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ORIGINAL ARTICLE

Cardioprotective Effect Of *Momordica Cymbalaria* Fenzl In Rats With Isoproterenol-Induced Myocardial Injury

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ABSTRACT

Momordica cymbalaria (M. *cymbalaria*) holds medicinal value and is used traditionally for treatment of various disorders. In the present study, we tested cardioprotective potential of M. *cymbalaria* against isoproterenol (ISO)-induced cardiac injury. Pretreatment with ethanolic extract of *M. cymbalaria* at 250 and 500 mg/kg prevented the elevation of serum marker enzymes, lactate dehydrogenase (LDH), creatinine kinase-MB Fraction (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and alterations in the oxidative stress markers like lipid peroxidase (LPO), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) caused by ISO (60 mg/kg s.c, 2days)- induced myocardial infarction in rats. The protective effect was confirmed by histological findings and was more prominent at 500 mg/kg. Hence, we conclude that pretreatment with ethanolic extract of *M. cymbalaria* protects against isoproterenol-induced myocardial injury.

Key words: Isoproterenol, cardioprotection, *Momordica cymbalaria,* myocardial ischemia, antioxidant.

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Introduction

Animals develop 'infarct-like' lesions when injected with isoproterenol (ISO), a potent synthetic catecholamine. These lesions are morphologically similar to those of 'coagulative myocytolysis' (COAM) or myofibrillar degeneration, one of the findings in acute myocardial infarction in humans [1]. Diabetic patients are more vulnerable to myocardial damage resulting in heart failure than nondiabetic patients [2]. It is suggested that heart failure subsequent to myocardial infarction may be associated with an antioxidant deficit, as well as increased myocardial oxidative stress [3]. Higher serum concentrations of enzymes aspartate transaminase (AST) and creatinine kinase (CK) act as markers and are associated with higher incidence of stroke after acute myocardial infarction [4]. Elevated serum uric acid lactate dehydrogenase (LDH), creatinine kinase-MB fraction (CK-MB) may also act as marker enzymes of tissue ischemia [5]. An increase in the concentration of total and LDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction [6].

Momordica cymbalaria Fenzl (Cucurbitaceae) is a species found in the Indian states of Karnataka and Andhra Pradesh. The tuber is traditionally used as an abortifacient and for the treatment of

diabetes mellitus [7]. We experimentally confirmed its antiovulatory and abortifacient activity [8]. Fruit powder and extract of *M. cymbalaria* are reported to possess antidiabetic and antihyperlipidemic activies in experimental diabetic models. [9],[10],[11]. The antidiabetic and antihyperlipidemic effects of this plant suggest a possible cardioprotective effect. Many herbal extracts [12],[13],[14],[15],[16] and formulations [17],[18],[19] are reported to have cardioprotective activity. However, our exhaustive literature review could not reveal any published data on the effect of *M. cymbalaria* on experimental-induced myocardial injury. Therefore, this study was carried out to elucidate the effect of *M. cymbalaria* on ISO- induced cardiac damage with reference to biochemical markers, antioxidant enzymes, lipid profile and histology.

Material and Methods

Experimental animals

Twenty four male Wistar rats weighing 120-150 g were purchased from NIMHANS (National Institute of Mental Health and Neuro Sciences), Bangalore (India). The animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12 h light and 12 h dark). They were fed with standard rat pellet diet (Amrut rat and mice feed, Pranav agro industries Ltd. Sangli, India) and water *ad libitum.* The animals were given a week time to get acclimatized with the laboratory conditions. All the animal procedures were performed according to the CPCSEA (Chennai, India) norms. The Institutional Animal Ethics Committee approved the experimental procedures.

Plant Material

The fresh roots of *M. cymbalaria* were collected from Gadag district, Karnataka. The powdered roots were soxhlet extracted with ethyl alcohol to get a yield of 14.1% w/w. Dried extract dissolved in distilled water was used for the study. Oral acute toxicity study was performed using the up and down procedure (OPPTS guidelines) [8].

Experimental Procedure

The rats were divided into four groups. Group I: control (distilled water p.o.), Group II: ISO (60 mg/kg, s.c.) at an interval of 24 h for two days [19]. Group III and IV: ethanolic extract of *M. cymbalaria* roots (250 and 500 mg/kg p.o., respectively) for 45 days followed by ISO (60 mg/kg, s.c.) at an interval of 24 h for two days.

Biochemical assessment

Marker Enzymes in serum

Twelve hours after the second injection of ISO, the animals were sacrificed by cervical decapitation, blood was collected and the heart was dissected out. The serum was separated immediately by cold centrifugation and used for determination of the myocardial infarction marker enzymes LDH, CK-MB, AST, ALT, and ALP along with serum uric acid, total cholesterol, triglycerides, LDL, and HDL. The enzymes, lipids and uric acid were estimated using commercial diagnostic kits (SPAN India Ltd, Surat, India).

Oxidative Stress in Heart tissue

Heart was immediately washed with ice-cold saline and a homogenate was prepared in 0.1 N Tris HCl buffer (pH 7.4). The homogenate was centrifuged and supernatant was collected, which was used for the assay of LPO, GSH, CAT, and SOD.

Estimation of LPO

The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde (MDA) as described by Wilbur et al [20]. Briefly, 1 ml of trichloroacetic acid (TCA) 20% and 2 ml of thiobarbituric acid (TBA) 0.67% were added to 2 ml of homogenate supernatant. The absorbance of the mixture was recorded at 530 nm and the values were expressed as ηM of MDA formed /mg of protein.

Estimation of GSH activity

GSH in the cardiac tissue was assayed by the method previously described by Ellman [21]. Briefly, 0.02 ml of the homogenate supernatant was added to 3 ml of Ellman reagent. The samples were mixed and kept at room temperature for at least 1 h. The changes in absorbance were read at 412 ηm. The amount of glutathione was expressed as µg of GSH/mg protein.

Estimation of SOD activity

The level of SOD was measured by the method of Kono [22]. Briefly, 1.3 ml of solution A (0.1 nM EDTA containing 50 nM Na_2CO_3, pH 10.3), 0.5 ml of solution B (90 M) nitro blue tetrazolium dye (NBT) and 0.1 ml of solution C (20mM Hydroxylamine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for 1 min at 560 ηm. SOD activity was expressed as unit/mg protein change in optical density per min.

Estimation of Catalase activity

Catalase activity was estimated by determining the decomposition of H_2O_2 at 240 ηm in an assay mixture containing phosphate buffer [23]. The activity was expressed in units as µM of H_2O_2 consumed/min/mg of protein.

Histopathology studies

A portion of the heart was fixed in formalin (10%) and subjected to histopathology studies. The section of the heart was processed and embedded in paraffin wax. Sections of about 4-6 µm were made and stained with hematoxylin and eosin and photographed.

Statistical Analysis: The data was analyzed using one-way ANOVA followed by Tukey Kramer multiple-comparison test and P values of 0.05 or less were considered significant.

Results

Mortality in the acute toxicity test was not seen in the limit test at the dose of 5000 mg/kg. Therefore, $1/10^{th}$ and $1/20^{th}$ of the dose were selected for the study.

ISO treatment caused significant elevation in the serum levels of marker enzymes AST, ALT, LDH, ALP, CPK and uric acid as compared to control group (Table/Fig 1). *M. cymbalaria* pretreatment at both doses significantly reduced the levels of the marker enzymes and uric acid. The effect was more prominent at the higher dose.

Table/Fig 1.
Effect of *M. cymbalaria*, on isoproterenol -induced alterations in serum levels of marker enzymes in rats.

Group	Treatment	AST U/L	ALT U/L	LDH U/L	ALP U/L	CPK U/L	Uric acid mg / dl
I	Normal control	75.91 ± 1.82	27.68 ± 0.86	48.03 ± 1.48	113.32 ± 2.22	260.07 ± 2.45	6.11 ± 0.72
II	ISO	171.76 ± 2.81†††	57.87 ± 0.45†††	58.15 ± 2.84††	197.21 ± 3.03†††	324.57 ± 2.08†††	10.51 ± 0.28†††
III	ISO + MC (250mg/kg)	123.64 ± 2.01***	38.86 ± 1.23***	51.84 ± 2.19*	150.54 ± 2.79***	281.4 ± 3.13***	7.75 ± 0.84*
IV	ISO + MC (500mg/kg)	78.5 ±1.66 ***	30.22 ± 1.49***	49.34 ± 1.08*	116.37 ± 3.66***	274.5 ± 2.14***	6.26 ± 0.45***

M. cymbalaria (MC) administered once daily p.o. for 45 days.
Values expressed as mean ±SEM, n=6
†††P<0.001, ††P<0.01, †P<0.05 vs. Group I.
***P<0.001,**P<0.01,*P<05 v Group II.

Table/Fig 2
Effect of *M. cymbalaria*, on isoproterenol -induced alterations in serum lipid parameters in rats

Group	Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Normal control	120.39 ±2.18	139.46 ±2.28	42.92 ±1.14	80.19 ±1.12	40.82 ±1.86
II	ISO	205.8 ±3.56^{+++}	177.20 ±2.51^{+++}	36.22 ±1.63^{+}	109.10 ±2.34^{+++}	58.16 ±1.72^{+++}
III	ISO + MC (250mg/kg)	141.87 ±2.67***	152.94 ±1.93***	38.36 ±1.18*	86.66 ±1.02***	50.32 ±1.23**
IV	ISO + MC (500mg/kg)	122.38 ±1.04***	141.16 ±1.39***	41.28 ±1.83*	80.80 ±1.82***	41.63 ±1.12***

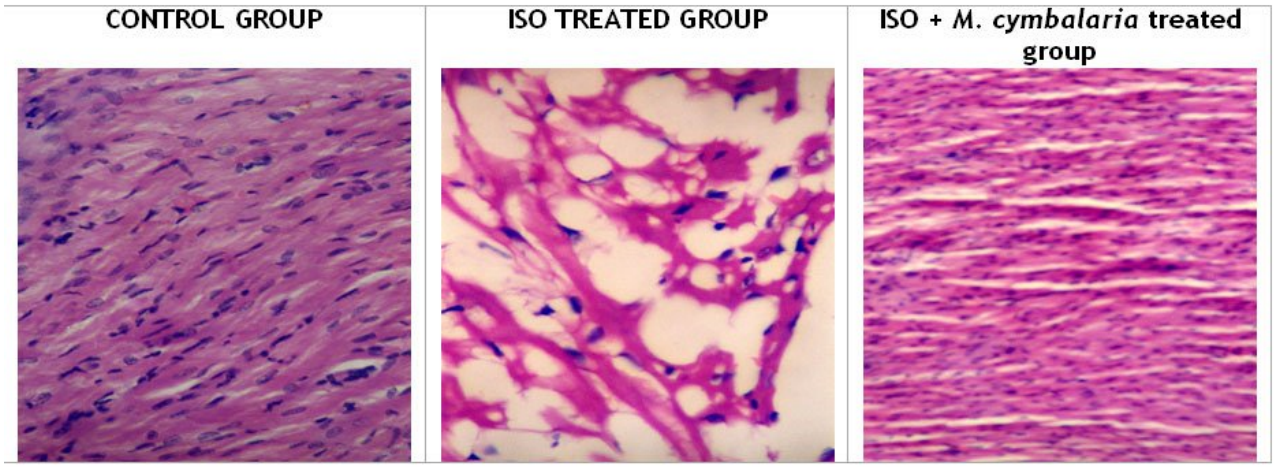
M. cymbalaria (MC) administered once daily p.o. for 45 days.
Values expressed as mean ±SEM, n=6
$^{+++}$P<0.001, $^{++}$P<0.01, $^{+}$P<0.05 vs. Group I.
***P<0.001, **P<0.01, *P<0.05 vs. Group II.

Table/Fig 3
Effect of *M. cymbalaria*, on isoproterenol -induced cardiac oxidative stress in rats.

Group	Treatment	LPO ηM of MDA / mg of protein	GSH µg/mg of protein	CAT µm H_2O_2/mg of protein	SOD U/mg of protein
I	Normal control	0.84±0.21	6.47±1.06	7.4±0.87	6.42±1.03
II	ISO	1.69±0.16^{+}	2.66±1.27^{+}	4.63±1.08^{+}	3.74±0.98^{+}
III	ISO + MC (250mg/kg)	1.40±0.07*	3.27±1.31*	5.8±0.95^{+}	4.48±0.74^{+}
IV	ISO + MC (500mg/kg)	0.89±0.23*	6.49±1.36*	7.52±1.13*	6.5±1.14*

M. cymbalaria (MC) administered once daily p.o. for 45 days.
Values expressed as mean ±SEM, n=6
$^{+}$P<0.05 vs. Group I.
*P<0.05 vs. Group II.

Table/Fig 4
Representative photomicrographs of cardiac sections demonstrating the cardioprotective effect of *M. cymbalaria*.



The serum lipid parameters- total cholesterol, triglyceride, HDL, LDL, & VLDL showed a significant elevation upon ISO treatment. *M. cymbalaria* pretreatment prevented the elevation of these parameters when compared to ISO treated rats. The effect was more prominent at 500 mg/kg (Table/Fig 2).

The levels of GSH, CAT, and SOD were significantly increased while malondialdehyde (MDA) which is a measure of LPO was significantly reduced following ISO treatment. Prophylactic treatment with *M. cymbalaria* at 500 mg/kg significantly ($P<0.001$) changed these oxidative marker levels when compared to ISO treated rats (Table/Fig 3).

The cardiac sections of the ISO–treated animals revealed degenerative changes in the muscle fiber, showing a coagulative necrosis characterized by more homogenous eosinophilic cytoplasm. The nuclei of myofibril revealed pyknotic nucleus. Interstitial edema was present in the connective tissue spaces. Pretreatment with *M. cymbalaria* (500 mg/kg) exerted a protective effect as evident from the normal myofibrillar structures with striations (Table/Fig 4).

Discussion

M. cymbalaria fruit powder and extract were previously reported to have antidiabetic activity. [9], [10], [11]. Diabetes is associated with a marked increase in the risk of coronary heart disease. It is recommended that patients with diabetes who do not have myocardial infarction should be treated as aggressively for cardiovascular risk factors as patients who had myocardial infarctions [24]. Hence antidiabetic medications with additional cardiovascular protective activity will have greater therapeutic advantage.

Reactive oxygen species (ROS) are formed at an accelerated rate in ISO-treated myocardium. Cardiac myocytes, endothelial cells, and infiltrating neutrophils contribute to this ROS production and can lead to cellular dysfunction and necrosis [25]. 'Infarct-like' lesions are produced in the myocardium when injected with ISO. These lesions are morphologically similar to those of 'coagulative myocytolysis' (COAM) or myofibrillar degeneration [1] Milei et al [26] suggested that myocardial necrosis induced by ISO is probably due to a primary action on the sarcolemmal membrane, followed by stimulation of adenylate cyclase, activation of Ca^{2+} and Na^{+} channels, exaggerated calcium inflow and excess of excitation-contraction coupling mechanism leading to energy consumption and cellular death. Free radicals generated by ISO [27], initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity. The metabolic damage of myocardium results in increase in the concentration of the marker enzymes like LDH, CK-MB, AST, ALP and uric acid. This was also evident in our findings. The GSH, CAT, and SOD were decreased while LPO

increased in the myocardial homogenate of ISO-administered rats indicating oxidative stress.
M. cymbalaria (500 mg/kg) prevented the alterations in marker enzymes of myocardial infarction, and oxidative stress along with uric acid. Myofilamental alterations such as myocytosis and myofibrillar degeneration are reported in ISO-treated rats [19]. Cardiac sections of the ISO-treated animals showed infiltration of inflammatory cells and continuity in the muscle fiber was lacking suggesting an irreversible cell injury. Rats pretreated with *M. cymbalaria* showed normal myofibrillar structures with striations and revealed a marked protection by the extract against myocardial necrotic damage.

Administration of ISO raised LDL cholesterol and decreased HDL cholesterol level in the serum. An increase in concentration of total cholesterol and LDL cholesterol, and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction [6]. High level of circulating cholesterol and its accumulation in heart tissue is accompanied with cardiovascular damage. *M. cymbalaria* elevated HDL level and decreased LDL cholesterol level. There is a growing body of evidence from epidemiologic, clinical, and laboratory data indicating that elevated triglyceride levels are an independent risk factor for cardiovascular disease. Hypertriglyceridemic patients at a risk for cardiovascular disease often develop a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol which causes myocardial membrane damage [28]. Hypertriglyceridemia observed in ISO- treated rats is clinically reported in ischemic heart disease. Pretreatment with *M. cymbalaria* prevented the elevation of triglycerides cholesterol and LDL in serum, signifying that the myocardial membrane is intact and not damaged. Antihyperlipidemic, antioxidant and antidiabetic activity along with cardioprotective properties of *Momordica cymbalaria* adds to the accumulating evidence for therapeutic potential of this plant.

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