

Cardioprotective Effects of Bromelain on Wistar Rat (*Rattus norvegicus*) Cardiomyocytes Exposed to Doxorubicin

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ABSTRACT

Introduction: Doxorubicin (Dox) is an effective anthracycline whose clinical use is limited by cardiotoxicity. Bromelain, a proteolytic enzyme from *Ananas comosus*, has antioxidant and anti-inflammatory properties and may confer cardioprotection. **Methods:** We conducted an in vivo posttest-only control group study in female Wistar rats (≈ 8 weeks, 150–200 g; $n=5$ /group) allocated to control (P1), Dox 15 mg/kg intraperitoneally (P2), or Dox 15 mg/kg plus bromelain 40 mg/kg orally 30 min prior (P3). Four hours post-treatment, serum CK-MB was measured by autoanalyzer and NT-proBNP by electrochemiluminescence immunoassay. Normality (Shapiro–Wilk) and homogeneity (Levene) were met; pairwise comparisons used independent t-tests ($\alpha=0.05$). **Results:** Mean \pm SD CK-MB (U/L) were control 0.3530 ± 0.0373 , Dox 1.3756 ± 0.0544 , Dox+bromelain 1.0218 ± 0.1086 ; p-values: control vs Dox 0.013, control vs Dox+bromelain 0.026, Dox vs Dox+bromelain 0.037. Mean \pm SD NT-proBNP (pg/mL) were control 0.3932 ± 0.0304 , Dox 1.3924 ± 0.0579 , Dox+bromelain 1.0826 ± 0.0647 ; p-values: control vs Dox 0.028, control vs Dox+bromelain 0.012, Dox vs Dox+bromelain 0.039. **Conclusion:** Bromelain significantly attenuated Dox-induced increases in CK-MB and NT-proBNP in Wistar rats but did not restore biomarker levels to control values, supporting a cardioprotective effect and warranting larger, longer-term studies to define dose–response, durability, and mechanisms.

KEYWORDS: Doxorubicin; Bromelain; Cardiotoxicity; CKMB; NT-proBNP; Wistar rats.

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INTRODUCTION

Breast cancer is one of the most common malignancies among women worldwide. It is estimated that approximately 1.67 million new cases are diagnosed annually, representing 25% of all newly diagnosed cancers (Siegel, Miller and Jemal, 2016; Tremont, Lu and Cole, 2017). According to the most recent Global Cancer Statistics 2022 (GLOBOCAN), there were an estimated 19.9 million new cancer cases and nearly 9.7 million cancer deaths globally in 2022. Among these, breast cancer in women, with around 2.3 million new cases, has now surpassed lung cancer as the most frequently diagnosed cancer (Sung et al., 2021; Tosello et al., 2018). Approximately 3.5% of all women with breast cancer in the United States already present with distant metastases at initial diagnosis, and 20–30% of them will eventually develop distant metastases, accounting for around 400,000 to 500,000 deaths annually worldwide. This proportion is even higher in low- and middle-income countries, with an estimated 50,000 women diagnosed with metastatic breast cancer each year (Pokrzywinski et al., 2018).

Patients with stage III–IV breast cancer often require chemotherapy, with doxorubicin as a mainstay drug. Despite its efficacy, doxorubicin is limited by its significant cardiotoxic effects, including left ventricular dysfunction, heart failure, and oxidative stress (Goyal et al., 2016). Strict cardiac monitoring is therefore necessary to reduce the risk of cardiovascular complications. Doxorubicin (Dox), an anthracycline antibiotic, is widely used as chemotherapy for breast cancer. Clinical evidence demonstrates that doxorubicin reduces mortality in ovarian cancer by 15% and improves survival in metastatic breast cancer by 13% (Belger et al., 2024; Linders et al., 2024). However, its cardiotoxicity is dose-dependent, with risks increasing significantly at cumulative doses exceeding 400–700 mg/m² (Goyal et al., 2016).

The underlying mechanisms of doxorubicin-induced cardiotoxicity (DIC) remain incompletely understood. Left ventricular dysfunction in DIC is thought to result from myocardial injury. Multiple molecular pathways have been proposed, including excessive generation of reactive oxygen species (ROS), apoptosis, ferroptosis, mitochondrial dysfunction, calcium overload, DNA damage, inflammatory mediator release, and autophagic flux disorders (Rawat et al., 2021). Early detection of cardiotoxicity biomarkers such as CKMB and NT-proBNP is crucial for timely intervention with cardioprotective strategies (Dulf et al., 2023). In recent years, research has increasingly focused on preventing and managing chemotherapy-induced cardiotoxicity. Strategies

have included dose limitation of doxorubicin and the use of cardioprotective agents. However, there is still no universally accepted solution for preventing or treating DIC (Dulf et al., 2023). On the other hand, natural plant-derived compounds have gained attention due to their relative safety, affordability, and accessibility. One such candidate is bromelain, a mixture of thiol endopeptidases derived from pineapple (*Ananas comosus*) fruit, stem, and roots. Bromelain contains thiol endopeptidases along with protease inhibitors, glucosidase, cellulase, and escharase (Varilla et al., 2021).

Bromelain has demonstrated beneficial cardiovascular effects, including the reduction of angina pectoris and transient cardiomyopathy through its ability to dissolve thrombi and reduce blood viscosity (Varilla et al., 2021). An experimental study in animals showed that bromelain's antioxidant activity could reduce cardiomyocyte damage from oxidative stress in atherosclerotic plaque formation (Chen et al., 2022). Furthermore, bromelain exerts immunomodulatory and anti-inflammatory effects, including modulation of T cells, stimulation of phagocytosis, secretion of interleukins (IL-1 β , IL-6, TNF α), and downregulation of inflammatory mediators (Varilla et al., 2021; Hikiş and Bernasinska-Slomczewska, 2021). Based on these considerations, the present study aims to evaluate the potential cardioprotective effect of bromelain extract against doxorubicin-induced cardiotoxicity in Wistar rat cardiomyocytes.

MATERIALS AND METHODS

This experimental study applied a post-test only control group design *in vivo* using female Wistar rats (*Rattus norvegicus*), aged eight weeks and weighing 150–200 g. The animals were divided into three groups: P1 (negative control, no treatment), P2 (positive control, doxorubicin 15 mg/kgBW), and P3 (doxorubicin 15 mg/kgBW plus bromelain 40 mg/kgBW). The doxorubicin dose was based on Podyacheva et al. (2021), while the bromelain dose followed Violita and Probosari (2015). The study was conducted at the Faculty of Veterinary Medicine Laboratory, Universitas Airlangga, Surabaya, over a three-month period.

Sample size was calculated using the E-ratio method (Charan and Biswas, 2013). With three treatment groups, the optimal range (E=10–20) required 5–8 rats per group. Six rats were allocated to each group, resulting in 18 animals in total. Rats were housed in polypropylene cages under controlled conditions (12-h light/dark cycle, 22 \pm 2 $^{\circ}$ C, ad libitum access to food and water) with three animals per cage (Varilla et al., 2021; Violita and Probosari, 2015). Doxorubicin was administered intraperitoneally as a single dose, while bromelain was given orally via nasogastric tube at 6.2 ml/day, 30 minutes before doxorubicin injection as shown in FIGURE 1.

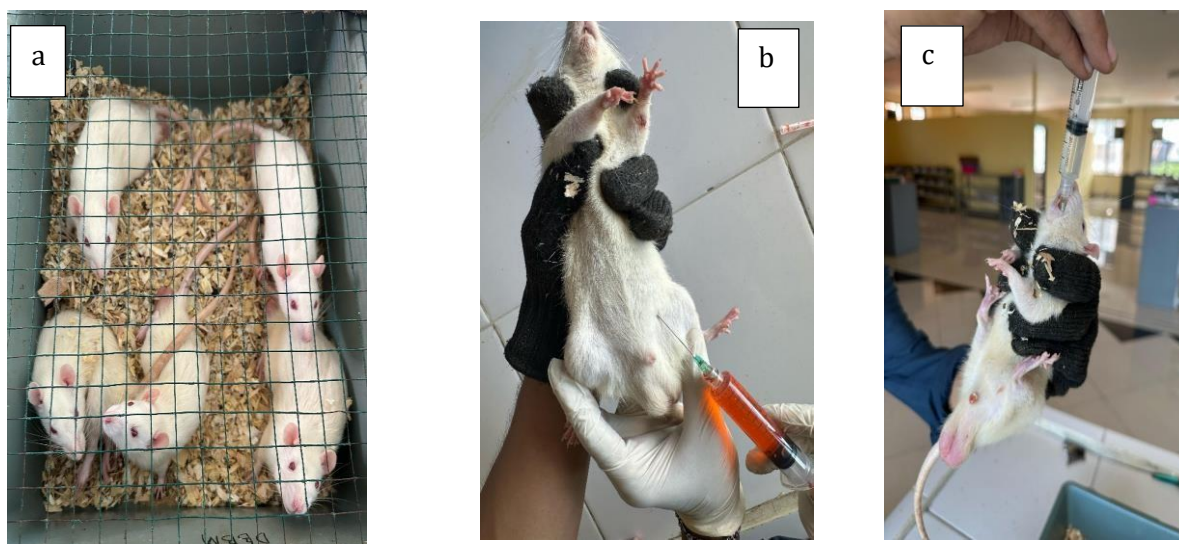


FIGURE 1. Wistar rats. (a) Adapted Wistar rats. (b) Wistar rats were given doxorubicin via intraperitoneum. (c) Wistar rats given bromelain orally.

Blood samples were collected four hours after treatment. For CKMB measurement, 5 ml of blood from the tail or heart was processed and analyzed using an autoanalyzer (Cobas c703, Roche Diagnostic). For NT-proBNP, 1 μ l of tail blood was diluted (1:4) and assayed by electro-luminescence immunoassay according to the manufacturer's protocol.

Data were processed using SPSS version 26.0 (SPSS Inc., Chicago, IL). Descriptive statistics were expressed as mean \pm standard deviation, with box plots for distribution. Comparative analysis between groups was performed using the independent t-test, and if normality assumptions were not met, the Mann–Whitney U test was used. All experimental protocols adhered to ethical standards for laboratory animal research.

RESULTS

A total of 15 Wistar rats (*Rattus norvegicus*) were included in this study and divided into three groups, each consisting of five animals: control (P1), doxorubicin (P2), and doxorubicin plus bromelain (P3). Serum CKMB and NT-proBNP levels were measured after 14 days of intervention.

Descriptive analysis showed that rats in the doxorubicin group (P2) had the highest mean CKMB and NT-proBNP levels, with mean values of 1.3756 \pm 0.05437 U/L and 1.3924 \pm 0.05787 pg/ml, respectively. In contrast, the control group (P1) exhibited the

lowest values (CKMB 0.3530 ± 0.03726 U/L, NT-proBNP 0.3932 ± 0.03043 pg/ml). Administration of bromelain in the treatment group (P3) significantly reduced biomarker levels compared to the doxorubicin-only group, with mean CKMB of 1.0218 ± 0.10858 U/L and NT-proBNP of 1.0826 ± 0.06471 pg/ml.

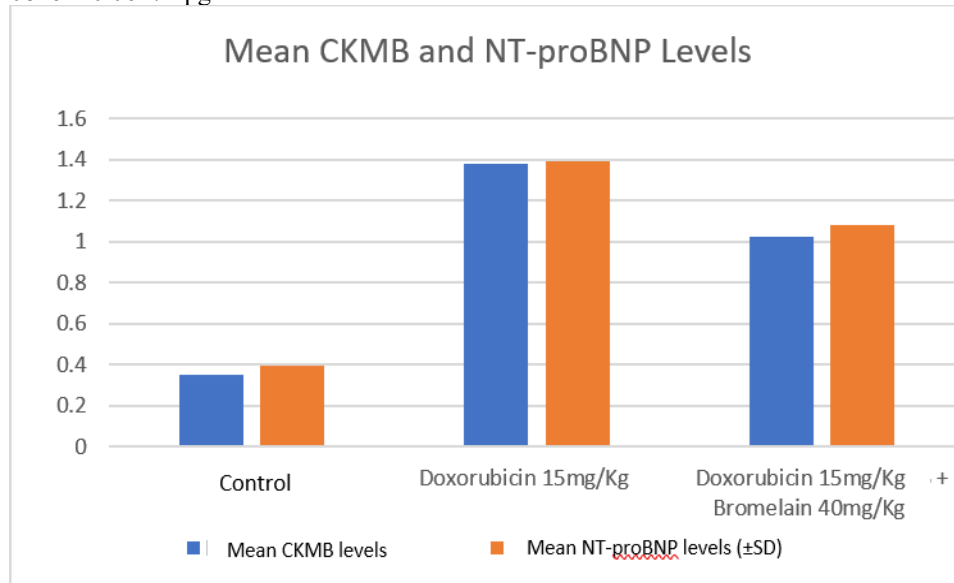


FIGURE 2. Graph of Average Levels of CKMB and NT-proBNP

Normality testing with the Shapiro-Wilk test indicated that all variables were normally distributed ($p > 0.05$), and homogeneity testing with Levene's test also confirmed homogeneous variances across groups ($p > 0.05$). Therefore, independent t-tests were performed for group comparisons.

The independent t-test revealed significant differences in CKMB and NT-proBNP levels between groups. CKMB levels were significantly higher in the doxorubicin group compared to control ($p = 0.013$), while bromelain administration significantly reduced CKMB compared to doxorubicin alone ($p = 0.037$) as shown in TABLE 1.

TABLE 1 CKMB Independent T-test

Group Comparison	n	Mean (±SD)	p-value
Control	5	0.3530 (0.03726)	0.013
Doxorubicin 15mg/kg	5	1.3756 (0.05437)	
Control	5	0.3530 (0.03726)	0.026
Doxorubicin 15mg/kg + Bromelain 40mg/kg	5	1.0218 (0.10858)	
Doxorubicin 15mg/kg	5	1.3756 (0.05437)	0.37
Doxorubicin 15mg/kg + Bromelain 40mg/kg	5	1.0218 (0.10858)	

Means in the same column/row with different superscript differ significantly ($p < 0.05$)

Similarly, NT-proBNP levels were significantly elevated in the doxorubicin group compared to control ($p = 0.028$), but bromelain administration significantly reduced NT-proBNP compared to doxorubicin alone ($p = 0.039$). Despite this reduction, NT-proBNP and CKMB levels in the bromelain group remained significantly higher than control values ($p < 0.05$) as shown in TABLE 2.

TABLE 2. T-test NT-proBNP Independent T-test

Group Comparison	n	Mean (±SD)	p-value
Control	5	0.3932 (0.03043)	0.028
Doxorubicin 15mg/kg	5	1.3924 (0.05797)	
Control	5	0.3932 (0.03043)	0.012
Doxorubicin 15mg/kg + Bromelain 40mg/kg	5	1.0826 (0.06471)	
Doxorubicin 15mg/kg	5	1.3924 (0.05797)	0.039
Doxorubicin 15mg/kg + Bromelain 40mg/kg	5	1.0826 (0.06471)	

Means in the same column/row with different superscript differ significantly ($p < 0.05$)

DISCUSSIONS

This study demonstrated that administration of doxorubicin significantly increased serum CKMB and NT-proBNP levels in Wistar rats, indicating myocardial injury and ventricular dysfunction. Conversely, bromelain supplementation attenuated these increases, supporting its potential role as a cardioprotective agent against doxorubicin-induced cardiotoxicity.

The findings align with existing evidence that doxorubicin, an anthracycline widely used in breast cancer chemotherapy, induces cardiotoxicity through oxidative stress, inflammation, and apoptosis of cardiomyocytes (Omran et al., 2022). Biomarkers such as CKMB and NT-proBNP serve as sensitive indicators of myocardial damage and ventricular wall stress, respectively (Hall, 2004). The elevated levels in the doxorubicin group confirm its cardiotoxic effects, while the attenuation in the bromelain group demonstrates partial protection.

The protective effects of bromelain observed here may be attributed to its anti-inflammatory and antioxidant properties. Bromelain has been reported to inhibit proinflammatory cytokines such as TNF- α and IL-6, while enhancing the activity of antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Khatun, Sindhu and Venkatesu, 2020). Such mechanisms likely reduce oxidative myocardial injury caused by doxorubicin. Additionally, the reduction in NT-proBNP levels suggests bromelain's potential role in preventing excessive ventricular remodeling and dysfunction, which commonly occurs in subclinical heart failure induced by anthracyclines (Sangweni et al., 2022).

These findings are consistent with other studies investigating natural compounds as cardioprotective adjuvants. Curcumin and resveratrol, for example, have been shown to attenuate anthracycline-induced cardiotoxicity by reducing oxidative stress and apoptosis, with significant decreases in cardiac biomarkers in animal models (Dulf et al., 2023). Bromelain, with its multi-target actions, may therefore represent a promising alternative or complementary therapeutic approach for patients undergoing anthracycline-based chemotherapy.

Nevertheless, this study has limitations. The small sample size may limit statistical power, and only biochemical markers were assessed. No histopathological or echocardiographic examinations were performed to confirm structural or functional cardiac changes. Further studies employing multimodal evaluation and larger sample sizes are warranted to strengthen clinical applicability.

Despite these limitations, the present findings support the hypothesis that bromelain exerts cardioprotective effects in doxorubicin-induced cardiotoxicity by reducing CKMB and NT-proBNP levels. These results encourage further exploration of bromelain as a potential adjuvant therapy to mitigate cardiovascular risks in cancer patients receiving doxorubicin.

CONCLUSIONS

Bromelain from pineapple (*Ananas comosus*) extract has been shown to have a cardioprotective effect on cardiomyocytes in Wistar rats (*Rattus norvegicus*), significantly preventing increases in CKMB and NT-proBNP levels caused by doxorubicin exposure. This study would be better served by adding echocardiography to assist in obtaining regular information every 1-2 weeks for non-invasive results and to analyze cardiac function during doxorubicin administration.

Conflict of Interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

Declaration Of Generative Ai And Ai-Assisted Technologies In The Writing Process

During the preparation of this work, the author(s) used ChatGPT in order to to enhance the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

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