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Anti Cancer Potential

RESEARCH ARTICLE

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Anti-Tumor and Apoptotic Potential of 4-hydroxy-2-Methylbenzothiazole on MDA-MB-231 Cell Line

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Abstract

Breast carcinoma is a prevalent and life-threatening disease in women, requiring early detection for effective treatment. Its development is influenced by genetic, hormonal, and lifestyle factors. The MDA-MB-231 cell line is widely used to investigate breast cancer biology and therapy. 4-Hydroxy-2-methylbenzothiazole has demonstrated promising cellular effects, including antioxidant, anti-proliferative, and pro-apoptotic activities. The present research assessed the apoptotic, anti-proliferative, and antioxidant effects of 4-hydroxy-2-methylbenzothiazole in MDA-MB-231 cells. The study was categorized into an untreated group and three experimental groups treated with different doses of 4-hydroxy-2-methylbenzothiazole. Cell viability was evaluated using MTT, crystal violet, and trypan blue assays. Angiogenesis and apoptosis were measured by ELISA of VEGF and p53, while antioxidants were assessed by GSH and SOD levels. A 50 µg/ml dose of 4-hydroxy-2-methylbenzothiazole showed significant effects compared to the control groups. MTT, crystal violet, and trypan blue assays revealed that 4-hydroxy-2-methylbenzothiazole reduced the viability of MDA-MB-231 cells and promoted cell death. ELISA results further demonstrated a decrease in angiogenic potential, elevated p53 levels, and a reduction in SOD and GSH antioxidant levels. Additionally, scratch assay and DAPI/PI staining confirmed apoptosis. These findings suggest that 4-hydroxy-2-methylbenzothiazole exerts significant apoptotic and anti-proliferative effects on MDA-MB-231 breast cancer cells.

Keywords: 4-hydroxy-2-methylbenzothiazole- MDA-MB-231 cells- anti-tumor activity- apoptosis- cell viability

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Introduction

Breast carcinoma is the most prevalent and aggressive tumor among women globally [1]. One in eight women is expected to be diagnosed with breast cancer during her lifetime [2]. Early detection is crucial for reducing the rate of mortality and improving the survival rate. Enhancing clinical outcomes and decreasing the recurrence rate are the main goals of treatment [3]. Risk factors for breast cancer include age, family history, hormonal influences, various epigenetic and environmental factors [4]. Genetic mutations like *BRCA1* and *BRCA2* in breast cancer significantly increase the incidence of syndromes like hereditary breast and ovarian carcinoma syndrome [5].

Among the various subtypes of breast cancer, triple-negative breast cancer (TNBC) is the most aggressive and most common tumor in reproductive-age women. Recent research indicates that TNBC has been linked to specific genetic types and mutations in genes responsible for DNA repair [6]. The MDA-MB-231 cell line, mimicking TNBC characteristics, is highly invasive, has high metastatic potential, and is resistant to conventional therapies. This cell line is specified by its lack of estrogen, progesterone, also *HER-2* receptors [7, 8].

Development of therapeutic resistance over time to recent breast therapy is a widely faced challenge. There is a critical demand for new and more effective agents capable of targeting tumor progression and overcoming resistance [9]. The most effective strategy involves targeting the apoptotic pathways in cancer cells [10].

Apoptosis is a crucial process that maintains cellular homeostasis by eliminating damaged or abnormal cells involving mitochondrial and extrinsic signaling

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pathways, and preventing tumorigenesis [11]. In cancer, dysregulations of this apoptotic pathway enable abnormal cells to become tumorigenic, which proliferate unregulated and hinder anticancer therapy by suppressing pro-apoptotic factors and promoting apoptotic inhibitors, so tumors become resistant to oncological treatment. Therefore, the restoration of apoptosis in tumor cells is a key strategy for therapeutic intervention [12, 13].

Generally, the tumor suppressor gene p53 is responsible for apoptosis in response to DNA damage, and when it gets mutated or non-functional, it leads to tumor proliferation and poor prognosis, with therapy resistance common in TNBC [14]. Meanwhile, p53 activation affects various transcriptional targets, promoting cell death and tumor inhibition [15]. Tumors with functional p53 respond better to cytotoxic therapies, like DNA-damaging drugs, and p53-mediated apoptosis contributes to tumor suppression and enhances treatment effectiveness [16].

Another critical process in cancer progression is angiogenesis. Vascular Endothelial growth factor [VEGF] is important for angiogenesis and is often overexpressed in tumors. Angiogenesis supports inflammation and also the progression of tumors and their metastasis. Autocrine VEGF reduces breast tumor chemokines, which hinders invasion and migration. Decrease in apoptosis is caused by reduced VEGF levels and decreased ability of tumor cells to metastasize [17].

Another important factor contributing to cancer progression and resistance to treatment is oxidative stress. Altered redox homeostasis causes increased production of reactive oxygen species ROS which damage the DNA. There is an imbalance between ROS production and antioxidant activity contributing to uncontrolled growth and resistance to therapy [18], thereby providing insight that cytotoxic drugs induce oxidative stress causing tumorous cell death via deprivation of GSH and SOD.

Among numerous compounds being researched for their anticancer potential explores thiazole derivatives are explored, having a vast range of biological activities, such as antimicrobial, anti-inflammatory, and anticancer effects [19]. One such compound, such as 4-hydroxy-2-methylbenzothiazole, featuring a thiazole scaffold, influences apoptosis and oxidative stress pathways. Given its high metastatic potential and therapy resistance, MDA-MB-231 is an ideal model for TNBC and testing new cancer therapies [19].

Latest studies have emphasized the importance of the apoptotic potential of thiazole derivatives in numerous cancer cell lines. These compounds induce anticancer effects by various mechanisms, like regulating pro-apoptotic and anti-apoptotic proteins, by caspase activation, and promoting oxidative stress [20]. H-2-methylbenzothiazole (4H2MBT), which has not been previously evaluated for its anticancer potential specifically against triple-negative breast cancer (TNBC). Unlike other thiazole derivatives studied primarily in hormone-positive or HER2+ subtypes, 4H2MBT exhibits distinct chemical features (such as the methyl substitution at position 2 and a non-substituted H at position 4 of the benzothiazole ring), which may enhance its lipophilicity and cellular permeability, making it particularly effective in TNBC cells that lack ER, PR, and HER2 targets.

Current research aimed to explore apoptotic, anti-proliferative, and anti-oxidative attributes of 4-hydroxy-2-methylbenzothiazole in MDA-MB-231 breast cancer cells.

Materials and Methods

Cell line sampling

MDA-MB-231 cells were taken from the cell culture lab of the Centre for Research in Molecular Medicine [CRiMM] and the Institute of Molecular Biology and Biotechnology [IMBB] established at the University of Lahore. This cell line was stored in cryotubes, maintained in a liquid nitrogen tank.

Cell cultivation

The cryovials obtained from liquid nitrogen cylinders were thawed. Then, the MDA-MB-231 cell line was grown in a culture flask containing DMEM-HG, with 10% [v/v] fetal bovine serum [FBS],100 mg/mL penicillin G, and 100 U/mL streptomycin. Cultures were maintained under aseptic conditions in a humidified cell incubator with 5% CO2 at 37°C [21].

Assays were executed in triplicate. When propagated, MDA-MB 231 cells attained 70-80% cell density, and their sub-culturing was carried out. Cells adherent to the walls of the culture flask were rinsed with phosphate-buffered saline (PBS) and incubated with 0.05% trypsin-EDTA until they detached from the surface of the culture flask. A few drops of FBS were added to the flask and thoroughly mixed. The combination was incorporated into a 15 ml tube and centrifuged at 2000 rpm for 5 minutes. After that supernatant was discarded, and the pellet was reintegrated into the culture medium [22].

Drug formulation

The drug 4-hydroxy-2-methylbenzothiazole was purchased and formulated at a concentration of 1mg/ml in plain media without FBS.

Cell line treatment using 4-hydroxy-2-methylbenzothiazole

For cell viability, MDA-MB-231 cells were cultivated in a 96-well plate, with 10,000 viable cells in each microplate well. Subsequently, the plate was incubated for 24 hours with 5% CO_2 at 37°C, and then the media was removed. The research work was categorized into: untreated, reference group treated using 50 µg/ml cisplatin, and three experimental groups treated with 4-hydroxy-2-methylbenzothiazole at levels of 10, 20, and 50 µg/ml, respectively. Cisplatin was used as a standard treatment drug to compare the effects of 4-hydroxy-2-methylbenzothiazole in MDA-MB-231 cells. All assays were performed in triplicate for 24 hours. 6-well microplates were used for the wound healing study. Cell viability assays

To analyze cell survival of the MDA-MB 231 cell line cultured in 96-well plates, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), trypan blue, and crystal violet assays were performed.

MTT assay

MTT assay was performed to evaluate the growth inhibition [5] of MDA-MB-231 cells by this drug. After incubating for 24 hours with different doses of 4-hydroxy-2-methylbenzothiazole, MDA-MB-231 cells were rinsed with phosphate buffer saline [PBS], then placed in an incubator with 100µl of Dulbecco's Modified Eagle Medium (DMEM) and 25µl MTT solution (Sigma) for 3-4 hrs. After 4 hours, formazan crystals were treated with 10% sodium dodecyl sulphate (SDS), and the absorbance was quantified at 570 nm [23].

Dead Cell Detection [Trypan Blue Assay]

Dead cell fraction and cell viability were evaluated by trypan blue, a marker for live and dead cells. The cell suspension from each group was incubated with trypan blue for 2-5 minutes. Thereafter, cells were rinsed with phosphate-buffered saline (PBS) and observed under an inverted microscope. Cells stained with trypan blue were considered dead, optical density at 570 nm [24].

Quantification [Crystal Violet Staining]

Cell viability was analyzed by crystal violet in treatment groups of the MDA-MB-231 cell line, which was performed on a 96-well plate. The secretome was discarded from the wells, and cells were washed with PBS. After that, 0.1% crystal violet dye in 2% ethanol was added to each well, ensuring that the surface was fully separated. Incubated the plate for 15 minutes at 37ºC. Then dye was removed, and the wells were washed to prevent the cells from dislodging. Then, 100 µL of 1% sodium dodecyl sulfate (SDS) was added to each well to solubilize the stain and left for 5-10 minutes. Finally, the absorbance at 595 nm was measured using by microtiter plate reader [25].

Enzyme-linked Immunosorbent Assay (ELISA)
The secretome and cell lysates of post-treated cells of the trial groups were

collected and analyzed for VEGF, p53, by performing solid-phase sandwich ELISA. The test was performed on a 96-well plate, coated with an antibody and incubated for 48 hours at 4°C, then rinsed with 1X TBS-T (washing solution). Then, 200µl of blocking solution was added to each plate well and incubated for 30 minutes. Then, removed blocking solution was removed, and 200 µL of secretomes from the different trial groups were added to each well. After 18 hours, the medium was discarded, and each well was rinsed. Then, 100 µL of HRP-conjugated secondary antibody was dispensed into each well, plate was placed in an incubator at 4°C for 12 hours. After that, the secondary antibody was removed, and the wells were again rinsed. Then, 100 µL of chromogenic solution 3,3',5,5; tetramethylbenzidine (TMB) was added and incubated for 20 minutes. Finally, 100 µL of stop solution was applied, and measured absorbance at 450 nm [26].

Evaluation of Antioxidative Enzymes
ELISA for Glutathione Reductase (GSH)

A 96-well plate with anti-GSH antibodies, incubated to allow antibodies to bind to the surface. After rinsing, standards are added and incubated. A secondary HRP-conjugated antibody is administered after infusing TMB (3,3',5,5'-Tetramethylbenzidine) substrate. The reaction was halted, and the absorbance at 450 nm was used to quantify GSH levels [26].

ELISA for Superoxide Dismutase (SOD)

A 96-well plate with anti-SOD antibodies, incubated for antibody binding. After washing, cell lysates were added and incubated for binding of SOD to antibodies. A secondary HRP-conjugated antibody is administered after adding TMB(3,3',5,5'-Tetramethylbenzidine) substrate. Then cessation of reaction and absorbance was calculated at 450 nm [27].

ELISA for p53

The ELISA kits were used to evaluate apoptotic markers by measuring p53 levels. Reagents, standard solutions, and samples were prepared at 37ºC. After adding 100 µL of samples to the pre-prepared micro-ELISA plate, the incubated for the recommended time and temperature as mentioned in the ELISA kit instructions. After that, a biotinylated detection antibody was added, and then, there HRP-conjugated secondary antibody was added. Rinsed the plate, TMB substrate reagent was added, and colour change was observed. The stop solution was used to stop the reaction, measured the optical density was measured at 450 nm [28].

DAPI and PI staining

DAPI and PI staining were performed to evaluate cell apoptosis [29]. For DAPI staining, cells were initially fixed with 4% paraformaldehyde for about 10 minutes at room temperature, then rinsed thrice with PBS to discard surplus fixative. Then, cells were stained with DAPI solution [1 µg/mL], placed in the dark for 10-15 minutes, then rinsed and observed under a fluorescence microscope [30]. PI staining, as described by [31], cells were stained with PI [5µg/ml], incubated for 20 min, then washed with PBS, and observed under a fluorescence microscope. For combined DAPI and PI staining, first, the cells were stabilized, then rinsed with PBS, and RNase was applied to eliminate RNA. After administering a combined solution of DAPI (1 µg/mL) and PI (5 µg/mL) for 10-15 minutes, the rinsed with PBS.

Scratch assay

A scratch wound assay was conducted to evaluate the cell proliferation and migration potential [5]. Create an artificial gap (wound) in a confluent cell monolayer on the base of a 6-well cell culture plate for both the experimental and control groups. To evaluate wound healing, the surface area of the wound in the pre-treated group was measured and compared to the healed treated area. Images of the wound were captured at 0, 48, and 72 hours. The wound closure rate was calculated by measuring the wound area covered by migrating cells and their healing abilities.

Statistical Analysis

All data from experimental groups were expressed as mean ± SEM. For statistical analysis, means were

compared by one-way ANOVA, and then Bonferroni's post hoc test to identify differences among groups. Quantitative data obtained were statistically analyzed using GraphPad software. A p-value of less than 0.05 was considered statistically significant.

Results

MTT Viability Assay

MTT assay was performed to assess cell survival in experimental groups [10 µg/ml, 20 µg/ml, 50 µg/ml] against breast cancer cell line MDA-MB-231 cells treated with 4-hydroxy-2-methylbenzothiazole. Cell viability was reduced in a dose-dependent manner; further, at the 50 µg/ml dose, a greater reduction in cell viability was observed in comparison to the untreated group (Table 1, Figure 1).

Crystal Violet Assay

Crystal violet staining was further performed to evaluate cell viability by live cell detection against MDA-MB-231 cells treated with 4-hydroxy-2-methylbenzothiazole. Cell viability was reduced in a dose-dependent manner; further, at the 50 µg/ml dose, increased reduction in cell viability was observed in comparison to the untreated group. At the 50 µg/ml dose, increased reduction in cell viability was observed compared to the untreated group (Table 2, Figure 2).

Trypan Blue Assay

Trypan blue staining was performed to assess the dead cell count in the experimental groups [10 µg/ml, 20 µg/ml, 50 µg/ml] of MDA-MB-231 cells treated with 4-hydroxy-2-methylbenzothiazole. At a dose of 50 µg/ml, a decline in cell viability was observed compared to the control groups (Table 3, Figure 3).

Evaluation of Antioxidative Enzymes

Glutathione Assay

The anti-oxidant, GSH, was evaluated in different experimental groups [10µg/ml, 20µg/ml, 50µg/ml] of MDA-MB-231 cells. At the dose of 50µg/ml of 4-hydroxy-2-methylbenzothiazole, there was a reduced level of GSH as compared to the control groups (Table 4, Figure 4).

Superoxide dismutase

The anti-oxidant, SOD was evaluated in different experimental groups [10µg/ml, 20µg/ml, 50µg/ml] of MDA-MB-231 cells. At the dose of 50µg/ml of 4 hydroxy-2-methyl benzothiazole, there was a reduced level of SOD as compared to control groups (Table 5, Figure 5).

p-53 by ELISA

The p-53 evaluation was conducted by ELISA to analyze apoptosis in different experimental groups [10µg/ml, 20µg/ml, 50µg/ml] of MDA-MB-231 cells. At the dose of 50µg/ml of 4-hydroxy-2-methylbenzothiazole, there was increased expression of p-53 in comparison to the control groups (Supplementary Figure 1).

VEGF by ELISA

The VEGF was performed to analyze angiogenesis in different experimental groups [10µg/ml, 20µg/ml, 50µg/

Table 1. MTT Mean Values by One-Way ANOVA & Multiple Comparison Tests

	Negative Control	Cisplatin [50 µg/ml]	Group 1 10µg/ml	Group 2 20µg/ml	Group 3 50µg/ml
Mean± Sd	0.96±0.03	0.55±0.03	0.72± 0.04	0.60± 0.01	0.47±0.008

Table 2. Crystal Violet Means Values by One-Way ANOVA & Multiple Comparison Tests

	Negative Control	Cisplatin [50 µg/ml]	Group 1 10µg/ml	Group 2 20µg/ml	Group 3 50µg/ml
Mean± Sd	1.17±0.04	0.61±0.06	0.83±0.04	0.64±0.03	0.51±0.02

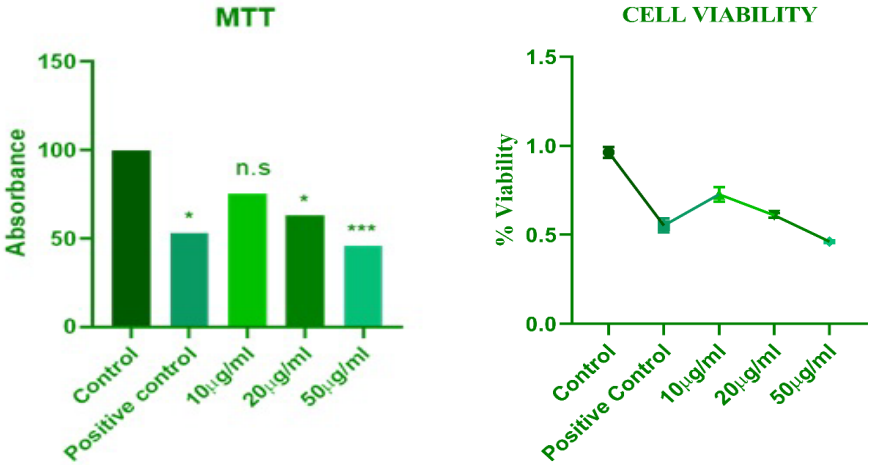

Figure 1. Reduction in Cell Survival of MDA-MB-231 Cells by the MTT Assay in Various Post-Treated Groups Relative to the Control Groups. Results were measured using one-way ANOVA and multiple comparison tests. P≤0.05. where *** represents a significant difference among groups.

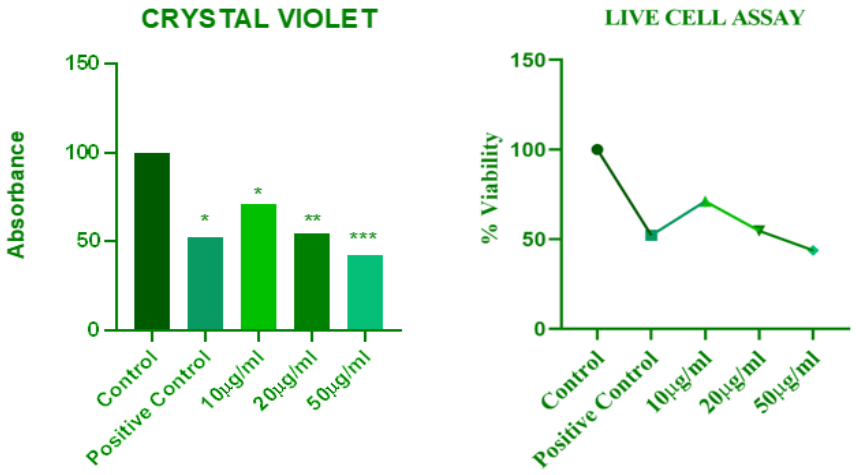

Figure 2. Reduction in Cell Viability of MDA-MB-231 Cells as Compared to the Control Groups. Results were measured using one-way ANOVA and multiple comparison tests. P≤0.05. where *** represents a significant difference among groups.

Table 3. Trypan Blue Mean Values by One Way ANOVA & Multiple Comparison Tests

	Negative Control	Cisplatin [50 µg/ml]	Group 1 10µg/ml	Group 2 20µg/ml	Group 3 50µg/ml
Mean± Sd	100.0±0.0	195 ±5.00	164.0±3.60	190.0±5.00	221.7±12.58

ml] of MDA-MB-231 cells. At the dose of 50µg/ml of 4-hydroxy-2-methylbenzothiazole, there was significantly decreased expression of VEGF in comparison to the control groups (Supplementary Figure 2).

DAPI and PI staining

Treated MDA-MB-231 cells with 4-hydroxy-2-methylbenzothiazole showed more dead cells in a dose-dependent manner (Supplementary Figure 3).

Scratch test

The results indicate that treatment with 4-hydroxy-2-methylbenzothiazole after the scratch inhibited cell growth and prevented wound healing compared to the control group. Findings demonstrated that non-treated cells were capable of regeneration, whereas treated cells with 4-hydroxy-2-methylbenzothiazole had decreased wound repair capacity (Supplementary Figure 4).

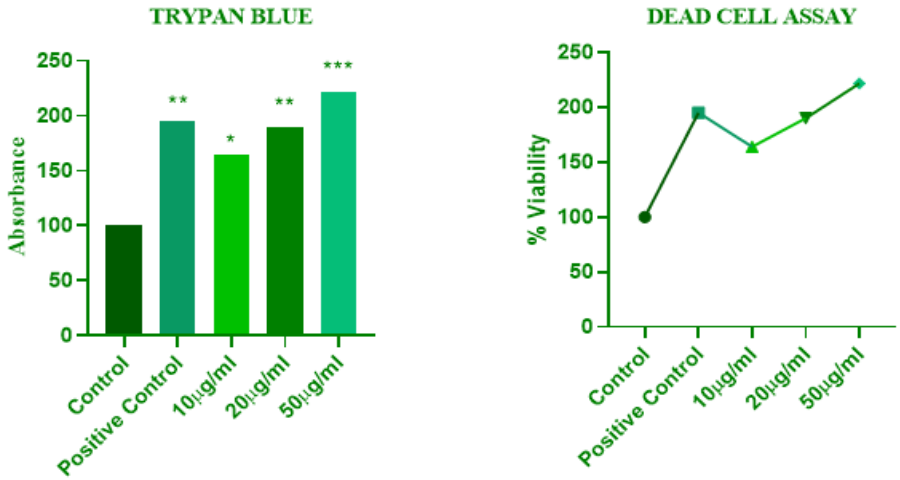

Figure 3. Cell Viability of MDA-MB231 Cells was Decreased in Different Treatment Groups. *,**,*** revealed a significant difference between the groups in comparison to untreated group. Results were measured using one-way ANOVA and multiple comparison tests. P≤0.05. where *** represents a significant difference among groups.

Table 4. GSH Mean Values by One Way ANOVA & Multiple Comparison Tests

	Negative Control	Cisplatin [50 µg/ml]	Group 1 10µg/ml	Group 2 20µg/ml	Group 3 50µg/ml
Mean± Sd	1.18±0.06	0.75±0.02	1.007±0.08	0.77 ±0.03	0.51±0.02

Table 5. SOD Mean Values by Using One Way ANOVA & Multiple Comparison Tests

	Negative Control	Cisplatin [50 µg/ml]	Group 1 10µg/ml	Group 2 20µg/ml	Group 3 50µg/ml
Mean± Sd	1.18±0.06	0.75±0.02	1.007±0.08	0.77 ±0.03	0.51±0.02

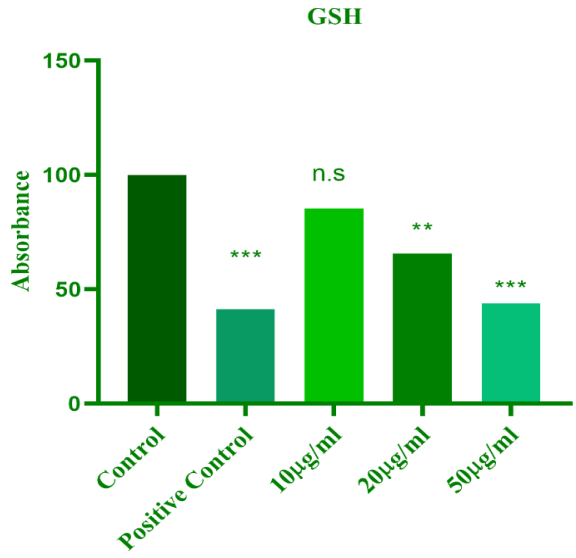

Figure 4: GSH is significantly decreased by ELISA in treatment groups compared to control group. Results were measured using one-way ANOVA and multiple comparison tests. P≤0.05. where *** represents a significant difference among groups.

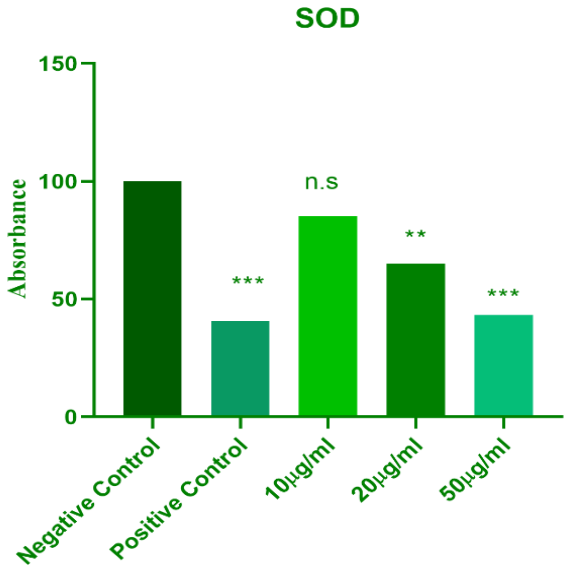

Figure 5. SOD is Significantly Decreased by ELISA in Treatment Groups Compared to Control Group. Results were measured using one-way ANOVA and multiple comparison tests. P≤0.05. where *** represents a significant difference among groups.

Discussion

Breast tumors are the most common cancer-related mortality and the leading malignancy in females. Currently, neoplasms are the primary cause of death and are expected to escalate over the next two decades. MDA-MB-231 is the most extensively researched breast tumor cell line globally, has a fundamental role in research, and influences cancer prognosis [19]. A thiazole derivative exhibits cytotoxic activity against tumors, including breast, colon, cervical, and ovarian cancer [12].

Research on thiazole has potential as an anti-glioma, demonstrating significantly enhanced cytotoxicity through antiproliferative and apoptotic induction in glial cells [13]. Tumor cells harbor mutations in both signaling pathways, which causes apoptosis. Targeting new approaches in apoptosis led to the discovery of new effective antitumor therapies [32].

Cell-based assays were used to evaluate cytotoxic effects, assess cell viability. It is an effective technique for measuring in vitro chemosensitivity and assessing drug efficacy in neoplasm cells [33]. In the current research, 4-hydroxy-2-methylbenzothiazole demonstrated significant inhibition of proliferation in MDA-MB-231 tumor cells. The IC50 values of 4-hydroxy-2-methylbenzothiazole were determined by MTT assay when the drug was used in doses of 10 µg/ml, 20 µg/ml, and 50 µg/ml to assess the limiting effect on cell viability. They exhibited anti-proliferative actions on MDA-MB-231 cells when compared to the control group, revealing cytotoxic effects of the drug.

The crystal violet assay evaluates the cell viability of live cells. Assessing cell viability in both treated and untreated groups confirms drug cytotoxicity. In this assay, dead cells no longer adhere to the walls of a multiwell plate [34]. This method is a standard protocol for assessing cell viability and growth inhibition while researching cytotoxic agents, such as 4-hydroxy-2-methylbenzothiazole, in MDA-MB-231 cells [35]. The research showed a decline in the viable cell count in the treated versus the untreated group.

A trypan blue assay was used to measure dead cells in the post-treated groups. Since their intact membranes prevent trypan blue from entering, live cells repel while dead cells uptake the dye, identifying dead cells [4]. In the recent research, an increased number of dead cells after treatment relative to the untreated group indicates that 4-hydroxy-2-methylbenzothiazole exerts a cytotoxic effect on MDA-MB-231 cells.

When antioxidants are used in conjunction with anti-proliferative therapies, they enhance the effectiveness of treatment by reducing ROS levels [36]. In our research, MDA-MB-231 cells treated with that drug revealed reduced the levels of SOD and GSH signifies that the drug induces oxidative stress or depletes cellular antioxidants. Decreased SOD levels make the cells more vulnerable to oxidative damage, ultimately leading to apoptosis.

Research on glutathione [GSH] in breast tumors has highlighted its role in cellular processes, particularly oxidative stress, apoptosis, and drug resistance. GSH

levels in breast tumors serve as potential biomarkers for diagnosing breast neoplasm and responses to therapy. GSH levels are often contributing to tumorigenesis and progression. Elevated oxidative stress in breast tissues results in lower GSH levels. This imbalance leads to increased DNA damage and promotes tumor development. Derivatives of benzothiazole revealed cytotoxic activity accompanied by decreased GSH activities, causing apoptosis as monitored by reduced protein synthesis and nucleic acids [37].

Angiogenesis is a complex process involving interactions between tumor cells and their environment, including adjacent endothelial cells, phagocytes, and various factors they secrete that can either promote or inhibit angiogenesis. Several studies have revealed that novel compounds play a crucial role in neoplasm treatment by suppressing tumor growth and halting both tumor spread and angiogenesis [38]. In numerous studies, angiogenesis suppression occurs by inhibiting VEGF, which blocks essential nutrients, and hindering tumor growth. As capillaries supply oxygen and essential nutrients for the growth of the tumor and its survival [39]. VEGF is important for angiogenesis and is often overexpressed in tumors [40]. Our research suggests that 4-hydroxy-2-methylbenzothiazole has anti-angiogenic potential as displayed by reduced VEGF levels.

The scratch assay is a wound-healing screening method for cell migration and invasion of tumor cells [41]. Making a "scratch" in a dense layer of cells and observing gap closure over time [42]. In this research, MDA-MB 231 cells of the control group closed the wound effectively through migration while the group treated with 4 hydroxy-2-methyl benzothiazole could not migrate, results in impaired/delayed wound healing, when images are captured at 0 & 24hrs which revealed that this drug is cytotoxic, helped in impairing cell migration, proliferation, or survival and involved in wound/tissue regeneration in vitro in MDA-MB 231 tumor cells.

During cell injury, p53 prevents cell differentiation, inhibits survival pathways, and halts the cell cycle, leading to cell death. It also regulates metabolic, autophagy, and cellular aging pathways [6, 22, 40]. Increased p53 levels after treatment with 4-hydroxy-2-methylbenzothiazole in MDA-MB-231 cells, compared to controls, suggest that this drug has a cellular stress response that activates p53, and apoptosis occurs in cancer.

Apoptosis and cell viability were further confirmed by DAPI and PI staining [5]. DAPI binds to DNA, giving blue colour to live cells, while PI only penetrates dead cells and gives red colour. In MDA-MB-231 cells, an increase in PI-positive cells with cytotoxic agents reflects compromised membrane integrity and cell death.

Thiazole derivatives exhibit an anti-tubercular role in drug-resistant strains [17], anti-tumor [3, 40]. Antiepileptic [26], diuretic, antidiarrheal, anti-allergic, analgesic, antiviral [42], antiretroviral [43], and platelet-activating factor antagonism [44]. In this research, treatment of MDA-MB-231 cells with 4-hydroxy-2-methylbenzothiazole resulted in reduced GSH levels in treated groups, suggesting that the drug induces oxidative stress or depletes antioxidant levels, more susceptible to oxidative damage, leading to apoptosis in MDA-MB-231 cells.

Cytotoxic drugs are effective in cancer therapy by suppressing tumor cell proliferation and triggering apoptosis, preventing angiogenesis, and tumor metastasis. These effects were specifically noted with 4-hydroxy-2-methylbenzothiazole in MDA-MB-231 breast tumor cells.

In this study, we evaluate the anti-tumor and growth-inhibitory properties of this compound by examining its apoptotic potential. Our findings highlight the promising potential of 4-hydroxy-2-methylbenzothiazole in inhibiting cell proliferation and angiogenesis while effectively triggering apoptotic pathways. This research could ultimately yield valuable understanding for the formulation of more focused and effective therapies that could enhance apoptosis and overcome the limitations of current treatment options, improving survival and outcomes for breast cancer patients. With the rise in breast tumor cases, identifying new anti-tumor agents is crucial.

In conclusion, 4-hydroxy-2-methylbenzothiazole at 50 µg/ml has been most effective in inhibiting proliferation in MDA-MB-231 cells by decreasing cell viability and increasing the oxidative stress levels in the cells, leading to apoptosis. Additionally, it enhanced suppression of MDA-MB-231 cell migration, decreased angiogenic potential compared to cisplatin.

Future Prospects

Future research should investigate how thiazole derivative 4-hydroxy-2-methylbenzothiazole may prove beneficial in TNBC. So, additional in vitro and in vivo investigations into its mechanism of action, potential drug interactions, and adverse effects are necessary to evaluate its potential for use in breast cancer treatment in the future. Ultimately, the aim is to introduce 4-hydroxy-2-methylbenzothiazole into clinical practice to enhance outcomes for patients with breast tumors.

Author Contribution Statement

MM, TM, and TMj conceived and designed the study, performed the experiments, and analyzed the data. MA and SD contributed to data interpretation and manuscript drafting. AB, AA, and SN assisted with laboratory work and literature review. MMd and MMA provided critical revisions and helped finalize the manuscript. All authors reviewed and approved the final version.

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Funding Statement

The study was self-supported by the authors

Ethical approval

This study was approved by the Institutional Research

Board, The University of Lahore, following ethical approval number Ref-IMBB/BBBC/23/22.

Availability of data

All the data is incorporated within the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

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