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Evaluation of the cardioprotective role of 7-hydroxy-4-methylcoumarin on isoproterenol induced myocardial infarction in rats

Abstract

Coumarins are the oldest class naturally occurring benzopyrene derivatives, associated with remarkable pharmacological activities, one of the most potent 7-hydroxy-4-methylcoumarin (7H4MC) shown the presence of various pharmacological activities. Thus, the present study aims to evaluate the cardioprotective effect of 7-hydroxy-4-methylcoumarin on isoproterenol (ISO) induced myocardial infarction in rats, experimental model. Myocardial infarction was induced by subcutaneous injection of ISO at the dose of (5.25 and 8.5mg kg^{-1}) on two consecutive days (29th day and 30th day), 7H4MC at two doses (100 and 200 mg kg^{-1}) was administered orally for 30days before Iso administration. Wistar rats were randomly assigned in experimental groups animals serve as a saline control, saline plus ISO-treated, standard drug (carvedilol 1mg kg^{-1} p.o.) plus ISO treatments, test drug only treated groups received 7H4MC (100 and 200mg kg^{-1} p.o) and test groups treated with the 7H4MC low dose and higher dose with ISO. The ISO-treated rats show a significant elevation in cardiac toxicity markers enzymes, lipid peroxidation; modulate oxidative enzyme level, infarct size, and hemodynamic parameters. The deformed by ISO was restored significantly by oral administration of 7H4MC. Test drug dose 200mg kg^{-1} is more effective and it was also shown nearly similar results as that of standard drug. Our study demonstrates that 7H4MC possessing antioxidant activity and has a significant protective effect against ISO-induced myocardial infarction. We believe that pre-treatment with 7H4MC may contribute to developing novel strategies in the prevention and treatment of cardiotoxic effects of elevated level of catecholamines.

Keywords: Isoproterenol, 7-Hydroxy-4-methylcoumarin, carvedilol, antioxidant activity, cardiac marker enzymes, catecholamines

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Eakta Sharma, Ajay Singh Kushwah, Taranbir Singh

Department of Pharmacology, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, India

Correspondence: Ajay Singh Kushwah, Department of Pharmacology, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, India, Tel +91 9417459195, Email kushwah_ph05@yahoo.co.in

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Introduction

Ischemic heart disease (IHD) is the underlying cause of stroke, heart attack, and renal failure. Myocardial infarction (MI) is a prevalent presentation of ischemic heart disease.[1] MI or heart attack is a therapeutic enigma which is the principal cause of the death in both developed and developing countries.[2] It is a clinical syndrome arising from rapid and persistent reduction of myocardial blood supply to an area of the heart resulting in necrosis of the myocardium. In MI, ischemic tissue generates oxygen-derived free radicals, making them chemically reactive and often leading to a chain reaction which contributes to cell death.[3] MI is the most dreaded sequel among IHD,[4] followed by various biochemical alterations such as lipid peroxidation, free radical damage, hyperglycemia, and hyperlipidemia, leading to qualitative and quantitative alterations of myocardium.[5]

Catecholamine plays a significant role in normal cardiac function. Their excessive release is responsible for the development of various cardiac dysfunctions like MI.[6] Isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride],[7] is a synthetic catecholamine and β-adrenergic agonist, which produce severe stress in the myocardium resulting in infarct-like necrosis of the heart muscle.[8] Isoproterenol (ISO) induced myocardial necrosis is a well standard model to study the beneficial effect of many drugs on cardiac dysfunction.[9] ISO-induced MI is a widely used experimental model for several reasons. The model is characterized by an extraordinary technical simplicity, an excellent reproducibility, as well as an acceptable low mortality.[10] Myocardial infarction induced by ISO, shows many metabolic and morphologic aberrations in the myocardium of experimental animals similar to those visualized in human myocardial infarction.[11]

Many modern drugs are effective in preventing cardiovascular diseases, but they are mostly accompanied by adverse effects.[2,12] Coumarins, is an old class naturally occurring benzopyrene derivative, associated with remarkable pharmacological activities. Coumarins (2H-1-benzopyran-2-ones) are important oxygen-containing fused heterocyclic.[13] Coumarins owe their class name to "coumarou" the vernacular name of the Tonka bean (Dipteryxodorata Wild (Fabaceae) from which coumarin itself was isolated in 1820.[14] In contrast, the substitution on coumarin masks the toxic nature of the parent compound. The pharmacological and therapeutic applications of simple coumarins depend upon the substitution. Chemically, coumarins and coumarin derivatives can be synthesized by various methods such as the Perkin, Pechmann reaction, Knoevenagel condensation, Reformatsky, Wittig, Claisen rearrangement, and catalytic cyclization reactions.[15] The literature review of 7-hydroxy-4-methylcoumarin (7H4MC) shown the presence of various pharmacological activities like antioxidant,[16] anti-cancerous,[17] anti- inflammatory,[18] antibacterial, anticoagulant, lipid-lowering, antimicrobial and anti-HIV activity.[15] Thus, the present study aims to evaluate the cardioprotective effect of 7-hydroxy-4-methylcoumarin on isoproterenol-induced myocardial infarction.

Materials and methods

Chemical reagent

The drug 7-hydroxy-4-methylcoumarin (7H4MC) was collected from Pharmaceutical chemistry department of ASBASJSM College of Pharmacy, Bela,(Ropar). Urethane, Dithiobis-2-nitrobenzoic acid, Potassium phosphate buffer, Trichloroacetic acid, HCl, Potassium dihydrogen phosphate, Thiobarbituric acid, and Pyridine, were purchased from Hi-media. Sodium Dihydrogen Phosphate, Triphenyltetrazolium Chloride, Formalin, Sodium dodecyl sulfate, Glacial Acetic Acid were purchased from Loba Chemie. Isoproterenol and Carvedilol were purchased from Sigma-Aldrich. Estimations kits Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were purchased from span diagnostic center.

Animal used

The research protocol of this study has been approved by the Institutional Animal Ethics Committee (IAEC) of ASBASJSM College of Pharmacy, Bela (Ropar) Punjab. Albino Wistar rats weighing 180-220g were used in the study. The animals were kept in the quarantine section till monitoring of health status of animals and subsequently transferred to the housing area. The animals were housed in standard size polypropylene cages lined with husk under the controlled conditions of temperature (23±2°C), humidity (40±10%) and 12:12h dark/light cycles. The animals were fed with standard diet and purified water was given ad libitum as per CPCSEA guideline.

Induction of myocardial injury

Isoproterenol was dissolved in normal saline and injected subcutaneously to rats at a dose 5.25 and 8.5mg kg^{-1} s.c. for 2 consecutive days to induce experimental myocardial infarction in rats.[19]

Experimental design

Forty- nine Wistar rats were randomly assigned in 7 groups (n=7 each) and grouping of animals was done on the basis of their average body weight and total duration of study 30days. All rats fasted overnight and had free access to water and pellet diet. Group, I rats received normal saline (10ml kg^{-1}p.o), Group II rats received normal saline with ISO (5.25 and 8.5mg kg^{-1}s.c) on 2 consecutive days (29^{th} and 30^{th} day). Group III rats treated with standard drug carvedilol (1mg kg^{-1} p.o.) with ISO. Group IV and V rats were received 7H4MC (100 and 200mg kg^{-1}p.o) respectively and Group VI and VII rats received 7H4MC low and high dose with ISO respectively.

Estimation of cardiotoxicity marker enzymes in plasma

At the end of the experimental period, the blood samples were taken and plasma was separated for analysis of different enzymes related to Myocardial infarction (MI) such as LDH, ALT, and ALP. All analysis was performed with commercially available kits using autobio analyzer (Reckon Diagnostics, Chandigarh and Span Diagnostics Ltd., Surat).

Measurement of hemodynamic parameters

Rats were anesthetized with 25% urethane (1.5g kg^{-1} i.p). Throughout the experimental protocol, the body temp of the animals was maintained at 37ºC. The neck was opened with a ventral midline incision to perform a tracheostomy. The left carotid artery was cannulated with a polyethylene tube (internal diameter 0.30mm; outer diameter 0.40mm) attached to a three-way cannula. The cannula was heparinized (Heparin 300IU/ml) and connected to POWER LAB 4/30 (AD Instruments, NSW, Australia) system using a pressure transducer for the measurement of systolic, diastolic, mean arterial pressures and heart rate.

Estimation of antioxidant enzymes in heart tissues

Animals were sacrificed by cervical dislocation and the heart tissues were removed, washed with the cold isotonic saline and dried with filter paper. After this hearts were diced and homogenized in 0.05M ice- cold phosphate buffer. After centrifugation supernatant was used for estimations of antioxidant enzymes GSH by Ellman's method and TBARS by Ohkawa methods.[20,21]

Evaluation of myocardial infarct size

The myocardial infarct size measured by using 2, 3, 5 tri-phenyl tetrazolium chloride (TTC) Staining method.[22] The heart was transversely cut to obtain slices no more than 0.1cm in thickness. The heart slices were placed in the glass dish containing pre-warmed (1%) TTC in phosphate buffer solution (PBS) (The 1% Triphenyl tetrazolium chloride powder diluted in a phosphate buffer).[23] The image of TTC slices was captured with a digital camera and analysed by Image J software.

Histopathology of rat heart

The excised heart was fixed in buffered formalin solution (10%). Heart samples were sent to diagnostic commercial lab histopathological slides were prepared and stained with hematoxylin and eosin (H and E) dyes and the pictures were taken from the prepared slides with the help of photomicroscope and changes in histology results were observed.

Statistical analysis

The data were expressed as mean±SEM was analyzed by one-way ANOVA followed by Turkey multiple comparison tests using Graph pad prism software package. A value of $P<0.05$ was considered to be significant.

Results

Effect of 7-hydroxy-4-Methylcoumarin treatment on the hematological parameter (LDH, ALT, and ALP) in plasma of Isoproterenol-induced myocardial infarction

A highly significant ($p<0.001$) elevation in the level of plasma LDH, ALP and ALT was observed in the positive control group (ISO) when compared to the control group. Standard drug (Carvedilol+ISO) treated a group of animal shows highly significant ($p<0.001$) reduction in the level of plasma LDH, ALP and ALT level when compared with positive control group. 7H4MC low dose+ISO-treated groups showed a significant ($p<0.01$) reduction in the level of ALT, at a similar dose, showed a significant ($p<0.001$) reduction in the level of LDH and ALT when compared with positive control group of animal. 7H4MC high dose + ISO-treated groups showed a significant ($p<0.001$) reduction in the level of LDH, ALT, and ALT when compared with positive control group (Table 1).

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Table 1 Effect of 7H4MC on various cardiotoxicity markers in ISO induced MI

Treatment groups	LDH (IU/L)	ALT (IU/L)	ALP (IU/L)
Saline control	393.2±1.68	83.33±1.22	116.7±2.05
ISO control	783.0±1.57c	134.5±4.7c	228.8±3.18c
Carve+ISO treated	401.5±0.76γ	87.50±0.34 γ	118.0±1.79 γ
7H4MC (100mg kg^{-1})	395.3±1.70	86.50±0.67	117.4±1.47
7H4MC (200mg kg^{-1})	393.8±0.83	85.67±0.66	117.0±0.72
7H4MC (100mg kg^{-1})+ ISO	458.0±1.63γ	119.8±3.1β	151.2±4.38 γ
7H4MC (200mg kg^{-1}) + ISO	430.5±1.80γ	97.17±1.74 γ	139.1±2.33 γ

Data expressed as mean ± SEM. (n=7)

$^{c}p<0.001$ when compared to saline control group, $^{\beta}p<0.01$ and $^{\gamma}p<0.001$ when compared to ISO treated group.

One way ANOVA followed by Tukey's Multiple Comparison Tests.

Effect of 7H4MC treatment on hemodynamic parameters (AP, MAP, DAP, SAP, and HR) by Isoproterenol-induced myocardial infarction.

It was observed in ISO-treated group a highly significantly ($p<0.001$) reduction in AP, MAP and DAP and highly significant ($p<0.001$) elevation in HR and SAP when compared to control groups of animal. Standard drug (Carvedilol+ISO) treated a group of animal shows a significant ($p<0.01$) maintain normal AP and highly significantly ($p<0.001$) maintain the normal value of MAP, DAP, SAP, and HR was observed when compared to positive control groups of animal. 7H4MC at low dose+ISO treated a group of animal show slightly modulation of AP and DAP and significantly ($p<0.001$) maintain normal of an HR, MAP and SAP maintain near to normal. 7H4MC at higher dose+ISO treated animal show slightly ($p<0.01$) modulation of AP rest of all hemodynamic parameters highly significantly ($p<0.001$) maintain normal when compared to positive control groups of animal (Table 2).

Table 2 Effect of 7H4MC on hemodynamic parameters in ISO induced MI in rats

Treatment	AP (mmHg)	HR (BPM)	MAP (mmHg)	SAP (mmHg)	DAP (mmHg)
Saline Control	115.0±2.21	370.9±0.16	109.4±0.50	125.7±0.10	88.24±0.38
ISO Control	95.10± 1.51c	432.5±0.74c	95.57±0.24c	137.7±0.20c	78.55±0.65c
Carvedilol + ISO	110.8±3.61β	379.2±1.47γ	105.3±0.48 γ	118.5±0.25γ	85.31±0.81γ
7H4MC (100mg kg^{-1})	105.2±2.22	364.0±0.50	104.1±0.35	120.8±0.04	83.64±0.16
7H4MC (200 mg kg^{-1})	112.9± 3.18	372.6±0.56	107.9±0.02	123.4±0.49	87.15±0.21
7H4MC (100 mg kg^{-1})+ ISO	104.0±1.53	404.1±1.95γ	98.90±0.32γ	133.9±1.00γ	80.41±0.66
7H4MC (200 mg kg^{-1})+ ISO	109.8±2.27β	367.2±1.74γ	103.9±0.02γ	130.8±0.12γ	84.64±0.19γ

Data expressed as Mean ± SEM (n=7)

$^{c}p<0.001$ when compared with control group, $^{\beta}p<0.01$and $^{\gamma}p<0.001$, when compared with ISO treated group.

One way ANOVA followed by Tukey's Multiple Comparison Test.

Effect of 7H4MC treatment on antioxidant parameters (GSH and TBARS) in tissue (rat heart) by Isoproterenol-induced myocardial infarction

A highly significant ($p<0.0001$) reduction in the level of GSH was observed whereas highly significant ($p<0.0001$) elevation in the level of TBA was observed in Iso treated group when compared to saline control group of animal. Standard drug (Carvedilol (1mg kg-1p.o.) Iso) treated a group of animal shows a highly significant ($p<0.0001$) elevation in the level of GSH whereas highly significant ($p<0.0001$) reduction in the level of TBA was observed when compared to positive control groups of animal. 7H4MC (100mg and 200mg kg-1p.o. + Iso) treated group of animal show a highly significant ($p<0.01$ and $p<0.0001$) elevation in the level of GSH whereas highly significant ($p<0.01$ and $p<0.0001$) reduction in level of TBA was observed when compared to positive control groups of animal (Figure 1).

TTC Staining

A high percentage of mean infarct size with increased staining was observed in group II (Iso treated) animals when compared to group I (saline control group). Animals in group III (Carvedilol+Iso) showed low infarct size with reduced staining when compared to group II (Iso treated) animals. Animals in group IV and group V (100 and 200mg kg^{-1}) both showed low infract size on heart tissue when compared to group I (saline control) animals. The animal in group VI and VII (100 and 200mg kg^{-1}+Iso) both showed a moderately low infarct size with reduced staining when compared to group II (Iso treated) animals. Normal myocardium turned to bright red (Figure 2).

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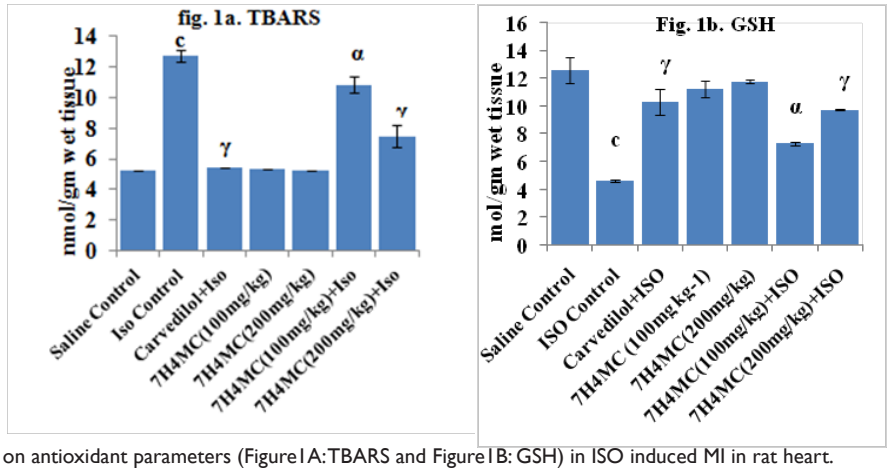

Figure.1 Effect of 7H4MC on antioxidant parameters (Figure1A: TBARS and Figure1B: GSH) in ISO induced MI in rat heart.

Data expressed as Mean ± SEM (n=7)

$^{c}p<0.001$ when compared with control group, $^{\alpha}p<0.01$ and $^{\gamma}p<0.001$, when compared with ISO treated group.

One way ANOVA followed by Tukey's Multiple Comparison Test.

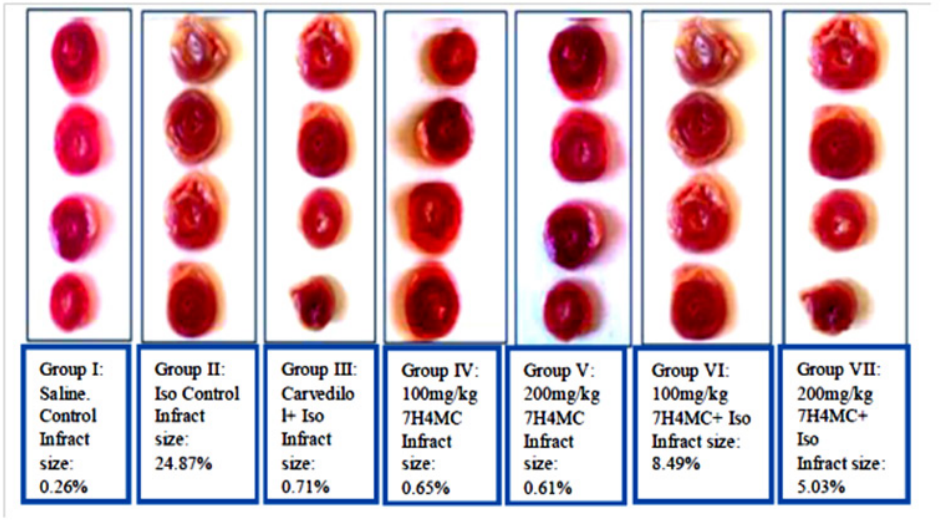

Figure 2 Effect of 7H4MC on % infarction in ISO induced MI in rat heart.

Histopathology

The histopathological examination of myocardial tissue shows normal morphology of cardiac muscle in the saline control group. But the Iso treated group shows an increase in infarct area, necrosis, and separation of myocardial fibers. Oral administration of Carvedilol shows a mild change in the infarct area, necrosis, and separation of myocardial fibers. At a dose of 100mg and 200mg kg^{-1}, 7H4MC+Iso showed mild infarct area, necrosis and separation of myocardial fibers, when compared to Iso treated group (Table 3).

Table 3 Effect of 7H4MC on Histopathological Grades MI in rat heart

Sign of Infraction	Saline Control	ISO Control	Carvedilol + ISO	7H4MC (100mg kg^{-1})	7H4MC (200 mg kg^{-1})	7H4MC (100 mg kg^{-1}) + ISO	7H4MC (200 mg kg^{-1}) + ISO
Infarct Area	0	4	2	0	0	3	1
Necrosis	0	3	1	0	0	2	1
Separation of myocardial fifers	0	4	2	0	0	2	1

0: No changes; **1:** Minimal; **2:** Mild; **3:** Moderate; **4:** Severe

Discussion

Isoproterenol (Iso) is a well-known cardiotoxic agent due to its ability to destruct myocardial cells. As a result of this, cytosolic enzyme LDH, ALT and ALP are released into the bloodstream and serve as the diagnostic markers of myocardial tissue damage.[24] In our findings Iso causes significant damage to the myocardium and increase the level of a diagnostic marker such as LDH, ALT, and ALP (49.78,

38.04 and 38.81%) respectively and cause necrotic damage of the myocardial membrane in Iso control group when compared to saline control group. The LDH, ALT and ALP level significantly decrease (48.72, 34.94 and 48.07%) respectively in Carvedilol+Iso treated a group of animals, when compared to group Iso treated group. 7H4MC (100 and 200mg kg^{-1}) +Iso treated group the LDH, ALP and ALT level decrease (41.50, 12.27 and 33.91%) and (45.08, 27.75 and 39.20%) with respect dose-dependently when compared to Iso control treated group. These results suggest that treatment with 7H4MC prevent the Iso induced leakage of the diagnostic marker.

Iso is a well-known inducer of myocardial necrosis and interstitial fibrosis at its dose,[25] have shown that Iso leads to myocardial necrosis characterized by increased end-diastolic volume, end-diastolic pressure and left ventricular wall thickness.[26] In the present investigation, administration of Iso shows significant reduction in the AP (17.30%), MAP (12.64%) and DAP (10.98%) whereas elevation in the SAP (8.71%) and HR (14.24%) in the heart was observed in Iso treated group, when compared to saline control group. Oral administration of carvedilol shows a significant elevation in the AP (13.54%), MAP (9.24%) and DAP (7.92%) whereas the reduction in SAP (13.94%) and HR (12.32%) in the heart, when compared to Iso treated group. 7H4MC (100mg and 200mg kg^{-1}) group of normal rats showed a significant reduction in the MAP (4.84 and 1.37%) respectively, DAP (5.21 and 1.23%) respectively, SAP (3.89 and 1.82%) respectively and HR (1.86%) respectively, when compared with saline control treated group of animals. 7H4MC (100mg and 200mg kg^{-1}. p.o.) +Iso show significant elevation in the AP (8.55 and 13.38%) respectively, MAP (3.36 and 8.01%) respectively and DAP (2.31 and 7.19 %) respectively whereas reduction in HR (6.56 and 15.09%) respectively and SAP (97.24 and 94.98%) respectively in the heart was observed in both groups, when compared with Iso treated group.

Iso induced MI, damages the cardiac myocytes which results in hypoxia, coronary hypertension, calcium overloading and depletion of energy reserve and excessive production of free radical due to oxidative metabolism of catecholamine.[25] GSH has a direct antioxidant function by reacting with superoxide radicals and singlet oxygen followed by the formation of oxidized GSH and other disulfides.[27]Glutathione plays an important role in the regulation of a variety of cell functions and in cell protection against oxidative injury.[28] Peroxidation of endogenous lipid has been shown to be a major risk factor in cardiotoxic action of isoproterenol.[29] There is a report showing that free radicals produced by Iso could initiate peroxidation of membrane-bound polyunsaturated fatty acids, leading to both functional and structural myocardial injury.[30] In the present investigation, administration of Iso shows a significant reduction (63.34%) in the level of GSH was observed whereas highly significant elevation (58.82%) in the level of TBA was observed in Iso treated group when compared to saline control group of animal. Standard drug (Carvedilol+Iso) treated a group of animal shows a highly significant elevation (55.17%) in the level of GSH whereas highly significant reduction (57.33%) in the level of TBA was observed when compared to positive control groups of animal. 7H4MC (100 and 200mg kg^{-1}+ Iso) treated a group of animal shows a highly significant elevation (36.81 and 52.53%) respectively in the level of GSH whereas highly significant reduction (14.68 and 41.25%) respectively in the level of TBA was observed when compared to positive control groups of animal.

A high percentage of mean infarct size with increased staining was observed in Iso treated group of animals when compared to the saline control group. Animals in group Carvedilol+Iso showed low infarct size with reduced staining when compared to group Iso treated animals. At a dose of 100 and 200mg kg^{-1}, 7H4MC both showed low infract size on heart tissue when compared to saline control group of animals. At a dose of 100 and 200mg kg^{-1}, 7H4MC+Iso both showed a moderately low infarct size with reduced staining when compared to group Iso treated animals. The biochemical findings were further confirmed by histopathological studies.[31]In the present investigation, the histopathological examination of myocardial tissue shows normal morphology of cardiac muscle in the saline control group. But the Iso treated group shows an increase in infarct area, necrosis, and separation of myocardial fibers. Oral administration of carvedilol shows a mild change in the infarct area, necrosis, and separation of myocardial fibers. At a dose of 100and 200mg kg^{-1}, 7H4MC+Iso showed mild infarct area, necrosis and separation of myocardial fibers, when compared to Iso treated group.

Conclusion

In conclusion, the conducted study showed that ISO injection in experimental animals induces MI which is confirmed by hemodynamic changes, biochemical changes. Treatment with 7H4MC prevents the ISO-induced MI by virtue of its potent antioxidative activity due to the ability to scavenge free radicals and improve the activity of cardiac marker (LDH, ALT, and ALP) and antioxidant (GSH), decrease the lipid peroxidation, in Iso treated rats. All these estimations (biochemical, hemodynamic, TTC Staining and histopathology) have shown that 200mg kg^{-1} dose of the drug (7H4MC) is more effective than 100 mg kg^{-1}. 200mg kg^{-1} dose has also shown nearly similar results as that of carvedilol (standard drug).On the basis of this study, it may be suggested that 7H4MC have a cardioprotective activity.

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Conflicts of interest

Authors declare that there is no conflicts of interest.

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