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CYTOTOXICITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF MOMORDICA CHARANTIA IN NEUROBLASTOMA CELLS

NÖROBLASTOMA HÜCRELERİNDE MOMORDİCA CHARANTİA EKSTRESİNİN SİTOTOKSİSİTESİ, TOTAL FENOLİK VE FLAVONOİD İÇERİĞİ

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Abstract

Momordica charantia has various bioactive compounds. In this study cytotoxic effects of the root, seed, leaf and fruit parts of the plant against neuroblastoma cells were investigated. Also total phenolic and flavonoid contents of different parts of Momordica charantia extract. According to cytotoxicity results, higher concentrations of all parts of Momordica charantia showed similar cytotoxicity against SH-SY5Y neuroblastoma cells. However, cytotoxicity of the root extract was higher than 50% in 500 µg/ml concentration, and also at the same concentration induces proliferation of ECV304 control cells. According to our results, the leaf part of Momordica charantia plant is rich with flavonoid and phenolic contents. However, its cytotoxic effect on SH-SY5Y cells is lower than the root extract. Also, while Momordica charantia root extract increased Akt1 gene expression, it decreased expression levels of BAD and caspase-3 genes in these cells. In conclusion, Momordica charantia root extract can induces apoptosis through p38-MAPK pathway in SH-SY5Y cells by considering the results.

Keywords: neuroblastoma, momordica charantia, cancer

Özet

Momordica charantia ekstresi çok sayıda biyoaktif maddelere sahiptir. Bu çalışmada, bitkinin kök, tohum, yaprak ve meyve kısımlarının nöroblastoma hücrelerine karşı sitotoksik etkileri ve bu ekstrelerin toplam fenolik ve flavonoid içeriği araştırılmıştır. Sitotoksisite sonuçlarına göre, Momordica charantia ekstresi tüm bölümlerinin yüksek konsantrasyonlarda, SH-SY5Y nöroblastoma hücrelerine karşı benzer düzeylerde sitotoksisite göstermiştir. Bununla birlikte, kök ekstresinin sitotoksisitesi, 500 ug / ml konsantrasyonda % 50'den fazladır ve aynı konsantrasyonda ECV304 kontrol hücrelerinde proliferasyona yol açtığı bulunmuştur. Çalışma sonuçlarına göre, Momordica charantia bitkisinin yaprak kısmı flavonoid ve fenolik içerik bakımından zengindir. Ancak, yaprak ekstresi SH-SY5Y hücrelerine karşı kök ekstresine göre daha düşük sitotoksisite göstermiştir. Ayrıca, Momordica charantia kök ekstresi SH-SY5Y hücrelerinde Akt1 gen ekspresyonunu arttırırken, BAD ve kaspaz-3 genlerinin ekspresyon seviyelerini azalttı. Bulunan sonuçlara göre, Momordica charantia kök ekstresi, SH-SY5Y nöroblastoma hücrelerinde p38 MAPK sinyal yolağı üzerinden apoptoza yol açabilir

Anahtar Kelimeler: *nöroblastoma, momordica charantia, kanser*

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1. Introduction

Neuroblastoma is a tumor that occurs in childhood period. It is responsible for the majority of pediatric cancers (Bernstein et al., 1992). Various studies showed that Momordica charantia has antidiabetic, antioxidant, anticancer, anthelmintic, antiviral, antibacterial, antiinflamatory, immunomodulator, hypoglycemic, and hypolipidemic effects. All parts of Momordica charantia has various bioactive compounds (Chao and Huang, 2003; Grover and Yadav, 2004; Nerurkar et al., 2008; Nerurkar and Ray 2010). However, its effects on neuroblastoma cells are unknown.

The root, seed, leaf and fruit parts of the plant were assessed for its possible anticancer drug potential for targeting malignant neuroblastoma cells. Cytotoxicity assay was applied to SH-SY5Y neuroblastoma cells and examined in comparison with ECV304 as the control. Also, intracellular apoptotic pathways of the root extract that show above 50% cytotoxic effect against SH-SY5Y cells was studied.

2. Materials and Methods

2.1. Plant material

Momordica charantia was collected from Istanbul-Beykoz with fruits, 2015. Voucher specimens has been identified by Professor E. Akalin and deposited in the Herbarium of the Pharmacy Faculty of Istanbul University (ISTE).

2.2. Extraction

The parts of Momordica charantia were extracted separately in 100 ml methanol by using a household blender. Then, extracts were centrifuged 600 g at 4ºC for 30 min by using Beckman Coulter Allegra X-30R refrigerated centrifuge. Supernatants of extracts were transferred in another falcon. Moreover, they were filtered by using an injection filter. Extracts that were prepared and filtered were stored in aliquots at -80ºC until further analysis.

2.3. Cell culture

SH-SY5Y neuroblastoma and ECV304 human umbilical vein endothelial cell lines were purchased from American Type Culture Collection (ATTC). ECV304 human umbilical vein endothelial cell line was used as control.

2.4. MTT viability test

The viability test was performed by using MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).

The solutions of the Momordica charantia extracts were prepared in methanol, 10 mg/mL. Cells were counted and they were suspended at 1 × 105 cells/mL in medium. Ninety µL of these cell suspensions were dispensed into 96-well plates that contained 10 µL of dilutions of Momordica charantia extracts. Thus, final concentrations of extracts were 500, 50, 5, 1 µg/mL, respectively. Ten µL medium without extract was used as a positive control. The viability test was repeated six times for each concentrations of extracts. These 96-well plates were incubated for 48 h. Then, 10 µL MTT (5 mg/mL) was added into every well of 96 well plates. After 4 h. incubation, 100 µL of 50% isopropanol and 50% DMSO mixture was added into every wells of 96 well plates. Colorimetric analysis was performed by ELISA spectrophotometry, and optical density (OD) was measured with a 570 nm test wavelength and a 630 nm reference wavelength (Atasever Arslan B et al., 2016). The cytotoxic activities of the extracts were calculated with the formula:

(Cytotoxicity index = 1 - [OD (treated well) / OD (control well)] × 100).

2.5. Real time PCR

Gene expression levels of p38, AKT1, BAD, caspase 3 and PARP were measured by using Roche LightCycler® FastStart DNA Master SYBR Green I kit. Firstly, reaction mix was melted then 10 µL of enzyme was added into this reaction mix. Then, according to the manufacturer's instructions of kit total volume 10 µL of PCR mix consisting of dH20, MgCl2, primer mix and master mix was prepared. Real time PCR was conducted with Roche LightCycler Nano machine by using modifiying protocols in accordance with primers. The fold change was analyzed 2- ΔΔCT method (Livak and Schmittgen, 2001).

2.6. Determination of total flavonoid and phenolic contents

To assess total flavonoid content of the extract, quercetin was used. Stock solution of quercetin was prepared as 0.1 mg/ml and then it was diluted as 6.25, 12.5, 25, 50, 80, 100 µg/ml. The extract (0.5 ml) incubated with 1.5 ml methanol, 0.1 ml 1M potassium acetate, 0.1 ml 10% aluminum chloride and 2.8 distilled water 30 minutes at room temperature. The resulting color was measured at 415 nm on an ELISA spectrometer and total flavonoid content calculated as quercetin equivalents from a calibration curve (Bag et al. 2015).

In order to measure total phenolic content of the extract, it was used gallic acide method. Stock solution of gallic acide as 0.1 mg/ml and then it was diluted as 6.25, 12.5, 25, 50, 80, 100 µg/ml. The extract (0.5 ml) incubated with 2.5 ml 10% Folin-Ciocalteau and 2.5 ml 75g/L sodium carbonate at 45°C, 45 minutes. Then it was measured at 765 nm on an ELISA spectrometer and total phenolic content was calculated as gallic acid equivalents from a calibration curve (Jaradat et al. 2017).

2.7. Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software. Results were expressed as the mean ± standard deviation

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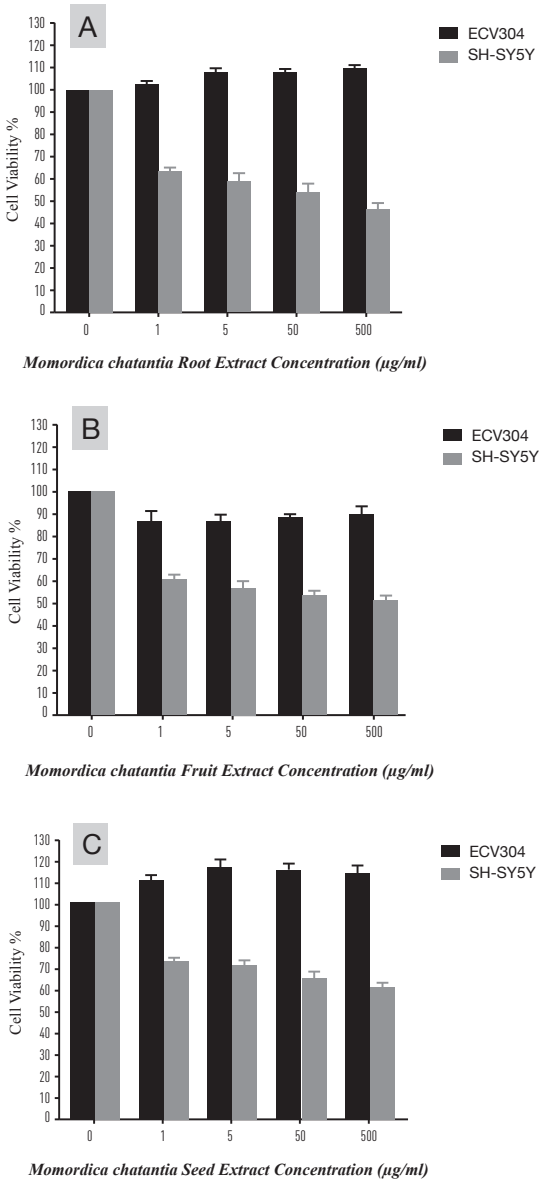
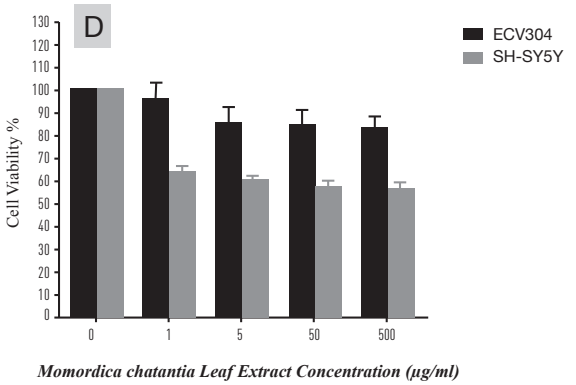
(SD). Statistical differences were assessed by Student's unpaired t-test, with $p < 0.05$ as a statistical significance cut-off.

3. Results

Cytotoxic effects of extracts of root, fruit, seed and leaf parts of Momordica charantia were investigated by using MTT colorimetric method. According to cytotoxicity results, higher concentrations of all parts of Momordica charantia showed similar cytotoxicity against SH-SY5Y neuroblastoma cells. However, cytotoxicity of the root extract was higher than 50% in 500 µg/ml concentration, and also at the same concentration induces proliferation of ECV304 control cells (Figure 1).

Figure 1. The cytotoxic activities of the Momordica charantia extracts against SH-SY5Y human neuroblastoma cells and ECV304 human endothelial cells. SH-SY5Y and ECV304 cells were treated with different concentration (1, 5, 50, 500 μg/mL) of Momordica charantia extracts. Error bars represent standard deviations.





Respectively these extracts; A: Momordica charantia root extract, B: Momordica charantia fruit extract, C: Momordica charantia seed extract, D: Momordica charantia leaf extract. Cell viability was measured by MTT assay. It was found the significant cytotoxic activity above %50 of Momordica charantia root extract against SH-SY5Y human neuroblastoma cell ($p < 0.05$).

Considering of the effects of the root extract, to understand its signaling pathways on SH-SY5Y cells, gene expression levels of some signaling nodes were measured. According to RT-PCR results, after treated with the root extract, expression levels of Akt1 and p38 genes significantly increased, whereas expressions of caspase3 and BAD genes decreased in SH-SY5Y cells. There was not found significant difference PARP gene expression in SH-SY5Y cells, before and after the root extract incubation (Figure 2).

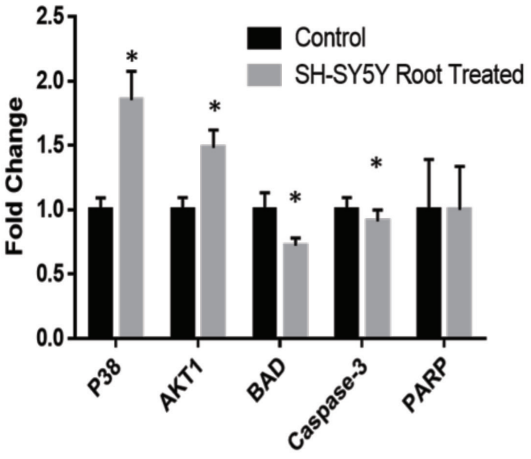


Figure 2. Real-time RT-PCR analysis of P38, AKT1, BAD, Caspase-3, and PARP gene expression in the SH-SY5Y control cell and Momordica charantia root extract treated SH-SY5Y cell. This bar graph representing the fold changes of gene expression quantified by normalization to the GAPDH (*: $p < 0.05$). Error bars represent standard deviations.

To assess total flavonoid content of root, leaf, fruit and seed parts of Momordica charantia extract, standard curve of quercetin was used (Figure 3). Also their total phenolic contents were measured by using standard curve of gallic acid (Figure 4). According to our results, the leaf part of Momordica charantia plant is rich with flavonoid and phenolic contents. However, its cytotoxic effect on SH-

SY5Y cells is lower than the root extract (Table 1).

Table 1. Mean concentrations ± standard deviation of total flavonoid content and total phenolic content of the studied samples.

Sample	Total flavonoid content in mg/g of Momordica charantia extract	Total phenolic content in mg/g of Momordica charantia extract
Roof	49±2.21	226±3.36
Leaf	489±5.6	430±5.83
Fruit	58±3.01	226±2.51
Seed	71±1.05	234±3.51

4. Discussion

Investigating of novel therapeutic agents for the treatment of neuroblastoma are very important. Because Momordica charantia has various bioactive compounds, we studied its possible anticancer potential on neuroblastoma cells. According to our results only Momordica charantia root extract showed above 50% cytotoxicity against SH-SY5Y cells. Chemical ingredients differences among each part of Momordica charantia may lead to change their anticancer efficiency on these cells.

Also, the root and seed extracts showed proliferative effect on ECV304 cells. Since endothelial tissue damage causes some diseases that have high morbidity and high mortality rate as diabetic micro-vasculopathies, atherosclerosis and hypertension. Therefore, proliferative effect of these extracts on ECV304 cells can be very important to develop new therapy approaches for the diseases. Further investigations as in vitro and in vivo are needed to understand underlying mechanisms of this effect.

Cucurbitacin B is one of the bioactive compound of Momordica charantia. It has a lot of biological activities and some studies showed their anticancer activity on different cell lines including breast, prostate, lung and brain cancers (Alghasham, 2013; Jayaprakasam et al., 2003; Miro, 1995). Moreover, trans-p-menth-2-ene and 2-menthene, the other its compounds, suppressed proliferation and progression of cancer (Kelloff et al., 1994). These studies suggest that the molecules can play a role in anticancer effect of Momordica charantia root extract against SH-SY5Y cells. Combinations of the molecules can be different in the parts of Momordica charantia plant.

According to our results, the leaf part of Momordica charantia plant is rich with flavonoid and phenolic contents. However, its cytotoxic effect on SH-SY5Y cells is lower than the root extract. Possible antagonistic or synergistic effects of total flavonoid and phenolic contents of the parts of Momordica charantia plant can lead to show different cytotoxic effects on SH-SY5Y cells. Therefore isolation of these contents from Momordica charantia extract and investigation of apoptotic effects of their various combinations can lead to discovery new anticancer agents.

While Momordica charantia root extract increased Akt1 gene expression, it decreased expression levels of BAD and caspase-3 genes. Increased Akt activation causes phosphorylation and inhibition of BAD protein (Datta et al., 1997; Hein et al., 2014; Zha et al., 1996). However, activation of Akt and inhibition of BAD can cause cell survival or this can indicate decreased apoptosis in this study. Moreover, significant decrease in gene expression of caspase3 was detected. Activation of Akt and inhibition of BAD can inhibit caspase3 activation (Datta et al., 1997). In addition, inhibition of caspase3 can be sign for caspase independent cell death (Tabata et al., 2012).

Momordica charantia root extract significantly increased p38 gene expression. p38 plays role in the regulation of apoptosis (Nebreda and Porras, 2000; Zarubin and Han, 2005). Ghatan et al. showed that p38 activity induces apoptosis in SH-SY5Y cells by stimulating Bax translocation (Ghatan et al., 2000). Therefore Momordica charantia root extract can trigger apoptosis via p38 MAPK pathway and activates cell survival mechanism via AKT signaling in neuroblastoma cells. Moreover, p38 MAPK and Akt signaling have a vital role in neuron because disrupted balance between pro-apoptotic and anti-apoptotic pathways trigger apoptosis (Jha et al., 2015). According to the results, balance between these p38 MAPK and Akt signaling can be disrupted by Momordica charantia root extract and apoptosis can be stimulated.

This study suggested that Momordica charantia root extract showing above 50% cytotoxicity against SH-SY5Y human neuroblastoma cells, and can induces apoptosis through p38 MAPK pathway in this cells. In conclusion, Momordica charantia root extract has anticancer drug potential on SH-SY5Y human neuroblastoma cells.

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