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Original research article

Anti-proliferative and apoptotic effects of ethanolic extracts of the seaweed *Caulerpa lentillifera* against human glioblastoma cells

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ABSTRACT

Caulerpa lentillifera (CL), or sea grapes, is a widely consumed as food in Asia. Our previous study showed the anti-proliferative and apoptosis effects of the hexane extract of this seaweed. We investigated the effects of the ethanolic extract of this seaweed (CLET) on the in vitro proliferation and apoptotic cell death in A172 human glioblastoma cells using MTT, CFSE, and Annexin V-FITC assays. CLET at 500 µg/ml reduced A172 cell viability by inducing apoptotic cell death, while lower concentrations of CLET reduced proliferation of A172 cells. The present study suggests that CLET could inhibit proliferation and promote apoptotic cell death in human GBM cells, suggesting its potential to be used as an alternative treatment for glioblastoma.

Keywords: *Caulerpa lentillifera,* seaweed, glioblastoma, proliferation, apoptosis

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1. Introduction

Marine seaweeds are valuable sources of nutrients, including proteins, vitamins, minerals, fatty acids and polysaccharides and are extensively used as functional foods.[1] Marine seaweeds synthesize various bioactive compounds with potential anticancer activity, including carotenoids, flavonoids, sulfated polysaccharides and terpenoids.[2,3] *Caulerpa lentillifera* (CL) is an edible green marine macroalga that is cultivated as food and for consumable products in tropical regions, including Thailand. CL is regarded as a valuable nutrition source of marine origin. The nutritional content of CL shows high amounts of essential amino acids, equivalent to egg proteins, vitamin B1, vitamin B2, vitamin E, palmitic acid, iodine, phosphorus, calcium, magnesium and copper.[4] CL extracts have shown high antioxidant activity determined by DPPH and ABTS assays.[5] CL extracts suppress inflammation mediators and induce DNA damage in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.[6]

Glioblastoma (GBM) is the most aggressive malignant tumor of the central nervous system. Although conventional chemotherapeutics for GBM are available, resistance to chemotherapy is still evident and may be due to the heterogeneity of GBM's cellular origin, leading to a low median survival time.[7] Recently, the discovery of novel bioactive components from natural sources has enabled alternative treatment or prevention of GBM to be investigated. Various metabolites from *Caulerpa* spp. have distinct anti-tumorigenic activities.[8] Our previous study presented the anti-proliferation and apoptosis-inducing effects of the CL extract in a hexane solvent fraction against A172 human GBM cells.[9] This study aimed to evaluate the in vitro anti-proliferation and cellular apoptosis effects of the ethanolic extracts of CL (CLET) in A172 human GBM cells.

2. Materials and Methods

2.1 Preparation of seaweed extracts

CL was cultivated and collected from the Phetchaburi Coastal Fisheries Research and Development Center, Phetchaburi, Thailand. Identification of species has been previously described.[6] Dried crude CL (1 kg) was macerated in 95% ethanol for a week and then evaporated. The extraction yield of the sample was 11.31%.

2.2 Cell culture

A172 human GBM cells were purchased from the American Type Culture Collection (ATCC, USA). Cells were cultured in DMEM containing 10% FBS and a 1% penicillin/streptomycin cocktail in an incubator with an atmosphere of 5% CO2 at 37°C.

2.3 Cytotoxicity

CLET was dissolved in dimethyl sulfoxide (DMSO). A172 cells were incubated for 24 h with CLET diluted in serum-free media at final concentrations of 10, 50, 100, 150, 200, 250, 500 and 1,000 µg/ml. The viability of treated cells was determined by methyl thiazolyl tetrazolium (MTT) assays. The reaction was measured by a spectrophotometer (Varioskan Flash Microplate Reader, Thermo Fisher Scientific) at the absorbance wavelengths of 562 and 630 nm.

2.4 Cell proliferation assay

A172 cells were labelled with CFSE dye (Sigma) in darkness at 37 °C for 10 minutes and then plated on 6-well plates. Cells were treated with CLET at 100 and 150 µg/ml for 48 h, then treated with the extract for another 48 h. The cells were trypsinized and resuspended in phosphate buffered saline (PBS). The fluorescence signal was measured using a Guava easyCyte flow cytometer (Merck Millipore, USA) at a minimum of 5,000 events/sample.

2.5 Apoptotic cell detection

A172 cells were treated with CLET

at a final concentration of 500 µg/ml for 24 h. Treated cells were collected and incubated with Annexin V-FITC (BD Biosciences) for 15 min in darkness at room temperature. The population of apoptotic cells was measured using a Guava easyCyte flow cytometer (Merck Millipore).

2.6 Statistical analysis

Data are expressed as mean ± SEM. Statistical variations of all experiments were analyzed by GraphPad Prism statistical analysis software (GraphPad Software Inc, USA) using one-way ANOVA test and a paired t-test. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Cytotoxicity

The effect of CLET on toxicity of A172 cells was analyzed by MTT assays. The maximal concentration was 1,000 µg/ml and the final concentration of DMSO was 0.25%. CLET at concentrations higher than 100 µg/ml showed mild toxicity to cells and reduced cell viability to 50% at the maximal tested concentration of 1,000 µg/ml (Fig. 1). Therefore, CLET at 100 and 150 µg/ml was selected for antiproliferative assays and CLET at 500 µg/ml was tested for apoptotic effects.

3.2 Cell proliferation

The effect of CLET on proliferation of A172 cells was analyzed by CFSE assays. The increased fluorescent signal detected in the assay indicated the disruption of cell division. At 96 h post-treatment, CLET at 100 and 150 µg/ml significantly increased the mean fluorescence intensity (MFI) of CFSE (Fig. 2). This suggested that low concentrations of CLET extract enhanced anti-proliferative effects against GBM cells.

3.3 Apoptosis induction

Apoptotic cell death after CLET exposure was determined by Annexin V staining. CLET treatment at 500 µg/ml in A172 cells significantly increased the apoptotic cell population, represented as Annexin V-stained cells compared to untreated and DMSO-treated cells (Fig. 3), indicating that high concentrations of CLET could induce apoptotic cell death in A172 cells.

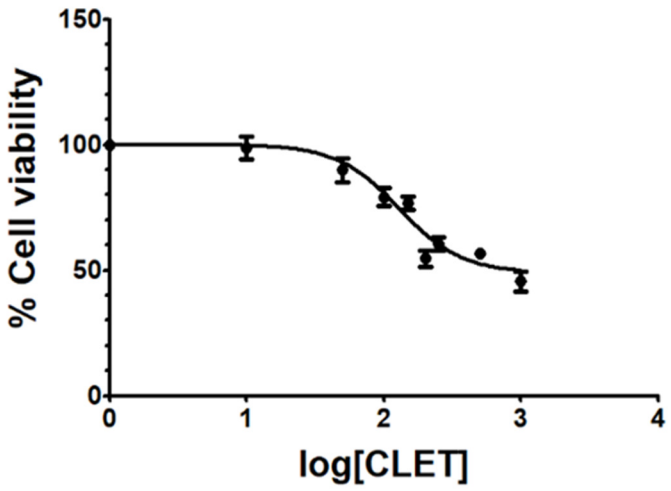

Fig. 1. Viability of A172 following CLET incubation for 24 h, detected by MTT assay (N = 5).

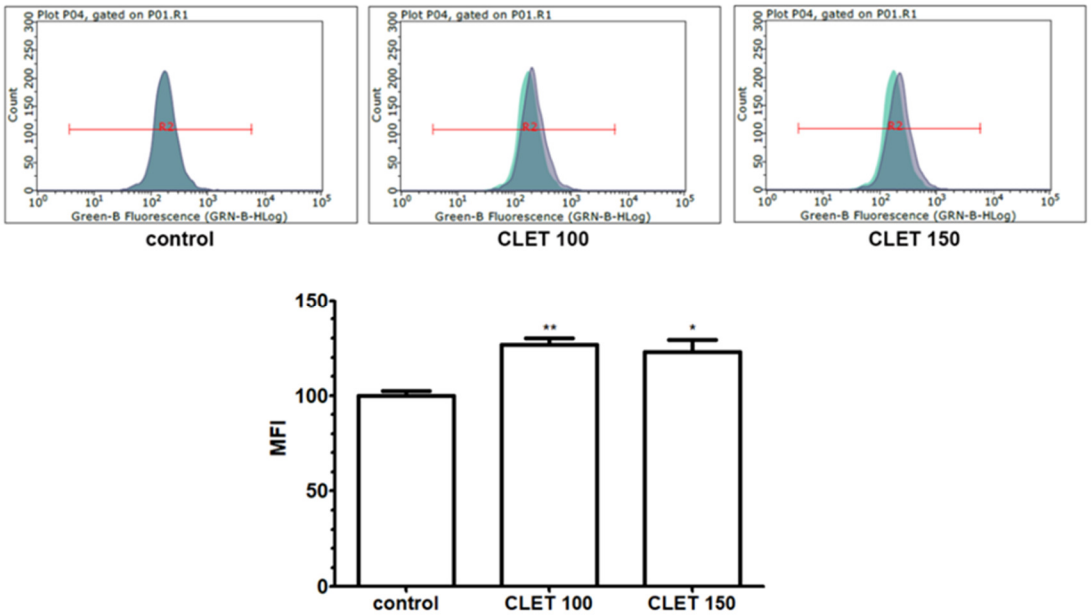

Fig. 2. Proliferation of A172 cells after CLET incubation at 100 and 150 µg/ml for 96 h measured by CFSE assay and flow cytometry. The bar graph presents the mean fluorescence intensities (MFI). * $p \leq 0.05$, ** $p \leq 0.01$ (N = 3).

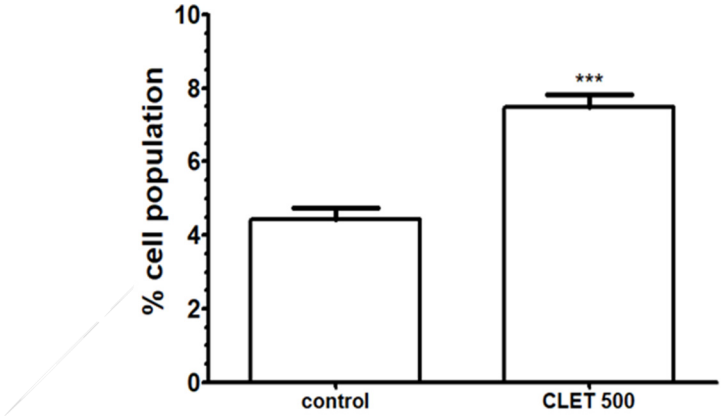

Fig. 3. Percentage of total apoptotic A172 cells after incubation with CLET for 24 h analyzed by Annexin V-FITC staining and flow cytometry (N = 3). ** $p \leq 0.01$.

4. Discussion

As algae have nutritional value for prevention of various chronic diseases, their bioactive metabolite may be beneficial by inhibiting cancer progression. Macroalgae *Caulerpa* spp., including CL, produced different secondary metabolites that interfered with multiple cancer hallmarks.[8]

We found that CLET decreased cellular proliferation and enhanced apoptotic cell death in A172 cells. CLET reduced the viability of A172 cells; however, the maximal effect was only an

approximately 50% decrease, indicating a lower cytotoxic effect than the hexane extract of CL.[9] The reduction of cell viability may be due to its antiproliferative effect. Low concentrations of CLET reduced the number of daughter cells, indicating the low dividing ability of the parent cells. This implied that CLET might interfere with the replication ability of A172 cells. On the other hand, the high concentration of CLET promoted cellular apoptotic changes in A172 cells. A previous study showed no cytotoxicity and genotoxicity of CLET in normal fibroblasts, keratinocytes, hepatocytes and macrophages up to 1,000 µg/ml.[5] This implied that CLET is safe for normal cells, although it is less toxic to A172 cancer cells than the hexane fraction.

Anticancer effects of bioactive metabolites from marine algae have been reported. Phenolic compounds are algal secondary metabolites enriched in C. *lentillifera* and other macroalgae species. These compounds promoted the major anticancer effects by inhibiting proliferation, enhancing antioxidation and inducing cell death.[10] Phenolic compounds in the methanolic extract of wireweed *Sargassum muticum* induced apoptosis, antioxidant and anti-angiogenesis effects in MCF-7 and MDA-MB-231 breast cancer cells.[11] The polyphenol derivative phloroglucinol, isolated from the brown seaweed *Ecklonia* Cava Kjellman, induced apoptosis in MCF-7 cells through the NF-κB pathway.[12] The total phenolic content in CLET consisted of 3.36 ± 0.52 mg gallic acid equivalents (unpublished data). The hexane extract of CL (CLHE), with an IC50 at 224.7 ± 5.21 µg/ml, contained higher amounts of total phenolic compounds (23.82 ± 0.32 mg GAE/g) than CLET.[8] The lower cytotoxicity of CLET compared to CLHE may be due to the lower amount of phenolic compounds. Hexane and ethanol have different polarities; therefore, bioactive compounds are likely to be different in terms of type and amount.

Polysaccharides are another bioactive compound of macroalgae and CL that affects cancer progression.[13] β-1,3-xylooligosaccharides isolated from CL reduced the viability of MCF-7 breast cancer cells and induced apoptosis by enhancing chromatin condensation and PARP expression.[14] Soluble polysaccharide fractions isolated from C. *racemosa* contained mainly sulfated polysaccharides and had antitumor activity against K562 and H22 tumors transplanted into mice.[15] Analysis of nutrient contents in CL from different regions, including Thailand, showed high amounts of total proteins and soluble dietary fibers.[16] It has been reported that algal proteins and dietary fibers have anticancer effects.[17] This implied that CL has various nutrients and secondary metabolites that may be beneficial as anticancer agents.

However, the maximal tested CLET concentration, which promoted 50% cytotoxicity, may be too high for further *in vivo* testing. The extract may instead be studied for synergistic combination with a conventional chemotherapeutic agent, possibly allowing the use of lower concentrations of the latter.

5. Conclusion

CLET could promote anti-proliferation and cellular apoptosis in A172 human GBM cells with less cytotoxic activity than CLHE. The seaweed may be used as a food supplement for the prevention of GBM cancer. The cellular and molecular effects of CLET on the inhibition of GBM proliferation and cell death, as well as tumor invasion, should be further investigated.

Acknowledgements

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Conflicts of Interest

The authors declare no conflict of interest.

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