Diabesity 2016; 2 (1): 12-18 doi: 10.15562/diabesity.2016.28
www.diabesity.ejournals.ca

ORIGINAL ARTICLE

Effect of cyclodextrin garcinol complex on isoproterenol - induced cardiotoxicity and cardiac hypertrophy in rats

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ABSTRACT

Background: *Garcinol is a polyisoprenylated benzophenone derivative present in the fruit rinds of Garcinia species namely Garcinia indica (common name 'Kokum') and Garcinia cambogia (common name 'Gombogee'). It appears to be involved in the regulation of oxidative stress and antioxidant capacity of heart tissue when the heart is subjected to oxidative stress in various pathogenic conditions/ chemical agent. But garcinol is associated with severe limitation of instability and poor bioavailability which can be improved complexing cyclodextrin with garcinol (garcinol complex).* ***Objective:*** *The objective of the present study was to investigate effect of cyclodextrin with garcinol complex (20 mg/kg), on Iso induced cardiotoxicity and cardiac hypertrophy in rats.* ***Methods:*** *Male Wistar rats (250-300g) were divided into following 4 groups of six animals each. Group 1 was control (distilled water 2 ml/kg/day orally for 18 days and water for injection by i.p. from day 9 to day 18), group 2 was cyclodextrin (cyclodextrin 2 ml/kg/day orally for 18 days and water for injection by i.p. from day 9 to day 18), group 3 Iso (distilled water 2 ml/kg/day orally for 18 days and isoproterenol 1 mg/kg by i.p. from day 9 to day 18), group 4 garcinol complex (20 mg/kg/day orally for 18 days and isoproterenol 1 mg/kg by i.p. from day 9 to day 18). After 24 hrs of last dose of isoproterenol, electrocardiogram (ECG) and heart rate were recorded in anaesthetized rats. The animals were sacrificed by overdose of ether. The hearts of animals were isolated for measurement of reduced glutathione (GSH) and lipid peroxidation (MDA).* ***Results:*** *Isoproterenol treated rats showed significant myocardial hypertrophy, decreased endogenous antioxidants when compared with the control group animals. The garcinol complex (20 mg/kg) treatment for 18 days showed significant cardioprotective activity by lowering the myocardial hypertrophy, level of lipid peroxidation (MDA content) as well as elevated the level of GSH. The results suggest pre-treatment of garcinol complex (20 mg/kg), may offer potential benefits in the management of cardiotoxicity and cardiac hypertrophy.* ***Conclusion:*** *It is thus concluded that Garcinol Complex (20 mg/kg) administration offered significant protection against isoproterenol induced cardiotoxicity and cardiac hypertrophy as well as decreased myocardial injury by preservation of endogenous antioxidants and reduction of lipid peroxidation in rat heart.*

Keywords: *Antioxidants, Cardiac hypertrophy, Garcinol Complex, Isoproterenol.*

Introduction

Cardiac hypertrophy is an adaptive response of heart muscle to a wide variety of intrinsic and extrinsic stimuli. It is defined as a physiological and pathological condition associated with changes in size, shape and function of heart. Although hypertrophy of the heart muscle is initially beneficial during early growth, but prolonged hypertrophy is potentially deleterious causing dilated cardiomyopathy and heart failure. It is a predictor of cardiovascular morbidity and mortality, independent of hypertension and coronary diseases.[1, 2]

Oxidative stress is one of the most important factors involved in pathological cardiac hypertrophy, some chemicals like isoproterenol are well known to generate free radicals and stimulate lipid peroxidation (LPO), which is a causative factor for irreversible damage to the myocardial membrane resulting in infract like necrosis and leads to deposition of lipids in myocardial muscles.[3,4,5]

The literature shows that chalcones having antioxidant, anti-inflammatory property can reverse the oxidative stress produced by various chemicals, pathogenic conditions and sedentary life style.[6,7] Garcinol is a polyisoprenylated benzophenone derivative present in the fruit rinds of Garcinia species namely Garcinia indica (common name 'Kokum') and Garcinia cambogia (common name 'Gombogee'). It is a potent antioxidant has a limitation of poor stability and bioavailability.[8] There are several methods to improve these limitations and complexation is one of them.

Literature shows that cyclodextrin is a (α-1, 4)-linked α-D-glucopyranose unit, which is able to form inclusion complexes with many drugs by taking up the drug molecule into the central cavity. No

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covalent bonds are formed or broken during complex formation. The complexed drug molecule remains in rapid equilibrium with free solvated and complexed forms in the solution. The complexation of garcinol with cyclodextrin is reported in literature. Cyclodextrin enhances the drug permeability by direct action on the biological membrane. It also increases the garcinol stability by restricting the drug solvent interaction. The overall result is increased bioavailability.[9]

The objective of present study was to investigate effect of cyclodextrin garcinol complex (garcinol complex) on the isoproterenol induced cardiotoxicity and cardiac hypertrophy in rats with a purpose to assess it's cardioprotective effect.

Materials and Methods

Animals

Male Wistar albino rats (250-300 g) were obtained from National Toxicological Centre (NTC) Pune. Rats were housed under standard housing conditions of temperature (25 ºC), relative humidity (60%) and photo period of 12 h dark/12 h light. Pellet diet (Chakan Oil Mills, Pune, India) and water were provided ad libitum. All the protocols of animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) (proposal no-CPCSEA/31/2008) in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi.

Chemicals and sample

Garcinol Complex were obtained as gift sample from Indus biotech private limited, Pune. Isoproterenol was procured from Samarth life sciences private limited, Mumbai. Epinephrine hydrochloride, and malondialdehyde were purchased from Sigma Chemical Co., USA. Reduced glutathione (GSH), 5,5´-dithiobis (2-nitro benzoic acid) (DTNB) and thiobarbituric acid (TBA) were obtained from Hi media; India. All chemicals used were of analytical grade.

Experimental Procedure

Male Wistar rats (250-300g) were divided into following 4 groups of six animals each. Group1 was control (distilled water, 2 mL/kg/day orally for 18 days and water for injection i.p. from day 9 to day 18), group 2 was cyclodextrin (cyclodextrin 2 mL/kg/day orally for 18 days and water for injection i.p. from day 9 to day 18), group 3 iso (distilled water, 2 mL/kg/day orally for 18 days and isoproterenol 1 mg/kg/day i.p. from day 9 to day 18), group 4 garcinol complex (garcinol complex 20 mg/kg/day orally for 18 days and isoproterenol 1 mg/kg/day by i.p. from day 9 to day 18). The changes in body weight were recorded daily. Garcinol complex (20 mg/kg/day) in distilled water was administered orally to group 4 rats daily for 18 days. The dose was selected on the basis of previous pillot dose response study. To group 1 (distilled water 2 mL/kg) and group 2 (cyclodextrin 2 mL/kg/day) was administered orally for 18 days. The control group received an intraperitoneal injection of sterile water (1 mL/kg for 10 days) from 9th to 18th day. While group 2 and group 4 received isoproterenol (1 mg/kg for 10 days) from 9th to 18th day. After 24 hours of the last injection of isoproterenol and vehicle, the rats were anaesthetized by anesthetic ether.

The ECG and heart rate were recorded using 8 channels Power Lab System (AD Instruments Pty Ltd, Unit 13, 18-22 Lexington Drive, Bella Vista NSW 2153, Australia). The animals were sacrificed with overdose of anesthetic ether; the hearts were isolated and weighed. Heart from four animals were randomly selected for tissue parameter measurement.

The heart of each animals were cut in to small pieces, placed in chilled 0.25 M sucrose solution and blotted on a filter paper. The tissues were homogenized in 10% chilled tris hydrochloride buffer (10 mM, pH 7.4) by tissue homogenizer (Remi Motors, Mumbai, India 400058) and centrifuged at 7500 rpm for 15 minutes at 0ºC using Eppendorf 5810-R high speed cooling centrifuge. The clear supernatant was used for the measurement of GSH and MDA content.

Tissue parameters

Lipid peroxidation assay (MDA content)

Thiobarbituric acid-reactive substances (TBARS) was measured by method of Slater and Sawyer.[10] To 2.0 mL of the tissue homogenate (supernatant) was added to 2.0 mL of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, followed by centrifugation at 2500 rpm for 10 minutes at 0 °C, two ml of clear supernatant solution was mixed with 2.0 ml of freshly prepared 0.67%w/v thiobarbituric acid. The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The absorbance of colour developed was measured against reagent blank by UV/VIS spectrophotometer (JASCO-V-530, Japan) at 532 nm using 1, 1, 3, 3-tetraethoxypropane as a standard.

Measurement of GSH

GSH was measured by method described by Moron et al.[11] 1.0 mL of tissue homogenate (supernatant) and 1 ml of 20% trichloroacetic acid (TCA) were mixed and centrifuged at 2500 rpm for 15 minutes at 0°C. In 0.25 mL of supernatant, 2 ml of 5, 5´-dithiobis (2-nitro benzoic acid) (0.6M) reagent was added. The final volume was made up to 3 mL with phosphate buffer (pH 8.0). The colour developed was read at 412 nm against reagent blank. Different concentrations (10-50µg) of standard glutathione were processed as mentioned above for constructing standard curve. The amount of reduced glutathione was expressed as µg of GSH /gm of protein.

Determination of organ weight ratio

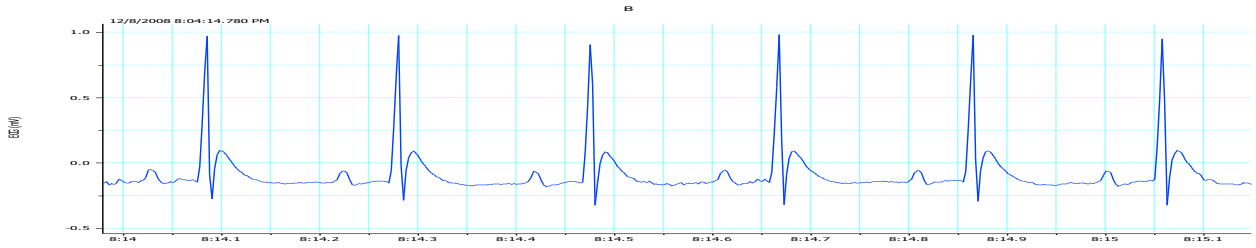
Body weight was the weight on the day of sacrifice of animal. Heart weight was recorded after keeping the heart in cold saline and squeezing out the blood. The weight of the left ventricle was recorded. The left ventricular weight/body weight ratio was calculated.

Statistical analysis

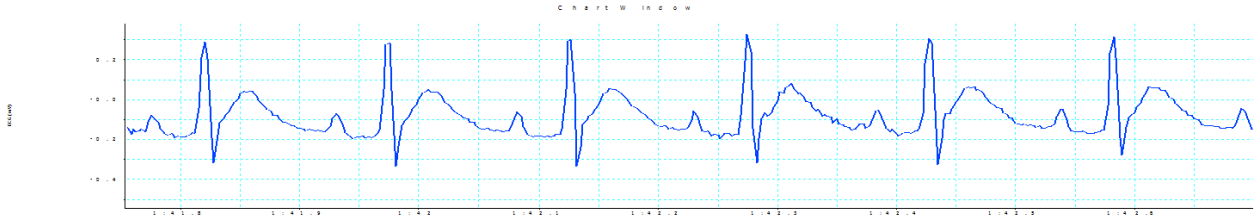
The statistical analysis was performed by software package of graph pad prism (version 4.03). All values were expressed as mean ± SEM. One way ANOVA was applied to test for significance of biochemical data of different groups and multiple comparisons were determined by the Bonferroni Post hoc test. $P < 0.05$ was considered statistically significant

Figure 1. Effect of garcinol complex (20 mg/kg) on QT and ST interval of male wistar rats in isoproterenol induced cardiotoxicity & cardiac hypertrophy.

1) ECG of rat (control gp) on 0th day



2) ECG of rat (Isoproterenol gp) on 18th day



3) ECG of rat garcinol complex (20 mg/kg) on 18th day

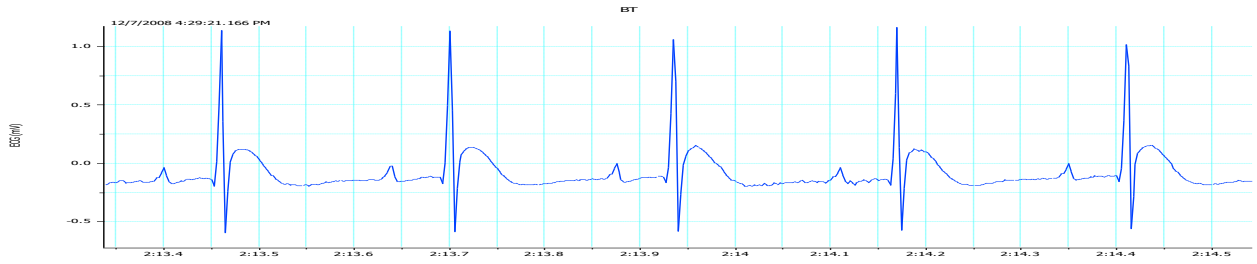
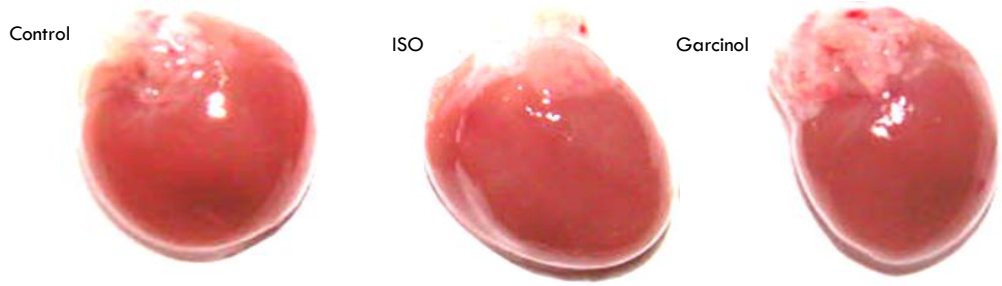


Figure 2. Hearts of rats from control, isoprotenol and garcinol complex treated groups



Results

Effect of garcinol complex on electrocardiograph of rats

At post treatment, the mean (SEM) QT interval was significantly higher in the iso group compared with the control group (16.8 ± 0.0021 vs 2.17 ± 0.0044 msec, respectively; P < 0.001); the ST interval was also significantly higher in the iso group (18 ± 0.0009 vs 1.7±0.0008 msec; P < 0.001). The QT and ST intervals were significantly lower in the garcinol complex (20 mg/kg) group compared with the iso group (5.4 ± 0.0013, P < 0.001 and 6.3 ± 0.0008, P < 0.001, respectively). (Table 1 and Figure 1).

Effect of garcinol complex on heart rate of rats

Heart rate was non-significantly higher in the iso group compared with the control group (43.66 ± 6.267 vs 16.68 ± 1.667 beats/min). Heart rate was non-significantly lower in the garcinol complex (20 mg/kg) group compared with the iso group (24.72 ± 4.819 beats/min. Table 2).

Effect of garcinol complex on myocardial MDA and GSH levels

Myocardial MDA concentration was significantly higher in the iso group compared with the control group (4.443 ± 0.0392 vs 2.690 ± 0.2154 nM MDA/mg protein, respectively; P < 0.001). The MDA concentration was significantly lower in the garcinol complex (20 mg/kg) group compared with the iso group (3.47 ± 0.1646 nM MDA/mg protein; P < 0.01). Myocardial GSH concentration was significantly lower in the ISO group compared with the control group (19.02 ± 0.8526 vs 27.50 ± 1.501µg GSH/mg protein; P < 0.01). Myocardial GSH concentration was significantly higher in the garcinol complex (20 mg/kg) group than in the iso group (23.49 ± 1.471 µg GSH/mg protein; P < 0.05), (Table 3).

Organ weight in treated with garcinol complex

Heart to body weight ratio was significantly higher in the iso group compared with the control group (3.84 ± 0.08864 vs 3.84 ± 0.08864 mg/g, respectively; P < 0.001). Heart to body weight ratio was non significantly lower in the garcinol complex (20 mg/kg) group compared with the iso group (3.58 ± 0.0966 mg/g), (Table 4 and Figure 2).

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Table 1. Effect of garcinol complex on ECG of rats

Interval	Control group (msec)	Cyclodextrin group (msec)	Iso group (msec)	Garcinol Complex+Iso (20 mg/kg) group (msec)
QT	2.17 ± 0.0044	0.88 ± 0.00085	16.8 ± 0.0021###	5.4 ± 0.0013***
ST	1.7±0.0008	4.2 ± 0.0008	18 ± 0.0009 ###	6.3 ± 0.0008***

Iso- Isoproterenol
[(n = 6), data are mean (SEM) msec.]
p<0.001 significant as compared with control group.
*** p<0.001 significant as compared with isoproterenol group.
p<0.001 significant as compared with control group.
*** p<0.001 significant as compared with Isoproterenol group.

Table 2. Effect of garcinol complex on myocardial MDA and GSH levels

Enzyme	Control group	Cyclodextrin group	Iso group	Garcinol Complex+ Iso (20 mg/kg) group
MDA, nM/mg protein	2.690 ± 0.2154	4.130 ± 0.1468	4.443 ± 0.0392 ###	3.47 ± 0.1646 **
GSH, µg/mg protein	27.50 ± 1.501	20.37± 0.8556	19.02 ± 0.8526 ##	23.49 ± 1.471 *

Iso- Isoproterenol, MDA- malondialdehyde, GSH- glutathione.
[(n = 6), data are mean (SEM)]
p<0.001 significant as compared with control group.
** p<0.01 significant as compared with isoproterenol group.
p<0.05 significant as compared with control group.
ns- non significant as compared with Isoproterenol group.

Table 3. Effect of garcinol complex on heart rate of rats

	Control group	Cyclodextrin group	Iso group	Garcinol Complex+ Iso (20 mg/kg) group
Heart rate (beats/min)	16.68 ± 1.667	9.117 ± 7.189	43.66 ± 6.267 ns	24.72 ± 4.819 ns

Iso: Isoproterenol.
[(n = 6), data are mean (SEM)]
ns: non significant as compared with control group.
ns: non significant as compared with Isoproterenol group.

Table 4. Heart to body weight ratio of rats

Ratio	Control group	Cyclodextrin group	Iso group	Garcinol Complex+Iso (20 mg/kg) group
Heart to body weight mg/g	3.19 ± 0.066	3.22 ± 0.0427	3.84 ± 0.08864 ###	3.58 ± 0.0966 ns

Iso: Isoproterenol.
[(n = 6), data are mean (SEM)]
p<0.001 significant as compared with control group.
ns: non significant as compared with isoproterenol group.

The results of present investigation clearly demonstrated that there was increase in myocardial injury as indicated by increase in QT and ST intervals of ECG pattern in isoproterenol treated group. Administration of garcinol complex along with isoproterenol (20 mg/kg) reduced QT and ST intervals close to control level.

The electrocardiographic recording from this study indicate that, isoproterenol treatment significantly prolonged QT and ST intervals as compared to control group. The treatment with garcinol complex (20mg/kg) resulted in significant reduction of the QT and ST interval prolongation as compared to isoproterenol group. Thus indicating the cardioprotective nature of the garcinol complex.

Administration of isoproterenol (1 mg/kg) caused a significant increase in MDA content (an index of lipid peroxidation) in cardiac tissues and decrease in myocardial GSH as compared to control group indicating an increase in oxidative stress. These results correlate with previous studies which have demonstrated the involvement of oxidative stress and lipid peroxidation in isoproterenol induced cardiac hypertrophy and cardiotoxicity.[12, 13, 14] Administration of garcinol complex (20 mg/kg) improved the biochemical marker levels indicating decrease in oxidative stress as evident by increased level of GSH with decreased lipid peroxidation (MDA value).

In isoproterenol (1 mg/kg for 10 days) treated rats there was significant increase in MDA content (an index of lipid peroxidation) and the significant reduction of biological antioxidant enzymes like GSH levels in cardiac tissues indicating the oxidative stress. The results showed that the treatment with garcinol complex significantly increased the GSH levels and

decreased the MDA level, thus indicating a cardioprotective effect of garcinol complex.

The plant phenolic compounds and flavonoids provide defense against oxidative stress.[15] Flavonoids act as good antioxidants because of their free radical scavenging activity and protect tissue against free radical mediated lipid peroxidation and also chelate metal ions.[16] Garcinol is a potent antioxidant present in *G. indica* which has structural similarity to curcumin as it contains both phenolic hydroxyl group as well as a β-diketone moiety. It is a free radical scavenger.[17, 18] In our study, administration of garcinol complex in a dose of 20 mg/kg for 18 days restored the altered parameters produced by isoprotenol.

A recently published study by Kumar and coworkers[19] showed cardio protective effect of ethanolic extract of G. indica in doses of 250 and 500 mg/kg.

Conclusion

It is thus concluded that garcinol complex (20 mg/kg) administration offered significant protection against isoproterenol induced cardiotoxicity and cardiac hypertrophy as well as decreased myocardial injury by preservation of endogenous antioxidants and reduction of lipid peroxidation in rat heart.

Acknowledgements

The authors acknowledge Dr. S.S. Kadam, vice chancellor, Dr. K.R. Mahadik, principal, Poona College of Pharmacy for keen interest for this work. We thank to Sunil Bhaskaran of Indus Biotech, Pune, for financial support and Dr. Manish Sinha for their help in writing of manuscript.

Conflict of interest

None Declared.

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