

Supplementary information to:

Original article:

**ANTIPROLIFERATIVE ACTIVITY OF TAMOXIFEN, VITAMIN D3
AND THEIR CONCOMITANT TREATMENT**

Derya Yetkin^{1*} , Ebru Ballı² , Furkan Ayaz^{3*} 

¹ Mersin University, Advanced Technology Education Research and Application Center, 33110, Mersin, Turkey

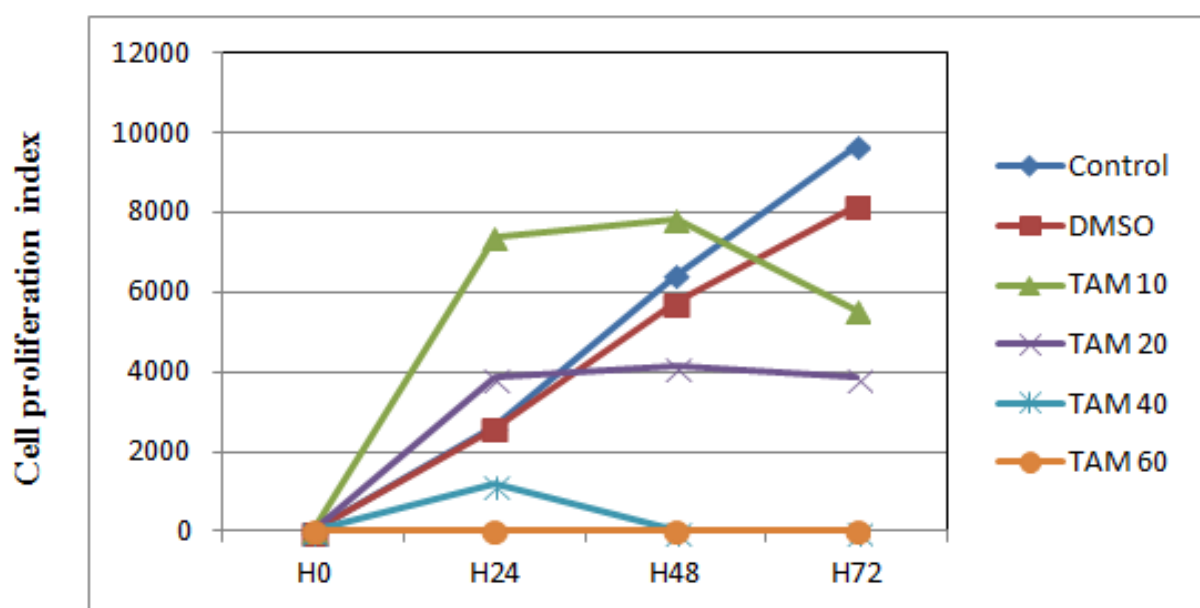
² Mersin University, Department of Histology and Embryology, 33110 Mersin, Turkey

³ Mersin University, Department of Biotechnology, Faculty of Arts and Science, 33110, Mersin, Turkey

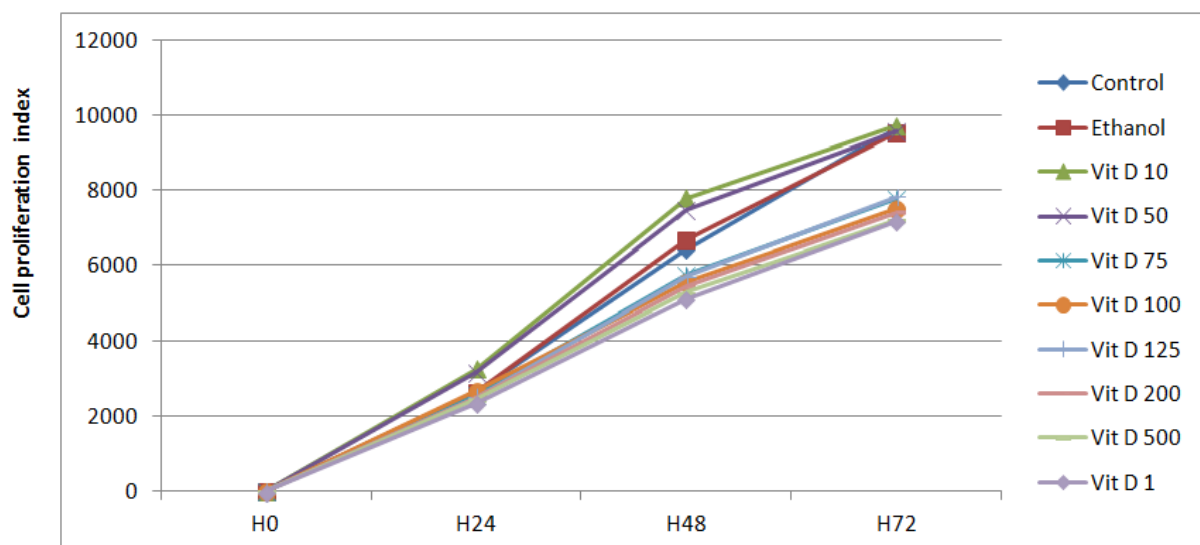
* **Corresponding authors:** Furkan Ayaz, Mersin University, Department of Biotechnology, Faculty of Arts and Science, 33110, Mersin, Turkey. Tel: 00 90-324-3610000, E-mail: furkanayaz@mersin.edu.tr;
Derya Yetkin, Mersin University, Advanced Technology Education Research and Application Center, 33110, Mersin, Turkey. Tel: 00 90-324-3610000, E-mail: deryayetkin@mersin.edu.tr

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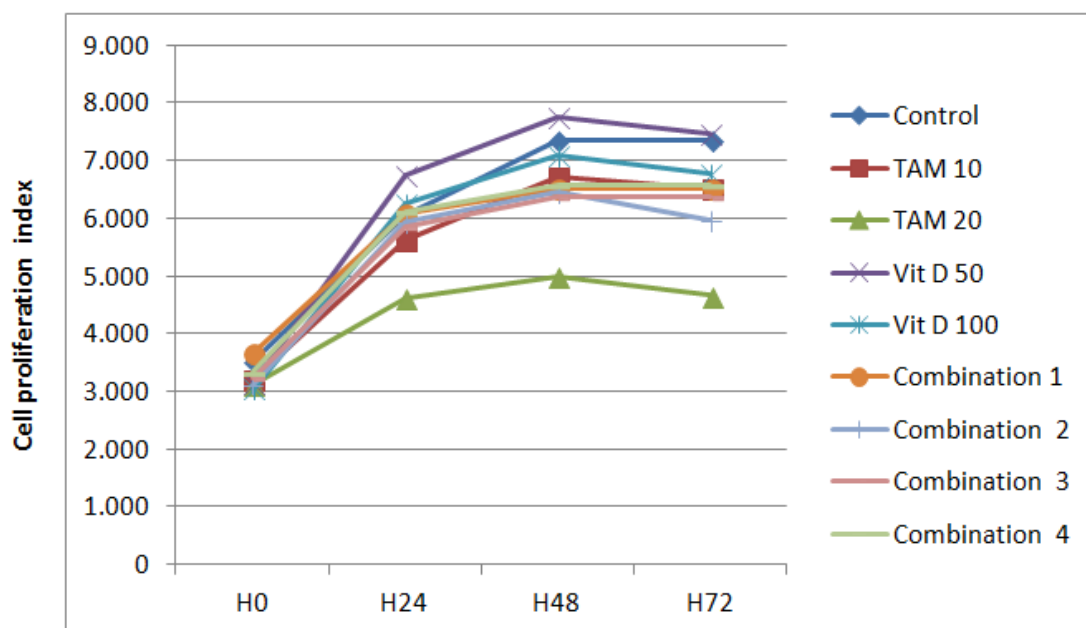
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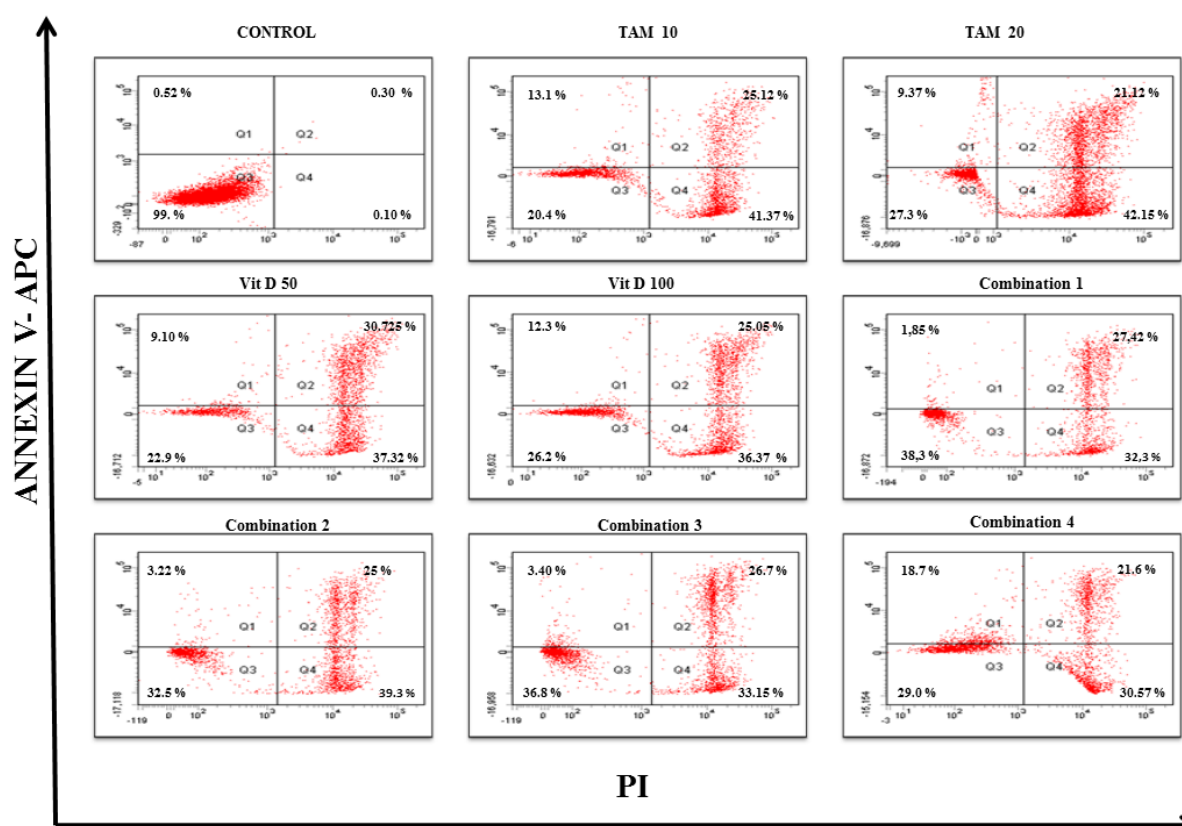
Supplementary Figure 1: Time dependent graph of cell index values of different tamoxifen concentrations in MCF-7 cells for 72 h calculated in RTCA Software 1.2.1.



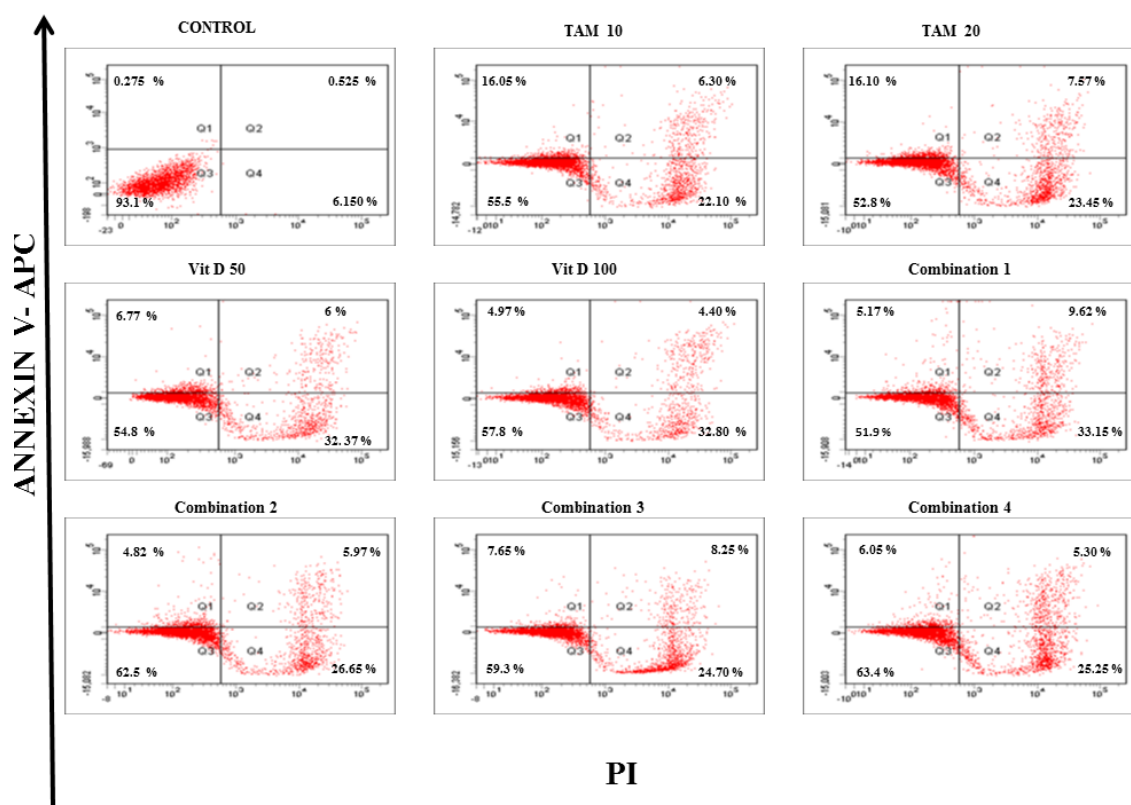
Supplementary Figure 2: Time dependent graph of cell index values of different Vitamin D₃ concentrations in MCF-7 cells for 72 h calculated in RTCA Software 1.2.1.



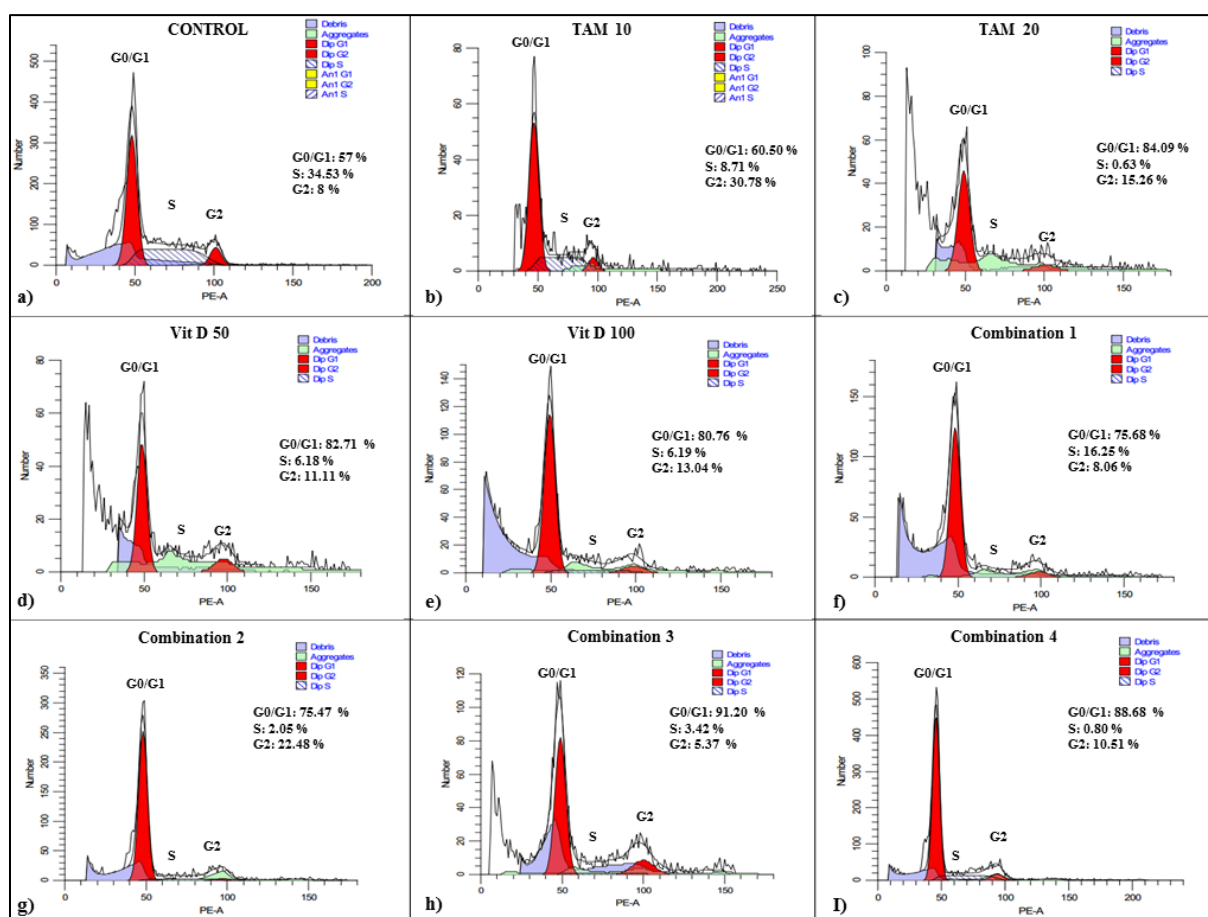
Supplementary Figure 3: Time dependent graph of cell index values of different concentration combinations of tamoxifen and Vitamin D₃ (Combination 1: TAM 10 μ M + vit D 50 nM, Combination 2: TAM 10 μ M + vit D 100 nM, Combination 3: TAM 20 μ M + vit D 50 nM and Combination 4: TAM 20 μ M + vit D 100 nM) in MCF-7 cells for 72 h calculated in RTCA Software 1.2.1.



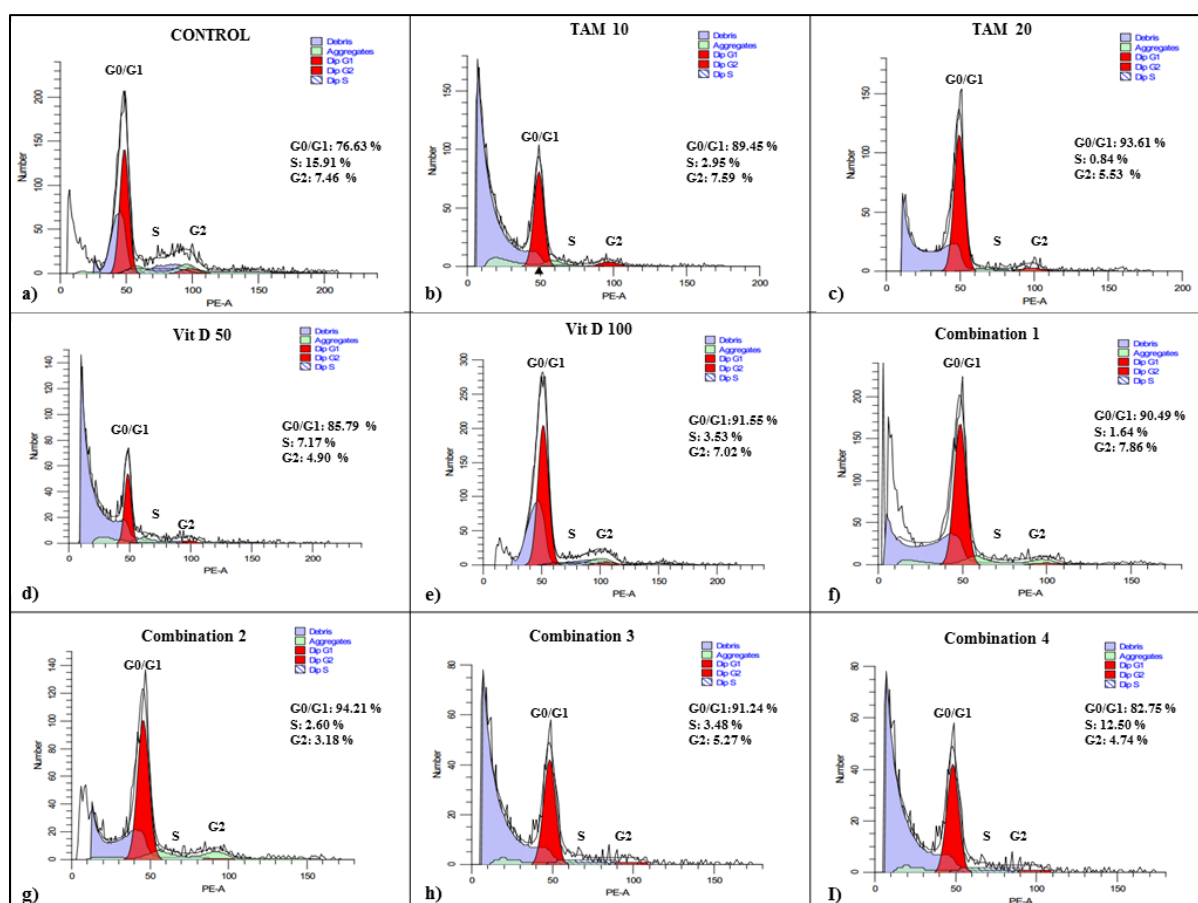
Supplementary Figure 4: Apoptosis and necrosis analysis of MCF-7 cells was evaluated in flow cytometry, after tamoxifen, vitamin D₃ and combination therapy for 48 h. Apoptosis data; live cells (Q3), early apoptosis (Q1), late apoptosis (Q2) and necrosis (Q4) populations were identified by plotting Annexin V staining intensity versus PI staining intensity.



Supplementary Figure 5: Apoptosis and necrosis analysis of MCF-7 cells was evaluated in flow cytometry, after tamoxifen, vitamin D₃ and combination therapy for 72 h. Apoptosis data; live cells (Q3), early apoptosis (Q1), late apoptosis (Q2) and necrosis (Q4) populations were identified by plotting Annexin V staining intensity versus PI staining intensity.



Supplementary Figure 6: Cell-cycle analysis by flow cytometry of MCF-7 cells treated with control (a), Tamoxifen (b: 10 μ M; c: 20 μ M), vitamin D₃ (d: 50 nM; e: 100 nM) and combination of both (f: Combination 1: TAM 10 μ M + vit D 50 nM, g: Combination 2: TAM 10 μ M + vit D 100 nM, h: Combination 3: TAM 20 μ M + vit D 50 nM and i: Combination 4: TAM 20 μ M + vit D 100 nM for 48 h.



Supplementary Figure 7: Cell-cycle analysis by flow cytometry of MCF-7 cells treated with control (a), Tamoxifen (b: 10 μ M; c: 20 μ M), vitamin D₃ (d: 50 nM; e: 100 nM) and combination of both (f: Combination 1: TAM 10 μ M + vit D 50 nM, g: Combination 2: TAM 10 μ M + vit D 100 nM, h: Combination 3: TAM 20 μ M + vit D 50 nM and i: Combination 4: TAM 20 μ M + vit D 100 nM for 72 h.

Supplementary Table 1: Average of cell proliferation index obtained between groups 24, 48 and 72 hours after tamoxifen treatment in MCF7 cells

Groups (n=4)	H 0	H 24	H 48	H 72
Control	0.772 ± 0.167	2.623 ± 0.546	6.445 ± 1.378	9.675 ± 0.789
DMSO	0.652 ± 0.435	2.600 ± 0.311	5.743 ± 0.745	8.165 ± 1.019
TAM 10	0.918 ± 0.125	7.350 ± 0.723	7.794 ± 0.520	5.531 ± 0.324 ^a
TAM 20	0.888 ± 0.101	3.863 ± 0.333 ^a	4.121 ± 0.421 ^a	3.854 ± 0.328 ^a
TAM 40	0.811 ± 0.094	1.166 ± 0.114 ^a	0.980 ± 0.084 ^a	0.7189 ± 0.0911 ^a
TAM 60	0.848 ± 0.136	0.725 ± 0.131 ^a	0.998 ± 0.172 ^a	0.6324 ± 0.094 ^a

^a p < 0.05 compared to the control group**Supplementary Table 2:** Cell proliferation index of treatment of MCF-7 cells with Vit D₃ administered at different concentrations

Groups (n=4)	H 0	H 24	H 48	H 72
Control	0.772 ± .167	2.623 ± .546	6.445 ± 1.378	9.675 ± .789
Ethanol	0.882 ± .010	2.623 ± .546	6.695 ± .936	9.549 ± 1.410
Vit D ₃ 10	0.926 ± .122	3.252 ± .567	7.791 ± 1.613	9.722 ± 1.418
Vit D ₃ 50	0.934 ± .056	3.165 ± .090	7.475 ± .896	9.576 ± .987
Vit D ₃ 75	0.790 ± .112	2.573 ± .420	5.742 ± .938	7.794 ± .917 ^a
Vit D ₃ 100	0.846 ± .101	2.660 ± .301	5.555 ± .478	7.512 ± .566 ^a
Vit D ₃ 125	0.877 ± .066	2.503 ± .153	5.710 ± .344	7.829 ± .545 ^a
Vit D ₃ 200	0.886 ± .053	2.476 ± .543	5.442 ± .348	7.427 ± .435 ^a
Vit D ₃ 500	.879 ± .048	2.454 ± .087	5.288 ± .568	7.218 ± .384 ^a
Vit D ₃ 1	.891 ± .072	2.335 ± .564	5.122 ± .479	7.195 ± .158 ^a

^ap < 0.05 is significantly different vs control with vitamin D groups