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Insulin Enhances NF-κB Activation in Luminal A T-47D Breast Cancer Cells Exposed to Low-Dose Doxorubicin

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Abstract: Doxorubicin is an effective chemotherapy agent for breast cancer; however, its efficacy is often limited by cellular resistance. Insulin is hypothesized to enhance anti-apoptotic activity through activation of Nuclear Factor Kappa-Beta (NF-κB), although its mechanism in the Luminal A subtype T-47D cell line has not been extensively studied. This analytical experimental study with a post-test only paired design used T-47D cells divided into insulin-treated and non-insulin groups. Both groups were exposed to various concentrations of doxorubicin, namely 0 (control), 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. NF-κB activation was measured as pNF-κB concentration using the ELISA method. Data were analyzed using the Shapiro–Wilk test for normality followed by a paired t-test. The insulin-treated group showed higher mean pNF-κB concentrations compared to the non-insulin group across all doxorubicin doses. Significant differences were observed at 0 µg/mL ($p = 0.008$), 3.125 µg/mL ($p = 0.009$), and 100 µg/mL ($p = 0.045$), whereas no significant differences were found at concentrations between 6.25 and 50 µg/mL ($p > 0.05$). Overall, increasing doxorubicin concentrations reduced pNF-κB levels; however, insulin treatment maintained higher pNF-κB levels compared to the non-insulin group. These findings indicate that insulin increases NF-κB activation in doxorubicin-treated T-47D breast cancer cells and may sustain NF-κB activity against the cytotoxic effects of doxorubicin.

Keywords: breast cancer, doxorubicin, insulin, NF-κB, luminal a, chemoresistance.

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Introduction

Breast cancer is the second most frequently diagnosed cancer worldwide. More than 2 million new cases were reported in 2022, and it was the fourth leading cause of cancer-related mortality globally, accounting for 666,103 deaths (WHO, 2022a). According to data from the Global Cancer Observatory (GLOBOCAN) 2022, breast cancer represented the highest number of new cancer cases in Indonesia, reaching 66,271 cases (16.2%) out of a total of 408,661 cancer cases. Breast cancer-related mortality exceeded 22,000 deaths, making it the third leading cause of cancer-related death in the country (WHO, 2022b).

Based on biomolecular characteristics, breast cancer is classified into four molecular subtypes: Luminal A, Luminal B, Human Epidermal Growth Factor Receptor 2-enriched (HER2-enriched), and Basal-like. Among these subtypes, Luminal A is the most prevalent, accounting for approximately 40–45% of all breast cancer cases. Luminal B contributes around 20–

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25%, followed by Basal-like at 15–20%, and HER2-enriched at 10–15% (Taurin & Alkhalifa, 2020).

Classification based on biomolecular characteristics plays an important role in determining appropriate therapeutic strategies in addition to cancer staging. Breast cancer treatment includes surgery for local tumor removal, radiotherapy following surgery, and systemic therapies such as chemotherapy. Chemotherapy remains an important systemic treatment option, particularly in advanced and metastatic breast cancer. Furthermore, chemotherapy can be administered across all breast cancer subtypes regardless of receptor status (Trayes & Cokenakes, 2021).

One chemotherapeutic agent with significant therapeutic potential in breast cancer is doxorubicin. This drug is recognized as one of the most effective chemotherapeutic agents and has been approved by the Food and Drug Administration (FDA). The effectiveness of doxorubicin is attributed to its complex mechanisms in inducing apoptosis, including inhibition of topoisomerase II activity and generation of reactive oxygen species (ROS) that damage cellular DNA. These mechanisms act synergistically to induce apoptosis in breast cancer cells (Kciuk et al., 2023).

Breast cancer frequently coexists with type 2 diabetes mellitus (T2DM), making diabetes an important clinical consideration in breast cancer management. Previous systematic reviews have reported that T2DM is associated with a 14–25% increased risk of developing breast cancer and a 37–61% higher risk of all-cause mortality among patients with breast cancer (Zhang et al., 2021). Hyperinsulinemia, a hallmark of T2DM, has been proposed as one of the biological mechanisms underlying these poorer clinical outcomes by promoting cancer cell survival and reducing treatment efficacy (Devanaboyina et al., 2022; Yee et al., 2020). These findings suggest that hyperinsulinemia may be an important contributor to treatment resistance, making insulin a potential factor involved in reduced responsiveness to chemotherapy.

Given the potential role of hyperinsulinemia in reducing treatment efficacy, insulin has emerged as one of the factors implicated in doxorubicin resistance (Devanaboyina et al., 2022; Yee et al., 2020). This is supported by a previous study conducted by Kheradmand et al. (2021) which demonstrated that insulin supplementation enhanced anti-apoptotic activity in Luminal A MCF-7 breast cancer cells and attenuated the cytotoxic effect of doxorubicin. Specifically, following doxorubicin treatment, cell viability increased from 62.9 ± 5.7% in untreated cells to 79.0 ± 7.2% in insulin-treated cells, suggesting that insulin promotes resistance by enhancing cancer cell survival.

Although insulin has been reported to enhance anti-apoptotic activity and reduce doxorubicin efficacy, the underlying mechanism has not been fully elucidated. A potential mechanism underlying this effect involves the activation of Nuclear Factor Kappa-Beta (NF-κB) by insulin (Yee et al., 2020). Several mechanistic studies have demonstrated that insulin activates the PI3K/AKT signaling pathway, which subsequently promotes NF-κB activation through phosphorylation of the IκB kinase (IKK) complex. Activated IKK phosphorylates IκB, leading to its ubiquitination and proteasomal degradation. The degradation of IκB releases NF-κB, allowing it to translocate into the nucleus, where phosphorylation of the NF-κB enhances its transcriptional activity. Consequently, phosphorylated NF-κB (pNF-κB) is widely used as a marker of activated NF-κB signaling. Activated NF-κB subsequently stimulates the transcription of anti-apoptotic genes, increasing the expression of proteins such as B-cell lymphoma 2 (BCL-2) and B-cell lymphoma-extra large (BCL-xL), thereby impairing chemotherapy-induced apoptosis and potentially contributing to doxorubicin resistance (Devanaboyina et al., 2022; Hassan et al., 2024; Pavitra et al., 2023).

To date, the MCF-7 cell line has commonly been used as a representative model of Luminal A breast cancer, whereas studies involving other Luminal A cell lines such as T-47D remain limited. T-47D cells exhibit higher mutant TP53 expression than MCF-7 cells, which may confer stronger anti-apoptotic properties and make them a valuable model for investigating therapy-resistant characteristics of Luminal A breast cancer (Cheng et al., 2022). Furthermore, no study has directly investigated whether insulin modulates NF-κB activation in T-47D breast cancer cells under doxorubicin exposure. This knowledge gap forms the basis of the present study.

Therefore, this study aimed to investigate whether insulin induces NF-κB activation in Luminal A T-47D breast cancer cells undergoing doxorubicin treatment and to evaluate its potential contribution to doxorubicin resistance.

Materials and Methods

Study Design

This study employed an analytical experimental study with a post-test only paired design to evaluate the effect of insulin supplementation on NF-κB activation in doxorubicin-treated Luminal A T-47D breast cancer cells. T-47D cells were assigned to paired treatment conditions consisting of doxorubicin treatment with and

without insulin supplementation. Following treatment, NF-κB activation was quantified and compared between the paired groups. A schematic overview of the experimental workflow is presented in Figure 1.

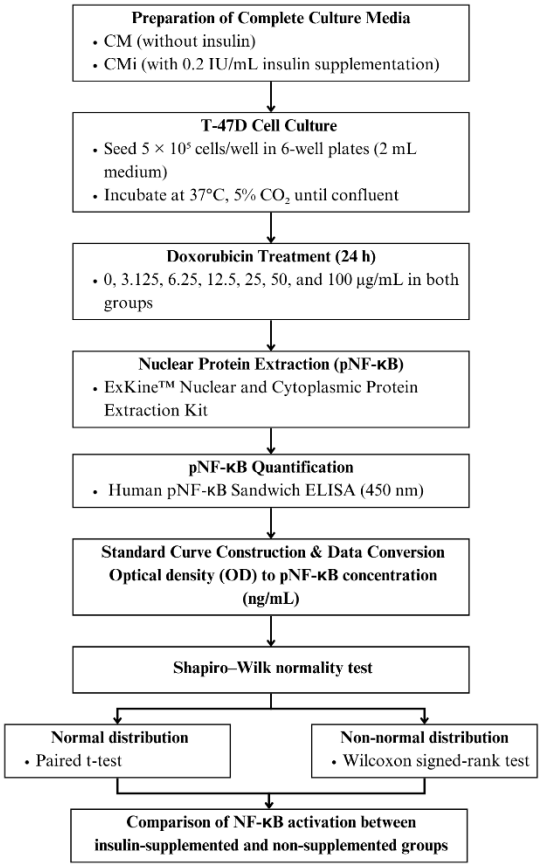

Figure 1. Experimental workflow of the study

Preparation of Culture Media

The culture media were divided into two groups: complete medium without insulin supplementation (CM) and complete medium with insulin supplementation (CMi). The CM was prepared by mixing RPMI-1640 basal medium to a final volume of 100 mL, followed by the addition of 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 0.5% fungizone. The CMi was prepared using the same composition with the addition of human recombinant insulin at a final concentration of 0.2 IU/mL.

Insulin conversion was calculated based on the assumption that 1 IU insulin is equivalent to 0.0347 mg, therefore 0.2 IU/mL corresponded to 0.00694 mg/mL. The insulin stock solution had a concentration of 5 mg/mL. The required insulin volume was determined using the dilution formula:

$$V_1 = \frac{C_2 \times V_2}{C_1}$$

where V_1 is the required stock insulin volume, C_1 is the stock insulin concentration, C_2 is the desired final concentration, and V_2 is the final medium volume. Based on the calculation, approximately 1.4 µL of insulin was required for every 1 mL of medium; therefore, 140 µL of insulin was added for the preparation of 100 mL CMi.

Cell Culture and Treatment

Each sample required a total of 2×10^6 cells for protein extraction. Since one well at confluency yielded approximately 1.2×10^6 cells, each sample was obtained by combining cells from two wells of a 6-well plate.

The experiment consisted of seven treatment conditions, including one control group without doxorubicin and six doxorubicin concentrations (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL). Each condition used two wells. Treatments were applied to two groups, namely cells cultured without insulin supplementation and cells cultured with insulin supplementation, resulting in a total of 28 wells. To minimize cross-contamination and treatment interference, the two groups were cultured on separate 6-well plates.

Each well was seeded with 5×10^5 cells in 2 mL of culture medium according to the designated group and incubated at 37°C in a humidified atmosphere containing 5% CO_2 until confluency was reached.

Doxorubicin working solutions were prepared from a 2 mg/mL stock solution by dilution with CM or CMi to a final volume of 4000 µL. The dilution was calculated using the formula:

$$V_1 = \frac{C_2 \times V_2}{C_1}$$

where C_1 represents the doxorubicin stock concentration (2000 µg/mL), C_2 the desired final concentration, and V_2 the final solution volume.

After preparation of the treatment solutions, the culture medium was carefully removed to prevent cell detachment and replaced with treatment medium containing the appropriate doxorubicin concentration according to the respective group. Each well received 2 mL of treatment medium, while the control group received medium without doxorubicin. Cells were subsequently incubated for 24 h at 37°C in 5% CO_2 to induce apoptosis.

Protein Extraction for pNF-κB Analysis

pNF-κB protein extraction was performed using the ExKine™ Nuclear and Cytoplasmic Protein Extraction Kit according to the manufacturer's instructions. Prior to extraction, working solutions were prepared. Cytoplasmic Extraction Solution A (CESA) and Nuclear Extraction Solution (NES) were supplemented with protease inhibitor and dithiothreitol

(DTT) and stored at 4°C. Cytoplasmic Extraction Solution B (CESB) was used without additional reagents.

A total of 2×10^6 cells were collected from two wells of a 6-well plate using a cell scraper. The cell suspension was centrifuged at 500 g for 5 min, and the supernatant was discarded. The cell pellet was washed with cold phosphate-buffered saline (PBS) and centrifuged again at 500 g for 2–3 min.

For cytoplasmic extraction, 200 µL of cold CESA was added to the cell pellet, vortexed for 5 s, and incubated on ice for 15 min. Subsequently, 10 µL of cold CESB was added, vortexed again for 5 s, and incubated on ice for 1–2 min. Samples were centrifuged at 16,000 g for 5 min at 4°C. The supernatant containing the cytoplasmic fraction was transferred into a new tube.

The remaining nuclear pellet was then resuspended in 100 µL NES and incubated on ice for 30 min with intermittent vortexing for 15 s every 3–5 min. Samples were centrifuged at 16,000 g for 15 min at 4°C. The supernatant containing nuclear proteins was collected and stored at −80°C until analysis.

Preparation of ELISA Standard Solutions

pNF-κB standard solutions were prepared by rehydrating the lyophilized standard with 1 mL of sample dilution buffer to obtain a 20 ng/mL stock solution. Serial dilutions were subsequently performed using sample dilution buffer to generate standard concentrations of 10, 5, 2.5, 1.25, 0.625, and 0.312 ng/mL, along with a blank containing only buffer.

All standard solutions were added into microtiter plate wells at 100 µL per well in duplicate. The resulting optical density (OD) values were used to construct the standard curve.

pNF-κB ELISA Assay

pNF-κB levels were measured using a Human pNF-κB ELISA Kit based on the sandwich ELISA principle. Prior to the assay, all kit components were allowed to equilibrate to room temperature. Wash buffer was prepared by diluting the concentrated wash buffer with distilled water according to the manufacturer's instructions.

A volume of 100 µL of standards or samples was added into each well and incubated for 90 min at 37°C. After washing, 100 µL of biotin-labeled detection antibody was added and incubated for 60 min at 37°C. The plate was washed again before the addition of 100 µL HRP-streptavidin conjugate and incubated for 30 min.

Subsequently, 90 µL tetramethylbenzidine (TMB) substrate solution was added and incubated for 10–20 min in the dark. The reaction was terminated by adding 50 µL stop solution. Absorbance was measured using a microplate reader at a wavelength of 450 nm. The absorbance values were converted into NF-κB concentrations based on the standard curve.

Standard Curve Construction and Data Conversion

The OD values of the standards were averaged from duplicate measurements and corrected against the blank. A standard curve was generated by plotting corrected OD values on the Y-axis against standard concentrations on the X-axis using FineTest ELISA analysis software. pNF-κB concentrations in the samples were calculated by interpolating corrected OD values onto the standard curve.

Data Analysis

All data were analyzed using Statistical Package for the Social Sciences (SPSS) version 29. Univariate analysis was performed descriptively to present the mean pNF-κB concentrations of each treatment group in tables and bar charts. Bivariate analysis was performed to assess the effect of insulin supplementation on NF-κB activation in doxorubicin-treated T-47D cells. Data normality was assessed using the Shapiro–Wilk test. If the data were normally distributed, comparisons between groups with and without insulin supplementation were analyzed using a paired t-test. Otherwise, the Wilcoxon signed-rank test was applied as a non-parametric alternative.

Result

The measurement of pNF-κB concentrations in T-47D breast cancer cells demonstrated differences in mean values between the insulin-supplemented group and the non-insulin group across all doxorubicin concentrations. Overall, the insulin-supplemented group showed higher pNF-κB concentrations compared with the group without insulin, as presented in Figure 1.

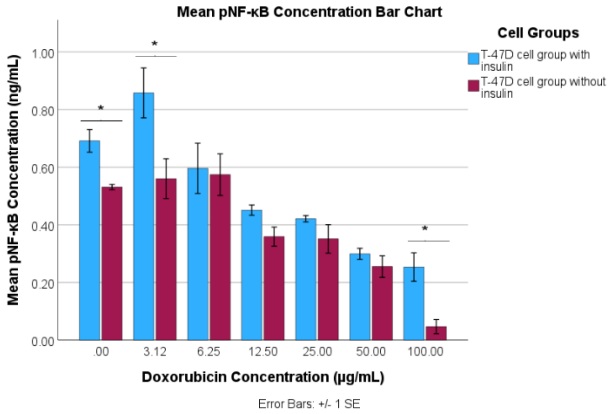

Figure 2. Bar chart of mean pNF-κB concentrations in T-47D breast cancer cells following exposure to different doxorubicin concentrations with or without insulin. *p<0.05, indicating a statistically significant difference between the insulin and non-insulin groups based on a paired-sample t-test.

Based on Figure 2, the largest differences in mean pNF-κB concentrations between the insulin-supplemented and non-insulin groups were observed at the 3.125 µg/mL doxorubicin concentration, followed by the 100 µg/mL concentration and the control group. At 3.125 µg/mL, the mean pNF-κB concentration in the insulin-supplemented group was 0.85767 ± 0.086490 ng/mL, whereas the non-insulin group showed a mean concentration of 0.56000 ± 0.069010 ng/mL. At 100 µg/mL, the mean pNF-κB concentration was 0.25333 ± 0,049431 ng/mL in the insulin-supplemented group and 0.04667 ± 0.024727 ng/mL in the non-insulin group. In the control group, the mean pNF-κB concentration was 0.7277 ± 0.02107 ng/mL in the insulin-supplemented group and 0.5480 ± 0.01178 ng/mL in the non-insulin group. The complete mean pNF-κB concentrations are presented in Table 1.

Table 1. Mean ± SE and p-values of pNF-κB

Doxorubicin Concentration (µg/mL)	With Insulin (ng/mL)	Without Insulin (ng/mL)	p-value
0	0.7277 ± 0.02107	0.5480 ± 0.01178	0.008*
3.125	0.85767 ± 0.086490	0.56000 ± 0.069010	0.009*
6.25	0.59600 ± 0.087123	0.57400 ± 0.072058	0.626
12.5	0.45100 ± 0.017776	0.35900 ± 0.033045	0.098
25	0.42100 ± 0.011136	0.35100 ± 0.049518	0.273
50	0.29900 ± 0.019287	0.25500 ± 0.037363	0.257
100	0.25333 ± 0.049431	0.04667 ± 0.024727	0.045*

*p<0.05, indicating a statistically significant difference between the insulin and non-insulin groups based on a paired-sample t-test.

The data demonstrated a similar trend in both groups, characterized by an initial increase in pNF-κB concentration at 3.125 µg/mL doxorubicin, followed by a gradual decrease at concentrations ranging from 6.25 to 100 µg/mL.

The association between insulin supplementation and pNF-κB concentration was analyzed using a paired t-test. Prior to comparative analysis, normality testing of the differences in pNF-κB concentrations between paired groups was performed using the Shapiro–Wilk test. All datasets showed significance values of $p > 0.05$, indicating normal data distribution. Therefore, parametric analysis using the paired t-test was subsequently performed. The results of the paired t-test are shown in Table 1.

The paired t-test analysis demonstrated statistically significant differences ($p < 0.05$) between the insulin-supplemented and non-insulin groups at doxorubicin concentrations of 0 µg/mL ($p = 0.008$), 3.125 µg/mL ($p = 0.009$), and 100 µg/mL ($p = 0.045$). In contrast, no statistically significant differences were observed at 6.25 µg/mL ($p = 0.626$), 12.5 µg/mL ($p = 0.098$), 25 µg/mL ($p = 0.273$), or 50 µg/mL ($p = 0.257$).

Discussion

Effect of Insulin on NF-κB Activation in T-47D Cells

The present study demonstrated that pNF-κB concentrations were consistently higher in the insulin-supplemented group than in the non-insulin group across all doxorubicin concentrations. These findings suggest that insulin may contribute to increased pNF-κB levels, even in the absence of cytotoxic stress, as indicated by the significant difference observed in the control group (0 µg/mL; $p = 0.008$). One plausible mechanism involves insulin-mediated activation of the PI3K/AKT signaling pathway, which has been reported to interact with the NF-κB pathway. Activation of AKT may stimulate the IKK complex, leading to phosphorylation and degradation of IκB, thereby allowing NF-κB activation. Activated NF-κB subsequently induces the expression of anti-apoptotic proteins, including BCL-2 and BCL-xL (Devanaboyina et al., 2022; Guo et al., 2024; Hassan et al., 2024; Yee et al., 2020).

Impact of NF-κB Activation on Doxorubicin Efficacy

Doxorubicin primarily exerts its cytotoxic effects through DNA intercalation, inhibition of topoisomerase II, and generation of reactive oxygen species (ROS), ultimately inducing apoptosis. However,

insulin-induced NF-κB activation may attenuate this apoptotic response by stimulating the expression of anti-apoptotic proteins such as BCL-2 and BCL-xL. Increased expression of these proteins enhances the protective mechanisms of cancer cells against the cytotoxic effects of doxorubicin. Therefore, elevated NF-κB activation may indicate reduced cellular sensitivity to doxorubicin treatment (Devanaboyina et al., 2022; Guo et al., 2024). Nevertheless, because cell viability and drug sensitivity were not directly evaluated in the present study, this proposed relationship should be considered a potential mechanistic explanation rather than definitive evidence of doxorubicin resistance.

Characteristics of T-47D Cells Related to the Findings

T-47D cells represent the Luminal A subtype of breast cancer and possess stronger pro-survival characteristics than MCF-7 cells due to a higher prevalence of *TP53* mutations, which contribute to anti-apoptotic tendencies (Cheng et al., 2022). The presence of *TP53* mutations in T-47D cells may confer intrinsic resistance to chemotherapy. The findings of the present study suggest that insulin supplementation may further strengthen these resistance mechanisms. Increased pNF-κB levels in the insulin-supplemented group may indicate enhanced pro-survival signaling in T-47D cells.

Effect of Doxorubicin Dose Variation on NF-κB Activation

At the doxorubicin concentration of 3.125 µg/mL, the difference in pNF-κB concentrations between the insulin-supplemented and non-insulin groups was statistically significant ($p = 0.009$), accompanied by the highest increase in pNF-κB concentration. According to Muna & Maulidina (2022), the IC_{50} value of doxorubicin in T-47D breast cancer cells is 8.17 µg/mL; therefore, 3.125 µg/mL can be classified as a low dose because it is below the IC_{50} value. At this concentration range, the cytotoxic effect of doxorubicin may not be sufficient to induce apoptosis optimally.

Under these conditions, cells may still activate compensatory mechanisms against genotoxic stress, including activation of the ATM–IKK signaling pathway in response to DNA damage (Bournique et al., 2025). Activation of this pathway subsequently induces NF-κB activation as an adaptive response to maintain cell survival. This effect may be enhanced in the insulin-supplemented group due to additional stimulation through the PI3K/AKT pathway, which further strengthens NF-κB activation (Bournique et al., 2025; Hassan et al., 2024; Yee et al., 2020). Consequently, pNF-κB concentrations were higher than those observed in the control group at this dose.

In addition to NF-κB activation, cancer cells may maintain survival through other compensatory mechanisms, such as increased expression of the efflux transporter P-glycoprotein, which reduces intracellular doxorubicin accumulation (Mirzaei et al., 2022). Furthermore, increased ROS production induced by doxorubicin may be counterbalanced through activation of antioxidant pathways mediated by the transcription factor Nrf2 (Mirzaei et al., 2021).

These interpretations are consistent with preliminary findings from an unpublished undergraduate thesis conducted under similar experimental conditions by Esthya et al. (2026), which demonstrated that T-47D cell viability remained relatively high at the 3.125 µg/mL doxorubicin concentration in both insulin-supplemented and non-insulin groups. The relatively high cell viability suggests that the cells were still in a compensatory phase and capable of activating multiple pro-survival pathways.

At intermediate doxorubicin concentrations (6.25–50 µg/mL), other anti-apoptotic pathways may become more dominant than NF-κB, resulting in non-significant changes in NF-κB activation. Insulin-mediated activation of the PI3K/AKT pathway may simultaneously activate several downstream targets. One such pathway involves Akt–mTORC1 activation, which enhances survivin protein translation and strengthens the anti-apoptotic phenotype of cancer cells (Warrier et al., 2020). AKT-activated mTORC1 phosphorylates 4E-BP1, leading to the release of eIF4E and subsequent translation of pro-survival proteins, including survivin, an anti-apoptotic protein (Böhm et al., 2021; Chen et al., 2023).

Additionally, Akt activation directly inhibits the FOXO1 and FOXO3a transcription factors, which normally function as negative regulators of survivin expression. Consequently, FOXO inactivation may lead to increased survivin expression (Warrier et al., 2020).

A different pattern emerged at the highest doxorubicin concentration (100 µg/mL), where the difference in pNF-κB concentrations between insulin-supplemented and non-insulin groups became statistically significant ($p = 0.045$). However, unlike the findings at 3.125 µg/mL, this significance was not caused by increased pNF-κB levels in the insulin-supplemented group, but rather by a marked reduction in pNF-κB concentration in the non-insulin group. One possible explanation is that the higher cytotoxic effect of doxorubicin at this concentration reduced the capacity of cells to maintain activation of pro-survival pathways, including NF-κB. Preliminary findings from an

unpublished undergraduate thesis conducted under similar experimental conditions suggested that cell viability was lower in the non-insulin group than in the insulin-supplemented group at 100 µg/mL doxorubicin (Esthya et al., 2026). However, because these data have not been peer-reviewed or independently validated, they should be interpreted with caution and are presented only as supportive evidence.

Pattern of pNF-κB Concentration Changes in Response to Increasing Doxorubicin Doses

The present study demonstrated a dose-dependent pattern of pNF-κB concentration changes in response to doxorubicin exposure. In general, pNF-κB concentrations initially increased at low doxorubicin doses, followed by a gradual decline as the doxorubicin concentration increased. One plausible explanation is that increasing cytotoxic stress at higher doxorubicin concentrations reduced the ability of surviving cells to sustain activation of pro-survival pathways, including NF-κB. This interpretation is consistent with preliminary observations from an unpublished undergraduate thesis conducted under similar experimental conditions, which reported progressively reduced T-47D cell viability with increasing doxorubicin concentrations in both insulin-supplemented and non-insulin groups (Esthya et al., 2026). Because these findings have not been peer-reviewed or independently validated, they should be regarded as supportive rather than conclusive evidence.

Conclusion

The present study demonstrated differences in pNF-κB concentrations between insulin-supplemented and non-insulin T-47D breast cancer cells following doxorubicin exposure. Overall, insulin supplementation was associated with higher mean pNF-κB concentrations across all doxorubicin concentrations tested (0, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL). Statistically significant differences between the insulin-supplemented and non-insulin groups were observed under control conditions (0 µg/mL; $p = 0.008$), at 3.125 µg/mL doxorubicin ($p = 0.009$), and at 100 µg/mL doxorubicin ($p = 0.045$), whereas no significant differences were detected at intermediate doxorubicin concentrations (6.25–50 µg/mL).

These findings suggest that insulin supplementation may be associated with increased NF-κB activation in T-47D breast cancer cells under both basal conditions and following doxorubicin exposure. Given the established role of NF-κB in promoting anti-apoptotic signaling, the observed increase in pNF-κB may represent a potential mechanism through which insulin contributes to reduced cellular responsiveness to doxorubicin. However, because cell viability and drug sensitivity were not directly assessed in this study, this interpretation should be considered a mechanistic hypothesis rather than direct evidence of doxorubicin resistance.

The absence of significant differences in pNF-κB concentrations at intermediate doxorubicin concentrations suggests that NF-κB activation alone may not fully explain the cellular response to doxorubicin across all dose ranges. As discussed, other pro-survival signaling pathways activated downstream of insulin may contribute to this response and warrant further investigation.

These findings may have potential implications for understanding the influence of hyperinsulinemia on chemotherapy response in patients with breast cancer. Future studies should directly evaluate cell viability, apoptosis, and doxorubicin sensitivity while simultaneously assessing additional pro-survival signaling pathways to clarify the role of insulin-induced NF-κB activation in doxorubicin resistance.

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References

Böhm, R., Imseng, S., Jakob, R. P., Hall, M. N., Maier, T., & Hiller, S. (2021). The dynamic mechanism of 4E-BP1 recognition and phosphorylation by mTORC1. Molecular Cell, 81(11), 2403-2416.e5. https://doi.org/10.1016/j.molcel.2021.03.031

Bournique, E., Sanchez, A., Oh, S., Ghazarian, D., Mahieu, A. L., Manjunath, L., Ednacot, E., Ortega, P., Masri, S., Marazzi, I., & Buisson, R. (2025). ATM and IRAK1 orchestrate two distinct mechanisms of NF-κB activation in response to DNA damage. Nature Structural and Molecular Biology, 32(4), 740–755. https://doi.org/10.1038/s41594-024-01417-0

Chen, X., An, Y., Tan, M., Xie, D., Liu, L., & Xu, B. (2023). Biological functions and research progress of eIF4E. In Frontiers in Oncology (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fonc.2023.1076855

Cheng, G. J., Leung, E. Y., & Singleton, D. C. (2022). In vitro breast cancer models for studying mechanisms of resistance to endocrine therapy. In Exploration of Targeted Anti-tumor Therapy (Vol.

3, Number 3, pp. 297–320). Open Exploration Publishing Inc. https://doi.org/10.37349/etat.2022.00084

Devanaboyina, M., Kaur, J., Whiteley, E., Lin, L., Einloth, K., Morand, S., Stanbery, L., Hamouda, D., & Nemunaitis, J. (2022). NF-κB Signaling in Tumor Pathways Focusing on Breast and Ovarian Cancer. In Oncology Reviews (Vol. 16). Page Press Publications. https://doi.org/10.3389/or.2022.10568

Esthya, F. R., Padauleng, N., & Prihatini, L. M. (2026). Pengaruh Insulin terhadap Viabilitas Sel Kanker Payudara Subtipe Luminal A (T-47D) yang diberi Doksorubisin. Skripsi (Unpublished), Universitas Mataram.

Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M., Zeng, C., Zhou, T., & Zhang, J. (2024). NF-κB in biology and targeted therapy: new insights and translational implications. In Signal Transduction and Targeted Therapy (Vol. 9, Number 1). Springer Nature. https://doi.org/10.1038/s41392-024-01757-9

Hassan, D., Menges, C. W., Testa, J. R., & Bellacosa, A. (2024). AKT kinases as therapeutic targets. In Journal of Experimental and Clinical Cancer Research (Vol. 43, Number 1). BioMed Central Ltd. https://doi.org/10.1186/s13046-024-03207-4

Kciuk, M., Gielecińska, A., Mujwar, S., Kołat, D., Kałuzińska-Kołat, Ż., Celik, I., & Kontek, R. (2023). Doxorubicin—An Agent with Multiple Mechanisms of Anticancer Activity. In Cells (Vol. 12, Number 4). MDPI. https://doi.org/10.3390/cells12040659

Kheradmand, P., Boroojeni, S. V., & Esmaeili-Mahani, S. (2021). MiR-221 expression level correlates with insulin-induced doxorubicin resistance in MCF-7 breast cancer cells. Cell Journal, 23(3), 329–334. https://doi.org/10.22074/cellj.2021.7153

Mirzaei, S., Gholami, M. H., Hashemi, F., Zabolian, A., Farahani, M. V., Hushmandi, K., Zarrabi, A., Goldman, A., Ashrafizadeh, M., & Orive, G. (2022). Advances in understanding the role of P-gp in doxorubicin resistance: Molecular pathways, therapeutic strategies, and prospects. Drug Discovery Today, 27(2), 436–455. https://doi.org/10.1016/j.drudis.2021.09.020

Mirzaei, S., Zarrabi, A., Hashemi, F., Zabolian, A., Saleki, H., Azami, N., Hamzehlou, S., Farahani, M. V., Hushmandi, K., Ashrafizadeh, M., Khan, H., & Kumar, A. P. (2021). Nrf2 signaling pathway in chemoprotection and doxorubicin resistance: potential application in drug discovery. Antioxidants, 10(3), 1–27. https://doi.org/10.3390/antiox10030349

Muna, L. N., & Maulidina, F. (2022). Penghambatan Proliferasi Sel Kanker Payudara T47D oleh Ekstrak Air Daun Kelor (Moringa oleifera) dan Doxorubicin dengan Metode MTT Assay. Jurnal Farmasi Sains Dan Terapan, 9(2), 99–104. https://doi.org/10.33508/jfst.v9i2.4361

Pavitra, E., Kancharla, J., Gupta, V. K., Prasad, K., Sung, J. Y., Kim, J., Tej, M. B., Choi, R., Lee, J. H., Han, Y. K., Raju, G. S. R., Bhaskar, L. V. K. S., & Huh, Y. S. (2023). The role of NF-κB in breast cancer initiation, growth, metastasis, and resistance to chemotherapy. In Biomedicine and Pharmacotherapy (Vol. 163). Elsevier Masson s.r.l. https://doi.org/10.1016/j.biopha.2023.114822

Taurin, S., & Alkhalifa, H. (2020). Breast cancers, mammary stem cells, and cancer stem cells, characteristics, and hypotheses. In Neoplasia (United States) (Vol. 22, Number 12, pp. 663–678). Elsevier Inc. https://doi.org/10.1016/j.neo.2020.09.009

Trayes, K. P., & Cokenakes, S. E. H. (2021). Breast Cancer Treatment. American Family Physician, 104(2), 171–178.

Warrier, N. M., Agarwal, P., & Kumar, P. (2020). Emerging Importance of Survivin in Stem Cells and Cancer: the Development of New Cancer Therapeutics. In Stem Cell Reviews and Reports (Vol. 16, Number 5, pp. 828–852). Springer. https://doi.org/10.1007/s12015-020-09995-4

WHO. (2022a). World Globocan 2022. https://gco.iarc.who.int/media/globocan/factsheets/populations/900-world-fact-sheet.pdf

WHO. (2022b). Indonesia Globocan 2022. https://gco.iarc.who.int/media/globocan/factsheets/populations/360-indonesia-fact-sheet.pdf

Yee, L. D., Mortimer, J. E., Natarajan, R., Dietze, E. C., & Seewaldt, V. L. (2020). Metabolic Health, Insulin,

and Breast Cancer: Why Oncologists Should Care About Insulin. In Frontiers in Endocrinology (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fendo.2020.00058

Zhang, F., de Haan-Du, J., Sidorenkov, G., Landman, G. W. D., Jalving, M., Zhang, Q., & de Bock, G. H. (2021). Type 2 diabetes mellitus and clinicopathological tumor characteristics in women diagnosed with breast cancer: A systematic review and meta-analysis. In Cancers (Vol. 13, Number 19). MDPI. https://doi.org/10.3390/cancers13194992