Shahid Bahonar University of Kerman

Agricultural Biotechnology Journal

Print ISSN: 2228-6705 Online ISSN: 2228-6500

Iranian Biotechnology Society

Combined inhibition of HMG-CoA reductase and the renin-angiotensin system attenuates doxorubicin-induced cardiotoxicity in an albino rat model

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Abstract

Objective

Doxorubicin-associated cardiotoxicity remains a significant clinical challenge in clinical settings. This study aimed to investigate the synergistic cardioprotective effects of combined simvastatin and losartan against doxorubicin-induced cardiac injury.

Materials and methods

A total of 42 Albino Wistar rats were enrolled in the preset study and subdivided into, control male and female groups (received distilled water orally for 7 days and IP normal saline dose at day 14), doxorubicin male and female groups (received distilled water orally for 13 days and single IP dose of doxorubicin 15 mg/kg at day 14), and simvastatin+losartan+doxorubicin male and female groups (received oral simvastatin dose of 10 mg/kg/day and losartan dose of 10 mg/kg/day for 13 days and single IP dose of doxorubicin 15 mg/kg at day 14)**.** The sampling for all group were conducted at day 16, including blood collection and tissue harvesting after the animal sacrificed. Initial blood sample collected at day 0 before starting any interventional products. Cardiac injury was assessed through histological examination and biochemical analysis of cardiac biomarkers including tumor necrosis factor-alpha (TNF-α), cardiac troponin (TNNI3), and heart-type fatty acid-binding protein (FABP3).

Results

Histological analysis revealed that combination therapy significantly attenuated doxorubicin-induced cardiac damage, showing only slight vascular congestion compared to severe endocardial

congestion, inflammatory cell infiltration, and myocyte necrosis observed in the doxorubicin-only group. The combination therapy group demonstrated localized thin fibrous tissue formation between muscle bundles and mild interstitial edema, suggesting active tissue remodeling and healing processes. Biochemically, control groups maintained stable baseline levels throughout the study (TNF-α: 49.3±2.5 to 48.5±1.2; troponin: 11±0.3 to 11.7±0.5; FABP3: 0.55±0.09 to 0.66±0.09). The combination therapy provided remarkable cardioprotection in both sexes, with male rats showing non-significant changes in TNF-α (56.3±4.1 to 59.4±6.3, p=0.53), troponin (12.2±1 to 11.8±0.9, p=0.22), and FABP3 (0.67±0.1 to 0.74±0.05, p=0.63). Female rats demonstrated similar protection with TNF-α levels (60.9±7.4 to 56.2±11.3, p=0.6), troponin (10.8±0.7 to 11.7±0.8, p=0.1), and FABP3 (0.6±0.11 to 0.68±0.08, p=0.9) remaining within normal ranges.

Conclusions

The combination of simvastatin and losartan demonstrated synergistic cardioprotective effects against doxorubicin-induced cardiotoxicity through dual mechanisms involving statin-mediated pleiotropic protection and angiotensin receptor blockade. This combination therapy preserved cardiac function, prevented elevation of cardiac injury biomarkers, and promoted beneficial tissue remodeling. These findings suggest that simvastatin-losartan combination therapy represents a promising cardioprotective strategy for patients receiving doxorubicin chemotherapy, potentially allowing for optimal oncological treatment while minimizing cardiac complications.

Keywords: Cardiac biomarkers, Cardiotoxicity, Doxorubicin, Losartan, Simvastatin

Paper Type: Research Paper.

Citation: Marwa, J. K., & Zeina, A. A. T. (2025). Combined inhibition of HMG-CoA reductase and the renin-angiotensin system attenuates doxorubicin-induced cardiotoxicity in an albino rat model. *Agricultural Biotechnology Journal,* 17(3), 235-252.

Agricultural Biotechnology Journal, 17(3), 235-252. DOI: 10.22103/jab.2025.25469.1722

Received: May 21, 2025. Received in revised form: July 16, 2025.

Accepted: July 17, 2025. Published online: August 30, 2025.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.

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Introduction

Doxorubicin is one of the most effective and extensively prescribed anthracycline antibiotics in oncology, demonstrating notable efficacy against a broad spectrum of malignancies including breast cancer, lymphomas, sarcomas, and acute leukemias (Hamaamin & Aziz, 2022). Since its introduction in the 1960s, this potent chemotherapeutic agent has protected lives and continues a cornerstone of several treatment protocols (Sinha et al., 2025). However, the clinical application of doxorubicin is markedly counteracted by its dose-dependent cardiotoxicity, a destructive side effects that can revealed as acute cardiac dysfunction during treatment or, more insidiously, as delayed-onset cardiomyopathy years after therapy completion (Bartlett et al., 2016). The pathophysiology of doxorubicin-induced cardiotoxicity is multifactorial and complex, involving oxidative stress, mitochondrial dysfunction, calcium dysregulation, and inflammatory cascades that ultimately lead to cardiomyocyte death and myocardial fibrosis (Hamaamin & Aziz, 2022). The heart restricted regenerative capacity makes it remarkably vulnerable to these toxic upsets, with cumulative doses exceeding 400-500 mg/m² carrying a remarkable risk of irreversible cardiac damage(Safra et al., 2000; Tian et al., 2020). This toxicity profile has forced clinicians into the difficult position of balancing oncological efficacy against cardiovascular safety, often necessitating dose limitations or treatment discontinuation that may compromise cancer outcomes. The renin-angiotensin-aldosterone system (RAAS) has emerged as a critical mediator of cardiac remodeling and dysfunction, with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers demonstrating cardioprotective effects in various cardiovascular conditions (Akolkar et al., 2015). Similarly, the HMG-CoA reductase pathway, beyond its well-established role in cholesterol synthesis, has been implicated in cellular stress responses, inflammation, and survival signaling (Bhasin et al., 2024). Statins, the primary inhibitors of HMG-CoA reductase, have shown pleiotropic effects including anti-inflammatory, antioxidant, and membrane-stabilizing properties that extend far beyond their lipid-lowering capabilities (Pecoraro et al., 2023). The convergence of these observations has led to the compelling hypothesis that simultaneous inhibition of both the angiotensin system and HMG-CoA reductase might provide superior cardioprotection against doxorubicin toxicity compared to either intervention alone. This dual-pathway approach represents a rational therapeutic strategy that addresses multiple mechanistic aspects of doxorubicin cardiotoxicity while potentially preserving the drug's anticancer efficacy. The present study was designed to investigate this novel combination approach and elucidate the underlying mechanisms of protection in a clinically relevant experimental model using losartan and simvastatin to block doxorubicin cardiotoxicity.

Materials and Methods

Study Time Frame: The current study was conducted in the animal house of the College of Veterinary Medicine/University of Mosul over a period of 29.10.2024 to 28.02.2025 with the support and assistance of the Department of Pharmacology and Toxicology, College of Pharmacy, University of Mosul.

Animals and housing: A total of 98 Albino Wistar rats (ten-week-old, ~200g average body weight) were kindly provided from the animal house at the College of Veterinary medicine, University of Mosul (Iraq) had been used in this experiment. The animals were housed in metallic cages and subjected to an adaptation period of two weeks with a photoperiod of (12 hr: 12 hr light/dark), 25°C ± 2°C temperature, and 45–50% humidity, while receiving the normal amount of water and food and all rats had been acclimatized for a period of 1 month before handling in experiments. Approval was obtained from the College Committee for Medical Research Ethics at the University of Mosul (Iraq), as well as the Graduate Studies Committee at the College of Pharmacy, to conduct this study. Ref: UM.VET.2024.132 on 02.12.2024.

Experimental design: The 42 rats were subdivided into six groups:

G1M=Control male group (7 rats): received distilled water orally for 7 days and IP normal saline dose at day 14.

G1F=Control female group (7 rats): received distilled water orally for 13 days and IP normal saline dose at day 14.

G2M=Dox male group (7 rats) received distilled water orally for 13 days and single IP dose of doxorubicin 15mg/kg at day 14.

G2F=Dox female group (7 rats) received normal saline orally for 13 days and single IP dose of doxorubicin 15mg/kg at day 14.

G3M= SMV+LST+Dox male group (7 rats): received oral simvastatin dose of 10mg/kg/day and losartan dose of 10mg/kg/day for 13 days and single IP dose of doxorubicin 15mg/kg at day 14

G3F= SMV+LST+Dox female group (7 rats): received oral simvastatin dose of 10mg/kg/day and losartan dose of 10mg/kg/day for 13 days and single IP dose of doxorubicin 15mg/kg at day 14.

The sampling for all groups was conducted at day 16, including blood collection and tissue harvesting after the animal was sacrificed. An initial blood sample was collected at day 0 before starting any interventional products.

Histological analysis: Tissue specimens from heart were collected immediately following euthanasia and processed for histopathological examination using standard protocols. The

specimens were fixed in 10% neutral buffered formalin (pH 7.2-7.5) for 24-48 hours to prevent autolysis and preserve cellular morphology. Following fixation, tissues underwent comprehensive processing through sequential dehydration using graded ethanol solutions (70%, 80%, 90%, and 100% ethanol, each for 2 hours), followed by clearing in xylene for 30 minutes to remove alcohol and prepare tissues for paraffin infiltration. The cleared specimens were then impregnated with molten paraffin wax at 58-60°C through two consecutive one-hour immersion cycles to ensure complete penetration and elimination of air bubbles. After embedding in paraffin blocks and cooling, the specimens were sectioned using a rotary microtome to produce 5-6 μm thick sections, which were subsequently mounted on glass slides and dried on a hot plate at 45°C. Histological evaluation was performed using standard hematoxylin and eosin staining following deparaffinization with xylene and rehydration through descending alcohol concentrations. Microscopic examination was conducted using light microscope equipped with a digital imaging system and specialized image analysis software for morphometric measurements and photodocumentation.

Biochemical Analysis: Comprehensive biochemical analysis was performed using multiple analytical platforms to evaluate cardiac injury biomarkers, using the CobasR-2000 Automatic Biochemistry Analyzer (Roche, Hitachi, Switzerland). Cardiac injury assessment was conducted through quantitative measurement of specific biomarkers using enzyme-linked immunosorbent assay (ELISA) techniques. Cardiac troponin I type 3 (TNNI3) levels were determined using the ELK Biotechnology Rat TNNI3 ELISA Kit, which employs a sandwich enzyme immunoassay principle. Heart-type fatty acid binding protein 3 (FABP3) was quantified using the corresponding ELK Biotechnology Rat FABP3 ELISA Kit. Inflammatory response evaluation was performed through measurement of tumor necrosis factor alpha (TNF-α) using the ELK Biotechnology Rat TNF-α ELISA Kit, utilizing sandwich enzyme immunoassay methodology with detection range of 15.63-1000 pg/mL. The TNF-α standard curve was generated through serial dilutions of reconstituted lyophilized standard (1000 pg/mL stock) across seven tubes to create concentrations ranging from 1000 pg/mL down to 15.63 pg/mL, with an eighth tube serving as blank. All ELISA procedures followed identical standardized protocols involving initial antigen capture on pre-coated microplates during 80-minute incubation at 37°C, followed by biotinylated antibody binding (50 minutes at 37°C), streptavidin-HRP conjugation (50 minutes at 37°C), and TMB substrate color development (20 minutes at 37°C in darkness). Critical quality control measures included equilibration of all reagents to room temperature (18-25°C), precise dilution of biotinylated antibodies and streptavidin-HRP conjugates (1:100 dilutions), rigorous washing protocols using 1× wash buffer (three washes after antibody steps, five washes after enzyme

conjugate), and careful handling of light-sensitive TMB substrate with reaction termination using stop reagent to convert blue color to stable yellow before optical density measurement at 450 nm.

Statistical analysis: Statistics of biochemical data was performed using GraphPad Prism software (Version 11.5, USA), employing one-way and two-way analysis of variance (ANOVA) with Tukey's multiple comparison post-hoc tests to identify significant differences between experimental groups, with statistical significance established at $P \leq 0.05$.

Results

Histological analysis: The histological analysis of cardiac section from male rats treated with the combination of simvastatin and losartan revealed the dual cardioprotective mechanisms against doxorubicin-induced cardiotoxicity. This microscopic observation perhaps indicated the synergistic effects of statin-mediated pleiotropic protection and angiotensin receptor blockade resulting in a distinctive pattern of cardiac improvement that differs from either agent used alone. The cardiac sections demonstrated slight congestion of blood vessels, showing significant recovery. This mild vascular deficit reflect that the combination therapy positively preserved cardiac perfusion via allowing for minimal, region-specific vascular adaptation. Perhaps the tissue analysis demonstrated the precipitation and formation of thin fibrous tissue localized between muscle bundles, reflecting tissue remodeling that was not adopted with either cardioprotective therapy alone. This fibrous tissue formation suggested that the dual therapy may have commenced protective fibroblast activation and collagen precipitation as part of a cardiac healing and resolution. The observation of edema between muscle bundles further reinforced the view of an active tissue response, possibly representing increased interstitial fluid associated with healing processes.

Biochemical analysis in doxorubicin group: The control groups showed stable TNFa concentration throughout the study period. Male control rats maintained consistent serum TNFa concentrations, with baseline levels of 49.5±2.7 and post-intervention levels of 49.3±2.5, demonstrating no significant change (p=0.8). Correspondingly, female control rats demonstrated stable proinflammatory markers, with pre-treatment levels of 49.3±2.5 and post-treatment levels of 48.5±1.2 (p=0.9). The control groups launched stable baseline cardiac troponin throughout the study period, acting as the standard for comparative analysis. Male control rats demonstrated sustained troponin levels, starting at 11±0.4 and demonstrating minimal change to 11.2±0.3 after the study period (p=0.26). Female control rats presented similar stability, with baseline troponin concentrations of 11±0.3 remaining essentially unchanged at 11.7±0.5 (p=0.88). The control groups established baseline cardiac parameters throughout the study period, male control rats

demonstrated baseline FABP3 levels of 0.62±0.1, which remained stable at 0.66±0.09 after the study period, with no statistical significance (p=0.66). Female control rats shown similar stability, with baseline FABP3 levels of 0.61±0.09 decreasing slightly to 0.55±0.09 (p=0.8). The normal FABP3 range seems to be approximately 0.55-0.66, providing a reference value for comparison with intervention groups.

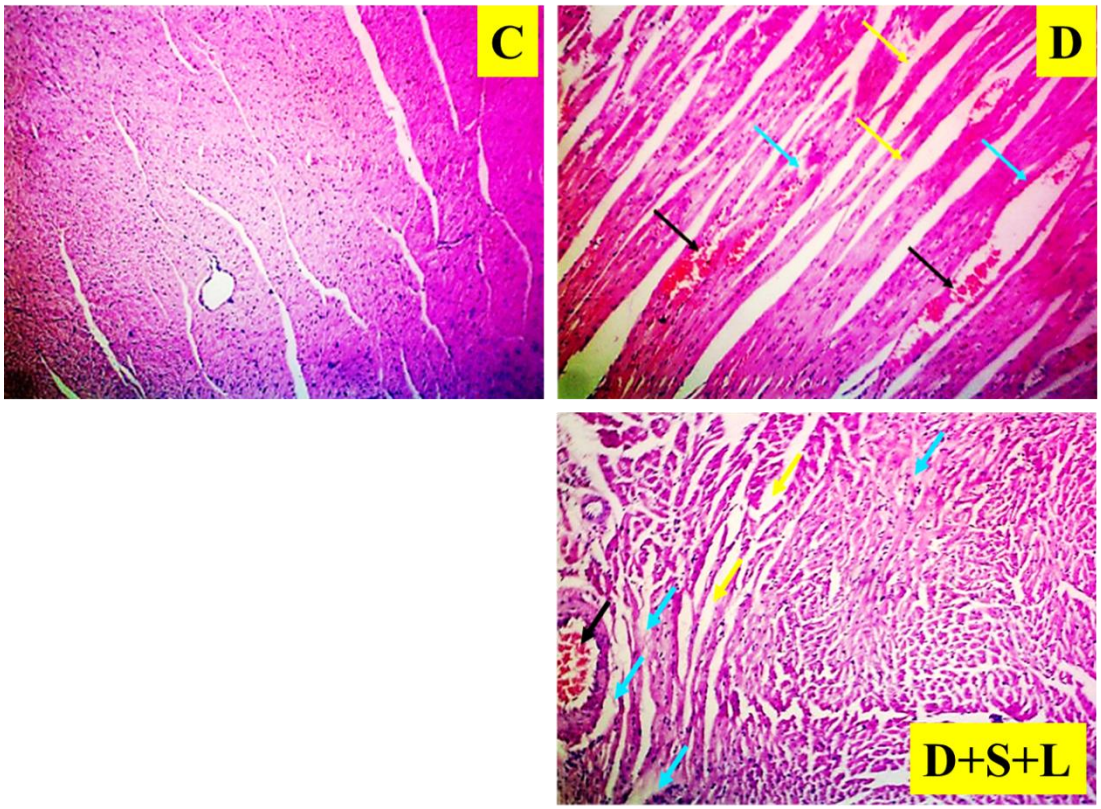

Figure 1. Histological section of male rat's heart tissue at the end of experiment. Control group (C) showing normal myocardium architectures, Doxorubicin group (D) revealed remarkable congestion of blood vessels in endocardium layers (black arrow) and sever infiltration of inflammatory cells (yellow arrow), sever necrosis of myocytes (blue arrows). Doxorubicin, losartan, and simvastatin group (D+L+S) revealed slight congested blood vessels in the ventricle (black arrows) deposition and formation of thin fibrous tissue (blue arrows) edema were detected as well between muscle bundles (Yellow arrows). H&E stain, scale-bar=100µm

Biochemical analysis in simvastatin, losartan, and doxorubicin group

Male rats receiving the combination of simvastatin, losartan, and doxorubicin demonstrated TNFa levels changing from 56.3±4.1 at baseline to 59.4±6.3 after treatment (p=0.53). Female rats in the triple combination group revealed TNFa levels decreasing slightly from 60.9±7.4 to 56.2±11.3 (p=0.6).

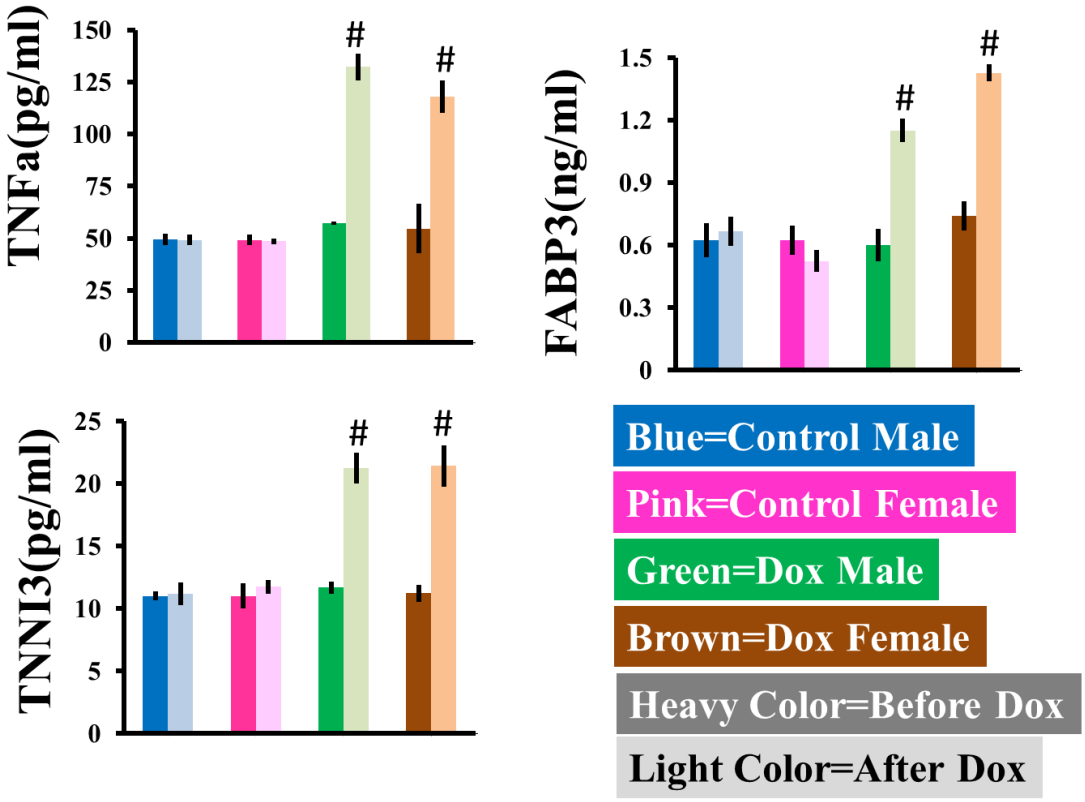

Figure 2. Cardiac biochemical parameters measured in the doxorubicin-exposed rats. The histogram bar represents mean±SD for each group. #indicates p value less than 0.05 using One-way ANOVA and Bonferroni tests to find the different group. TNFa=tumour necrosis factor alpha, TNNI3=cardiac troponin, FABP3=Heart-type fatty acid-binding protein

The combination of simvastatin, losartan, and doxorubicin demonstrated remarkable cardioprotective effects that appeared to provide near-complete cardioprotection. Male rats administered the triple combination demonstrated baseline troponin levels of 12.2±1, which decreased to 11.8±0.9 after treatment (p=0.22), indicating that the combination maintained normal serum troponin levels despite doxorubicin exposure. Female rats in the simvastatin, losartan, and doxorubicin group demonstrated comparable cardioprotective findings, with troponin baseline levels of 10.8±0.7, these animals showed minimal change to 11.7±0.8 after treatment (p=0.1). The final troponin level was markedly lower than the 21.4±1 observed in doxorubicin monotherapy females, confirming that the combination therapy prevented approximately 100% of the cardiac injury. The results in Figure 3.9 and Table represents an evaluation of serum Fatty Acid Binding Protein 3 (FABP3) concentration in rats to assess the cardioprotective effects of combination therapy using simvastatin and losartan against doxorubicin-induced cardiac injury, including results of both male and female rats over multiple

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

treatment groups, quantifying FABP3 levels before and after interventions to evaluate the protective efficacy of this dual-drug approach. Male rats receiving the triple agents showed baseline FABP3 levels of 0.67±0.1, which increased minimally to 0.74±0.05 after treatment. This slight elevation was not statistically significant (p=0.63), indicating that the combination therapy effectively prevented the dramatic FABP3 elevation typically associated with doxorubicin exposure. The final FABP3 level of 0.74±0.05 remained well within the normal range observed in control animals, demonstrating that the combination successfully protected against myocardial cellular damage. Female rats in the combination therapy group demonstrated parallel cardioprotective outcomes with FABP3 values, with baseline FABP3 levels of 0.6±0.11, these animals showed minimal change to 0.68±0.08 after treatment (p=0.9). The p-value suggesting complete protection against doxorubicin-induced myocardial injury, with final FABP3 level was markedly lower than the 1.42±0.05 observed in doxorubicin-only treated females, highlighting that the combination therapy prevented the expected cardiac cellular damage.

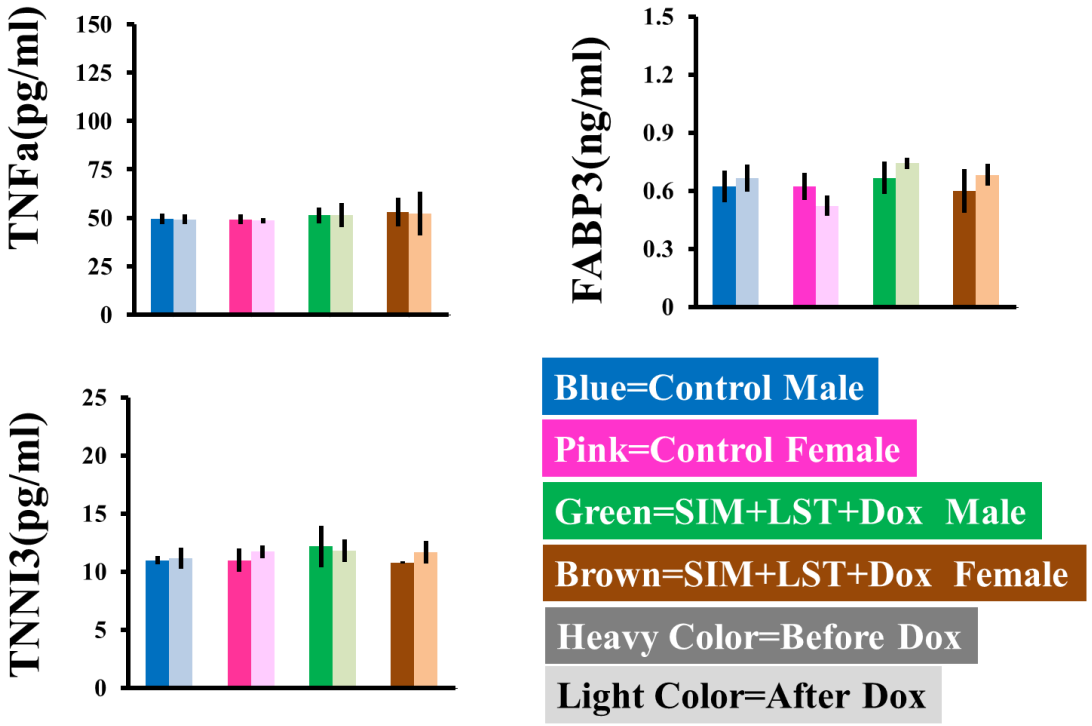

Figure 3. Cardiac biochemical parameters measured in the SMV+LST+Dox-exposed rats. The histogram bar represents mean±SD for each group. p value was more than 0.05 using One-way ANOVA and Bonferroni tests to find the different group. TNFa=tumour necrosis factor alpha, TNNI3=cardiac troponin, FABP3=Heart-type fatty acid-binding protein

Discussion

The present study demonstrated that simvastatin and/or losartan have provided cardioprotection against doxorubicin induced tissue damage confirmed by histological and biochemical parameters. Both drugs have mitigated the cardiac tissue damage alone or combined treated group, perhaps a combination provided more efficient restoration against doxorubicin-induced cardiac damage. The combination of simvastatin and losartan have reduced rat serum TNNI3, this troponin reduction by combined ARBs coadministration with simvastatin against doxorubicin injury has been reported in earlier evidence which confirmed that troponin reduction to nearly normal level after coadministration candesartan and rosuvastatin (Cho et al., 2020). Simvastatin, an HMG-CoA reductase blocker, participates to cardiac protection through its capacity to mitigate oxidative stress by decreasing NADPH oxidase activity, enhancing endothelial function, offering anti-inflammatory impacts, and perhaps boosting cellular autophagy while reducing apoptosis. Synergistically, angiotensin II receptor blockers (such as, losartan) acts by blocking angiotensin II-mediated cardiac tissue remodeling and fibrosis, mitigating cardiac hypertrophy and inflammation, enhancing coronary perfusion, and offering additional antioxidant action. The combination seems to highlight doxorubicin sophisticated cardiac damage through an integrated approach that targets oxidative stress, inflammation, and pathological cardiac remodeling concurrently. Perhaps what makes this study particularly compelling is that both medications are repurposed with well-established safety profiles, potentially speeding the translation from preclinical findings to clinical application. In agreement with this study, Hadi and Yousif et al. (2013), confirmed that simvastatin has induced a notable histological protective effect through its anti-inflammatory properties against doxorubicin in rats undergoing coronary artery ligation, significantly has attenuated the inflammatory pathways triggered by ischemia-reperfusion injury. Simvastatin has reversed the elevation of proinflammatory marker, interleukin-1β (IL-1β). This anti-inflammatory action translates into noticeable histological protection, with treated animals showing markedly reduced severity of cardiac injury compared to controls exposed to the same ischemic injury (Hadi NR & Yousif FG, 2013). In addition to these studies, clinical trials using combined statins with anticancer have promising outcomes through statins-induced cardioprotection. In meta-analysis (887 patients) evidences demonstrating a noticeable 54% reduction in the rate of cancer therapy-related cardiac dysfunction when statins are prescribed prophylactically to patients receiving anthracycline-based chemotherapy regimens (Felix et al., 2024), establishing a new indication as clinical foundation for the routine combination of statin therapy as a cardioprotective strategy in oncological settings. The outcomes from a detailed meta-analysis of 1,839 breast cancer patients treated with

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

anthracyclines and/or trastuzumab, revealed that statin treatment offered remarkable protection against chemotherapy-induced cardiac injury, with patients administered statins facing a 55% reduction in cardiotoxicity consequences compared to statin-free group (Lingamsetty et al., 2024). This protective effect confirmed via clinical benefits demonstrated by the restoration of left ventricular ejection fraction, since statin-treated patients maintained cardiac function with 4.11% positive restoration of cardiac function compared to untreated group (Lingamsetty et al., 2024). In systematic review, the analysis of 33 published studies of total 3000 patients revealed that simvastatin offered cardioprotection during cancer therapy in preserving left ventricular ejection fraction reduction, with a difference of 6.72% compared to control groups (Bhasin et al., 2024). The action being explained in the context of the simvastatin anti-inflammatory, antioxidant, and pleiotropic effects shaping unique aspects of chemotherapy-induced cardiac injury that extend beyond hemodynamic considerations alone. In the line with the present study, in vivo study conducted on male Sprague-Dawley rat model exposed to doxorubicin with losartan used as cardiprotective agent. The results have confirmed that co-administration of doxorubicin with losartan offered cardioprotective impact by attenuating left ventricular hypertrophy, pro-inflammatory factors, and apoptosis in left ventricular tissue (Kim et al., 2023). In alternative study conducted on male Wistar rats losartan improved diastolic but not systolic dysfunction and decreased cardiac remodeling due to hypertrophy of left ventricle (Freiwan et al., 2022). In contrast, a study conducted by Freiwan et al. (2021) using male Wistar rats, losartan revealed partial cardioprotective role against doxorubicin-induced cardiotoxicity when administered as an interventional therapy following doxorubicin exposure. Losartan showed partial impact on the major echocardiographic parameters of cardiac dysfunction. Losartan failed to significantly enhance the key markers of doxorubicin cardiotoxicity at week 8 assessment. Specifically, losartan-treated animals continued to exhibit significantly reduced systolic septal and anterior wall thicknesses, decreased fractional shortening and ejection fraction, and increased left ventricular end-systolic diameter compared to controls, indicating persistent systolic dysfunction. However, losartan did provide some beneficial effects by significantly reducing isovolumic contraction time compared to the doxorubicin-only group, suggesting modest improvement in cardiac contractility dynamics, while losartan exerted some hemodynamic effects and slight improvements in some cardiac hemodynamic parameters, it failed to improve the principle pathological steps of doxorubicin cardiotoxicity, including systolic dysfunction, cardiac atrophy, and fibrotic remodeling (Freiwan et al., 2022). The role of losartan in minimizing cardiotoxicity associated with anthracyclines including doxorubicin has been explained in the light of the role of renin angiotensin system involvement in this context. The renin-angiotensin system (RAS) is principle involved in the pathogenesis of cardiac hypertrophy, heart failure, and ischemia-

reperfusion injury (Pinter et al., 2018; Zablocki & Sadoshima, 2013). Inhibiting this system through angiotensin-converting enzyme (ACE) inhibitors has been shown to uplift cardiac remodeling processes and prolong survival in both animal models and clinical studies affected by these cardiovascular conditions (Akolkar et al., 2015; Iqbal et al., 2008). From a mechanistic outcome, angiotensin II suppresses the neuregulin-1/ErbB signaling pathway, which is fundamental for cardioprotection. This integration suggests that the therapeutic benefits plausible with ACE inhibition may originated from preserving or enhancing NRG-1/ErbB system function (Lemmens et al., 2006). Additionally, a study of telmisartan demonstrated significant cardioprotective efficacy against doxorubicin-induced cardiac toxicity through integrated mechanisms that target the key pathways of chemotherapy-related heart damage, with mechanisms involved tissue protection, antioxidants effects, and suppressing the expression of inducible nitric oxide synthase, moreover, histologically telmisartan prevented fibrosis, a hallmark of doxorubicin cardiotoxicity that leads to progressive cardiac dysfunction and heart failure (Ibrahim et al., 2009). In the present study, rat serum TNFa was greatly reduced after losartan exposure indicating that losartan possesses anti-inflammatory role. This anti-inflammatory activity has been described earlier in vitro study involving nucleus pulposus cells donated by male patients with mild disc degeneration, losartan at low concentration reduced TNFa expression in culture media (Saravi et al., 2021). Alternatively, cultured macrophage model THP-1 cell line have expressed less TNFa after stimulation when exposed to losartan compared to losartan free group (An et al., 2010). Similarly, in clinical study involving losartan treatment for heart failure has demonstrated significant impact in reducing TNFa levels indicating the relationship between angiotensin pathway and inflammatory markers expression (Gurlek et al., 2001). In the present study, rat serum TNNI3 was greatly reduced after losartan exposure indicating that losartan possesses cardioprotective role. This troponin reduction by ARBs coadministration with doxorubicin has been reported in earlier evidence which confirmed that troponin reduction to nearly normal level after coadministration with candesartan. Similarly, valsartan has reduced TNNI3 when coadministered with trastuzumab reducing trastuzumab-Mediated Cardiotoxicity in in vivo rat model (Olorundare et al., 2021).

Conclusions: This study demonstrates that the combination of simvastatin and losartan provides exceptional cardioprotective efficacy against doxorubicin-induced cardiotoxicity through complementary mechanisms of action. The comprehensive evaluation of both histological and biochemical parameters revealed that this dual therapeutic approach achieved near-complete prevention of cardiac injury, representing a significant advancement in cardio-oncology. These findings have significant clinical implications for cancer patients requiring

doxorubicin therapy. The development of an effective cardioprotective strategy that maintains the oncological efficacy of doxorubicin while preventing cardiac complications could transform treatment protocols in cardio-oncology. The combination of simvastatin and losartan, both well-established medications with favorable safety profiles, presents a practical and immediately implementable therapeutic option that could reduce treatment-related morbidity and improve long-term outcomes for cancer survivors. Future research should focus on translating these preclinical findings to clinical trials, investigating optimal dosing regimens, and evaluating the long-term cardiovascular outcomes in cancer patients. Additionally, mechanistic studies exploring the molecular pathways underlying the synergistic cardioprotective effects could provide insights for developing next-generation cardioprotective strategies. The potential for this combination therapy to be extended to other cardiotoxic chemotherapeutic agents also warrants investigation.

Author Contributions

Conceptualization: ZAA; Data curation: MJK; Formal analysis: ZAA; Investigation: MJK; Methodology: MJK; Project administration: ZAA; Software: ZAA; Resources: MJK and ZAA; Supervision: ZAA; Validation: ZAA; Visualization: ZAA; Writing – original draft: MJK and ZAA; Writing – review & editing: ZAA. All authors contributed equally to the conceptualization of the article and the writing of the original and subsequent drafts.

Data Availability Statement

Data is available on request from the authors.

Acknowledgements

The authors are grateful to the University of Mosul for providing the facilities necessary to conduct this research.

Ethical Considerations

Approval was obtained from the College Committee for Medical Research Ethics at the University of Mosul (Iraq), as well as the Graduate Studies Committee at the College of Pharmacy, to conduct this study. Ref: UM.VET.2024.132 on 02.12.2024.

Funding

The study was funded by the University of Mosul, Iraq.

Conflict of Interest

The authors declare no conflict of interest.

References

Akolkar, G., Bhullar, N., Bews, H., Shaikh, B., Premecz, S., Bordun, K.-A., Cheung, D. Y., Goyal, V., Sharma, A. K., Garber, P., Singal, P. K., & Jassal, D. S. (2015). The role of renin angiotensin system antagonists in the prevention of doxorubicin and trastuzumab induced cardiotoxicity. *Cardiovascular Ultrasound*, *13*(1), 18. https://doi.org/10.1186/s12947-015-0011-x

An, J., Nakajima, T., Kuba, K., & Kimura, A. (2010). Losartan inhibits LPS-induced inflammatory signaling through a PPARγ-dependent mechanism in human THP-1 macrophages. *Hypertension Research*, *33*(8), 831–835. https://doi.org/10.1038/hr.2010.79

Bartlett, J. J., Trivedi, P. C., Yeung, P., Kienesberger, P. C., & Pulinilkunnil, T. (2016). Doxorubicin impairs cardiomyocyte viability by suppressing transcription factor EB expression and disrupting autophagy. *Biochemical Journal*, *473*(21), 3769–3789. https://doi.org/10.1042/BCJ20160385

Bhasin, V., Vakilpour, A., & Scherrer-Crosbie, M. (2024). Statins for the Primary Prevention of Anthracycline Cardiotoxicity: A Comprehensive Review. *Current Oncology Reports*, *26*(10), 1197–1204. https://doi.org/10.1007/s11912-024-01579-6

Cho, D.-H., Lim, I.-R., Kim, J.-H., Kim, M.-N., Kim, Y.-H., Park, K. H., Park, S.-M., & Shim, W. J. (2020). Protective Effects of Statin and Angiotensin Receptor Blocker in a Rat Model of Doxorubicin- and Trastuzumab-Induced Cardiomyopathy. *Journal of the American Society of Echocardiography*, *33*(10), 1253–1263. https://doi.org/10.1016/j.echo.2020.05.021

Felix, N., Nogueira, P. C., Silva, I. M., Costa, T. A., Campello, C. A., Stecca, C., & Lopes, R. D. (2024). Cardio-protective effects of statins in patients undergoing anthracycline-based chemotherapy: An updated meta-analysis of randomized controlled trials. *European Journal of Internal Medicine*, *126*, 43–48. https://doi.org/10.1016/j.ejim.2024.04.007

Freiwan, M., Kovács, M. G., Kovács, Z. Z. A., Szűcs, G., Dinh, H., Losonczi, R., Siska, A., Kriston, A., Kovács, F., Horváth, P., Földesi, I., Cserni, G., Dux, L., Csont, T., & Sárközy, M. (2022). Investigation of the Antiremodeling Effects of Losartan, Mirabegron and Their Combination on the Development of Doxorubicin-Induced Chronic Cardiotoxicity in a Rat Model. *International Journal of Molecular Sciences*, *23*(4), 2201. https://doi.org/10.3390/ijms23042201

Gurlek, A., Kilickap, M., Dincer, I., Dandachi, R., Tutkak, H., & Oral, D. (2001). Effect of Losartan on Circulating TNF Levels and Left Ventricular Systolic Performance in Patients with Heart Failure. *European Journal of Cardiovascular Prevention & Rehabilitation*, *8*(5), 279–282. https://doi.org/10.1177/174182670100800506

Hadi NR & Yousif FG. (2013). The effect of Simvastatin in attenuation of Myocardial Ischemia/Reperfusion injury. *Kufa Journal For Veterinary Medical Sciences.*, *4*(2), 8–17.

Hamaamin, K. S., & Aziz, T. A. (2022). Doxorubicin-Induced Cardiotoxicity: Mechanisms and Management. *Al-Rafidain Journal of Medical Sciences (ISSN: 2789-3219)*, *3*, 87–97. https://doi.org/10.54133/ajms.v3i.90

Ibrahim, M. A., Ashour, O. M., Ibrahim, Y. F., EL-Bitar, H. I., Gomaa, W., & Abdel-Rahim, S. R. (2009). Angiotensin-converting enzyme inhibition and angiotensin AT1-receptor antagonism equally improve doxorubicin-induced cardiotoxicity and nephrotoxicity. *Pharmacological Research*, *60*(5), 373–381. https://doi.org/10.1016/j.phrs.2009.05.007

Iqbal, M., Dubey, K., Anwer, T., Ashish, A., & Pillai, K. K. (2008). Protective effects of telmisartan against acute doxorubicin-induced cardiotoxicity in rats. *Pharmacological Reports: PR*, *60*(3), 382–390.

Kim, H. A., Kim, K. C., Lee, H., & Hong, Y. M. (2023). Losartan Reduces Remodeling and Apoptosis in an Adriamycin-Induced Cardiomyopathy Rat Model. *Journal of Chest Surgery*, *56*(5), 295–303. https://doi.org/10.5090/jcs.23.044

Lemmens, K., Segers, V. F. M., Demolder, M., & De Keulenaer, G. W. (2006). Role of Neuregulin-1/ErbB2 Signaling in Endothelium-Cardiomyocyte Cross-talk. *Journal of Biological Chemistry*, *281*(28), 19469–19477. https://doi.org/10.1074/jbc.M600399200

Lingamsetty, S. S. P., Kritya, M., Huang, W., Fatima, S. R., Gewehr, D. M., Doma, M., Pastrana, S. H., Hemdanieh, M., Naji, Z., Kuhar, K., & Martignoni, F. (2024). Cardioprotective role of statins in breast cancer patients treated with anthracyclines and/or trastuzumab: A systematic review and meta-analysis. *European Heart Journal*, *45*(Supplement_1), ehae666.3218. https://doi.org/10.1093/eurheartj/ehae666.3218

Olorundare, O. E., Adeneye, A. A., Akinsola, A. O., Ajayi, A. M., Agede, O. A., Soyemi, S. S., Mgbehoma, A. I., Okoye, I. I., Albrecht, R. M., Ntambi, J. M., & Crooks, P. A. (2021). Therapeutic Potentials of Selected Antihypertensive Agents and Their Fixed-Dose Combinations Against Trastuzumab-Mediated Cardiotoxicity. *Frontiers in Pharmacology*, *11*, 610331. https://doi.org/10.3389/fphar.2020.610331

Pecoraro, M., Marzocco, S., Belvedere, R., Petrella, A., Franceschelli, S., & Popolo, A. (2023). Simvastatin Reduces Doxorubicin-Induced Cardiotoxicity: Effects beyond Its Antioxidant

Activity. *International Journal of Molecular Sciences*, *24*(8), 7573. https://doi.org/10.3390/ijms24087573

Pinter, M., Kwanten, W. J., & Jain, R. K. (2018). Renin–Angiotensin System Inhibitors to Mitigate Cancer Treatment–Related Adverse Events. *Clinical Cancer Research*, *24*(16), 3803–3812. https://doi.org/10.1158/1078-0432.CCR-18-0236

Safra, T., Muggia, F., Jeffers, S., Tsao-Wei, D. D., Groshen, S., Lyass, O., Henderson, R., Berry, G., & Gabizon, A. (2000). Pegylated liposomal doxorubicin (doxil): Reduced clinical cardiotoxicity in patients reaching or exceeding cumulative doses of 500 mg/m2. *Annals of Oncology*, *11*(8), 1029–1034. https://doi.org/10.1023/A:1008365716693

Saravi, B., Li, Z., Pfannkuche, J., Wystrach, L., Häckel, S., E. Albers, C., Grad, S., Alini, M., Richards, R. G., Lang, C., Südkamp, N., Schmal, H., & Lang, G. (2021). Angiotensin II Type 1 Receptor Antagonist Losartan Inhibits TNF-α-Induced Inflammation and Degeneration Processes in Human Nucleus Pulposus Cells. *Applied Sciences*, *11*(1), 417. https://doi.org/10.3390/app11010417

Sinha, S., Kumar, B., Prasad, C., Chauhan, S., & Kumar, M. (2025). Emerging Research and Future Directions on Doxorubicin: A Snapshot. *Asian Pacific Journal of Cancer Prevention*, *26*(1), 5–15. https://doi.org/10.31557/APJCP.2025.26.1.5

Tian, Z., Yang, Y., Yang, Y., Zhang, F., Li, P., Wang, J., Yang, J., Zhang, P., Yao, W., & Wang, X. (2020). High cumulative doxorubicin dose for advanced soft tissue sarcoma. *BMC Cancer*, *20*(1), 1139. https://doi.org/10.1186/s12885-020-07663-x

Zablocki, D., & Sadoshima, J. (2013). Angiotensin II and Oxidative Stress in the Failing Heart. *Antioxidants & Redox Signaling*, *19*(10), 1095–1109. https://doi.org/10.1089/ars.2012.4588

مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸

مهار ترکیبی HMG-CoA ردوکتاز و سیستم رنین-آنژیوتانسین، کاردیوتوکسیسیتی ناشی از دوکسوروبیسین را در مدل موش صحرایی آلبینو کاهش می‌دهد

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تاریخ دریافت: ۱۴۰۴/۰۲/۳۱ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۴/۰۴/۲۵ تاریخ پذیرش: ۱۴۰۴/۰۴/۲۶

چکیده

هدف: سمیت قلبی مرتبط با دوکسوروبیسین همچنان یک چالش بالینی مهم محسوب می‌شود. هدف این مطالعه بررسی اثرات هم‌افزای محافظت قلبی ناشی از ترکیب سیمواستاتین و لوزارتان در برابر آسیب قلبی القا شده توسط دوکسوروبیسین بود.

مواد و روش‌ها: در این مطالعه، ۴۲ موش ویستار آلبینو وارد شدند و به گروه‌های نر و ماده کنترل (دریافت آب مقطر خوراکی به مدت ۷ روز و تزریق داخل صفاقی نرمال سالین در روز چهاردهم)، گروه‌های نر و ماده دوکسوروبیسین (دریافت آب مقطر خوراکی به مدت ۱۳ روز و تزریق داخل صفاقی تک‌دوز دوکسوروبیسین با دوز ۱۵ میلی‌گرم بر کیلوگرم در روز چهاردهم) و گروه‌های نر و ماده دریافت‌کننده سیمواستاتین + لوزارتان + دوکسوروبیسین (دریافت خوراکی سیمواستاتین با دوز ۱۰ میلی‌گرم/کیلوگرم/روز و لوزارتان با دوز ۱۰ میلی‌گرم/کیلوگرم/روز به مدت ۱۳ روز و تزریق داخل صفاقی تک‌دوز دوکسوروبیسین در روز چهاردهم) تقسیم شدند. نمونه‌برداری در روز ۱۶ شامل جمع‌آوری خون و برداشت بافت پس از کشتار حیوان انجام شد. نمونه خون اولیه در روز صفر، پیش از هرگونه مداخله، جمع‌آوری شد. آسیب قلبی از طریق بررسی‌های بافت‌شناسی و تحلیل بیوشیمیایی نشانگرهای قلبی از جمله فاکتور نکروز توموری-آلفا (TNF-α)، تروپونین قلبی (TNNI3) و پروتئین متصل‌شونده به اسید چرب نوع قلبی (FABP3) ارزیابی شد.

نتایج: تحلیل بافت‌شناسی نشان داد که درمان ترکیبی به طور قابل‌توجهی آسیب قلبی القا شده توسط دوکسوروبیسین را کاهش داده و تنها احتقان خفیف عروقی را در مقابل احتقان شدید آندوکارد، نفوذ سلول‌های التهابی و نکروز سلول‌های ماهیچه‌ای در گروه

دریافت‌کننده دوکسوروبیسین به تنهایی نشان داد. در گروه درمان ترکیبی، تشکیل بافت فیبری نازک بین دسته‌های عضلانی و ادم بینابینی خفیف مشاهده شد که حاکی از فرایند بازسازی و ترمیم فعال بافتی است. از نظر بیوشیمیایی، گروه‌های کنترل سطح پایه پایداری را در طول مطالعه حفظ کردند (TNF-α: 49.3±2.5 to 48.5±1.2; troponin: 11±0.3 to 11.7±0.5; FABP3: 0.55±0.09 to 0.66±0.09). درمان ترکیبی حفاظت قابل‌توجهی از قلب در هر دو جنس فراهم کرد؛ به‌طوری‌که در موش‌های نر تغییرات معناداری در TNF-α (56.3±4.1 to 59.4±6.3, p=0.53)، تروپونین (12.2±1 to 11.8±0.9, p=0.22) و FABP3 (0.67±0.1 to 0.74±0.05, p=0.63) مشاهده نشد. موش‌های ماده نیز حفاظت مشابهی با سطوح TNF-α (60.9±7.4 to 56.2±11.3, p=0.6)، تروپونین (10.8±0.7 to 11.7±0.8, p=0.1) و FABP3 (0.6±0.11 to 0.68±0.08, p=0.9) نشان دادند و در محدوده‌های طبیعی باقی ماندند.

نتیجه‌گیری: ترکیب سیمواستاتین و لوزارتان اثرات محافظت قلبی هم‌افزایی در برابر سمیت قلبی ناشی از دوکسوروبیسین نشان داد که از طریق مکانیسم‌های دوگانه شامل حفاظت پلی‌تروپیک ناشی از استاتین و مهار گیرنده آنژیوتانسین عمل می‌کند. این درمان ترکیبی عملکرد قلب را حفظ کرده، از افزایش نشانگرهای آسیب قلبی جلوگیری نموده و بازسازی مفید بافتی را ترویج داد. این یافته‌ها نشان می‌دهند که درمان ترکیبی سیمواستاتین–لوزارتان می‌تواند یک راهبرد محافظت قلبی امیدبخش برای بیماران دریافت‌کننده شیمی‌درمانی با دوکسوروبیسین باشد و امکان دستیابی به درمان بهینه سرطان را با کاهش عوارض قلبی فراهم آورد.

کلمات کلیدی: دوکسوروبیسین، سمیت قلبی، سیمواستاتین، لوزارتان، نشانگرهای قلبی

نوع مقاله: پژوهشی.

استناد: مروه ج. خلیل، زینا ا. الثنون (۱۴۰۴). مهار ترکیبی HMG-CoA ردوکتاز و سیستم رنین–آنژیوتانسین، کاردیوتوکسیسیتی ناشی از دوکسوروبیسین را در مدل موش صحرایی آلبینو کاهش می‌دهد. *مجله بیوتکنولوژی کشاورزی*، ۱۷(۳)، ۲۳۵–۲۵۲.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.

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