Ain Shams University
Faculty of Science
Botany Department

Evaluation of some coumarin derivatives as potential anticancer drugs

Thesis

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of Science in Botany

(Cytology and Genetics)

By

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

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Abstract

Thousands of coumarin compounds have been isolated and identified in different plant species. These compounds play an important role in anticancer drug development due to their wide range of biological activities and their effect against different types of cancer cells. Therefore, this study was carried out to gain information about the mechanism of these compounds and searching for novel cytotoxic agents. Seven plants containing coumarin derivatives and ten new furocoumarin analogues were used in this work. HepG-2 cells (liver cancer cell line) were treated with concentrations ranged from 0.1 -1000 µg/ml for 72 hours. Compound number 9 showed highest reduction in cell viability percentage, and exhibited the highest inhibitory activity with IC_{50} (inhibitory concentration for 50% of cells) equal 11.97 µg/ml, and fall within the American National Cancer Institute criteria; while the other compounds recorded medium or weak effect as compared with the standard drug Doxorubicin (IC_{50}=0.87 µg/ml). The plant extracts also reduced the viability of cancer cells in a dose dependent manner. Their cytotoxic effect was more observed at the higher concentrations. IC_{50} of the seven plant extracts was ranged from 121.4 µg/ml for *Verbascum thapsus* to 511.8 µg/ml for *Ammi majus*.

Root tip cells of *Pisum sativum* plant were treated with concentrations ranged from 0.05–1.0 mg/ml of compounds number 9 and 8 and with 2.19 – 35 mg/ml for the natural plant extracts of *Achillea millefolium* and *Verbascum thapsus*. Results showed significant decrease in mitotic index that increased gradually by increasing the concentration and the time of treatments; in addition to different types of mitotic abnormalities were recorded such as stickiness and chromosome breakage. The expression level of two cell cycle regulatory genes; cyclin B_1 and cyclin D_1 were examined using RT-PCR techniques. The results indicated that cyclin B_1 was down regulated in response to treatment of *Pisum sativum* roots with the concentrations 0.5 mg/ml of compound 9 and 1.0 mg/ml of compound 8, and with 17.5, 35 mg/ml of the extracts of *Verbascum thapsu*s and *Achillea millefolium* as compared with the reference gene β-tubulin-3. Moreover, only compound number 9 caused decrease in cyclin D_1 in response to treatment

with 0.5 mg/ml, as compared with compound number 8; extracts of *Verbascum thapsus* and *Achillea millefolium* which showed no effect, indicating the effect of compound number 9 on the cell cycle arrest at the transition point G_1/S. The genomic stability percentage in *Pisum sativum* plant cells treated with the previous materials using ISSR-DNA fingerprints showed change in band number and decrease in genomic template stability (GTS) values in all treatments. Compound number 9 recorded the lowest GTS (46%) while compound number 8 recorded 64% in comparison to the untreated control, indicating that compound 9 is more effective on genome stability. The GTS value of plant extracts decreased with increasing the concentrations. The lowest GTS (76.12%) recorded with the highest concentration (35 mg/ml) of *Verbascum thapsus*; while recorded 88.06% in samples treated with the same concentration of *Achillea millefolium*. Thus, 0.5 mg/ml of compound 9 have greater damage on cell's genetic material indicative of the genotoxicity at this concentration. On the other hand, the molecular docking technique showed that this compound has a good ability to inhibit topoisomerase I which in turn will lead to cancer cell inhibition through the inhibition of DNA replication.

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1. Introduction

The plant kingdom is gaining global recognition as unique and renewable resource for the discovery of phytochemicals that may represent pharmacological active compounds (**Brower, 2008 and Newman and Cragg, 2012)**. Plants are important sources of medicinal compounds worldwide. Plant materials have a long history of being successfully used in the treatment of cancer, both as chemotherapeutic agents and as complementary treatments (**Newman *et al*., 2003)**. According to **Newman and Cragg (2012)**, 40% of all anticancer drugs are developed from natural products while 20% are synthetic derived from natural ones. Continuous efforts have been made for discovering bioactive compounds or designing new drugs with more potent effect against cancer.

Coumarins are class of natural compounds widely distributed in plants, having multiple biological activities and anti-oxidant properties. About 1300 coumarin compounds have been identified, principally as secondary metabolites in green plants (**Hoult and Paya, 1996**). They found free or as hetero-sides in many