RESEARCH ARTICLE

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# Nisin Induces Cytotoxicity and Apoptosis in Human Asterocytoma Cell Line (SW1088)

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## Abstract

**Background:** Nisin is a member of the group of anti-microbial peptides which are considered as bacteriocins, but it possesses a vast range of activities. Astrocytoma is among the most prevalent types of brain tumor globally. Considering all facts about this peptide, the aim of the present study was the evaluation of any impact of nisin on proliferation and apoptosis of an astrocytoma cell line (SW1088). **Methods:** The SW1088 cell line was purchased from the Pasteur Institute of Iran and treated with various concentrations of Nisin. Nisin-induced cell toxicity and apoptosis were detected by both MTT assay and annexin V-FITC /propidium iodide (PI) staining. **Result:** In current study we observed that the cell death and apoptosis were significantly increased following nisin treatment, as compared to the control group. **Conclusion:** These results open a new window for establishment promising approaches with the concept of anti-cancer therapy by nisin in the future.

**Keywords:** Nisin- SW1088 tumor cell line- cell viability- apoptosis

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## Introduction

Although, cancer has faced remarkable improvements in treatment during recent years, but it is still be considered as a major source of mortality, worldwide. Within the USA, cancer is considered as the most leading cause of death in particular amongst people aged less than 85 years old (Siegel et al., 2012; Mohammadizadeh et al., 2018). Furthermore, the prevalence of several cancer types, such as skin, prostate, breast, and kidney is increasing worldwide (Kohler et al., 2011; Hosseini et al., 2017). In fact, "Cancer" is a more popular word which is used for explaining more than hundred various malignancies in which several various tissues and cell types are involved. All of cancer types appear to occur due to uncontrolled cell growth which is induced both inheritance and environmentally-induced genetic variations (Balmain et al., 1993; Karimabad et al., 2017; Sheikhrezaei et al., 2018).

Astrocytoma (a type of the brain cancer originated from either neural stem cells or progenitor cells located within the central nervous system) is the most prevalent priority tumor types of brain and contains around 60% of all brain involving tumors (Yi et al., 2013). According to the WHO data, astrocytoma is categorized into four various subgroups, based on certain possessive characteristics such as mitosis, atypia, necrosis, and endothelial proliferation (Bresalier et al., 1997). In spite of low frequency of astrocytoma in comparison to other human cancers, its mortality is still significant. The most important issue which could be referred to is the fact that although the availability of combined treatment strategies, including surgery, radiotherapy, and chemotherapy, there are difficulties in the improvement of the prognosis for high-grade astrocytoma. Both clinical signs and prognosis are tightly associated with tumor site, size as well as histopathological grade. The diffuse form of astrocytoma is most commonly found in children and young adults (Schweizer, 2009).

Most chemotherapeutic reagents, which are applied in the treatment of cancers, affect normal cells as well and consequently cause severe disadvantages and side effects. Thus, the developments of new anticancer medications with lack of toxicity non-toxicity for normal cells are urged to be considered. A possible group of which are under investigation includes cationic antimicrobial peptides (AMPs), that are involved in innate immunology of a wide spectrum of organisms, vary from insects, amphibians, to

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mammals (Hoskin and Ramamoorthy, 2008). The AMPs have amino acids sequences with highly heterogeneous and massive variation in their secondary structures. Most of AMPs show cationic properties (i.e., the net charge at normal pH ranges from +2 to +9) and amphipathic. In such condition, peptides are able to interact with lipid membranes and cause their disruptions (Papo and Shai, 2005; Huang et al., 2010).

Nisin as one of the natural AMPs is generated by strains of Lactococcus lactis subsp. lactis which effectively acts as an inhibitor for both gram-positive and gram-negative bacteria. Nisin is also applied as a food preservative as well as a promising target in pharmaceutical, veterinary and products relating to health care. Furthermore, nisin claimed to have any toxic effect on animals and hence, WHO approved its safety for human usage in 1969. Considering all facts about nisin, in the present study, the impacts of nisin on proliferative behavior and apoptosis was examined in an astrocytoma cell line (SW1088) (Hancock and Lehrer, 1998).

## Materials and Methods

*SW1088 Cells culture and maintenance*

The SW1088 human tumor cell line was purchased from the Iranian national cell bank (Pasteur Institute, Tehran, Iran). Cell were cultivated within RPMI-1640 medium (Gibco, UK), supplemented by 10 % fetal bovine serum (FBS) (Gibco, UK) and treated with 100 u/mL penicillin,100 µg/mL streptomycin, at 37°C in a humidified condition with 95% $O_2$ and 5% $CO_2$ (Helgason and Miller, 2005).

*MTT assay and Apoptosis analysis*

The percentage of viable cells was examined employing 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium (MTT) technique (Mosmann, 1983; Sharifi et al., 2005). Approximately $1\times10^3$ cells (in 200-µl of media) were cultured in 96-well culture plates and were then treated by different concentrations of nisin solution (1 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL) for 24, 48 and 72 hours at 37°C. The supernatant was discarded and the cellular fraction was then labeled with MTT solution (5 mg/mL in PBS) for 4 hours and then resulting formazan was solubilized in 50 µL of Dimethyl sulfoxide (DMSO). Subsequently, the absorption was read at 570 nm (620 nm as a reference) employing an ELISA (Enzyme-Linked-Immuno-Sorbent Assay) reader.

In order to detect apoptosis, cells ($2\times10^3$) were cultured onto a 6-well cell culture plate and treated with 10 µg/mL nisin. Further 24 hours of culture, cells were harvested and subjected to resuspend in an appropriate volume of cold phosphate buffer saline (PBS) for analysis. Treated cells were stained using an annexin V-FITC apoptosis kit, which was purchased (BD Pharmingen, San Diego, CA, USA), for monitoring of apoptotic cells and propidium iodide (PI) for detection of necrotic cells(Van Engeland et al., 1998). Collected data were obtained and analyzed using a BD FACS Calibur flow cytometer (San Jose, CA, USA). For an individual concentration and each time of the investigation, an untreated control sample was examined that only received untreated medium. All of the various treatments were performed in triplicate.

*Statistical analysis*

The results of this study were expressed as mean ± SD in the triplicate experiment. Differences were determined by ANOVA comparisons test at significant difference level of $p< 0.05$.

## Results

*The effects of nisin on cell viability*

This was an experimental study in which the cytotoxic

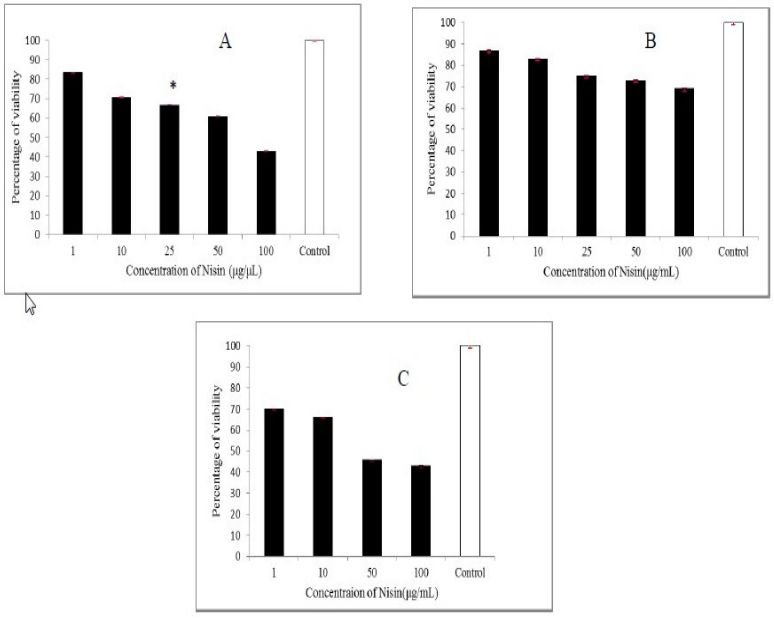
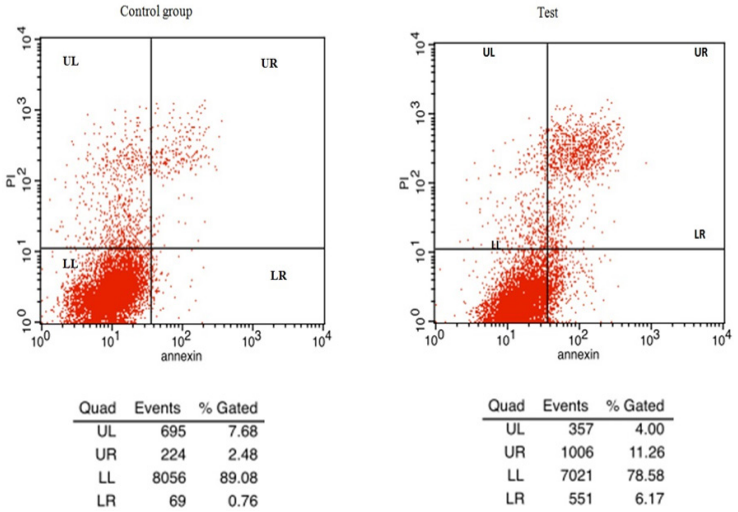



Figure 1. Demonstrates the Effect of Nisin on of SW1088 Cells Viability. Cells were treated with different con¬centrations of the peptide for 24 (A), 48 (B) and 72 hours (C) and then the cell survival rate (%) was determined by the MTT assay. The average of each triplicate experiment is presented in individual column as mean ± SD.

Table 1. Exhibits the Cell Proliferation Following Treatment with Different con-Centrations of Nisin (x±s, %) for 24h, 48h and 72h

| | Nisin Concentrations (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 1 | 10 | 25 | 50 | 100 |
| 24 h | 0.391±0.017 | 0.376±0.007 | 0.364±0.026 | 0.36±.0.037* | 0.359±0.032 | 0.339±0.027 |
| 48 h | 0.321±.0.024 | 0.342±0.024 | 0.333±0.023 | 0.311±0.033 | 0.292±0.011 | 0.295±0.024 |
| 72 h | 0.325±0.014 | 0.28±0.015 | 0.258±0.033 | | 0.265±0.028 | 0.256±0.02 |

*, Significantly different with control




| Quad | Events | % Gated |
|---|---|---|
| UL | 695 | 7.68 |
| UR | 224 | 2.48 |
| LL | 8056 | 89.08 |
| LR | 69 | 0.76 |

| Quad | Events | % Gated |
|---|---|---|
| UL | 357 | 4.00 |
| UR | 1006 | 11.26 |
| LL | 7021 | 78.58 |
| LR | 551 | 6.17 |

Figure 2. Demonstrates Flow Cytometric Analysis of Nisin Effect on SW1088 Tumor Cell Line. Test, treated SW1088 cells with nisin; Control, Non-treated SW1088 cells; LL, live cell percentage; LR, apoptotic cell; UR, the necrosis percent of cells. In control, the majority (89.08%) of cells were viable and non-apoptotic, and by adding 10 µg/ml of nisin, there was a decrease in the Annexin V–PI– population and an increase in cells in early apoptosis (Annexin V+PI–). As clear from figure, when cells were treated with 10 µg/mL nisin for 24 hours, 78.58 % of Annexin V–PI– cells were observed.

effect of nisin has been evaluated against human astrocytoma cell line (SW1088). Various concentrations of nisin were used as following: 1µg/mL, 10µg/mL, 25µg/mL, 50µg/mL, and 100 µg/mL at three different time points of 24, 48, and 72 hour. Findings obtained in the present study have demonstrated that nisin down-regulated cell viability in SW1088 cells, in a dose-dependent fashion (Table 1). The statistical analysis of MTT assay confirmed that the proliferative function of SW1088 cells was remarkably inhibited by nisin. Our results showed that the growth rate of nisin treated SW1088 cells was decreased when compared to cells to nisin untreated cells. As shown in Figure 1, the IC50 value of nisin for SW1088 cells was 50 µg/mL, 75 µg / mL, and 50 µg/mL at 24 hours, 48 hours and 72 hours, respectively (Figure 1).

*Flowcytometric analysis of nisin impact on SW1088 cells*

The annexin test was performed by 10 µg/mL nisin and control group during 24 hours in order to evaluate the apoptosis and necrosis which were occurred. Flow cytometric analysis demonstrated that treated cells encountered apoptosis during the test as opposed to the cell which did not receive any treatment. The compartment of apoptotic SW1088 cells of nisin -induced cytotoxicity is shown in Table 2.

The normal alive cells were observed in LL region (Figure 2). The immediate fraction of apoptotic cells was determined by the sum of the cells in LR while the late apoptotic and necrosis cells were observed mainly in the UR and UL regions, respectively (figure 2). The percentages of early apoptotic, necrotic and late apoptotic cells following treatment by 10µg/mL were 6.17%, 11.26%, and 4%, respectively (Figure 2).

## Discussion

Cancer is defined as a growing challenge for health worldwide. Products with natural origins have long been utilized for the prevention and treatment of several diseases, including cancers. Thus, they are well-introduced as promising candidates for the development of anti-cancer compounds serving against cancer (Smith-Warner et al., 2000). In the present study, we have demonstrated that nisin causes a moderate apoptosis in SW1088 tumor cell line. Our results showed that the cell viability depends directly on the concentration of nisin and the duration of incubation. On the other hand, the highest rate of cell toxicity has occurred once cells were treated with the highest concentration of nisin over 72 hours. Moreover, nisin was able to induce a marked apoptosis in SW1088 cell line, as opposed to control group. Theoretically, nisin can be used for future development as a therapeutic agent for cancer.

Antimicrobial peptides, known as bacteriocins, are produced by various types of bacteria. Studies in the late 70s decade confirmed anti neoplastic properties of bacteriocins. This was achieved by using their crude preparations in mammalian cell lines. In the most recent years, these peptides were gene ordered and purified and following being used, they have revealed such a property to be applied against cancerous line cells. Pyocin, colicin, pediocin, and microcin are of the bacteriocins which claimed to represent these types of activities. Furthermore, modified bacteriocins were approved to positively affect glioblastoma xenograft mouse model. Investigating regarding the presence of bacteriocin was screened in the colon cancer patients resulted in controversial reports. Applying bacteriocin either for therapy or for preventive demands is the main issue in evaluating bacteriocins biochemical properties (Breukink and de Kruijff, 1999; Lagos et al., 2009; Yates et al., 2012). Cancer cells represent highly rate of apoptotic threshold and the induction of apoptosis in cancer cells has been extremely affected them. Specific characteristics of cationic peptides enable them to induce apoptosis in tumor cells, therefore; they are increasingly regarded as potential agents for the improvement of anticancer therapy (Bhutia and Maiti, 2008).

However to the best of our knowledge there exists no available study within the database on the biological effects of nisin on SW1088 cells and therefore our results are novel in this field, but there are some evidence on head and neck cancer cell line as well as colorectal cancer cells. Joo et al., (2012) claimed that nisin can be considered as a promising treatment potential for therapy of head / neck squamous cell carcinoma (HHNCC). Nisin causes programmed cell death, cell cycle arrest, and decreases cell proliferation in HNSCC cells, in comparison with the normal cell line. Moreover, HNSCC tumorgenesis was decreased by nisin in vivo. This study revealed that nisin performs these duties on HNSCC, at least, in part, via a pro-apoptotic cation transport regulator which is called cation transport regulator homolog 1 (CHAC1). In concert with our study, Kamarajan and his colleagues examined the effects of different types of nisin including nisin ZP (95%), nisin AP (95%) and low content nisin (2.5%) on HNSCC cells. They indicated that the rate of apoptosis was significantly increased in HNSCC cells, which were treated with both nisin ZP from (95%) and nisin AP from (95%) in comparison with treatment with low content nisin (2.5%). The above mentioned study showed and supported the theory that nisin ZP may be a beneficial and promising treatment for HNSCC, since nisin ZP promotes HNSCC apoptosis, suppresses HNSCC cell proliferation, and blocks processes of the formation of new blood vessels along with tumorgenesis in vivo (Kamarajan et al., 2015). Ahmadiet al., (2017) revealed that nisin possesses the cytotoxic effect on colorectal cancer cells and induces apoptosis via intrinsic pathways. In another study, the anti-melanoma potential of Nisin Z was investigated. Their results revealed that Nisin Z was able to induce toxicity in melanoma cells in comparison with the normal cells (Lewies et al., 2018).

*Conflict of interest*

We declare that we have no conflict of interest.

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