RESEARCH ARTICLE

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Effect of Curcuma Longa Extract to Granzyme Expression and Tumor Mass Diameter of Mammary Adenocarcinoma with Chemotherapy Adriamycin Cyclophosphamide: An Animal Study in Rats

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Abstract

Objective: This study aimed to prove the effectiveness of the curcuma longa extract to mammary adenocarcinoma with chemotherapy. **Methods:** This research employs an in vivo experimental laboratory design with a post-test-only control group. Thirty female Balb/c mice with mammary adenocarcinoma were randomly assigned to five groups: K- (without chemotherapy), K+ (with chemotherapy), P1 (chemotherapy with 100 mg *C. longa*), P2 (chemotherapy with 150 mg *C. longa*), and P3 (chemotherapy with 200 mg *C. longa*). After a five-week treatment period, granzyme expression was examined by immunohistochemistry, and tumor diameter was measured. Data normality test using the Shapiro-Wilk test, then homogeneity test using the Levene test. Tumor diameter use test with One Way Anova followed by the Post Hoc LSD test to determine differences between groups. Statistical significance was defined as $p < 0.05$. **Result:** The highest levels of granzyme expression were observed in the P3 group, with the results P3 were 51.83±19.66 and data was found to be normally homogeneous (p: 0,137) and distributed (p: 0,486). A comparative analysis revealed notable disparities between K-vsP3 (p=0.001), K+vsP1 (p=0.028), K+vsP2 (p=0.016), K+vsP3 (p=<0.001), and P1vsP3 (p=0.037). The smallest tumor diameter was observed in the P3 group, with the results for group P3 were 10.55±2.33 and and data was found to be normally homogeneous (p: 0,667) and distributed (p: 0,796). **Conclusion:** The administration of Curcuma longa extract in conjunction with chemotherapy has been demonstrated to enhance granzyme expression and reduce tumor diameter in mammary adenocarcinoma.

Keywords: Breast Cancer- Curcuma Longa- Granzyme- Tumor Diameter- Mammary Adenocarcinoma

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Introduction

Breast cancer is a malignancy that may originate from either the ductal epithelium or the lobules. These malignant cells proliferate rapidly and uncontrollably, subsequently infiltrating the surrounding tissue and metastasizing. This gene mutation is initiated by the presence of a foreign substance that enters the body, such as radioactivity, free radicals, or carcinogenic substances originating from external and internal sources within the body. The prevalence of cancer is on the rise, largely due to the adoption of unhealthy lifestyle habits, including smoking, the consumption of fast food, environmental pollution, and the depletion of the ozone layer [1].

Breast cancer is currently one of the most common types of cancer suffered by women with the highest prevalence in all countries in the world. Globally, breast cancer has an incidence rate of 11.6% of all new cancer cases and causes 6.6% of all cancer deaths [2]. WHO recorded that as many as 2.3 million women were diagnosed with breast cancer in 2020, so in the last 5 years there were 7.8 million living women diagnosed with breast cancer, making it the cancer with the greatest prevalence [3]. Breast cancer in Indonesia was reported to

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have reached 68,858 out of 396,914 new cases of cancer with an incidence rate of 16.6% based on 2020 Globocan data [4]. Early detection results reached 1,925,943 or 5.2% using the Acetic Acid Visual Inspection method to detect cervical cancer and clinical mammary examination to detect mammary cancer until 2017. Based on statistics from the Indonesia Social Security Administering Agency, cancer has cost 2.1 trillion rupiah as of September 2017 [5].

Management of breast cancer depends on the type and stage experienced by the patient. Surgery, radiotherapy, cytostatic, immunotherapy, and hormonal therapy are breast cancer treatment modalities. Chemotherapy for breast cancer is carried out by providing a systemic regimen, such as Fluorouracil, Adriamycin, and Cyclophosphamide (FAC); Fluorouracil, Epirubicin and Cyclophosphamide (FEC); Adriamycin and Cyclophosphamide (AC); And Cyclophosphamide, Methotrexate, And Fluorouracil (CMF) is the most frequently used combination regimen. This chemotherapy is given at three to four weeks intervals intermittently. FAC, FEC, and CMF are divided into six categories (given over 18-24 months), while AC is divided into 2 categories (given over 6 weeks) [6].

The results of a meta-analysis of studies with a total of 2,732 patients using standard agents in late-stage solid cancers suggest a rate of Complete Response for chemotherapy treatment is generally low, about 7.4% [7]. On the other hand, chemotherapy has side effects that damage the liver, kidneys, heart, and other organs of the body, as well as immunosuppressive effects. Apart from that, the high cost of cancer treatment means that people are starting to abandon conventional cancer treatment modalities. People try looking for other methods of treatment or complementary therapy for psychological, and economic reasons, minimal side effects in dealing with cancer. Complementary therapy or Complementary Alternative Medicine (CAM) is a therapy that is used as a "complement" or as an additional therapy to conventional therapy. Additionally, researchers are exploring methods to enhance the efficacy of chemotherapy, with a particular focus on dietary modifications and immune system optimization. Several studies have demonstrated that dietary modifications in cancer patients undergoing chemotherapy can yield favorable outcomes. However, which diet strategy is best cannot be determined, considering that this will vary greatly depending on the patient, type of cancer, and treatment regimen [8]. The combination and integration of complementary therapies and conventional therapies is called integrative therapy. One type of complementary therapy is herbal therapy. Many efforts have been made to explore new natural ingredients that are thought to improve the immune system against tumors, including Mahkota Dewa, Phylantus niruri, honey, Artemisia, and turmeric or Curcuma longa [6].

The herb Curcuma longa has been demonstrated to possess several beneficial properties, including the capacity to inhibit the development of cancerous cells by suppressing key processes involved in carcinogenesis, angiogenesis, and tumor growth [9]. Curcuma longa is a readily available, inexpensive, and generally well-tolerated plant [8]. Curcumin is one of the phytochemical components of *C. longa* which has an effect as a chemo-preventive agent. This natural compound extracted from *C. longa* is capable of suppressing, retarding, or inverting carcinogenesis [10]. Curcumin has anti-carcinogenic, anti-migration, anti-oxidant, anti-inflammatory, anti-metastatic, anti-angiogenic, apoptotic, radioprotective, and chemo-sensitizing properties [11]. Granzyme is a serine protein that has a role in apoptosis. In cancer, granzyme has been widely used as a representative marker of immune enhancement and efficient killing of tumor cells [12]. A study was conducted on triple-negative breast cancer (TNBC) to ascertain whether granzyme expression could serve as a marker of therapeutic and prognostic effects. The results of this study suggest that granzyme expression, especially granzyme B, can be used as a prognostic marker and therapeutic response in patients with breast cancer [12]. Further research is required to elucidate the effect of administering *C. longa* extract on granzyme expression and tumor mass diameter in mice mammary adenocarcinoma models receiving Adriamycin and Cyclophosphamide chemotherapy. It is anticipated that the findings of this study will provide evidence to support the use of *C. longa* as an adjunct to chemotherapy in the treatment of breast cancer. This research aimed to prove the effectiveness of the curcuma longa extract to mammary adenocarcinoma with chemotherapy adriamycin cyclophosphamide.

Materials and Methods

Animal Study

The research uses experimental animals and data collection was conducted for 25 weeks. The preparation of *C. longa* extract was conducted at the Laboratory of the Functional Implementation Unit of Traditional Health Services Tawangmangu Dr. Sardjito General Hospital. Treatment of mice at the Stem Cell and Cancer Research Laboratory Gajah Mada University. Subjects in this study were females of the Balb/c strain mice with DMBA-induced mammary adenocarcinoma nodules. Inclusion criteria include female mice with mammary adenocarcinoma nodules, 18 weeks old, and body weight 100-150 grams; Exclusion criteria in this study included anatomical defects, illness, and inactivity; while the dropout criteria in this study was death during the study.

Trial Design

This research is an experimental laboratory in vivo with Post Test Only Control Group, randomized controlled trials used to demonstrate clinical benefit between groups in terms of efficacy, which uses experimental animals as research objects. There were 30 subjects in this study with required this study based on Federer's Formula. The subject was divided into 5 research groups; the positive control group was given chemotherapy, the negative control group without chemotherapy, the group with a combination of chemotherapy and oral *C. longa* extract at a dose of 100 mg/kgBW, the group with a combination of chemotherapy and oral *C. longa* extract at a dose of 150mg/kgBW, and the group with a combination chemotherapy

and oral *C. longa* extract at a dose of 200mg/kgBW. The variables in this study include independent variables, namely administration of graded doses of *C. longa* extract; and dependent variables, namely granzyme expression and tumor mass diameter.

This research has received ethical clearance from the Health Research Ethics Commission (KEPK) of the Faculty of Medicine Diponegoro University, Indonesia (protocol number: No.133/EC-H/KEPK/FK-UNDIP/XI/2023) and we used the CONSORT reporting guidelines for reporting parallel group randomized trials.

Curcuma Longa

The dried turmeric simplicia to be extracted is cleaned from dirt and contaminants. Then the turmeric simplicia is cut into pieces and dried until it reaches a low water content (around 10-12%). The turmeric simplicia is ground with a mesh size of 60-80 to become a simplicia powder to increase the extraction surface area. 400 g of turmeric simplicia powder is dissolved with 3600 mL of 70% Ethanol into a 5L glass container with a ratio of 1:10 (weight/volume). Make sure the turmeric simplicia powder is completely submerged. Then the container is closed and left for 48 hours at room temperature, while stirring periodically using a head stirrer at a speed of 2000 rpm for 30 minutes every 12 hours to ensure an even extraction process. After the maceration period, it is then filtered with filter paper to separate the liquid extract from the dregs of the simplicia. Furthermore, the liquid extract is evaporated using a rotary evaporator at a temperature of 50-60ºC until concentrated into a thick extract. Concentration of the extract can be continued with a drying process at a temperature of 50 ºC until the extract weight is constant. The thick turmeric extract is ready to be used for further analysis with a curcumin content of 8.42%.

Granzyme Expression and Tumor Diameter Analysis

The subjects (30 mice) were given standard food and drink ad libitum and adapted for 1 week in the laboratory. DMBA induction 20 mg/kgBW 2 times a week, for 13 weeks through subcutaneous injection in the lateral mammary part which was carried out on all research subjects. Following 13 weeks of DMBA induction and nodule assessment, the mouse model with tumors was randomly divided into six groups, and the tumor diameter was evaluated before intervention. Curcuma longa extract and a combination of Adriamycin and Cyclophosphamide were given at the recommended dosage, graded doses of *C. longa* extract 100 mg/kgBW, 150 mg/kgBW, 200 mg/kgBW, Adriamycin 2 mg/kgBW and Cyclophosphamide 50 mg/BW. Re-evaluation after 5 weeks of treatment by measuring tumor diameter and granzyme expression. Data analysis was carried out for 2 weeks.

Tumor mass diameter was measured using a digital caliper (Calipro Digital Caliper, China) with 0.01 mm precision to ensure consistent and accurate readings (Figure 1). Measurements were performed at the widest point of the tumor in a single dimension to maintain consistency across all subjects. This procedure was applied uniformly throughout the study. The figure below illustrates the measurement process performed on a mammary adenocarcinoma-bearing mouse.

Measurement of granzyme expression by immunohistochemical staining with Streptavidin-Biotin, reading of the preparation by counting the cytoplasm or that attached to the brown tumor cells per 100 tumor cells with a magnification of 400x. Preparation was examined in five fields of view, and then the average presentation was calculated.

Statistical Analysis

The collected data in the form of measurement results of granzyme expression and tumor mass diameter were processed through editing, coding, entry, and cleaning data, then analyzed using SPSS Ver 21.0 for Windows. Data analysis was carried out in the form of descriptive analysis and hypothesis testing. Data on Granzyme expression and tumor mass diameter were presented in the form of tables of mean and SD. Data normality test using the Shapiro-Wilk test. The results of the normality test of granzyme expression and tumor diameter showed normal data distribution, the data was then tested for homogeneity with the Levene test. Tumor diameter data was then tested for One-Way ANOVA followed by the Post Hoc LSD test to determine differences between groups.

Results

Granzyme Expression

The granzyme expression level in group K+ was 15.33±1.21; in group K- was 20.63±8.80; in the treatment group P1 was 34.07±11.49; in the treatment group P2 was 36.17±19.40; and in the treatment group P3 was 51.83±19.66 is listed in Table 1. The obtained Levene test results indicate a homogeneous data variance of 0.137. Observations were made at 400x magnification with readings of the percentage of area fraction using Image software. Visible cells that express granzyme will be darker in color. Immunohistochemical staining of granzyme expression indicated by yellow arrows in mammary adenocarcinoma mice tissues is listed in

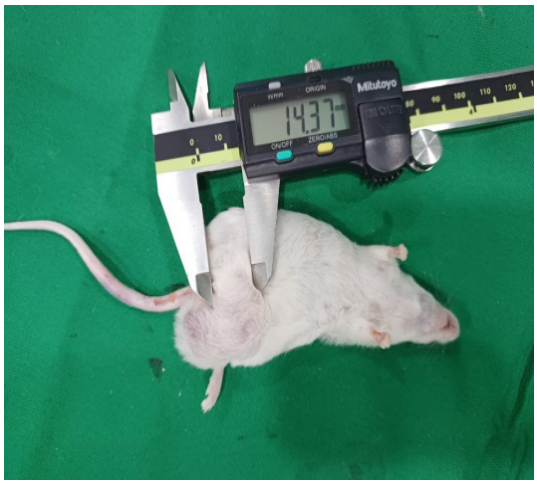

Figure 1. Procedure for Measuring Tumor Diameter Using a Digital Caliper (CaliPro®) on a Mouse Model with Mammary Adenocarcinoma

Table 1. Baseline characteristic Data

Variable	Group	N	Mean ± SD	p
Granzyme expression	K+	6	15.33 ± 1.21	0.415*
	K–	6	20.63 ± 8.80	0.516*
	P1	6	34.07 ± 11.49	0.813*
	P2	6	36.17 ± 19.40	0.305*
	P3	6	51.83 ± 19.66	0.486*
Tumor Diameter	K–	6	17.11 ± 3.28	0.988*
	K+	6	12.93 ± 2.03	0.504*
	P1	6	12.21 ± 1.75	0.543*
	P2	6	12.15 ± 2.81	0.476*
	P3	6	10.55 ± 2.33	0.798*

Description: *normality test (p>0.05)

Figure 2.

The normality test showed that the distribution of the granzyme expression data was normal (p>0.05). Different test results show significant differences between treatment groups. An LSD post hoc test was performed to evaluate the differences in granzyme expression between groups, with the result that there were significant differences between K+vsP1 (p=0.028), K+vsP2 (p=0.016), K+vsP3 (p=<0.001), K-vsP3 (p=0.001), and P1vsP3 (p=0.037) is listed in Table 2.

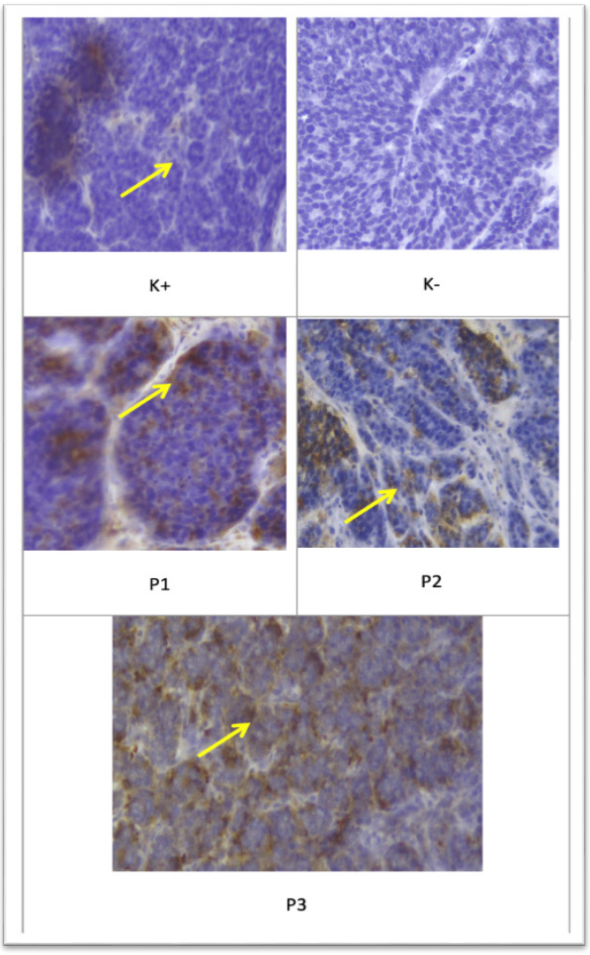

Figure 2. Immunohistochemical Staining of Granzyme Expression, Cells that Express Granzyme will Appear Darker. The darker cells increased gradually from the control group to the treatment group. It appears that the P3 group has a greater number of granzyme-expressing cells.

Tumor Diameter

The tumor diameter in group K+ was 12.93±2.03; in group K- was 17.11±3.28; in the treatment group P1 was 12.21±1.75; in the treatment group P2 was 12.15±2.81; and in the treatment group P3 was 10.55±2.33 is listed in Table 1. The tumor diameter data was found to be normally distributed (p>0.05), and the homogeneity test with the Levene test revealed that the data was homogenous (p=0.687). The LSD post hoc test showed that there were significant differences between the K+vsK- (p=0.008), K-vsP1 (p=0.002), K-vsP2 (p=0.002), and K-vsP3 (p=<0.001) is listed in Table 2.

Discussion

Breast cancer is a cancer that causes of high prevalence rate and high mortality rate among women. In the last few decades, several therapies such as surgery, radiotherapy, chemotherapy, hormonal therapy, and targeted therapy have been widely used. Heterogeneity in presentation and treatment outcomes has led to a multimodal and multidisciplinary approach involving the use of complementary therapies that are expected to improve patients's quality of life. Herbal medicines, medicines that use natural plants as basic ingredients, have shown promising results as anti-tumor and anti-cancer agents. Herbal medicines are known to have chemopreventive properties that can be prophylactic and therapeutic and

Table 2. Comparison of each Group for Granzyme Expression and Tumor Diameter

Variable	Group		p
Granzyme expression	K+	K–	0.359027778
		P1	0.028*
		P2	0.016*
		P3	<0.001*
	K–	P1	0.075
		P2	0.065
		P3	0.001*
	P1	P2	0.552777778
		P3	0.037*
	P2	P3	0.063
Tumor diameter	K+	K-	0.008*
		P1	0.431944444
		P2	0.411805556
		P3	0.077083333
	K-	P1	0.002*
		P2	0.002*
		P3	<0.001*
	P1	P2	0.671527778
		P3	0.180555556
	P2	P3	0.192361111

Description: *Significant (p<0.05)

are safe for long-term use [13].

Turmeric (Curcuma longa) is a plant belonging to the Zingiberaceae family. This plant has the main active ingredient in the form of curcuminoids. Curcuminoids are natural polyphenolic compounds, and there are three types, namely diferuloylmethane (curcumin I), desmetoxy curcumin (curcumin II), and bisdemethoxycurcumin (curcumin III). The main curcuminoid is diferuloylmethane which has the highest concentration (77%) and gives turmeric its yellow color. Turmeric also contains sugar, resin, protein, and three main essential oils (zingiberene, tumerone and atlatone) which have pharmacological activity [13].

Curcumin is a chemopreventive agent. Curcumin has antioxidant, anti-inflammatory, antiseptic, and anticancer properties. The role of curcumin as an anticancer agent targets several biological pathways and processes, including mutagenesis, oncogene expression, angiogenesis, metastasis, apoptosis, and autophagy. Curcumin is known to cause inhibition of cell proliferation, initiation of cell cycle arrest in the G2/M phase, upregulation of TIMP 1 and 4 expression, suppression of the FABP5/PPARβ/δ pathway, inactivation of the Akt/mTOR pathway, and EGFR/PEGFR pathway signaling [12]. The combination of curcumin with conventional cancer therapy, such as chemotherapy or radiation, has been proven to increase the effectiveness of cancer cell sensitivity to cytocidal effects and reduce treatment-related side effects [14].

Curcuma longa also affects the pathological characteristics of breast cancer, in a study of breast cancer induced by polycyclic aromatic hydrocarbons (PAH) namely benzo (a) pyrene showed an abnormal microscopic picture, namely the discovery of early signs of growth and development towards malignant tumors in the form of hyperplasia of cuboidal epithelial cells lining the walls of the lactiferous ducts accompanied by a picture of rough nuclear chromatin so that the duct walls appear thicker (> 4 cell layers) and darker in color. PMN inflammatory cells were also found spreading around the connective tissue. Changes in the appearance of the mouse breast can occur due to benzo (α) pyrene which is induced and then becomes active in the form of an epoxide reaction after being metabolized in the body. Different results show that giving curcuma longa on the pathological picture of breast cancer in mice. Microscopically, the breasts of mice in this group still showed PMN inflammatory cells in the surrounding connective tissue, but the number of layers of cuboidal epithelial cells with coarse nuclear chromatin surrounding the lactiferous ducts was reduced (2-3 layers) compared to that seen in the group of mice not given turmeric extract after being induced by benzo(α) pyrene. This indicates inhibition of cell proliferation and induction of elimination of damaged/abnormal cells (apoptosis) [15].

Granzyme Expression

Granzyme is a serine protein that is naturally expressed by cytotoxic T-lymphocytes and NK cells. There are 5 types of granzyme in humans; granzyme A, granzyme B, granzyme H, granzyme K, and granzyme K. Granzyme plays a role in modulating various cellular pathways, namely inducing apoptosis in the cleavage of intracellular substrates [16]. The apoptosis mechanism is through two main pathways, through the introduction of a series of proteases or granzymes into the cytosol of target cells or through a TNF-dependent apoptosis mechanism [17]. In breast cancer immunotherapy, the role of granzyme in the tumor microenvironment is also important. Granzyme has also been reported as a prognostic marker for cancer. Research with the title "Granzyme B is correlated with clinical outcome after PD-1 blockade in patients with stage IV non-small-cell lung cancer" by Hurkmans et al. [18] stated that lower granzyme levels were found in patients with metastatic cancer, indicating that the immune microenvironment of the tumor supports tumor growth by inhibiting the antitumor response of cytotoxic immune cells [19].

In this study, an intervention was carried out in the form of administering a combination of *C. longa* extract and chemotherapy to find out whether *C. longa* extract could be used as a complementary therapy for breast cancer chemotherapy. Curcumin is known to perform autophagy and induce apoptosis in MCF-7 breast cancer cells by downregulating the Bcl-2 signaling cascade and blocking the PI3K/Akt signaling pathway [13]. This study showed that granzyme expression was found to be higher in the group given *C. longa* extract compared to the negative control group. The groups given the intervention, namely groups P1, P2, and P3, had the highest granzyme expression in group P3, followed by P2, and P1. This shows that the combination of Adriamycin cyclophosphamide and *C. longa* extract has a positive effect on mammary carcinoma, especially at a dose of 200mg/kgBW *C. longa* extract.

Liu et al, in their research on the role of curcumin in neck and head cancer, stated that the administration of curcumin increased the ability of effector T-cells to kill cancer cells [20]. The study also stated that the combination of immunotherapy and curcumin caused an increase in granzyme secretion. Bhattacharyya et al. [2] researched curcumin on T-cell mediated adaptive immunity in tumor-bearing hosts. This study measured granzyme and perforin levels in CD8. Higher intracellular granzyme levels were obtained in the group with curcumin intervention. The results showed that curcumin not only prevented tumor-induced loss of effector T cell populations, but also contributed to immune-mediated killing of tumor cells [21].

Tumor Diameter

The prognosis of breast cancer depends on patient and tumor characteristics, including tumor size, largest diameter on TNM staging, nodules, and metastases. Tumor size and diameter are known to have a linear correlation with patient survival [22]. Tumor size is directly related to an increased likelihood of regional metastasis, an increased mean number of involved axillary lymph nodes, and an increased likelihood of recurrence and death. Lymph node status and primary tumor size were independent influences on the survival of 24,740 breast cancer patients. These two variables identify prognostic

groups with five-year relative survival rates from 99.2 to 45.5 percent [9].

In addition to tumor diameter and granzyme expression, pathological differences were also observed among groups receiving different doses of Curcuma longa extract compared to the control groups. In the negative (K−) and positive (K+) control groups, histopathological examination revealed densely packed tumor cells with high nuclear pleomorphism and minimal inflammatory infiltration. These features indicated aggressive tumor growth with limited immune-mediated cytotoxicity, supported by low granzyme expression. Conversely, in the treatment groups (P1, P2, and P3), especially those receiving higher doses of *C. longa*, there were marked improvements in pathological features. Microscopic evaluation showed reduced cellular atypia, increased apoptotic features such as chromatin condensation and nuclear fragmentation, and elevated granzyme expression. The P3 group (200 mg/kgBW) exhibited the most prominent immune cell infiltration, suggesting enhanced immunomodulatory activity. These findings support a dose-dependent pathological improvement, consistent with *C. longa*'s known anti-inflammatory, antiproliferative, and pro-apoptotic effects.

Furthermore, the smaller tumor diameter observed after administration of curcumin is strongly associated with its antiproliferative properties. The largest tumor diameter was noted in the positive control group, while the smallest was found in the P3 treatment group, which received Adriamycin cyclophosphamide in combination with *C. longa* extract at 200 mg/kgBW. Curcumin's antiproliferative mechanisms are multifaceted, involving induction of cell cycle arrest and p53-dependent apoptosis, alteration of key signaling proteins such as Ras, PI3K, protein kinase B, mTOR, and Wnt/β-catenin, as well as the downregulation of transcription factors. These molecular actions contribute to the inhibition of tumor growth, suppression of angiogenesis, and overall tumor regression. The combination of chemotherapy and high-dose Curcuma longa extract thus demonstrates a synergistic effect, not only by reducing tumor mass but also by enhancing histopathological and immunological responses.

Studies on experimental animals that were induced by ovarian cancer and intervention in the form of administering curcumin and a combination of curcumin with docetaxel showed that curcumin could reduce tumor growth. In this study, it was found that intervention with curcumin induced an average reduction in tumor growth of 49-55% compared to control animals, while the combination of curcumin with docetaxel resulted in an average reduction in tumor growth of 77% compared to controls [9].

Mansouri et al in their research on the clinical effects of curcumin as an adjuvant for cancer therapy, it was stated that curcumin has preventive and therapeutic effects for various types of cancer [7]. Curcumin can prevent and reduce the formation or spread of tumors. Curcumin inhibits the proliferation of various cancer cells by reducing the modulation of antiapoptotic gene products, activating caspases, and upregulating cancer suppressor genes. These mechanisms are related to the size of the tumor diameter [23]. Another study conducted by Liu et al. [20] showed that in the treatment group treated with curcumin there was significant inhibitory effect on tumor growth [18]. The results of their study are in accordance with this study, where a lower tumor diameter was found in the treatment group compared to the negative control group.

Recent clinical studies have underscored the significance of pCR. For instance, a study by wang et al. [24, 25] demonstrated that specific MRI-based tumor shrinkage patterns after early neoadjuvant therapy are predictive of pCR, highlighting the nuanced relationship between imaging findings and pathological outcomes. Furthermore, research in Gentile et al indicated that achieving pCR is associated with significant improvements in disease-free and overall survival, emphasizing its prognostic value.

In our preclinical model, direct assessment of pCR was not feasible due to methodological constraints. However, the observed increase in granzyme expression, particularly in higher-dose Curcuma longa extract groups, suggests enhanced cytotoxic immune activity, which may parallel mechanisms leading to pCR in clinical settings. Future studies should aim to incorporate comprehensive histopathological evaluations, including assessments of residual viable tumor cells, to align preclinical findings more closely with clinical endpoints like pCR.

In conclusion, the results of this study show granzyme expression in mammary adenocarcinoma mice models with Adriamycin cyclophosphamide chemotherapy and turmeric extract (Curcuma longa) was higher than the negative control group, while the tumor diameter was lower than the negative and positive control groups.

Author Contribution Statement

SB, BEN, YWP, NS, LA, and EM conceived the study idea and contributed to its overall conceptualization. Study design was conducted collaboratively by SB, BEN, YWP, MM, LA, and SPP. The intellectual content was defined and developed by SB, BEN, NS, EM, and SPP. All authors participated in the literature search. Clinical trials and experimental procedures were carried out by SB, BEN, YWP, NS, and EM. Data acquisition was performed by SB, NS, and LA. Data analysis was conducted by SB, BEN, YWP, and NS, with statistical analysis completed by SB, BEN, EM, MM, LA, and SPP. Manuscript drafting and editing were completed by SB, BEN, EM, MM, LA, and SPP. All authors reviewed and approved the final manuscript and accept responsibility for the integrity and content of the work.

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General

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throughout the project.

Study Registration

The experiment was approved by the Research and Ethics Committee of the Faculty of Medicine Diponegoro University, Indonesia (protocol number: 133/EC-H/KEPK/FK-UNDIP/XI/2023), and explains that there are no issues.

Conflict of Interest

All authors declare no conflicts of interest.

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