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Capsanthin induces death in human prostate cancer cell lines by inducing DNA damage

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Abstract

There is a relationship between a person's diet and the development and prevention of some cancers. Carotenoids are found as various natural pigments in many fruits and vegetables. Studies on carotenoids and their potential roles in carcinogenesis are increasing in importance day by day. In this study, we aimed to determine the cytotoxic and genotoxic effects of capsanthin, a carotenoid compound, in human prostate cancer cell lines.

After different concentrations of capsanthin were applied to human prostate cancer cell lines (LNCaP and PC-3), the effects of the compound on cell viability were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. The single-cell gel electrophoresis (Comet) assay was then used to reveal the genotoxic effects of probable cytotoxic dosages on cell DNA. After the treatments, apoptotic cell death levels were determined by Tunel staining. At high concentrations, capsanthin dramatically reduced PC-3 and LNCaP cell viability ($p<0.05$). In addition, capsanthin caused DNA damage and apoptotic cell death in the prostate cancer cells. The results show that capsanthin reduces cell viability by causing genotoxicity in prostate cancer cells.

Keywords: Capsanthin, Prostate cancer, Cell Culture, DNA damage, Cell Viability

Introduction

Cancer is a group of diseases characterized by uncontrollable cell proliferation and the spread of abnormal cell structures throughout the body. The high heterogeneity of cancer cells reduces the success of treatment and as a result, cancer is accepted as the leading cause of death in every country in the world. In 2020, it is reported that 19.3 million new cancer cases and 10 million cancer-related deaths occurred worldwide [1]. It is stated that prostate cancer is the most common type of cancer among men after lung cancer. This group of cancers has the highest heritability compared to other types and is most common in middle-aged and elderly men in general [2]. In addition, the incidence of prostate cancer shows widespread regional variations, possibly due to differences in dietary habits and due to race (eg, highest in African-Americans) [3,4]. Although there is evidence that some compounds, such as 5α-reductase inhibitors, can safely reduce the incidence of prostate cancer [5,6], chemoprevention for prostate cancer is limited. Therefore, it is important to identify new approaches to increase the success rate in treatment.

Plants contain phytochemicals with antioxidant and anti-inflammatory properties. These compounds generally exhibit low toxicity and can be used for the treatment of many metabolic diseases such as cancer [7]. Carotenoids, which form a subfamily of isoprenoids, cover more than 700 characterized structures [8]. These lipophilic compounds are found in photosynthetic plants, algae, and microorganisms, and they play a part in the production of colors that are unique to each of these creatures. These compounds play protective roles against light and oxygen-induced deformations to which tissues are exposed in animals [9,10]. In addition, carotenoids contribute to the normal metabolism and function of the human body. For example, a diet rich in carotenoids plays a role in the prevention

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of different chronic disorders such as cardiovascular disease, some types of cancer, and age-related macular degeneration [11-13]. Capsanthin and capsorubin are mainly abundant in red pepper and are the major carotenoids responsible for the red color of this fruit [14,15]. This compound is one of the most resistant to oxidation secondary metabolites [16] and exhibits significant antioxidant properties [17]. Studies have shown that capsanthin increases plasma high-density lipoprotein (HDL), which is associated with a lower incidence of cardiovascular disease [18]. Tomatoes contain abundant lycopene, which is known as one of the most powerful antioxidant compounds [19]. It has been reported that lycopene reduces the risk of ischemic stroke [20], prevents bacterial infection [21] and cancer cell proliferation [22]. Many studies explain the strong relationship between human health and carotenoids and recommend consuming foods rich in these compounds [23,24].

Despite improved treatment options and the development of early diagnosis systems, cancer remains one of the most significant health issues of our time. Although hereditary factors are at the forefront of cancer types, environmental factors (such as lifestyle and diet) can have a substantial impact. Dietary choice is a factor that can alter the risk of developing cancer by about 40% [25]. In this study, we aimed to determine the cytotoxic and genotoxic effects of capsanthin, one of the main carotenoids of paprika, on human prostate cancer cell lines PC-3 and LNCaP.

Materials and Methods

Cell culture

Human prostate cancer cell lines PC-3 and LNCaP were used in the study. Cells were fed with RPMI-1640 medium (doped with 10% FBS, 100 U/mL penicillin and 0.1 mg/mL streptomycin). The medium of the cells was changed twice a week and the cells were incubated at 37°C (Thermo Forma II CO_2 Incubator, USA) in a %5 CO_2 environment throughout the experimental period. Confluent cells were removed with the trypsin-EDTA solution and counted after staining with 0.4% trypan blue. For cytotoxicity studies, 96-well plates were used and approximately $15x10^3$ cells were seeded in each well [26].

Cytotoxicity analysis

Cells seeded with different concentrations of capsanthin (Abcam, ab142638, USA) and the same amount of solvent (DMSO) were treated (final volume 1 µL). Changes in cell viability after 24 h of incubation were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Briefly, after the treatments, the medium from the 96-well plates was aspirated and 50 µL of MTT solution (0.5 mg/ml) was added to each well. After 3 h of incubation, the MTT solution in the wells was aspirated and 100 µL of DMSO was added to each well. Finally, the optical densities of the cells in the well were read on an ELISA plate reader (Thermo MultiskanGo, USA) at a wavelength of 570 nm [27]. The absorbance values obtained from the control wells (wells with medium only) were averaged and this value was accepted as 100% viable cells. The viability levels in the other groups were proportioned to the control absorbance value and the percent viability values were calculated. These experiments were repeated independently at least 10 times on different days.

Genotoxicity analysis

The "Comet Assay", also known as single-cell gel electrophoresis, is a widely used method to determine DNA damage (Genotoxicity) in mammals [28]. The alkaline Comet Assay technique was used in the study. For genotoxicity analysis, cells were grown in 6-well plates and incubated for 24 h with two different doses of capsanthin. The wells were then rinsed twice with PBS after the medium in the wells was removed. Cells were removed with a scraper and $1x10^4$ cells were mixed with 1% low melting agarose. Cell + LMA suspension was added to microscope slides covered with normal melting agarose, the slides were closed and the preparations were made. Slides were left in lysis solution for 1 h. The preparations placed in the horizontal electrophoresis tank on the same plane were processed at 25 V (maximum 300 mA) for 20 minutes. Finally, lampar, which was kept in neutralization solution for 3x5 minutes, was stained with Ethidium bromide and the samples were viewed under a fluorescence microscope (Carl Zeiss / Scope A1, Germany). Scoring was done using Comet Score software (TriTek Corp, Sumerduck, VA). The tail DNA (%) parameters of the groups were determined by randomly counting at least 250 cells from each slide [29,30] .

Tunel assay

Cells were seeded in 8-well culture plates and treated with the low concentration of capsanthin used in the Comet assay. After 24 h treatment, the medium was removed and the wells were washed twice with PBS. The cells were fixed for 20 minutes by adding 4% paraformaldehyde solution onto the slide and wells were washed twice with PBS. To detect DNA fragmentation, the Tunel mix was used (Abcam, ab66108, UK). Cell nuclei were stained with propidium iodide (PI) and the slides were viewed under a fluorescence microscope. The number of Tunel positive cells was expressed as %.

Statistical analysis

The conformity of the groups to the normal distribution was evaluated with the Kolmogorov Smirnov test. One-way analysis of variance was used to compare the groups. The homogeneity of variances was analyzed by Levene's test. TAMHANE T2 test was used in cases where variances were not homogeneous after one-way analysis of variance and for multiple comparisons. Values $p<0.05$ were expressed as mean±standard deviation (SD). A value of $p<0.05$ was considered statistically significant.

Results

Capsanthin treatment reduced viability in prostate cancer cells

The viability changes in PC-3 and LNCaP cell lines 24 h after capsanthin treatment are shown in Figure 1. Capsanthin con-

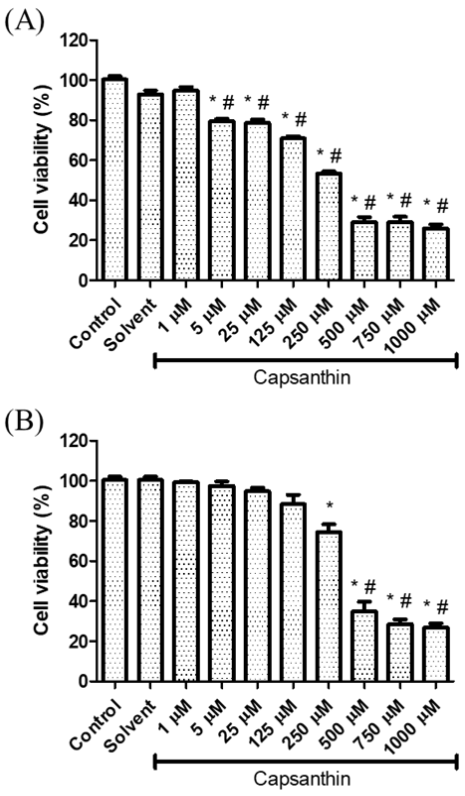

Figure 1. Changes in PC-3 **(A)** and LNCaP **(B)** cells viability after capsanthin treatment. Data were expressed as mean±SD. *p<0.05 compared to control; #p<0.05 compared to the solvent group.

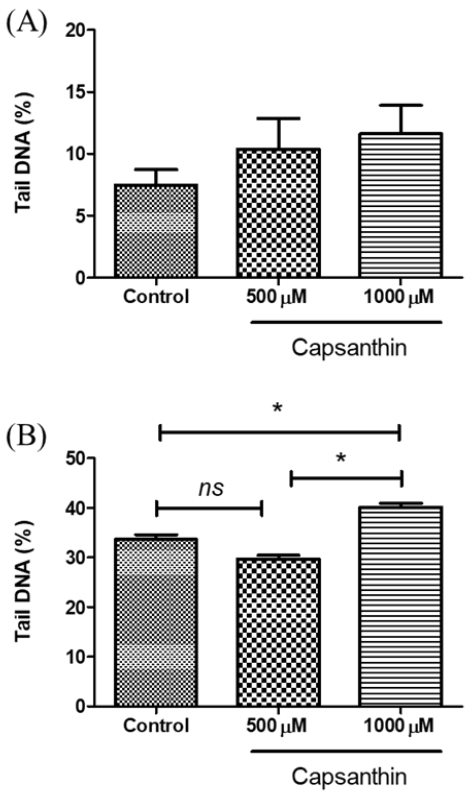

Figure 2. DNA damage level in PC-3 **(A)** and LNCaP **(B)** cells after capsanthin treatment. Data were expressed as mean ± SD. *p<0.05 compared to control; ns: no significant.

centrations of 5 µM and above caused a significant decrease in viability compared to the solvent and control groups ($p<0.05$). No significant change was detected between the control, solvent, and 1 µM capsanthin groups (Figure 1A). In LNCaP cells, on the other hand, concentrations of 250 µM and above of the test compound significantly reduced cell viability (Figure 1B; $p<0.05$). It was observed that concentrations of 500 µM and above had a similar effect on viability in both cell lines.

Capsantin caused DNA damage in cell lines

DNA damage levels in PC-3 and LNCaP cells 24 h after capsanthin treatment are shown in Figure 2. Accordingly, although there was dose-related increased damage to PC-3 cell DNA after Capsanthine treatment, this increase was not statistically significant ($p>0.05$, Figure 2A). On the other hand, DNA damage at 1000 µM concentration applied to LNCaP cells was significantly higher compared to the control and 500 µM capsanthin groups ($p<0.05$, Figure 2B). These results show us that the test compound can cause DNA damage, resulting in a decrease in cell viability.

Apoptotic effect of capsanthin in prostate cell lines

Tunel analysis, which is widely used in the determination of apoptotic cell death, was also performed in the study. 24 h after capsantin treatment, the number of apoptotic cells in prostate cell lines was found to be significantly higher than in the control group (Figure 3B, $p<0.05$). This increase was more pronounced in the LNCaP cell line. These results show that capsanthin induces apoptotic cell death in prostate cancer cells by causing DNA damage.

Discussion and Conclusion

Carotenoids are important compounds that play protective roles in human health after dietary intake. These compounds exhibit chemopreventive, anticarcinogenic, resistance-modifying, and apoptosis-inducing effects depending on their chemical structure. The pharmacological roles of carotenoids in the prevention and reduction of cancer incidence have received increasing attention with increasing evidence from epidemiological studies, tissue culture studies using human cancer cell lines, animal studies as well as human clinical studies.

Epidemiological studies have shown that the consumption of foods rich in flavonoids and carotenoids plays a protective role against various cancer risks [31]. Carotenoids, which are important compounds in vegetables and fruits, are taken in significant amounts almost daily [32]. Although many other carotenoids other than beta- and alpha-carotene have been chemically characterized, their effects on cancer cells have still

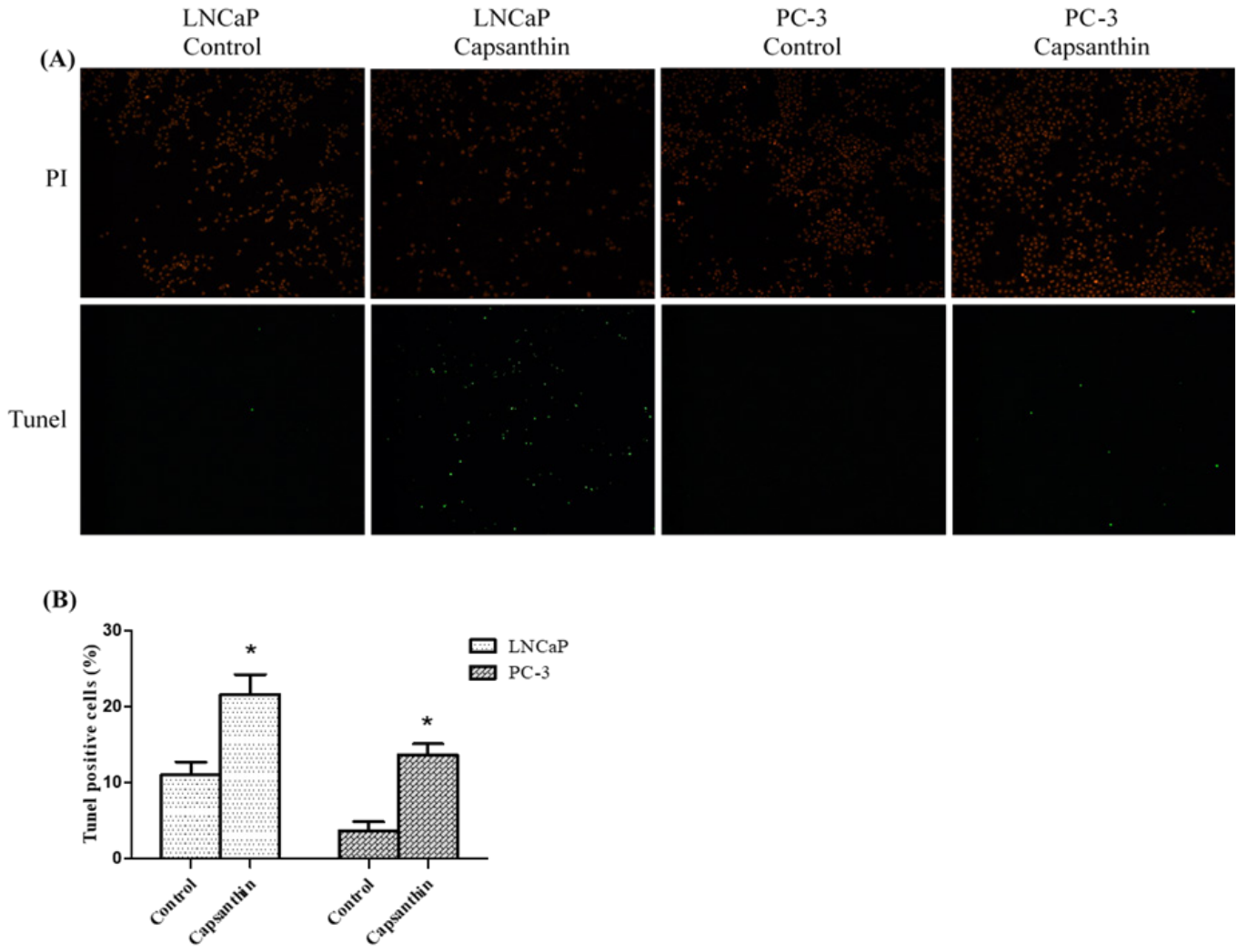

Figure 3. Tunel assay results of groups after capsanthin treatment. **(A)** Microscope image of Tunel staining in cell preparations 24 h after 500 μM treatment of capsanthin (100×) and **(B)** comparison of Tunel positive cells of groups. Values are given as mean ± SD. *$p < 0.05$ value was considered significant.

not been studied in detail. It has been reported that carotenoids exhibit anticarcinogenic effects in various tumor types [33-36]. Zhang et al. reported that capsanthin, β-carotene, astaxanthin, and bixin inhibited the proliferation and decreased the viability of leukemia K562 cells in dose- and time-dependent manners, induced cell apoptosis, and interfered with cell cycle progression [32]. Lycopene inhibits growth in MCF-7 cells by reducing cyclin D1, slowing cell cycle progression through the G1-S phases [37]. Molnár et al. reported that the application of lycopene, zeaxanthin, and capsanthin stimulated apoptosis in human MDR1-transfected mouse lymphoma cells and human breast cancer MDA-MB-231 (HTB-26) cell lines. Researchers report that cells treated with capsanthin and capsorubin have a 30-fold increase in rhodamine 123 accumulation compared to untreated lymphoma cells. It has been shown that the carotenoids used in the study, lycopene, zeaxanthin, and capsorubin, have a high effect on the induction of early apoptosis, whereas lutein and capsanthin have a slightly lower effect [31].

In this study, we determined that capsanthin significantly reduced cell viability in human prostate cancer cell lines, compared to control groups. In particular, the compound significantly reduced viability in both cell lines (PC-3 and LNCaP) after 250 μM concentration. Moreover, capsanthin treatment caused mild to moderate cell DNA damage and apoptotic cell death. This cytotoxic and genotoxic effect of capsanthin on human prostate cancer cells supports the current studies. The pathways through which this effect of capsanthin application on cell lines is exerted is also a subject of research. New studies to be planned in line with the data obtained from this study will help to illuminate the said effect.

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Conflict of Interest

There is no conflict of interest between the authors in the article.

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