Amlodipine and Carvedilol Exhibits Cardioprotective Effect on Cyclophosphomide Induced Cardiotoxicity in Rats.

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Abstract

Cardiotoxicity is a well-known side effect of several cytotoxic drugs, especially of the anthracyclines and can lead to long term morbidity. The mechanism of anthracycline induced cardiotoxicity seems to involve the formation of free radicals leading to oxidative stress. This may cause apoptosis of cardiac cells or immunologic reactions. CYP has been recorded to be carditoxic. Amlodipine and carvedilol has been exploited to test its cardioprotective activity on CYP induced cardiotoxicity.

Objective: the aim of this study was assessment of cardioprotective effect of Amlodipine and carvedilol on CYP induced cardiotoxicity in albino wistar rats by measuring the enzymatic, non-enzymatic antioxidant levels, serum enzyme levels and study of ECG alteration. **Materials Methods:** Albino wistar rats were allotted in to 4 groups (6 rats/group), normal control: (i.p. injection with normal saline), CYP group (200 mg/kg ip), Amlodipine and CYP group (Aml-10 mg/kg oral & CYP- 200 mg/kg ip), carvedilol and CYP group (Carve-3mg/kg oral & CYP- 200 mg/kg ip) for 10 days of duration and ECG was measured using power lab software.

Results: cardioprotects of amlodipine and carvedilol significantly reduced the elevated levels of serum biomarkers like CK, CK-MB, LDH, calcium when compare to CYP induced cardiotoxicity. Amlodipine and carvedilol has shown significant increase in the levels of tissue biomarkers such as SOD, GSH, catalase when compare to CYP induced cardiotoxicity.

In Histopathological studies, the group treated with amlodipine + CYP and carvedilol + CYP has shown intact arrangement of cardiac muscle fibres, intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils.

Considering improvement in the serum biomarker levels and tissue biomarker levels amlodipine and carvedilol showed cardioprotective activity.

Finally it concluded that amlodipine and carvedilol has cardio protective effect on cyclophosphamide induced rats.

Introduction

Cardiovascular disease (CVD) remains the principle cause of death in both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year. CVD in India cause 3 million deaths per year, accounting for 25% of all mortality. the World Health Organization (WHO) predicts that deaths due to circulatory system disease are projected to double between 1985 and 2015.¹

Cyclophosphamide is one of the most widely used antitumor and immunosuppressant drug. Cyclophosphamide has wide spectrum of clinical uses and is an essential component of numerous combination chemotherapeutic regimens. Cyclophosphamide is activated by the cytochrome P450 oxidase system and it decomposes to phosphoramidate mustard and acrolein. Phosphoramidate mustard is linked to cyclophosphamide therapeutic effect while acrolein associated with the side effect. Acrolein interfere with the tissue antioxidant defense system and induces reactive oxygen species which causes cardiac injury, arrhythmias, congestive heart failure.² Amlodipine is a intrinsically long acting dihydropyridine calcium channel blocker. It is used for the treatment systolic hypertension and it is most commonly prescribed branded cardiovascular agent.³ Carvedilol is third- generation, nonselective β -adrenoceptor antagonist and it is a calcium channel blocker.⁴

Materials And Methods

Experimental animals:

Albino Wistar rats were purchased from Venkateshwara Enterprises Bengaluru. Animal procedure were performed in accordance with the declaration of Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy was taken prior to the animal experiments (Reference NO. - PESCP/IAEC/02/13-12-2014). All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Study of Cardio protective effect of Amlodipine and Carvedilol in cyclophosphamide induced cardio toxicity.⁵

Group 1: Normal control (vehicle) for 10 days.

Group 2: Cyclophosphamide (200mg/kg ip) at single dose on 1st day. Vehicle was administered only for 10 days except 1st day.

Group 3: Amlodipine (10 mg/kg) orally for 10 days and cyclophosphamide (200mg/kg ip) on 1st day

Group 4: Carvedilol (3mg/kg) orally for 10 days and Cyclophosphamide (200mg/kg ip) on 1st day.

After 10 days of experimental duration at 11th day rats were anaesthetized and ECG was recorded using power lab instrument. Blood samples from retro-orbital venous plexus were collected and serum was separated for the estimation of serum biomarkers Creatinine, Creatinine Kinase, Creatinine Kinase-MB, LDH. Animals were sacrificed by cervical dislocation and heart was immediately isolated and used for Histopathological studies and also for tissue biochemical estimations SOD, LPO, catalase & GSH.

Recording of ECG.⁶

At the end of experimental period animals were restrained by ketamine 30 mg/kg ip. The positive and negative electrodes were attached to the surface of left and right limbs and earth electrodes were attached to ECG was recorded the surface of right limb and surface of the posterior limb of the animal.

Biochemical analysis.⁷

After recording of ECG, blood samples were collected from retro-orbital plexus, serum was separated for the estimation of enzyme marker. The activities of CK, CK-MB, LDH, calcium were measured by using standard kits.

Histopathology studies.

Animals were sacrificed by injecting higher dose of thiopental sodium. The hearts were removed and washed immediately with saline and then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Then heart were then routinely embedded in paraffin and stained with Hematoxylin-Eosin. These sections were then examined under a light microscope for histological changes.

Statistical analysis:

Values were expressed as Mean±SEM from 6 animals. The results were subjected to statistical analysis by using one way ANOVA followed by Bartlett's test for to calculate significance. P<0.05 was considered as significant.

Results

Table NO.1- Effect of Amlodipine & Carvedilol on ECG parameters in Cyclophosphamide induced cardiotoxicity in rats.

Groups	Treatment	P wave (sec)	QRS complex (sec)	QT interval (sec)	RR interval (sec)	HR
Group-I	Control	0.024±0.0021	0.02617±0.0019	0.13±0.092	0.3042±0.020	200.8±11.1
Group-II	Cyclophosphamide	0.065±0.0072***a	0.22±0.0033***a	0.50±0.080**a	0.51±0.067**a	97.26±19.4***a
Group-III	Amlodipine+ Cyclophosphamide	0.028±0.0047***b	0.038±0.017***b	0.06±0.0045***b	0.29±0.008***b	208.8±6.32***b
Group-IV	Crvediole + Cyclophosphamide	0.021±0.0011***b	0.02±0.0009***b	0.07±0.025***b	0.30±0.013**b	200.3±9.13***b

The data were expressed as Mean ± S.E.M for six rats in each group by One Way ANOVA followed by Bartlett's test for equal variances. The ECG parameters were expressed in seconds (sec) and the heart rate as Beats per Minute (BPM). Where ***a P<0.001; compared with normal control group. **b P<0.01, *** b P<0.001; compared to toxic group. Normal rats showed normal ECG wave patterns whereas animals treated with Cyclophosphamide alone showed significant alterations in QRS complex, ST segment and RR interval.

Table NO.2- Effect of Amlodipine & Carvedilol on serum biochemical parameters in Cyclophosphamide induced cardiotoxicity in rats.

ups	Drug treatment	CK-MB (IU/L)	CK (IU/L)	LDH (IU/L)	Calcium mg/dl
up-I	Normal control	22.10 ± 6.830	22.70 ± 8.640	103.9 ± 31.09	1.288± 0.2835
up-II	Cyclophosphamide	101.5±11.36***a	290.9±27.03***a	3256± 572.5***a	6.042± 1.073***a
up-III	Amlodipine + Cyclophosphamide	36.40±11.77***b	55.71±20.84***b	89.04±63.47***b	0.9467±0.2037***b
up-IV	Carvediol + Cyclophosphamide	18.22±3.608***b	59.84±17.11***b	95.79±32.21***b	1.397±0.4021***b

The data were expressed as Mean ± S.E.M for six rats in each group. Statistical Comparisons were performed by One Way ANOVA followed by Bartlett's test for equal variances. Where ***a P<0.001; compared with normal control group **b P<0.01, *** b P<0.001; compared to toxic group.

Table NO.3- Effect of Amlodipine & Carvedilol on Tissue biochemical parameters in Cyclophosphamide induced cardiotoxicity in rats.

ups	Drug treatment	Catalase µMole of H ₂ O ₂ / min	GSH Units/mg of protein	LPO ¶moles/MDA/min/mg of protein	SOD Units/mg of protein
up-	Normal control	153.8± 3.478	173.3± 17.83	7.433 ± 1.366	29.83 ± 1.721
up-	Cyclophosphamide (200 mg/kg ip)	20.31± 4.362***a	14.73± 1.705***a	14.43 ± 0.53***a	8.77 ± 1.5** a
up-	Amlodipine(10 mg/kg oral) + Cyclophosphamide (200 mg/kg ip)	76.17± 5.770***b	129.6± 29.24**b	5.422 ± 0.507***b	44.21 ± 3.09*** b
up-	Carvediol(3 mg/kg oral)+ Cyclophosphamide (200 mg/kg ip)	69.13±4.900***b	133.3±30.27**b	9.120 ± 1.263**b	32.76± 6.04***b

The data were expressed as Mean ± S.E.M for six rats in each group. Statistical Comparisons were performed by One Way ANOVA followed by Bartlett's test for equal variances. Where ***a P<0.001; compared with normal control group **b P<0.01, *** b P<0.001; compared to toxic group.

Discussion

Cyclophosphamide induced cardio toxicity is not yet fully unravelled. However, toxicity of CYP was postulated to be mediated by oxidative stress which may have deleterious effects on the heart. Moreover, it is thought to involve direct endothelial damage, with extravasation of plasma proteins, high concentration of cyclophosphamide and erythrocytes into the myocardial interstitium and muscle cells, resulting in damage of myocardial cells.

ECG studies showed significant alterations in group-II rats (Cyclophosphamide treated) rats as compared group-I (normal rats). There was a change in ECG pattern such as decrease in the heart rate, P wave inversion, prolongation of QT interval, QRS complex and decreased in R wave amplitude. CYP causes

cardiac dysfunction through impaired mitochondrial metabolism. Bradycardia observed in CYP treated animals which may be due to release of significant amount of acetylcholine (Ach) which is also linked with the genesis of myocardial damage. CYP is known to increase the cellular Na⁺ content and decreases in K⁺ content. Hypokalemia may be associated with QT interval elongation⁸.

Rats treated with Amlodipine (10 mg/kg oral) + Cyclophosphamide (200 mg/kg ip) and Carvedilol (3 mg/kg oral)+ Cyclophosphamide (200 mg/kg ip) showed significant changes in the P wave, QRS complex, reduction in QT interval, RR interval and normal heart rate when compared to DOX group. This indicates the normal heart condition. This may support as cardio protective⁹.

In this study, a significant increase in the serum LDH, Calcium, Creatinine kinase, Creatinine kinase-MB was observed in CYP treated rats (Group II) when compared with Amlodipine (10 mg/kg)+ CYP (200 mg/kg ip) & Carvedilol (3 mg/kg) + CYP (200 mg/kg ip). Treatment with Amlodipine (10 mg/kg)+ CYP (200 mg/kg ip) & Carvedilol (3 mg/kg) + CYP (200 mg/kg ip) reduced the levels of LDH, calcium, Creatinine kinase, Creatinine kinase-MB when compared with CYP treated group.

Rats treated with CYP (200 mg/kg ip) showed increase in the level of LPO when compared to group I, III & IV respectively when compared with CYP treated rats (Group II). This is due to LPO enhances in disease state due to decreased antioxidant defence mechanism Free radicals generated during treatment with CYP causes membrane injury indicated by the lipid peroxidation resulting in the loss of function and integrity of myocardial membrane. Rats treated with Amlodipine (10 mg/kg oral) + Cyclophosphamide (200 mg/kg ip) and Carvedilol (3 mg/kg oral)+ Cyclophosphamide (200 mg/kg ip) showed a significant decreased levels of LPO when compared with Cyclophosphamide treated group(Group II).

Rats treated with Amlodipine (10 mg/kg)+ CYP (200 mg/kg ip) & Carvedilol (3 mg/kg) + CYP (200 mg/kg ip) showed significant increase in the level of catalase, GSH, SOD in group I, III & IV respectively when compared with CYP treated rats (Group II)¹⁰.

Conclusion

Significant reduction in the elevated level of Creatinine kinase, calcium, creatine kinase MB, LDH when compared to CYP induced cardiotoxicity in rats.

Amlodipine & carvedilol showed significant significant increase in the level of tissue biomarker such as SOD, GSH, catalase.

Protective Histopathological changes confirmed the cardioprotective activity of Amlodipine & Carvedilol on CYP induced cardiotoxicity in rats.

Declarations

CONFLICT OF INTERESTS

Declared none

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Figures

Figure 1
Effect of Amlodipine & Carvedilol on ECG pattern of rats.

A. P Wave

B. QRS Complex

C. T Wave

D. RR Interval

Figure 2

Effect of Amlodipine & Carvedilol on ECG waves of rats.

E. Heart Rate

Figure 3

Effect of Amlodipine & Carvedilol on ECG waves of rats.

A. CKMB

B. Creatinine Kinase

C. Serum Calcium

D. LDH

Figure 4

Effect of Amlodipine & Carvedilol on CYP induced cardiotoxicity in serum parameters in rats.

A. Catalase

B. GSH

C. SOD

D. LPO

Figure 5

Effect of Amlodipine & Carvedilol on CYP induced cardiotoxicity in tissue parameters in rats.

Figure 6

Effect of Amlodipine & Carvedilol on Histopathological changes in rat cardiotoxicity [H & E 10X].