Finanziato dall'Unione europea NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

THE Tuscany Health Ecosystem

Cardioprotective effects of Simvastatin in a Doxorubicin-induced acute cardiotoxicity model

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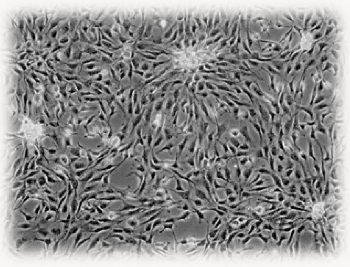
SITOX 22° Congresso Nazionale – Il rapporto dose-effetto nella valutazione del rischio tossicologico – BOLOGNA 10-12 Febbraio 2025

Introduction

Cardiotoxicity is the main side effect of Doxorubicin (Doxo) and cardiomyocytes damage can occur as early as the first administration of drug [1,2,3]. Current research is focused on identifying potential drugs that can mitigate cardiac side effects without compromising Doxorubicin's anti-tumor efficacy and statins are particularly promising in this field since their pleiotropic biological effects in addition to their cholesterol lowering activity [4]. Moreover, statins may influence the expression of Cx43, a protein member of the Gap Junctions (GJS) family that plays a crucial role in the early adaptative response to Doxorubicin-induced stress [5].

Aim

The purpose of this study was to evaluate the protective effects of Simvastatin in a cellular model of Doxorubicin-induced acute cardiotoxicity.



Human Cardiomyocytes

→ 4h pre-treatment Simvastatin (Sim) [10 μM]
20h co-administration Doxo[1μM] + Sim [10μM].

Results

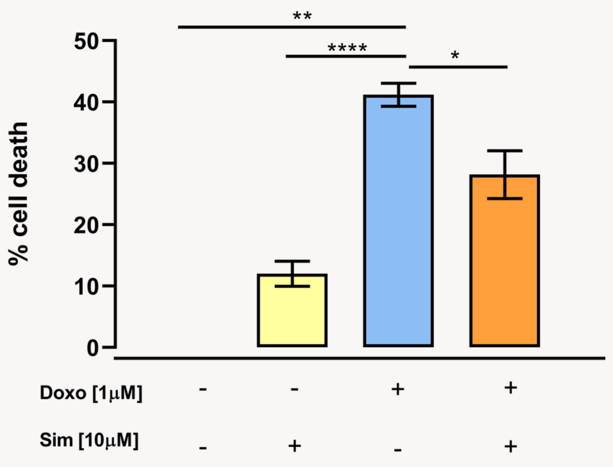


Figure 1. Cellular viability was assessed by MTT assay. Cell viability was calculated as % of dead cells = 100 - ([OD treated/ OD control] x 100). Data were analyzed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of % cell death. * p<0.05 ** p<0.01, **** p<0.001

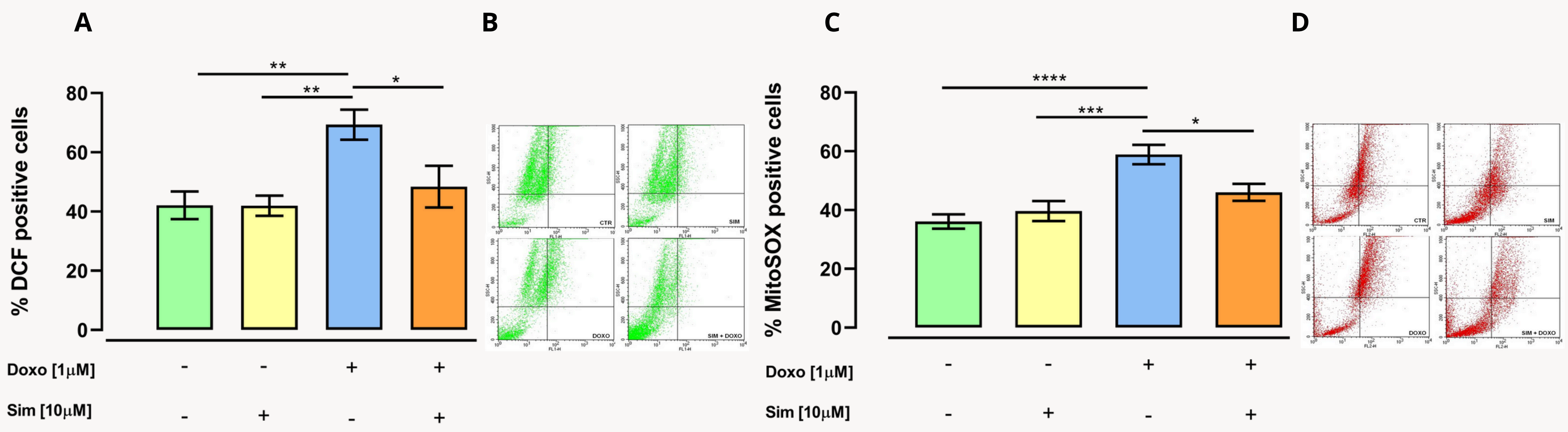


Figure 2. The fluorescent probes 2'-7'dichlorofluorescein diacetate (H2DCF-DA) and MitoSOX Red, a Mitochondrial Superoxide Indicator, were used to evaluate cytosolic ROS and mitochondrial superoxide generation, respectively. Data were analyzed by flow cytometry. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of the percentage of DCF and MitoSOX positive cells. *p<0.05, ** p<0.01, ***p<0.005, **** p<0.001 (A,C). Representative histograms for the flow cytometry analysis are reported in Panels (B,D).

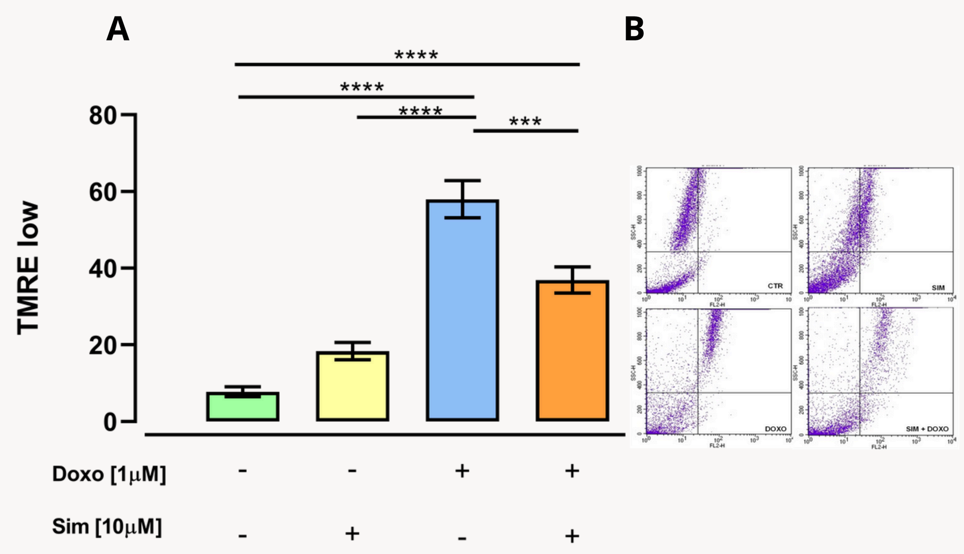


Figure 3. The fluorescent dye tetramethylrhodamine methyl ester (TMRE) was used to evaluate mitochondrial membrane potential. Data were analyzed by flow cytometry. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM TMRE-positive cells percentage. *** p<0.005, **** p<0.001 (A). Representative histograms for the flow cytometry analysis are reported in Panel (B).

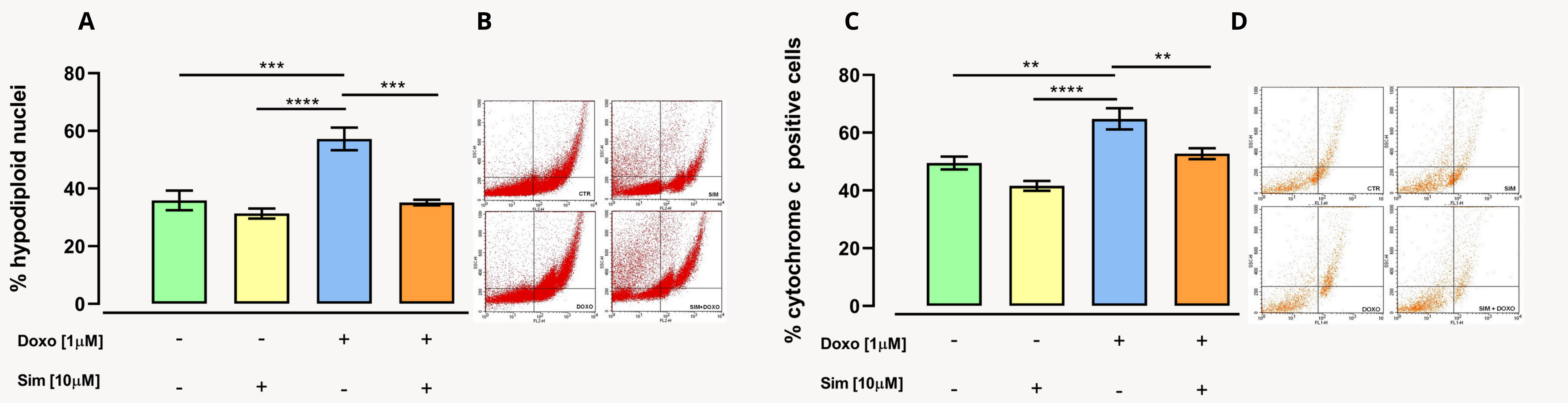


Figure 4. HCM were stained by propidium iodide and fluorescence of individual nuclei was measured by flow cytometry. To evaluate cytosolic cytochrome c levels flow cytometry analysis was used. Statistical analysis was performed using the One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of % hypodiploid nuclei and cytochrome c positive cells (Panel A and C). ** p<0.01, *** p<0.005, **** p<0.001. Representative histograms for the flow cytometry analysis are reported in Panels (B, D)

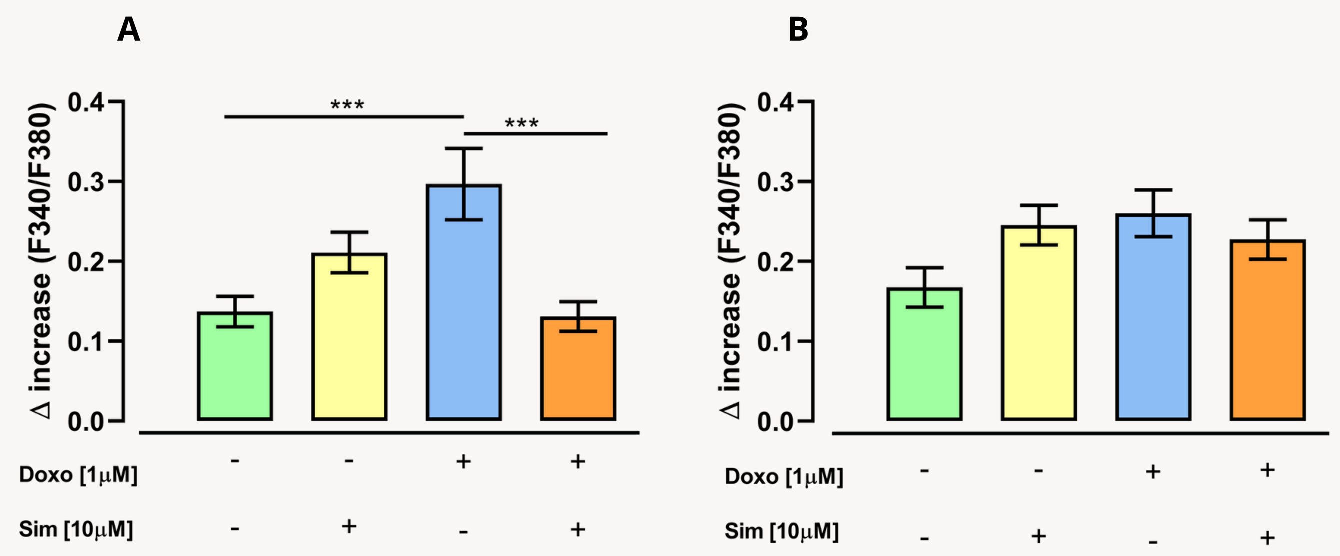


Figure 5. FCCP in calcium-free medium was used to evaluate mitochondrial calcium levels and Ionomycin in calcium-free medium was used to evaluate intracellular calcium levels (Panels A, B). Values are reported as mean ± S.E.M. of percentage of delta increase in FURA2 ratio fluorescence (340/380 nm). *** p<0.05

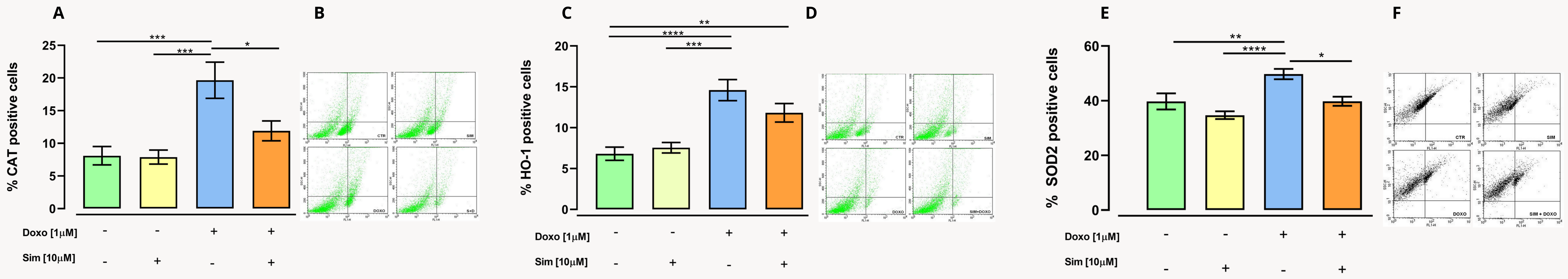


Figure 6. To evaluate CAT, HO-1 and SOD2 level flow cytometry analysis was used. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of % of CAT, HO-1 and SOD2 positive cells (Panel A, C, E). * p<0.05, ** p<0.01, *** p<0.005, **** p<0.001. Representative histograms for the flow cytometry analysis are reported in Panels (B,D,F).

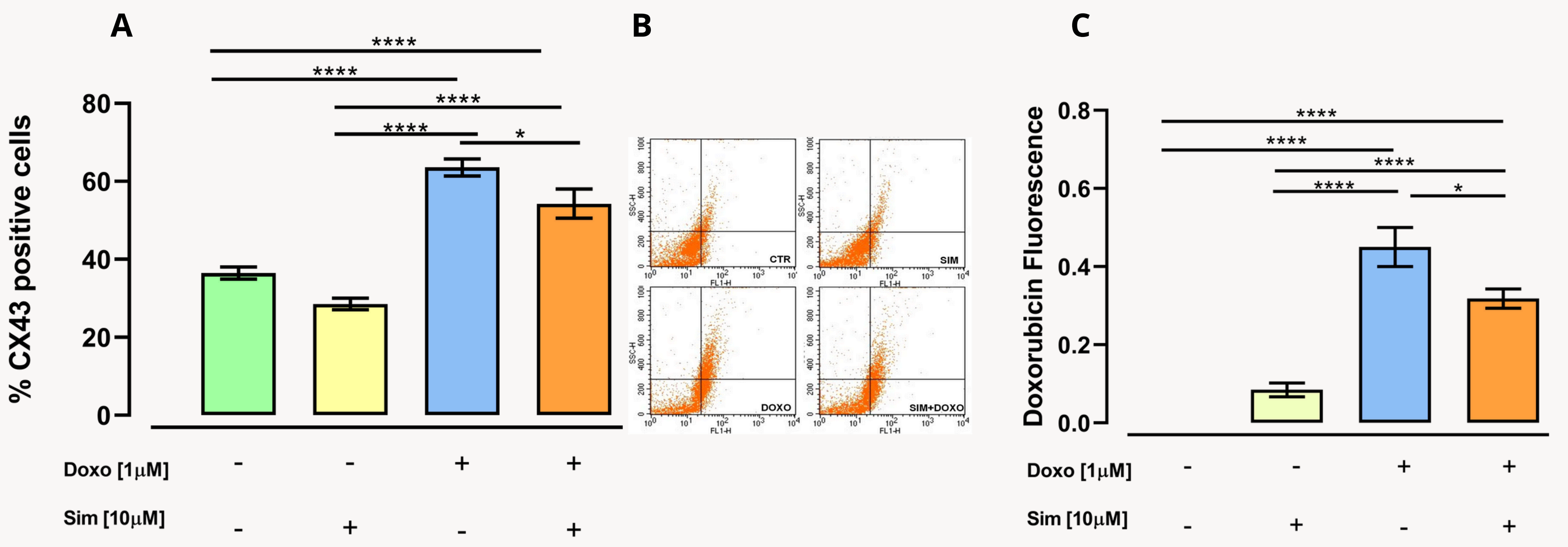


Figure 7. To evaluate membrane level of Cx43 flow cytometry analysis was used. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni multiple comparisons test. Values are expressed as mean ± SEM of % Cx43 positive cells (A). Spectrofluorimetric analysis was used to evaluate Doxorubicin uptake in HCM cells. Values are reported as mean ± S.E.M. of Fluorescence intensity of Doxorubicin (C). Data were analyzed by variance test analysis and multiple comparisons were made by Bonferroni's test. * p<0.05 *** p<0.005, **** p<0.001. Representative histograms for the flow cytometry analysis are reported in Panel (B)

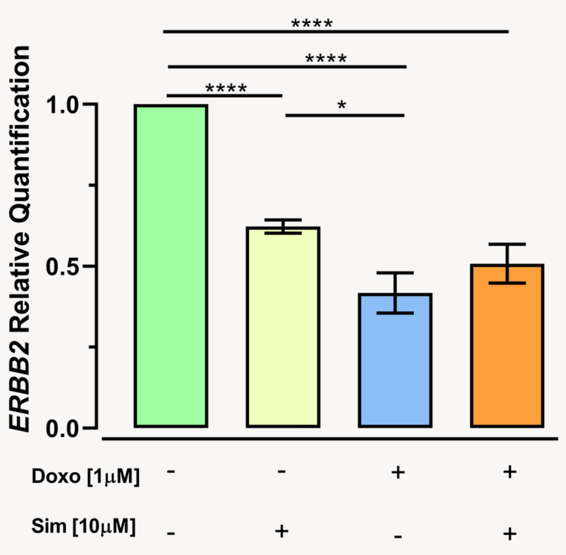


Figure 8. Effects of Sim, Doxo and Sim co-treatment on *ERBB2* relative gene expression in a Human Cardiomyocyte cell line, as determined by real-time RT PCR. Data were calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to GAPDH cDNA levels and then expressed as relative to control (calibrator sample, defined as 1.00). Values are expressed as means ± SD and were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. * p<0.05, **** p<0.001

Conclusion

Simvastatin co-treatment, in our experimental model, was shown to alleviate oxidative stress and reduce apoptosis, thus leading human cardiomyocytes to reduce their defense responses.

These data indicate that Simvastatin could be a valuable therapeutic approach to mitigate or prevent Doxorubicin-induced acute cardiotoxicity.

Funded by the European Union - Next Generation EU. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them. PNRR MUR M4 C2 Inv. 1.5 CUP B83C22003920001

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