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Research Article

Synergistic Anti-Migration Effects of *Garcinia cowa* and Doxorubicin in T47D Breast Cancer Cells: A Scratch Assay Analysis

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Abstract

Objective: Breast cancer metastasis is a major cause of mortality, highlighting the need for effective anti-migratory therapies. This study investigated the synergistic anti-migratory effects of *Garcinia cowa* bark ethanol extract and doxorubicin on T47D breast cancer cells, aiming to explore its potential as a combination therapy to inhibit cancer cell migration

Methods: T47D cells were treated with *Garcinia cowa* bark ethanol extract (GCBEE) (130 µg/mL), doxorubicin (Dox) (0.026 µg/mL), and their combination. Cell migration was evaluated using the scratch assay, with scratch closure monitored at 0, 24, and 48 hours.

Results: The combination of GCBEE (130 µg/mL) and Dox (0.026 µg/mL) significantly inhibited T47D cell migration at both 24 and 48 hours, compared to the individual treatments of DOx (0.026 µg/mL) alone and GCBEE (130 µg/mL) alone.

Conclusions: The combination of Garcinia cowa ehtanol extract and doxorubicin demonstrates synergistic anti-migratory effects on T47D breast cancer cells, suggesting its potential as an adjuvant therapy to enhance the efficacy of doxorubicin in preventing metastasis.

Keywords: *Garcinia cowa*, doxorubicin, T47D cells, anti-migration, scratch assay, combination therapy.

INTRODUCTION

Breast cancer remains one of the most prevalent and deadly malignancies worldwide, with metastasis being the primary cause of mortality among patients[1]. Metastasis is a complex process involving the migration and invasion of cancer cells into surrounding tissues and distant organs[2]. Despite advances in chemotherapy, the development of resistance and severe side effects associated with conventional drugs, such as doxorubicin, underscores the urgent need for novel therapeutic strategies[3,4]. Natural products, particularly plant-derived compounds, have gained significant attention due to their potential to enhance the efficacy of existing chemotherapeutic agents while minimizing adverse effects[5]. Among these, *Garcinia cowa*, a tropical plant traditionally used in Southeast Asian medicine, has shown promising bioactive properties, including anticancer activities[6].

Recent studies have highlighted the anti-migratory and anti-invasive potential of natural compounds in breast cancer. For instance, research by Guan et al. (2016) demonstrated that curcumin, a polyphenolic compound from turmeric, significantly inhibited the migration of MDA-MB-231 breast cancer cells by modulating the PI3K/AKT/mTOR pathway[7]. Similarly, Maugeri et al. (2023) reported that quercetin, a flavonoid found in fruits and vegetables, suppressed the migration of T47D cells through the downregulation of matrix metalloproteinases (MMPs)[8]. These findings underscore the potential of natural compounds as adjuvants to conventional therapies. However, the specific mechanisms by which *Garcinia cowa* exerts its anti-migratory effects, particularly in combination with doxorubicin, remain poorly understood.

Doxorubicin, a widely used chemotherapeutic agent, is known for its potent cytotoxic effects against cancer cells[9]. However, its clinical utility is often limited by dose-dependent toxicity and the development of drug resistance[10]. Recent studies have explored combination therapies to enhance the efficacy of doxorubicin while

reducing its side effects. For example, Suh et al. (2018) demonstrated that the combination of doxorubicin with resveratrol, a natural polyphenol, synergistically inhibited the migration and invasion of MCF-7 breast cancer cells[11]. Similarly, Khalki et al. (2020) found that combining doxorubicin with berberine, an alkaloid from Berberis species, significantly reduced the metastatic potential of triple-negative breast cancer cells[12]. These studies provide a strong rationale for investigating the synergistic effects of *Garcinia cowa* extract and doxorubicin in breast cancer treatment.

In this study, we aimed to evaluate the anti-migratory potential of *Garcinia cowa* stem bark ethanol extract in combination with doxorubicin on T47D breast cancer cells using the scratch assay. The T47D cell line, a model for luminal A breast cancer, was selected due to its relevance in studying hormone receptor-positive breast cancer, which accounts for a significant proportion of breast cancer cases. By investigating the synergistic effects of this combination, we seek to provide a scientific basis for the development of *Garcinia cowa* as a potential adjuvant therapy to enhance the efficacy of doxorubicin in preventing breast cancer metastasis.

MATERIALS AND METHOD

Plant Materials

The bark of *Garcinia cowa* Roxb. was collected in December 2022 at Kudu Gantiang, Pariaman City, West Sumatra. The plant material has been deposited at the Herbarium of Andalas University, West Sumatra, Indonesia, under the voucher number 556-ID/ANDA/XII/2022. Dr. Nurainsa, a botanist from Andalas University's Herbarium, identified the *Garcinia cowa* Roxb. bark.

Preparation of plant material and extraction

Plant materials were sliced into tiny pieces (3-5 mm thick) and allowed to air-dry in a shaded area for 7 days. The dried bark of *Garcinia cowa* was ground into a powder using a traditional grinder. The materials were then soaked for 24 hours at room temperature in 70% ethanol with intermittent stirring and then filtered. This process was repeated thrice. The filtrates were combined and concentrated under a vacuum using a rotary evaporator at 45°C till a brownish semisolid extract was formed. The extract was kept in a refrigerator at 4°C for further pharmacological testing.

Cell culturing procedure

T47D cells, a kind of human breast cancer, were provided for the study by Prof. Masashi Kawaichi of the Nara Institute of Science and Technology in Japan. T47D cells was grown in Dulbecco's modified Eagles medium, which contained 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 1% penicillin-1% streptomycin (Gibco, Grand Island, NY, USA), and 0.5% fungizon (Gibco, Grand Island, NY, USA), in a flask in a humidified atmosphere (5% CO2) at 37°C

Scratch assay

T47D cells were grown into 24 well plates. Cells were observed using an inverted microscope to see their distribution, then incubated for one night until the cells reached 80% confluence. After that, a scratch was made on each well using a sterile yellow tip. All media in each well was removed using a pipette and the cells were washed using PBS. Medium containing each test sample was added to each well, then the plate was incubated in an incubator at 37° C, 5% CO_2 for 48 hours. Cell migration observations were carried out using an inverted microscope at 0, 24 and 48 hours. Then changes in the scratch area over time as a percentage of scratch closure were measured using ImageJ software.

Data Analysis

The scratch closure results were presented as a percentage, with the area measured at zero time set as 0%. Treatment comparisons were conducted using analysis of variance (ANOVA) followed by the Tukey test (evaluating three data sets), where untreated cells served as the control. Data are expressed as mean ± SEM, and a p-value <0.05 was considered statistically significant. GraphPad Prism Software, version 8.0 (San Diego, CA, USA), was used for generating graphs and performing statistical analyses.

RESULT AND DISCUSSION

Scratch assay

To determine whether GCBEE and Dox inhibit cell migration, a scratch assay was performed using T47D breast cancer cells. The effects of *Garcinia cowa* bark ethanol extract (GCBEE), doxorubicin (Dox), and their combination on cell migration were evaluated. Cells were treated with GCBEE (130 µg/mL), Dox (0.026 µg/mL), or a combination of GCBEE and Dox (130 µg/mL + 0.026 µg/mL), and scratch closure was observed at 0, 24, and 48 hours. The untreated group served as a control. **Figure 1A** shows an optimal result of the scratch assay. **Figure 1B** illustrates the scratch area (in μm^2) at 0, 24, and 48 hours across different treatment groups: untreated, GCBEE (130 µg/mL), Dox (0.026 µg/mL), and the combination of GCBEE and Dox. While all groups showed a reduction in scratch area over time, the combination treatment resulted in a significantly slower decrease in scratch area compared to single treatments. This indicates a stronger inhibition of cell migration in the combination group. **Figure 1C** depicts the percentage of scratch closure at 24 and 48 hours. At 24 hours, the combination treatment exhibited a marked reduction in scratch closure compared to the untreated group and single treatments. By 48 hours, the inhibitory effect of the combination was even more pronounced, demonstrating its superior efficacy in suppressing T47D breast cancer cell migration.

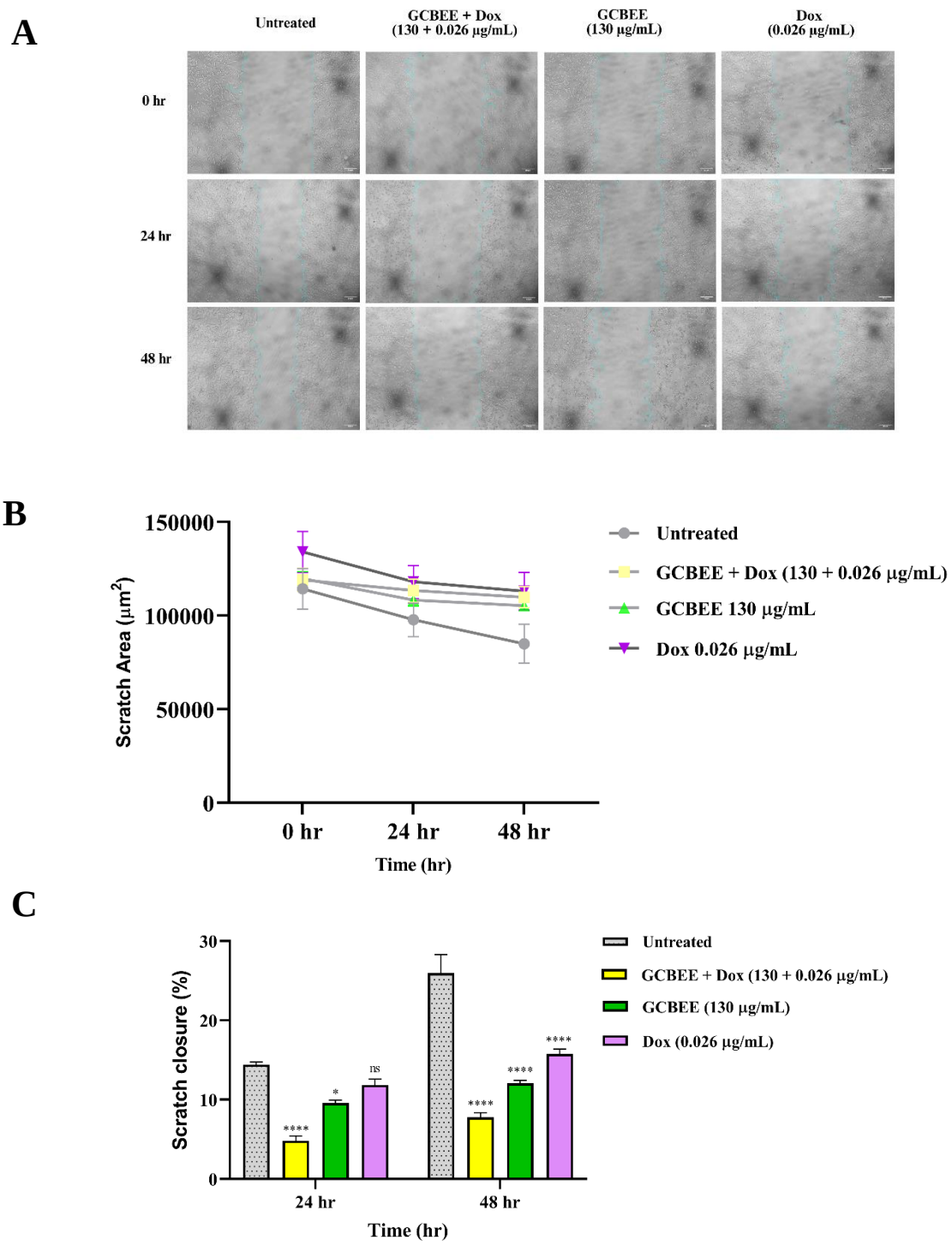

Figure 1: Inhibitory Effects of *Garcinia cowa* Bark Ethanol Extract (GCBEE) and Doxorubicin (Dox) on T47D Breast Cancer Cell Migration by Scratch Assay. **A**. Representative scratch assay images from each of the four experimental cell groups observed at times 0, 24, and 48 hr (Scale bar = 50 µm). **B**. Graphical representation of Scratch area (µm²). **C**. Graphical representation of Scratch closure percentage (%). Results are presented as mean ± SEM (n = 3). ns non-significant, * $p < 0.05$, **** $p < 0.0001$ compared to the untreated group.

This study highlights the synergistic anti-migratory effect of combining *Garcinia cowa* bark ethanol extract (GCBEE) with doxorubicin (Dox) on T47D breast cancer cells. The inhibition of cell migration is a critical step in preventing metastasis, which remains the primary cause of mortality in breast cancer patients[13]. The observed synergy between GCBEE and Dox suggests that the combination not only enhances the anti-migratory activity but also provides a potential strategy to overcome the limitations of conventional chemotherapy. This is particularly significant given the high prevalence of drug resistance and severe side effects associated with doxorubicin monotherapy[10,14]. The findings of this study align with the growing body of evidence

supporting the use of natural compounds as adjuvants to enhance the efficacy of chemotherapeutic agents[15].

The synergistic effects observed in this study are supported by previous research on the mechanisms by which natural compounds enhance chemotherapy. For instance, Chowchaikong et al. (2018) demonstrated that cowanin, such as those found in *Garcinia cowa*, enhance the efficacy of chemotherapy by inhibiting key migration-related signaling pathways, including PI3K/Akt and MAPK[16]. These pathways play a crucial role in regulating cell motility and invasion, and their inhibition can significantly reduce metastatic potential[17]. Additionally, *Garcinia cowa* is rich in bioactive compounds like xanthones and flavonoids, which are known for their anti-inflammatory and anti-proliferative properties[18]. These compounds likely contribute to the enhanced cytotoxic effects of doxorubicin by modulating multiple molecular targets involved in cancer cell migration and survival. Furthermore, other studies have shown that combining plant extracts with doxorubicin reduces the expression of matrix metalloproteinases (MMP-2 and MMP-9), enzymes that are critical for extracellular matrix degradation and cancer cell migration[19,20]. The observed reduction in T47D cell migration in this study may be attributed to similar mechanisms, where GCBEE potentiates the inhibitory effects of doxorubicin on these key enzymes.

Beyond efficacy, the safety profile of combination therapy is a critical consideration. Doxorubicin, while effective, is associated with significant toxicity, including cardiotoxicity and myelosuppression, which limit its clinical utility[10]. Combining doxorubicin with plant extracts like GCBEE not only enhances therapeutic efficacy but may also allow for dose reduction, thereby minimizing adverse effects. This approach aligns with the findings of Khaledifar et al. (2023), who reported that combining doxorubicin with natural compounds like berberine reduced the required dose of doxorubicin while maintaining its anticancer effects[21]. The potential of GCBEE to mitigate the toxicity of doxorubicin while enhancing its anti-migratory activity underscores its promise as a complementary therapy. Future studies should focus on elucidating the precise molecular mechanisms underlying this synergy and evaluating the in vivo efficacy and safety of the GCBEE-Dox combination in preclinical models. Such investigations will provide a stronger foundation for translating these findings into clinical applications, ultimately improving outcomes for breast cancer patients.

CONCLUSIONS

In conclusion, the combination of GCBEE and Dox shows potential as an adjuvant therapy to enhance the efficacy of doxorubicin in preventing breast cancer metastasis. Further studies, including in vivo experiments and molecular mechanism analyses, are recommended to validate these findings and support their clinical application.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Author's Declaration: The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethical approvals: This study does not involve experiments on animals or human subjects.

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