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# Diallyl disulfide-induced apoptosis in a breast-cancer cell line (MCF-7) may be caused by inhibition of histone de-acetylation

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**Abstract**

The health benefits of garlic have been proven by epidemiological and experimental studies. Diallyl disulphide (DADS), the major organosulfur compound found in garlic oil, is known to lower the incidence of breast cancer both *in vitro* and *in vivo*. The studies reported here demonstrate that DADS induces apoptosis in the MCF-7 breast-cancer cell line through interfering with cell-cycle growth phases in a way that increases the sub-$G_0$ population and substantially halts DNA synthesis. DADS also induces phosphatidylserine (PS) translocation from the inner to the outer leaflet of the plasma membrane and activates caspase-3. Further studies revealed that DADS modulates the cellular levels of Bax, Bcl-2, Bcl-xL and Bcl-w in a dose-dependent manner, suggesting the involvement of Bcl-2 family proteins in DADS induced apoptosis. Histone deacetylation inhibitors (HDACi) are known to suppress cancer growth and induce apoptosis in cancer cells. Here it is shown that DADS has HDACi properties in MCF-7 cells as it lowers the removal of an acetyl group from an acetylated substrate and induces histone-4 (H4) hyper-acetylation. The data thus indicate that the HDACi properties of DADS may be responsible for the induction of apoptosis in breast cancer cells.

**Keywords**

Breast cancer, Diallyl disulphide, Apoptosis, Histone deacetylation inhibitor.

## Introduction

Worldwide, breast cancer comprises 22.9% of all non-melanoma skin cancers and 13.7% of cancer deaths in women (1). Some of the primary risk factors for female breast cancer include age (2), lack of childbearing or breastfeeding (3), high hormone levels (*e.g.* estrogen) (4) and ethnicity (5). However, diet is another important factor that contributes to the development of breast cancer (6-8). The impact of diet on the incidence of breast cancer is indicated by geographic differences in the occurrence of the disease (9), and by the many dietary components experimentally proven to act as anti-breast cancer agents. One such dietary factors is diallyl disulphide (DADS), which is a major (~60%) organosulfur compound found in garlic oil (10). DADS inhibits the growth of breast cancer cell lines (MDA-MB-231, KPL-1, MKL-F and MCF-7) (11) and reduces the incidence of *N*-methyl-*N*-nitrosourea (MNU) and 2-amino-1-methyl-6-phenylimidazo-4-5-*b*-pyridine (PhIP)-induced mammary tumours (12, 13). The anti-cancerous effect of DADS is often attributed to its ability to induce apoptosis in cancer cells via cell-cycle arrest and modulation of the activity of Bcl-2 family proteins (14-16).

The difference between normal and cancerous cells is that the later lack the ability to die. In normal tissue, a balance between cell proliferation and cell death must be maintained, otherwise cells display uncontrolled growth and thereby cancer arises (17). Cellular apoptosis or programmed cell death is the key process in maintaining this balance. Apoptosis naturally occurs in mammalian tissues and is regulated by a cascade of cellular proteins, such as pro- and anti-apoptotic molecules. Prominent among such factors are the Bcl-2 family proteins which act as signalling molecules in cellular apoptosis and survival pathways. Bcl-2 family proteins include both pro-apoptotic (Bax, Bak, Bok, Bad, Bid, Bim and Bmf) and anti-apoptotic members (Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and Bfl-1/A1) (18, 19). Such pro-apoptotic

proteins function by promoting mitochondrial permeability, resulting in the release of apoptogenic factors (*e.g.* cytochrome *c* and apoptosis inducing factor [AIF]) from the mitochondrial inter-membrane space into the cytosol (20). The presence of such factors in the cytosolic compartment induces the activation of protease activators (caspases) that ultimately lead to apoptosis. Either increasing levels of pro-apoptotic Bcl-2 proteins or decreasing levels of anti-apoptotic Bcl-2 proteins (or a combination of both) can result in activation of the apoptotic machinery, thereby initiating apoptotic cell death. In this context, DADS up-regulates pro-apoptotic Bcl-2 proteins in many types of cancerous cells. For example, DADS-induces Bax expression and triggers the apoptotic mitochondrial pathway in MCF-7 human breast cancer cells (14), and is known to induce apoptosis through down-regulation of anti-apoptotic Bcl-2 in A549 human lung adenocarcinoma cells (21). DADS also caused down-regulation of the anti-apoptotic proteins Bcl-2 and Bcl-xL in a human colon cancer cell line (COLO 205), whereas it induced up-regulation of the pro-apoptotic Bak and Bax proteins (22). Furthermore, DADS treatment of a human prostate cancer cell line (PC-3) resulted in increased cellular levels of pro-apoptotic BAX and BAD, but decreased Bcl-2 levels (23). In a similar study by Arunkumar *et al*. (2007), the apoptotic impact of DADS in PC-3 cells were accompanied by histone (H3 and H4) hyper acetylation (24).

Aberrant gene transcription is common in malignant cells, resulting in activation of some genes and silencing of others (25, 26). Such changes in transcriptional status are often correlated with alterations in histone acetylation with hyper-acetylation mediating activation whilst de-acetylation promotes gene silencing (27, 28). Histone acetylation controls transcription by facilitating or restraining the recruitment of transcription factors on to associated promoter (29, 30). Histone acetylation has received considerable interest of late because of its link to the pathogenesis of cancer. The progression of carcinogenesis involves the transcriptional activation of 'inappropriate' genes (e.g. oncogenes, RAS, WNT, MYC,

ERK, TRK and Bcr-Abl) at the expense of tumour suppressor genes (e.g. TP53, CDKN1B, HNPCC, MEN1, APC, CD95, ST5, ST7, ST14 and BRCA) and pro-apoptotic genes (e.g. pro-apoptotic Bcl-2 proteins) (31-35). Since aberrant transcription of Bcl-2 family genes is often observed in breast cancer cells (36, 37), this raises the possibility that the modulation of expression of such genes by DADS is mediated by DADS-induced histone deacetylation inhibition (HDACi).

This possibility raised above is addressed here through the investigation of the effect of DADS on the acetylation status of histones in the breast-cancer cell line, MCF-7. The data confirm that DADS strongly induces apoptosis in MCF-7 cells, which is apparently caspase dependent. DADS also enhances histone hyper-acetylation in MCF-7 cells, through its HDACi activity, and promotes pro-apoptotic Bax but depresses anti-apoptotic Bcl-2, Bcl-xL and Bcl-w. Thus, the anti-cancerous activity of DADS could arise from its HDACi effect.

## Materials and Methods

### Cell line and treatment

The human mammary gland adenocarcinoma cell line (MCF-7) (38) was kindly provided by Professor A El-Tayeb (Assiut University) and cells were used following 43–51 passages. Cells were cultured in Dulbecco's modified Eagles's minimum essential medium (CAMBREX) supplemented with 10% heat-inactivated (60 °C, 30 min) fetal bovine serum (FBS, CAMBREX) and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin), in six-well tissue culture plates in a concentration of $10^5$ cells/ml at 37 ˚C in a 5% $CO_2$/95% humidified atmosphere. Culturing media were supplemented with DADS (Sigma, purity ≤ 80%) dissolved in dimethylsulfoxide [(DMSO), Aldrich, purity ≤ 95.5%)] in final concentrations of 1, 10 or 100 μM DADS. The DMSO final concentration did not exceed 0.1% in all treatments

including control cultures. Trichostatin A (TSA), at 1 μM final concentration, was used to inhibit histone deacetylase activities in positive control samples (39)

**Cell cycle analysis**

MCF-7 cells, treated and control, were harvested by trypsinization. The cells were washed with phosphate buffered saline (PBS). Cells were then fixed by pipetting 1 ml of cell suspension (approximately $10^6$ cells/ml) was on to 4 ml of absolute ethanol at -20 °C in a Falcon tube while vortexing at top speed. Fixed cells were re-hydrated in 5 ml PBS for 15 min at room temperature (RT). Finally, 3 mM propidium iodide (PI) in staining buffer (100 mM Tris; pH 7.4, 150 mM NaCl, 1 mM CaCl2, 0.5 mM MgCl2, 0.1% NP-40) was added and mixed by gentle pipetting. Pancreatic RNAase (50 μl of 10 μg/ml stock; Sigma) was added and the cells were incubated for 2 h at 4 °C. Cell cycle analysis was performed using a FACScan Flow Cytometer (Becton Dickson) according to the manufacturer's protocol.

**Annexin V/PI test**

MCF-7 cells were tested for apoptosis induction by DADS using Annexin V-FITC/PI double staining. Briefly, approximately $10^6$ cells were washed in cold PBS followed by two washes in binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, pH 7.4). Cells were then re-suspended in 100 μl Annexin V-FITC (4 μg/ml in binding buffer) and incubated on ice for 10 min. An additional 400 ml of binding buffer, containing PI (2 μg/ml), were added and the cellular suspension was incubated on ice for a further 15 min. Cells thus treated were then analysed by flow cytometry.

**Caspase-3 activation**

MCF-7 cells were seeded in 96-well plates at a density of $10^5$ cells/well. Plates were then incubated overnight at 37˚C in a '5% $CO_2$/95% humidity' atmosphere. DADS was added at final concentrations of 1, 10 and 100 μM and cells were incubated for an additional 24 h. For

positive controls, cells were treated with staurosporine (3 µg/ml) for 4 h (40) prior to performing the assay. Caspase-3 activation upon DADS treatment was measured using the Caspase-3 Fluorescence Assay Kit (Cayman chemicals) according to the manufacturer's instructions. In order to test assay specificity, negative control samples were prepared by adding 10 µl/well of caspase-3 inhibitor solution (provided with the kit). The plates were read on a FLUOstar OPTIMA fluorescent plate reader at 485 nm excitation and 535 nm emission wavelengths.

**Cellular lysate preparation and Western blot**

Cells were lysed with Radio-Immuno Precipitation Assay (RIPA) lysis buffer (50 mM Tris–HCl, pH 8, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 100 µg/ml PMSF [phenylmethylsulphonyl fluoride], 1 µg/ml aprotinin, 1% NP-40) for 30 min on ice. The cellular lysate was then centrifuged at 12,000×*g* for 2 min at 4 °C. The supernatant was then transferred to a fresh Eppendorf tube, and total protein concentrations were determined using the BioRad protein assay (41). Cellular lysates were then denatured with an equal volume of loading buffer (0.125 M Tris–HCl, 4% SDS, 20% v/v glycerol, 0.2 M dithiothreitol, 0.02% bromophenol blue, pH 6.8) at 100 °C for 10 min. Protein samples (50 µg) were then immediately subjected to SDS-PAGE (BioRad Mini Protein II Electrophoresis gel) and trans-blotted onto a nitrocellulose membrane (Amersham). The membranes were blocked in 5% non-fat dry milk in TBS (20 mM Tris–HCl, 500 mM NaCl, pH 7.5) overnight at 4 °C. The membranes were immuno-stained for pro-apoptotic (Bax, Bak and Bid) and anti-apoptotic Bcl-2 proteins (Bcl-2 and Bcl-xL [rabbit polyclonal, Abcam] and Bcl-w [Cell Signaling Technology®]). β-Actin primary antibody (mouse monoclonal, Abcam) was used as a loading control. Immuno-detected band visualisation was carried out using the chemiluminescent alkaline phosphatase substrate (Immobilom™ Western).

**Histone extraction and electrophoresis**

Histone extraction was performed as previously published (42). Approximately $10^7$ cells, treated and control, were resuspended in 1 ml nuclear extraction buffer (60 mM KCI, 15 mM NaCl, 3 mM $MgCl_2$, 15 mM piperazine- N/N' bis [2 ethanesulfonic acid]; pH 6.5, 0.1% NP-40, 0.5 mM phenyl methyl sulfonyl fluoride, 1 mM tetrathionate) and kept on ice for 20 min, and then centrifuged at 1300 x*g* and 4 °C for 10 min. The resulting nuclear pellet was suspended in $H_2SO_4$ (0.2 M final concentration) for 2 h and centrifuged at 10,000 x*g* and 4 °C for 10 min. The supernatants containing the histones were removed, and dissolved histones were precipitated with absolute alcohol at -20 °C. The precipitant was suspended in water and quantified by Bio-Rad protein assay (41). A 1 ml volume of sample buffer was prepared by mixing 7.7 mg dithiothreitol (DTT), 900 µl of 8 M urea, 50 µl phenolphthalein [1% w/v in 95% ethanol] and 50 µl of 30% $NH_4OH$. The sample buffer was then added to the dissolved histones in a 1:1 v/v ratio, and kept for 5 min at RT. Samples were then acidified by adding 5 µl of glacial acetic acid. Histones were electrophoresed in a Triton-Aced-Urea (TAU) gel as described previously (43).

**Nuclear extract preparation and DADS-HDACi activity analysis**

Nuclear extracts were prepared as described before (44). Approximately $10^7$ cells were lysed in 1 ml of ice cold lysis buffer (10 mM Tris-HCl, 10 mM NaCl, 15 mM $MgCl_2$, 250 mM sucrose, 0.1 mM ethylene glycol bis-[beta-aminoethylether] N,N,N[1]-tetraacetic acid [EGTA], 0.5% NP-40; pH 7.5), and kept on ice for 15 min. The suspension was then carefully pipetted over a 4 ml layer of sucrose buffer (30% sucrose, 10 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, pH 7.5), and centrifuged at 1300 x*g* and 4 °C for 10 min. The pellet was resuspended in Tris-HCl buffer (10 mM Tris-HCl, 10 mM NaCl, pH 7.5) and centrifuged at 1300 x*g*, 4 °C for 10 min. The pellet (nuclear fraction) was re-suspended in extraction buffer (50 mM HEPES, 420 mM NaCl, 0.5 mM EDTA, 0.1 mM EGTA, 10% glycerol, pH 7.5) and

ultrasonicated for 30 s and kept on ice for 30 min. The mixture was then centrifuged at 10,000 x*g* and 4 °C for 10 min. The supernatant (nuclear extract) was collected and kept at -80 °C for HDAC activity analysis. Different concentrations of DADS were tested for HDACi activity, using the fluor de lys™ fluorescent assay system (Drug discovery kit-AK-500; BIOMOL) according to the manufacture instructions. The kit includes deacetylases inhibitor (Trichostatin A [TSA]) for use as positive control. The samples were quantified on a FLUOstar OPTIMA fluorescent plate reader at 360 nm excitation and 460 nm emission wavelengths.

**Statistical Analysis**

All data were obtained from three independent experiments, and all results are expressed as mean±SE. We employed the two-tailed (or paired) Student's *t*-test, using Microsoft Excel, to determine significant differences. In all analysis, differences with probability values $\leq 0.05$ were considered significant.

## Results

### DADS induce cell-cycle arrest and apoptosis in MCF-7 cells

Cell-cycle analysis of MCF-7 cells treated with 0-100 μM DADS showed that such treatment causes changes in the occupation of the various stages of the cell cycle (Fig. 1). DADS stimulated a concentration-dependent increase in the sub-$G_0$ population (representing apoptotic bodies and/or cells with fragmented DNA) combined with a corresponding decrease in the S-phase population. Thus, 100 μM DADS induces a 56.9% ($p \leq 0.05$) reduction in the proportion of cells engaging in DNA synthesis (the S-phase population) coupled with more than a tenfold increase (from 3.16 to 31.1%; $p \leq 0.05$) in the number of apoptotic, sub-$G_0$ cells (Fig. 1). In contrast, the $G_0/G_1$ and $G_2/M$ populations were little

affected by DADS (Fig. 1). The DADS induction of apoptosis was confirmed by Annexin V/PI double staining which detects both early and late apoptotic cells (AV and PI stained cells, respectively). Annexin V binds to phosphatidylserine (PS) translocated from the inner leaflet of the plasma membrane (considered a hallmark of early apoptosis (45)). DADS increased the number of early apoptotic cells from 11.0% (in untreated cells) to 18.2 ($p \leq 0.05$), 23.2 ($p \leq 0.05$) and 34.8% ($p \leq 0.05$) in 1, 10 and 100 μM treated cells, respectively. Late apoptotic cells numbers were also increased in a dose-dependent manner from 7.17% in untreated cells to 11.2 ($p \leq 0.05$), 16.3 ($p \leq 0.05$) and 24.9% ($p \leq 0.05$) in 1, 10 and 100 μM DADS treated cells (Fig. 2). Thus, the double-staining analysis indicates that 100 μM DADS increased total apoptotic cell numbers by more than threefold from 18.2 to 59.7% ($p \leq 0.05$) (Fig. 2), a result which closely matches that obtained by single labeling (Fig. 1).

**DADS increases caspase-3 activity in MCF-7 cells**

In order to determine whether activation of caspase-3 might be involved in the observed DADS-induced apoptosis of MCF-7 cells, such cells were tested for caspase-3 activity following DADS treatment, by fluorescence assay. The results show that caspase-3 activity increased by 80.8% ± 24.9 and 270% ± 13.6 in cells treated with 10 and 100 μM DADS, respectively, compared to untreated cells (Fig. 3). Indeed, the caspase-3 induction achieved by the highest dose of DADS (370%) was close to that obtained by staurosporine (500%) (the positive control). However, low concentrations (1 μM) of DADS had no significant impact on caspase-3 activation. 'Caspase-3 inhibitor' eliminated the effects of DADS on caspase-3 activity indicating that the increases in activity observed in the absence of inhibitor were indeed caspase-3 specific (Fig. 3). These results show that DADS elicits a dose-dependent stimulation of caspase-3 activity in MCF-7 cells (Fig. 3) which suggests that caspase-3 activation is a component of the DADS-dependent induction of apoptosis in MCF-7 cells.

**DADS modulates the expression of Bcl-2 proteins in MCF-7 cells**

In order to further confirm that DADS induces apoptosis in breast cancer cells and to determine the likely regulatory pathway involved, Western blotting was used to examine the effect of DADS on the levels of three pro-apoptotic proteins (Bax, Bak and Bid) in MCF-7 cells. The results show that cellular levels of Bax were raised by 23.0% ± 8.15 and 32.4% ± 7.83, respectively, by treatment with 10 and 100 μM DADS, although levels were not notably affected by DADS at 1 μM (Fig. 4). In contrast, DADS failed to significantly increase the levels of either Bak or Bid in MCF-7 cells (Fig. 4). However, all three of the anti-apoptotic Bcl-2 proteins examined here were reduced by DADS treatment: 10 and 100 μM DADS decreased Bcl-2 cellular levels by 42.2% ± 9.13 and 64.4% ± 9.7; Bcl-xL cellular levels were decreased by 33.3% ± 8.7 and 57.7% ± 7.7; and Bcl-w cellular levels were decreased by 38.1% ± 8.4 and 68.6% ± 7.4, respectively (Fig. 4). Thus, DADS at 10-100 μM raises the MCF-7 cellular levels of at least one pro-apoptotic Bcl-2 family protein (Bax) and decreases those of at least three anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-xL and Bcl-w). These effects are again fully consistent with the DADS-induced apoptosis reported above, and roles for Bcl-2 proteins in modulating DADS-stimulated apoptosis in MCF-7 cells.

**DADS induces $H_4$ acetylation and inhibits the deacetylation activity of MCF-7 nuclear extract**

The above studies show that the apoptosis effect generated by DADS in MCF-7 cells correlates with appropriate changes in the levels Bcl-2 family proteins. Previous work suggests that such changes in protein levels may arise from alterations in expression caused by modifications of histone acetylation status (46, 47). To determine whether the effects of DADS might arise through alterations in histone acetylation, global changes in overall

histone acetylation levels were assayed using TAU gel electrophoresis (Fig.5 I). This analysis showed that histone 4 is primarily in the unacetylated and mono-acetylated forms in untreated MCF-7 cells. However, following treatment with 1 μM DADS, the di-acetylated form of histone 4 became apparent as a minor species. As the DADS concentrations were raised more so, the acetylation profiles were further altered as tri- and tetra-acetylated histone 4 became increasingly apparent (Fig. 5I). Thus, DADS treatment raises the histone-4 acetylation status of MCF-7 nuclear DNA.

To determine whether the increased histone acetylation levels are caused by DADS-mediated inhibition of deacetylase activity, the effect of DADS on the ability of MCF-7 nuclear extracts to remove acetyl groups from an acetylated substrate was investigated (Fig.5 II). The addition of DADS, at 10 and 100 μM, to MCF-7 nuclear extracts diminished deacetylation activities to 85.7% ± 17.6 and 64.2% ± 12.9 of that of controls, although 1 μM DADS had no significant effect. TSA, a potent deacetylases inhibitor (39), reduced deacetylation to 24.77% ± 7.6 of that of the control, showing that the assay was effective in reporting deacetylation inhibition. The results therefore suggest that the DADS-induced increase in acetylation of histone 4 is caused by the ability of DADS to act as a direct HDAC inibitor.

**Discussion**

The health benefits and medicinal properties of garlic have long been known. Records dating back to 1550 BC describe garlic as a "wonder drug" for a variety of diseases (48). Epidemiological studies report that high consumption of garlic decreases the risk of breast cancer (49, 50). This effect has been attributed to DADS, which is present in garlic cloves at ~140 mM as the major organosulfur compound of garlic oil (51), since it inhibits the growth of breast cancer cells *in vitro* through induction of apoptosis (14, 16). The effects of DADS are not confined to cancerous cells; it has been shown that DADS suppresses hepatic P450

2E1 protein expression and *N*-nitrosodimethylamine demethylase activity (52, 53). It is suggested that this may affect the metabolic activation of procarcinogens and the clearance rate of toxic metabolites (54).

It should be stressed that human studies on garlic consumption and cancer risk provide limited evidence of any relationship between garlic intake and protection against cancer (55-58). However, the value of such human studies is often limited by variability in the garlic preparations taken and uncertainty concerning the amounts of garlic consumed (58), and it is suggested that further human trials are required in order to establish whether dietary garlic exhibits any anti-cancerous effects (59). Important factors affecting DADS availability in food include alkalinity and processing, which highlights the need to consider various aspects of diet, as well as the composition of the garlic, when assessing any health benefit of garlic in clinical trials (60-63). Although the direct exposure of human breast cancer cells to DADS (as performed here) fails to replicate the complexity of the whole human system, such studies have the advantage of avoiding the problems raised above that are associated with diet composition effects.

The studies reported here confirm that DADS is able to induce apoptosis in the breast cancer cell line, MCF-7, by interfering with the cell cycle. DADS increased the sub-$G_0$, apoptotic population in a dose-dependent manner and at the same time decreased the occupancy of the S phase of the cycle, indicating an inhibition of DNA synthesis had occurred. These findings are consistent with those reported previously (11, 16, 64-67) where the growth inhibitory properties of DADS were also attributed to its induction of apoptosis and reduction in DNA synthesis in KPL-1, MCF-7, COLO 205, HCT-116 and B16F-10 cells. Examination of both early (*e.g.* PS translocation) and late (*e.g.* caspase-3 activation) molecular events characteristic of apoptosis confirmed the ability of DADS, at physiologically relevant concentration (10–100 μM), to induce apoptosis in MCF-7 cells.

Bcl-2 family proteins play an important role in the progression of apoptosis through controlling the release of apoptogenic factors, *e.g.* cytochrome *c* and 'apoptosis-inducing factor' (AIF), from the mitochondrial inter-membrane space into the cytosol (20, 68). Released cytochrome *c* binds to cytosolic Apaf-1 to form the 'apoptosome' in a reaction that eventually leads to the autoactivation of pro-caspase-9 (69) which in turn activates the effector caspases (-3, -6 and -7) leading to apoptosis (70). Released AIF is translocated to the nucleus where it induces DNA fragmentation (71). The balance between the levels of pro- and anti-apoptotic proteins within the cell is crucial in determining whether apoptosis progresses (72). The involvement of Bcl-2 family proteins in DADS-induced apoptosis in MCF-7 cells was suggested (Fig. 4) by the DADS-dependent increase of the cellular levels of pro-apoptotic Bax and concomitant decreases of anti-apoptotic Bcl-2, Bcl-xL and Bcl-w. The results therefore suggest the involvement of Bcl-2 family proteins in the progression of DADS-induced caspase-dependent apoptosis in MCF-7 cells. The results reported here are in accordance with previous work (14), where DADS was shown to cause caspase-dependent apoptosis in human breast cancer cells MCF-7 through the Bax-triggered mitochondrial pathway. The involvement of Bcl-2 family proteins in DADS-induced apoptosis was also shown in human lung adenocarcinoma A549 cells (21), where a decrease in the expression of Bcl-2 (but no change of Bax levels) was elicited by treatment with 200 μM DADS for 24 h.

Interest in HDAC inhibitors has risen recently because of their anticancer potential; various HDACi have the ability to suppress cancer growth and induce apoptosis *in vitro* in cancer cell cultures and *in vivo* in tumor bearing animal models (73-76). In this study, it was shown that DADS has HDACi properties when presented at relatively low concentrations (10-100 μM) that are likely to be well within dietary concentrations ranges (51). Low concentrations of DADS were found to inhibit the removal of the acetyl group from an acetylated substrate through reducing the HDAC enzymatic activity of MCF-7 nuclear extracts by up to 35.8%.

Furthermore, the appearance of di-, tri- and tetra-acetylated H4 in DADS-treated cells demonstrated the induction of histone hyper-acetylation by DADS. Induction of cellular changes characteristic of apoptosis, such as cell cycle arrest, cellular senescence, and activation of cell death in cancerous cells upon the treatment with a variety of HDAC inhibitors, has been confirmed in many studies, although the underlying mechanisms are not entirely clear (77-79). Such apoptotic changes were clearly seen here in DADS-exposed MCF-7 cells. Previous studies have shown that modulation of gene expression occurs upon alteration of the acetylation status of associated histones. It is thought that histone hyper-acetylation promotes localised chromatin de-condensation (80) which thereby facilitates binding of transcriptional factors at cognate promoters, leading to induction of gene expression. DADS is likely to operate similarly by inducing histone hyper-acetylation through its HDACi activity resulting in alterations in the expression of various apoptosis factors, such as the pro- and anti-apoptotic Bcl-2 family proteins as observed here. However, the precise mechanism by which Bcl-2 family protein expression is modulated and apoptosis is induced by DADS remains unclear. Similar effects were obtained when a renal tubular cell line (RPTC) was treated with a histone deacetylase inhibitor (suberoylanilide hydroxamic acid). This caused a decrease in Bcl-xL levels, while the expression levels of Bax and Bak remained relatively constant (81). It is unlikely that all of the expression changes observed here are a direct effect of histone acetylation status, since levels of Bcl-2, Bcl-xL and Bcl-w were decreased (rather than increased) by DADS. Thus, further work is required to decipher the precise manner in which DADS promotes apoptosis in MCF-7 breast cancer cells.

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**Abbreviations**

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| DADS | Diallyl disulphide |
| DMSO | Dimethylsulfoxide |
| FBS | Fetal bovine serum |
| HDAC | Histone deacetylation |
| HDACi | Histone deacetylation inhibitor |
| PBS | Phosphate buffered saline |
| PI | Propidium iodide |
| PS | phosphatidylserine |
| RIPA | Radio-Immuno Precipitation Assay |
| RT | Room temperature |
| TSA | Trichostatin A |

## References


1. WHO. *World most frequent cancers*. 2008 [cited; Available from: http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900.
2. Schonberg M A ,Marcantonio E R ,Ngo L ,Li D ,Silliman R A*, et al.*s. Causes of Death and Relative Survival of Older Women After a Breast Cancer Diagnosis. *J Clin Oncol*. 2011;
3. Daniilidis A ,Giannoulis C ,Sardeli C ,Dinas K ,Nasioutziki M*, et al.*s. Pregnancy-associated breast cancer--a review analysis. *Eur J Gynaecol Oncol*. 2011; 31: 485-90.
4. Yager J D and Davidson N Es. Estrogen carcinogenesis in breast cancer. *N Engl J Med*. 2006; 354: 270-82.
5. Reeder-Hayes K E ,Bainbridge J ,Meyer A M ,Amos K D ,Weiner B J*, et al.*s. Race and age disparities in receipt of sentinel lymph node biopsy for early-stage breast cancer. *Breast Cancer Res Treat*. 2011;
6. Spector D, Deroo L A and Sandler D Ps. Lifestyle Behaviors in Black and White Women With a Family History of Breast Cancer. *Prev Med*. 2011;
7. Moral R ,Escrich R ,Solanas M ,Vela E ,Costa I*, et al.*s. Diets High in Corn Oil or Extra-Virgin Olive Oil Provided From Weaning Advance Sexual Maturation and Differentially Modify Susceptibility to Mammary Carcinogenesis in Female Rats. *Nutr Cancer*. 2011; 1.
8. Stoddard F R, 2nd, Brooks A D, Eskin B A and Johannes G Js. Iodine alters gene expression in the MCF7 breast cancer cell line: evidence for an anti-estrogen effect of iodine. *Int J Med Sci*. 2008; 5: 189-96.
9. Hill M Js. Nutrition and human cancer. *Ann N Y Acad Sci*. 1997; 833: 68-78.
10. Dausch J G and Nixon D Ws. Garlic: a review of its relationship to malignant disease. *Prev Med*. 1990; 19: 346-61.
11. Nakagawa H ,Tsuta K ,Kiuchi K ,Senzaki H ,Tanaka K*, et al.*s. Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines. *Carcinogenesis*. 2001; 22: 891-7.
12. Schaffer E M, Liu J Z, Green J, Dangler C A and Milner J As. Garlic and associated allyl sulfur components inhibit N-methyl-N-nitrosourea induced rat mammary carcinogenesis. *Cancer Lett*. 1996; 102: 199-204.
13. Suzui N ,Sugie S ,Rahman K M ,Ohnishi M ,Yoshimi N*, et al.*s. Inhibitory effects of diallyl disulfide or aspirin on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats. *Jpn J Cancer Res*. 1997; 88: 705-11.
14. Nagaraj N S, Anilakumar K R and Singh O Vs. Diallyl disulfide causes caspase-dependent apoptosis in human cancer cells through a Bax-triggered mitochondrial pathway. *J Nutr Biochem*. 2011; 21: 405-12.
15. Milner J As. Preclinical perspectives on garlic and cancer. *J Nutr*. 2006; 136: 827S-831S.
16. Jun Z ,Suzuki M ,Xiao J ,Wen J ,Talbot S G*, et al.*s. Comparative effects of natural and synthetic diallyl disulfide on apoptosis of human breast-cancer MCF-7 cells. *Biotechnol Appl Biochem*. 2009; 52: 113-9.
17. Melet A ,Song K ,Bucur O ,Jagani Z ,Grassian A R*, et al.*s. Apoptotic pathways in tumor progression and therapy. *Adv Exp Med Biol*. 2008; 615: 47-79.
18. Zhou F, Yang Y and Xing Ds. Bcl-2 and Bcl-xL play important roles in the crosstalk between autophagy and apoptosis. *Febs J*. 2011; 278: 403-13.
19. Zong W X, Lindsten T, Ross A J, MacGregor G R and Thompson C Bs. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. *Genes Dev*. 2001; 15: 1481-6.

20. Murphy E, Imahashi K and Steenbergen Cs. Bcl-2 regulation of mitochondrial energetics. *Trends Cardiovasc Med*. 2005; 15: 283-90.
21. Wu X J, Hu Y, Lamy E and Mersch-Sundermann Vs. Apoptosis induction in human lung adenocarcinoma cells by oil-soluble allyl sulfides: triggers, pathways, and modulators. *Environ Mol Mutagen*. 2009; 50: 266-75.
22. Yang J S ,Chen G W ,Hsia T C ,Ho H C ,Ho C C*, et al.*s. Diallyl disulfide induces apoptosis in human colon cancer cell line (COLO 205) through the induction of reactive oxygen species, endoplasmic reticulum stress, caspases casade and mitochondrial-dependent pathways. *Food Chem Toxicol*. 2009; 47: 171-9.
23. Gayathri R ,Gunadharini D N ,Arunkumar A ,Senthilkumar K ,Krishnamoorthy G*, et al.*s. Effects of diallyl disulfide (DADS) on expression of apoptosis associated proteins in androgen independent human prostate cancer cells (PC-3). *Mol Cell Biochem*. 2009; 320: 197-203.
24. Arunkumar A, Vijayababu M R, Gunadharini N, Krishnamoorthy G and Arunakaran Js. Induction of apoptosis and histone hyperacetylation by diallyl disulfide in prostate cancer cell line PC-3. *Cancer Lett*. 2007; 251: 59-67.
25. Jing G J ,Xu D H ,Shi S L ,Li Q F ,Wang S Y*, et al.*s. Aberrant expression and localization of hnRNP-A2/B1 is a common event in human gastric adenocarcinoma. *J Gastroenterol Hepatol*. 2011; 26: 108-15.
26. Kanai M ,Hamada J ,Takada M ,Asano T ,Murakawa K*, et al.*s. Aberrant expressions of HOX genes in colorectal and hepatocellular carcinomas. *Oncol Rep*. 2011; 23: 843-51.
27. Hashimoto Y, Akiyama Y, Otsubo T, Shimada S and Yuasa Ys. Involvement of epigenetically silenced microRNA-181c in gastric carcinogenesis. *Carcinogenesis*. 2011; 31: 777-84.
28. Takai N ,Kawamata N ,Walsh C S ,Gery S ,Desmond J C*, et al.*s. Discovery of epigenetically masked tumor suppressor genes in endometrial cancer. *Mol Cancer Res*. 2005; 3: 261-9.
29. Harrison M R, Georgiou A S, Spaink H P and Cunliffe V Ts. The epigenetic regulator Histone Deacetylase 1 promotes transcription of a core neurogenic programme in zebrafish embryos. *BMC Genomics*. 2011; 12: 24.
30. Haddad F, Jiang W, Bodell P W, Qin A X and Baldwin K Ms. Cardiac myosin heavy chain gene regulation by thyroid hormone involves altered histone modifications. *Am J Physiol Heart Circ Physiol*. 2011; 299: H1968-80.
31. Mellert H S ,Stanek T J ,Sykes S M ,Rauscher F J, 3rd ,Schultz D C*, et al.*s. Deacetylation of the DNA-binding domain regulates p53-mediated apoptosis. *J Biol Chem*. 2011; 286: 4264-70.
32. Liu S ,Klisovic R B ,Vukosavljevic T ,Yu J ,Paschka P*, et al.*s. Targeting AML1/ETO-histone deacetylase repressor complex: a novel mechanism for valproic acid-mediated gene expression and cellular differentiation in AML1/ETO-positive acute myeloid leukemia cells. *J Pharmacol Exp Ther*. 2007; 321: 953-60.
33. Ohshiro K ,Rayala S K ,Wigerup C ,Pakala S B ,Natha R S*, et al.*s. Acetylation-dependent oncogenic activity of metastasis-associated protein 1 co-regulator. *EMBO Rep*. 2011; 11: 691-7.
34. Cheong J W ,Chong S Y ,Kim J Y ,Eom J I ,Jeung H K*, et al.*s. Induction of apoptosis by apicidin, a histone deacetylase inhibitor, via the activation of mitochondria-dependent caspase cascades in human Bcr-Abl-positive leukemia cells. *Clin Cancer Res*. 2003; 9: 5018-27.

35. Kai L, Samuel S K and Levenson A Ss. Resveratrol enhances p53 acetylation and apoptosis in prostate cancer by inhibiting MTA1/NuRD complex. *Int J Cancer*. 2011; 126: 1538-48.
36. Wang X ,Belguise K ,Kersual N ,Kirsch K H ,Mineva N D*, et al.*s. Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. *Nat Cell Biol*. 2007; 9: 470-8.
37. Fahy B N, Schlieman M G, Mortenson M M, Virudachalam S and Bold R Js. Targeting BCL-2 overexpression in various human malignancies through NF-kappaB inhibition by the proteasome inhibitor bortezomib. *Cancer Chemother Pharmacol*. 2005; 56: 46-54.
38. McLeskey S W ,Zhang L ,Kharbanda S ,Kurebayashi J ,Lippman M E*, et al.*s. Fibroblast growth factor overexpressing breast carcinoma cells as models of angiogenesis and metastasis. *Breast Cancer Res Treat*. 1996; 39: 103-17.
39. Duong V ,Bret C ,Altucci L ,Mai A ,Duraffourd C*, et al.*s. Specific activity of class II histone deacetylases in human breast cancer cells. *Mol Cancer Res*. 2008; 6: 1908-19.
40. Xue L Y, Chiu S M and Oleinick N Ls. Staurosporine-induced death of MCF-7 human breast cancer cells: a distinction between caspase-3-dependent steps of apoptosis and the critical lethal lesions. *Exp Cell Res*. 2003; 283: 135-45.
41. Bradford M Ms. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72: 248-54.
42. Cousens L S and Alberts B Ms. Accessibility of newly synthesized chromatin to histone acetylase. *J Biol Chem*. 1982; 257: 3945-9.
43. Waterborg J Hs. Dynamics of histone acetylation in vivo. A function for acetylation turnover? *Biochem Cell Biol*. 2002; 80: 363-78.
44. Waldecker M ,Kautenburger T ,Daumann H ,Veeriah S ,Will F*, et al.*s. Histone-deacetylase inhibition and butyrate formation: Fecal slurry incubations with apple pectin and apple juice extracts. *Nutrition*. 2008; 24: 366-74.
45. van Heerde W L ,Robert-Offerman S ,Dumont E ,Hofstra L ,Doevendans P A*, et al.*s. Markers of apoptosis in cardiovascular tissues: focus on Annexin V. *Cardiovasc Res*. 2000; 45: 549-59.
46. Fortson W S ,Kayarthodi S ,Fujimura Y ,Xu H ,Matthews R*, et al.*s. Histone deacetylase inhibitors, valproic acid and trichostatin-A induce apoptosis and affect acetylation status of p53 in ERG-positive prostate cancer cells. *Int J Oncol*. 2011; 39: 111-9.
47. Kim H ,Kim S N ,Park Y S ,Kim N H ,Han J W*, et al.*s. HDAC inhibitors downregulate MRP2 expression in multidrug resistant cancer cells: implication for chemosensitization. *Int J Oncol*. 2010; 38: 807-12.
48. Block Es. The chemistry of garlic and onions. *Sci Am*. 1985; 252: 114-9.
49. Levi F, La Vecchia C, Gulie C and Negri Es. Dietary factors and breast cancer risk in Vaud, Switzerland. *Nutr Cancer*. 1993; 19: 327-35.
50. Challier B, Perarnau J M and Viel J Fs. Garlic, onion and cereal fibre as protective factors for breast cancer: a French case-control study. *Eur J Epidemiol*. 1998; 14: 737-47.
51. Athanasios C ,Kimbaris N G ,Siatis C S ,Pappas P A ,Tarantilis D J*, et al.*s. Quantitative analysis of garlic (Allium sativum) oil unsaturated acyclic components using FT-Raman spectroscopy *Food Chemistry*. 2006; 94: 287-295.
52. Wu C C ,Sheen L Y ,Chen H W ,Kuo W W ,Tsai S J*, et al.*s. Differential effects of garlic oil and its three major organosulfur components on the hepatic detoxification system in rats. *J Agric Food Chem*. 2002; 50: 378-83.

53. Brady J F, Li D C, Ishizaki H and Yang C Ss. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. *Cancer Res*. 1988; 48: 5937-40.
54. Melino S, Sabelli R and Paci Ms. Allyl sulfur compounds and cellular detoxification system: effects and perspectives in cancer therapy. *Amino Acids*. 2011; 41: 103-12.
55. Dorant E, van den Brandt P A and Goldbohm R As. Allium vegetable consumption, garlic supplement intake, and female breast carcinoma incidence. *Breast Cancer Res Treat*. 1995; 33: 163-70.
56. Galeone C ,Pelucchi C ,Levi F ,Negri E ,Franceschi S*, et al.*s. Onion and garlic use and human cancer. *Am J Clin Nutr*. 2006; 84: 1027-32.
57. Franceschi S ,Parpinel M ,La Vecchia C ,Favero A ,Talamini R*, et al.*s. Role of different types of vegetables and fruit in the prevention of cancer of the colon, rectum, and breast. *Epidemiology*. 1998; 9: 338-41.
58. Kim J Y and Kwon Os. Garlic intake and cancer risk: an analysis using the Food and Drug Administration's evidence-based review system for the scientific evaluation of health claims. *Am J Clin Nutr*. 2009; 89: 257-64.
59. Rivlin R Ss. Can garlic reduce risk of cancer? *Am J Clin Nutr*. 2009; 89: 17-8.
60. Kice J L and Rogers T Es. Mechanisms of the alkaline hydrolysis of aryl thiolsulfinates and thiolsulfonates. *J.Am.Chem.Soc*. 1974; 96: 8009-8015.
61. Müller Bs. Analytische Bewertung von Knoblauchpräparaten *Dtsch.Apoth.Ztg* 1989; 129: 2500-2504.
62. Kerckhoffs D A, Brouns F, Hornstra G and Mensink R Ps. Effects on the human serum lipoprotein profile of beta-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *J Nutr*. 2002; 132: 2494-2505.
63. Lawson L Ds. Garlic for total cholesterol reduction. *Ann Intern Med*. 2001; 35: 65-66.
64. Tsubura A, Lai Y C, Kuwata M, Uehara N and Yoshizawa Ks. Anticancer effects of garlic and garlic-derived compounds for breast cancer control. *Anticancer Agents Med Chem*. 2011; 11: 249-53.
65. Lai K C ,Hsu S C ,Kuo C L ,Yang J S ,Ma C Y*, et al.*s. Diallyl sulfide, diallyl disulfide, and diallyl trisulfide inhibit migration and invasion in human colon cancer colo 205 cells through the inhibition of matrix metalloproteinase-2, -7, and -9 expressions. *Environ Toxicol*. 2011;
66. Pratheeshkumar P, Thejass P and Kutan Gs. Diallyl disulfide induces caspase-dependent apoptosis via mitochondria-mediated intrinsic pathway in B16F-10 melanoma cells by up-regulating p53, caspase-3 and down-regulating pro-inflammatory cytokines and nuclear factor-kappabeta-mediated Bcl-2 activation. *J Environ Pathol Toxicol Oncol*. 2010; 29: 113-25.
67. Song J D ,Lee S K ,Kim K M ,Park S E ,Park S J*, et al.*s. Molecular mechanism of diallyl disulfide in cell cycle arrest and apoptosis in HCT-116 colon cancer cells. *J Biochem Mol Toxicol*. 2009; 23: 71-9.
68. Reed J C, Jurgensmeier J M and Matsuyama Ss. Bcl-2 family proteins and mitochondria. *Biochim Biophys Acta*. 1998; 1366: 127-37.
69. Nakabayashi J and Sasaki As. A mathematical model for apoptosome assembly: the optimal cytochrome c/Apaf-1 ratio. *J Theor Biol*. 2006; 242: 280-7.
70. Onuki R, Kawasaki H, Baba T and Taira Ks. Analysis of a mitochondrial apoptotic pathway using Bid-targeted ribozymes in human MCF7 cells in the absence of a caspase-3-dependent pathway. *Antisense Nucleic Acid Drug Dev*. 2003; 13: 75-82.
71. Delavallee L, Cabon L, Galan-Malo P, Lorenzo H K and Susin S As. AIF-mediated caspase-independent necroptosis: a new chance for targeted therapeutics. *IUBMB Life*. 2010; 63: 221-32.

72. Ploner C, Kofler R and Villunger As. Noxa: at the tip of the balance between life and death. *Oncogene*. 2008; 27 Suppl 1: S84-92.
73. Bhalla K Ns. Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. *J Clin Oncol*. 2005; 23: 3971-93.
74. Vanhaecke T, Papeleu P, Elaut G and Rogiers Vs. Trichostatin A-like hydroxamate histone deacetylase inhibitors as therapeutic agents: toxicological point of view. *Curr Med Chem*. 2004; 11: 1629-43.
75. Bolden J E, Peart M J and Johnstone R Ws. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov*. 2006; 5: 769-84.
76. Marks P A and Breslow Rs. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. *Nat Biotechnol*. 2007; 25: 84-90.
77. Zhao X ,Yang W ,Shi C ,Ma W ,Liu J*, et al.*s. The G1 phase arrest and apoptosis by intrinsic pathway induced by valproic acid inhibit proliferation of BGC-823 gastric carcinoma cells. *Tumour Biol*. 2011; 32: 335-46.
78. Chuang J Y and Hung J Js. Overexpression of HDAC1 induces cellular senescence by Sp1/PP2A/pRb pathway. *Biochem Biophys Res Commun*. 2011; 407: 587-92.
79. Thangaraju M ,Cresci G A ,Liu K ,Ananth S ,Gnanaprakasam J P*, et al.*s. GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. *Cancer Res*. 2009; 69: 2826-32.
80. Kurdistani S K and Grunstein Ms. Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol*. 2003; 4: 276-84.
81. Dong G ,Wang L ,Wang C Y ,Yang T ,Kumar M V*, et al.*s. Induction of apoptosis in renal tubular cells by histone deacetylase inhibitors, a family of anticancer agents. *J Pharmacol Exp Ther*. 2008; 325: 978-84.

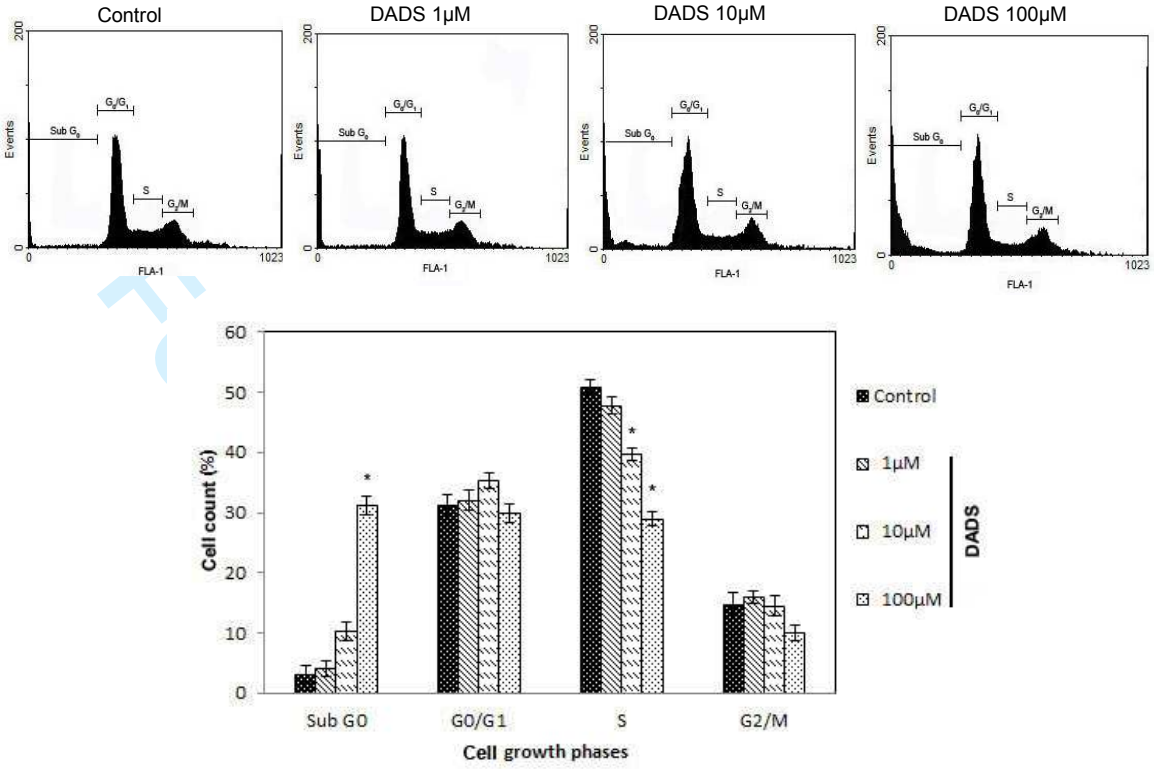


**Figure 1:** Cell-cycle analysis of PI labeled MCF-7 cells following treatment with 1, 10 and 100 μM DADS for 24 h. The results are reported as mean ± SE of three independent experiments. *, significance ($p \leq 0.05$).

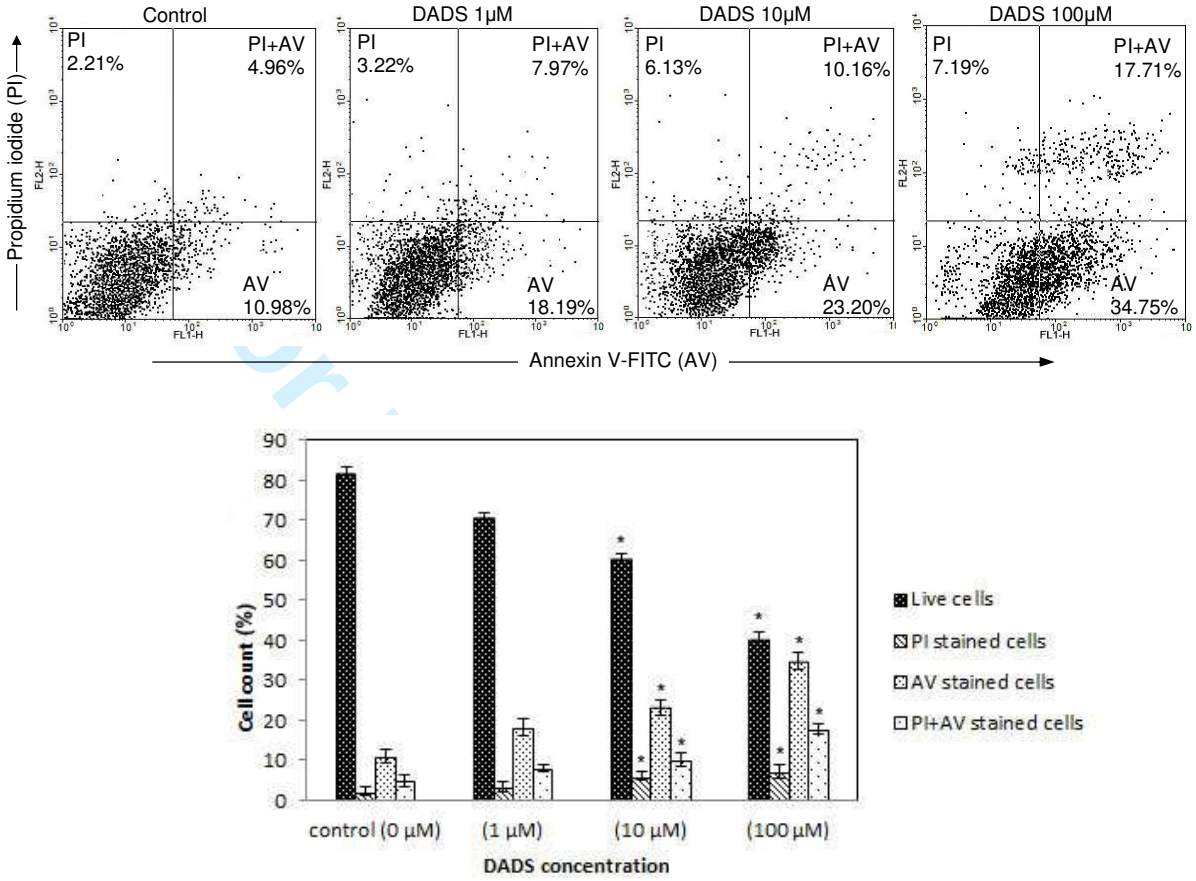


**Figure 2:** Estimation of the effect of DADS on the proportion of MCF-7 cells in late and early apoptotic states. The analysis was performed by flow-cytometry of Annexin V/PI-labeled cells following DADS (1, 10 and 100 μM) treatment for 24 h. The results are reported as mean ± SE of three independent experiments. *, significant ($p \leq 0.05$).

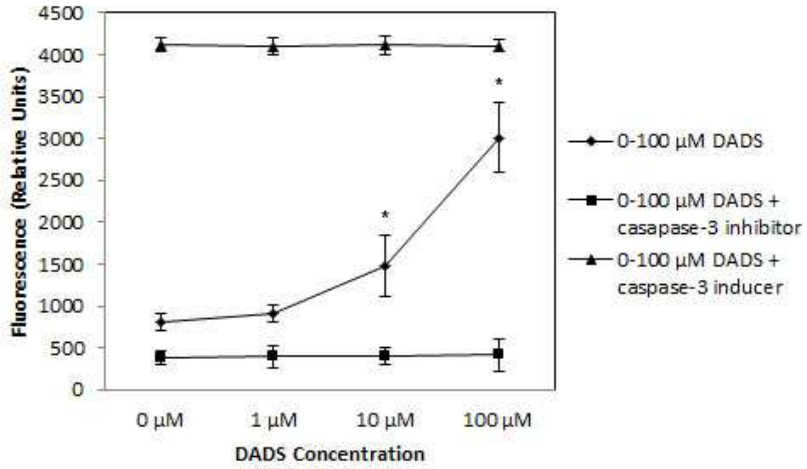


**Figure 3:** Fluorescence assay of caspase-3 activity in MCF-7 cells following DADS (1, 10 and 100 µM) treatment for 24 h. The positive control consists of MCF-7 cells treated as above except for the inclusion of the caspase-3 inducer, staurosporine (3 µg/ml), for the final 4 h. Negative controls were also obtained as above except that the inducer was replaced by a caspase-3 inhibitor solution (10 µl; see Methods) which was present for the entire 24 h growth period. The results are reported as mean ± SE of three independent experiments. *, significant ($p \leq 0.05$).

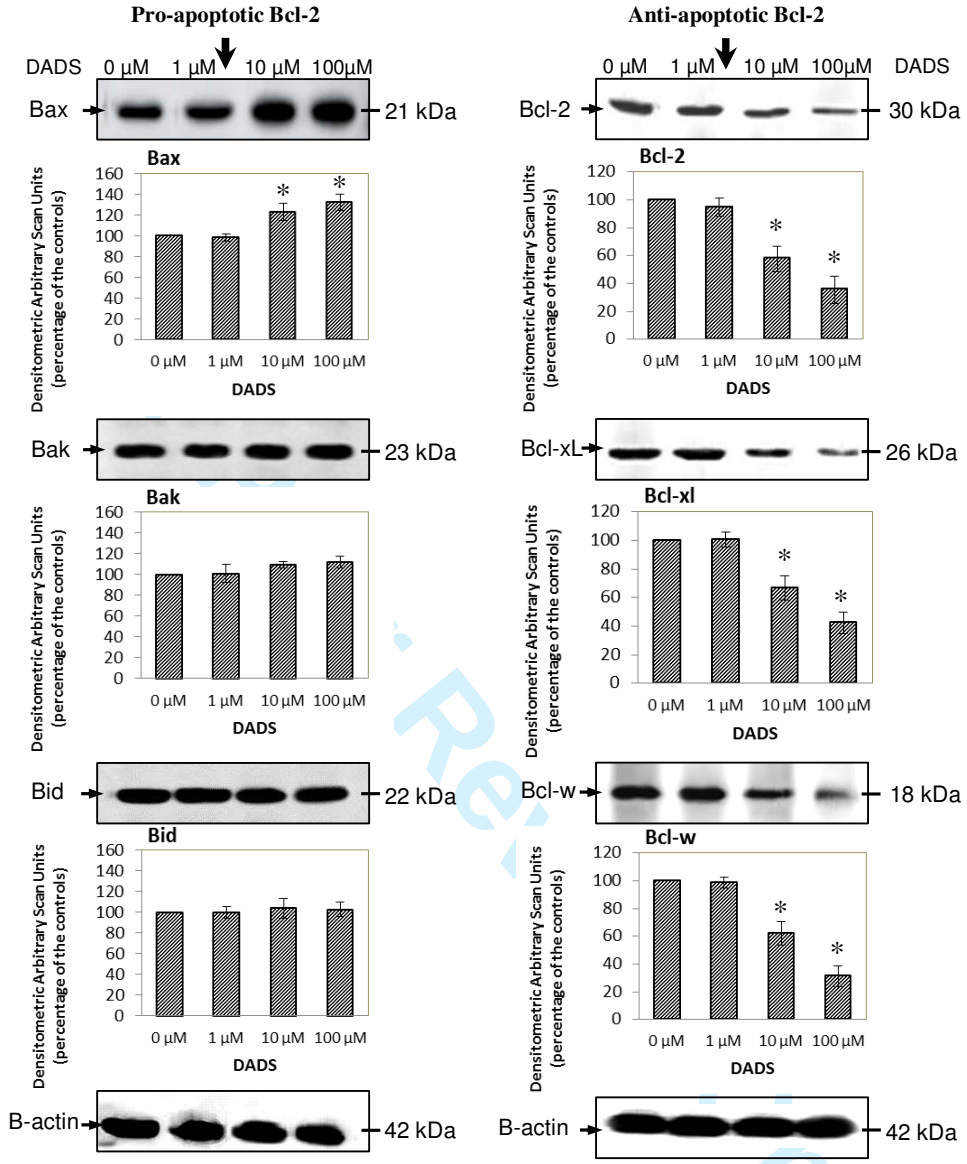


**Figure 4:** Western blot and densitometric analysis showing the effect of DADS on the levels of pro- and anti-apoptotic Bcl-2 proteins in MCF-7 cells. Cell cultures were treated with DADS for 24 h. Loading control, β-actin detection. Densitometric analysis was performed using TotaLabTM software. The results are reported as mean ± SE of three independent experiments. *, significant ($p \leq 0.05$).

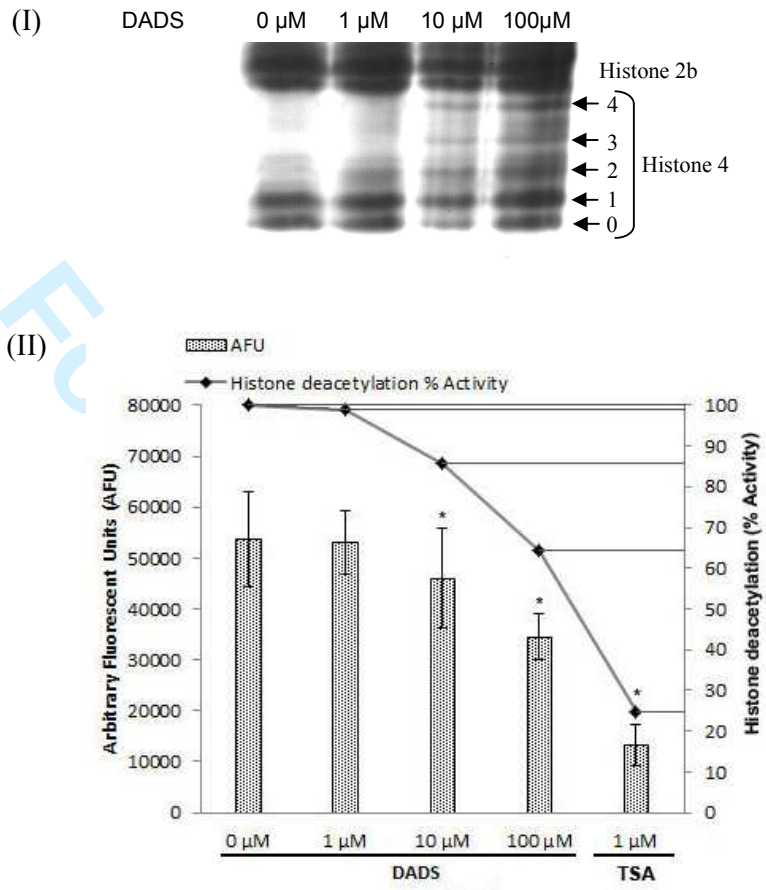


**Figure 5:** Effect of DADS on histone acetylation in Mcf-7 cells. **(I)** DADS induction (24 h) of histone 4 hyper-acetylation in MCF-7 cells, assessed by TAU gel electrophoresis as in materials and methods. **(II)** DADS inhibition of histone deacetylation in MCF-7 nuclear extracts as determined using the Fluor de Lys substrate (Fluorimetric Assay/Drug Discovery Kit; BIOMOL); data is represented as arbitrary fluorescent units and measured as a percentage of the fluorescence signal obtained with no addition of DADS or TSA to the reaction mixture. The results are reported as mean ± SE of three independent experiments. *, significant ($p \leq 0.05$).