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Research Article

PRE-TREATMENT WITH CUCURBITACIN TRITERPENOIDS RICH BIOFRACTION OF *MOMORDI CADIOICA* ROXB. FRUIT PROTECTED THE CARDIOTOXICITY INDUCED BY ISOPROTERENOL AND STRESS IN RATS

Chinmaya Narayana K, Chandan HM, K.L. Krishna *, Seema Mehdi, Nandini HS, Abhinav Raj Ghosh, Bhooshitha A.N
Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India
*Corresponding Author Email: klkrishna@jssuni.edu.in

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ABSTRACT

Cardiovascular diseases are the leading cause of death globally and the increase in cardiovascular mortality and heart failure are common in all age groups. This may be due to accelerated atherosclerosis and compelling epidemiological and clinical data indicate that diabetes mellitus increases the risk of cardiac dysfunction and heart failure. The present study was conceived to explore the cardioprotective activity of cucurbitacin enriched fraction of *Momordica dioica* Roxb (MDR) fruit (CEFMD) against isoproterenol (ISO) and stress-induced cardiotoxicity on normal rats. CEFMD was prepared and standardized by precipitation test and TLC fingerprint. The fraction was evaluated for *in vitro* antioxidant and free radical scavenging activities like DPPH and reducing power. The *in vivo* cardioprotective activity of CEFMD was done at three doses (100, 200 and 400 mg/kg) for 15 days and activity was compared with that of carvedilol. The various parameters like non-serum parameters- ECG, heart rate, wet heart weight, serum biomarkers (CKMB, LDH, SGOT), endogenous antioxidant enzymes like SOD, CAT, GSH and MDA were determined in heart tissue homogenate. To support the activity histopathological observations were also done. The results of our study reveal the cardioprotective activity of CEFMD and significant activity was observed at low dose and activity was decreased as the dose increases. The same was observed in all parameters and further research is required to find out the exact reason for it.

Keywords: CVD, *Momordica dioica,* Cucurbitacins, Triterpenoids, Isoproterenol

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in India as well as the globe. Indians are affected at least a decade earlier and in their most productive midlife years when compared with Europeans. As per the report, 52% of Indians die due to CVD before the age of 70, whereas it is 23% in the Western population. Case fatality attributable to CVD is much higher in low-income countries like India than the high-income countries. It is now typically considered a disease of developed countries and also developing countries because of the increase in major risk factors such as obesity and diabetes. As per the estimates of the World Health Organization, with the current burden of CVD, India would lose $ 237 billion from the loss of productivity and spending on healthcare.[1]

There is a major risk factor behind the CVD is diabetes. Diabetes is a well- recognized cause of premature death and disability, increasing the risk of cardiovascular disease, kidney failure, blindness, and lower-limb amputation. People with impaired glucose tolerance and impaired fasting glycemia are also at risk of future development of diabetes and cardiovascular disease.[2]

The existence of diabetic cardiomyopathy is becoming increasingly recognized. As the mechanisms responsible for diabetic cardiomyopathy continue to be elucidated, it is hoped that these insights will provide the impetus for novel therapies that are adapted to reduce the risk of heart failure in individuals with diabetes mellitus.[3]

Herbal formulations are becoming increasing in recent days for many diseases as they are having less toxicity and adverse effects. They are also used to treat cardiovascular diseases (CVD's) since ancient times in many different ways. Ayurveda has mentioned few important herbs; they can be used as cardioprotective agents.[4] Although, there is more research being taking place throughout the world on the cardioprotective activity of the herbs. Scientists are interested to find out the major chemical constituent responsible for the cardioprotective activity of a particular herb so that they can formulate it and develop an efficacious composition of lead compound herbs.

Momordica dioica Roxb. (MDR) is a perennial, dioecious climber belongs to family Cucurbitaceae There are 80 different species are present in the *Momordica* genus among which six species are well-identified in India. Common names of MDR are spine gourd, teasel gourd or small bitter gourd worldwide and in India as kankro, kartoli, kantoli, kantola, kantroli, ban karola, or janglee karel.[5] *Momordica charantia* another species belongs to the same genus and popularly known as a bitter guard is well reported as antidiabetic as well as cardioprotective. But there is not much information available on MDR regarding its cardioprotective activity. The phytochemical responsible for the cardioprotective activity of MDR needs to be identified and characterized. Based on the above facts, the present study is conceptualized to evaluate the cardioprotective activity of

Cucurbitacins rich fraction of MDR (CEFMD) on normal and hyperglycaemic Wistar albino rats.

MATERIAL AND METHODS

Animals

Albino rats of either sex weighing 150-250 g were used and animals used in the study were procured from JSS Medical College, animal facility Centre, Mysore. Animals used in the study were procured from CPCSEA registered breeder. The animal care and handling was carried out in accordance to CPCSEA guidelines issued by the Institutional Animal Ethics Committee, JSS College of Pharmacy, Mysore, and Karnataka. Animals were acclimatized to the experimental condition for one week prior to the experiment. Animals were maintained under controlled conditions of temperature (23 ± 30ºC) and humidity (50 ± 5 %) and were caged in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of four animals in each cage. The rats were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethical Committee, JSS College of Pharmacy, Mysore, Karnataka (Approval no. 176/2015)

Plant material

The fresh fruits of MDR were collected from local, identified, authenticatedby Dr. J. Suresh, Professor, Department of Pharmacognosy, JSS College of Pharmacy, Mysuru and a specimen sample was deposited in the Dept. of Pharmacognosy, JSSCP, Mysore. The fruits of plants were cleaned to remove impurities, washed with tap water, cut into pieces and shade dried. The dried fruits were then coarsely powdered, weighed and stored in airtight containers.

Preparation of CEFMD

The dried powder of MDR fruits were extracted in 5 batches by soxhlation using methanol. Then the solvent was removed by distillation using a rotary evaporator, the obtained extract was concentrated by heating them on a water bath. The concentrated thick sticky extraction was later macerated successively with the dichloromethane and then it was concentrated using a water bath to get an enriched fraction of cucurbitacins.[6,7]

Standardization of CEFMD

A. Precipitate test

CEFMD (4 ml) + 1 g triphenyltetrazolium chloride; occurrence of red precipitate (formazin) indicates presence of cucurbitacins.[8]

B. Characterization of Triterpenoid by TLC method

Presence of Cucurbitacins in CEFMD was ascertained by TLC using Ether: hexane: methanol in the ratio of (70:30:5).[9,10]

In- vitro antioxidant activity of different extracts of CEFMD

The CEFMD was subjected to *in-vitro* antioxidant models like DPPH free radical scavenging assay,[11-13] Reducing Power[14] and IC_{50} of each extract was determined.

Acute Toxicity study

Acute toxicity was performed according to OECD guidelines 425 and the dose for cardioprotective activity was selected accordingly.

In-vivo cardioprotective activity

Isoproterenol and stress-induced cardiotoxicity on normal rats

The study protocol was followed as shown in Table 1. Blood was collected by carotrid bleeding at the end of the study.[15]

RESULTS

CEFMD biofraction of plant material

The percentage yield of the biofraction was calculated based on the amount of the dried powdered fruit taken.

Standardization of CEFMD

Precipitate test

There was a red precipitate shows the presence of cucurbitacins in CFEMD when the test sample was treated with triphenyltetrazolium chloride.

TLC

TLC enables the qualitative, semi qualitative and quantitative evaluation of phytochemical constituents of herbal drugs. The initial primary phytochemical screening of the bio-fractions revealed the presence of cucurbitacins in the CEFMD. TLC profiling results with 4 different spots, one is visible in UV light i.e. $R_f = 0.78$ and other three are visible in fluorescent light i.e. $R_f = 0.26, 0.28, 0.61$. As cucurbitacins are having the λmax at the range of 200-290 nm and the spots are visible only in UV and fluorescent light in comparison with the study carried out by Kamel *et. al*.[16]

In- vitro antioxidant activity of CEFMD

In DPPH free radical scavenging activity CEFMD has shown moderate activity with an IC_{50} of 1005.23 ± 26.75 µg/ ml. The IC_{50} of ascorbic acid was found to be 3.48 ± 0.0087 µg/ml.

Acute Toxicity Studies

Acute toxicity studies carried out under OECD guidelines 425 and not show mortality at 2000 mg/kg and accordingly the doses of 100, 200 and 400 mg/kg were fixed for cardioprotective activity.

Cardioprotective activity

Non-serum parameters

A. ECG changes

The ECG parameters revealed a significant increase ($p < 0.05$) in heart rate, while a decreased ($p < 0.05$) RR interval in the control group when compared with normal. These changes are almost similar to the indications of myocardial infarction. Pre- and co-treatment with the CEFMD resulted in decreased heart rate ($p < 0.05$) and increased RR and QT interval when compared with the control. Out of 3 doses,and only a lower dose (100 mg) has decreased the heart rate

significantly. (Table 6 and Figure 2)

B. Heart weight changes

There was a significant increase ($p < 0.05$) in the heart weight of the control group when compared to the normal group. A significant decrease ($p < 0.05$) in heart weight was seen in treatment groups.

Biochemical parameters

Blood was collected after anaesthesia by carotid bleeding method and kept aside for 15- 30 mins. undisturbed at room temperature. The clot was removed by centrifuging at 1000-2000 rpm for 10 mins., in cooling centrifuge and the supernatant serum was collected. Serum biochemical markers for a myocardial injury like CK-MB, LDH and SGOT were determined and the activities of these enzymes were increased significantly ($p < 0.05$) in the control group when compared to the normal group. The treatment groups and carvedilol groups showed a significant decrease ($p < 0.05$) in the enzyme levels when compared to the control group. Only the CEFMD 100 mg/kg group has shown the activity very much similar to the carvedilol administered the group.

Evaluation of endogenous antioxidant enzymes

Significantly decreased ($p < 0.05$) levels of superoxide dismutase, catalase, and glutathione reductase in the heart tissue homogenate were observed in the control group when compared to the normal group, whereas an increased level of lipid peroxidation. Pre and co-treatment with the different doses of CEFMD and carvedilol showed an increase in the levels of superoxide dismutase, catalase and glutathione reductase when compared to control and a decrease in lipid peroxidation enzyme level. Only the low dose i.e.CEFMD 100 mg/kg group has shown the significant activity very much similar to the carvedilol administered group.

Histopathological studies

The animals (36) were sacrificed by anaesthetizing with ketamine followed by cervical dislocation. Hearts were collected and stored in 10% formalin and sent for histopathological studies to Tissue Tech. Lab, Agrahara road, Mysore.

A. Histology of normal heart tissue treated with vehicle exhibited normal myocardial cells each with well-defined myoplasm, prominent nucleus, and nucleolus.
B. Histology of heart section treated with isoproterenol showed the damage of myocardial architecture with myocardial necrosis.
C. Histology of heart tissue treated with carvedilol clearly showed a protective effect with normal myocyte when compared to ISO treated group.
D. Histology of heart tissue treated with CEFMD (100 mg/kg) group shows good protection of the heart against ISO as there was very less myocardial necrosis.
E. Histology of heart tissue treated with CEFMD (200 mg/kg) group protected the heart against ISO but not good as Carvedilol and CEFMD (100 mg/kg) group.
F. Histology of heart tissue treated with CEFMD (400 mg/kg) group shows very less protection of the heart against ISO but not well as Carvedilol and CEFMD (100 mg/kg) group.

Table 1: Treatment Schedule to evaluate CEFMD on Isoproterenol induced cardiotoxicity in Normal Rats

Group	Treatment, dose, duration, and induction of cardiotoxicity	Evaluation
Normal	0.5% Na CMC (vehicle) 1 ml/kg body weight, for 15 days	**Non-serum Parameters-** ECG Recording (16,17) **Morphological Parameters-** Bodyweight, Wet heart weight (18) **Serum Parameters-** Serum CK-MB (Creatinine phosphokinase–MB) (18) Serum LDH (18) **Endogenous antioxidant enzymes-** Superoxide dismutase (18) Catalase (19) Glutathione reductase (20) Lipid peroxidation (21) **Histopathological studies of heart**
Control + ISO	Vehicle for 15 days and 85 mg/kg of Isoproterenol was administered subcutaneously on 14th and 15th day	
Carvedilol + ISO	2 mg/kg B.W p.o in-vehicle daily for 15 days and 85 mg/kg of Isoproterenol was administered subcutaneously on 14th and 15th day	
CEFMD (100 mg/kg) +ISO	100 mg/kg B.W p.o in vehicle daily for 15 days and 85 mg/kg of Isoproterenol was administered subcutaneously on 14th and 15th day	
CEFMD (200 mg/kg) + ISO	200 mg/kg B.W p.o in vehicle daily for 15 days and 85 mg/kg of Isoproterenol was administered subcutaneously on 14th and 15th day	
CEFMD (400 mg/kg) +ISO	400 mg/kg B.W p.o in vehicle daily for 15 days and 85 mg/kg of Isoproterenol was administered subcutaneously on 14th and 15th day	

ISO = Isoproterenol

Note: All animals were given water restrained stress on 14th and 15th day of treatment for

Table 2: The Percentage yield of MDR

Parts of plant	% Yield w/w of CEFMD
Fruit of MDR	7.4%

Table 3: TLC Profile of CEFMD

S. No	Rf value	Colour	Visualization
1.	0.78	Blue	UV light
2.	0.26	Violet	Fluorescent light
3.	0.61	Violet	Fluorescent light
4.	0.28	Violet	Fluorescent light

Table 4: Antioxidant and free radical scavenging activity of CEFME by DPPH free radical scavenging activity

S. No	Con µg/ml	% Scavenging CEFMD	Concentration µg/ml	%scavenging Ascorbic acid
1	200	8.26 ± 2.36	2	33.46 ± 0.27
2	400	14.23 ± 1.80	4	52.59 ± 0.19
3	600	30.87 ± 0.80	6	62.87 ± 0.31
4	800	39.45 ± 0.90	8	79.92 ± 0.23
5	1000	50.27 ± 0.76	10	93.77 ± 0.31
6	**IC_{50}**	**1005.23 ± 26.75**	**IC_{50}**	**3.48±0.0087**

All values are expressed in Mean ± SEM, n = 3

Table 5: Antioxidant and free radical scavenging activity of CEFME by reducing the Power Method

S. No	Con µg/ml	% Reduction CEFMD	Con µg/ml	% Reduction Ascorbic acid
1	200	53.09 ± 0.98	10	30.32 ± 1.08
2	400	57.96 ± 1.05	20	55.09 ± 0.91
3	600	61.50 ± 1.14	30	71.76 ± 0.78
4	800	71.23 ± 0.85	40	82.61 ± 0.89
5	1000	78.76 ± 1.02	50	92.80 ± 1.11
6	**IC_{50}**	**302.08 ± 0.97**	**IC_{50}**	**18.23 ± 0.96**

All values are expressed in Mean ± SEM, n = 3

Table 6: Effect of different doses of CEFMD on ECG Parameters

Groups	RR interval (mS)	QT interval (mS)	Heart Rate (BPM)
Normal	201.02 ± 7.01	109.20 ± 11.57	298.72 ± 4.44
Control	165.05 ± 5.38[a]	68.33 ± 2.39[a]	365.23 ± 11.07[a]
Carvedilol	196.31 ± 6.97[b]	101.01 ± 5.58[b]	307.32 ± 11.52[b]
CEFMD 100 mg	184.23 ± 2.39[b,c]	80.83 ± 3.25 [b]	325.95 ± 2.86 [b]
CEFMD 200 mg	174.94 ± 1.51	61.00 ± 5.13	344.04 ± 3.33
CEFMD 400 mg	167.78 ± 1.12	57.00 ± 2.62	357.80 ± 2.40

All values are expressed as Mean ± SEM, n = 6. Data were analyzed by one- way ANOVA followed by post-Tukey's multiple comparison test.
a Significant when compared to normal ($p < 0.05$) b Significant when compared to control ($p < 0.05$)
c Significant when compared to Carvedilol ($p < 0.05$)

Table 7: Effect of different doses of CEFMD on Heart weight

Groups	Wet heart weight (gm)
Normal	1.03 ± 0.04
Control	1.41 ± 0.01[a]
Carvedilol	1.06 ± 0.05[b]
CEFMD 100 mg	1.13 ± 0.02[b,c]
CEFMD 200 mg	1.25 ± 0.03
CEFMD 400 mg	1.32 ± 0.08

All values are expressed as Mean ± SEM, n = 6. Data were analyzed by one- way ANOVA followed by post-Tukey's multiple comparison test.
a Significant when compared to normal ($p < 0.05$)
b Significant when compared to control ($p < 0.05$)
c Significant when compared to Carvedilol ($p < 0.05$)

Table 8: Effect of different doses of CEFMD on Serum Parameters

Groups	CK-MB U/L	LDH U/L	SGOT U/L
Normal	12.73 ± 0.20	113.52 ± 0.68	56.50 ± 0.29
Control	28.12 ± 0.36[a]	145.21 ± 1.38[a]	90.49 ± 0.28[a]
Carvedilol	13.63 ± 0.30[b]	116.14 ± 1.15[b]	63.06 ± 0.60[b]
CEFMD 100 mg	14.43 ± 0.27[b]	120.80 ± 1.4[b,c]	68.08 ± 0.78[b]
CEFMD 200 mg	16.53 ± 0.28[b]	141.07 ± 1.17	84.45 ± 1.03
CEFMD 400 mg	18.54 ± 0.28	143.53 ± 1.41	91.87 ± 1.15

All values are expressed as Mean ± SEM, n = 6. Data were analyzed by one- way ANOVA followed by post-Tukey's multiple comparison test.
a Significant when compared to normal ($p < 0.05$)
b Significant when compared to control ($p < 0.05$)
c Significant when compared to Carvedilol ($p < 0.05$)

Table 9: Effect of different doses of CEFMD on Endogenous antioxidant enzymes in Heart tissue homogenate

GROUPS	Lipid peroxidation MDA nmol/g protein	Superoxide dismutase U/mg protein	Catalase U/mg protein	Glutathione reductase U/mg protein
Normal	0.25 ± 0.02	10.63 ± 0.06	6.62 ± 0.56	6.65 ± 0.23
Control	1.02 ± 0.04[a]	7.87 ± 0.18[a]	1.59 ± 0.22[a]	2.84 ± 0.06[a]
Carvedilol	0.31 ± 0.06[b]	10.12 ± 0.06[b]	6.46 ± 0.59[b]	6.82 ± 0.03[b]
CEFMD 100 mg	0.41 ± 0.05[bc]	9.92 ± 0.03[b]	5.55 ± 0.14[b]	5.27 ± 0.28[b]
CEFMD 200 mg	0.63 ± 0.05[b]	9.70 ± 0.04	4.56 ± 0.35	5.04 ± 0.21
CEFMD 400 mg	0.82 ± 0.04	9.12 ± 0.04	4.02 ± 0.20	4.22 ± 0.22

All values are expressed as Mean ± SEM, n = 6. Data were analyzed by one- way ANOVA followed by post-Tukey's multiple comparison test.
a Significant when compared to normal ($p < 0.05$)
b Significant when compared to control ($p < 0.05$),
c Significant when compared to Carvedilol ($p < 0.05$).

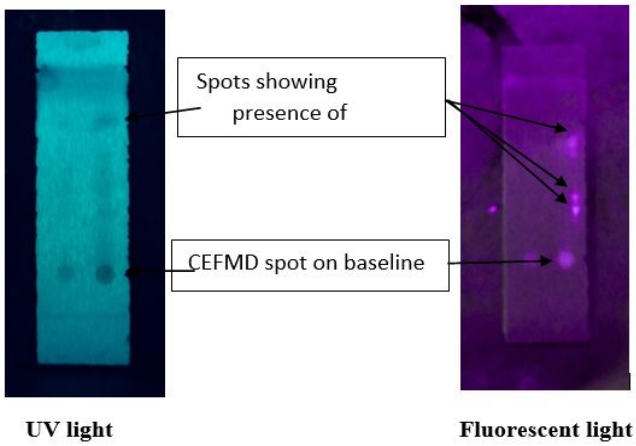
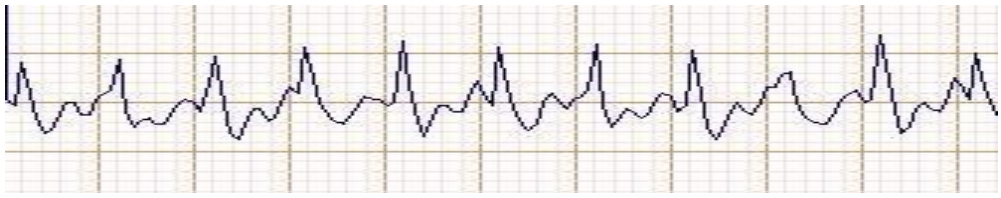
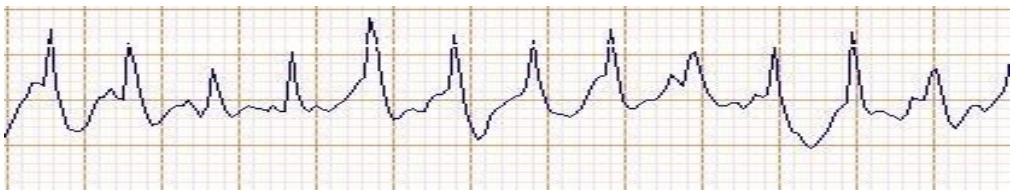


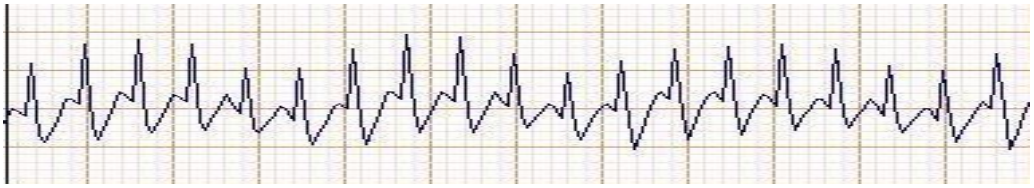
Figure 1: TLC profile of CEFMD showing the presence of cucurbitacin spots in UV and Fluorescent light



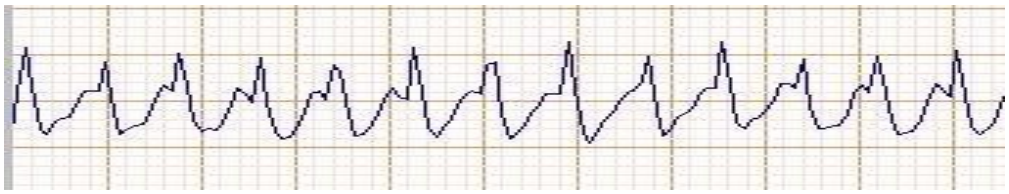
Normal



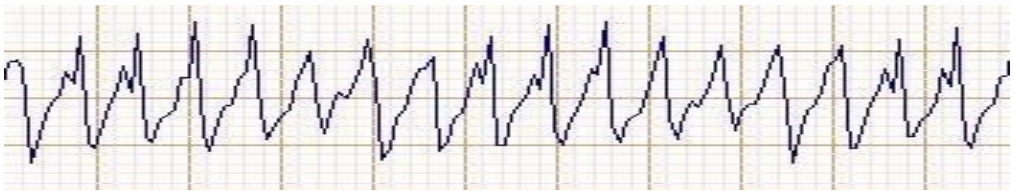
Carvedilol



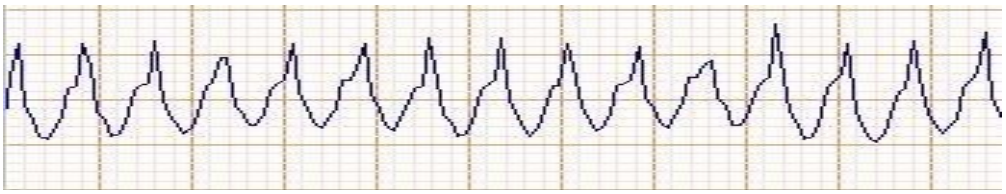
Control



CEFMD 100 mg



CEFMD 200 mg



CEFMD 400 mg

Figure 2: ECG recordings of animals treated with different doses of CEFMD (Paper speed: 50 mm/sec Scale: 0.1 sec/div, 5 mm/div, 2 sec of ECG data)

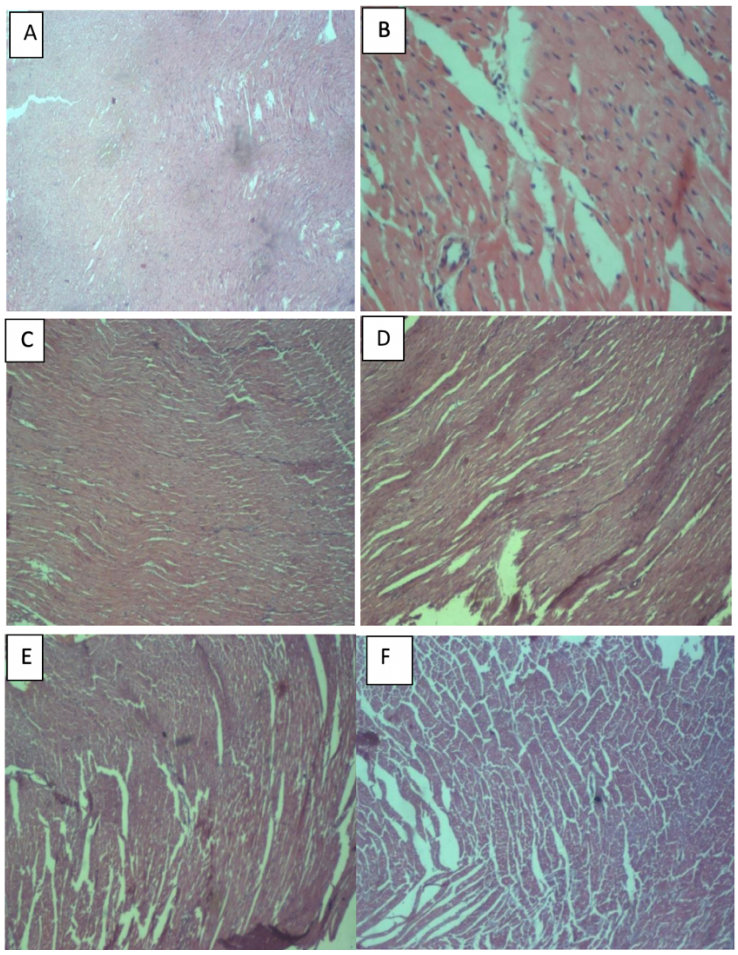


Figure 3: Effect of different doses of CEFMD on heart histology in isoproterenol-induced cardiotoxicity in normal rats

DISCUSSION

Cardiovascular diseases (CVD) have been the leading cause of morbidity and mortality in India. Recent trends indicate that the disease has escalated to younger age groups also. It has a significant presence in males and females in both urban and rural populations.[17] The prevalence of its associated risk factors has been found to exist increasingly in the population. With such a fast pace of increasing incidence, a number of epidemiological studies have been carried out in India to trace the prevalence of CVD over time. Some of them have forecasted the future incidence and prevalence of CVD in India. The studies referred to indicate an alarming rate of prevalence of CVDs in India. In fact, the prevalence in India is higher than in other countries of the same region. The escalation in the prevalence rates have been observed since the last decade and are expected to continue with the same pattern if the current situation prevails. Previously thought to affect only high-income countries, CVD burden is now being transferred to the developing countries as evident by its presence inIndia.[18]

Multifactorial risk factor modification and control, especially interventions designed to reduce total cholesterol, systolic blood pressure, smoking prevalence, overweight/obesity, diabetes mellitus, and physical inactivity, can have a profound and favorable impact on decreasing the incidence of initial and recurrent cardiovascular events.[19]

Currently, herbs are applied to the treatment of chronic and acute conditions and various ailments and problems such as cardiovascular disease, prostate problems, depression, inflammation, and to boost the immune system, to name but a few. Plants are rich in a variety of compounds. Many are secondary metabolites and include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins.[20,21] Many of these compounds have antioxidant properties.[22]

Ethnobotanical and phytotherapeutic studies show MDR has almost all the pharmacological properties along with the anti-diabetic, anti-inflammatory activity. Traditionally juices of fruits were used to treat hypertension and diabetes. As it comes under the family Cucurbitaceae, it has an abundant amount of cucurbitacins and cucurbitacin glycosides, which are present along with other constituents. Cucurbitacins are the triterpenoids, which mainly present the plants of family Cucurbitaceae. These cucurbitacins are the principle constituents for most of their pharmacological activities, especially for their anti-cancer, anti-diabetic and anti-inflammatory activities.

MDR fruits have already reported having good anti-diabetic anti-hyperlipidemic activity as that of *Momordica charantia.* Cucurbitacin glycosides are the major constituents present in *Momordica* species and they are called Momordicoside. Tan *et al.* reported that four cucurbitane glycosides, momordicosides Q, R, S, and T, and stereochemistry-established karaviloside XI, were isolated from the vegetable bitter melon acts as anti-diabetic agents by activation on AMPK pathway.[23] Rahman *et al*. reported that bitter melon reduces the serum salicylic acid in Type 2 Diabetics, so it is believed to delay the process of atherosclerosis.[24] In the present study, the CEFMD of MDR fruit was taken to evaluate their cardioprotective activity against isoproterenol-induced myocardial infarction.

The precipitate test and the TLC show that cucurbitacins are present in the biofraction CEFMD. In TLC all the spots which were elucidated are visible in UV and fluorescent light. Cucurbitacins are reported to have λmax between 224-230 nm. Often invariable presence of α, β unsaturated ketones either in the side chain or in the A ring of cucurbitane skeleton results in the UV absorbance at 230 nm for most cucurbitacins, yet for many other cucurbitacin analogs, UV absorbance does not go above 210 nm (Table 3).[25]

The *in-vitro* anti-oxidant assays like DPPH assay and reducing power assay shows that CEFMD has anti-oxidant property. CEFMD exhibited good free radical scavenging and antioxidant activity by DPPH and reducing power activity. This supports the findings of Tannin-spitz*et al.*, Bernard *et al.* report that cucurbitacin glycosides are having good antioxidant activity.[26,27]

Catecholamines are important regulators of myocardial contractility and metabolism. However, it has been known for a long time that excess catecholamines are responsible for cellular damage, observed in clinical conditions like angina, transient myocardial hypoxia, acute coronary insufficiency and sub-endocardial infarct.[28] The oxidation of hydroxyl groups in catecholamines leading to the conversion into quinones and the subsequent formation of adrenochromes most probably account for the hazardous effects of catecholamines.[29]

ISO a synthetic catecholamine and β-adrenergic agonist has been documented to produce myocardial infarction in large doses.[30] ISO acts both on β1 and β2 adrenoceptors, activation of which leads to positive inotropic and chronotropic effects. Thus, isoproterenol produces relative ischemia due to myocardial hyperactivity and coronary hypotension. Other probable mechanisms include increased cyclic adenosine monophosphate, increased intracellular Ca^{++} overload, depletion of high energy phosphate stores and oxidative stress.[31-35] On auto-oxidation, ISO generates highly cytotoxic free radicals known to stimulate peroxidation of membrane phospholipids and cause severe damage to the myocardial membrane.

Myocardial necrosis of uniform severity was produced in the rat by the subcutaneous administration of ISO (85 mg/kg of body weight) on two consecutive days.[36] On the other hand, stress also plays an important role in the induction and development of cardiovascular diseases, especially restraint (immobilization) of the rat has been widely employed to observe the effect of psychological stress.[37,38] So, in our present study, we applied the water restrained stress to elevate the ISO induced cardiotoxicity. Where water restrained stress act as environmental stress which elevates the heart rate, concentrations of hormones and catecholamines, and changes in the hypothalamic-pituitary-adrenal axis. Clinical reports now suggest that mental stress during daily life, including feelings of tension, frustration, and sadness, can more than double the risk of myocardial ischemia in the subsequent hour.[39] The administration of different doses of CEFMD i.e. 100 mg/kg, 200 mg/kg and 400 mg/kg shows cardioprotective activity against the ISO induced MI in normal rats. The ECG parameters show a good picture of the heart's function. In disease alone induced group i.e. control, there was a decrease in RR and QT interval and increase in HR. It has been demonstrated that an increase in heart rate is responsible for increased oxygen consumption leading to accelerated myocardial necrosis.[36] The CEFMD treated animals were not showing such a significant reversal of cardiotoxicity except the 100 mg/kg dose of CEFMD by reducing the HR 11% compared to control. This response shows that 100 mg/kg CEFMD has cardioprotective activity when compared to the other two doses. Even in the case of a standard group (Carvedilol 2 mg/kg) the RR interval and HR were maintained at a normal level against ISO induced cardiotoxicity by reducing the HR 16% (Table 6).

ISO administration causes fibroblastic hyperplasia which causes an increase in the heart weight. It has been proposed that a 1% increase in myocardial water content could be expected to result in possibly a 10% reduction in myocardial function.[40] The comparison of the heart weight parameters revealed that there was an increase in the heart weight of the control animals which were treated with ISO, while there was a marked decrease in the heart weights in treated groups especially CEFMD 100 mg, where the % difference of wet heart weight between the control and CEFMD 100 mg/kg was 13% (Table 7).

The elevation of biochemical parameters like CK-MB, LDH and SGOT helps to substantiate cardiotoxicity by ISO. CK-MB is an isoenzyme of creatinine kinase specifically present in the heart. Whenever there is damage to the myocardium, there would be an increase in the CK-MB level in blood. This is a specific marker to identify cardiac damage. In the control group there was an increase in the CK-MB level, the different doses CEFMD has shown a decrease in CK-MB level with CEFMD 100 mg/kg showing a significant decrease by maintaining the release of CK-MB to the serum up to 50.23% when control was showing 100% release. Even serum levels of LDH and SGOT also were increased in the control group but CEFMD 100 mg/kg decreased its level significantly against ISO induced MI (Table 8). Endogenous antioxidant enzymes such as SOD, catalase, and GSH are the first lines of cellular defense system against oxidative stress, eliminating reactive oxygen radicals such as superoxide and hydrogen peroxide (H_2O_2) and preventing the formation of more deteriorating hydroxyl radicals.[41] The evaluation of endogenous antioxidant enzymes revealed that there was a marked increase in Lipid peroxidation levels while SOD, Catalase and Glutathione reductase levels markedly decreased in the control group. This was reversed in the treatment groups especially CEFMD 100 mg/kg by reducing the lipid peroxidation 60% and increasing the availability of SOD, catalase and GSH upto 94%, 83.83% and 79.2% respectively when compared with control (Table 9).

The histopathological study of heart tissue taken from different groups substantiates all the above parameters. In ISO alone treated group (Control) heart section damage of myocardial architecture with myocardial necrosis was visible with cells are separated apart. This shows there was MI in the control group this effect was prevented by the CEFMD 100 mg/kg group (Figure 3).

CONCLUSION

The present study was carried out to evaluate the possible cardioprotective activity of CEFMD. We conclude in the present investigation that, the low dose of CEFMD i.e 100 mg/kg has shown better cardioprotective activity against ISO induced MI in normal rats when compared with the other two high doses (200 and 400 mg/kg). CEFMD has shown significant cardioprotective activity when tested on ISO and stress-induced cardiotoxicity on normal. The activity was found to be as similar to that of carvedilol (standard drug) in all parameters employed to assess the activity. Interestingly the activity was decreased as the dose increase and shown maximum effect at a dose of 100 mg/kg. Some literature says that cucurbitacin triterpenoids exhibits toxicity at high doses and safe at lower doses. This may be one of the reasons for the findings of our study. However further research is required to establish the cardioprotective activity and its mechanism of cucurbitacin in higher animal models. Our study gives a lead for further research on this plant leading to isolation and characterization of cardioprotective bioactive from MDR fruit.

REFERENCES

1. Dorairaj Prabhakaran, Panniyammakal Jeemon, Ambuj Roy. Cardiovascular Diseases in India Current Epidemiology and Future Directions. Circulation Journal 2016; 133: 1605-1620. DOI: 10.1161/CIRCULATIONAHA.114.008729
2. Levitan B, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. Archives of internal medicine 2004; 164: 2147−55.
3. Boudina S, Abel ED. NIH Public Access 2010; 11(1): 31–9.
4. Vikrant A, Kumar GV. Review on some cardioprotective plants from Ayurveda. International Journal of Research in Ayurveda and Pharmacy 2011; 2(1): 80–3.
5. Sattya Narayan Talukdar and Mohammad Nazir Hossain. Phytochemical, Phytotherapeutical and Pharmacological Study of *Momordica dioica*. Evidence-Based Complementary and Alternative Medicine; 2014. p. 1-11. http://dx.doi.org/10.1155/2014/806082.
6. Dinan L, Harmatha J, Lafont R. Chromatographic procedure for the isolation of plant steroids. Journal of Chromatography A 2001; 935: 105-23.
7. Gry J, Søborg I, Andersson HC. Cucurbitacinsin plant food. Nordic Council of Ministers; 2006.
8. Njoroge GN, Newton LE. Edible and Poisonous Species of Cucurbitaceae in the Central Highlands of Kenya. Journal of the East Africa Natural History Society and National Museum 1994; 115(2): 101–15.
9. Aeri V, Kaushik U, Mir S. Cucurbitacins - An insight into medicinal leads from nature. Pharmacognosy Reviews 2015; 9(17): 12. Available from:http://www.phcogrev.com/text.asp?2015/9/17/12/156314
10. Halaweish F., Tallamy DW. Quantitative Determination of Cucurbitacins by High Perforamance Liquid Chromotography and High Performance Thin Layer Chromotography. Journal of Liquid Chromatography and Related Technologies 1993; 16(2): 497–511.
11. Gülçin I. Antioxidant activity of food constituents: an overview. Archives of toxicology 2012; 86(3): 345-91.
12. Karadag A, Ozcelik B, Saner S. Review of Methods to Determine Antioxidant Capacities. Food Analytical Methods 2009; 2: 41–60.
13. Dimitrova DZ, Zhelev I, Dyulgerova ID. Antioxidant Activity of Some *Carduus* Species Growing in Bulgaria. Free Radical Biology and Medicine. Vol 1, Issue 4, Oct- Dec; 2011.
14. Amir M, Ahsanullah K, Mujeeb M, Ahmad M, Usmani S, Akhtar M. Phytochemical Analysis and *in vitro* Antioxidant Activity of *Zingiber officinale*. Free Radical Biology and Medicine 2011; 1(4): 75-81.
15. Georg R, Davn SK, Clifford IC. Study on the heating of cardiac necrosis in the rat. The American Journal of Pathology 1961; 39(4): 473-89.
16. Kamel AM, EL-Gengaihi S. Secondary and Primary Plant Metabolites as Chemical Markers for Resistance of Bitter Candytuft (*Iberis amara*) Plant against Insect Attack. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2008 Jul 1; 36.
17. Pachauri SD, Tota S, Khandelwal K, Verma PR, Nath C, Hanif K, Shukla R, Saxena JK, Dwivedi AK. Protective effect of fruits of *Morinda citrifolia* L. on scopolamine induced memory impairment in mice: a behavioral, biochemical and cerebral blood flow study. Journal of Ethnopharmacology 2012 Jan 6; 139(1): 34-41.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 1979; 95(2): 351–8.
19. Naseem Shah. Oral and Dental Diseases. National Commission on National Commission on Macroeconomics and Health Macroeconomics and Health; 2005. p. 1–388.

20. Chauhan S, Aeri B. Prevalence of cardiovascular disease in India and it is economic impact-A review. International Journal Of Science 2013; 3(10): 1–5.
21. Franklin BA, Cushman M. Recent advances in preventive cardiology and lifestyle medicine: A Themed series. Circulation Journal 2011; 123(20): 2274–83.
22. De Smet PAGM. Herbal Medicine in Europe-Relaxing Regulatory Standards. Massachusetts Medical Society - New England Journal 2005; 352(12): 1176-8. Available from: http://dx.doi.org/10.1056/NEJMp048083
23. Tilburt J. C, Kaptchuk T. J. J. Herbal medicine research and global health: An ethical analysis. Bull. World Health Organ 2008; 86(8): 594–9.
24. Paur I, Carlsen MH, Halvorsen BL, *et al*. Antioxidants in Herbs and Spices: Roles in Oxidative Stress and Redox Signaling. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor and Francis; 2011. Chapter 2. Available from: http://www.ncbi.nlm.nih.gov/books/NBK92763/
25. Tan MJ, Ye JM, Turner N, Hohnen-Behrens C, KeCQ, Tang CP. Antidiabetic Activities of Triterpenoids Isolated from Bitter Melon Associated with Activation of the AMPK Pathway. Chemistry and Biology 2008; 15(3): 263- 73.
26. Rahman IU. Bitter Melon (*Momordica charantia*) Reduces Serum Sialic Acid in Type 2 Diabetics: Evidence to Delay the Process of Atherosclerosis. Chinese Medicine 2011; 02(04): 125–9.
27. Lavie D, Glotter E. The Cucurbitacins, a group of tetracyclic triterpenes. Progress in the Chemistry of Organic Natural Products. Journal of the American Chemical Society 1971; 29: 307-62.
28. Tannin-Spitz T, Bergman M, Grossman S. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. Biochemical and Biophysical Research Communications 2007; 364(1): 181–6.
29. Bernard SA, Olayinka OA. Search for a novel antioxidant, anti inflammatory/analgesic or anti-proliferative drug: Cucurbitacins hold the ace. Journal of Medicinal Plants Research 2010 Dec 29; 4: 2821-6.
30. Patel V, UpaganlawarA, Zalawadia R, Balaraman R. Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitectural evaluation. European Journal of Pharmacology. Elsevier B.V. 2010; 644(1- 3): 160–8. Available from: http://dx.doi.org/10.1016/j.ejphar.2010.06.065
31. Rajadurai M, Stanely Mainzen Prince P. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistar rats. Toxicology 2007; 230(2-3): 178–88.
32. Yeager JC, Iams SG. The haemodynamicsof isoproterenol induced cardiac failure in rats. Circulatory shock 1981; 81: 151–63.
33. Bhagat B, Sullivan JM, Fischer VW, Nadel EM, Dhalla NS. cAMPactivity and isoproterenol-induced myocardial injury in rats. Recent advances in studies on cardiac structure and metabolism 1978; 12: 465–470.
34. Bloom S, Davis DL. Calcium as a mediator of isoproterenol induced myocardial necrosis. American Journal of Pathology 1972; 69: 459–470.
35. Rajadurai M, Prince PSM. Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats: biochemical and histopathological evidences. Toxicology 2006; 228: 259–268.
36. Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhalla NS. Role of free radicals in catecholamine induced cardiomyopathy. Canadian Journal of Physiology and Pharmacology 1982; 60: 1390–7.
37. Georg R, Davn SK, Clifford IC. Study on the heating of cardiac necrosis in the rat. The American Journal of Pathology 1961; 39(4): 473-89.
38. Yoshida KI. Pursuing enigmas on ischemic heart disease and sudden cardiac death. Journal of Legal Medicine 2009; 11(2): 51-8.
39. Scheuer DA. Regulation of the stress response in rats by central actions of glucocorticoids. Experimental Physiology 2010; 95(1): 26-31.
40. Gullette EC, Blumenthal JA, Babyak M, Jiang W, Waugh RA, Frid DJ, O'Connor CM, Morris JJ, Krantz DS. Effects of mental stress on myocardial ischemia during daily life. Journal of the American Medical Association 1997; 277(19): 1521-6.
41. Laine GA, Allen SJ. Left ventricular myocardial edema. Lymph flow, interstitial fibrosis, and cardiac function. Circulation Journal 1991; 68: 1713- 21.

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