	77
(C)- Methods:	80
<i>Results</i>	94
<i>Discussion</i>	121
<i>Summary and Conclusion</i>	130
<i>References</i>	134

List of figures:

Figure 1: The Natural History of HCV Infection and Its Variability from Person to Person	7
Figure 2: Chronologic sequence of cellular lesions culminating in the development of HCC	8
Figure 3: Signaling pathways in HCC	16
Figure 4: Cellular functions of survivin that contribute to tumor development and metastasis.....	24
Figure 5: Diagram showing cell-cycle phases	25
Figure 6: Chemical structure of Sorafenib Tosylate	32
Figure 7 : Structure of Common Isoflavones.....	46
Figure 8: Biochanin-A chemical structure	57
Figure 9: Fenton reaction.....	62
Figure 10: Chemical structures of naturally occurring isoflavones, from soybean and red clover.	65
Figure 11: Flavonoids can block or suppress multistage carcinogenesis	69
Figure 12: Bio-A concentration-response plot in HepG2 liver cancer cell line after 72h treatment.	96
Figure 13: Concentration-response plot of SOR/Bio-A combination ratio 1:4 in HepG2 liver cancer cell line after 72h treatment.	97
Figure 14: Concentration-response plot of Bio-A/SOR combination ratio 1:16 in HepG2 liver cancer cell line after 72 h treatment.	98
Figure 15: Effect of Bio-A on the cytotoxicity of SOR at combination ratio 1:50 in HepG2 liver cancer cell line after 72 h treatment.	99
Figure 16: Effect of Bio-A on DNA-ploidy flow cytometric analysis of HepG2 cells treated with SOR. The cells were treated with SOR (3 μ M), Bio-A (22 μ M), and SOR/Bio-A combination (0.3 μ M+ 15 μ M) for 24 h.	104
Figure 17: Effect of Bio-A on the protein abundance of cyclin D1 in HepG2 cells treated with SOR for 72 h using immunocytochemistry.	106
Figure 18: Effect of Bio-A on the gene expression of Ki-67 proliferation marker in HepG2 cells treated with SOR for 48 h.	108
Figure 19: Effect of Bio-A on the protein expression of Bax and Bcl-2 apoptotic markers in HepG2 cells treated with SOR for 72 h.	110
Figure 20: Effect of Bio-A on Bcl-2/Bax ratio in HepG2 cells treated with SOR for 72 h.	111
Figure 21: Effect of SOR/Bio-A on the mRNA level of caspase-9 apoptotic marker in HepG2 cells treated for 24 h.	113

Figure 22: Effect of SOR/Bio-A on the mRNA level of caspase-9 apoptotic marker in HepG2 cells treated for 48 h.	114
Figure 23: Effect of Bio-A on the gene expression of apoptotic regulatory molecule caspase-3 in HepG2 cells treated with SOR for 24 h.	116
Figure 24: Effect of Bio-A on the gene expression of apoptotic regulatory molecule caspase-3 in HepG2 cells treated with SOR for 48 h.	117
Figure 25: Effect of Bio-A on caspase-3 activity in HepG2 cells treated with SOR for 72 h.	118
Figure 26: Effect of Bio-A on the gene expression of survivin proliferating marker in HepG2 cells treated with SOR for 48 h.	120
Figure 27: Schematic diagram of the probable mechanism of synergy between SOR and Bio-A through mitochondrial apoptosis signaling pathway.	132

List of Abbreviations:

5-FU	5-fluorouracil
AFB1	Aflatoxin B1
AFP	Alpha-feto protein
AJCC	American Joint Committee Cancer
Bio-A	Biochanin-A
CI	Combination index
CT	Cycle threshold
DAB	3,3'-diaminobenzidine
DCR	Disease control rate
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DRI	Dose Reduction Index
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
Fa	Fraction of cells affected
HBsAG	Hepatitis-B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HFSR	Hand-foot skin reaction
HMEC	Primary human mammary epithelia cells
IACT	Intra-artery chemotherapy
IL-2	Interleukin 2
NASH	Non-alcoholic steatohepatitis
OS	Overall survival
PBS	Phosphate-buffered saline
PDGFR	Platelet-derived growth factor receptor
PEI	Percutaneous ethanol injection
PET	Positron emission tomography
PVT	Portal vein thrombosis
qRT-PCR	Quantitative real-time polymerase chain reaction

RECIST	Response evaluation criteria for solid tumours
RNA	Ribonucleic acid
SHARP	Sorafenib hepatocellular carcinoma assessment protocol
SHBG	Sex hormone binding globulin
SOR	Sorafenib
TACE	Trans-arterial chemoembolization
TTP	Time to progression
US	United States
VEGFR	Vascular endothelial growth factor receptor

Abstract

Biochanin-A, a promising isoflavone, is a natural chemopreventive with selective toxicity towards cancer cells only. Sorafenib is considered as the first-line therapy for patients with advanced hepatocellular carcinoma (HCC). The current study was designed to investigate the synergistic cytotoxicity of biochanin-A (Bio-A) combined with sorafenib (SOR) in HepG2 human hepatocellular carcinoma cell line through modulating pro-survival and apoptotic pathways. Bio-A alone showed cytotoxic potentiality against HepG2 cells at relatively lower potency ($IC_{50}=22\mu M$) compared to SOR ($IC_{50}=3\mu M$) using sulforhodamine-B cytotoxicity assay. CalcuSyn analysis indicated that the concurrent treatment of Bio-A and SOR at 10% of IC_{50} was synergistic ($CI<1$). Cell cycle analysis indicated a significant cellular arrest at pre-G and G_0/G_1 phases and subsequent decreased percentage of HepG2 cells in the other cell cycle phases by SOR/Bio-A co-treatment. Concomitantly, the expression of cyclinD1, a cell cycle regulatory protein, was down-regulated. Further mechanistic studies in HepG2 cells showed that the gene expression pattern of Ki-67, a proliferation marker, was down-regulated upon combination of SOR and Bio-A. Apoptotic pathway signaling investigation for this co-treatment

showed decreased Bcl-2/Bax ratio as well as time-dependant elevation of both, caspases 3, 9 gene expression and caspase 3 activity, compared to either drug alone. Additionally, this combination exhibited a significant down-regulation of survivin gene which is an inhibitor of apoptosis. In conclusion, Bio-A synergistically enhanced the cytotoxic and apoptotic effects of SOR on HepG2 cells. Our findings suggest that Bio-A may be a potential adjuvant to SOR as a new therapeutic regimen for treating HCC.

Introduction

Hepatocellular Carcinoma

Epidemiology:

Liver cancer is the fifth most common cancer in men worldwide (523,000 cases per year, 7.9% of all cancers) and the seventh in women (226,000 cases per year, 6.5% of all cancers) according to the International Agency for Research on Cancer (**El-Serag, 2012**). An estimated 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008 (**Ferlay *et al.*, 2010**). In men, it is the second most frequent cause of cancer death. In women, it is the sixth leading cause of cancer death (**Jemal *et al.*, 2011; Globocan, 2012**).

Among primary liver cancers, hepatocellular carcinoma (HCC) represents the major histological subtype accounting for 85%-90% of primary liver cancers (**Kim and Ho-Lim, 2011**). Most cases of HCC (>80%) occur in sub-Saharan Africa and in Eastern Asia, with typical incidence rates of > 20 per 100,000 individuals (**El-Serag, 2012**), whereas rates are low in South-Central and Western Asia, as well as Northern and Eastern Europe (**Kim and Ho-Lim, 2011**).

Men have a higher prevalence of HCC than women; the ratio of affected men to affected women varies between 2:1 and 4:1, depending on the geographic region **(El-Serag, 2012)**.

The reasons for the disparity between men and women are obscure, but they may include environmental factors such as a higher prevalence of persistent hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcohol abuse, smoking in men than in women, genetic and hormonal factors **(Naugler *et al.*, 2007)**.

The incidence of HCC increases with age, reaching its highest prevalence among those aged over 65 years **(Motola-Kuba *et al.*, 2006)**. Although HCC is rare before the age of 50 years in North America and Western Europe **(Yu *et al.*, 2003)**, a shift in incidence towards younger persons has been noted in the last two decades **(El-Zayadi *et al.*, 2005)**, and this may be attributed to emergence of HCV infection **(Montalto *et al.*, 2002)**, as well as to acquisition of both hepatitis B and C virus infection at younger age **(Velazquez *et al.*, 2003)**.

Hepatocellular carcinoma in Egypt:

In Egypt, liver cancer constitutes 1.68% of total malignancies. Liver tumors were mostly hepatocellular carcinoma (70.48%) **(Mokhtar *et al.*, 2007)**. HCC was reported to account for about 4.7% of chronic liver disease patients **(El-Zayadi *et al.*,**