Analyzing the apoptotic pathways in cell lines treated with some pure natural compounds

A thesis

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Prepared by

Doaa Taha Ramadan

Demonstrator at Department of Zoology – Faculty of Science – Ain Shams University

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Under supervision of

Dr. Wael M. El-Sayed

Professor of Physiology

Department of Zoology – Faculty of Science – Ain Shams University

Dr. Shaymaa M. Yahya

Assistant Professor of Biochemistry

Hormones Department – Medical Division – National Research Centre

Dr. Mohamed A. M. Ali

Assistant Professor of Biochemistry

Department of Biochemistry – Faculty of Science – Ain Shams University

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بسم الله الرحمن الرحيم

(..... وَأَنزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا)

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Dedication

I would like to dedicate my humble effort to my sweet and loving

Father and mother

Along with all hard working and respected

Teachers

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List of Abbreviations

Abbreviation	Full-term
AIF	Apoptosis-inducing factor
Apaf-1	Apoptotic protease-activating factor-1
ANOVA	One-way analysis of variance
ATCC	American Type Culture Collection
Bad	Bcl-2-associated death promoter
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma-extra large
BH3	Bcl-2 homology domains
Bid	BH3 interacting-domain death agonist
Caspases	Cysteine-aspartic proteases
cDNA	Complementary DNA
Ct	Threshold cycle
DISC	Death inducing signaling complex
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

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Abstract

Analyzing the apoptotic pathways in cell lines treated with some pure natural compounds

Doaa Taha Ramadan

Keywords: Amygdalin; Naringenin; Ellagic acid; antioxidant activity; antimutagenicity

The need for effective treatment and prevention of cancer is very important as cancer continues to be a major health problem worldwide. Chemotherapeutic drugs have high toxicity associated with undesirable side-effects. Natural products are the most important anti-cancer agents because of their low toxicity and potential effectiveness. The aims of the present study were finding a relation between the antioxidant properties (free radical, superoxide radical, and hydroxyl radical scavenging activities, total antioxidant capacity, and metal chelating activity) of three phytochemicals and their antiproliferative activity against human cancer cell lines, analyzing the apoptotic pathways through which these phytochemicals act, understanding the antimutagenic activity of these phytochemicals and its link to their antiproliferative effect. We investigated three natural compounds; amygdalin, naringenin and ellagic acid. Ellagic acid showed the highest antioxidant and antiproliferative activities. Amygdalin and naringenin with low and moderate antioxidant profiles, respectively showed a corresponding low and moderate cytotoxicity against cancer cell lines. There was a direct relationship between the antioxidant / antiproliferative activities of phytochemicals and the number of hydroxyl groups on B ring of these botanicals. Naringenin and ellagic acid had an antimutagenic activity which was detected by the *Salmonella* test. Ellagic acid offered much better antimutagenic activity than naringenin. The apoptotic pathway evoked by ellagic acid in HepG2 and MCF-7 cells was investigated. The results showed that a caspase-dependent and caspase-independent apoptosis occurred in MCF-7 and HepG2, respectively. Taken together, these phytochemicals have

antioxidant, anticancer and antimutagenic activities that make them good therapeutic agents. This study proved that some easy, quick, and cheap assays could predict the antiproliferative activity of many nutraceuticals. Finally, this platform could help in the discovery of new anticancer agents where hundreds or even thousands of compounds are investigated in the pipeline of drug discovery.

1. Introduction

Cure rates are still extremely bad for some cancers. In addition, the high toxicity usually associated with some cancer chemotherapeutic drugs and their undesirable side-effects increase the demand for novel anti-tumour drugs with fewer side-effects and with greater therapeutic efficiency. Doxorubicin is one of the most effective anticancer drugs to date against a wide number of human cancers **(Chen *et al.*, 2011)**. However, it induces serious toxic effects in brain, kidney, lung and small intestine and leads to dose-dependent irreversible cardiomyopathy and severe heart failure **(Balli *et al.*, 2004)**. At the cellular level, it is incorporated between two nitric bases of double DNA helix, thus causing the inhibition of DNA-dependent DNA and RNA polymerases. This results in the suppression of replication and transcription and damage to DNA repair mechanisms in cancer cells as well as in normal cells. Doxorubicin also alters topoisomerase II activity **(Czeczuga-Semeniuk *et al.*, 2004)**.

Various plant products have been reported to possess chemopreventive properties. Approximately, 20% of cancer incidents are preventable by consuming more vegetables and fruits which may potentially prevent approximately 200,000 cancer-related deaths annually **(Pratheeshkumar *et al.*, 2012)**. Amygdalin is a natural glycoside, also called vitamin B17. It is

composed of two molecules of glucose, one molecule of benzaldehyde and one molecule of hydrocyanic acid. Hydrocyanic acid is an antitumor compound and benzaldehyde can induce an analgesic action, therefore it can be used for the treatment of cancer and it can also relieve the associated pain. Cancer cells are deficient in rhodanese enzyme that detoxifies hydrocyanic acid into non-toxic thiocyanate. The antitumor effect of amygdalin is one of the hottest topics in the recent years **(Song and Xu, 2014)**. Naringenin is one of the naturally occurring flavonones present in citrus fruits and grape fruit. It exhibits extensive pharmacological effects as free radical scavenging, antioxidant and anti-proliferative activities. Naringenin plays an important role in cancer prevention and treatment by inducing apoptosis, reversing drug resistance and promoting DNA repair **(Li *et al.*, 2015)**. Ellagic acid is a polyphenolic compound present in green tea, strawberries, blackberries and raspberries. Ellagic acid has antioxidant, anti-carcinogenic and chemopreventive activities **(Zhang *et al.*, 2014)**.

The three natural compounds exhibit anticarcinogenic activities probably through enhancing apoptosis in cancer cells. There are two well characterized apoptotic pathways in cells. The extrinsic pathway is initiated by ligand-induced activation of the death receptors at the plasma membrane. The intrinsic pathway is triggered by cellular stress signals such as DNA damage and

oncogene activation. The mechanisms involve a cascade of successive activation of initiator and effector caspases **(Kalimuthu and Se-Kwon, 2013)**.

Aim of the study

The main aim of the study was the investigation of the ability of pure natural products such as amygdalin, naringenin and ellagic acid in comparison to doxorubicin to induce apoptosis in human colon epithelial (HCT 116) cell line, liver epithelial (HepG2) cell line and breast epithelial (MCF-7) cell line. It included the following assays:

1. Determination of total antioxidant activity, metal chelating activity and free radical, hydroxyl and superoxide scavenging activities of the natural products.
2. Determination of cell viability by neutral red uptake assay.
3. Using Ames test to determine the anti-mutagenic activity of the natural products.
4. RNA isolation and quantitative real-time PCR analysis of apoptotic genes:
    - Tumour suppressor genes such as p53
    - Apoptosis promoting factors such as bax and bid
    - Anti-apoptotic factors such as bcl-2
    - Death ligand such as fasl

- Initiator caspases such as caspase-9
- Effector caspases such as caspase -3 and caspase -7

2. Review of Literature

2.1 Cancer statistics

Cancer is one of the leading causes of death worldwide and the incidence is on the rise in both developing and developed countries. It is the primary reason of mortality in developing countries **(Jemal *et al.*, 2011).** Globally, more than 70% of all cancer deaths occur in low- and middle-income countries, which have few or no resources for the prevention, diagnosis and treatment of cancer. Based on GLOBOCAN estimates, about 14.1 million new cancer cases and 8.2 million deaths existed worldwide in 2012 **(Torre *et al.*, 2015).** This number is expected to increase to 22 million per year within the successive two decades. Developing countries have 60% of the world's total cases of cancer and 70% of world's deaths due to the lack of accessibility to treatment and failure of early detection. This increases the economic and social burdens due to cancer incidence in such countries. Thus, the need for effective treatment and prevention of cancer is important as cancer continues to be a major health problem worldwide. Human cancer comprises more than 200 different types of cancer. Carcinomas are cancers arising from epithelial cells which are the most prevalent cancers overall **(Suvarna *et al.*, 2017)**. Four types of carcinomas are important with regard to incidence as well as mortality. In contrast, in developing countries, lung, stomach and liver cancers are predominant in