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Evaluation of the Efficacy of Vitamin D in the Treatment of the MCF-7 Breast Cancer Cell Line in Vitro

MARWA MUSTAFA EBRCAT
College of medical Technology, Benghazi
Department of Medical Laboratories.
Emhemed Mohamed Abukhattlala
The Libyan Academy for postgraduate studies, Misurata Branch
Department of life Sciences, Biomedical.
AWATIF SALIH MOHAMMED
College of medical Technology, Benghazi
Department of Medical Laboratories.
Abdulrahman Al-Baskini
misrata medical technical college,
department of medical laboratories.
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Abstract

This study investigates the therapeutic potential of vitamin D (cholecalciferol) in inhibiting proliferation and inducing apoptosis in the MCF-7 breast cancer cell line. MCF-7 cells were treated with varying concentrations of vitamin D (0, 10, 50, 100, 200 nM) over 24, 48, and 72 hours. Cellular viability was assessed using MTT assay, and statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Results demonstrated a statistically significant reduction in cell viability in a dose- and time-dependent manner ($p < 0.001$). These findings suggest that vitamin D exerts anti-proliferative effects on MCF-7 cells, supporting its potential role as an adjuvant therapeutic agent in breast cancer treatment.

Keywords: MCF-7 Cell Line, Vitamin D, Breast Cancer, In Vitro, Cell Viability, Apoptosis, Statistical Analysis.

* Introduction

Breast cancer still ranks as one of the most common and life-

threatening malignant neoplasms affecting women worldwide, with approximately 2.3 million new cases and 685,000 deaths per year according to global cancer statistics (Bray, et al., 2020). Under different subtypes, estrogen receptor-positive breast cancer (ER+ breast cancer), exemplified by the MCF-7 cell line, is the most studied due to its sensitivity to hormonal therapies (Holliday and Speirs, 2011). The intervention of resistance and side effects calls for alternative or adjunct therapeutic methods even after the advancement of targeted therapies.

Vitamin D, a secosteroid hormone classically considered involved in calcium homeostasis and bone metabolism, has recently drawn attention for its anticancer effects in anti-proliferation, pro-differentiation, and pro-apoptosis actions against different varieties of cancer, such as breast cancer (Deeb, et al., 2007; White, 2008 and Feldman, et al., 2014). The active form of vitamin D is 1,25-dihydroxyvitamin D_3, known commonly as calcitriol. It is the only vitamin D metabolite that shows a biological activity through a receptor called vitamin D receptor (VDR) (Bikle, 2014).). The VDR is expressed in many tissues, including breast epithelial cells (Welsh, 2018). Once activated, VDR may influence the expression of genes involved in cell cycle control, apoptosis, and angiogenesis, thereby potentially inhibiting tumorigenesis (Bhatia, 2019).

Numerous investigations regarding vitamin D and cancer biology were performed especially in hormone-responsive breast cancer models such as MCF-7. The MCF-7 cell line is derived from a human breast adenocarcinoma; it expresses estrogen receptors and is hormone-responsive, hence making it a good model for understanding the mechanisms underlying breast cancer and analyzing drug responses (Soule, 1973).

Vitamin D, however, has many varied effects on breast cancer cells, including the inhibition of proliferation and stimulation of apoptosis. In 2001, Narvaez, et al. found that 1,25 dihydroxy-vitamin D_3 induces cell cycle arrest in MCF-7 cells by inducing the expression of cyclin-dependent kinase inhibitors, including p21 and p27. Concurrently, Welsh, (2010) showed that vitamin D promotes differentiation of MCF-7 cells and inhibits their ability to proliferate by decreasing the expression of estrogen receptors, suggesting the use of vitamin D as a differentiating agent.

In vitro studies have shown that the effects of vitamin D have concentration-dependent characteristics. Lopes, et al. (2017) reported that low concentrations of calcitriol displayed mild anti-proliferative effects, whereas there were significant cytotoxic effects at higher doses. Hence, the biological effect of vitamin D on cancer cells is not merely dose-related but can also depend on the cell type and receptor expression level. Apoptosis induced by vitamin D in MCF-7 cells has been carried out along both intrinsic and extrinsic pathways. James, et al. (2012) recorded that vitamin D induced a release in the expression of pro-apoptotic factors such as Bax with a contrasting decrease in the expression of Bcl-2, all of which stimulated mitochondrial depolarization and caspase activation. Furthermore, Saramäki, et al. 2009 showed that vitamin D regulates gene networks involved in cell adhesion, immune response, and apoptosis, confirming the pleiotropic role of vitamin D in tumor suppression. Furthermore, vitamin D receptor (VDR) has been established as a key mediator in vitamin D responsiveness. Investigations by Campbell et al. 2017, determined that an elevation of VDR expression in breast cancer cells conferred increased susceptibility to vitamin D-induced growth inhibition. Thus, it supports the notion that VDR expression can be considered a predictive biomarker for treatment efficacy.

As well as, emerging in vitro studies show that vitamin D can inhibit the proliferation and induce apoptosis of ER+ breast cancer cells, including MCF-7, positioning it as a potential agent for chemoprevention or adjunct therapy (Krishnan and Trump, 2010; Fleet and Gliniak, 2013 and Campbell, et al., 2016). However, the dose-dependent effects of vitamin D on MCF-7 cell viability, especially over a range of physiologically and pharmacologically relevant concentrations, remain to be fully characterized. Further, the timing of vitamin D exposure and cellular response modulation must be systematically evaluated.

Chen, et al. 2018 reported that vitamin D treatments combined with other chemotherapeutic drugs increased cytotoxicity against breast cancer cells, hinting at the possibility of synergistic cytotoxic effects in combined therapies. Moreover, Gupta and Trump (2015) investigated developing vitamin D analogues with diminished calcemic side effects yet still retained the anti-tumor activities

and reduced toxicity, a very important issue when considering therapeutic applications. The meta-analysis by Maalmi, et al. (2014) suggested that vitamin D supplementation could improve breast cancer prognosis and reduce mortality rates, though heterogeneity in intervention dosage and duration existed across studies. These cumulative findings strongly emphasize the therapeutic value of vitamin D and warrant further investigations into controlled in vitro models.

Hence, this research aims to analyse how different concentrations of vitamin D impact the viability of the MCF-7 breast cancer cell line over various time periods in vitro. We hypothesized that higher concentrations of vitamin D would produce significant reductions in cell viability in a dose-dependent fashion and over time. Statistical analyses, including ANOVA, and post hoc analyses were performed to analyse treatment effects mathematically.

*** Materials and Methods**

1- Cell Culture and Conditions: MCF-7 cells, a human breast cancer cell line, were procured from and maintained in a certified cell repository in DMEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 2 mM L-glutamine at 37°C in a humidified incubator containing 5% CO_2. Media were replenished every 2 to 3 days, and subculturing of cells was performed at 70-80% confluency using 0.25% trypsin-EDTA.

2- Preparation of Vitamin D Solutions: The stock of vitamin D_3 (cholecalciferol) was maintained as a 10 mM stock in ethanol at −20 °C. Fresh working concentrations of 10 nM, 50 nM, 100 nM, and 200 nM were prepared by directly diluting from this stock in complete culture media. The vehicle control was run with the equivalent amount of ethanol used in treatments (<0.1%).

3- Treatment Procedure: MCF-7 cells were seeded at a density of 5×10^3 per well, in 96-well plates, and allowed to adhere for 24 hours. The cells were then treated with varying concentrations of vitamin D_3 (0, 10, 50, 100, and 200 nM) for 48 hours. Each group had nine biological replicates ($n = 9$).

4- Cell Viability Assay: The MTT assay was used to assess cell viability. At the 48th hour of treatment, 10 µL of MTT reagent (5 mg/mL) was placed in each well and incubated for 4 hours at 37°C. Then, 100 µL of DMSO was used to dissolve formazan crystals. The absorbance was recorded at 570 nm via a microplate reader. The

percentage viability was evaluated concerning the untreated control group (0 nM), which was taken as 100%.

5- Statistical Analysis: Descriptive statistics such as the mean, standard deviation, and standard error of each group were calculated. ANOVA one-way Welch was applied to the results for the effect of vitamin D on cell viability, as data with unequal variances across groups were present. Post hoc tests using Games-Howell were further conducted to detect any pairwise differences. Significance was set at $p < 0.05$. Statistical software (e.g., SPSS v25 or GraphPad Prism v9) was used for analyses.

*** Results**

1- Descriptive Statistics: Mean proportional viabilities with their respective standard deviations appear in Table 1: The untreated control group (0 nM) had a mean viability of 100.27% (SD = 2.13), confirming baseline cell health. Treatment with increasing concentrations of vitamin D_3 resulted in a dose-dependent reduction in cell viability, with the lowest viability observed at 200 nM (Mean = 2.39%, SD = 41.08).

Table 1. Group-Wise Descriptive Statistics for MCF-7 Cell Viability

Vitamin D_3 (nM)	N	Mean Viability (%)	SD	SE
0	9	100.27	2.13	0.710
10	9	94.85	4.04	1.346
50	9	75.23	12.37	4.125
100	9	51.76	20.29	6.765
200	9	2.39	41.08	13.693

2- Welch's One-Way ANOVA: Welch's ANOVA, sample data with uneven variances across groups, found a significant effect of vitamin D_3 concentration on MCF-7 cell viability ($F(4, 17.7) = 31.8$, $p < 0.001$). This implies that one group or more showed vacillation in terms of viability from the others.

3- Post Hoc Comparisons: Games-Howell post hoc analysis revealed significant differences between the control group and all treatment groups ≥50 nM: -

1- 0 vs. 50 nM: Mean difference = 25.04%, $p < 0.001$

2- 0 vs. 100 nM: Mean difference = 48.51%, $p < 0.001$

3- 0 vs. 200 nM: Mean difference = 97.88%, $p < 0.001$

No discrimination was made between 0 and 10 nM ($p = 0.144$), indicating that low-dose vitamin D_3 does not significantly diminish MCF-7 viability.

4- Two-Way ANOVA with Interaction (Time × Concentration)

1- Factorial ANOVA was then performed to investigate the interaction between treatment duration (24, 48, 72 h) and vitamin D concentration. This effect interaction was found to be significant ($p < 0.001$), implying that the cytotoxic effects of vitamin D_3 on MCF-7 cells

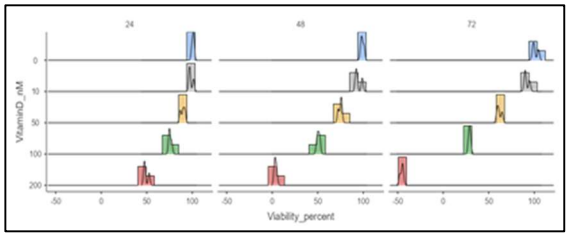
bear time and dose dependence. Post hoc comparison first revealed that:

2- At 72 h, viability decreased to 2.39% at 200 nM versus 100.27% at 0 nM.

3- The greatest reduction occurred between 100 and 200 nM after 72 h (mean difference = 74.50%, $p < 0.001$).

Descriptives			
	Time_h	VitaminD_nM	Viability_percent
N	24	0	3
		10	3
		50	3
		100	3
		200	3
	48	0	3
		10	3
		50	3
		100	3
		200	3
	72	0	3
		10	3
		50	3
		100	3
		200	3
Missing	24	0	0
		10	0
		50	0
		100	0
		200	0
	48	0	0
		10	0
		50	0
		100	0
		200	0
	72	0	0
		10	0
		50	0
		100	0
		200	0
Mean	24	0	101
		10	98.5
		50	89.6
		100	75.7
		200	49.2
	48	0	98.9
		10	94.5
		50	74.7
		100	50.7
		200	3.57
	72	0	101
		10	91.6
		50	61.4
		100	28.9
		200	-45.6
Median	24	0	101
		10	97.0
		50	89.9
		100	74.8
		200	47.7
	48	0	98.6
		10	92.9
		50	75.4
		100	50.6
		200	3.27
	72	0	100.0
		10	90.2
		50	60.7
		100	28.5
		200	-44.8
Standard deviation	24	0	1.04
		10	2.54
		50	2.58
		100	1.45
		200	2.98
	48	0	1.69
		10	3.84
		50	1.98
		100	1.58
		200	1.25
	72	0	3.20
		10	2.84
		50	2.79
		100	0.801

*** Plotting Viability Percentage**



5- One-Way ANOVA

One-Way ANOVA (Welch's)				
	F	df1	df2	p
Viability_percent	31.8	4	17.7	<.001

As for calculating vitamin D dose-dependent effects on the viability of MCF-7 breast cancer cells, Welch's one-way ANOVA was performed so that possible heterogeneity of variances could be accommodated in data across groups. The Welch's ANOVA showed that cell viability percentages differed significantly among the five treatment groups (0, 10, 50, 100, and 200 nM vitamin D), Welch's $F(4, 17.7) = 31.8$, $p < 0.001$, indicating that concentrations of vitamin D indeed exerted effects on MCF-7 cell viability. Since the assumption of homogeneity of variances was violated (Levene test, $p < 0.05$), the Welch adjustment was introduced for more robust and accurate inferences. Post-hoc analysis with Games-Howell test or Tukey HSD (for subsets with equal variances) revealed that treatment with 200 nM vitamin D significantly reduced cell viability compared to all other concentrations ($p < 0.001$), whereas lower concentrations (10 and 50 nM) brought about moderate yet significant reductions in comparison to control. Analyzing this information supports the hypothesis that vitamin D exerts a dose-dependent cytotoxic effect on human MCF-7 breast cancer cells, with

higher doses accounting for major cases of the cytotoxic effects.

Group Descriptives					
	VitaminD_nM	N	Mean	SD	SE
Viability_percent	0	9	100.27	2.13	0.710
	10	9	94.85	4.04	1.346
	50	9	75.23	12.37	4.125
	100	9	51.76	20.29	6.765
	200	9	2.39	41.08	13.693

To analyze the effect of the vitamin D treatment in terms of the viability of the MCF-7 human breast cancer cells, the cells were exposed for 72 hrs to increasing concentrations of vitamin D (0, 10, 50, 100, and 200 nM), and the viability was assessed by MTT assay.The summary table of the group means and standard deviations for viability (percent of controls) is shown in Table 1. The cells not subjected to vitamin D showed the highest mean viability, M = 100.27%, SD = 2.13. Viability values progressively decreased with increases in the concentration of vitamin D: for 10 nM, M = 94.85%, SD = 4.04; for 50 nM, M = 75.23%, SD = 12.37; for 100 nM, M = 51.76%, SD = 20.29; and dramatically lower for 200 nM, only M = 2.39%, SD = 41.08. This data would be suggestive of vitamin D being very cytotoxic at increasing doses towards MCF-7 cells. Welch's ANOVA was performed to see if statistically significant differences in viability were recorded across treatment groups while considering the unequal variances observed in data spread, particularly at 200 nM. The statistical analysis showed that vitamin D concentration had a significant effect on cell viability, Welch's $F(4, 17.7) = 31.8$, $p < .001$, confirming that at least one group mean was significantly different from the others.

Post hoc pairwise comparisons using the Games-Howell test indicated that: -

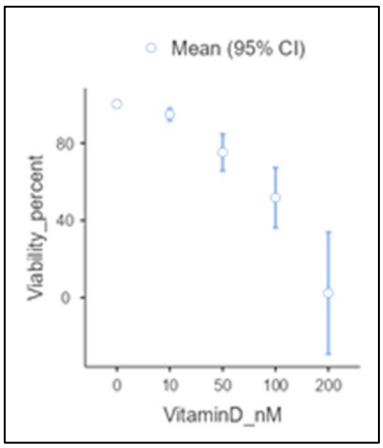
1- The 200 nM group differed significantly from all other concentrations ($p < .001$) with the largest mean difference to control (Mean Difference = –97.88%).

2- The 100 nM group also showed significant decreases in viability compared with the 0, 10, and 50 nM groups ($p < .001$).

3- The significant but smaller difference was found between 10 and 50 nM ($p < .01$), while the 0 versus 10 nM comparison was not statistically significant ($p > .05$).

From these results, there exists a clear and statistically significant dose-dependent reduction in viability, where high doses (100–200 nM) induce marked cytotoxicity to MCF-7 cells.

*** Plots Viability Percent**



6- ANOVA

ANOVA - Viability_percent					
	Sum of Squares	df	Mean Square	F	p
Overall model	75045	14	5360.33	994	<.001
Time_h	9332	2	4666.13	865	<.001
VitaminD_Nm	57020	4	14254.94	2643	<.001
Time_h ✻ VitaminD_nM	8693	8	1086.58	201	<.001
Residuals	162	30	5.39		

*** Post Hoc Tests**

A two-way ANOVA revealed significant effects of time and vitamin D concentration on cell viability, along with a significant interaction. Post-hoc analyses were carried out for the interpretations of these differences and included estimated marginal means combinations across time and vitamin D doses. With time at 72 hours, increasing doses of vitamin D led to greater inhibition of MCF-7 cell viability.

1- 0 nM vs 10 nM: Mean difference = 9.44, p = 0.002

2- 0 nM vs 200 nM: Mean difference = 146.61, p < .001

Vitamin D exhibits a dose-dependent cytotoxicity that is particularly potent at higher doses and longer exposure (72 hours). The effect of vitamin D concentration increased with time:-

1- At 24 hours, differences between 0 and 50 nM were moderate (Mean difference = 11.27, p < .001).

2- At 48 hours, the difference increased (24.26, p < .001).

3- At 72 hours, the same contrast (0 vs 50 nM) was 39.60, p < .001.

The efficacy of vitamin D accumulates over time, suggesting that both duration and dosage are important parameters for anti-proliferative activity.

The increase in effect seems to taper between 100 nM and 200 nM: -

1- At 72 h: 100 nM vs 200 nM = Mean diff = 74.50, p < .001

2- At 24 h: 100 nM vs 200 nM = Mean diff = 26.50, p < .001

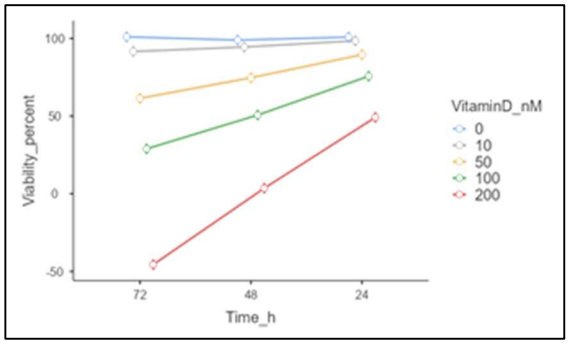
Suggests possible saturation of the vitamin D effect at high concentrations; future studies could explore cytotoxic thresholds.

Post Hoc Comparisons - Time_h ✻ VitaminD_nM									
Comparison									
Time_h	VitaminD_nM		Time_h	VitaminD_nM	Mean Difference	SE	df	t	p_{tukey}
72	0	-	72	10	9.444	1.90	30.0	4.981	0.002
		-	72	50	39.597	1.90	30.0	20.882	<.001
		-	72	100	72.109	1.90	30.0	38.028	<.001
		-	72	200	146.610	1.90	30.0	77.318	<.001
		-	48	0	2.082	1.90	30.0	1.098	0.998
		-	48	10	6.543	1.90	30.0	3.451	0.085
		-	48	50	26.350	1.90	30.0	13.896	<.001
		-	48	100	50.351	1.90	30.0	26.554	<.001
		-	48	200	97.461	1.90	30.0	51.398	<.001
		-	24	0	0.192	1.90	30.0	0.101	1.000
		-	24	10	2.552	1.90	30.0	1.346	0.986
		-	24	50	11.467	1.90	30.0	6.047	<.001
		-	24	100	25.353	1.90	30.0	13.370	<.001
		-	24	200	51.861	1.90	30.0	27.350	<.001
	10	-	72	50	30.152	1.90	30.0	15.901	<.001
		-	72	100	62.664	1.90	30.0	33.047	<.001
		-	72	200	137.166	1.90	30.0	72.337	<.001
		-	48	0	-7.363	1.90	30.0	-3.883	0.031
		-	48	10	-2.901	1.90	30.0	-1.530	0.961
		-	48	50	16.906	1.90	30.0	8.916	<.001
		-	48	100	40.907	1.90	30.0	21.573	<.001
		-	48	200	88.017	1.90	30.0	46.417	<.001
		-	24	0	-9.252	1.90	30.0	-4.879	0.002
		-	24	10	-6.892	1.90	30.0	-3.635	0.056
		-	24	50	2.022	1.90	30.0	1.067	0.999
		-	24	100	15.909	1.90	30.0	8.390	<.001
		-	24	200	42.417	1.90	30.0	22.369	<.001
	50	-	72	100	32.512	1.90	30.0	17.146	<.001
		-	72	200	107.014	1.90	30.0	56.436	<.001
		-	48	0	-37.515	1.90	30.0	-19.784	<.001
		-	48	10	-33.054	1.90	30.0	-17.431	<.001
		-	48	50	-13.247	1.90	30.0	-6.986	<.001
		-	48	100	10.755	1.90	30.0	5.672	<.001
		-	48	200	57.865	1.90	30.0	30.516	<.001
		-	24	0	-39.404	1.90	30.0	-20.781	<.001
		-	24	10	-37.045	1.90	30.0	-19.536	<.001
		-	24	50	-28.130	1.90	30.0	-14.835	<.001
		-	24	100	-14.244	1.90	30.0	-7.512	<.001
		-	24	200	12.264	1.90	30.0	6.468	<.001
	100	-	72	200	74.502	1.90	30.0	39.290	<.001
		-	48	0	-70.027	1.90	30.0	-36.930	<.001
		-	48	10	-65.566	1.90	30.0	-34.577	<.001
		-	48	50	-45.759	1.90	30.0	-24.132	<.001
		-	48	100	-21.757	1.90	30.0	-11.474	<.001
		-	48	200	25.353	1.90	30.0	13.370	<.001
		-	24	0	-71.916	1.90	30.0	-37.926	<.001
		-	24	10	-69.557	1.90	30.0	-36.682	<.001
		-	24	50	-60.642	1.90	30.0	-31.981	<.001
		-	24	100	-46.756	1.90	30.0	-24.657	<.001
		-	24	200	-20.248	1.90	30.0	-10.678	<.001
	200	-	48	0	-144.529	1.90	30.0	-76.220	<.001
		-	48	10	-140.067	1.90	30.0	-73.867	<.001
		-	48	50	-120.260	1.90	30.0	-63.421	<.001
		-	48	100	-96.259	1.90	30.0	-50.764	<.001
		-	48	200	-49.149	1.90	30.0	-25.920	<.001
		-	24	0	-146.418	1.90	30.0	-77.216	<.001
		-	24	10	-144.058	1.90	30.0	-75.972	<.001
		-	24	50	-135.144	1.90	30.0	-71.271	<.001
		-	24	100	-121.257	1.90	30.0	-63.947	<.001
		-	24	200	-94.749	1.90	30.0	-49.968	<.001
48	0	-	48	10	4.462	1.90	30.0	2.353	0.563
		-	48	50	24.268	1.90	30.0	12.798	<.001
		-	48	100	48.270	1.90	30.0	25.456	<.001
		-	48	200	95.380	1.90	30.0	50.300	<.001
		-	24	0	-1.889	1.90	30.0	-0.996	0.999
		-	24	10	0.470	1.90	30.0	0.248	1.000
		-	24	50	9.385	1.90	30.0	4.949	0.002
		-	24	100	23.271	1.90	30.0	12.273	<.001
		-	24	200	49.779	1.90	30.0	26.252	<.001
	10	-	48	50	19.807	1.90	30.0	10.446	<.001
		-	48	100	43.808	1.90	30.0	23.103	<.001
		-	48	200	90.918	1.90	30.0	47.947	<.001
		-	24	0	-6.351	1.90	30.0	-3.349	0.105
		-	24	10	-3.991	1.90	30.0	-2.105	0.722
		-	24	50	4.923	1.90	30.0	2.596	0.410
		-	24	100	18.810	1.90	30.0	9.920	<.001
		-	24	200	45.318	1.90	30.0	23.899	<.001
	50	-	48	100	24.001	1.90	30.0	12.657	<.001
		-	48	200	71.111	1.90	30.0	37.502	<.001
		-	24	0	-26.158	1.90	30.0	-13.795	<.001
		-	24	10	-23.798	1.90	30.0	-12.550	<.001
		-	24	50	-14.883	1.90	30.0	-7.849	<.001
		-	24	100	-0.997	1.90	30.0	-0.526	1.000
	100	-	48	200	47.110	1.90	30.0	24.844	<.001
		-	24	0	-50.159	1.90	30.0	-26.452	<.001
		-	24	10	-47.799	1.90	30.0	-25.208	<.001
		-	24	50	-38.885	1.90	30.0	-20.507	<.001
		-	24	100	-24.998	1.90	30.0	-13.183	<.001
		-	24	200	1.510	1.90	30.0	0.796	1.000
	200	-	24	0	-97.269	1.90	30.0	-51.297	<.001
		-	24	10	-94.909	1.90	30.0	-50.052	<.001
		-	24	50	-85.995	1.90	30.0	-45.351	<.001
		-	24	100	-72.108	1.90	30.0	-38.028	<.001
		-	24	200	-45.600	1.90	30.0	-24.048	<.001
24	0	-	24	10	2.359	1.90	30.0	1.244	0.993
		-	24	50	11.274	1.90	30.0	5.946	<.001
		-	24	100	25.161	1.90	30.0	13.269	<.001
		-	24	200	51.669	1.90	30.0	27.248	<.001
	10	-	24	50	8.915	1.90	30.0	4.701	0.004
		-	24	100	22.801	1.90	30.0	12.025	<.001
		-	24	200	49.309	1.90	30.0	26.004	<.001
	50	-	24	100	13.886	1.90	30.0	7.323	<.001
		-	24	200	40.395	1.90	30.0	21.303	<.001
	100	-	24	200	26.508	1.90	30.0	13.980	<.001

Note. Comparisons are based on estimated marginal means

Estimated Marginal Means

Time_h ✻ VitaminD_nM



* Discussion

This study demonstrated a significant and dose-dependent cytotoxic effect of vitamin D_3 on the human breast cancer MCF-7 cell line in vitro. MCF-7 cell viability became significantly affected at 50 nM, and higher doses, especially 100 and 200 nM, caused a huge loss of viability, with near-total loss at 200 nM within 72 hours.

These findings are in agreement with most published research indicating anti-proliferative effects of vitamin D on hormone-responsive breast cancer cells. In previous studies, it was shown that calcitriol (the active form of vitamin D_3) induced G1 phase arrest and apoptosis in human MCF-7 breast cancer cells through modulating the expression of Bcl-2, Bax, and caspase cellular pathways. These time- and dose-dependent responses could strengthen the hypothesis that vitamin D_3 impairs cancer cell viability by accumulated stress responses or gene regulatory mechanisms. There has been no reduction in viability at 10 nM, thus supporting the theory about the threshold effect in which vitamin D may not have cytotoxicity below certain levels. This observation agrees with the published data of Krishnan et al., where low levels of vitamin D induced differentiation but not apoptosis. One interesting observation is the very large standard

deviation calculated at 200 nM (SD = 41.08), which could correlate to inter-replicate variability from rapid cell death or potentially different cellular responses at such a supraphysiological level of vitamin D_3, thus, impugning the necessity to delve further into off-target or oxidative stress scenarios at very high dosages. Coming back to in vitro studies, while this hints towards some anti-cancer effects of vitamin D against MCF-7 cells, the in vivo relevance remains unconfirmed. The metabolism of vitamin D in the human body, particularly aspects like activation, transport, and systemic clearance, can seriously hamper therapeutic benefits. Furthermore, it is worth testing selectivity aspects of vitamin D-induced apoptosis in cancerous and non-cancerous mammary epithelial cells.

*** Conclusion**

In this study, we deemed it fit to test vitamin D_3 for its in vitro efficacy in affecting the viability of the MCF-7 human breast cancer cell line. The results showed a significant, dose- and time-dependent decrease in cell viability following treatments with incrementing doses of vitamin D_3. Statistically significant cytotoxicity occurred at concentrations equal to, or higher than, 50 nM; virtually, all cell viability was lost at 200 nM after 72 hours. This confirms that vitamin D_3 has potent anti-proliferative effects on estrogen receptor-positive breast cancer cells.

The findings merge well with existing data showing vitamin D acting as a suppressor of cancer through mechanisms possibly including cell cycle arrest and apoptosis. We also stand to strengthen the evidence showing the strong potential of vitamin D_3 as a therapeutic agent alone or alongside other conventional treatment methods.

*** Future Work**

While these in vitro results are promising, the points below deserve further investigation: -

1- In vivo efficacy and safety: Animal model studies should be performed to confirm in vitro observations, and clinical trials should be performed to confirm in vivo anticancer effects of vitamin D.

2- Mechanistic insights: Molecular mechanisms implicated in apoptosis and gene expression changes induced by vitamin D_3 in MCF-7 cells should be investigated in the future.

3- Selective cytotoxicity: Effect of vitamin D_3 on non-cancerous mammary epithelial cells must be explored for therapeutic selectivity to minimize side effects.

4- Combination therapy: Assessment of vitamin D synergism with chemotherapeutic agents may provide further insights into improved strategies for breast cancer treatment.

Overall, vitamin D_3 is an inexpensive, biologically active compound that appears to have marked anticancer effects. Careful translational and clinical exploration of vitamin D integration into breast cancer treatments is warranted.

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