Trop J Nat Prod Res, August 2024; 8(8): 7996-8000 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Effect of Diallyl Trisulfide Administration on The Viability of MDA-MB-231 Cell Lines

Lysa Veterini[1,2], Gondo Mastutik[3], Nora Ertanti[4], Mohammad QB Zulfikar[5], Akbar R Muhammad[6], Rizky F Meirawan[7], Soetjipto[8]*

[1]*Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, 60132 Surabaya, Jawa Timur, Indonesia.*
[2]*Department of Anatomical Pathology, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, 60237 Surabaya, Jawa Timur, Indonesia.*
[3]*Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, 60132 Surabaya, Jawa Timur, Indonesia.*
[4]*Stem Cell Research and Development Center, Universitas Airlangga, 60132 Surabaya, Jawa Timur, Indonesia.*
[5]*Master of Public Health, Faculty of Medicine, University of Liège, 4000 Liège, Belgium.*
[6]*Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, 60237 Surabaya, Jawa Timur, Indonesia.*
[7]*Public Health Undergraduate Program Lecturer, Universitas Indonesia Maju, 12610 Jakarta Selatan, Indonesia.*
[8]*Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, 60132 Surabaya, Jawa Timur, Indonesia.*

ARTICLE INFO

Article history:
Received 8 May 2024
Revised 10 May 2024
Accepted 11 August 2024
Published online 01 September 2024

ABSTRACT

Diallyl trisulfide or DATS is an important organic sulphur compound (OSCs) of garlic that has been recognized for its ability to inhibit migration and invasion processes, promote programmed cell death, and impede the proliferation of breast cancer cells. However, the most effective dosage of DATS to decrease survival rate and cell viability of triple-negative breast cancer cell line has been undetermined. The present study aimed to discover the MDA-MB-231 (triple-negative breast cancer cell line) viability differences due to the addition of DATS in several doses. DATS was administered to 6 groups of MDA-MB-231 cell cultures at different concentrations (20, 40, 80, 160, and 320 µM), with a control group. Cell viability was evaluated using colorimetric MTT assay. The study reported that DATS effectively inhibited the cell line survival rate. Treatment with DATS at a dose of 20 µM or higher led to cell death and a significant MDA-MB-231 cell line viability reduction compared to the control group but not between the intervention groups. The findings demonstrated that DATS had the ability to trigger cell death and possess potential anti-cancer properties particularly in triple-negative breast cancer. A novel observation was made when low dose of DATS exhibited comparable efficacy to the large dose in reducing MDA-MB-231 cell line viability, while minimizing the potential for negative side effects.

Keywords: Breast Cancer, *Diallyl trisulfide*, Antioxidants, Cancer prevention

Introduction

Allium sativum (garlic) was commonly utilized in ancient times to treat many health problems including cancer.[1] The beneficial effects of garlic on health are attributed to various organic sulphur compounds (OSCs) including allicin, *Diallyl sulfide* (DAS), *Diallyl disulfide* (DADS), and *Diallyl trisulfide* (DATS).[2,3] Moreover, research suggests that the biological activity of OSCs is influenced by the number of sulphur atoms enclosed. It has been proven that DATS, which has the highest number of sulphur atoms, is the most efficient chemical.[4] The previous *in silico* research has shown its ability as an anti-angiogenesis especially in breast cancer.[5] A prior study found a correlation between the decrease of breast cancer risk and the consumption of garlic. This study demonstrates that women who consume high quantities of garlic have a significantly lower probability of experiencing breast cancer than those who consume a modest number of garlic.[6]

*Corresponding author. Email: soetjipto@fk.unair.ac.id
Tel: + 62 813-3134-0518

Citation: Veterini L, Mastutik G, Ertanti N, Zulfikar MQB, Muhammad AR, Meirawan RF, Soetjipto. The Effect of Diallyl Trisulfide Administration on The Viability of MDA-MB-231 Cell Lines. Trop J Nat Prod Res. 2024; 8(8):7996-8000. https://doi.org/10.26538/tjnpr/v8i8.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Women's breast cancer (BC) rises to the top of the global cancer incidence list. According to epidemiological data, in 110 out of 185 nations, women have died from cancer due to breast cancer.[7] Triple negative breast cancer (TNBC) is considered the highly malignant and aggressive mammary epithelial tumor due to its rapid proliferation, swift spread, and frequent incidence of aggressive, heterogeneous, and metastatic tumours.[8] The primary challenge of TNBC is the lack of therapeutic targets for conventional treatments which result in limited therapy options. This is further exacerbated by the frequent occurrence of TNBC metastases, the development of resistance to regular chemotherapy, and poor prognoses with short survival rates despite receiving normal therapy.[9] To prevent the development of chemotherapy resistance and increase the success of TNBC therapy, previous review study postulates that DATS and DADS compounds are sufficiently good to enhance therapeutic outcome and decrease the drug resistance process especially in TNBC woman.[10]

Cellular viability and proliferation assays are widely employed to evaluate the impact of potential anti-cancer treatments including substances that inhibit cell growth and those causing cell death.[11] DATS is an eminent chemical commonly utilized as a potential candidate for anti-cancer therapy.[12] Previous similar studies have used DATS in MDA-MB-231 cells as an anti-cancer therapy without focusing on cellular viability assesement,[13] or using a high dose of DATS (320 µM).[14,15]

Despite the reported benefits of DATS treatment in the symptomatic treatment of malignancy especially in breast cancer *in vitro* or *in silico*, their efficacy and safety are still questionable. Therefore, the present study aimed to evaluate the MDA-MB-231 cell viability

differences due to DATS treatment in dissimilar doses to determine the efficacy and optimal dose especially for discontinuing breast cancer cell lineage.

Materials And Methods

Methods

A quantitative longitudinal experiment was used as the research design along with a completely randomized design (CRD) to address the research objectives. *Diallyl trisulfide* (DATS) treatment was applied to MDA-MB-231 cultures as part of the experiment. DATS doses were separated into five groups: 20 µM (K1), 40 µM (K2), 80 µM (K3), 160 µM (K4), 320 µM (K5), and control group (K0). Six samples of observations were made in each group. The study received ethical clearance from the Medical Faculty Ethics Committee of Airlangga University (Number 39/EC/KEPK/FKUA/2023).

Materials

Diallyl trisulfide (DATS) (GlpBio Technology Inc, Montclair, CA, USA) (Synonyms: DATS, NSC 651936) was isolated from garlic extract with the purity of 98% as determined by HPLC. DATS was dissolved in 100% DMSO and stored at a temperature of -20°C. The administration of DATS was accomplished by delivering a pre-mixed combination of DATS and a substitute medium into a specified group cell. Heat-Inactivated Fetal bovine serum (FBS) and Thiazolyl Blue Tetrazolium Bromide (MTT) assay were obtained from Sigma (St. Louis, Missouri, USA).

Cell lines and cell culture

The MDA-MB-231 cell lines, derived from human breast cancer, were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were grown in DMEM medium supplemented with 10% foetal bovine serum, 100 IU/mL penicillin, and 100 µg/mL streptomycin. The cells were maintained in a humidified incubator at 37°C with an atmosphere of 5% CO_2.[16] The cell lines were cultured in the Stem Cell Laboratory of Universitas Airlangga.

Thiazolyl Blue Tetrazolium Bromide (MTT) Assay

Cells were seeded in 96-well microplates ($5x10^3$ cells/well of 200 µL) and routinely cultured in a controlled environment for 24 hours to make a suspension of MSCs cells. This controlled environment was an ESCO CelCulture (Xiupu Road, Shanghai China) 5% CO2 atmosphere incubator, that maintained at 37ºC. After a 24-hour pre-culture, the media was removed and replaced with medium containing different concentrations of DATS, ranging from 20 to 320 µM. Cells were then re-incubated for 24 hours. Afterwards, 25 µL of MTT solution was added each well and re-incubated for 4 hours. After the incubation period, it was carefully aspirating the MTT solution from each well. The formazan crystals formed by viable cells would be visible as purple precipitates when observed under an inverted microscope (Olympus CKX-53). A total of 50 µL /well solution of DMSO was added. The Optical Density (OD, Absorbance) was quantified using a microplate reader (GloMax® Discover Microplate Reader, Promega) at a specific wavelength (595 nm) and monitored 24 hours after the treatment or exposure to DATS.

Viability cell calculation

The viability of MDA-MB-231 cell lines was determined using viability calculation formula.[17] The cell viability proportion was expressed as a percentage, representing the numerical data scale of viability for MDA-MB-231 cell lines. This study used a nominal data scale with DATS doses divided into six groups as the independent variables.

The calculation of live cells was conducted using the following formula:[17]

$$Percentage\ of\ cell\ viability = \frac{A_{Treatment} - A_{Blank}}{A_{Control} - A_{Blank}} x\ 100\%$$

Note:

$A_{Treatment}$ = OD value of test material sample
$A_{Control}$ = OD value of cell control
$A_{Treatment}$ = OD value of growth medium without cells

Statistical analysis

The study hypothesis posited a difference in the viability capacity of MDA-MB-231 cell lines when exposed to varied doses of DATS. The acceptance of a hypothesis was determined by a statistical test's *p*-value, which was significant at below α ($p<\alpha$). The study used a 95% of accuracy threshold, leading to an α value of 0.05. The statistical test was performed by the assistance of IBM SPSS 27.

Results and Discussion

Treatment with DATS at a concentration of 20 µM or higher led to cell death and decreased MDA-MB-231 cell viability. Based on the findings from Figure 1 and Figure 2, it was observed that the administration of DATS at a greater dosage resulted in a significant drop in both cell viability and the percentage of living cells. Treatment with DATS at a concentration of 20 µM or higher led to a mortality rate of 94.02% or greater in MDA-MB-231 cell lines. As compared to the baseline, cell live count also significantly decreased (see Figure 3). The figure also demonstrated that an increase in DATS doses resulted in a major decrease in the quantity of the cancer cell lines. Furthermore, Table 1 illustrates that the mortality of the MDA-MB-231 cell line rose as the dose of DATS increased. The data indicated a dosage response between the DATS dosages and the death of the MDA-MB-231 cell line.

Based on the Kruskal Wallis statistical test portrayed in Table 1, the statistical value was 26.024 with $p<0.001$ ($p<\alpha$). Therefore, it was hypothesized that there was a difference in the response of MDA-MB-231 cell lines to different doses of DATS, leading to a decrease in cell viability. The study verified that DATS possessed the capacity to eliminate cancer cells and might potentially be used as an anti-cancer agent.

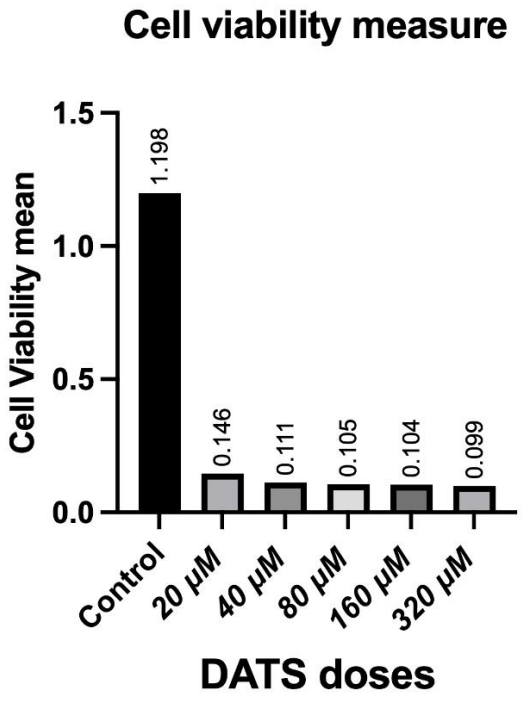

Figure 1: Column diagram illustrating cell viability measurement (mean) between DATS doses determined by optical density. The diagram illustrates that treatment with DATS at a concentration of 20 µM or higher decreases breast cancer cell line viability

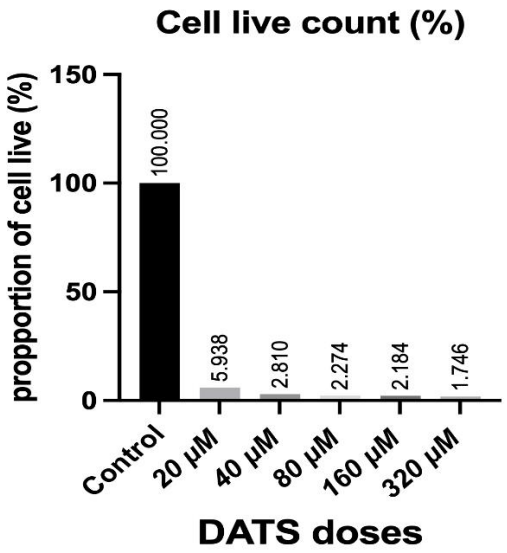

Figure 2: Column diagram presenting the percentage (%) of live cells between DATS doses determined by an Automated Cell Counter. The diagram illustrates that treatment with DATS at a concentration of 20 µM or higher leads to breast cancer cell line death

DATS, a constituent found in garlic, had been identified as a potential anti-carcinogenic agent with the capacity to impede the growth,[18] migration, and invasion of cancer cells.[19] Numerous investigations provided evidence of the anti-carcinogenic and anti-metastatic properties of DATS.[4,18,20] Nevertheless, it was hypothesized that variations in the DATS dosage might impact its efficacy in manifesting its anti-cancer characteristics. Previous research indicated that the administration of DATS at moderate concentrations of 40 µM, 60 µM, and 80 µM, or similar to the daily intake limit in humans, specifically 3.5 mg, 5.2 mg, and 7.07 mg, effectively exhibited anti-carcinogenic properties by inducing DNA damage, arresting the cell cycle, and causing cell death, which then resulted in reduced viability of cancer cells[21]. The present study was objected to evaluate the efficacy of different dosages of DATS on cell viability and the decrease in the percentage of viable MDA-MB-231 cells. Several commonly employed doses would be examined including low-dose DATS at 20 µM,[4] moderate dose at 40 µM, 80 µM,[21,22] and 160 µM,[4,23] and high-dose at 320 µM.[24] It was anticipated that the administration of several doses of DATS would yield preliminary findings regarding the most effective dosage for reducing cell viability and their proportion of living cells. The study demonstrated that the administration of DATS at low, moderate, or high doses dramatically lowered the viability of the MDA-MB-231 cell line. Conversely, no appreciable variations were revealed between the low-dosage group and the other treatment groups, which were the moderate and high dose groups. Thus, it may be postulated that the administration of DATS at a low concentration (20 µM) was sufficient to exhibit its anti-cancer properties, leading to a reduction in cell viability and the number of viable cancer cells.

Two processes were thought to be responsible for the decrease in viability of the MDA-MB-231 cell line following the DATS administration, even at a low dose. The first mechanism was based on the ability of DATS to suppress the metastasis process of breast cancer tissue, particularly the MDA-MB-231 and HS 578T cell lines. According to the previous study, DATS could prevent metastasis by reducing the activity and production (down-regulating) of ERK/NFκB/MMP-2/MMP-9.[4]

Second, the Reactive Oxygen Species (ROS) pathway was thought to be the reason why DATS was able to reduce the viability of cancer cells.[25] In many forms of cancer, alterations in the dynamics and function of the mitochondria were linked to malignancy.[26] The formation of ROS was one of the consequences of mitochondrial malfunction, which was involved in various aspects of carcinogenesis and an elevated amount of ROS had been detected in tumor cells.[27,28] In comparison to normal cells, cancer cells had an elevated concentration of ROS because of hyper-metabolism. Nevertheless, cancer cells were able to maintain redox balance as a result of their substantial antioxidant capability. The rapid buildup of ROS disturbed the balance of redox reactions and caused significant harm to cancer cells.[29]

Furthermore, the results of this study showed a decrease in the number of viable cells in the low, moderate, and high-dose groups. The low-dose group (20 µM) exhibited a significant decrease of over 90% of MDA-MB-231 viable cells, indicating the DATS potential in inducing cell death and exhibiting anti-cancer properties. One important type of cell death in cancer cells was apoptosis. Research had revealed a strong correlation between apoptosis and the survival of cancer cells. Thus, this made it a key focus for the identification and advancement of novel anti-cancer medications. Various studies had shown that targeting the apoptosis signaling pathway by anti-cancer drugs was a crucial mechanism in anti-cancer therapy.[30]

According to previous study,[21] the intrinsic apoptosis pathway, had been extensively studied as the most well-documented cell death process in response to genotoxic stress. DATS was found to induce a dose-dependent decrease of anti-apoptotic Bcl-2 protein and had a minor impact on the production of the pro-apoptotic protein Bak (Bcl-2 homologue antagonist). In addition, DATS had been observed to enhance the permeability of the mitochondrial membrane and induced the release of cytochrome c by dose-dependent manner.[21,31] In the end, these activities resulted in the creation of the apoptosome, which was composed of an Apaf-1 and cytochrome c complex.[32,33] This complex was responsible for activating apoptosis executioner caspases 3 and 7 by causing more cleavage of procaspase forms.[21,32]

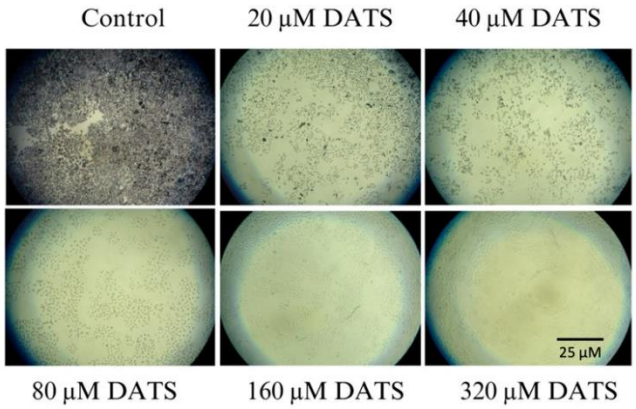

Figure 3: A visible decrease in breast cancer cell line following an increase in DATS dose using an inverted microscope at a magnification of 40x**.** The image portrays a negative correlation between DATS dosage and the count of visible MDA-MB-231 cell lines. This indicates that higher DATS dosage results in a drop in cell line count.

Diallyl trisulfide, which became the predominant bioactive molecule present in garlic, had been scientifically demonstrated to exhibit antioxidant characteristics, mitigate double-strand DNA damage, and trigger cell cycle arrest and apoptosis.[18] The induction of cell cycle arrest mediated by reactive oxygen species (ROS) and apoptosis, as well as the activation of caspases, had been provided by DATS in various cancer models, including breast cancer.[34] Numerous studies have demonstrated diverse inhibitory effects of DATS on tumor growth through multiple mechanisms, including the induction of

ROS, cell cycle arrest, promotion of apoptosis, suppression of proliferation, and inhibition of tumor cell invasion and metastasis in a dose-dependent manner.[21,24] These findings aligned with our research's finding that higher doses led to reduced cell viability and a decrease in viable cells.

Table 1: Comparison of Average Viability of MDA-MB-231 Cells

Group	Average Viability of MDA-MB-231 Cells (MTT Assay)	P Values (Kruskal Wallis test)
Control (K0)	1.198	
DATS Dose of 20 μM (K1)	0.146	
DATS Dose of 40 μM (K2)	0.111	
DATS Dose of 80 μM (K3)	0.105	p<0.001
DATS Dose of 160 μM (K4)	0.104	
DATS Dose of 320 μM (K5)	0.099	

p-value obtained from Kruskal Wallis test and the average viability of live cell MDA-MB-231 in each group showed a significant result. An observed disparity in the mean viability of MDA-MB-231 cells was found between the intervention group and the baseline, as indicated by the data.

Additionally, it was important to note that administering DATS in large doses did not come without risks and side effects. An overview of *in vitro* toxicity experiments conducted on MDA-MB-231 cells revealed that DATS had more potency compared to DADS and DAS in the induction of mitochondria-mediated cell death and the formation of ROS.[35] The cytotoxicity of DATS was enhanced when taken in specific doses due to its dose-dependent,[21,36] and concentration-dependent,[37] characteristics. According to a previous study, the administration of DATS within the concentration range of 50-80 μM to MDA-MB-231 cells led to an elevation in intracellular ROS, which then triggered its cytotoxic properties.[36] An In-vivo study also discovered that consuming garlic over a period of 4 weeks could have a detrimental impact on the gastrointestinal tract.[38] A toxicity study of DATS on animal models (mice) showed that the LD50 of DATS was 100mg/bw.[39] A systematic literature review assessing *in vitro* toxicity studies of DATS,[35] explained that there was no conclusive evidence of potential DATS toxicity due to the variety of cell models used and the justification of DATS potential as a chemoprotective agent against carcinogenesis rose the need for more genotoxicity studies on DATs in the future.

Conclusion

Regarding the present study's findings, administration of *Diallyl trisulfide* (DATS) at a dose over 20 μM effectively decreases the viability of the MDA-MB-231 breast cancer cell line. However, doses greater than 20 μM do not demonstrate significant differences from other treatment groups and may have unknown effects on cells. The findings of this study suggest that low-modest intake of DATS may help to reduce the viability of MDA-MB-231 breast cancer cell line to minimize unpredicted side effects. While the *in vitro* findings have been established, further investigation is required through *in vivo* or *in vitro* studies to explore additional cellular parameters that contribute to the decline in cell viability. Additional research is required to demonstrate its genotoxicity and safety in normal cells at certain dosages.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

The authors would like to sincerely thank the advising professors for their invaluable help and support during this research endeavor. The authors would like to express their gratitude to the workers and scientists in the Stem Cell Laboratory of Universitas Airlangga for their valuable help. The authors would also like to thank Universitas Nahdlatul Ulama Surabaya for their moral and material support in the success of this research.

References

1. Farhat Z, Hershberger PA, Freudenheim JL, Mammen MJ, Hageman Blair R, Aga DS, Mu L. Types of garlic and their anticancer and antioxidant activity: a review of the epidemiologic and experimental evidence. Eur J Nutr. 2021;60(7):3585-3609. doi:10.1007/s00394-021-02482-7
2. Lawson L, Hunsaker S. *Allicin* Bioavailability and Bioequivalence from Garlic Supplements and Garlic Foods. Nutrients. 2018;10(7):812. doi:10.3390/nu10070812
3. Almatroodi SA, Alsahli MA, Almatroudi A, Rahmani AH. Garlic and its Active Compounds: A Potential Candidate in The Prevention of Cancer by Modulating Various Cell Signalling Pathways. Anticancer Agents Med Chem. 2019;19(11):1314-1324. doi:10.2174/1871520619666190409100955
4. Liu Y, Zhu P, Wang Y, Wei Z, Tao L, Zhu Z, Sheng X, Wang S, Ruan J, Liu Z, Cao Y, Shan Y, Sun L, Wang A, Chen W, Lu Y. Antimetastatic Therapies of the Polysulfide *Diallyl trisulfide* against Triple-Negative Breast Cancer (TNBC) via Suppressing MMP2/9 by Blocking NF-κB and ERK/MAPK Signaling Pathways. Paulmurugan R, ed. PLoS One. 2015;10(4):e0123781. doi:10.1371/journal.pone.0123781
5. Veterini L, Savitri AD, Widyaswari MS, Muhammad AR, Fairus A, Zulfikar MQB, Astri M, Ramasima NA, Anggraeni DP, Nainatika RSA. *In Silico* Study of the Potential of Garlic *Allicin* Compound as Anti-Angiogenesis in Breast Cancer. TJNPR. 2021;5(11):1995-1999. doi:10.26538/tjnpr/v5i11.17
6. Desai G, Schelske-Santos M, Nazario CM, Rosario-Rosado R V., Mansilla-Rivera I, Ramírez-Marrero F, Nie J, Myneni AA, Zhang ZF, Freudenheim JL, Mu L. Onion and Garlic Intake and Breast Cancer, a Case-Control Study in Puerto Rico. Nutr Cancer. 2020;72(5):791-800. doi:10.1080/01635581.2019.1651349
7. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249. doi:10.3322/caac.21660
8. Matou-Nasri S, Aldawood M, Alanazi F, Khan AL. Updates on Triple-Negative Breast Cancer in Type 2 Diabetes Mellitus Patients: From Risk Factors to Diagnosis, Biomarkers and Therapy. Diagnostics. 2023;13(14):2390. doi:10.3390/diagnostics13142390
9. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Research. 2020;22(1):1-13. doi:10.1186/S13058-020-01296-5/TABLES/3
10. Malla RR, Marni R, Chakraborty A, Kamal MA. *Diallyl disulfide* and *Diallyl trisulfide* in garlic as novel therapeutic agents to overcome drug resistance in breast cancer. J Pharm Anal. 2022;12(2):221-231. doi:10.1016/j.jpha.2021.11.004

11. Gordon J, Brown M, Reynolds M. Cell-Based Methods for Determination of Efficacy for Candidate Therapeutics in the Clinical Management of Cancer. Diseases. 2018;6(4):85. doi:10.3390/diseases6040085
12. Subramanian MS, Nandagopal GMS, Nordin SA, Thilakavathy K, Joseph N. Prevailing knowledge on the bioavailability and biological activities of Sulphur compounds from *Alliums*: A potential drug candidate. Molecules. 2020;25(18). doi:10.3390/molecules25184111
13. Liu Y, Zhao Y, Yingyu W, Zhu P, Wei Z, Siliang W, Tao L, Liu Z, Wu H, Sheng X, Lu Y. Suppressive role of *Diallyl trisulfide* in the activated platelet-mediated hematogenous metastasis of MDA-MB-231 human breast cancer cells. Int J Mol Med. 2017;39(6):1516-1524. doi:10.3892/ijmm.2017.2953
14. Kanga KJW, Mendonca P, Soliman KFA, Ferguson DT, Darling-Reed SF. Effect of *Diallyl trisulfide* on TNF-α-induced CCL2/MCP-1 release in genetically different triple-negative breast cancer cells. Anticancer Res. 2021;41(12):5919-5933. doi:10.21873/anticanres.15411
15. Marni R, Kundrapu DB, Chakraborti A, Malla RR. Insight into drug sensitizing effect of *Diallyl disulfide* and *Diallyl trisulfide* from *Allium sativum L*. on paclitaxel-resistant triple-negative breast cancer cells. J Ethnopharmacol. 2022;296. doi:10.1016/j.jep.2022.115452
16. Gest C, Joimel U, Huang L, Pritchard LL, Petit A, Dulong C, Buquet C, Hu CQ, Mirshahi P, Laurent M, Fauvel-Lafève F, Cazin L, Vannier JP, Lu H, Soria J, Li H, Varin R, Soria C. Rac3 induces a molecular pathway triggering breast cancer cell aggressiveness: differences in MDA-MB-231 and MCF-7 breast cancer cell lines. BMC Cancer. 2013;13(1):63. doi:10.1186/1471-2407-13-63
17. Lee D, Lee J, Vu-Huynh KL, Van Le TH, Tuoi Do TH, Hwang GS, Park JH, Kang KS, Nguyen MD, Yamabe N. Protective Effect of *Panaxynol* Isolated from *Panax vietnamensis* against Cisplatin-Induced Renal Damage: *In Vitro* and *In Vivo* Studies. Biomolecules. 2019;9(12):890. doi:10.3390/biom9120890
18. Darling-Reed SF, Nkrumah-Elie Y, Ferguson DT, Flores-Rozas H, Mendonca P, Messeha S, Hudson A, Badisa RB, Tilghman SL, Womble T, Day A, Jett M, Hammamieh R, Soliman KFA. *Diallyl Sulfide* Attenuation of Carcinogenesis in Mammary Epithelial Cells through the Inhibition of ROS Formation, and DNA Strand Breaks. Biomolecules. 2021;11(9):1313. doi:10.3390/biom11091313
19. Cheng SY, Yang YC, Ting KL, Wen SY, Viswanadha VP, Huang CY, Kuo WW. Lactate dehydrogenase downregulation mediates the inhibitory effect of *Diallyl trisulfide* on proliferation, metastasis, and invasion in triple-negative breast cancer. Environ Toxicol. 2017;32(4):1390-1398. doi:10.1002/tox.22333
20. El-Saber Batiha G, Magdy Beshbishy A, G. Wasef L, Elewa YHA, A. Al-Sagan A, Abd El-Hack ME, Taha AE, M. Abd-Elhakim Y, Prasad Devkota H. Chemical Constituents and Pharmacological Activities of Garlic (*Allium sativum L.*): A Review. Nutrients. 2020;12(3):872. doi:10.3390/nu12030872
21. Stan SD, Abtahi M. *Diallyl Trisulfide* Induces Apoptosis in Breast Ductal Carcinoma *In Situ* Derived and Minimally Invasive Breast Cancer Cells. Nutrients. 2022;14(7):1455. doi:10.3390/nu14071455
22. Ferguson DT, Taka E, Messeha S, Flores-Rozas H, Reed SL, Redmond B V., Soliman KFA, Kanga KJW, Darling-Reed SF. The Garlic Compound, *Diallyl trisulfide*, Attenuates *Benzo[a]Pyrene*-Induced Precancerous Effect through Its Antioxidant Effect, AhR Inhibition, and Increased DNA Repair in Human Breast Epithelial Cells. Nutrients. 2024;16(2):300. doi:10.3390/nu16020300
23. Sun S, Liu X, Wei X, Zhang S, Wang W. *Diallyl trisulfide* induces pro-apoptotic autophagy via the AMPK/SIRT1 signalling pathway in human hepatocellular carcinoma HepG2 cell line. Food Nutr Res. 2023;67. doi:10.29219/fnr.v67.8981
24. Jiang X, Zhu X, Liu N, Xu H, Zhao Z, Li S, Li S, Cai J, Cao J. *Diallyl trisulfide* Inhibits Growth of NCI-H460 *in Vitro and in Vivo*, and Ameliorates Cisplatin-Induced Oxidative Injury in the Treatment of Lung Carcinoma in Xenograft Mice. Int J Biol Sci. 2017;13(2):167-178. doi:10.7150/ijbs.16828
25. Hecht F, Pessoa CF, Gentile LB, Rosenthal D, Carvalho DP, Fortunato RS. The role of oxidative stress on breast cancer development and therapy. Tumor Biology. 2016;37(4):4281-4291. doi:10.1007/s13277-016-4873-9
26. Sarmiento-Salinas FL, Delgado-Magallón A, Montes-Alvarado JB, Ramírez-Ramírez D, Flores-Alonso JC, Cortés-Hernández P, Reyes-Leyva J, Herrera-Camacho I, Anaya-Ruiz M, Pelayo R, Millán-Pérez-Peña L, Maycotte P. Breast Cancer Subtypes Present a Differential Production of Reactive Oxygen Species (ROS) and Susceptibility to Antioxidant Treatment. Front Oncol. 2019; 9:480. doi:10.3389/fonc.2019.00480
27. Barrera G. Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy. ISRN Oncol. 2012; 2012:1-21. doi:10.5402/2012/137289
28. Gu H, Huang T, Shen Y, Liu Y, Zhou F, Jin Y, Sattar H, Wei Y. Reactive Oxygen Species-Mediated Tumor Microenvironment Transformation: The Mechanism of Radioresistant Gastric Cancer. Oxid Med Cell Longev. 2018; 2018:1-8. doi:10.1155/2018/5801209
29. Kim SJ, Kim HS, Seo YR. Understanding of ROS-Inducing Strategy in Anticancer Therapy. Oxid Med Cell Longev. 2019; 2019:1-12. doi:10.1155/2019/5381692
30. An W, Lai H, Zhang Y, Liu M, Lin X, Cao S. Apoptotic Pathway as the Therapeutic Target for Anticancer Traditional Chinese Medicines. Front Pharmacol. 2019; 10:758. doi:10.3389/fphar.2019.00758
31. Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol. 2020;21(2):85-100. doi:10.1038/s41580-019-0173-8
32. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev. 2001;15(22):2922-2933. http://www.ncbi.nlm.nih.gov/pubmed/11711427
33. Ravagnan L, Roumier T, Kroemer G. Mitochondria, the killer organelles and their weapons. J Cell Physiol. 2002;192(2):131-137. doi:10.1002/jcp.10111
34. Puccinelli MT, Stan SD. Dietary Bioactive *Diallyl trisulfide* in Cancer Prevention and Treatment. Int J Mol Sci. 2017;18(8):1645. doi:10.3390/ijms18081645
35. Cascajosa-Lira A, Andreo-Martínez P, Prieto AI, Baños A, Guillamón E, Jos A, Cameán AM. *In Vitro* Toxicity Studies of Bioactive Organosulfur Compounds from *Allium* spp. with Potential Application in the Agri-Food Industry: A Review. Foods. 2022;11(17):2620. doi:10.3390/foods11172620
36. Lee BC, Park BH, Kim SY, Lee YJ. Role of bim in *Diallyl trisulfide*-induced cytotoxicity in human cancer cells. J Cell Biochem. 2011;112(1):118-127. doi:10.1002/jcb.22896
37. Sielicka-Dudzin A, Borkowska A, Herman-Antosiewicz A, Wozniak M, Jozwik A, Fedeli D, Antosiewicz J. Impact of JNK1, JNK2, and ligase Itch on reactive oxygen species formation and survival of prostate cancer cells treated with *Diallyl trisulfide*. Eur J Nutr. 2012;51(5):573-581. doi:10.1007/s00394-011-0241-0
38. Ikele BC, Okoye CK, Ikele FC, Obiezue RN. Effects of Garlic (*Allium sativum*) on Serum Biochemical Parameters and Histopathological Changes in Wistar Rats (*Rattus norvegicus*). TJNPR. 2022;6(3):371-375. doi:10.26538/tjnpr/v6i3.12
39. Dutta A, Dahiya A, Prakash A, Agrawala PK. Acute toxicity of *Diallyl sulfide* derived from *Allium sativum* (garlic) in mice and its possible mechanisms. Phytomedicine Plus. 2021;1(3):100084. doi:10.1016/j.phyplu.2021.100084

