ORIGINAL RESEARCH

Harnessing the Anti-Migratory Potential of Gingerol against Oral Cancer Cells: An *In vitro* Approach

Prathiyangara[1], Anitha Roy[1*], Jospin Sindya[2], Elumalai Perumal[2]

Abstract

Background: Gingerol, the principal bioactive constituent of *Zingiber officinale*, possesses documented anti-inflammatory, antioxidant, and anticancer activities, supporting its evaluation as a candidate for oral cancer therapeutics.

Aim: To evaluate gingerol for its cytotoxic, antimigratory and CXCL-8 and CXCR2 expression in oral cancer cell line.

Materials and Methods: KB -1oral cancer cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics at 37°C in a humidified 5% CO_2 atmosphere. The cytotoxic effects of gingerol (10–100 µM) were quantified by MTT assay, and morphological alterations were examined using phase-contrast microscopy. Cell migration was assessed by a scratch wound healing assay following treatment with the IC50 concentration of gingerol (76.97µM). Expression of the metastasis-associated molecules CXCL-8 and CXCR2 was analyzed by real-time PCR. Statistical significance was set at $p<0.05$.

Results: Gingerol reduced KB-1 cell viability in a dose-dependent manner, with significant cytotoxicity observed across the tested concentration range ($p<0.05$). Gingerol not only showed morphological changes such as cell shrinkage and membrane blebbing but also induced apoptosis and markedly inhibited cell migration in the scratch assay ($p<0.05$). It also significantly downregulated CXCL-8 and CXCR2 expression.

Conclusion: Gingerol exerts pronounced anticancer effect on oral cancer cells by decreasing cell viability, suppressing CXCL-8/CXCR2 signaling, and inducing apoptosis-associated morphological changes. Its anti-migratory effects evidenced from scratch wound assay limits migratory potential of cancer cells and there by inhibiting metastasis. These data underscore gingerol's promise as a potential adjuvant or complementary agent in oral cancer management and support further preclinical and clinical investigation.

Key words: Cell Line, Chemokine CXCL8, CXCR, Chemokine Receptors, Tumor, Gene Expression Regulation

Introduction

Oral squamous cell carcinoma is characterized by aggressive local invasion and a high tendency for regional and distant metastasis, and cell migration is a critical early step in this process.[1] Limiting migratory and invasive behavior of oral cancer cells can therefore reduce metastatic spread and improve therapeutic outcomes.[1]

Many natural compounds have the ability to fight cancer and have little side effects, making them an excellent alternative to chemotherapy drugs.[2] Gingerol, the main bioactive ingredient in ginger *(Zingiber officinale)*[3]. It has garnered significant attention because of its several pharmacological properties, which include effects that are anti-inflammatory, antioxidant, and anti-cancer.[4,5] It is suggested for cancer prevention and treatment.[6] Recent research has demonstrated gingerol's ability to alter a number of signaling pathways that are essential for the invasion and migration of cancer cells such as activation of AMPK and suppression of the AKT/mTOR Signaling Pathway.[7] Gingerol again demonstrated

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How to cite the article: Prathiyangara, Roy A., Sindya J., Perumal E. Harnessing the Anti-Migratory Potential of Gingerol against Oral Cancer Cells: An In Vitro Approach. Oral Maxillofac Pathol J 2026; 17(1); 48-52.

Source of Support: Nil

Conflict of Interest: None

Acknowledgement: The authors are thankful to Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences for providing the research facilities.

to disrupt the epithelial-mesenchymal transition (EMT) in prostate cancer.[8] Gingerol's therapeutic potential is further highlighted by its influence on the nuclear factor-kappa B (NF-κB) pathway, which is crucial for inflammation and cancer growth.[9] Antioxidants are known for their anticancer properties.[10] Additionally, gingerol's antioxidant qualities further contribute to its anticancer benefits as oxidative stress can lead to cancer.

A complete understanding of these molecular pathways is necessary for the development of gingerol-based therapies for oral cancer. As research advances, including gingerol in treatment regimens may provide a novel, healthful, and effective means of treating oral cancer as well as perhaps other malignancies with similar migration tendencies. Gaining insight into the molecular mechanisms underlying gingerol›s anti-migrative actions may pave the way for novel therapeutic approaches targeted at halting the spread of oral cancer. Hence, the current study set out to assess gingerol's cytotoxic and anti-migratory effects along with gene expression of chemokine signaling by CXCR-8 and CXCR2 pathway

Materials and Methods

The present study was conducted in the Cancer Genomics Laboratory, Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences from May 2024 -July 2024 after approval from the Scientific Review Board (SMC /R02/2024).

Maintenance of the cell line

The cell lines (KB-1) for oral cancer were donated by the NCCS in Pune. The cells were cultured in T25 culture flasks supplemented with 1% antibiotics and 10% FBS (Dulbecco›s Modified Eagle Medium). The cells were kept moist with 5% CO2 at 37°C. After the cells were confluent and trypsinized, they were passed.

Cell viability assay

After receiving gingerol (G5798-5MG, Sigma) therapy, the malignant cells› viability was evaluated using the MTT assay.[11,12] The theory behind the procedure is that soluble yellow tetrazolium salt is converted into insoluble purple formazan crystals by metabolically active cells. 96-well plates containing 5x103 KB cells/well were used for plating. Following plating, cells were cultivated for three hours at 37°C in serum-free medium to starve them. A full day later, two flushes using 100µl of medium without serum were carried out. Cells were starved for 24 hours and then treated to different dosages of gingerol. Following the completion of the treatment, 100µl of the medium from the treated and untreated cells was added to each well, along with 0.5 mg/ml of DMEM containing MTT. The cells were then maintained for four hours at 37°C in the CO2 incubator. After removing the MTT-containing medium, the cells were cleaned with a single PBS wash. After the formazan crystals dissolved in 100µl of DMSO, the mixture was allowed to settle in the dark for one hour. Next, a Micro ELISA plate reader was used to measure the color intensity at 570 nm. The approximate total number of living cells is determined by taking the percentage of control cells that are cultivated in serum-free media. All of the cells in the control media were alive when no treatment was administered. The following formula is used to determine the cell viability:

Percentage of viable cells = A570 nm of treated cells×100 / A570 nm of control cells

Cell Morphology Study

The MTT experiment results were utilized to establish the ideal dosage (IC50: 76.97µM) for additional research. Phase contrast microscopy is employed to examine alterations in cell morphology. Subsequently, 6 well plates with 2×10^5 cells each had a full day of Gingerol treatment. After the incubation period, the media was removed from the cells, and they were given one pH 7.4 PBS wash. To inspect the plates, a phase contrast microscope was employed.

Scratch wound healing assay

Oral cancer cells (2×105 cells/well) cells were seeded onto six-well culture plates. The cell monolayer was scratched using a 200µl tip to create a wound, washed with PBS and photographed in an inverted microscope. IC-50 dose 76.9µM of Gingerol was treated for 24 h and control cells were received with serum free culture medium, after the treatment period, the wounded area was photographed using the same microscope. And the experiments were repeated in triplicate for each treatment group.[13]

Real Time PCR

Real-time PCR was used to examine the gene expression of chemokine signaling and CXCR-8 and CXCR2 pathway anti-metastatic signaling molecules. Using Trizol Reagent (Sigma), a standardized procedure was used to separate the total RNA. Employing a PrimeScript first strand cDNA synthesis kit (TakaRa, Japan), 2µg of RNA was used for the reverse transcription process to synthesize cDNA. Specific primers were utilized to enhance the amplification of the targeted genes. The PCR reaction was conducted using GoTaq® qPCR Master Mix (Promega), which contains all necessary PCR components along with SYBR green dye. Real-time PCR was conducted

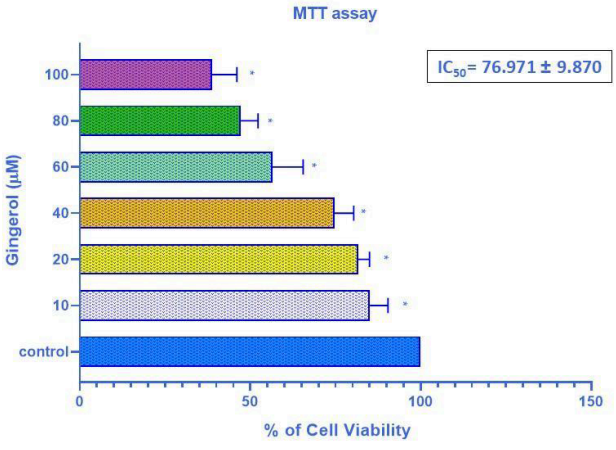

Fig. 1: The cytotoxic effects of Gingerol on Oral cancer cells at different concentration of Gingerol (10 – 100 µM) for 24 hours by MTT assay. '*' denotes statistical significance ($p<0.05$) between the control and drug treatment groups.

using a Biorad CFX96 PCR instrument. The comparative CT approach was employed for result analysis, and the 2−ΔΔCT method by Schmittgen and Livak was used to determine the fold change.

Statistical analysis

SPSS was used for the complete data analysis by Student t-test and One-Way ANOVA. The results were shown as mean ± SD in triplicate. The statistical significance was set at $p<0.05$.

Results

The cytotoxic effects of gingerol on the oral cancer cell line KB-1 was shown in Figure 1. Data was expressed as

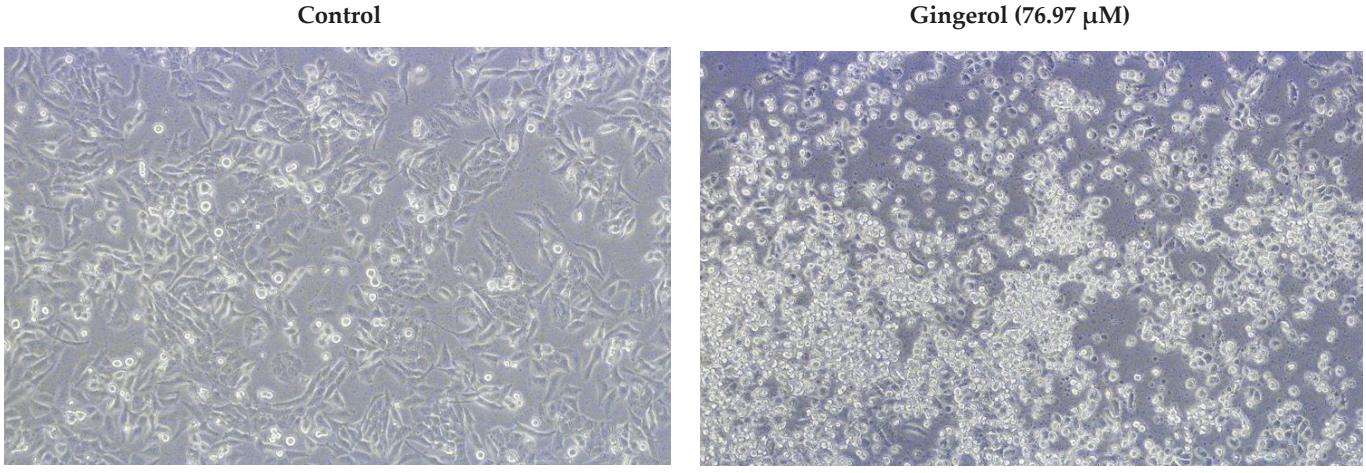

Fig. 2: Impact of Gingerol on the cellular structure of the oral cancer cell line (KB-1). Cells underwent treatment with Gingerol (76.97µM) for a duration of 24 hours, followed by observation using an inverted phase contrast microscope.

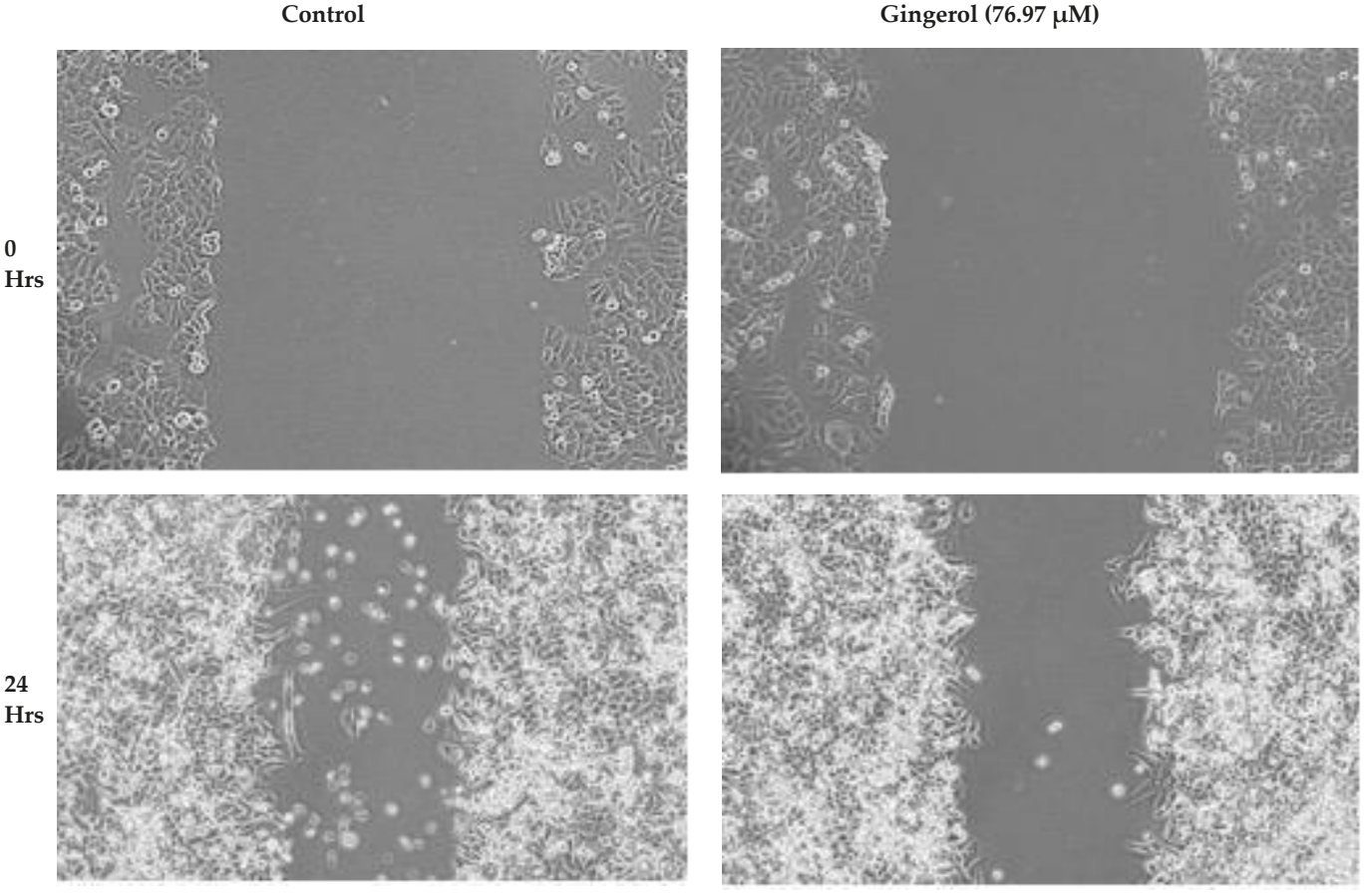

Fig. 3: Image of Scratch wound assay showing the anti-migratory potential of Gingerol (76.9µM) against oral cancer cells.

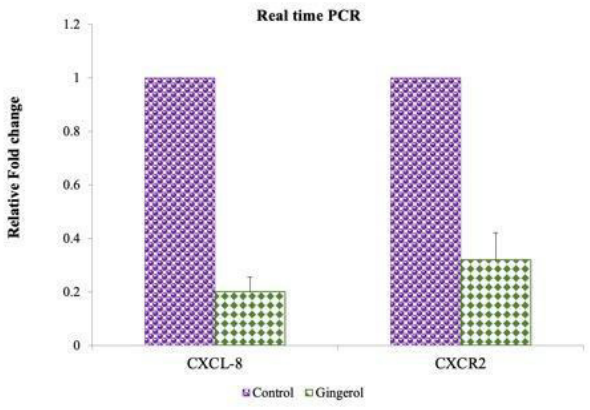

Fig. 4: Impact of gingerol (76.97μM) in CXCL-8 CXCR2 expression on oral cancer cell line.

mean ± SD (n = 3). The treatment with Gingerol resulted in a reduction in cell count. The number of viable cells dropped as concentration rose and 50% cytotoxicity was observed at 76.97μM concentration, which was considered as IC-50 concentration and this particular concentration was used for further research such as morphological studies, scratch wound assay and expression of CXCL8 and CXCR2.

Considering the morphological evaluation, after exposure with 76.97μM of gingerol for 24 hours, the treated KB-1 oral cancer cell line exhibited notable morphological changes in comparison to the untreated cells (Figure 2). The cells showed observable cell shrinkage and formation of blebs on the cytoplasmic membrane. Apoptotic cells exhibited a variety of changes, such as decreased cell number and cell atrophy.

The scratch wound assay showed the anti-migratory potential of Gingerol (76.97μM) against oral cancer cells compared to control (Figure 3).

Gingerol exposure significantly decreased the expression of CXCL8 and CXCR2 in oral cancer cells (Figure 4). The results were expressed as a fold change from the control after the target gene expression was normalized to GAPDH mRNA expression. The mean ± SEM of three separate observations was represented by each bar and * denotes statistical significance at the $p<0.05$ level between the drug treatment and control groups.

Discussion

KB-1 cell lines are commonly utilized to explore the anticancer potential of phytochemicals as well as nano formulation with phytochemicals for oral cancer.[14,15,16] The present study also used KB-1 cells to study the anticancer potential of gingerol and it showed a dose dependent cytotoxicity and the IC50 value was estimated as 76.97μM. Metastasis represents a key characteristic of cancer progression and depends on multiple processes. Among these, the ability of cancer cells to evade apoptosis is crucial because apoptosis restrict cancer cells from surviving and disseminating.[17]

Here, the IC50 concentration was used to assess the apoptotic effect of gingerol in oral cancer cell line and it showed clear morphological changes indicating apoptosis. Recent research also reported very good cytotoxic and apoptotic effect of hesperetin in KB cells.[18] [6]-gingerol demonstrated many antiproliferative mechanism such as activation of caspase, upregulation of apoptotic protease activation factor inducing apoptosis and release of Cytochrome c and so on.[19] Here gingerol also showed antimigratory potential as proven by scratch wound healing assay. The control cells showed more migratory cells showing chances of metastasis.

Further, gingerol also reduced the expression or down regulated CXCL8 (Interleukin-8), as well as CXCR2 in the study. CXCL8 is a human Chemokine which is agonist on CXCR2 receptors.[20] CXCL8 has many roles in the tumor micro environment and it acts as a prognostic biomarker and its inhibition can produce antitumor effect.[21] It is believed that CXCL8 is a proinflammatory chemokine which promotes angiogenesis, tumor growth, invasion and metastasis and activation of CXCR2. Anti-apoptosis is thought to be a crucial step in the metastasis of cancer cells because it allows them to move and invade. Activation of CXCR2 can modulate many different signalling pathways leading to different biological responses including cancer.[22]

However, the work is limited by its reliance on in vitro models with one type of cell lines. It also lacks pharmacokinetic and toxicity evaluation, and incomplete characterization of all possible molecular targets, which restricts direct clinical translation. Future research should therefore include in vivo experiments and rigorously designed clinical trials to investigate optimal dosing and safety so as to explore combination regimens with standard chemoradiotherapy, and develop improved delivery systems to enhance bioavailability and tumor targeting of gingerol in oral cancer patients.

Conclusion

This study demonstrates that gingerol significantly reduced the migratory capacity of oral cancer cells by modulating key signaling pathways such as CXCL-8 CXCR2 expression on oral cancer cell line supporting its potential as a therapeutic adjunct in oral cancer management.

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