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Ameliorative Effect of Acarbose Against Isoproteronol Induced Cardiotoxicity in Swiss Albino Mice

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ABSTRACT

Cardiovascular diseases involve myocardial injury associated with oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis. This study investigated the cardioprotective effects of Acarbose against isoproterenol (ISO)-induced cardiotoxicity in male Swiss albino mice. Animals were divided into six groups (n=6): normal control, ISO (150 mg/kg), Acarbose (25, 50, and 100 mg/kg) plus ISO, and propranolol (20 mg/kg) plus ISO. Cardiotoxicity was evaluated using heart weight/body weight ratio, serum CK-MB, LDH and cTn-T, cardiac MDA, SOD, CAT, GSH and TAC, TUNEL assay, and histopathology. ISO significantly increased cardiac biomarkers, MDA, and myocardial apoptosis while reducing antioxidant status and disrupting cardiac architecture. Acarbose dose-dependently reversed these changes, with 100 mg/kg showing the greatest protective effect. Histopathology confirmed reduced myocardial damage and preservation of tissue architecture, comparable to propranolol. Overall, Acarbose demonstrated dose-dependent cardioprotective activity against ISO-induced myocardial injury, potentially through antioxidant and anti-apoptotic mechanisms.

KEYWORDS: Cardiovascular diseases, Cardiotoxicity, Isoproterenol, Acarbose, Anti-Apoptotic

INTRODUCTION

Cardiovascular diseases (CVDs) are a major global health burden, with myocardial injury being an important pathological feature of several cardiovascular disorders. Myocardial damage may result from oxidative stress, inflammation, mitochondrial dysfunction, altered calcium homeostasis and apoptosis. Excessive reactive oxygen species (ROS) can overwhelm endogenous antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH), leading to lipid, protein and cellular membrane damage. Persistent oxidative and inflammatory injury can impair cardiac structure and function and contribute to cardiomyopathy, arrhythmias, ischemia and heart failure. [1,2]

Isoproterenol (ISO), a synthetic non-selective β-adrenergic receptor agonist, is widely used to induce experimental myocardial injury. Excessive β-adrenergic stimulation increases cardiac workload and oxygen demand, promoting ROS generation, calcium imbalance, mitochondrial dysfunction and inflammation. ISO-induced myocardial injury can be evaluated using biochemical and structural parameters, including heart weight-to-body weight (HW/BW) ratio, CK-MB, LDH and cardiac troponin-T (cTn-T), together with oxidative stress markers and histopathological examination. Acarbose is an orally active α-glucosidase inhibitor used for the management of type 2 diabetes mellitus. By delaying carbohydrate digestion and reducing postprandial glucose excursions, Acarbose may influence metabolic and oxidative processes. Its hydroxyl and glycosidic groups also provide potential hydrogen-bonding sites for interaction with selected molecular targets. [3,4]

The present study was designed to investigate the potential cardioprotective and neuroprotective effects of Acarbose against ISO-induced injury. Acarbose was evaluated at 25, 50 and 100 mg/kg, with propranolol (20 mg/kg) as the positive control and ISO (150 mg/kg) used to induce experimental cardio- and neurotoxicity. Cardioprotective activity was assessed using HW/BW ratio, CK-MB, LDH, cTn-T and cardiac oxidative stress parameters, while neuroprotective activity was evaluated using behavioral tests, brain antioxidant parameters, AChE activity, histopathology and immunohistochemistry. Molecular docking was additionally performed with selected protein targets (4KV1, 4FLIP, 1G5MP and 4S0OP), with 4S0OP showing the most favorable predicted Vina score. Collectively, these approaches were used to provide preliminary experimental and computational evidence for the protective potential of Acarbose against ISO-induced cardiac and neurological injury. [4,10]

Methods

Animals

Male Swiss albino mice (35–50 g) were kept in an SPF facility at 23 ± 2 °C, 60 ± 5% humidity, and a 12/12 h light/dark cycle, with free access to food and water. All procedures followed CCSEA guidelines (IAEC No. 2308/PO/Re/S/2024/CCSEA, Pallavan Pharmacy College) and mice were acclimatized for one week before experiments. Mice were divided into six groups (n = 6): Group I (normal control, vehicle), Group II (ISO 150 mg/kg), Groups III–V (Acarbose 25, 50, 100 mg/kg + ISO), and Group VI (Propranolol 20 mg/kg + ISO, positive control). Treatments followed the experimental protocol. [13]

Effect of Acarbose on isoproterenol-induced cardiotoxicity.

Evaluation of the heart weight: body weight ratio (HW/BW ratio)

The body weight of each mouse was measured 24 hours after the final dose. The mice were then sacrificed by cervical dislocation under anesthesia. After euthanasia, the hearts were removed and rinsed with normal saline. Any remaining connective tissue and blood vessels were carefully removed, and the hearts were dried. Heart weight was then recorded, and the heart weight to body weight (HW/BW) ratio was calculated.

Collection of blood and serum preparation

Collected 0.2 mL of blood from the retro-orbital venous plexus of each anesthetized mouse using capillary tubes. The blood was placed in microcentrifuge tubes. After the blood clotted, we separated the serum by centrifuging at 3,000 rpm for 20 minutes at 4°C with a Remi C-24 refrigerated centrifuge. The serum was then stored at −80°C for later biochemical analysis.

Evaluation of creatine kinase-MB (CK-MB)

The CK-MB level in serum was measured using a colorimetric kit from Reckon Diagnostics Pvt. Ltd., Baroda, India. The kit included CK-MB-I (Buffer Antibody) and CK-MB-II (Enzyme Activator) reagents. To prepare the working reagent, the enzyme activator was combined with the buffer antibody and incubated at 37°C for five minutes. After incubation, 1 mL of the working reagent was added to 50 µL of serum, mixed, and incubated again at 37°C for five minutes. The first absorbance was measured after five minutes, with subsequent readings at 30, 60, 90, and 120 seconds at 340 nm. CK-MB values are expressed in IU/L.

Evaluation of lactate dehydrogenase (LDH)

LDH levels were measured using a colorimetric kit from Span Diagnostics, Surat, India. The kit included two reagents: reagent 1 (Coenzyme) and reagent 2 (Buffered substrate). To create the working reagent, reagent 1 was mixed with 1.1 mL of reagent 2, gently combined, and allowed to stand for five minutes. Then, 1 mL of this working reagent was added to 50 µL of serum sample, mixed, and the absorbance was measured at 340 nm after one minute, with further readings at 30, 60, and 90 seconds. LDH values are expressed in IU/L.

Evaluation of cardiac troponin T (cTn-T)

cTn-T was evaluated using a sandwich ELISA kit purchased from Krishnan Biosystems, Mumbai, India. All reagents were used at room temperature. Firstly, wash buffer and standard were prepared according to the given method. 50 µL of standard was added to the standard well. 40 µL of samples and then 10 µL of biotinylated Tn-T antibody were pipetted into sample wells. After this, 50 µL of streptavidin-HRP conjugate was pipetted into all the wells (sample wells as well as standard wells). Then, the plate was incubated for 1 h at 37°C. After one hour, the plate was aspirated and washed using the prepared wash buffer (4 times) and blotted with the remaining buffer. 50 µL of substrate A and B was pipetted in all the wells and incubated at 37 °C for 10 min.

Stop solution (50 µL) was pipetted into all the wells, and absorbance was recorded at 450 nm.

Evaluation of MDA.

Cardiac malondialdehyde (MDA) levels were detected biochemically. Trichloroacetic acid was added to the sample for protein precipitation, and then thiobarbituric acid was added. The mixture was heated for 10 min in a boiling water bath. One molecule of MDA in the homogenized heart samples reacted with two molecules of thiobarbituric acid, and the resulting chromogen was centrifuged. The intensity of the color developed in the supernatant was measured spectrophotometrically at 535 nm. [19]

Evaluation of the SOD.

Cardiac superoxide dismutase (SOD) activity was detected biochemically. The method used to determine SOD activity in homogenized heart samples is based on the fact that the autoxidation of pyrogallol is inhibited by SOD. One unit of SOD is generally defined as the amount of enzyme that inhibits the autoxidation of pyrogallol by 50%. The activity of SOD was monitored spectrophotometrically at 420 nm.

Evaluation of catalase activity (CAT)

Catalase level was evaluated using a previously given method. Homogenate of the heart tissue was prepared in potassium phosphate buffer at a ratio of 1:10 w/v. The buffer pH was maintained at 7.4. The supernatant was collected from the homogenate after centrifugation. Supernatant (50 µL) was added to 2.95 mL of 19 mM/L H2O2 solution made in potassium phosphate buffer. The optical density change was recorded for three minutes at a wavelength of 240 nm at one-minute intervals.Values of catalase were indicated as n moles of H2O2/min/mg protein. [17]

Evaluation of reduced glutathione (GSH)

Reduced glutathione activity has been determined using a previously described method. The basic principle of this method is based on the formation of one mole of 5-Mercapto-2-nitro-benzoic acid/mole of SH from the reduction of Ellman's Reagent (5,5′-dithiobis-(2-nitrobenzoic acid) by a thiol. Heart tissue homogenate was prepared in 0.02 M EDTA. To the mixture of double-distilled water (4 mL) and 50% TCA (1 mL), 5 mL of the homogenate was added. After centrifugation of this homogenate, 2 mL of supernatant was collected and added to the mixture of 4 mL of Tris buffer and 0.1 mL of DTNB. Optical density was observed at 410 nm against a blank (without homogenate). Data are represented as µmol/mg of protein. [18]

Evaluation of the total antioxidant capacity (TAC)

The TAC was determined using a previously described method. 10 µL of cytosolic supernatant was mixed with the solution of sodium phosphate buffer (0.49 mL), sodium benzoate (0.50 mL), Fe-EDTA (0.20 mL), and H2O2 (0.20 mL). The mixture was incubated for 60 min at 37°C. Acetic acid (1 mL) and thiobarbituric acid (1 mL) were added to this mixture, incubated for 10 min at 100°C, and cooled. After this, absorbance was recorded at 532 nm against deionized water. TAC values were expressed as mmol/L. [20]

Effect of Acarbose on Myocardial Apoptosis (TUNEL Assay)

Compared with the normal control group (Group I), the isoproterenol-treated group (Group II) showed a significant increase in the number of TUNEL-positive nuclei, indicating enhanced myocardial apoptosis. Treatment with acarbose at 25 mg/kg (Group III), 50 mg/kg (Group IV), and 100 mg/kg (Group V) showed a dose-dependent reduction in the number of TUNEL-positive nuclei compared with the isoproterenol-treated group. The positive control group treated with propranolol (20 mg/kg) along with isoproterenol (150 mg/kg) (Group VI) also showed a significant reduction in TUNEL-positive nuclei compared with Group II. [14,15]

Among the acarbose-treated groups, the 100 mg/kg dose (Group V) showed greater protection against isoproterenol-induced myocardial apoptosis, whereas the 25 mg/kg dose (Group III) exhibited comparatively less protective activity. These findings suggest that acarbose, particularly at higher doses, attenuates isoproterenol-induced myocardial apoptosis and may contribute to its cardioprotective effect.

Histopathological Evaluation

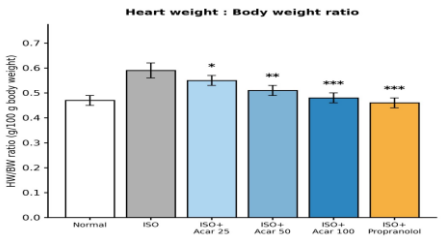
Heart tissues were fixed in formalin for a week. Then, the formalin-fixed tissues were processed following a series of exchanges in different solvents. Finally, they were embedded in paraffin wax. The formalin-fixed paraffin-embedded tissues were serially sectioned in 5–10 µm thickness using a microtome (Leica Biosystems, Nussloch, Germany). The tissue sections were stained with hematoxylin and eosin (H&E). Thereafter, these were mounted on gelatin-coated glass slides. The mounted sections were visualized under a light microscope BX41 obtained from Olympus, Center Valley, PA, USA, and images were captured for histopathological assessment.

Result

Effect of Acarbose on isoproterenol-induced cardiotoxicity.

Effect on heart weight to body weight ratio

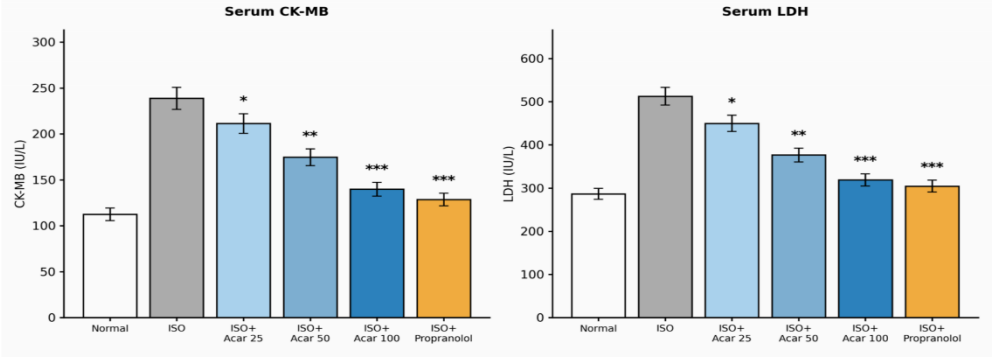
The results showed a modest increase in the HW/BW ratio in the isoproterenol control group compared with the normal control group. Acarbose treated groups showed a progressive reduction in the HW/BW ratio toward the normal control value.



Results of Acarbose on heart weight to body weight ratio. The Data are organized as the mean ± SD. * p < 0.05, ** p 0.01, *** p <0.001 for the control Isoproteronol group.

Effect on serum CK-MB and LDH

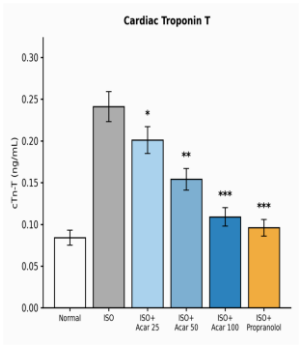
Isoproterenol markedly increased serum CK-MB and LDH levels, indicating myocardial cellular injury. Treatment with acarbose attenuated these elevations, with the high-dose group showing the greatest reduction.



Results of Acarbose on serum Ck-MB and LDH levels. The Data are organized as the mean ± SD. * p < 0.05, ** p 0.01, *** p <0.001 for the control Isoproteronol group.

Effect on cardiac troponin T

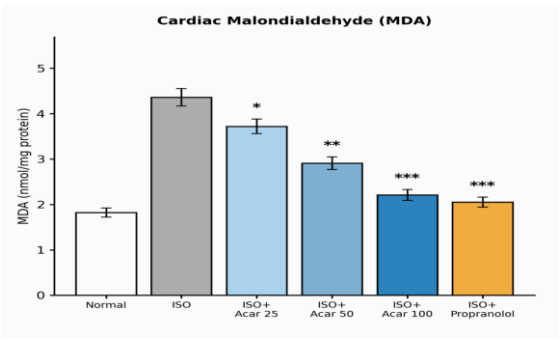
The cTn-T concentration was substantially elevated in the isoproterenol control group. Acarbose treatment reduced the cTn-T concentration in a progressive manner, suggesting attenuation of myocardial injury.



Results of Acarbose on cTn-T concentration. The Data are organized as the mean ± SD. * p < 0.05, ** p 0.01, *** p <0.001 for the control Isoproteronol group.

Effect on cardiac MDA

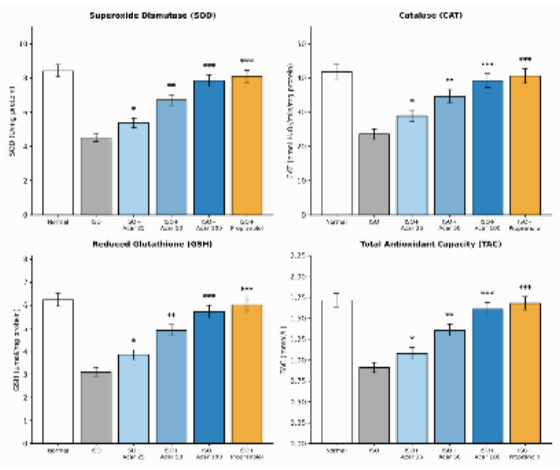
Isoproterenol exposure increased cardiac MDA levels compared with the normal control group, indicating enhanced lipid peroxidation. Acarbose significantly reduced the elevated MDA level in the treated groups.



Results of Acarbose on Cardiac MDA levels. The Data are organized as the mean ± SD. * $p < 0.05$, ** p 0.01, *** $p < 0.001$ for the control Isoproteronol group.

Effect on antioxidant parameters

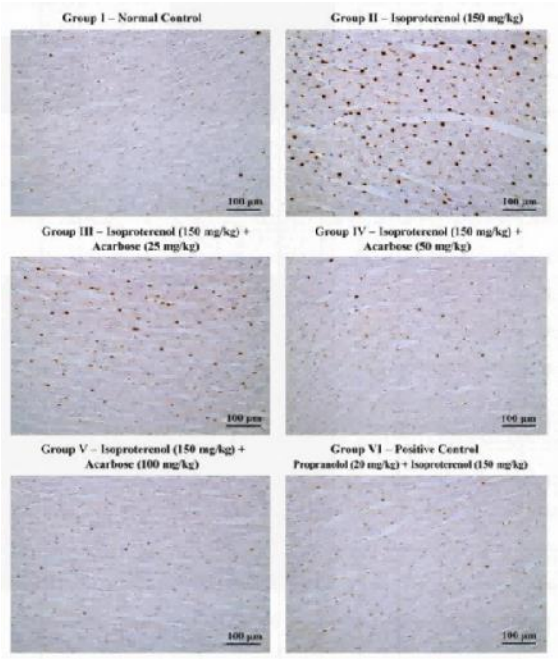
The isoproterenol control group showed decreased SOD, CAT, GSH and TAC compared with the normal control group. Acarbose treatment restored these antioxidant parameters toward normal values, with the greatest improvement observed in the high-dose treatment group.



Results of Acarbose on Cardiac SOD, CAT, GSH and TAC. The Data are organized as the mean ± SD. * $p < 0.05$, ** p 0.01, *** $p < 0.001$ for the control Isoproteronol group

Effect of Acarbose on Myocardial Apoptosis (TUNEL Assay)

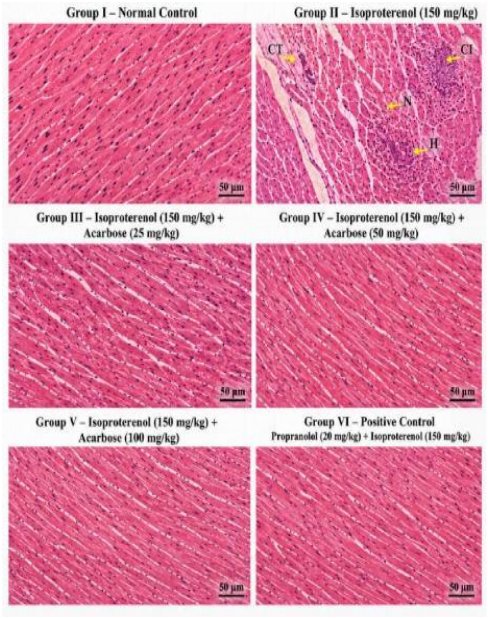
The TUNEL assay showed increased myocardial apoptosis in Group II compared with control. Acarbose treatment (25, 50, and 100 mg/kg) progressively reduced TUNEL-positive nuclei, with the greatest reduction at 100 mg/kg. Propranolol (20 mg/kg) also markedly reduced apoptosis. Overall, acarbose demonstrated a dose-dependent anti-apoptotic effect against isoproterenol-induced myocardial injury. [14,15]



Representative images showing effect of Acarbose on apoptosis. TUNEL assay was used to detect myocardial apoptosis. TUNEL-positive nuclei were stained dark brown because of the HRP-DAB reaction [Scale bar 100 µm].

Histopathological evaluation of cardiac tissue

Histopathological examination of the normal control group showed preserved myocardial architecture with regularly arranged cardiac muscle fibers and intact nuclei. The isoproterenol control group showed prominent myocardial damage, including myofibrillar disruption, edema, inflammatory cell infiltration and focal necrotic changes. Acarbose-treated groups showed progressive preservation of myocardial architecture, with reduced edema, inflammatory infiltration and necrotic changes. The high-dose treatment group showed comparatively near-normal myocardial morphology



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Histopathological changes induced by isoproterenol and the protective effect of acarbose in mouse heart (H&E, 40×). Group 1: Control showing normal myocardial architecture. Group 2: Isoproterenol showing hypertrophy, necrosis, and cellular infiltration. Groups 3–5: Acarbose (25, 50, and 100 mg/kg) showing dose-dependent improvement and protection. Group 6: Propranolol (20 mg/kg) showing protection against isoproterenol-induced cardiac injury. H: Hypertrophy; N: Necrosis; CI: Cellular infiltration.

DISCUSSION

ISO (150 mg/kg) induced marked cardiotoxicity, as evidenced by increased HW/BW ratio, CK-MB, LDH, cTn-T and MDA levels, along with decreased SOD, CAT and GSH. Acarbose treatment (25, 50 and 100 mg/kg) progressively attenuated these changes, with the 100 mg/kg dose showing the greatest improvement. These findings suggest that Acarbose reduced ISO-induced myocardial injury and oxidative stress while restoring endogenous antioxidant defenses. [3,4,6]

The improvement in cardiac biomarkers and oxidative stress parameters indicates a potential cardioprotective effect of Acarbose against ISO-induced myocardial damage. The greater protection observed at 100 mg/kg suggests a dose-dependent response. Overall, Acarbose demonstrated promising cardioprotective activity, possibly through attenuation of oxidative stress and preservation of cardiac tissue integrity. [4,10]

CONCLUSION

The present study evaluated the ameliorative effect of Acarbose against isoproterenol (ISO)-induced cardiotoxicity in experimental animals. Administration of ISO at 150 mg/kg produced marked myocardial injury, as evidenced by alterations in cardiac injury biomarkers and increased oxidative stress. Treatment with Acarbose at doses of 25, 50 and 100 mg/kg progressively attenuated the ISO-induced cardiac alterations. Acarbose treatment reduced the elevated levels of CK-MB, LDH and cardiac troponin-T (cTn-T), indicating a reduction in myocardial cellular injury and preservation of cardiomyocyte integrity. The ISO-induced alteration in the heart weight-to-body weight (HW/BW) ratio was also improved following Acarbose treatment. Furthermore, Acarbose reduced lipid peroxidation and restored the endogenous antioxidant defense system, as evidenced by improvement in superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) levels in cardiac tissue. The 100 mg/kg dose of Acarbose produced the most pronounced protective effect among the tested doses. Overall, the findings demonstrate that Acarbose attenuated ISO-induced cardiotoxicity by reducing cardiac injury biomarkers, oxidative stress and lipid peroxidation while restoring endogenous antioxidant defenses. These findings suggest that Acarbose possesses potential cardioprotective activity against ISO-induced myocardial injury, although further studies are required to establish the precise molecular mechanisms underlying this protective effect.

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