Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.2486135790-246

Regulation of IL-10 by Herbal and Hormonal Therapy: The Impact of *Eleutherine Bulbosa* and Tamoxifen in an Experimental Breast Cancer Model

Rahmi[a], Andi Nilawati Usman*[a], Risfah Yulanty[c], Stang[d], Andi Ariyandy[e]

[a]*Department of Midwifery, Graduate School, Hasanuddin University, Makassar, South Sulawesi, Indonesia*

[c]*Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia*

[d]*Department of Public Health, Faculty of Public Health, Hasanuddin University, Makassar, South Sulawesi, Indonesia*

[e]*Department of Physiology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia*

ARTICLE INFO

Received:
4 August 2025
Revised:
14 November 2025
Accepted:
13 December 2025

Keywords:
interleukin-10, mice, inbred BALB/c, ethanol, animals, cytokines, immunosuppression therapy

ABSTRACT

Background: Breast cancer is the most common cancer affecting women worldwide, with the number of cases and deaths continuing to rise. This study evaluated the single and combined effects of Eleutherine bulbosa ethanol extract and tamoxifen in reducing interleukin-10 (IL-10) levels in BALB/c mice with breast cancer models.

Methods: This study used a laboratory experimental approach with a post-test-only control group design, using 36 female Mus musculus BALB/c strain mice (aged 8-10 weeks), randomly divided into six groups: negative and positive control groups. The intervention groups were given *Eleutherine bulbosa* ethanol extract at a dose of 180 mg/kg BW for 14 days, tamoxifen at a dose of 10 mg/kg BW every 2 days for 7 days within a 14-day period, a combination of *Eleutherine bulbosa ethanol* extract and tamoxifen, and tamoxifen and *Eleutherine bulbosa* ethanol extract. Effectiveness was evaluated based on IL-10 levels in serum.

Results: This study found that the combination of tamoxifen and ethanol extract from *Eleutherine bulbosa* significantly reduced IL-10 levels in BALB/c mice with a breast cancer model. The extract *Eleutherine bulbosa* ethanol extract reduced IL-10 by 31.4%, while tamoxifen reduced it by 59.1%. The strongest synergistic effect was observed when combined with tamoxifen, reducing IL-10 levels by 64.6%, indicating its effectiveness in suppressing the immunosuppressive response supporting tumor growth.

Conclusion: Tamoxifen and *Eleutherine bulbosa* extract showed synergistic effects in reducing IL-10 levels, suggesting enhanced immunomodulatory action compared to monotherapy. This combination can enhance breast cancer treatment efficacy by diminishing immunosuppression.

INTRODUCTION

Breast cancer is the most common cancer among women worldwide, with incidence rates continuing to rise each year.[1] In 2024, the American Cancer Society estimated 313,510 new cases and 42,780 deaths from breast cancer globally. [2] In Indonesia, breast cancer is a serious health problem, with 66,271 new cases and 22,598 deaths reported in 2020, underscoring the need for effective treatment strategies. [3]

Breast cancer is caused by various complex factors, including genetic mutations, hormonal

***Address for correspondence:**
Dr. Andi Nilawati Usman, SKM., M.Kes
Postgraduate Education
Hasanuddin University, Makassar, 90245
Email: andinilawati@pasca.unhas.ac.id

imbalance, and oxidative stress. [4,5] IL-10 plays an important role in breast cancer pathogenesis. Despite its anti-inflammatory properties, IL-10 can support cancer cell proliferation and migration and is associated with a poor prognosis. [6] IL-10 acts through the JAK1-TYK2-STAT3 signaling pathway, which reduces the production of proinflammatory cytokines and T cell proliferation. [7] In the context of tumors, IL-10 promotes carcinogenesis by affecting neutrophils, making it a biomarker for cancer diagnosis, prognosis, and therapy. [8,9]

Tamoxifen is the primary therapy for ER+ breast cancer. [10,11] This drug inhibits estrogen action in breast tissue to reduce cancer cell proliferation, although long-term use can cause adverse effects. [12] As an alternative, natural compounds from herbal plants have potential as anticancer agents. *Eleutherine bulbosa*, rich in bioactive compounds such as flavonoids, polyphenols, alkaloids, quinones, and saponins, [13,14] exhibits antioxidant, antitumor, and antimetastatic activities. Its active compounds, such as avenasterol, inhibit the proliferation of MCF-7 breast cancer cells. [15,16]

Given the challenges and limitations of conventional therapy, this study investigated the potential of combination therapy with tamoxifen and *Eleutherine bulbosa* ethanol extract to reduce IL-10 levels. An in vivo approach using DMBA-induced BALB/c mice (*Mus Musculus*) was chosen to comprehensively evaluate the efficacy and mechanism of action. [17] The main objective was to demonstrate the stronger synergistic effect of combination therapy compared to single therapy in reducing IL-10 levels, thereby potentially improving the efficacy of breast cancer treatment. This study is part of a comprehensive research project that also analyzed TP53 apoptosis, TNF-α, COX-2, and Interleukin-6.

METHODS

Study Design

We employed a completely randomized design (CRD) with a post-test-only control group to minimize selection bias by ensuring equal treatment assignment probability for all subjects.

Healthy and energetic female mice (*Mus musculus*), aged 8–10 weeks and weighing 18–25 g, were included in the study.

Criteria for Exclusion

Mice that refused food/water, died during the study, or were found to be pregnant at any point were excluded from the analysis. The dropout criteria included mice that perished prior to the conclusion of the study. The mice were sourced from the Healthy Animal Laboratory in Malang, Indonesia.

Eleutherine Bulbosa Extract Preparation

Eleutherine bulbosa, a medicinal plant indigenous to Kalimantan, was harvested in amounts totaling 40 kg from the Kalimantan area. The reddish-purple tubers were segregated, cleansed, sliced, and dehydrated at 40°C for one week at the Women's Health Laboratory of Hasanuddin University. The desiccated tubers were pulverized and macerated with 96% ethanol over three periods of 24 hours at the Phytopharmaceutical Laboratory. The filtrate was subjected to filtration, evaporation, and subsequent drying to yield a concentrated dark crimson extract for further pharmacological evaluation.

Breast Cancer Model Induction

This study adhered to ethical animal procedures. Breast cancer was induced in 36 BALB/c mice using the carcinogenic substance 7, 12-dimethylbenz [a] anthracene (DMBA). Each mouse weighing 20 g was administered a dose of 1 mg of DMBA per day for 42 days, resulting in a total DMBA dose of 42 mg per mouse (1 mg/day * 42 days = 42 mg). Before administration, DMBA was dissolved in sesame oil to form a 1% solution, and each mouse received 0.1 ml of this solution per day via an oral tube. This procedure aimed to induce breast cancer in vivo as an experimental model and was performed at Satwa Sehat, Malang. The breast cancer model in mice was validated by examining histopathological changes (cell and tissue structure) and using immunohistochemistry (IHC) techniques. In addition, the presence of lumps and tumor masses physically detected in DMBA-induced mice was also an indication of the success of the model.

Experimental ProceduresExperimental Groups

This study employed a laboratory experiment utilizing a post-test-only control group design within a completely randomized design framework. This approach was implemented to mitigate selection bias and ensure each subject had an equal probability of being assigned to any treatment group. The study included 36 female BALB/c mice (*Mus musculus*), aged 8–10 weeks and weighing approximately 18–25 g. Female BALB/c strain mice that satisfied the inclusion and exclusion criteria were allocated to two control groups and four intervention groups, with 6 mice in each group. These mice were randomly allocated to groups. The negative control group consisted of healthy mice that were only fed and given water without any treatment. The positive control group consistsed of breast cancer model mice that did not receive *Eleutherine bulbosa* ethanol extract or tamoxifen. Intervention Group 1 was given *Eleutherine bulbosa* ethanol extract at 180 mg/kg daily for 14 days, [18] while Intervention Group 2 was

given tamoxifen at 10 mg/kg every two days for seven doses. Intervention Group 3 received a combination of *Eleutherine bulbosa* extract and tamoxifen simultaneously, and Intervention Group 4 received a combination of tamoxifen followed by *Eleutherine bulbosa* extract sequentially for 14 days.

Fourier Transform Infrared Spectroscopy Analysis

FTIR analysis was performed at the Analytical Chemistry and Food Control Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, to non-destructively identify the functional groups of active chemicals in the concentrated E. bulbosa ethanol extract. A droplet of the extract was deposited onto the surface of an ATR crystal, and the instrument was scanned across the range of 4000–400 cm^{-1}. The resultant spectrum was examined to identify the –OH, –C=O, and –C–O functional groups, which are typically present in flavonoids and phenolic compounds. This technique is rapid, precise, and suitable for phytochemical analysis of plant extracts. Here is the image:

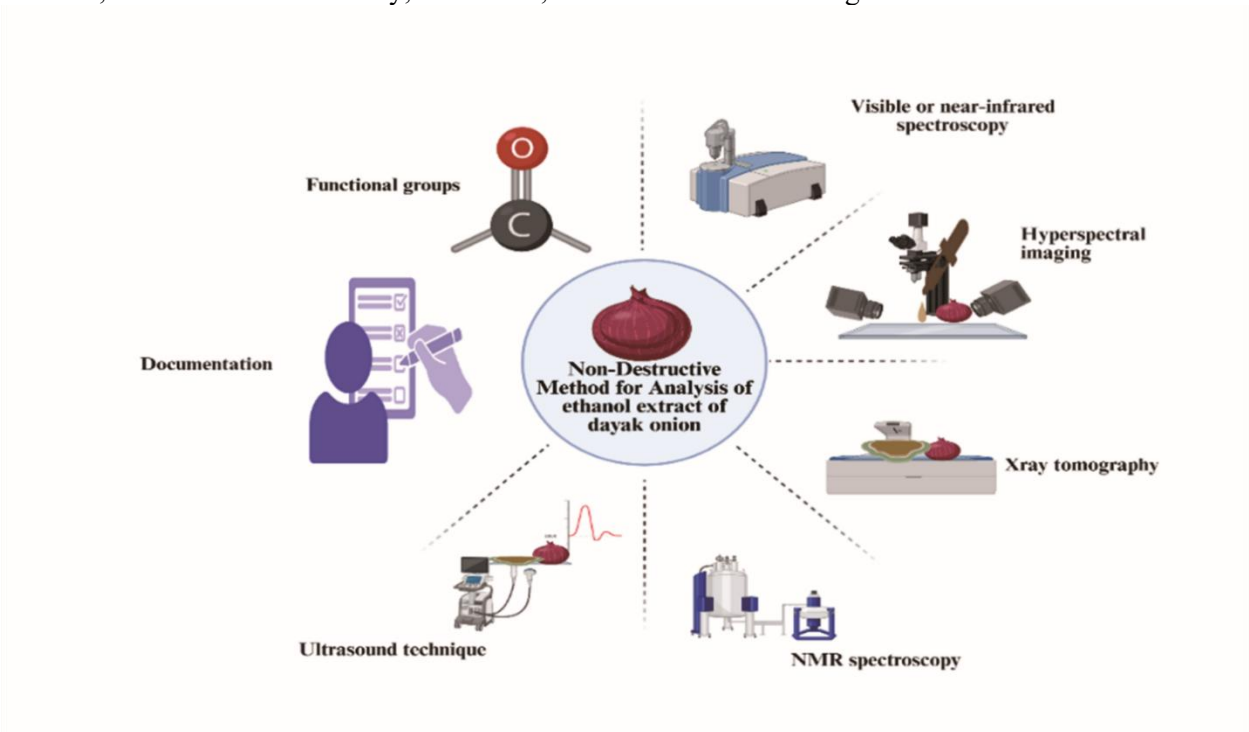

Figure 1. Examination Process of Fourier Transform Infrared Spectroscopy of Ethanol Extract of Eleutherine Bulbosa. This image shows various non-destructive techniques, such as FTIR, NMR, ultrasound, X-ray tomography, and hyperspectral imaging, which are used to analyze bioactive compounds without damaging the sample. FTIR was used to identify the functional groups of phenol, alcohol, and alkyl from organic compounds, flavonoids, aldehydes, ketones, esters, and glycosides that play a role in pharmacological activity.(BioRender)

Hematoxylin-Eosin Analysis

Histopathological examination was performed at the Satwa Sehat Laboratory in Malang using hematoxylin-eosin staining as the conventional method for breast cancer diagnosis. Tissue was acquired via biopsy, preserved in 10% formalin, embedded in paraffin blocks, and finely sectioned using a microtome. Hematoxylin staining highlights cell nuclei, whereas eosin imparts color to the cytoplasm. The data were analyzed microscopically to evaluate cell differentiation, mitotic activity, and invasion patterns, and to ascertain the malignancy grade using the Nottingham approach to inform the treatment plan.

Imunohistokimia (IHK)

The immunohistochemistry (IHC) procedure on the blood plasma of breast cancer mice was performed by collecting blood into an EDTA tube and centrifuging it at 1,500 × g for 10 min at 4°C to obtain plasma. A total of 100 µL of plasma was placed on a glass slide using the cytospin method (600 rpm, 4 min) and fixed with 4% paraformaldehyde for 10 min. After rinsing with PBS, the sample was treated with 0.1% Triton X-100 for 10 min for permeabilization and blocked with 5% normal serum for 45 min. Next, the slides were incubated with p53 primary antibody overnight at 4°C, followed by incubation with HRP-labeled secondary antibody for 1 h. Staining was performed with DAB substrate until a brown color appeared, followed by staining with hematoxylin contrast dye, gradual dehydration, and permanent mounting. Positive and negative controls, as well as four intervention groups, were used to ensure specific results were obtained.

ELISA Examination

This test was conducted by the Healthy Animal Laboratory in Malang using the ELISA method, a quantitative immunological technique used to measure IL-10 levels in biological samples such as serum, plasma, or tissue homogenates. The ELISA method is a sandwich technique, wherein the IL-10 antigen in the sample binds to specific antibodies coated on a microtiter plate, followed by the addition of biotinylated antibodies and streptavidin-HRP enzyme. The enzymatic reaction produces a color change proportional to the concentration of IL-10, which was read using a spectrophotometer at 450 nm, as shown in the figure below. The findings indicated a reduction in IL-10 levels, signifying an increase in the inflammatory response. This result corresponds with findings in the journal Biomedical Research and Therapy, highlighting the significant function of IL-10 as an anti-inflammatory cytokine in the pathology of chronic illnesses.

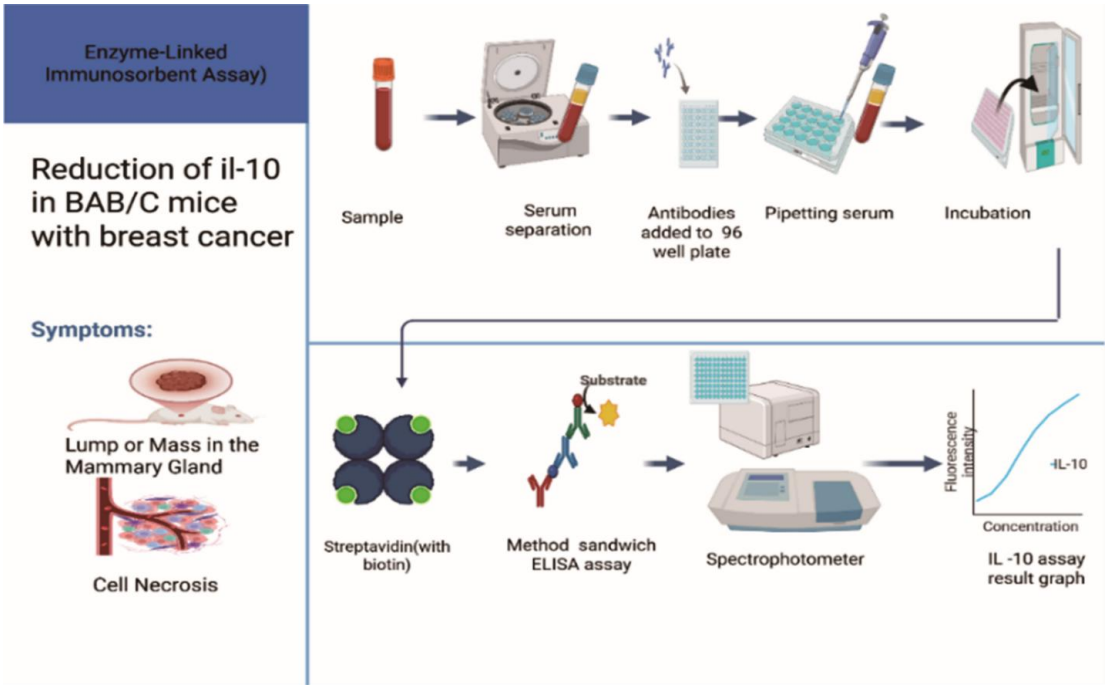

Figure 2. IL-10 ELISA Assessment Procedure in Each Treatment Cohort of Experimental Animals Using Blood Samples. [19] This image depicts the phases of interleukin-10-level assessment using the sandwich ELISA technique in BAB/C mice with breast cancer, marked by tumors in the mammary glands and cellular necrosis. The findings indicated a reduction in interleukin-10 levels, signifying an increase in the inflammatory response. This result corresponds with findings in the journal Biomedical Research and Therapy, highlighting the significant function of interleukin-10 as an anti-inflammatory cytokine in the pathology of chronic illnesses.

Statistical Analysis

In the data analysis using SPSS version 25, the normality test was performed first, as indicated by the results of the Kolmogorov-Smirnov and Shapiro-Wilk tests ($P > 0.05$), indicating that the data were normally distributed. Next, a homogeneity test was performed, and because the results were not homogeneous, a Brown-Forsythe test was performed to determine whether there were significant differences between the intervention groups. Subsequently, to identify the specific intervention groups that had significant differences, a post-hoc Games Howell test was used, which showed significant differences between the intervention groups.

Table 1. FTIR analysis of the extract revealed the presence of several functional groups that significantly contributed to its biological activity.

RESULTS

Analysis Results of Eleutherine Bulbosa Ethanol Extract

Table 1. Analysis of Eleutherine Bulbosa Ethanol Extract

Cluster	Peak	Compound
O-H (Hidroksil)	3348.85 cm^{-1}	Phenol or Alcohol
C-H (Aliphatic)	2927.28 cm^{-1}	Alkyl of Organic Compounds
C=O (carbonyl)	1649.65 cm^{-1}	Flavonoids, Aldehydes, or Ketones
C=C (Aromatics)	1451.43 cm^{-1}	Flavonoids
C-O	1055.00 cm^{-1}	Alcohols, Esters, or Glycosides

The absorption spectra at approximately 3348.85 cm^{-1} signify the presence of hydroxyl (O-H) groups in phenolic chemicals or alcohols, which are known

for their antioxidant properties. The absorption peak at 2927.28 cm^{-1} signifies aliphatic C-H groups from alkyl chains, whereas the peak at 1649.65 cm^{-1} denotes carbonyl groups (C=O), typically present in flavonoids, aldehydes, or ketones, which possess significant anti-inflammatory and anticancer properties. The detection of aromatic C=C bonds at 1451.43 cm^{-1} corroborates the existence of aromatic chemicals such as flavonoids, and the peak at 1055.00 cm^{-1} signifies C-O groups from alcohols, esters, or glycosides. These findings suggest that the extract contains bioactive chemicals that can be developed as pharmacological agents for cancer therapy.

Results of Histopathological Examination

Histopathological analysis of breast tissue from a murine model of breast cancer using hematoxylin-eosin staining revealed distinct cellular morphological variations among the different treatment groups. The positive control group exhibited predominant and dense proliferation of cancer cells, which displayed distinct malignant characteristics, such as hyperchromatic nuclei, pleomorphism, and an elevated nucleus-to-cytoplasm ratio. The tissue architecture was disordered, exhibiting stromal invasion with few signs of apoptosis. Concurrently, the cohorts administered tamoxifen or *Eleutherine bulbosa* ethanol extract exhibited a reduction in tumor cell density, expansion of intercellular gaps, and indications of apoptosis, including pyknosis, karyorrhexis, and cytoplasmic vacuolization. The combination of tamoxifen and *Eleutherine bulbosa* extract exhibited the most significant therapeutic effect, characterized by an extensive area of focal necrosis and a marked decrease in the number of active cancer cells, suggesting the synergistic potential of these agents in suppressing proliferation and inducing tumor cell apoptosis (Figure 3).

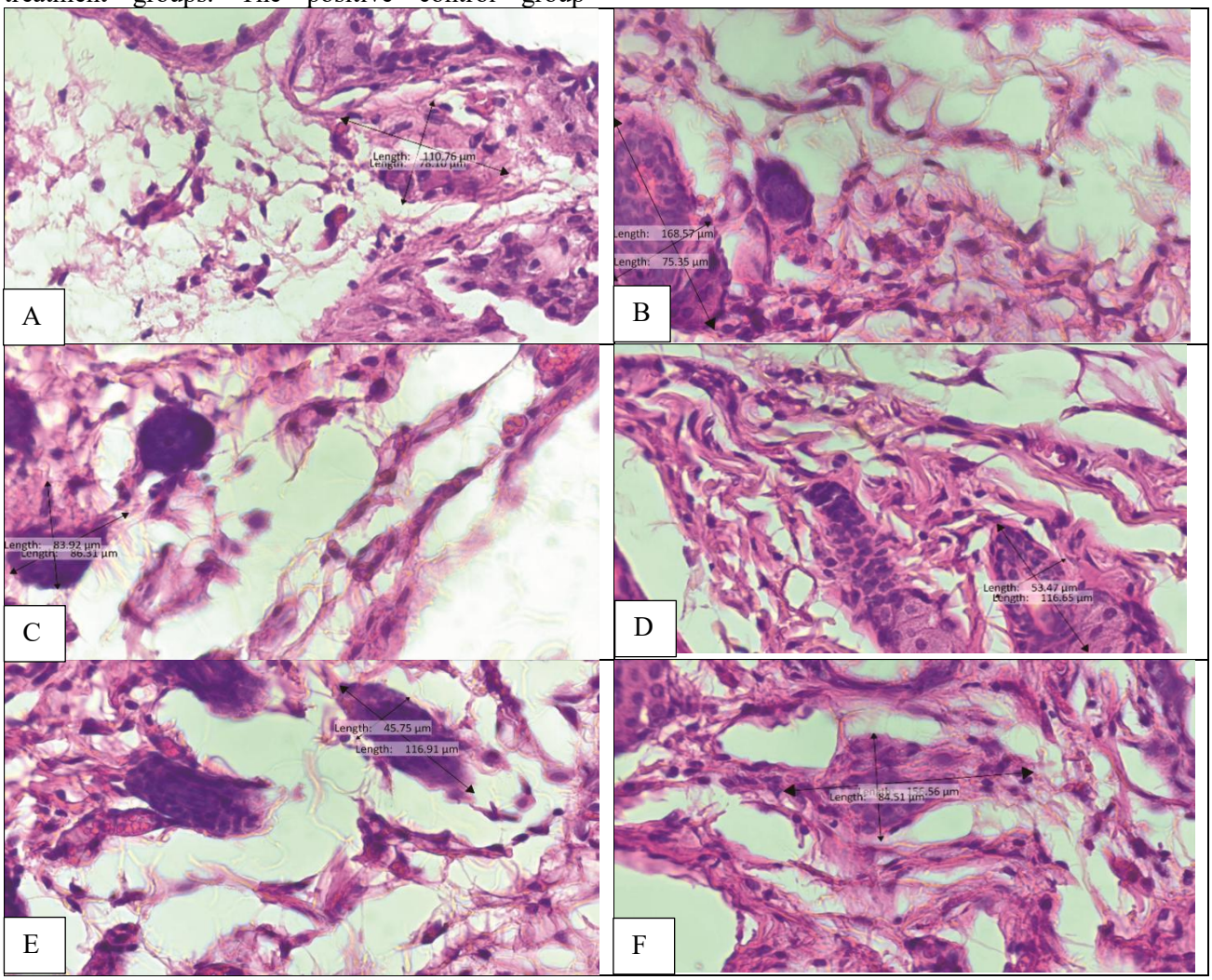

Figure 3. The results of histopathological examination in each treatment of experimental animals in mammary tissue. A: Negative control; B: Positive control; C: Intervention 1(treatment group *Eleutherine bulbosa*); D: Intervention 2(treatment group tamoxifen); E: Intervention 3(combination treatment group of *Eleutherine bulbosa* and tamoxifen); F: Intervention 4(combination treatment group of tamoxifen and *Eleutherine bulbosa*)

Imunohistokimia (IHK)

Immunohistochemical examination of breast tissue in experimental animals was performed to evaluate the expression of markers associated with breast cancer growth and responses. The tissue examination results showed differences in the responses of each group. In the control group (Figure A), there were no signs of cancer; therefore, the staining of cancer markers was almost invisible. In contrast, in the cancer-induced group (Figure B), cancer markers were clearly visible, indicating rapid cancer cell growth. After treatment, *Eleutherine bulbosa* (Figure C), tamoxifen (D), and *Eleutherine bulbosa* and tamoxifen (Figure E) suppressed cancer growth, although tamoxifen showed a stronger reduction. The best results were observed in the group treated with a combination of tamoxifen and *Eleutherine bulbosa* (Figure F), where cancer marker expression decreased drastically, approaching normal conditions. This indicates that the combination of both is far more effective in combating cancer than when they are used separately. And here is the image:

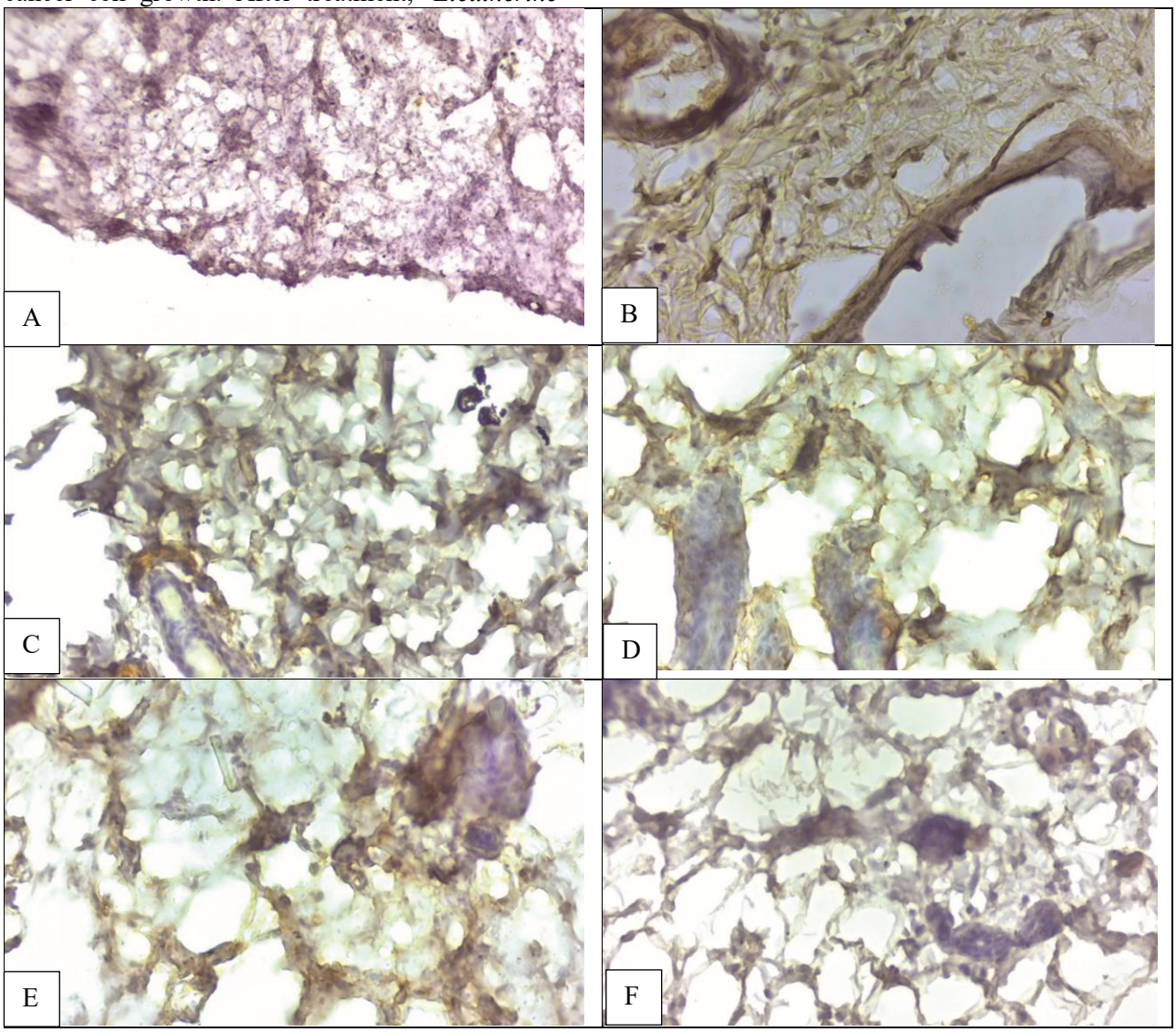

Figure 4. The results of histopathological examination in each treatment of experimental animals in mammary tissue. A: Negative control; B: Positive control; C: Intervention 1(treatment group *Eleutherine bulbosa*); D: Intervention 2(treatment group tamoxifen); E: Intervention 3(combination treatment group of *Eleutherine bulbosa* and tamoxifen); F: Intervention 4(combination treatment group of tamoxifen and *Eleutherine bulbosa*) .

Results from the ELISA analysis of Interleukin-10 concentrations are presented below.

Normality test results

The normality test results showed that all treatment groups had a normal data distribution, as indicated by a p-value greater than 0.05. Thus, the data meets the requirements to proceed to the homogeneity test stage to assess variance uniformity, so that the analysis of interleukin-10 levels between groups can be carried out more accurately and validly

Homogeneity test result

Levene's test for homogeneity of variance on the Interleukin-10 variable yielded a significance value

of 0.014 (based on the mean), which is below the threshold α (0.05). This finding indicates that the variances between groups are not homogeneous ($p < 0.05$); therefore, the assumption of variance homogeneity as a key prerequisite for the use of standard parametric tests, such as Analysis of Variance (ANOVA), is not met. Therefore, for further comparative analysis, it is recommended to use alternative statistical tests that are more robust to variance inequality, such as the Brown-Forsythe test.

Brown Forsythe test result

The Brown–Forsyth test results for the Interleukin-10 variable showed a statistical value of F(df1 = 5, df2 = 18.607) = 527.534, with a significance value of $p < 0.001$. A minimal significance value ($p < 0.05$) indicated a statistically significant difference in the mean Interleukin-10 levels between the treatment groups. Thus, it can be concluded that the treatment given to each group affects the Interleukin-10 level. The observed variation in Interleukin-10 levels between the groups was statistically significant and not due to chance. These findings provide the basis for continuing the Games-Howell post-hoc test analysis to identify which pairs of groups specifically show significant differences.

Games-Howell test result

Table 2. Results of Games-Howell test of interleukin-10 levels in mice (Mus musculus) induced with DMBA by administration of tamoxifen and Eleutherine bulbosa ethanol extract

Interleukin-10

Games-Howell

Treatment Group	n	α = 0.05				
		1	2	3	4	5
Negative control	6	0.4140				
Intervention 4	6	0.4834				
Intervention 2	6				0.5595	
Intervention 3	6					0.7543
Intervention 1	6			0.9377		
Positive control	6		1.3668			

Based on the results of the Games-Howell , which was used as a post hoc test after it was found that the data did not meet the assumption of variance homogeneity, most of the comparisons between treatment groups showed statistically significant differences in interleukin-10 levels ($p < 0.05$). This test was chosen because it accommodates conditions of non-homogeneous variance and unequal sample sizes, making it suitable for continuing the analysis after the Brown-Forsythe test.

The results showed that treatment 4, which is a combination of tamoxifen and *Eleutherine bulbosa* therapy (0.4834), was close to the value in the negative control group (0.4140). This indicates that Intervention 4 is more effective in significantly reducing the number of active cancer cells than the other treatments. Meanwhile, Intervention 2 using tamoxifen (0.5595) showed almost equivalent effectiveness to Intervention 4. In Intervention 3, a combination therapy of *Eleutherine bulbosa* and tamoxifen (0.7543), and Intervention 1 with Eleutherine bulbosa therapy (0.9377), there was a decrease in tumor cell density, widening of the intercellular space, and signs of apoptosis, such as pyknosis, karyorrhexis, and cytoplasmic vacuolization. However, these changes were not significant and were still close to the conditions in the positive control group (1.3668)

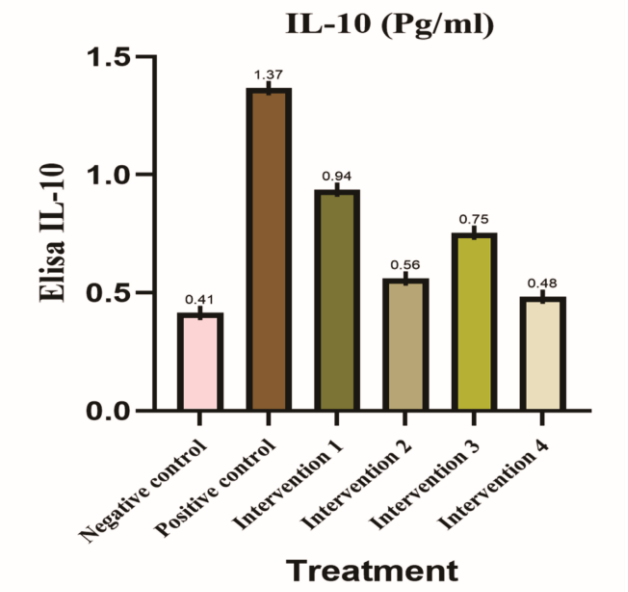

Figure 5. Interleukin-10 levels in BALB/c strain mice (Mus musculus) induced with breast cancer using DMBA after administration of tamoxifen and Eleutherine bulbosa. Note: NC: Negative control; PC: Positive control; I1: *Eleutherine bulbosa* treatment group; I2: Tamoxifen treatment group; I3: Combined *Eleutherine bulbosa* and tamoxifen treatment group; I4: Combined tamoxifen and *Eleutherine bulbosa* treatment group

Figure 5 shows the results of IL-10 level measurements (pg/ml) using the ELISA method in various treatment groups. The highest IL-10 level was found in the positive control group at 1.37 pg/ml, followed by intervention group 1 at 0.94 pg/ml, intervention group 3 at 0.75 pg/ml, intervention group 2 at 0.56 pg/ml, and intervention group 4 at 0.48 pg/ml, and the lowest level in the negative control group at 0.41 pg/ml. These results indicate that the treatment in Intervention 1 group significantly increased IL-10 levels compared to the negative control, although it was still lower than that of the positive control.

DISCUSSION

The results showed that the combination of tamoxifen therapy and *Eleutherine bulbosa* extract had the most significant effect on reducing interleukin-10 levels in mice with breast cancer. Group I4 showed a 64.6% decrease in interleukin-10 levels compared to the positive control (100%) receiving a combination of tamoxifen and *Eleutherine bulbosa*, and only a slight difference was observed from the negative control group, which experienced a 69.7% decrease. This is likely due to the action of tamoxifen as an antiestrogenic agent that inhibits cancer cell proliferation and reduces excessive immune activation, as well as the contribution of *Eleutherine bulbosa*, which contains active compounds such as flavonoids, polyphenols, alkaloids, quinones, and saponins, which are known to have antioxidant and anti-inflammatory activities. The combination of these two agents works synergistically by suppressing the expression of proinflammatory cytokines, such as interleukin-10, and by enhancing the regulation of the immune system, resulting in a more optimal anti-inflammatory effect than the administration of *Eleutherine bulbosa* and tamoxifen alone or in combination. Therefore, the combination of tamoxifen and Eleutherine bulbosa has the potential to be more effective in controlling inflammatory biomarkers such as interleukin-10 in animal models of breast cancer. [14,20,21]

A comparison of interleukin-10 levels between Intervention 3 (*Eleutherine bulbosa* and tamoxifen) and Intervention 4 (tamoxifen and *Eleutherine bulbosa*) showed a significant difference. The mean interleukin-10 level in Intervention 3 was 0.7543 pg/mL, while in Intervention 4, it was 0.4834 pg/mL, representing a decrease of 0.2709 pg/mL or 35.9% when the sequence of administration began with tamoxifen followed by Eleutherine bulbosa. Conversely, the interleukin-10 level in Intervention 3 was 56.0% higher than that in Intervention 4, indicating that the sequence of administration potentially affects the combined immunomodulatory effect. Intervention 4 (tamoxifen and *Eleutherine bulbosa*) was more effective in reducing interleukin-10 than Intervention 3 (*Eleutherine bulbosa* and tamoxifen) due to a combination of pharmacodynamic and pharmacokinetic factors influenced by the sequence of administration. First, tamoxifen has an "off-target" immunomodulatory effect on macrophages that tends to reset the tumor microenvironment toward a pro-inflammatory response (e.g., increased M1 macrophage activation, reduced interleukin-10), so that administering tamoxifen first can reduce the initial immunosuppressive pressure and open a "window" in which the immunostimulatory action of *Eleutherine bulbosa* compounds can work more effectively. [22,23] Second, the phytochemical compounds in *Eleutherine bulbosa* (flavonoids, polyphenols, alkaloids, quinones, and saponins) are known to suppress the NF -κB pathway and increase the expression of pro-immune cytokines that support T cell activity when administered after tamoxifen, the inhibitory effect of interleukin-10 by both agents can be synergistic, resulting in a greater reduction in interleukin-10. [24,25] Third, from a pharmacokinetic perspective, administering *Eleutherine bulbosa* before tamoxifen risks reducing tamoxifen availability or altering its metabolism through enzymatic (CYP) interactions or protein binding mechanisms, as reviewed in studies on the interaction between *Eleutherine Bulbosa* and tamoxifen, thereby reducing tamoxifen efficacy if *Eleutherine Bulbosa* is administered first. Conversely, administering tamoxifen first reduces this risk and allows *Eleutherine Bulbosa* to act as an adjuvant. [26] Overall, modern literature supports the notion that the sequence of administration of systemic drugs and natural agents influences immunological and therapeutic outcomes, such that protocols in which tamoxifen is administered before *Eleutherine Bulbosa* biologically result in a greater reduction in interleukin-10.[24,26]

The challenges, safety, and clinical implications of combining *Eleutherine bulbosa* ethanol extract and tamoxifen should be considered before use in patients. Although animal studies have shown a decrease in interleukin-10 levels, human studies are still needed to confirm safe and effective dosages that do not cause organ damage. The active compound content of *Eleutherine bulbosa* may also vary depending on the growing location and extraction method, necessitating the standardization of products. Additionally, interactions with tamoxifen via metabolic enzymes may affect efficacy or increase side effects, therefore, the order of administration must be considered. Clinically, these findings support more personalized cancer treatment by combining conventional therapy with natural compounds to enhance therapeutic outcomes, and improve the immune response.[27–29]

CONCLUSION

This study revealed that the combination of tamoxifen and ethanol extract from *Eleutherine bulbosa* significantly reduced interleukin-10 levels and improved breast tissue architecture in a mouse model of breast cancer. The combination group had the most favorable outcomes, with interleukin-10 levels nearing normalcy and tissue architecture similar to that of healthy tissue, suggesting a synergistic effect on immune modulation and tissue

preservation. These findings support the possibility of this combination as adjunctive therapy. nevertheless, additional research is required for clinical implementation.

ACKNOWLEDGMENTS

We would like to express our gratitude to all parties who have supported this research, especially the Central Sulawesi Provincial Hospital, various laboratories at Hasanuddin University, the Malang Healthy Animal Clinic, and the Hasanuddin University Graduate School for their permission, facilities, and academic support.

CONFLICT OF INTEREST

The writers assert that they possess no conflicts of interest to reveal.

ETHICAL CONSIDERATIONS

This study was conducted following the acquisition of ethical approval (number 049/UN4.14.1/TP.01.02/2024) from the Health Research Ethics Committee (KEPK) of the Faculty of Public Health, Hasanuddin University.

FUNDING

None.

DATA AVAILABILITY

Data can be obtained upon request from the corresponding authors.

AUTHOR CONTRIBUTIONS

R: led the conceptualization and design of the study and took the lead in drafting the manuscript. ANU: contributed to the study design, managed data curation and project administration, and was actively involved in data interpretation and manuscript revision. supported data curation, provided critical revisions to the manuscript, and offered supervisory guidance throughout the project. RY: involved in data curation, data analysis, and interpretation, and also contributed to manuscript revisions. S and AA: responsible for data acquisition and participated in the manuscript revision process. All authors reviewed and approved the final version of the manuscript.

AI DISCLOSURE

Artificial intelligence–assisted tools were used during manuscript preparation to improve language clarity and grammar. These tools were not involved in study design, experimental procedures, data analysis, or interpretation of results. The authors reviewed and approved the final manuscript and take full responsibility for its content..

REFERENCES

1. Bakhsh T, Alyami NM. Inducing breast cancer cell death: The impact of taxodone on proliferation through apoptosis. *Heliyon*. 2024;10(13):e34044. doi:10.1016/j.heliyon.2024.e34044.
2. Breast. American Cancer Society. Available from: https://cancerstatisticscenter.cancer.org/types/breast
3. Katalog Data- Layanan Permintaan Data | Kementerian Kesehatan RI. Available from: https://layanandata.kemkes.go.id/katalog-data/ski/ketersediaan-data/ski-2023
4. Vilchis-Landeros MM, Vázquez-Meza H, Vázquez-Carrada M, Uribe-Ramírez D, Matuz-Mares D. Antioxidant Enzymes and Their Potential Use in Breast Cancer Treatment. *Int J Mol Sci*. 2024 23;25(11):5675. doi:10.3390/ijms25115675.
5. Dubey A, Sharma B. Targeting Oxidative Stress Biomarkers in Breast Cancer Development and the Potential Protective Effect of Phytochemicals. *Drugs Drug Candidates*. 2025 23;4(2):23. doi:10.3390/ddc4020023.
6. Carlini V, Noonan DM, Abdalalem E, Goletti D, Sansone C, Calabrone L, et al. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. *Front Immunol*. 2023 8;14:1161067. doi:10.3389/fimmu.2023.1161067.
7. Islam H, Chamberlain TC, Mui AL, Little JP. Elevated Interleukin-10 Levels in COVID-19: Potentiation of Pro-Inflammatory Responses or Impaired Anti-Inflammatory Action? *Front Immunol*. 2021 21;12:677008. doi:10.3389/fimmu.2021.677008.
8. Sun H, Wu Y, Zhang Y, Ni B. IL-10-Producing ILCs: Molecular Mechanisms and Disease Relevance. *Front Immunol*. 2021 29;12. doi:10.3389/fimmu.2021.650200.
9. Heimes AS, Shehaj I, Almstedt K, Krajnak S, Schwab R, Stewen K, et al. Prognostic Impact of Acute and Chronic Inflammatory Interleukin Signatures in the Tumor Microenvironment of Early Breast Cancer. *Int J Mol Sci.* 2024 16;25(20):11114. doi:10.3390/ijms252011114.
10. Raheem F, Karikalan SA, Batalini F, El Masry A, Mina L. Metastatic ER+ Breast Cancer: Mechanisms of Resistance and Future Therapeutic Approaches. *Int J Mol Sci.* 2023; 11;24(22):16198. doi:10.3390/ijms242216198.
11. Zhuo Z, Zhang D, Lu W, Wu X, Cui Y, Zhang W, et al. Reversal of tamoxifen resistance by artemisinin in ER+ breast cancer: bioinformatics analysis and experimental validation. *Oncol Res*. 2024;32(6):1093–107. doi:10.32604/or.2024.047257.
12. Zhang J, Liu J, Yue Y, Wang L, He Q, Xu S, et al. The immunotoxin targeting PRLR increases tamoxifen

sensitivity and enhances the efficacy of chemotherapy in breast cancer. *J Exp Clin Cancer Res*. 2024 20;43(1):173. doi:10.1186/s13046-024-03099-4.
13. Nurkolis F, Kurniatanty I, Wiyarta E, Permatasari HK, Mayulu N, Taslim NA, et al. Identification of novel functional compounds from forest onion and its biological activities against breast cancer. *J Agric Food Res*. 2024;18(101362). doi:10.1016/j.jafr.2024.101362.
14. Silva RMG da, Alves CP, Barbosa FC, Santos HH, Adão KM, Granero FO, et al. Antioxidant, antitumoral, antimetastatic effect and inhibition of collagenase enzyme activity of Eleutherine bulbosa (Dayak onion) extract: In vitro, in vivo and in silico approaches. *J Ethnopharmacol*. 2024;10;318(Pt B):117005. doi:10.1016/j.jep.2023.117005.
15. Arbain D, Sriwahyuni K, Susanti D, Taher M. Genus Eleutherine: A review of its distribution, traditional uses, phytochemistry, biological activities and its interchangeable common names. *South Afr J Bot*. 2022;150:731–43. doi:10.1016/j.sajb.2022.08.022.
16. Kamarudin AA, Sayuti NH, Saad N, Razak NAA, Esa NM. Eleutherine bulbosa (Mill.) Urb. Bulb: Review of the Pharmacological Activities and Its Prospects for Application. *Int J Mol Sci*. 2021;23;22(13):6747. doi:10.3390/ijms22136747.
17. Laskar YB, Bhattacharjee K, Nath M, Choudhury Y, Mazumder PB, Talukdar AD. Protective Effects of Pelargonidin against DMBA-Induced Mammary Tumorigenesis in BALB/c Mice through Reduced Oxidative Stress and Lipid Anomalies. *Nutr Cancer*. 2023;9;75(7):1579–99. doi:10.1080/01635581.2023.2219027.
18. Efrem DG, Ilmiawan MI, Pratiwi SE. Chemopreventive effect of dayak onion [Eleutherine bulbosa, Mill. (Urb)] against 7,12-dimethylbenz [α] anthracene (DMBA)-induced breast cancer in rats: study on cancer antigen 15-3 (CA 15-3). *Indones J Biomed Clin Sci*. 2024;21 ;56(3). doi:10.22146/inajbcs.v56i3.12245.
19. Moghadam RH, Rouzbahani M, Salehi N, Sadeghi M. Association between blood Interleukin-10 level in coronary artery disease: A systematic review, meta-analysis, and network analyses. *Biomed Res Ther*. 2024;31;11(7):6603–21. doi:10.15419/bmrat.v11i7.907.
20. Matariek G, Teibo JO, Elsamman K, Teibo TKA, Olatunji DI, Matareek A, et al. Tamoxifen: The Past, Present, and Future of a Previous Orphan Drug. *Eur J Med Health Sci*. 2022;24;4(3):1–10. doi:10.24018/ejmed.2022.4.3.1124.
21. Zhang L, Virgous C, Si H. Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. *J Nutr Biochem*. 2019;69:19–30. doi:10.1016/j.jnutbio.2019.03.009.
22. Huang H, Zhou J, Chen H, Li J, Zhang C, Jiang X, et al. The immunomodulatory effects of endocrine therapy in breast cancer. *J Exp Clin Cancer Res*. 2021;7;40(1):19. doi:10.1186/s13046-020-01788-4.
23. Sfogliarini C, Pepe G, Dolce A, Della Torre S, Cesta MC, Allegretti M, et al. Tamoxifen Twists Again: On and Off-Targets in Macrophages and Infections. *Front Pharmacol*. 2022;30;13:879020. doi:10.3389/fphar.2022.879020.
24. Kubatka P, Koklesova L, Mazurakova A, Brockmueller A, Büsselberg D, Kello M, et al. Cell plasticity modulation by flavonoids in resistant breast carcinoma targeting the nuclear factor kappa B signaling. *Cancer Metastasis Rev*. 2024;43(1):87–113. doi:10.1007/s10555-023-10134-x.
25. Wang S, Wang J, Chen Z, Luo J, Guo W, Sun L, et al. Targeting M2-like tumor-associated macrophages is a potential therapeutic approach to overcome antitumor drug resistance. *Npj Precis Oncol*. 2024;10;8(1):31. doi:10.1038/s41698-024-00522-z.
26. Yen C, Zhao F, Yu Z, Zhu X, Li CG. Interactions Between Natural Products and Tamoxifen in Breast Cancer: A Comprehensive Literature Review. *Front Pharmacol*. 2022;2;13:847113. doi:10.3389/fphar.2022.847113.
27. Sun Z, Li Q, Zhang J, Sun Z, Wu H, Liang H, et al. Phenolic constituents with antibacterial activity from *Eleutherine bulbosa*. Fitoterapia. 2024;1;172:105787. doi:10.1016/j.fitote.2023.105787.
28. Zakaria NH, Saad N, Che Abdullah CA, Mohd. Esa N. The Antiproliferative Effect of Chloroform Fraction of Eleutherine bulbosa (Mill.) Urb. on 2D- and 3D-Human Lung Cancer Cells (A549) Model. *Pharmaceuticals*. 2023;28;16(7):936. doi:10.3390/ph16070936.
29. Mulder TAM, de With M, del Re M, Danesi R, Mathijssen RHJ, van Schaik RHN. Clinical CYP2D6 Genotyping to Personalize Adjuvant Tamoxifen Treatment in ER-Positive Breast Cancer Patients: Current Status of a Controversy. *Cancers*. 2021;13(4):771. doi:10.3390/cancers13040771.

How to Cite This Article

Rahmi, Usman AN, Yulanty R, Stang, Ariyandy A. Regulation of IL-10 by Herbal and Hormonal Therapy: The Impact of Eleutherine Bulbosa and Tamoxifen in an Experimental Breast Cancer Model. Arch Breast Cancer. 2025; 13(1):50-9.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/1174