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Investigate if Metoprolol and Troxerutin Could Prevent Cardiotoxicity Mitigated by Doxorubicin

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ABSTRACT

Doxorubicin, which is widely used as an anti-tumour drug, it associated with Cardiotoxic problems. Previous studies suggested that each of the metoprolol and troxerutin alone could provide potential protection against this cardiotoxic effect. This study was aimed to investigate if the combination of these two drugs could provide superior protection against the cardiotoxic effect of doxorubicin. Fifty male Wistar rats were divided into five groups; each group had 10 animals. Both control negative and control positive groups, a treatment group for each drug and a combination group. The blood samples were collected from the animals to extract the serum, and the serums were used in Elisa technique for investigation the concentration of CK-MB, SOD, MDA, IL-6 and TNF-Alpha for all groups to study the effect of the drugs to these markers. The combination treatment group provided a superior effect in almost all groups, The combination treatment group achieved nearly the levels of the control negative group in 2 markers, which were CK-MB and TNF. In oxidative stress markers (MDA) and antioxidant (SOD), it had the lowest concentration of MDA compared to positive group. While highest concentration of antioxidant (SOD) compared to positive group suggesting a slightly better effect of the combination. In conclusion, Combination therapy provides a superior effect on key markers, suggesting its benefits by working on various pathways to protect the heart. Although the effect of the combination was still comparable in oxidative stress markers to other treatment groups, the combination therapy totally outperformed monotherapies.

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KEYWORD: Cancer, Cardiotoxicity, Doxorubicin, Metoprolol, Troxerutin.

INTRODUCTION

The cardiotoxicity of doxorubicin is considered to be the main reason for its narrower use in the medical field. Doxorubicin is a well-known drug used to treat various types of cancers, including lymphoma, breast cancer, and bladder cancer [1]. Although the cardiotoxic effect of the

doxorubicin limits its use. [2,3]. The cardiotoxic effect often reaches life-threatening levels; the pathway in which doxorubicin causes cardiotoxicity is through oxidative stress since it can produce reactive oxygen species (ROS); the cardiac disorders associated with doxorubicin could be moderate as in arrhythmias or severe as in irreversible damage to heart cells, those damages could be chronic or acute [1,4-6]. The heart is highly susceptible to adverse effects of doxorubicin since the harmful pathway of doxorubicin is through oxidative stress and reactive oxygen species [7]. These effects, when associated with the heart's elevated demand for oxygen and its high susceptibility to oxidative stress leads to cardiotoxic effect [8]. However, doxorubicin is still one of the main drugs used as an anti-tumor drug, although the patients who receive this drug need special care [9].

The cancer is considered to be the second cause of death worldwide after cardiomyopathies [10]. Cancer characterized by abnormal growth and distribution of undifferentiated cells [11]; cancer chemotherapy generally has harmful side effects, which includes depression of the immune system and damaging normal tissue of the body. [12], Due to these effects, many patients and medical centers seek alternative ways to treat cancers or drugs that could decrease the harmful side effects of the old treatments. [13].

Troxerutin, also known as vitamin P4, is a natural flavonoid drug in fruits [14], vegetables, tea, and coffee. Troxerutin gained attention because of its therapeutic properties, which include anti-oxidant, anti-inflammatory, prevent thrombogenesis, enhance microcirculation, and anti-DNA damage effects. All those properties make Troxerutin one of the drugs of choice to prevent Doxorubicin-induced cardiotoxicity [15]. While metoprolol is a drug that belongs to the β1-blocker family, β1 drugs are known to be effective against cardiomyopathies, Metoprolol, which is one of the most common drugs used for managing acute myocardial infarction. It is also effective in treating hypertension and tachycardia [16].

Cardiotoxicity induced by Doxorubicin leads to limitation in the use of it for treatment cancers because the long-term cardiac complications. Previous studies have shown that troxerutin and metoprolol individually offer some degree of cardiac protection against this toxicity. However, their combined effect has not been studied thoroughly yet. This study aims to investigate if the combination of troxerutin and metoprolol provides enhanced cardiac protection compared to either drug alone.

MATERIALS AND METHODS

Ethical approval

All procedures used in this study were reviewed and approved by the Scientific Committee of the Faculty of Veterinary Medicine, University of Kufa, in compliance with the ethical principles of animal welfare, with reference number UK.VET.2025.2105.

Experimental design

This study consisted of 50 male Wistar rats (n=50) that were divided into five groups, as each group had 10 animals. Each group was divided into 2 cages to avoid overcrowding and keep the best conditions for the animals. The conditions of the animals were ideal for the study as the temperature was 22±4, the humidity was 40-60%, light/dark hours were 14/10, and animals had free access to food and drink, fig. 1.

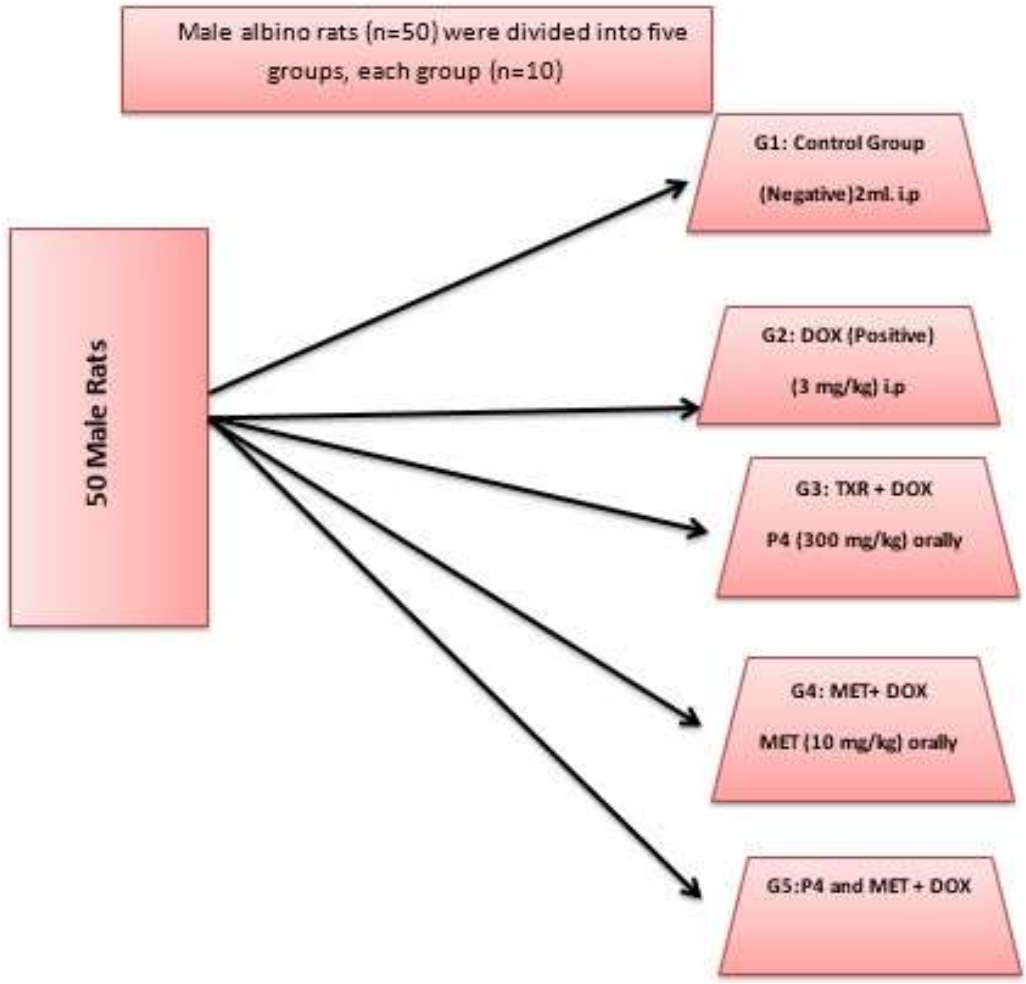

Fig. 1. The experimental design

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and metoprolol were given was (300mg/kg BW and 10mg/kg BW), respectively.

At the end of the study, the animals were anesthetized using ketamine and xylazine in doses of (10 mg/kg BW and 40 mg/kg BW), respectively [20]. Then, the blood was collected directly from the heart without opening the animal's chest to ensure that the blood was without hemolysis and to cause the little harm possible to the animals to extract the serum was for use in the ELISA technique. The blood was put in Gel tubes and centrifuged for 15 minutes with 3500 rounds per minute [21]. The parameters measured using the ELISA technique were Creatinine Kinase MB (CK-MB), Malondialdehyde (MDA), Superoxide Dismutase (SOD), Interleukin-6 (IL-6), and Tumor Necrosis Factor-α (TNF-α).

RESULTS

The Fig. 2 showed there was an increase in Ck-MB in the control positive group, which was administrated with only doxorubicin, when compared to the control negative group, which administrated with only saline, the increase was statistically significant ($P \leq 0.05$). However, all protection groups demonstrated a decrease in Ck-MB compared to the control-positive group. All of the protection groups' decreases were statistically significant ($P \leq 0.05$) when compared to the control-positive group, also the differences between the protection groups were statistically significant ($P \leq 0.05$), as the group that received troxerutin+doxorubicin were significantly lower than the group that received only doxorubicin it was also significantly higher than the group which received metoprolol+doxorubicin. However, the combination group had a lower concentration of CK-MB among all protection groups, almost the same concentration as the control-negative group, and there wasn’t a statistically significant difference between the control-negative group and the combination group.

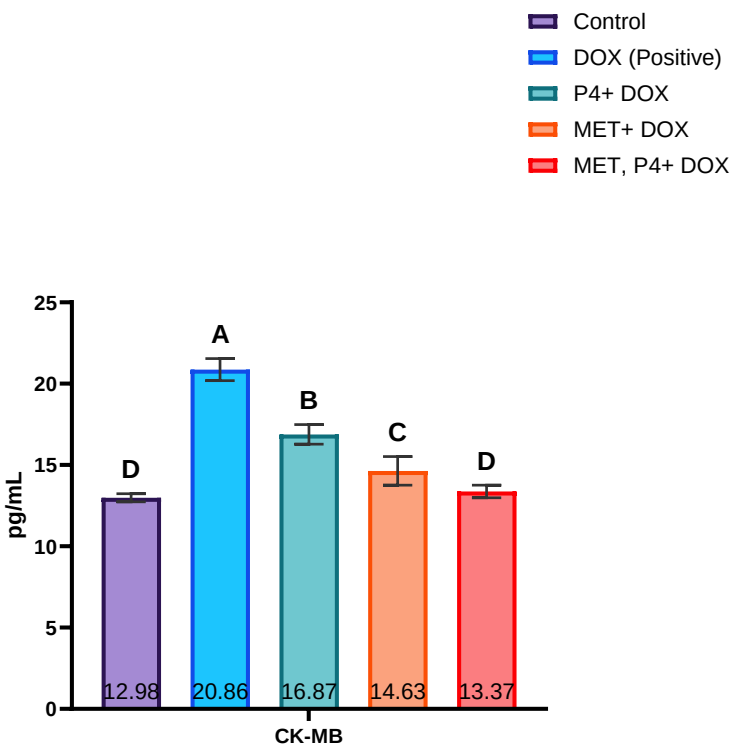

Fig. 2. Concentration of CK-MB among all groups

The fig. 3 and 4 showed the oxidative stress markers among all groups. Fig. 2 shows MDA results, as it demonstrated that the MDA increased significantly (P≤0.05) in Control-positive groups when compared to the control-negative group. All the groups of the treatment were statistically different from the control positive group (P≤0.05). However, the group that received Doxorubicin+Troxerutin wasn't significantly different from the group that received Doxorubicin+Metoprolol. Still, The Combination Group wasn't significantly different from the Doxorubicin+Troxerutin Group or Doxorubicin+Metoprolol Group. Also The combination Group wasn't significantly different from the control negative Group. Still, Both treatment groups, rather than the combination group, were significantly different (P≤0.05) when compared to the control negative group.

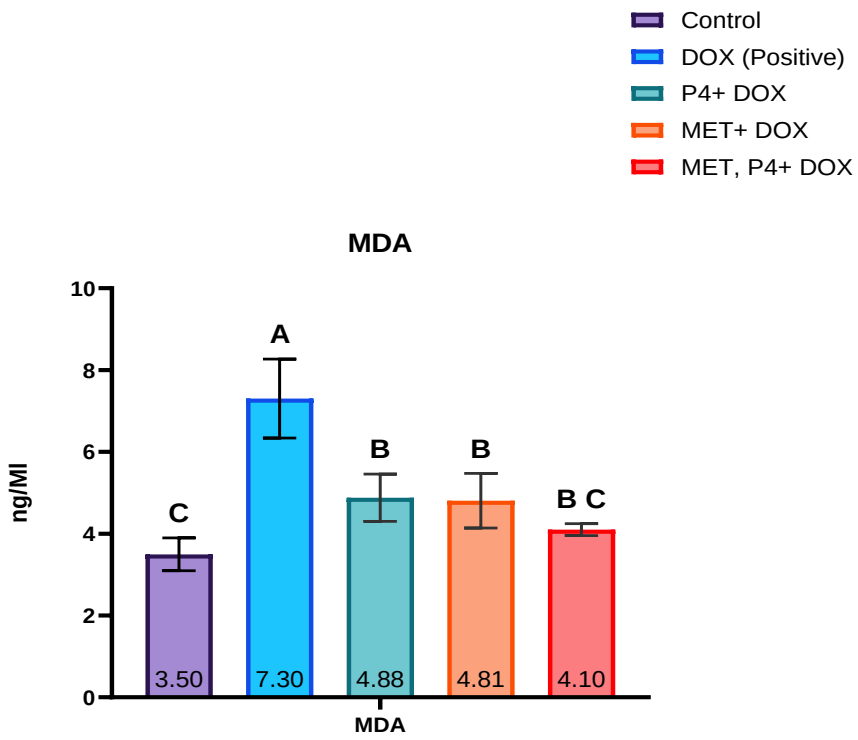

Fig. 3. MDA concentration among all groups.

Fig. 4 shows SOD concentrations among all groups; it demonstrates that the SOD decreased significantly (P≤0.05) in the control positive group, which received only Doxorubicin when compared to the control-negative group. The results were somehow inverse to the results of the MDA, as both of the treatment groups that received Doxorubicin+Troxerutin and Doxorubicin+Metoprolol, respectively, had significantly higher (P≤0.05) concentrations of SOD when compared to control positive group. Still, the results of these two groups weren't significantly different from the control negative group or the combination group. However, the combination group had a significantly lower (P≤0.05) concentration of SOD compared to the Control negative group.

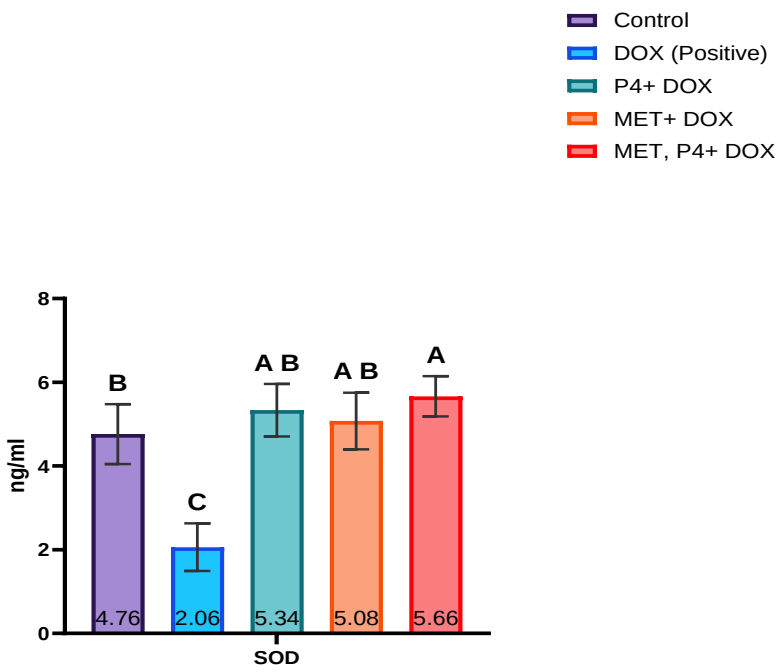

Fig. 4. Concentration of SOD among all groups

The fig. 5 showed concentrations of IL-6 Among all groups, the results of IL-6 Concentrations demonstrate that control positive group, which received only Doxorubicin, have significantly higher (P≤0.05) concentrations when compared to the control negative group, however, all the treatment groups have lower concentrations of IL-6 when compared to control positive group, the results of protection groups were significantly different (P≤0.05) from the control positive group. Also, there wasn't any statistical difference (P≤0.05) between treatment groups. Also, the control negative group had the lowest concentration of IL-6; the results of IL-6 showed the concentration of the control negative group was significantly different (P≤0.05) when compared to the treatment groups.

Fig. 6 showed the concentration of TNF-α among all groups. The results of TNF-α demonstrate that TNF-α increased significantly (P≤0.05) in the control positive group when compared to the control negative group; however, The treatment group that received Troxerutin+doxorubicin had significantly (P≤0.05) lower concentration of TNF-α when compared to the control positive group, Still, the results of the Group that received Metoprolol+Doxorubicin, the combination group, and control negative group were significantly (P≤0.05) lower than the group that received Troxerutin+doxorubicin. However, there wasn't any significant difference (P≤0.05) between these three groups.

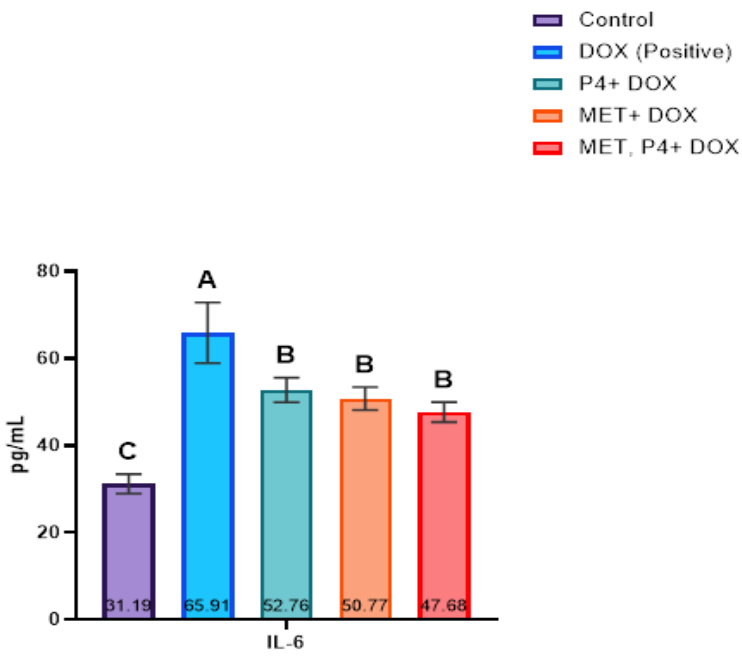

Fig. 5. Concentration of IL-6 among all groups

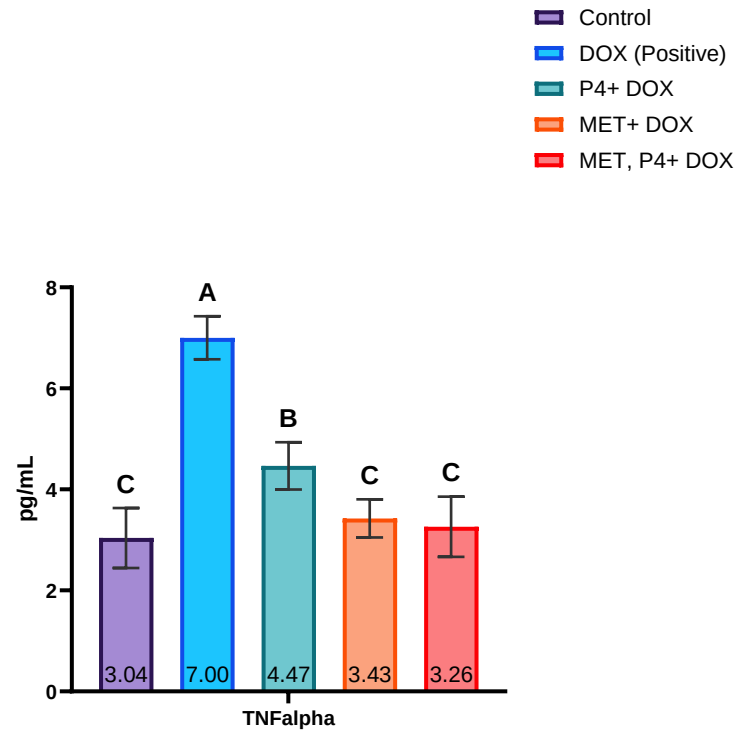

Fig. 6. Concentration of TNF-alpha among all groups.

DISCUSSION

Doxorubicin induced cardiotoxicity primarily occurs with mitochondrial dysfunction, inflammatory signaling pathways and most importantly oxidative stress mechanisms, which accounts for the toxic effect of this drug, this mechanism involves the excessive generation of reactive

oxygen species (ROS), mainly by redox cycling of mitochondria, once doxorubicin enters cardiomyocytes it undergoes redox reaction and releasing and production of superoxide free radicals onset of multiple pathologic events among them the disruption of the electron transport chain, injuries of mitochondrial membrane and initiate lipid peroxidation that mainly affecting directly some key biomarkers like malondialdehyde (MDA), a byproduct of lipid peroxidation, that increased significantly in the group receiving a positive and control of DOX confirming oxidative damage [22,23].

The most important of the many antioxidant defence mechanisms conferred by these major antioxidant defence mechanisms is catalysing the conversion of reactive oxygen species (ROS) to free radicals in superoxide dismutase (SOD). The site of doxorubicin apparent activation is in the SOD group. The decrease, however, relies on oxidative damage. Oxidative stress also triggers the transcription factor nuclear factor kappab N (NF-kappab) and inflammatory cytokines, tumor necrosis factor alpha (tumor necrosis factor alpha). When this upregulated factor is present, it compounds myocardial fibrosis, heart dysfunction, and cell apoptosis. Overall, doxorubicin set the stage for the inflammatory response that, together with all other factors, did even more harm to the heart. The result of oxidative stress and inflammation is damage to the cardiomyocyte membrane causing the release of CK-MB. Increased significantly in the doxorubicin treated group was this myocardium injury biomarker. Moreover, the NF-κB activation leads to upregulation of STAT along with detrimental cardiac dysfunction. Although, in conclusion, we demonstrate that the cardiotoxicity induced by doxorubicin occurs through a high level of interaction between oxidative stress and inflammatory response. Together, oxidative damage and high pro-inflammatory cytokines increase heart injury [24,25].

Troxerutin function as a cardioprotective agent because it downregulates oxidative stress, mitochondrial dysfunction, and inflammation through modulation of a few of the pathways. Proanthocyanidins possess some mechanism of action such as serving as ROS scavenger. Superoxide dismutase (SOD) activity was significantly increased in troxerutin treated group due to Troxerutin increases in the activity of antioxidant enzymes. The further enzyme mediated conversion of superoxide radicals by the increased activity of SOD would have facilitated less oxidative damage. The reduced MDA levels indicated that the mitochondrial membrane damage as well as lipid peroxidation had been adequately minimized [26].

Troxerutin also blocks mPTP, a critical event toward mitochondrial depolarization and the initiation of apoptotic signalling, at the mitochondria during doxorubicin effect Troxerutin protects ATP generation and prevents the release of cytochrome c, a pro-apoptotic factor that triggers caspase-dependent pathways of programmed cell death, by stabilizing mitochondrial membrane potential. This mitochondrial protective effect is associated with less cardiomyocyte apoptosis and lower CK-MB levels reflecting less myocardial damage [27].

Troxerutin also modulates the inflammatory signaling pathways. Troxerutin inhibits NF-κB activation and suppresses the transcription of pro – inflammatory cytokines, resulting in a significant decrease of TNF-α and IL-6 levels. Prevention of macrophages recruitment to the site of damage and limited degree of cardiac fibrosis, due to suppression of inflammatory mediators. Below, it has been additionally demonstrated that troxerutin upregulates nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that

controls the expression of antioxidant genes. Activation of Nrf2 confers resistance of cells to oxidative stress through enhancing the production of glutathione and other detoxifying enzymes. This additional antioxidant support also contributes to the increased effect of troxerutin on reducing oxidative stress markers as well [28-30].

Metoprolol provides cardioprotection through β1-adrenergic antagonist that decreases myocardial strain, oxidative stress and inflammation. Increased intracellular calcium and mitochondrial calcium overload leads to β1-adrenergic receptor overload with transport of doxorubicin. The consequence of overproduction of this free calcium, and an overload of it, causes not only mitochondrial dysfunction but there an increase in oxidative stress, and these withered mitochondria ultimately can't avoid oxidisation by the reactive oxygen species (ROS) so they release vast amounts of free calcium. In addition, it inhibits beta 1 adlnergic receptors and prevents high influx of calcium and stabilizes mitochondrial effector function thereby reducing production of reactive oxygen species (ROS) [31,32].

For oxidative discharging damage, the protection of SOD was dramatically increased and the antioxidation damage was declined in metoprolol-treated group. Similar to metoprolol, blunting of caspase-3 activation in the apoptotic signaling pathway has been described as having a similar type of cardioprotective effect. Doxorubicin induced the loss of mitochondrial function which released cytochrome c that then activated caspase 3 and cardiomyocyte apoptosis. The reduction in cardiomyocyte loss is a result of metoprolol's unintended effect of preventing mitochondrial stress and calcium overload and consequently reducing cytochrome c release, caspase-3 activation, and apoptosis [33,34].

The myocardial injury decreased in the metoprolol-treated group because they presented lower CK-MB levels than the doxorubicin group. The anti-inflammatory properties in metoprolol treatment occur mainly because the drug blocks the activation of NF-κB by adrenergic stimulation. While activated NF-κB transcription is reduced by metoprolol treatment the drug blocks pro-inflammatory cytokine production to substantially lower TNF-α and IL-6 levels. The reduction of inflammatory markers accomplished through metoprolol treatment blocks immune cell entry into cardiac tissue [35,36].

The inflammatory markers reduction through metoprolol administration impedes immune cell entry into cardiac tissue while decreasing fibrosis which enables protection of heart function. The administration of metoprolol has been documented to boost endothelial nitric oxide synthase (eNOS) activity in the body leading to better nitric oxide (NO) output. The enhanced availability of NO through metoprolol therapy works to better both endothelial function and decreases vascular inflammation to contribute additional cardioprotective benefits [37,38].

CONCLUSION

The combination of Metoprolol with troxerutin shows superior cardio protective activity against doxorubicin-induced cardiotoxicity than each of drugs alone. This combination treatment notably decreases the levels of oxidative stress, inflammation, and myocardial injury markers, bringing levels closer to that of controls negative rats. These results indicate that dual therapy may be an effective strategy for limiting the cardiac side effects of doxorubicin.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Sheibani M, Azizi Y, Shayan M, Nezamoleslami S, Eslami F, Farjoo MH, Dehpour AR. Doxorubicin-induced cardiotoxicity: an overview on pre-clinical therapeutic approaches. Cardiovasc Toxicol. 2022;22(4):292–310. Doi: https://doi.org/10.1007/s12012-022-09721-1
2. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol. 2012;52(6):1213–22. Doi: https://doi.org/10.1016/j.yjmcc.2012.03.006
3. Wenningmann N, Knapp M, Ande A, Vaidya TR, Ait-Oudhia S. Insights into doxorubicin-induced cardiotoxicity: molecular mechanisms, preventive strategies, and early monitoring. Mol Pharmacol. 2019;96(2):219–32. Doi: https://doi.org/10.1124/mol.119.115725
4. Belger C, Abrahams C, Imamdin A, Lecour S. Doxorubicin-induced cardiotoxicity and risk factors. IJC Heart Vasc. 2023; 50:101332. Doi: 10.1016/j.ijcha.2023.101332
5. Ghigo A, Mingchuan LI, Hirsch, E. New signal transduction paradigms in anthracycline-induced cardiotoxicity. Biochimica et Biophysica Acta Molecul Cell Res, 2016, 1863.7: 1916-1925. https://doi.org/10.1016/j.bbamcr.2016.01.021
6. Zhu H. Doxorubicin-induced cardiotoxicity. In: InTech eBooks. 2018. Doi: https://doi.org/10.5772/intechopen.78791
7. He H, Wang L, Qiao Y, Zhou Q, Li H, Chen S, et al. Doxorubicin induces endotheliotoxicity and mitochondrial dysfunction via ROS/ENOS/NO pathway. Front Pharmacol. 2019; 10:1531. Doi: https://doi.org/10.3389/fphar.2019.01531
8. Münzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC. Impact of oxidative stress on the heart and vasculature. J Am Coll Cardiol. 2017;70(2):212–29. Doi: https://doi.org/10.1016/j.jacc.2017.05.035
9. Cappetta D, De Angelis A, Sapio L, Prezioso L, Illiano M, Quaini F, et al. Oxidative stress and cellular response to doxorubicin: a common factor in the complex milieu of anthracycline cardiotoxicity. Oxid Med Cell Longev. 2017; 2017:1521020. Doi: 10.1155/2017/1521020
10. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis. 2017;9(3):448–51. Doi: https://doi.org/10.21037/jtd.2017.02.75
11. Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the definition of cancer. Mol Cancer Res. 2023;21(11):1142–7. Doi: https://doi.org/10.1158/1541-7786.mcr-23-0411
12. Fakhri SA, Latif ARA, Khafaji BAA. Potentiate the anticancer effect of methotrexate by Zingiber officinale Roscoe extract. Kufa J Vet Med Sci. 2011;2(2):67–78. Doi: https://doi.org/10.36326/kjvs/2011/v2i23907
13. Aqubi AE, Al-Naimi RS, Al-Taee EH. Comparative pathological and cytogenetical study of ethanolic extract of Vinca rosea L. and vinblastine in treating mammary gland adenocarcinoma implanted mice. Kufa J Vet Med Sci. 2011;2(1):136–46. Doi: https://doi.org/10.36326/kjvs/2011/v2i14035

14. Zamanian M, Bazmandegan G, Sureda A, Sobarzo-Sanchez E, Yousefi-Manesh H, Shirooie S. The protective roles and molecular mechanisms of troxerutin (Vitamin P4) for the treatment of chronic diseases: a mechanistic review. Curr Neuropharmacol. 2020; 19(1):97–110. Doi: https://doi.org/10.2174/1570159x18666200510020744
15. Babaei-Kouchaki S, Babapour V, Panahi N, Badalzadeh R. Effect of troxerutin on oxidative stress and expression of genes regulating mitochondrial biogenesis in doxorubicin-induced myocardial injury in rats. Naunyn Schmiedebergs Arch Pharmacol. 2020; 393(7): 1187–95. Doi: 10.1007/s00210-020-01818-0
16. Georgakopoulos P, Roussou P, Matsakas E, Karavidas A, Anagnostopoulos N, Marinakis T, et al. Cardioprotective effect of metoprolol and enalapril in doxorubicin-treated lymphoma patients: a prospective, parallel-group, randomized, controlled study with 36-month follow-up. Am J Hematol. 2010;85(11):894–6. Doi: 10.1002/ajh.21840
17. Pan D, Li B, Wan S. Evaluation of biomarkers for doxorubicin induced cardiac injury in rats. Exp Ther Med. 2022;24(6):11648. Doi: https://doi.org/10.3892/etm.2022.11648
18. Babri S, Amani M, Mohaddes G, Alihemmati A, Ebrahimi H. Protective effects of troxerutin on β-amyloid (1–42)-induced impairments of spatial learning and memory in rats. Neurophysiology. 2012;44(5):387–93. Doi: https://doi.org/10.1007/s11062-012-9309-6
19. Fowler ED, Drinkhill MJ, Norman R, Pervolaraki E, Stones R, Steer E, et al. Beta1-adrenoceptor antagonist, metoprolol attenuates cardiac myocyte Ca2+ handling dysfunction in rats with pulmonary artery hypertension. J Mol Cell Cardiol. 2018; 120:74–83. Doi: https://doi.org/10.1016/j.yjmcc.2018.05.015
20. Sotoudeh N, Namavar MR. Optimisation of ketamine-xylazine anaesthetic dose and its association with changes in the dendritic spine of CA1 hippocampus in young and old male and female Wistar rats. Vet Med Sci. 2022;8(6):2545–52. Doi: 10.1002/vms3.936
21. Lozano NA, Malvar NWJD, Mana NBB, Mercado NFV, Queddeng NMB, Tejano NSPP, et al. Effect of centrifugation speed and time on the blood chemistry values of healthy individuals. Vector. 2023;32(1). Doi: 10.69566/ijestm.v32i1.301
22. Wang T, Xing G, Fu T, Ma Y, Wang Q, Zhang S, et al. Role of mitochondria in doxorubicin-mediated cardiotoxicity: from molecular mechanisms to therapeutic strategies. Int J Med Sci. 2024;21(5):809–16. Doi: https://doi.org/10.7150/ijms.94485
23. Kong C, Guo Z, Song P, Zhang X, Yuan Y, Teng T, et al. Underlying the mechanisms of doxorubicin-induced acute cardiotoxicity: oxidative stress and cell death. Int J Biol Sci. 2021;18(2):760–70. Doi: https://doi.org/10.7150/ijbs.65258
24. Liu X, Huang K, Zhang RJ, Mei D, Zhang B. Isochlorogenic acid A attenuates the progression of liver fibrosis through regulating HMGB1/TLR4/NF-κB signaling pathway. Front Pharmacol. 2020; 11:582. Doi: https://doi.org/10.3389/fphar.2020.00582
25. Dellafiora L, Galaverna G, Dall'Asta C. In silico analysis sheds light on the structural basis underlying the ribotoxicity of trichothecenes—a tool for

supporting the hazard identification process. Toxicol Lett. 2017; 270:80–7. Doi: https://doi.org/10.1016/j.toxlet.2017.02.015

26. Armin R, Zühlke S, Grunewaldt-Stöcker G, Mahnkopp-Dirks F, Kusari S. Production of siderophores by an apple root-associated Streptomyces ciscaucasicus strain GS2 using chemical and biological OSMAC approaches. Molecules. 2021;26(12):3517. Doi: https://doi.org/10.3390/molecules26123517
27. Bernardi P, Di Lisa F. The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. J Mol Cell Cardiol. 2014; 78:100–6. Doi: https://doi.org/10.1016/j.yjmcc.2014.09.023
28. Wu Q, Yao J, Xiao M, Zhang X, Zhang M, Xi X. Targeting Nrf2 signaling pathway: new therapeutic strategy for cardiovascular diseases. J Drug Target. 2024;32(8):874–83. Doi: https://doi.org/10.1080/1061186x.2024.2356736
29. Feng Y, Ye D, Wang Z, Pan H, Lu X, Wang M, et al. The role of interleukin-6 family members in cardiovascular diseases. Front Cardiovasc Med. 2022; 9:818890. Doi: https://doi.org/10.3389/fcvm.2022.818890
30. Rolski F, Błyszczuk P. Complexity of TNF-α signaling in heart disease. J Clin Med. 2020;9(10):3267. Doi: https://doi.org/10.3390/jcm9103267
31. Bernstein D, Fajardo G, Zhao M. The role of β-adrenergic receptors in heart failure: differential regulation of cardiotoxicity and cardioprotection. Prog Pediatr Cardiol. 2011;31(1):35–8. Doi: https://doi.org/10.1016/j.ppedcard.2010.11.007
32. Nicol M, Sadoune M, Azibani F, Polidano E, Launay J, Samuel J, et al. Effect of metoprolol in a chronic murine model of cardiotoxicity induced by doxorubicin and trastuzumab. Arch Cardiovasc Dis Suppl. 2018;11(1):109. Doi: https://doi.org/10.1016/j.acvdsp.2018.10.241
33. Rajgarhia A, Ayasolla KR, Zaghloul N, Da Re JML, Miller EJ, Ahmed M. Extracellular superoxide dismutase (EC-SOD) regulates gene methylation and cardiac fibrosis during chronic hypoxic stress. Front Cardiovasc Med. 2021; 8:669975. Doi: https://doi.org/10.3389/fcvm.2021.669975
34. Yang B, Ye D, Wang Y. Caspase-3 as a therapeutic target for heart failure. Expert Opin Ther Targets. 2013;17(3):255–63. Doi:https://doi.org/10.1517/14728222.2013.745513
35. Matsumori A. Nuclear factor-κB is a prime candidate for the diagnosis and control of inflammatory cardiovascular disease. Eur Cardiol Rev. 2023; 18:10. Doi: https://doi.org/10.15420/ecr.2023.10
36. Zhang H, Dhalla NS. The role of pro-inflammatory cytokines in the pathogenesis of cardiovascular disease. Int J Mol Sci. 2024;25(2):1082. Doi: https://doi.org/10.3390/ijms25021082
37. Frangogiannis NG. Cardiac fibrosis: cell biological mechanisms, molecular pathways and therapeutic opportunities. Mol Aspects Med. 2018; 65:70–99. Doi: https://doi.org/10.1016/j.mam.2018.07.001
38. Roy R, Wilcox J, Webb AJ, O'Gallagher K. Dysfunctional and dysregulated nitric oxide synthases in cardiovascular disease: mechanisms and therapeutic

potential. Int J Mol Sci. 2023;24(20):15200. Doi: https://doi.org/10.3390/ijms242015200