International Journal of Advances in Pharmacy Medicine and Bioallied Sciences

An International, Peer-reviewed, Indexed, Open Access, Multi-disciplinary Journal

www.biomedjournal.com

ISSN 2348-2109

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Original Research Article

Alpha lipoic acid and nebivolol protect doxorubicin-induced cardiotoxicity and oxidative stress in mice

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ARTICLE INFO

Article History:
Received 03 July 2018
Revised 20 July 2018
Accepted 5 Aug 2018

Keywords:
Doxorubicin,
Oxidative Stress,
Alpha Lipoic Acid
Nebivolol.

ABSTRACT

Background: Objectives: The present study was designed to evaluate the effect of alpha lipoic acid and nebivolol on doxorubicin (DOX)-induced cardiotoxicity and oxidative stress in mice.

Materials and Methods: Mice were pre-treated with alpha lipoic acid (16 mg/kg, i.p.) and nebivolol (1 mg/kg, p.o.) for 7 days and received a single dose of DOX (15 mg/kg, i.p.) on day 8. After 36 h, serum chemistry and relevant enzymes in cardiac tissues were assessed. Histopathology of the heart tissues was performed.

Results: Alpha lipoic acid and Nebivolol produced significant reduction in serum lactate dehydrogenase (LDH), creatine kinase-muscle/brain (CK-MB), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and cardiac lipid peroxides (MDA) levels, and elevation in cardiac antioxidant enzymes [glutathione (GSH), and superoxide dismutase (SOD) and catalase (CAT)] levels. These results were also supported by the histological findings.

Conclusion: 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.

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INTRODUCTION

Doxorubicin (DOX) is a broad spectrum agent from anthracycline class used for cancer chemotherapy of solid tumors and hematologic malignancies. However, the use of DOX has been associated with myocardial injury (Khan et al, 2014). The cardiac injury becomes more obvious after several years of DOX treatment that leads to dilated cardiomyopathy and ultimately congestive heart failure (Ghibu et al, 2012). Oxidative stress is a biochemical disequilibrium occurring due to excessive production of reactive oxygen species (ROS). Increased oxidative stress aggravates oxidative damage to tissues that cannot be countered by the antioxidative defense (Foyer et al, 2009; Minibayeva et al, 2009). Several mechanisms (oxidative stress, inflammation, and apoptosis) have been proposed for the DOX-induced cardiotoxicity. Increased oxidative stress and continuous ROS generation are the main factors responsible for DOX-induced cardiotoxicity (Shaker et al, 2018).

Alpha lipoic acid is a naturally occurring antioxidant (Ayaz et al, 2005). Alpha-lipoic acid's function is in the same manner as Vitamin B complex (Ghibu et al, 2012). Various studies have reported the beneficial

antioxidant effects of alpha lipoic acid (Al-Majed et al, 2002; Ghibu et al, 2012). Nebivolol is a third generation cardioselective β-blocker used for the treatment of hypertension (de Nigris et al, 2008). Nebivolol has been reported to reduce ROS-mediated target organ damages (Coats and Jain, 2017). The purpose of the present study was to investigate the protective effect of alpha lipoic acid and nebivolol on the biochemical and histopathological changes in DOX-induced cardiotoxicity and oxidative stress in mice through evaluation of serum cardiac enzymatic parameters such as lactate dehydrogenase (LDH) and creatine kinase-muscle/brain (CK-MB) activities as well as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) level. In addition, oxidative stress biomarkers such as lipids peroxides (MDA) level, glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity and histopathological changes were evaluated in cardiac tissues of mice.

MATERIALS AND METHODS

Drugs and chemicals

Alpha lipoic acid (Himedia Pvt. Ltd., Mumbai, India), Nebivolol (Nebicard tablets of Torrent Pharmaceuticals, Gujarat, India), DOX (ADRIM vial of Fresenius oncology, Kabi, India), LDH and CK-MB (Reckon Diagnostics Pvt. Ltd. Baroda, India), SGOT and SGPT (Span Diagnostics Ltd., Vadodara, Gujarat, India) were used in the study. All chemicals were of analytical grade.

Experimental design

The experimental protocol was approved by the Institutional Animal Ethics Committee of Maharishi Dayanand University, Rohtak, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration no. 134/99/CPCSEA). Swiss albino mice weighing (20-30 g) of either sex were housed in polypropylene cages under controlled conditions (room temperature 25 ± 2°C, natural light-dark cycle) and had free access to the commercial pellet diet and water ad libitum. The animals were randomly divided into 4 groups (n = 16 mice in each group). Group 1 (normal control) and Group 2 (DOX group) received saline for 7 consecutive days. Group 3 (alpha lipoic acid 16 mg/kg, i.p.) and Group 4 (nebivolol 1 mg/kg, p.o.) received respective drugs for 7 consecutive days and a single dose of DOX (15 mg/kg, i.p.) on day 8.

Determination of biochemical parameters

Thirty-six hours after DOX administration, body weight was measured for each mice. Percent survival of mice was recorded. Then mice were sacrificed using ether and blood samples were collected by cardiac puncture. Serum was obtained by centrifugation at 3000 rpm for 10 min at 4 oC. Serum was kept frozen at -20ºC and was used later for the estimation of serum LDH, CK-MB, SGOT, and SGPT levels. Hearts were rapidly dissected out, blotted dry, weighed and kept frozen at -20 ºC till further use for biochemical estimations of cardiac MDA (Okhawa et al, 1979), GSH (Ellman, 1959), SOD (Kakkar et al, 1984), and CAT (Sinha, 1972) levels.

Histopathological analysis

For histological examination, the heart tissue was fixed in 10% neutral buffered formalin, and embedded in paraffin. Standard sections of 3-5 μm thickness were cut and stained with hematoxylin and eosin (H & E). The slides were examined by light microscopy.

Statistical analysis

The data are expressed as means ± S.E.M. All statistical analyses were performed using Graph-Pad InStat version 3.06 (Graph Pad Software). All data were analyzed using 1-way analysis of variance (ANOVA) followed by the Tukey's post hoc test. Results were considered statistically significant when $p < 0.05$.

RESULTS

Effect of alpha lipoic acid and nebivolol on survival rate and heart to body weight ratio

DOX control group showed a 75% survival on day 8 while normal control (NC) group or nebivolol showed 100% survival rate. Alpha lipoic acid group showed a 93.7% survival rate (Figure 1). There was a statistically significant decrease in heart weight/body weight ratio DOX-treated group as compared with the NC group ($p < 0.001$). Pre-treatment with nebivolol significantly ($p < 0.05$) increased the heart to body weight ratio compared to DOX control group (Figure 2).

Effect of alpha lipoic acid and nebivolol on serum biochemical parameters

The serum LDH, CK-MB, SGOT and SGPT levels are depicted in Table 1. DOX control group showed a significant ($p < 0.001$) increase in serum LDH, CK-MB, SGOT, and SGPT level when compared with the NC group. Administration of alpha lipoic acid or nebivolol for a period of 7 days caused a significant ($p < 0.05$) decrease in serum LDH, CK-MB, SGOT and SGPT levels as compared to the DOX control group.

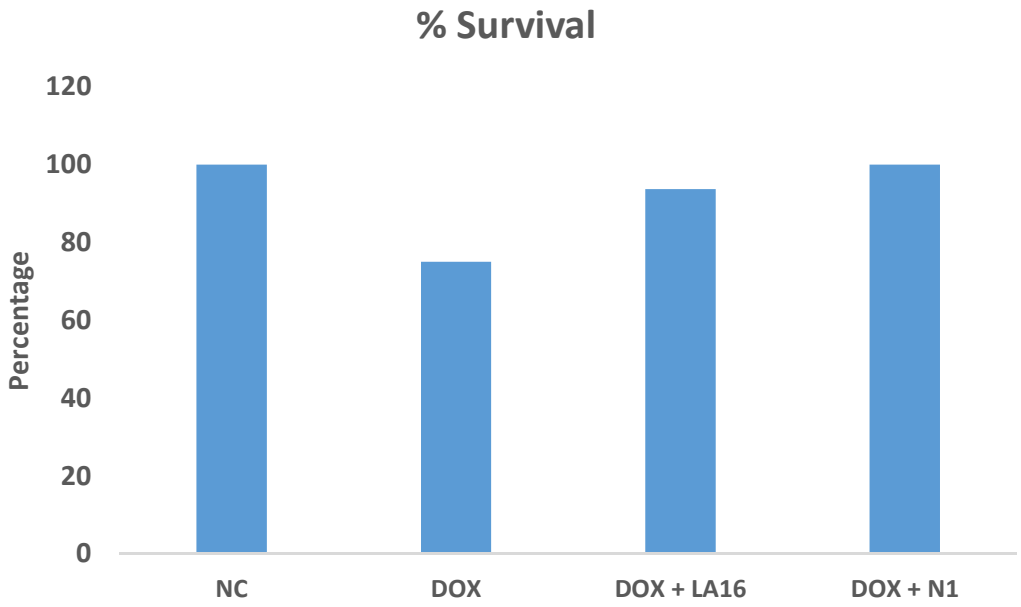

Figure 1: Effect of alpha lipoic acid and nebivolol on survival rate in DOX-treated mice.

Abbreviations denotes: *DOX: doxorubicin, AL16: alpha lipoic acid (16 mg/kg, i.p.), N1: Nebivolol (1 mg/kg, p.o.); NC: normal control.*

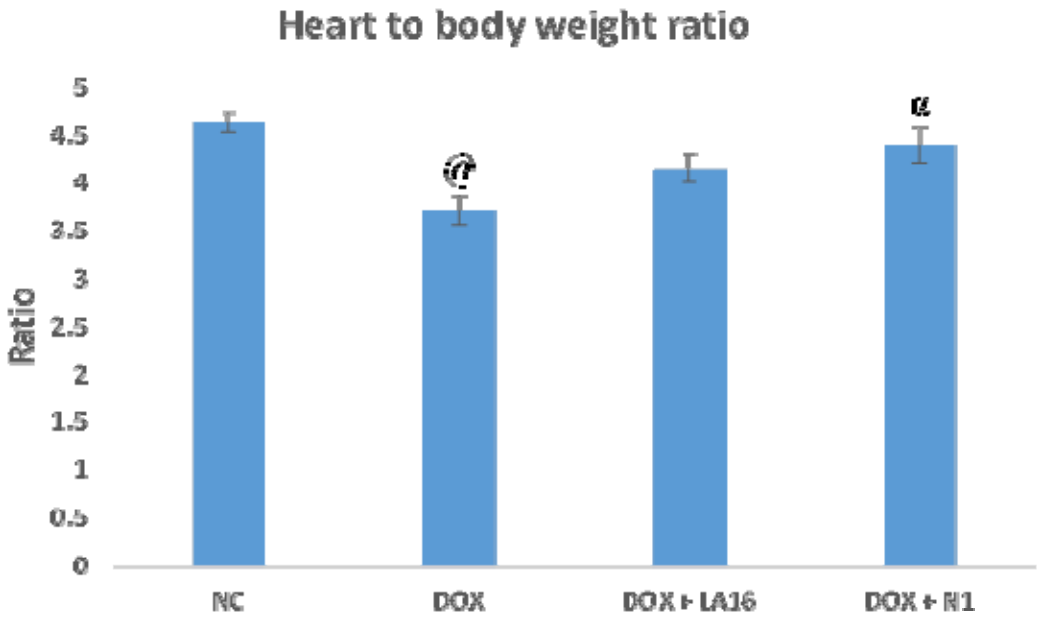

Figure 2: Effect of alpha lipoic acid and nebivolol on heart to body weight ratio in DOX-treated mice.

Results are expressed as mean ± S.E.M. for 10 separate observations (n = 10); the data were analyzed with One-way ANOVA followed by Tukey test; @p < 0.001 vs compared to normal control group; ∝p < 0.05 compared to DOX treated group; Abbreviations denotes: DOX: doxorubicin, AL16: alpha lipoic acid (16 mg/kg, i.p.), N1: Nebivolol (1 mg/kg, p.o.); NC: normal control.

Table 1: Effect of alpha lipoic acid and nebivolol on serum LDH, CK-MB, SGOT and SGPT levels in DOX-treated mice.

Groups	LDH (IU/L)	CK-MB (IU/L)	SGOT (IU/L)	SGPT (IU/L)
NC	165.30 ± 2.72	134.75 ± 3.65	25.34 ± 1.99	46.20 ± 4.03
DOX	367.50 ± 4.80$^{@}$	308.23 ± 9.90$^{@}$	59.75 ± 5.49$^{@}$	154.97 ± 6.43$^{@}$
DOX + AL16	294.76 ± 5.42$^{\gamma}$	221.67 ± 6.43$^{\gamma}$	36.83 ± 4.64$^{\beta}$	124.83 ± 6.18$^{\beta}$
DOX + N1	230.85 ± 4.16$^{\gamma}$	197.09 ± 8.44$^{\gamma}$	35.92 ± 5.00$^{\beta}$	121.38 ± 4.32$^{\gamma}$

Results are expressed as mean ± S.E.M. for 10 separate observations (n = 10); the data were analyzed with One-way ANOVA followed by Tukey test; $^{@}p < 0.001$ vs compared to normal control group; $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ compared to DOX treated group;
Abbreviations denotes: DOX: doxorubicin, AL16: alpha lipoic acid (16 mg/kg, i.p.), N1: Nebivolol (1 mg/kg, p.o.), LDH: lactate dehydrogenase, CK-MB: creatine kinase-muscle/brain, SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase.

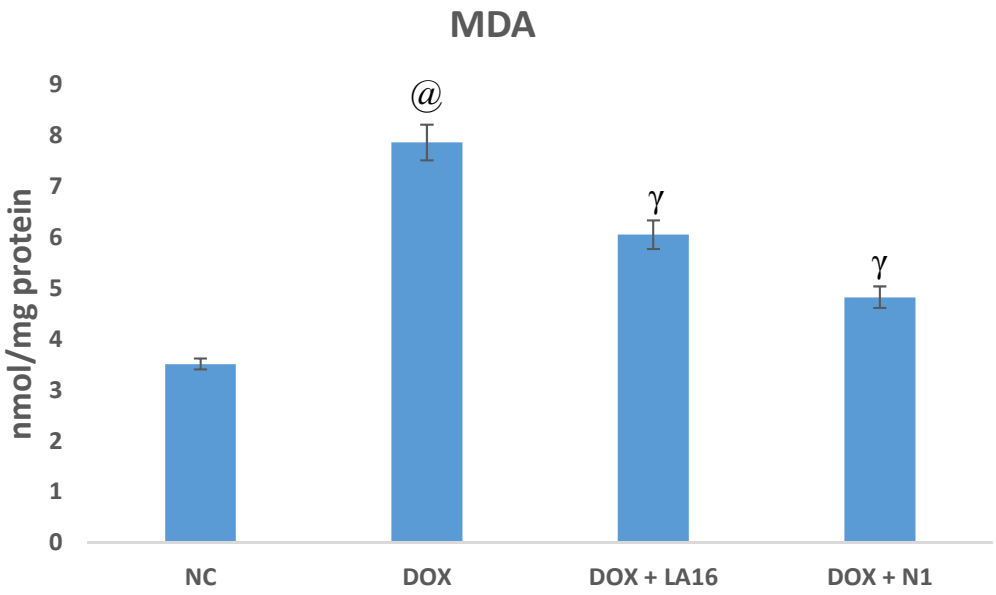

Figure 3: Effect of alpha lipoic acid and nebivolol on cardiac MDA levels in DOX-treated mice.

Results are expressed as mean ± S.E.M. for 10 separate observations (n = 10); the data were analyzed with One-way ANOVA followed by Tukey test; $^{@}p < 0.001$ vs compared to normal control group; $^{\gamma}p < 0.001$ compared to DOX treated group; *Abbreviations denotes*: DOX: doxorubicin, AL16: alpha lipoic acid (16 mg/kg, i.p.), N1: Nebivolol (1 mg/kg, p.o.), MDA: malondialdehyde; NC: normal control.

Table 2: Effect of alpha lipoic acid and nebivolol on cardiac GSH, SOD and CAT levels in DOX-treated mice.

Groups	GSH (µg/mg protein)	SOD (Units/mg protein)	CAT (µmol of H_2O_2 consumed/mg protein)
NC	24.30 ± 1.08	12.92 ± 0.69	62.15 ± 1.19
DOX	8.41 ± 0.92$^{@}$	6.43 ± 0.94$^{@}$	27.43 ± 2.11$^{@}$
DOX + AL16	17.60 ± 1.67$^{\gamma}$	10.45 ± 0.65$^{\beta}$	41.98 ± 3.04$^{\beta}$
DOX + N1	20.95 ± 1.00$^{\gamma}$	10.97 ± 0.60$^{\gamma}$	54.74 ± 3.96$^{\gamma}$

Results are expressed as mean ± S.E.M. for 10 separate observations (n = 10); the data were analyzed with One-way ANOVA followed by Tukey test; $^{@}p < 0.001$ vs compared to normal control group; $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ compared to DOX treated group;
Abbreviations denotes: DOX: doxorubicin, AL16: alpha lipoic acid (16 mg/kg, i.p.), N1: Nebivolol (1 mg/kg, p.o.), GSH: glutathione, SOD: superoxide dismutase, CAT: catalase.

Effect of alpha lipoic acid and nebivolol on MDA, GSH, SOD and CAT levels

DOX control mice showed a significant ($p < 0.001$) increase in cardiac MDA levels as compared to the NC group. Administration of alpha lipoic acid or nebivolol caused a significant ($p < 0.001$) decrease in cardiac MDA levels as compared to the DOX control group (Figure 3).

DOX control group showed a significant ($p < 0.001$) depletion in cardiac GSH, SOD and CAT levels as compared to NC group. Administration of alpha lipoic acid or nebivolol caused a significant ($p < 0.05$) increase in these levels as compared to DOX control group (Table 2).

Cardiac histopathology

The histopathological examination of the NC group showed normal myocardium morphology and normal myofibrillar structure, while DOX control group showed marked tissue injury with myofibril loss and focal cytoplasmic vacuolization and infiltration of cells. On the other hand treatment with alpha lipoic acid-treated heart showed lesser focal fibrillar loss than DOX-treated mice and slight inflammatory cellular infiltration. Nebivolol treated heart showed regular myofibril arrangement and better-preserved appearance of cardiac muscle fibers with very few infiltrative cells (Figure 4).

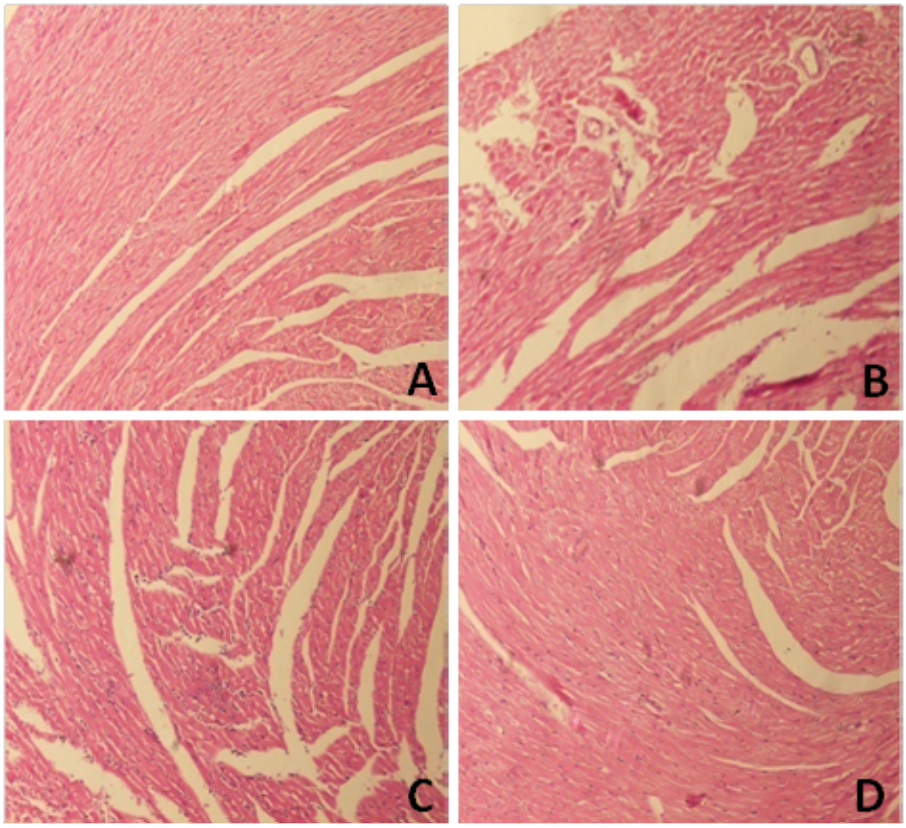

Figure 4: Effect of alpha lipoic acid and nebivolol on cardiac histopathological changes in DOX-treated mice.

Group 1 (A): Normal control, showing normal myocardium morphology and normal myofibrillar structure; Group 2 (B): DOX control, showing marked tissue injury with myofibril loss and focal cytoplasmic vacuolization and infiltration of cells; Group 3 (C): DOX + AL16, showing lesser focal fibrillar loss than DOX treated mice and slight inflammatory cellular infiltration; Group 4 (D): DOX + N1, showing regular myofibril arrangement and better-preserved appearance of cardiac muscle fibers with very few infiltrative cells; Abbreviations denotes: DOX: doxorubicin, AL16: alpha lipoic acid (16 mg/kg, i.p.), N1: Nebivolol (1 mg/kg, p.o.), MDA: malondialdehyde; NC: normal control.

DISCUSSION

In the present study, the DOX control mice showed cardiotoxicity perhaps due to the oxidative stress, inflammation, and apoptosis, which is in agreement with previous reports (Abdel-Raheem and Abdel-Ghany, 2009; Palani et al, 2012).

DOX-treated mice in our study led to cardiotoxicity as indicated by the increase in serum activities of cardiac enzymes such as LDH and CK-MB. LDH and CK-MB enzymes are present in high amount in myocardial tissue and the myocardial injury results in a substantial increase in measured enzyme activity in serum (Yagmurca et al, 2003; Khan et al, 2014; Zhang et al, 2017). Administration of alpha lipoic acid or nebivolol resulted in a significant reduction in levels of LDH and CK-MB which is similar to other findings (Al-Majed et al, 2002; Ayaz et al, 2005; Imbaby et al, 2014).

As per earlier reports, SGOT and SGPT enzymes are the sensitive markers of tissue damage and their levels are predictive of damage to the organs with high metabolic activity (liver, brain, heart, and lungs) (Kumar and Bhandari, 2012). The results of our study showed that DOX-treated mice were more prone to cardiotoxicity as evidenced by increased levels of serum SGOT and SGPT. Administration of alpha lipoic acid and nebivolol significantly reduced the elevated SGOT and SGPT levels, which could be attributed to the protective effect on cardiac tissue.

It is well known that generation of ROS leads to oxidative damage of membrane lipids and other cellular components which is the major mechanism for DOX-induced cardiotoxicity (Al-Majed et al, 2002). Our findings are consistent with previous observations (Abdel-Raheem and Abdel-Ghany, 2009; Khan et al, 2014) who reported that DOX administration to animal models significantly increased lipid peroxides (MDA) and decreased GSH, SOD, and CAT in cardiac tissues. The serum MDA is a marker of lipid peroxidation and it increases in DOX-treated animals (Shah et al, 2013; Zhang et al, 2017). GSH protects the cells against free radicals and other toxic compounds (Harlan et al, 1984; Hiraishi et al, 1994). SOD mediates the breakdown of superoxide anion into hydrogen peroxide and oxygen (Zelko et al, 2002). CAT mediates the conversion of hydrogen peroxide to water and oxygen (Chelikani et al, 2004). The decreased activities in GSH, SOD and CAT levels in our study are in agreement with previous reports (Ayaz et al, 2005; Imbaby et al, 2014) who suggested that DOX-induced lipid peroxidation contributed to the depletion of tissue levels of these enzymes. Treatment with alpha lipoic acid or nebivolol caused a significant reduction in MDA levels and elevation in antioxidant enzymes (GSH, SOD, and CAT) indicating the protection of the cardiac tissues from the damaging effect of DOX. The results of our study show that the indices of cardiac risk biomarkers (LDH, CK-MB, SGOT, and SGPT), indices of lipid peroxidation (MDA) and the cardiac tissue antioxidant enzymes (GSH, SOD, and CAT) were significantly restored towards normal levels with alpha lipoic acid or nebivolol treatment.

Furthermore, in this study, the biochemical alterations induced by DOX in mice were further confirmed by histopathological studies of heart tissues which showed marked tissue injury with myofibril loss and focal cytoplasmic vacuolization and infiltration of cells. Treatment with alpha lipoic acid or nebivolol was associated with regular myofibril arrangement and better-preserved appearance of cardiac muscle fibers.

It is well-established that alpha lipoic acid and nebivolol are known antioxidants in animal species (Ayaz et al, 2005; de Nigris et al, 2008; Ghibu et al, 2012; Imbaby et al, 2014). Alpha lipoic acid and nebivolol seems to have an important role in preventing the development of DOX-induced cardiotoxicity and oxidative stress. The protective effect of alpha lipoic acid and nebivolol may be related to their antioxidant property and may be helpful in protection from the myocardial injury caused by DOX.

CONCLUSION

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.

CONFLICT OF INTEREST

None declared.

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