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Exploring Anticancer Potential of *Anethum graveolens*: Effects on Apoptosis and *HER2* Expression in Human Gastric Adenocarcinoma

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ABSTRACT

Gastric cancer is among the most prevalent malignancies worldwide, with a poor prognosis in advanced stages. Overexpression of human epidermal growth factor receptor 2 (HER2, also known as ERBB2) and heat shock protein 90 alpha (HSP90AA1, also known as HSP90α) has been associated with tumor progression. *Anethum graveolens*, a medicinal plant from the *Apiaceae* family, has demonstrated anticancer properties in previous studies. This study aimed to evaluate the effects of *A graveolens* methanolic extract on apoptosis and the expression of *HER2*, *HSP90a*, *tumor protein p53* (*TP53*), and *caspase-3* genes in the human gastric adenocarcinoma cell line (AGS).

A methanolic extract of the aerial parts of A graveolens was prepared. Cytotoxicity was assessed in AGS and human gingival fibroblast (HUGU) cell lines using the -(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Gene expression was measured by real-time PCR, and apoptosis was evaluated using Annexin V/propidium iodide (PI) staining followed by flow cytometry.

The methanolic extract exhibited dose-dependent cytotoxicity, with a half-maximal inhibitory concentration (IC50) of 1280 µg/mL in AGS cells. Gene expression analysis revealed significant downregulation of *HER2* and upregulation of *HSP90a*, *TP53*, and *caspase-3* in treated AGS cells compared to controls. Apoptosis rates were significantly higher in treated AGS cells, confirming the proapoptotic effect of the extract. *A graveolens* exerts cytotoxic and proapoptotic effects in AGS cells and modulates key genes involved in gastric cancer progression.

These findings suggest its potential as a natural therapeutic candidate for gastric cancer, warranting further preclinical and clinical investigations.

Keywords: Anethum graveolens; Apoptosis; Cell line, Gastric cancer; Plant extracts; Tumor

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INTRODUCTION

Gastric cancer ranks as the fifth most frequently diagnosed cancer and the fourth leading cause of cancer mortality globally. It is also the most common malignant tumor of the digestive system.[1] Numerous factors, including genetic and epigenetic changes, environmental influences, pathogens, inflammation, and oxidative stress, contribute to the development of gastric cancer.[2] The activation of oncogenes and the loss of function in tumor suppressor genes are key factors that lead to defects in cancer cells.[3] A better understanding of the molecular basis of cancer aids in developing treatments that specifically target the factors involved in cell differentiation, proliferation, and survival pathways.[4]

Despite advances in therapeutic approaches, late-stage diagnosis and resistance to existing therapies have led to reduced life expectancy and poor prognosis for gastric cancer patients. Common treatment methods such as chemotherapy, radiotherapy, and surgery often face challenges like severe adverse effects and limited effectiveness in advanced stages of cancer.[5,6] This situation has further emphasized the need for new and more effective therapies.

In recent years, the use of medicinal plants as natural and complementary therapeutic options for combating cancers has gained widespread attention. *Anethum graveolens* (dill), from the Apiaceae family, has demonstrated anti-inflammatory, antioxidant, and anticancer properties due to its various bioactive compounds, including flavonoids, terpenoids, and essential oils. This plant has been used in traditional medicine to treat various diseases, including gastrointestinal and inflammatory disorders. Recent evidence suggests that its extract may have positive effects in reducing tumor growth and inducing cell death in various types of cancer.[7-12] However, the effects of this plant on gastric cancer and its molecular mechanisms have yet to be fully elucidated.

Human epidermal growth factor receptor 2 (HER2) and heat shock protein 90 alpha (HSP90α) genes are among the most important molecular factors involved in gastric cancer, and targeting these 2 molecules could serve as a potential therapeutic strategy.[13-15]

HER2, also known as Neu or ERBB2, is a proto-oncogene overexpressed in many cancers, including breast, lung, pancreatic, colorectal, and gastric cancers. This protein is a member of the epidermal growth factor receptor (EGFR) family encoded by the *ERBB2* gene on chromosome 17 and acts as a cell growth and survival promoter. In HER2-positive cancers, this gene is associated with poor prognosis due to its role in accelerating cell proliferation and contributing to resistance to conventional treatments.[16-19]

On the other hand, HSP90 is a molecular chaperone with 2 main isoforms, HSP90α and HSP90β, which play essential roles in protecting against stressful conditions and are critical for maintaining intracellular protein homeostasis.[20] This protein helps stabilize oncogenic proteins such as HER2 in cancer cells, thereby preventing cell death and enabling tumors to evade therapeutic pathways.[21,22]

Apoptosis, or programmed cell death, is a fundamental biological process that eliminates unnecessary, damaged, or potentially harmful cells, thereby contributing to the maintenance of tissue homeostasis. Various internal and external stimuli, including DNA damage and uncontrolled cell proliferation, can initiate this precisely regulated mechanism. Dysregulation of apoptosis is closely associated with the onset and progression of numerous diseases, particularly cancer, where cells evade death and continue to divide abnormally.[23-27]

The tumor protein p53, one of the most important tumor suppressors, is frequently mutated or inactivated in many cancers. In response to DNA damage, this protein regulates processes such as cell cycle arrest, DNA repair, and the induction of apoptosis. In cancers, reactivation of TP53 or upregulation of its expression can lead to tumor growth inhibition and the induction of cell death.[28-31] In this regard, caspase-3, a member of the cysteine protease family, is recognized as a key enzyme in the execution of apoptosis, which induces programmed cell death by cleaving other cellular proteins.[32] Investigating the changes in the expression of these 2 genes in response to new treatments can provide valuable insights into the molecular mechanisms involved in cancer therapy, particularly in gastric cancer.

This study's main objective was to investigate the effect of a methanolic extract of *A graveolens* on apoptosis and the expression changes of *HER2*, *HSP90α*, *TP53*, and caspase-3 genes in the human gastric adenocarcinoma cell line (AGS). The findings of this research may provide new evidence supporting the use of *A graveolens* as a natural therapeutic option in combating gastric cancer.

MATERIALS AND METHODS

Preparation of Methanolic Extract from *A graveolens*

The aerial parts of the plant (IBRC P1000802) were acquired in a dried, powdered form from the Plant Bank of the Iranian Biological Resources Center (IBRC), Karaj, Iran. To prepare the extract, 10 g of the powder was dissolved in 80% methanol (Merck, Germany) and incubated in a shaker at 40°C for 24 hours. The extract was then stored at 2°C to 8°C for the next experiments.

Assessment of Antioxidant Activity by the DPPH Method

The DPPH (2, 2-diphenyl-l-picrylhydrazyl, Sigma-Aldrich, St. Louis, MO, USA) method was used to evaluate the plant's antioxidant activity. Different concentrations of the extract were mixed with a 1 mM methanolic DPPH solution and incubated in the dark for 20 minutes. The absorbance was subsequently measured at 517 nm using a spectrophotometer (PG Instruments, UK). Ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) served as the reference antioxidant.

Cell Culture

Two cell lines, including Human Gastric Adenocarcinoma (AGS; IBRC C10071) and Human Gum Fibroblast (HUGU; IBRC C10459), were obtained from the Human and Animal Cell Bank of the IBRC, Tehran, Iran. The cells were cultured in DMEM: F12 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Examining Cell Viability Using the MTT Assay

AGS and HUGU cell lines were cultured in 96-well plates at densities of 1×10^4 cells/mL. After 24 hours of incubation, the cells were treated with various concentrations of the methanolic extract (ranging from 20 to 10 240 µg/mL) and incubated for an additional 48 hours at 37 °C with 5% CO_2. A 5 mg/mL MTT solution (Sigma-Aldrich, St. Louis, MO, USA) was then dispensed into each well, and the plates were incubated for 3 hours. After incubation, dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added to solubilize the formazan crystals, and the plates were placed on a shaker in the dark for 30 minutes. Absorbance was subsequently measured at 570 nm using a microplate reader (Biotek ELX800, Winooski, VT, USA). The MTT assay was conducted in triplicate.

Apoptosis Assay by Flow Cytometry

AGS and HUGU cell lines were cultured in 35-mm Petri dishes and incubated for 24 hours. The culture medium was then replaced, and the cells were treated with a medium containing 1280 µg/mL of the methanolic plant extract for 48 hours. After treatment, cells were washed with phosphate-buffered saline (PBS; Sigma-Aldrich, USA) and detached using trypsin (0.25%) / EDTA (0.02%) solution (Sigma-Aldrich, USA). Apoptosis was assessed using an Annexin V-FITC/PI kit and analyzed with a BD FACS-Calibur flow cytometer (Becton Dickinson, USA). Data were analyzed using FlowJo software (Version X; TreeStar Inc, Ashland, OR, USA).

Gene Expression Analysis by Real-Time PCR

AGS and HUGU cell lines were treated with 1280 µg/mL of the plant extract for 48 hours. Total RNA was extracted using the RNX-PLUS kit (Tehran Cavosh Clon, Tehran, Iran). Following quantitative and qualitative assessment of the RNA, cDNA synthesis was performed using a Roje Technologies kit (Alborz, Iran). Primers were designed using Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). The expression levels of the *HER2*, *HSP90α*, *TP53*, and caspase-3 genes were analyzed by real-time PCR using the Real Q Plus 2x Master Mix Green kit (Amplicon, Odense, Denmark) on an ABI StepOne Real-time PCR system (Applied Biosystems, Foster City, CA, USA). *GAPDH* was used as the internal reference gene due to its stable expression across experimental conditions. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, in which Ct values of target genes were first normalized to *GAPDH* ($\Delta Ct = Ct\ target - Ct\ GAPDH$), followed by comparison with the untreated control group ($\Delta\Delta Ct = \Delta Ct\ treated - \Delta Ct\ control$). All reactions were performed in triplicate, and results are expressed as mean ± SD. The primer sequences used for real-time PCR are provided in Supplementary Table 1.

Statistical Analysis

All statistical analyses were performed using SPSS software (version 25, IBM Corp, Armonk, NY, USA). Data are expressed as mean ± standard deviation (SD) derived from at least 3 independent experiments. For the DPPH assay, differences among multiple concentrations were analyzed using 1-way analysis of variance (1-way ANOVA) followed by Tukey post hoc test. In the MTT

assay, to assess the effects of both extract concentration and cell type, a 2-way ANOVA was applied followed by Sidak multiple comparisons test. For the apoptosis assay and gene expression analysis, comparisons between the treatment and control groups were conducted using an unpaired Student *t* test. The relative gene expression was initially calculated using Genex 6 software (MultiD Analyses AB, Gothenburg, Sweden), and the resulting values were statistically analyzed in SPSS. Graphs were generated using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). A $p<0.05$ was considered statistically significant.

RESULTS

DPPH Assay

The antioxidant activity of the *A graveolens* extract was evaluated using different concentrations (500, 1000, and 2000 µg/mL) in comparison with ascorbic acid as a positive control. As observed in Figure 1, the plant extract exhibits a concentration-dependent antioxidant activity, such that increasing the concentration of the extract leads to a higher DPPH radical scavenging effect. The results indicate that at the highest tested concentration (1000 µg/mL), more than 62% of the DPPH free radicals were scavenged.

Cytotoxic Effects of *A graveolens* Methanolic Extract

MTT assay results demonstrated a concentration-dependent inhibitory effect of *A graveolens* methanolic extract on the proliferation of AGS cells compared to the normal HUGU cell line. After 48 hours of treatment, the IC_{50} value was 1280 µg/mL for AGS cells and 2560 µg/mL for HUGU cells. Figure 2 represents a comparative analysis of the extract's cytotoxic effects on AGS and HUGU cells at 24 and 48 hours. These findings indicate that the methanolic extract of *A graveolens* exerts greater cytotoxicity against AGS cells than against normal HUGU cells.

Investigating Apoptosis by Flow Cytometry Method

Forty-eight hours after treatment, the morphology of AGS (Figure 3A–B) and HUGU (Figure 3C–D) cells in both the treatment and control groups was evaluated using an inverted microscope (Figure 3). Subsequently, the cells were analyzed for apoptosis after detachment by flow cytometry. Flow cytometric analysis of cells treated with 1280 µg/mL of *A graveolens* methanolic extract revealed distinct differences compared to the control group. In AGS cells, 33.9% early apoptosis, 0.055% late apoptosis, and 0.120% necrosis were observed (Figure 4A–B). In contrast, treated HUGU cells exhibited 6.04% early apoptosis and 0.252% necrosis (Figure 4C–D). Overall, the apoptosis rate in treated samples was significantly higher than in the control group, with AGS cells showing a markedly higher apoptosis rate than HUGU cells following treatment.

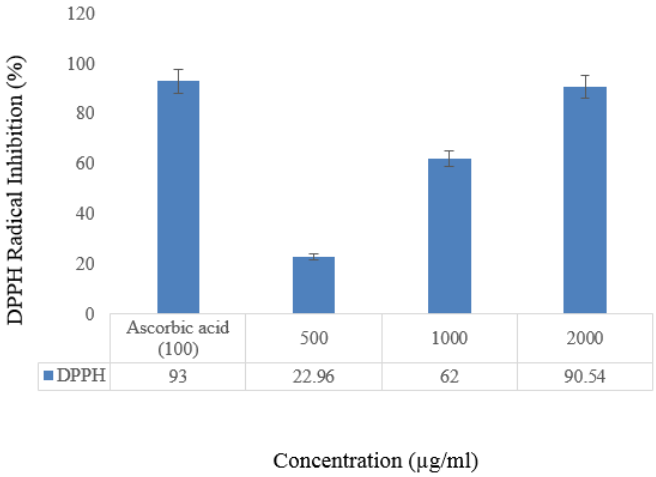

Figure 1. Free radical scavenging activity of *Anethum graveolens* extract assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different concentrations of the extract (500, 1000, and 2000 µg/mL) were tested and compared with ascorbic acid as a positive control. The extract exhibited a concentration-dependent antioxidant effect, with the highest scavenging activity observed at 1000 µg/mL, resulting in more than 62% inhibition of DPPH radicals. Data represent mean ± SD of 3 independent experiments.

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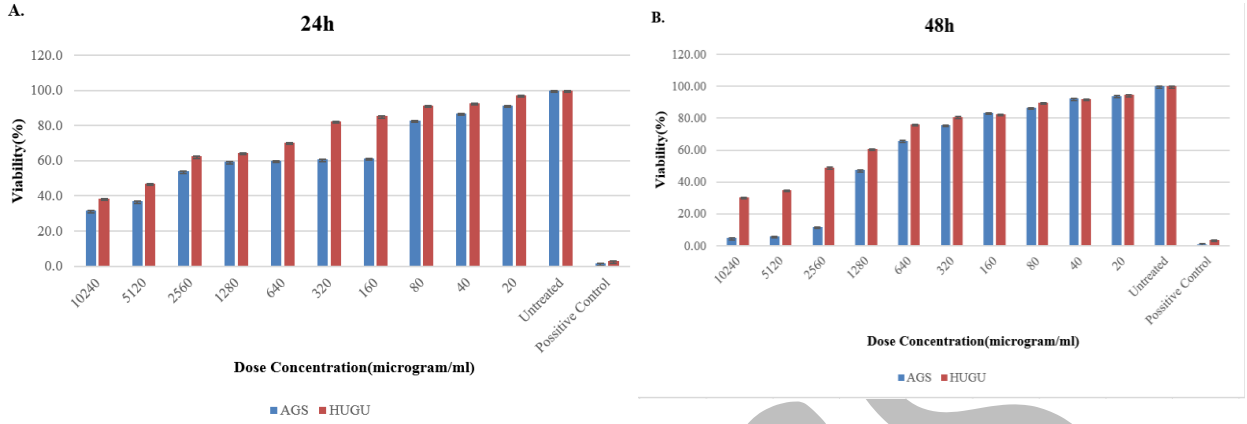

Figure 2. Cytotoxic effects of *Anethum graveolens* methanolic extract on human gastric adenocarcinoma (AGS) and human gingival fibroblast (HUGU) cells assessed by MTT assay. Cells were treated with various concentrations of the extract for 24 hours (A) and 48 hours (B). The extract exhibited a concentration-dependent inhibitory effect, with AGS cancer cells showing higher sensitivity than normal HUGU cells. The half-maximal inhibitory concentration (IC_{50}) values after 48 hours were 1280 µg/mL for AGS cells and 2560 µg/mL for HUGU cells. Data represent mean ± SD of 3 independent experiments.

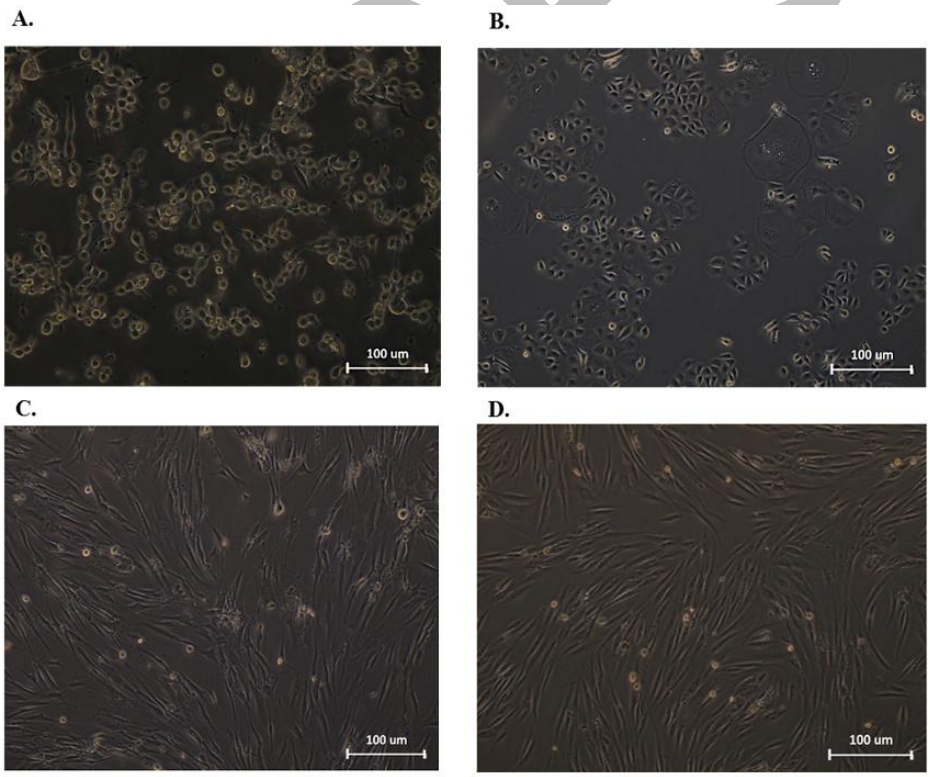

Figure 3. Morphological changes in human gastric adenocarcinoma (AGS) and human gingival fibroblast (HUGU) cells 48 hours after treatment with 1280 µg/mL of *Anethum graveolens* methanolic extract. A, Treated AGS cells exhibit granular and vacuolated cytoplasm with reduced adherence to the substrate. B, Untreated AGS cells (control) show normal morphology. C, Treated HUGU cells and D, untreated HUGU cells (control) display no notable morphological alterations. Cells were observed using an inverted microscope. These results indicate that AGS cancer cells are more sensitive to the extract-induced morphological changes than normal HUGU cells.

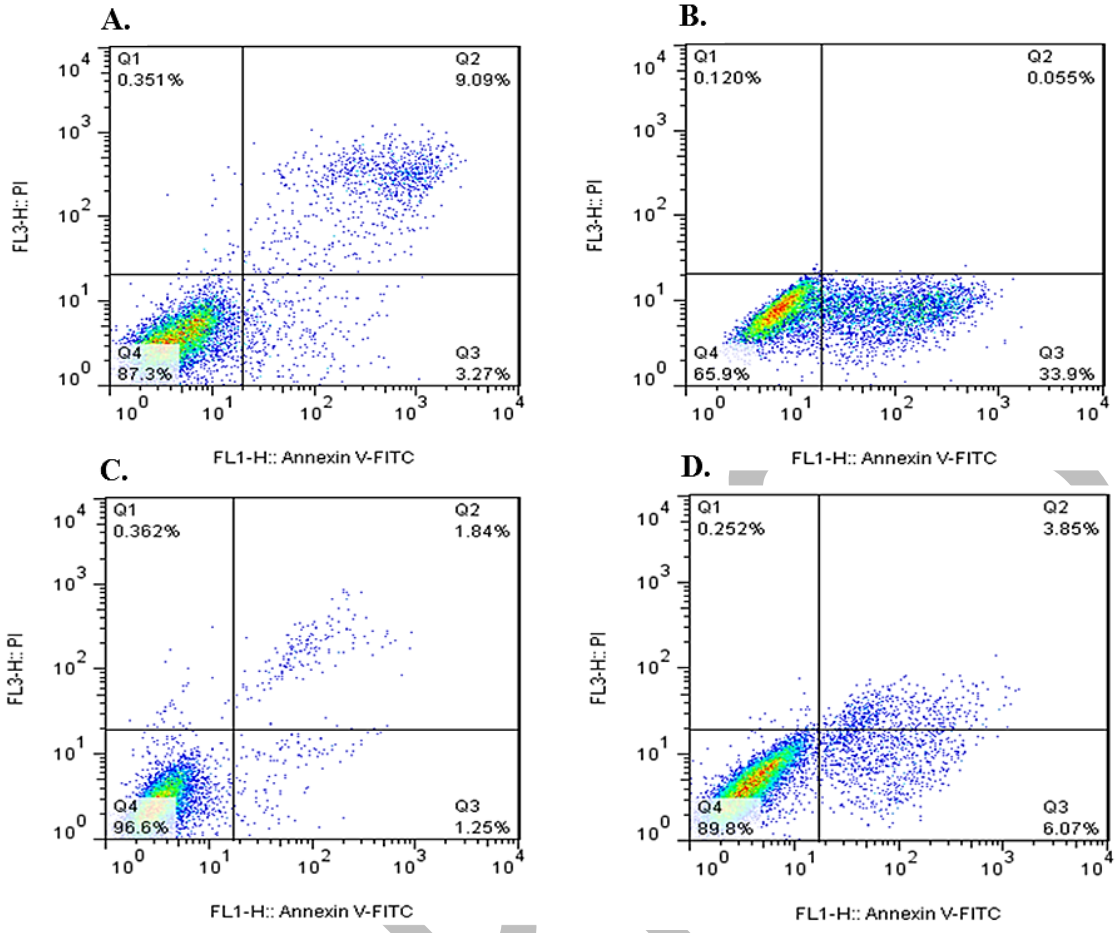

Figure 4. Flow cytometry analysis of apoptosis in human gastric adenocarcinoma (AGS) and human gingival fibroblast (HUGU) cells after 48 hours of treatment with 1280 µg/mL of *Anethum graveolens* methanolic extract. A, Untreated AGS cells (control); B, AGS cells treated with the extract; C, untreated HUGU cells (control); and D, HUGU cells treated with the extract. The results indicate that AGS cancer cells are more sensitive to extract-induced apoptosis than normal HUGU cells.

Quantitative Gene Expression Analysis by Real-time PCR

To evaluate the effect of *A graveolens* on the expression of *HER2*, *HSP90α*, *TP53*, and caspase-3 genes, mRNA levels were analyzed in AGS and HUGU cells. As shown in Figure 5A–D, treatment with the extract significantly reduced *HER2* expression in AGS cells ($p<0.01$), while the expression levels of *TP53* ($p<0.0001$), *HSP90α* ($p<0.05$), and caspase-3 ($p<0.0001$), were significantly increased compared to untreated AGS cells. In the treated HUGU cells, caspase-3 ($p<0.0001$) expression level was significantly higher compared to untreated HUGU cells (Figure 5D). No significant changes were observed in expression levels of *HER2*, *HSP90α*, and *TP53* genes in treated HUGU cells compared to untreated HUGU cells (Figure 5A–C).

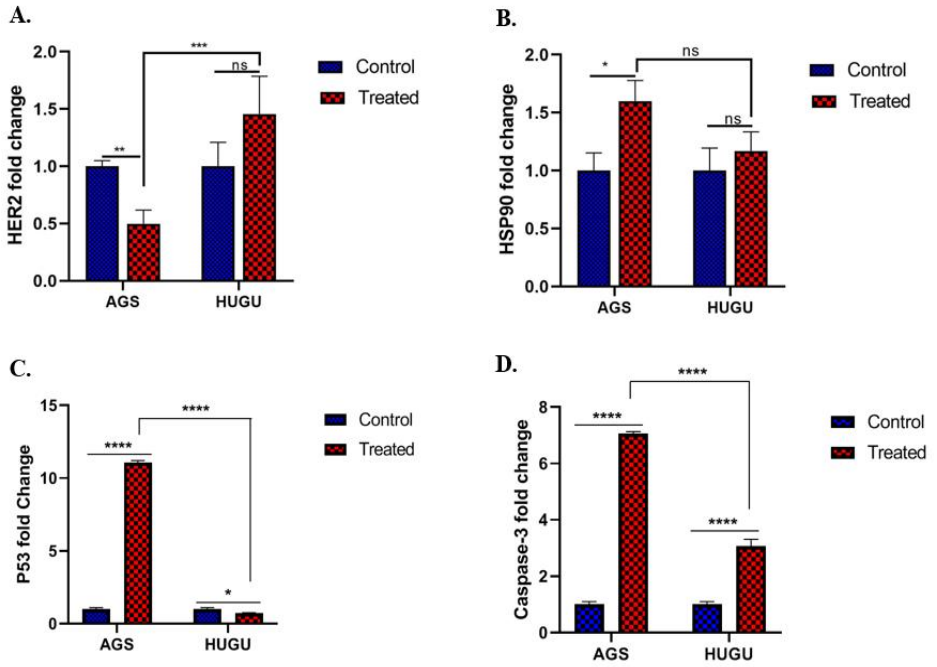

Figure 5. Quantitative analysis of gene expression in human gastric adenocarcinoma (AGS) and human gingival fibroblast (HUGU) cells 48 hours after treatment with 1280 µg/mL of *Anethum graveolens* methanolic extract. mRNA levels of human epidermal growth factor receptor 2 (*HER2*; also known as ERBB2) (A), heat shock protein 90 alpha (*HSP90α)* (B), tumor protein p53 (*TP53)* (C), and caspase-3 (D) were measured by real-time PCR. Treatment significantly decreased *HER2* and increased *HSP90α*, *TP53*, and caspase-3 expression in AGS cells, while in HUGU cells only caspase-3 expression was notably elevated. Data represent mean ± SD of 3 independent experiments performed in triplicate. Statistical significance is indicated as **p*<0.05, ***p*<0.01, ****p*<0.001, and *****p*<0.0001.

DISCUSSION

Gastric cancer remains one of the most prevalent cancers worldwide and represents a significant public health challenge due to its associated complications and high mortality rates.[33] Standard treatment options, including surgery, chemotherapy, and radiotherapy, often come with a range of adverse effects, and their effectiveness can vary depending on the cancer's stage and type.[34] Consequently, there has been increasing interest in the use of natural and plant-based substances as adjunctive and preventive treatments for cancer. One such plant, *A graveolens*, is known for its considerable medicinal properties and has recently emerged as a potential anticancer agent.

This study aimed to investigate antioxidant and anticancer properties of dill on gastric cancer. Antioxidant activity was confirmed through the DPPH assay, with results suggesting that antioxidant mechanisms may play a complementary role in cancer prevention. Numerous studies have demonstrated that various plants exhibit antioxidant effects that can mitigate oxidative stress and reduce cancer risk. A study by Zhang et al[35] evaluated the antioxidant activity of different plant extracts, including *Curcuma longa* and *Cinnamomum cassia*, which exhibited significant antioxidant activity in the DPPH assay. The present study found similar IC_{50} values for *A graveolens* (ranging from 500 to 2000 µg/mL), indicating strong antioxidant potential in dill. The similarity in IC_{50} values across different plant extracts underscores the potential of *A graveolens* as a rich source of antioxidant compounds, which could be beneficial in preventing oxidative damage.

The results of this study further suggest that the extract of *A graveolens* exerts significant cytotoxic effects on the AGS cell line, effectively inducing apoptosis. The methanolic dill extract demonstrated an IC_{50} of 1280 µg/mL in AGS cells, indicating a marked inhibitory effect on cell proliferation. It should also be noted that the relatively high IC_{50} value (1280 µg/mL) observed for AGS cells is likely due to the use of a crude

methanolic extract, which contains a mixture of both active and inactive compounds. This may reduce the apparent potency of the extract compared to isolated compounds. Fractionation or isolation of bioactive constituents could yield more potent effects with lower IC_{50} values and help identify the specific molecules responsible for the observed cytotoxicity and apoptotic activity. Despite the high IC_{50}, the extract demonstrated selective cytotoxicity toward AGS cells compared to normal HUGU cells and effectively modulated apoptosis-related gene expression, indicating that it still possesses therapeutic potential worth further investigation. These findings are consistent with a study by Hossen et al[36] which reported that various plant extracts can inhibit cancer cell growth in a concentration-dependent manner. Similarly, a study by Mohammad et al[37] indicated that *A graveolens* has cytotoxic effects on the hepatocellular carcinoma cell line (HepG2), with MTT assay results showing concentration-dependent inhibition of cell growth.

Morphological and flow cytometric analysis revealed a significant increase in apoptosis among AGS cells treated with dill extract. Flow cytometric results showed 33.9% early apoptosis in AGS cells treated with the extract. Morphological changes, including granulation and cytoplasmic vacuolation, were observed, suggesting structural alterations that could lead to cell death. These findings are consistent with those of Khan et al[38] who observed similar morphological changes in cancer cells under the influence of plant extracts. In another study, Enogieru et al[39] demonstrated that plant extracts could enhance cell death in cancer cells by modulating apoptotic signaling pathways. Additionally, Bortolin et al[40] showed that *Hypericum perforatum* (St John's wort) extract increased apoptosis in gastric cancer cells. Studies on *Curcuma longa* indicated that curcumin, its active compound, can inhibit growth and promote apoptosis in gastric cancer cells.[41] Comparable findings have been reported by Gholami et al[3] who showed that *Alkanna bracteosa* extract induced cell death in AGS cells. Such effects are generally attributed to bioactive phytochemicals in plant-derived extracts, which can act as potent apoptosis inducers. In the case of *A graveolens*, compounds such as flavonoids and terpenoids are believed to play a critical role in suppressing cancer cell proliferation and promoting apoptosis, possibly through the modulation of key signaling pathways involved in tumor progression and cell survival.[3]

This study also investigated the impact of *A graveolens* on the expression of genes associated with apoptosis and gastric cancer. The results showed that treatment with this plant had distinct effects on the expression of *HER2*, caspase-3, *TP53*, and *HSP90α* genes in AGS and HUGU cell lines. In AGS cells, treatment with *A graveolens* resulted in a significant reduction in *HER2* expression, which was not observed in HUGU cells, where only a nonsignificant increase in *HER2* expression occurred. The decrease of *HER2* expression in AGS cells may indicate the anticancer properties of this plant. Numerous studies have shown that decreasing *HER2* expression can reduce tumor growth and progression in HER2-related cancers, including breast and gastric.[42,43] In a study conducted by Gupta et al[42] the anticancer properties of extracts from 10 different medicinal plants were evaluated in HER2-positive cancer cells. The results showed that the extracts of *Terminalia chebula*, *Berberis aristata*, and *Mucuna pruriens* could reduce *HER2* expression in cancer cells, consistent with our findings.

A significant increase in caspase-3 expression in AGS cells suggests that *A graveolens* induces apoptosis. Caspase-3 is a key enzyme in the apoptosis pathway, and its activation leads to programmed cell death. These results are consistent with previous studies, such as that by Ray et al[44] which demonstrated that *Hedychium spicatum* essential oil can activate caspase-3 and induce apoptosis in human prostate adenocarcinoma (PC3) cells. Furthermore, an increase in caspase-3 expression was less pronounced in HUGU cells than AGS cells, likely due to differences in cellular responses between these 2 cell types.

The significant increase in *TP53* expression in AGS cells following treatment with *A graveolens* suggests the activation of this tumor suppressor gene, which may enhance the ability of cancer cells to repair DNA damage and regulate abnormal cell growth. In a study conducted by Nam et al[45] investigating the effects of *Euryale ferox* Salisb extract on lung cancer cells (A549), it was demonstrated that the anticancer activity of the extract is mediated in a *TP53*-dependent manner. Similarly, in another study conducted by Fouzat et al[46] it was shown that the extract of *Elaeagnus angustifolia* induces apoptosis in breast cancer cells through the activation of *TP53*. The results obtained from these studies were consistent with our findings. In contrast, no significant changes in *TP53* expression were observed in HUGU cells, which may reflect the inherent

characteristics of HUGU cells and their differential sensitivity to *A graveolens* treatment.

HSP90α is a molecular chaperone involved in protein stability and tumor growth. The results of this study showed that *A graveolens* significantly increased *HSP90α* gene expression in AGS cells. These findings are consistent with a similar study conducted by Gholami et al[3] which investigated the effect of *Alkanna bracteosa* extract on AGS cells and also observed an increase in *HSP90α* gene expression. Although the observed upregulation of *HSP90α* mRNA in AGS cells is interesting, it appears contradictory to conventional cancer therapies, in which HSP90 is often targeted for inhibition. This increase may reflect a cellular stress response induced by the dill extract, as HSP90α plays a key role in protein stabilization and maintaining cellular homeostasis under stress conditions. In other words, cells may upregulate HSP90α to cope with damage caused by bioactive compounds in the extract. This response could serve as a protective mechanism against oxidative and cytotoxic stress, while apoptotic pathways are simultaneously activated. These findings indicate that the effects of *A graveolens* are complex and multidimensional, and further studies are warranted to clarify the precise role of HSP90α and its interactions with apoptotic signaling pathways in cancer cells.[3]

The findings of this study suggest that *A graveolens* extract has significant therapeutic potential against gastric cancer, demonstrating both cytotoxic and proapoptotic effects on AGS cancer cells. However, these results are limited to in vitro conditions; factors such as the tumor microenvironment, pharmacokinetics, and bioavailability of the extract were not evaluated, and its efficacy in in vivo models remains unverified. Therefore, further preclinical, and clinical studies are necessary to clarify the underlying molecular mechanisms, assess safety, and determine therapeutic effectiveness. These findings may provide a scientific foundation for developing natural compound-based strategies as adjuncts to conventional gastric cancer treatments.

STATEMENT OF ETHICS

The present study was conducted using commercially available cell lines obtained from the Iranian Biological Resources Center (IBRC). As the study did not involve human participants or animals, and utilized only established cell lines, formal approval from an institutional ethics committee was not required at the time of the study. However, all experiments were performed in accordance with the relevant guidelines and regulations for the handling and use of biological materials.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

The authors used Grammarly (https://www.grammarly.com) for language polishing and grammar checking. No AI tools were used for data analysis, figure generation, or scientific writing.

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