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iNOS-Dependent Cardioprotection by Cilostazol in Ischemia-Reperfusion Injury: Evidence from Isolated Rat Heart Model

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Abstract

Myocardial Ischemia-Reperfusion (I/R) injury remains a major clinical challenge, and pharmacological strategies that mimic Ischemic Preconditioning (IPC) are of significant therapeutic interest. This study explored the cardioprotective potential of cilostazol and examined whether its effects are mediated through Inducible Nitric Oxide synthase (iNOS) derived from Nitrous Oxide (NO) signalling. Isolated rat hearts were subjected to 20 min of global ischemia followed by 40 mins of reperfusion using the Langendorff perfusion model. Animals were divided into six groups: Control (I/R), IPC (4 cycles of 5-min ischemia/reperfusion), and Cilostazol (50 µmol/L), Cilostazol + L-NAME (30 µmol/L; selective iNOS inhibitor). Cilostazol markedly reduced infarct size (21.167± 1.352%), comparable to IPC (20.167±0.792%) relative to the control group (66.667±2.539%). There were notable reductions in cardiac injury biomarkers, such as LDH (34.167±1.352 U/L) and CK-MB (1.667±0.494 U/L), reflecting the protective effects mediated by IPC. However, when L-NAME or aminoguanidine was administered alongside cilostazol, its protective effects were entirely nullified, causing the infarct size and biomarker levels to revert to those seen in untreated I/R hearts.

Major Findings: Cilostazol's cardioprotection is mediated primarily via iNOS/NO pathways, as evidenced by reversal with NOS inhibitors. Its efficacy parallels IPC, suggesting cilostazol as a non-invasive alternative to surgical preconditioning in procedures like Coronary Artery Bypass Grafting (CABG), enhancing patient compliance.

Keywords: Cardioprotection, Cilostazol, Ischemic Reconditioning (IPC), Ischemia-Reperfusion (I/R), Nitric Oxide Synthase (iNOS).

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1. Introduction

The Sustainable Development Goals (SDGs) were endorsed by UN member states in 2015 as part of a pledge to enhance global population health[1]. A major goal of the SDGs is to decrease Early death due to chronic illnesses by 1/3rd by 2030 through improved preventive measures and therapy[1]. Cardiovascular disease (CVD) is the leading cause of death and disability worldwide[2,3]. The Global Burden of Disease (GBD) claims Cardiovascular Disease (CVD) was responsible for 438 million disability-adjusted life years lost and 19 million deaths worldwide in 2021[4]. Preventing and managing CVD requires comprehensive, multilevel measures significantly reduce premature mortality linked to non-communicable diseases[5]. Many open-heart procedures, such as coronary artery bypass surgery, are typically carried out on a bloodless, immobile heart; in order to reach these conditions, global cardiac ischemia must be induced. However, negative outcomes could occur when performing global ischemia of the heart. Ischemic necrosis, or permanent cardiac damage, occurs after 20 mins of a bloodless period[6]. Four hours after myocardial infarction, histopathological analyses of cardiac tissue have shown the presence of coagulative necrosis, oedema, and neutrophil infiltration. Both during the reperfusion phase, after the infarction site's circulation is restored, myocardial cells die. Myocardial stunning, arrhythmias, and a rise in tissue impact (infarction)

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may all result from such cell injury. One major factor contributing to reduced cardiac output, which might result in death, is myocardial injury[7].

Reactive Oxygen Species (ROS) and disrupted calcium homeostasis in cardiomyocytes, the enhanced neutrophil infiltration, and the upregulation of adhesion molecules due to endothelial cells or macrophages releasing cytokines and interleukins are the primary causes of ischemia-reperfusion damage[8,9]. The chemical name for cilostazol is 6-[4-(1-cyclohexyl-5-tetrazole) butoxy]-1, 2, 3, 4-tetrahydro-2-oxoquinoline. It exhibits antiplatelet aggregation and vasodilator properties[10]. By Antagonising Phosphodiesterase (PDE) activity in platelets/vascular smooth muscle or impeding Thromboxane 2 (TXA2) formation in lipid bilayers, it primarily raises the concentration of cyclic adenosine monophosphate (cAMP) or liberates Adenosine Diphosphate (ADP) and 5-hydroxytryptamine (5-HT)[11]. Cilostazol is commonly used to treat recurrent isolation, and it is also infrequently used to prevent stroke or cerebral atherosclerosis. A growing body of research indicates that cilostazol may protect several organ systems from ischemia-reperfusion damage[12]. Another research demonstrated the benefits of pre-treating cold hepatic ischemia reperfusion damage with Cilostazol, attributing its effectiveness to preventing endothelial inflammation and apoptosis. Furthermore, pre-treatment Cilostazol improved neurological functioning results[12,13].

The study explores the cardioprotective effects of Cilostazol, with a specific focus on the function of Inducible Nitric Oxide synthase (iNOS) as a key mediator. Cilostazol, a phosphodiesterase-3 inhibitor, is known or its antiplatelet and vasodilator properties, which contribute to improved blood flow and reduced thrombotic events. The rationale behind this research lies in the potential of cilostazol to upregulate iNOS, leading to increased NO production. Elevated NO levels can promote vasodilation, reduce oxidative stress, and attenuate ischemia-reperfusion injury, thereby protecting the heart during ischemic events. By investigating the iNOS pathway, the study aims to elucidate the molecular mechanisms underlying cilostazol's therapeutic benefits, offering insights into its potential as a targeted treatment for ischemic heart conditions. This could pave the way for novel strategies to enhance myocardial survival and improve outcomes in patients with cardiovascular disease.

2. Methods and Materials

2.1 Animals

The Wistar Albino Rats employed in this study were sourced from the Animal Facility at NIMS University Rajasthan, Jaipur, Rajasthan. For this research, rats weighing 125-150 g (both sexes) were selected. The animals were maintained on standard rodent chow with ad libitum access to purified water. The study protocol received ethical clearance from the Institutional Animal Ethics Committee (IAEC, 2022) [Approval Nos. 01, 02, 03, 05 and 08] at NIMS University Rajasthan, Jaipur, India, which operates under the regulatory oversight of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration No. 1203/PO/Re/S/09/CPCSEA).

2.2 Chemicals and Drugs

Cilostazol was procured from Niksan Pharmaceuticals, Gujarat, India. Ketamine was obtained from Troika Pharmaceuticals Limited, Uttarakhand, India. L-NAME (Nω-nitro-L-arginine methyl ester) was purchased from Tokyo Chemical Industry Co. Ltd., Toshima, Kita-Ku, Tokyo, Japan. Aminoguanidine was obtained from BLD Pharmatech (India) Pvt. Ltd., Hyderabad, Telangana, India. Heparin was procured from BE Pharmaceuticals Private Limited, Telangana, India.

2.3 Biomarkers-LDH, CK-MB and Troponin I

Notably, the observed biomarker elevations (CK-MB, LDH, and troponin I) mirror those typically seen in perioperative myocardial infarction[14,15].

2.4 Measurement of Infarct Size [16,17]

The measurement of infarct size was performed using the volume method, in which the heart tissue slices were sandwiched between two glass plates to ensure uniform thickness and accurate area determination. A translucent acrylic grid consisting of 100 squares, measuring each 1 cm^2, was placed over the slices for precise quantification of the infarcted and non-infarcted areas. The total quantity of squares on each side of each slice was counted to determine its mean area. Similarly, the quantity of

squares that fell on the yellow area was likewise tallied. A proportion of the whole area was used to represent the infarct surface[17].

2.5 Troponin I

In skeletal and cardiac muscle tissue, troponin, a protein complex consisting of three subunits (I, C, and T), influences the calcium-mediated interaction between actin and myosin[18]. The majority of cardiac troponins in myocardial cells are affixed to the contractile apparatus, although a portion—3–8% of cTnI and 6–7% of cTnT, respectively—are free in the cytoplasm[20-23].

2.5.1 Cardiac Troponin to Estimate Infarct Size

An early cytosolic release peaking at the event of reperfusion and a gradually diminishing stabilisation period representing deterioration from the bound fraction are characteristics of the Liberation dynamics of cardiac troponins, setting of cardiac cell death[24,25]. Research indicates that in reperfused patients, cTnT and cTnI both peak early (at 12 h), but cTnI's disappearance is quicker than cTnT's, even though both are still noticeably increased after 72 h[26-28]. Because of the characteristics mentioned, the cardiac troponin is particularly well-suited for both early infarction diagnosis and, later on, determining the degree of myocardial necrosis[28,29].

2.6 Surgical Setup

Equipment – Langendorff system (INCO, Ambala, India), electrocardiograph (BPL CARDIAART 108TDIGI, New Delhi, India)[30].

2.7 Experimental Procedure

The experimental procedure was conducted as follows: Rats were first anesthetised using ketamine at a dosage of 100 mg/kg, followed by the placement of limb lead II Electrocardiogram (ECG) electrodes to monitor cardiac activity. The thoracic cavity was then surgically opened to expose the heart. Approximately 15 min prior to euthanasia, heparin (500 IU) was administered intraperitoneally to prevent blood clotting. The heart was carefully excised after removing the pericardium and promptly transferred to a Langendorff perfusion system for retrograde aortic perfusion. The heart was excised after removal of the pericardium and mounted on a Langendorff apparatus for retrograde aortic perfusion, where it was continuously perfused with oxygenated Krebs–Henseleit solution (pH 7.4; 95% O_2 and 5% CO_2) at a constant pressure of 70 mmHg, a level that approximates physiological coronary perfusion pressure and ensures stable myocardial function and coronary flow[31]. To maintain physiological temperature, the heart was enclosed in a double-jacketed chamber circulated with warm water set at 37°C. Global ischemia was induced for 20 min, followed by a 40-min reperfusion period. Cardiac electrical activity was recorded using a digital ECG system with silver electrodes placed at the left ventricular apex and right atrial appendage. Continuous monitoring was performed during baseline stabilisation, throughout the ischemic phase (at 0, 10, and 20 min), and during reperfusion (at 0, 10, 20, 30, and 40 min). Regional ischemia was achieved by ligating the left anterior descending coronary artery with a 4/0 silk suture using a slipknot technique. Following the ischemia-reperfusion protocol, coronary effluent samples were collected to assess myocardial injury markers, including Lactate Dehydrogenase (LDH), creatine kinase -MB (CK-MB) and Troponin I.

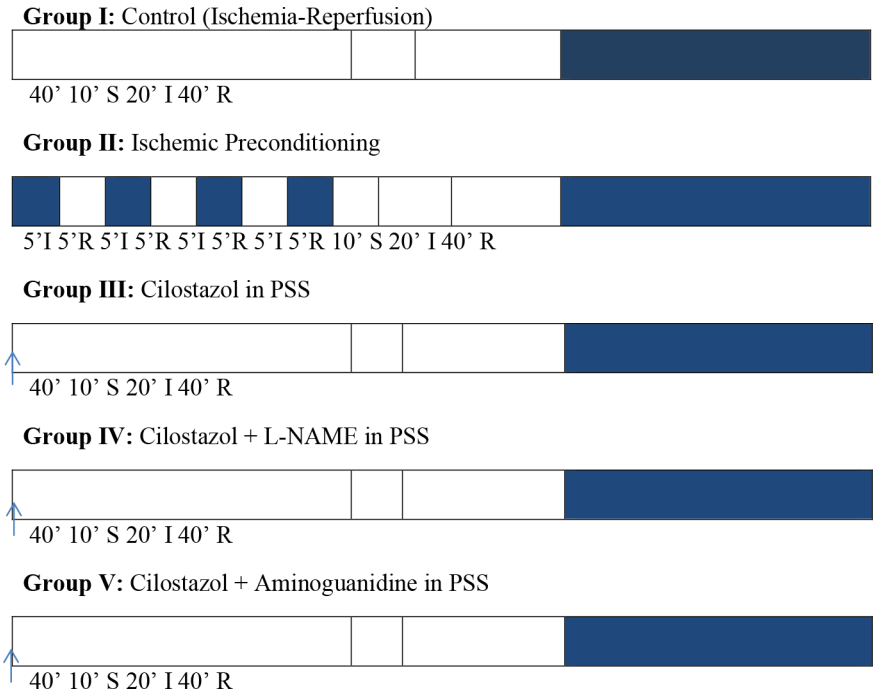
Subsequently, the heart was excised, the aorta and auricles were removed, and the tissue was stored in a deep freezer at −20°C. After 24 h, the frozen heart was sectioned into 1 mm thick transverse slices, which were then incubated with 1% Triphenyltetrazolium Chloride (TTC) at 37°C for 10 min. The stained sections were rinsed with saline and sandwiched between glass slides to differentiate viable and infarcted myocardium. This standardised protocol allowed for the evaluation of cardio-protective interventions against ischemia-reperfusion injury[32-35]. Graph transparency was put on the glass plate, and infarct size was calculated by the volume method. Drug treatment in Krebs-Henseleit Physiological Salt Solution (PSS) was administered prior to induction of ischemia-reperfusion injury to elucidate the underlying mechanism of action[32-35].

2.8 Experimental Protocol

There were 30 Wistar Albino Rats (15 male and 15 female): utilised in this investigation, divided into 5 groups with an average of 6 animals per group (n = 6). Diagrammatic representation of experimental protocol, Table 1, Figure 1.

Table 1. Different types of groups with a number of animals

Group	Treatment Description	Intervention Details
Group I	Control-Ischemia Reperfusion (I/R)	Subjected to standard I/R injury protocol (20 min ischemia + 40 min reperfusion) with no pretreatment in PSS.
Group II	Ischemic Preconditioning (IPC)	Brief cycles of ischemia/reperfusion prior to sustained I/R to induce protective adaptation with no pretreatment in PSS.
Group III	Cilostazol Treatment	Administered cilostazol (50 µmol/L) in PSS solution (Krebs–Henseleit) (50 µmol/L) with pretreatment in PSS.
Group IV	Cilostazol + L-NAME (Non-selective NOS inhibitor)	Cilostazol (50 µmol/L) co-administered with L-NAME to assess nitric oxide synthase (NOS) dependency with pretreatment in PSS.
Group V	Cilostazol + Aminoguanidine (iNOS inhibitor)	Cilostazol (50 µmol/L) with selective iNOS blockade to determine iNOS-specific mechanisms with pretreatment in PSS.



Note: I, R, S denotes ischemia, reperfusion and stabilisation, respectively, at ‘mins-time interval.

Figure 1. Diagrammatic representation of experimental protocol.

2.8.1 Group I Control-Ischemia Reperfusion (I/R)

Experimental rats were anesthetised with ketamine and monitored using limb lead II ECG (Figure 2), with heparin given beforehand to prevent clotting. The heart was then rapidly excised and cannulated through the aorta for retrograde perfusion using the Langendorff apparatus (Figure 3) to induce ischemia–reperfusion injury.

To induce regional ischemia, the left anterior descending coronary artery was occluded for 20 min by passing a 4-0 silk suture around the vessel and securing it with a slipknot. This was followed by a 40-min period of reperfusion, during which the ligature was released to restore coronary blood flow, as shown in Figure 3. This standardised protocol created a well-established model of myocardial ischemia-reperfusion injury for subsequent experimental investigations.

2.8.2 Group II Ischemic Preconditioning (IPC)

Rats were anesthetised, ECG-monitored, heparinised, and their hearts were rapidly excised and mounted on a Langendorff apparatus for ischemic preconditioning.



Figure 2. Anaesthesia and pre-surgical preparation of experimental rats.

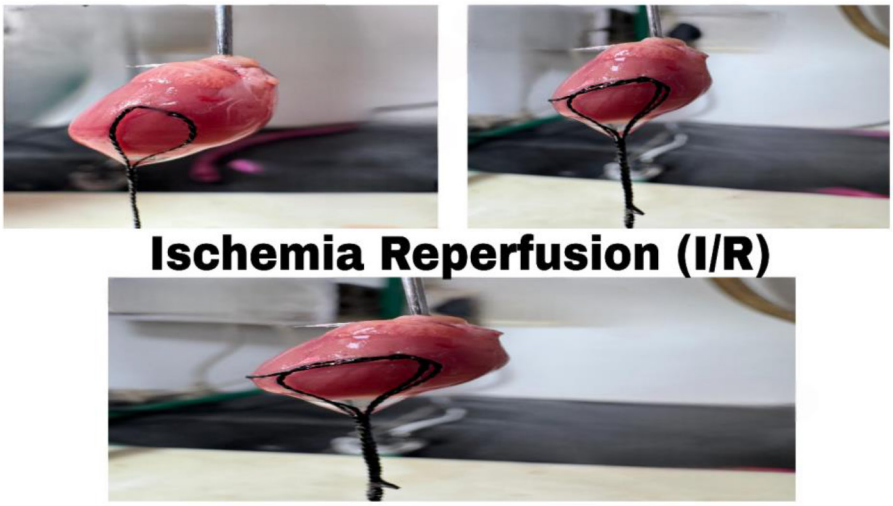


Figure 3. Showing Ischemia Reperfusion (I/R) of the heart excised and cannulated via the aorta for retrograde perfusion using the Langendorff apparatus. To induce regional ischemia, the left anterior descending coronary artery was occluded for 20 min by passing a 4-0 silk suture.

2.8.3 Group III Cilostazol Treatment

Rats were anesthetised, ECG-monitored, heparinised, and hearts were excised and mounted on a Langendorff apparatus, with cilostazol added to the perfusion solution before ischemia.

2.8.4 Group IV Cilostazol + L-NAME (Non-Selective NOS Inhibitor)

Rats were anesthetised, ECG-monitored, heparinised, and hearts were excised and mounted on a Langendorff system perfused with PSS containing cilostazol and L-NAME.

2.8.5 *Group V Cilostazol + Aminoguanidine (iNOS Inhibitor)*

Rats were anesthetised, ECG-monitored, heparinised, and the hearts were excised and mounted on a Langendorff system perfused with PSS containing cilostazol and aminoguanidine.

2.9 Assessment of LDH, CK-MB and Troponin I Levels

Lactate Dehydrogenase (LDH), creatine kinase-MB (CK-MB), and troponin I levels were assessed as biochemical markers of myocardial injury (Figures 4-6). LDH and CK-MB activities were determined using standard enzymatic assay kits, while cardiac-specific troponin I was quantified through an immunoassay method. These biomarkers provided a quantitative measure of cardiac cell damage following ischemia–reperfusion injury.

2.10 Statistical Analysis

All data were expressed as mean ± Standard Error of the Mean (SEM) for both enzymatic parameters and infarct size. Statistical significance among groups was evaluated using one-way Analysis of Variance (ANOVA). Post hoc analysis for comparisons with the control group was performed using Dennett's test, while Student's test was applied for multiple intergroup comparisons. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were conducted using Sigma Plot version 15.

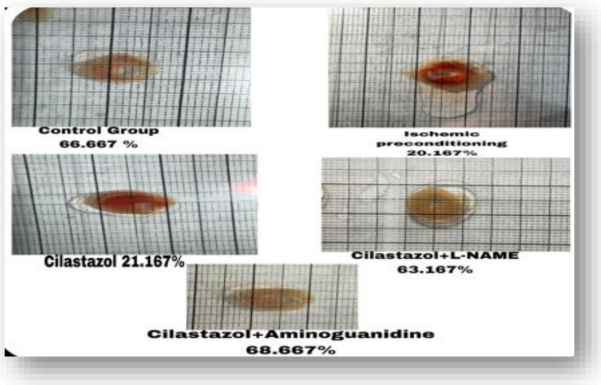


Figure 4. Effect of cilostazol on myocardial infarct size in isolated rat hearts subjected to ischemia–reperfusion (I/R) injury. Control (I/R) group, Ischemic preconditioning (IPC) group, Cilostazol-treated group, Cilostazol + L-NAME group, Cilostazol + Aminoguanidine group.
Values represent mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA, followed by Dunnett's test and Student's t-test where appropriate. A p value < 0.05 was considered statistically significant.

3. Results

3.1 Impact of the Ischemia Preconditioning Group and the Control Group (Ischemia-Reperfusion) on Myocardial Infarction, LDH, CK-MB, and Troponin I

Myocardial infarct size decreases markedly in the ischemic preconditioning group relative to the control (ischemia-reperfusion) group. The extent of myocardial infarction in the control group was determined to be of 66.667±2.539 %, and the extent of myocardial infarction in the ischemic preconditioning group was determined to be 20.167±0.792 %. The amount of LDH in the control group was measured as 104.833±1.558 (U/L), while LDH was determined that the ischemic preconditioning group was 33.833±1.249 (U/L). The CK-MB in the control group was assessed to be of 9.500±0.764 (U/L), and CK-MB in the ischemic preconditioning group was found to be 1.500±0.563 (U/L). In the Control (ischemia-reperfusion), the troponin I values were found to be 95.333±7.689 (pg/ml) and troponin I in the ischemic preconditioning group was found to be 22.333±3.721 (pg/ml) (Figures 4-8), (Tables 2-5).

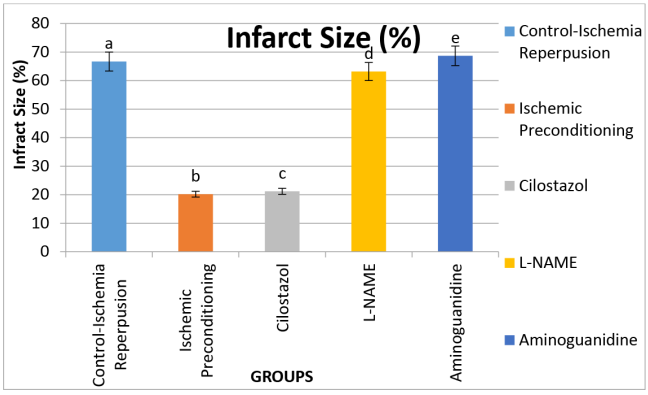


Figure 5. Effect of cilostazol, ischemic preconditioning, and iNOS inhibition on myocardial infarct size following ischemia–reperfusion (I/R) injury.
Values are expressed as mean ± SEM (n = 6). Groups labelled with different superscripts (a–e) differ significantly from one another ($p<0.05$) as determined by one-way ANOVA followed by Dunnett's and Student's *t*-tests

Table 2. Size of myocardial infarct in several animal groups (%)

S. No.	Groups	Infarct Size (%)
1	Group I: Control-Ischemia Reperfusion	66.667±2.539
2	Group II: Ischemic Preconditioning	20.167±0.792***a
3	Group III: Cilostazol	21.167±1.352***b
4	Group IV: Cilostazol + L-NAME	63.167±3.135***c
5	Group V: Cilostazol + Aminoguanidine	68.667±2.155***d

Here, the *** denotes extreme significance, meaning that, in comparison with the Cilostazol standard, p<0.01-0.001. One-way ANOVA was used for the statistical analysis. Student's t-test was applied for evaluating differences across groups, while Dunnett's post hoc Test was applied to compare findings with the control group. SigmaPlot v15 software was used for all statistical analyses.

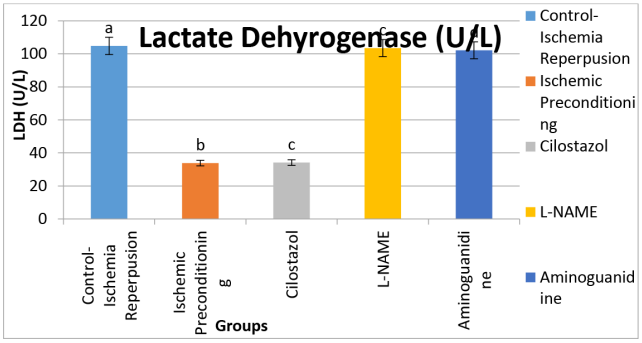


Figure 6. Lactate Dehydrogenase (U/L).
Values are expressed as mean ± SEM (n = 6). Groups labelled with different superscripts (a–e) differ significantly from one another (p<0.05) as determined by one-way ANOVA followed by Dunnett's and Student's *t*-tests.

3.2 Effect of Cilostazol Treatment Group on Myocardial Infarction, LDH, CK-MB and Troponin I

The control group exhibited a greater degree of myocardial infarction, while treatment with Cilostazol markedly reduced the infarct size to 21.167 ± 1.352%. The LDH in the control group was found to be 34.167±1.352 (U/L). The CK-MB in the control group was found to be 1.667±0.494 (U/L), and Troponin I values were found to be 21.667±2.155 (pg/ml) (Figures 4-8), (Tables 2-5).

Table 3. Release of Lactate Dehydrogenase (LDH) in the isolated rat heart's coronary effluent during 40 min of reperfusion (U/L)

S. No.	Groups	LDH (U/L)
1	Group I: Control-Ischemia Reperfusion	104.833±1.558
2	Group II: Ischemic Preconditioning	33.833±1.249***a
3	Group III: Cilostazol	34.167±1.352***b
4	Group IV: Cilostazol + L-NAME	103.500±2.217***c
5	Group V: Cilostazol + Aminoguanidine	102.167±2.197***d

Here, the *** denotes extreme significance, meaning that, in comparison with the Cilostazol standard, p<0.01-0.001. One-way ANOVA was used for the statistical analysis. Student's t-test was applied for evaluating differences across groups, while Dunnett's post hoc Test was applied to compare findings with the control group. SigmaPlot v15 software was used for all statistical analyses.

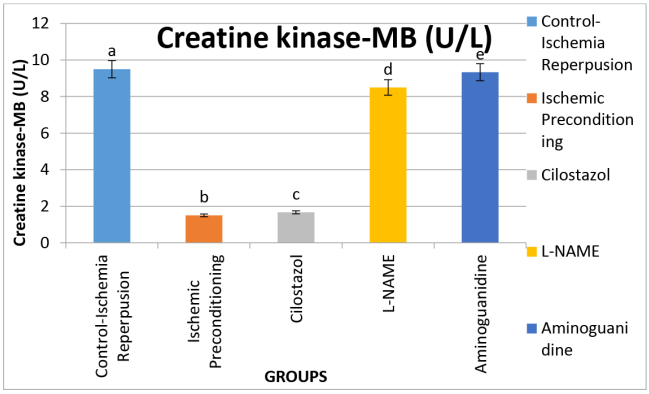


Figure 7. Creatine kinase-MB (U/L).
Values are expressed as mean ± SEM (n = 6). Groups labelled with different superscripts (a–e) differ significantly from one another (P < 0.05) as determined by one-way ANOVA followed by Dunnett's and Student's *t*-tests.

3.3 Effect of L-NAME (Nitric Oxide Synthase Inhibitor) on Cilostazol Treatment on Myocardial Infarction, LDH, CK-MB and Troponin I

The L-NAME-treated group showed a marked increase in myocardial infarction (63.167 ± 3.135%) along with elevated LDH levels (103.500 ± 2.217 U/L). In comparison, the control group demonstrated CK-MB values of 8.500 ± 0.764 U/L and Troponin I levels of 93.333 ± 8.028 pg/ml (Figures 4-8, Tables 2-5).

Table 4. Release of creatine kinase myoglobin binding (CK-MB) in the isolated rat heart's coronary effluent during 40 mins of reperfusion (U/L)

S. No.	Groups	Creatine kinase MB (U/L)
1	Group I: Control-Ischemia Reperfusion	9.500±0.764
2	Group II: Ischemic Preconditioning	1.500±0.563***a
3	Group III: Cilostazol	1.667±0.494***b
4	Group IV: Cilostazol + L-NAME	8.500±0.764***c
5	Group V: Cilostazol + Aminoguanidine	9.333±1.054***d

Here, the *** denotes extreme significance, meaning that, in comparison with the Cilostazol standard, p<0.01-0.001. One-way ANOVA was used for the statistical analysis. Student's t-test was applied for evaluating differences across groups, while Dunnett's post hoc Test was applied to compare findings with the control group. SigmaPlot v15 software was used for all statistical analyses.

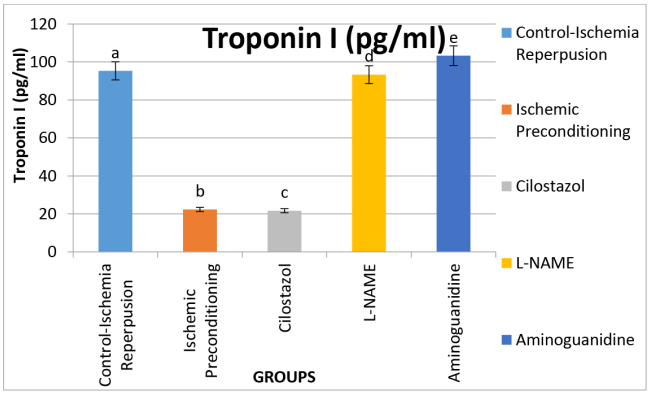


Figure 8. Troponin I (pg/ml).
Values are expressed as mean ± SEM (n = 6). Groups labelled with different superscripts (a–e) differ significantly from one another (P < 0.05) as determined by one-way ANOVA followed by Dunnett's and Student's *t*-tests.

3.4 Impact of Cilostazol and Cilostazol +Aminoguanidine (Inducible Nitric Oxide Synthase Inhibitor) on Myocardial Infarction, LDH, CK-MB and Troponin I

The aminoguanidine group exhibited a marked myocardial infarction of 68.667 ± 2.155% along with elevated LDH levels (102.167 ± 2.197 U/L). Meanwhile, CK-MB and Troponin I values in the control group were found to be 9.333 ± 1.054 U/L and 103.333 ± 8.028 pg/ml, respectively (Figures 4-8, Tables 2-5).

Table 5. Troponin I (pg/ml)

S. No.	Groups	Troponin I (pg/ml)
1	Group I: Control-Ischemia Reperfusion	95.333±7.689
2	Group II: Ischemic Preconditioning	22.333±3.721***a
3	Group III: Cilostazol	21.667±2.155***b
4	Group IV: Cilostazol + L-NAME	93.333±8.028***c
5	Group V: Cilostazol + Aminoguanidine	103.333±8.028***d

Here, the *** denotes extreme significance, meaning that, in comparison with the Cilostazol standard, p<0.01-0.001. One-way ANOVA was used for the statistical analysis. Student's t-test was applied for evaluating differences across groups, while Dunnett's post hoc Test was applied to compare findings with the control group. SigmaPlot v15 software was used for all statistical analyses.

4. Discussion

The present study demonstrates that ischemic preconditioning (IPC) significantly reduces myocardial infarct size and attenuates cardiac injury markers—LDH, CK-MB, and troponin I—compared with the control (I/R) group (Figures 4–8, Tables 2-5). The infarct size in the IPC group was markedly decreased (20.2% vs 66.7% in control), confirming the well-established cardioprotective effects of IPC in experimental models of ischemia–reperfusion injury. This protection is mediated through activation of intracellular signalling pathways such as mitochondrial K^+ ATP channels and protein kinase C, which limit apoptosis and preserve cardiomyocyte integrity[36,37].

Cilostazol treatment produced comparable reductions in infarct size and biomarkers (21.2% infarct size; LDH 34.2 U/L; CK-MB 1.7 U/L; troponin I 21.7 pg/ml), suggesting that it confers significant cardioprotection in I/R injury (Figures 4-8, Tables 2-5). These effects align with previous studies demonstrating that cilostazol attenuates myocardial injury by modulating oxidative stress, enhancing NO signalling, and preserving mitochondrial function[38].

The role of NO signalling was confirmed by the co-administration of L-NAME, a nitric oxide synthase inhibitor, which significantly reversed cilostazol-mediated protection (infarct size 63.2%; LDH 103.5 U/L; CK-MB 8.5 U/L; troponin I 93.3 pg/ml) (Figures 4-8, Tables 2-5). Similarly, combining cilostazol with aminoguanidine, an inducible NOS inhibitor, abolished cardioprotection (infarct size 68.7%; LDH 102.2 U/L; CK-MB 9.3 U/L;

troponin I 103.3 pg/ml), indicating that iNOS-mediated NO production is essential for cilostazol's beneficial effects. These findings are consistent with previous research showing that modulation of NOS isoforms influences myocardial susceptibility to I/R injury[39,40].

In summary, the results indicate that cilostazol mimics or complements IPC, likely via preservation of NO pathways and attenuation of oxidative and cellular injury, as evidenced by reductions in LDH, CK-MB, and troponin I. These findings highlight the potential of cilostazol as a pharmacological cardioprotective agent during ischemic events.

5. Conclusion

The equivalent reversal of protection observed with both L-NAME and aminoguanidine suggests that compensatory mechanisms involving other NOS isoforms play a minimal role in cilostazol's effects under these experimental conditions. According to our study, iNOS is implicated in the cardioprotective action of Cilostazol. These findings strongly support the hypothesis that cilostazol mimics the adaptive mechanisms of IPC through the activation of iNOS and subsequent NO production, which may influence oxidative stress, mitochondrial function, calcium homeostasis, and inflammatory signalling pathways during reperfusion.

Research identifies a new mechanistic connection between Cilostazol and the iNOS/NO pathway in the setting of myocardial I/R injury. The results hold significant transnational importance, indicating that cilostazol might be repurposed or further developed as a treatment approach to safeguard the heart during plant ischemic events, such as cardiac surgery, angioplasty or thrombolytic therapy. Nonetheless, additional *in vivo* studies, dose optimisation trials, and clinical research are necessary to confirm these cardioprotective effects and to clarify the intricate molecular signalling pathway involved. In summary, cilostazol emerged as a promising pharmacological mimic of ischemic preconditioning, with it with its cardio-protective effect heavily reliant on iNOS-derived nitric oxide (iNOS/NO) signaling making it a potential candidate for managing myocardial ischemia reperfusion injury.

6. Acknowledgement

I would like to share my gratitude to my guide, Prof. (Dr.) Ashish Kumar Sharma for helping me to complete the study, and secondly, to NIMS University for providing the lab and animals to complete this study.

7. Ethical Approval

The experimental research protocol has been ethically approved by the Institutional Animal Ethics Committee (IAEC, 2022) [Protocols No. 01, 02, 03, 05, and 08], NIMS University Rajasthan, Jaipur, Rajasthan, India, Registration No. 1203/PO/Re/S/09/CPCSEA, under the supervision of CPCSEA, Government of India, New Delhi, India.

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Author Contributions

JT: Experimental work, writing whole draft, AKS: Original concept, writing original draft.

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