Interdisciplinary Journal of Acute Care
Volume 3, January/June 2022, Issue 1, pp.11-15
Journal homepage: www.ijac.lums.ac.ir

Antioxidant Activity of Capsaicin on LS-180 Cell line

Parastoo Baharvand[1], Maryam Hormozi [2]

[1]Department of Social Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[2*] Razi Herbal Medicines Research Center, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

ABSTRACT

Cancer is one of the main causes of premature death in the world, and oxidative stress plays an important role in the pathogenesis of this disease. Since there is much evidence about the anticancer effect of antioxidants, this study aims to investigate the effect of capsaicin on the activity of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase in the LS-180 human colorectal cancer cell line.In this study, the human colorectal cancer cell line (LS-180) was treated with different concentrations of capsaicin for 24 hours, and then the activities of the antioxidant enzymes of superoxide dismutase, catalase, and glutathione peroxidase were determined by calorimetric methods. Analysis of the results showed that the activities of superoxide dismutase, catalase, and glutathione peroxidase enzymes increased in capsaicin-treated groups compared to the control group in the LS-180 cell line (p<0.05). Capsaicin inhibits cell proliferation by increasing the activity of antioxidant enzymes.

Keywords: Capsaicin; Antioxidant activity; LS-180 cell line

Introduction:

Cancer is one of the health problems of society, which, in addition to inflicting pain and mental pressure on the sufferers and their families, also has expensive treatments. Oxidative stress and the production of free radicals through different metabolic pathways play a key role in the initiation and progression of all types of cancer; Free radicals produced through different signaling pathways can affect many cellular processes, such as growth and proliferation, which are important in the initiation and progression of cancer. The cancer process plays an important role. Reducing the power of the body's antioxidant defense system is one of the main factors in the spread and development of cancer. The antioxidant defense system in the body is provided by enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase and non-enzymatic antioxidants such as vitamins E, C, and A, flavonoids, and chelating agents. Metal ions and polyphenol compounds weaken free radical reactions [1-3]. Colorectal cancer is one of the most common cancers in the world, and based on various studies, it seems that oxidative stress may increase the risk of developing colorectal cancer [4].

Capsaicin is a phenolic compound responsible for red pepper's spicy taste. It has antioxidant, antimicrobial, and anticancer properties. This compound is used in weight loss, diabetes control, and metabolic syndrome treatment. It reduces the progression of leukemia and stomach, prostate, and liver cancer [5]. Capsaicin also prevents the activity of carcinogenic compounds in the body and prevents the formation of malignant tumors [6].

Various studies have shown the effect of capsaicin on inducing apoptosis in different cancer cells, such as pancreatic cells [7], colon [8], liver [9], skin [10], leukemia [11], lung [12], and endothelial cells [13]. However, it does not have a cytotoxic effect on normal cells [14]. Various mechanisms have been proposed for

* *Corresponding* author: Maryam Hormozi
Razi Herbal Medicines Research Center, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran E-mail: maryamhormozi@yahoo.com
DOI: 10.22087/ijac.2022.470227.1017

the antitumor effect of capsaicin, such as disruption of mitochondrial membrane electron flow, induction of apoptosis through oxidative stress, and effect on signaling pathways related to cell proliferation. [7, 11, and 15], but the exact mechanism is unknown. This study aimed to investigate the effect of capsaicin on the activity of antioxidant enzymes in the LS-180 colorectal cancer cell line.

Materials and Methods

Material

Capsaicin (M2028), and phosphate buffer saline (PBS) (SLBJ2117V) tablets were achieved from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide (H2O2) (107209), pyrogallol, Tris-HCl buffer, and Tris-EDTA buffer were obtained from Merck (Merck, Germany). Dulbecco's Modified Eagle's Medium (DMEM) (1791923) high glucose media, Trypsin/EDTA 0.25 % (1726653), and Fetal Bovine Serum (FBS) (42Q7363K) were obtained from Gibco (Maryland, USA).

Cell culture and treatment

This study obtained the human colorectal cancer cell line LS-180 from the Institute Pasteur Cell Bank in Tehran. They were cultured in a DMEM culture medium containing 10% FBS, 100 IU/ml penicillin, and 100 µg/ml streptomycin in an incubator with a temperature of 37°C, 5% CO2, and appropriate humidity. After treating the cells with concentrations of 50, 100, and 200 µM capsaicin for 24 hours, the cells were collected by trypsinization.

Measuring the protein content of cells:

To check the activity of antioxidant enzymes, after cells were treated and collected, their protein content was extracted using lysing buffer, and the total protein content of each sample was measured using the Bradford method (16).

Measurement of cellular antioxidant enzyme activity

To measure catalase activity, 50 µl of the sample was added to 1000 µl of potassium phosphate buffer (50 mM), and then 50 µl of H2O2, and the sample's absorption was read against the blank at 0, 30, and 60 seconds at 240 nm wavelength. The specific activity of the catalase enzyme was obtained in terms of unit/mg protein (17).

For superoxide dismutase activity measurement, 25 µl of the sample was added to 725 µl of Tris-EDTA buffer; after that, 80 µl of pyrogallol was added to it. The sample's absorption was read against the blank at 90 seconds and 210 seconds at 420 nm wavelength. The specific activity of the superoxide dismutase enzyme was obtained in terms of unit/mg protein (18).

To measure glutathione peroxidase activity, 200 µl of the sample was added to Tris-HCl buffer (0.4 M), sodium azide (1 mM), glutathione (2 mM), and hydrogen peroxide (0.2 mM). After that, 0.4 ml of TCA (10%) was added, the sample was centrifuged for 3 minutes at 2000 rpm, and the supernatant was separated. 25 µl supernatant was added to 140 µl of Tris-EDTA (0.2 M), and DTNB, and the sample's absorption was read against the blank at 420 nm wavelength. The specific activity of glutathione peroxidase activity was obtained in unit/mg protein (19).

Statistical analysis

SPSS software was used to analyze the data on the activity of antioxidant enzymes. Mean, standard deviation and frequency tables were used to describe the data, and one-way analysis of variance and Tukey's post hoc test were used to analyze the data.

Ethical Consideration

This study was approved by the ethics committee of Lorestan University of Medical Sciences, Khorramabad, Iran, with the ethics number IR.LUMS.REC.1399.389.

Results

The results show that the level of catalase enzyme activity in all concentrations treated with capsaicin showed a significant increase compared to the control group ($P < 0.05$) (Fig 1A). The activity of the superoxide dismutase enzyme in the groups treated with capsaicin increased significantly compared to the control group ($P < 0.05$) (Fig 1B). The activity of glutathione peroxidase showed a significant increase in the groups treated with concentrations of 100 and 200 µM capsaicin compared to the control group ($P < 0.05$) (Fig 1C).

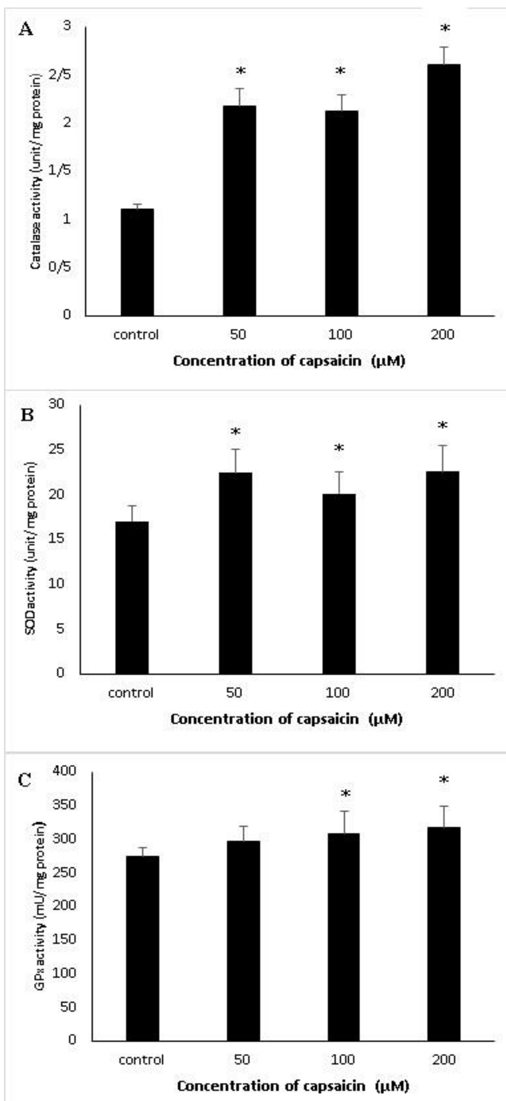

*Figure 1: Catalase (A), Enzymatic activity of SOD (B), and GPX (C) (mean ± S.D) in groups treated with 50, 100, and 200 µM capsaicin and control in LS-180 cell line. *p < 0.05.*

Discussion

Many researchers have focused on natural compounds and food products due to their effectiveness in treating diseases and side effects [12]. Capsaicin is a natural alkaloid, one of the main components of hot peppers. Various studies have shown that capsaicin induces apoptosis in various cancer cells, such as gastritis, colon, prostate, etc., without cytotoxic effects on normal cells [7-14].

This research showed that capsaicin can increase the activity of the antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) in the LS-180 cell line.

Various studies have shown the effect of capsaicin on the production of reactive oxygen species [12, 13]. It seems that the production of reactive oxygen species by capsaicin leads to an increase in the expression of Nrf2 and then PPARγ. Studies have shown a mutual relationship between the expression of these two factors, and increasing the expression of PPARγ can lead to increasing the expression of Nrf2 [20]. Increasing the expression of these factors plays an important role in increasing the activity of antioxidant enzymes [20]. Also, increasing the expression of PPARγ can lead to the induction of the expression of genes such as Bax, caspase 3, and P53 [20].

On the other hand, reactive oxygen species also play a role in inducing the expression of P53. Although P53 is a tumor suppressor and plays an important role in inhibiting carcinogenesis, it has been found that this factor also reduces mitochondrial respiration, increases the production of reactive oxygen species, and causing oxidative stress. This factor also increases the expression and activity of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase [21]. Also, this factor plays a role in increasing the expression of effective apoptosis genes, such as Bax and caspase 3 [22].

Different research has shown that capsaicin plays an important role in inducing cell apoptosis by causing oxidative stress [13]. It seems that capsaicin, through competition with coenzyme Q, inhibits complexes I and III of the mitochondrial electron transport chain, disrupts the electron flow of the mitochondrial membrane, produces reactive oxygen species, and creates a pro-oxidant environment in the plasma membrane [23].

Also, various studies have shown that oxidative stress affects many signaling pathways related to cell proliferation, among which we can mention the epidermal growth factor receptor (EGFR) signaling pathway, in which proteins such as nuclear factor-erythroid-related factor (Nrf2) and Raf are involved. In addition, the methanogen-activating protein kinases (MAPKs), phosphatidyl-inositol 3-kinase (PI3K), phospholipase C, and protein kinase C are affected by oxidative stress. Also, reactive oxygen species can suppress the P53 gene involved in programmed death [24].

It has been shown that capsaicin in HepG2 cells activates AMPK through binding to the TRPV1 receptor, increases cytosolic calcium, and activates calcium/calmodulin-dependent protein kinase β (Ca MKKβ), AMPK activation also occurs through the production of reactive oxygen species, and inhibition of Akt/mTOR decreases cell survival and induces apoptosis [12]. Also, capsaicin can bind to TRPV6; this action may stimulate apoptosis through increasing cytosolic calcium and stimulating the apoptotic regulatory factor calpains and calcium-dependent cysteine proteases in small-cell lung cancer [22]. These changes by capsaicin may effectively inhibit growth and induce apoptosis in cancer cells.

Conclusion

According to the results of this study, it seems that the oxidative stress caused by capsaicin can lead to an increase in the activity of antioxidant enzymes, which may be effective in inducing apoptosis and inhibiting colorectal cancer cells.

Conflict of Interests

Authors declare that they do not have any conflict interests.

Acknowledgment

This work was supported by the Lorestan University of Medical Sciences, Khoramabad, Iran [grant number 1397-1-99-1756].

References

1. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative stress in cancer. Cancer cell. 2020 Aug 10;38(2):167-97.

2. Jelic MD, Mandic AD, Maricic SM, Srdjenovic BU. Oxidative stress and its role in cancer. Journal of cancer research and therapeutics. 2021 Jan 1;17(1):22-8.

٣. Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J, Roychoudhury S, Rathi B, Kumar D. Oxidative stress in cancer cell metabolism. Antioxidants. 2021 Apr 22;10(5):642.

4. Bardelčíková A, Šoltys J, Mojžiš J. Oxidative stress, inflammation and colorectal cancer: an overview. Antioxidants. 2023 Apr 9;12(4):901.

5. Adaszek Ł, Gadomska D, Mazurek Ł, Łyp P, Madany J, Winiarczyk S. Properties of capsaicin and its utility in veterinary and human medicine. Research in Veterinary Science. 2019 Apr 1;123:14-9.

6. Clark R, Lee SH. Anticancer properties of capsaicin against human cancer. Anticancer research. 2016 Mar 1;36(3):837-43.

7. Pramanik KC, Boreddy SR, Srivastava SK. Role of mitochondrial electron transport chain complexes in capsaicin mediated oxidative stress leading to apoptosis in pancreatic cancer cells. PloS one. 2011 May 25;6(5):e20151.

8. Kim YM, HWANG JT, Kwak DW, Lee YK, Park OJ. Involvement of AMPK signaling cascade in capsaicin-induced apoptosis of HT-29 colon cancer cells. Annals of the New York Academy of Sciences. 2007 Jan;1095(1):496-503.

9. Lee YS, Kang YS, Lee JS, Nicolova S, Kim JA. Involvement of NADPH oxidase-mediated generation of reactive oxygen species in the apototic cell death by capsaicin in HepG2 human hepatoma cells. Free radical research. 2004 Apr 1;38(4):405-12.

10. Hail Jr N, Lotan R. Examining the role of mitochondrial respiration in vanilloid-induced apoptosis. Journal of the National Cancer Institute. 2002 Sep 4;94(17):1281-92.

11. Ito K, Nakazato T, Yamato K, Miyakawa Y, Yamada T, Hozumi N, Segawa K, Ikeda Y, Kizaki M. Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. Cancer research. 2004 Feb 1;64(3):1071-8.

12. Athanasiou A, Smith PA, Vakilpour S, Kumaran NM, Turner AE, Bagiokou D, Layfield R, Ray DE, Westwell AD, Alexander SP, Kendall DA. Vanilloid receptor agonists and antagonists are mitochondrial inhibitors: how vanilloids cause non-vanilloid receptor mediated cell death. Biochemical and biophysical research communications. 2007 Mar 2;354(1):50-5.

13. Min JK, Han KY, Kim EC, Kim YM, Lee SW, Kim OH, Kim KW, Gho YS, Kwon YG. Capsaicin inhibits in vitro and in vivo angiogenesis. Cancer research. 2004 Jan 15;64(2):644-51.

14. Mori A, Lehmann S, O'Kelly J, Kumagai T, Desmond JC, Pervan M, McBride WH, Kizaki M, Koeffler HP. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Cancer research. 2006 Mar 15;66(6):3222-9.

١٥. Bley K, Boorman G, Mohammad B, McKenzie D, Babbar S. A comprehensive review of the carcinogenic and anticarcinogenic potential of capsaicin. Toxicologic pathology. 2012 Aug;40(6):847-73.

16. Noble JE, Bailey MJ. Quantitation of protein. Methods in enzymology. 2009 Jan 1;463:73-95.

17. Hadwan MH. Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC biochemistry. 2018 Dec;19:1-8.

18. Peskin AV, Winterbourn CC. Assay of superoxide dismutase activity in a plate assay using WST-1. Free Radical Biology and Medicine. 2017 Feb 1;103:188-91.

19. Vives-Bauza C, Starkov A, Garcia-Arumi E. Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase. Methods in cell biology. 2007 Jan 1;80:379-93.

20. Lee C. Collaborative power of Nrf2 and PPARγ activators against metabolic and drug-induced oxidative injury. Oxidative medicine and cellular longevity. 2017;2017(1):1378175.

21. Chikara S, Nagaprashantha LD, Singhal J, Horne D, Awasthi S, Singhal SS. Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. Cancer letters. 2018 Jan 28;413:122-34.

22. Mohamad N, Gutiérrez A, Núñez M, Cocca C, Martín G, Cricco G, Medina V, Rivera E, Bergoc R. Mitochondrial apoptotic pathways. Biocell. 2005 Aug;29(2):149-61.

23. Jaganathan R, Purushotham B, Radhakrishnan N, Swamy MK. Capsaicin and its potential anticancer mechanisms of action. Plant-derived Bioactives: Chemistry and Mode of Action. 2020:301-21.

24. Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression?. Cell death & differentiation. 2018 Jan;25(1):104-13.