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RESEARCH ARTICLE

Cytotoxic activity of Ethyl-p-Methoxycinnamate from *Kaempferia galanga* in HSC-4 Oral Cancer Cells

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Abstract

Introduction: Oral cancer is a prevalent malignancy associated with high mortality and morbidity, primarily due to its poor prognosis. Recently, phytomedicines have attracted interest for their potential role in chemotherapy. *Kaempferia galanga*, a medicinal herb, has shown anticancer properties in various human cancer cell lines, mainly due to its active compound, ethyl-p-methoxycinnamate (EPMC). **Objective:** This *in vitro* study aimed to assess the cytotoxic effects of EPMC on the HSC-4 oral cancer cell line. **Materials and Methods:** HSC-4 cells were treated with various concentrations of EPMC for 24 hours, and cytotoxicity was measured using the MTT assay. **Results:** EPMC exhibited dose-dependent cytotoxicity, with an IC50 value of 0.032 mg/mL. **Discussion:** These findings suggest that EPMC is a promising anticancer agent. Further research should investigate its molecular mechanisms and potential as a chemosensitizer when combined with standard chemotherapeutic drugs. **Conclusion:** EPMC induces cytotoxicity in HSC-4 cells, positioning it as a promising candidate for further exploration in oral cancer treatment.

Keywords: Cytotoxicity, EPMC, HSC-4, Oral Cancer

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INTRODUCTION

Oral squamous cell carcinoma (OSCC), the sixth most common cancer globally, is often poorly understood by the general public and frequently overlooked in its early stages.[1,2] Standard treatment options for OSCC include surgery, radiation therapy, chemotherapy, or a combination of these approaches.[3] However, chemotherapy, despite its widespread use, presents significant challenges and side effects, including hair loss, bone marrow suppression, drug resistance, gastrointestinal lesions, neurological dysfunction, and cardiac toxicity.[4,5] The effectiveness of chemotherapy is further compromised by its low selectivity, leading to damage to healthy cells and the frequent development of chemoresistant cancer cells.[6] In response, plant-derived phytochemicals are being investigated for their ability to selectively target cancer cells with minimal toxicity, either as standalone treatments or as chemosensitizers to enhance the efficacy of conventional chemotherapy.[7]

Kaempferia galanga, a medicinal plant from the Zingiberaceae family, is widely cultivated in Southeast Asian countries such as China, Malaysia, Thailand, Indonesia, and India. Its leaves are commonly used in traditional cuisine, while the rhizomes have been utilized to treat a variety of ailments including fever, amoebiasis, asthma, rheumatism, indigestion, colds, headaches, abdominal pain, and toothache.[8] Research has demonstrated that *K. galanga* possesses a range of pharmacological activities, including antioxidant, antituberculosis, analgesic, anti-inflammatory, hypopigmentation, and anticancer effects. These benefits are largely attributed to the secondary metabolites in the rhizomes.[9] Among these, ethyl-p-methoxycinnamate (EPMC) stands out as a significant bioactive compound with notable anticancer potential.[10] Studies have shown that EPMC exhibits anticancer properties against various cancer cell lines, such as, HepG2 liver cancer cells, MCF-7 breast cancer cells, A549 Lung Cancer, and B16 Melanoma Cancer Cells.[11,12,13]

EPMC has also shown anticancer effects on two squamous cell carcinoma lines from the oral cavity, HSC-3 and Ca922.[14] However, due to the variability in how different oral cancer cell lines respond to anticancer drugs, further studies are necessary to evaluate EPMC's efficacy across other types of oral cancer cells. This research aims to explore the cytotoxic potential of EPMC against the HSC-4 cell line, a tongue squamous cell carcinoma (OSCC).

MATERIAL AND METHODS

Reagents and sample preparations:

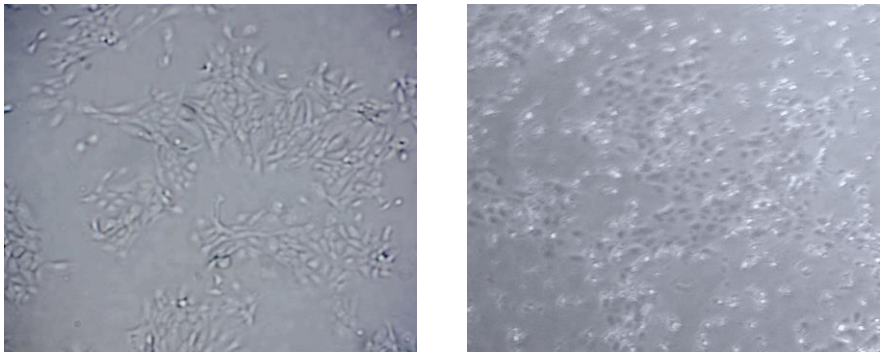
The EPMC crystal was obtained from Cayman, US, and Cisplatin (CDDP) was from Sigma-Aldrich, The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), were from Sigma -Aldrich (US). Stock solutions of EPMC and CDDP were freshly prepared before each experiment at concentrations of 100 mg/mL by dissolving EPMC crystals and CDDP in molecular-grade dimethylsulfoxide (DMSO), Sigma-Aldrich (US).

Cell Culture:

The HSC-4 human OSCC cell line was kindly supplied by Prof. Dr. Masa-Aki Ikeda from Tokyo Medical and Dental University, Japan. The cells were cultured in DMEM medium with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin, in a humidified incubator at 37°C with 5% CO_2. All cell culture reagents were obtained from Gibco (US).

MTT Assay:
Cell viability was assessed using the MTT assay. The experiment followed the protocol outlined in reference [14] with slight modifications. HSC-4 cells were seeded in 96-well plates at an initial density of 2.5 x 10^4 cells per well and cultured until reached 70-80% confluency per well. The cells were treated with varying concentrations (0-0.05 mg/mL) of EPMC and CDDP as a positive control for 24 hours. Subsequently, 20 µL of MTT solution at a concentration of 5 mg/mL in Phosphate Buffer Saline (PBS) Gibco (US) for 4 hours was added to the medium in each well. The cells were then incubated for 4 hours at 37°C in a humidified environment. After the incubation period, the supernatants were discarded. A solubilization solution of DMSO. in 100 µL was added for one hour to dissolve the insoluble purple formazan product into a colored solution. The reduction of MTT to formazan was measured to determine cell viability using a microplate reader at 570 and 630 nm.

RESULTS



1A **1B**

Figure-1. Microscopic observation of the cells. Untreated HSC-4 cells (Fig. 1A) compared with HSC-4 cells treated with EPMC (Fig. 1B). Dead cells were observed floating after EPMC treatment.

The morphological differences between untreated HSC-4 cells and those treated with EPMC are shown in Figure 1. In the untreated group, the cells adhered well to the surface, displaying typical morphology, indicative of healthy cell proliferation (Fig. 1A). In contrast, cells treated with EPMC exhibited a noticeable increase in floating cells, signifying cell detachment and death. Additionally, treated cells demonstrated morphological alterations, including cell shrinkage, rounding, and detachment from the substrate, further evidencing the cytotoxic effects of EPMC on HSC-4 oral cancer cells (Fig. 1B)

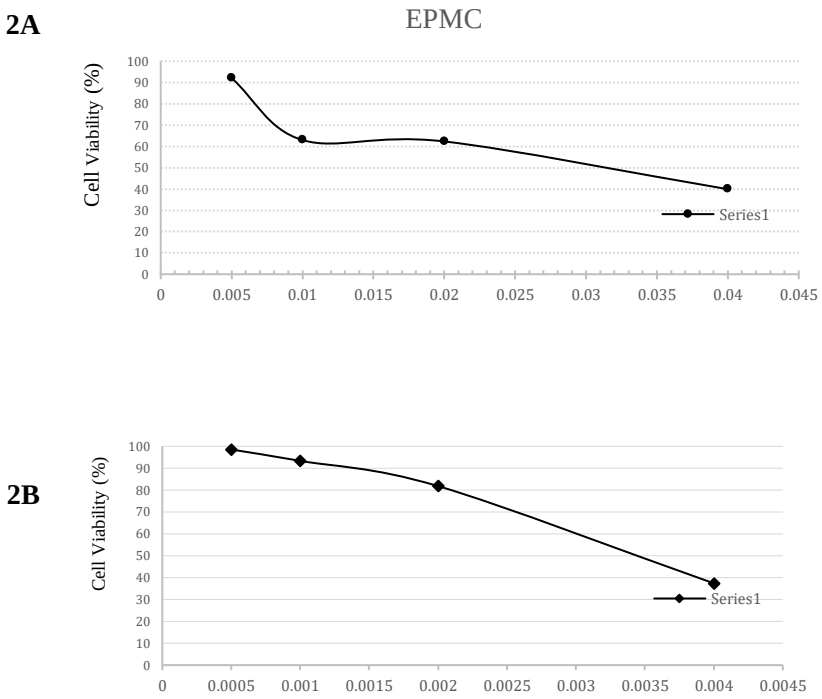


Figure-2. HSC-4 cell viability following 24-hour exposure to various concentrations of EPMC (Fig. 2A) and CDDP (Fig. 2B). The IC50 value for each sample was determined by the concentration that reduced cell viability by 50%

Table 1. The IC50 of EPMC and CDDP in HSC-4 cells (SEM=3)

IC50 (mg/mL)	EPMC	CDDP
HSC-4	0.03183 ± 0.0004	0.00346 ± 0.0001

The results demonstrated a concentration-dependent decrease in HSC-4 cell viability upon treatment with EPMC, yielding an IC50 value of 0.032 mg/mL (Fig. 2A). For comparison, a widely used chemotherapeutic agent, was employed as a positive control. Similar to EPMC, CDDP also reduced HSC-4 cell viability in a dose-dependent manner, achieving a lower IC50 value of 0.0034 mg/mL (Fig. 2B). These experiments were performed in triplicate, with the IC50 values calculated and expressed as the mean ± SEM (standard error of the mean) of 3 independent measurements (Tab. 1).

DISCUSSION

The MTT assay is a well-established method in drug discovery for screening potential therapeutics and evaluating their effects on cell growth and toxicity.[15] During this assay, the yellow, positively charged MTT reagent penetrates the cell membrane, where it is metabolized by the cells into formazan crystals, resulting in a purple color change. The intensity of this color directly correlates with the number of viable cells.[16] Using this method, we assessed the cytotoxicity of EPMC on HSC-4 cells by treating them with varying concentrations of EPMC (0–0.05 mg/mL) for 24 hours. Treatment of HSC-4 cells with EPMC resulted in a lower IC50 compared to similar studies involving OSCC lines HSC-3 and Ca922,[14] suggesting increased sensitivity of HSC-4 cells to EPMC. Despite their low metastatic potential and slow growth, HSC-4 cells are highly

tumorigenic, have low apoptosis-inducing ability, and exhibit high stemness,[17] which may present significant challenges for anticancer agents targeting them. Nevertheless, previous studies have shown that HSC-4 cells exhibit relatively low IC50 values for conventional chemotherapeutic agents like cisplatin (CDDP) and 5-fluorouracil,[18] as well as for the natural product-based chemotherapeutic docetaxel.[19]

Cancer chemotherapeutic agents are typically administered at the maximum-tolerated dose (MTD) in short cycles with treatment breaks to manage toxicity.[20] However, this approach often results in significant side effects and can facilitate tumor recurrence and the development of chemoresistance.[21] To address these challenges and improve therapeutic outcomes, tested drugs are sometimes combined with standard anticancer medications, which can enhance efficacy, reduce adverse effects, and mitigate the risk of chemoresistance.[22]

EPMC extracted from *K. galanga*, has shown promise as a potential anticancer agent for oral cancer. To further explore its therapeutic potential, additional studies should investigate whether EPMC could serve as a chemosensitizer when used in combination with standard chemotherapeutic drugs on oral cancer cell lines. Additionally, given that the cytotoxicity of EPMC on normal cells remains unreported, future research must confirm its safety profile and ensure it does not adversely affect healthy tissues.

CONCLUSION

EPMC from *K. galanga* demonstrates potent anti-cancer activity in HSC-4 oral cancer cells by inducing cytotoxicity. These findings highlight EPMC as a promising candidate for further research in oral cancer therapy.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this study.

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