International Islamic Medical Journal
Journal Homepage ://journal2.unusa.ac.id/index.php/IIMJ

The Pro-Apoptotic Effect of Diallyl Trisulfide (DATS) on MDA-MB-231 Breast Cancer Cells

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DOI : 10.33086/iimj.v7i1.7314

ARTICLE INFO

Keywords:
Diallyl Trisulfide, ROS, Apoptosis, MDA-MB-231, Breast Cancer.

Submitted: May 18th 2025
Reviewed: June 10th 2025
Accepted: July 2nd 2025

ABSTRACT

Introduction: Breast cancer is the most prevalent cancer globally, impacting almost 2.1 million people each year. The MDA-MB-231 exemplifies the aggressive nature of the triple-negative breast cancer (TNBC) subtype. Diallyl Trisulfide (DATS) in Allium sativum (garlic) demonstrates potential anticancer characteristics primarily by causing the generation of ROS that may trigger apoptosis.
Objective: The study evaluates the effect of DATS on apoptosis in MDA-MB-231 cells via the ROS pathway.
Methods: An in vitro study utilizing MDA-MB-231 cells, categorized into four groups: (1) Medium control, (2) Negative control, (3) 20 µM DATS after 24 hours, and (4) 40 µM DATS after 24 hours. The study used Annexin V-FITC/PI flow cytometry to measure apoptotic cell death, the MTT assay to measure cell viability, and DCFH-DA fluorescence to measure ROS production.
Results: Cell viability was decreased after DATS treatment and a substantial increase in intracellular ROS production was observed that varies with time and dose ($p < 0.001$). In comparison to the other groups, the group that received 40 µM DATS for 24 hours had the highest rate of apoptosis and the largest accumulation of reactive oxygen species (ROS).
Conclusions: DATS significantly elevates intracellular ROS levels to promote apoptosis in MDA-MB-231 cells as a pro-oxidant therapy for aggressive breast cancer.

Introduction

Breast cancer becomes a significant global issue, impacting over 2.1 million women annually, and is the most often diagnosed cancer as well as the world's fifth most common cause of cancer-related fatalities (Sung et al., 2021). Breast cancer is highly heterogeneous, with subtypes distinguished by the presence or absence of the HER2 and HR. Basal-like or TNBC has become the worst prognosis and the most aggressive subtype among these subtypes, as it lacks HR and HER2 expression. Chemotherapy continues to be the

conventional method for treating TNBC, yet, the effectiveness of treatment is sometimes compromised by the emergence of multidrug resistance (MDR), leading to therapeutic failure in advanced stages (Malla et al., 2022). Hence, the search for novel, more effective therapeutic agents, particularly for advanced TNBC unresponsive to chemotherapy, is urgently needed.

The GLOBOCAN 2020 database indicates that breast cancer resulted in 2.3 million new cases and 684,996 fatalities globally yielding a mortality-to-incidence ratio (MIR) of 0.30, which highlights inequalities in access to Early diagnosis and therapy (Sung et al., 2021; WHO, 2020). Almost 63% of worldwide breast cancer fatalities in 2020 transpired in Asia and Africa (WHO, 2020). In Indonesia, breast cancer represents a significant proportion of the national cancer burden, with 68,858 new cases (16.6% of all cancers) and over 22,000 deaths recorded in 2020 (Sung et al., 2021). National health data from Riskesdas (2018), ranked breast cancer as Indonesia's seventh most prevalent cancer to be diagnosed. and its prevalence is on the rise from 1.4 to 1.79 per 1,000 population between 2013 and 2018 (Kemenkes RI, 2019). In East Java, Breast cancer is the second most common malignancy, impacting roughly 0.5% of the population (Riskesdas, 2013), while in Surabaya City, 1.93% of suspected cases were identified in 2018 (Dinas Kesehatan Kota Surabaya, 2019).

Current dietary recommendations for breast cancer patients emphasize the consumption of garlic and vegetables, which are believed to offer broad anticancer benefits (Gan et al., 2017). Diallyl trisulfide (DATS) has demonstrated significant anticancer efficacy (De Gianni & Fimognari, 2015) and modulate critical cancer pathways by inhibiting phospholipase A2 and mitogen-activated protein kinases (MAPKs), cytochrome P450, and glutathione-S-transferase. Its effect on the redox-sensitive cellular microenvironment interferes involving cancer cell proliferation, tumor angiogenesis, and metastasis. Furthermore, DATS and its analogs, including DADS, modulate apoptotic and mitotic signals in breast cancer cells (Malla et al., 2022).

In vivo studies have demonstrated that DATS reduces TNBC metastasis via decreasing MMP-2/9 through the suppression of signaling pathways (Liu et al., 2018). DATS further inhibits proliferation and metastasis by targeting the STAT3 signaling pathway of cyclin D1, VEGF, BCL2, BCL-xL, and MMP-2 expression (Kim et al., 2020).

According Kiesel and Stan (2017), DATS is most effective in the first phases of breast carcinogenesis due to its focus on

Notch ligands (Jagged-1 and Jagged-2) and α-secretases (ADAM10 and ADAM17) in MDA-MB-231 cells. Conversely, other studies have reported that DATS is also effective in later stages by suppressing the Wnt/β-catenin pathway (Liu et al., 2018).

Given these findings, further research is essential to determine the mechanism by which Diallyl trisulfide causes apoptosis in TNBC. This study aims to assess the effect of DATS on the MDA-MB-231 breast cancer cell line, thorugh apoptosis mechanism by ROS.

Methods

Study Design

This experimental study was conducted at the Stem Cell Research and Development Center, Universitas Airlangga, Surabaya. Immunocyto-chemistry and TUNEL staining procedures were performed at the Department of Anatomical Pathology, Dr. Soetomo General Hospital, and the Laboratory of the Surabaya Infectious Disease Hospital (Ethical Clearance No. 0105/EC/KEPK/UNUSA/2024).

Cell culture

MDA-MB-231 cells line were used in this study, purchased from ATCC® HTB-26™, grown in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO_2.

Cell line revival and maintenance

Frozen MDA-MB-231 cells were quickly thawed in a 37°C water bath and moved into a 15 mL tube with 10 mL of warm RPMI-1640 medium. The cells were spun at 1200 rpm for 5 minutes, then the liquid was removed. The cells were mixed with 10 mL of RPMI-1640 with 10% FBS and placed in a T-25 flask. They were grown at 37°C with 5% CO_2. When about 80% full, the cells were split using 0.25% trypsin-EDTA, spun at 180 × g for 7 minutes, and moved to new flasks at 2,500–5,000 cells/cm² in fresh medium.

DATS preparation and treatment

Diallyl trisulfide (DATS) was obtained in pure form and dissolved in dimethyl sulfoxide (DMSO). In order to mitigate solvent-induced cytotoxicity, the ultimate concentration of DMSO in the culture medium was limited to 0.1% (v/v). Cells were divided into four experimental groups (each in duplicate wells, a total of 8 wells):

1. Medium Control**:** Wells with complete medium only, no cells.
2. Negative Control**:** MDA-MB-231 cells without any treatment.
3. DATS 20 μM (K24–20 μM)**:** Cells treated with 20 μM Diallyl Trisulfide for 24 hours.
4. DATS 40 μM (K24–40 μM)**:** Cells treated with 40 μM Diallyl Trisulfide for 24 hours.

Cells were seeded into plates, allowed to adhere overnight, and subsequently treated

with the designated concentrations of DATS for 24 hours.

Cell viability assay (MTT)

The study measured cell viability using the MTT assay in 96-well plates and incubated for 24 hours. Living cells produced purple formazan crystals, and disintegrated in 100 µL of DMSO.

Statistical Analysis

The study utilized Welch's ANOVA, followed by the Games–Howell post hoc test to ascertain statistically significant pairwise differences. Statistical significance was defined as a p-value of less than 0.05.

Results and Discussion

Comparison of the effect before DATS treatment on the cell death mechanism of MDA-MB231 via ROS pathway

The effect of diallyl trisulfide on the cell death mechanism of the MDA-MB231 cell line is shown in Figure 1. The post hoc analysis test revealed significant differences between the control and both DATS treatment groups (Table 1).

Additionally, a significant difference was observed between the two DATS concentrations, indicating a dose-dependent effect of DATS on MDA-MB231 cell death via the ROS pathway. Notably, the number of viable cells in the DATS-treated groups decreased significantly, approaching the level observed in the medium control group. This suggests that DATS effectively induces cell death in MDA-MB231 cells, potentially restoring cell viability closer to baseline conditions.

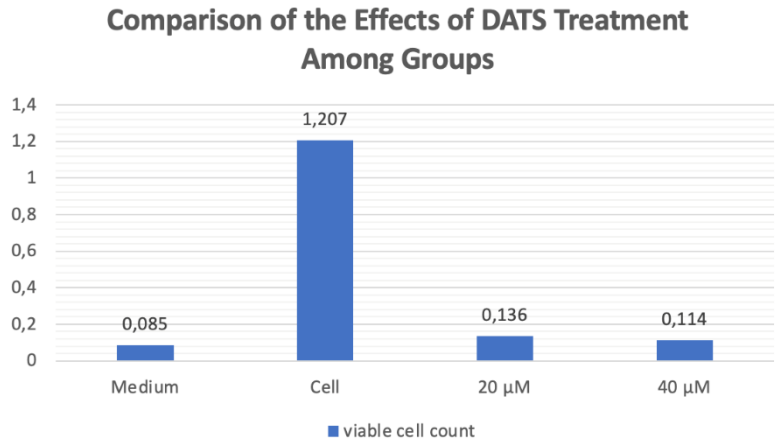

Figure 1. Comparison of DATS treatment versus control group

Table 1. Comparison test results for the effect of DATS on MDA-MB231 cell death via the ROS pathway

Group	Mean	Min	Max	p-value (Welch Anova test)
Medium Control	0.085	0.078	0.092	<0.001
Negative Control	1.207[a]	1.200	1.214	
DATS 20 µM	0.136[b]	0.129	0.143	
DATS 40 µM	0.114[c]	0.107	0.121	

Note: Data are presented as mean ± SD. Different superscript letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$)

Comparison of pre-treatment and post-treatment effects of DATS on MDA-MB-231 cell death via the ROS pathway

This study illustrates DATS impact in inducing MDA-MB-231 apoptosis through the ROS pathway, as depicted in Figure 5.1. The findings indicated a significant decrease of viable cells following DATS exposure, highlighting its cytotoxic potential and its promise as a future therapeutic option for inhibiting cell and inducing apoptosis in TNBC cells line.

These findings are consistent with prior research reporting that DATS at concentrations above 20 µM can reduce viable cell numbers by more than 50%, with the maximum effect observed after 72 hours of treatment (Kanga et al., 2021). Another study demonstrated that 40 µM DATS was more effective than 20 µM in suppressing MDA-MB-231 cell viability, with ROS production significantly increasing within the first 3 hours post-treatment, followed by a decrease after 6 hours (Chandra-Kuntal et al., 2013). The observations substantiate the essential function of ROS in facilitating apoptosis.

Prior studies indicate that DATS demonstrates significant cytotoxicity in breast cancer cell line, triggering apoptosis via caspase -3 and -9 (Marni et al., 2022). DATS is recognized for its inhibition of critical enzymes, including phospholipase A2, MAPKs, cytochrome P450s, and glutathione S-transferases, indicating its potential in cancer theraphy.

OSC-induced apoptosis include the activation of apoptotic protein. These effects are mediated by mitochondrial membrane disruption and enhanced ROS production (Na et al., 2012). The chemopreventive effects of DATS are thought mediated by interference with signaling pathways involved in inflammation, angiogenesis, invasion, and metastasis.

The effect of diallyl trisulfide on cell death mechanisms in MDA-MB-231 cells via the ROS pathway

The study demonstrated a substantial impact of DATS therapy and apoptosis in the breast cancer cell line that demonstrated by a reduction in the quantity of viable cells in the DATS-treated groups relative to the controls. These findings are verified by past studies that suggested a similar outcome as the study (Chandra-Kuntal et al., 2013).

DATS at concentrations of 20 μM and 40 μM were shown to trigger cell death and reduce cell viability, partly through the ROS-mediated apoptotic pathway. Additional studies have also demonstrated that 40 μM DATS can reduce cell viability by more than 50%, and the IC_{50} of DATS has been reported to be in the range of 30–40 μM (Kiesel & Stan, 2017).

DATS increases ROS production by influencing various cellular signaling pathways that which play a role in tumor growth including Akt, NF-κB, JNK, and ERK. DATS exhibits multiple biological functions, including anti-inflammatory activity, enhanced detoxification, histone acetylation, ROS generation, and ER stress (Shigemi et al., 2016). It is crucial to note that DATS has the ability to directly produce ROS by homolytically cleaving its intramolecular trisulfide bond. This process results in oxidative and ER stress, which in turn induces apoptosis in a variety of cancer cell types.

The JNK signaling axis is crucial in modulating cell survival and death during ROS generation triggered by DATS. This route is intricately connected to ferritin stability, which governs the release of labile iron and the ensuing production of deleterious reactive oxygen species (ROS) (Na et al., 2012).

The production of ROS by DATS is crucial for cancer cell migration and apoptosis. Activation of Bak, but not Bax, has been found to be ROS-dependent and crucial for apoptosis induction by DATS. Moreover, the pro-apoptotic protein Bim was found to be phosphorylated upon DATS treatment, further linking DATS exposure to ROS production and apoptosis induction (Puccinelli & Stan, 2017).

Conclusion

This study shown that Diallyl Trisulfide (DATS) can reduce MDA-MB-231 viability by inducing cell death via the ROS pathway with a dose-dependent effect. This substantiates the notion that reactive oxygen species (ROS) are pivotal in the mechanism by which DATS induces apoptosis in cancer cells. DATS exhibits promise as a natural anticancer agent, particularly against aggressive breast malignancies such as triple-negative breast cancer. Additional study is required to

investigate its application in animal studies and potential clinical uses.

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