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Investigating the Effect of Capsaicin on The Indicators of Oxidative Stress and Apoptosis Caused by Hydrogen Peroxide in Human Neuroblastoma BE(2)-C Cell Line

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Abstract

Background: Capsaicin exhibits free radical scavenging activity. In the present study, we evaluated the impact of capsaicin on the expression level of apoptosis-related genes (*CASP3*, *BCL2*, *BAX*, *TP53*), the transcription factor NRF2 (which regulates the antioxidant response), and the level and activity of antioxidant enzymes (SOD, CAT, GPx) in the human cell line of neuroblastoma BE(2)-C.

Methods: BE(2)-C neuroblastoma cells were administered with capsaicin at the amounts of 25, 50, and 75 µM, or with 50 µM ascorbic acid, for 24 hours. Subsequently, the cells were exposed to oxidative stress by treatment with hydrogen peroxide for two hours. A gene expression study was performed by real-time PCR, and antioxidant enzyme activity was evaluated by colorimetric methods.

Results: Gene expression analysis indicated that capsaicin significantly decreased the level of pro-apoptotic genes *CASP3* and *BAX*, while *TP53* expression remained unchanged. Capsaicin also upregulated NRF2 and *PPAR* gene expression ($P<0.05$). Notably, vitamin C reduced Caspase-3 and Bax levels but did not significantly affect Bcl-2 or p53. *SOD* gene expression was reduced in the capsaicin-treated groups, whereas antioxidant enzyme activity, including SOD, CAT, and GPx, significantly increased in all treatment groups. Malondialdehyde (MDA) levels, a marker of oxidative stress, were significantly lower in capsaicin-treated groups than in the H2O2 group ($P<0.05$).

Conclusion: Capsaicin modulated the expression of apoptosis-related genes and enhanced antioxidant defense mechanisms. While it inhibited key apoptotic markers, it had no significant effect on *TP53* expression but increased *PPAR* expression, indicating a shift toward a regulated antioxidant and antiapoptotic cellular state.

Keywords: Antioxidants, Gene expression, Cell culture, Herbal medicines, Capsaicin, Apoptosis

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Introduction

Oxidative stress modulates cell function, especially in astrocytes and microglia (1). It can cause necrosis or apoptosis (2). Excess free radicals damage nucleotides, proteins, and lipids. This leads to neurological complications (3). Free radicals activate glial cells and receptors. They also activate caspases and Bcl-2 family proteins (4). In addition, free radicals cause mitochondrial and p53 dysfunction (5). NRF2 is a transcription factor that regulates antioxidant responses and can protect neurons (6). The phosphorylated form of NRF2 regulates the expression of protective genes such as superoxide dismutase. NRF2 levels decrease with oxidative stress (7).

Hydrogen peroxide (H2O2) is destructive to cells. H2O2 produces reactive oxygen species (ROS) in the brain, which can lead to ischemia. ROS production involves lipid peroxidation, protein modification, and activation of signaling pathways. These processes induce oxidative stress and cell death. H2O2 in the nerve culture medium decreases gene expression and cell proliferation (8). The biological system has endogenous defense mechanisms against free radicals. Glutathione peroxidase, catalase, and superoxide dismutase are antioxidant enzymes. These enzymes neutralize oxidative intermediates (9). Exogenous antioxidants, such as vitamin C, are obtained through dietary intake. Vitamin C is an antioxidant found in body fluids, such as the lungs and retina. It neutralizes free radicals in aqueous environments, often with the help of vitamin E. Vitamin C also indirectly impedes lipid peroxidation. Ascorbate preserves vitamins A and E and fatty acids in the body (10). Capsaicin is the main pungent compound in red pepper, which gives it its spicy

taste (11), and is mainly used to control oxidative stress. This substance is effective across a variety of physiological processes, including inhibition of the JAK/STAT pathway, calcium ion influx, and the JNK–ROS–CHOP pathway leading to apoptosis (12, 13).

In a study conducted on the cell lines of HepG2 and SK–N–SH, reactive oxygen species (ROS) levels increased during HepG2 apoptosis. At the same time, SK–N–SH apoptosis was reduced following capsaicin treatment. In both cell lines, apoptosis decreased, particularly in the neuronal cell line. Most antioxidant enzymes increase in response to capsaicin in SK–N–SH cells (14). A study on phospholipid hydroperoxide glutathione peroxidase (PHGPx) in spermatogenic cells shows that capsaicin protects spermatogenic cells through antioxidant and antiapoptotic activities (15).

Capsaicin has dual roles in apoptosis and in modulating oxidative stress in other cancers. However, its effects on apoptosis-related gene expression and antioxidant enzyme activity in H2O2-treated BE(2)-C cells remain poorly understood. It is unclear whether pro-apoptotic or antioxidant mechanisms are dominant in this cell line.

Despite extensive research on the antioxidant and apoptotic effects of capsaicin in various cell types, significant gaps remain in fully elucidating its comprehensive molecular mechanisms. Most existing studies have primarily concentrated on measuring changes in the expression of a limited panel of apoptosis-related genes such as *BCL2*, *BAX*, and *CASP3*. However, these studies often do not examine whether these transcriptional changes lead to corresponding alterations at the protein level or result in functional cellular effects, such as activation of apoptotic enzymes, DNA fragmentation, or mitochondrial dysfunction. The lack of assessment of downstream molecular events limits understanding of how gene expression changes translate into actual cell-fate decisions. Furthermore, the majority of experiments have been conducted in a single cell line or a narrow range of model systems, which limits the generalizability of findings and the ability to determine whether the observed mechanisms are consistent across different tissues, disease models, or physiological conditions. This limitation also overlooks potential cell-type-specific responses to capsaicin treatment. Together, these gaps hinder a complete and integrative understanding of capsaicin's dual roles in modulating apoptosis and oxidative stress pathways and prevent clear delineation of its therapeutic potential or toxic effects across diverse biological contexts.

Despite the studies conducted, there has not been much research on the impact of capsaicin on the expression level of genes effective in programmed cell death and the activity of antioxidant enzymes in the BE(2)-C human neuroblastoma cell line treated with H2O2, so the objective of the present study was to investigate the effect of these substances.

Methods

Study Design

An experimental in vitro study was performed on a human cell line. This study consisted of an analysis of gene expression, including apoptosis- and antioxidant-related genes, and measurement of antioxidant enzyme activity. The genes related to apoptosis were *BAX*, *BCL2*, *CASP3* and *TP53*. The antioxidant-related genes were catalase (*CAT*), superoxide dismutase (*SOD1*), and glutathione peroxidase (*GPX1*). Also, the level of malondialdehyde (MDA) was studied. The protocol of this study was approved by the Ethics Committee of Lorestan University of Medical Sciences under the registration number IR.LUMS.REC.1398.210.

Groups and Treatments

The study was conducted on the human neuroblastoma cell line BE(2)-C, obtained from Pasteur Institute (Tehran, Iran). After cell preparation and passage, the cells were divided into six groups. The first group: control group (no treatment); the second group: co-treated with hydrogen peroxide at a concentration of 400 μM for 2 hours; the third group: treated with vitamin C (Vit. C) at the amount of 50 μM as a positive control before exposure to hydrogen peroxide at 400 μM as a co-treatment. The fourth, fifth, and sixth cells were respectively exposed to 25, 50, and 75 μM capsaicin before being exposed to 400 μM H2O2 as a co-treatment. The cells were exposed to capsaicin or antioxidants for 24 hours. The cells were then collected to assess the factors under study.

Gene Expression Study

Total RNA extraction was performed using a column-based RNA extraction kit (Jena Bioscience, Germany). RNA quality was evaluated by agarose gel electrophoresis (28S and 18S rRNA bands) and quantified using a NanoDrop spectrophotometer. cDNA synthesis was performed using random hexamer primers and a commercial kit (Jena Bioscience, Germany). Gene expression analysis was performed with real-time RT-PCR using SYBR Green Master Mix (Jena Bioscience, Germany). The reference gene was *GAPDH*. Primer sequences were obtained from published sources (Table 1).

Enzyme Activity Study

Total protein assay: For this aim, the Bradford method was used to measure the total protein content of cell lysates, and bovine serum albumin was used to construct a standard curve via serial dilutions.

MDA assay: MDA was measured by a spectrophotometric method based on its reaction with thiobarbituric acid. The absorbance of the resulting complex was read at 532 nm. The outputs were reported as nmol MDA/mg protein (16).

CAT activity assay: CAT activity was evaluated spectrophotometrically by measuring the enzyme-

mediated reaction of methanol in the presence of hydrogen peroxide. The absorbance change was monitored, and activity was reported as units/mg protein (17).

SOD activity assay: SOD activity was determined by spectrophotometry, measuring the enzyme's ability to inhibit the pyrogallol autoxidation reaction. The results were expressed as units/mg protein (18).

How to measure GPx activity: GPx activity was measured spectrophotometrically by monitoring the enzyme-catalyzed reaction and calculating the production rate per mg protein. Results were reported as units/mg protein (19).

MTT Assay

After cell culture, the MTT assay was used to determine the optimal dose and incubation time. 10^4 cells/well were seeded in 96-well plates. After 24 hours, treatments were added and incubated for 24, 48, or 72 hours. 20 µL MTT solution (5 mg/mL) was added, incubated for 5 hours in the dark, then the medium was removed and 200 µL DMSO was added to dissolve the formazan crystals. After 15 minutes, absorbance was measured at 570 nm (reference 690 nm) using an ELISA reader.

Statistical Analysis

The REST 2009 program was used for the gene expression study. It calculates relative expression (RE) by comparing the study groups to the control group. The enzyme levels and MTT assay results were compared using one-way analysis of variance (ANOVA) and Tukey's post hoc test in SPSS version 24 (IBM Corp., NY, US). Normality was assessed using the Kolmogorov–Smirnov test.

Table 1. The primer sequences of the study

Gene	Primer	Sequence (5' to 3')	Size
BAX	Forward	TGGCAGCTGACATGTTTTCTGAC	195
	Reverse	TCACCCAACCACCCTGGTCTT	
BCL2	Forward	TCGCCCTGTGGATGACTGA	134
	Reverse	CAGAGACAGCCAGGAGAAATCA	
CASP3	Forward	TACCTGTGGCTGTGTATCCG	134
	Reverse	TCAGTGTTCTCCATGGATACCT	
NRF2	Forward	ACGGTCCACAGCTCATCAT	147
	Reverse	TCCGTCGCTGACTGAAGT	
TP53	Forward	GGACACTTTGCGTTCGGG	118
	Reverse	CTAGGATCTGACTGCGGCTC	
GAPDH	Forward	CTCTCTGCTCCTCCTGTTCG	108
	Reverse	ACGACCAAATCCGTTGACTC	
SOD1	Forward	ACGGTGGGCCAAAGGATGAA	151
	Reverse	TCATGGACCACCAGTGTGCG	
CAT	Forward	CGTGCTGAATGAGGAACAGA	119
	Reverse	AGTCAGGGTGGACCTCAGTG	
GPX1	Forward	TCGGTGTATGCCTTCTCGGC	150
	Reverse	CCGCTGCAGCTCGTTCATCT	

Results

Primary Findings and Viability Test

According to the results obtained using the MTT method, the 25, 50, and 75 µM capsaicin doses and 24 hours were the most suitable treatment options (Figure 1). After treatment and cell collection, RNA extraction was performed, and the products were analyzed on a 1.5% agarose gel. Bands 28s rRNA and 18s rRNA were visible in the extracted RNA samples.

The expression levels of the *NRF2*, *BAX*, *BCL2*, *CAS3*, *CAT*, *GPX1*, and *SOD1* genes in the BE(2)-C human neuroblastoma cell line were investigated using real-time PCR. Agarose gel electrophoresis of real-time PCR products showed that the expected products were obtained during amplification of the target genes. In addition to electrophoresis of the products, the melting curves were examined after 40 cycles, indicating the absence of specific product or primer dimer formation.

Gene Expression Results

Examining the expression level of the *BAX* gene across different treatment groups relative to the H2O2 group showed that its expression was significantly down-regulated in all treatment groups (Table 2).

The level of *BCL2* gene expression in capsaicin-treated groups showed significant downregulation compared with the H2O2 group, although its expression in the Vit. C-treated group did not change significantly (Table 2).

Examining CASP3 expression across different treatment groups relative to the H2O2 group showed that it was significantly down-regulated in all treatment groups (Table 2).

Examining *NRF2* gene expression across treatment groups relative to the H2O2 group showed that its expression was significantly upregulated in all treatment groups (Table 2).

Examining CAT gene expression across different treatment groups relative to the H2O2 group showed that its expression was significantly upregulated in all

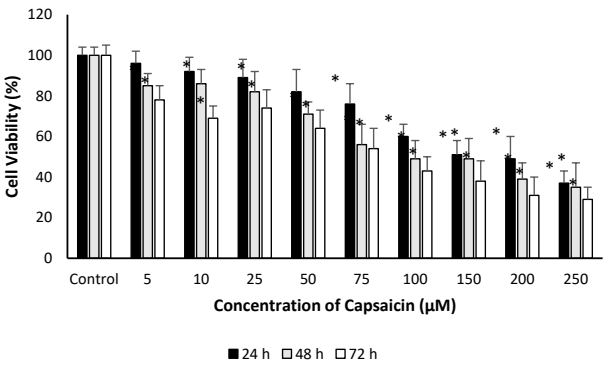

Figure 1. Cell survival rate (percentage) of BE(2)C human neuroblastoma cell line exposed to capsaicin treatment in 24 h, 48 h, and 72 h time periods by the MTT method. *: Significant at $P<0.05$ compared with the control

treatment groups (Table 2).

Examining GPX1 expression across different treatment groups relative to the H2O2 group showed that it was significantly upregulated in all treatment groups (Table 2).

Investigations showed that *SOD1* gene expression in the capsaicin-treated groups was significantly down-regulated compared with the H2O2 group, but it was upregulated in the Vit. C group (Table 2).

Enzyme Activity Study

The antioxidant role of capsaicin and the effect of H2O2-induced oxidative stress on neuronal death were studied. The activities of catalase, glutathione peroxidase, and superoxide dismutase were measured in all groups. Tukey's post hoc test showed that catalase activity increased significantly in all capsaicin-treated groups compared to the H2O2 group. The highest increase in catalase activity was observed in the 75 µM capsaicin group (Table 3).

The results of our study showed that the MDA index in the treated groups decreased significantly compared with the H2O2 group. It was also observed that MDA levels increased significantly in the H2O2 group. However, no statistically significant difference was observed between the treated groups (Table 3).

The results of our study showed that superoxide dismutase activity increased significantly in the treated groups compared with the H2O2 group (Table 3). Regarding glutathione peroxidase, all treatment doses showed a statistically significant difference compared with the H2O2 group (Table 3).

Discussion

In this study, the effects of capsaicin on oxidative stress markers and apoptosis-related gene expression in the BE(2)-C human neuroblastoma cell line were investigated under hydrogen peroxide-induced oxidative stress. The results demonstrate that capsaicin, particularly at 25 µM, significantly decreased *CASP3* expression and increased *NRF2* and *PPAR* gene expression. Furthermore, capsaicin enhanced the activity of antioxidant enzymes SOD, CAT, and GPx, and reduced MDA levels, indicating a protective antioxidant effect.

The intracellular process of apoptosis begins after DNA damage due to factors such as oxidative stress. Following DNA damage, the transcription of the *TP53* gene increases, which in turn upregulates the transcription of the *BAX* gene. This cascade leads to the release of cytochrome C from the apoptosis-inducing factor 1 (APAF1) and caspases from the active mitochondrial pathway (20). In the present study, *BAX* gene expression in the treated groups showed a statistically significant decrease compared with the control group in cells under H2O2 stress, indicating reduced activation of the apoptotic pathway.

Many intracellular proteins support the cell against apoptosis. Bcl-2 inhibits cytochrome C release from mitochondria. Bcl-2 also binds to sites that generate free radicals, including the mitochondria, the endoplasmic reticulum, and nuclear membranes. However, Bcl-2

Table 2. The results of gene expression for the groups of study reporting relative expression

Group	*BAX* RE, SE (P)	*BCL2* RE, SE (P)	*CASP3* RE, SE (P)	*NRF2* RE, SE (P)	*SOD1* RE, SE (P)	*GPX1* RE, SE (P)	*CAT* RE, SE (P)	*TP53* RE, SE (P)	*PPAR* RE, SE (P)	*BAX/BCL2* ratio
H_2O_2 (reference)	1	1	1	1	1	1	1	1	1	1
Control	0.44, 0.06 (0.02*)	1.11, 0.16 (0.74)	0.15, 0.03 (<0.01*)	2.55, 0.54 (0.03*)	1.84, 0.24 (0.03*)	3.53, 0.51 (0.03*)	4.73, 1.37 (0.03*)	2.51, 0.52 (<0.01*)	0.95, 0.15 (0.77)	0.40
Vit. C	0.44, 0.06 (0.03*)	1.18, 0.17 (0.55)	0.15, 0.03 (0.02*)	3.10, 0.71 (0.02*)	1.93, 0.25 (<0.01*)	3.63, 0.49 (0.01*)	4.78, 1.70 (<0.01*)	0.23, 0.04 (0.01*)	1.26, 0.17 (0.01*)	0.37
CAP 25µM + H_2O_2 400 µM	0.47, 0.05 (0.02*)	0.38, 0.07 (0.01*)	0.07, 0.02 (<0.01*)	14.21, 2.90 (0.03*)	0.25, 0.04 (0.01*)	3.51, 0.51 (<0.01*)	12.49, 5.45 (0.02*)	0.82, 0.07 (0.37)	2.09, 0.41 (0.02*)	1.24
CAP 50 µM + H_2O_2 400µM	0.40, 0.06 (0.01*)	0.30, 0.06 (0.01*)	0.16, 0.04 (<0.01*)	13.50, 2.42 (0.03*)	0.44, 0.06 (0.02*)	1.54, 0.22 (0.02*)	4.45, 0.96 (<0.01*)	0.68, 0.07 (0.19)	1.19, 0.21 (0.27)	1.33
CAP 75 µM + H_2O_2 400 µM	0.35, 0.09 (0.03*)	0.29, 0.09 (0.02*)	0.10, 0.03 (0.02*)	12.25, 4.22 (0.03*)	0.58, 0.12 (0.04*)	2.64, 0.70 (0.01*)	10.63, 3.39 (<0.01*)	2.03, 0.65 (0.01*)	1.95, 0.42 (0.01*)	1.21

* Significant at $P<0.05$ according to *t*-test compared with the H2O2 group (REST 2009) normalized with GAPDH

Table 3. Enzyme activity in groups treated with capsaicin, ascorbic acid, hydrogen peroxide and the control group

Group	CAT (unit/mg protein)	MDA (nMol/mg protein)	GPx (unit/mg protein)	SOD (unit/mg protein)
H_2O_2	1.95 ± 0.08	0.83 ± 0.01	49.97 ± 4.61	2.70 ± 0.46
Control	3.77 ± 0.09*	0.75 ± 0.01*	87.83 ± 1.59*	7.82 ± 0.10*
Vit. C	5.94 ± 0.06*	0.6 ± 0.01*	92.00 ± 3.38*	10.50 ± 0.70*
CAP 25 µM + H_2O_2 400 µM	4.54 ± 0.06*	0.64 ± 0.01*	88.03 ± 3.05*	12.73 ± 0.21*
CAP 50 µM + H_2O_2 400 µM	6.94 ± 0.06*	0.6 ± 0.01*	97.33 ± 3.62*	13.90 ± 0.26*
CAP 75 µM + H_2O_2 400 µM	8.47 ± 0.06*	0.6 ± 0.01*	101.70 ± 4.22*	13.87 ± 0.21*

The results are reported as mean ± SD. *: $P<0.05$ (Tukey's test, compared with H2O2 group).

does not destroy antioxidants such as acetylcysteine and glutathione peroxidase. Instead, Bcl-2 may affect the cellular environment in which these antioxidants function. It protects the cell from reactive oxygen species, especially H2O2 (21). Nevertheless, this study showed down-regulation of Bcl-2 in the treatment groups.

The results of our study showed that *CASP3* expression at 25 μM capsaicin was significantly lower than in the H2O2 group. Regarding apoptosis-related genes, the antiapoptotic effect of capsaicin was mediated by down-regulation of *BAX* and *CASP3*.

Based on the data presented in Table 2, capsaicin significantly upregulated *PPAR* expression. It downregulated or had no significant effect on *TP53* gene expression in BE(2)-C neuroblastoma cells under oxidative stress conditions. The upregulation of *PPAR*, a key transcription factor involved in lipid metabolism and cellular differentiation, suggests that capsaicin may promote neuroblastoma cell differentiation and exert anti-proliferative effects in a protective, non-toxic manner. Conversely, the absence of a significant increase in *TP53* expression, a major pro-apoptotic gene activated by DNA damage, indicates that capsaicin might help maintain cellular homeostasis by preventing excessive activation of apoptotic pathways. Together, these findings highlight capsaicin's dual role in enhancing antioxidant defense and modulating gene expression to reduce apoptosis under oxidative stress exposure.

Other studies have shown conflicting results regarding the apoptotic and antiapoptotic activity of capsaicin. For example, in PHGPx spermatogenic cells, capsaicin showed a protective effect against spermatogenic cell death through its antioxidant and antiapoptotic activities. Bcl-2 significantly increased, and Bax decreased (15). However, the opposite action of capsaicin results in the release of mitochondrial cytochrome C, the activation of Caspase-3, and the cleavage of poly (ADP-ribose) polymerase in a dose-dependent manner. In addition, the expression of *BCL2* in cells treated with capsaicin was slightly decreased. On the other hand, there was no change in the Bax levels in cells treated with capsaicin (22). This function of capsaicin is related to its anticancer activity, and the heterogeneous results are due to its toxicity.

Ascorbic acid, as an effective substance in cell regeneration, did not significantly change *BCL2* gene expression. However, under vitamin C treatment, *BAX* and *CASP3* gene expressions were reduced, and *NRF2* expression also increased in the ascorbic acid-treated group. However, as pointed out, the effect of vitamin C on genes involved in apoptosis remains controversial. On the one hand, it did not alter BCL2 expression, an antiapoptotic gene; on the other hand, it reduced BAX expression, an apoptotic gene. The effect of vitamin C on apoptosis appears to be context-dependent. In the study by Nematollahi et al, vitamin C increased the Bax/Bcl-2 ratio (23).

To investigate the antioxidant role of capsaicin and the effect of oxidative stress on H2O2-induced neuronal death, we measured the activities of antioxidant enzymes CAT, GPx, and SOD in all experimental groups. The relative expression of the catalase gene increased at all capsaicin concentrations. At 25 μM capsaicin, *GPX1* gene expression increased. Glutathione peroxidase activity increased at all concentrations compared to the H2O2 group. At 25 and 75 μM, *SOD1* gene expression increased. *SOD1* activity increased at all concentrations compared to the control group. Capsaicin has a protective effect, especially in neuronal and hepatic cells, which may be partially explained by reduced MDA production, induction of antioxidant systems, and inhibition of active caspases (24). Exposing red blood cells to oxidative stress increases MDA levels and elevates protein carbonyl content above baseline values. The presence of capsaicin in the medium protects red blood cells from oxidant-induced oxidative stress, as evidenced by reduced MDA levels and protein carbonyl content. Ascorbic acid also demonstrated a similar protective influence. The results consequently support the antioxidative efficacy of capsaicin. This evidence suggests that foods that act as antioxidants, reducing membrane lipid peroxidation and protein carbonyl formation, may help protect against atherosclerosis, cancer, and some age-related diseases. Oxidant/antioxidant imbalance is considered a cause of endothelial dysfunction. Upon exposure to chemical, physical, or oxidative stress, endothelial cell integrity and the balance between vasoconstriction and vasodilation are disrupted, leading to inflammation, altered permeability, platelet aggregation, thrombosis, and subsequent pathological outcomes. MDA is a well-established marker of oxidative stress. It has been shown that capsaicin can reduce MDA levels (25). In the present experiments, compared with the control group, capsaicin reduced MDA concentration at all concentrations in the treated groups. It should be noted that the experiment shows that ascorbic acid also has a similar protective effect. In comparing the mean increase in MDA across the control groups, a significant difference was observed, with greater enzyme activity in H2O2, confirming its pro-oxidant effect.

This study has several important limitations. First, we measured only the expression of apoptosis-related genes, such as *BCL2* and *BAX*. We did not assess whether these changes led to functional outcomes, such as activation of downstream proteins or DNA fragmentation. Gene expression does not always correlate with protein synthesis or activity. Therefore, our findings provide only a partial view of cellular processes. Second, we did not directly measure cell viability or cell death after treatment. This makes it challenging to link gene expression changes to cellular outcomes. Third, we did not evaluate parameters of mitochondrial damage or oxidative stress, such as

mitochondrial membrane potential or lipid peroxidation. This limits our understanding of how hydrogen peroxide induces cytotoxicity. We also did not assess the impact of capsaicin on antioxidant enzyme activity. This leaves unanswered questions about its protective effects against oxidative stress. We did not use confirmatory assays for cell death, such as staining techniques or protein-level analyses. This limits mechanistic insight. Finally, all experiments were done in a single neuroblastoma cell line. This limits the generalizability of our results to other cancer types or non-cancerous cells. Future research should include genetic, proteomic, and functional assays for a more comprehensive understanding.

This study has several notable strengths that enhance its scientific rigor and relevance. Primarily, the experimental design using human neuroblastoma BE2-C cells allowed for precise investigation of capsaicin's effects on apoptosis-related gene expression and antioxidant enzyme activity under controlled conditions. The study's comprehensive approach included measuring not only gene expression but also the enzymatic activities of key antioxidants, including superoxide dismutase, catalase, and glutathione peroxidase, providing a more complete picture of capsaicin's protective mechanisms. Testing multiple capsaicin concentrations alongside a positive control (vitamin C) strengthens the dose-response analysis and comparative evaluation. The use of reliable, sensitive methods such as real-time PCR and the MTT assay to assess cellular viability and molecular changes adds robustness to the results. Additionally, integrating molecular and biochemical assays provides a comprehensive understanding of capsaicin's dual role in reducing oxidative stress and modulating programmed cell death. These factors collectively ensure the validity and applicability of the findings for future therapeutic research involving capsaicin.

Conclusion

The findings of this study suggest that capsaicin protects BE(2)-C neuroblastoma cells against hydrogen peroxide-induced oxidative damage. This protection is mainly due to upregulation of *NRF2* expression. Increased NRF2 is associated with higher expression and activity of antioxidant enzymes and inhibition of apoptosis. Laboratory analyses show that capsaicin acts as an effective antioxidant. It preserves lipids, proteins, and nucleic acids against oxidative stress from exogenous oxidants. Capsaicin is naturally occurring and easy to include in the diet. It is a promising candidate for antioxidant supplementation. Developing capsaicin-loaded nanoparticles may enhance their bioavailability and therapeutic efficacy. Capsaicin may serve as a dietary antioxidant for chemoprevention of oxidative damage in neuroblastoma cells. It could also help reduce treatment-related oxidative stress. Future studies should focus on nanoparticle-based delivery systems and evaluate capsaicin's effectiveness in broader biological contexts.

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Authors' Contribution

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Software: Maryam Hormozi.
Supervision: Maryam Hormozi.
Validation: Maryam Hormozi.
Visualization: Maryam Hormozi.
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Competing Interests

The authors declare that they have no conflicts of interest.

Data availability

Data are available by corresponding author on a reasonable request.

Ethical Approval

The protocol for this study was approved by the Ethics Committee of Lorestan University of Medical Sciences, with registration number IR.LUMS.REC.1398.210.

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