RESEARCH ARTICLE

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Inhibitory Effect of Melatonin on the Proliferation of Breast Cancer Cells MDA-MB-468 by Antioxidants Superoxide Dismutase and Glutathione Peroxidase

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Abstract

Objective: The purpose of this study was to determine the inhibitory effect of melatonin on breast cancer cells MDA-MB-468 by the antioxidant superoxide dismutase and glutathione peroxidase. **Methods:** This research was an in-vitro experimental study using MDA-MB-468 breast cancer cells from ATCC, treated with melatonin at concentrations of 1.5 mM, 2 mM, and 2.5 mM, with a 24-hour incubation. Cytotoxicity was examined using WST-8. SOD and GPx levels were measured using ELISA. Data analysis was performed using SPSS 27.0 with a one-way ANOVA parametric test, followed by post-hoc LSD analysis. **Result:** The IC_{50} of melatonin in MDA-MB-468 breast cancer cells was 1.9 mM. There was a significant increase in SOD antioxidant levels ($p = 0.000$) in the melatonin concentration groups of 1.5 mM, 2 mM, and 2.5 mM. An increase in GPx antioxidant levels was also observed at the same concentrations (1.5 mM, 2 mM, and 2.5 mM), but it was not statistically significant ($p > 0.05$). **Conclusion:** Melatonin at concentrations of 1.5 mM, 2 mM, and 2.5 mM acts as an antiproliferative agent in MDA-MB-468 cells by significantly increasing the antioxidant activity of SOD and increasing GPx activity, although not significantly.

Keywords: Glutathione peroxidase- Malondialdehyde- MDA-MB-468- Melatonin- superoxide dismutase

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Introduction

Breast cancer is the type of cancer with the highest incidence in women. It is estimated that the number of cases worldwide exceeds 1,000,000 per year. Based on the International Agency for Research on Cancer, it is estimated that in 2020, there will be 1.15 million cases of breast cancer, with more than 400,000 deaths in the world [1]. According to WHO, breast cancer makes up 16.7% of all cancer cases in Indonesia [2].

In breast cancer, reactive oxygen species (ROS) and oxidative stress are involved in Deoxyribonucleic acid (DNA) damage that can inhibit or induce transcription, signal transduction pathways, replication errors, genome instability, and oncogene activation [3]. There are several breast cancer risk factors associated with ROS induction, such as aging, menopause, genetic predisposition or estrogen, resulting in DNA damage, and chromosomal abnormalities that favor the development and progression of the disease [4]. Under conditions of excessive stress, the resulting ROS causes an imbalance between free radicals and antioxidant defenses also known as oxidative stress [5].

Biomarkers of oxidative stress have been observed to be associated with the development and progression of all types of cancer [6]. Carcinogenic compounds can contribute to increasing the formation of ROS in the body [7]. ROS can interact with biomolecules, including DNA, lipids, and proteins [8]. Reactive oxygen species and nitrogen species resulting from oxidative stress can damage the structure of biomolecules, including fats, proteins, and polynucleotides [9]. To protect against the overproduction of ROS, cells protect themselves by forming antioxidant compounds [10].

Several therapy strategies are being undertaken to overcome resistance in the treatment of breast cancer. However, chemotherapy treatment has side effects that limit the dose and duration of treatment. Previous research has reported that chemoresistance increases in early treatment of triple-negative breast cancer [11]. Therefore, the search for complementary therapies to chemotherapy to prevent chemoresistance and reduce chemotherapy side effects is an important area in cancer treatment research. One of the treatments that helps inhibit oxidative stress is the provision of antioxidants.

The administration of antioxidants has been widely

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used as an adjunct therapy in cancer patients [12]. One of these is melatonin. Melatonin is a nocturnally released pineal hormone and has attracted attention for its pleiotropic role. The hormone melatonin is essential for counteracting free radicals, which can induce the synthesis of antioxidant enzymes, and is involved in inhibiting tumor growth, angiogenesis, and metastasis in cancer [13, 14].

Melatonin can be found in various foods, including fruits and vegetables. Grao-Cruces et al. [15] report that related foods, such as tomatoes, olive oil, red wine, beer, nuts, and vegetables, contain high levels of melatonin. Data regarding research on melatonin on triple-negative breast cancer cells is available, but there is no literature that examines the effect of melatonin on MDA-MB-468 breast cancer cells [16]. The melatonin to be studied is melatonin purchased from Sigma. MDA-MB-468 breast cancer is a type of breast cancer cell with a triple-negative biomolecular profile (ER negative, PR negative, HER2 negative), where the therapy is only in the form of chemotherapy and is often resistant to chemotherapy.

The purpose of this study was to determine the inhibiting effect of melatonin on breast cancer cells MDA-MB-468 by antioxidant superoxide dismutase and glutathione peroxidase. The results of this study can make melatonin a supportive therapy in chemotherapy.

Materials and Methods

Research design

This research was an in vitro experimental study at YARSI University integrated laboratory using MDA-MB-468 breast cancer cells (ATCC HTB-132). MDA-MB-468 breast cancer is a type of breast cancer cell with a triple negative biomolecular profile. MDA-MB-468 cells were cultured using complete Dubelco's minimum essential medium (DMEM) (Gibco), Fetal Bovine Serum or FBS (Gibco), Antibiotic-Antimycotic (Gibco), and Insulin Transferrin Selenium or ITS (Gibco), and Melatonin (Sigma) (M5250). The research flow begins with MDA-MB-468 cell culture, followed by a cytotoxicity test to determine the IC_{50} of melatonin using WST-8. Based on IC_{50}, the concentration of melatonin to be tested is obtained. Furthermore, SOD and GPx examinations are carried out using the ELISA method. All experimental assays were conducted using cells derived from the same culture batch to ensure consistency across tests. The MTT assay and antioxidant enzyme assays (SOD and GPx) were performed sequentially using separate sets of wells prepared from the same passage and treatment conditions. All analyses were completed within the same 24-hour treatment period to minimize variability. All experiments were performed in triplicate and repeated independently at least three times using cells from different passages to ensure biological reproducibility and data reliability. This research was approved by Ethical clearance from the YARSI University research institute No: 127/KEP- UY/BIA/IV/2021.

Cell Culture

MCF-7 and MDA-MB-468 cells were routinely cultured in a tissue flask containing DMEM with 10% FBS, 5% penicillin/streptomycin, and 5% amphotericin at 370C in a humidified atmosphere with 5% CO2. The identity of the MDA-MB-468 cell line was verified by short tandem repeat (STR) profiling. All cultures were routinely tested for mycoplasma contamination using PCR-based detection to ensure cell line authenticity and purity.

The MDA-MB-468 breast cancer cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA), 1% penicillin-streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin), and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were used between passages 5 and 15 to ensure consistency and reproducibility across all experiments. To determine the effects of melatonin on cell proliferation, cells from stock plates were suspended by treatment with 0.25% trypsin, buffered with 0.2% EDTA (pH 7.3), and counted using a hemocytometer.

MTT Assay

Melatonin was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) and diluted with culture medium to obtain final concentrations of 1.5, 2.0, and 2.5 mM. The final concentration of DMSO in all groups did not exceed 0.1% (v/v). A solvent control group containing 0.1% DMSO without melatonin was included to evaluate the possible effect of the vehicle on cell viability and antioxidant enzyme activity. Cell viability was assessed using the MTT assay (Sigma-Aldrich, USA). Briefly, MDA-MB-468 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and treated with melatonin (1.5–2.5 mM) for 24 hours. After treatment, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours at 37 °C. The formazan crystals formed were dissolved in 100 µL DMSO, and absorbance was measured at 570 nm using a microplate reader (Bio-Rad, USA). Cell viability was expressed as a percentage relative to the untreated control. In the preliminary phase, melatonin concentrations ranging from 0 to 10 mM were initially screened to determine the half maximal inhibitory concentration (IC_{50}). Based on the viability curve, the concentrations of 1.5, 2.0, and 2.5 mM were selected for subsequent analyses of antioxidant enzyme activities (SOD and GPx), as they were within the range surrounding the IC_{50} value (1.9 mM) and produced measurable cellular responses without causing excessive cytotoxicity. Thus, the concentration range presented in the Results section corresponds to the effective doses identified from the initial screening. The IC_{50} value was determined using nonlinear regression (curve fitting) analysis from the dose–response curve generated in GraphPad Prism 9. All experiments were performed in triplicate and repeated three times independently to ensure reproducibility. The MTT assay protocol was validated through internal consistency and solvent control testing, confirming that DMSO at 0.1% had no cytotoxic effect on the cells.

Cytotoxicity examination

The cytotoxicity examination with (2-(2-methoxy-4-

nitrophenyl)-3-(4-nitrophenyl)-5-(2,4- disulfophenyl)-2H-tetrazolium, monosodium salt)/Cell counting kit-8 (WST-8). MDA-MB 468 cells were planted at 20,000 cells/well in a 96-well plate, then incubated for 24 hours at 37 °C and 5% CO2. Next, additional treatment with melatonin concentrations of 2.5 mM, 5 mM, 7.5 mM, and 10 mM in the same 96-well plate. As a negative control, the melatonin concentration was 0 mM. After incubation for 24 hours, 10 µl of CCK-8 was added to each well. Cells were incubated for 1 hour at 37° C. Next, the absorbance was read using a Benchmark Plus microplate spectrophotometer at a wavelength of 450 nm. IC_{50} value was calculated, and the percentage of viable cells (C) in different treatments was determined using Eq. 1.

$$C\ (\%) = (At/Ac)100 \quad (1)$$

where at is the mean absorbance of treated cells and Ac is mean absorbance of control cells. There is no melatonin IC_{50} data on MDA-MB-468 cells as a reference.

SOD examination

The MDA-MB-468 breast cancer cells were planted in 24-well plates at 40,000 cells/well. Then the cells were treated with melatonin concentrations of 0 mM, 2.5 mM, 5 mM, 7.5 mM, and 10 mM. Next, incubate in a 5% CO2 incubator, temperature 37°C for 24 hours. The activity of antioxidant enzyme SOD from Elabscience (E-BC-K019-S) was measured by the ELISA method. The absorbance was measured with the colorimetric method at 550 nm. Tests are made in triplicate and triplicate for each melatonin treatment, where one run is carried out 3 times for each melatonin concentration and refining is carried out 3 times on different days.

GPx examination

The MDA-MB-468 breast cancer cells were planted in 6-well plates at 90,000 cells/well, then the cells were treated with melatonin concentrations of 0 mM, 2.5 mM, 5 mM, 7.5 mM, and 10 mM. Next, incubate in a 5% CO2 incubator, temperature 37°C for 24 hours. The inspection is carried out in accordance with the protocol outlined in the datasheet GPx from Elabscience (E-BC-K096-M) using the ELISA method. Optical density was measured at a wavelength of 412 nm on a microplate reader (Tecan infinite M200). Tests are made in triplicate and triplicate for each melatonin treatment, where one run is carried out 3 times for each melatonin concentration and refining is carried out 3 times on different days. The SOD and GPx assays were validated according to the manufacturer's ELISA kit guidelines (Elabscience), including standard curve generation ($R^2 > 0.98$) and intra-assay reproducibility checks. Each concentration was measured in triplicate, and consistent results across independent runs confirmed assay reliability. Each assay included a positive control provided by the kit standards and a negative control without enzyme substrate to verify assay specificity and accuracy.

Statistical analysis

Statistical analysis to test the relationship between SOD and GPx with melatonin using SPSS 27.0 with the one-way Anova test followed by post hoc analysis of LSD. All experiments were performed in triplicate (n = 3) and repeated independently at least three times. Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS software version 27.0 (IBM, USA). One-way analysis of variance (ANOVA) was applied to compare the means among groups, followed by the least significant difference (LSD) post hoc test to determine intergroup differences. A p-value of less than 0.05 was considered statistically significant. Confidence intervals (95% CI) were calculated for all mean values and comparisons to provide estimates of the precision of the statistical results.

Limitation

This research was limited to antioxidant testing only. No examination of pro-oxidants and the mechanism of melatonin in inhibiting cell proliferation was carried out

Results

The solvent control group (0.1% DMSO) showed no significant difference in cell viability or antioxidant enzyme levels (SOD and GPx) compared with the untreated control group ($p > 0.05$), indicating that DMSO at this concentration had no cytotoxic or antioxidant-modulating effects.

Cytotoxicity tests were conducted to determine the optimal melatonin (IC_{50}) concentration for use in this study. The concentration used consists of 3 concentrations, which refer to concentrations around IC_{50}, below IC_{50} and above IC_{50}. The results of the cytotoxicity test were calculated by determining the percentage of cell proliferation inhibition, as well as the concentration of melatonin required to inhibit 50% of cell proliferation (IC_{50}) (Table 1). The percentage of dead cells for each concentration is presented as mean ± standard deviation (SD) from three independent experiments. Statistical analysis was performed using one-way ANOVA followed by LSD post hoc test ($p < 0.05$). The data are also visualized in graphical form to facilitate comparison between treatment groups.

In Table 1, it can be seen that the number of dead cells at a melatonin concentration of 2.5 mM is 61.35%. Based on the trendline formula, the IC_{50}% is obtained at a melatonin concentration of 1.9 mM. Based on the IC_{50} results, the MDA-MB-468 cell research will be given melatonin concentration groups of 1.5 mM, 2 mM, and 2.5 mM. The concentration range of melatonin (1.5–2.5 mM) used in this study was selected based on previous in vitro research in breast cancer cell lines that demonstrated effective antiproliferative activity within the millimolar range. Although these concentrations are higher than physiological levels, in vitro experiments often require higher doses due to the absence of metabolic degradation, plasma binding, and enzymatic conversion that occur in vivo. Therefore, the range applied in this study is within a validated experimental window for assessing melatonin's cellular effects.

After treatment, changes in the morphology of

MDA-MB-468 cells were seen (Figure 1). In addition to qualitative morphological observations, quantitative analysis of cell viability was performed based on the MTT assay results to support the visual findings presented in Figure 1. It was observed that the decrease in viable MDA-MB-468 cancer cells was inversely proportional to the melatonin concentration, indicating that the higher the melatonin concentration, the lower the number of viable MDA-MB-468 cancer cells.

Examination of Superoxide Dismutase (SOD) Antioxidant level

The results of statistical tests show that the higher the concentration of melatonin, the higher the SOD level significantly (p=0.000) (Figure 2). It appears that the higher the concentration of melatonin, the higher the SOD level in MDA-MB-468 breast cancer cells (p=0,005).

Examination of Glutathione Peroxidase (GPx) Antioxidant Level

The results of statistical tests show that the higher the concentration of melatonin, the higher the GPx level although not significantly (p=0.067) (Figure 3). It appears that the higher the concentration of melatonin, the higher the GPx level in MDA-MB-468 breast cancer cells; however, this difference is not statistically significant (p = 0.067).

Discussion

At a melatonin concentration of 1.5 mM, the cells still appear to be alive, as evidenced by their basal-like morphology and the presence of minimal debris around them. This study demonstrated that melatonin inhibited the proliferation of MDA-MB-468 cells in a dose-dependent manner. Although the IC_{50} concentration of melatonin observed in this study (1.9 mM) appears high compared to physiological concentrations in humans, this finding aligns with previous in vitro studies that required millimolar concentrations to achieve similar antiproliferative effects. In cell culture systems, high concentrations are necessary due to the absence of

Table 1. Inhibition of Melatonin Proliferation in MDA-MB-468 Cells

	2.5 mM	5 mM	7,5 mM	10 mM
Dead cells (%)	61.35	68.27	76.73	76.45

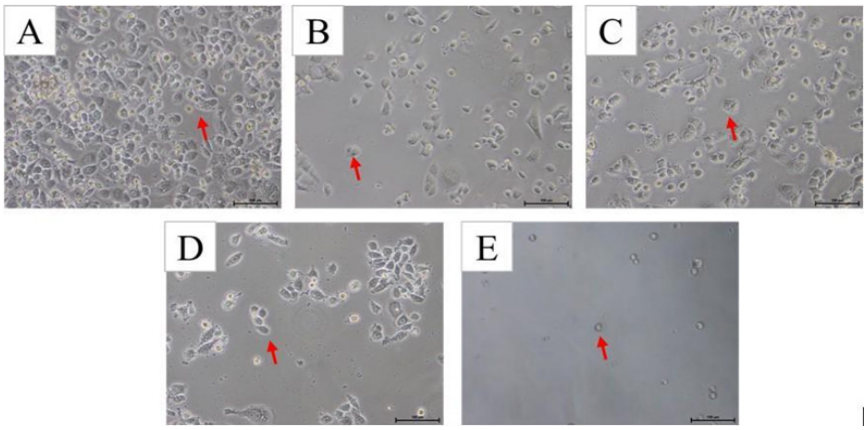

Figure 1. MDA-MB-468 Cells after 24-hour Melatonin Administration (Light Microscope, 10x). (a) control groups (Melatonin concentration 0 mM); (b) solvent control group; (C) Melatonin Group Concentration 1.5 mM; (D) Melatonin group concentration of 2 mM; (E) Melatonin group concentration 2.5 mM.

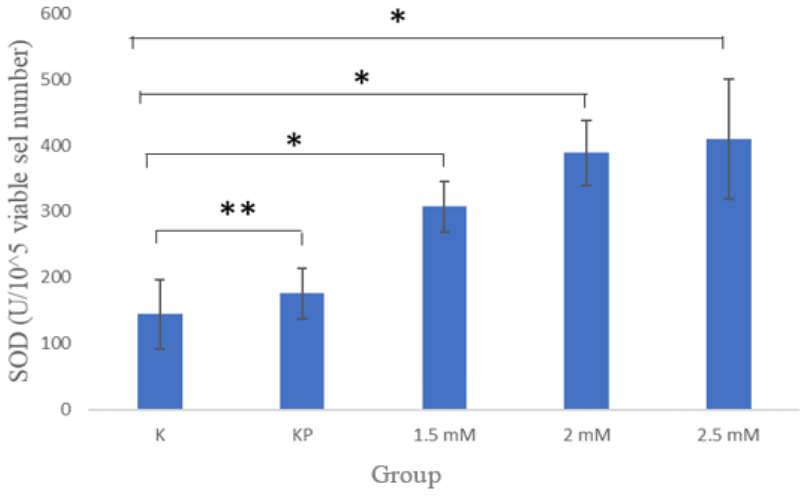

Figure 2. SOD Test Results on MDA-MB-468 Cells Given Melatonin

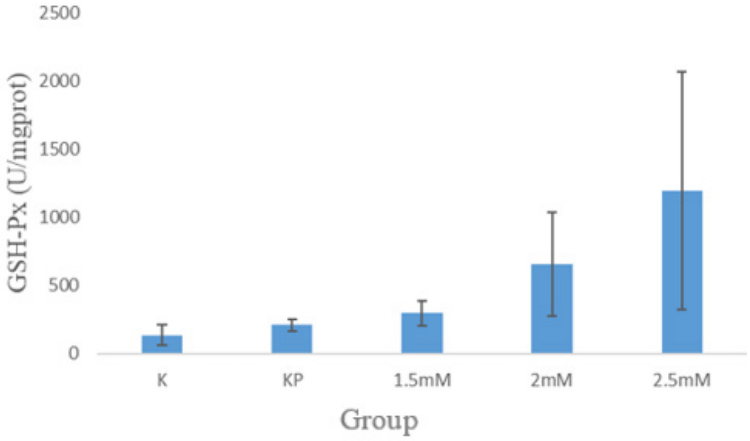

Figure 3. GPx Test Results on MDA-MB-468 Cells Given Melatonin

metabolism, plasma protein binding, and melatonin's short half-life. Previous research on breast cancer cell lines, such as MDA-MB-231 and MCF-7, has also reported effective concentrations ranging from 1 to 2.5 mM [17, 18]. Therefore, the concentration used in this study remains within an acceptable experimental range for mechanistic evaluation. Although the concentrations used in this study are relatively high compared to physiological melatonin levels in humans, such doses are commonly required in in vitro systems because of the absence of metabolic degradation, serum protein binding, and enzymatic conversion. In clinical settings, melatonin is typically administered at lower doses (1–20 mg/day), resulting in plasma concentrations in the micromolar range. Therefore, the present findings primarily describe cellular mechanisms rather than direct clinical effects. These results, however, may provide a basis for future in vivo and clinical studies to determine therapeutic concentrations relevant to breast cancer treatment. At a melatonin concentration of 2 mM, cells still appeared alive, but their amount was reduced by surrounding debris, and 2.5 mM cells appeared to be less likely to die, with fewer living cells compared to both the Control and Solvent Control groups (Figure 1). In that picture, it can be seen that the number of living MDA-MB-468 cells decreased, with the lowest number observed in the 2.5 mM melatonin concentration group.

Cytotoxicity Test

The IC_{50} of melatonin in MDA-MB-468 cells in this study was 1.9 mM (Table 1) Melatonin inhibited the proliferation of MDA-MB-468 cells in a dose-dependent manner The concentration range of melatonin used in this study (1.5–2.5 mM) was selected based on previous in vitro research showing that relatively high concentrations are required to achieve measurable antiproliferative and antioxidant effects in breast cancer cell models. Although these concentrations are above the physiological levels observed in plasma (nanomolar to micromolar), they are commonly used in cell culture systems where melatonin's short half-life, lack of metabolic conversion, and absence of serum-binding proteins reduce its bioavailability. Similar effective ranges (1–3 mM) have been reported in MDA-MB-231, MCF-7, and T47D breast cancer cells [17-19]. Therefore, the 1.5–2.5 mM range applied in this experiment represents an optimal in vitro window to assess melatonin's mechanistic impact on oxidative stress and proliferation inhibition. There is no literature that presents morphological images of MDA-MB-468 cells treated with melatonin. This happened due to more research on MCF-7 breast cancer cells (Luminal A; ER+, PR+, HER2-), and MDA-MB-231 cells (mesenchymal-like; ER-, PR-, HER2-). IC_{50} of melatonin for MCF- 7 cells was 4.52 mM [20]. Meanwhile, there is no research on melatonin in MDA-MB-468 cells (Basal- like; ER negative, PR negative, HER2 negative).

Superoksidase Dismutase (SOD) Antioxidant level

In the SOD level test, a significant increase in SOD levels was observed in MDA-MB-468 cells at melatonin concentrations of 1.5 mM (307), 2 mM (388,26), and 2.5 mM (409,42) (Figure 2). This research is in line with the research of Shi et al. [21], who reported that exogenous melatonin can increase SOD enzyme activity, reduce abiotic stress-induced ROS accumulation, and oxidative damage . The increase in SOD levels after melatonin treatment indicates that melatonin may reduce oxidative stress and protect cells from ROS-induced damage. This antioxidant effect may contribute to its ability to inhibit the growth of cancer cells. Besides its antioxidant action, melatonin may also show anticancer activity through other mechanisms, such as inducing apoptosis, regulating the p53 and Bcl- 2 pathways, and inhibiting NF-κB activation. These alternative pathways may explain melatonin's anticancer effects even when direct antioxidant tests were not performed. In addition to these mechanisms, several studies have reported that melatonin exerts anticancer activity through other molecular pathways. Melatonin has been shown to downregulate TRPC6 channel expression, leading to reduced calcium influx and inhibition of cancer cell proliferation and migration. It also modulates the circadian rhythm by influencing clock genes such as BMAL1 and PER2, which are involved in tumor suppression and cellular homeostasis. Furthermore,

melatonin can induce epigenetic modifications, including changes in DNA methylation and histone acetylation, that regulate gene expression linked to cell cycle control and apoptosis. These multifaceted mechanisms highlight melatonin's potential as a pleiotropic anticancer agent.

SOD levels in this study increased significantly when compared to controls (0 mM (144,65)) p=0.005. This indicates that low-dose absolute ethanol and short-term administration do not affect MDA-MB- 468 cells [18]. These results are in line with research reporting that MCF-7 breast cancer cells given ethanol long-term (more than 1 week), at a dose of 2 mM, exert a malignant effect on these cancer cells [22].

Elevated SOD levels can occur through pathways that rely on the protein prion cellular (PrPC). Normal PrPC is a ubiquitous glycoprotein involved in a variety of physiological cellular processes, including proliferation, differentiation, stress protection, and signal transduction regulation. PrPC also protects cells against oxidative stress by increasing the activity of antioxidant enzymes such as SOD and catalase, whereas low PrPC activity decreases the activity of these antioxidants [23]. In the study by Lee et al. [24], it was reported that increased PrPc activity leads to increased antioxidant activity and higher SOD levels and catalase activity. However, research by Florido et al. [23] reports that the higher the melatonin levels, the lower the SOD levels. This result is consistent with Florida et al. (2022), who reported that melatonin enhanced antioxidant enzyme activity in breast cancer models. The findings of this study are supported by Florida et al. [24], who demonstrated that melatonin enhanced the activity of antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, thereby reducing oxidative stress and suppressing tumor cell proliferation. This agreement suggests that melatonin's antioxidant mechanism is consistent across different breast cancer models.

Glutation Peroksidase (GPx) Antioxidant level

The GPx level test in this study showed an increase in GPx levels at melatonin concentrations of 1.5 mM (296.8), 2 mM (657.43), and 2.5 mM (1192.93) (Figure 3). GPx levels in this study increased but were not significant when compared to controls (0 mM (134.81), p=0.067). The results of this study differ from those of Chen et al. [25], Huang et al. [26], and Gelfand et al. [27], who reported that exogenous melatonin significantly increases GPx activity. The results indicate that melatonin administration at concentrations above the IC_{50} increases both SOD and GPx activity, leading to an imbalance where antioxidants dominate over free radicals, resulting in a reduction in viable MDA-MB-468 cells. This suggests that melatonin enhances the activity of antioxidant enzymes, thereby contributing to its antiproliferative effect.

This study focused on SOD and GPx as representative antioxidant enzymes that play central roles in protecting cells from oxidative stress. Although other assays such as catalase, malondialdehyde (MDA), reactive oxygen species (ROS), and apoptosis markers (e.g., caspase-3 activity) were not performed, SOD and GPx were selected as primary indicators of melatonin-induced redox modulation. These enzymes reliably reflect the antioxidant response as shown in prior studies [18, 24]. Future research should include additional oxidative and apoptotic markers to further elucidate the anticancer mechanisms of melatonin.

This study demonstrated the inhibitory and antioxidant effects of melatonin on MDA-MB-468 breast cancer cells. These findings are limited to in vitro observations but provide preliminary evidence of melatonin's potential as an adjunct or complementary agent. Further studies involving in vivo and clinical models are required to confirm its therapeutic applicability. Previous studies have also indicated melatonin's ability to enhance chemotherapy response and modulate redox balance in breast cancer cells [18, 27, 28].

This study did not include a direct comparison between melatonin and standard chemotherapeutic agents such as doxorubicin. Future research should incorporate combination models (e.g., melatonin with doxorubicin or paclitaxel) to assess possible synergistic or adjuvant effects. Several reports have suggested that melatonin can enhance the efficacy of chemotherapy while reducing oxidative toxicity [27-29]. Therefore, this limitation should be considered when interpreting the current results and designing future experiments.

The present study evaluated melatonin's effects after 24 hours of treatment; thus, temporal variations in antioxidant enzyme activity and cell viability were not assessed. Future studies with multiple time points are needed to determine whether melatonin's effects are time-dependent and to gain a better understanding of its dynamic cellular responses.

In conclusion, melatonin exhibits significant antiproliferative effects in MDA-MB-468 breast cancer cells by enhancing SOD activity and increasing GPx levels, although the changes are not statistically significant. Melatonin showed antioxidant and antiproliferative effects on breast cancer cells in vitro,

suggesting its potential as a complementary therapeutic candidate. However, further investigations using in vivo and clinical models are required to validate these preliminary findings. The findings of this study suggest that melatonin has potential as an adjuvant therapeutic agent in breast cancer, as it enhances antioxidant defense and inhibits cancer cell proliferation. Although further validation in clinical settings is needed, these results provide a scientific basis for considering melatonin as a complementary approach in breast cancer management.

Recommendation

Future studies are also recommended to explore melatonin using lower and more clinically relevant concentration ranges to better reflect physiological conditions. In vivo studies are also necessary to evaluate the therapeutic potential of melatonin in various breast cancer models.

Author Contribution Statement

Bevita Bevita: Conceptualization, Methodology, Data curation, Investigation, Writing- Original draft preparation. Nunung Ainur Rahmah: Conceptualization, Methodology,

Data curation, Investigation, Writing- Original draft preparation. Insan Sosiawan Tunru: Visualization, Writing- Reviewing and Editing. Harliansyah: Validation, Writing- Reviewing and Editing. Fatimah Eliana: Validation, Writing- Reviewing and Editing.

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Ethical Declaration

This research was approved by Ethical clearance from the YARSI University research institute No: 127/KEP-UY/BIA/IV/2021

Conflict of Interest

The authors declare no conflict of interest.

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