Lycopene Potentiates the Protective Effect of Aliskiren on Doxorubicin-Induced Cardiomyopathy in Rats

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Abstract: *Objective*: The present study was designed to explore the combination therapy of lycopene with aliskiren in doxorubicin induced cardiomyopathy.

Methods: Cardiomyopathy was induced in Wistar rats by i.p. administration of Doxorubicin (DOX) (15 mg/kg, single dose). Haemodynamic parameters (Systolic, diastolic blood pressure, Heart rate), heart weight, heart weight/body weight ratio, serum lactate dehydrogenase (LDH), oxidative stress (TBARS) and antioxidant enzymes (Glutathione, SOD and catalase) as well as transmission electron microscopein heart tissue were carried out.

Results: There was significant increase in blood pressure, serum, lactate dehydrogenase (LDH), oxidative stress (TBARS) and significant decrease in heart weight, heart weight/body weight ratio and antioxidant enzymes in cardiomyopathic rats. Combined therapy of lycopene (2 and 4mg/kg) with aliskiren (100 mg/kg) treatment showed pronounced beneficial effect on above parameters. Furthermore lycopene with aliskiren significantly improves the antioxidant defense by increasing reduced glutathione, SOD, catalase, heart size and heart weight/body weight ratio. It is clearly observed from Transmission electron microscopic slides of DOX treated rats, there was swelling of mitochondria with disruption of cristae, rupture of nuclear membrane, condensation and margination of nuclear chromatins which were well protected by lycopene along with aliskiren treatment.

Conclusion: Therefore, combination therapy of lycopene with aliskiren offers better treatment for DOX-induced cardiomyopathy than ALK alone.

Keywords: Aliskiren, lycopene, doxorubicin, cardiomyopathy, antioxidant enzymes.

INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic, is widely used cytotoxic agent [1] for the treatment of human neoplasm such as breast cancer, ovarian cancer, leukemias and sarcomas [2]. However, the clinical use of DOX proved to be hampered because it produces chronic cardiomyopathy and congestive heart failure (CHF) [3]. The mechanism by which cardiomyopathy induced by DOX is not completely clear. On the other hand, different mechanisms have been involved in DOX-induced cardiomyopathy, such as release of vasoactive amines [4] generation of reactive oxygen species (ROS) [5], induction of apoptosis [6], oxidative DNA damage [7], lipid peroxidation [8], impairment of enzymatic activity of creatine kinase [9], and induction of renin angiotensin system (RAS) activity [10]. A number of agents have been screened to treat DOX-induced cardiomyopathy including probucol, amifostine, and dexrazoxane with some protection [11]. But, all these agents have well defined clinical disadvantages, including a significant decline in HDL levels, an inability to prevent DOX-induced mortality and weight loss, and potentiation of DOX-induced myelosuppression [12]. Several studies have suggested that angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) have protective effects against DOX-induced cardiomyopathy [8]. However, the valuable effect of ACE inhibitors and ARBs in DOX-induced cardiomyopathy is only some scope [13]. Thus it was assumed that the inhibition of renin at the first step in RAS cascade may protect the heart from DOX toxicity.

Aliskiren (ALK) is a nonpeptide direct acting renin inhibitor and has a number of advantages over existing RAS blockers such as; it does not have ACE-escape like activity [14], prevents the development of both angiotensin I and angiotensin II and produces effective blockade of RAS without the compensatory augment in plasma renin activity (PRA) [15].

Lycopene is one of the most effective antioxidants amongst the dietary carotenoids and has the strongest singlet oxygen quenching capacity [16]. The singlet quenching capability of lycopene is twice as high as β-carotene and 10 times higher that of α-tocopherol [17]. Hence, it has protective ability to myocardium from oxidative damage [18]. Additionally, it has potential to stimulate antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase [19]. It protects isoproterenol and

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doxorubicin-induced cardiotoxicity, coronary artery disease and possesses antihyperlipidemic properties [20].

The aim of this study was to evaluate combination therapy of lycopene with aliskiren on doxorubicin-induced cardiomyopathy in rats.

MATERIALS AND METHODS

Experimental Animals

Male albino Wistar rats (b.wt. 180 and 200 g) were used in the present study. The animals were kept in polypropylene cages under standard laboratory conditions (12 h light and 12 h dark cycle), and had a free access to commercial pellet diet (Pranav Agro Industries Ltd., New Delhi) and water *ad libitum*. The animal house temperature was maintained at 25 ± 2°C. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of KIET School of Pharmacy (Registration number: 1099/07/ CPCSEA, dated 27.07, 2007), Ghaziabad (UP).

Drugs and Chemicals

DOX, ALK and LYC were procured as a gift sample from Fresenius Kabi Oncology Ltd., Gugaon, Haryan, India; Novartis Healthcare Private Ltd, Mumbai, India; Krishgir Pharmaceuticals, Kangra, India respectively. Lactate dehydrogenase assay kit was purchased from Crest Biosystems, Goa, India. All the other chemicals used were of AR grade.

Experimental Schedule

After acclimatization, all the animals were randomly allocated into five groups of six animals each and treated as follows:

Group I: received Normal saline (2ml/kg, i.p) single dose and served as control; Group II: received DOX, Doxorubicin (15 mg/kg, i.p.) single dose; Group III: received DOX+ALK (Aliskerin 100mg/kg/day, p.o.) for 7 days; Group IV: received DOX+ALK100+LYC2 (ALK100mg/kg + LYC2 mg/kg, p.o.l) for 7days; Group V: received DOX+ALK100+LYC4 (ALK100mg/kg +LYC4mg/kg.p.o.) for 7days.

After 24 hrs of last dose of drugs the rats were anesthetized by diethyl ether and the blood was collected from retro orbital plexus. The enzymatic parameter (LDH) was estimated in serum. Heart tissue was dissected out, washed with ice-cold physiological saline and weighed. A small portion of heart was homogenized for biochemical estimations (malondial-dehyde [MDA], glutathione [GSH], superoxide dismutase [SOD] and catalase [CAT]). The left ventricular heart tissue was trimmed into 1.0-1.5mm thick pieces and immediately fixed in 0.1 M sodium phosphate buffer (pH 7.2) preserved for transmission electron microscopic studies.

Hemodynamic Measurements

Hemodynamic parameters were measured using tail cuff method by Non-Invasive Blood Pressure instrument (AD Instruments, Australia). All the rats were initially trained in the restrainer for a period of 10 days at least 15 min every day prior to the day of measurement of the hemodynamic parameters (systolic, diastolic, mean blood pressure and heart rate).

Biochemical Estimation in Serum

Lactate dehydrogenase (LDH), was estimated in serum by enzymatic kit (Reckon Diagnostics Pvt. Ltd., Baroda, India) using an UV-Visible spectrophotometer (Shimadzu, Tokyo, JapanUV-1601, Japan).

Biochemical Assay in Cardiac Tissues Homogenates

Measurement of lipid peroxidation was performed by determination of cardiac malondialdehyde (MDA) content by the method of Ohkawa *et al*. [21]. Antioxidant enzymes viz. GSH, SOD and CAT were estimated in cardiac tissue as per standard protocol. GSH was estimated by the Sedlack and Lindsay method [22]. The activity of SOD was measured according to the method of Marklund and Marklund [23]. CAT activity was measured according to Clairbone [24].

Ultra Structural Studies in Cardiac Tissues

The left ventricular heart tissue was trimmed into 1-1.5mm thick pieces and immediately fixed in 0.1 M sodium phosphate buffer (pH 7.2) containing 2.5% glutaraldehyde and 2% paraformaldehyde for 12 h, post fixed in 1% osmium tetroxide. After it was dehydrated in a series of graded ethanol solutions, then embedded in araldite mixture CY212 (TAAB Laboratories Equipment, Berks, United Kingdom). Ultrathin (70 to 80 nm) sections were cut and the grids containing sections were stained with 2% uranyl acetate and 0.2% lead acetate. The sections were observed by Transmission Electron Microscope (2100F, JEOL, Tokyo, Japan) operating at 120 kV.

Statistical Analysis

The results data was expressed as mean ± standard error of mean (SEM). Groups data were compared with the analysis of variance (ANOVA) followed by Dunnett's t test to identify significance among groups. Values are considered statistically significant at $p < 0.05$.

RESULTS

Effect on Haemodynamic Parameters

The systolic, diastolic, mean BP and heart rate were significantly increased in DOX treated rats (i.e. Group II) as compared to the normal control rats (i.e. Group I). Treatment with LYC (2 & 4 mg/kg) along with ALK (100 mg/kg) showed significant ($p<0.05$) reduction in the systolic, diastolic, mean BP and heart rate in dose dependant manner as compared to the DOX treated group (Table **1**).

Effect on Heart Weight (g), Heart Weight/Body Weight Ratio

Mean heart weight and heart weight/body weight ratio were a significantly ($p<0.05$) decreased in the DOX treated group as compared to normal control group (Figures **1** and **2**). The mean heart weight, heart weight/body ratio in the DOX+ ALK (100mg/kg), DOX+ ALK+LYC (2 mg/kg) treated groups were significantly restored ($p<0.05$) as compared with DOX treated group. While the mean heart weight and heart weight/body weight ratio was most significantly ($p < 0.01$) increased in ALK+LYC (4 mg/kg) treated group.

Effect on Oxidative Stress and Antioxidant Enzyme Levels

Oxidative stress was estimated by determining myocardial MDA content (Table **2**). MDA level was significantly ($p<0.05$) augmented in the DOX treated

Table 1: Effect of Lycopene and Aliskiren on Systolic, Diastolic, Mean BP and Heart Rate of Wistar Rats

Groups	Heart Rate (BPM)	Systolic BP (mmHg)	Diastolic BP (mm Hg)	Mean BP (mmHg)
Normal Control Group (saline 2ml/kg, i.p) single dose	397.89±7.6	128.56±3.96	101.96±1.98	115.26±2.69
Doxorubicin Group (DOX 15 mg/kg, i.p.) single dose	558.7±20.43[a]	168.07±5.69[a]	122.95±2.9[a]	145.51±3.17[a]
DOX+ALK (100 mg/kg, p.o.) for 7 days	491.09±18.07[b]	148.09±4.62[b]	113.0±3.17[b]	130.55±2.56[c]
DOX+ALK100+LYC2 (2mg/kg, p.o.) for 7days	445.09±15.37[c]	136.02±3.56[c]	108.48±2.22[c]	122.25±2.85[c]
DOX+ALK100+LYC4(LYC 4mg/kg, p.o.) for 7days	431.21±12.15[c]	132.98±3.33[c]	107.95±2.53[c]	12.47±2.08[c]

DOX: doxorubicin; ALK: aliskiren; LYC: lycopene; BPM: beats per minute; BP: blood pressure.
All values were expressed as Mean ± S.E.M. (n=6), [a]P<0.01 as compared to the normal control group, [b]P<0.05 and [c]P<0.01 as compared to the DOX control group (ANOVA followed by Dunnett's test).

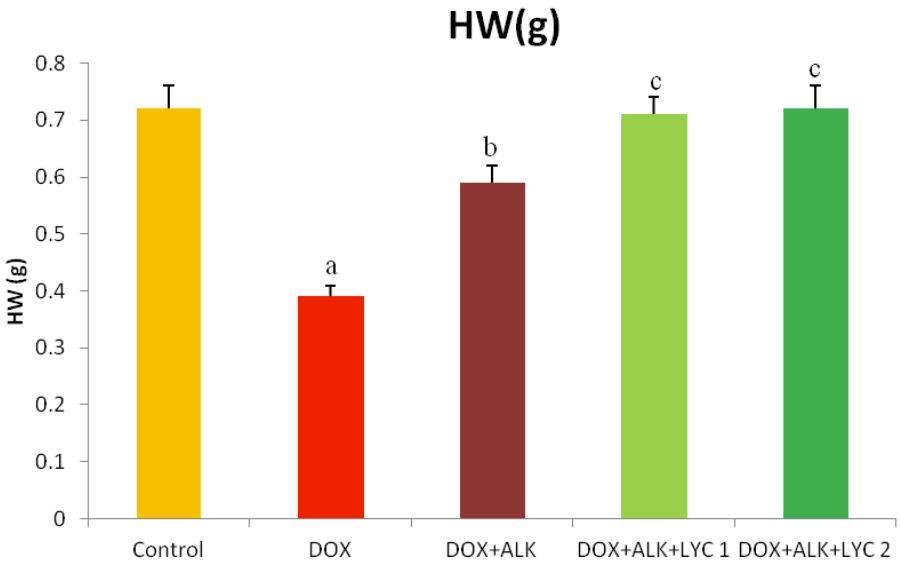


Figure 1: Effect of lycopene and aliskiren treatment on heart weight.
All values were expressed as Mean ±S.E.M. (n=6),[a]P<0.01 as compared to the normal control group, [b]P<0.05 and [c]P<0.01 as compared to the DOX control group (ANOVA followed by Dunnett's test).

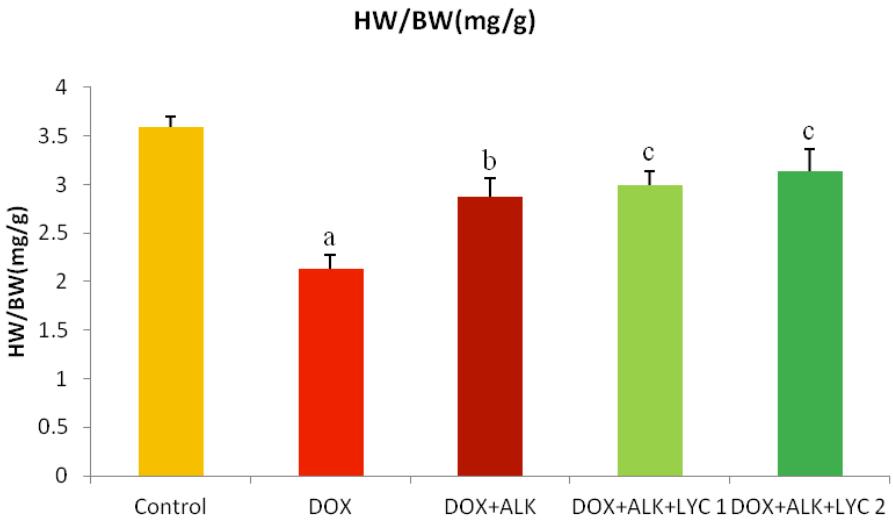


Figure 2: Effect of lycopene and aliskiren treatment on heart weight and body weight ratio.
All values were expressed as Mean ± S.E.M. (n=6), [a]P<0.01 as compared to the normal control group, [b]P<0.05 and [c]P<0.01 as compared to the DOX control group (ANOVA followed by Dunnett's test).

group as compared to the normal control group. MDA level was significantly (p<0.05) decreased in DOX+ALK (100 mg/kg), DOX+ ALK +LYC (2 and 4 mg/kg) treated groups compared with the DOX treated group. In DOX treated group antioxidant enzymes viz. CAT, GSH and SOD were significantly decreased as compared to normal control group. While the antioxidant enzyme levels were significantly increased in DOX+ALK (100 mg/kg) and DOX+ ALK +LYC (2 and 4 mg/kg) treated groups. Lycopene showed antioxidant effect in a dose dependent manner (Table **2**).

Effect on Lactate Dehydrogenase (LDH) Activities

Treatment of rats with DOX (15 mg/kg, i.p.) resulted significant (p< 0.05) raise in the LDH enzyme activity as compared to the normal control group. ALK (100 mg/kg) treatment for 7 days non-significantly (P>0.05) decline the serum LDH levels as compared to DOX control group. Coadministration of ALK with LYC (2 and4 mg/kg) with DOX resulted in significant (p<0.01) decrease in LDH activity dose dependent manner as compared to DOX control group (Figure **3**).

Effect on Transmission Electron Microscopic Studies

There was significant myocardial ultrastructural damage manifested as vacuolation of the cytoplasmic reticulum, swelling of mitochondria with disruption of cristae, rupture of nuclear membrane, condensation and margination of nuclear chromatin at the nuclear membrane due to administration of DOX (15 mg/kg, i.p.) (Figure **4B**, **B1**). ALK (100 mg/kg) protects the

Table 2: Effect of Lycopene and Aliskiren on Lipid Peroxides (TBARS), Superoxide Dismutase (SOD), Catalase (CAT) & Reduced Glutathione (GSH) Levels in Heart Tissue of Wistar Rats

Groups	TBARS (nmol MDA/mg protein)	SOD (IU/mg protein)	CAT (nmole H_2O_2 consumed/min/mg protein)	GSH (nmole/mg protein)
Normal Control Group (saline 2ml/kg, i.p) single dose	0.64±0.63	608.35±55.31	52.15±1.18	1.012±0.06
Doxorubicin Group (DOX 15 mg/kg, i.p.) single dose	3.99±0.26[a]	265.05±29.12[a]	24.99±1.28[a]	0.355±0.06[a]
DOX+ALK (100 mg/kg, p.o.) for 7 days	2.51±0.30[b]	415±44.34[b]	30.58±1.62[b]	0.616±0.07[b]
DOX+ALK100+LYC2 (2mg/kg, p.o.) for 7days	1.29±1.33[c]	498.5±21.21[c]	47.35±1.52[c]	0.899±0.08[c]
DOX+ALK100+LYC4 (LYC 4mg/kg, p.o.) for 7days	1.11±0.17[c]	533.4±29.17[c]	48.13±1.72[c]	0.95±0.08[c]

All values were expressed as Mean ± S.E.M. (n=6), [a]P<0.01 as compared to the normal control group, [b]P<0.05and [c]P<0.01 as compared to the DOX control group (ANOVA followed by Dunnett's test).

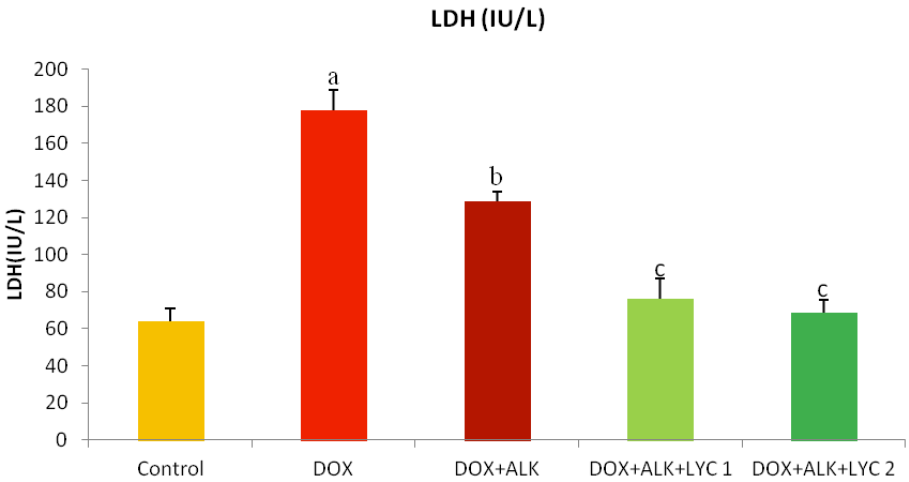
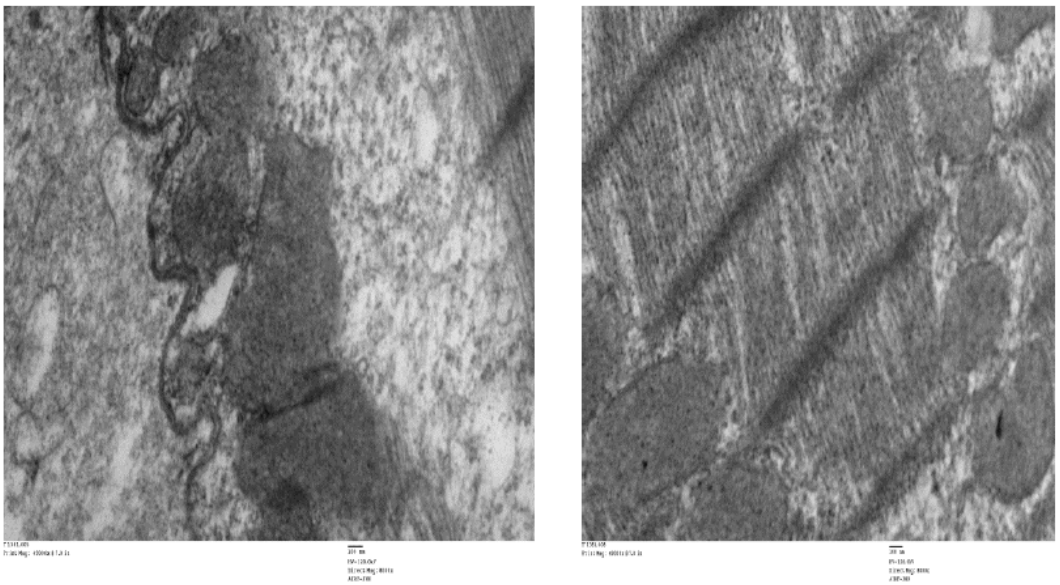
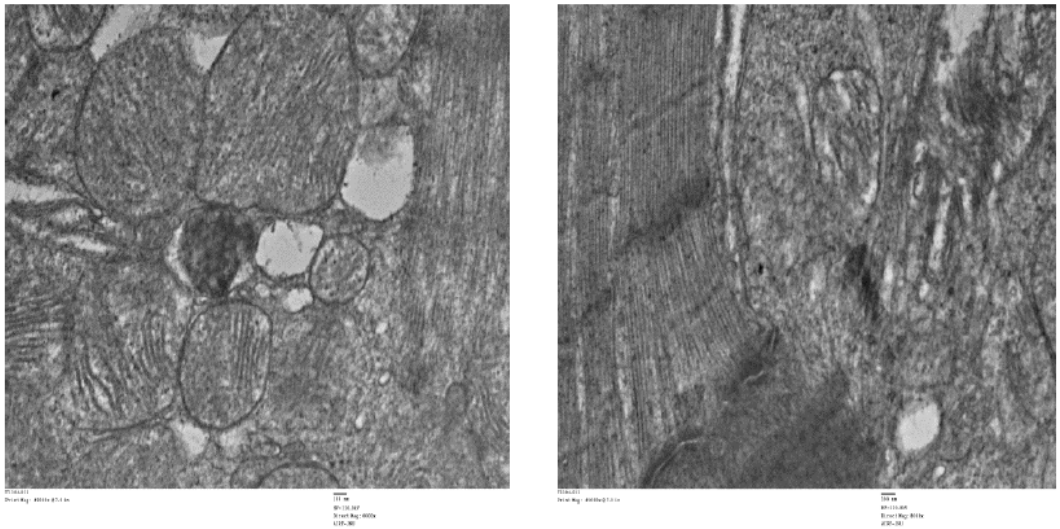


Figure 3: Effect of lycopene and aliskiren treatment on serum Lactate dehydrogenase (LDH) activity.
All values were expressed as Mean ±S.E.M. (n=6), [a]P<0.01 as compared to the CONTROL group, [b]P<0.05and [c]P<0.01 as compared to the DOX control group (ANOVA followed by Dunnett's test).



A **A1**



B **B1**

(Figure 4). Continued.

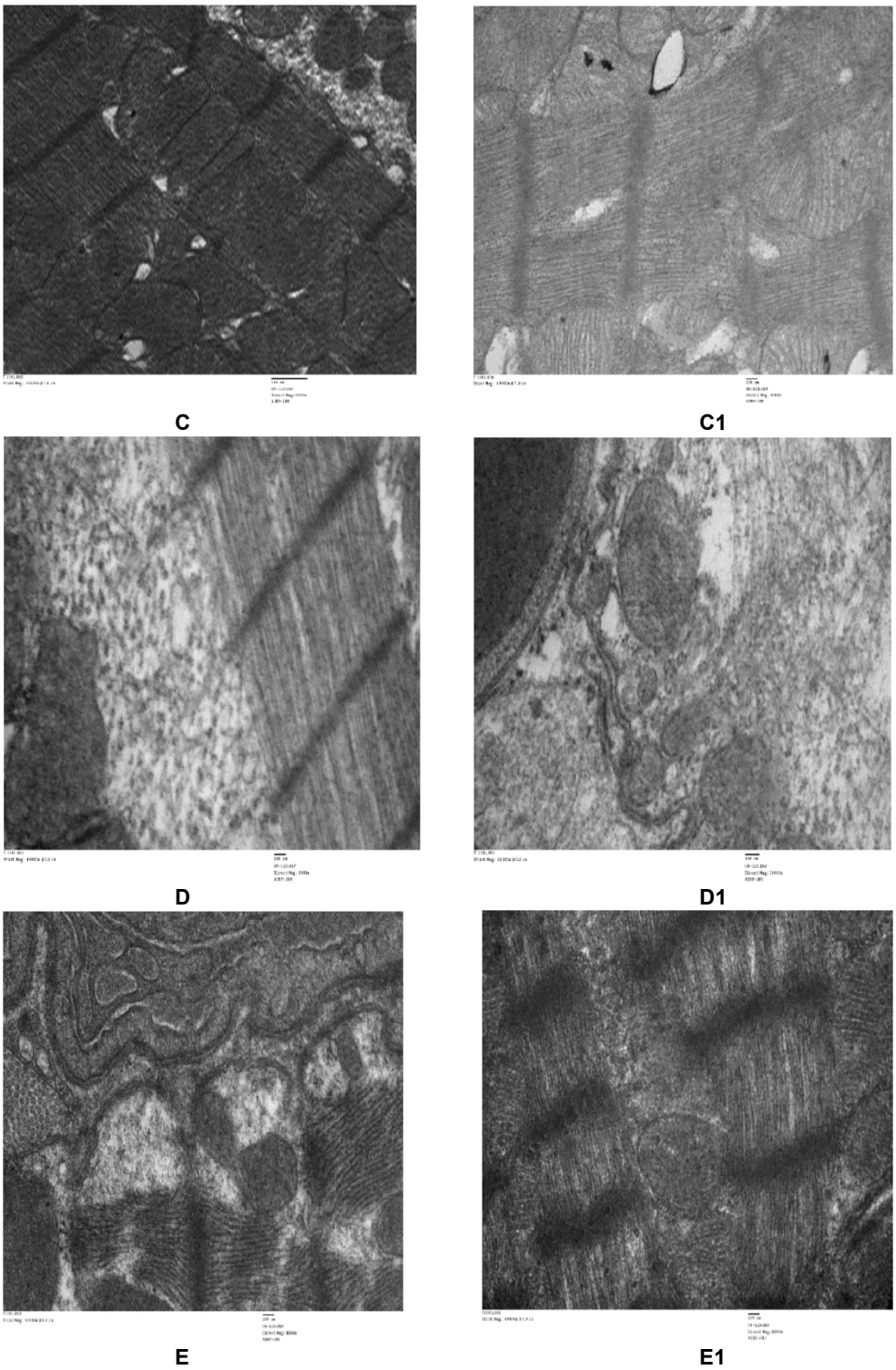


Figure 4: A, **A1**. Normal control group showed normal mitochondria, nucleus with intact myofibril.

B, **B1**. DOX treated rats showed cytoplasmic vacuolization, myofibril loss, swelling of mitochondrial with disruption of cristae, **B1** DOX alone treated showed rupture of nuclear membrane, condensation and margination of nuclear chromatin at the nuclear membrane.

C, **C1**. DOX+ALK treated showed margination of nuclear chromatin at nuclear membrane and vacuolation of cytoplasmic reticulum but structure of mitochondria and myofibrils near normal.

D, **D1**. DOX+ALK+LYC (2 mg/kg) showed homogeneous chromatin, nuclear membrane and vacuolation of cytoplasmic reticulum and **D1** normal mitochondria architecture.

E, **E1**. DOX+ALK+LYC (4 mg/kg) showed homogeneous chromatin, normal structure of nucleus, nuclear membrane and vacuolation of cytoplasmic reticulum and **E1** normal mitochondria architecture with myofibrils.

myocardium but not significant manner from DOX toxicity, as apparent by margination of chromatin at the nuclear membrane and vacuolation of the cytoplasmic reticulum (Figure **4C**, **C1**). Treatment of DOX treated rats with ALK (100 mg/kg) with lycopene (2 mg/kg) showed homogeneous chromatin, nuclear membrane and vacuolation of cytoplasmic reticulum and normal mitochondria architecture (Figure **4D**, **D1**). In contrast, treatment of DOX treated rats with ALK (100 mg/kg) along with LYC (4mg/kg) notably suppressed the membrane damage, homogeneous chromatin in the nucleus, structure of mitochondria and myofibrils were found normal (Figure **4E**, **E1**).

DISCUSSION

The pathogenesis of cardiomyopthy has not been fully known yet, however DOX-induced oxidative stress and heart failure provide a good insight in to this pathology and clearly indicate the involvement of lipid peroxidation and reactive oxygen species. In the present study, it was found that aliskiren and lycopene exerted a strong cardioprotective effect against DOX-induced cardiomyopathy in Wistar rats by attenuation of lipid peroxidation, augmentation of antioxidant enzymes and normal structure of myocytes.

DOX is one of the most important anthracycline for the treatment of cancer patients. Effective anticancer therapy with DOX is restricted due to side effects such as cardiomyopathy and congestive heart failure [25]. DOX administration caused a significant increase ($P < 0.01$) in the systolic, diastolic and mean blood pressure as well as heart rate in the DOX treated rats as compared to normal control rats. The raise in BP and heart rate in DOX treated rats could be due to increased release of renin and catecholamines that leads to formation of angiotensin I (Ang I) and finally angiotensin II (Ang II) [25]. Lycopene along with ALK prevented the increase in heart rate and blood pressure in the present study and showed encouragement in its use as antihypertensive drug.

HW and HW/BW ratio are important parameters for assessment of hypertrophic index. In the present study, there was significant ($P < 0.01$) decrease in HW and HW/ BW ratio in DOX treated rats which were markedly raised on treatment with aliskiren and lycopene combination, suggesting their cardiac connective tissue protective effect. DOX administration caused a decrease in HW and HW/BW ratio which support the results of previous studies [26]. Alteration in HW and HW/BW ratio could be due to the consecutive loss of myocardium connective tissue in damaged myocardium, deleterious action on intestinal mucus membrane, which might have reduced the food intake and a decrease in secretion of intestinal hormones [27].

Cytosolic enzyme LDH is main cardiac enzymes in the evaluation of cardiac injury and congestive heart failure. It leaks out from the damaged tissue to the blood stream when there is rupture of cell membrane [28]. The results of present study are in corroborate with the previous findings that have shown significant elevation in the level of serum LDH in DOX treated rats [29], which indicate lesion of the myocardial membrane. Combination therapy of lycopene with aliskiren significantly reduced the DOX- induced elevated level of serum LDH. Hence demonstrates that these drugs could maintain membrane integrity, thereby restricting the leakage of this enzyme.

The oxidative stress was assessed by measurement of MDA activity and level of endogenous antioxidant enzyme (GSH, SOD and CAT). In the present study, MDA concentration was significantly ($P < 0.01$) elevated and decreased activity of GSH, Catalase and SOD in cardiac tissue of the DOX treated rats, which are in agreement with earlier studies [8]. Lipid peroxidation is an signal of the severity of DOX-induced cardaic damage and has been concerned in alteration of membrane structure and enzyme inactivation [30]. Malondialdehyde is a main lipid peroxidation derivative. The raised level of TBARS may contribute to increased generation of free radical and /or decreased activities of antioxidant enzymes [29]. The results of the present study showed that combined treatment with lycopene and aliskiren significantly decreased the DOX induced elevated TBARS level probably due to restoration of antioxidant enzymes (GSH, SOD and CAT), that scavenged the free radicals.

DOX-induced myocardial apoptosis was established by ultrastructural changes in the myocyte, manifested as margination of thick nuclear chromatin near nuclear membrane, disruption in cristae of mitochondria and spreading of myofibrils as shown Figure **4B**. These ultrastructural changes are consistent with preceding studies reported by other researchers [26, 27]. Treatment with lycopene with ALK for 7 days prevented the myocardium from margination and thickening of chromatin and rupture of nuclear membrane (Figure **4E**) while ALK partly confined the myocardium from DOX-induced toxicity, as apparent by margination of chromatin at only some spaces (Figure **4C**).

CONCLUSION

It can be concluded that DOX-induced cardiomyopathy has rennin angiotensin system involvement on the basis of observations and findings. Combination therapy of lycopene with ALK with attenuated the DOX- induced raise in blood pressure, serum LDH and oxidative stress. This combination restored the DOX- induced heart weight, heart weight/body weight ratio and antioxidant enzyme levels.

The combination of ALK and lycopene has good protective effect at cellular level i.e. prevention of mitochondrial damage, harmonized chromatin, regular structure of nucleus and nuclear membrane. But still more studies are required to find out the involvement between DOX- induced cardiomyopathy, and exact mechanism of lycopene in cardiomyopathy.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

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REFERENCES

[1] Sacco G, *et al*. Cardioprotective effects of zofenopril, a new angiotensin-converting enzyme inhibitor, on doxorubicin-induced cardiotoxicity in the rat. European Journal of Pharmacology 2001; 414: 71-78. http://dx.doi.org/10.1016/S0014-2999(01)00782-8

[2] Luca G, *et al*. Anthracycline cardiomyopathy in breast cancer patients: synergism with trastuzumab and taxanes. Cardiovascular Toxicology 2007; 7: 67-71. http://dx.doi.org/10.1007/s12012-007-0013-5

[3] Hiona A, *et al*. Pre-treatment with ACE inhibitor attenuates doxorubicin-induced cardiomyopathy via preservation of mitochondrial function. J Thorac Cardiovac Surg 2011; 142: 396-403. http://dx.doi.org/10.1016/j.jtcvs.2010.07.097

[4] Siveski-Iliskovic N, *et al*. Probucol promotes endogenous antioxidants and provides protection against adriamycin-induced cardiomyopathy in rats. Circulation 1994; 89: 2829-2835. http://dx.doi.org/10.1161/01.CIR.89.6.2829

[5] Sharma H, *et al*. Anti-apoptotic potential of rosuvastatin pretreatment in murine model of cardiomyopathy. Int J Cardiol 2011; 150: 193-200. http://dx.doi.org/10.1016/j.ijcard.2010.04.008

[6] Ferreira AL, *et al*. Doxorubicin as an antioxidant: maintenance of myocardial levels of lycopene under doxorubicin treatment. Free Radic Biol Med 2007; 43: 740-51. http://dx.doi.org/10.1016/j.freeradbiomed.2007.05.002

[7] Myers CE, *et al*. Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. Science 1977; 197: 165-167. http://dx.doi.org/10.1126/science.877547

[8] Rashikh A, *et al*. Aliskiren attenuates myocardial apoptosis and oxidative stress in chronic murine model of cardiomyopathy. Biomed Pharmacother 2012; 5: 529-535. http://dx.doi.org/10.1016/j.biopha.2011.11.020

[9] Toko H, *et al*. Angiotensin II: type 1a receptor mediates Doxorubicin-induced cardiomyopathy. Hypertens Res 2002; 25: 597-603. http://dx.doi.org/10.1291/hypres.25.597

[10] Nazeyrollas P, *et al*. Effects of amifostine on perfused isolated rat heart and on acute doxorubicin-induced cardiomyopathy. Cancer Chemother Pharmacol 1999; 43: 227-32. http://dx.doi.org/10.1007/s002800050888

[11] Liu X, *et al*. Melatonin as an effective protector against doxorubicin-induced cardiomyopathy. Am J Physiol: Heart Cir Physiol 2002; 283: H254-63. http://dx.doi.org/10.1152/ajpheart.01023.2001

[12] Xu Z, *et al*. Ghrelin prevents doxorubicin-induced cardiotoxicity through TNF-alpha/NF-kB pathways and mitochondrial protective mechanisms. Toxicol 2008; 247: 133-138. http://dx.doi.org/10.1016/j.tox.2008.02.018

[13] Soga M, *et al*. Effects of angiotensin II receptor blocker in daunorubicin-induced cardiomyopathic rats. Intern J Cardiol 2006; 110: 378-385. http://dx.doi.org/10.1016/j.ijcard.2005.08.061

[14] Hollenberg NK, *et al*. Pathways for angiotensin II generation in intact human tissue: evidence from comparative pharmacological interruption of the renin system. Hypertens 1998; 32: 387-92. http://dx.doi.org/10.1161/01.HYP.32.3.387

[15] Young DS. Effects of drugs on clinical laboratory tests. Washington: AACC press, 1990; 120-22.

[16] Stahl W, Sies H. Physical quenching of singlet oxygen and cis-trans isomerization of carotenoids. Annals New York Academy of Science 1993; 691: 10-19. http://dx.doi.org/10.1111/j.1749-6632.1993.tb26153.x

[17] Di Mascio P, *et al*. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989; 274: 532-538. http://dx.doi.org/10.1016/0003-9861(89)90467-0

[18] Ojha S, *et al*. Cardioprotective effect of lycopene against isoproterenol-induced myocardial infarction in rats. Human and Experimental Toxicology 2013; 32: 492-503. http://dx.doi.org/10.1177/0960327112454890

[19] Pennathur S, *et al*. Potent antioxidative activity of lycopene: A potential role in scavenging hypochlorous acid. Free Radic Biol Med 2010; 49(2): 205-13. http://dx.doi.org/10.1016/j.freeradbiomed.2010.04.003

[20] Ferreira AL, *et al*. Tomato-oleoresin supplement prevents doxorubicin-induced cardiac myocyte oxidative DNA damage in rats. Mutat Res 2007; 631: 26-35. http://dx.doi.org/10.1016/j.freeradbiomed.2010.04.003

[21] Ohkawa H, *et al*. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 359-64. http://dx.doi.org/10.1016/0003-2697(79)90738-3

[22] Sedlack J, Lindsay RH. Estimation of total, protein bound and non-protein bound sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25: 192-205. http://dx.doi.org/10.1016/0003-2697(68)90092-4

[23] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469-74. http://dx.doi.org/10.1111/j.1432-1033.1974.tb03714.x

[24] Clairbome A. Assay of catalase. In: Greenwald RA, editor. Handbook of Methods of Oxygen Free Radical Research. Boca Raton: CRC Press, 1985; 283-84.

[25] Singal PK, Iliskovic N. Adriamycin cardiomyopathy. N Engl J Med 1998; 339: 900-905. http://dx.doi.org/10.1056/NEJM199809243391307

[26] Pathan RA, *et al*. Naproxen aggravates Doxorubicininduced cardiomyopathy in rats. Indian J Pharmacol 2010; 42: 44-49. http://dx.doi.org/10.4103/0253-7613.62411

[27] Zhu W, *et al*. MAPK superfamily plays an important role in daunomycin-induced apoptosis of cardiac myocytes. Circ 1999; 100: 2100-07. http://dx.doi.org/10.1161/01.CIR.100.20.2100

[28] Goto T, Takase H, Toriyama T. Circulating concentrations of cardiac proteins indicate the severity of congestive heart failure. Heart 2003; 89: 1303-7. http://dx.doi.org/10.1136/heart.89.11.1303

[29] Singh BK, *et al*. Diclofenac sodium, a nonselective nonsteroidal anti-inflammatory drug aggravates doxorubicin-induced cardiomyopathy in rats. J Cardiovascul Pharmacol 2010; 55; 139-14. http://dx.doi.org/10.1097/FJC.0b013e3181c87e17

[30] Yilmaz S, *et al*. Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology 2006; 218: 164-171. http://dx.doi.org/10.1016/j.tox.2005.10.015

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