Research in Molecular Medicine

Cytotoxic Effects of Methanolic and Ethanolic Extracts of *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Nostoc* on Human Gastric Cancer Cell Line

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ABSTRACT

Background: Gastric cancer is among the most frequently diagnosed and deadliest cancers worldwide. Current treatments often exhibit limited efficacy and are associated with considerable side effects.

Objective: This study aimed to evaluate the effects of methanolic and ethanolic extracts of *Chlorella vulgaris* (CV), *Scenedesmus obliquus* (SO), and *Nostoc* (NSC) on apoptosis in the AGS gastric cancer cell line.

Materials and Methods: The cytotoxic effects of the extracts at various concentrations were assessed using the MTT assay. Total RNA was extracted to measure BAX gene expression using quantitative real-time PCR.

Results: Extracts from all three microalgae enhanced cell death in the AGS cell line. Notably, the ethanolic extract of NSC significantly upregulated BAX gene expression.

Conclusion: The ethanolic extract of NSC shows promising anticancer potential and may serve as a candidate for future gastric cancer therapies.

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Introduction

Gastric cancer is among the most commonly diagnosed and deadliest cancers worldwide, accounting for approximately 1.1 million new cases and 770,000 deaths in 2020 [1]. In recent years, several therapeutic strategies have been developed to combat this disease, including chemotherapy, targeted therapies, and immunotherapy [2]. However, drug resistance and adverse side effects remain major obstacles to effective treatment [3]. Consequently, there is a growing need to explore alternative, more effective therapeutic options.

Microalgae are photosynthetic microorganisms that constitute a significant portion of freshwater and marine phytoplankton [4]. Their adaptability to extreme environments, such as high temperatures and hydrothermal vents, makes them attractive candidates for drug discovery. Microalgae produce a diverse array of secondary metabolites that allow them to thrive in various ecological conditions while exhibiting significant medicinal potential [5]. These organisms are also valued for their nutritional and pharmacological properties, owing to their rich content of proteins with balanced amino acid profiles, polyunsaturated fatty acids, carbohydrates, minerals, vitamins, pigments, and bioactive compounds [6]. Numerous studies have reported their anticancer, antiviral, antibacterial, antifungal, cytotoxic, immunomodulatory, and enzyme-inhibitory properties [4].

Scenedesmus obliquus (SO) is a common green alga from the genus Scenedesmus, typically found in planktonic communities [7, 8]. *Chlorella vulgaris* (CV) is a unicellular eukaryotic green microalga considered one of the earliest plants with a distinct nucleus [9]. It is particularly rich in β-carotene and chlorophyll and is widely recognized as a nutrient-dense superfood due to its high concentrations of protein, vitamins, and minerals such as iron, potassium, and calcium [10, 11]. Both SO and CV have demonstrated notable antimicrobial and anticancer properties [5, 8, 11].

Cyanobacteria, also known as blue-green algae, are Gram-negative photosynthetic prokaryotes found in both freshwater and marine ecosystems. They produce a wide range of secondary metabolites, including phenolic compounds and chlorophyll. *Nostoc* (NSC), a genus within this group, has been shown to possess antimicrobial, anticancer, immunosuppressive, and anti-inflammatory activities, and has demonstrated the ability to overcome multidrug resistance [12-15].

To date, the effects of these three microalgal species on gastric cancer cell lines have not been thoroughly investigated. Therefore, the present study aims to evaluate the cytotoxic effects of methanolic and ethanolic extracts of SO, CV, and NSC on the AGS gastric cancer cell line, with a particular focus on the expression of the apoptosis-related gene BCL2-associated X protein (BAX).

Materials And Methods

Chemicals and reagents

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and dimethyl sulphoxide (DMSO) were procured from Sigma (St. Louis, MO). Roswell Park Memorial Institute (RPMI)-1640 and fetal bovine serum were procured from Gibco, USA. Penicillin-streptomycin and trypsin were obtained from Biowest, Germany. All primers were designed and obtained from Metabion, Germany. The commercial kits for RNA extraction were obtained from Favorgen, Taiwan, and cDNA synthesis kits were purchased from Addbio, South Korea. Z8 culture medium was purchased from NORCCA, Bulgaria. All flasks and plates were purchased from SPL, South Korea.

Preparation of microalgal extracts

A total of 250 mL of water was collected from the Khazar Sea. Thirty microliters of the sample were inoculated into 100 µL of Z8 culture medium and incubated under optimal light and temperature conditions. Figure 1 shows morphology of three microalgae of CV, SO, and NSC using an inverted phase-contrast microscope. The culture medium was filtered, and the resulting algal biomass was dissolved in 40 ml of ethanol or methanol. The mixture was incubated in a water bath at 37 ˚C to evaporate the solvent and obtain the crude extract.

Cell culture

The human gastric cancer cell line (AGS) was obtained from the Pasteur Cell Line Bank in Tehran, Iran. Cells were cultured in RPMI-1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (10,000 U/mL each). Cultures were maintained at 37 ˚C in a humidified atmosphere containing 5% CO_2. Once cells reached confluence in 25 cm^2 flasks, they were prepared for the MTT assay.

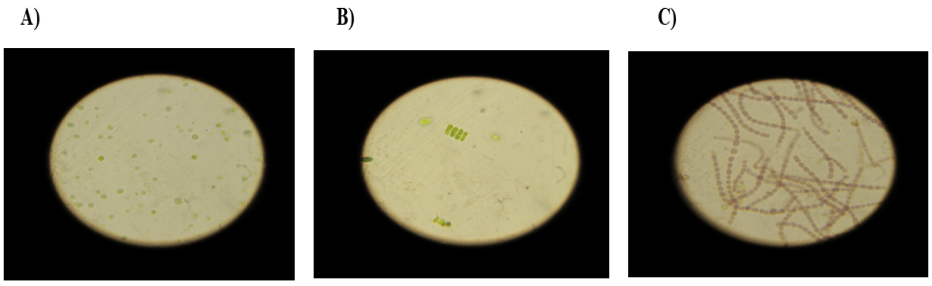

Figure 1. Representative images of three microalgae of (A) *Chlorella vulgaris, Scenedesmus obliquus* (B) and C) *Nostoc*
Note: Images were taken at 400× magnification using an inverted phase-contrast microscope.

MTT assay for cell proliferation

Cell proliferation and viability were assessed using the MTT assay. AGS cells were seeded into 96-well plates at a density of 1×10^4 cells per well and incubated with various concentrations of algal extracts for 24, 48, and 72 hours. Untreated cells served as the control group. Following treatment, 10 µL of MTT solution (5 mg/mL) was added to each well, and cells were incubated for an additional 24 hours. Absorbance was measured at 490 nm using an ELISA microplate reader [16, 17].

Quantitative real-time PCR (qRT-PCR)

AGS cells were seeded in 6-well plates at a density of 2×10^5 cells per well and treated with IC_{50} concentrations of NSC, SO, and CV extracts for 24 hours. Untreated cells served as controls. Total RNA was extracted using a commercial RNA extraction kit (Favorgen, Taiwan), according to the manufacturer's instructions. cDNA synthesis was carried out using a reverse transcription kit (Addbio, South Korea). BAX gene expression was quantified using a stem-loop TaqMan-based real-time PCR assay with USI barcodes and probes [18]. GAPDH was used as the housekeeping gene for normalization. Primers were designed using AlleleID 6.0 software and referenced from previous studies [16, 19]. PCR amplification conditions included an initial denaturation at 95 ˚C for 5 minutes, followed by 40 cycles of 95 ˚C for 15 seconds and 60 ˚C for 60 seconds.

Statistical analysis

MTT assay results were presented as Mean±SD, and gene expression data were reported as Mean±SE. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test in SPSS version software, version 23.0. A $P<0.05$ was considered statistically significant. All experiments were performed in triplicate.

Data description

Inhibition of gastric cancer cell line growth

The cytotoxic effects of varying concentrations (400, 500, 600, and 700 µg/mL) of methanolic and ethanolic extracts of NSC, SO and CV were assessed on the AGS gastric cancer cell line using the MTT assay. Results demonstrated a dose-dependent decrease in cell viability at all three time points: 24, 48, and 72 hours (Figures 2, 3 and 4).

Extracts from all three microalgal species exhibited cytotoxic effects, with SO and CV extracts showing half-maximal inhibitory concentration (IC_{50}) values below 700 µg/mL (Table 1), indicating moderate to strong anticancer activity.

BAX expression

A relative quantitative real-time PCR (qRT-PCR) method was employed to assess BAX gene expression levels in AGS cells treated for 24 hours with the IC50 concentrations of ethanolic extracts from CV, SO, and NSC. The results demonstrated a significant upregulation of BAX mRNA in AGS cells treated with the NSC ethanolic extract, whereas no significant changes were observed following treatment with CV or SO extracts (Figure 5).

Discussion

In the present study, the effects of methanolic and ethanolic extracts of CV, SO, and NSC on the viability of the AGS cell line were investigated. The findings showed that all ethanolic and methanolic extracts of the three algae inhibited the growth of the AGS gastric cancer cell line. Subsequently, the ethanolic extracts were used to evaluate BAX expression. BAX is a pro-apoptotic protein, and its mRNA expression increases following treatment with anticancer agents. The results illustrated that BAX mRNA levels increased after treatment with NSC, but not with CV or SO. In conclusion, NSC may activate the expression of the BAX gene in cancer cells.

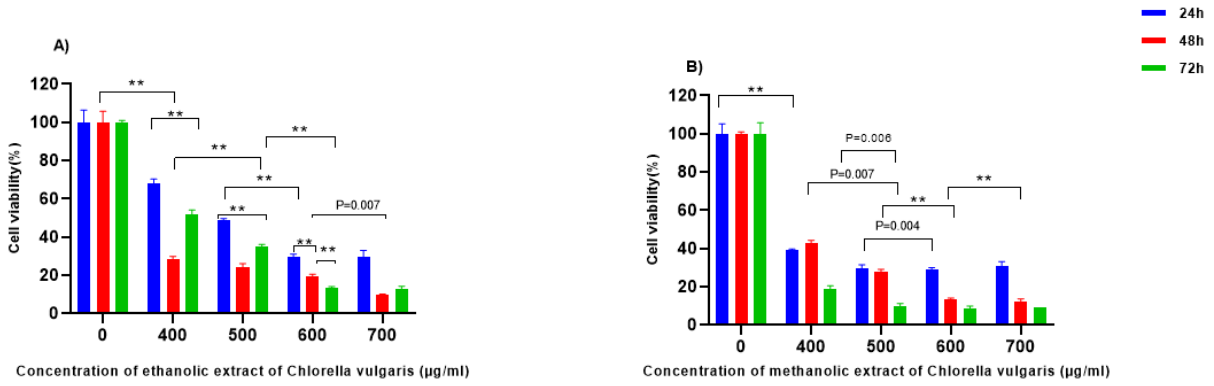

Figure 2. Effect of different concentrations of ethanolic (A) and methanolic (B) *Chlorella vulgaris* extract on the growth of gastric cancer cell lines (AGS), as measured by the (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) MTT assay

Data are shown as Mean±SD; significant differences were considered significant at P<0.05, **P<0.0001.

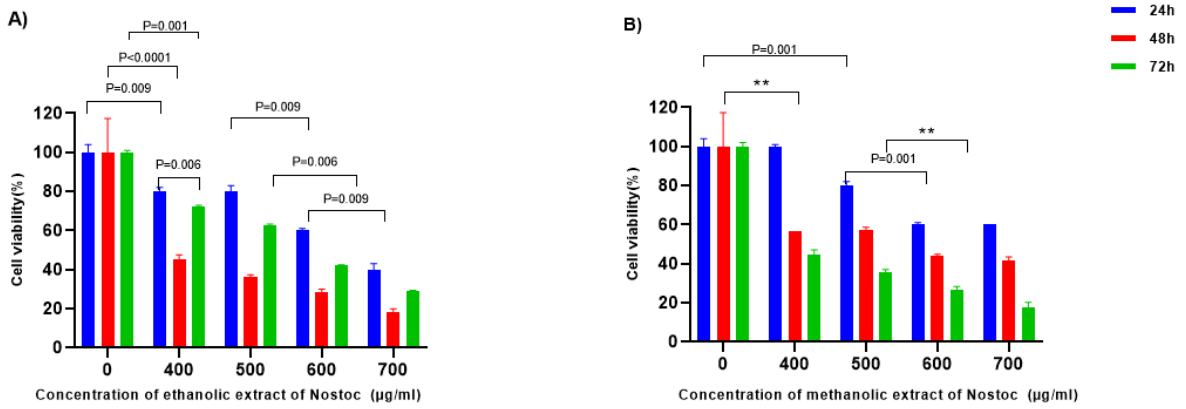

Figure 3. Effect of different concentrations of ethanolic (A) and methanolic (B) *Nostoc* extract on the growth of gastric cancer cell lines (AGS), as measured by the (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) MTT assay

Data are shown as Mean±SD; significant differences were considered significant at P<0.05, **P<0.0001.

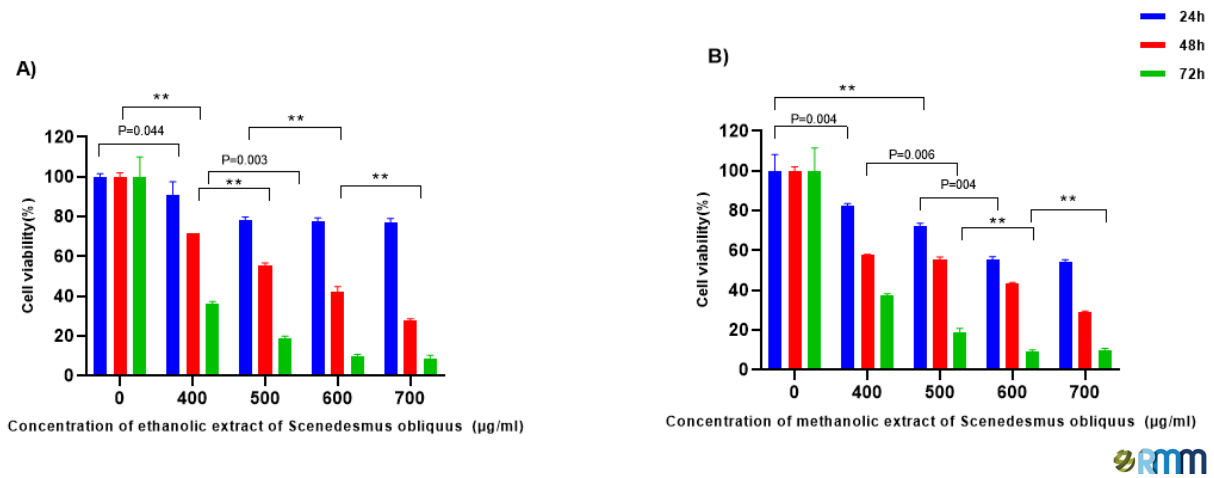

Figure 4. Effect of different concentrations of ethanolic (A) and methanolic (B) *Scenedesmus obliquus* A extract on the growth of gastric cancer cell lines (AGS) as measured by the (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) MTT assay

Data are shown as Mean±SD; significant differences were considered significant at P<0.05, **P<0.0001.

Table 1. IC50 values of each extract for the gastric cancer cell line

Time (hours)	Type of Extraction	Extract	IC_{50} (µg/mL)
24	Ethanolic	*Chlorella vulgaris*	502.8
		Scenedesmus obliquus	1472
		Nostoc	337.1
	Methanolic	*Chlorella vulgaris*	198.3
		Scenedesmus obliquus	753.4
		Nostoc	392.8
48	Ethanolic	*Chlorella vulgaris*	346.1
		Scenedesmus obliquus	463
		Nostoc	406.6
	Methanolic	*Chlorella vulgaris*	306.5
		Scenedesmus obliquus	335.3
		Nostoc	277.2
72	Ethanolic	*Chlorella vulgaris*	266.5
		Scenedesmus obliquus	357.5
		Nostoc	418.5
	Methanolic	*Chlorella vulgaris*	195.4
		Scenedesmus obliquus	361.7
		Nostoc	400.9

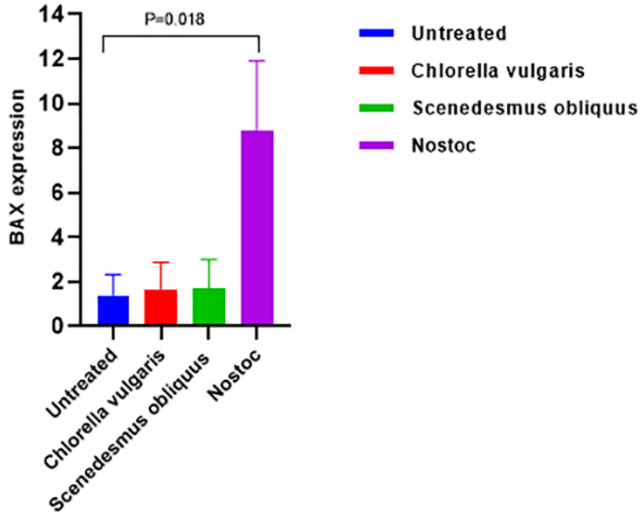

Figure 5. Effect of ethanolic extracts of *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Nostoc* microalgae on gene expression profiling of BAX in C26 and L929 cells

Data are shown as Mean±SD; significant differences were considered significant at P<0.05.

These three microalgae contain components such as alkaloids, carotenoids, and phenols, which may have antioxidant, antimicrobial, and anticancer effects [13, 20, 21]. To date, the effects of these three algae on gastric cancer cell lines have not been reported. However, other studies have documented the anticancer effects of compounds from these algae on various cell lines, including liver [20, 22], lung [20, 22], breast [22], and colon cancers [23]. Our study is consistent with these findings.

Our study has some limitations. We did not assay other related apoptotic genes, such as BCL-2 and Caspase-3. Additionally, we did not evaluate other cell death pathways, such as necrosis. Further studies should be performed to address these limitations.

Conclusion

This is the first report investigating the effects of methanolic and ethanolic extracts of three algae—CV, SO, and NSC—on the proliferation of gastric cancer cell lines. The results demonstrated that both methanolic and ethanolic extracts from these algae exert cytotoxic effects on gastric cancer cells. Additionally, the NSC ethanolic extract induced the expression of the apoptosis-related gene BAX. Therefore, these extracts may have potential as therapeutic agents for gastric cancer. However, further studies are necessary to clarify and validate these findings.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran.

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Authors contribution's

Study design and supervision: Alireza Rafiei and Seyed Abbas Hosseini; Data collection and data analysis: Fatemeh Khajehpour; Writing the original draft: Zahra Yazdani; Review and editing: Alireza Rafiei.

Conflict of interest

The authors declared no conflict of interest.

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