Investigation on Cytotoxic and Genotoxic Effects of Capsanthin, the Major Carotenoid of Paprika, on Human Colon Cancer Cells

Sena Davran Bulut[1], Yavuz Erden[2], Buse Nur Derebasi[1], Merve Kubra Gonen[1], Ahmet Karakus[1], Hasan Ufuk Celebioglu[1*]

[1]Bartın University, Faculty of Science, Department of Biotechnology

[2]Bartın University, Faculty of Science, Department of Molecular Biology and Genetics

*Corresponding Author: ufukcelebioglu@gmail.com

Abstract

Cancer, one of the leading causes of death worldwide, continues to pose a significant challenge to public health. Nowadays, natural compounds obtained from plants have become important to use for the prevention and treatment of cancer. One of the promising natural compounds is capsanthin, which is a compound found abundantly in paprika and has significant bioactive properties such as antioxidant, anti-inflammation, and anti-cancer. This study explores the diverse biological activities of capsanthin. Employing *in vitro* methodologies, we investigated the cytotoxic and genotoxic effects on the human colon cancer cell line Caco-2, alongside examining the antimicrobial and antioxidant properties of capsanthin. In antimicrobial studies, concentrations ranging from 125 to 1000 μM of capsanthin displayed a significant ($p<0.05$) reduction in the viability of both *E. coli* and *S. aureus*. The antioxidant potential of capsanthin was evaluated through the determination of DPPH radical scavenging, revealing substantial inhibition at concentrations of 750 and 1000 μM. Cytotoxicity assessments on Caco-2 cells using the MTT method demonstrated a concentration-dependent decrease in viability. Furthermore, genotoxic effects were evidenced by a significant increase in DNA damage in Caco-2 cells treated with capsanthin for 24 hours, compared to controls ($p<0.05$). Even though no statistical difference was observed between applied doses, these findings suggest a potential contribution of capsanthin to cell death through DNA damage. In conclusion, capsanthin exhibited promising antimicrobial, antioxidant, cytotoxic, and genotoxic effects in *in vitro* assessments. These findings provide valuable insights into the multifaceted bioactivity of capsanthin, warranting further exploration for potential therapeutic applications.

Keywords: Antimicrobial, Anti-cancer, Antioxidant, Caco-2 cell line, Capsanthin, Colon cancer, Cytotoxicity

1. INTRODUCTION

Cancer, one of the leading causes of death worldwide, continues to pose a significant challenge to public health. In recent years, researchers have intensified their efforts to explore alternative therapeutic approaches to combat this complex disease (Zugazagoitia et al., 2016). Natural compounds derived from plants have gained considerable attention due to their potential health benefits and fewer side effects compared to traditional chemotherapy agents. Among these natural compounds, carotenoids have emerged as a promising group of bioactive molecules with diverse biological activities, including antioxidant, anti-inflammatory, and anticancer properties (Amengual, 2019).

One such carotenoid that has garnered interest in the scientific community is capsanthin, which is abundantly present in paprika, a widely used spice derived from red peppers. Capsanthin is responsible for the vibrant red color of paprika and possesses potent antioxidant properties (Furubayashi et al., 2021). While previous studies have suggested its ability to protect against oxidative stress and inflammation, the specific effects of capsanthin on cancer cells, particularly human colon cancer cells, remain poorly understood (Eraslan et al., 2022).

The human colon is a crucial site where the development and progression of colorectal cancer occur. Given the increasing incidence and mortality rates associated with this type of cancer, investigating potential therapeutic strategies becomes paramount (Halbrook & Crawford, 2019). Understanding the impact of capsanthin on human colon cancer cells could provide valuable insights into its cytotoxic and genotoxic effects, thus contributing to the development of novel therapeutic interventions.

The present study aims to present a comprehensive investigation into the cytotoxic and genotoxic effects of capsanthin on human Caco-2 colon cancer cells. By employing advanced cell culture techniques, we evaluated the viability and proliferation rates of colon cancer cells upon exposure to different concentrations of capsanthin. Furthermore, we assessed the potential genotoxicity of capsanthin by examining DNA damage and mutations induced by this carotenoid in these cancer cells. The findings of this study may shed light on the underlying mechanisms through which capsanthin exerts its anticancer effects and provide a foundation for further research in the field.

2. MATERIALS AND METHODS

2.1. Antimicrobial Assay

Broth Micro-dilution Assay was used to investigate anti-bacterial activities of the capsanthin, with some modifications (Brandt et al., 2010). Gram (-) *Escherichia coli* and Gram (+) *Staphylococcus aureus* bacterial cultures were inoculated into Nutrient Broth (NB) cultures from frozen stocks for 24 h at 37ºC. Then, sub-culturing was performed, and new cultures were incubated at 37ºC until 0.5 McFarland Unit. 20 µL of bacterial cultures were added in microtiter plate wells and the volumes were completed to 200 µL with NB containing different concentrations of the compounds ranging from 0 to 1000 µg/mL. Negative controls were prepared using LB without bacteria. Absorbances of microtiter plates at 600 nm were read using an ELISA reader before (0th h) and after (24th h) incubation at 37ºC. Percentage inhibition vs. compound concentration plot were drawn and the Minimum Inhibitory Concentrations (MICs) of the compounds were calculated.

2.2. DPPH Scavenging Antioxidant Assay

Antioxidant effects of LGG grown in the presence of cinnamic acid were examined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995). After growth of probiotic bacteria, cultures were centrifuged (3200xg, 15 min) and the supernatant was remover and filtered (0.22 µm syringe-filters). The cell-free supernatants (CFS) were stored at -80ºC until use. For DPPH scavenging assay, 25 mg/L DPPH was dissolved in methanol. CFS (100 µL) and DPPH (100 µL) were added to a 96-well plate, incubated (30 min, RT, in the dark) and the absorbance measured spectrophotometrically at 517 nm. Decreased absorbance, so the remaining amount of DPPH was determined as the amount of free radical scavenging, i.e. antioxidant activity. Relative antioxidant activities were calculated as percentage of re-duction in the absorbances to the absorbance of initial DPPH solution.

2.3. *In vitro* Cytotoxicity Assay for Caco-2 Cells

Human colon cancer cell line (Caco-2) was used to determine the anticancer properties of the capsanthin. Caco-2 cells were cultured in DMEM medium (Gibco, UK). This medium supplemented with 20% fetal bovine serum (Biowest, USA) and 1% penicillin/streptomycin solution (Gibco, UK). Cell plates were maintained in 75-cm^2 culture flasks (TPP) and were placed in 5% CO_2 humidified atmosphere at 37°C (Thermo Forma II CO2 incubator, USA).

The cytotoxicity of capsanthin was tested using 3-(4,5-dimethyl thiazol-2-il)-2,5-diphenyltetrazolium bromide (MTT) assays.

Caco-2 cells were seeded in 96-well microplate (15×10^3) and cultured for 24 h. Cells were treated with 5-2000 µM concentrations of capsanthin compound (DMSO for Vehicle control) at 24 h. After incubated, 50 µL of MTT (0.5 mg/mL) solution was added to the wells and incubated further for 3 h. Then medium was removed and 100 µL of DMSO was added to each well. A microplate reader was used for measurement of absorbance of each plate at 550 nm (Thermo MultiskanGo, USA). Absorbances from the control wells (only cells and culture medium) were measured and the mean absorbance values obtained were considered as 100% viable cells. Viability values in treated groups were calculated according to the control group. These experiments were repeated 5 times independently on different days (Kenan & Su, 2016) (Mosmann, 1983).

2.4. *In vitro* Genotoxicity Assay (Comet Analysis)

The "Comet Assay," also known as single-cell gel electrophoresis, is a widely used method for assessing DNA damage (genotoxicity) in mammals (Singh et al., 1988). In this study, the Alkaline Comet Assay technique, as described by Nandhakumar et al., was performed with minor modifications (Nandhakumar et al., 2011). Initially, microscope slides were coated with 1% high melting agarose (HMA) dissolved in phosphate buffer and allowed to dry in the dark for one day. Caco-2 cells cultured in 6-well plates were treated with the test compound at concentrations of 1000 and 2000 µM and incubated for 24 hours. After incubation, the cells were mixed with low melting agarose at a temperature of 40-42 °C and spread onto the agarose-coated slides, which were rapidly covered with coverslips. The prepared slides were then chilled at +4 °C in the dark for 10-15 minutes to allow the agarose to solidify. Subsequently, the slides were immersed in a freshly prepared working solution (composed of stock lysis solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, supplemented with 1% Triton X-100 and 10% DMSO) and incubated at +4 °C for 1 hour. Following the lysis process, the slides were placed in an electrophoresis tank (Bio-Rad, USA) in the same orientation, and electrophoresis was performed at a constant voltage of 25 V and current of 300 mA for 30 minutes. After electrophoresis, the slides were washed three times for 5 minutes each at +4 °C using neutralization buffer (0.4 M Tris, pH 7.5). Finally, the slides were stained with 50 µL of ethidium bromide (20 µg/mL), covered with coverslips, and photographed using a fluorescence microscope (Carl Zeiss / Scope A1, Germany). Randomly selected at least 25 cells from each slide and a minimum of 100 cells from each group were analyzed. Scoring was performed using the CometScore software (TriTek, Sumerduck, USA). Increased tail DNA (%) was considered as an indicator of DNA damage.

2.5. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.0.0 software package. Homogeneity of variance was evaluated by Shapiro-Wilk test. Kruskal-Wallis H test was used to determine differences between the two groups and Tukey's test was used for multiple comparisons. Quantitative data were expressed as the mean of standard deviation (mean±SD) and were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

In this study, some biological activities of capsanthin, a carotenoid in red peppers, have been determined. For this purpose, the cytotoxic and genotoxic effects on the human colon cancer cell line Caco-2, as well as the antibacterial properties of capsanthin, were investigated under *in vitro* conditions.

3.1. Antimicrobial Effects of Capsanthin

As a result of antimicrobial effect studies conducted using the micro-dilution method, concentrations of 125-1000 µM of capsanthin have led to a significant ($p<0.05$) decrease in the viability of both *E. coli* and *S. aureus* bacteria compared to the control (Figure 1). The IC50 value of capsanthin was found to be 1000 µM for both bacteria.

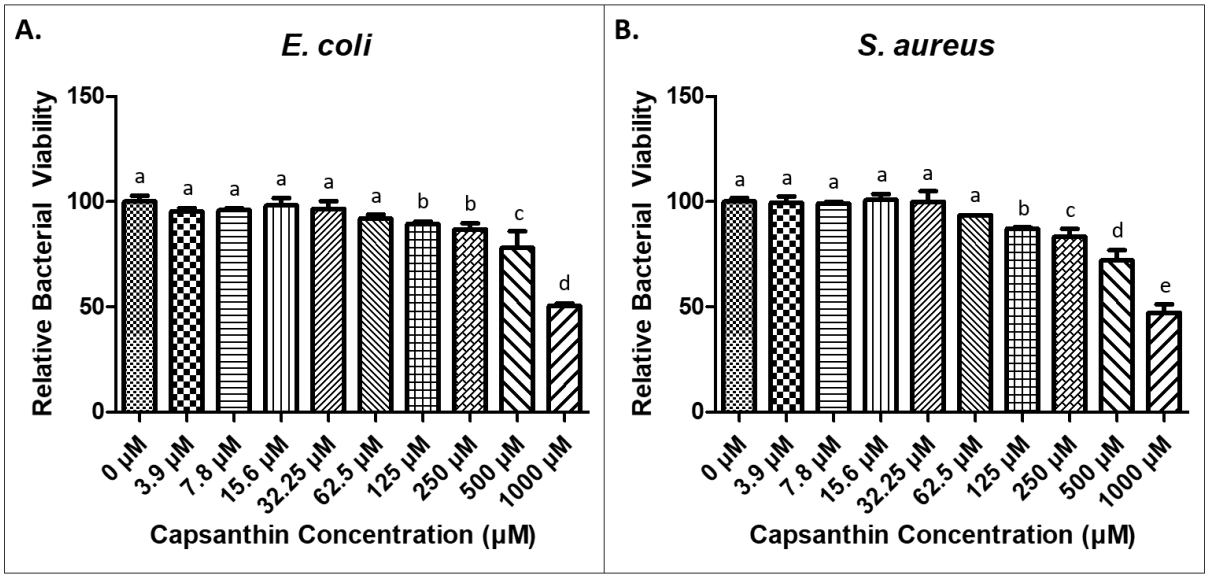

Figure 1: Antimicrobial effects of capsanthin on *E. coli* and *S. aureus.*

3.2. Antioxidant Capacity of Capsanthin

The antioxidant effects of carotenoids are well-known, and in this study, the antioxidant effect of capsanthin was determined through the determination of DPPH radical scavenging.

Accordingly, DPPH inhibition was observed at concentrations of 750 and 1000 µM of capsanthin (Figure 2).

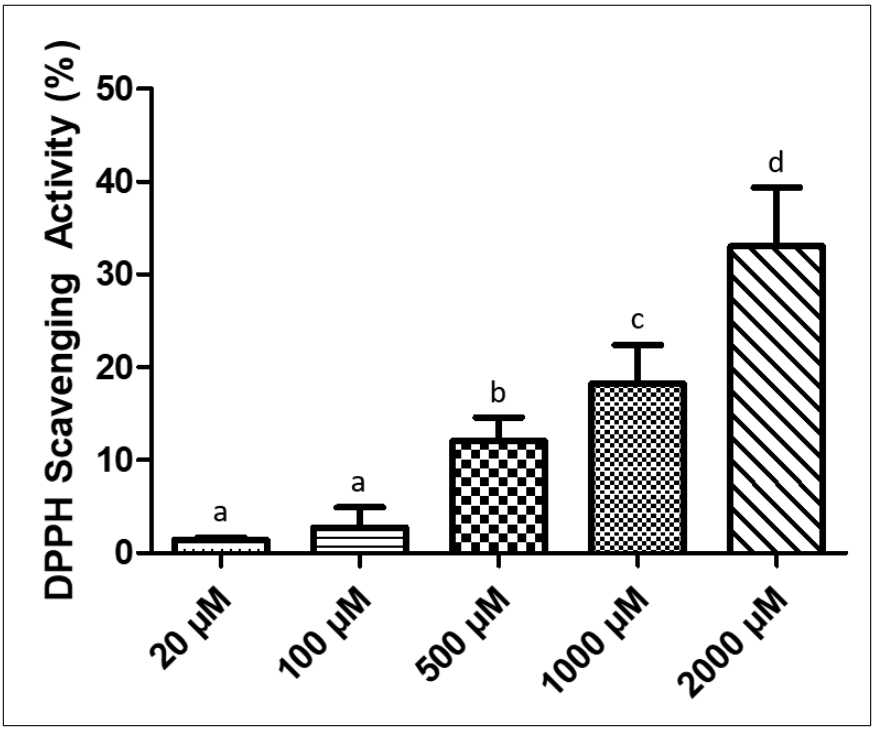

Figure 2: Antioxidant capacity of capsanthin.

3.3. Cytotoxic and Genotoxic Effects of Capsanthin for Caco-2 Cell Line

The cytotoxic effects of capsanthin were determined using the human colon cancer cell line Caco-2 through the MTT method. As a result of the conducted studies, it has been observed that capsanthin exerts cytotoxic effects on Caco-2 cells when tested at different concentrations (Figure 3). Particularly, the concentrations of 1000, 1500, and 200 µM of capsanthin have significantly ($p<0.05$) reduced the viability of Caco-2 cells. The $LogIC_{50}$ value for capsanthin was calculated as 3.35±0.04 µM. In previous studies, the cytotoxic effects of carotenoids on human colon cancer have been investigated, and many carotenoids have shown positive effects (Hsu et al., 2017)(Grudzinski et al., 2018)(Rowles & Erdman, 2020). In this study, capsanthin has also dose-dependently reduced the viability of colon cancer cells.

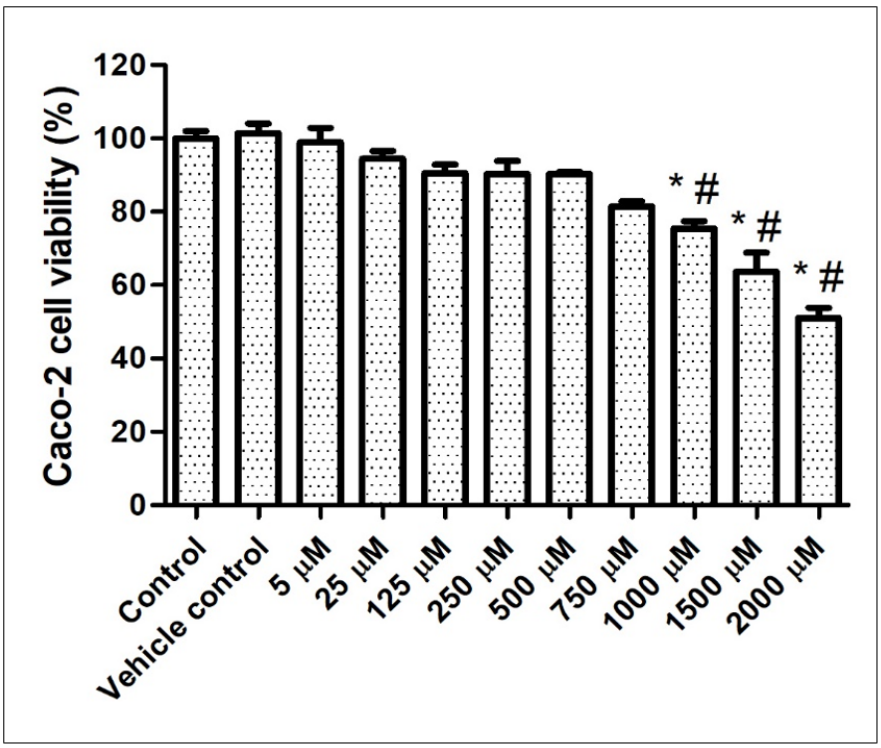

Figure 3: Cytotoxic effects of capsanthin on Caco-2 cells.

The DNA damage occurring in Caco-2 cells treated with the capsanthin compound for 24 hours is presented in Figure 4. Both applied doses of capsanthin have caused a significant increase in DNA damage compared to the control group ($p<0.05$). However, there was no statistical difference observed between the applied doses of the compound. These results indicate that capsanthin may contribute to cell death by causing damage to cellular DNA.

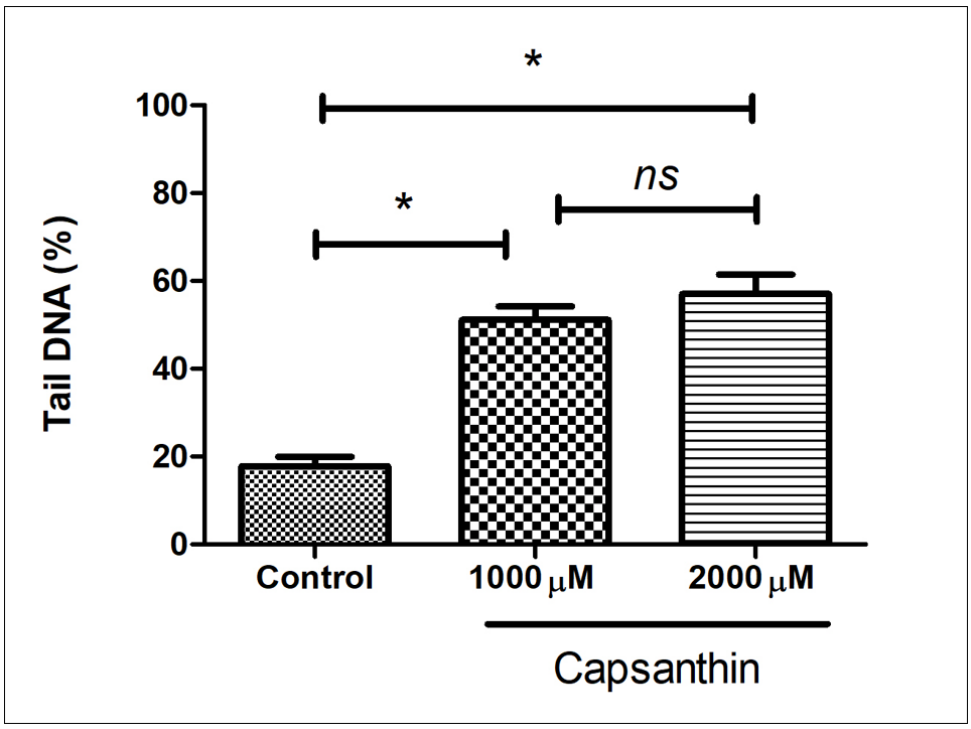

Figure 4: Genotoxic effects of capsanthin on Caco-2 cells.

In conclusion, this comprehensive investigation sheds light on the multifaceted biological activities of capsanthin, encompassing antimicrobial, antioxidant, cytotoxic, and genotoxic effects. These findings contribute valuable insights into the potential therapeutic applications of capsanthin, particularly in the context of cancer treatment and antimicrobial interventions. Further studies are warranted to elucidate the underlying mechanisms and to explore the translational implications of these observations.

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