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Research Article
Celastrol Improves Isoproterenol-Induced Heart Failure by Reducing Inflammation, Apoptosis and Oxidative Stress

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Abstract

Background and Objective: Celastrol is a pentacyclic triterpenoid with a long history of therapeutic potential. Unfortunately, their prime mechanism for myocardial infarction was unknown. Therefore, the current study has investigated celastrol's efficiency and its cardioprotective role in isoproterenol-induced heart injury. **Materials and Methods:** The animals have been separated into 4 groups (n = 6). The test group was pretreated with celastrol on the 7th day at the same time as isoproterenol-induced heart damage on the 6th-7th days. The biochemical parameters were determined in serum and heart tissue homogenates. **Results:** Celastrol significantly decreased the myocardial infarction markers concentration in serum and increased the antioxidant concentration in isoproterenol-induced heart tissue. In addition to this, celastrol also regulates the membrane-bound as well as lysosome enzymes located in the heart tissue. Furthermore, the elevation of pro-inflammatory cytokines and NF-kB mRNA was lessened by celastrol administration. Celastrol also reduced isoproterenol-induced programmed cell death, by altering the Bcl-2, Bax and caspase-3 levels in cardiac tissue. **Conclusion:** Current findings suggested that administering celastrol may help to reduce cardiac damage in myocardial infarction by reducing inflammation, apoptosis and oxidative stress.

Key words: Isoproterenol, celastrol, heart failure, anti-oxidant, inflammatory markers, apoptosis, pathophysiology, myocardial cells

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heart failure is a major cardiovascular disease triggered either by structural or functional cardiac irregularities which point toward high intra-cardiac pressure or decreased functional cardiac output during rest or stress. The occurrence of heart failure is dependent on age whereby 2% of the adult population and 10% of older people ranging from 60-75 years are affected globally[1]. The pathophysiology of heart failure remains ambiguous and moderately studied. The progression of heart failure mainly leads to cardiac injury and myocardial dysfunction. It develops together with one or more pathological conditions like chronic kidney disease, deficiency of iron, increased blood pressure and diabetes which initiates a systemic pro-inflammatory state. According to new studies, inflammation may influence the progression of heart failure[2]. C-reactive protein, Tumour Necrosis Factor-α (TNF-α), interleukins (IL-1 and IL-6) and monocyte chemoattractant protein-1 are all overexpressed in heart failure patients. These mediators promote apoptosis in myocardial cells and lead to heart failure[3]. Tissue hypoxia can be induced by a decrease in cardiac function or a decrease in sympathetic vasoconstriction and it subsequently promotes free radical production and inflammation. As a result, inflammation and oxidative stress are closely linked to heart failure[4]. Continuous and excessive release of cytokines leads to immune system-mediated damage to the myocardium and stimulates cardiac remodelling all of which lead to heart failure. Fundamentally, the pro-inflammatory cytokines and reactive oxygen species (ROS) stimulate the stress response[5].

The biological procedure of apoptosis sometimes referred to as "programmed cell death", is started and ends via internal and external signalling pathways. Intracellular stress, particularly oxidative stress and activates the intrinsic pathway, whereas extracellular stress, specifically the TNF-signal, activates the signalling pathway including death receptors. These pathways trigger caspase 3 cleavage and result in apoptotic cell death[6]. There are two major types of genes, such as B-Cell Lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) that regulate apoptosis incidence and progression[7,8].

Celastrol is a pentacyclic triterpenoid categorized under triterpene quinine methides. It is an isolate from *Tripterygium wilfordii* and is traditionally used as a medicine[9]. Celastrol has been widely documented as an anti-obesity, anti-diabetic, neuroprotective, anti-cancer and anti-inflammatory agent[10]. Several studies have documented that celastrol can potentially quell inflammation via attenuation of the production of several mediators of inflammation and enzymes such as nitric oxide synthase (NOS) and cyclooxygenases (COX-1 and COX-2) as well as caspase-1[11-13]. Additionally, it was revealed that celastrol inhibits ischemic/thermal conditioning via the activation of heat shock factor 1 and promotes cardio-protective heme oxygenase-1 expression[14]. Celastrol has been demonstrated to diminish transverse aortic constriction stress and overload-induced myocardial hypertrophy and heart injury in mice by suppressing miR-21 levels and decreasing MAPK/ERK signaling[15]. Regarding the above observations, celastrol appears to diminish the inflammatory mediators and oxidative stress in various pathological conditions. Unfortunately, till now, the connection between inflammation, oxidative stress and apoptosis in myocardial tissue has not been well studied.

So, this study will assess the effectiveness and efficacy of the drug and its involvement in protecting against isoproterenol-induced myocardial damage by suppressing caspase-3 and inflammatory and oxidative stress mediators.

MATERIALS AND METHODS

Study area: The present study was carried out in the Jinling Hospital, Medical School, Nanjing University in January to April, 2022.

Chemicals: Celastrol and Isoproterenol were purchased from PayPay Technologies, Inc., in Shenzhen, China. Additional chemicals were purchased from Merck, USA.

Animal husbandry: Totally 24 male Wistar rats (165-185 g) were procured from the SPF Animal Center at Dalian Medical University. During the testing period, test animals were isolated, in wide, clean cages with a constant temperature of 23±1°C and exposed to a 12 hrs dark-light sequence. The animals were allowed 7 days to adapt to laboratory circumstances. Animal experiments were followed by the guidelines of guide for the care and use of laboratory animals (NRC 2011). Our institutional ethics review board approved this study (Reg. No. 31201/2022/CPC/FTULC/12.01.2022).

Experimental design: The animals have been split into 4 groups (n = 6) after becoming acclimated to the laboratory setting, as shown below:

- **Group I (control):** Over 7 days, the animals were given 3 mL kg^{-1} of normal saline oral
- **Group II (ISO):** The animals were administered an oral dosage of 3 mL kg^{-1} of normal saline for 7 days. They also received via i.p., of isoproterenol (ISO), 85 mg kg^{-1} on the 6th and 7th day
- **Group III (CEL only):** Celastrol (5 mg kg^{-1}) was given i.p., for 7 days
- **Group IV (CEL+ISO):** Celastrol (5 mg kg^{-1}) was given i.p., for 7 days. These animals also received an i.p., of isoproterenol, (85 mg kg^{-1}) on the 6th and 7th day

Food access was denied overnight following the final doses of celastrol and isoproterenol. The animals were decapitated after being profoundly sedated with phenobarbital sodium (35 mg kg^{-1}, i.p.). The blood was drawn from the jugular vein and kept in heparinized tubes. The blood was centrifuged to get the serum, which was then utilised to evaluate the heart failure marker enzymes.

Each animal's body weight was examined in each group. The phenobarbital sodium (35 mg kg^{-1}, i.p.) was used to anaesthetize the animals before their decapitation. Animals' hearts were removed, cleansed and cleaned in cold saline water before being dried. The heart tissues were weighed and 100 mg was homogenised in a 10% w/v pre-chilled Tris-HCl buffer. The supernatant was used to analyse several biochemical indicators.

Assessment of the body-to-heart weight ratio: Each animal's body weight was noted before decapitation and the animals' hearts' weight after decapitation was analyzed and the ratio was calculated.

Determination of the infarct size: The size of the infarct was measured using the 2, 3 and 5-triphenyl tetrazolium chloride (TTC) stain. After quick transcardial perfusion, the animals were injected with 5 mL of 1% Evans blue dye into their femoral veins, where it was allowed to circulate throughout the animals' bodies. The heart was collected and put in a -80°C fridge for 20 min. The frozen hearts were then individually sliced (~2 mm). The 2 mm slices were immersed in 1% TTC for 30 min at 37°C before being fixed in paraformaldehyde (4%). The infarction area (off-white) and normal area (deep red) on each slice were measured by Image-Pro Plus 6.0 software (Media Cybernetics, USA). The following formula was used to measure the size of the infarct in percent[16]:

$$\frac{\text{Infarct area}}{\text{Normal+Infarct area}} \times 100$$

Hemodynamic parameters: The animals were sedated by using phenobarbital sodium (35 mg kg^{-1}, i.p.) after the dosing procedure. Electrocardiography was done by inserting an electrode needle beneath the skin of the animal's limbs to detect the heartbeat and systolic and diastolic blood pressures.

ELISA of circulating markers of cardiac injury: Lactate dehydrogenase and creatine kinase-MB levels were measured in collected serum using commercially available kits and performed as per the manufacturer's guidelines. The cardiac Troponin T expression level was evaluated with an ELISA kit from Roche Diagnostics, Germany according to their instructions.

Assessment of lysosomal enzymes: Lysosomal fractions were assessed as described by Wattiaux *et al.*[17]. Following lysosomal enzymes, such as β-D-N-acetyl glucosaminidase, acid phosphatase, β-D-galactosidase, β-D-glucuronidase and cathepsin-D were measured followed by the modified process of Kawai and Anno[18], Levvy and Conchie[19], Moore and Morris[20], Gopalakrishnan *et al.*[21] and Sapolsky *et al.*[22].

Determination of the membrane-bound phosphatases: The heart tissue supernatant was utilised to estimate the Na^+-K^+ ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase using the techniques of Bonting by Sathishsekar and Rajasekaran[23], Hjerten and Pan[24] and Ohnishi *et al.*[25].

Assessment of cardiac antioxidant levels: The levels of glutathione (GSH) were assessed as described by Moron *et al.*[26] and catalase (CAT) levels were determined by Takahara *et al.*[27], while glutathione peroxidase and glutathione S-transferase (GST) were analysed using the modified process of Habig *et al.*[28] and superoxide dismutase (SOD) levels were estimated using a modified process developed by Marklund and Marklund[29].

Assessment of oxidative stress measures: The malonaldehyde levels were determined using the methodology developed by Ohkawa *et al.*[30] and the NO concentrations were determined using the methods developed by Green[31].

Determination of the serum TNF-α and IL-6: The TNF-α and IL-6 levels in serum were determined by ELISA kit as directed by the manufacturer instructions.

Western blot technique: The levels of Bcl-2, Bax and caspase-3 in cardiac tissue extract were examined using a Western blot, as described by Kumas *et al.*[32]. In this study, the primary antibodies such as Rabbit anti-Bcl-2 (1:400, Santa Cruz, USA), Rabbit anti-Bax (1:400, Santa Cruz, USA), Rabbit anti-caspase-3 (1:400) and rabbit anti-β-actin (control) (1:400, Santa Cruz, USA) were used. As a secondary antibody, goat anti-rabbit that had been treated with horseradish peroxidase (Santa Cruz, USA) was used and maintained at room temperature for 2 hrs. The protein bands were analyzed using an ECL kit and the FR-200 system was used.

RT-PCR assay of the NF-κB gene: In this study, the Takara RNA Isolation Kit's instructions were followed to separate and purify total RNA using RNAase. To create cDNA, 1 g of RNA underwent reverse transcription. Following primers were used. Forward-5′ CCTATCCACGACAACCTTGC 3′, Reverse-5′ CATA GATGCTGCTGACCCAAC 3′; β-actin (493 bp): Forward-5′ -GT GGGGCGCCCAGGCACCA-3′ and Reverse-5′-GCTCGGCCGTGG TGGTGAAGC-3′. Flour Chen v. 2 was used to collect the integrated density values of the western blot bands.

Statistical analysis: The result was evaluated using ANOVA in the Statistic Tools for the Social Sciences v22. The outcomes of the study were shown as a Mean±Standard Error Mean (SEM) and $p<0.05$ was statistically variation.

RESULTS

Analysis of celastrol on the heart-to-body weight ratio in isoproterenol-induced heart failure in test animals: In comparison to the control group, the isoproterenol-treated animals exhibited a substantial improvement (a*-$p<0.05$) in the heart-to-bodyweight ratio (Fig. 1). Celastrol and isoproterenol-administrated animals exhibited a substantially decrease (b*-$p<0.05$) in heart-to-bodyweight ratio than the isoproterenol-administered animals.

Analysis of celastrol on infarct size in isoproterenol-induced animals: The isoproterenol-induced heart failure animals expressed considerably higher (a*-$p<0.05$) infarct sizes than Group I animals as shown in Fig. 2. Same time, isoproterenol and celastrol-administered group animals exhibited substantially decreased infarct sizes (b*-$p<0.05$) than the isoproterenol-only administrated animals.

Analysis of celastrol on hemodynamic measures in animals with heart failure caused by isoproterenol: The hemodynamic measures, such as heartbeat rate, systolic, diastolic and mean arterial blood pressure, were analyzed (Fig. 3a-b). In this study, isoproterenol-induced heart failure animals expressed considerably lower systolic and diastolic blood pressure (Fig. 3a) and higher heartbeat rates (Fig. 3b) than control animals. However, the celastrol treatment effectively restored these hemodynamic measures. Compared to the isoproterenol-induced heart failure animals treated with celastrol expressed moderate heart rate and systolic and diastolic blood pressure (b*-$p<0.05$) with control animals.

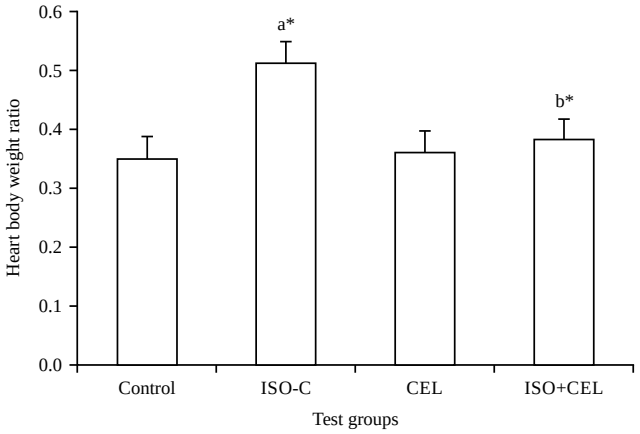

Fig. 1: Analysis of celastrol on the heart-to-body weight ratio

Data from the tested groups are shown as Mean±Standard deviation (a* and b*-$p<0.05$) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol, CEL: Celastrol and X-axis: Tested groups

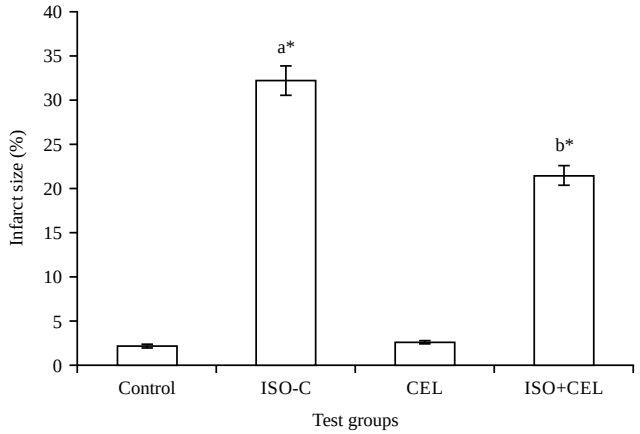

Fig. 2: Analysis of infarct size in isoproterenol-induced heart failure in animals

Data from the tested groups are shown as Mean±Standard deviation (a* and b*-$p<0.05$) where a* denotes comparison between ISO-C vs Control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol, CEL: Celastrol and X-axis: Tested groups

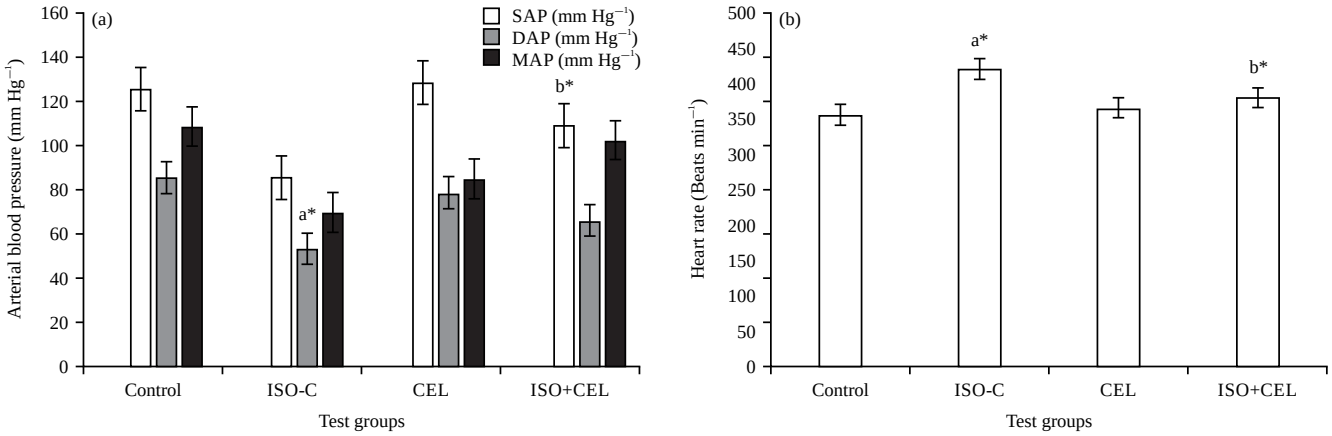

Fig. 3(a-b): Analysis of celastrol on hemodynamic parameters, (a) Effect of celastrol on arterial blood pressure and (b) Effect of celastrol on heart rate

Data from the tested groups are shown as Mean±Standard deviation (a* and b*-p<0.05) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol, CEL: Celastrol, SAP: Systolic arterial blood pressure, DAP: Diastolic arterial blood pressure, MAP: Mean arterial blood pressure and X-axis: Tested groups

Analysis of celastrol on myocardial infarction marker enzymes in isoproterenol-induced heart failure animals: Elevations of enzymes like lactate dehydrogenase, troponin T and creatine kinase were used as cardiac injury markers (Table 1). In isoproterenol-induced heart failure animals expressed significantly increased expression of lactate dehydrogenase, troponin T and creatine kinase (a*-p<0.05) was observed, indicating myocardial infarction. In contrast, celastrol treatment of isoproterenol-induced heart failure animals considerably diminished (b*-p<0.05) the expression of myocardial infarction marker enzymes than heart failure animals that only received vehicle treatment.

Analysis of celastrol on lysosomal enzymes in isoproterenol-induced heart failure animals: The levels of lysosomal enzymes (-D-glucuronidase, -D-galactosidase, -D-N-acetylglucosaminidase, acid phosphatase and cathepsin-D) in isoproterenol-induced heart failure animals were significantly lower (a*-p<0.05) than in control animals. Celastrol treatment, on the other hand, significantly reduced lysosomal enzyme levels (b*-p 0.05) in isoproterenol-induced animals (Table 2).

Analysis of celastrol on membrane-bound enzymes in heart failure animals: NA^+/K^+ ATPase activity was dramatically reduced (a*-p<0.05) in isoproterenol-induced heart failure animals, which was restored by celastrol treatment. Same time, celastrol pretreatment animals (group IV) significantly prevented (b*-p<0.05), Ca^{2+} and Mg^{2+} ATPases hyperactivity by restoring them to near control levels (Table 3).

Analysis of celastrol on antioxidants in heart tissue of isoproterenol-induced heart failure animals: The following antioxidants, SOD, CAT, GPx, GST and GSH were detected in the myocardial tissues of all groups (Table 4). There was a considerable decrease (a*-p<0.05) in the concentrations of antioxidant enzymes in heart failure animals (Group II) compared to the control animals (Group I). Pre-treatment with celastrol in isoproterenol-induced heart failure animals (Group IV) substantially (b*-p<0.05) boosted the dwarf antioxidant defence enzymes.

Analysis of celastrol on oxidative stress measures in isoproterenol-induced heart failure animal's heart tissue: Malondialdehyde (MDA) and nitric oxide (NO) levels were assessed in the test animals (Fig. 4a-b). This study revealed that animals receiving isoproterenol administered (Group II) expressed elevated MDA levels (a*-p<0.05), while those of NO were considerably lower (a*-p<0.05) in the control animals. Furthermore, compared to isoproterenol-induced heart failure animals, celastrol-treated animals had significantly lower MDA levels (b*-p<0.05) and greater NO concentrations (b*-p<0.05).

Analysis of celastrol on inflammatory markers in isoproterenol-induced heart failure in animals: The serum TNF- α and IL-6 levels are considerably greater (a*-p<0.05) in isoproterenol-induced heart failure animals than in control animals as shown in Fig. 5a-b. Moreover, pre-treated with celastrol significantly suppresses the levels of inflammatory markers (b*-p<0.05).

Table 1: Analysis of celastrol on serum markers during isoproterenol-induced heart failure in animals

Groups	LDH (IU L^{-1})	CK-MB (IU L^{-1})	Troponin T (ng mL^{-1})
Group I (Control)	146.25±5.43	140.25±11.56	0.25±0.03
Group II (ISO C)	564.98±10.76^{a*}	302.02±20.65^{a*}	0.55±0.10^{a*}
Group III (CEL)	150.85±4.62	141.33±12.23	0.26±0.02
Group IV (ISO+CEL)	256.87±63.38^{b*}	197.10±14.56^{b*}	0.32±0.05^{b*}

Data represented as Mean±Standard deviation (a* and b*-p<0.05) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol, CEL: Celastrol, LDH: Lactate dehydrogenase and CK-MB: Creatine kinase-MB

Table 2: Analysis of celastrol on lysosomal enzymes in the heart during isoproterenol-induced heart failure in animals

Groups	β-D-glucuronidase	β-D-galactosidase	β-D-N-acetylglucosaminidase	Acid phosphatase	Cathepsin-D
Group I (Control)	50.23±4.54	32.45±3.15	55.72±3.89	140.58±8.56	67.45±5.36
Group II (ISO C)	32.56±3.45^{a*}	22.78±2.45^{a*}	36.63±2.99^{a*}	90.45±10.24^{a*}	48.78±7.24^{a*}
Group III (CEL)	49.54±4.01	33.72±3.01	54.12±3.72	139.78±8.12	66.36±5.12
Group IV (ISO+CEL)	46.56±4.10^{b*}	30.36±2.75^{b*}	51.35±3.25^{b*}	131.56±9.56^{b*}	62.53±6.10^{b*}

Data represented as Mean±Standard deviation (a* and b*-p<0.05) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol and CEL: Celastrol. β-D-galactosidase, β-D-glucuronidase and β-DN-acetyl glucosaminidase: Measured as µmol of p-nitrophenol liberated/min/mg of protein with different p-nitrophenol derivatives as substrates. µmole of tyrosine liberated/hrs/100 mg of protein for Cathepsin-D and µmole of phenol released/hrs/100 mg of protein for acid phosphatase

Table 3: Analysis of celastrol on membrane-bound phosphatases in the heart during Isoproterenol-Induced heart failure in animals

Groups	NA^+K^+ ATPase	Ca^{2+} ATPase	Mg^{2+} ATPase
Group I (Control)	0.36±0.01	0.75±0.05	4.80±0.25
Group II (ISO C)	0.19±0.05^{a*}	1.65±0.14^{a*}	5.65±0.82^{a*}
Group III (CEL)	0.34±0.03	0.73±0.04	4.79±0.33
Group IV (ISO+CEL)	0.33±0.02^{b*}	0.78±0.08^{b*}	4.83±0.21^{b*}

Data represented as Mean±Standard deviation (a* and b*-p<0.05) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol and CEL: Celastrol

Table 4: Analysis of celastrol on antioxidants in heart tissue of isoproterenol-induced heart failure in animals

Groups	GST (mmol L^{-1})	GSH (nM mL^{-1})	SOD (U^A mg^{-1} protein)	CAT (U^B mg^{-1} protein)	Gpx (U^c mg^{-1} protein)
Group I (Control)	0.23±0.11	0.83±0.13	10.23±0.11	1.52±0.11	0.79±0.33
Group II (ISO C)	0.01±0.11^{a}	0.19±0.33^{a}	4.16±0.11^{a}	0.27±0.13^{a}	0.10±0.01^{a}
Group III (CEL)	0.19±0.13	0.92±0.01	10.14±0.19	1.20±0.11	0.81±0.13
Group IV (ISO+CEL)	0.21±0.11^{b}	0.52±0.04^{b}	7.11±0.11^{b}	1.00±0.66^{b}	0.70±0.31^{b}

Data represented as Mean±Standard deviation (a* and b*-p<0.05) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol and CEL: Celastrol

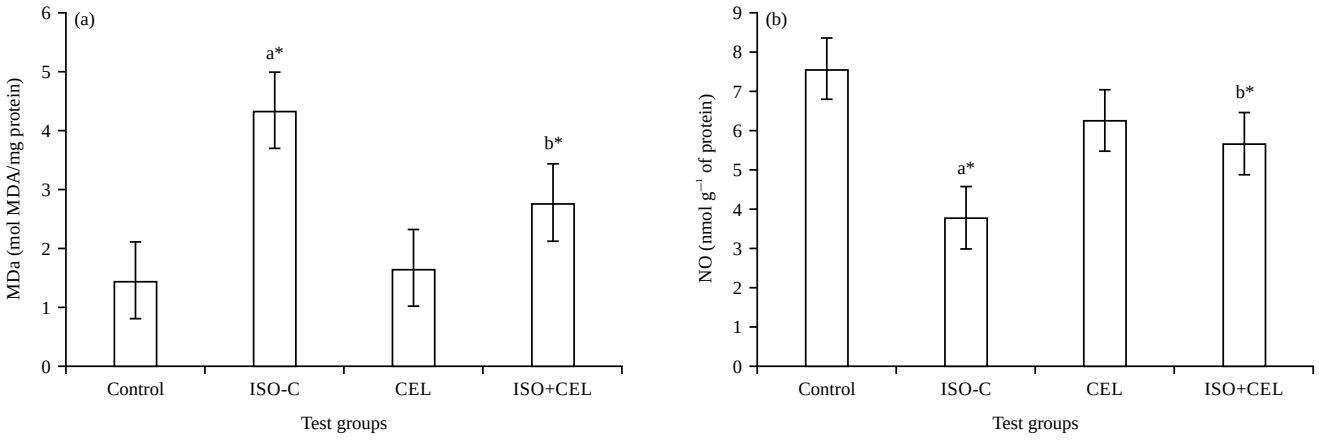

Fig. 4(a-b): Effect of celastrol on oxidative stress parameters, (a) Effect of celastrol on MDA and (b) Effect of celastrol on NO

Data from the tested groups are shown as Mean±Standard deviation (a* and b*-p<0.05) where a* denotes comparison between ISO-C vs control and b* denotes CEL vs ISO+CEL, respectively. ISO: Isoproterenol, CEL: Celastrol, MDA: Malondialdehyde, NO: Nitric oxide and X-axis: Tested groups

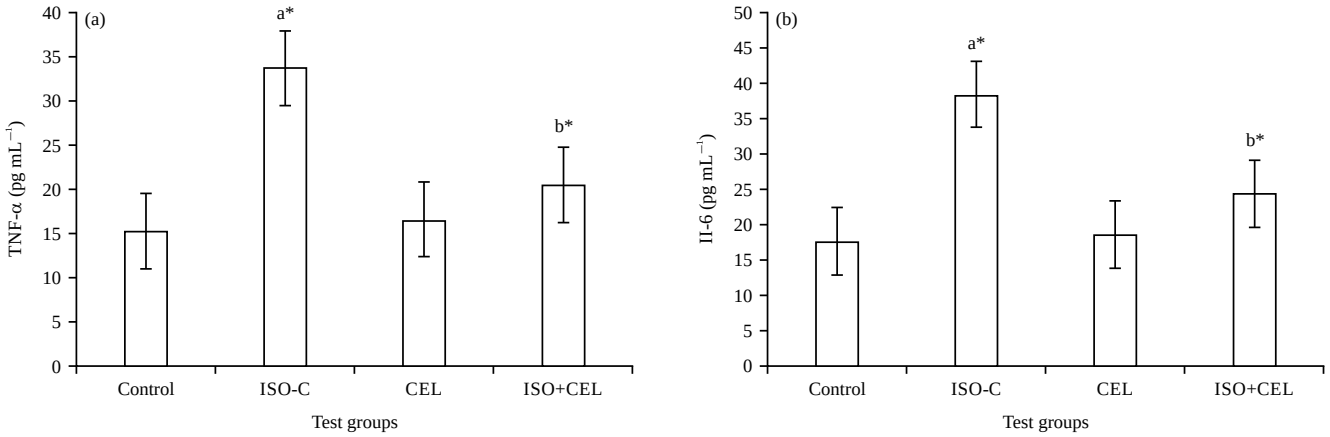

Fig. 5(a-b): Effect of celastrol on inflammatory markers, (a) TNF-α and (b) IL-6

Data from the tested groups are shown as Mean±Standard deviation (a* and b*-p<0.05) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO: Isoproterenol, CEL: Celastrol, TNF-α: Tumor necrosis factor-alpha, IL-6: Interleukine-6 and X-axis: Tested groups

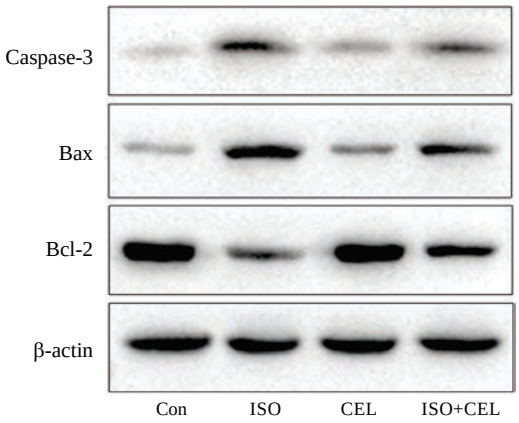

Fig. 6: Effect of Celastrol on apoptotic protein expression during Isoproterenol induced heart failure in animals (Western blot analysis for apoptosis)

Data represented as mean ± standard deviation (a* and b* -p < 0.05) where a* denotes comparison between ISO-C vs control and b* denotes ISO-C vs ISO+CEL, respectively. ISO, Isoproterenol; CEL, celastrol. Bax, BCL2-associated X protein; Bcl-2, B-cell lymphoma 2

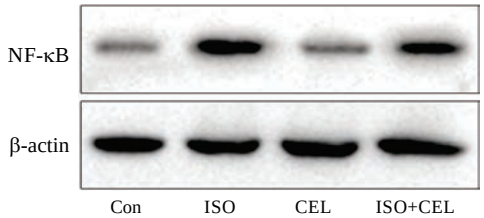

Fig. 7: Effect of celastrol on NF-κB protein expression during isoproterenol-induced cardiac failure in rats (RT – PCR for Inflammatory Response)

Data represents as mean ± standard deviation (a* and b* -p < 0.05) where a* denotes comparison between ISO-C vs Control and b* denotes ISO-C vs ISO+CEL respectively. ISO, Isoproterenol; CEL, Celastrol; NF-κB, Nuclear factor kappa light-chain-enhancer of activated B cells

Analysis of celastrol on pro-and anti-apoptotic protein expression in isoproterenol-induced heart failure animals: The apoptotic protein levels in the cardiac tissue homogenates were shown in Fig. 6. Caspase-3 and Bax levels were elevated in isoproterenol-administrated animals, but Bcl-2 levels were significantly suppressed (a*-p<0.05) in control animals. When compared to isoproterenol-only treated animals, pre-treatment with celastrol significantly reduced caspase-3 and Bax levels while significantly increasing Bcl-2 levels (b*-p<0.05).

Analysis of celastrol on NF-κB protein expression in isoproterenol-induced heart failure animals: A considerable increase in NF-κB expression in isoproterenol-induced heart failure animals (Group II) (a*-p<0.05), with a signal of serious inflammation, was depicted in Fig. 7. This was significantly attenuated by celastrol pre-treatment to isoproterenol-induced heart failure animals (Group IV) (b*-p<0.05).

DISCUSSION

Myocardial infarction is a dreaded pathological change that induces heart failure and it is considered the most severe pathological sequela among cardiovascular diseases[33]. At present, the development of some medical interventions has decreased the occurrences of cardiovascular diseases. However, heart failure remains a common cause of death across world populations. Therefore, it is necessary to understand the pathogenesis of heart failure for effective treatment design. Previously several research activities have proved that celastrol has beneficial potential such as anti-oxidant and anti-inflammatory actions against the

development of cardiovascular disease. For example, celastrol was found to regulate high blood pressure triggered by inflammation and oxidative stress in vascular smooth muscle cells[34] and to inhibit LOX-1 in aortic atherosclerotic lesions in mice[35]. Additionally, celastrol was also reported for its cardioprotective activity against ischemia[14]. Based on these results, we designed to partially evaluate whether celastrol could inhibit the isoproterenol-induced oxidative damage to the heart.

Isoproterenol has been associated with structural and functional cardiac abnormalities. It causes hypoxia, myocardial ischemia and necrosis all of which disrupt diastolic and systolic pressure among other pathological changes closely related to the myocardial-infarction[14]. Isoproterenol-induced heart failure animal models have been studied widely to test the efficacy of many drugs in treating heart failure[36]. When compared to the control groups, animals in the isoproterenol groups exhibited a substantial increase in heart-body weight ratio and infarct size. This rise in heart weight may be an increased accumulation of water in the edematous intramuscular space in heart tissue[37]. However, pretreatment with celastrol to isoproterenol-induced heart failure animals effectively lessened the heart-body weight ratio and also decreased the infarct size. This outcome was consistent with prior research outputs presented by Tong *et al.*[38]. Similarly, the animals pretreated with celastrol considerably lowered the otherwise elevated heartbeat rate and also regulated the systolic and diastolic arterial pressure of the isoproterenol-induced heart failure animals.

Cardiac damage causes the production of greater amounts of cardiac injury biomarkers (lactate dehydrogenase, creatine kinase-MB and cardiac troponin T). These cardiac enzymes are the greatest indicators for identifying tissue damage because of their specificity, sensitivity and catalytic activity.

They are mainly responsible for damaging the functional stability and permeability of cellular membranes. The leakage of these enzymes may also be due to an insufficient supply of oxygen or glucose. Thus, in turn, cells become more permeable or rupture and finally causing the leakage of cardiac injury biomarkers. Low molecular weight contractile protein called cardiac troponin T was identified as a significant cardiac marker that exists only in myocardial damage[39,40]. In this experiment, isoproterenol-induced heart failure animals were found with a significant increase in these enzymes in the serum as evidence of cardiac damage. However, this effect was diminished by the administration of celastrol. Furthermore, celastrol administration generated a similar pattern of lactate dehydrogenase and creatine kinase-MB expression in the research described by Li *et al.*[41].

Apoptosis in the heart, inflammatory responses and oxidative stress are assumed to be important factors for the aetiology of myocardial infarction[40]. According to research, oxidative stress causes cardiomyocyte apoptosis by destroying DNA, altering proteins via enzymatic activities and oxidation[42]. SOD, CAT, GPx, GST and GSH are strong free radical antagonists that act as the first line of antioxidant defence. As a result, the presence of such enzymes is required for the deactivation of superoxide anions and other reactive oxygen species generated during isoproterenol-induced myocardial infarction or heart failure[40,43]. In this study, isoproterenol-induced heart failure animals showed decreased antioxidant enzymes such as SOD, CAT, GPx, GST and GSH. However, as compared to isoproterenol-induced animals that did not receive vehicle treatment with normal saline, celastrol dramatically increased the levels of antioxidant enzymes. On the other hand, sustained oxidative stress can occur if the antioxidant defence enzymes are decreased. The inability to manage the elevated ROS levels leads to membrane damage to lipids, proteins and nucleic acids. This, together with toxic and other reactive aldehyde metabolites (MDA, the final product in the process of lipid peroxidase), is released from the interaction between ROS and polyunsaturated fatty acids[44]. Interestingly, the increased MDA levels in isoproterenol-induced heart failure animals were significantly reduced by celastrol.

The ATPases are in charge of moving sodium, potassium, calcium and magnesium across the cytoplasmic membrane to meet cellular energy demands. Under pathological conditions, these membrane-bound enzymes (ATPases) contribute to the changes in the membrane. According to a prior study, abnormal functions of Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase result in increases in intracellular sodium and calcium levels, resulting in heart dysfunction[43]. Patients with myocardial infarction showed increased extracellular and decreased intracellular potassium levels which eventually leads to tissue acidosis as well as changes in the electrophysiological pattern of cardiac cells[44]. The present study found that Na^+/K^+ ATPases were less active, while Ca^{2+} and Mg^{2+} ATPases were more active. Physiologically, in the myocardium, Na^+/K^+ ATPase actively participates in the regulation of intracellular Na^+ and K^+ levels. However, increased lipid peroxidation from isoproterenol-induced heart failure leads to the inactivation of Na^+/K^+ ATPase. As a result, the Na^+/Ca^{2+} exchange pathway is activated. Pretreatment of celastrol significantly enhanced the Na^+/K^+ ATPase while reducing the Ca^{2+} and Mg^{2+} ATPases levels which control intracellular Ca^{2+} concentration. Therefore, isoproterenol protects myocardial tissue from damage. The MDA activity rose considerably in isoproterenol-induced heart failure

animals whereas, the levels of Na^+-K^+-ATPase decreased compared to the control. Yin and colleagues reported similar correlations between the MDA and Na^+-K^+-ATPase[45]. Furthermore, alterations in the activity of lysosomal enzymes constitute a clinical characteristic in patients with cardiovascular disease. Production of oxygen free radicals in heart failure may affect lysosomal membranes and lysosomal enzymes hence the observed reduction in certain enzymes in the lysosomal part of heart tissue. These sequelae can either be due to the penetration of inflammatory cells or the overload of Ca^{2+} [46]. On this note, in our study, we observed reduced levels of β-glucuronidase, β-N-acetyl glucosaminidase, β-galactosidase, acid phosphatase and cathepsin-D in isoproterenol-induced heart failure animals, an indication of cardiac tissue damage which was reversed by administration of celastrol.

Myocardial infarction also triggers the immune system response and causes inflammation. The production of cytokines and the responses they elicit are always implicated in the pathophysiology of heart injury. Elevated cytokine levels in patients are directly associated with the degree of myocardial injury. It was reported that a less severe form of inflammation may be involved in repairing the cardiac tissue as well as driving angiogenesis. However, overexpression of inflammatory mediators may invariably lead to the development of scar tissue and fibrosis, remodelling of the ventricular system and eventually disturbing the function of the heart[47,48]. This study clearly showed that celastrol therapy dramatically reduced blood levels of TNF-α and IL-6 in isoproterenol-induced inflammation.

Furthermore, nitric oxide generated from the myocardium are tend to positively regulate the function of cardiac muscles. In animals with heart failure caused by isoproterenol, nitric oxide production was decreased, but celastrol treatment reversed it. However, NO has multifaceted actions that depend upon the NO synthase[49]. The NF-κB, a transcription factor, helps to keep the immune response in check and limit the expression of some inflammatory cytokines. TNF-α and IL-6 are released as a result of NF-κB activation[50].

Increased expression of NF-κB was found in isoproterenol-induced heart failure animals and this was attenuated by celastrol therapy. Furthermore, celastrol was shown to reduce NF-κB mRNA- expression in recent research[8]. An NF-κB transduction pathway has also been connected to cell death and cardiac remodelling[51]. Myocardial apoptosis occurs as a result of cardiac overload or ischemia and it plays a crucial part in cardiac cell death after myocardial injury. Many studies have documented that the Bax/Bcl-2 ratio may increase caspase-3 activation and modify the apoptosis-related disease progression. The activation of the pro-apoptotic signalling pathway was regulated via caspase-3. Bax can cause cell death by activating other molecules in the cytoplasm, whereas Bcl-2 functions as an anti-apoptotic protein[52,53]. The current study found that untreated isoproterenol-induced heart failure animals had increased caspase-3 and Bax and decreased Bcl-2 expression. However, administering celastrol downregulated pro-apoptotic proteins caspase-3 and Bax and at the same time upregulated Bcl-2 expression.

CONCLUSION

Celastrol therapy significantly reduced the pathological processes underpinning isoproterenol-induced heart failure by downregulating cardiac damage indicators, oxidative stress, inflammation and apoptosis. This cardioprotective activity of celastrol appears to be due to its antioxidant, anti-inflammatory and anti-apoptotic activities.

SIGNIFICANCE STATEMENT

Celastrol is a pentacyclic triterpenoid with a strong history of clinical use. However, the primary cause of their myocardial infarction was unclear. Therefore, the current study investigated the effectiveness of celastrol and its cardioprotective effects in isoproterenol-induced heart injury. According to the outcomes of the study, celastrol treatment dramatically decreased the pathological processes that underlay isoproterenol-induced heart failure by downregulating cardiac damage markers, oxidative stress, inflammation and apoptosis. Celastrol's cardioprotective action appears to be attributable to its antioxidant, anti-inflammatory and anti-apoptotic properties.

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