Original Article

Nebivolol and Atenolol Roles in Doxorubicin-Induced Cardiotoxicity

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Abstract

Background: This study investigated the potential role of ivabradine (Nebivolol and Atenolol) in the attenuation of doxorubicin induced cardiotoxicity in mice. So we will seek the role of nebivolol and atenolol in reducing cardiotoxicity induced by doxorubicin in this experimental study. **Aims:** To investigate the potential roles of nebivolol and atenolol in the attenuation of doxorubicin (DXR)-induced cardiotoxicity in mice. **Materials and Methods:** A total of 42 Swiss-Albino male and female mice were used, which were divided into six equal groups: A negative control, a group 1 not received any agents, group 2 (DXR group) received a single dose of DXR 15 mg/kg, treated group 3 was pretreated with nebivolol 15 mg/kg plus DXR. Treated group 4 was pretreated with nebivolol 30 mg/kg plus DXR. Treated group 5 was pretreated with Atenolol 45 mg/kg plus DXR, and treated group 6 was pretreated with atenolol 90 mg/kg plus DXR. The duration of the study was 10 days. Inflammatory biomarkers including tumor necrosis factor-alpha (TNF-α), lactate dehydrogenase (LDH), malondialdehyde (MDA), and cardiac troponin (cTn-I) serum levels were measured. SPSS version 28.00 was used for data analysis. **Results:** TNF-α, LDH, MDA, and cTn-I serum levels were higher in the DXR-treated mice as compared to the control ($P < 0.05$). Nebivolol and atenolol produced a dose-dependent effect in the reduction of TNF-α, LDH, MDA, and cTn-I serum levels as compared to the DXR-treated mice ($P < 0.05$). **Conclusion:** Atenolol and nebivolol were effective agents in the mitigation of DXR-induced cardiotoxicity by their anti-inflammatory effects of both atenolol and nebivolol and antioxidant effects of nebivolol. Atenolol and nebivolol illustrated a dose-dependent effect in the attenuation of DXR-induced cardiotoxicity through inhibition of lipid peroxidation and cardiomyocyte injury.

Keywords: Atenolol, cardiotoxicity, doxorubicin, nebivolol

Introduction

Doxorubicin (DXR) is a cytotoxic drug belonging to an anthracycline antibiotic used in the treatment of different malignancies including leukemia, lymphoma, breast, and ovarian cancers.[1] DXR is a hydroxylated form of daunorubicin, which is abundant as a natural product produced by many strains of Streptomyces. In contrast, only *Streptomyces cesius* can produce DXR, and genetic modification of Streptomyces strains can produce DXR.[2] Later one in 1996, a gene encoding for the conversion of daunorubicin to DXR had been identified.[2] Acute or prolonged use of DXR is associated with different complications including cardiotoxicity, nephrotoxicity, immunosuppression, bone marrow suppression, and gastrointestinal disorders. DXR-induced cardiotoxicity is the life threatening complication linked with the use of DXR.[3]

Access this article online

Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_18_22

DXR-induced cardiotoxicity is thought to be mediated by mechanisms including the generation of reactive oxygen species (ROS), lipid peroxidation, endoplasmic stress, mitochondrial dysfunction, and alteration of membrane integrity.[4,5]

DXR-induced cardiotoxicity is presented in different clinical forms such as hypotension, arrhythmia, and acute heart failure.[6] Chronic use of DXR may lead to the development of congestive heart failure due to the propagation of dilated cardiomyopathy.[7] Epidemiological and clinical trial studies

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Submitted: 23-Jun-2022 **Revised:** 13-Jul-2022 **Accepted:** 21-Jul-2022 **Published:** 07-Aug-2023

How to cite this article: Issa HK, Al-Gareeb AI. Nebivolol and atenolol roles in doxorubicin-induced cardiotoxicity. Mustansiriya Med J 2023;22:13-9.

indicated that DXR causes dose-dependent irreversible cardiotoxicity.[7] In this state, various therapeutic strategies were applied to attenuate the development of DXR-induced cardiotoxicity using antioxidants, β-adrenoceptor blockers, and renin–angiotensin system modulators.[8] However, some of these cardioprotection agents may interfere with the anticancer effect of DXR. Notably, phosphodiesterase 5 inhibitors such as tadalafil and sildenafil can reduce DXR-induced cardiotoxicity without interfering with the anticancer effect of DXR.[9,10]

Thus, the objective of the present study was to investigate the potential role of atenolol and nebivolol in the attenuation of DXR-induced cardiotoxicity in rats.

Materials and Methods

This study was performed at the Department of Clinical Pharmacology and Therapeutics, College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq, from December 2021 to February 2022. This study was allowed and approved by the Scientific Jury and Editorial Board, College of Medicine, Al-Mustansiriyah University.

Animals

A total of 42 Swiss-Albino male mice weighing 250–500 g were obtained from the National Center for Drug Control and Research, Baghdad, Iraq. All experimental mice were kept in sterilized cages (3 per cage) under 12-h/12-h light–dark, respectively, with standard and appropriate atmospheric conditions. All mice had free access to water and food *ad libitum*. All mice were permitted to stay in the cages for 1-week duration to ensure adaptation.

Induction of doxorubicin-induced cardiotoxicity

In this experimental study, each mouse was anesthetized with the use of isoflurane 2 mg/kg, and then, DXR 15 mg/kg was injected intraperitoneally (IP) using 26G sterile syringes. The mice were observed prudently for any deterioration of general health. This method was done according to the previous study.[2]

Study design

A total of 42 mice were allocated into six groups: The negative control not received any agent, DXR group received a single dose of DXR 15 mg/kg on the 8th day. Treated group 3 was pretreated with nebivolol 15 mg/kg orally for 10 days plus DXR 15 mg/kg on the 8th day. Treated group 4 was pretreated with nebivolol 30 mg/kg orally for 10 days plus DXR 15 mg/kg on the 8th. Treated group 5 was pretreated with atenolol 45 mg/kg orally for 10 days plus DXR 15 mg/kg on the 8th day. And treated group 6 was pretreated with atenolol 90 mg/kg orally for 10 days plus DXR 15 mg/kg on the 8th day.

The duration of the study was 10 days. Each experiment was done according to the guidelines for the use and care of laboratory animals.

Biochemical variables

At the end of the study, the mice were sacrificed under general anesthesia, and blood samples were drained from heart chambers. The blood samples were immediately centrifuged at 3000/rpm. The sera were stored at −20°C in a specific refrigerator till the time of analysis.

Inflammatory biomarkers including tumor necrosis factor-alpha (TNF-α) were assessed by ELISA kit methods (MyBio Source, San Diego, USA). In addition, serum lactate dehydrogenase (LDH), malondialdehyde (MDA), and cardiac troponin (cTn-I) levels were measured by the ELISA kit method (MyBio Source, San Diego, USA). Procedures of ELISA kits were performed according to the manufacture's protocol and instructions. All of the drugs and reagents were purchased from private pharmaceutical companies.

Data analysis of the present study was performed using SPSS (Statistics for Window version 28.00, 2022, Armonk, NY, IBM, Corp., USA). The presented data were expressed as a mean ± standard deviation. An unpaired *t*-test was applied to detect the differences between unrelated groups. Besides, a one-way analysis of variance was used to detect the differences among unrelated groups. The level of significance was applied when $P < 0.05$.

Results

The mean serum TNF-α level of the DXR group was (350.85 ± 148.59) as compared to the control group, which was with serum TNF-α mean (44.91 ± 18.45) ($P < 0.05$).

TNF-α serum level was reduced in both doses of nebivolol 15 mg/kg and 30 mg/kg to (139.99 ± 21.34 ng/mL) and (135.18 ± 57.84 ng/mL) respectively as compared to DXR-treated mice ($P < 0.05$) [Figure 1].

TNF-α serum level was reduced in both doses of atenolol 45 mg/kg and 90 mg/kg to (185.51 ± 68.05 ng/mL) and (189.81 ± 50.03 ng/mL) respectively as compared to DXR-treated mice ($P < 0.05$) [Figure 2].

Cardiac troponin (cTn-I) level was higher in the DXR-treated mice (148.29 ± 37.44 pg/mL) as compared to the control (49.64 ± 12.62 ng/mL) ($P < 0.05$).

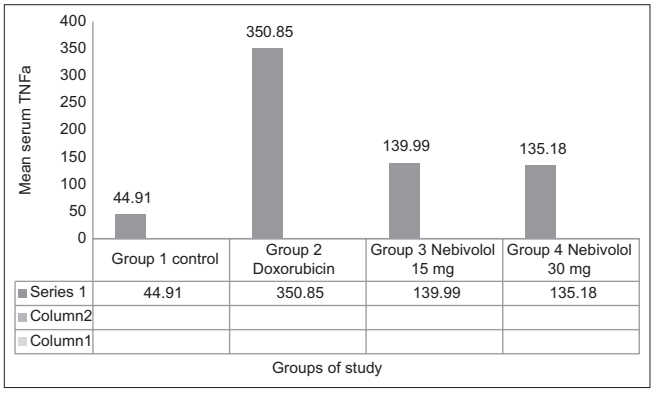

Figure 1: Comparison between means of serum level TNFa for group 1 (control), group 2 (Doxo), group 3 (nebivolol 15 mg), and group 4 (nebivolol 30 mg). TNFa: Tumor necrosis factor-alpha

As well, cTn-I serum level was reduced in both doses of nebivolol 15 mg/kg and 30 mg/kg to (83.15 ± 11.23 pg/mL) and (75.14 ± 17.04 pg/mL) respectively as compared to DXR-treated mice ($P < 0.05$) [Figure 3].

As well, cTn-I serum level was reduced in both doses of atenolol 45 mg/kg and 90 mg/kg to (77.52 ± 11.97 pg/mL) and (76.58 ± 15.40 pg/mL) respectively as compared to DXR-treated mice ($P < 0.05$) [Figure 4].

MDA serum level was higher in the DXR-treated mice (1.91 ± 0.42 ng/mL) as compared to the control (1.15 ± 0.34 ng/mL) ($P = 0.05$).

MDA serum level was reduced in both doses of nebivolol 15 mg/kg and 30 mg/kg to (1.67 ± 0.31 ng/mL) and (1.30 ± 0.37 ng/mL) respectively as compared to DXR treated mice ($P < 0.05$) [Figure 5].

MDA serum level was reduced in both doses of atenolol 45 mg/kg and 90 mg/kg to (1.59 ± 0.13 ng/mL) and (1.45 ± 0.23 ng/mL) respectively as compared to DXR treated mice ($P < 0.05$) [Figure 6].

LDH serum level was higher in the DXR-treated mice (17.53 ± 3.84 IU/L) as compared to the control (7.95 ± 2.56 IU/L) ($P = 0.01$).

LDH serum level was reduced in both doses of nebivolol 15 mg/kg and 30 mg/kg to (14.75 ± 1.97 IU/L) and (13.29 ± 6.07 IU/L) respectively as compared to DXR treated mice ($P < 0.05$) [Figure 7].

LDH serum level was reduced in both doses of atenolol 45 mg/kg and 90 mg/kg to (14.08 ± 1.82 IU/L) and (14.68 ± 0.56 IU/L) respectively as compared to DXR treated mice ($P < 0.05$) [Figure 8].

Discussion

The present study illustrated that nebivolol and atenolol produced a significant dose-dependent effect in the attenuation of DXR-induced cardiotoxicity.

In this study, DXR has a cardiotoxic effect, which is a serious effect that should be avoided, and this effect is primarily due to oxidative stress.

Nebivolol and atenolol have a protective effect. Moreover, these drugs significantly reduce the cardiotoxic effects of DXR, as evidenced by serum biomarkers such as MDA, TNFa, LDH, with CTn-1 being the most important biomarker.

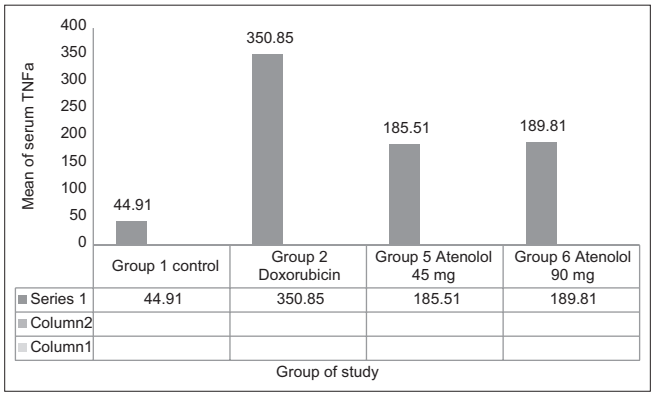

Figure 2: Comparison between means of serum TNFa level for group 1 (control), group 2 (Doxo), group 5 (atenolol 45 mg), and group 6 (atenolol 90 mg). TNFa: Tumor necrosis factor-alpha

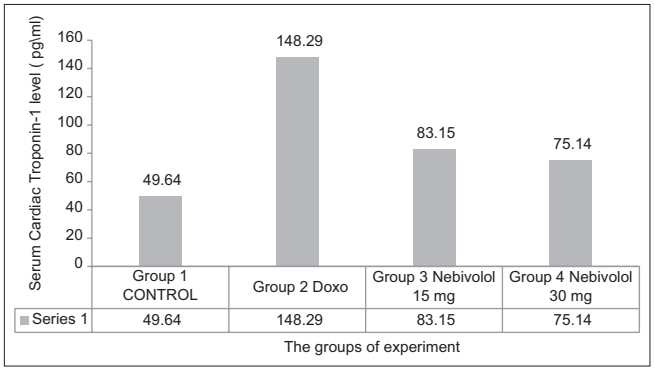

Figure 3: Comparison between means of serum CTn1 level for group 1 (control), group 2 (Doxo), group 3 (nebivolol 15 mg), and group 4 (nebivolol 30 mg)

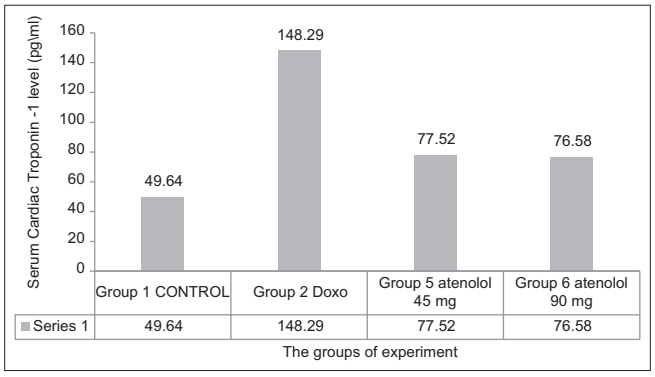

Figure 4: Comparison between means of serum CTn1 level for group 1 (control), group 2 (Doxo), group 5 (atenolol 45 mg), and group 6 (atenolol 90 mg)

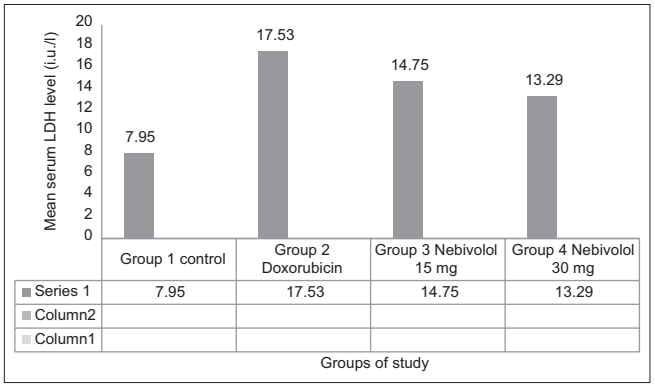

Figure 5: Comparison between means of serum level LDH for group 1 (control), group 2 (Doxo), group 3 (nebivolol 15 mg), and group 4 (nebivolol 30 mg)

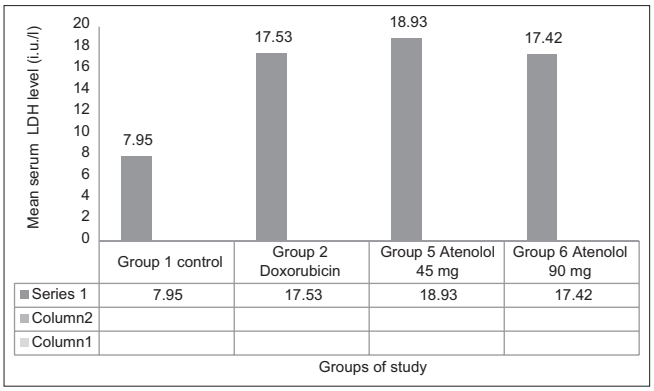

Figure 6: Comparison between means of serum LDH level for group 1 (control), group 2 (Doxo), group 5 (atenolol 45 mg), and group 6 (atenolol 90 mg). LDH: Lactate dehydrogenase

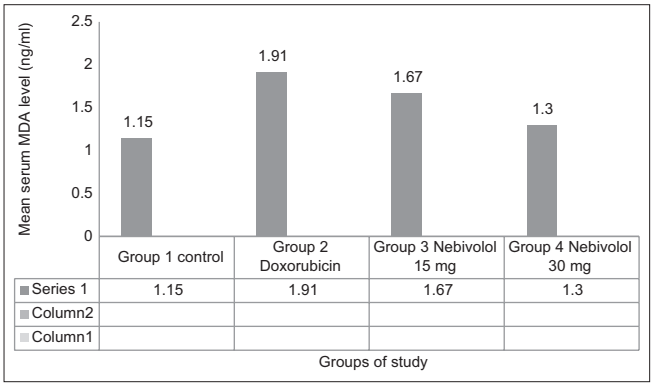

Figure 7: Comparison between means of serum level MDA for group 1 (control), group 2 (Doxo), group 3 (nebivolol 15 mg), and group 4 (nebivolol 30 mg). MDA: Malondialdehyde

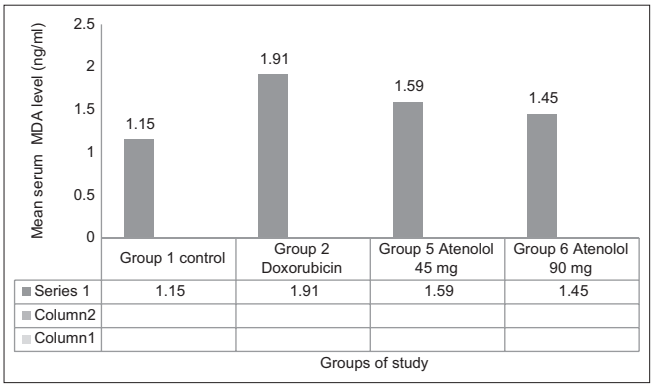

Figure 8: Comparison between means of serum MDA level for group 1 (control), group 2 (Doxo), group 5 (atenolol 45 mg), and group 6 (atenolol 90 mg). MDA: Malondialdehyde

This study shows that the effects of atenolol and nebivolol are not significantly different in protecting the heart from DXR's cardiotoxic effect.

The underlying mechanism of the cardioprotective effect of atenolol and nebivolol might be linked to the anti-inflammatory effect of atenolol and nebivolol and the antioxidant effects of nebivolol.

There are several proposed mechanisms for DXR-induced cardiotoxicity. DXR primarily targets topoisomerase IIβ (Top2β) and induces DNA double-strand breaks. It also disrupts the cardiac prosurvival pathway, the neuregulin/ErbB signaling, which results in mitochondrial dysfunction and apoptosis. Moreover, the most pronounced mechanism responsible for DXR-induced cardiotoxicity is the formation of ROS, leading to oxidative stress.[11-15]

β-Adrenergic blockers can provide some protection from this toxicity; particularly, third-generation β-blockers are appealing. Nebivolol has an antioxidant effect and vasodilating properties ascribed to its interface with the L-arginine/nitric oxide pathway. Some researchers have discussed the protective effect of nebivolol on DXR-induced cardiotoxicity both in rats and humans. Previous study found that administration of nebivolol attenuated both the functional and histopathological picture of DXR-induced cardiotoxicity. Previous study found that nebivolol treatment protects the myocardium against anthracycline-induced cardiotoxicity in breast cancer patients.[16-21]

The nebivolol group had lower systolic and diastolic dimensions, lower levels of natriuretic peptide, and better ejection fraction, which all prove the protective effect of nebivolol.

When comparing the nebivolol group to the DXR-only group in this study, the effect of nebivolol on myocardial injury was clear.

When comparing the nebivolol low and high dose received groups to the DXR only received group, the finding of cardiac MDA was partially reduced in serum results in the nebivolol low and high dose received groups.

This result of the present study agrees with the previous studies.[17,19,22]

Nebivolol can act as a nitric oxide donor. Mercanoglu *et al*. discovered that nebivolol reduces myocardial apoptosis by preventing ROS formation, thus limiting infarct zone expansion and helping to maintain left ventricular function after myocardial infarction in rats.[23,24]

When comparing the atenolol group to the DXR-only group in this study, the effect of atenolol on the myocardial injury was clear.

The most important biomarker for detecting cardiac injury is MDA.

When comparing the atenolol low and high dose received groups to the DXR only received group, we found that cardiac MDA was partially reduced in serum and resulting in the atenolol low and high dose received groups.

This result of the present study agrees with the previous studies.[25,26]

Our study appeared that β-blockers have a protective effect on anthracycline cardiotoxicity, and this agrees with the previous study. Carvedilol preserved the diastolic functions and left ventricular diameters as compared to placebo in 25 patients treated with DXR.[27,28]

The reduction of MDA was because of the effect of nebivolol and atenolol on reducing ventricular function after myocardial infarction.

When comparing the nebivolol group to the DXR-only group in this study, the effect of nebivolol on the myocardial injury was clear.

When comparing the nebivolol low and high dose received groups to the DXR only received group, we found that CTn-1 was partially decreased in serum results in the nebivolol low and high dose received groups.

This study's findings are consistent with previous research.[17]

The previous study demonstrated that DOX increased the most specific highly sensitive markers for myocardial cell injury; cardiac troponin I as well as cardiac toxicity enzymatic indices (CPK and CK-MB).[18,29]

When comparing the atenolol group to the DXR-only group in this study, the effect of atenolol on the myocardial injury was clear. When comparing the atenolol low and high dose received groups to the DXR only received group, we found that cardiac CTn-1 was partially decreased in serum results in the Atenolol low and high dose received groups.

However, this study found no significant differences between groups receiving high and low doses of atenolol.

This result of the present study agrees with previous studies.[25,26]

The present study findings showed that in the DXR-only group, cardiac LDH levels were significantly higher in serum.

This study's findings are consistent with previous research.[30]

The certain enzyme (LDH) are known to be released from injured heart muscle cells, and the magnitude of LDH activities in the blood following myocardial injury reflects the extent of musculature damage.

When comparing the nebivolol group to the DXR-only group in this study, the effect of nebivolol on the myocardial injury was clear.

When comparing the nebivolol low and high dose received groups to the DXR only received group, our study found that cardiac LDH was partially decreased in serum results in the Nebivolol low and high dose received groups.

This study's findings are consistent with previous research.[31]

Results provide clear evidence that alpha-lipoic acid and nebivolol offered significant protection against DOX- induced cardiotoxicity in mice. The cardioprotective effects are attributed to the antioxidant effects of alpha-lipoic acid and nebivolol.[32]

When comparing the atenolol group to the DXR-only group in this study, the effect of atenolol on the myocardial injury was clear.

When comparing the atenolol low and high dose received groups to the DXR only received group, the present study found that cardiac LDH was partially reduced in serum results in the atenolol low and the high dose received groups.

This result of the present study is in agreement with the previous studies.[25,26]

Oral administration of atenolol every day for 10 days reduced the serum LDH and levels.[33]

However, when compared to each other, there is no significant difference in the reduction of LDH between nebivolol and atenolol low and high doses, and between each nebivolol and atenolol received groups when given to reduce the cardiotoxic effect of DXR.

DOX-induced inflammatory and oxidative injury in cardiac tissue were the main findings of the current study.

Inflammation, in addition to oxidative stress, is another important factor in the pathogenesis of DOX-induced cardiotoxicity. Overproduction of free radicals causes an increase in the production of inflammatory mediators, which in turn triggers inflammatory processes in the heart.[15,34]

DXR induces apoptosis in cardiomyocytes and causes significant changes in TNF-receptor levels. Furthermore, exogenous TNF significantly enhances the DXR-induced apoptosis in cardiomyocytes. TNF boosts the effect of DXR on cancer cells by inducing caspase-8 cleavage, which leads to cell death. TNF-producing cells killed by DXR could also be a source of more TNF, amplifying the inflammatory response.[35]

When comparing the nebivolol group to the DXR-only group in this study, the effect of nebivolol on myocardial injury was clear.

When comparing the nebivolol low and high dose received groups to the DXR only received group, we found that cardiac TNFa was partially reduced in serum results in the Nebivolol low and high dose received groups.

This result of the present study agrees with the previous studies.[35]

The present study findings contradict those of previous study who found that nebivolol had no effect on TNF levels in hypertensive rats.

Furthermore, in heart failure patients treated with nebivolol, previous study found no change in serum cytokine concentrations, including TNF. The differences could be due to the fact that the plasma was sampled rather than the myocardium.[35]

When comparing the atenolol group to the DXR-only group in this study, the effect of atenolol on the myocardial injury was clear.

When comparing the atenolol low and high dose received groups to the DXR only received group, we found that cardiac TNFa was partially reduced in serum results in the Atenolol low and high dose received groups.

This result of the present study agrees with previous studies.[25,26]

This study's findings contradict a study that said atenolol did not influence TNF-alpha and IL-1.[16,36]

In the present study, atenolol and nebivolol reduced TNF-a, cTn-I, MDA, and LDH serum levels as compared to the induced group.

Levels of increased oxidative stress and antioxidant deficits are believed to play an important role in DOX-induced cardiotoxicity. Previous study showed that DOX treatment caused an increase in the mean blood pressure in an experimental model. This important finding could be of importance for the pathogenesis and therapy of DOX-induced cardiotoxicity.

This hypothesis is also in line with the results of the studies, which suggest that increased heart rate is a risk factor for cardiovascular morbidity and mortality both in primary prevention and in patients with hypertension, coronary artery disease, and myocardial infarction.[11,13]

Nebivolol reduced apoptosis, prevented maladaptive eNOS/iNOS activation and TNF-α expression, and helped reduce extracellular matrix deposition.

This study consist of previous studies which appear that β blockers were associated with reduced risk of heart failure, decreased left ventricular diameter, improved left ventricular systolic function, and alleviative cardiomyocyte injury.[11]

Conclusion

Nebivolol and atenolol have an effective agent in the mitigation of DXR-induced cardiotoxicity of their anti-inflammatory and antioxidant effects. Remarkably, nebivolol and atenolol illustrated a dose-dependent effect in the attenuation of DXR-induced cardiotoxicity through inhibition of lipid peroxidation and cardiomyocyte injury. In this state, large-scale experimental, preclinical, and clinical studies are warranted in this regard.

Acknowledgment

The authors would like to thank Mustansiriyah University, Baghdad, Iraq, for the great support of scientific research.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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