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Research Article

In vitro anticancer effects of a marine-derived glycosaminoglycan-containing extract from *Dicentrarchus labrax* scales on human glioblastoma cells

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Abstract

Background: Marine ecosystems are an underexplored source of structurally diverse bioactive compounds with therapeutic potential. Among these, glycosaminoglycans (GAGs) have gained attention for their anticoagulant, antioxidant, anti-inflammatory, and antitumor properties. Glioblastoma, the most aggressive malignant primary brain tumor in adults, remains largely incurable due to its high invasiveness, therapeutic resistance, and the protective blood–brain barrier. Therefore, the ongoing need to identify new treatment candidates persists.

Aims: This study aimed to investigate the potential anticancer effects of *Dicentrarchus labrax* (European seabass) scale extract (DLSE) on human glioblastoma U-87MG cells.

Methods: GAG-containing extracts were prepared from fish scales, and their chemical structures were characterized using Fourier-transform infrared (FTIR) spectroscopy. Cell viability was determined by the MTT assay, migration by scratch wound-healing assay, apoptosis by Hoechst/PI staining, and molecular changes by immunoblotting.

Results: FTIR analysis confirmed the key functional groups consistent with a GAG-like polysaccharide profile. DLSE reduced cell viability in a dose- and time-dependent manner, with an IC_{50} value of 993 µg/mL at 72 hours. Treatments with 600 and 1000

Özet

Dayanak: Deniz ekosistemleri, yapısal olarak çeşitli, terapötik potansiyele sahip biyoaktif bileşikler açısından yeterince araştırılmamış bir kaynaktır. Bunlar arasında glikozaminoglikanlar (GAG'lar), antikoagülan, antioksidan, antiinflamatuar ve antitümör özellikleri nedeniyle dikkat çekmiştir. Yetişkinlerde en agresif malign primer beyin tümörü olan glioblastoma, yüksek invazivliği, tedaviye direnci ve koruyucu kan-beyin bariyeri nedeniyle büyük ölçüde tedavi edilemez durumdadır. Bu nedenle, yeni tedavi adaylarını belirleme ihtiyacı devam etmektedir.

Amaçlar: Bu çalışma, *Dicentrarchus labrax* (Avrupa levreği) pul ekstresinin (DLSE) insan glioblastoma U-87MG hücreleri üzerindeki potansiyel antikanser etkilerini araştırmayı amaçlamıştır.

Yöntemler: Balık pullarından GAG içeren özütler hazırlandı ve kimyasal yapıları Fourier dönüşümlü kızılötesi (FTIR) spektroskopisi kullanılarak karakterize edildi. Hücre canlılığı MTT testi ile, göç yara iyileşme testi ile, apoptoz Hoechst/PI boyama ile ve moleküler değişiklikler immünoblotlama ile belirlendi.

Bulgular: FTIR analizi, GAG benzeri bir polisakkarit profiliyle tutarlı temel fonksiyonel grupları doğruladı. DLSE, hücre canlılığını doz ve zamana bağlı olarak azalttı; 72 saatte IC_{50} değeri 993 µg/mL idi. 72 saat boyunca 600 ve 1000 µg/mL DLSE ile yapılan tedaviler hücre göçünü engelledi, apoptozu tetikledi, Bax/Bcl-2 oranını artırdı

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µg/mL DLSE for 72 hours inhibited cell migration, induced apoptosis, increased the Bax/Bcl-2 ratio, and elevated levels of cleaved caspase-3 and PARP1. DLSE also activated p53/p21-mediated cell-cycle regulatory pathways, and induced ER stress through dose-dependent increases in Grp78, PERK, IRE1α, and cleaved ATF6 levels.

Conclusion: Our findings indicate that GAG-containing DLSE stimulates multiple antitumor pathways in glioblastoma cells. Although further *in vivo* research and compound fractionation are necessary, marine-derived GAG-rich extracts could be promising adjuncts for glioblastoma therapy.

ve parçalanmış kaspaz-3 ve PARP1 seviyelerini arttırdı. DLSE ayrıca p53/p21 aracılı hücre döngüsü düzenleyici yolları aktive etti ve Grp78, PERK, IRE1α ve kesilmiş ATF6 düzeylerini doza bağlı olarak artırarak ER stresini indükledi.

Sonuç: Bulgularımız, GAG içeren DLSE'nin glioblastoma hücrelerinde birden fazla antitümör yolunu uyardığını göstermektedir. Daha fazla *in vivo* araştırma ve bileşik fraksiyonlaması gerekli olsa da deniz kaynaklı GAG açısından zengin özütler, glioblastoma tedavisi için umut vadeden yardımcı maddeler olabilir.

Keywords: Fish scale extract, marine bioactive compounds, apoptosis, anti-tumoral activity

Introduction

Marine ecosystems, which cover nearly 70% of Earth's surface, remain comparatively underexplored for biotechnological applications, whereas terrestrial environments have long dominated natural product research (Gerwick & Moore, 2012; Wang et al., 2020). Marine organisms possess unique evolutionary adaptations that give rise to structurally diverse and biologically active metabolites rarely found in terrestrial species, creating valuable opportunities for pharmaceutical and cosmetic innovation (Nhani et al., 2024). Increasing consumer demand for natural alternatives and evolving regulatory frameworks further highlight the importance of marine-derived compounds (Augusto et al., 2024). Within this context, marine glycosaminoglycans (GAGs) have attracted growing interest due to their broad biological activities, including anticoagulant, anti-inflammatory, antioxidant, and antitumor effects (Damonte et al., 2004; Chavante et al., 2014). Distinct sulfation motifs and structural features differentiate marine GAGs from their terrestrial counterparts, endowing them with unique biological properties. Importantly, rather than acting through a single mechanism, GAGs interact with multiple cellular and extracellular components, modulating the tumor microenvironment, influencing cell–matrix interactions, and affecting key signaling processes involved in tumor progression (Afratis et al., 2012; Morla, 2019).

Cancer remains a major global health issue as a leading cause of illness and death (Bray et al., 2024). Despite progress in diagnosis and treatment, some cancers continue to have very poor outcomes. Among these, glioblastoma (GB) is the most common and aggressive primary brain cancer in adults, classified by the World Health Organization as a grade IV astrocytoma, with a median survival time of less than 15 months (Grochans et al., 2022; Price et al., 2024). Even with combined treatments, including surgery, radiation, and chemotherapy, outcomes remain generally poor because of the tumor's high invasiveness, natural resistance to therapy, and the protective blood–brain barrier (Arvanitis et al., 2020; Tan et al., 2020). These challenges highlight the urgent need for new therapeutic strategies. Natural products offer a promising source of bioactive compounds because they can target multiple pathways at once and often have lower systemic toxicity than conventional chemotherapies (Newman & Cragg, 2020). Notably, compounds from marine sources are especially interesting, as marine organisms have unique biochemical pathways that produce structurally distinct and biologically potent metabolites not typically found in land-based species (Gerwick & Moore, 2012; Wang et al., 2020). Although marine-derived polysaccharides such as fucoidan have shown promising anti-glioblastoma activity in vitro, including induction of apoptosis and inhibition of cell proliferation (Liao et al., 2019; Cabral et al., 2021), the effects of GAGs derived specifically from fish scales on glioblastoma remain entirely unexplored, and the molecular mechanisms by which such compounds may influence glioblastoma cell behavior have not been characterized.

Marine-derived GAG-like compounds have been studied for various biological activities, but the anticancer potential of fish scale-derived GAG-rich extracts remains poorly characterized, particularly in glioblastoma. In this context, because *Dicentrarchus labrax* (European seabass) is a widely farmed aquaculture species, its scales may provide an accessible and sustainable source of marine bioactive compounds, thereby increasing its potential for use in drug development studies. Based on this rationale, this study aimed to evaluate the therapeutic potential of *Dicentrarchus labrax* scale extract (DLSE), including GAGs following the extraction process, in human glioblastoma U-87MG cells. The biological effects were assessed by examining cell viability, proliferation, migration, apoptosis, and endoplasmic reticulum (ER) stress responses. Results showed that GAG-enriched extracts from marine organisms can affect key cellular and molecular pathways in glioblastoma, indicating that marine bioactive compounds could be promising candidates for future glioblastoma treatments.

Materials and Methods

Extraction of GAG-like compounds from fish scales

Glycosaminoglycan (GAG)-like compounds were extracted from the scales of *Dicentrarchus labrax* (European seabass) using the method described by de Moura et al. (2021) with

minor modifications. In the modified protocol, the enzymatic hydrolysis duration was reduced, and the mechanical disruption and the second papain addition from the reference method were omitted. In brief, fish scales were defatted with acetone, dried at 60 °C for 24-h, and enzymatically digested in sodium acetate buffer (pH 5.5) containing EDTA, cysteine, and papain at 60 °C for 24-h. After heat inactivation and centrifugation, the supernatant was precipitated with ethanol. The resulting pellet was dried and stored at –20 °C. For experiments, the dried *Dicentrarchus labrax* scale extract (DLSE) was dissolved in DMSO (#D8418, Merck/Germany) to a concentration of 100 mg/mL and stored at –20 °C until use.

FTIR analysis

Fourier-transform infrared (FTIR) spectroscopy was performed to evaluate the chemical structure of the GAG-like compounds extracted from fish scales and verify the presence of specific functional groups. The FTIR spectra were recorded using a Bruker Alpha-P FTIR spectrometer (Bruker, Germany), scanning from of 4000–5000 cm^{-1}. Dried samples of the GAG-like compounds were prepared using the standard solid-sample protocol prior to collecting spectral data.

Cell culture and conditions

MG cells were obtained from the Istanbul University Cell Culture Collection, and maintained in Dulbecco's Modified Eagle Medium (DMEM, #41966) supplemented with 10% fetal bovine serum (FBS, #10500), 1% antibiotic–antimycotic solution (100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin-B, #15240062), and 1% non-essential amino acids (NEAA). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. All experiments were carried out with logarithmically growing cells between passages 3 and 10. Standard culture reagents were purchased from Gibco (Carlsbad, USA).

In vitro cytotoxicity assay and DLSE treatments

The cytotoxic effects of DLSE on glioblastoma cells were evaluated using the MTT assay as previously described (Pekmez et al., 2025). One day after seeding in 96-well plates (1×10^4 cells/well), cells were treated with increasing concentrations of the extract (1–1000 µg/mL) for 24-, 48-, and 72-h. After treatments, MTT solution (#1334, NeoFroxx, Einhausen, Germany; 5mg/mL in D-PBS, #21600069, Gibco, Carlsbad, USA) was added and incubated for 4-h. Formazan crystals were dissolved in DMSO, and absorbance was measured at 540nm using a microplate reader (EON, BioTek Instruments Inc., Winooski, USA). To evaluate cytotoxicity, the percentage of cell viability was calculated using the following formula: (absorbance of treated cells/absorbance of untreated cells) × 100.

Based on the sigmoidal dose–response curves, DLSE treatment doses and application time were selected. Doses of 600 µg/mL (IC_{20}, 80% cell viability) and 1000 µg/mL (IC_{50}, 50% cell viability) of DLSE for 72-h were used for further analysis. The final DMSO concentration in the culture medium did not exceed 1% (v/v), serving as the vehicle control for the experiment.

Morphological cell changes

To assess the impact of DLSE treatments on glioblastoma cell morphology, both untreated and treated U-87MG cells were imaged using an inverted light microscope (Olympus CKX31, Tokyo, Japan) equipped with a digital camera (ToupTek Photonics XCAM-1080PHD, Zhejiang, China).

Cell migration assay

Glioblastoma cells (6×10^4 cells/well) were seeded in 24-well plates and incubated for 24-h to allow monolayer formation. A linear scratch was created in the cell layer using a sterile 1000µL pipette tip. Cells were washed with D-PBS to remove debris and treated with DLSE. Images were captured at 0-, 24-, and 48-h using an inverted microscope with a digital camera. Scratch closure was quantified using ImageJ software (NIH, USA), and the migration rate was calculated as: [(Area(0-h) − Area(48-h))/Area(0-h)] × 100.

Apoptosis assay: Hoechst/propidium iodide double staining

Cell apoptosis was assessed as previously described (Pekmez et al., 2025) by double staining with Hoechst-33342 (HO, #H-1399, Invitrogen, Carlsbad, USA) and propidium iodide (PI, #P1304MP, Invitrogen, Carlsbad, USA). Briefly, one-day after seeding (2×10^4 cells/well) into 8-well chamber slides (Nunc Lab-Tek II, Thermo-Invitrogen, Carlsbad, USA), cells were treated with DLSE. After 72-h of incubation, cells were stained with HO/PI solution (5 µg/mL, 30-min at 37°C) and visualized under an Olympus BX53 fluorescence microscope equipped with a DP73 digital camera (Tokyo, Japan). Viable, apoptotic, and necrotic cells were distinguished based on nuclear morphology, manually counted, and quantified using ImageJ software (NIH, USA).

Protein extraction and Western blot analysis

Western blot analysis was performed as previously described (Aksüt et al., 2025) to evaluate proteins associated with apoptosis and ER stress in glioblastoma cells treated with DLSE. Briefly, one-day after seeding (3×10^5 cells/well) in 6-well plates, cells were treated with DLSE. After 72-h incubation, cells were collected by trypsinization, washed with D-PBS, and lysed in ice-cold RIPA buffer (#89901, Thermo, Kwartsweg, Holland) supplemented with EDTA-free protease inhibitor (#11873580, Roche, Darmstadt, Germany) and PMSF (#A0999, AppliChem, Germany). Lysates were centrifuged (20000rpm, 20min, 4°C), and protein concentrations were determined using the BCA assay (iNtRON Biotechnology, Seongnam, Korea). Equal assay (iNtRON Biotechnology, Seongnam, Korea). Equal protein amounts (30µg) were resolved by SDS-PAGE and transferred onto PVDF membranes (Bio-Rad, Hercules, USA). After

Table 1. Antibodies used for Western blot analysis.

Antibody	Host	Dilution	Catalog No	Company*
Anti-p53	Mouse	1:1000	NB200-103	Novus
Anti-p21	Rabbit	1:1000	10355-1-AP	Proteintech
Anti- PERK	Mouse	1:1000	NBP1-51661	Novus
Anti-GRP78	Rabbit	1:1000	NBP2-16749	Novus
Anti-IRE1α	Rabbit	1:1000	NB100-2324	Novus
Anti-ATF6	Mouse	1:1000	NBP1-40256	Novus
Anti-Bax	Rabbit	1:1000	50599-2-Ig	Proteintech
Anti-Bcl-2	Rabbit	1:1000	12789-1-AP	Proteintech
Anti-Caspase-3$^{pro\&cleaved}$	Rabbit	1:1000	19677-1-AP	Proteintech
Anti-β-actin	Mouse	1:5000	NB600-501	Novus
Anti-Mouse IgG	Goat	1:5000	BA1050	Boster
Anti-Rabbit IgG	Goat	1:5000	BA1054	Boster

* Antibodies used for immunoblotting were from Novus (St.Louis, USA), Boster (Pleasanton, USA), and Proteintech (Chicago, USA).

blocking with 5% non-fat skim milk (Sigma 70166), membranes were incubated overnight at 4 °C with primary antibodies (Table-1), followed by HRP-conjugated secondary antibodies (2-h, RT). β-actin was used as a loading control. Protein bands were detected using an ECL substrate (#34580, SuperSignal West-Pico, Thermo) and visualized with a ChemiDoc-XRS imaging system (Bio-Rad, USA). Band intensities were quantified using ImageLab software (Bio-Rad).

Statistical analysis

All quantitative results were presented as mean ± standard deviation (SD) from at least three independent experiments. Statistical analyses and graph creation were conducted using GraphPad Prism v10.3 (GraphPad Software, San Diego, CA, USA). Data normality and homogeneity of variance were confirmed using the Shapiro–Wilk and Levene's tests, respectively; therefore, a parametric analysis of variance (ANOVA) was used. Half-maximal inhibitory concentration (IC_{50}) values were determined by nonlinear regression analysis of sigmoidal dose–response curves. One- or two-way ANOVA, followed by Tukey's post hoc test, was used for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Abbreviations: Bax – Bcl-2–associated X protein; **Bcl-2** – B-cell lymphoma 2; **Cl-Cas-3** – Cleaved caspase-3; **DLSE –** *Dicentrarchus labrax* scale extract; **GAGs –** Glycosaminoglycans; **Grp78** – Glucose-regulated protein 78; **IC_{50}** – Half-maximal inhibitory concentration; **IRE1α** – Inositol-requiring enzyme 1 alpha; **PARP1** – Poly (ADP-ribose) polymerase 1; **PERK** – Protein kinase RNA-like endoplasmic reticulum kinase.

Results

FTIR characterization of GAG-like compounds

The chemical features of the extract, including GAGs from *D. labrax* scales, were analyzed using FTIR spectroscopy (Figure 1). The spectrum showed a broad, intense band at 3060–3280 cm^{-1}, attributed to the stretching vibrations of hydroxyl (–OH) and amine (–NH) groups, indicating the hydrophilic nature and hydrogen-bonding capacity of the GAG structure (Lungu et al., 2011; Brézillon et al., 2014). Peaks around 2935 cm^{-1} were assigned to the asymmetric and symmetric stretching vibrations of methyl ($–CH_3$) and methylene ($–CH_2$) groups. Prominent bands at 1633 and 1404 cm^{-1} were attributed to COO^- stretching vibrations, whereas the signal at ~1530 cm^{-1} corresponded to N–H bending and C–N stretching (amide II band), supporting the presence of N-acetylated sugar residues and uronic acid units within the GAG chain (Zahari & NS, 2016; Lettow et al., 2020). Notably, the band at ~1245 cm^{-1} was consistent with sulfate ($–SO_3^-$) groups, suggesting a sulfated polysaccharide structure

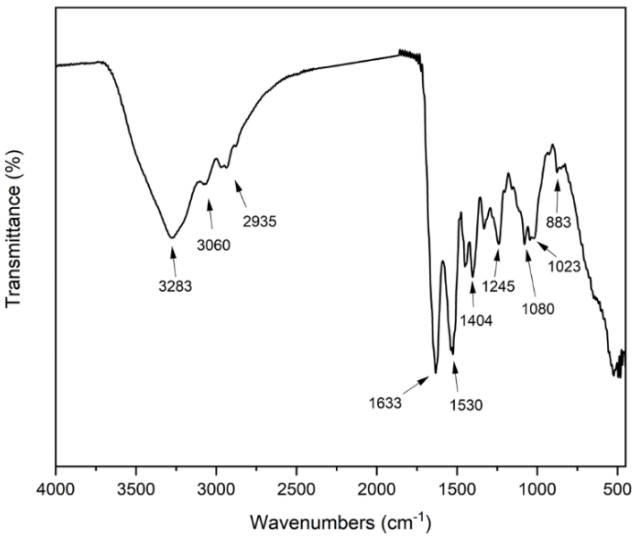

Figure 1. FTIR spectrum of the GAG-like compounds isolated from *Dicentrarchus labrax* scales. The broad band at 3283–3060 cm^{-1} indicates –OH/–NH stretching, and the peak at 2935 cm^{-1} corresponds to –CH stretching. Bands at 1633 and 1404 cm^{-1} are assigned to COO^- stretching, while the peak at 1530 cm^{-1} represents the amide II band. The signal at 1245 cm^{-1} suggests sulfate ($–SO_3^-$) groups, and peaks at 1080–1023 cm^{-1} reflect carbohydrate ring (C–O/C–O–C) vibrations, supporting a GAG-like polysaccharide structure.

(Zahari & NS, 2016). Finally, peaks in the 1023–1080 cm^{-1} region were attributed to C–O–C, C–C–O, and C–O stretching vibrations in pyranose ring structures, confirming the characteristic carbohydrate backbone of GAGs (Lungu et al., 2011; Brézillon et al., 2014). Overall, these FTIR findings confirmed the successful isolation of GAG compounds, potentially a sulfated polysaccharide fraction from fish scales.

Cytotoxic effects of *Dicentrarchus labrax* scale extracts on glioblastoma cells

The cytotoxic effects of DLSE on U-87MG cells were assessed using an MTT assay. Cells were treated with different concentrations of the extract (1–1000 µg/mL) for 24-, 48-, and 72-h. Results indicated that cell viability decreased in a dose- and time-dependent manner, with an IC_{50} at 72-h calculated as 993.10±10.57 µg/mL (Figure 2a). Based on these findings, concentrations of 600 µg/mL and 1000 µg/mL were selected for further testing. After 72-h, these doses reduced cell viability by 19.60% ($p < 0.001$) and 52.35% ($p < 0.001$), respectively, compared to vehicle control. Phase-contrast images visually confirmed the reduction in cell density in the treated groups (Figure 2b).

Effect of *Dicentrarchus labrax* scale extract on glioblastoma cells migration

The effect of DLSE on glioblastoma cell migration was assessed using an *in vitro* scratch assay (Figure 3). In vehicle control group, the scratch area was nearly fully closed within 48-h, indicating a high migratory capacity of U-87MG cells. In contrast, treatment with DLSE significantly inhibited migration in a dose-dependent manner. At 48-h, cells treated with 600 µg/mL reduced closure to 67.65% ($p < 0.001$), compared to nearly complete closure in vehicle control. The reduction in scratch closure (36.28%, $p < 0.001$), particularly at 1000 µg/mL, may reflect both decreased cell viability and impaired cell migration. These results suggest that GAG-like extracts markedly suppress the migratory ability of glioblastoma cells.

Induction of apoptosis by *Dicentrarchus labrax* scale extract in glioblastoma cells

The apoptotic effects of DLSE on U-87MG cells were first assessed using HO/PI double staining (Figure 4a). HO staining showed that the nuclei (blue-fluorescence) of the vehicle control group were round and intact, but treatment with 600 and 1000 µg/mL DLSE significantly increased the

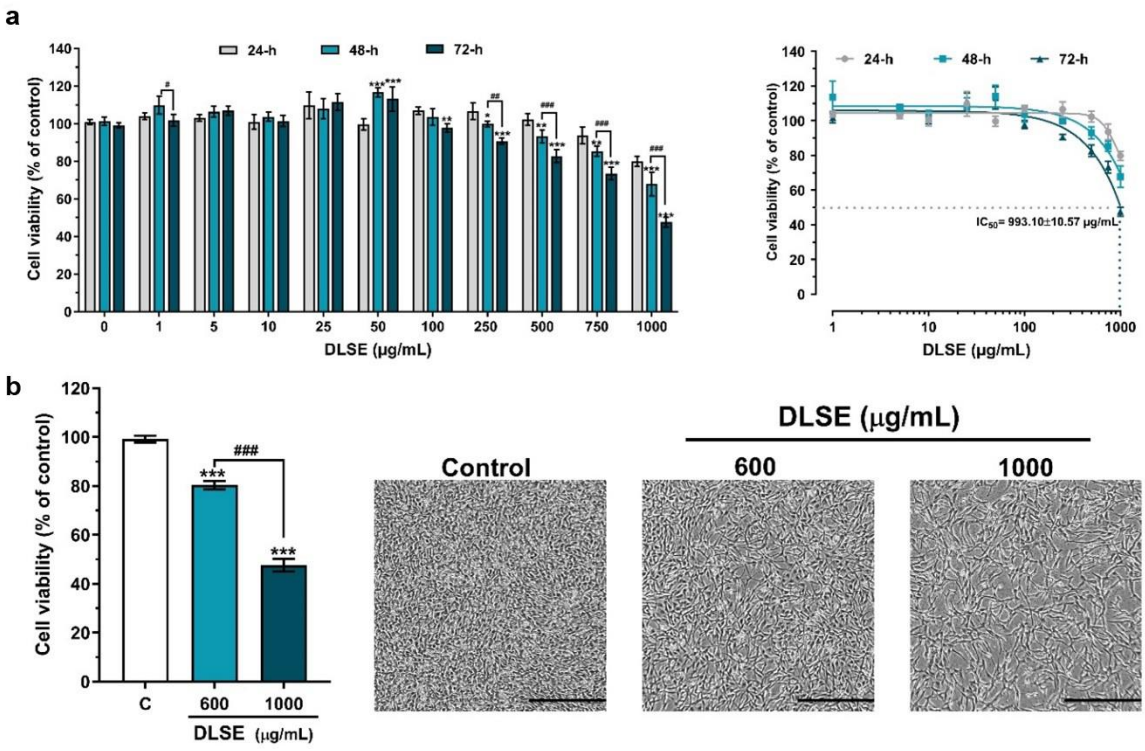

Figure 2. Cytotoxic effects of *Dicentrarchus labrax* scale extract (DLSE) on glioblastoma cells. (a); U-87MG cells were exposed to increasing concentrations of DLSE (0–1000 µg/mL) for 24-, 48-, and 72-h, and an MTT assay was performed to assess cell viability and determine IC_{50} (the half-maximal inhibitory concentration) value (n=4) (b); Selected concentrations of DLSE (600 and 1000 µg/mL) were applied for 72-h, and cell viability was assessed (n=4). Representative phase-contrast images show cellular morphology in vehicle control and treated groups. Data are expressed as mean±SD. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ versus vehicle control; $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ indicate comparison between different groups. Statistical comparisons were performed using one- or two-way ANOVA, followed by Tukey's post hoc test.

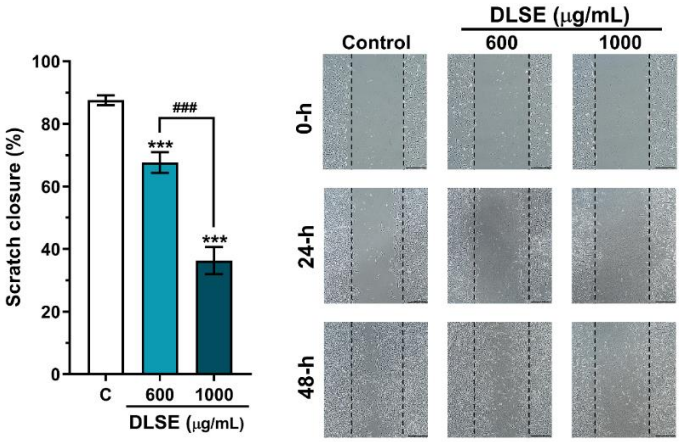

Figure 3. Effects of *Dicentrarchus labrax* scale extract (DLSE) on glioblastoma cell migration. U-87MG cells were treated with DLSE (600 and 1000 μg/mL), and wound closure was evaluated at 0- and 48-h (n=4). Representative images show the scratch area in vehicle control and treated groups at 0-, 24-, and 48-h points. Data are expressed as mean±SD. ***$p < 0.001$ versus vehicle control; $^{###}p < 0.001$ indicate comparison between different groups. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test.

Bax/Bcl-2 ratio (1.28-fold, $p < 0.001$, and 1.36-fold, $p < 0.001$, respectively). Similarly, the levels of cleaved caspase-3 were notably higher in treated cells compared to vehicle control (6.17-fold, $p < 0.001$, and 7.23-fold, $p < 0.001$, respectively). Additionally, the cleaved form of PARP1 (p89) increased in treated cells (1.98-fold, $p < 0.001$, and 4.17-fold, $p < 0.001$, respectively), indicating impaired DNA repair activity and increased induction of apoptosis.

The expression of cell cycle–related regulatory proteins p53 and p21 was also examined (Figure 4b). Treatment with DLSE increased p53 expression by 1.48-fold ($p < 0.01$) at 600 μg/mL and 2.32-fold ($p < 0.001$) at 1000 μg/mL compared with vehicle control, with the difference between doses being statistically significant. Similarly, p21 expression levels increased 1.54-fold ($p < 0.01$) and 2.19-fold ($p < 0.001$) following treatment with 600 and 1000 μg/mL, respectively. These results demonstrate that DLSE not only promotes apoptosis in U-87MG cells but also enhances p53 and p21 expression, suggesting their involvement in the anti-cancer mechanism of action

Induction of ER stress by *Dicentrarchus labrax* scale extract in glioblastoma cells

To investigate the involvement of endoplasmic reticulum (ER) stress in the cellular response to DLSE treatments, we assessed the expression levels of Grp78, PERK, IRE1α, and ATF6 p50 in U-87MG cells (Figure 5). Treatment with 600 μg/mL extract significantly increased Grp78 expression by 1.62-fold ($p < 0.001$) compared to vehicle control, while the more cytotoxic dose of 1000 μg/mL further elevated Grp78 by 2-fold ($p < 0.001$). Similarly, PERK and IRE1α protein levels, respectively, increased dose-dependently, rising by 1.81- and 1.67-fold at 600 μg/mL ($p < 0.001$), and 2.06- and 2.53-fold at 1000 μg/mL extract ($p < 0.001$). The active ATF6 fragment (p50) increased significantly after treatments, with both doses raising levels compared to vehicle control (3.23-fold, $p < 0.001$; 6.53-fold, $p < 0.001$), and the higher dose showed a greater effect.

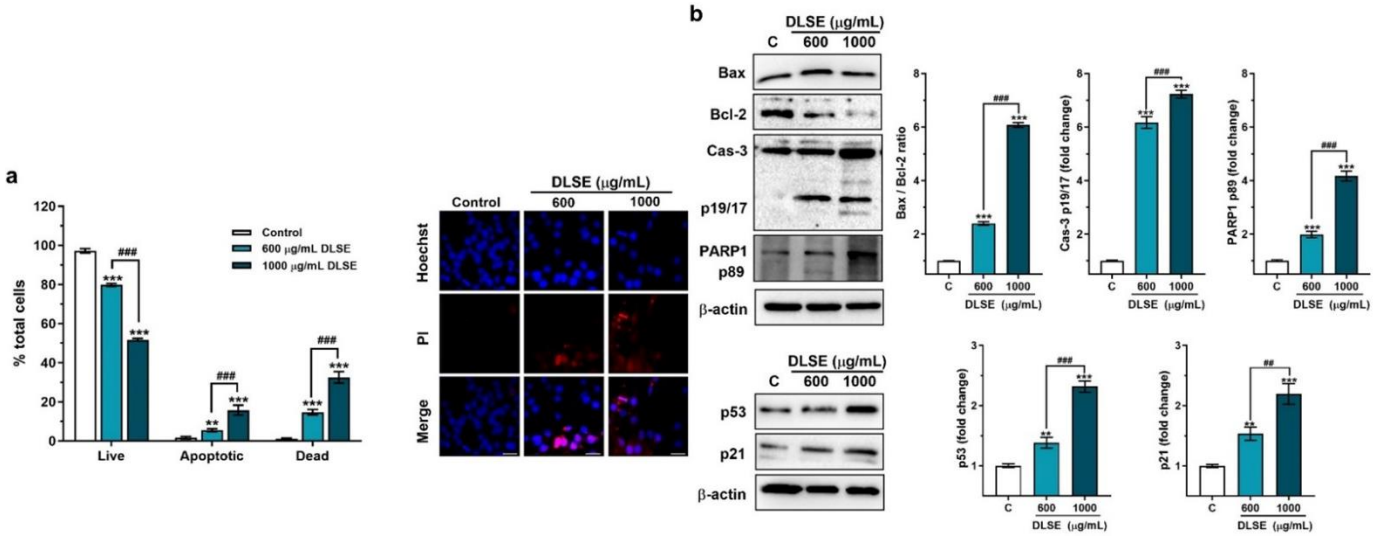

Figure 4. Effects of *Dicentrarchus labrax* scale extract (DLSE) on apoptosis-related pathways in glioblastoma cells**.** U-87MG cells were treated with DLSE (600 and 1000 μg/mL) for 72-h. (a); hoechst (blue-fluorescence) and PI (red-fluorescence) double staining was performed to evaluate live, apoptotic, and dead cells, with quantitative analysis (n=4) (b); western blot analysis of apoptosis-related proteins, including Bax, Bcl-2, caspase-3, and PARP-1 (n=3) (c)**;** Western blot analysis of p53 and p21 expression (n=3). β-actin was used as a loading control. Data are expressed as mean±SD. ***$p < 0.001$, **$p < 0.01$ versus vehicle control; $^{###}p < 0.001$, $^{##}p < 0.01$ indicate comparison between different groups. Statistical comparisons were performed using one- or two-way ANOVA, followed by Tukey's post hoc test.

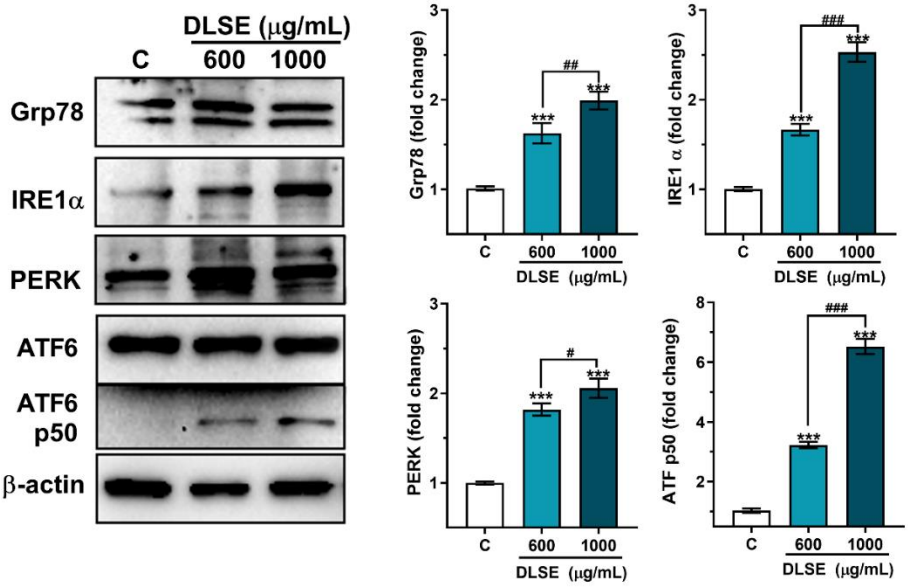

Figure 5. Effects of *Dicentrarchus labrax* scale extract (DLSE) on endoplasmic reticulum (ER) stress-related proteins in glioblastoma cells. U-87MG cells were treated with DLSE (600 and 1000 μg/mL) for 72-h. Protein levels of Grp78, IRE1α, PERK, and ATF6 (full-length and cleaved ATF6 p50) were analyzed by Western blotting (n=3). β-actin was used as a loading control. Data are expressed as mean±SD. ***$p < 0.001$ versus vehicle control; $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ indicate comparison between different groups. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test.

Discussion

Marine-derived natural products are largely unexplored but promising sources of bioactive compounds, known for their anticoagulant, antioxidant, and antitumor effects. These substances hold considerable promise for the development of new therapeutic treatments (Gerwick & Moore, 2012; Wang et al., 2020). Thanks to their diverse structures and multiple functions, they might help develop alternative treatments for difficult-to-treat cancers like glioblastoma, which is often resistant to standard therapies and leads to poor patient outcomes (Singh et al., 2025). In this study, GAG-containing *Dicentrarchus labrax* scale extracts (DLSE) demonstrated anticancer activity against U-87MG cells by reducing cell viability, inhibiting migration, and inducing apoptosis. The extracts also increased p53/p21 expression and activated ER stress pathways. These findings suggest that GAG-containing DLSE is a promising marine-derived option that could complement existing cancer treatments.

GAG-containing DLSE treatments resulted in a marked decrease in glioma cell viability and migratory capacity, consistent with previous studies demonstrating that marine-derived extracts exhibit antiproliferative and anti-motility effects against cancer cells. Extracts from the skins of *Balistes capriscus* (gray triggerfish) and *Mustelus* species have been shown to inhibit the growth of HCT116 colon cancer cells by more than 70% (Krichen et al., 2017). Similarly, extracts from sea cucumber body walls were found to suppress the proliferation of A549 lung adenocarcinoma cells (Lin et al., 2020). hGAG from *Holothuria leucospilota* inhibited platelet-mediated adhesion and migration of MDA-MB-231 cells, supporting its anti-metastatic potential (Qian et al., 2015). Compared with our findings, the lower IC_{50} values or effective concentrations reported in these studies may be attributed to the use of purified or highly enriched GAG fractions, whereas DLSE is a GAG-containing extract. In addition, both doses of DLSE blocked glioma cell migration; however, the effect observed at the IC_{50} dose may be partly due to cytotoxicity as well as the anti-motility effect. An evaluation of these study findings, along with supporting reports, suggests that marine-derived extracts might influence cell-matrix interactions and signaling pathways that control cellular invasion.

Beyond their cytostatic and anti-migratory properties, DLSE induced apoptosis, as shown by Hoechst/PI staining and changes in apoptotic marker expression. The increased Bax/Bcl-2 ratio, along with caspase-3 and PARP-1 cleavage, confirms activation of the intrinsic apoptotic pathway, a therapeutically advantageous mechanism in glioblastoma, given the tumor's significant resistance to apoptosis (Houy & Le Grand, 2018). Additionally, the observed upregulation of p53 and its downstream effector p21 suggests that DLSE not only triggers cell death but may also promote cell-cycle arrest, thereby reducing tumor growth. Supportively, several marine-derived compounds have been reported to exhibit anticancer effects: sea cucumber extract promotes apoptosis in lung cancer cells by regulating Bax, caspase-3, Bcl-2, and survivin (Lin et al., 2020); fucoidan from brown seaweed induces p53-mediated apoptosis in gastric and colorectal

cancers (Atashrazm et al., 2015); sturgeon-derived chondroitin sulfate (CS) inhibits colorectal cancer cell growth via the mitochondrial Bcl-2 pathway (Wu et al., 2022); and a chitosan-CS scaffold impacts prostate cancer by modulating epithelial-to-mesenchymal transition markers (Xu et al., 2020). Together, these studies emphasize the therapeutic potential of marine-derived polysaccharides in targeting multiple tumor-promoting mechanisms, supporting the promise of DLSE as a candidate for glioblastoma therapy (Newman & Cragg, 2020; Carrasqueira et al., 2025).

Another important finding was that DLSE induces ER stress, indicated by higher levels of Grp78, PERK, IRE1α, and the cleaved form of ATF6. Activation of these unfolded protein response (UPR) sensors suggests that glioblastoma cells experience ER stress after treatment, which could lead to apoptosis if the UPR's adaptive capacity is overwhelmed. This mechanism is especially significant because targeting ER stress pathways has been proposed as a promising therapeutic approach for glioblastoma, where metabolic and proteostatic imbalances are common (Morla, 2019). Similar observations have been documented for other marine-derived compounds. For example, the small molecule MHO7 was shown to induce immunogenic cell death in triple-negative breast cancer through the ER stress–CHOP axis (Wen et al., 2022). Additionally, a nudibranch extract was found to selectively chemosensitize colorectal cancer cells via ROS-mediated ER stress and UPR activation (Ruiz-Torres et al., 2021). Likewise, extracts from the starfish *Marthasterias glacialis* promoted both apoptosis and cell cycle arrest in neuroblastoma cells by increasing ER stress and CHOP expression (Pereira et al., 2013). The dose-dependent activation of ER stress markers observed in our study further supports the idea that marine GAG-like extracts may target this cellular vulnerability to induce tumor cell death; however, their causal role in apoptosis should be confirmed by inhibitor or gene-silencing studies.

Although DLSE showed anticancer activity in glioblastoma cells, several limitations should be considered. Because DLSE was used as a crude GAG-rich extract, the active GAG fraction remains unidentified, an important limitation. The relatively high IC_{50} value also underscores the need for further purification, fractionation, and structural characterization. In addition, the study was limited to a single glioblastoma cell line (U-87MG). Because U-87MG cells are considered p53 wild-type, it remains unclear whether these findings can be extrapolated to p53-mutant glioblastoma, a clinically more challenging subgroup. Future studies should therefore include additional glioblastoma models, particularly p53-mutant cells. Moreover, the absence of a non-cancerous control cell line, such as human fibroblasts or HEK293 cells, limits the evaluation of DLSE selectivity and safety; demonstrating tumor-specific cytotoxicity would increase the clinical relevance of the findings. Since glioblastoma is a central nervous system tumor, BBB permeability represents a major translational limitation for DLSE (Jin et al., 2021). Therefore, future studies should evaluate BBB permeability and investigate specialized brain-targeted delivery strategies, including nanoparticle-based formulations and exosome-mediated delivery. Furthermore, mechanistic studies using pathway inhibitors, gene silencing, or knockdown approaches are needed to clarify the causal roles of ER stress, apoptosis, and p53/p21-mediated cell-cycle pathways. Finally, validation in three-dimensional cultures, BBB models, and *in vivo* glioblastoma models will be necessary to strengthen the translational relevance of DLSE.

Conclusion

This study demonstrates that a GAG-rich *Dicentrarchus labrax* scale extract exerts anticancer effects on glioblastoma cells by reducing viability and migration, inducing apoptosis, activating caspase-3 and PARP1 cleavage, upregulating p53/p21 signaling, and triggering ER stress-related pathways. These findings indicate that marine-derived GAG-like extracts may serve as valuable bioactive sources for glioblastoma-related anticancer research. Although further studies on confirmation and molecular-mechanism experiments in 2D- and 3D-cultures, as well as *in vivo* validation, are required, the present results provide an important preliminary basis for the future development of marine-derived GAG-based products with potential biomedical and therapeutic applications.

Ethics

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: Data available upon reasonable request.

Footnotes

Authorship Contributions: Conceptualization: M.P.; Design/methodology: Y.S. and M.P.; Execution/ investigation: Y.S., N.B.A., A.Ş., Er.Y.; Resources/materials: Em.Y.; Data acquisition: Y.S., N.B.A., A.Ş., Er.Y.; Data analysis/interpretation: Y.A. and A.Ş.; Writing – original draft: Y.A., N.B.A., Er.Y.; Writing – review & editing/critical revision: Y.A., A.Ş, Em.Y., M.P.

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