

Cytotoxic Profiling of Some Compounds of Natural Origin against HepG2

Liver Cancer Cell Line in-vitro

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Abstract: Cancer is so far a national and international health problem. WHO reports showed constant rates of mortality caused by various types of cancer through the years 1950-2000. There is a wide gap between the disease incidence (0.16%), disease burden (5.6%) and the mortality caused by cancer (12.5%) worldwide. These gaps denote clearly the lack of effective treatment for cancer compared to other mortality causes. Despite the availability of several anticancer agents, the treatment of cancer remains medical hurdle in the developed and developing countries. Discovery of natural products with potential anti cancer activity is very initiative trend in countries with rich botanical flora. Liver cancer is very serious solid tumor which is highly abundant in areas endemic with hepatitis viruses such as middle and Far East. Herein, we have assessed the cytotoxic characteristics of several natural molecules (cerulinin, chrysin, honikiol, limonin, mevinolin, resveratrol, salicin, retinol, ascorbic acid and calciferol) against HepG2 liver solid tumor cell line. After exposure to serial concentrations of the test compounds for 72 h, SRB-u assay was undertaken and viability assessment was performed via fitting to Emax model to identify the cytotoxicity parameters such as, IC50 and resistant fraction (R-value). Most of tested compound showed sort of cytotoxic effects against HepG2 cell line with IC50's ranged from 1.1 to 33.1 µg/ml. Cerulinin and mevinolin showed the highest potency with IC50 less than 5 µg/ml. Chrysin, honikiol and resveratrol showed moderate potency with IC50 ranging from 5-10 µg/ml. Ascorbic acid was weak cytotoxic agent against HepG2 cell line. Limonine, salicin and retinol failed to exert any cytotoxicity against liver cancer cell line in-vitro. Impressively, molecules of potent and moderate potency (except chrysin) showed low resistant fraction in HepG2 liver cancer cell line. In conclusion, our data showed wide range of variable efficacy of several molecules of natural origin against liver solid tumor.

Key Words: Anti-cancer screening, L cytotoxicity, Liver cancer, Natural products

1. Introduction

Natural products from plants have been valuable sources for anticancer drug discovery (Schwartzmann *et al.*, 2002). A screening program was initiated by Leven *et al.* (1979) that identified many antibacterial antifungal, antiviral, antiparasitic, and other pharmacologically active substance activities in higher plants (Jang *et al.*, 1997).

Traditionally, resveratrol of red grape wine was considered as general anti-aging material with classical antioxidant properties due to its polyphenolic composition (Yao *et al.*, 2009). Further studies revealed anti-inflammatory, vascular endothelial protective and lipid metabolism enhancement effects for Resveratrol. Resveratrol has been found to interfere with the expression/activity of several inflammatory/immunoregulatory cytokines and enzymes such as cyclooxygenase, TNF- α , NF- κ B, INF- γ and IL-1, IL-4 and IL-6 (Han *et al.*, 2008). Cancer preventive property of resveratrol has been attributed mainly due to its antioxidant anti-inflammatory/immunoregulatory profile (Yang *et al.*,

2009). In addition, several reports have indicated potential anti-proliferative or anti-cancer effect of resveratrol per se against many types of human cancers (Alfaras *et al.*, 2009; Cui *et al.*, 2010 ; Cui *et al.*, 2010 ; Gagliano *et al.*, 2010).

Resistance of solid tumor to anticancer treatment has been attributed currently to pharmacokinetic reasons rather than resistance at the cellular level. Anticancer agent need to be introduced to each single tumor cell in a cytotoxic concentration to generate tumor killing effect (Tredan *et al.*, 2007; Hicks *et al.*, 2003). This study aim to measure the activities of some natural compounds as anticancer (liver).

2. Materials and Methods

2.1. Chemicals and drugs

Pure compounds were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI-164 media, fetal bovine serum and other cell culture materials were purchased from AATC (Houston, TX). Other reagents were of the highest analytical grade.

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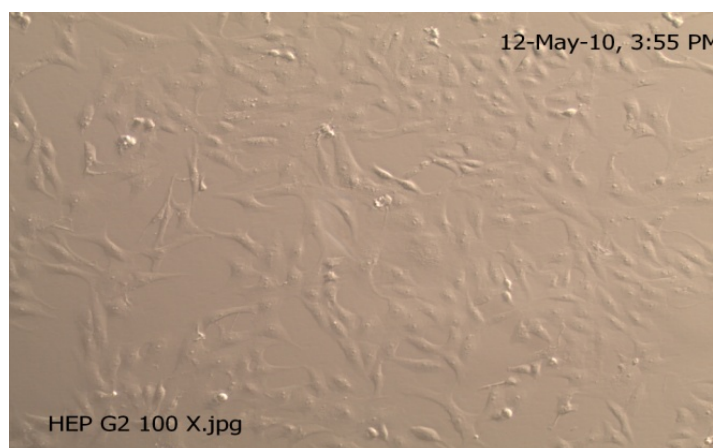


Fig.1. HepG2 monolayer culture at 70-80% confluent ratio.

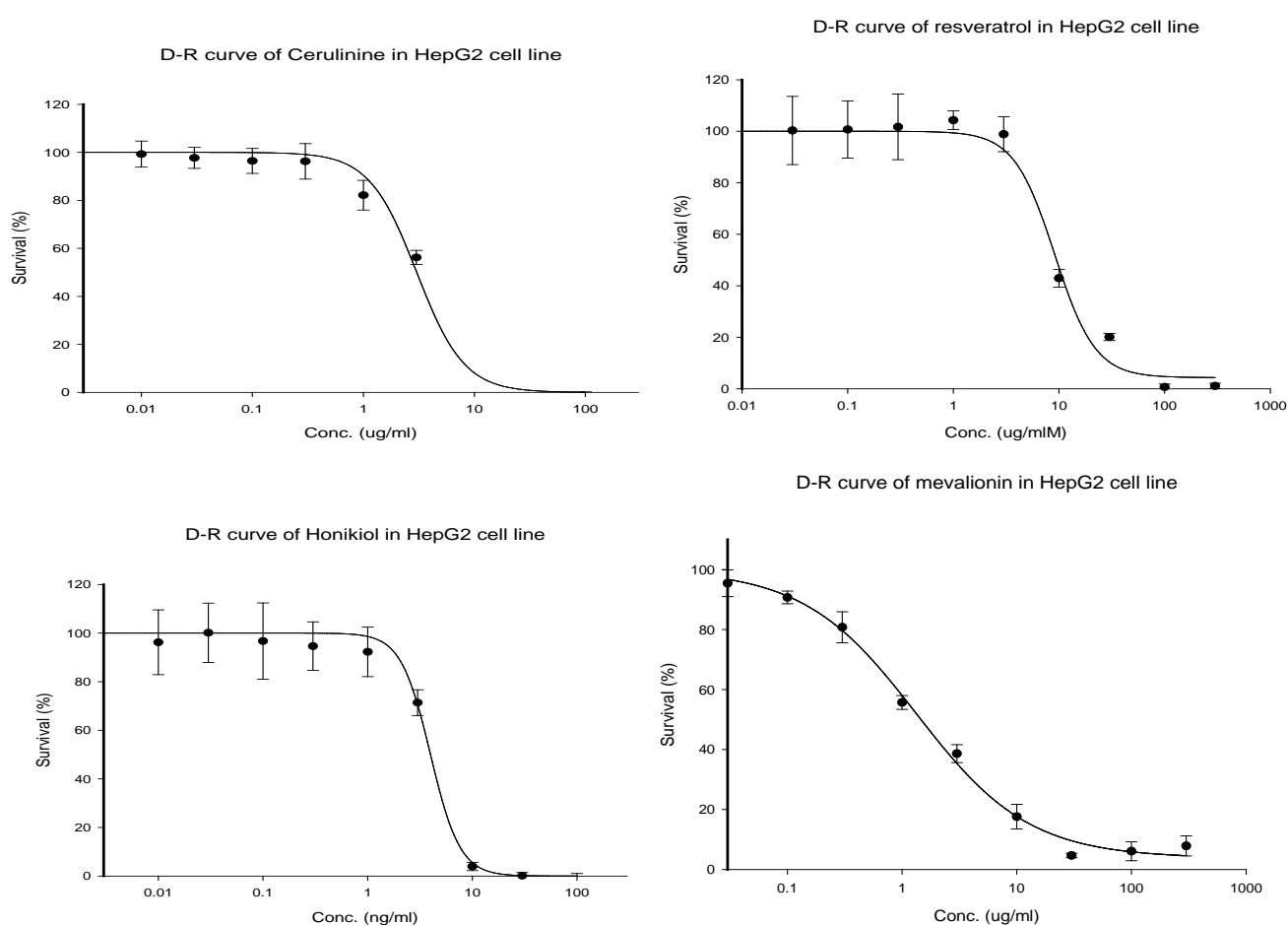


Fig.2. The effect of natural compounds on the dose response curve (D-R) of HepG₂ liver cancer cells.

Table 1. Effect of natural compounds on the cytotoxicity parameters of Hep G2 cell line.

Compounds	Resveratrol	Cerulinine	chrysin	Honikiol	Limonene	Mevinolin	Salicin	VITA	Vit-C	Vit D
Parameter										
IC ₅₀	8.93±0.12 μg/ml	2.97±0.15 μg/ml	8.70±2.65 μg/ml	4.60±0.35 μg/ml	--	1.06±0.12 μg/ml	--	---1	33.10±2.36 μg/ml	10.04±1.83 μg/ml
Resistance Fraction	4.70±0.23%	0.00±0.00 %	16.93±16.93%	0.00±0.00 %	> 100%	3.74±1.67 %	>100%	>100%	0.27±0.2 %	0.00±0.00 %

2.2. Cell culture

Human hepatocellular cancer cell line (HepG-2) was obtained from the Vaccera (Giza, Egypt). Cells were maintained in RPMI-1640 supplemented with 100 µg/mL streptomycin, 100 units/mL penicillin and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO₂ atmosphere at 37°C (Fig. 1).

2.3. Cytotoxicity assays

The cytotoxicity of crude extract and the fractionated compounds was tested against HepG-2 cells by SRB assay as previously described (Skehan *et al.*, 1990). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to each test compound for 72 h and subsequently fixed with TCA (10%) for 1 h at 4°C. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm (Skehan *et al.*, 1990).

2.4. Data analysis

The dose response curve of compounds was analyzed using Emax model (Eq. 1).

$$\% \text{ Cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d^m + [D]^m} \right) + R \quad \dots \text{ (Eq. 1)}$$

Where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC₅₀ was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e., K_d = IC₅₀ when R=0 and E_{max} = 100-R) (Al-Abd *et al.*, 2008).

3. Results and Discussion

Cerulinine, Vit D and Honkiol were showed moderately active as single agent for the liver cancer.

Mevenoline was moderately active as single agent for the liver cancer (Table 1, Fig. 2).

Vitamin A and C were showed weak activities for liver cancer.

Additionally, to study the effect of RES on the cytotoxic profile of two corner stone chemotherapeutic agents (Table 1) (Fig. 2) for 72 h incubation period.

Several studies have demonstrated that mixtures in extracts from herbal medicines had an anticancer potential in vitro or in

vivo (Bonham *et al.*, 2002; Hu *et al.*, 2002; Chen *et al.*, 2005; El-Shemy, *et al.*, 2007). Aqueous extracts from willow (*Salix* sp.; Saliceae) leaves prevented proliferation of three cancer cell types acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and Ehrlich ascites carcinoma cells (EACC; El-Shemy *et al.*, 2007). Alcohol extracts of *Ganoderma lucidum* (Hu *et al.*, 2002) induced apoptosis in MCF-7 human breast cancer cells. Three of ten sesquiterpene lactone compounds from *Vernonia chinensis* were active against P-388 and A-549 tumor cell lines (Chen *et al.*, 2005). Therefore, the complex mixtures in crude extracts may be more effective than single purified compounds.

The safety profile of Mevinolin (MVN) both in experimental and clinical stages are encouraging further clinical trials for the treatment of various types of tumors. The dose of MVN suggested for anti-cancer treatment is believed to be clinically safe 6,7. Therefore, very high doses of MVN administered every four hours to patients have been found very tolerable (Holstein *et al.*, 2006). MVN and other natural statins are believed to be better treatment option for cancer than synthetic statins (Ahn *et al.*, 2008). MVN was shown to be a clinically safe drug of natural origin that is known to inhibit the HMGCo-A reductase activity and interfere with steroidogenesis (Kumari *et al.*, 2009). In future, gene silencing studies would be recommended to understand the exact mechanism of different compounds on HepG-2 cell line.

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