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Original Article

Suppression of MCF-7 Breast Cancer Cell Multiplication by *Alismatis rhizoma* Extract

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The aim of this study was to investigate the inhibitory effects of *Alismatis rhizoma* extract on breast cancer cell growth and elucidate the underlying mechanisms. The growth inhibitory effects of *Alismatis rhizoma* extract on breast cancer cells were measured using the WST-1 assay, while its impact on relevant proteins and genes was analyzed using real-time polymerase chain reaction. The results demonstrated significant inhibition of breast cancer cell growth by *Alismatis rhizoma*, with the inhibitory effect showing a positive correlation with the dosage, treatment duration, and quantity. Furthermore, *Alismatis rhizoma* extract markedly decreased the expression levels of cell cycle protein D1 and suppressed cell migration and invasion. In conclusion, *Alismatis rhizoma* exhibits the potential to inhibit breast cancer cell growth, possibly by regulating cell cycle progression and inhibiting cell migration and invasion. These findings suggest that *Alismatis rhizoma* extract possesses anticancer properties against human breast cancer cells. Further investigation of its reuglation of action will provide foundational data, potentially paving the way for its development as an anticancer therapeutic agent.

Key Words: *Alismatis rhizome* Extracts, Breast cancer, MCF7, Cell growth

INTRODUCTION

Cancer is a serious disease with an increasing incidence worldwide (Giaquinto et al., 2022). According to the World Health Organization, approximately 18 million people are diagnosed with cancer each year, resulting in approximately nine million deaths (Frick et al., 2023). Cancer has emerged as a major health priority worldwide because of its high incidence and mortality rates (Ji et al., 2023). Breast cancer is one of the most common malignancies in women and poses a significant health threat (Baskar et al., 2012; Xu et al., 2023). Traditional cancer treatments include surgical resection, radiation therapy, and chemotherapy. However, these methods have limitations (Baskar et al., 2012). Although surgical resection can completely remove tumors, it often fails to completely eradicate metastatic tumors (Elkaeed et al., 2021). Chemotherapy and radiation therapy are the mainstays of breast cancer treatment, effectively killing cancer cells; however, they have side effects and drug resistance issues, potentially causing damage to healthy cells and triggering various adverse effects (Kim et al., 2020a;

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Nawara et al., 2021). Therefore, identifying safer and more effective anticancer agents is imperative. *Alismatis rhizoma* is a natural medicine that is widely used not only for health maintenance but also for clinical treatment (Zhang et al., 2017). It possesses various pharmacological properties, such as diuretic, antiedematous, anti-inflammatory, and antioxidant effects, and is known to have significant efficacy in the treatment of renal diseases and edema (Wang et al., 2014). Furthermore, some studies have suggested the potential tumor-suppressive effects of *Alismatis rhizoma*. However, the inhibitory effects of *Alismatis rhizoma* on breast cancer cells and its mechanisms of action remain unclear (Kim et al., 2022). Therefore, the aim of this study was to evaluate the anticancer effects of *Alismatis rhizoma* on breast cancer cells and to elucidate its possible mechanisms of action. Experimental treatment of breast cancer cells with *Alismatis rhizoma* allowed us to understand its impact on cell proliferation and survival, as well as to analyze the expression levels of relevant proteins and genes to elucidate its mechanism of action. Thus, a deeper understanding of the potential efficacy of *Alismatis rhizoma* in breast cancer treatment could be achieved, laying the groundwork for the development of new therapeutic strategies. This study aimed to present new ideas and possibilities for breast cancer treatment, with the expectation of providing safer and more effective treatment options for patients. Moreover, a better understanding of the anticancer mechanism of *Alismatis rhizoma* could broaden its application to other tumor treatments. Therefore, the results of this study have significant clinical relevance and potential applications. In this study, we experimentally validated the anticancer effects of *Alismatis rhizoma* on breast cancer cells and elucidated its regulation of action to propose new ideas and possibilities for breast cancer treatment.

MATERIALS AND METHODS

Cell culture and preparation of *Alismatis rhizoma* extract

The MCF7 cancer cell line was acquired from the American Type Culture Collection (Rockville, MD, USA). MCF7 cells were cultured in RPMI1640 (Welgene, Korea) and Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum (Corning Cellgro, USA) and 1% antibiotics (Invitrogen). The cells were detached using Trypsin-EDTA (Sigma) and maintained at 37℃ in a humidified atmosphere containing 5% CO_2. Cancer cells were plated in six-well plates and treated with *Alismatis rhizoma* extract (0~1,200 μg/mL). *Alismatis rhizoma* was extracted using methanol as the solvent. The *Alismatis rhizoma* extract was obtained from the Korea Plant Extract Bank (Cheongju, Korea).

WST analysis

MCF7 cells were seeded in 96-well culture plates at a density of 3×10^3 cells per well. After 24 h, cells were treated with ARE for an additional 24 h. Subsequently, the WST-1 plus cell proliferation assay reagent (GenDEPOT, TX, USA) was added to each well. The cell viability was assessed using an ELISA plate reader at a wavelength of 450 nm.

Total RNA extraction and reverse transcription-polymerase chain reaction

RNA extraction was conducted using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturers protocol. Reverse transcription polymerase chain reaction was performed using a reverse transcription system (TOYOBO, Tokyo, Japan). PCR amplification was performed according to instructions provided in the Ex Taq manual (TaKaRa Bio, Kyoto, Japan). Real-time PCR was performed using SYBR Premix Ex Taq (Clontech Laboratories, Mountain View, CA, USA) on an ABI instrument (Applied Biosystems). β-actin was used for normalization of all results.

Statistical analysis

The data were presented as mean ± SEM for statistical evaluation. Student's *t*-test and one-way analysis of variance were used to analyze the statistical significance of differences among treatment groups using GraphPad Prism software (version 6; GraphPad Software Inc., La Jolla, CA). Statistical significance was denoted as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

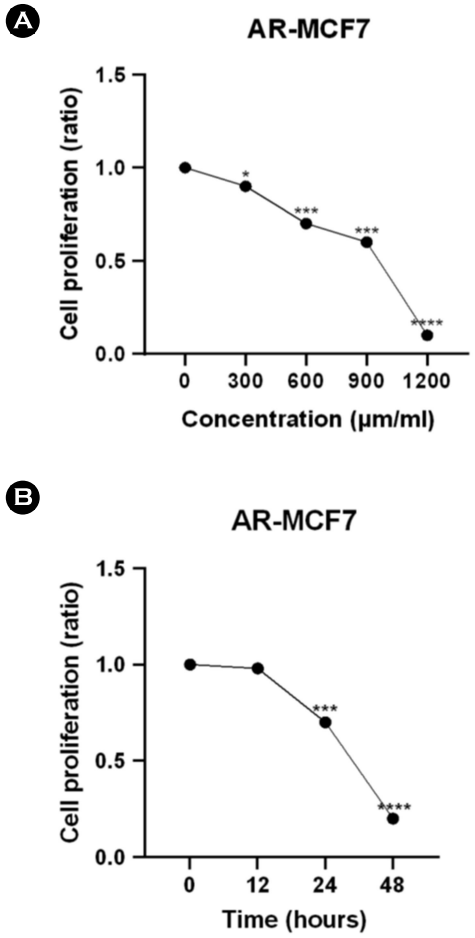

Fig. 1. Effect of *Alismatis rhizome* extract on proliferation of MCF-7 in dose dependent manner and a time dependent manner. (A-B) The cells were exposed to varying concentrations of the extract, including 0 μg/mL (control group), 300 μg/mL, 600 μg/mL, 900 μg/mL and 1,200 μg/mL. Cells were exposed to this concentration at different time points (0 hours, 12 hours, 24 hours and 48 hours). Cell metabolic activity was measured using the WST-1assay, revealing time-dependent variations.

RESULTS

The dose-dependent and time-dependent impact of *Alismatis rhizoma* extract on MCF-7 proliferation

To observe the inhibitory effect of *Alismatis rhizoma* extract on MCF-7 cell proliferation, we used the WST-1 assay, a commonly used method for assessing cell viability and cytotoxicity. In this study, the WST-1 assay was used to evaluate the inhibitory effects of the natural compounds on breast cancer cells (Fig. 1). A significant dose-dependent inhibition of breast cancer cells treated with *Alismatis rhizoma* extract was observed. As the concentration of *Alismatis rhizoma* extract increased in the treatment groups of 0, 300, 600, 900, and 1,200 μg/mL, a gradual decrease in cell viability was noted. A time-course experiment was conducted to evaluate the inhibitory effects of *Alismatis rhizoma*. The WST-1 assay was performed at 0, 12, 24, and 48 hours. The results revealed that the inhibitory effects of *Alismatis rhizoma* on breast cancer cells gradually increased over time. The WST-1 assay results demonstrated a significant inhibitory effect of *Alismatis rhizoma* on breast cancer cells, with the inhibitory effect showing a close association with the concentration and duration of the *Alismatis rhizoma* treatment. These findings provide an important groundwork for further research on the anticancer mechanisms of *Alismatis rhizoma*.

Analysis of tumor suppressor and cytokine gene expression in MCF-7 cells treated with *Alismatis rhizoma* extract

In the present study, real-time PCR was used to investigate the inhibitory mechanisms of *Alismatis rhizoma* against cancer cells. Initially, we selected seven genes associated with various biological processes pertinent to cancer, including vascular endothelial growth factor (VEGF) essential for vascular growth and maintenance; epidermal growth factor receptor (EGFR) involved in cell growth, differentiation, and cancer; cell cycle protein D1 (Cyclin D1) closely related to cell growth, division, and regulation; (Montalto and De Amicis, 2020). Myc protein factor associated with cell growth and regulation; as well as cytokine groups related to cell growth, movement, immune regulation, and tissue repair (TGF-β and TNF-α) (Shibuya, 2011; Batlle and Massague, 2019; Kim et al., 2020b). Subsequently, we analyzed the expression levels of these genes in breast cancer cells after treatment with *Alismatis rhizoma* extract using qPCR. We investigated the expression levels of seven genes at 0, 6, 12, 24, and 48-hour intervals at five-hour intervals. As shown in Fig. 2, the expression levels of VEGF, EGFR, Myc, TGF, and F4/80 significantly decreased in breast cancer cells after treatment with *Alismatis rhizoma* extract. By contrast, Cyclin D1 and TNF-α exhibited an overall decreasing trend, although their relative expression

levels at 48 h were slightly higher than those at 24 h (Fig. 2). Overall, these tumor-suppressive proteins and tumor-related cytokines exhibited a decreasing trend over time, indicating that *Alismatis rhizoma* may suppress the growth of MCF-7 cells by promoting apoptotic pathways.

Inhibitory effects were observed at varying concentrations.

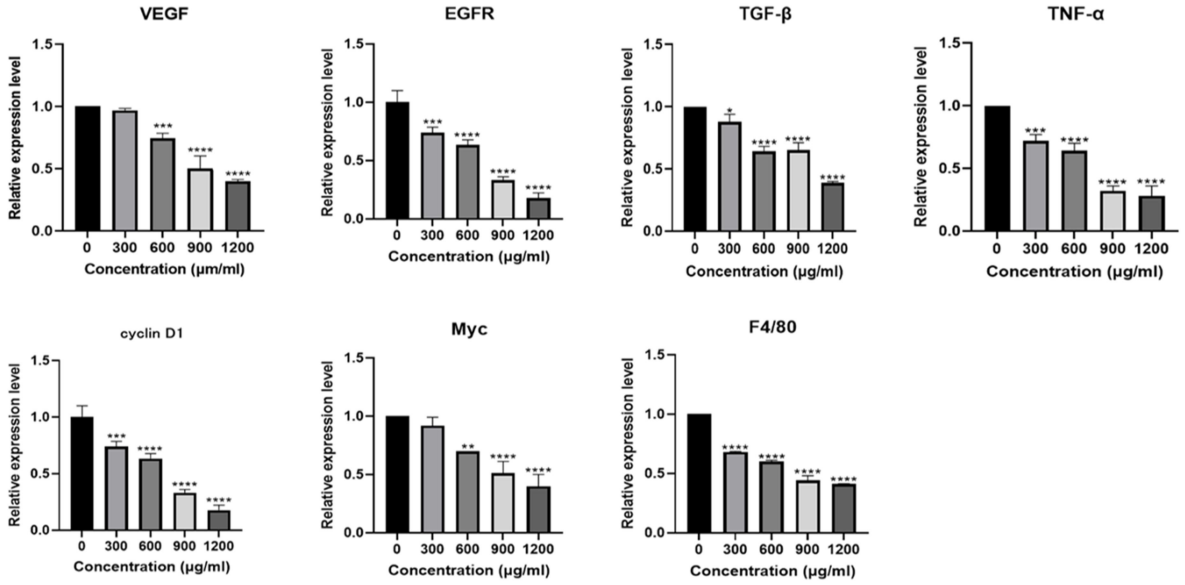

Fig. 2. Analysis with bar graph of tumor suppressor protein and tumor-related cytokine gene expression in MCF-7 cells treated with *Alismatis rhizome* extract in a dose dependent manner. *Alismatis rhizome* treatment on gene expression in cells. Following a 24-hour treatment of MCF-7 cells with *Alismatis rhizoma* extract at concentrations ranging from 0 to 1,200 μg/mL.

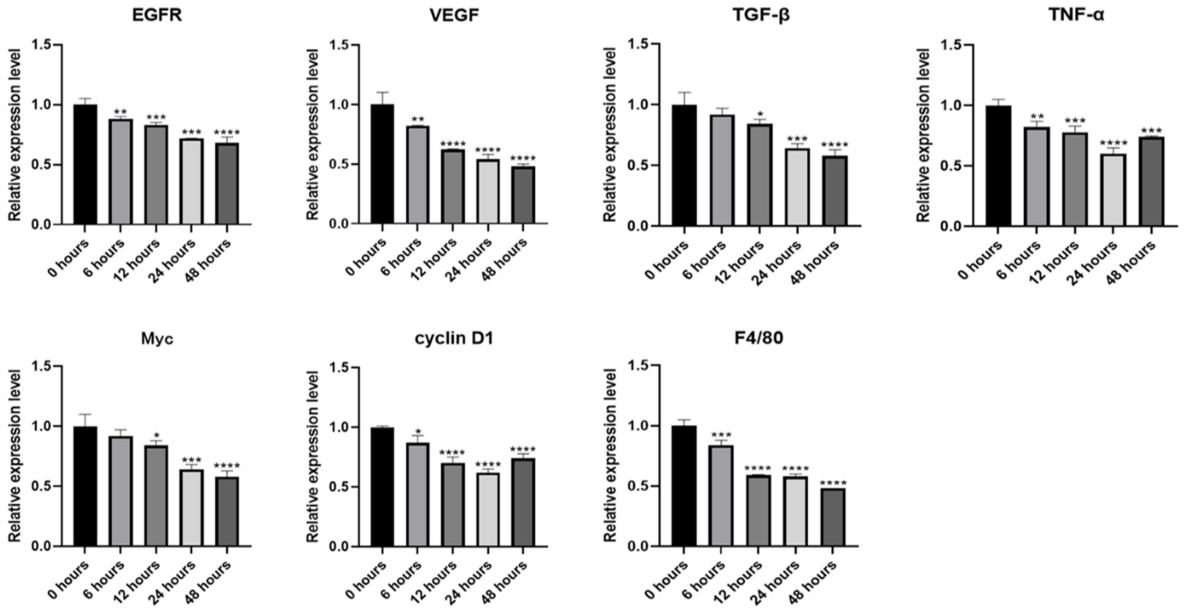

Fig. 3. Analysis with bar graph of tumor suppressor protein and tumor-related cytokine gene expression in MCF-7 cells treated with *Alismatis rhizome* extract in a time dependent manner. Through qPCR analysis, investigated the impact of *Alismatis rhizome* treatment on gene expression in cells. By comparing the gene expression levels between the experimental and control groups at different time points with AR 1,000 μg/mL.

As illustrated in Figs. 3, an increase in the concentration of *Alismatis rhizoma* extract resulted in a decrease in the expression of tumor-suppressive proteins and tumor-related cytokine genes, such as VEGF, EGFR, Cyclin D1, Myc, TGF-β, TNF-α, and F4/80. Using qPCR analysis, following a 24-hour treatment of MCF-7 cells with *Alismatis rhizoma* extract at concentrations ranging from 0 to 1,200 μg/mL, we observed significant changes in gene expression (Fig. 3).

DISCUSSION

The aim of this study was to evaluate the anticancer effects of *Alismatis rhizoma* on breast cancer cells and to elucidate its possible mechanisms of action. In the WST-1 assay, *Alismatis rhizoma* significantly inhibited the proliferation and survival of breast cancer cells. Real-time PCR analysis revealed alterations in the expression levels of relevant proteins and genes in breast cancer cells following treatment with *Alismatis rhizoma*.

Alismatis rhizoma has been widely used in clinical treatments as a herbal medicine and is known for its various pharmacological effects, including anti-inflammatory, antioxidant, and antitumor properties (Zhang et al., 2017; Bailly, 2022; Zhou et al., 2023). These findings suggest that the inhibitory effects of *Alismatis rhizoma* on breast cancer cells may be mediated by the regulation of protein and gene expression.

In this study, significant inhibition of proliferation and survival of breast cancer cells was observed following treatment with *Alismatis rhizoma*. This suggests that *Alismatis rhizoma* disrupts cell proliferation and survival by inhibiting cell cycle progression. Additionally, alterations in the expression of relevant proteins and genes were observed in breast cancer cells after treatment with *Alismatis rhizoma*. These changes may be associated with processes, such as cell death, cell cycle regulation, and cell signaling.

Further research is required to explore the molecular mechanisms and therapeutic potential of *Alismatis rhizomes*. A thorough investigation of the mechanism of action of *Alismatis rhizoma* could provide a better understanding of its inhibitory effects on breast cancer cells, thereby offering more evidence for its clinical application. Additionally, researchers could explore the efficacy of treatment with *Alismatis rhizoma* in combination with other anticancer agents to enhance efficacy and mitigate drug side effects.

However, it is important to note the several limitations of this study, as it primarily investigated the anticancer effects of *Alismatis rhizoma* on MCF7 cells and preliminarily explored its inhibitory mechanism.

First, this study exclusively examined the effects of *Alismatis rhizoma* on a single breast cancer cell line and did not assess its effects on other breast cancer cell lines. Furthermore, the inhibitory effects of *Alismatis rhizoma* have been limited to *in vitro* cell experiments and lack validation through *in vivo* animal studies and clinical trials. Therefore, additional research is needed to validate the *in vivo* inhibitory effects and safety profile of *Alismatis rhizoma*. Future studies should aim to further elucidate the *in vivo* inhibitory effects and safety of *Alismatis rhizoma*, validate its anticancer activity through animal models and clinical trials, expand the scope of research to evaluate the inhibitory effects of *Alismatis rhizoma* on different types of cancer cells, and explore the differences in their mechanisms of action.

Second, this study only observed cell proliferation and survival after treatment with *Alismatis rhizoma* extract, and further research is needed to investigate its effects on cell death, cell cycle regulation, and other aspects to elucidate the specific mechanisms of action. For instance, RNA sequencing or proteomic analysis is necessary to confirm protein changes and assess the regulatory effects of *Alismatis rhizoma* on key genes and proteins.

Thirdly, the concentration of *Alismatis rhizoma* was typically set at a high level. Since methanol was used as the solvent, the experiment was conducted by diluting it with the medium used for culturing MCF7 cells to reduce toxicity. When measuring cell viability, the experiment was initially conducted at a low dose, but cell viability remained high. For IC50 determination, the dose was set at 300 μg/mL, and since the IC50 was determined to be around 600 μg/mL, the range was extended to 1,200 μg/mL. However, when conducting experiments with mouse diets, the doses were limited to 600 μg/mL and 900 μg/mL to minimize potential *in vivo* toxicity.

Finally, it is necessary to explore the enhanced anticancer

effects through synergistic effects with other compounds or drugs by combining *Alismatis rhizoma* with different anticancer agents. In conclusion, the findings of this study suggested that *Alismatis rhizoma* has the potential to inhibit the proliferation and survival of breast cancer cells. Despite these limitations, this study provides a foundation for further in-depth research on the anticancer activity and regulation of action of *Alismatis rhizoma*. Future studies aimed at refining and expanding these findings will provide more reliable evidence for the clinical application of *Alismatis rhizoma*.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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