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Original Article

Study of *mTOR*/*PTEN* gene expression in gastric cancer cell line treated with ethanolic extract of *Tetraselmis suecia* microalgae

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Abstract: The second leading cause of death worldwide is cancer, and the fourth most prevalent cancer is stomach cancer. Natural seafood products include a variety of biologically active compounds with medicinal properties that may be useful in cancer therapy. This study aimed to assess the growth-inhibitory impact of the ethanolic extract of *Tetraselmis suecica* algae on the expression of mTOR/PTEN genes using the MTT assay in the cell line. The optimal IC50 dosage of this extract was 1000 and 750 µg/mL at 48 and 72 hours, respectively. *Tetraselmis suecica* extract dramatically lowered the survival rate of gastric cancer cells, increased the apoptotic PTEN gene expression, and decreased the mTOR gene expression in the cell line. The extract of *T. suecica*, as shown in the current study, is potentially useful in the treatment of stomach cancer.

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PTEN Phosphohydrolase

Introduction

Cancer is an abnormal accumulation of cells that occurs due to an imbalance between cell proliferation and cell death. Mutations in genes that regulate cell growth and programmed cell death cause cancer (Glaviano et al., 2023). Cancer is not just one type of illness; it is a term that refers to more severe forms of neoplasia. A neoplasm that progresses to cancer must be malignant, meaning its growth is no longer controlled, and the tumor must spread to distant sites by invading nearby tissues (metastasize), or both (Glaviano et al., 2023). Gastric cancer is the second leading cause of cancer death worldwide, and the fourth most prevalent malignancy. Several factors, including *Helicobacter pylori* infection, as well as genetic and environmental factors, contribute to the development of this cancer. The highest incidence of this cancer was observed in Japan, China, and Russia, and the lowest incidence was in developed Western countries. Due to variations in the genetic makeup and lifestyle, particularly diet habits like salt intake and processed meals, the incidence of gastric cancer differs greatly between populations. Of course, the prevalence of gastric cancer differs from its mortality rate, and the latter phenomenon is mostly due to late diagnosis in advanced stages of the disease (Sah et al., 2019).

The PAM signaling route, which mediates the effects of external stimuli on cell survival and growth (Manning and Cantley 2007, Ahmad et al., 2023; Tian et al., 2023). Aberrant expression or mutation of many components of this pathway has been linked to human oncogenesis (Vivanco and Sawyers, 2002; Yu et al., 2022). One such component is the tumor suppressor protein PTEN, whose reduced expression or inactivation (Dillon et al., 2007) may potentially contribute to cancer initiation and/or progression (Hennessy et al., 2005; Ko et al., 2019; Ashrafizadeh et al., 2020). Loss of PTEN function due to PTEN mutations has been detected in 45-60% of breast cancers (Sidorov et al., 2023). Several tumors contain PTEN loss-of-function mutations (Fusco et al., 2020; Vidotto et al., 2023). Hyperactivation of the PAM pathway, leading to increased cell proliferation, is a

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common consequence of PTEN loss in both primary and metastatic breast cancer (Song et al., 2012; Bergholz et al., 2023). The mechanism of antiestrogen therapy resistance is linked to both PAM pathway activation and PTEN downregulation (Miller et al., 2009).

Gastric malignancies frequently show mutations in PTEN (Fang et al., 2015). In addition, dephosphorylating and inhibiting the expression of PTEN (Li et al., 2020) indirectly causes an increase in signals via the PAM pathway in human gastric cancer (Ooki et al., 2011). Mutations in PTEN are also seen in colorectal cancer (Jin et al., 2020). Mutations in PTEN are also reported in bladder cancer because a loss of PTEN coupled with an altered TP53 has a detrimental impact, accelerating the growth of the tumor (Puzio-Kuter et al., 2009). In addition to brain cancer (Fusco et al., 2020), glioblastoma multiforme (Verhaak et al., 2010), anaplastic/poorly differentiated thyroid cancer (Landa et al., 2016), SCLC, NSCLC (Pérez-Ramírez et al., 2015), melanoma (Jiang et al., 2020), esophageal cancer (Zhou et al., 2010), gallbladder cancer (Li et al., 2019), pancreatic cancer (Zhu et al., 2016), renal cell carcinoma (Lee et al., 2003), prostate cancer (Li et al., 1997), testicular germ cell tumors (Feldman et al., 2014), cervical cancer (Hsieh et al., 2007), ovarian cancer, and a variety of sarcomas, PTEN loss of function mutations, particularly deletion, have also been discovered (Glaviano et al., 2023).

The mTOR protein kinase plays a crucial role in regulating metabolism, cell growth, survival, immune responses, and the aging process. It responds to various factors, including hormones, nutrients, growth signals, and stress indicators (Leung and Rangamani, 2023). Dysregulation of mTOR signaling has also been linked to several cancers, where abnormal cell proliferation and division directly contribute to tumor development and progression (Glaviano et al., 2023; Yan et al., 2024). It has been noted that about 70% of breast malignancies have a dysregulated mTOR. Research indicates that the creation of human prostate cancer cell lines depends on mTORC2 (Marafie et al., 2024).

Living in both freshwater and saltwater settings, microalgae are solitary photosynthetic phytoplankton. Through photosynthesis, microalgae can transform sunlight, water, and carbon dioxide into algal biomass (Khan et al., 2018; Masoumi et al., 2025). Because it minimizes toxicity to healthy cells and targets only cancer cells, therapy based on photosynthetic microorganisms is considered a unique method for cancer prevention (Kucerova and Cervinkova, 2016). Due to their bioactive components, which are used to treat cancer, microalgae are unique organisms. Microalgae are composed of substances like lipids, vitamins, polysaccharides, antioxidants, and proteins. Microalgae can enhance the protection of host cells. In other words, they activate the immune system, boost the activity of natural killer cells, and inhibit the growth of cancer cells. For this reason, microalgae are considered a promising anticancer therapy. Despite the abundance of research on the biological effects of plant-derived phytochemicals, there are few reports on microalgae-based phytochemicals. Importantly, microalgae-derived phytochemicals are more effective biological agents than those from terrestrial sources (Prabakaran et al., 2018). For this reason, the current research investigated the impact of *Tetraselmis suecica* algae extract on the *mTOR*/*PTEN* gene expression in the gastric cancer cell line.

Materials and Methods

***Tetraselmis suecica* microalgae culture:** A sterile supply of the microalgae *T. suecica* was provided by Shiraz Provincial Science and Technology Park. The algae were cultivated in TMRL media with a salinity of 40 ppm. Using 90% culture media and 5% algal stock, the inoculation was performed in a carefully regulated environment with a consistent temperature (25°C) and pH of 7. The photoperiod consisted of 18 hours of light (irradiance, 40 μmol photons m^{-2} s^{-1}) and 6 hours of darkness (18:6). The microalgae culture tanks were maintained by a continuous air bubble system to ensure a consistent distribution of nutrients and irradiance throughout each cell, thereby preventing sedimentation. Daily monitoring of the cells continued until they reached their highest

Table 1. Specific primers used in the current study.

Gene	Primers
m-TOR	Forward: 5'- GCTTGATTTGGTTCCCAGGACAGT -3'
	Revers: 5'- GTGCTGAGTTTGCTGTACCCATGT -3'
PTEN	Forward: 5'- TGGGCCCTGTACCATCCCAAGT -3'
	Revers: 5'- TGTGGCAACCACAGCCATCGT -3'
β-actin	Forward: 5'- TCCTCCTGAGCGCAAGTAC-3'
	Revers: 5'- CCTGCTTGCTGATCCACATCT-3'

concentration of 10^6 cells/mL.

Ethanol extraction of *T. suecica*: The sediment from the cultured microalgae was dried for 24 hours at 50°C following centrifugation for 10 minutes at 3500 rpm. The collected biomass was stored at 4°C until it was extracted. Using a Soxhlet extractor, the ethanol extract was produced, and a 12.5% ethanol solution (Merck, Germany) was made. The supernatant from this extraction was used in the subsequent procedures of the experiment after it was centrifuged at 30°C and 3500 rpm for 10 minutes. The ethanolic extract was concentrated using a freeze dryer. The ethanolic extract ultimately had a concentration of 8.7 mg/mL.

Cell line culture: In a humidified atmosphere containing 5% CO_2, cells were grown at 37°C in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (FBS; Gibco).

Cell viability assay (MTT): Each well of a 96-well plate was seeded with 10^4 in 100 µL of media. The cells were treated with serial concentrations of *T. suecica* alcoholic extract (0-1000 µg/mL) for 48 and 72 hours after the medium was changed to the new medium. After the therapy, the cells were rinsed with PBS, and each well was incubated for three hours at 37°C with 100 µL of new media containing 10 µL of MTT (5 mg/mL). After the medium was removed, 100 µL of isopropanol was added. A microplate reader (Elx808, Biotek, USA) was used to measure the optical density at 570 nm. In triplicate, all tests are performed and repeated at least three times.

Real Time-qPCR analyses: Using RT-qPCR, the expression levels of the *mTOR* and *PTEN* genes were determined. The Revert AidTM First Strand cDNA Synthesis Kit (Fermentas) was used to generate cDNA in accordance with the process outlined earlier. Using an ABI StepOneTM instrument (Applied Biosystems) and AccuPower® 2X GreenStar™ qPCR Master Mix (Bioneer, Korea). Real-Time qPCR was then performed according to the following protocol: one minute at 95°C, followed by 45 cycles of 95°C for 20 seconds, 52°C for 20 seconds, and 72°C for 20 seconds. The primers used are shown in Table 1. The gene for βactin served as the internal control gene. Using the $2^{-\Delta\Delta Ct}$ technique, the relative expression of possible targets was assessed.

Results

The MTT assay, a colorimetric method, was used to assess the cytotoxic impact of ethanol extract from *T. suecica* on cells. In triplicate, the bioactivity of *T. suecica*'s alcoholic extract was assessed by determining the concentration that caused 50% inhibition (IC50) in the growth of treated cells compared to the controls. Different concentrations of the alcoholic extract of *T. suecica* (0-1000 g/mL) were applied to cells for 48 and 72 hours. For a 48-hour exposure, the IC50 value was 1245 g/mL. According to the comparison of various concentrations of the ethanolic extract of *T. algae*, raising the concentration of the extract had a greater effect on controlling cancer cells. The findings also demonstrated a notable difference in controlling cancer cells when the cell line was treated with 1000 µg/mL of the microalgae's ethanolic extract (Fig. 1A).

In the 72-hour treatment, a decreasing trend in viable cells was observed with increasing concentration of *T. algae* extract from 250 to 1000 µg/mL. However, the comparison test between different concentrations showed that 750 µg/mL can

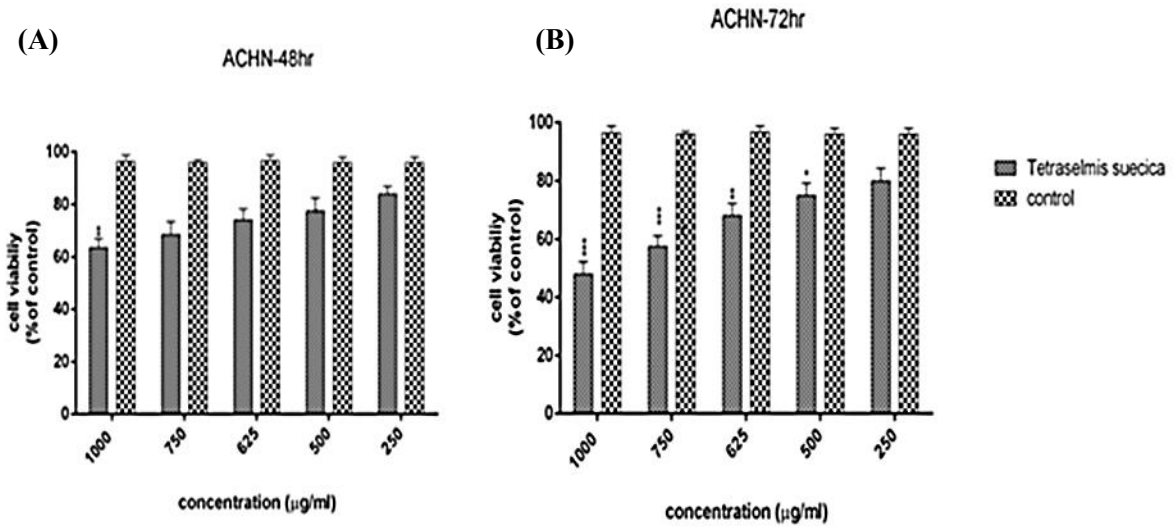

Figure 1. (A) The 48-hour and (B) 72-hour effect of *Tetraselmis suecia* extract on the vital activity of cancer cells (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$).

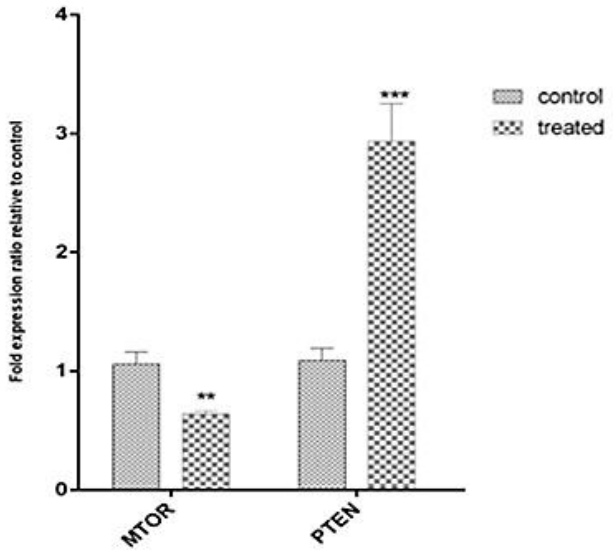

Figure 2. Analysis of the expression of *m-TOR* and *PTEN* genes in the cell line treated with *Tetraselmis suecica* microalgae compared to the control group in RT-PCR reaction. *T. Suecica* extract led to a significant increase in the expression of PTEN, and a decrease in the *m-TOR* gene compared to the control group (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$).

be considered the most effective concentration. With increasing exposure time of cancer cells to algae extract, the percentage of cell viability decreased further beyond 48 hours, resulting in an IC50 value of 816 µg/mL (Fig. 1B).

According to the results, the activity of the *MTOR* gene, which stimulates the growth of gastric cancer cells, was inhibited by the ethanolic extract of seaweed. However, the expression of the apoptotic genes PTEN and casp3 was significantly increased (Fig. 2).

Discussions

Microalgae are crucial to cancer research, as they contain a wide variety of bioactive compounds that may have anticancer effects. The bioactive compounds found in various microalgal species differ in their concentration and exhibit a range of anticancer properties. Due to their diversity, researchers have a wide range of options for diagnosing and treating specific types of cancer. Microalgae are an excellent source for discovering novel anticancer substances, as they are also known to exhibit a distinctive range of metabolic and biochemical properties. The importance of microalgal diversity in cancer prevention research has been emphasized in many publications. For instance, Martínez et al. (2022) demonstrated that bioactive compounds derived from microalgae exhibit a broad spectrum of anticancer activity against various cancer cell lines due to their diverse chemical compositions. Similarly, Chu et al. (2020) found that microalgal extracts from different species exhibited varying anticancer effects against prostate cancer cells. As a result, research on the diversity of microalgae and their bioactive compounds has the

potential to lead to the development of novel and potent anticancer therapies.

In comparison to the control group, the MTT test used in the current investigation showed a significant decrease in cell viability at a T. suecica concentration of 1000 µg/mL after 48 hours of therapy. Additionally, the 72-hour treatment at extract concentrations of 500, 625, 750, and 1000 µg/mL showed a significant decrease in cell viability. Therefore, it can be concluded that increasing the treatment time, even at lower concentrations of Tetraselmis algae extract, can achieve the desired result. In confirming the obtained data, Asoudeh-Fard et al. (2025) demonstrated that a concentration of 75 mg/mL of *T. suecica* therapy dramatically decreased the viability of HeLa cells to 25%. Furthermore, *T. suecica* extract was found by Davoodi and Mabudi (2024) to have a considerable impact on the survival rate of the HeLa cell line (Davoodi and Mabudi, 2024). In Cheragh and Mabudi (2023) work, concentrations of 500 and 1000 g/mL of *T. suecica* extract markedly decreased the viability of cancer cells in a 48-hour culture. Additionally, the 72-hour treatment significantly reduced cell viability at concentrations of 250, 500, and 1000 g/mL (Cheragh and Mabudi, 2023). The polysaccharides of *T. suecica* exhibit potent antioxidant and cytotoxic activities, and marine microalgae may be utilized as functional food components or as a potential source of nutrients to help mitigate the risk of tumor growth and spread in the human body (Parra-Riofrío et al., 2020). Hussein et al. (2020) found that a novel anticancer drug, composed of silver nanoparticles and *T. suecica* microalgal extract, displayed significant cytotoxicity against MCF7 and 4T1 cancer cells (Hussein et al., 2020).

Gene expression analysis in the current study's findings showed that in cells treated with *Tetraselmis* algae extract, the expression of the apoptotic PTEN gene was significantly higher. In comparison, the expression of the antiapoptotic *MTOR* gene was much lower than that in the control group. As a result, the anticancer effect of *Tetracelmis* algae on cells may be related to the MTOR/PTEN signaling cascade. Asoudeh-Fard et al. (2025) demonstrated that treatment with *T. suecica* on HeLa cells at a concentration of 75 mg/mL increased the expression of proapoptotic genes while decreasing the levels of antiapoptotic markers. The ethanolic extract of *T. suecica* significantly reduced the expression of antiapoptotic *AKT* and *mTOR* genes in the Huh7 cell line, as reported by Cheragh and Mabudi (2023).

Conclusion

In this study, *Tetraselmis susica* extract showed potential effects against the gastric cancer cell line by reducing the survival rate, increasing apoptosis, increasing PTEN and casp3 gene expression, and decreasing MTOR gene expression. Therefore, *T. susica* extract may have potential therapeutic effects against gastric cancer.

List of abbreviations: FBS: Fetal Bovine Serum**; MTOR:** Mammalian Target Of Rapamycin**; MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide**; PAM:** PI3K/AKT/Mtor**; PTEN:** Phosphatase and Tensin Homolog.

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