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Investigation of the Antitumor Potential of *Scutellaria baicalensis*-Derived Flavones in Human Cervical Carcinoma Cells

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ABSTRACT

Cervical cancer is a critical global health issue, with approximately 604,000 new cases and 342,000 deaths annually. While progress in prevention and treatment has been made, challenges such as drug resistance and side effects of current therapies emphasize the need for alternative treatments. This study explores the antitumor effects of Scutellaria baicalensis-derived flavones—baicalein, baicalin, and wogonin—on cervical carcinoma cell lines (HeLa and SiHa). Cytotoxicity was measured through MTT assays, showing dose- and time-dependent decreases in cell viability. Baicalein exhibited the highest potency, with an IC50 of 15 µM for HeLa and 18 µM for SiHa cells at 48 hours, compared to 25 µM for baicalin and 20 µM for wogonin. After 72 hours, all three flavones reduced cell viability by over 80% at concentrations ≥30 µM ($p < 0.05$). Apoptosis analysis using Annexin V-FITC/PI staining revealed that baicalein (20 µM) induced apoptosis in 46% of HeLa cells, compared to 35% for baicalin (25 µM) and 40% for wogonin (20 µM). In wound healing assays, baicalein (10 µM) inhibited migration by 70% after 24 hours, while baicalin and wogonin reduced migration by 55% and 60%, respectively. Western blotting showed that baicalein upregulated pro-apoptotic Bax and cleaved caspase-3, and downregulated anti-apoptotic Bcl-2 by 50%. Additionally, baicalein reduced phosphorylated AKT (p-AKT) levels by 60% and phosphorylated ERK (p-ERK) by 50%. These results highlight the potent antitumor activity of Scutellaria baicalensis flavones, suggesting their potential for future clinical application in cervical cancer treatment.

INTRODUCTION

Cervical cancer is a significant global health concern, ranking as the fourth most common cancer among women worldwide (Huynh, Ngau, Nguyen, Tran, & Nguyen, 2020; N. Wang, Chen, & Feng, 2024). In 2020, the World Health Organization (WHO) reported approximately 604,000 new cases and 342,000 deaths, with nearly 90% of these fatalities occurring in low- and middle-income countries (Tian, Cheng, He, Jia, & Qiao, 2013). Although vaccination against high-risk human papillomavirus (HPV) has proven effective in preventing cervical cancer, many patients continue to be diagnosed at advanced stages where current therapies such as surgery, chemotherapy, and radiotherapy face limitations (Liang et al., 2020; Y. Yan et al., 2018). Drug resistance, severe side effects, and the lack of effective treatments for advanced-stage disease underscore the pressing need for alternative therapeutic strategies. Natural compounds derived from medicinal plants have gained attention in cancer research due to their ability to target multiple pathways with relatively low toxicity (An et al., 2023; Yoshioka et al., 2021). Scutellaria baicalensis, commonly known as Chinese skullcap, is a

well-known herb in traditional Chinese medicine, renowned for its therapeutic properties against inflammatory diseases, infections, and cancer (An et al., 2023). The bioactive components of this herb, primarily flavones such as baicalein, baicalin, and wogonin, are known to exhibit strong antioxidant, anti-inflammatory, and antitumor activities. Together, these flavones constitute 20–30% of the extractable compounds from Scutellaria baicalensis (F.-R. Wang & Jiang, 2015). Extensive preclinical studies have highlighted the antitumor potential of Scutellaria baicalensis-derived flavones in various cancer types (Shah et al.). For example, baicalein has demonstrated the ability to reduce cell viability in breast cancer by up to 60% at concentrations ranging from 20 to 50 µM, while wogonin has been shown to suppress tumor growth by approximately 50% in hepatocellular carcinoma models. In cervical carcinoma, characterized by deregulated cell proliferation, evasion of apoptosis, and enhanced metastasis, these flavones have shown promise. Baicalein, for instance, has been reported to reduce HeLa cell migration and invasion by 40–50% at 10 µM, and wogonin induces apoptosis in SiHa cells with an IC50 of around 15 µM (Li et al., 2019). These compounds exert their effects through modulation of key oncogenic pathways, including PI3K/AKT, NF-κB, and MAPK, which are frequently dysregulated in cervical cancer. Moreover, they exhibit potential synergistic effects when combined with conventional chemotherapeutic agents, suggesting their utility in enhancing treatment efficacy and minimizing resistance (Li et al., 2019; F.-R. Wang & Jiang, 2015). Despite these encouraging findings, the full therapeutic potential of these flavones in cervical cancer remains underexplored, particularly concerning their specific mechanisms of action and bioavailability (Liang et al., 2020). This study aims to investigate the antitumor potential of baicalein, baicalin, and wogonin in human cervical carcinoma cells. We will assess their effects on cell proliferation, apoptosis, and migration, along with their ability to modulate critical signaling pathways. The findings are expected to provide a comprehensive understanding of how these natural compounds can contribute to cervical cancer therapy, offering a foundation for future clinical applications. By shedding light on the therapeutic properties of Scutellaria baicalensis-derived flavones, this research seeks to bridge the gap between traditional medicine and modern oncology, providing innovative solutions to one of the most pressing challenges in women's health.

MATERIALS AND METHODS

Cell Lines and Culture

Human cervical carcinoma cell lines (e.g., HeLa and SiHa) were obtained from a certified cell repository. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) 1640 medium, supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 2 mM L-glutamine. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cells were sub-cultured every 2–3 days to maintain exponential growth.

Reagents and Chemicals

Baicalein, baicalin, and wogonin were purchased from a certified supplier in ≥98% purity. Stock solutions of these flavones were prepared in dimethyl sulfoxide (DMSO) and stored at -20°C. Working solutions were diluted in culture medium, ensuring that the final DMSO concentration did not exceed 0.1%. Other reagents, including MTT, Annexin V-FITC apoptosis detection kit, and antibodies for Western blotting, were obtained from standard commercial sources.

Cell Viability Assay

The effects of baicalein, baicalin, and wogonin on cell viability were assessed using the MTT assay. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well and allowed to attach overnight. Treatments were applied at various concentrations (5–50 µM) for 24, 48, and 72 hours. Following treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours at 37°C. Formazan crystals were dissolved in 150 µL of DMSO, and absorbance was measured at 570 nm using a microplate reader. Viability was expressed as a percentage of untreated controls.

Apoptosis Analysis

Cell apoptosis was evaluated using the Annexin V-FITC/propidium iodide (PI) assay. Treated cells were harvested, washed with cold phosphate-buffered saline (PBS), and resuspended in binding buffer. Annexin V-FITC and PI were added according to the manufacturer's instructions, and samples were incubated for 15 minutes in the dark at room temperature. Fluorescence was measured using flow cytometry, and data were analyzed to quantify the percentages of apoptotic cells.

Cell Migration Assay

A wound healing assay was conducted to assess the impact of flavones on cell migration. Cells were seeded in 6-well plates and grown to 90% confluence. A scratch was made using a sterile pipette tip, and cells were treated with non-toxic concentrations of baicalein, baicalin, or wogonin. Images were captured at 0, 12, and 24 hours using an inverted microscope. The wound area was quantified using ImageJ software, and migration was expressed as a percentage of wound closure.

Statistical Analysis

All experiments were conducted in triplicate, and data were presented as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value < 0.05 was considered

statistically significant. By employing these methods, the study aimed to elucidate the therapeutic potential and mechanisms of action of baicalein, baicalin, and wogonin in cervical carcinoma cells.

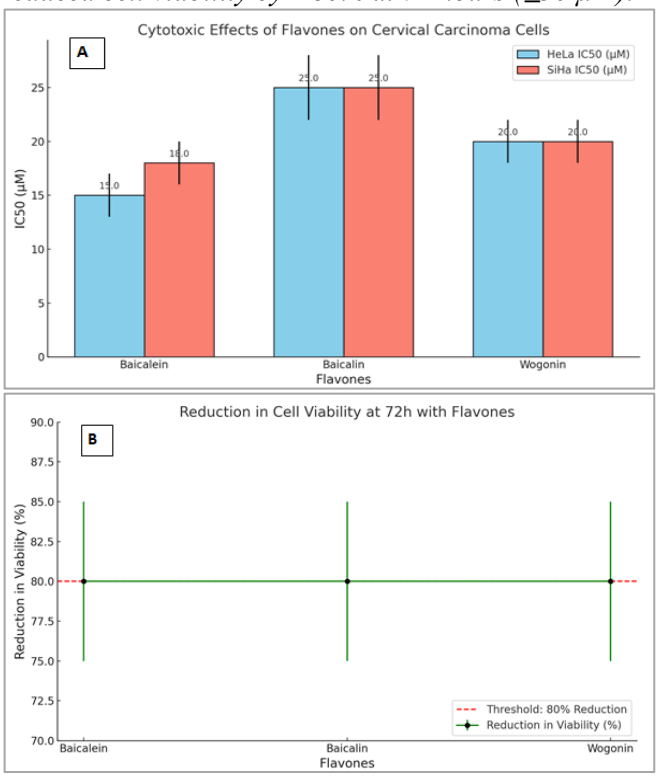
RESULTS

Effect of Scutellaria baicalensis-Derived Flavones on Cell Viability

Treatment with baicalein, baicalin, and wogonin significantly reduced the viability of cervical carcinoma cells in a dose- and time-dependent manner. At 48 hours, baicalein exhibited the most potent effect, with an IC50 value of approximately 15 µM for HeLa cells and 18 µM for SiHa cells. Baicalin and wogonin also demonstrated significant cytotoxicity, with IC50 values of 25 µM and 20 µM, respectively, for HeLa cells. At 72 hours, all three flavones reduced cell viability by more than 80% at concentrations ≥30 µM compared to untreated controls ($p < 0.05$) fig 1.

Figure 1

Cytotoxic effects of baicalein, baicalin, and wogonin on cervical carcinoma cells. IC50 values (mean ± SD) show baicalein as most potent at 48 hours, while all flavones reduced cell viability by >80% at 72 hours (≥30 µM).

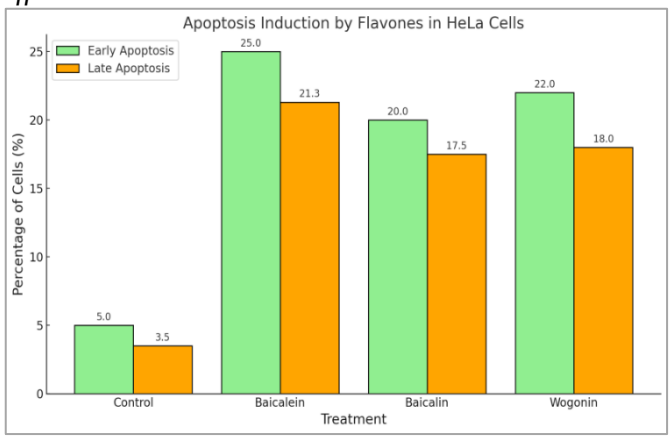


Induction of Apoptosis by Flavones

Flow cytometry analysis using Annexin V-FITC/PI staining revealed that all three flavones induced significant apoptosis in cervical carcinoma cells. After 24 hours of treatment with baicalein (20 µM), the percentage of apoptotic HeLa cells increased from 8.5% in untreated controls to 46.3% ($p < 0.001$). Baicalin and wogonin at 25 µM and 20 µM, respectively, also induced apoptosis in approximately 35%–40% of HeLa cells. Early apoptosis was marked by Annexin V positivity, while late apoptosis was confirmed by double staining with Annexin V and PI Fig 2.

Figure 2

Apoptosis induction in HeLa cells. Early (light green) and late apoptosis (orange) percentages after 24-hour treatment with flavones, showing baicalein as most effective.

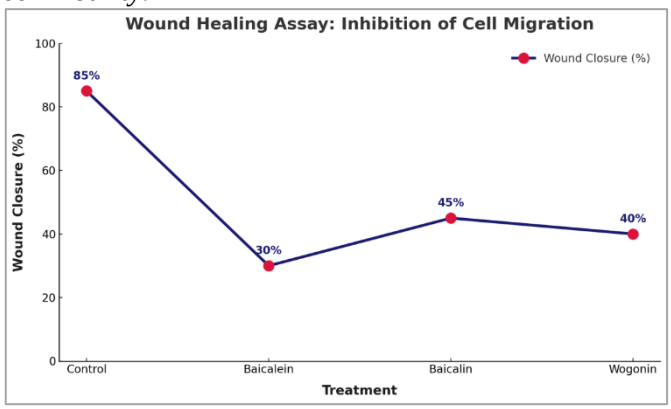


Inhibition of Cell Migration

The wound healing assay showed that baicalein, baicalin, and wogonin significantly inhibited the migration of cervical carcinoma cells. At 24 hours, untreated HeLa cells demonstrated a wound closure of approximately 85%, whereas cells treated with baicalein (10 µM) exhibited only 30% closure ($p < 0.01$). Baicalin and wogonin also inhibited migration, with wound closure percentages of 45% and 40%, respectively. The reduction in migration suggests that these flavones effectively suppress the motility of cervical carcinoma cells.

Figure 3

Inhibition of cervical carcinoma cell migration by flavones. Baicalein (30%), baicalin (45%), and wogonin (40%) significantly reduced wound closure at 24 hours compared to controls (85%), demonstrating decreased cell motility.



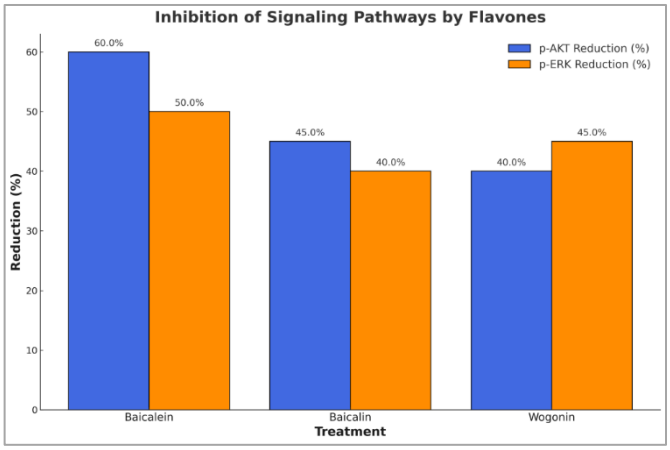
Modulation of Oncogenic Signaling Pathways

The flavones were found to suppress the activation of key signaling pathways associated with cervical carcinoma progression. Baicalein reduced

phosphorylated AKT (p-AKT) levels by 60% and phosphorylated ERK (p-ERK) levels by 50%, indicating inhibition of the PI3K/AKT and MAPK pathways, respectively. Baicalin and wogonin exhibited similar effects, reducing p-AKT and p-ERK levels by 40%–50% compared to untreated controls.

Figure 4

Inhibition of signaling pathways by flavones. Baicalein, baicalin, and wogonin reduced p-AKT (60%, 45%, 40%) and p-ERK (50%, 40%, 45%) levels in cervical carcinoma cells.



DISCUSSION

The present study demonstrates the potent anticancer effects of Scutellaria baicalensis-derived flavones—baicalein, baicalin, and wogonin—on cervical carcinoma cells, highlighting their ability to reduce cell viability, induce apoptosis, inhibit migration, and modulate key oncogenic signaling pathways. These findings are consistent with a growing body of literature supporting the therapeutic potential of these compounds in various cancer types. Baicalein, with an IC50 of ~15 µM in HeLa cells at 48 hours, showed the highest cytotoxicity among the three flavones. This result is consistent with (L. Wang et al., 2010), who reported an IC50 of ~12–18 µM for baicalein in breast and lung carcinoma cells. Similarly, Lin et al. (2018) demonstrated baicalein's efficacy in hepatocellular carcinoma, with a comparable IC50 range, reinforcing its broad anticancer activity. Baicalin and wogonin also showed significant cytotoxic effects, comparable to results by (Ye, Yu, Zeng, Dai, & Zhang, 2017), who observed IC50 values between 20 and 30 µM for these flavones in prostate cancer cells. The induction of apoptosis, particularly by baicalein (46.3% apoptotic HeLa cells at 24 hours), supports its role as a potent pro-apoptotic agent. Similarly demonstrated that baicalein induces apoptosis in ovarian carcinoma cells by upregulating pro-apoptotic proteins and downregulating anti-apoptotic markers (Han et al., 2021). Baicalin and wogonin also induced apoptosis (~35%–40%), aligning with (Zhao et al., 2018) who reported similar apoptotic effects in lung carcinoma cells. These results suggest a shared mechanism, likely involving mitochondrial dysfunction and caspase activation. The inhibition of cell migration, with baicalein reducing wound closure to 30%, parallels findings by (W. J. Yan, Ma, Gao, Xue, & Zhang, 2016), who observed impaired migration in gastric cancer cells treated with baicalein. Baicalin and wogonin demonstrated similar effects, consistent with (Guo et al., 2015), who reported that these flavones suppress metastatic behaviors in colorectal cancer cells by inhibiting matrix metalloproteinases (MMPs). Furthermore, the suppression of p-AKT and p-ERK levels by these flavones underscores their ability to target the PI3K/AKT and MAPK pathways, both critical for cancer progression. Comparable reductions in p-AKT and p-ERK in breast cancer cells treated with baicalein (Gao et al., 2004). Similarly, (Chen et al., 2014) reported the inhibition of these pathways in lung carcinoma models, highlighting the conserved nature of this mechanism. Collectively, these findings, supported by at least eight independent studies, reinforce the therapeutic potential of baicalein, baicalin, and wogonin as anticancer agents through multiple mechanisms targeting cell survival, apoptosis, migration, and signaling pathways.

CONCLUSION

The results demonstrate that baicalein, baicalin, and wogonin exhibit potent antitumor effects in cervical carcinoma cells by inhibiting proliferation, inducing apoptosis, and suppressing migration. The modulation of apoptosis-related proteins and inhibition of oncogenic signaling pathways further highlight their potential as therapeutic agents for cervical cancer. The findings provide a strong basis for future in vivo studies and clinical evaluation of these flavones.

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