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Evaluation of PPAR-Ɣ Agonist Aliskiren in Myocardial Fibrosis

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ABSTRACT

Peroxisome proliferator activated receptor gamma agonists are new class of compounds for the treatment of fibrotic diseases. In the present investigation, the effect of PPAR-Gamma agonist, Aliskiren was studied in experimental model of myocardial fibrosis. Myocardial fibrosis was induced by administration of doxorubicin (2.5 mg/kg i.p thrice a week with a cumulative dose of 15 mg/kg for 2 weeks). The most widely accepted mechanism of myocardial toxicity is through generation of reactive oxygen species (ROS), which causes mitochondrial cell death. The cardiac functional measurements and the left and right ventricular weight indices (LVWI and RVWI respectively) were analyzed. The administration of Aliskiren resulted in significant improvement in cardiac function, decrease in cardiac weight indices, reduced fibrous tissue proliferation with normalization of electrocardiographic measurement. The results obtained in this study provide evidence for the usefulness of Aliskiren as a cardioprotective agent.

INTRODUCTION

Myocardial fibrosis is defined by a significant increase in the collagen volume fraction of myocardial tissue but more commonly refer to as net accumulation of extracellular matrix in the myocardium. Myocardial fibrosis occurs when fibroblasts are activated to myofibroblasts and produce elevated amounts of ECM proteins that form scar tissue and alter normal degradation of ECM. Both processes lead to a buildup of collagen, which impacts both systolic and diastolic function. The anthracycline drug doxorubicin (DOX) is one of the most effective antineoplastic agent and widely used. However its use has been restricted due to dose dependent cardiotoxicity and which may result myocardial damage, resulting in dilated cardiomyopathy with fatal congestive heart failure. Multiple mechanisms are involved in doxorubicin induced cardiac failure. Doxorubicin-induced cardiomyopathy is strongly linked to an increase in cardiac oxidative stress, as evidenced by reactive oxygen species (ROS) induced damage such as lipid peroxidation, along with reduced levels of antioxidants and sulfhydryl groups. It is generally accepted that the oxidative stress evoked by doxorubicin activates apoptotic signaling leading to cardiomyocyte apoptosis, and that both extrinsic and intrinsic apoptotic pathways are involved. It is the need of the time that investigating and examining the agents which can result effective against myocardial fibrosis be found out. Dut to the fact that the clinical estimation of myocardial fibrosis is unusual in normal scenario, one of the approaches should be utilization of agents which are either regularly used or indicated in various disorders culminating myocardial fibrosis in later, such as use of PPAR-Gamma agonist, Aliskiren. Therefore, in this research work an attempt has been made to study the cardioprotective effect of Aliskiren in doxorubicin-induced myocardial fibrosis.

OBJECTIVES

To study the effect of Aliskiren in doxorubicin induced myocardial fibrosis in experimental wistar rats.

To induce myocardial fibrosis by administration of Doxorubicin (2.5mg/kg i.p).

To confirm the progression of cardiac fibrosis by diagnosing and analysing the electrocardiogram patterns in rats.

To evaluate the effect of Aliskiren as a single drug by targeting antioxidant pathway. (Heart weight, Heart weight to body weight ratio, Left Ventricular Weight Index and Right Ventricular Weight Index).

To evaluate histologically the effect of Aliskiren on myocardium of rats using H&E staining.

To assess the effect of Aliskiren on rat heart antioxidant enzymes system.

MATERIALS AND METHODS

Experimental Animals

The animals were procured from National Institute of Biosciences, Pune. CPCSEA Reg.No. : 1091/PO/07/abc. Rats of Wistar strain (150-200g) of either sex were used for the study. Animals were housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature: 25 C and 50+-5 % RH with free access to food and water ad libitum. Animals were acclimatized to laboratory conditions before the test. All the experiments were carried out during the light period (8:00-16:00 h).

The studies were carried out in accordance with the guidelines given by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institutional Animal Ethical Committee of MVP's College of Pharmacy, Nashik-02 approved the protocol of study (IAEC/FEB/2016/01).

Chemicals and drugs used are mentioned in Table. 1.

Table 1: Chemicals & Drugs Used

Name of Chemical	Manufacturer/ Source
Nitrobluetetrazolium Chloride (NBT)	Alfa Aesar, A Johnson Mathey Company, Chennai, India)
2-Thiobarbituric Acid (TBA)	Research Lab , Fine Chem, Mumbai, India
Ethylenediamine tetea-acetic acid(EDTA)	Research Lab , Fine Chem, Mumbai, India
Sterile Saline Solution	Fresenius Kabi Pvt. Ltd., India
Picric Acid	Modern Industies, Nashik, India
Potassium Chloride	Fine Chem Industry, Mumbai, Ondia
Doxorubicin	Avanscure Life Sciences Pvt Ltd, India
Aliskiren	Joshi Pharma, Gurgaon West, Mumbai.

METHOD

The anthracycline drug doxorubicin (DOX) is one of the most effective antineoplastic agent. It has its own side effects like cardiac dysfunction and cardiac toxicity. Doxorubicin induces myocardial fibrosis via Reactive Oxygen Species (ROS) which induces myocardial damage, DNA damage, DNA strand break and altered Gene expression which leads to fibrotic tissue formation.

Experiment protocol:-

Experiment protocol is mentioned in Table. 2

Table-2: Experimental Protocol

Groups	Treatment
I	Vehicle: Saline solution (n=6
II	Doxorubicin (2.5mg/kg/day, i.p) 3 times a week (n=6)
III	Doxorubicin (2.5mg/kg/day, i.p) + Aliskiren (30 mg/kg, p.o) (n=6)
IV	Doxorubicin (2.5 mg/kg/day, i.p) + Aliskiren (40 mg/kg, p.o) (n=6)
V	Doxorubicin (2.5 mg/kg/ day, i.p) + Aliskiren (50 mg/kg, p.o) (n=6)

Procedure:-

(1) Animals were divided in five groups as given above. Each group having 6 animals weighing 150-200g and allowed free access to standard laboratory diet.

(2) Body weight of each rat were measured before and at the end of treatment.

(3) Intra peritoneal dose of Doxorubicin 2.5mg/kg were started accordingly and continued for 2 weeks.

(4) Drug treatment in different animal groups were started in groups II to V after pretreatment period.

(5) Heart rate and ECG were recorded in rats to check the development of cardiac fibrosis.

(6) H&E staining was carried out for examination of tissue architecture.

(7) Antioxidant activity was evaluated for presence of catalase emzyme, superoxide dismutase and malonaldehyde.

RESULTS

(1) Effect on body weight:-

DOX (2.5 mg/kg) treated rats showed significant ($p < 0.0001$) decrease in body weight as compared to vehicle treated groups in 14 days of study. Aliskiren (30, 40, 50 mg/kg/day) treated rats did not show significant decline in body weight compared to DOX (2.5 mg/kg) treatment in 14 days. The results are shown in Table 3.

Table 3: Effect on body weight

Groups Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Vehicle (n=6)	161	161	161	161	162	162	162	163	163	163	163	164	164	164	164
DOX(2.5 mg/kg) 3 times a week	159	159	158	158	157	156	156	155	155	155	154	153	152	151	151
DOX+ALI (30 mg/kg)	160	160	160	160	159	159	159	159	159	158	158	158	158	158	158
DOX+ALI (40 mg/kg)	161	161	161	161	161	160	160	160	160	160	160	160	159	159	159
DOX+ALI (50 mg/kg)	160	160	160	160	160	160	159	159	159	159	159	159	159	159	159

(2) Effect on Heart Rate:-

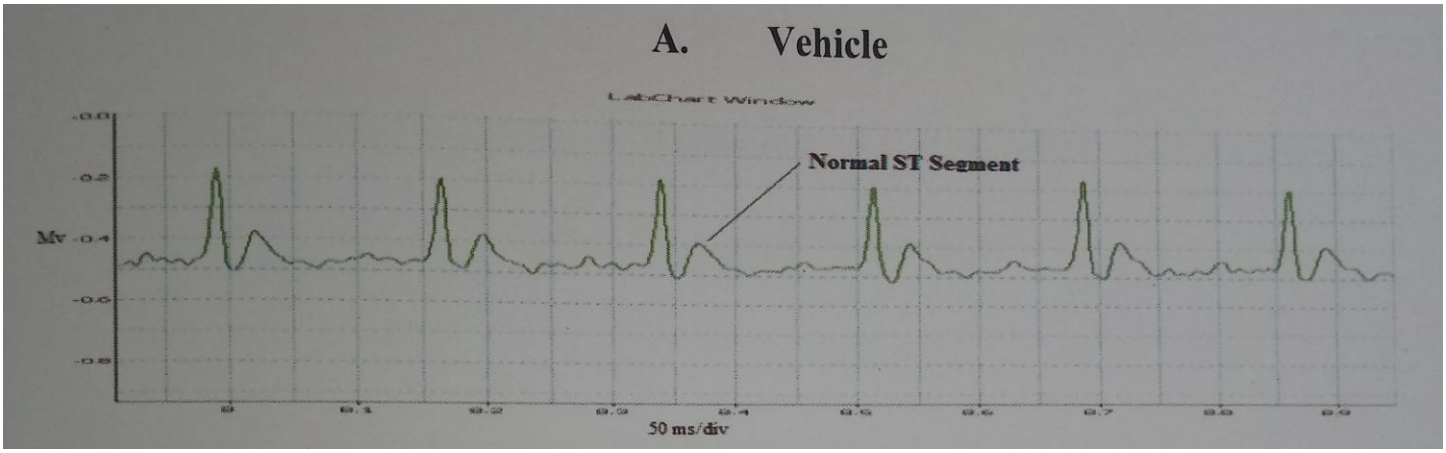
DOX (2.5 mg/kg) treated rats showed significant ($p < 0.0001$) increase in heart rate compared to vehicle treated group during treatment schedule. Aliskiren (30, 40, 50 mg/kg/day pretreated DOX (2.5 mg/kg) showed significant ($p < 0.0001$) decrease in heart rate compared to DOX (2.5 mg/kg) treated rats during treatment schedule. The results are shown in Table 4.

Table 4: Effect on heart rate

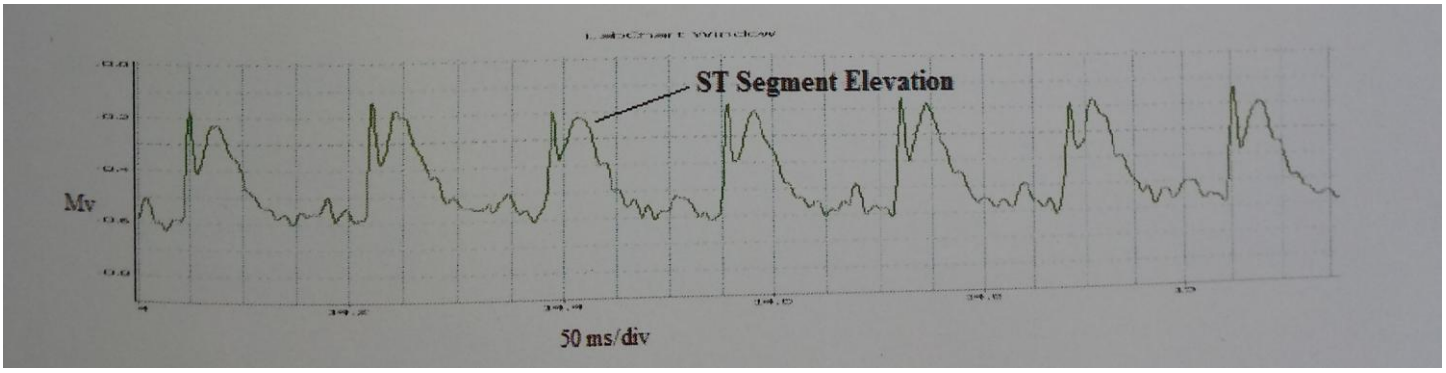
Treatment Groups	Heart Rate Measurement (Beats/min)
Vehicle (n=6)	381± 0.2582
Doxorubicin (2.5 mg/kg) 3 times a week (n=6)	423.8± 0.4773
Doxorubicin+ Aliskiren(30 mg/kg) (n=6)	388±0.6831
Doxorubicin+Aliskiren (40 mg/kg) (n=6)	387±0.2582
Doxorubicin+Aliskiren (50 mg/kg) (n=6)	389.3±0.2108

(3) Effect on electrocardiographic recording:-

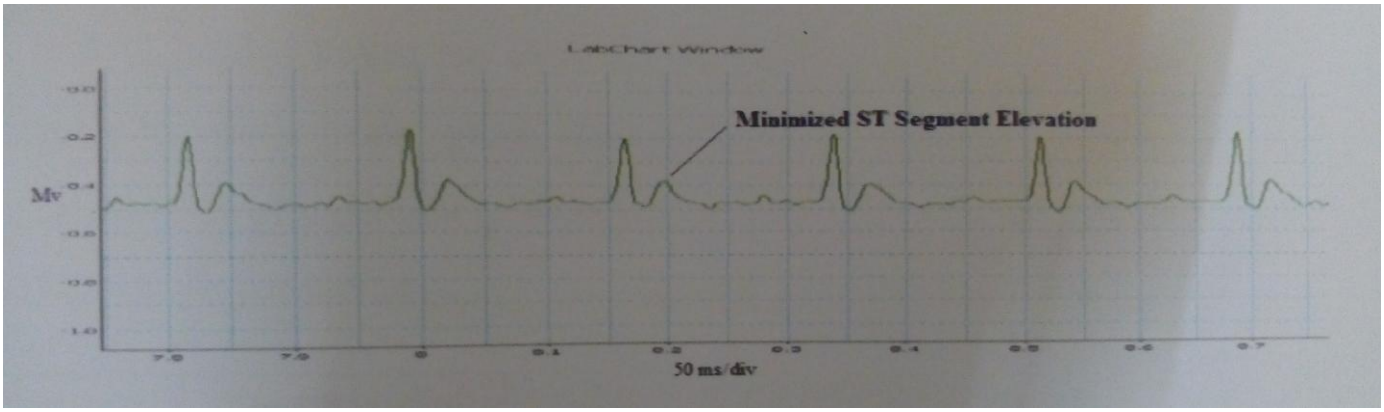
A. VEHICLE



B. DOXORUBICIN



C. DOXORUBICIN+ALISKIREN



(A) Electrocardiographic pattern of vehicle treated rat's heart showed normal ST-segments.

(B) Electrocardiographic pattern of DOX (2.5 mg/kg) induced cardiac dysfunction heart showed elevated

(C) Electrocardiographic pattern of Aliskiren (pretreated DOX 2.5 mg/kg) showed rat's heart showing minimized ST- segment elevation.

4) Effect on heart weight:-

DOX (2.5 mg/kg) treated rats showed significant ($p<0.0001$) increase in heart weight compared to vehicle treated rats. Aliskiren post-treated DOX (2.5 mg/kg) rats showed significant ($p< 0.0001$) decrease in heart weight compared to DOX (2.5 mg/kg) treated rats. The results are shown in Table 5.

Table 5: Effect on heart weight

Treatment Groups	**Heart Weight (g)**
Vehicle (n=6)	0.94± 0.0031
Doxorubicin (2.5 mg/kg) 3 times a week (n=6)	1.37± 0.0042
Doxorubicin+Aliskiren (30 mg/kg) (n=6)	0.98± 0.0031
Doxorubicin+Aliskiren (40 mg/kg) (n=6)	0.96±0.0031
Doxorubicin+Aliskiren (50 mg/kg) (n=6)	0.99± 0.0037

(5) Heart Weight to Body Weight Ratio

DOX treated rats showed significant ($p<0.0001$) increase in the HW/BW compared to vehicle treated group. Aliskiren (30, 40, 50 mg/kg) post treated DOX (2.5 mg/kg) rats showed significant ($p<0.0001$) decrease in HW/BW compared to DOX (2.5 mg/kg) treated rats. The results are shown in Table 6.

Table 6: Heart weight to body weight ratio

Treatment Groups	Heart Weight/ Body Weight (g)
Vehicle (n=6)	0.0047
Doxorubicin (2.5 mg/kg) 3 times a week (n=6)	0.0064
Doxorubicin+Aliskiren (30 mg/kg) (n=6)	0.0045
Doxorubicin+Aliskiren (40 mg/kg) (n=6)	0.0043
Doxorubicin+Aliskiren (50 mg/kg) (n=6)	0.0044

(6) Effect on Right Ventricle (RVWI) and Left Ventricle Weight Indices (LVWI)

DOX treated rats showed significant ($p<0.0001$) increase in the LVWI as well as RVWI compared to vehicle treated groups. Aliskiren (30, 40, 50 mg/kg) post treated DOX (2.5 mg/kg rats showed significant ($p<0.0001$) decrease in both LVWI and RVWI compared to DOX (2.5 mg/kg) treated rats. The results are shown in Table 7.

Table 7: Effect on Left Ventricle (LVWI) and Right Ventricle Weight Indices (RVWI)

Treatment Groups	LVWI	RVWI
Vehicle (n=6)	0.002637	0.002512
Doxorubicin (2.5 mg/kg) 3 times (n=6)	0.004955	0.003802
Doxorubicin+Aliskiren (30 mg/kg) (n=6)	0.003255	0.002937
Doxorubicin+Aliskiren (40 mg/kg) (n=6)	0.003177	0.002978
Doxorubicin+Aliskiren (50 mg/kg) (n=6)	0.003025	0.003080

(7) Effect on Biochemical Parameters in Rat Heart

Levels of MDA were significantly increased in Doxorubicin treated group, as compared to vehicle treated group. Aliskiren (30, 40, 50 mg/kg) significantly lowered levels of LPO as compared to Doxorubicin treated group. Significant ($p<0.0001$) decrease in levels of CAT

and SOD enzymes were observed after Doxorubicin administration as compared to vehicle treated group, indicating induction of cardiotoxicity in rats. Treatment with Aliskiren (30, 40, 50 mg/kg) showed significant ($p<0.0001$) rise in levels of CAT and SOD as compared to rats treated with Doxorubicin. The content of GSH was depleted significantly ($p<0.0001$) in Doxorubicin treated group as compared to vehicle treated group. Aliskiren (30, 40, 50 mg/kg) treated groups showed significant ($p<0.0001$) elevated cardiac GSH levels. The results are shown in Table 8.

Table 8: Effect on Biochemical Parameters

Treatment Groups	Catalase activity (u mole of H202 decomposed/mg Protein/min	SOD level (% inhibition of reduction of NBT)	GSH levels (u mole of GSH/mg protein)	Lipid peroxidation (n mole of MDA/mg protein)
Vehicle (n=6)	11.00±0.1461	83.13± 0.2728	9.300±0. 0967	17± 0.8563
Doxorubicin (2.5 mg/kg) 3 times a week (n=6)	7.603± 0.1476	59.07± 0.1838	7.850±0. 0224	25.17±0.3073
Doxorubicin+Aliskiren (30 mg/kg) (n=6)	8.467± 0.1229	69.48± 0.1195	8.217± 0.0477	22.67± 0.2108
Doxorubicin+Aliskiren (40 mg/kg) (n=6)	7.950± 0.1893	70.82±0.22 72	8.617± 0.0307	21±0.3651
Doxorubicin+Aliskiren (50 mg/kg) (n=6)	9.717± 0.0792	72.38±0.27 86	8.783 ± 0.0477	19.83± 0.3073

(8) Histological staining: H&E Staining Study

DOX (2.5 mg/kg) treated rats showed hard tissue compared to vehicle treated group. Aliskiren (30, 40, 50 mg/kg/day) post treated DOX (2.5 mg/kg) rats showed significant change in tissue architecture (mild hard and scar tissue). The result are shown in Fig.2

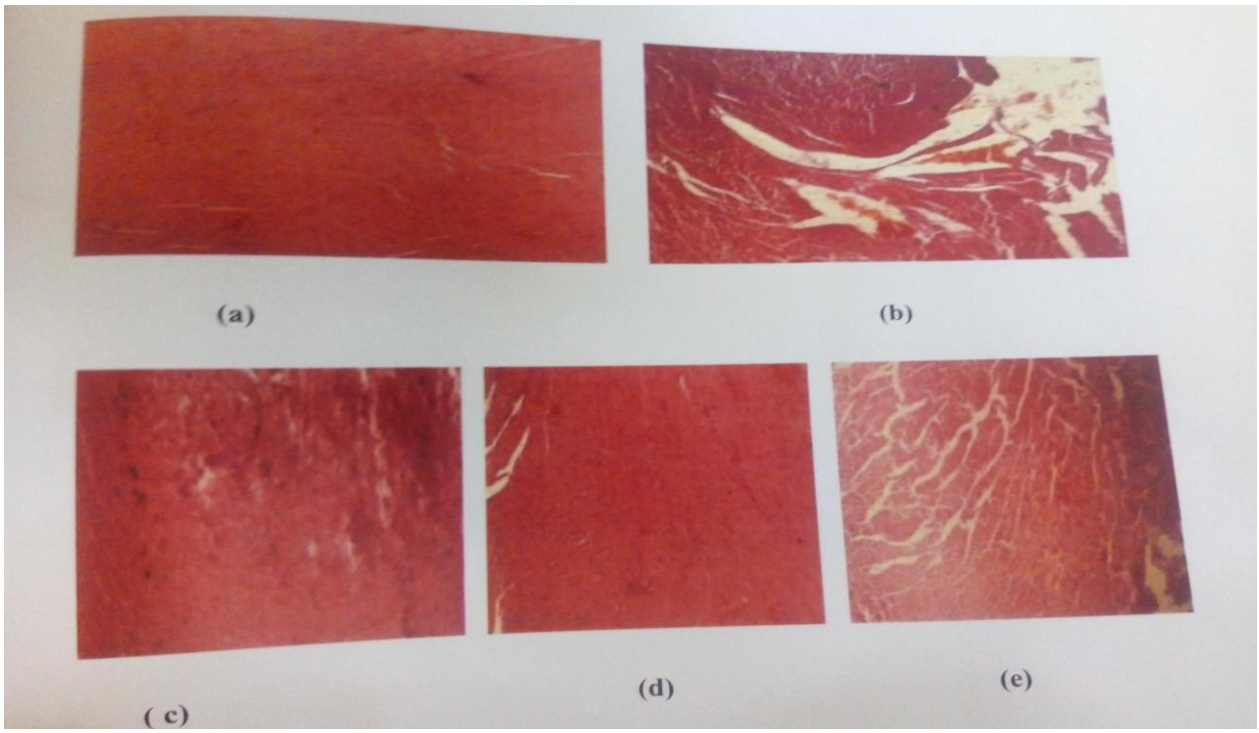


Figure 2. Histological study: H&E Staining

(a) Histological section of vehicle treated rat's heart showed normal tissue architecture.

(b) Histological section of doxorubicin (2.5 mg/kg) induced rat's heart showed abnormal tissue architecture (Hard and scar tissues)

(c) Histological section of Aliskiren (30 mg/kg) post treated doxorubicin (2.5 mg/kg) induced rat's heart showed mild hard and scar tissues.

(d) Histological section of Aliskiren (40 mg/kg) post treated doxorubicin (2.5 mg/kg) induced rat's heart showed mild hard and scar tissues.

(e) Histological section of Aliskiren (50 mg/kg) post treated doxorubicin (2.5 mg/kg) induced rat's heart showed mild hard and scar tissues

CONCLUSION

The result of present study clearly indicated the cardioprotective role of Aliskiren in treatmemt of myocardial fibrosis as a single drug regimen.In doxorubicin treated animals, body weight decreased over the period of 2 weeks of study indicating change in body weight after the administration of doxorubicin. The DOX treated rats receiving Aliskiren for 2 weeks did not show any decline in body weight over thepruod of study indicating protective effect. Moreover in Doxorubicin treated groups elevation in ST segment was observed compared to normal rats indicating myocardial fibrosis. Aliskiren successfully minimized the elevated ST-segment in Doxorubicin induced myocardial fibrosis indicating its cardioprotective properties. Hence aliskiren is drug of choice for treatment of myocardial fibrosis.

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