

Anticancer activity of methanolic extract of *Momordica charantia* against human colon, liver and breast cancer cell lines- In vitro

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Abstract

Natural products are the best source for various medicinal drugs. Regardless investigate the toxic effect of these plant extracts, the results will still unsafe and unacceptable. This study aimed to identify anti-cancer activity and cytotoxicity effect of *Momordica charantia* extract on different cancer cell line. Materials and Methods: to archive the aim of this study 3 different cancer cell lines (HCT116, MCF-7, and HepG2) were treated with different *Momordica charantia* extract doses (from 0-100 μ g) for each cell line. IC₅₀, cell viability, apoptosis, were evaluated. Results: The effect of *Momordica charantia* extract was highly significant in HepG2 cells than HCT116 cell as well as MCF-7 which showing the IC₅₀ of *Momordica charantia* extract in HepG2 was 0.77 μ g/ml while in HCT116 was 0.81 μ g/ml and was 1.35 μ g/ml in MCF-7 cells respectively. Also, the effect of the *Momordica charantia* extract was more potent in HCT116 compared to MCF-7 cells. Conclusions: in the light of these results *Momordica charantia* extract may use as anticancer pro-drug at a specific type of cancer (Liver HepG2 cell line) but still need further studies to explore the mechanism of action that led to observe different results from different cell lines despite the same extract.

Keywords: *Momordica charantia*, Apoptosis, MCF-7, HepG2, HCT116.

1. Introduction

Natural products possess valuable, essential molecules and chemical groups which form the pool for the different drugs.

With the steady increase of the importance of natural products to use in different purposes, for pharmaceutical, food, antibiotics, insecticide, etc. the anti-cancer drug research and development field acquired new and strong momentum. So far, there are many anti-cancer drugs have been discovered. Despite, they have an anti-cancer effect on different cancer types, the mechanism of action of many drugs still unknown.

One of the most simple, least expensive, reliable, short-time in-vitro anticancer activity screening tests is apoptotic, IC₅₀, and cell viability. All of these tests can use together as different parameters useful to screen positive and negative effects of many plant extracts, potential anticancer drugs and so on (Wang et al., 2006) many societies (Indian and Turkish) used *Momordica charantia* in their own folk medicine for treating different antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies) (Zhao et al., 2015). (Licastro et al., 1980) have investigated the toxicity of *Momordica charantia* lectin as an inhibitor for human normal and leukemic lymphocytes, while (Pongnikorn et al., 2003), studied the effect of the plant (MC) on level and function of natural killer cells in cervical cancer patients with radiotherapy. With the continued interest, many researchers showed different effect of (MC) plant on different type of cancers in vivo and in vitro (Brennan et al., 2012; Fang et al., 2012a; Fang et al., 2012b; Weng et al., 2013; Yung et al., 2015).

The splendor of the impact of this plant makes the researchers going to do more investigations on different cancer types.

Under the influence of previous research, cytotoxicity effect of (MC) plant on HepG2, MCF7 and HCT116 cancer cells were evaluated by means of IC₅₀. Consequently, our study has been designed to fill the avoid data regarding the effect of (MC) plant extract.

2. Materials and Methods:

2.1 Plant materials

Momordica charantia fresh aerial parts were purchased from locally market.

2.2 Chemicals and drugs:

SulphoRhodamine-B (SRB), ethanol and methanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Culture media and growth supplements were purchased from Gibco / Life Technologies Co, (Carlsbad, CA, USA). Cell culture vessels were purchased from Nunc Co. (Roskilde, Denmark).

2.3 Cell culture

Human colorectal carcinoma (HCT 116), Human hepatic carcinoma (HEPG-2) and Human breast cancer (MCF-7) were obtained from Vacsera (Giza, Egypt). Cells were maintained in RPMI media 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, the cells were sub-cultured two times in a week.

2.4 Preparation of Plant extract

The aqueous plant extract was prepared by washed in distilled water and air-dried overnight then the fleshy parts were cut into small pieces and fine powdered. The aqueous plant extract was left in the water bath (Fisher Scientific-2232, USA) for 1 hr. at 60 °C then leave overnight at room temperature. The extract was filtered with filter paper. The filtrates were combined and concentrated using vacuum rotatory evaporator system (HB-10, IKA) and the temperature was maintained at 45 °C. The extract was dried in an oven. The dried extract was stored at -20 °C until use (Nawwar *et al.*, 2012)

2.5 Cytotoxicity assays against tumor cells

The cytotoxicity of the extracts was tested against the HCT116, HEPG-2 and MCF-7 tumor cell lines by sulforhodamine B (SRB). Exponentially growing cells were collected using 0.25% trypsin - EDTA and plated in 96-well plates at 1000 cells/well. Cells were exposed to the extracts for 72h and subsequently fixed with TCA (10%) for 1h at 4°C. After many washings, cells were exposed to 0.4% SRB solution for 10min in a dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris - HCl was used to dissolve the SRB-stained cells and the color intensity was measured at 540nm. The data are analyzed using SigmaPlot version 12.0. (Skehan *et al.*, 1990)

2.6 Cell viability

sulforhodamine B (SRB) stain, allowed us to distinguish viable and dead cells from each other. Viable and dead cells were detected using density measured at 450 nm using microplate reader (Anthos Zenyth-200RT, Cambridge, England) (Tolba *et al.*, 2013)

2.6 Apoptosis

Using fluorescein stain, condensed or fragmented nucleus was counted with the assistance of the fluorescent microscope (Nikon).

2.8 Statistical Analysis

The data are analyzed using SigmaPlot version 12.0. (Skehan *et al.*, 1990)

3. Results

3.1 Cytotoxicity Profile of *Momordica charantia* against HCT116, MCF-7 and HepG2 cell lines

SRB assay was used to assess the anti-proliferative effect of the *Momordica charantia* extract against three different cell lines (HCT116, MCF-7 and HepG2). The extracts showed considerable anti-proliferative activity selectively against MCF-7, HCT116 and HepG2. The effect of the *Momordica charantia* extract was highly significant in HepG2 cells than HCT116 cell as well as MCF-7 which showing the IC₅₀ of *Momordica charantia* extract in HepG2 was 0.77 µg/ml while in HCT116 was 0.81 µg/ml and was 1.35 µg/ml in MCF-7 cells respectively. Also the effect of the *Momordica charantia* extract was more potent in HCT116 compared to MCF-7 cells (table 1).

In the other hand the cell viability parameter showed rise line with increasing dose, striking that the curve of cell viability percent gone very slowly with small concentrations (0.01, 0.01, and 1 µg/ml) while the difference between the concentration 1 µg/ml and concentration 10 µg/ml was highly significant in all cancer cell lines (Table 2 figure 1., 2, 3). HepG2 cells are still the most sensitive cancer cell line to (MC) plant extract.

4 Discussion:

Although the way to discover new anticancer drugs seems long, hard and expensive, it is important to give people living with cancer longer survival, better quality of life, less of pain and suffering. Just as cancer has the

potential to have a variety of types, the plants which produce natural products also have the potential to produce different molecules and chemical groups withstand or resist cancer.

Cancer which considered as complicated disorder or devastating disease characterized by transformation led to uncontrolled cell growth which involves, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis (Ichikawa et al., 2006). Consequently, there are two ways trying to keep cancer cell under control, Drug design by synthetic chemistry or natural products using plant, bacterial or algae extract. Drugs design by synthetic chemistry generally suffering from unwanted side effects because they have been produced under conditions far away from living materials. While natural products, produced inside the cell itself which may give it an advantage than synthetic products. The anticancer activity of *Momordica charantia* extract has been demonstrated in vitro and in vivo in prostate, breast, ovary and pancreatic, breast cancers in vivo. (Srinivasan et al., 2007; Pitchakarn et al., 2010; Fang et al., 2012a; Yung et al., 2015). Our study focused on the effect of crude extract of *Momordica charantia* on liver cancer (HepG2), Human colon cancer (HCT116) and breast cancer (MCF-7) in vitro because it had not been reported especially in MCF-7 and HCT116 while HepG2 used as a control to compare our results with elder research and publications. Our observations showed that the (MC)plant methanolic extract has a potential effect to decrease the proliferation rate of different cancer cell lines specially HepG2, liver cancer. The mechanism of action of MC plant extract may due to their chemical contents like triterpenoid, which confirmed as anti-proliferative ingredient (Akihisa et al., 2007). It seems that triterpenoid showed its effect on the liver, breast and colon cancer in vitro.

5.CONCLUSION:

There is no doubt that this plant gained very high attention to be one of the strong candidate anticancer drugs. Despite that, needs further studies to screen and to investigate the mechanism of action in details.

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Table 1: The IC₅₀ as µg of *Momordica charantia* extract HCT116, MCF-7 and HepG2 cancer cell.

Plant extract	MCF-7	HCT116	HepG2
<i>Momordica charantia</i>	1.358	0.8105	0.777

Table (2): The viability % of HCT116, MCF-7 and HepG2 cancer cell after treatment with *Momordica charantia* extract

Concentration µg/ml	Viability%		
	HCT116	MCF-7	HepG2
0	100	100	100
0.01	96.53397392	99.48811096	95.00102438
0.1	92.50171585	97.98546896	87.89182545
1	92.27865477	96.31770145	79.38946937
10	2.848318463	6.819682959	3.56484327
100	2.127659574	1.882430647	2.315099365

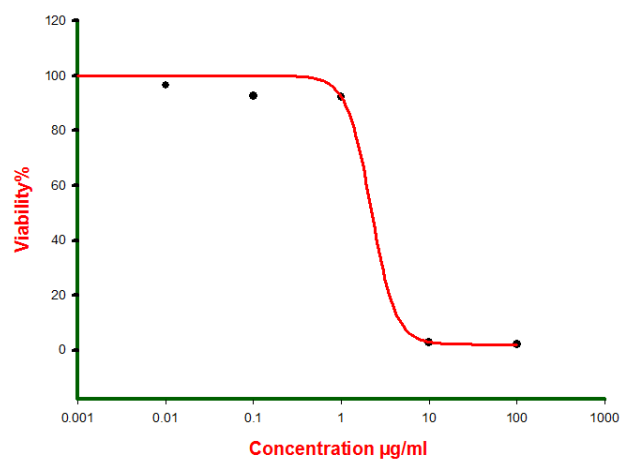


Fig: (1) Dose response curves of *Momordica charantia* extract against HCT 116 cells by SRB assay. Cells were treated with various concentrations of *Momordica charantia* for 72 hrs.

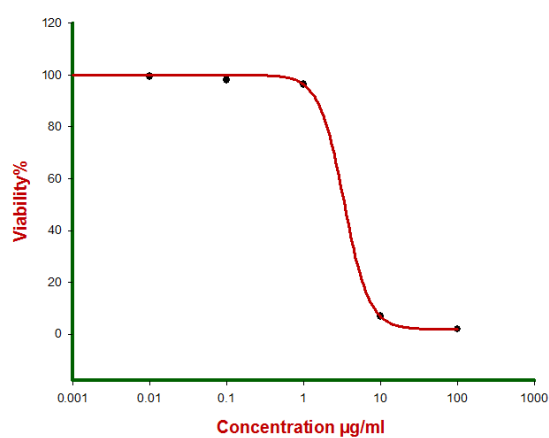


Fig: (2) Dose response curves of *Momordica charantia* extract against MCF-7 cells by SRB assay. Cells were treated with various concentrations of *Momordica charantia* for 72 hrs.

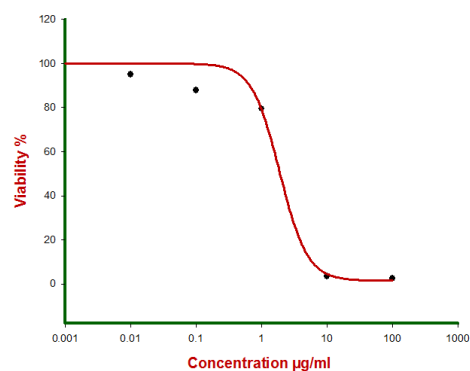


Fig: (3) Dose response curves of *Momordica charantia* extract against HEPG2 cells by SRB assay. Cells were treated with various concentrations of *Momordica charantia* for 72 hrs.