Alqalam Journal of Medical and Applied Sciences. 2026;9(4):1001-1009
https://doi.org/10.54361/ajmas.269432

Original article

Rotenone and Diallyl Disulfide–Induced Mitochondrial Apoptosis in MCF-7 Cells: A Combination Study

Ahmed Kareem Hussein Alatafi

Department of Biology, College of Science, University of Diyala, Iraq
Email. ahmedkareemalatafi@uodiyala.edu.iq

Abstract
Cancer is among the most serious problems affecting the health of people across the globe. It is therefore important to come up with better treatment options that work effectively without causing any side effects. This study examines the effect of rotenone (ROT) and Diallyl disulfide (DADS) on the MCF-7 breast cancer cells through apoptosis. Cytotoxicity of the drugs was determined by measuring IC_{50} at 48 hours using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; their interactions were estimated using the Chou-Talalay protocol. QRT-PCR was used to analyze changes in gene expression of Bax and Bcl-2, and cell distribution was quantified by flow cytometry. Both substances (IC_{50} values of 4.62 µM for rotenone and 14.2 µM for DADS) exhibited dose-dependent cytotoxicity. The combination effects were determined by the Chou–Talalay approach, which indicated dose-dependent synergy, additivity, and antagonism at low, medium, and high doses, respectively, based on CI < 1, CI = 1, and CI > 1, where CI was the combination index. A significant change in the expression levels of Bax and Bcl-2 genes, and their ratio were observed in treatment groups. There were more apoptotic cells for rotenone than for both DADS and the combination treatment group. The percentage of cell population was as follows: rotenone (UR~69%), DADS (UR~63%), and combination treatment (moderate). In conclusion, it could be seen that while rotenone had a greater effect on the induction of apoptosis, the effectiveness was inhibited in the case of the combination treatment group.
Keywords. Rotenone, Diallyl Disulphide, MCF-7, Cell Viability, Bax, Bcl-2, Flow Cytometry.

Introduction

Cancer is one of the severe diseases that has plagued people all over the world, so it is important to find new ways to treat it. Despite immense progress made in the discovery of newer forms of therapies like chemotherapy, radiation therapy, and targeted therapy strategies, several difficulties, such as drug resistance, toxicity, and tumor recurrence, have continued to occur [1-2]. Against this background, research has been conducted to identify agents effective at eliminating cancer cells. Recent cancer treatments use selective mitochondrial drugs known as "mitocans" because mitochondria play an important role in the life and death of cancer cells. Most cancer cells exhibit mitochondrial dysfunction and hence produce more ROS than normal cells [3].

Rotenone, a natural hydrophobic insecticide derived from roots, has shown great potential as an anticancer agent due to its ability to block the electron transport chain at complex I of the mitochondria. By interfering with complex, I, Rotenone causes production of ROS, which leads to activation of stress response pathways such as JNK and p38 MAP kinase and initiates mitochondria-dependent apoptosis in many forms of cancer cells, including breast cancer cells (MCF-7) [4], colon cancer [5], osteosarcomas [6], and B-cell lymphoma [7]. Various studies have shown that rotenone-induced apoptosis is strongly correlated with high levels of mitochondrial ROS. There is a direct relationship between ROS and apoptosis, which can be prevented by antioxidant treatments, including glutathione and N-acetylcysteine [8]. Recent research has proven that rotenone is effective against cancer proliferation and metastasis and potentiates chemotherapy drugs such as doxorubicin [9].

Another example of a natural anticancer compound is an organosulfur compound, diallyl disulfide (DADS), derived from garlic (*Allium sativum*) [10]. This compound is known to have antitumor potential in several cancers like breast, prostate, gastric, colorectal, and hepatocellular carcinoma [11]. Anticancer properties of DADS include induction of caspase-mediated apoptosis, changes in the Bax/Bcl-2 ratio, cell cycle arrest, inhibition of metastasis, and increased sensitivity to chemotherapy [12]. More interestingly, DADS is selective toward cancer cells [13]. It has been shown that DADS leads to cell death via apoptosis, where Bax activation triggers the mitochondrial pathway, causing the release of cytochrome c, thereby activating caspases 9 and 3 [14]. In addition to this, the oil-soluble sulfur components found in garlic, such as diallyl disulfide, possess high levels of anti-cancer characteristics through several mechanisms like enzyme induction, prevention of adducts, antioxidant effects, regulation of the cell cycle, apoptosis, angiogenesis, and metastasis [15]. DADS were found to induce apoptosis in MCF-7 cell lines via ERK inhibition and SAPK/JNK, P38 activation [16] or via inhibition of histone deacetylase (HDAC) [17].

There is no evidence to support their combination effects; however, both compounds have been reported to exhibit synergistic or additive effects when combined with other chemotherapeutic drugs. Rotenone has a synergistic effect with doxorubicin in osteosarcoma [6] and with staurosporine, resulting in G1/G2/M cell cycle phase arrest in thyroid papillary carcinoma cells [18]. It works additively when used together with TRAIL (TNF-related apoptosis-inducing ligand) in the A549 cell line [3], and with 5-fluorouracil in colon

Received: *22-02-2026* - ***Accepted***: *18-04-2026* - ***Published***: *23-04-2026*

cancer [19]. In combination with 5-fluorouracil (at a concentration of 100 µM for both DADS and 5-FU), DADS is synergistic in the colorectal cancer model (Caco-2 and HT-2) [20] and gastric cancer cell lines [21]. DADS in combination with sorafenib has an additive effect in hepatocellular carcinoma [22].
While both compounds were found to have anti-tumor or anticancer activity, they were also found to have some level of toxicity. Rotenone is a known toxin mainly used to induce Parkinson's disease in vivo rat models and in vitro in human neuroblastoma cell lines SH-SY5Y [23-24] and SK-N-MC [25]. However, the toxicity is dose-dependent, with toxicity higher than 100 nM. DADS are relatively non-toxic with minimal or less toxicity against normal cells. However, they were reported to induce hepatic toxicity at high doses [26], while a low dose of 30–60 mg/kg of body weight per day was rather protective for hepatocytes [27]. DADS was found to be cytotoxic to ovary cells in Chinese hamsters [28].
Indeed, rotenone coupled with DADS could be an exciting but less explored approach. Since both molecules are involved in targeting pathways of mitochondria and apoptosis induced via ROS, there might be some synergy. An understanding of the mechanisms by which rotenone and DADS exert their effects individually and when combined is necessary in order to explore new therapeutic regimens that take advantage of their mitochondrial targeting abilities without any antagonistic effects. The rotenone and DADS may have enhanced mitochondrial stress, high ROS in cancer cells, apoptosis, and enhanced selectivity towards cancer cell lines. The combination may have some possible antagonistic effects, as H2S released by DADS may reduce the mitochondrial dysfunction induced by rotenone and thus may decrease the rotenone cytotoxic effect [23]. The study thus focuses on their separate and combination effect on the MCF-7 cell line via MTT assay, gene expression analysis (Bax, Bcl-2), Bax/Bcl-2 apoptosis marker, and flow cytometry for their functional correlation.

Methods

Material

Rotenone, Diallyl disulfide was procured from Sigma-Aldrich, MTT (Thermo Fisher), QIAGEN RNeasy Mini Kit, Thermo Scientific RevertAid Reverse Transcriptase, Fetal bovine serum (Gibco), Dimethyl sulfoxide (Sigma-Aldrich), DMEM (Thermo Fisher), Trypsin-EDTA (Thermo Fisher).
The breast cancer MCF-7 cell lines were purchased from the National Center for Cell Science (NCCS), Pune, India.

Methods

Cell viability assay

Viability tests were conducted to estimate the half maximal concentration (IC_{50}) of the compound at varying concentrations through the Mosmann (1983) cell cytotoxicity assay [29]. Briefly, varying concentrations of the compounds were added to 24 h-grown cells for an additional 48 hours. Control groups were given culture media with the same amount of DMSO (≤0.1%). After a 48-hour incubation, 25 µL of MTT solution (0.5 mg/mL) was added to each well and incubated for 4 hours to convert MTT into formazan crystals, which reflect metabolic activity in living cells. The medium was aspirated from the wells, following 0.1 mL DMSO addition to each well; then the wells were shaken for 10 minutes to dissolve the purple formazan crystals. Optical density was then noted at 570 nm using the microplate reader.

Combination study of rotenone and diallyl disulfide using the Chou–Talalay method

The combined effect of rotenone and diallyl disulfide was estimated using the Chou–Talalay method of fixed ratio combinatorial approach. [30]. MCF-7 cells at a density of $5*10^3$ were seeded in each well of a 96-well microtitre plate, and incubated for 24 h under optimized growth conditions of 37^0 and 5% CO_2. The stock solutions of rotenone (10 mM) and DADS (100 mM) were prepared in DMSO and diluted in culture media to the desired concentrations. First, the dose-response experiments for each substance alone were conducted to estimate their individual IC_{50} values. A drug mixture was prepared in a fixed IC_{50} combination. A concentration was derived from IC_{50} values of rotenone and DADS to get a consistent ratio of drugs in a 1:3 proportion. Increasing amounts of drugs respective to 0.25X, 0.5X, 1X, 2X, and 4X times their relevant IC_{50} values, were added to each respective well and incubated for 24 h. Following the incubation, the cytotoxicity assay was performed. In order to determine whether there is any interaction between rotenone and DADS, the CI was evaluated following the Chou-Talalay equation.

$$CI = \left(\frac{D1}{Dx1}\right) + \left(\frac{D2}{Dx2}\right)$$

Where: D1: Rotenone (combo), Dx1: Rotenone IC_{50}, D2: DADS (combo), Dx2: DADS IC_{50}
with CI < 1 signifying synergism, CI = 1 additive effect, and CI > 1 antagonism.

Qualitative and Quantitative analysis of RNA

IC_{50} concentrations of rotenone, DADS, and the combination thereof were incubated with MCF-7 cells for 48 h before RNA isolation. Isolation of total RNA from the samples was performed with the aid of the QIAGEN RNeasy Mini Kit following the manufacturer's instructions with slight modifications. Cell lysis and homogenization were performed using the RLT buffer containing β-mercaptoethanol (10 µL/mL). The

Received*: 22-02-2026 -* ***Accepted****: 18-04-2026 -* ***Published****: 23-04-2026*

resulting cell lysates were diluted with 70% ethanol and applied to RNeasy spin columns for the binding of total RNA. RNA was then collected in 50 µL of RNase-free water and immediately used or frozen at −80^0 C.

First-strand cDNA synthesis

The first-strand cDNA was synthesized by reverse transcription using standard protocols. In other words, the RNA template (1 µg, or 10^{-5} µg) was combined with 1 µL of the oligo(dT)$_{18}$ primer. The reaction mixture was prepared by adding 4 µL of 5X reaction buffer together with 1 µL of RiboLock RNase inhibitor at 20 U/µL and 2µL of 10 mM dNTPs solution, and 1µL of RevertAid reverse transcriptase at 200 U/µL, which combined to create a 20 µL volume. The reaction mixture was incubated at 42°C for 60 minutes, followed by brief incubation for 5 minutes at 70^0 C. The first strand cDNA was stored in -20^0 C till further use.

Gene expression analysis

Bax and Bcl-2 gene primer sequences for QRT-PCR were designed using the NCBI primer blast tool. The primer sequences are tabulated in Table 1.

Table 1: Primer sequences for gene expression analysis

Gene	Primer Type	Sequence (5′ → 3′)	Annealing Temp (°C)
β-Actin	Forward	TCCTCCTGAGCGCAAGTAC	58–60
	Reverse	CCTGCTTGCTGATCCACATCT	58–60
BAX	Forward	TCAGGATGCGTCCACCAAGAAG	60–62
	Reverse	TGTGTCCACGGCGGCAATCATC	60–62
Bcl-2	Forward	GGTGGGGTCATGTGTGTGG	58–60
	Reverse	CGGTTATCGTACCCGTTGGC	58–60

A reaction mixture of 10 µL of SYBR Green mix, having 1 µL of each forward and reverse primer, 2 µL of cDNA template, and 6 µL of nuclease-free water was prepared. The amplification process used these specific conditions, which included initial denaturation for 3 minutes at 95^0 C; 40 cycles, including 15 sec 95^0 C (denaturation); 30 sec at 60^0 C (annealing); 30 sec at 72^0 C (extension). The comparative Ct ($2^{-\Delta\Delta Ct}$) method was used to calculate target gene expression levels for Bax and Bcl-2 genes after normalizing them to the housekeeping gene β-actin.

Cell population distribution study

To detect cell distribution, the PI staining technique was performed as described by Riccardi & Nicoletti (2006) [31]. Rotenone IC$_{50}$, DADS IC$_{50}$, and their concentration sum (Rotenone IC$_{50}$ + DADS IC$_{50}$) were employed in the assay for 48 hours, whereas the control cells were not subjected to any treatment. The test group and the control samples were removed from the wells, washed with cold PBS, and resuspended in buffer at a concentration of 1 × 10^5 cells/mL, followed by the addition of 5 µL of PI dye and 15-minute incubation in the dark. Data were analysed to distinguish PI-negative (viable) and PI-positive (membrane-compromised/dead) cell populations, as described in standard cell viability assays [32].

Statistical analysis

All the experiments were performed in triplicate. Significance was estimated by one-way ANOVA following Dunnett's post hoc test using GraphPad Prism 8.0v. Flow cytometry data were retrieved, analysed by FlowJo v11, and plots were created using RStudio.

Result and discussion

Cell viability assay and half maximal inhibition (IC$_{50}$) of a single compound

Rotenone was found to have high cytotoxicity to the cells. The results indicate a significant reduction in cell viability at different concentrations of rotenone. However, the response was not completely linear; it showed a biphasic pattern and was dose-dependent. There is a steep drop in viability at the concentration of 0.625 µM and then partial recovery of viability at the intermediate concentration levels of 1.25-2.5 µM (Figure 1). The biphasic response highlights the beneficial or adaptive effect of rotenone in the cell line at low concentrations, while the toxic effect occurs in higher concentrations. Similar results were found in SH-SY5Y neural cells [33]. The IC$_{50}$ was 4.62 µM in MCF-7 cells, consistent with the 2.5–5 µM range reported by Srivastava et al. (2007) [34]. Some studies have reported higher IC$_{50}$ values (>50 µM) in MCF-7 cell lines [35], which may be due to differences in incubation time. The partial restoration seen in the 1.25 to 2.5 µM range is most likely an indication of the activation of AMPK/AMPKα/CncC/GPX4 pathway to compensate for the stresses experienced by the mitochondria, which has been proven in rotenone treatment; thus, the optimal range of rotenone treatment is considered to be that where the protective pathways are activated, which means that when rotenone is applied together with another substance like DADS, a synergistic effect may occur.

Received*: 22-02-2026 -* ***Accepted****: 18-04-2026 -* ***Published****: 23-04-2026*

The current findings indicate that DADS exerts a nonlinear, two-phase influence on cell survival. The survival rate increased gradually with an increase in dose, starting from around 7% to 49% for low concentrations (0.625 µM - 2.5 µM) and peaked at 5 µM (87%) before a drastic drop was observed in high concentrations (10 µM – 20 µM), where survival rates fell to 63% and 21%, respectively (Figure 2). However, the observed increase in cellular viability at moderate concentrations cannot be interpreted as increased cell proliferation; rather, it reflects cellular adaptation to mild stress. Indeed, it has previously been demonstrated that allicin acts as a redox modifier; at low concentrations, it induces oxidative or electrophilic stress, activating defense mechanisms, such as Nrf2 pathway induction, thereby temporarily enhancing cellular viability [36-37].

On the other hand, high concentrations lead to ROS overproduction, which disrupts mitochondrial integrity by facilitating Bax protein representation, cytochrome c release, and the subsequent activation of caspase-3 and caspase-9, resulting in apoptosis [38-39]. All these data are consistent with numerous earlier publications demonstrating that DADS exerts mitochondria-related and caspase-dependent anticancer effects, including modulation of MAPK signaling pathways, particularly through JNK, p38 MAPK activation, and ERK1/2 inactivation [38–40]. In addition, garlic-derived organosulfur compounds, including DADS, exhibit dose-dependent hormetic responses: low concentrations activate defensive mechanisms, whereas elevated concentrations cause irreversible cellular damage [41]. Therefore, the observed dose-dependent effect proves the potential usefulness of DADS as an effective anticancer drug. The IC_{50} of DADS was found to be 14.2 µM in MCF-7 cell lines after two days of treatment. The result is found to be within the range of other reported findings of 2 to 18 µM in different breast cancer cell lines after 72 hrs. of incubation [42].

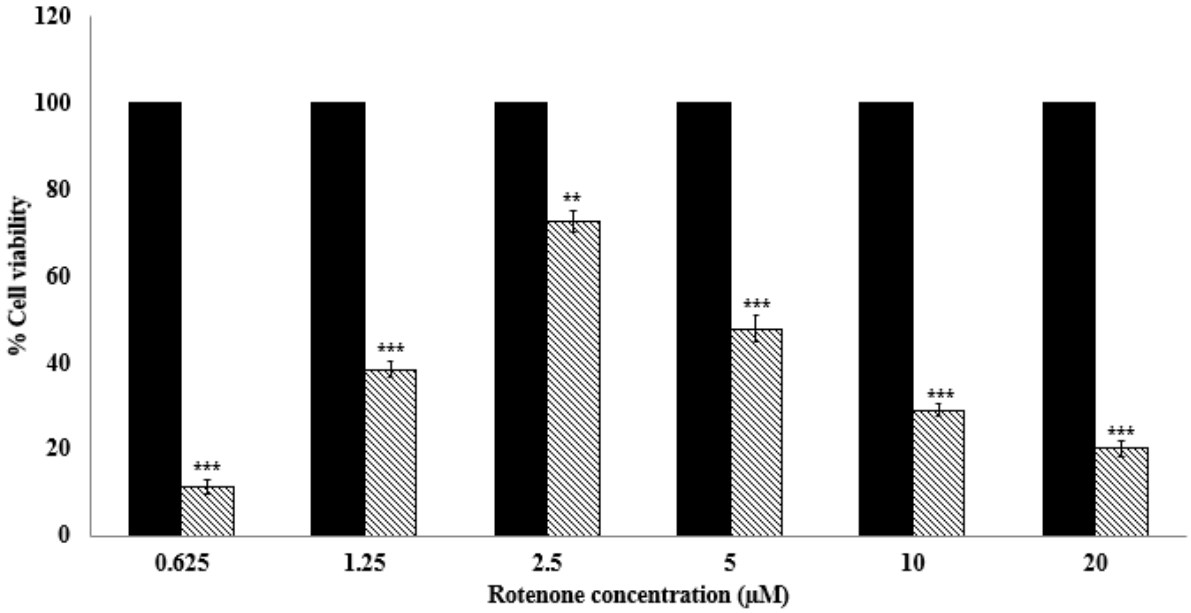

Figure 1: Cell viability assay of rotenone at different concentrations in MCF-7 cell lines. Data are expressed as mean ± SD. Statistical significance was determined by one-way ANOVA followed by Dunnett's test. *p < 0.05, **p < 0.01, ***p < 0.001 as compared to control.

Received: *22-02-2026* - ***Accepted***: *18-04-2026* - ***Published***: *23-04-2026*

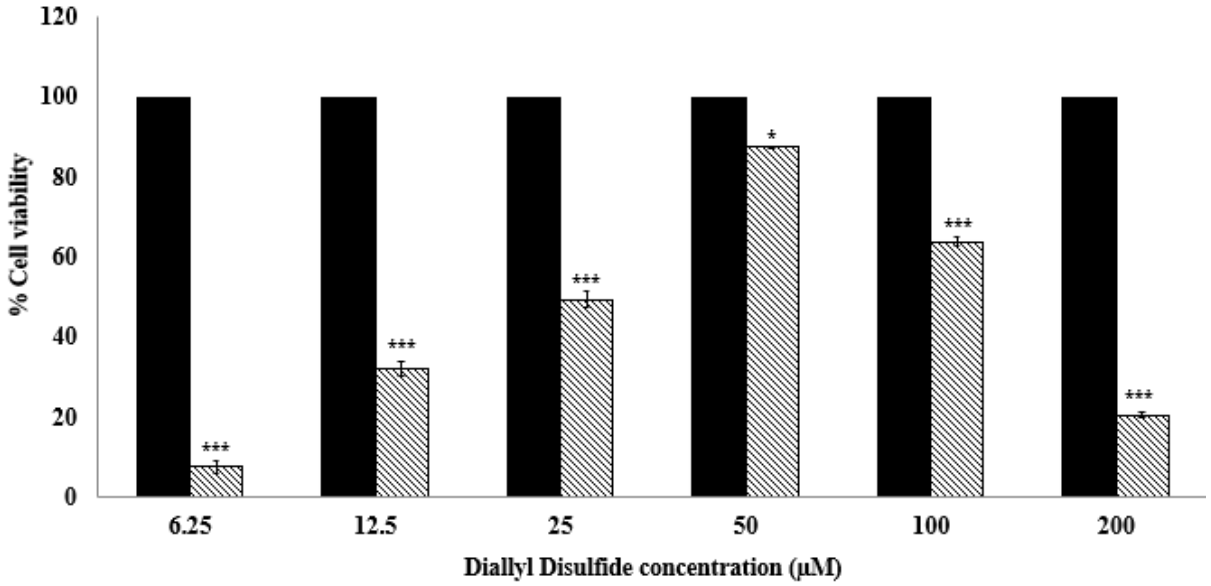

Figure 2: Cell viability assay of diallyl disulfide at different concentrations in MCF-7 cell lines. The data were presented as mean values with standard deviation. The analysis used one-way ANOVA. The statistical results showed significant differences between groups, which was followed by Dunnett's test showing ***p < 0.001.

Combination effect of drugs

In the first experiment with the ROT+DADS mixture, a biphasic dose-response curve was seen with lower cytotoxicity at intermediate dosages, suggesting antagonistic behavior (CI > 1). The reason for such a response may be the antioxidative function of DADS at certain dosages, which mitigates rotenone-induced cellular oxidation. In the following experiment, with the optimal selection of dosage and fixed ratio approach, an evident decrease in cell viability was observed, accompanied by a shift in the interaction to additive (CI = 1, Fa=0.545 (as 50% inhibition was selected). Such results suggest the importance of dosage selection to reduce the adaptation effects associated with DADS treatment. The values of Fa and CI at various concentrations showed that the effect of the ROT+DADS combination depends on the concentration. The effect can be either synergistic (CI < 1), additive (CI = 1), or antagonistic (CI > 1) (Table 2).

The value of Fa increased with concentration, suggesting greater cytotoxicity of the drugs when used in combination. Synergistic interactions were observed when the level of Fa was about 0.33, and the value of CI was less than one, whereas additivity of drug effect was observed when the value of Fa was 0.5, and CI was equal to one. On the other hand, antagonism was observed when Fa was greater than 0.7.

Table 2: Combination analysis of rotenone (ROT) and diallyl disulfide (DADS) using the Chou–Talalay method

Drug combinations	Fa (Fraction affected)	CI (Combination Index)	Interpretation
ROT (1.15 µM) + DADS (3.55 µM)	0.336	0.5	Synergistic
ROT (2.31 µM) + DADS (7.1 µM)	0.545	1	Additive
ROT (4.62 µM) + DADS (14.2 µM)	0.746	2	Antagonistic
ROT (9.24 µM) + DADS (28.4 µM)	0.808	4	Strong antagonism
ROT (18.48 µM) + DADS (56.8 µM)	0.809	8	Very strong antagonism

 # Fa: Fraction affected, which represents the proportion of the cell inhibition (e.g., Fa=0.5 and 0.8 indicate 50 and 80% inhibition, respectively); #CI: Combination Index, indicates drug interaction; ROT: Rotenone; DADS: Diallyl disulfide

Received*: 22-02-2026 -* ***Accepted****: 18-04-2026 -* ***Published****: 23-04-2026*

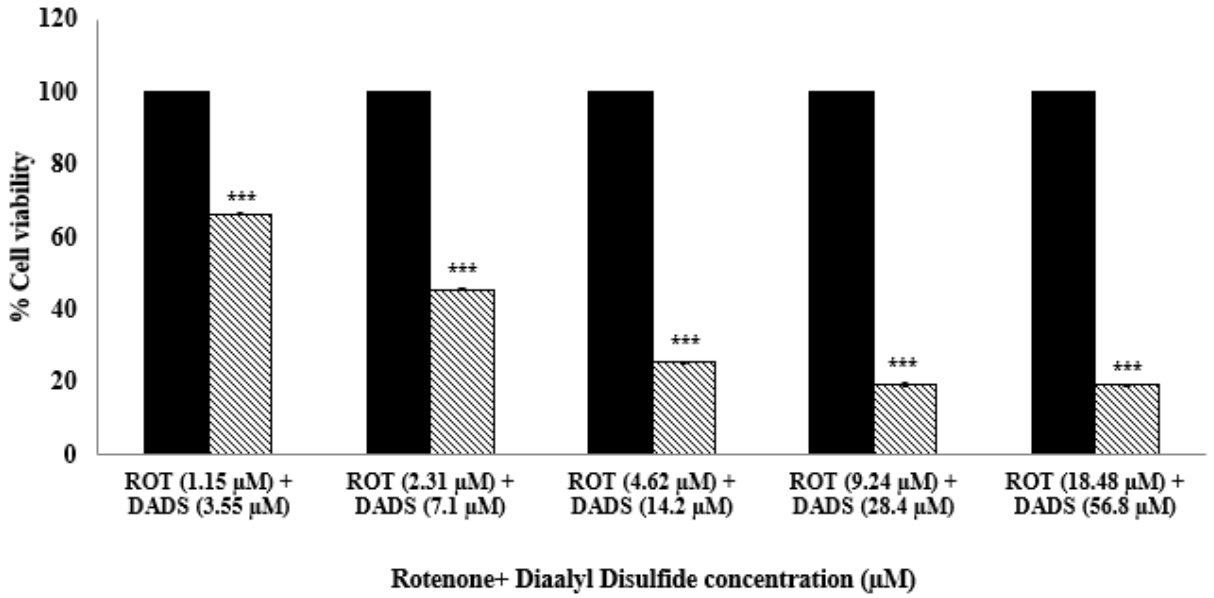

Figure 3: Combination effect of rotenone and diallyl disulfide on MCF-7 breast cancer cell lines. The data were presented as mean values with standard deviation. The analysis used one-way ANOVA. The statistical results showed significant differences between groups, which was followed by Dunnett's test showing ***p < 0.001.

Gene expression analysis of Bax and Bcl-2

Gene expression was analyzed by treating MCF-7 cells with 0.5x IC_{50} (2.31 µM ROT and 7.1 µM DADS), which provided an acceptable toxicity-to-viability balance for studying the Bax and Bcl-2 genes, which play an important role in apoptosis. Intact RNA with a purity of 1.9-2.1 (Figure 4a) and a total amount of 87-94.3 µg was extracted from MCF-7 (control), MCF-7 (untreated), MCF-7/ROT_IC_{50}, MCF-7/DADS_IC_{50}, and MCF-7/ROT+DADS_0.5× IC_{50}.

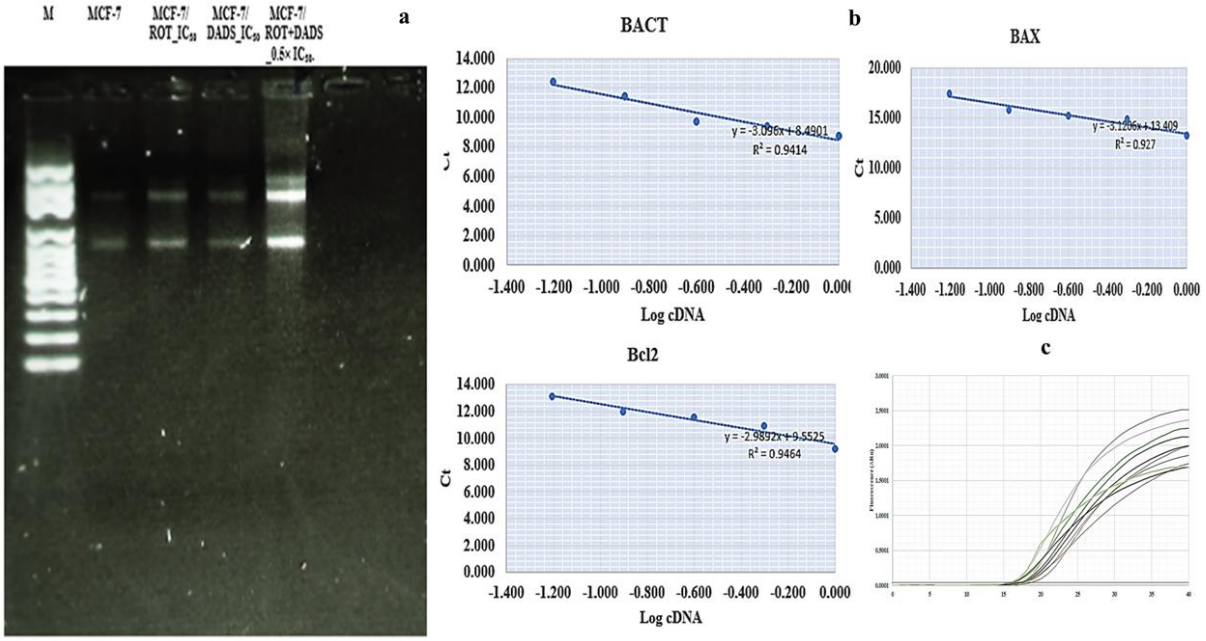

Figure 4: Gene expression analysis. a: Qualitative analysis of RNA, b: Standard curves of β-actin, Bax, and Bcl-2 genes, c: Amplification plot of gene expression analysis.

All the treated groups underwent a significant change towards apoptosis. Indeed, rotenone significantly elevated Bax expression (9.19-fold increase) while Bcl-2 expression was greatly inhibited (0.20-fold decrease), leading to a high Bax/Bcl-2 ratio (45.95), indicating a pro-apoptotic tendency. In its turn, DADS increased Bax expression (6.50-fold) and inhibited Bcl-2 expression (0.35-fold). Interestingly, the group that was subjected to the combined action of both agents showed a much more remarkable result since Bax expression was greatly induced (84.45-fold increase) while Bcl-2 expression was significantly inhibited (0.03-fold), thus providing a very high Bax/Bcl-2 ratio (2815.00) (Figure 5). Though cytotoxic assay of the combination exhibited an additive effect on the MCF-7 cell line, they showed a strong apoptotic effect, indicating strong mitochondrial apoptotic activation

***Received**: 22-02-2026 - **Accepted**: 18-04-2026 - **Published**: 23-04-2026*

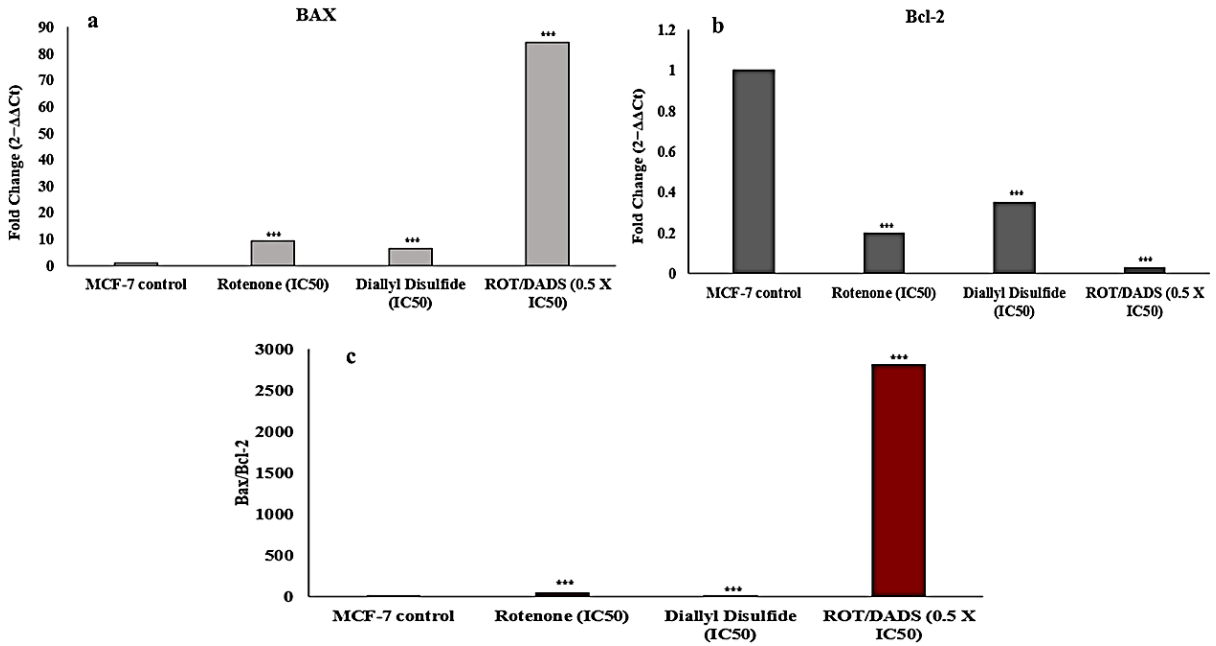

Figure 5: Gene expression levels of Bax and Bcl-2 and their corresponding ratio. The data were presented as mean values with standard deviation. The analysis used one-way ANOVA. The statistical results showed significant differences between groups, which was followed by Dunnett's test showing ***p < 0.001.

Analysis of apoptosis

It has been observed that in terms of quadrant distribution, there is the maximum presence of cells in the upper right quadrant (~69%) in MCF-7/ROT samples, followed by MCF-7/DADS (~63%), whereas, in the case of MCF-7 and MCF-7/ROT+DADS treatment, it is comparatively lower (58–59%). Increase in UR values signify greater presence of positively stained or affected cells, implying increased efficacy of the treatment in the order MCF-7/ROT > MCF-7/DADS > MCF-7 (untreated) ≈ MCF-7/ROT+DADS. The results indicate that rotenone acts more effectively than DADS; however, DADS acts in a controlled manner. Intracellular marker expression or fluorescence intensity (FL3-A) is maximum in case of MCF-7/DADS (~28,000), followed by MCF-7/ROT+DADS treatment (~16,000); on the other hand, MCF-7 (untreated) and MCF-7/ROT have comparatively lower fluorescence intensity.

It can be seen that MCF-7/ROT shows maximum UR population but lower fluorescence intensity. According to the quadrant analysis, the majority of cells fell into the UR region; hence, treatment had an impact on them. However, the cells in the combined treatment sample (MCF-7/ROT+DADS) contained the largest population of cells in the upper left region (~36%). The results indicated that the combination therapy caused delayed progression of the cells' response to treatment. As seen from morphological analysis (FSC-A and SSC-A), the sample MCF-7/ROT+DADS had the highest values of both characteristics, which implied significant changes in cell sizes and granularity caused by the treatment's cytotoxic effect. MCF-7/ROT had the highest SSC-A value among MCF-7/ROT and MCF-7/DADS, indicating greater cellular granularity due to the presence of more organelles. MCF-7, the untreated sample, had the lowest FSC and SSC values, thus demonstrating no change in morphological characteristics. Higher SSC implies apoptosis; higher FSC, on the other hand, means swelling/stress in cells. According to the cluster analysis, MCF-7 (untreated) and MCF-7/DADS had similar biological responses since they clustered together; at the same time, MCF-7/ROT and MCF-7/ROT+DADS clustered separately from others.

***Received**: 22-02-2026 - **Accepted**: 18-04-2026 - **Published**: 23-04-2026*

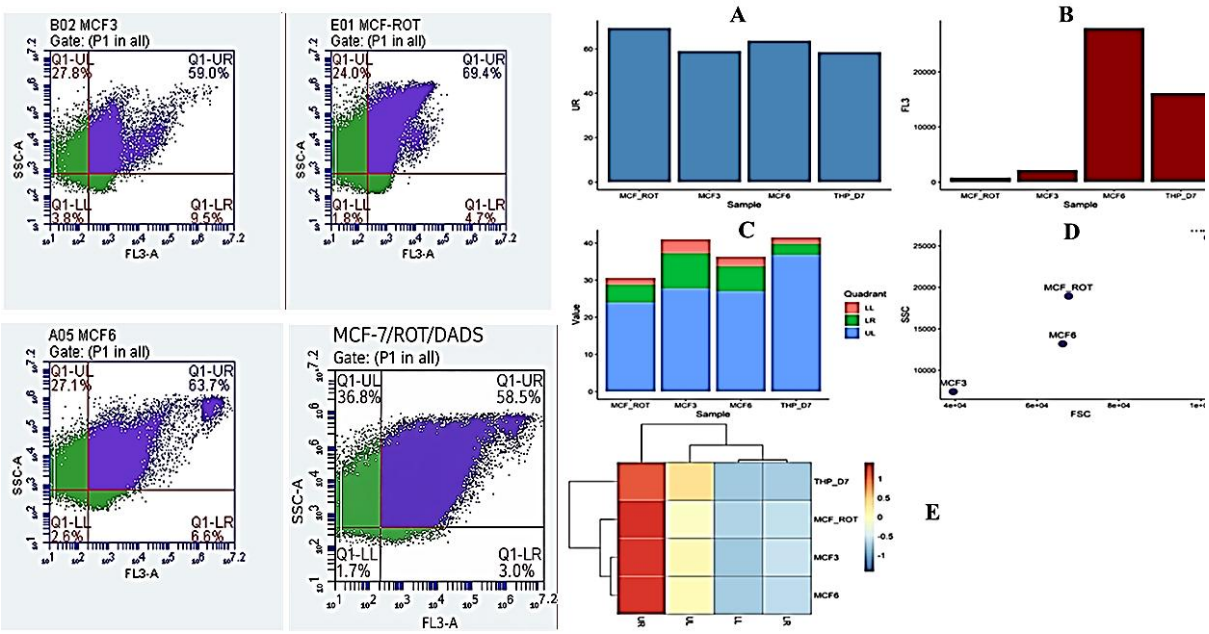

Figure 6: Flow cytometric analysis showing (A) UR population, (B) fluorescence intensity, (C) quadrant distribution, (D) cell morphology (FSC vs SSC), and (E) heatmap of cell populations across samples.

Conclusion

The combined use of the MTT assay, gene expression analysis, and flow cytometry demonstrates that the rotenone-DADS combination, despite exhibiting only additive cytotoxicity, can elicit highly synergistic apoptosis. The rotenone-DADS combination increased Bax gene expression and the apoptotic index (Bax/Bcl-2). These results were further validated using flow cytometry; rotenone induced the maximum percentage of apoptosis, whereas the combination caused a delay in apoptosis along with distinct morphological differences. Thus, this inconsistency highlights the limitation of viability tests in estimating apoptosis efficiency, whereas gene expression analysis and flow cytometry reflect the actual pro-apoptotic capacity. Therefore, it can be concluded that the optimum concentration of rotenone (2.31 µM) and DADS (7.1 µM) combination elicits mitochondria-mediated apoptosis through Bax/Bcl-2 imbalance and exhibits potential as an anticancer agent.

Conflict of interest. Nil

References

1. Paz GS, Fernandes J. Natural compounds targeting mitochondrial metabolism in cancer therapy: a literature review. Chin Clin Oncol. 2025;14(5):56.
2. Mani S, Swargiary G, Singh KK. Natural agents targeting mitochondria in cancer. Int J Mol Sci. 2020;21(19):6992.
3. Shi YL, Feng S, Chen W, Hua ZC, Bian JJ, Yin W. Mitochondrial inhibitor sensitizes non-small-cell lung carcinoma cells to TRAIL-induced apoptosis. Cell Death Dis. 2014; 5(12):e1579.
4. Deng Y, Huang H, Lin J. Rotenone induces apoptosis in MCF-7 human breast cancer cells via ROS through JNK and p38 signaling. Mol Carcinog. 2009;49(2):141–151.
5. Shi Y, Cao Z, Ge L, Lei L, Tao D, Zhong J, et al. Rotenone adaptation promotes migration and invasion of colon cancer. Clin Transl Oncol. 2024;27(6):2557–2567.
6. Ma X, Li Z, Ma H, Jiang K, Chen B, Wang W, et al. Rotenone inhibited osteosarcoma metastasis via ROS/Ca2+/AMPK pathway. Redox Rep. 2025;30(1):2493556.
7. Armstrong JS, Hornung B, Lecane P, Jones DP, Knox SJ. Rotenone-induced apoptosis in human B lymphoma cells. Biochem Biophys Res Commun. 2001;289(5):973–978.
8. Li N, Ragheb K, Lawler G, Sturgis J, Rajwa B, Melendez JA, et al. Rotenone induces apoptosis via mitochondrial ROS. J Biol Chem. 2003;278(10):8516–8525.
9. Kciuk M, Gielecińska A, Mujwar S, Kołat D, Kałuzińska-Kołat Ż, Celik I, et al. Doxorubicin: multiple mechanisms of anticancer activity. Cells. 2023;12(4):659.
10. Mitra S, Das R, Emran TB, Labib RK, Noor-E-Tabassum, Islam F, et al. Diallyl disulfide: a garlic-derived anticancer compound. Front Pharmacol. 2022; 13:943967.
11. Zhou YF, Zhu YW, Wang YW, Liang XY, Jiang QY, Wu DD. Diallyl disulfide in oncotherapy. Apoptosis. 2025;30(7-8):1730–1750.
12. Yi L, Su Q. Molecular mechanisms of diallyl disulfide anticancer effects. Food Chem Toxicol. 2013; 57:362–370.
13. Knowles LM, Milner JA. Allyl sulfides suppress neoplastic cell proliferation. J Nutr. 2001;131(3):1061S–1066S.
14. Nagaraj NS, Anilakumar KR, Singh OV. Diallyl disulfide induces Bax-mediated apoptosis. J Nutr Biochem. 2010;21(5):405–412.

Received: *22-02-2026* - ***Accepted***: *18-04-2026* - ***Published***: *23-04-2026*

15. Xu L, Li Q, Wang H, et al. Progress of diallyl disulfide in anticancer. Chemother Open Access. 2017;6(4).
16. Lei X, Yao S, Zu X, Huang Z, Liu L, Zhong M, et al. Apoptosis induced by DADS in MCF-7 cells. Acta Pharmacol Sin. 2008;29(10):1233–1239.
17. Altonsy MO, Habib TN, Andrews SC. DADS-induced apoptosis via HDAC inhibition. Nutr Cancer. 2012;64(8):1251–1260.
18. Goncalves AP, Videira A, Maximo V, Soares P. Synergistic growth inhibition by rotenone. J Biosci. 2011;36(4):639–648.
19. Denise C, Paoli P, Calvani M, Taddei ML, Giannoni E, Kopetz S, et al. 5-FU resistant colon cancer metabolism. Oncotarget. 2015;6(39):41706–41721.
20. Treviso EM, Sanchez CA, Rocha CCS, Aissa AF, Antunes LMG. DADS with 5-FU in colorectal cancer. Nutrients. 2025;17(15):2412.
21. Zhang Y, Li J, Liu H, Xia H, Su J, et al. DADS regulates EMT in gastric cancer. Oncol Res. 2025;33(12):3869–3886.
22. Machado ART, Tuttis K, Santos PWS, Aissa AF, Antunes LMG. DADS enhances sorafenib activity. Pharmaceutics. 2022;14(12):2582.
23. Hu LF, Lu M, Wu ZY, Wong PT, Bian JS. Hydrogen sulfide protects against rotenone apoptosis. Mol Pharmacol. 2009;75(1):27–34.
24. Shin WH, Chung KC. Rotenone-induced α-synuclein aggregation. Exp Neurobiol. 2020;29(3):207–218.
25. Sherer TB, Richardson JR, Testa CM, Seo BB, Panov AV, Yagi T, et al. Mechanism of rotenone toxicity. J Neurochem. 2007;100(6):1469–1479.
26. Truong D, Hindmarsh W, O'Brien PJ. DADS-induced hepatocyte toxicity. Chem Biol Interact. 2009;180(1):79–88.
27. Kim JW, Kim JH, Kim CY, Jeong JS, Lim JO, Kim JC, et al. DADS prevents hepatotoxicity. Food Chem Toxicol. 2022; 160:112814.
28. Musk SR, Clapham P, Johnson IT. Cytotoxicity of diallyl sulfide. Food Chem Toxicol. 1997;35(3-4):379–385.
29. Mosmann T. Rapid colorimetric assay for cell viability. J Immunol Methods. 1983;65(1–2):55–63.
30. Chou TC, Talalay P. Quantitative analysis of drug combinations. Adv Enzyme Regul. 1984; 22:27–55.
31. Riccardi C, Nicoletti I. Analysis of apoptosis by propidium iodide staining and flow cytometry. Nature Protocols, 2006; 1(3):1458–1461.
32. Krishan A. Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. The Journal of cell biology, 1975; 66(1):188–193.
33. Borland MK, Trimmer PA, Rubinstein JD, Keeney PM, Mohanakumar K, Liu L, et al. Rotenone and neurodegeneration. Mol Neurodegener. 2008;3:21.
34. Srivastava P, Panda D. Rotenone inhibits microtubule assembly. FEBS J. 2007;274(18):4788–4801.
35. Siddiqui MA, Saquib Q, Ahamed M, Ahmad J, Al-Khedhairy AA, Abou-Tarboush FM, et al. Resveratrol and rotenone cytotoxicity. Toxicol Int. 2011;18(2):105–110.
36. Song X, Yue Z, Nie L, Zhao P, Zhu K, Wang Q. Biological functions of DADS. Evid Based Complement Alternat Med. 2021; 2021:5103626.
37. Anantharaju P, Veeresh P, Dey S, Reddy Bovilla V, Madhunapantula SV. DADS inhibits breast cancer growth. Biomed Pharmacol J. 2017;10(4):1619–1630.
38. Yin X, Zhang J, Li X, Liu D, Feng C, Liang R, et al. DADS suppresses tumor growth. Int J Mol Sci. 2014;15(7):12422–12441.
39. Yin X, Zhang R, Feng C, Zhang J, Liu D, Xu K, et al. DADS induces apoptosis in carcinoma. Oncol Rep. 2014;32(4):1748–1756.
40. Ling H, Zhang LY, Su Q, Song Y, Luo ZY, Zhou X, et al. ERK involvement in DADS response. Cell Mol Biol Lett. 2006;11(3).
41. Cheng F, Cheng Z, Meng H, Tang X. DADS affects plant root growth. Front Plant Sci. 2016;7.
42. Nakagawa H, Tsuta K, Kiuchi K, Senzaki H, Tanaka K, Hioki K, et al. DADS inhibits breast cancer cells. Carcinogenesis. 2001;22(6):891–897.

Received*: 22-02-2026 -* ***Accepted****: 18-04-2026 -* ***Published****: 23-04-2026*