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Evaluation of anti-metastatic potential of mangosteen extract via targeting the CXCL-8/CXCR2 chemokines signalling in oral cancer cell line - An Ex vivo study

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Abstract

Background: Oral cancer is a significant global health challenge, with high mortality rates primarily due to late diagnosis and limited treatment options. The CXCL-8/CXCR2 chemokine signaling pathway is known to play a crucial role in cancer metastasis. This study evaluates the anti-metastatic potential of mangosteen (Garcinia mangostana) extract by targeting this pathway in an oral cancer cell line (KB).

Methods: Oral cancer cells were treated with varying concentrations of mangosteen extract. Cell viability was assessed using an MTT assay, while migration was evaluated through a scratch wound healing assay. Gene expression of CXCL-8 and CXCR2 was analyzed using real-time PCR. Morphological changes indicative of apoptosis were observed via phase contrast microscopy.

Results: The mangosteen extract exhibited a dose-dependent cytotoxic effect, with an IC50 value of 12.531 ± 1.139 µg/ml. Treated cells displayed significant apoptosis, including cell shrinkage and membrane blebbing. The extract also inhibited cell migration and downregulated CXCL-8 and CXCR2 gene expression ($p<0.05$), indicating its anti-metastatic potential.

Conclusion: Mangosteen extract shows promising anti-metastatic and pro-apoptotic effects in oral cancer cell lines by modulating the CXCL-8/CXCR2 pathway. Further in vivo studies and clinical trials are necessary to validate these findings and explore its potential as a natural adjunct therapy for oral cancer.

Introduction:

Oral cancer, encompassing malignancies in the oral cavity and oropharynx, represents a critical public health issue worldwide. This cancer type predominantly arises from squamous cells, leading to squamous cell carcinoma,

which accounts for over 90% of all oral malignancies (1). The global burden of oral cancer is significant, with an estimated 377,000 new cases and 177,000 deaths annually, making it the sixth most common cancer globally (2). Several risk factors contribute to the development of oral cancer. Tobacco use, including smoking and smokeless forms, remains the leading cause, responsible for approximately 75% of cases (3, 4). Alcohol consumption, often in conjunction with tobacco, further amplifies the risk, creating a synergistic effect that significantly increases the likelihood of developing oral cancer (5). Additionally, human papillomavirus (HPV) infection, particularly HPV-16, has emerged as a significant etiological factor, especially in oropharyngeal cancers, including those affecting the tonsils and base of the tongue (6, 7).

Despite advances in medical technology, the prognosis for oral cancer remains poor, with a five-year survival rate hovering around 50% (8). This low survival rate is often due to late-stage diagnosis, as early-stage oral cancers are frequently asymptomatic or mistaken for benign lesions (9). Early detection and accurate diagnosis are crucial for improving outcomes, as they allow for timely intervention and treatment (10).

Current treatment options for oral cancer include surgery, radiation therapy, and chemotherapy, often used in combination depending on the stage and location of the tumor (11). However, these treatments can be associated with significant side effects and morbidity, highlighting the need for more targeted and less toxic therapeutic strategies (12).

Recent research has focused on understanding the molecular mechanisms underlying oral cancer progression and identifying potential therapeutic targets. One such target is the CXCL-8/CXCR2 chemokine signaling pathway, which plays a crucial role in tumor growth, angiogenesis, and metastasis (13, 14). Inhibition of this pathway may offer a novel approach to controlling the spread of oral cancer and improving patient outcomes (13).

Natural compounds are being investigated for their potential anti-cancer properties, and mangosteen extract, known for its anti-inflammatory and antioxidant effects, has gained attention in this context (15). This ex vivo study aims to evaluate the anti-metastatic potential of mangosteen extract in oral cancer cell lines by targeting the CXCL-8/CXCR2 chemokine signaling pathway. The study seeks to explore new therapeutic avenues that could complement existing treatments and enhance the overall management of oral cancer.

One promising area of research involves targeting specific molecular pathways involved in cancer progression. The CXCL-8/CXCR2 chemokine signaling pathway has been identified as a key contributor to the processes of tumor growth, angiogenesis, and metastasis in various cancers, including oral cancer. This pathway's role in facilitating the spread of cancer cells makes it an attractive target for therapeutic intervention.

Natural compounds are increasingly being explored for their potential anti-cancer properties, offering a less toxic alternative to conventional treatments. Mangosteen (Garcinia mangostana), known for its potent antioxidant and anti-inflammatory properties, has shown promise in preliminary studies for its ability to inhibit cancer cell proliferation and induce apoptosis. However, the effects of mangosteen extract on the CXCL-8/CXCR2 signaling pathway in oral cancer have not been extensively studied.

This study aims to explore the anti-metastatic potential of mangosteen extract in oral cancer cell lines by focusing on its effects on the CXCL-8/CXCR2 chemokine signaling pathway. Understanding how mangosteen extract influences this pathway could lead to the development of new therapeutic strategies that specifically target the mechanisms driving oral cancer progression. This research not only seeks to find more effective treatments but also aims to provide options with fewer side effects, potentially improving patient outcomes and quality of life. The insights gained could lay the groundwork for future clinical trials and the inclusion of natural compounds like mangosteen in oral cancer treatment protocols.

MATERIALS AND METHODOLOGY

Maintenance of the Cell Line

Oral cancer KB cell lines were procured from the National Centre for Cell Science (NCCS), Pune. The cells were cultured in T25 flasks containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution. The cells were maintained at a temperature of 37°C in a humidified atmosphere with 5% CO_2. Upon reaching confluence, the cells were trypsinized and subcultured.

Cell Viability Assay

To evaluate the viability of the oral cancer cells following treatment with mangosteen extract, the MTT assay was employed. This assay relies on the conversion of the yellow tetrazolium salt (MTT) into purple formazan crystals by metabolically active cells. Cells were plated in 96-well plates at a density of 5x10^3 KB cells per well. After

plating, cells were subjected to serum starvation in a serum-free medium for three hours at 37°C. Post-starvation, the cells were treated with varying concentrations of mangosteen extract for 24 hours. Following the treatment, 100µl of media from both treated and untreated wells was replaced with DMEM containing MTT at a concentration of 0.5 mg/ml. The plates were then incubated at 37°C for four hours. After incubation, the medium containing MTT was removed, and the cells were washed with phosphate-buffered saline (PBS). The formed formazan crystals were dissolved in 100µl of dimethyl sulfoxide (DMSO) and allowed to stand in the dark for one hour. The absorbance was measured at 570 nm using a Micro ELISA plate reader. The percentage of cell viability was calculated using the formula:

% cell viability = A570 nm of treated cells/A570 nm of control cells×10

Control cells cultured in serum-free medium without treatment were considered 100% viable.

Scratch Wound Healing Assay

To assess the impact of mangosteen extract on cell migration, a scratch wound healing assay was performed. Oral cancer cells were seeded at a density of 2×10^5 cells per well in six-well culture plates. Once the cells formed a monolayer, a scratch was made using a 200µl pipette tip to simulate a wound. The cells were then washed with PBS, and the initial wound area was photographed using an inverted microscope. The cells were treated with the IC-50 dose of mangosteen extract (12.5µg/ml) for 24 hours, while control cells received serum-free culture medium. After the treatment, the wounded areas were re-photographed using the same microscope. Each experiment was conducted in triplicate for consistency.

Real-Time PCR

The expression of anti-metastatic signaling molecules and CXCL-8/CXCR2 chemokines was analyzed using real-time PCR. Total RNA was extracted using Trizol Reagent (Sigma) following a standardized protocol. Subsequently, 2µg of RNA was used for cDNA synthesis with the PrimeScript 1st strand cDNA synthesis kit (TakaRa, Japan). Specific primers were employed to amplify the target genes. The PCR reactions were performed using GoTaq® qPCR Master Mix (Promega), which includes SYBR green dye and all necessary components for PCR. Real-time PCR was carried out on a CFX96 PCR system (Bio-Rad). The data were analyzed using the comparative CT method, and fold changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ method as described by Schmittgen and Livak.

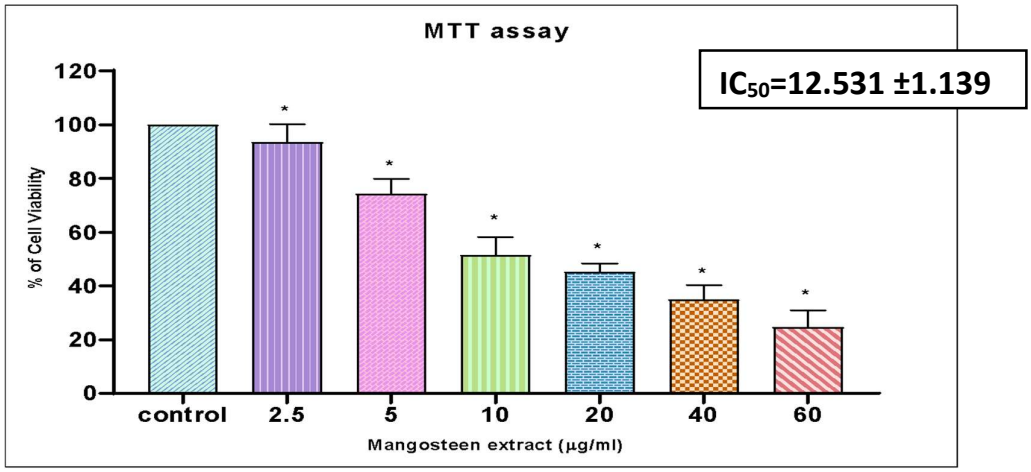
Statistical Analysis

All data were statistically analyzed using SPSS software. A one-way ANOVA was first performed, followed by Student's t-test. Results were presented as mean ± standard deviation (SD) in triplicate. A p-value of less than 0.05 was considered statistically significant.

RESULTS:

MTT ASSAY:

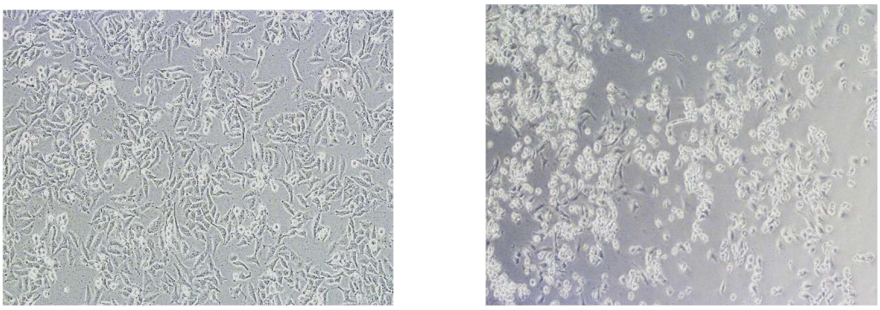
The MTT assay was used to assess the cytotoxicity of mangosteen extract on oral cancer (KB) cells. The cells were treated with varying concentrations of mangosteen extract ranging from 2.5 to 60 µg/ml for 24 hours. The results indicated a dose-dependent reduction in cell viability, with significant cytotoxic effects observed at concentrations above 12.5 µg/ml. The half-maximal inhibitory concentration (IC50) was determined to be 12.531 ± 1.139 µg/ml, indicating the concentration at which the extract reduced cell viability by 50%. Statistical analysis showed that the differences between the control and treatment groups were significant ($p<0.05$) at all tested concentrations (Figure 1).



CELL MORPHOLOGY:

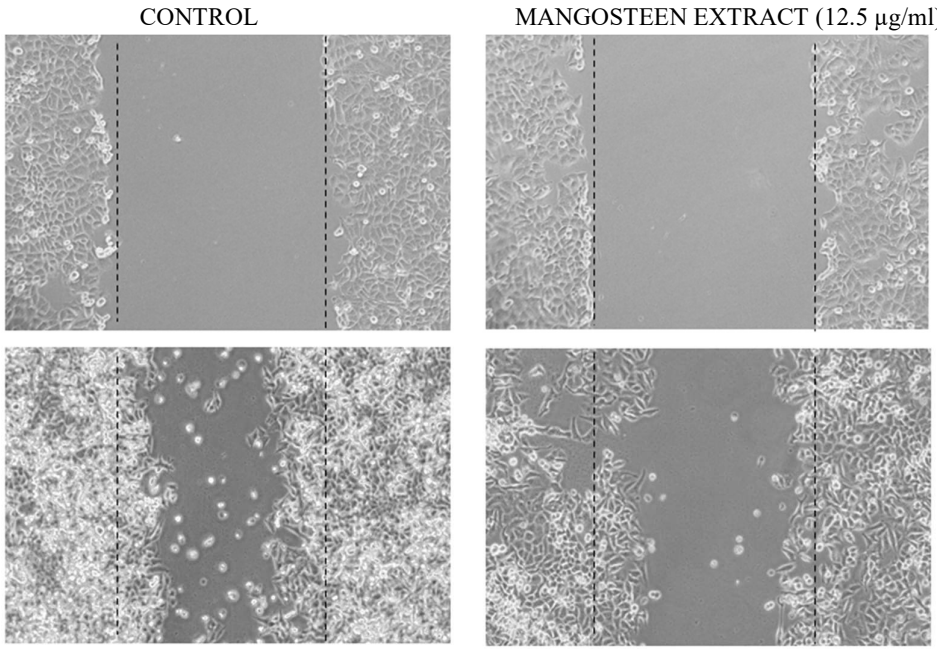
The morphological changes in KB cells treated with mangosteen extract were observed using an inverted phase contrast microscope. At the IC50 concentration (12.5 µg/ml), treated cells exhibited noticeable morphological alterations, including cell shrinkage and cytoplasmic membrane blebbing, indicative of apoptosis. In contrast, the control cells maintained a typical morphology with no signs of apoptosis (Figure 2).

CONTROL MANGOSTEEN EXTRACT (12.5 µg/ml)



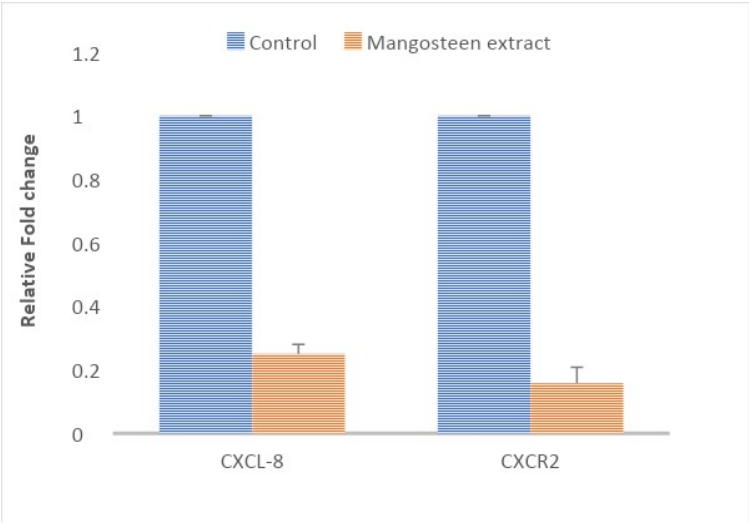
SCRATCH WOUND HEALING ASSAY:

The anti-migrative effects of mangosteen extract were evaluated using a scratch wound healing assay. Oral cancer cells were treated with 12.5 µg/ml of mangosteen extract for 24 hours. The control group displayed substantial wound closure, indicative of cell migration, whereas the treated group showed significantly reduced migration, suggesting that the extract effectively inhibits cell movement (Figure 3).



GENE EXPRESSION:

The expression levels of CXCL-8 and CXCR2, key genes involved in the chemokine signaling pathway, were analyzed using real-time PCR. The results demonstrated that treatment with mangosteen extract (12.5 µg/ml) significantly downregulated the expression of both genes compared to the control group. The relative fold change in gene expression was normalized to GAPDH mRNA, with the treated group showing a notable decrease in CXCL-8 and CXCR2 expression levels ($p<0.05$) (Figure 4).



Discussion:

Oral cancer remains a significant global health challenge due to its high prevalence and often late diagnosis, which contributes to a poor prognosis and high mortality rates. It is one of the most common cancers worldwide, particularly affecting populations in regions with high tobacco and alcohol use (16). The standard treatments for oral cancer, including surgery, chemotherapy, and radiation, are associated with severe side effects and often lead to a diminished quality of life for patients. This has driven the search for novel, less toxic therapeutic agents, including natural compounds with potential anti-cancer properties.

Mangosteen (Garcinia mangostana) pericarp extract has garnered attention for its potent antioxidant, anti-inflammatory, and anti-cancer properties. The pericarp of mangosteen contains a variety of xanthones, which are bioactive compounds known to exhibit anti-cancer activities in various cancer types (17). Previous studies have highlighted the potential of mangosteen extract in inhibiting cancer cell proliferation and inducing apoptosis, but

its specific effects on oral cancer cells and the mechanisms involved, particularly through the CXCL-8/CXCR2 chemokine signaling pathway, remain underexplored.

In this study, we investigated the effects of mangosteen extract on the viability, morphology, migration, and gene expression in oral cancer cell lines. Our findings revealed that mangosteen extract significantly reduced cell viability in a dose-dependent manner, with an IC50 value of 12.531 ± 1.139 µg/ml. This cytotoxic effect is consistent with previous research by Chitchumroonchokchai et al. (18), who reported similar IC50 values in colon cancer cell lines treated with mangosteen extract, highlighting its broad-spectrum anti-cancer activity.

Morphological observations in our study indicated apoptosis in treated cells, characterized by cell shrinkage and membrane blebbing. These findings align with the work of Chantarasriwong et al. (19), who found that mangosteen extract induced apoptosis in human leukemia cells, suggesting a common mechanism of action across different cell types. The induction of apoptosis by mangosteen extract is likely mediated through the modulation of apoptotic pathways, including the activation of caspases and the regulation of Bcl-2 family proteins, as also observed in other studies (20).

The scratch wound healing assay demonstrated a significant inhibition of cell migration in the presence of mangosteen extract. This result supports the findings of Akao et al. (21), who showed that mangosteen extract inhibits the migration and invasion of prostate cancer cells. The inhibition of cell migration is particularly important in the context of cancer metastasis, as it suggests that mangosteen extract could potentially prevent the spread of cancer cells to other parts of the body.

Moreover, our study showed a significant downregulation of CXCL-8 and CXCR2 gene expression in treated cells. The CXCL-8/CXCR2 axis is known to promote angiogenesis, tumor growth, and metastasis by enhancing the invasive capabilities of cancer cells (22). The downregulation of these genes by mangosteen extract indicates a possible mechanism through which it exerts its anti-metastatic effects. Similar findings were reported by Hung et al. (23), who observed that targeting the CXCR2 pathway inhibited the metastasis of breast cancer cells.

Furthermore, the study by Lee et al. (24) demonstrated that mangosteen extract could suppress the expression of another chemokine receptor, CXCR4, in ovarian cancer cells, indicating that mangosteen extract may have a broader impact on chemokine signaling pathways involved in cancer progression. This suggests that mangosteen extract could be an effective agent in targeting multiple pathways involved in tumor metastasis, making it a promising candidate for further research and development.

The results of this study are consistent with the anti-cancer properties of mangosteen extract observed in other studies. Moongkarndi et al. (25) also reported that mangosteen extract inhibited the proliferation of hepatocellular carcinoma cells and induced apoptosis. The consistency of these findings across different types of cancer cells suggests that mangosteen extract may have a universal anti-cancer effect, possibly due to its ability to modulate key signaling pathways involved in cell survival and metastasis.

In conclusion, our study supports the potential of mangosteen pericarp extract as a natural therapeutic agent for oral cancer. The extract's ability to reduce cell viability, induce apoptosis, inhibit migration, and downregulate pro-metastatic gene expression underscores its therapeutic potential. Further in vivo studies and clinical trials are warranted to explore the efficacy and safety of mangosteen extract in the treatment of oral cancer and potentially other cancers. The integration of mangosteen extract into existing cancer treatment regimens could provide a novel, less toxic alternative that enhances patient outcomes and quality of life.

LIMITATIONS:

This study is limited by its ex vivo design, as the findings from a single oral cancer cell line (KB) may not fully reflect in vivo conditions, necessitating broader validation. Additionally, the focus on the CXCL-8/CXCR2 pathway leaves other potential mechanisms unexplored.

FUTURE SCOPE:

Future research should include in vivo studies and clinical trials to confirm efficacy and safety, while also exploring additional molecular pathways. The potential for combining mangosteen extract with conventional therapies and addressing resistance development should also be investigated to enhance its therapeutic relevance in oral cancer treatment.

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