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Original article

Investigation of the Anticancer and Proliferative Effect of Broccoli Extract on Du145 Prostate Cancer and MEF Healthy Fibroblast Cell Lines

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Abstract

In recent years, many researchers have focused on the health effects of plant-derived foods. In this context, foods with high content of flavonoids and phenolic substances have received a great deal of attention as potential agents for cancer prevention and treatment. Studies on the broccoli plant have revealed that broccoli has antioxidant and anti-carcinogenic properties. However, effective dose of broccoli needs to be determined for the cancer treatment. In this study, the effects of broccoli extracts on du145 prostate cancer and Mef fibroblast healthy cells were investigated at different doses for 48 hours.

Du145 and Mef cells were grown with Dulbecco's Minimum Essential Medium (DMEM) and HAMS F 12 (1: 1) supplemented with 2% FBS. Broccoli extracts at ten different doses (0,19% - 100%) were added into cultures and incubated at 37°C for 24 and 48 h in 5% CO2. The viability of the cells was determined by the MTT method (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide). Probit analysis by SPSS software revealed that the broccoli extract at a dose of 4.070 % dose killed du145 cancer cells at 48 h. At the same time, MTT results showed that the viability of Mef cells was increased during 48 h of incubation.

As a result, broccoli extract showed a significant level of anticancer activity in Du145 cells, while increasing the viability of Mef healthy cells. This result suggests that broccoli extract is a potential candidate for cancer treatment

Keywords: Broccoli, Cancer, Prostate Cancer, Fibroblast Cell.

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INTRODUCTION

Since the cancer is a disease caused by uncontrolled proliferation of body cells, the factors that cause uncontrolled proliferation of the cells must be determined. In this case, the researchers first went to illuminate the normal reproduction mechanisms. As a result of the studies conducted, normal cell cycle processes and understanding of how these processes went wrong have provided important information about the mechanisms that trigger cancer. As a result of the studies, the results are not the result of a single event or factor (Chan-Smutko et al., 2008; Kristen and Devanshi, 2010).

Although there are many different types of cancer, the most common common feature of all cancer cells is the presence of abnormal cells in which the processes governing the division of normal cells are impaired (Lynn et al., 1995). The prostate is a small gland located in the pelvis only in men. It is located between the penis and the urinary bladder and wraps the urethra (the urine-carrying channel from the bladder). The main function of the prostate is to help with semen production. Prostate cancer is the most common type of cancer in men. In 1980, it accounted for 18% of cancers and now accounts for 28% (Murphy et al., 1997). The likelihood of developing prostate cancer increases with age, most of the cases occur in men aged 65 and over. Although its treatment seems relatively difficult, the general picture in the prostate is often favorable. The reason for this is that prostate cancer progresses very slowly, unlike other cancers. If diagnosed early enough, prostate cancer can be treated. Prostate cancer treatments include prostate removal, hormone therapy and radiotherapy.

Many researchers have focused on the health effects of plant-derived foods. In this context, foods with high content of flavonoids and phenolic substances have received a great deal of attention as potential agents for cancer prevention and treatment. Studies on the broccoli plant have revealed that broccoli has antioxidant and anti-carcinogenic properties.

Material and Methods

Production of Broccoli Extract

Broccoli (*B. oleracea* var. *italica*) was purchased from local farmers in Karaagac region of Edirne province of Turkey. Broccoli juice was prepared by squeezing broccoli with commercial 700 W juice extractor (Philips-HR1861, China) with a yield of 35%. The extracted juice was centrifuged at 9000 g for 10 min at 25 °C to reduce fouling in UF process. Total soluble solid content (TSS) of broccoli juice remained unchanged after centrifugation. The centrifuged broccoli juice sample was pretreated by using 100 kDa PES membrane in order to remove suspended solids and macromolecules. The ultrafiltered broccoli juice was submitted to Osmotic Distillation (OD) process using a capillary membrane module (MD 020 CP 2 N, Microdyn, Germany). In OD process, the ultrafiltered juice was pumped in the shell side and calcium chloride dihydrate at 65% (w/w) used as stripping solution was pumped in the tube

side of the membrane in a counter-current mode by using peristaltic pumps (Masterflex, l/s, USA) at flow rate of 30 l/h on both sides.

Determination of total antioxidant activity

The total antioxidant activity (TAA) was determined by Trolox Equivalent Antioxidant Capacity (TEAC) method according to Re et al. (1999). ABTS+ radical cation was generated by mixing 7 mM ABTS and 2.45 mM potassium persulfate and the mixture was kept in the dark at room temperature for 16 h. The solution was diluted with ethanol to an absorbance reading of 0.70 (±0.02) at 734 nm. Three mL of ABTS+ cation solution was mixed with 30 µL methanol extract and the decrease of absorbance was recorded after 6 min. TAA was expressed as TEAC (mmol Trolox/l juice).

Culturing Cells

DU-145 prostate cancer and MEF healthy fibroblast cells; Dulbecco ,s Minimum Essential Medium (DMEM) containing 5% fetal bovine serum (FBS), 1% L-glutamine and 1% penicillin-streptomycin was incubated in HAMS F 12 (1:1) medium containing 5% CO_2 at 37°C. The culture medium was renewed every three days.

Determination of Substance Concentration to be Applied to Cell Lines by MTT (3- (4,5-dimethylthiazol-2-yl) -2,5 diphenyltetrazolium bromide) Method

Dulbecco's Minimum Essential Medium (DMEM), HAMS F 12 (1:1), Du145 and Mef cell lines grown with 5% FBS were seeded to 96 well cell culture plates, 180 DML for each item to be applied. It was cultured for 48 hours in an incubator containing 5% CO 2 at 37°C. For 48 hours cultured cells; Broccoli extract was applied between 19% and 100% and incubated for 48 hours. After 48 hours, the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed to test cell viability and proliferation. As a result of the MTT test, the IC50 dose was determined.

Results

Broccoli Concentration and Antioxidant Capacity

The ultrafiltered broccoli juice with an initial TSS of 7.1 °Brix was concentrated up to 42.6 °Brix by OD process, where juice and brine temperature were maintained constant at 25 ± 2 °C throughout the operation. Phenolic compounds, ascorbic acid and sulforaphane may contribute to antioxidant activity of broccoli juice. The TAA of ultrafiltered broccoli juice was maintained constant during subsequent 6- fold concentration by OD up to a TSS of 42.6 ± 0.9 °Brix. And by concentration, the antioxidant activity was increased to 9.46 ± 0.02 from 1.90 ± 0.09 mM.

Broccoli's anticancer effect

The effects of broccoli on cancer and healthy cells were determined by MTT test. Cells were incubated with broccoli extract at increasing concentrations for 48 hours. Statistical analysis revealed significant differences between control and broccoli groups in Du145 cells ($p < 0.05$). After 48 hours, probit analysis was performed to calculate the IC50 value (4.07%), which resulted in 50% death of the cells (Fig. 1). As a result of the application, no cytotoxic effect of broccoli was found on Mef cells (Fig. 2).

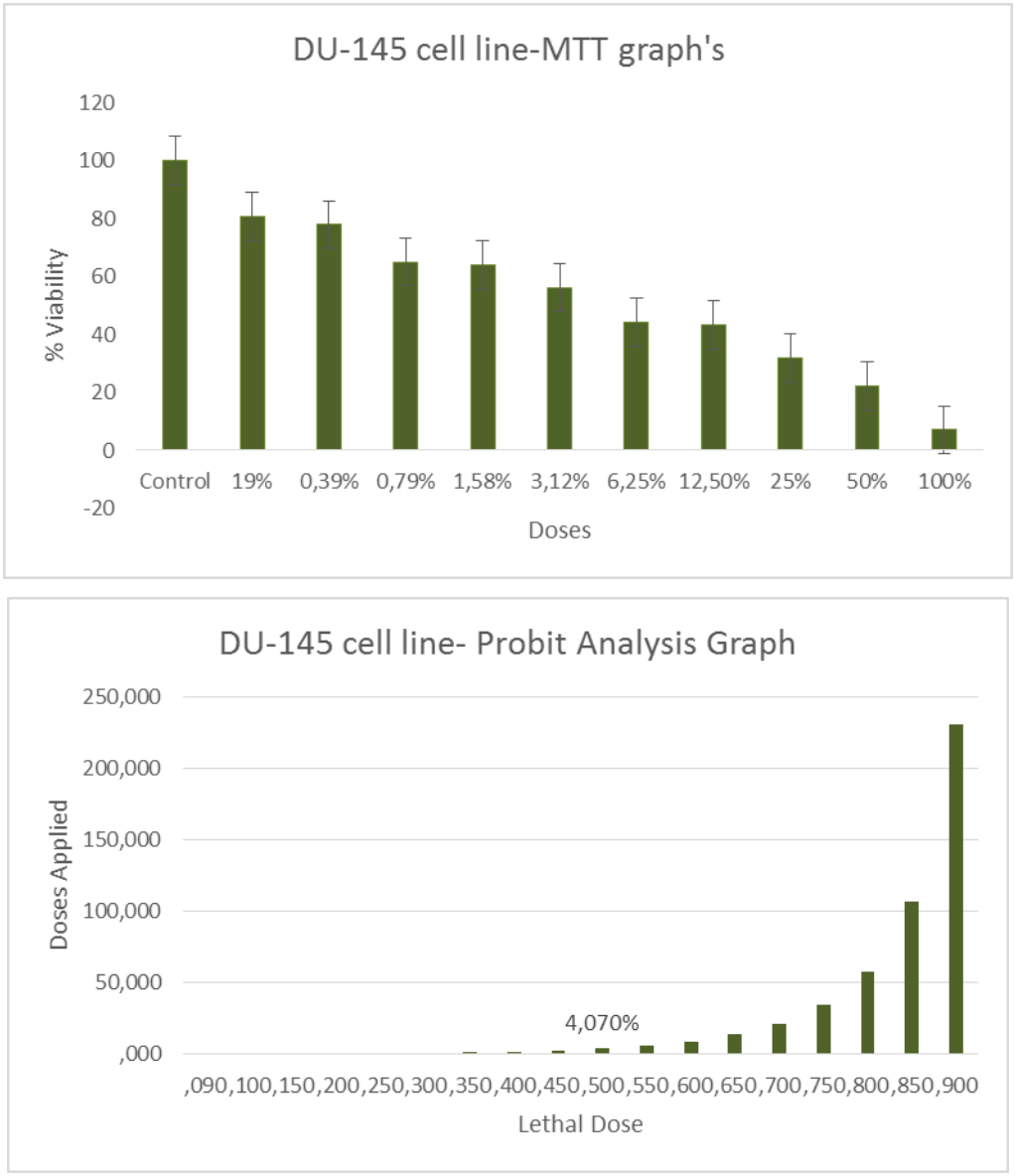

Figure 1. IC_{50} Value of Du145 Cell Line Using MTT Test Results of Broccoli Application for 48 Hours (MTT graphs and Probit Analysis)

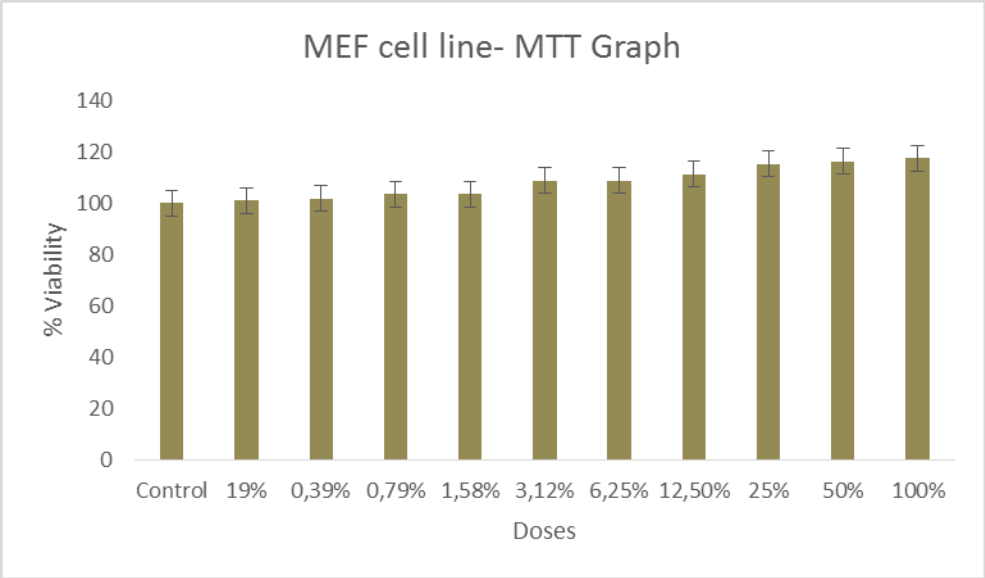

Figure 2. MEF Cell Line Using MTT Test Results of Broccoli Application for 48 Hours (MTT graphs)

Discussion

Prostate cancer is the second most common cause of cancer in the United States (Lynn and Reis, 1995). Advanced prostate cancer is somehow treated by treatment with androgen deprivation (Dawson, 1993; Catalona, 1994). Today, researchers suggest that hormone therapy for prostate cancer is not a very effective treatment for resistant prostate cancer (Laufer et al., 2000). There is a growing belief that selective pressure on prostate cancer cells can accelerate the development of more aggressive and rapidly growing clones of these tumor cells.

Researchers have recently turned to alternative models of vegetative origin, including nutritional modification as an adjuvant therapy for prostate cancer.

Broccoli, which is one of the most important health food developments in recent years, besides intense vitamins and minerals, It is stated that it is one of the most powerful cancer fighters with its strong anti-carcinogenic substance Sulphoraphane. In 2010, Qazi and et al., in their study named "Sulforafan caused both time and dose dependent decrease in cell survival, cell cycle and apoptosis"; With the application of sulforafan, anticancer activity was suppressed. This anticancer activity was thought to be due to the induction of caspase 8 and p21, a molecular chaperone required for the activity of various proliferation-dependent proteins and down-regulation of hsp90.

In many studies, broccoli was analyzed for its antioxidant content and anticancer activities. However, in the studies, leaf roots and trunks of broccoli were emphasized at different harvest times. In 2015, Joon-Ho Hwang and et al., Human colon cancer HT29 and lung cancer have investigated the

anticancer effects of broccoli in NCI-H1299 cell lines, and in all varieties observed higher impact on leaves. The effect dose was 2 mg/ml.

In 2007, Canene-Adams et al. (2007) reported that broccoli extract decreased tumor activity on rats prostate cancer in Their study.

In 2005, Rose et al. Showed that a significant loss of cell viability was not observed in cells incubated with increased broccoli extract concentrations (0, 0.1, 0.2, 0.5 and 1 mg/ml) as a result of their studies in MDA-MB-231 breast cancer cells.

Conclusions

Broccoli is among the most rinsed anticancer natural products. The natural compound called sulforafan in the sprout kills cancer stem cells as well as cancer cells. In our study, the effects of broccoli on prostate cancer were investigated and high killer effect was found on prostate cancer. Using advanced molecular studies, the use of broccoli can be expanded in cancer treatment.

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