Research Square

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Differential Oxidative Stress and Cytotoxic Responses to 5- Hydroxymethylfurfural (5-HMF) in Breast Cancer Cell Lines: Regulatory Effects of Vitamin E

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Abstract

5-Hydroxymethylfurfural (5-HMF) is a compound formed by heat treatment of carbohydrate-rich foods and is widely used to assess food quality. However, increasing research indicates that 5-HMF can exert toxic effects on cells through mechanisms involving oxidative stress. Since different breast cancer subtypes have differing baseline oxidative balances and capacities to neutralize free radicals, how these cells respond to 5-HMF exposure and whether antioxidants like vitamin E can modify these responses remains unclear. In this study, researchers evaluated the toxic, oxidative, and cell-death properties of 5-HMF and the protective potential of vitamin E using two human breast cancer cell lines (MCF-7 and MDA-MB-231) and, for comparison, normal human bronchial epithelial cells (BEAS-2B). Cell viability (IC_{50}) was determined via SRB assay at 24 and 48 hours. Oxidative parameters evaluated through various markers and enzyme activities, while Western blot analysis of caspase-3 and cleaved caspase-8 confirmed the initiation of cell death. The data showed that 5-HMF increased the rate of cell death in all examined cell types, especially with prolonged exposure and higher concentrations. When we examined the oxidative stress response, we observed that each cell type reacted differently. BEAS-2B and MCF-7 cells exhibited an antioxidant/adaptive redox response characterized by a decrease in oxidative stress markers and lipid peroxidation. In contrast, MDA-MB-231 cells, with elevated oxidative stress markers, increased lipid peroxidation, and decreased antioxidant enzyme activity, demonstrated a limited capacity to counteract oxidative stress. These results demonstrate that intracellular defense mechanisms play a critical role in shaping cell-type-specific responses to 5-HMF.

1. Introduction

Breast cancer is the most found cancer in women in the world. It causes a big health problem for many, even though we have made big steps in finding it early and treating it in new ways. We know that genes and body hormones can lead to breast cancer. Still, more and more proof now shows that things around us and the food we eat matter, too. These can help initiate cancer, help it grow, and change how it reacts when we try to treat it [1–4]. In particular, exposure to food-derived toxic compounds and redox-active substances has become a significant regulator of cancer cell survival, oxidative stress equilibrium, and apoptosis-related signaling pathways [5–7].

5-hydroxymethylfurfural (5-HMF) is a chemical compound generated from diet, produced through the Maillard reaction during the heat processing and extended storage of carbohydrate-rich foods such as honey, fruit juices, baked goods, and coffee [8,9]. Although 5-HMF has traditionallly been regarded as an indicator of food quality and processing intensity, increasing experimental evidence indicates that it exhibits biological activity with toxicological significance, particularly at heightened exposure levels [8,10]. Recent in vitro and in vivo investigations have shown that 5-HMF may induce oxidative stress, facilitate lipid and protein oxidation, and potentially activate apoptosis-related signaling via redox-dependent processes [10,12]. From a toxicological perspective, these results suggest that 5-HMF should not be considered as an inert dietary byproduct but rather a physiologically active molecule that can alter cellular redox balance, especially in cells with diminished antioxidant defense capabilities. Especially,

cancer cells with restricted redox adaptability or increased basal oxidative stress may demonstrate enhanced sensitivity to dietary-derived factors, hence increasing cytotoxic and apoptotic responses [11,13].

Oxidative stress is an important part of cancer biology and notably contributes to genomic instability, metabolic reprogramming, cellular proliferation and regulation of apoptosis [5]. Cellular redox homeostasis is preserved through a meticulously regulated equilibrium between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms [6]. Breast cancer is distinguished by considerable variability in redox regulation and sensitivity to oxidative stress, with different molecular subtypes exhibiting varying abilities to handle reactive oxygen species and dependence on antioxidants [14]. This diversity may noticeably influence cellular susceptibility to oxidative damage induced by environmental and dietary toxicants, such as 5-HMF.

Vitamin E, among dietary and endogenously produced antioxidants, has been extensively studied for its potential to protect cell membranes from lipid peroxidation and oxidative damage [15,16]. In addition to its classic chain-breaking antioxidant role, vitamin E is increasingly being studied for its potential to regulate redox-sensitive signaling pathways, modulate apoptosis-related molecular events, and regulate the cellular response to toxin-induced oxidative stress [15]. These properties position vitamin E as a promising redox-modulating agent for addressing oxidative damage mediated by dietary pro-oxidant compounds. Nonetheless, the biological efficacy of vitamin E may vary depending on the cellular context, redox status, and the nature of the oxidative damage [16].

Despite significant research on oxidative stress and antioxidant defense mechanisms in cancer, investigations focusing on dietary toxins like 5-HMF and antioxidant-based interventions simultaneously in breast cancer models are limited. In particular, there is insufficient and fragmented evidence regarding vitamin E's ability to modulate 5-HMF-induced cytotoxicity, oxidative stress, and apoptosis across various breast cancer subtypes, as well as in normal epithelial cells for comparative analysis [10,12–15]. This signifies a crucial deficiency in the existing literature, considering the notable redox heterogeneity identified among breast cancer subtypes.

Therefore, the present study aimed to evaluate the cell line–specific effects of Vitamin E on 5-HMF–induced cytotoxicity and oxidative stress–related imbalance in oxidant/antioxidant status by assessing cell viability, oxidative stress status (TOS, TAS, OSI), lipid peroxidation (MDA), antioxidant enzyme activities (CAT, MPO), and apoptosis-related markers in MCF-7 and MDA-MB-231 cells, with comparative analysis in BEAS-2B cells.

2. Materials And Methods

2.1 Cell Culture

Human breast cancer cell lines MCF-7 (RRID:CVCL_0031) and MDA-MB-231 (RRID:CVCL_0062) were used as experimental breast cancer cells, while the BEAS-2B cell line (RRID:CVCL_0168) served as

human bronchial epithelial. All cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. Cells were maintained at 37°C in a humidified incubator containing 5% CO_2. Cell morphology and growth were routinely monitored using an inverted microscope. Upon reaching an 70–80% confluence level, cells were collected using trypsin–EDTA (0.25%) solution and subcultured for subsequent experimental procedures [17].

2.2 Cell Viability Analysis by Sulforhodamine B (SRB) Assay

Cell viability was evaluated using the sulforhodamine B (SRB) assay, a well-established colorimetric method based on the quantification of total cellular protein content, which is directly proportional to cell number [18]. Cells were seeded into 96-well plates at a density of 5,000 cells per well and allowed to attach for 24 h prior to treatment.

Two experimental designs were employed, and all experiments were performed in triplicate.

Experimental Design I:

To determine the half-maximal inhibitory concentration (IC_{50}) of 5-HMF, MCF-7, MDA-MB-231, and BEAS-2B cells were treated with increasing concentrations of 5-HMF (0.2–2 mM). Following 24 and 48 h of incubation, cell viability was assessed. IC_{50} values were calculated using nonlinear regression analysis based on dose–response curves.

Experimental Design II:

To evaluate the modulatory effects of Vitamin E on 5-HMF–induced cytotoxicity, MCF-7 and MDA-MB-231 cells were co-treated with the IC_{50} concentration of 5-HMF and varying concentrations of Vitamin E (50–400 µM) for 48 h. [19].

Cell viability was assessed using the sulforhodamine B (SRB) assay as previously described [20]. Cell viability of treated cells was calculated using the formula: viability (%) = 100 × (sample absorbance)/(control absorbance).

2.3 Preparation of Cell Lysates and Protein Quantification

Control and treatment groups were harvested, and cell lysates were obtained according to a previously described protocol [21]. Total protein content was quantified using the Pierce™ BCA Protein Assay Kit in accordance with the manufacturer's instructions. Lysates were aliquoted and stored at − 80°C until use for oxidative stress marker measurements (TAS, TOS, OSI, MDA, MPO, and CAT) and Western blot analyses. All measurements were performed in triplicate.

2.4 Determination of Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI)

Total antioxidant status (TAS) and total oxidant status (TOS) were measured using commercially available colorimetric assay kits (Rel Assay Diagnostics, Türkiye) following the methods described by Erel [20,21].

TAS values were calculated based on the inhibition of hydroxyl radical–mediated oxidative reactions, whereas TOS values reflected the oxidation of ferrous ions to ferric ions by oxidant molecules present in the samples. Absorbance was measured at 660 nm for TAS and 530 nm for TOS.

Results were expressed as mmol Trolox equivalent/L for TAS and μmol H_2O_2 equivalent/L for TOS. The oxidative stress index (OSI) was calculated as the ratio of TOS to TAS and expressed in arbitrary units.

2.5 Measurement of Oxidative Stress Markers and Antioxidant Enzymes

Levels of malondialdehyde (MDA), catalase, and myeloperoxidase (MPO) in cell lysates were measured using commercially available ELISA-based kits. These parameters were selected as indicators of lipid peroxidation, antioxidant defense, and oxidative stress–related cellular responses [22,23]. Absorbance was read at 450 nm using a microplate reader.

2.6 Western Blot Analysis

Protein expression levels of apoptosis-related markers were analyzed by Western blotting. Following protein quantification, equal amounts of protein (30 μg) were separated on 10% SDS–polyacrylamide gels and transferred onto nitrocellulose membranes. Membranes were blocked with 5% bovine serum albumin in TBS-T for 1 h at room temperature.

Membranes were incubated overnight at 4°C with primary antibodies against caspase-3 (CST, # 9665) and cleaved caspase-8 (CST, # 9496), followed by incubation with HRP-conjugated secondary antibody (CST #7074). Protein bands were visualized using a chemiluminescence detection system as previously described [24] β-actin (sc-47778, Santa Cruz Biotechnology, Inc) was used as an internal loading control.

2.7 Statistical analyses

Statistical analyses were performed using GraphPad Prism 9.4.1 (GraphPad Software, USA). Data are presented as mean ± SD from at least three independent experiments. One-way ANOVA was used for single-factor comparisons, while two-way ANOVA was applied for cell viability assays. Multiple comparisons were performed where appropriate. A p value < 0.05 was considered statistically significant (*p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001).

3. Results and Discussion

3.1 Effect of 5-HMF on Cell Viability and Determination of IC_{50} Values

5-HMF is a product of the Maillard reaction and has been described in the literature as a compound capable of modulating both cytotoxicity and redox homeostasis in biological systems [25,26–27]. In this study, the effects of 5-HMF were comparatively investigated in the healthy BEAS-2B cell line and the breast cancer cell lines MCF-7 and MDA-MB-231 following 24- and 48-hour exposures. In addition, under cytotoxic conditions induced by 5-HMF at the IC_{50} level, the potential modulatory effects of Vitamin E co-treatment were evaluated with respect to cell viability, oxidative stress/antioxidant defense parameters, and apoptosis-related markers.

At 24 h, BEAS-2B cells did not exhibit a significant loss of viability, and intermediate concentrations (250–500 μM) were associated with a modest increase in viability. In contrast, both breast cancer cell lines demonstrated a clearer dose-dependent susceptibility at this time point. Specifically, MCF-7 cells showed a significant reduction in viability at concentrations ≥ 1000 μM, whereas MDA-MB-231 cells displayed significant decreases starting at substantially lower concentrations (≥ 250 μM). Collectively, these data indicate that BEAS-2B cells largely maintain tolerance to low-to-moderate 5-HMF doses during short-term exposure, while 5-HMF elicits an earlier and more pronounced cytotoxic response in MDA-MB-231 cells compared with MCF-7 cells.

The 48-hour results further highlighted both dose and time dependence. Low-to-medium doses again tended to increase viability in BEAS-2B cells; however, viability declined at high concentrations (particularly ≥ 1000 μM), indicating that higher dose levels and prolonged exposure can overcome cellular tolerance and cause toxicity in healthy cells. This biphasic dose–response pattern reflects transient metabolic or adaptive changes at moderate doses, while cytotoxic effects become dominant at higher concentrations. Dose-related changes in metabolic activity that affect viability assay signals may also have an impact on such increases in viability. Overall, these results are consistent with research indicating that 5-HMF can exhibit hormetic behavior under specific circumstances, encouraging adaptive responses at low doses but exhibiting negative effects at higher doses [28,29].

The type of breast cancer cell line affected the degree and timing of cytotoxicity. Longer exposure greatly increases cytotoxicity in MCF-7 cells, as demonstrated by a significant and consistent decline in viability at ≥ 500 μM at 48 hours, even though the 24-hour effect is limited to higher concentrations. MDA-MB-231 cells demonstrated a more pronounced decrease in viability at earlier dose levels and after 48 hours in comparison to MCF-7 cells, suggesting higher sensitivity and more potent time-amplified cytotoxicity. This divergence is consistent with reports that the TNBC model MDA-MB-231's aggressive phenotype and differences in metabolic/redox adaptations can lead to different reactions to toxic agents [30,31–32].

IC_{50} values were computed from the 48-hour SRB data to measure sensitivity. For MCF-7, MDA-MB-231, and BEAS-2B cells, the IC_{50} of 5-HMF was 746.6 μM, 230.3 μM, and 1756.9 μM, respectively. A relatively selective cytotoxic profile of 5-HMF toward malignant cells is indicated by the significantly lower IC_{50} values in cancer cells when compared to the healthy cell line. A notable sensitivity in the TNBC model is

highlighted by the significantly lower IC_{50} in MDA-MB-231, indicating that 5-HMF's cytotoxic effect is stronger in this more aggressive breast cancer subtype under the tested conditions.

3.2 Effect of Vitamin E on 5-HMF–Induced Cytotoxicity

MCF-7 and MDA-MB-231 breast cancer cells were co-treated for 48 hours with increasing concentrations of Vitamin E (50–400 µM) and the IC_{50} concentration of 5-HMF to determine whether Vitamin E modulates 5-HMF–induced cytotoxicity. The SRB assay was used to evaluate cell viability after co-treatment.

In contrast to the control group that received the IC_{50} dose of 5-HMF alone, Fig. 2 shows that all Vitamin E co-treatment groups had decreased cell viability. The interaction between vitamin E and 5-HMF does not follow a simple concentration-dependent trend in these conditions, as evidenced by the notable lack of a dose-response relationship across the range of tested vitamin E concentrations. The magnitude of the responses varied between the two cell lines as well. At all vitamin E doses, MDA-MB-231 cell viability was markedly reduced, indicating a strong and consistent sensitization to cytotoxicity during co-treatment. However, MCF-7 cells only showed a marked decline in viability at higher vitamin E concentrations (200 and 400 µM), indicating a more limited or threshold-like response pattern.

These findings demonstrate that adding vitamin E to the cytotoxicity condition caused by 5-HMF at the IC_{50} level further decreases the viability of breast cancer cells in addition to the effect of 5-HMF alone (Fig. 2). Despite its well-known antioxidant qualities, vitamin E can also make cancer cells more cytotoxic by altering redox balance and stress-response pathways in a dose-dependent and cell-type-dependent way [33,34]. MDA-MB-231 cells were sensitive to all tested doses of vitamin E, while MCF-7 cells only showed significant susceptibility at higher concentrations. The co-treatment response profile in this study differed significantly between cell lines. This implies that the cytotoxic contribution of vitamin E may cause some cell types to display threshold-dose characteristics.

Based on these combination results, 200 µM Vitamin E was selected for subsequent experiments because it produced a pronounced reduction in viability in both cell lines, providing a consistent experimental condition for downstream analyses. Finally, it should be emphasized that results derived from *in vitro* models should not be directly extrapolated to clinical supplementation outcomes; Vitamin E's effects are highly dependent on dose, context, and cell type. Nevertheless, even though direct clinical translation is not appropriate, the response patterns observed under the tested experimental conditions appear broadly compatible with general trends discussed in the clinical literatüre [35–36].

3.3 Alterations in Oxidative Stress Parameters Following 5-HMF and Vitamin E Treatment

To clarify the biological mechanisms associated with 5-HMF-induced cytotoxicity, oxidative stress and antioxidant defense status were assessed after 48 hours of treatment by measuring total oxidant status

(TOS), total antioxidant status (TAS), and calculating the oxidative stress index (OSI; expressed as TOS/TAS, TOS/TAS×100). The effects of 5-HMF, both alone and in together with vitamin E, on redox balance was evaluated in BEAS-2B, MCF-7, and MDA-MB-231 cells, with results displayed in Fig. 3 (A-C).

In generally, the redox response to 5-HMF depended significantly on the cell line. In BEAS-2B and MCF-7 cells, 5-HMF treatment diminished TOS, elevated TAS, and led to a substantial decrease in OSI, signifying a comprehensive transition of the redox balance towards antioxidant capacity rather than oxidative burden. Vitamin E co-treatment significantly diminished the already decreased OSI in these cells, indicating an enhanced antioxidant dominance under the experimental conditions (Fig. 3C). This pattern suggests that in BEAS-2B and MCF-7 cells, 5-HMF did not primarily induce a “pro-oxidant toxicity” profile; rather, it seemed to stimulate redox regulation marked by an adaptive/antioxidant response.

In contrast, MDA-MB-231 cells displayed an opposite redox pattern. Following 5-HMF exposure, TOS increased, TAS decreased, and OSI rose, reflecting a shift toward oxidative stress. When Vitamin E was added, OSI was partially reduced; however, it was not restored to the level observed in the control group, indicating only a limited correction of the redox imbalance. Taken together, these findings suggest that 5-HMF induces a more oxidative, stress-promoting environment in MDA-MB-231 cells, whereas it promotes an antioxidant-adaptive redox response in BEAS-2B and MCF-7 cells.

On the other hand, MDA-MB-231 cells showed a different redox pattern. Following 5-HMF exposure, TOS increased, TAS decreased, and OSI rose, reflecting a shift toward oxidative stress. When Vitamin E was added, OSI was partially reduced; however, it was not restored to the level observed in the control group, indicating only a limited correction of the redox imbalance. These results indicate that 5-HMF induces a more oxidative and stress-inducing environment in MDA-MB-231 cells, while it promotes an antioxidant-adaptive redox response in BEAS-2B and MCF-7 cells.

These results align with previous reports indicating that 5-HMF may stimulate adaptive/antioxidant responses rather than exerting pro-oxidant toxicity under certain experimental conditions [27,28–37].

In our comparison investigation, we assessed the cytotoxic threshold of 5-HMF relative to standard human exposure levels. The IC50 values for MDA-MB-231, MCF-7 nd BES-2B cells after 48 hours were found to be 230.3 µM, 746.6 µM, and 1756.9 respectively (around 29.04; 94,15 and 221,56 mg/L). On the other hand, pharmacokinetic data shows that there is a big difference in how the body processes drugs, even if the average daily human diet ranges from 5 to 30 mg (up to 150 mg in extreme circumstances). After taking doses of up to 4000 mg by mouth, the highest plasma concentration (Cmax) in humans was just 0.699 mg/L. Even though erythrocytes had higher concentrations (around 3.64 mg/L), the concentrations employed in this study (29.04; 94,15 and 221,56 mg/L) are still 8 to 40 times higher than the values found in vivo [38].

3.4 Effects on Lipid Peroxidation and Antioxidant Enzyme Activities

To better understand the oxidative damage profile related to 5-HMF exposure and the possible modulatory effect of Vitamin E, lipid peroxidation and antioxidant enzyme activities were assessed post-treatment. Figure 4 shows that catalase (CAT) activity and myeloperoxidase (MPO) activity were used as markers of enzymatic antioxidant/defense, and malondialdehyde (MDA) levels were used as a marker of lipid peroxidation. The results from both the treatment and control groups generally corroborated the redox balance findings derived from observable OSI variations.

In BEAS-2B and MCF-7 cells, treatment with 5-HMF resulted in a non-significant increase in CAT (Fig. 4A) and MPO (Fig. 4B) activities, indicating a slight inclination towards the activation of enzymatic defense mechanisms. Nonetheless, these alterations did not reach statistical significance, suggesting that the principal protective shift may not be contingent upon robust enzyme induction. MDA levels, which show lipid peroxidation and oxidative membrane damage, were consistently much lower after 5-HMF exposure in both BEAS-2B and MCF-7 cells [39]. This drop was even bigger when Vitamin E was added (Fig. 4C). The suppression of MDA and the absence of significant enzyme upregulation support the interpretation that oxidative damage did not escalate in these cell lines; rather, damage-related markers were reduced, likely indicating an overall redox shift towards the antioxidant direction at the TOS/TAS level, rather than a substantial induction of CAT and MPO activity [40].

Conversely, MDA-MB-231 cells displayed a markedly different pattern indicative of a damage-dominant oxidative response. In this cell line, exposure to 5-HMF diminished CAT and MPO activities, signifying a compromised antioxidant/defense capacity, while concurrently elevating MDA levels (Fig. 4C). This finding corresponds with the notable elevation of OSI previously recorded in MDA-MB-231 cells, indicating that antioxidant defenses were inadequately activated to mitigate the heightened oxidative burden. Vitamin E co-treatment somewhat improved the profile, as shown by a slight rise in CAT and MPO activities and a drop in the high MDA levels, but these values did not go back to normal. Notably, even though MDA levels were lower in the combination group than in the 5-HMF group alone, they were still much higher than in the control group. This means that oxidative damage was only partially reduced, not completely reversed.

These data collectively substantiate that the redox effect of 5-HMF is significantly dependent on the cell line. In BEAS-2B and MCF-7 cells, there was a clear antioxidant-adaptive response profile. In contrast, MDA-MB-231 cells showed a pattern that was more pro-oxidant and focused on damage. The partial normalization with Vitamin E indicates that, within these experimental parameters, Vitamin E may have limited capacity to fully restore redox balance in the MDA-MB-231 model [41,42]. Overall, the findings indicate understanding 5-HMF's redox behavior requires consideration of each cell line's basal redox state, antioxidant capacity, and metabolic characteristics, rather than assuming a single "unidirectional" oxidative effect across cell types [6,43].

3.5 Analysis of Apoptosis-Related Protein Expression

In our study, total caspase-3 was evaluated as an indicator of apoptotic involvement in the execution phase[44] while cleaved caspase-8 was examined to investigate the potential activation of the extrinsic (death receptor-mediated) pathway.[45] Together, these markers provided an initial framework for distinguishing general apoptotic signaling from death receptor-associated contributions.

To explore whether 5-HMF–induced cytotoxicity and the modulatory effect of Vitamin E are associated with apoptotic signaling, apoptosis-related protein expression was evaluated by densitometric analysis. Total caspase-3 and cleaved caspase-8 levels were assessed in BEAS-2B and MDA-MB-231 cells following treatment (Fig. 5A–B). MCF-7 cells were not included in this analysis because they lack caspase-3 expression [24].

Overall, the apoptosis-related findings differed markedly by cell line. In BEAS-2B cells, densitometric analysis showed no significant differences in total caspase-3 levels among the experimental groups (Fig. 5A). This lack of caspase-3 modulation supports the interpretation that 5-HMF, either alone or in combination with Vitamin E, did not trigger notable apoptotic activation in the healthy cell model and is consistent with the more limited toxicity profile observed in BEAS-2B cells in earlier assays.

In contrast, MDA-MB-231 cells exhibited an apoptosis-associated response. Total caspase-3 expression increased following 5-HMF exposure, and this increase was further enhanced in the 5-HMF + Vitamin E co-treatment group (Fig. 5A). When interpreted together with the oxidative stress and damage patterns observed in the same cell line—particularly increased OSI and elevated lipid peroxidation (MDA)—these results suggest that 5-HMF–induced cytotoxicity in MDA-MB-231 may be linked to cell death processes involving apoptotic mechanisms, and that Vitamin E co-treatment may potentiate this signaling under the tested conditions [46, 47].

Cleaved caspase-8 levels did not differ significantly between groups (Fig. 5B), indicating that there is no strong evidence for dominant activation of the extrinsic (death receptor–mediated) apoptotic pathway. Accordingly, the presence of increased caspase-3 in MDA-MB-231 alongside unchanged cleaved caspase-8 may support the possibility that apoptotic signaling proceeds predominantly via intrinsic/mitochondrial mechanisms rather than through extrinsic pathway activation [48].

(B–C) Densitometric quantification of caspase-3 and cleaved caspase-8 protein expression levels.

β-actin was used as the loading control.

4. Conclusion

In conclusion, this study demonstrates that the effects of 5-HMF differ significantly depending on the cell line, and that the redox response is related to cellular defense capacity. For a correct physiological interpretation of these data, the concentrations employed in vitro must be compared with human daily exposure levels. Our investigation employed an IC_{50} values of (29.04; 94,15 and 221,56 mg/L) for cells, which is markedly elevated (about 8 to 40 times) compared to the peak plasma concentrations (0.699

mg/L) recorded in people following oral administration. This quantitative discrepancy suggests that in vitro conditions may not accurately reflect metabolic and detoxification processes in the human body; thus, care is advised in directly linking these cytotoxic effects to dietary 5-HMF intake. In BEAS-2B cells, 5-HMF exposure resulted in a shift in redox balance towards the antioxidant/adaptive direction and a decrease in lipid peroxidation markers without causing significant cytotoxicity. Under the same conditions, co-treatment of 5-HMF and vitamin E further supports and stabilizes this antioxidant orientation.

In MCF-7 cells, 5-HMF-induced cytotoxicity was more pronounced with high dose/long exposure; an antioxidant/adaptive trend in redox parameters and a decrease in MDA levels were observed. Similarly, co-treatment of vitamin E promote a stabilizing antioxidant orientation in MCF-7 cells; however, its effect on viability followed a duration- and dose-dependent profile. In MDA-MB-231 cells, the response to 5-HMF manifested as an earlier and more pronounced cytotoxicity, along with a pro-oxidant/damage-dominant redox profile; this was supported by increased OSI and MDA and decreased catalase. Although 5-HMF + Vitamin E co-treatment provided a partial recovery in redox balance, it did not fully return to control levels; consequently, it may have increased viability loss. The observation of a pro-oxidant/damage-dominant response accompanying 5-HMF in MDA-MB-231 cells, along with apoptotic signals, points to a significant redox fragility in the context of the TNBC model and suggests that this axis may be therapeutically targeted.

In general, 5-HMF has shown cell line-specific effects due to differences in intracellular redox and defense capacity. Vitamin E co-treatment tends to support/stabilize antioxidant orientation across cell types; however, in redox-sensitive TNBC-like phenotypes, antioxidant support may be insufficient to completely eliminate 5-HMF-associated toxicity.

To interpret these findings more clearly, it is important to perform detailed confirmation of the type of cell death (apoptosis/necrosis), confirmatory analyses such as caspase activity and PARP cleavage; and to add complementary readouts such as ROS species, mitochondrial function indicators, and the Nrf2-antioxidant response axis to elucidate the source of the redox response [49].

This study demonstrates that the biological effects of 5-HMF are highly cell line–dependent and closely associated with differences in intracellular redox balance and defense capacity. In BEAS-2B cells, 5-HMF exposure shifted the redox state toward an antioxidant/adaptive profile, accompanied by reduced lipid peroxidation and minimal cytotoxicity. Under the same conditions, vitamin E co-treatment further supported this antioxidant-oriented response.

In MCF-7 cells, 5-HMF-induced cytotoxicity became evident primarily at higher concentrations and longer exposure durations, while redox parameters indicated an overall antioxidant/adaptive trend together with decreased MDA levels. Vitamin E co-treatment similarly promoted stabilization of redox balance in MCF-7 cells, although its impact on cell viability remained dose- and time-dependent.

In contrast, MDA-MB-231 cells exhibited an earlier and more pronounced sensitivity to 5-HMF, characterized by a pro-oxidant, damage-dominant redox profile, increased OSI and lipid peroxidation, and reduced catalase activity. Although vitamin E co-treatment partially attenuated redox imbalance in this cell line, it did not fully restore control conditions and may have contributed to enhanced loss of viability. The coexistence of oxidative damage and apoptotic signaling in MDA-MB-231 cells highlights a pronounced redox vulnerability in the TNBC model and suggests that redox-related pathways may represent potential therapeutic targets.

Overall, these findings underscore that the cellular response to 5-HMF is determined by intrinsic redox homeostasis rather than a uniform toxic mechanism. While vitamin E generally exhibits an antioxidant-oriented redox profile, its protective capacity appears to be limited in redox- fragile TNBC-like phenotypes such as MDA-MB-231.

In this study, vitamin E did not exhibit a dominant pro-oxidant or redox cycle behavior. Instead, its activity varied depending on the cell's baseline antioxidant defense capacity and appeared to act as a redox modulator and membrane-associated antioxidant. Accordingly, antioxidant support alone may be insufficient to reverse 5-HMF-associated damage in highly oxidative cellular environments.

Further studies incorporating direct ROS measurements, mitochondrial function analyses, and detailed characterization of cell death pathways are warranted to clarify the mechanistic basis of these cell type–specific responses.

Declarations

Funding Declaration

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Conflicts of Interest:

The authors declare no conflict of interest

Author Contribution

G.V. conceived the study and performed the literature search. H.Ş., S.A., and H.D. carried out the experiments. H.Ş. and G.V. prepared the figures and visualized the data. T.G. assisted in the writing of the manuscript. G.V. and H.D. provided supervision throughout the research process. All authors reviewed and approved the final manuscript.

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Data Availability

All data generated or analyzed during this study are included in this published article [and its supplementary information files]. Further datasets are available from the corresponding author on reasonable request

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Figures

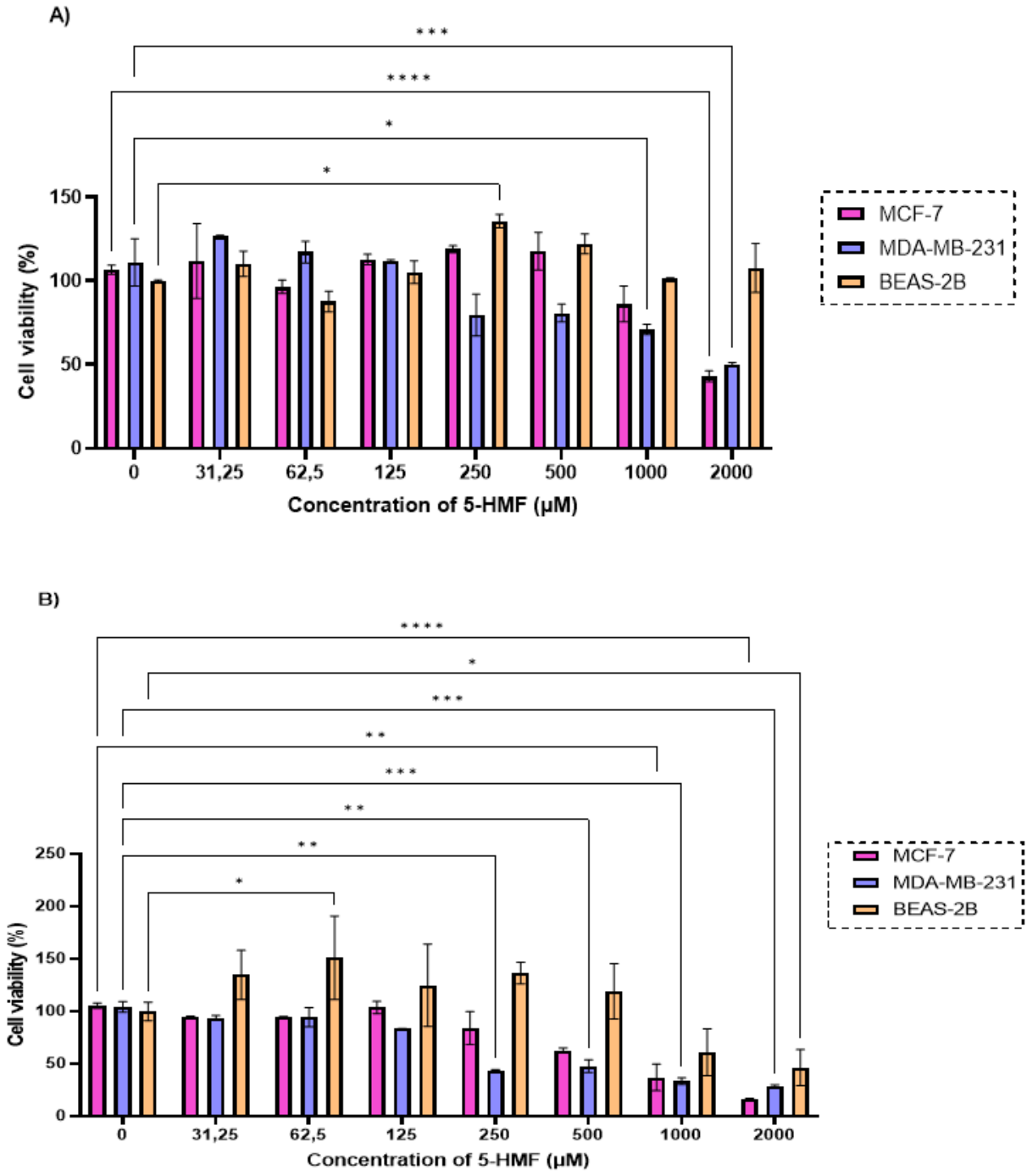


Figure 1

Cell viability of MCF-7, MDA-MB-231, and BEAS-2B cells after 24-hour (A) and 48-hour (B) exposure to 5-hydroxymethylfurfural (5-HMF).Cell viability is expressed as a percentage relative to untreated control cells.

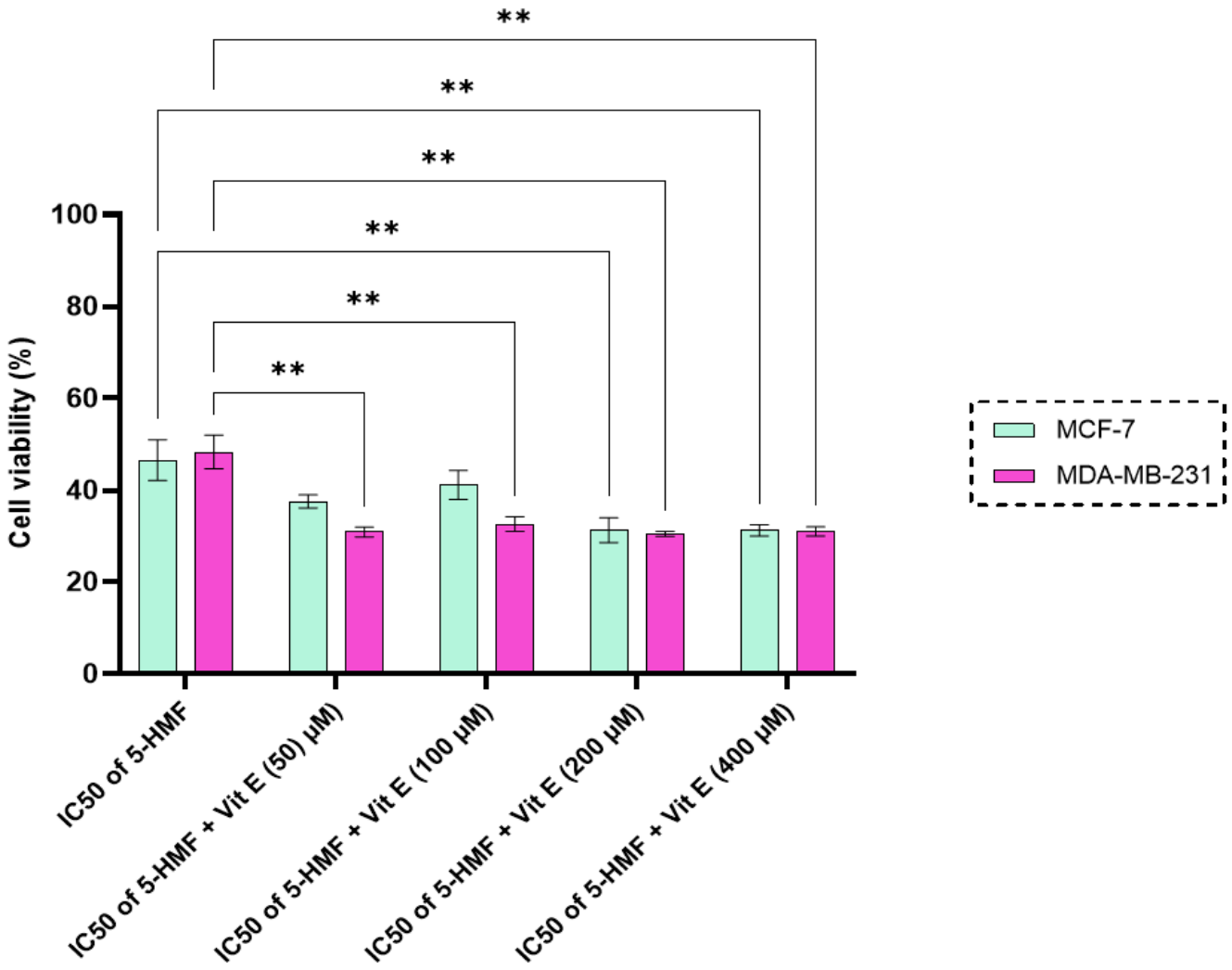


Figure 2

Effects of vitamin E on cell viability in MCF-7 and MDA-MB-231 cells treated with the IC_{50} concentration of 5-hydroxymethylfurfural (5-HMF). Cell viability was assessed using the sulforhodamine B (SRB) assay after 48 hours of treatment and expressed as a percentage of the control.

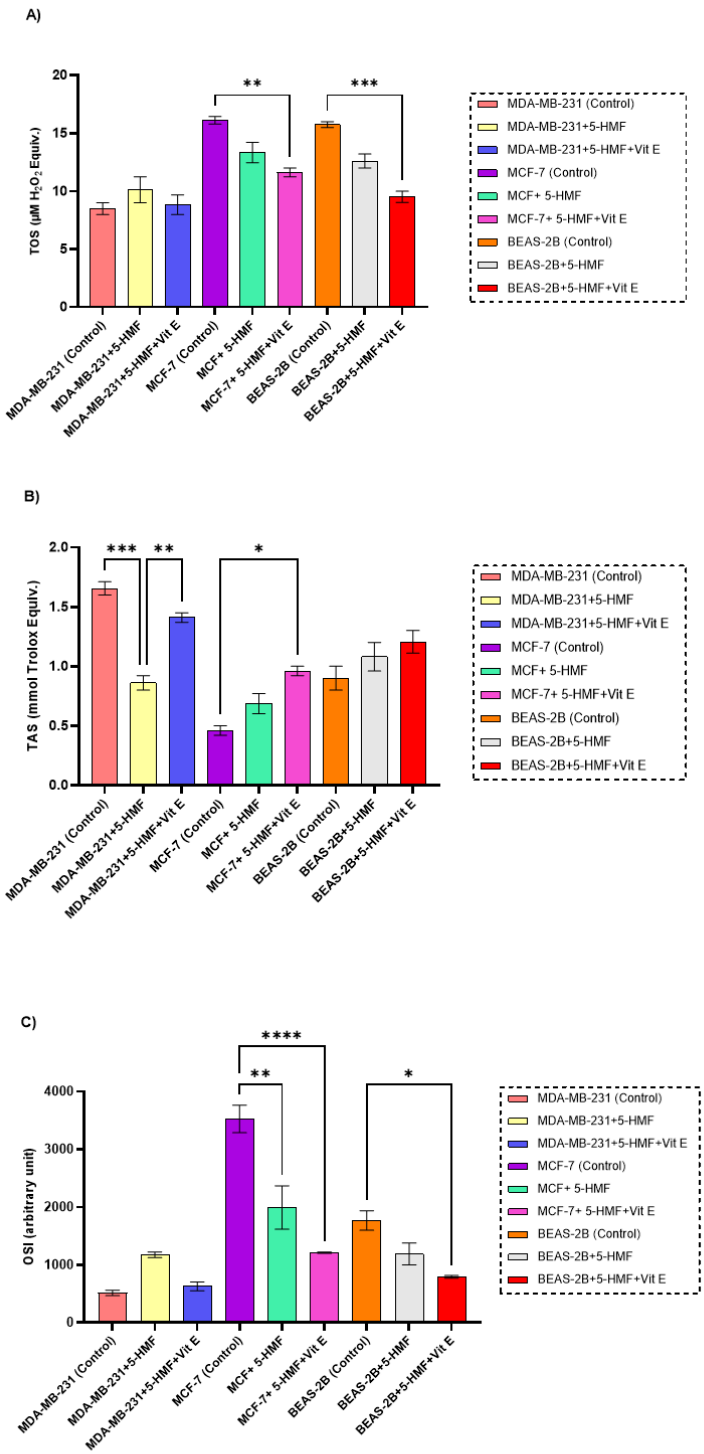


Figure 3

Effects of 5-hydroxymethylfurfural (5-HMF) and vitamin E treatments on A) total oxidant status (TOS), B) total antioxidant status (TAS), and C) oxidative stress index (OSI) levels in MCF-7, MDA-MB-231, and BEAS-2B cells.

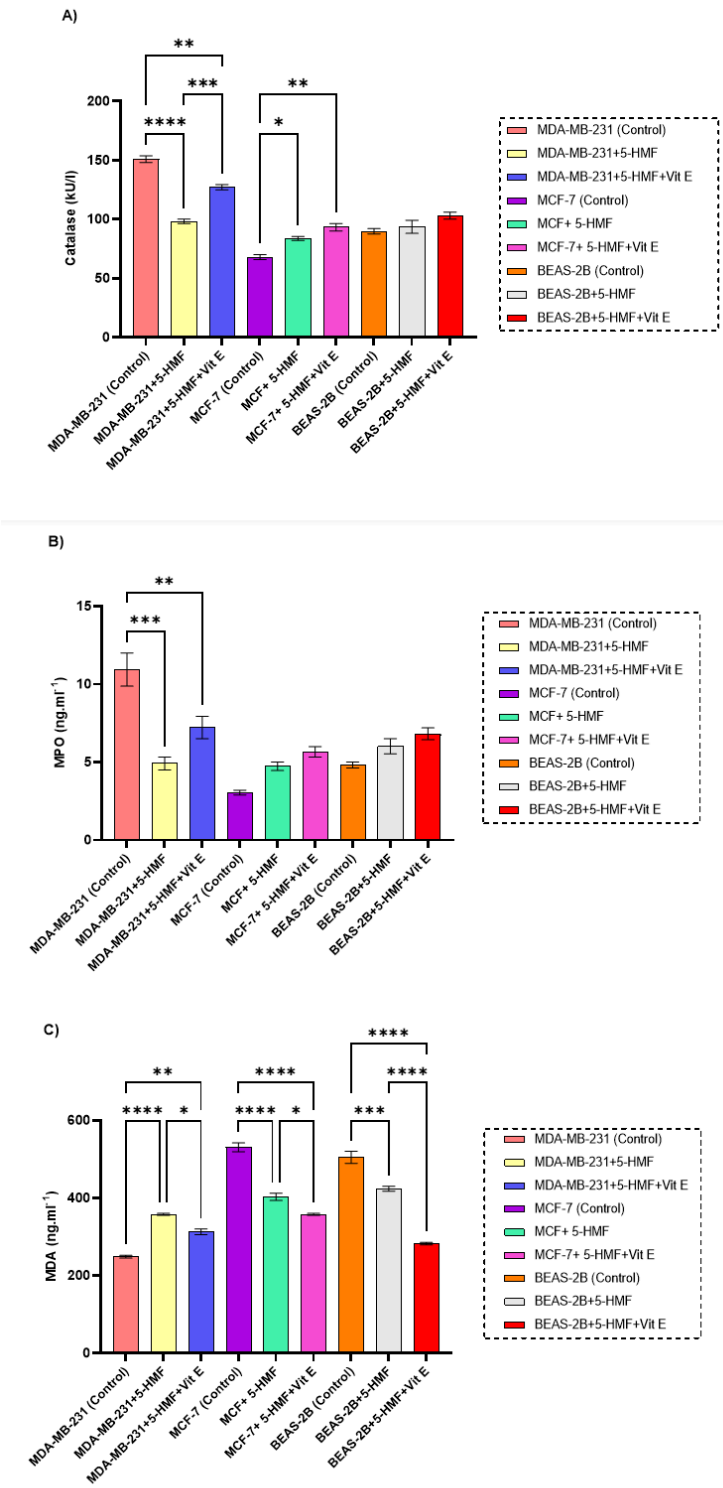


Figure 4

Effects of 5-hydroxymethylfurfural (5-HMF) and vitamin E co-treatments on A) catalase, B) myeloperoxidase (MPO), and C) malondialdehyde (MDA) levels in MCF-7, MDA-MB-231, and BEAS-2B cells.

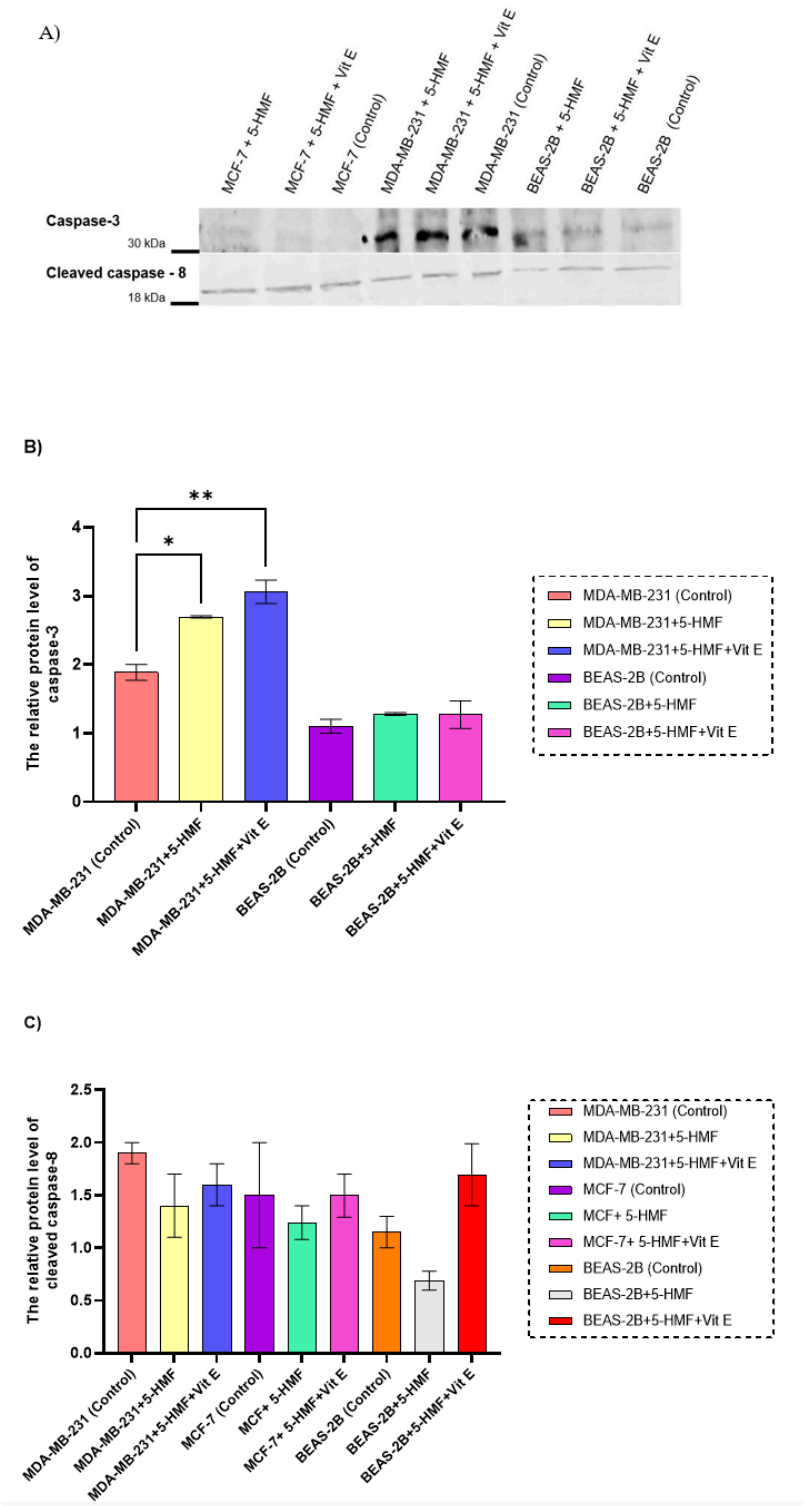


Figure 5

Effects of 5-hydroxymethylfurfural (5-HMF) and 5-HMF plus vitamin E treatments on caspase-3 and cleaved caspase-8 protein expression in MCF-7, MDA-MB-231, and BEAS-2B cells. **(A)** Representative Western blot membrane images of caspase-3 and cleaved caspase-8.
(B–C) Densitometric quantification of caspase-3 and cleaved caspase-8 protein expression levels. β-actin was used as the loading control.

Supplementary Files

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- SupplementaryMaterial.xlsx