Original Article

Growth Inhibitory Activity of *Brassica oleracea* var. Alboglabra on Human Gastric Cancer Cells

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Abstract

Objectives: The aim of this study is to investigate anticancer activity of *Brassica oleracea* var. *alboglabra* (BOA) against the proliferation of BGC-823 human gastric cancer cells. **Materials and Methods:** *B. oleracea* var. *alboglabra* was extracted by ethanol 98% at a solid-to-liquid ratio of 1:8, (w/v) for 24h at room temperature. The cytotoxic effect of vegetables was examined by MTT assay. The migration of the cancer cells was conducted by wound healing assay and visualized under an inverted microscope. The mRNA expression level was quantified by real time PCR. **Major Findings:** It was found that ethanol extract of BOA exhibited the inhibitory activity against the proliferation of BGC-823 cells at IC_{50} value of 217.6 ± 2.8 µg/ml. Moreover, the treatment of BOA extract at concentration of 100 µg/ml for 24 h significantly suppressed the migration of gastric cancer cells into the gap as compared to the untreated cell group. Notably, the cytotoxic effect of BOA extract on human gastric cancer cells was found due to induction of apoptosis, mediating the up-regulation of caspase-8, -9, -3, and Bax in cancer cells. **Conclusion:** These results indicated that *B. oleracea* var. *alboglabra* have the potential inhibitory activity against the development of gastric cancer.

Keywords: *Anticancer, apoptosis, BGC-823 cells, Brassicaceae, vegetables*

Introduction

In last decades, several types of cancers have been the top cause of death all around the world. Cancer treatment involves time, money and endures that can last for months or even years, and requires several types of drugs, therapies, surgeries. Therapies such as radioactive and chemotherapy not only destroy cancer cells but also damage healthy cells and tissues, which undoubtedly weaken the patients and worsen their overall health status.[1] Thus, scientists and physicians have been studying new methods with higher efficiency, lower cost, and lower damage to patients. Dietary products are considered as potential factors in preventing, suppressing cancers, and supporting treatments.[2] Especially, vegetables plants have a various nutrients and secondary metabolites which give additional benefits to human health status and prevent numerous chronic diseases, such as cancer.[3]

Brassicaceae vegetables, a common part of human diet, are consumed by people around the world. Especially, *Brassicaceae* vegetables contain various phytochemicals such as amino acids, minerals, vitamins, polyphenols, flavonoids, carotenoids, alkaloids, phytosterols chlorophyll, glucosinolates, terpenoids, and glucosides.[4] Thus, high intake of these vegetables can attenuate the cancer risk, cardiovascular, and other chronic diseases.[5,6] Among *Brassicaceae* vegetables with health benefits, *Brassica oleracea* var. *alboglabra* has been focused to study its health-promoting phytochemicals and antioxidant capacity.[7,8] *B. oleracea* var. *alboglabra* has been found as a potential source of glucosinolates, which exhibit anti-carcinogenicity by inhibiting the production of endogenous and exogenous carcinogens as well as inducing antioxidant.[7-9] Thus, *B. oleracea* var. *alboglabra* may be suggested to possess the inhibitory activity against several types of cancer. However, the cytotoxicity of these vegetables on gastric cancer cells should be further investigated. For the first time, this study was proposed to investigate the anticancer activity of *B. oleracea* var. *alboglabra* against gastric cancer cells. Its activity was evidenced via inhibition of cell proliferation, suppression of cell migration, and induction of cell apoptosis. The result of this study will indicate new target of

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B. oleracea var. *alboglabra* toward gastric cancer cells, supporting its potential role in prevention and treatment of various cancers.

Materials and Methods

Materials

B. oleracea var. *alboglabra* was obtained from local greengrocer in Ho Chi Minh city, Vietnam. Human gastric cancer cells (BGC-823 cells) were donated by Chinese Academy of Sciences (Shanghai, China). Reagents for real time PCR were purchased from Qiagen (Hilden, Germany). Primers for qPCR were purchased from Intergrated DNA Technologies, Iowa, USA. The other one were purchased from Sigma-Aldrich (MO, USA).

Extraction

B. oleracea var. *alboglabra* (BOA) was air-dried under shade and the powder was extracted by ethanol 98% at a solid-to-liquid ratio of 1:8, (w/v) for 24h at room temperature. The ethanol extract with the humidity level of less than 12% were then hold at 4°C for further investigation.

Cytotoxic assay

The inhibitory effect of ethanol extract of BOA on BGC-823 cells was investigated by MTT method.[10] Briefly, BGC-823 cells were cultured into 96-well plates (2×10^5 cells/ml) before in

cubated with the extract for 24 h at different concentrations. The medium was then changed by 100 µl of MTT solution at final concentration of 0.5 mg/ml. After 4 h of incubation, the formed formazan salt was solubilized by DMSO (100 µl), and optical density was measured by a microplate reader (BioTek Instruments, USA) at 540 nm.

Inverted microscope observation

BGC-823 cells were cultured at density of 2×10^5 cells/ml before treated with 200 µg/ml of BOA extract for one day. The cells were washed by PBS and the morphology was visualized under an inverted microscope (Oxion, Euromex, Netherlands).

Cell migration assay

This assay was conducted as reported by Kwak and Ju.[11] Briefly, a monolayer of BGC-823 cells were scratched by a yellow tip. Subsequently, the cells were treated with BOA extract at concentration of 100 µg/ml for one day. Gap area was visualized under an inverted microscope (Oxion, Euromex, Netherlands).

Real time PCR

BGC-823 cells (2×10^5 cells/ml) were treated with 100 µg/ml of BOA extract for a day. Total RNA from the treated cells was isolated by a commercial kit (Qiagen, Hilden, Germany). Subsequently, cDNA synthesis was performed according protocol of NEB (MA, USA). Each cycle of qPCR was conducted under the conditions of denaturation of 94 °C (30 s), annealing of 60 °C (30 s), and extension of 72 °C (30 s). The mRNA expression level of relative gene in the treated cells was respectively compared with that of the blank (without treatment). Primer for caspase-3 (F: TCGCTTTGTGCCATGCTGAA and R: ACTCAAATTCTGTTGCCACC); for caspase-8 (F: AATGGAACACACTTGGATGC and R: GCTCTACTGTGCAGTCATCG); for caspase-9 (F: TTGAGGACCTTCGACCAGCT and R: CAACG TACCAGGAGCCACTC); for Bax (F: CTGACGG CAACTTCAACTGG and R: CCAATGTCCAG CCCATGATG); for GAPDH (F: GGGCTCTCCA GAACATCATC and R: GGTCCACCACTGACA CGTTG).

Statistical analysis

The ANOVA test of SPSS was used for analysis of data. Tukey's multiple range test was further assessed to identify statistical differences among groups at $p < 0.05$.

Results

The inhibitory effect of BOA extract on cell proliferation

To determine the inhibition of BOA extract on proliferation of human gastric cancer cells, BGC-823 cells were exposed by different dose of BOA extract for one day and MTT assay was conducted to identify the cytotoxic effect of BOA extract on the cells. The treatment of BOA extract caused a substantial suppression of cell growth at a concentration-dependent manner [Figure 1A]. The inhibitory effect of BGC-823 cell growth was determined IC_{50} value of 217.6 ± 2.8 µg/ml. Moreover, cell morphology was observed to be changed as compared with the cells in blank group [Figure 1B]. The cell size was reduced, and cell boundary was disrupted as compared with the blank cells. It indicated that BOA extract could inhibit proliferation and cause injury and death in gastric cancer cells.

The suppression of BOA extract on cancer cell migration

In order to determine the inhibition of BOA extract on the migration of cancer cell, a cell-free area was conducted by a yellow pipette tip on a monolayer of BGC-823 cells. It was observed that the induction of the cell-free area caused the migration of the gastric cancer cells onto the gap area in the untreated cell group after 24 h culture [Figure 2A & B]. However, the treatment of BOA extract at concentration of 100 µg/ml meaningfully suppressed the migration of gastric cancer cells onto the gap area as compared to the untreated cell group [Figure 2C & D]. Thus, BOA extract was suggested to inhibit extracellular matrix production, causing suppression of the migration of gastric cancer cells.

Effect of BOA extract on apoptotic signaling molecules

To further clarify the inhibition of BOA extract on gastric cancer cell proliferation, expression of genes involved in

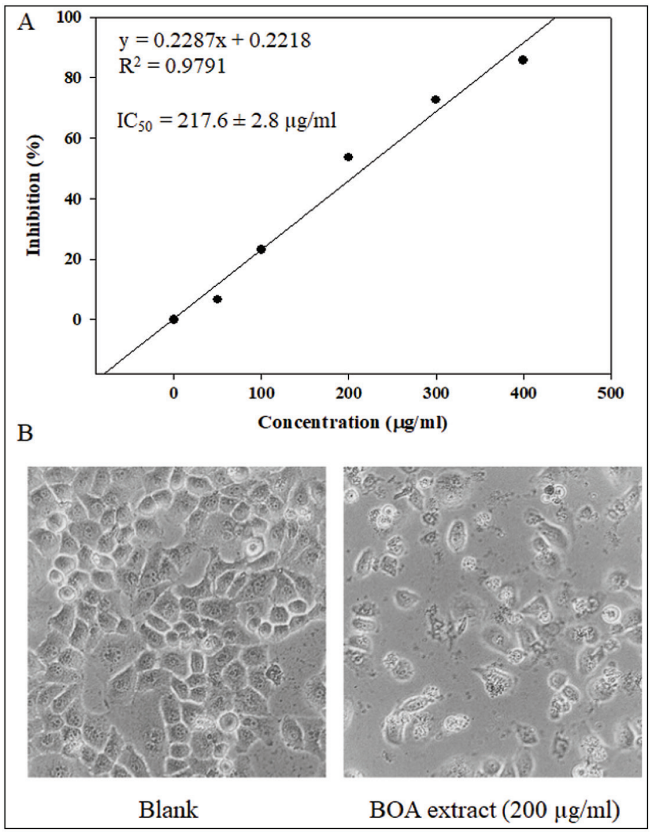

Figure 1: The inhibition of BOA extract on BGC-823 cell proliferation (A) and cell morphological change (B). (A) BGC-823 cells were exposed by various doses of BOA extract for one day and the IC_{50} value was indicated by a linear regression equation. (B) BGC-823 cells were treated with BOA extract for one day and cell morphology was then visualized under an inverted microscope (10x magnification).

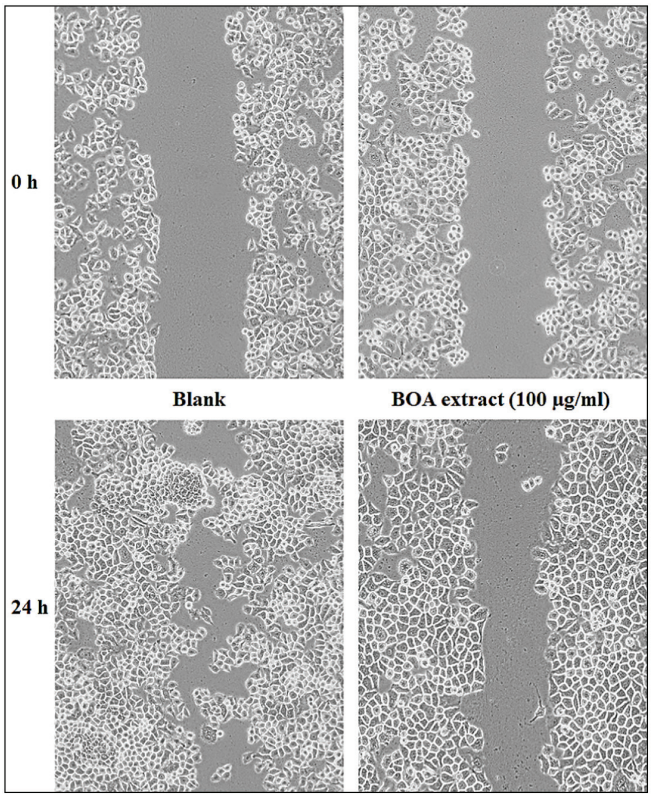

Figure 2: The suppression of BOA extract on cell migration. BGC-823 cells were cultured on plates before a gap space was made by a yellow pipette tip. Cells were subsequently exposed by BOA extract for one day and the images of gap space were visualized under an inverted microscope (10x magnification)

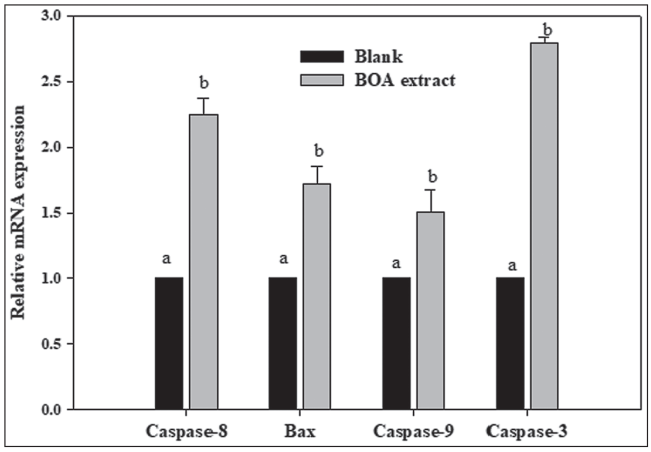

Figure 3: Apoptosis induction in BGC-823 cells by BOA extract. The cells were exposed by BOA extract for one day and total RNA was then collected. The expression levels of caspase-8, -9, -3, and Bax was quantified by qPCR. GAPDH was considered as an internal control. For each group, different letter (a-b) indicates significant difference at p<0.05

apoptosis process was measured by qPCR. The treatment of BOA extract at concentration of 100 µg/ml for one day up-regulated the mRNA expression level of caspase-8, -9, -3, and Bax [Figure 3]. The expression levels of these genes in the treated cells were 2.2-fold, 1.7-fold, 1.5-fold, and 2.8-fold higher than that of the untreated cells, respectively. These findings suggested that BOA extract possesses the inhibitory effect on cell proliferation via induction of apoptosis process in gastric cancer cells.

Discussion

Dietary products play an significant role in prevention as well as management of cancers.[12] For the past decades, numerous edible plants have been found due to their potential anticancer activity.[13] It has evidenced that a healthy diet rich in vegetables and fruits can lower the risk of various cancers.[14,15] Notably, *Brassicaceae* plants, common vegetables consumed worldwide, are known to contain various micronutrients, macronutrients, and secondary metabolites.[4,16] These phytochemicals can contribute to their inhibitory activity against a broad range of cancer cells.[17,18] In this study, ethanol extract from *B. oleracea* var. *alboglabra* extract was determined to suppress the growth of gastric cancer cells at IC_{50} value of 217.6 ± 2.8 µg/ml. So far, several other *Brassicaceae* vegetables have been reported for their anticancer effect. Particularly, the methanol extract of cabbage leaves (*B. oleracea* L. var. *capitata* f. *rubra*) was shown to inhibit Hela cells and HepG2 at IC_{50} values of 23.4 mg/ml and 28.7 mg/ml, respectively.[19] According to Barakat and colleagues, aqueous extract of *B. rapa*

roots inhibited against the growth of Hela cells (64%) and murine adenocarcinoma cells (63%) at concentrations of 10 mg/ml and 1.25 mg/ml, respectively.[20] Ethanol extract of *B. oleracevaar acephala* inhibited HeLa cells growth at IC_{50} value of 170.7 µg/ml.[21] *B. nigra* ethanolic extract was found to inhibit A549 and H1299 at IC_{50} values of 32 and 25.4 µg/ml for 72 h treatment, respectively.[22] Besides, the 70% ethanol extract of *B. juncea* leaves also inhibited HCT116 human colon cancer cells at IC_{50} value of 253 µg/ml at 72 h time point.[23] Accordingly, anticancer activity of BOA extract is more potential than that of *B. oleracea* L. var. *capitata* f. *rubra*, *B. rapa*, and *B. juncea*. Notably, vegetables species of *Brassicaceae* was reported to contain various vital compounds, especially glucosinolates and phenolics. These compounds may be responsible for their inhibitory activity against a wide range of cancer types.[17] Thus, *B. oleracea* var. *alboglabra* was suggested to comprise of these secondary metabolites that promote its inhibition on the growth of human gastric cancer cells.

Anti-cancer therapies not only target to cell proliferation, but also focus on suppression of cell migration and invasion. The cancer metastasis is due to moving away from initial side and invading blood circulation that is responsible for 90% of cancer deaths.[24,25] Thus, the prevention of cancer cell migration significantly contributes to the blockade of cancer metastasis. Herein, *B. oleracea* var. *alboglabra* also exhibited suppressive effect on migration of gastric cancer cells at a dose of 100 µg/ml. It was evidenced that downregulation of matrix metalloproteinase expression leads to the inhibition of extracellular matrix degranulation, and subsequent suppression of cancer cell migration.[26-28] Moreover, the inactivation of transcription factors including nuclear factor (NF)-κB and activator protein-1 via blocking mitogen-activated protein kinase (MAPKs) and focal adhesion kinase (FAK) pathways also interferes cancer cell migration..[29] As the result, the inhibition of BOA extract on gastric cancer cell migration may be suggested due to downregulation of matrix metalloproteinase expression as well as MAPKs and FAK pathways.

Apoptosis is a caspase-mediated programmed cell death to eliminate selectively unnecessary cells.[30] The activation of caspase (-8, -9, and -3) and Bax initiates the process of cell apoptosis.[31,32] Therefore, trigger of apoptosis process is regarded as a promising strategy for cancer chemotherapy and is useful indicator for screening of potential anticancer agents derived from natural products.[33] In this study, the treatment of BOA extract for 24 h could augment the mRNA expression level of caspase-8, -9, -3, and Bax in human gastric cancer cells. It was reported that the anti-cancer effect of various plant extracts, such as *Brucea javanica*, *Camellia sinensis*, *Cinnamomum kanehirai* Hayata, *Corni Fructus*, *Cucurbita ficifolia*, *Cyperus rotundus* L. was due to induction of apoptosis via up-regulating the expression level of caspase-8, -9, -3, and Bax in cancer cells.[34] Hence, the inhibition of BOA extract against gastric cancer cell growth was suggested due to promotion of apoptosis in gastric cancer cells, thus resulting in the cell death.

Conclusion

In this study, anticancer activity of *B. oleracea* var. *alboglabra* againt human gastric cancer cells has been evidenced on *in vitro* experimental model. These results suggested that crude extract of this vegetable can be developed as a novel component of potential products that is used for the prevention and/or treatment of gastric cancer. However, the further studies should be proposed due to identification of bioactive compounds as well as evaluation of its mechanism of action on cell migration inhibition.

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Conflict of interest

There is no conflicting interest among authors.

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