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Original Article

Hemin-induced HO-1 protects isolated rat hearts from ischemia-reperfusion injury by activation of pro-survival signalling pathways

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Abstract: Objectives: Myocardial ischemia-reperfusion (I/R) injury significantly contributes to cardiac dysfunction and increased mortality following myocardial infarction (MI). Heme oxygenase-1 (HO-1), a stress-inducible enzyme playing a key role in degrading heme into biliverdin, carbon monoxide (CO), and free iron, all of which possess cytoprotective properties. This study aimed to investigate the effects of pharmacological modulation of HO-1 on myocardial I/R injury in isolated rat hearts and to elucidate the underlying mechanisms of HO-1-mediated cardioprotection. Methods: Using a Langendorff perfusion system, rat hearts were subjected to controlled I/R in the presence and absence of HO-1 modulators such as Hemin and Zinc Protoporphyrin IX. To check the possible involvement of various cardioprotective signalling pathways, their inhibitors were used. Hemodynamic and cardiodynamic data, cardiac injury markers, oxidative stress markers, and histopathologic changes were evaluated. Results: Our results reveal that HO-1 induction by Hemin confers substantial protection against I/R injury by preserving cardiac function, and reducing myocardial injury and oxidative stress. However, this cardioprotective effect of HO-1 was either partially or completely abolished upon inhibition of specific pro-survival pathways, including phosphoinositide 3-kinase (PI3K), protein kinase C (PKC), nitric oxide synthase (NOS), and ATP-sensitive potassium (K_{ATP}) channels. Furthermore, the HO-1 inhibitor Zinc Protoporphyrin IX (ZnPP IX) significantly reversed the protective effects, confirming a central role of HO-1 activity in mediating myocardial protection. Conclusion: HO-1 induction by Hemin protects the myocardium against I/R injury through activation of multiple pro-survival signalling pathways, supporting its potential as a therapeutic target for myocardial infarction.

Keywords: Heme oxygenase-1 (HO-1), myocardial ischemia-reperfusion injury, cardioprotection, oxidative stress, hemodynamic and cardiodynamic data

Introduction

Myocardial infarction (MI) remains a global health concern, primarily caused by restricted blood flow due to obstruction in coronary circulation. Although timely restoration of blood flow is essential to salvage ischemic myocardium, the process itself can paradoxically result in further myocardial injury, referred to as ischemia-reperfusion injury (IRI). IRI is characterized by a rapid surge in reactive oxygen species (ROS), elevated pro-inflammatory cytokine release, calcium overload, and the triggering of apoptotic signalling pathways. These pathologic events ultimately culminate in irreversible injury and adverse clinical outcome [1].

Heme oxygenase-1 (HO-1) is an enzyme induced following stress conditions, known for its protective cellular properties. It facilitates the catalytic degradation of heme into biliverdin, carbon monoxide (CO), and free iron - each playing distinct roles in modulating oxidative stress, inflammation, and apoptotic processes. Importantly, HO-1 expression is upregulated under stress conditions, reinforcing its potential protective role in myocardial injury [2].

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HO-1 expression can be induced by its natural substrate heme, as well as by various stressors such as ultraviolet radiation, hypoxia, heavy metals, hydrogen peroxide, and nitric oxide (NO). These regulatory mechanisms involve transcription factors such as nuclear factor kappa B (NF-κB), nuclear factor erythroid 2-related factor 2 (Nrf2), and activator protein-1 (AP-1), all of which are central to HO-1 gene induction during cellular stress [3]. Among pharmacologic agents, Hemin is a well-established HO-1 inducer that activates Nrf2, thereby enhancing HO-1 expression and promoting cytoprotection [4]. Conversely, Zinc protoporphyrin IX (ZnPP IX) is a competitive inhibitor of HO-1 and is frequently used to elucidate the enzyme's functional role by blocking its activity [5]. These agents provide useful tools for mechanistic investigations into HO-1 mediated tissue protection.

Several studies have demonstrated the therapeutic potential of HO-1 for reducing cellular damage associated with IRI. For instance, CO exhibits anti-apoptotic and anti-inflammatory activity that could be helpful in neutralizing the harmful effects of reperfusion injury [6]. Moreover, bilirubin is a potent antioxidant capable of neutralizing oxidative stress and mitigating IRI-induced damage [7]. There are further supportive evidences of HO-1 mediated protection in the form of its genetic and pharmacologic upregulation which improve cardiac function following I/R injury [8]. Together, these observations indicate a protective function of HO-1 in cardiac injury that could serve as a viable therapeutic target.

This study aimed to explore the cardioprotective effects of pharmacological modulation of HO-1 in isolated rat hearts subjected to IRI and to delineate the pro-survival mechanisms involved in HO-1 mediated protection.

Materials and methods

Drugs, chemicals and reagents

Hemin, Chelerythrine, and Zinc protoporphyrin IX were obtained from Sigma-Aldrich. L-NAME (N(ω)-nitro-L-arginine methyl ester) and Wortmannin were procured from Tokyo Chemical Industry India Pvt. Ltd. Biochemical kits for CK-MB (creatine kinase-myocardial band) and LDH (lactate dehydrogenase) were acquired from ARKRAY, Inc. An ELISA (enzyme-linked immunosorbent assay) kit for HO-1 was sourced from Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd.

Animals

Male Wistar albino rats weighing between 250-300 g were procured from Jai Research Foundation. Animals were maintained in a controlled laboratory environment under standard conditions, and all experimental procedures were performed following the established guidelines set by CCSEA (Committee for Control and Supervision of Experiments on Animals) and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The Institutional Animal Ethics Committee (IAEC) reviewed and approved the experimental protocol under approval number RPCP/IAEC/2020-2021/R16.

Isolated perfused rat heart preparation

The study employed an isolated rat heart model using the Langendorff perfusion technique for the ex vivo experiment [9]. Rats were heparinised and anaesthetised intraperitoneally with Ketamine (75 mg/kg) and Xylazine (10 mg/kg). Once deep anaesthesia was confirmed, hearts were excised (No additional euthanasia procedure was required, as the hearts were excised under deep anesthesia in accordance with CPCSEA guidelines and IAEC-approved protocol) in accordance with the ethical guidelines and promptly transferred into in chilled Krebs-Henseleit (K-H) buffer composed of potassium chloride (4.7 mM), sodium chloride (118 mM), magnesium sulphate (1.2 mM), sodium bicarbonate (25 mM), sodium dihydrogen phosphate (1.2 mM), calcium chloride (2.5 mM) and glucose (11 mM) and adjusted to pH 7.4. Cannulation of the hearts was performed through the aorta and mounted onto a Langendorff apparatus. The hearts were retrogradely perfused with Krebs-Henseleit buffer at a constant flow rate of 15 ml/min, maintained at 37°C, and continuously aerated with carbogen (95% O_2 and 5% CO_2 mixture). A fluid-filled latex balloon, connected to a pressure transducer (SS13L), was positioned into the left ventricle to monitor cardiac function, with

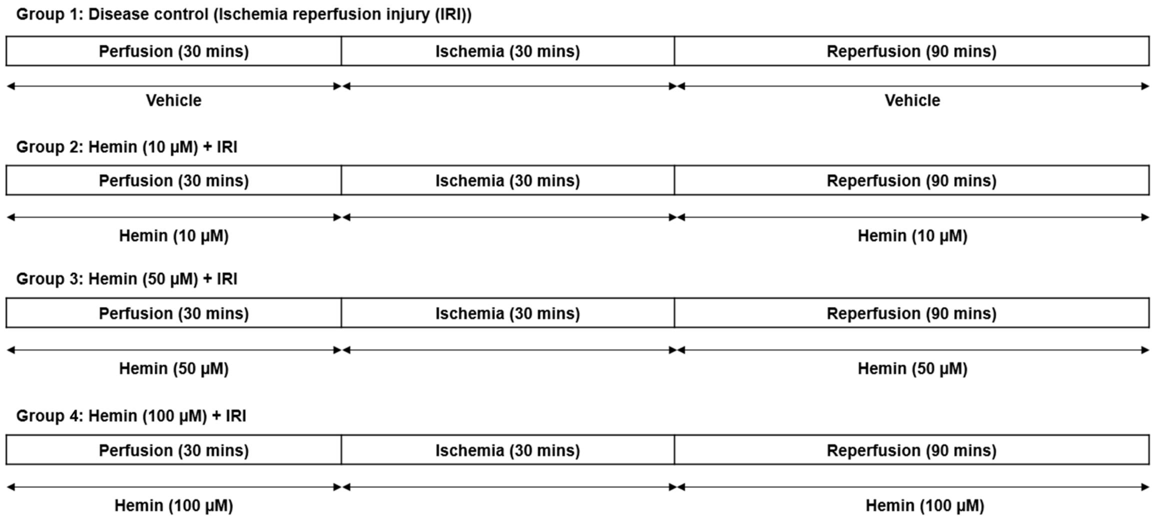

Figure 1. Experimental protocol (Part-1).

preload adjusted to 5-10 mm Hg through balloon inflation. The cannulated hearts were allowed to stabilized. Ischemia-reperfusion injury (IRI) was then induced by subjecting the hearts to 30 minutes of ischaemia, followed by 90 minutes of reperfusion.

Experimental protocol

The experiment was conducted in two parts, involving a total of nine groups with six rats per group.

*Part 1: Dose optimization of Hemin: (**Figure 1**)*

The first part of the study was performed to find out the optimal dose of Hemin for the HO-1 induction and its associated cardioprotection.

Group 1: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer and, then subjected to the induction of ischemia reperfusion injury.

Group 2: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 10 μM Hemin (HO-1 inducer) and, then subjected to the induction of ischemia reperfusion injury.

Group 3: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 50 μM Hemin (HO-1 inducer) and, then subjected to the induction of ischemia reperfusion injury.

Group 4: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 100 μM Hemin (HO-1 inducer) and, then subjected to the induction of ischemia reperfusion injury.

*Part 2: Mechanistic study using pathway inhibitors: (**Figure 2**)*

The second part of the study was performed to investigate the involvement of pro-survival signalling pathways in HO-1 mediated cardioprotection. Specific pharmacological inhibitors were selected based on their established roles in myocardial ischemia-reperfusion injury. Chelerythrine, L-NAME (L-arginine methyl ester) inhibitor, Wortmannin, and Glibenclamide were used to inhibit PKC (Protein kinase C), PI3K (Phosphatidylinositol 3-kinase), NOS (Nitric oxide synthase), and K_{ATP} (ATP-dependent potassium channel), respectively. Their inhibition allowed us to evaluate the involvement of these pathways in mediating cardioprotective effects of HO-1 activation.

Group 5: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 50 μM Zinc Protoporphyrin IX (HO-1 inhibitor) and, then subjected to the induction of ischemia reperfusion injury.

Group 6: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 10 μM Chelerythrine (PKC inhibitor) and

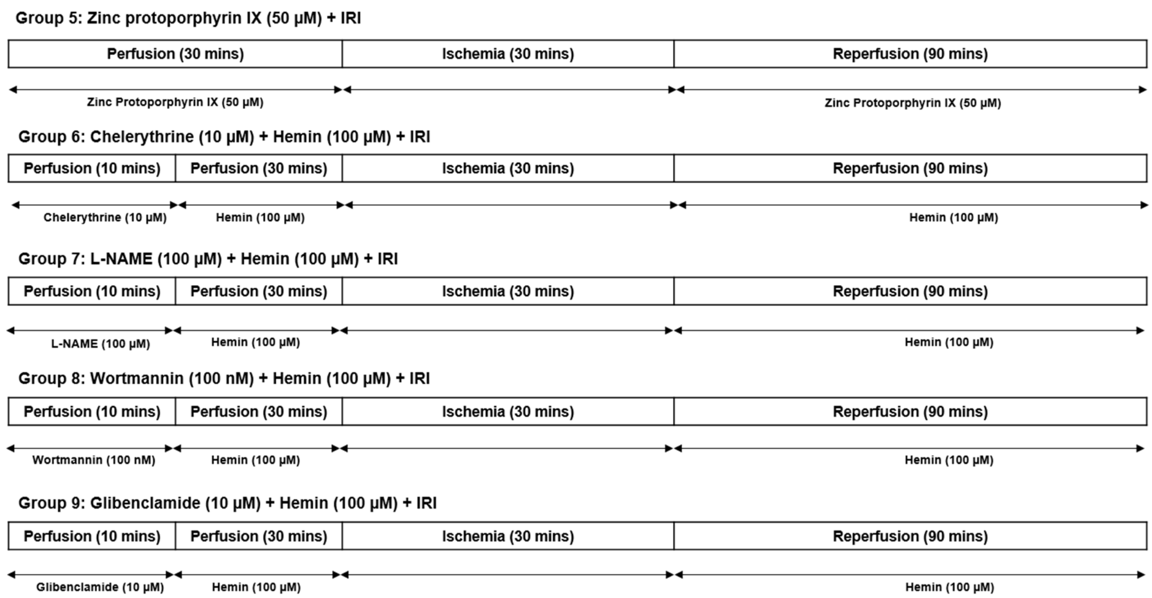

Figure 2. Experimental protocol (Part-2).

Hemin (HO-1 inducer) and, then subjected to the induction of ischemia reperfusion injury.

Group 7: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 100 μM L-NAME (NOS inhibitor) and Hemin (HO-1 inducer) and, then subjected to the induction of ischemia reperfusion injury.

Group 8: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 10 nM Wortmannin (PI3K inhibitor) and Hemin (HO-1 inducer) and, then subjected to the induction of ischemia reperfusion injury.

Group 9: The perfusion of isolated hearts was carried out with Krebs-Henseleit buffer containing 10 μM Glibenclamide (K_{ATP} blocker) and Hemin (HO-1 inducer) and then subjected to the induction of ischemia reperfusion injury.

HO-1 activity assay

Following the experiment, heart tissues were homogenized, and HO-1 activity was assessed using a commercial ELISA kit as per the manufacturer's protocol.

Assessment of haemodynamic and cardiodynamic parameters

Throughout the experiment, cardiac functional data such as LVDP (left ventricular developed pressure), LVEDP (left ventricular end-diastolic pressure), dp/dt_{max} (peak rate of contraction), dp/dt_{min} (peak rate of relaxation), BPM (Beats per minute, heart rate), and coronary flow were recorded using Biopac data acquisition system. Data were evaluated before ischemia and after reperfusion. % recovery was calculated as the ratio of post-ischemia-reperfusion (post-I/R) values to the corresponding baseline (pre-ischemic) values, expressed as a percentage. The formula used was: (Post-I/R value ÷ Baseline [pre-ischemic] value) × 100. This calculation reflects the proportion of cardiac function retained after I/R injury due to Hemin treatment [10, 11].

Measurement of myocardial injury markers

Levels of CK-MB and LDH in coronary effluent were quantified before ischaemia and immediately after reperfusion according to the protocols of commercially available kits using biochemical analyser.

Determination of oxidative stress and antioxidant enzyme activity

At the end of the reperfusion period, hearts were rinsed with Phosphate buffered saline (PBS) and homogenized in 10 mM Tris-HCl buffer. Homogenates were centrifuged at 5000 rpm for 10 minutes at 4°C. MDA

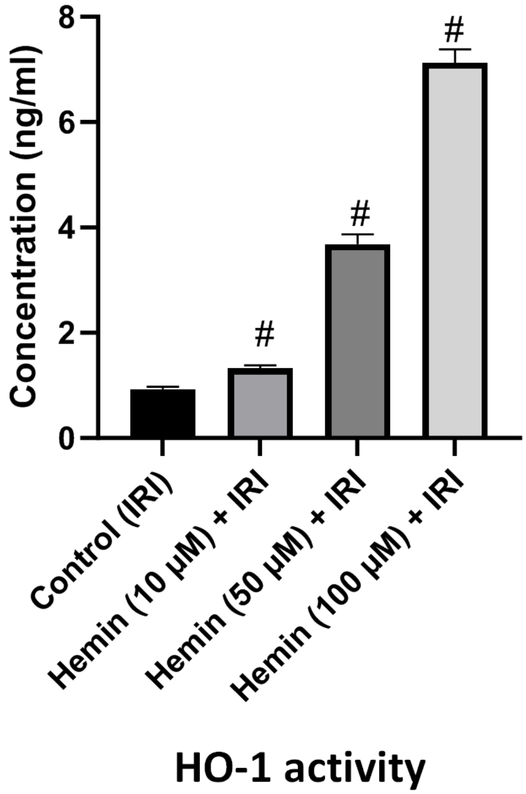

Figure 3. Effects of Hemin on HO-1 activity. HO-1 activity was assessed following IRI. Data are presented as mean ± SEM (n=6). '#' denotes P<0.05 compared to the control (IRI) group. IRI, Ischemia reperfusion Injury.

(Malondialdehyde) and SOD (Superoxide dismutase) were assessed to evaluate oxidative stress and antioxidant defence [12, 13].

Histopathologic analysis

After the experiment, hearts were rinsed with PBS and fixed in 10% formalin for the purpose of cell fixation. The formalin-fixed samples were submitted to an external pathology laboratory for standard histological processing. Tissue sections for the histopathological evaluation were stained with hematoxylin and eosin (H&E), which is commonly used for evaluating cardiac tissue morphology. The stained sections were assessed for features of ischemia-reperfusion injury, including myocardial necrosis (eosinophilic fibers with nuclear loss), interstitial edema (widened spaces between fibers), contraction band formation (dense eosinophilic transverse bands), and separation of myocardial fibers.

Statistical analysis

Result data are presented as mean ± standard error of the mean (SEM). Statistical comparison between multiple groups were performed using one-way analysis of variance (ANOVA), followed by Turkey's multiple comparison test as the post hoc analysis. A *p*-value of less than 0.05 was considered significant. All statistical analyses were performed using GraphPad Prism software.

Results

Part 1: Determination of optimal dose of Hemin for the HO-1 induction and cardioprotection

Effects of Hemin on HO-1 activity: HO-1 activity elevated in a dose dependent manner following Hemin treatment. Hearts treated with 10 μM hemin, 50 μM hemin and 100 μM hemin showed significantly elevated HO-1 activity compared to the control group (P<0.05), indicating successful enzyme induction (**Figure 3**).

Effects of Hemin induced HO-1 on cardiodynamic and hemodynamic parameters: Hemin treatment resulted in significant improvement in cardiac function following ischemia-reperfusion. Significant recovery (P<0.05) was observed in LVDP and LVEDP in hearts treated with different doses of hemin when compared to the hearts of the untreated group (**Figure 4A** and **4B**). Similarly, dP/dt_{max} significantly improved in the 100 μM hemin group, and dP/dt_{min} improved significantly in the 50 μM and 100 μM hemin groups (P<0.05) (**Figure 4C** and **4D**). BMP and coronary flow also improved in a dose-dependent manner, with significant recovery in the coronary flow (CF) of hearts treated with 100 μM hemin (P<0.05) (**Figure 4E** and **4F**). These findings confirm the improvement in cardiodynamic and hemodynamic data due to HO-1 induction following hemin treatment.

Effects of Hemin induced HO-1 on myocardial injury markers: CK-MB and LDH levels were significantly reduced following hemin treatment in a dose-dependent manner. CK-MB was significantly lower in the 50 μM and 100 μM hemin groups (P<0.05), while LDH levels were significantly reduced in all the hemin treated groups

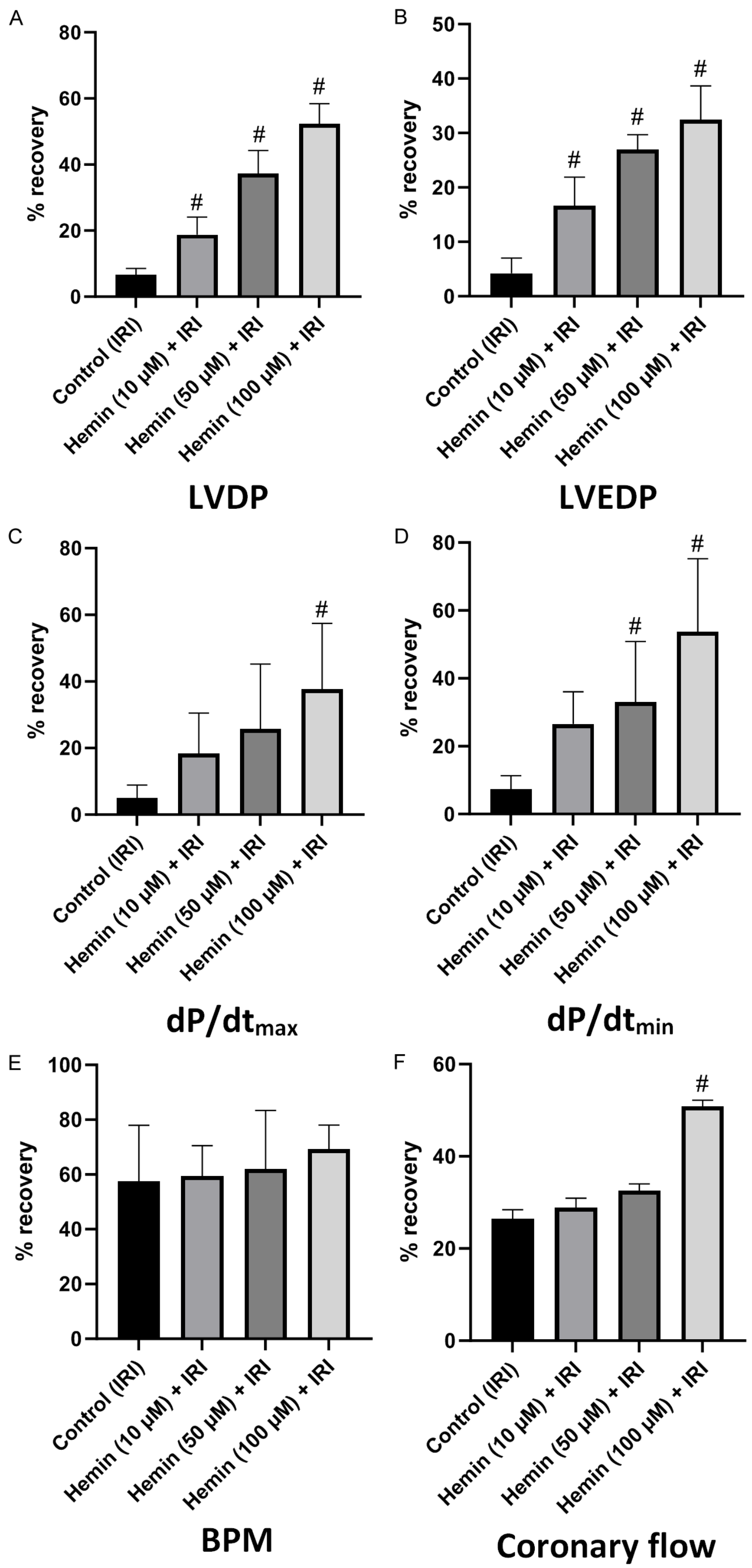

Figure 4. Effects of HO-1 induction by Hemin on LVDP, LVEDP, dP/dt_{max}, dP/dt_{min}, BPM, and Coronary flow in terms of % recovery. All the data were measured before ischemia and after reperfusion. Data are presented as mean ± SEM (n=6). '#' denoted P<0.05 compared to the control (IRI) group. IRI, Ischemia reperfusion Injury.

compared to controls (P<0.05) (**Figure 5A** and **5B**).

Effects of Hemin induced HO-1 on oxidative stress and antioxidant activity: Hemin treatment mitigated oxidative stress, indicated by reduced MDA levels and enhanced SOD activity in heart tissue homogenates. MDA levels significantly reduced across all hemin-treated groups (P<0.05), while SOD activity increased significantly in the 10 μM, 50 μM and 100 μM hemin treated groups in a dose-dependent manner (P<0.05) (**Figure 6A** and **6B**).

Effects of Hemin induced HO-1 on histopathology: Histopathologic analysis revealed that IRI caused notable structural alterations, including myocardial necrosis, interstitial edema, contraction bands, and fibre separation. These changes were markedly reduced in hemin treated groups, indicating structural preservation and reduced tissue damage (**Figure 7**).

Part 2: Evaluation of pro-survival signalling pathways involved in HO-1 mediated cardioprotection

Effects of pharmacological inhibitors of pro-survival signaling pathways on HO-1 activity: Hearts treated with hemin showed significantly elevated HO-1 activity compared to control group (P<0.05). Treatment with the HO-1 inhibitor ZnPP IX significantly reduced HO-1 activity (P<0.05 vs. Hemin). Groups treated with hemin and various pathway inhibitors maintained elevated HO-1 activity, confirming that the inhibitors did not directly affect HO-1 induction **Figure 8**.

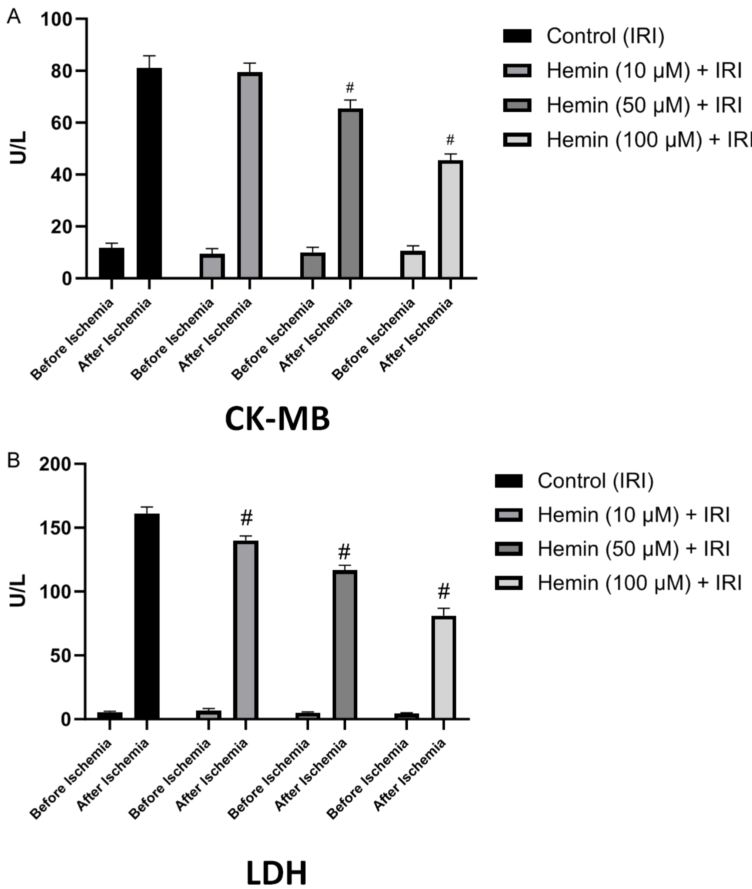

Figure 5. Effects of HO-1 induction by Hemin on CK-MB and LDH. CK-MB and LDH were measured before ischemia and after reperfusion. Data are presented as mean ± SEM (n=6). '#' denotes P<0.05 compared to the control (IRI) group. IRI, Ischemia reperfusion Injury.

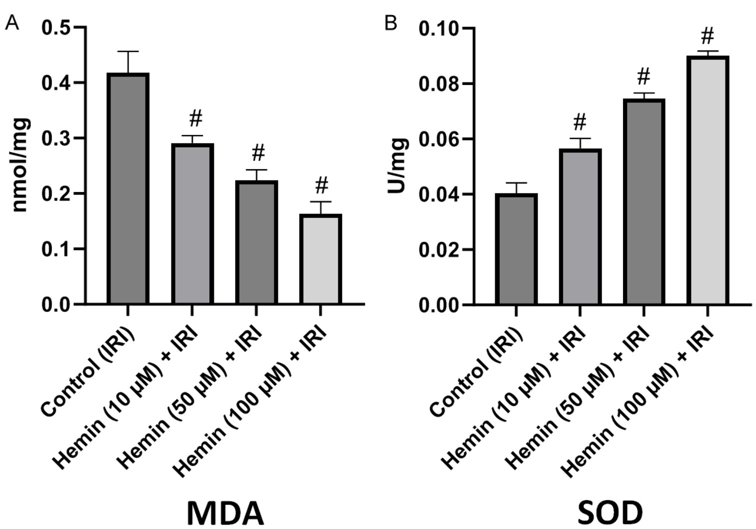

Figure 6. Effects of HO-1 induction by Hemin on MDA and SOD. MDA and SOD were measured after IRI. Data are presented as mean ± SEM (n=6). '#' denotes P<0.05 compared to the control (IRI) group. IRI, Ischemia reperfusion Injury.

Effects of pharmacological inhibitors of pro-survival signaling pathways on cardiodynamic and hemodynamic data: Hemin treatment significantly improved dP/dt_{max}, dP/dt_{min}, LVDP, LVEDP and coronary flow compared to control (P<0.05). Although BPM was improved in hemin treated hearts, the difference was not statistically significant. ZnPP IX abolished these improvements, indicating that these effects were mediated through HO-1 activity. Moreover, co-administration of hemin with specific inhibitors of signaling pathways-namely Wortmannin (PI3K inhibitor), Chelerythrine (PKC inhibitor), L-NAME (NOS inhibitor), and Glibenclamide (K_{ATP} inhibitor)-led to either partial or complete attenuation of these functional improvements.

For instance, the recovery of LVDP was significantly impaired in the groups treated with PKC an PI3K inhibitors and the recovery of LVEDP was significantly impaired in the groups treated with hearts treated with PKC, NOS an PI3K inhibitors (P<0.05 vs. Hemin) (**Figure 9A** and **9B**). Similarly, dP/dt_{max} was significantly reduced in the NOS inhibited group, while dP/dt_{min} declined in both NOS and PI3K inhibitor treated hearts (P<0.05 vs. Hemin) (**Figure 9C** and **9D**). BPM did not exhibit a significant change in any of the inhibitor treated groups while, coronary flow was significantly reduced upon inhibition of PKC, NOS, PI3K and K_{ATP} channels, further confirming involvement of these pathways in HO-1 mediated cardioprotection (P<0.05 vs. Hemin) (**Figure 9E** and **9F**).

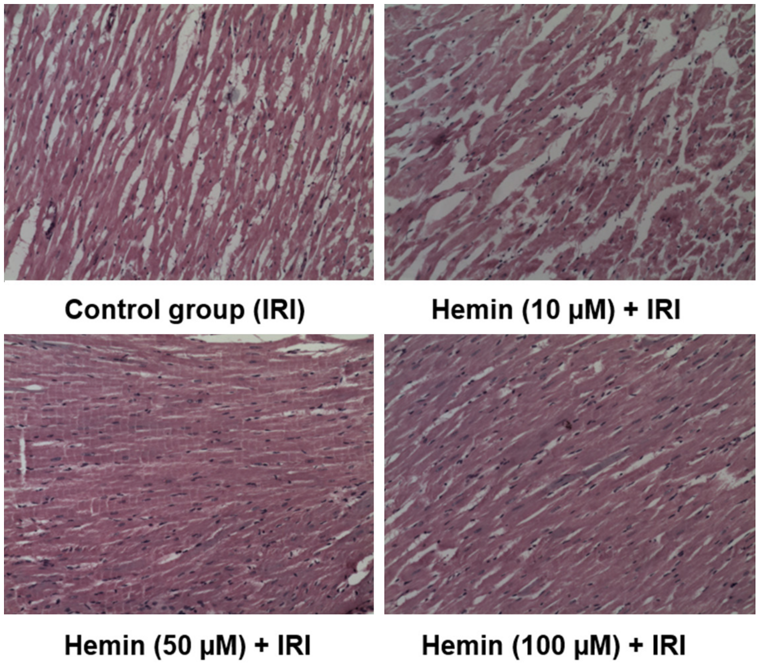

Figure 7. Effects of Hemin-induced Heme oxygenase-1 on histopathology (H&E stain, 400×). Control group (IRI - ischemia Reperfusion Injury), Hemin (10 μM) + IRI, Hemin (50 μM) + IRI, Hemin (100 μM) + IRI.

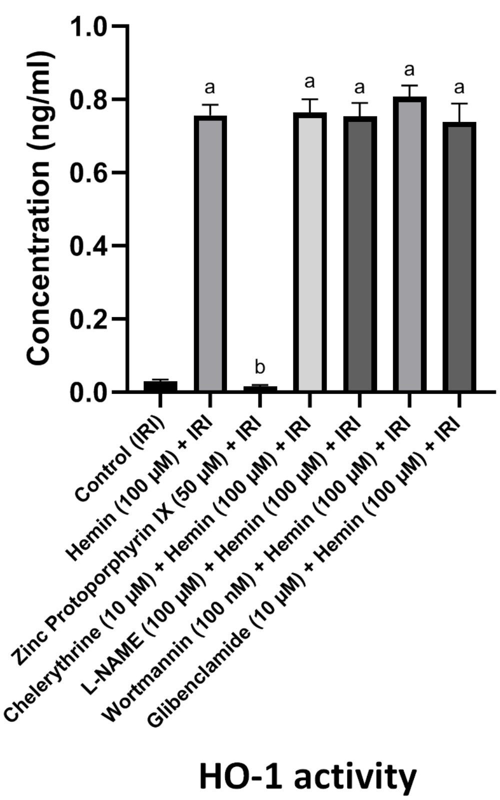

Figure 8. Effects of pharmacologic inhibitors of various cardioprotective mechanisms on HO-1 activity. HO-1 activity was assessed following IRI. Data are shown as mean ± SEM (n=6). 'a' denotes $P<0.05$ compared to the control (IRI) group, while 'b' signifies $P<0.05$ relative to the Hemin treated group. IRI, ischemia Reperfusion Injury.

Effects of pharmacologic inhibitors of pro-survival signaling pathways on myocardial injury markers: Levels of LDH as well as CK-MB, which were significantly reduced by hemin ($P<0.05$), increased significantly upon co-treatment with ZnPP IX or pathway inhibitors ($P<0.05$ vs. Hemin), suggesting that the cardioprotective effects of HO-1 involve these signaling pathways. This trend was observed across all groups treated with pathway inhibitors (**Figure 10A** and **10B**).

Effects of pharmacologic inhibitors of pro-survival signaling pathways on oxidative stress and antioxidant activity: MDA levels, which were lowered by hemin, increased significantly with ZnPP IX and other pathway inhibitors ($P<0.05$ vs. Hemin), indicating enhanced lipid peroxidation due to oxidative stress (**Figure 11A**). In parallel, SOD activity, which was upregulated with hemin treatment, decreased significantly in ZnPP IX and inhibitor treated groups ($P<0.05$ vs. Hemin), reflecting diminished antioxidant defense (**Figure 11B**).

Effects of pharmacologic inhibitors of prosurvival signaling pathways on histopathology: Histologic assessment revealed minimal tissue injury in Hemin-treated group, characterized by preserved myocardial architecture and reduced signs of necrosis and edema. In contrast, hearts treated with ZnPP IX or specific pathway inhibitors exhibited prominent features of IRI, including myocardial necrosis, interstitial edema, contraction band and fibre separation. These histological alterations were consistent with the biochemical and functional data, further affirming the contribution of pro-survival pathways in mediating HO-1-induced protection (**Figure 12**).

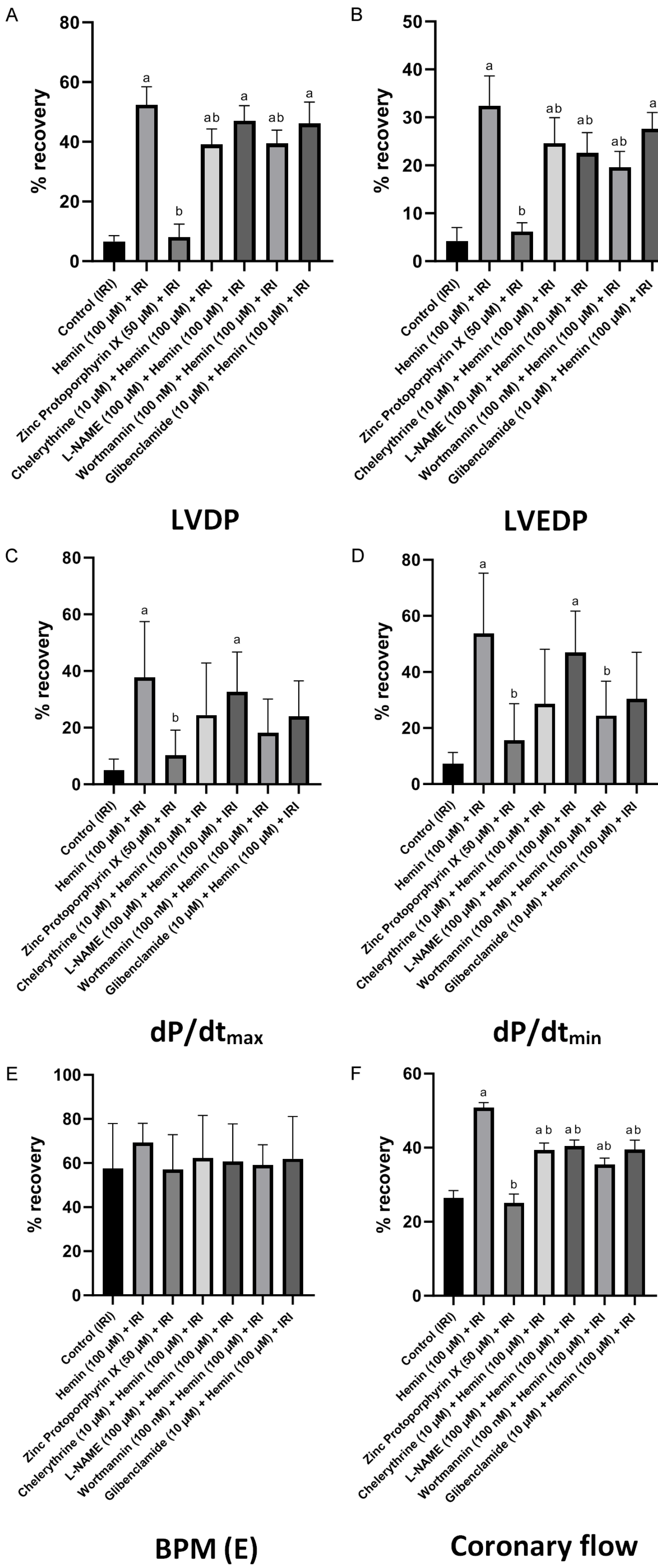

Figure 9. Effects of pharmacologic inhibitors of various cardioprotective mechanisms on LVDP, LVEDP, dP/dt_{max}, dP/dt_{min}, BPM, and Coronary flow in terms of % recovery. All the data were measured before ischemia and after reperfusion. Data are shown as mean ± SEM (n=6). 'a' denotes P<0.05 compared to the control (IRI) group, while 'b' signifies P<0.05 relative to the Hemin-treated group. IRI, ischemia Reperfusion Injury.

Discussion

The current study investigated the cardioprotective effects of Heme Oxygenase-1 modulation by its inducer Hemin [14], and the underlying mechanisms of HO-1 mediated cardioprotection. Three doses of HO-1 inducer Hemin - low (10 μM), medium (50 μM), and high (100 μM) were selected based on prior studies and subsequent standardization. The investigation was carried out in two parts. The first part evaluated cardioprotective effect of HO-1 induced by different doses of Hemin in isolated rat hearts subjected to ex vivo ischemia reperfusion injury. The second part investigated the underlying mechanism of HO-1 mediated cardioprotection.

Hemin induced HO-1 in a dose-dependent manner, as evidenced by increased HO-1 activity in cardiac tissue homogenate. HO-1 catalytically degrades heme into carbon monoxide (CO), biliverdin, and iron which are proven to have antioxidant, anti-inflammatory, vasodilatory and anti-apoptotic effects essential for protection during ischemia reperfusion injury [15]. HO-1 induction had also been reported to confer direct cardioprotection inde-

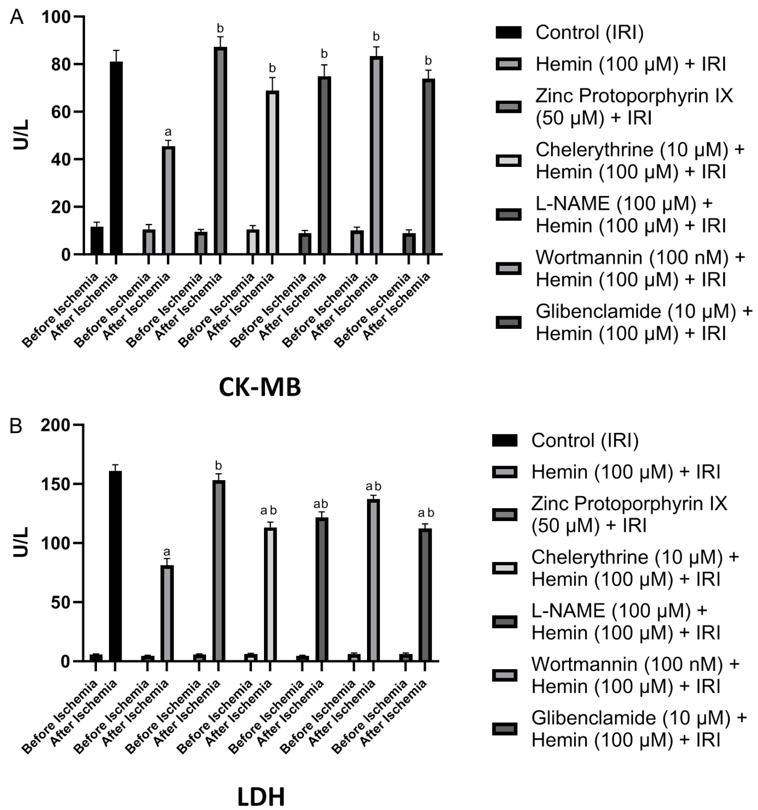

Figure 10. Effects of pharmacologic inhibitors of various cardioprotective mechanisms on CK-MB and LDH. CK-MB and LDH were measured before ischemia and after reperfusion. Data are shown as mean ± SEM (n=6). 'a' denotes P<0.05 compared to the control (IRI) group, while 'b' signifies P<0.05 relative to the Hemin-treated group. IRI, ischemia Reperfusion Injury.

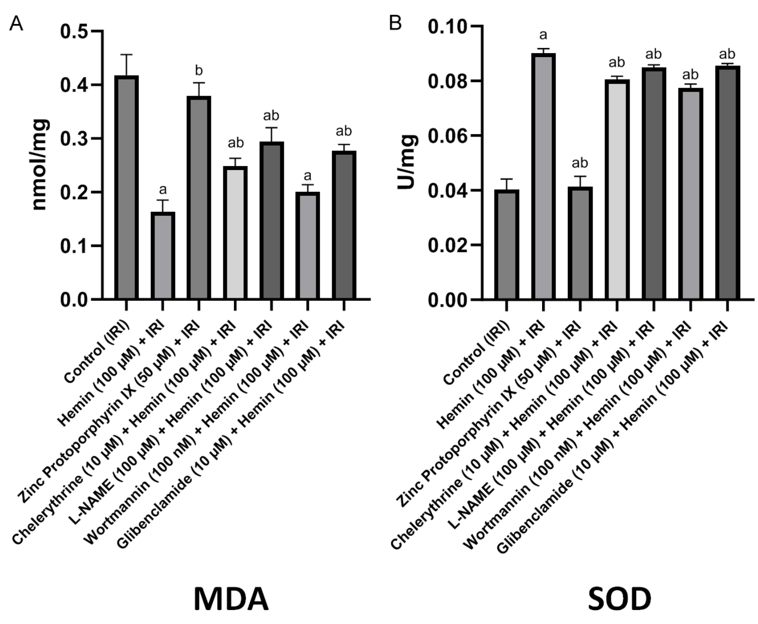

Figure 11. Effects of pharmacologic inhibitors of various cardioprotective mechanisms on MDA and SOD. MDA and SOD were measured after IRI. Data are shown as mean ± SEM (n=6). 'a' denotes P<0.05 compared to the control (IRI) group, while 'b' signifies P<0.05 relative to the Hemin-treated group. IRI, ischemia Reperfusion Injury.

pendent of its enzymatic products [16]. In this study, HO-1 mediated cardioprotection was reflected in the recovery of several cardiodynamic and haemodynamic measures (LVEDP, LVDP, dP/d_{tmax}, dP/dt_{min} and coronary flow), reduction in cardiac injury markers (LDH and CK-MB), and improved histopathologic findings.

Cardiac contractile function and performance are determined by the ventricle's ability to generate pressure. Reduced LVDP indicated impaired contractility [17]. LVEDP reflects preload, ventricular compliance, and overall cardiac function. It reflects the volume and pressure in the left ventricle just before contraction. Abnormal LVEDP may signal diastolic dysfunction [18]. Hemin-treated hearts showed improvements in LVDP and LVEDP, indicating preserved cardiac function during IRI. dP/d_{tmax} and dP/dt_{min} reflects the heart's ability to contract and relax, respectively [19]. Both dP/d_{tmax} and dP/dt_{min} improved with hemin treatment. The heart rate influences the balance between myocardial oxygen demand and supply. Injury to myocardium results in difficulty in maintaining adequate perfusion and a mismatch between myocardial perfusion andcontraction. The complex process of IRI results in fluctuations in the heart rate such as initial bradycardia, followed by arrhythmias, and potentially sustained tachycardia

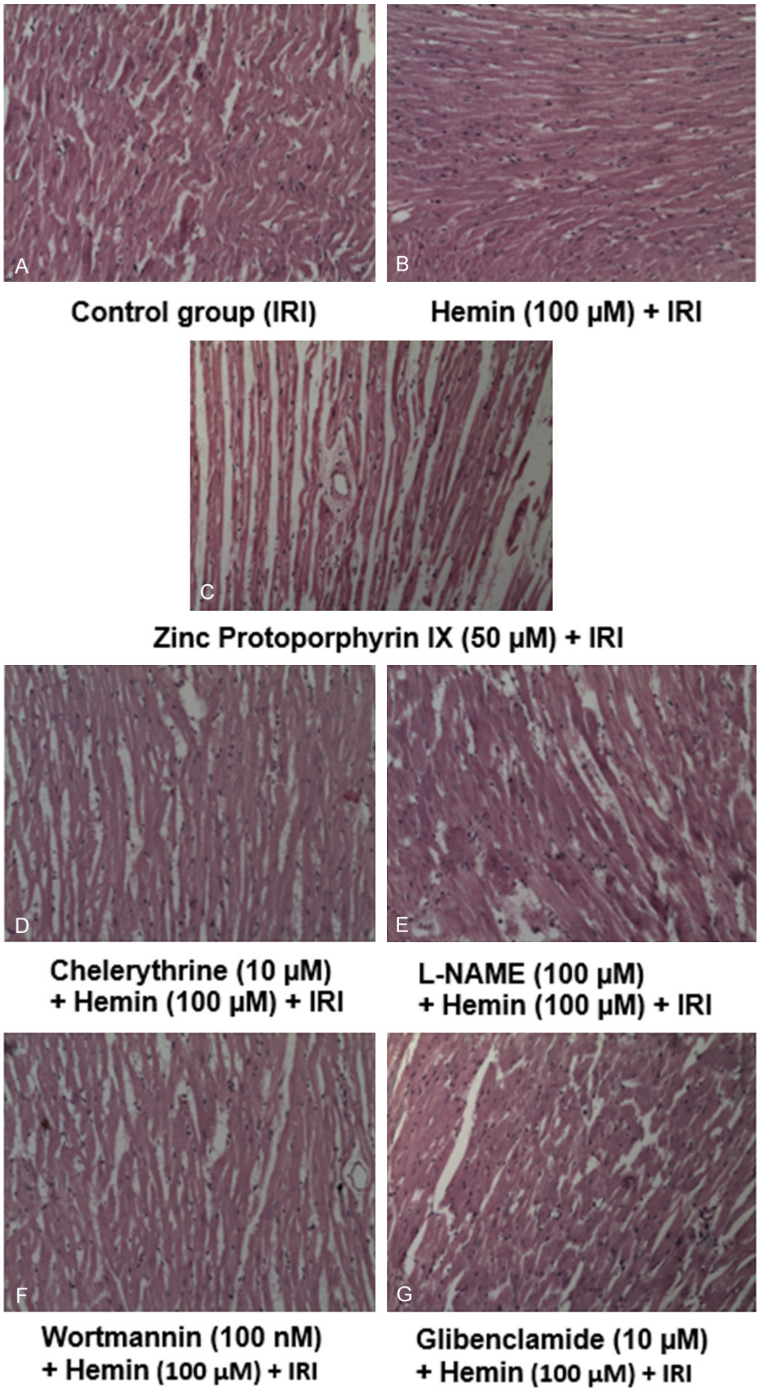

Figure 12. Effects of pharmacologic inhibitors of various cardioprotective mechanisms on histopathology (H&E stain, 400×). (A) Control group (IRI, ischemia Reperfusion Injury), (B) Hemin (100 μM) + IRI, (C) Zinc Protoporphyrin IX (50 μM) + IRI, (D) Chelerythrine (10 μM) + Hemin (100 μM) + IRI, (E) L-NAME (100 μM) + Hemin (100 μM) + IRI, (F) Wortmannin (100 nM) + Hemin (100 μM) + IRI, (G) Glibenclamide (10 μM) + Hemin (100 μM) + IRI.

[20]. Similar trends were observed in this study as indicated by BPM in various treatment groups. Coronary circulation impaired during ischemic phase resulted in myocardial injury. During reperfusion, sudden reinstatement of perfusion leads to several changes such as endothelial dysfunction, increased vascular permeability, and microvascular obstruction [21]. There was improvement in coronary circulation in hemin-treated hearts, as indicated by enhanced coronary flow. Cardiac biomarkers are crucial for detecting myocardial injury. Lower CK-MB and LDH levels in hemin-treated groups reflected reduced myocardial injury [22]. IRI results in oxidative stress and subsequent overwhelming of the antioxidant defence system and damage to the structural and functional components of the myocardium [23]. Hemin treatment reduced oxidative stress and enhanced antioxidant capacity, as seen in MDA and SOD levels, respectively. Various histopathologic changes occur during IRI such as myocardial necrosis, interstitial edema, contraction bands, and fiber separation [24]. These damaging histopathologic changes were also ameliorated in hemin-treated hearts.

Multiple cellular and molecular pathways govern survival of myocardium during IRI. Targeting these pro-survival pathways may reduce IRI associated damage and may exert cardioprotection [25]. Several studies have reported PI3K, PKC, NOS, and K_{ATP} channels as important pro-survival signaling during myocardial IRI. PI3K signaling preserves the myocardium during ischemic reperfusion injury by halting the process of apoptosis, reducing oxidative stress, suppressing inflammation, and promoting angiogenesis and tissue repair [26]. PKC exerts cardioprotection in myocardial ischemia/reperfusion injury by regulating redox signaling, calcium homeostasis, cell death, oxidative stress, and mitochondrial function [27]. Nitric oxide produces vasodilation, anti-inflammatory actions, and mitochondrial stabilization and thus exerts a protective effect during IRI [28]. ATP-sensitive potassium channels protect the heart during IRI by preventing calcium overload, reducing oxidative stress, conserving energy, protecting mitochondrial function, improving blood flow, and modulating inflammation [29]. The current study explored a possible role of above pro-survival signaling pathways in cardioprotection exerted by HO-1 induction. In this study, co-treatment with specific inhibitors of these pathways such as Chelerythrine, L-NAME, Wortmannin and Glibenclamide abolished or attenuated the protective effects of HO-1 induction, supporting the contribution of these pathways in cardioprotection.

An important contribution of this work was the demonstration that HO-1 mediated cardioprotection involves the interplay of multiple pro-survival signaling pathways in an ex vivo model of myocardial ischemia-reperfusion injury. Our findings established that these pathways function collectively downstream of HO-1 activation. Attenuation of protective effects upon inhibition of each pathway supports their functional relevance. Notably, administration of Zinc Protoporphyrin IX, a selective HO-1 inhibitor, completely abolished the protective effects of Hemin, confirming the central role of HO-1 activity in mediating cardioprotection. The most marked loss of protection was observed with PI3K inhibition, suggesting that this pathway may play a dominant role in the cardioprotective mechanism. These results support the concept that HO-1 acts as a central upstream regulator that orchestrates a network of pro-survival responses, including attenuation of oxidative stress, reduction in myocardial injury, and preservation of cardiac function and architecture following ischemia-reperfusion.

Overall, our findings show that HO-1 induction offers cardioprotection during myocardial ischemia-reperfusion injury by activating multiple pro-survival signaling pathways. The varying degrees of protection loss seen with each pathway inhibitor suggest that all these mechanisms contribute to the overall effect, with PI3K playing a particularly important role. Further studies are needed to better understand how these pathways interact and to identify the specific downstream targets through which HO-1 exerts its protective action.

Conclusion

HO-1 induction by Hemin provides significant protection against IRI in isolated rat hearts. This protection is linked to improved cardiac function, reduced oxidative stress and myocardial injury, and preservation of tissue structure. The involvement of PI3K, PKC, NOS, and K_{ATP} pathways suggests that HO-1 acts through multiple pro-survival mechanisms. These findings support further exploration of HO-1 as a therapeutic target for managing myocardial ischemic injury.

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Disclosure of conflict of interest

None.

Abbreviations

ATP, Adenosine triphosphate; ANNOVA, One way analysis of variance; ARRIVE, Animal Research: Reporting of In Vivo Experiments; BPM, Beats per minute; CCSEA, Committee for the control and supervision of experiments on animals; CK-MB, Creatine kinase - myocardial band; CO, Carbon monoxide; CF, Coronary flow; dp/dt_{max}, peak rate of contraction; dp/dt_{min}, peak rate of relaxation; ELISA, Enzyme - linked immunosorbent assay; HO-1, Heme oxygenase-1; IR, Ischemia reperfusion; IRI, Ischemia - reperfusion injury; NOS, Nitric oxide synthase; IAEC, Institutional Animal Ethic Committee; K_{ATP}, ATP-sensitive potassium channel; KH, Krebs-Henseleit; L-NAME, N(ω)-nitro-L-arginine methyl ester; LDH, Lactate dehydrogenase; LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; MDA, Malondialdehyde; MI, Myocardial infarction; ROS, Reactive oxygen species; PBS, Phosphate-buffered saline; PI3K, Phosphoinositide-3 kinase; PKC, Protein kinase C; SD, Standard deviation; SOD, Superoxide dismutase; ZnPP IX, Zinc Protoporphyrin IX.

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