

***Orostachys Japonicus* Promotes Apoptosis in Cervical Cancer Cells through Modulation of NF- κ B and ERK1/2 Pathways**

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Received November 15, 2025; Revised December 17, 2025; Accepted December 23, 2025

Abstract Cervical cancer remains a significant global health burden, necessitating the development of safer and more effective therapeutic agents. *Orostachys japonicus*, a traditional medicinal herb, has shown promising anti-cancer activity, but its molecular mechanisms in cervical cancer cells have not been fully elucidated. We investigated the pro-apoptotic effects of the ethyl acetate fraction of *O. japonicus* (E-OJ) on HeLa cells and compared its activity to known phenolic constituents (kaempferol, quercetin, and gallic acid). Apoptosis was assessed by Annexin V/PI flow cytometry and DAPI staining. Western blot analysis was used to examine NF- κ B and MAPK signaling pathways, as well as caspase-3 activation. The role of ERK signaling was further evaluated using the ERK-specific inhibitor U0126. E-OJ treatment induced significant, dose-dependent apoptosis in HeLa cells, accompanied by nuclear fragmentation and chromatin condensation. Compared to individual compounds, E-OJ exhibited greater apoptotic efficacy. Mechanistically, E-OJ suppressed NF- κ B signaling by blocking nuclear translocation of the NF- κ B p65 subunit. Among the MAPK pathways, only ERK1/2 was activated by E-OJ. Inhibition of ERK1/2 with U0126 attenuated caspase-3 cleavage, indicating that ERK signaling mediates E-OJ-induced apoptosis through a caspase-3-dependent mechanism. These findings suggest that E-OJ induces apoptosis in cervical cancer cells by suppressing NF- κ B activation and promoting ERK1/2-mediated caspase-3 activation. The superior efficacy of E-OJ compared to its individual phenolic components highlights its therapeutic potential as a natural anti-cancer agent.

Keywords: *Orostachys japonicus*, cervical cancer, apoptosis, NF- κ B, ERK1/2

Cite This Article: Seon-Hee Kim, and Dong Seok Lee, “*Orostachys Japonicus* Promotes Apoptosis in Cervical Cancer Cells through Modulation of NF- κ B and ERK1/2 Pathways.” *Journal of Food and Nutrition Research*, vol. 13, no. 12 (2025): 455-461. doi: 10.12691/jfnr-13-12-3.

1. Introduction

Cervical cancer remains one of the most common malignancies affecting women worldwide, particularly in low- and middle-income countries [1,2]. Despite considerable advances in surgical resection, radiotherapy, and chemotherapeutic strategies, the prognosis for patients with advanced or recurrent cervical cancer is still poor [3,4]. This is primarily due to the development of drug resistance and the toxic side effects associated with conventional treatments [5,6]. These challenges highlight the urgent need for safer and more effective therapeutic alternatives, particularly those derived from natural products with multi-targeted anti-cancer activities.

Apoptosis, or programmed cell death, is a fundamental process for maintaining tissue homeostasis and eliminating damaged or malignant cells [7,8,9]. Defects in apoptotic signaling pathways are recognized as hallmarks of cancer, contributing to tumor progression and resistance to therapy. Among the critical molecular pathways

regulating apoptosis are the nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling cascades [10,11]. NF- κ B is a key transcription factor that regulates cell survival and proliferation, and its constitutive activation has been frequently observed in various cancer types [12]. In contrast, MAPK signaling pathways—including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38—can mediate either pro-survival or pro-apoptotic responses depending on the context [13].

Orostachys japonicus, a perennial herb widely used in traditional East Asian medicine, has been reported to possess anti-inflammatory, antioxidant, and anti-tumor properties [14,15,16,17,18,19]. Several studies have demonstrated that extracts of *O. japonicus* exert cytotoxic effects in various cancer cell lines, such as liver, breast, and gastric cancers [18,19,20]. In our previous study, we reported that an extract of *O. japonicus* induces caspase-dependent apoptosis in HeLa cervical cancer cells [21]. However, the precise molecular mechanisms underlying these effects remained unclear, necessitating further investigation.

Therefore, the present study was designed as a follow-up to our earlier findings, aiming to elucidate the apoptotic mechanisms induced by the ethyl acetate fraction of *O. japonicus* (E-OJ) in cervical cancer cells. Specifically, we focused on the involvement of NF- κ B and MAPK signaling pathways and their downstream effector caspase-3. In addition, we compared the pro-apoptotic effects of E-OJ with its major phenolic constituents—kaempferol, quercetin, and gallic acid—to assess whether E-OJ exhibits enhanced or synergistic activity. This study provides new insights into the therapeutic potential of *O. japonicus* as a natural anti-cancer agent targeting apoptotic pathways in cervical cancer.

2. Materials and Methods

2.1. Plant Extracts

Dried *O. japonicus* material was obtained from Geobugiwasang Ltd. (Miryang, Republic of Korea). The plant was air-dried under natural conditions and subsequently pulverized into a fine powder. To prepare the ethyl acetate (EtOAc) fraction, 200 g of the powdered sample was extracted with 1 L of 95% ethanol (EtOH) by performing three rounds of reflux extraction using a reflux condenser (SciLab®, Seoul, Republic of Korea), as adapted from previously established protocols [17,18,19,21]. The ethanol extract was concentrated under reduced pressure using a rotary evaporator (IKA-Werke GmbH, Co. KG, Staufen, Germany).

The concentrated crude extract was further partitioned sequentially with solvents in the following order: n-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), n-butanol (BuOH), and distilled water. Among the resulting fractions, the EtOAc fraction (E-OJ) was selected for further experimentation. This fraction was evaporated to dryness at 40°C using a rotary evaporator and stored at -20°C until use. For in vitro treatments, E-OJ was dissolved in dimethyl sulfoxide (DMSO) to prepare working concentrations.

2.2. Cell Line and Reagents

HeLa human cervical cancer cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Republic of Korea). Culture media (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Hyclone Laboratories (Logan, UT, USA). Primary antibodies against NF- κ B p65, phosphorylated I κ B α , total and phosphorylated forms of ERK1/2, p38, JNK, pro-caspase-3, and cleaved caspase-3, as well as GAPDH, were sourced from Cell Signaling Technology (Beverly, MA, USA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). U0126 (ERK pathway inhibitor), kaempferol, quercetin, and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Cell Culture

HeLa cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cultures

were incubated at 37°C in a humidified atmosphere containing 5% CO₂. The medium was refreshed every two days, and cells were passaged when they reached approximately 80% confluence.

2.4. Nuclear Morphology Analysis by DAPI Staining

To examine nuclear changes, cells were treated with kaempferol, quercetin, gallic acid, or E-OJ. After treatment, cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, rinsed with PBS, and stained with 1 μ g/ml DAPI (Vector Laboratories, CA, USA) for 10 minutes. After three PBS washes, nuclear morphology was observed using a confocal fluorescence microscope (LSM510 Meta, Carl Zeiss, Jena, Germany) with excitation at 350 nm.

2.5. Apoptosis Assay

Apoptotic cell death was assessed using the Annexin V-FITC/PI double staining method and an apoptosis detection kit (BD Biosciences, NJ, USA), following the manufacturer's instructions. HeLa cells (4×10^5 cells/ml) were seeded in 24-well plates and incubated overnight. Serum deprivation was used for synchronization, followed by treatment with kaempferol, quercetin, gallic acid, or varying concentrations of E-OJ for 12 hours. After treatment, cells were harvested, washed twice with cold PBS, and resuspended in 100 μ l of 1 $\times$ binding buffer. Subsequently, cells were stained with Annexin V-FITC and PI for 15 minutes in the dark at room temperature. Finally, samples were analyzed immediately using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA).

2.6. Western Blot Analysis

HeLa cells were exposed to E-OJ, kaempferol, quercetin, or gallic acid for specified durations. For ERK pathway inhibition experiments, cells were pretreated with 25 μ M U0126 for 30 minutes prior to compound exposure. After treatment, cells were rinsed with PBS, lysed on ice for 1 hour in cold lysis buffer, and centrifuged to collect the supernatant. Protein concentrations were determined using a BCA assay (Pierce, IL, USA). Equal amounts of protein (40 μ g) were separated on 10–15% SDS-PAGE gels and transferred onto PVDF membranes using a semi-dry transfer apparatus (Bio-Rad, CA, USA). Membranes were blocked in 5% non-fat dry milk prepared in PBS-T (PBS + 0.1% Tween-20) and incubated overnight at 4°C with primary antibodies. After washing, membranes were incubated with HRP-conjugated secondary antibodies for 2 hours. Protein bands were visualized using an enhanced chemiluminescence detection kit (Santa Cruz Biotechnology) and quantified using ImageJ software.

2.7. Statistical Analysis

Statistical analyses were conducted using GraphPad Prism v5.01 (GraphPad Software, CA, USA). Results are expressed as mean $\pm$ standard deviation (SD). Differences between control and treatment groups were evaluated

using Student's *t*-test. Statistical significance was considered at $p < 0.05$, with values denoted as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

3. Results

3.1. The EtOAc Fraction of *O. japonicus* Induces Apoptosis in HeLa Cells

To evaluate the pro-apoptotic effects of the EtOAc fraction of *O. japonicus* (E-OJ) and its major phenolic components (kaempferol, quercetin, and gallic acid), HeLa cells were subjected to Annexin V/PI staining followed by flow cytometric analysis [15]. As illustrated in Figure 1, cell populations were classified as follows: lower-left (LL), viable cells (Annexin V⁻/PI⁻); lower-right (LR), early apoptotic cells (Annexin V⁺/PI⁻); and upper-right (UR), late apoptotic or necrotic cells (Annexin V⁺/PI⁺).

Treatment with E-OJ at concentrations of 0, 5, 7.5, and 10 µg/ml for 12 hours resulted in a dose-dependent increase in apoptotic cell populations, with total apoptosis rates of 14.72%, 15.45%, 21.79%, and 51.84%, respectively. Similarly, treatment with 80 µM of kaempferol, quercetin, and gallic acid induced apoptosis at

rates of 19.87%, 16.34%, and 18.79%, respectively.

These findings indicate that E-OJ, as well as its phenolic constituents, promotes apoptosis in HeLa cells. Notably, E-OJ exhibited a stronger pro-apoptotic effect than the individual compounds, suggesting potential synergistic or additive effects of its constituents.

3.2. *O. japonicus* Induces Nuclear Morphological Changes

Morphological changes in the apoptotic cells, such as the presence of nuclear apoptotic bodies, were analyzed using confocal microscopy with a laser confocal fluorescence. Nuclear DAPI staining was used to examine any morphologic changes in the nuclei of HeLa cells treated with kaempferol, quercetin, gallic acid, and E-OJ. To investigate the formation of nuclear apoptotic bodies, HeLa cells were treated with 0, 5, 7.5, 10 µg/mL of E-OJ and 80 mM of kaempferol, quercetin, and gallic acid for 12 h, respectively (Figure 2). The percentage of apoptotic cells treated with E-OJ increased in a dose-dependent manner. We found a significant increase in chromatin condensation and the number of apoptotic bodies in HeLa cells treated with E-OJ, suggesting its apoptotic activity.

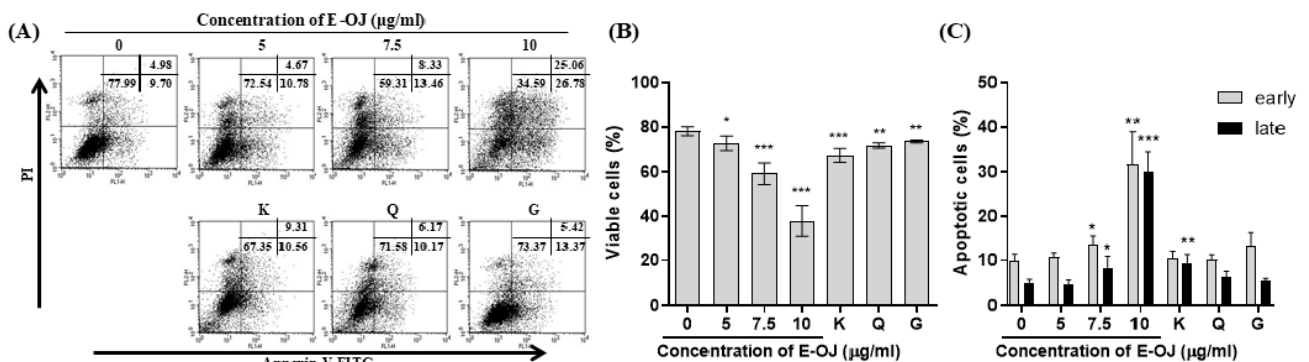


Figure 1. Induction of apoptosis in HeLa cells by E-OJ and phenolic compounds. HeLa cells were treated for 12 hours with the ethyl acetate fraction of *Orostachys japonicus* (E-OJ) at concentrations of 0, 5, 7.5, and 10 µg/ml, or with 80 µM of kaempferol (K), quercetin (Q), or gallic acid (G). (A) Representative Annexin V-FITC/PI flow cytometry plots. (B) Quantification of viable cells (Annexin V⁻/PI⁻). (C) Percentage of early (Annexin V⁺/PI⁻) and late (Annexin V⁺/PI⁺) apoptotic cells. Data are presented as mean ± standard deviation (SD) from three independent experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to control

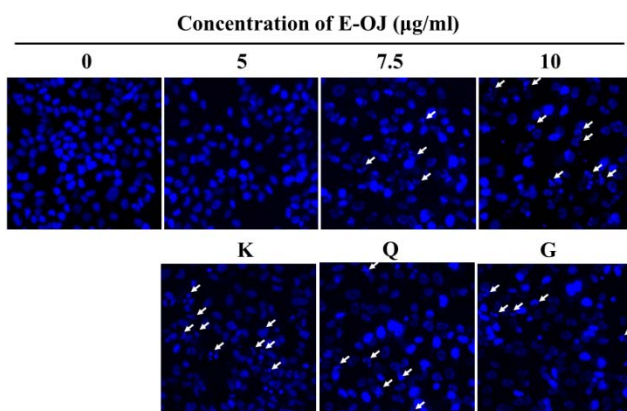


Figure 2. Nuclear morphological changes indicating apoptosis in HeLa cells. HeLa cells were treated for 12 hours with E-OJ (0, 5, 7.5, or 10 µg/ml), kaempferol, quercetin, or gallic acid. Nuclei were stained with DAPI to visualize chromatin structure. Apoptotic features, including chromatin condensation and nuclear fragmentation, were observed by fluorescence microscopy. Apoptotic bodies are indicated with white arrows

3.3. *O. japonicus* Suppresses NF-κB Signaling

Our previous study demonstrated that E-OJ induces apoptosis in HeLa cells via a caspase-dependent mechanism [21]. To further elucidate the upstream events contributing to this effect, we investigated whether E-OJ modulates NF-κB signaling, a pathway closely associated with cell survival and apoptosis regulation.

NF-κB is a key transcription factor that regulates the expression of genes involved in anti-apoptotic responses [12]. To assess the involvement of NF-κB signaling in E-OJ-induced apoptosis, we examined the expression and subcellular localization of the NF-κB p65 subunit and phosphorylated IκBα (p-IκBα) following treatment with E-OJ, kaempferol, quercetin, or gallic acid.

Western blot analysis of cytoplasmic and nuclear protein fractions revealed that treatment with E-OJ resulted in a dose-dependent decrease in nuclear p65 levels, accompanied by a corresponding increase in

cytoplasmic p65 expression (Figure 3A and 3B). These findings suggest that E-OJ inhibits the nuclear translocation of NF- κ B p65. The inhibitory effect was more pronounced at higher E-OJ concentrations.

To further explore the regulatory mechanism, we examined the cytoplasmic levels of phosphorylated I κ B α . As shown in Figure 3C, E-OJ treatment led to a dose-dependent increase in cytoplasmic p-I κ B α levels. These results indicate that E-OJ suppresses NF- κ B signaling by preventing I κ B α degradation and inhibiting the nuclear translocation of the NF- κ B p65 subunit.

Collectively, these findings suggest that E-OJ promotes apoptosis in HeLa cells by downregulating NF- κ B activity, thereby disrupting a key pro-survival signaling pathway.

3.4. *O. japonicus* Selectively Activates the ERK1/2 Pathway

To investigate whether the pro-apoptotic effect of E-OJ involves modulation of MAPK signaling upstream of the NF- κ B pathway, we examined the activation status of

three major MAPK subfamilies: p38, JNK, and ERK1/2 [13]. HeLa cells were treated with E-OJ, and the expression levels of total and phosphorylated forms of each MAPK protein were assessed by Western blotting.

As shown in Figure 4, phosphorylation of p38 and JNK was not observed following E-OJ treatment, suggesting that these signaling pathways are not significantly activated under the current experimental conditions. In contrast, phosphorylated ERK1/2 (p-ERK1/2) levels were markedly elevated as early as 15 minutes after E-OJ exposure, indicating rapid activation of the ERK1/2 pathway.

Furthermore, E-OJ treatment at increasing concentrations (5, 7.5, and 10 μ g/ml) led to a dose-dependent increase in p-ERK1/2 levels, while total ERK1/2 expression remained constant. These data suggest that E-OJ specifically activates the ERK1/2 pathway, but not p38 or JNK, in HeLa cells.

Taken together, these results imply that ERK1/2 plays a central role in mediating the cellular response to E-OJ, and may function as a key upstream regulator in its apoptosis-inducing mechanism.

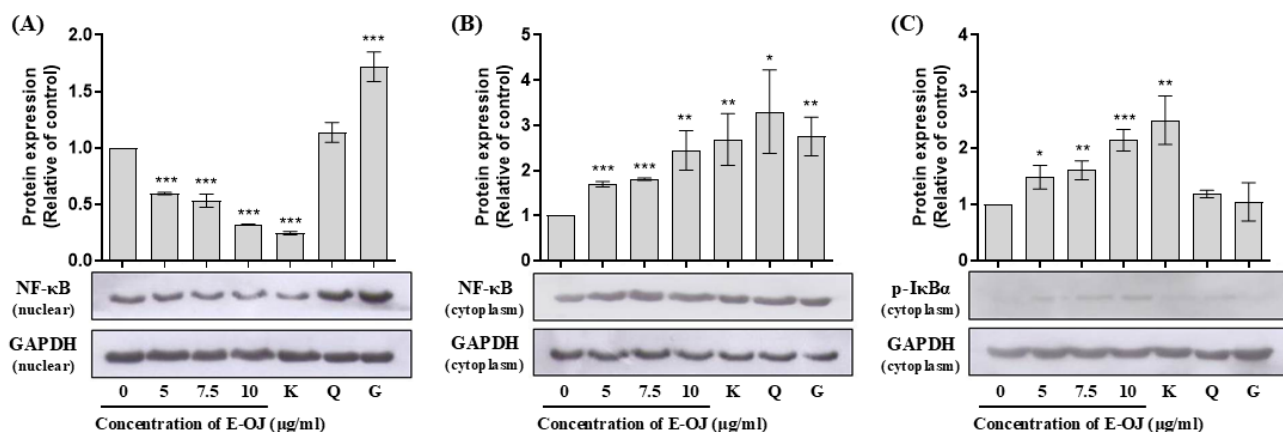


Figure 3. E-OJ and phenolic compounds modulate NF- κ B p65 localization and I κ B α phosphorylation. HeLa cells were treated for 12 hours with E-OJ (5, 7.5, or 10 μ g/ml), kaempferol (K), quercetin (Q), or gallic acid (G). (A) Nuclear and (B) cytoplasmic fractions were analyzed by Western blotting for NF- κ B p65 expression. (C) Phosphorylated I κ B α (p-I κ B α) levels in cytoplasmic fractions were also evaluated. GAPDH was used as a loading control. Band intensities were quantified using densitometric analysis from three biologically independent replicates. Data are shown as mean $\pm$ standard deviation (SD). * p < 0.05; ** p < 0.01; *** p < 0.001.

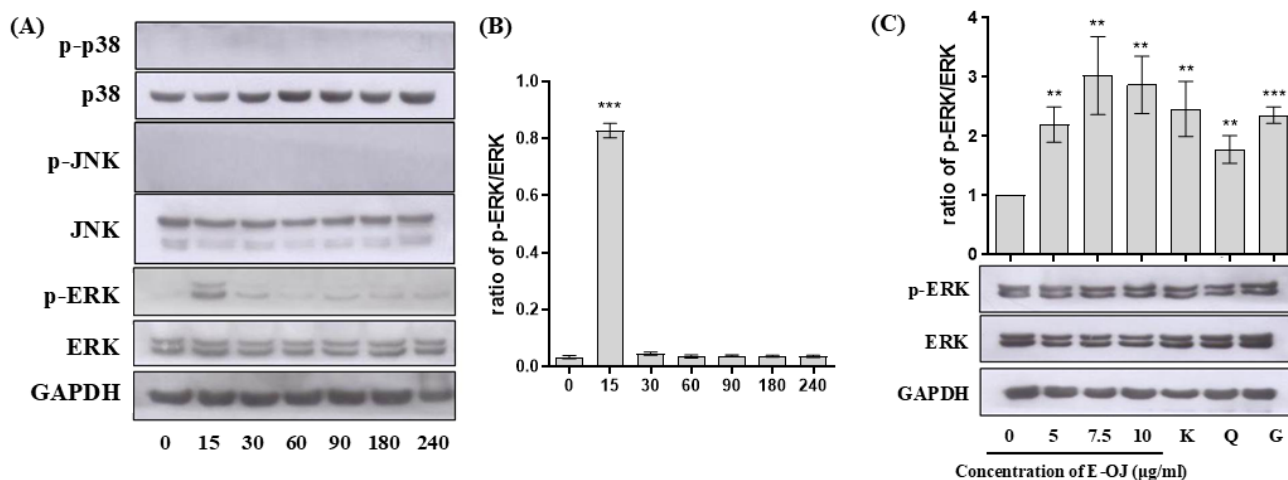


Figure 4. E-OJ modulates MAPK signaling pathways in HeLa cells. (A) HeLa cells were treated with 10 μ g/ml of E-OJ for the indicated time points, and total and phosphorylated forms of p38, JNK, and ERK1/2 were analyzed by Western blotting. (B) ERK1/2 activation was quantified as the ratio of phospho-ERK1/2 to total ERK1/2. (C) Cells were exposed to E-OJ (5, 7.5, or 10 μ g/ml), kaempferol (K), quercetin (Q), or gallic acid (G) for 15 minutes, and ERK1/2 phosphorylation was assessed. GAPDH served as a loading control. Data were obtained from three independent experiments. * p < 0.05; ** p < 0.01; *** p < 0.001.

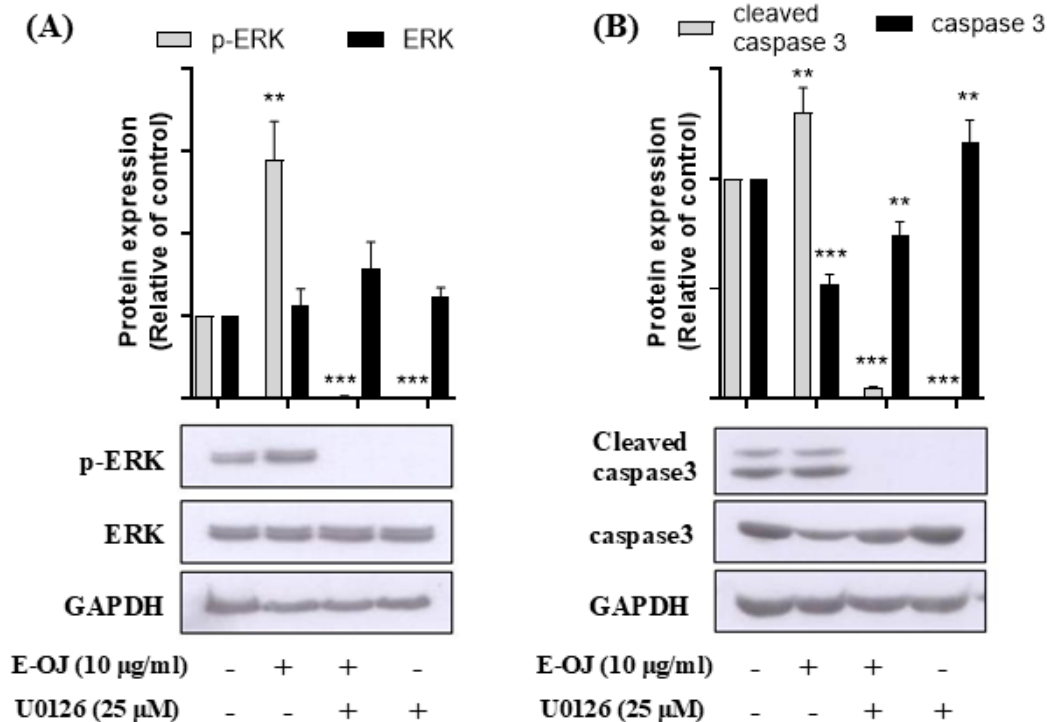


Figure 5. ERK1/2 activation by E-OJ and its role in apoptosis in HeLa cells. (A) HeLa cells were pretreated with U0126, a selective ERK1/2 inhibitor, followed by treatment with 10 µg/ml E-OJ for 15 minutes. Phospho-ERK1/2 and total ERK1/2 protein levels were assessed by Western blotting. (B) Cells were treated with 10 µg/ml E-OJ for 12 hours in the presence or absence of U0126, and expression of cleaved caspase-3 and caspase-3 was evaluated. GAPDH was used as a loading control. Densitometric analysis was conducted based on three independent experiments. Data are presented as mean $\pm$ SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

3.5. ERK1/2 Activation Contributes to Caspase-3-mediated Apoptosis

Previous study demonstrated that E-OJ induces apoptosis via a caspase-dependent mechanism. To further validate the involvement of caspase-3 and to clarify its upstream regulatory signals, we investigated whether ERK1/2 activation contributes to caspase-3 processing during E-OJ-induced apoptosis.

HeLa cells were pretreated with U0126, a selective ERK1/2 inhibitor, prior to E-OJ exposure. As shown in Figure 5A, U0126 effectively blocked E-OJ-induced phosphorylation of ERK1/2, confirming successful inhibition of the ERK signaling cascade. Importantly, inhibition of ERK1/2 by U0126 markedly affected caspase-3 activation. Specifically, the level of cleaved caspase-3 was significantly reduced, while pro-caspase-3 levels were elevated in cells co-treated with U0126 and E-OJ (Figure 5B). These findings suggest that caspase-3 activation occurs downstream of ERK1/2 signaling.

Taken together, these results indicate that E-OJ-induced apoptosis in HeLa cells is at least partially mediated through ERK1/2 activation, which in turn promotes caspase-3-dependent apoptotic signaling.

4. Discussion

This study was conducted as a follow-up to our previous finding that *O. japonicus* induces caspase-dependent apoptosis in cervical cancer cells [21]. While that study confirmed the extract's apoptotic potential, the molecular mechanisms driving this effect remained largely

undefined. In the present study, we demonstrated that the ethyl acetate fraction of *O. japonicus* (E-OJ) induces apoptosis in HeLa cells through modulation of both the NF- κ B and MAPK signaling pathways and subsequent activation of caspase-3.

Annexin V/PI staining revealed that E-OJ induces apoptosis in HeLa cells in a dose-dependent manner, with higher concentrations resulting in a marked increase in early and late apoptotic populations. These effects were more pronounced than those observed with individual phenolic compounds such as kaempferol, quercetin, and gallic acid, suggesting that E-OJ may contain synergistic constituents that enhance apoptotic efficacy. Morphological analysis by DAPI staining further supported these findings, with evident chromatin condensation and apoptotic body formation in E-OJ-treated cells [22,23].

Interestingly, among the three major MAPK signaling pathways, only ERK1/2 was significantly phosphorylated in response to E-OJ treatment, whereas JNK and p38 MAPKs remained unchanged. This selective activation suggests that the cellular response to E-OJ is pathway-specific rather than a general MAPK activation.

Although all three MAPKs are activated in response to extracellular stimuli, they are functionally and contextually distinct. The ERK1/2 pathway is primarily activated by growth factors and mitogens and is often associated with cell proliferation and differentiation [13,24]. However, under certain stress conditions or in response to phytochemicals, sustained ERK activation can paradoxically lead to apoptosis [25,26,27,28]. In contrast, the JNK and p38 pathways are more commonly activated by inflammatory cytokines, oxidative stress, and

environmental stressors, such as UV radiation or osmotic shock [29]. These differences may explain why E-OJ, which likely contains compounds mimicking mitogen-like signals or modulating redox balance, selectively targets ERK1/2 without significantly impacting JNK or p38 pathways.

Additionally, the phenolic components within E-OJ, such as kaempferol and quercetin, are known to interact with upstream regulators of ERK signaling, including MEK1/2 and Ras-related proteins [30,31]. The enhanced and rapid activation of ERK1/2 within 15 minutes of E-OJ treatment further supports a mechanism involving receptor-mediated or redox-sensitive ERK pathway activation, independent of the classical stress-activated MAPKs. Therefore, the selective activation of ERK1/2 observed in this study appears to reflect the specific chemical composition of E-OJ and its targeted modulation of intracellular signaling.

Moreover, we observed that E-OJ significantly downregulated NF- κ B signaling, a pathway known to confer anti-apoptotic protection in cancer cells. E-OJ treatment effectively inhibited NF- κ B activity by suppressing the degradation of I κ B α phosphorylation and reducing the nuclear translocation of NF- κ B p65 subunit. Given the well-established role of NF- κ B in promoting tumor cell survival and resistance to apoptosis, its inhibition may sensitize HeLa cells to ERK1/2-mediated apoptotic signaling [10,32,33].

5. Conclusions

Taken together, our results suggest that the pro-apoptotic effects of E-OJ in HeLa cells are mediated by a dual mechanism: suppression of NF- κ B-dependent survival signaling and activation of ERK1/2-mediated apoptotic signaling. The selective engagement of ERK1/2, in the absence of p38 or JNK activation, underscores the specificity of E-OJ's molecular effects. These findings provide a compelling rationale for further investigation of *O. japonicus* as a natural therapeutic agent for cervical cancer, particularly in cases where NF- κ B-mediated resistance is a concern.

ACKNOWLEDGEMENTS

This work was supported by the 2024 Inje University research grant.

References

- [1] Hull, R., Mbele, M., Makhafola, T., Hicks, C., Wang, S. M., Reis, R. M., Mehrotra, R., Mkhize-Kwitshana, Z., Kibiki, G., Bates, D. O., and Dlamini, Z. "Cervical cancer in low and middle-income countries", *Oncol Lett*, 20(3), 2058-2074, Sep. 2020.
- [2] Vale, D. B., Teixeira, J. C., Braganca, J. F., Derchain, S., Sarian, L. O., and Zeferino, L. C., "Elimination of cervical cancer in low- and middle-income countries: Inequality of access and fragile healthcare systems", *Int J Gynaecol Obstet*, 152(1), 7-11, Jan. 2021.
- [3] Tsuda, N., Watari, H., and Ushijima, K., "Chemotherapy and molecular targeting therapy for recurrent cervical cancer", *Chin J Cancer Res*, 28(2), 241-253, Apr. 2016.
- [4] Chao, X., Fan, J., Song, X., You, Y., Wu, H., Wu, M., and Li, L. "Diagnostic strategies for recurrent cervical cancer: A cohort study", *Front Oncol*, 10, 591253, Dec. 2020.
- [5] George, I. A., Chauhan, R., Dhawale, R. E., Iyer, R., Limaye, S., Sankaranarayanan, R., Venkataramanan, R., and Kumar, P. "Insights into therapy resistance in cervical cancer", *Advances in Cancer Biology - Metastasis*, 6, 100074, Dec. 2022.
- [6] Burmeister, C. A., Khan, S. F., Schafer, G., Mbatani, N., Adams, T., Moodley, J., and Prince, S. "Cervical cancer therapies: Current challenges and future perspectives", *Tumour Virus Res*, 13, 200238, Apr. 2022.
- [7] Gavrilescu, L. C., and Denkers, E. Y. "Apoptosis and the balance of homeostatic and pathologic responses to protozoan infection", *Infect Immun*, 71(11), 6109-6115, Nov. 2003.
- [8] Pistrutto, G., Trisciuglio, D., Ceci, C., Garufi, A., and D'Orazi, G., "Apoptosis as anticancer mechanism: Function and dysfunction of its modulators and targeted therapeutic strategies", *Aging (Albany NY)*, 8(4), 603-619, Apr. 2016.
- [9] Fernald, K., and Kurokawa, M., "Evading apoptosis in cancer", *Trends Cell Biol*, 23(12), 620-633, Dec. 2013.
- [10] Verzella, D., Pescatore, A., Capece, D., Vecchiotti, D., Ursini, M. V., Franzoso, G., Alesse, E., and Zazzeroni, F., "Life, death, and autophagy in cancer: NF- κ B turns up everywhere", *Cell Death & Disease*, 11(3), 210, Mar. 2020.
- [11] Yue, J., and López, J. M., "Understanding MAPK signaling pathways in apoptosis", *Int J Mol Sci*, 21(7), Mar. 2020.
- [12] Gaur, U., and Aggarwal, B.B., "Regulation of proliferation, survival and apoptosis by members of the TNF superfamily", *Biochem Pharmacol*, 66(8), 1403-1408, Oct. 2003.
- [13] Zhang, W., and Liu, H. T., "MAPK signal pathways in the regulation of cell proliferation in mammalian cells", *Cell Research*, 12(1), 9-18, Mar. 2002.
- [14] Jeong, J. H., Ryu, D. S., Suk, D. H., and Lee, D. S., "Anti-inflammatory effects of ethanol extract from *Orostachys japonicus* on modulation of signal pathways in LPS-stimulated RAW 264.7 cells", *BMB Rep*, 44(6), 399-404, Jun. 2011.
- [15] Ryu, D. S., Lee, H. S., Lee, G. S., and Lee, D. S., "Effects of the ethylacetate extract of *Orostachys japonicus* on induction of apoptosis through the p53-mediated signaling pathway in human gastric cancer cells", *Biol Pharm Bull*, 35(5), 660-665, 2012.
- [16] Park, H. J., Yang, H. J., Kim, K. H., and Kim, S. H., "Aqueous extract of *Orostachys japonicus* A. Berger exerts immunostimulatory activity in RAW 264.7 macrophages", *J Ethnopharmacol*, 170, 210-217, Jul. 2015.
- [17] Lee, K. S., Kim, S. W., and Lee, H. S., "*Orostachys japonicus* induce p53-dependent cell cycle arrest through the MAPK signaling pathway in OVCAR-3 human ovarian cancer cells", *Food Sci Nutr*, 6(8), 2395-2401, Oct. 2018.
- [18] Kim, J. H., Nam, G. S., Kim, S. H., Ryu, D. S., and Lee, D. S., "*Orostachys japonicus* exerts antipneumonia cancer activity through induction of apoptosis and cell cycle arrest in PANC-1 cells", *Food Sci Nutr*, 7(11), 3549-3559, Sep. 2019.
- [19] Kwon, J. H., Kim, J. H., Ryu, D. S., Lee, H. J., and Lee, D. S., "Anticancer effect of the ethyl acetate fraction from *Orostachys japonicus* on MDA-MB-231 human breast cancer cells through extensive induction of apoptosis, cell cycle arrest, and antimetastasis", *Evid Based Complement Alternat Med*, 2019, 8951510, Nov. 2019.
- [20] Ryu, D.S., Baek, G.O., Kim, E.Y., Kim, K.H., and Lee, D.S., "Effects of polysaccharides derived from *Orostachys japonicus* on induction of cell cycle arrest and apoptotic cell death in human colon cancer cells", *BMB Rep*, 43(11), 750-755, 2010.
- [21] Kim, S. H., and Lee, D. S., "*Orostachys japonicus* induce caspase-dependent apoptosis in HeLa human cervical cancer cells", *Nutr Res Pract*, 19(1), 131-142, Feb. 2025.
- [22] Dmitrieva, N. I., and Burg, M. B., "Analysis of DNA breaks, DNA damage response, and apoptosis produced by high NaCl", *Am J Physiol Renal Physiol*, 295(6), F1678-F1688, Dec. 2008.
- [23] Mandelkow, R., Gumbel, D., Ahrend, H., Kaul, A., Zimmermann, U., Burchardt, M., and Stope, M. B. "Detection and quantification of nuclear morphology changes in apoptotic cells by fluorescence microscopy and subsequent analysis of visualized fluorescent signals", *Anticancer Res*, 37(5), 2239-2244, May 2017.
- [24] Zou, J., Lei, T., Guo, P., Yu, J., Xu, Q., Luo, Y., Ke, R., and Huang, D. "Mechanisms shaping the role of ERK1/2 in cellular senescence (Review)", *Mol Med Rep*, 19(2), 759-770, Nov. 2019.
- [25] Cagnol, S., and Chambard, J. C., "ERK and cell death: Mechanisms of ERK-induced cell death—apoptosis, autophagy and senescence", *FEBS J*, 277(1), 2-21, Jan. 2010.

- [26] Wang, X., and Tournier, C., "Regulation of cellular functions by the ERK5 signalling pathway", *Cell Signal*, 18(6), 753-760, Jun 2006.
- [27] Liu, J., Ma, L., Chen, X., Wang, J., Yu, T., Gong, Y., Ma, A., Zheng, L., and Liang, H., "ERK inhibition sensitizes cancer cells to oleanolic acid-induced apoptosis through ERK/Nrf2/ROS pathway", *Tumour Biol*, 37(6), 8181-8187, Jun 2016.
- [28] Jung, G. H., Lee, J. H., Han, S. H., Woo, J. S., Choi, E. Y., Jeon, S. J., Han, E. J., Jung, S. H., Park, Y. S., Park, B. K., Kim, B. S., Kim, S. K., and Jung J, Y., "Chrysin induces apoptosis via the MAPK pathway and regulates ERK/mTOR-mediated autophagy in MC-3 cells", *Int J Mol Sci*, 23(24), 15747, Dec. 2022.
- [29] Kyriakis, J. M., and Avruch, J., "Mammalian MAPK signal transduction pathways activated by stress and inflammation: A 10-year update", *Physiol Rev*, 92(2), 689-737, Apr. 2012.
- [30] Kim, B. W., Lee, E. R., Min, H. M., Jeong, H. S., Ahn, J. Y., Kim, J. H., Choi, H. Y., Choi, H., Kim, E. Y., Park, S. P., and Cho, S. G., "Sustained ERK activation is involved in the kaempferol-induced apoptosis of breast cancer cells and is more evident under 3-D culture condition", *Cancer Biol Ther*, 7(7), 1080-1089, Jul. 2008.
- [31] Yufang, W., Mingfang, L., Nan, H., and Tingting, W., "Quercetin-targeted AKT1 regulates the Raf/MEK/ERK signaling pathway to protect against doxorubicin-induced nephropathy in mice", *Tissue and Cell*, 85, 102229, Dec. 2023.
- [32] Luo, J. L., Kamata, H., and Karin, M., "IKK/NF- κ B signaling: Balancing life and death—a new approach to cancer therapy", *J Clin Invest*, 115(10), 2625-2632, Oct. 2005.
- [33] Chen, B., Liu, J., Ho, T. T., Ding, X., and Mo, Y. Y., "ERK-mediated NF- κ B activation through ASIC1 in response to acidosis", *Oncogenesis*, 5(12), e279, Dec. 2016.



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