Advances in Applied and Pharmaceutical Sciences Journal

Volume 1, Issue 1 (2017), 24-32
Available online at www.aapsj.in

Research Article

Tempol Ameliorates Oxidative Stress, Apoptosis in Doxorubicin Induced Cardiotoxicity

Syed Ayaz Ali[1], Uma Bhandari [2], K K Pillai [2]*
[1]Department of Pharmacology, Y.B.Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad.*
[2] Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062

Article information

Received: 08 August 2017
Received in revised form: 19 August 2017
Accepted: 25 August 2017
Available online: 01 September 2017

Subject: Pharmaceutical Sciences
Branch: Pharmacology

∗ Corresponding author
Dr. Syed Ayaz Ali
Department of Pharmacology,
Y.B.Chavan College of Pharmacy,
Dr. Rafiq Zakaria Campus,
Aurangabad.
Email: ayazpharm@gmail.com

DOI: 10.26768/AAPSJ.1.1.24-32

Quick Response Code

Abstract

The purpose of this study was to investigate protective effect of tempol as cardioprotective and antioxidant, on damage caused by doxorubicin (DOX). Wistar albino rats were used in this experiment. Doxorubicin was administered intraperitoneally in six equal injections (each containing 2.5 mg/kg doxorubicin at 48 h interval) to a total cumulative dose of 15 mg/kg over a period of 2 weeks to produce cardiotoxicity. Tempol was administered as pretreatment and post-treatment. The biochemical parameters such as tissue glutathione (GSH), malondialdehyde (MDA), lactate dehydrogenase (LDH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), and glucose-6-phosphate dehydrogenase (G6PD) were monitored after 30 days. Pre-treatment and Post treatment with tempol significantly protected the myocardium from the toxic effects of doxorubicin, by increasing the levels of GSH and reducing the levels of antioxidant enzymes such as CAT, SOD, GPx, GR, GST, and G6PD towards normal and decreased the increased levels of MDA and LDH. It has also reduced the severity of cellular damage of the myocardium. The restoration of the endogenous antioxidant system clearly depicts that tempol has produced its protective effect by scavenging the reactive oxygen species (ROS). Our study shows that pretreatment with tempol as cardioprotective and antioxidant is more beneficial as compared to post treatment against DOX-induced cardiac toxicity.

Keywords: *Doxorubicin, Cardiotoxicity, Tempol, Antioxidant.*

Cite this article
S A Ali, U Bhandari , K K Pillai" Tempol Ameliorates Oxidative Stress, Apoptosis in Doxorubicin Induced Cardiotoxicity"Advances in Applied and Pharmaceutical Sciences Journal 2017, 1 (1);24-32

e-ISSN No 2457-0842, [Available online at aapsj.in]

Introduction

Doxorubicin (DOX), an anthracycline antibiotic, is a potent, broad spectrum chemotherapeutic agent that has been found to be highly effective in treating patients with leukemias, lymphomas and a variety of soft and solid tumors. [1, 2] However, it has been reported that it causes a cumulative dose-dependent cardiac toxicity that is characterized by an irreversible dilated cardiomyopathy and congestive heart failure. [3, 4] Several mechanisms for the DOX induced cardiotoxicity have been proposed including membrane lipid peroxidation, free radical formation, mitochondrial damage, and iron dependent oxidative damage to macromolecules. [5-7] Oxygen radicals are apparently involved in all of the mechanisms proposed. Cardiac tissue which has a less developed antioxidant defense mechanism is highly susceptible to injury by anthracycline induced oxygen radicals. [8]

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-4-hydroxy-tempo) is a water soluble and stable piperidine nitroxide. It has a relatively low molecular weight (172) and it permeates biological membranes. [9] Studies have shown that tempol is a superoxide anion scavenger in vitro [9] and may show effect as a genuine SOD-mimetic. [10] It was a recent discovery that tempol reduced the renal dysfunction and injury caused by endotoxin in the rat [11] and during hemorrhagic shock [12]; where increased ROS formation are involved in its pathogenesis. Tempol has been reported experimentally to ameliorate oxidative stress mediated renal dysfunction and glomerular injury. [13] Also, tempol relieved the endothelial cell dysfunction in diabetic rats [14] and in an experimental model of regional myocardial ischemia/reperfusion, reduced the infarct size. [15]

Till date, there has been relatively less research into the protective effects shown by tempol with respect to anticancer drugs such as doxorubicin induced cardiotoxicity in reference to the biochemical and histological examinations. In this study, we propose the potent antioxidant role of tempol in doxorubicin induced cardiac damage in rats.

Materials and Methods

Chemicals

Doxorubicin was a generous gift from Dabur Research Laboratories (Ghaziabad, India). Tempol was obtained from Sigma-Aldrich Chemical Co. (St.Louis, MO, USA). LDH kits were purchased from Reckon Diagnostic Pvt. Ltd. (Baroda, India). All other chemicals and solvents used were of highest purity and analytical grade.

Animals

Adult Wistar rats of either sex (weighing 150-200 g), bred in the central animal house of the Hamdard University (New Delhi, India) were used. The animals were housed under standard light/dark cycles with free access to food (Amrut Laboratory Rat feed, Navmaharashtra Chakan Oil Mills Ltd., Pune, India) and water. Experiments on animals were conducted after obtaining approval from Hamdard University Animal Ethics Committee.

Experimental protocols

After acclimatization, the animals were randomly divided into five groups, each group comprising of 10 animals. Eight animals from each group were used for biochemical estimations and the remaining rats were used for the histopathological studies. The animals were allowed free access to food and water.

Group I animals served as normal control and received lactose 75 mg/kg in saline intraperitoneally (i.p) in the same regimen as doxorubicin.

Group II animals received doxorubicin alone (2.5 mg/kg, body weight in normal saline (i.p.) in six equal injections for a period of 2 weeks for a total cumulative dose of 15 mg/kg, body weight).

Group III animals received tempol 100 mg/kg, per oral (p.o.) for 15 days as a pretreatment followed by doxorubicin administration (dosage and duration were as in Group II).

Group IV animals received doxorubicin (dosage and duration were as in Group II) and after 15 days tempol 100 mg/kg, p.o. for 15 days.

Group V animals received only tempol 100 mg/kg, p.o. for 15 days.

Assessment of nonezymatic and enzymatic parameters

Control, as well as treated animals were observed for a period of 4 weeks, and their body weights were checked. At the end of the fourth week, the animals were killed under ether anesthesia and a midline abdominal incision was performed and the hearts tissue were quickly dissected out, washed in ice-cold saline, dried on a filter paper, and weighed. For histopathological studies, heart tissues of each group were stored in 10% formalin in saline before processing.

A portion of each heart was taken from all the groups and a 10% w/v homogenate was prepared in 0.9% buffered potassium chloride (pH 7.4) for the estimation of glutathione [16, 17] and malondialdehyde. [18]

The remaining portion of the heart tissue was used for the assay of cardiac damage marker enzyme and antioxidant enzymes. A 10% w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7.4). The homogenate was subjected to cold centrifugation at 4 °C for 20 min and used for estimation of lactate dehydrogenase [19], catalase [20], superoxide dismutase [21], glutathione peroxidase [22], glutathione reductase [23], glutathione-S-transferase [24], glucose-6-phosphate dehydrogenase [25] and protein content. [26]

Histopathological studies

The hearts were fixed in 10% formalin. The specimens were processed by standard procedure and embedded in paraffinwax. The blocks were sectioned from the ventricular portion and 5-micron thick sections were stained according to the hematoxylin and eosin (H & E) method given by Smith and Burton. [27] The sections were examined by light microscopy.

Statistical analysis

Data were expressed as the mean ± SEM. For a statistical analysis of the data, group means were

compared by one-way analysis of variance (ANOVA) followed by Dunnett's t test, which was used to identify differences between groups. *P* value <0.05 was considered significant.

Results

Effect of tempol pretreatment

The chronic treatment with doxorubicin injection significantly decreased the levels of tissue glutathione. Treatment with tempol (100 mg/kg) has produced a significant increase in the levels of tissue glutathione. Doxorubicin treatment produced a significant increase in malondialdehyde and LDH levels as compared with the control group. Tempol pretreatment has produced a significant reduction in tissue malondialdehyde and lactate dehydrogenase levels in doxorubicin-treated rats. Doxorubicin significantly increased the activities of antioxidant defense enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione–s-transferase, and glucose-6-phosphate dehydrogenase. Tempol treatment has produced a significant reduction in the levels of these enzymes towards normal.

Effect of tempol post-treatment

Tempol post treatment significantly reduced MDA levels as compared to the doxorubicin treated group. Tempol post treatment did not significantly reduced the levels of the enzyme lactate dehydrogenase. Tempol treatment has significantly reduced the activities of the antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-s-transferase, and glucose-6-phosphate dehydrogenase towards normal.

Histopathological Findings

The vehicle treated group (i.e. group) 1 did not show any morphological changes (Figure A). On the contrary, the hearts of rats treated with doxorubicin (i.e. group 2) showed marked histological changes in endocardium. The heart sections showed severe changes with varying degrees of vacuolar changes in the endocardial surface of the heart, necrosis of cardiac muscle fibers with isolated cells showing features of hypertrophy in between the necrotic and fragmented muscle fibers (Figure Bi, Bii). Pretreatment with tempol (i.e. group 3) completely reverted the morphological changes produced by doxorubicin (Figure C). Post treatment with tempol (i.e. group 4) did partially reverted the morphological changes and vacuolated cells were seen scattered in subendocardial layers (Figure D). The per se treatment of tempol (i.e. group 5) has no effect as such on the myocardium (Figure E).

Discussion

The clinical use of doxorubicin is limited by its dose dependent cardiac damage and repeated administration of doxorubicin beyond a certain dose has been shown to cause cardiomyopathic changes in patients[4] as well as in a variety of animal species. [28, 29] Studies have shown that tempol is a superoxide anion scavenger in vitro [9] and may show effect as a genuine SOD-mimetic. [10] Tempol has been reported experimentally to ameliorate oxidative stress mediated renal dysfunction and glomerular injury [13] and in an experimental model of regional myocardial ischemia/reperfusion, reduced the infarct size. [15] The purpose of this study was to prove that tempol as the currently available cardioprotective and antioxidant which can be used in protecting the cancer patients from doxorubicin induced cardiotoxicity.

The doxorubicin-induced cardiomyopathy in rats in the present studies was evaluated by the myocardial damage and the destabilization of the antioxidant defense enzyme system. Many researchers have shown the role of reactive oxygen species including hydroxyl radical in the DOX-induced cardiomyopathy models. [31-33] DOX is capable of generating oxygen radicals, it decreases the synthesis of glutathione, and increases the production of MDA in humans and experimental animals. [32] It has been shown that marked decreases in GSH pool occur in many tissues after acute and chronic DOX toxicities. [33-35] Treatment of rats with tempol produced a significant reduction of MDA levels produced by ischemia/reperfusion. [36] The evidence in the present study with respect to the MDA levels are in agreement with the above study.

The DOX induced cardiotoxicity is secondary evident following lipid peroxidation of cardiac membrane leads to increase in leakage of LDH and CPK from cardiac myocytes into plasma. [37] Our findings are consistent with above mentioned studies and *treatment with tempol was* found to inhibit the DOX-induced CK-MB release in serum. It is widely reported that DOX-induced free-radical generation triggers membrane peroxidation and disruption of cardiac myocytes, which can lead to increased release of CKMB in the serum. [38] Our study shows that *tempol and* Vitamin E led to decrease in CKMB release in doses used. Also the administration of DOX induced cardotoxicity manifested significant increase in serum LDH levels. The LDH levels are significantly decreased in tempol and Vitamin E treated group.

DOX significantly decreased the level of tissue GSH in accordance with the previous studies. [39] Decrease in the levels of GSH represents its increased utilization by myocardial cells due to oxidative stress. Treatment with tempol & Vitamin E has significantly restored the GSH levels, this effect could be attributed either to increased biogenesis of GSH or the reduction in oxidative stress levels leading to decreased generation of toxic free-radical species. The protective activity was further supported by increased myocardial antioxidant enzyme activity and decrease extent of lipid peroxidation. The decrease in antioxidant enzymes and lipid peroxidation are known to cause cellular damage and responsible for reactive oxygen species (ROS) induced organ damage. The antioxidant enzymes such as catalase, SOD, glutathione peroxidase, glutathione reductase, glutathione-s-transferase and G-6-PD and all of them constitute the major supportive team of defense against free radicals. The equilibrium between these enzymes is an important process for the effective

removal of ROS in intracellular organelles. [40] In present study, a significant decrease in levels of catalase, SOD. GPx, GR, GST and G-6-PD enzymes in DOX treated group was observed. Tempol and Vitamin E treatment significantly increased the antioxidant enzymes levels induced by DOX. A decrease in the activity of SOD can result in the decreased removal of superoxide ion, which can be harmful to the organs.

Moreover, the enhanced SOD activity in the tempol and Vitamin E treated groups might be involved in the scavenging of O2 - generated from DOX. tempol and Vitamin E efficiently counteracted the DOX induced cardiac tissue damage by significantly decreasing the MDA levels and increasing the GSH, catalase, SOD, GPx, GR, GST and G-6-PD enzymes activities.

The present study shows that a simultaneous treatment with tempol could attenuate the doxorubicin-induced cardiomyopathic effects as shown by the improved myocardial structures and stabilization of the antioxidant defense enzyme system in the pretreated tempol group and to a lesser extent in the post treatment with tempol. The results of the present study markedly indicate that pretreatment with tempol offered protection against the chronic administration of doxorubicin in rats as compared to doxorubicin treated group. The evidence for protective effect of tempol was seen with a significant increase in the antioxidant defense enzyme system, reduction in MDA production and restoration of tissue glutathione content in heart tissue. The cardiotoxic effects of DOX are directly related to oxidative stress and many studies have confirmed this finding. Tempol increased the levels of the above enzymes. Thus, our results showed that the stress on the cardiac cells during the generation of ROS could be prevented by tempol. The biochemical changes and the histological changes are relatively confirming that DOX has indeed caused the free radical injury in the myocardium of the rats to which it was administered showing the marked changes such as necrosis and hypertrophied cells as compared to vehicle treated rats. Our study shows that pre treatment with tempol is more beneficial as compared to post treatment against DOX-induced cardiac toxicity

.

Table 1: Effects of Tempol on antioxidant enzyme catalase, glutathione peroxidase and superoxide dismutase activity in the heart of control and experimental animal groups

Groups	Catalase	GPx	SOD
Normal Control	126.17 ± 5.82	179.59 ± 7.03	140.95 ± 23.81
Doxorubicin (15 mg/kg)	32.99 ± 4.05**	49.89 ± 2.20**	66.22 ± 2.28 **
Tempol (100 mg/kg) +DOX	90.13 ± 1.36α	115.19 ± 3.22α	118.03 ± 1.42α
DOX +Tempol (100 mg/kg)	79.27 ± 10.87α	87.52 ± 48.77β	103.54 ± 37.36β
Tempol *per se* (100 mg/kg)	132.26 ± 6.79α	186.60 ± 3.4 α	154.91 ± 3.19α

The data are expressed as mean □ SEM for five rats in each group. ** $p < 0.01$ versus normal control. 'a' is $p < 0.01$ versus DOX toxic group, 'b' is $p < 0.05$ versus DOX toxic group. The enzyme activities are expressed as nmol of H_2O_2 consumed/min/mg protein for Catalase, nmol of NADPH oxidized/min/mg protein for GPx, amount of enzyme required to give 50% inhibition of pyrogallol auto oxidation, Unit/mg protein for SOD.

Table 2: Effects of Tempol on antioxidant enzyme GR, GST and G6PD activity in the heart of control and experimental animal groups

Groups	GR	GST	G6PD
Normal Control	32.59 ± 2.55	91.94 ± 7.05	11.90 ± 4.85
Doxorubicin (15 mg/kg)	15.65 ± 1.09**	28.31 ± 4.20**	1.69 ± 0.33**
Tempol (100 mg/kg) + DOX	27.54 ± 4.21α	79.16 ± 3.03α	6.29 ± 0.22α
DOX + Tempol (100 mg/kg)	22.40 ± 2.60β	58.67 ± 3.55β	4.85 ± 1.02β
Tempol *per se* (100 mg/kg)	31.07 ± 0.84α	90.48 ± 4.63α	12.52 ± 0.42α

The data are expressed as mean ± SEM for five rats in each group. ** $p < 0.01$ versus normal control. 'a' is $p < 0.01$ versus DOX toxic group, 'b' is $p < 0.05$ versus DOX toxic group. The enzyme activities are expressed as nmol of NADPH oxidized/min/mg protein for GR, nmol of 1-chloro 2, 4 dinitrobenzene (CDNB) conjugate formed/min/mg protein for GST, nmol of reduced NADP oxidized/min/mg protein for G6PD.

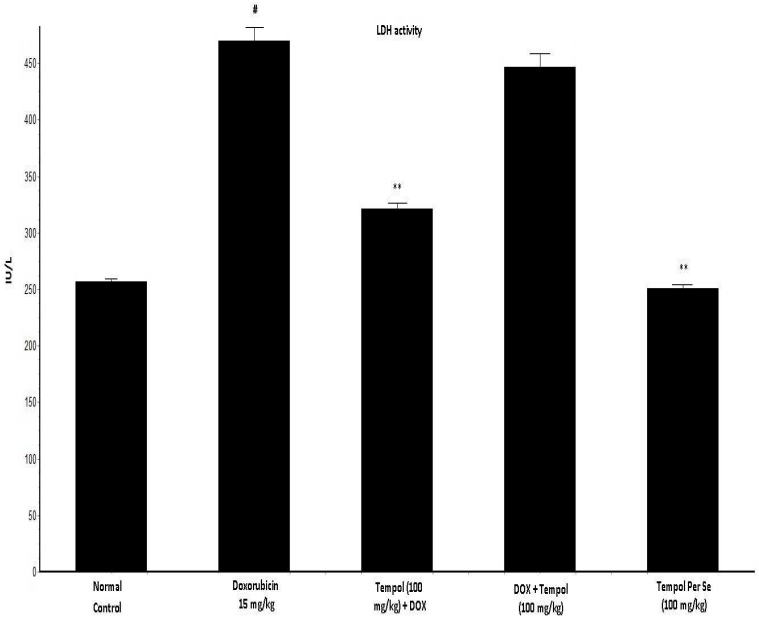

Figure 1. Level of LDH in serum. Effect of tempol pretreatment, post treatment and per se (100 mg/kg, p.o. respectively) and toxic group induced by DOX (15 mg/kg, i.p.) on serum of rat. Values are mean ± SEM from a group of five animals. # $p < 0.01$ when control group was compared with toxic group. ** $p < 0.01$ when treated groups were compared with toxic group.

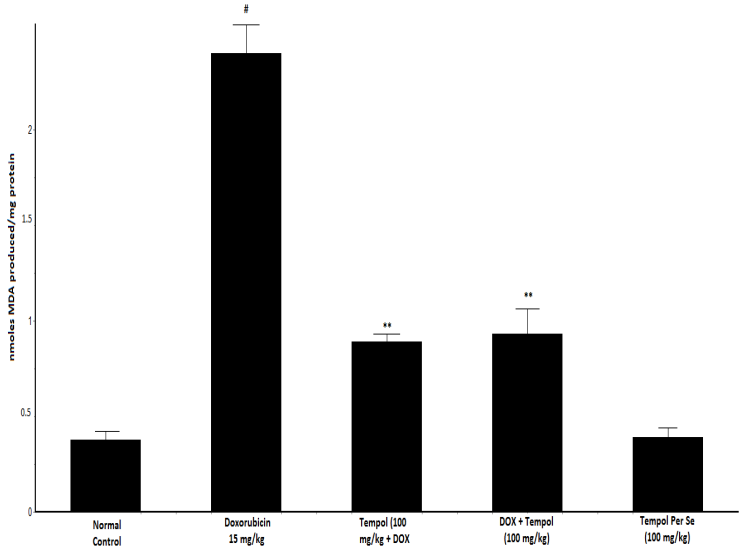

Figure 2. Level of MDA in cardiac tissue. Effect of tempol pretreatment, post treatment and per se (100 mg/kg, p.o. respectively) and toxic group induced by DOX (15 mg/kg, i.p.) on serum of rat. Values are mean ± SEM from a group of five animals. # $p < 0.01$ when control group was compared with toxic group. ** $p < 0.01$ when treated groups were compared with toxic group.

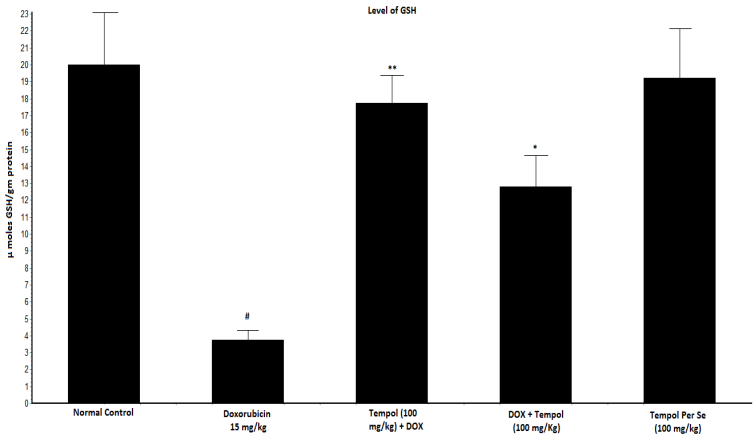

Figure 3. Level of GSH in cardiac tissue. Effect of tempol pretreatment, post treatment and per se (100 mg/kg, p.o. respectively) and toxic group induced by DOX (15 mg/kg, i.p.) on serum of rat. Values are mean ± SEM from a group of five animals. # $p < 0.01$ when control group was compared with toxic group. ** $p < 0.01$, * $p < 0.05$ when treated groups were compared with toxic group.

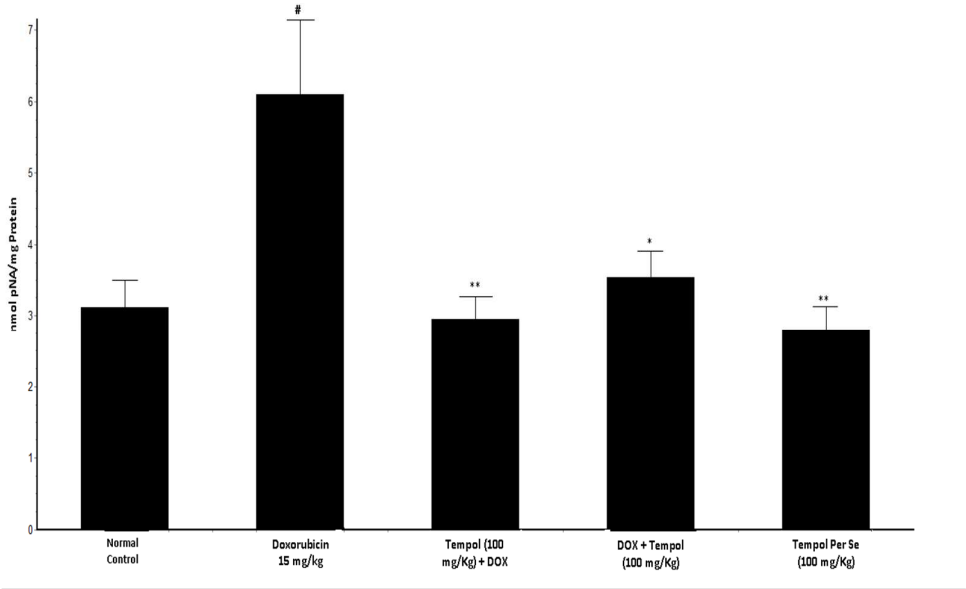

Figure 4. Level of caspase-3 in cardiac tissue. Effect of tempol pretreatment, post treatment and per se (100 mg/kg, p.o. respectively) and toxic group induced by DOX (15 mg/kg, i.p.) on serum of rat. Values are mean ± SEM from a group of five animals. # $p < 0.01$ when control group was compared with toxic group. ** $p < 0.01$, * $p < 0.05$ when treated groups were compared with toxic group.

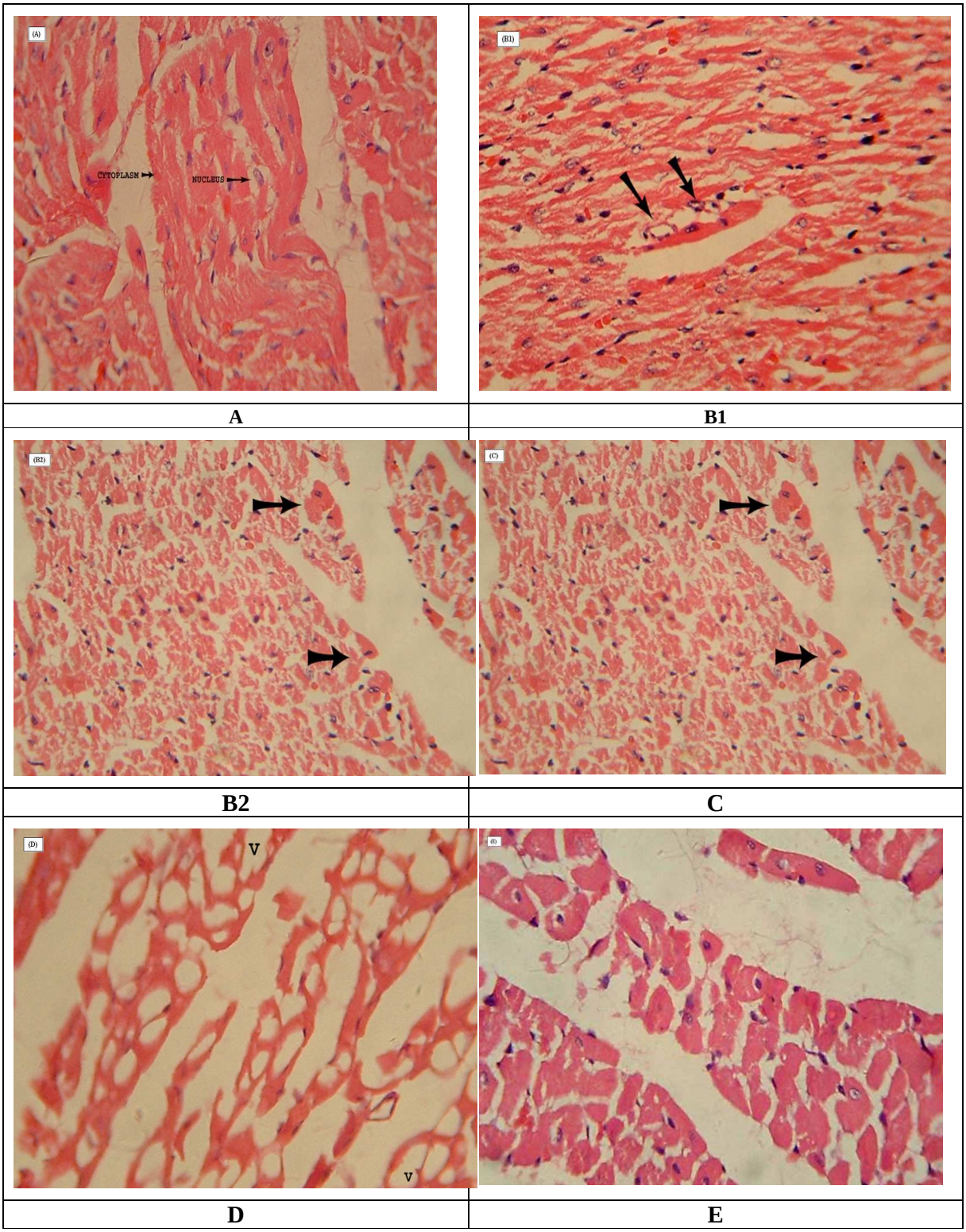

Figure 5. Photomicrograph of rat heart (A) normal control group showing normal architecture of the myocardial fibers. (B1 & B2) Doxorubicin-treated (15 mg/kg, i.p.) showing patchy necrosis and hypertrophied myocardial fibers, patchy necrosis and a single vacuolated hypertrophied myocardial fiber marked by arrows. (C) Tempol 100 mg/kg, p.o. + DOX showing normal architecture of myocardial cells. (D) DOX + Tempol 100 mg/kg, p.o., showing vacuolated cells marked by V. (E) *Per Se* group (Normal + Tempol 100 mg/kg, p.o.) showing normal architecture of myocardial cells.

Conclusion

In conclusion, the cardiotoxicity induced by DOX is in relation with oxidative stress. Our study suggests that tempol could be used as a cardioprotective and antioxidant as preventive therapy rather than curative therapy against doxorubicin-induced cardiac toxicity.

Acknowledgements

This study is supported by University Grants Commission (UGC) New Delhi, India. We are thankful to the Incharge, Central Animal House Facility, Jamia Hamdard, for providing the animals. We thank Mr. Mushtaq Ahmad for assisting in the experimental work.

References

1.Young, R.C., Ozols, R.F., Myers, C.E., The anthracyclines antinoeplastic drugs, N. Engl. J. Med., 1981, 305, 139-53

2.Booser, D.J., Hortobagyi, G.N., The anthracycline antibiotic in cancer therapy focus on drug resistance, Drugs., 1994, 47, 223-58

3.Allen, A., The cardiotoxicity of chemotherapeutic drugs, Semin. Oncol., 1992, 19, 529- 42

4.Lefrak, E. A., Pitha, J., Rosenheim, S., Gottlieb, J. A.,A Clinicopathologic analysis of adriamycin cardiotox icity, Cancer., 1973, 32, 302-14

5. Yin, X., Wu, H., Chen, Y., Kang, Y.J., Induction of antioxidants by adriamycin in mouse heart, Biochem. Pharmacol., 1998, 56, 87-93

6.Hershko, C., Link, G., Tzahor, M., Pinson, A., The role of iron and iron chelators in anthracic-line cardiotoxicity, Leuk. Lymphoma., 1993, 11, 207-14

7.Thomas, C.E., Aust, S.D., The release of iron from ferritin by cardiotoxic anthracycline antibiotic, Arch. Bio. Chem. Biophys., 1986, 248, 684-89

8.Olson, R.D., Mushlin, P.S., Doxorubicin cardiotoxicity: analysis of prevailing hypotheses, FASEB. J., 1990, 4, 3076-86.

9.Laight, D.W., Andrews, T.J., Haj-Yehia, A.I., Microassay of superoxide anion scavenging activity in vitro, Environ. Toxic. Pharm., 1997, 3, 65– 68

10. Krishna, M.C., Russo, A., Mitchell, J.B., Goldstein, S., Dafni, H., Samuni, A., Do nitroxide antioxidants act as scavengers of superoxide anions or as SOD mimetics? J. Biol. Chem., 1996, 271, 26026–26031.

11. Leach, M., Frank, S., Olbrich, A., Pfeilschifter, J., Decline in the expression of copper/zinc superoxide dismutase in the kidney of rats with endotoxic shock: Effects of the superoxide anion radical scavenger, tempol on organ injury, Br. J. Pharmacol., 1998, 125, 817–825

12. Mota-Filipe, H., McDonald, M.C., Cuzzocrea, S., Thiemermann, C., A membrane permeable radical scavenger reduces the organ injury in hemorrhagic shock, Shock., 1999, 12, 255–261

13. Shah, S.V., Baliga, R., Rajapurkar, M., Fonseca, V.A., Oxidants in chronic kidney disease., J. Am. Soc. Nephrol., 2007, 18, 16–28

14. Nassar,T., Kadery, B., Lotan, C., Da'as, N., Kleinman, Y., et al., Effects of superoxide dismutase-mimetic compound tempol on endothelial dysfunction in streptozotocin induced diabetic rats,. Eur. J. Pharmacol., 2002, 436, 111–118

15. McDonald, M.C., Zacharowski, K., Bowes, J., Cuzzocrea, S., Thiemermann, C., Tempol reduces infract size in rodent models of regional myocardial ischemia and reperfusion., Free. Radic. Biol. Med., 1999, 27, 493– 503

16. Sedlak, J., Lindsay, R.H., Estimation of total, protein bound, and nonprotein sulfhydryl Groups in tissue with Ellman's reagent., Anal. Biochem., 1968, 25, 192-205

17. Ellman, G. L., Tissue sulfhydryl groups, Arch. Biochem. Biophys., 1959, 82, 70-77

18. Ohkawa, H., Ohish, N., Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid., Anal. Bio. Chem., 1979, 95, 351-58

19. Lum, G., Gambino, S. R., A comparison of serum versus heparinised plasma for routine chemistry tests, Am. J. Clin. Pathol., 1974, 61, 108-1320. Clairborne, A., Handbook of methods for oxygen radical research. CRC Press; Boca Raton; Florida; 1985.

21. Marklund, S.L., Handbook of methods for oxygen radical research. CRC Press; Boca Raton; lorida;1985.

22. Paglia, D. E., Valentine, W. N., Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase., J. Lab. Clin. Med., 1967, 70, 158-69

23. Carlberg, I., Mannerviek, B., Purification and characterization of the flavoenzymes glutathione reductase from rat liver, J. Biol. Chem., 1975, 250, 5475-80

24. Habig, W.H., Pabst, M. J., Jakoby, W. B., Glutathione S-transferase. The first enzymatic step in mercapturic acid formation., J. Biol. Chem., 1974, 249, 7130-39

25. Zaheer, N., Tewari, K. K., Krishnan, P. S., Exposure and solubilisation of hepatic mitochondrial shunt dehydrogenases., Arch. Biochem. Biophys., 1965, 109, 646-48

26. Lowry, O. H., Rosenbrough, N. T., Farr, A. L., Randall, A. T., Protein measurement with the Folins phenol reagent. J. Biol. Chem., 1951, 193, 265-75

27. Smith, A., Burton, J., A color atlas of histological staining techniques. Wolfe Medical Publications; London; 1977.

28. Buja, L.M., Ferrans, V.J., Mayer, R.J., Roberts, W.C., Henderson, E.S., Cardiac ultrastructural changes induced by daunorubicin therapy., Cancer., 1973, 32, 771-88

29. Sarvazyan, N.A., Askari, A., Huang, W.H., Effects of doxorubicin on cardiomyocytes with reduced level of superoxide dismutase, Life. Sci., 1995, 57, 1003-10

30. Singal, P.K., Siveski-Iliskovic, N., Hill, M., Thomas, T.P., Li, T., Combination therapy with probucol prevents adriamycin induced cardiomyopathy., J. Mol. Cell. Cardiol., 1995, 27, 1055-63

31.Dorr, R.T., Cytoprotective agents for anthracyclines., Semin. Oncol., 1996, 23, 23-34

32. Doroshow, J.H., Locker, G.Y., Ifrim, I., Myers, C.E., Prevention of doxorubicin cardiac toxicity in the mouse by N-acetylcysteine, J. Clin. Invest., 1981, 68, 1053-64

33. Ayaz, S.A., Bhandari, U., Pillai, K.K., Influence of alpha Lipoic acid and vitamin E against doxorubicin Induced biochemical and histological changes in the cardiac tissue of rats., Ind. J. Pharmacol., 2005, 37, 294-99

34. Mohamed, H.E., El-Swefy, S.E., Hagar, H.H., The protective effect of glutathione administration on adriamycin

induced acute cardiac toxicity in rats., Pharmacol. Res., 2000, 42, 115-21

35. Neha, N.Q., Ayaz, S.A., Subur, K., Cardioprotective and Antioxidant activity of Onion (Allium cepa) Leaves Extract in Doxorubicin Induced Cardiotoxicity in Rats., Ann. Expt. Biol., 2014, 2, 37-42

36. Kaithwas, G., Dubey, K., Pillai, K.K., Effect of Aloe vera (Aloe barbadensis Miller) gel on doxorubicin- induced myocardial oxidative stress and calcium overload in albino rats, Ind. J. Exp. Biol., 2011; 44: 260-68

37. Liu, X., Chen, Z., Chua, C.C., et al., Melatonin as an effective protector against Doxorubicin induced cardiotoxicity., Amer. J. Physiol., Heart Cir. Physiol., 2002, 283, 254-63

38. Hino, Y., Yoo, S.B., Kajima. K., et al., Effect of riboflavin-butyrate on cardiac glutathione reductase affected by adriamycin, J. Nutr. Sci. Vitaminol., 1985, 31, 139

39. Bertzoli, C., Dell'oro, L., Ferni, G., et al., Adriamycin experimental cardiotoxicity in the rabbit, Int. Res.Commun. Sys. Med. Sci., 1974, 2, 145-62