

Hemin-Induced Heme Oxygenase-1 Induction Preserves Myocardial Architecture and Functions Via PI3K Signalling in Isoproterenol-Induced Myocardial Infarction in Rats

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ABSTRACT

Introduction: Myocardial infarction (MI) is a major cause of cardiac morbidity and mortality, driven by oxidative stress, inflammation, and cell death. Heme oxygenase-1 (HO-1), a stress-responsive enzyme, is known for its cytoprotective and antioxidant properties. **Objective:** The objective of this study was to investigate the cardioprotective potential of HO-1 induction by hemin and to explore the involvement of the PI3K signalling pathway in a rat model of isoproterenol-induced myocardial infarction. **Methods:** Rats were divided into three groups: Normal control., Disease control, and Hemin-treated. ECG parameters, myocardial injury markers (CK-MB and LDH), oxidative stress (MDA), antioxidant enzyme activities (SOD, catalase), HO-1 and PI3K activity, and histopathological changes were evaluated to assess the effect of hemin-induced HO-1 activation. **Results:** Isoproterenol administration induced significant myocardial injury, as evident by ST-segment elevation, pathological Q-waves, increased CK-MB and LDH levels, elevated MDA, suppressed SOD and catalase activities, and histopathological changes. Hemin treatment significantly attenuated these pathological changes, improved antioxidant defenses, and preserved cardiac tissue architecture. Hemin induced HO-1 activation offers significant cardioprotection against isoproterenol-induced myocardial injury. HO-1 activity and PI3K signalling were significantly enhanced in the hemin-treated group, suggesting an association between HO-1 induction and PI3K pathway activation. **Conclusion:** Hemin induced HO-1 induction appears to confer cardioprotection, possibly mediated by PI3K signalling.

Keywords: Heme Oxygenase-1, Hemin, Myocardial Infarction, Cardioprotection, Oxidative Stress, Isoproterenol and PI3K Signaling.

INTRODUCTION

Cardiovascular diseases (CVDs), especially ischemic heart disease (IHD) and stroke, remain the primary cause of mortality worldwide and are significant contributors to long-term disability [1]. Among these, myocardial infarction (MI) stands out as a life-threatening cardiac event and represents the most severe clinical manifestation of coronary artery disease [2]. MI is characterized by the sudden death of myocardial tissue resulting from ischemia, which triggers adverse remodelling of the affected myocardium and causes metabolic and ionic disturbances at the cellular level, ultimately leading to cell death [3]. Although recent advancements in early reperfusion techniques, pharmacotherapy, and standardized care protocols have substantially improved the prognosis of acute myocardial infarction over the past decades, MI continues to be a major global cause of death, highlighting the need for further therapeutic improvements [4]. Recent cardioprotective strategies are aimed at deriving benefits by exerting protection as early as possible during the ongoing ischaemic phase and continued protection upon reperfusion. To get the optimal level of protective stimulus, multi targeted approach may be used directed at diverse intracellular signalling and molecular mechanisms by combining proven conventional and newer approaches [5]. Heme oxygenase-1 (HO-1), metabolic enzyme and a stress protein, has attracted the attention of research community involved in basic and translational research due to its significant control over various cellular, molecular and tissue homeostatic processes [6]. HO-1, a significant inducible isozyme, catalytically breakdown the heme to

various products namely biliverdin (BV) which is subsequently converted to bilirubin BR by the action of an enzyme biliverdin reductase (BVR), carbon monoxide (CO), and ferrous iron (Fe²⁺). The enzyme HO-1 and catalytic breakdown products of heme have various beneficial effects such as immunomodulatory, anti-inflammatory, and cytoprotective effects which could have significance in targeting the pathogenesis of cardiovascular diseases, especially MI and its complications [7]. Various synthetic and natural agents are used as pharmacological inducers of HO-1, including Hemin [8, 9]. Hemin induces HO-1 by activating redox sensitive transcription factors and upstream signalling pathways resulting in transcriptional upregulation and subsequent cytoprotection [10]. Various signalling pathways are involved in the pathogenesis and survival of various cells of cardiac importance such fibroblasts, cardiomyocytes, stem cells, endothelial cells, and monocytes. These intracellular signalling pathways regulate various processes such as inflammation, oxidative stress, apoptosis, myocardial fibrosis, and angiogenesis which are of prime importance in MI and associated complications [11]. Among various signalling pathways, the phosphoinositide 3-kinase (PI3K) signalling pathway stands out for its central role in promoting cardiomyocyte survival, making it a key target for cardioprotective interventions [12]. The PI3K signalling pathway activation provides cardio protection by increasing antioxidant, anti-inflammatory, and autophagy activities and decreasing mitochondrial dysfunction and cardiomyocyte apoptosis and thus plays a significant role in

cardiovascular health and disease [13]. Considering the substantial role of oxidative stress and apoptosis in the pathogenesis of MI, modulation of endogenous protective systems such as HO-1 offers the promising strategy especially due to its potential to activate pro-survival signalling pathways. The current study was designed to evaluate if the cardio protection exerted by HO-1 induction by Hemin could be attributed to the activation of PI3K signalling, thereby offering mechanistic insights into HO-1 mediated cardio protection.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents

Hemin and Isoproterenol (ISO) were procured from Tokyo Chemical Industry India Pvt. Ltd. The biochemical kits of creatine kinase-myocardial band (CK-MB) and lactate dehydrogenase (LDH) were purchased from ARKRAY, Inc. HO-1 enzyme-linked immunosorbent assay (ELISA) kit was purchased from BT LAB, Shanghai Korain Biotech Co., Ltd. PI3K ELISA kit was received from Allianz Biosciences Pvt Ltd.

Animals

Wistar albino rats (Male) weighing 250-300 grams were procured from Jai Research Foundation. Animals were maintained and all the experimental procedures were performed according to the and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA). Institutional Animal Ethics Committee (IAEC) approved the research protocol with protocol approval number RCP/IAEC/2020-2021/R16.

Experimental Design

In the present study, Animals were randomly divided in three groups containing six animals in each group.

- Normal control group: Animals were given Dimethyl Sulfoxide (DMSO) through intraperitoneal (IP) route on alternate days for 7 days. Animals also received normal saline through subcutaneous (SC) route for the last two days of the study.
- Disease control group: Animals were given DMSO through intraperitoneal (IP) route on alternate days for 7 days. Isoproterenol (ISO) (45 mg/kg) was administered via SC route for the last 2 days of the study.
- Hemin treated group: Hemin (4mg/kg, IP) was administered on alternate days for 7 days and ISO (45 mg/kg) was administered via SC route for the last 2 days of the study.

ECG Analysis

Electrocardiogram (ECG) analysis was performed to assess cardiac abnormalities induced by isoproterenol (ISO). Anaesthesia was induced in the experimental rats via intraperitoneal administration of ketamine (75 mg/kg) and xylazine (10 mg/kg). The ECG recordings were obtained using the BIOPAC MP36 System. Electrodes were positioned on the right forelimb, left forelimb, and left hind limb to capture the ECG signals. Key parameters including the ST segment, Q wave and beats per minute (BPM) were evaluated [14].

Detection of Serum Cardiac Injury Markers

Anaesthesia was induced in the experimental rats using intraperitoneal administration of ketamine (75 mg/kg) and xylazine (10 mg/kg) prior to blood collection from retro-orbital plexus [15]. The collected blood samples were centrifuged at 5000 rpm for 10 minutes at 4°C, and the separated serum was stored at -20°C until further analysis. Cardiac injury biomarkers were quantified using commercially available assay kits, following manufacturer's protocols.

Estimation Oxidative Stress and Antioxidant Parameters

For euthanasia, rats were administered a high dose of barbiturates, after which the hearts were excised and rinsed with phosphate-buffered saline (PBS). The cardiac tissues were then homogenized in 10 mM Tris-HCl Buffer and centrifuged at 5000 rpm for 10 minutes at 4°C. The resulting supernatant was used to evaluate oxidative stress marker malondialdehyde (MDA) along with anti-oxidant enzyme, including catalase (CAT), and superoxide dismutase (SOD) [16, 17].

HO-1 Activity Assay

Homogenate of the hearts from each experimental groups were estimated for the HO-1 activity. For the same, ELISA method was employed [18] according to the protocol of commercial kit provided by manufacturer (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd.).

Estimation of Phosphoinositide-3-Kinase (PI3K)

The levels of PI3K in heart tissue homogenates from each experimental group were quantified using an enzyme-linked immunosorbent assay (ELISA) kit [19] following the protocol provided by Allianz Biosciences Pvt Ltd.

Histopathological Analysis

After euthanasia, hearts from each group were excised and rinsed with normal saline to eliminate residual blood. The cardiac tissues were fixed in 10% formalin for preservation. The fixed samples were submitted to a pathology laboratory for histological examination to assess structural alterations in the cardiac tissue [20].

Statistical Analysis

All experimental results were expressed as Mean $\pm$ SEM. Data from various parameter estimations were analysed using one-way ANOVA followed, followed by Turkey's multiple comparison test. Statistical analysis was conducted using GraphPad Prism software, and a p-value of less than 0.05 was considered statistically significant.

Results

Effects of HO-1 Induction by Hemin on ECG Parameters

Isoproterenol administration in disease control group led to significant ST-segment elevation, appearance of pathological Q waves, and an increased heart rate (BPM) compared to the normal control group. HO-1 induction by Hemin notably reduced the ST-segment elevation and normalised the Q-wave morphology, along with a significant reduction in heart rate compared to the disease control, suggesting an improvement in myocardial electrical stability (Figure 1).

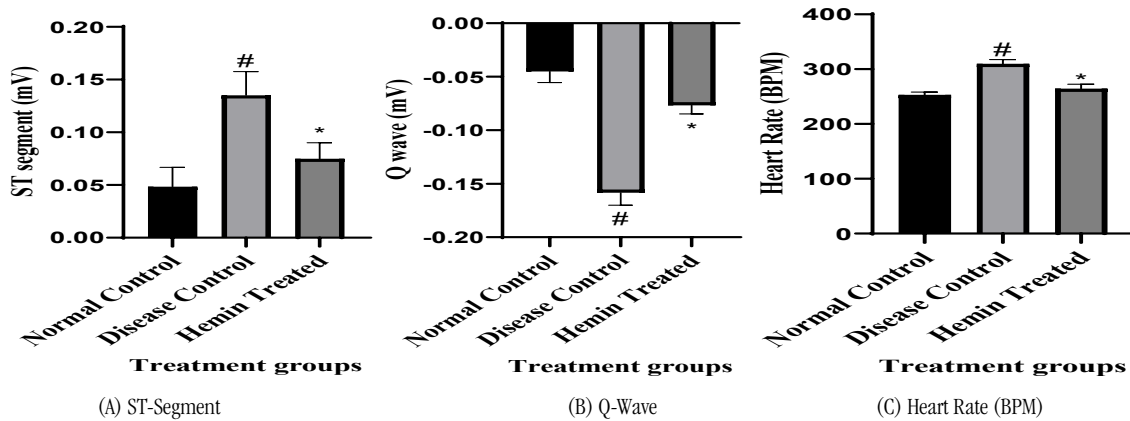


FIGURE 1: Effects of HO-1 Induction by Hemin on Ecg Parameters. All Data are Presented as Mean $\pm$ Sem (N=6). ‘#’ Denotes a Statistically Significant Difference (P<0.05) Compared to the Normal Control Group, while ‘*’ Indicates a Significant Difference (P<0.05) Compared to the Disease Control Group.

Effects of HO-1 Induction by Hemin on Myocardial Injury Markers

LDH and CK-MB, which are the markers of cardiac injury, were measure in the blood serum. The levels of LDH and CK-MB were markedly elevated in disease control group, confirming the extent of

cardiac injury induced by isoproterenol. HO-1 induction by Hemin significantly reduced both CK-MB and LDH levels compared to the disease control group, indicating the protective effect against myocardial injury (Figure 2 (A) and (B)).

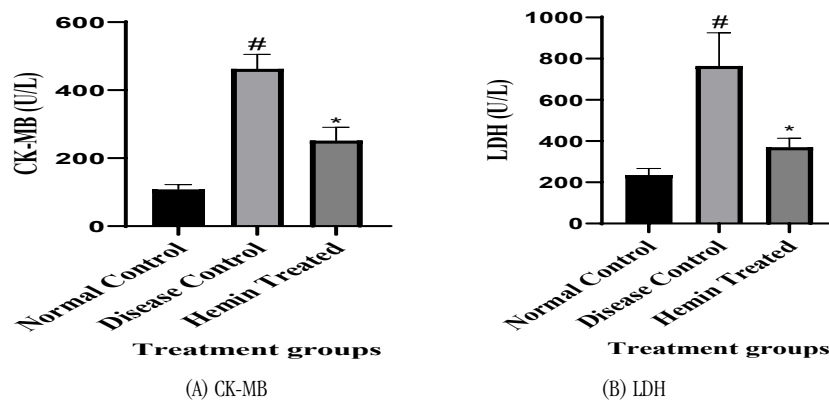


FIGURE 2: Effects of HO-1 Induction by Hemin on CK-MB and LDH. All Data are Presented as Mean $\pm$ SEM (N=6). ‘#’ Denotes a Statistically Significant Difference (P<0.05) Compared to the Normal Control Group, while ‘*’ Indicates a Significant Difference (P<0.05) Compared to the Disease Control Group.

Effects of HO-1 Induction by Hemin on Oxidative Stress Marker and Antioxidant Enzyme Activity

Isoproterenol exposure significantly elevated malondialdehyde (MDA) levels in the cardiac tissue, indicating lipid peroxidation and oxidative stress. Hemin treatment and subsequent HO-1 induction led to a significant decrease in MDA levels, demonstrating its role in

mitigating oxidative damage through HO-1 induction. A substantial decline in the superoxide dismutase (SOD) and catalase activity was observed in the disease control group, reflecting impaired antioxidant defence. Hemin treatment restored both SOD and catalase activities near to normal values, supporting its role in enhancing endogenous antioxidant mechanisms (Figure 3).

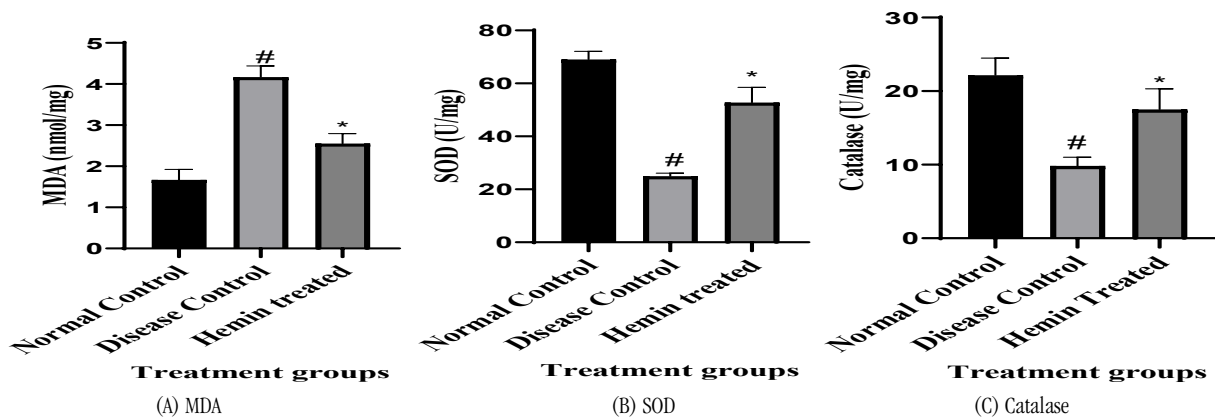


FIGURE 3: Effects Of HO-1 Induction by Hemin on Oxidative Stress Marker and Antioxidant Enzyme Activity. All Data are Presented as Mean $\pm$ SEM (N=6). ‘#’ Denotes A Statistically Significant Difference (P<0.05) Compared to the Normal Control Group, while ‘*’ Indicates a Significant Difference (P<0.05) Compared to the Disease Control Group.

Effects of Hemin on HO-1 Activity

HO-1 activity, as quantified by ELISA was significantly elevated in the Hemin treated group compared to both normal and disease control

groups, confirming the induction of HO-1 enzyme by Hemin (Figure 4).

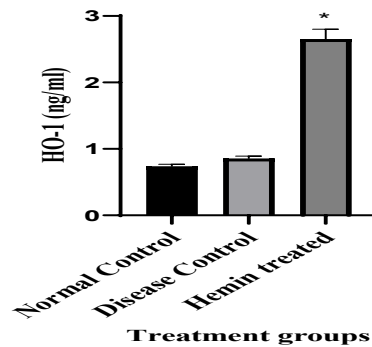


FIGURE 4: Effects of Hemin on HO-1 Activity. All the Values are Expressed as Mean $\pm$ SEM (n=6). '*' indicates $P < 0.05$ Versus Disease Control Group.

Effects of HO-1 Induction by Hemin on PI3K Pathway

PI3K activity was significantly reduced in the disease control group following isoproterenol administration. Hemin pretreatment

significantly enhanced PI3K activity compared to the disease control group, suggesting a potential role of PI3K signalling in HO-1 mediated cardio protection (Figure 5).

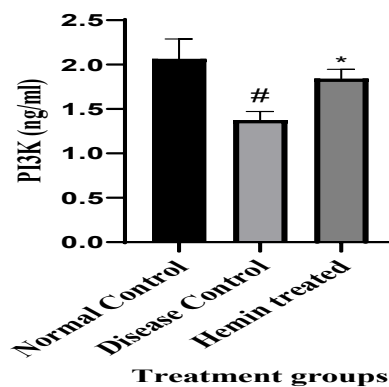


FIGURE 5: Effects of HO-1 Induction by Hemin on PI3K Pathway. All Data are Presented as Mean $\pm$ SEM (N=6). '#' Denotes a Statistically Significant Difference ($P < 0.05$) Compared to the Normal Control Group, while '*' Indicates a Significant Difference ($P < 0.05$) Compared to the Disease Control Group.

Effects of HO-1 Induction on Histopathology of Heart

Histopathological examination of the cardiac tissue from the disease control group revealed typical features of the myocardial infarction, including myocardial fibre disruption, necrosis,

inflammatory cell infiltration, and edema. In contrast, heart sections from the Hemin treated group displayed remarkably preserved myocardial architecture, reduced necrosis, and minimal inflammatory infiltration, supporting other findings of HO-1 mediated cardio protection (Figure 6).

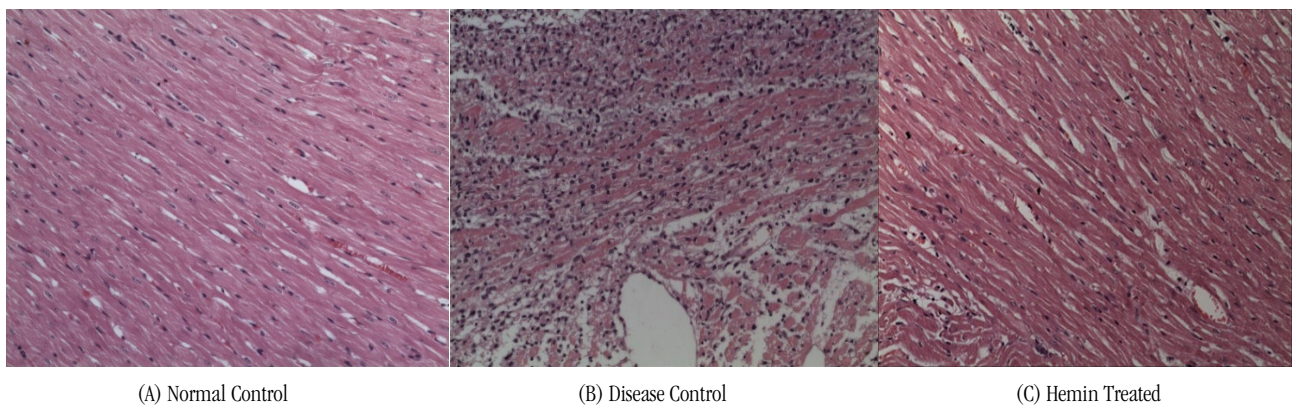


FIGURE 6: Effects of HO-1 Induction by Hemin on Histopathology.

Discussion

In present study, we evaluated cardioprotective potential of Heme oxygenase-1 induced by Hemin using well established model of

Isoproterenol induced MI in rats, with a particular focus on the involvement of the PI3K signalling pathway [21]. Our results demonstrated that HO-1 induction exerted cardio protection, which was evident through improvements in ECG patterns. cardiac enzyme

markers, oxidative stress profile, anti-oxidant enzyme activities and histopathological integrity. Our findings also highlighted a potential mechanistic link between HO-1 mediated cardio protection and PI3K pathway activation, offering further insight into the cellular basis of HO-1 mediated cardio protection. Isoproterenol induced myocardial infarction in rats has long been validated model for mimicking human cardiac injury, as it triggers both electrical conduction abnormalities, biochemical and structural derangements comparable to clinical MI [22]. In present study, disease control group exhibited characteristic ST-segment elevation, pathological Q-wave formation, and increased heart rate, reflecting acute myocardial damage and electrical stability. These observations align with classical MI pathophysiology, where ischemia induced ion channel disruption and infarct related conduction block manifest as ECG abnormalities [23, 24]. In contrast, the Hemin treated group showed marked normalization of these abnormalities, suggesting a preservation of myocardial electrical stability. The observed cardioprotective electrophysiological effect can be mechanistically linked to PI3K pathway activation. The PI3K pathway is known to modulate ion channel activity and maintain membrane potential stability in cardiomyocytes under stress conditions [25]. The activation of PI3K signalling promotes the phosphorylation of downstream targets such as GSK-3 β , eNOS, and FOXO, which regulate calcium homeostasis, myocardial contractility, and resistance to apoptotic stimuli [26]. The improved ECG profiles in the Hemin group may therefore results from PI3K-mediated stabilization of these ion transport and signalling systems, facilitating improved electrical conduction and rhythm preservation during ischemic stress. Serum levels of CK-MB and LDH are sensitive markers of myocardial membrane injury and cellular necrosis. Isoproterenol-induced elevation of these markers, as seen in the disease group, reflects irreversible cellular damage and loss of membrane integrity [27]. This pattern is consistent with the earlier studies highlighting CK-MB and LDH as indicators of myocardial injury [28]. Hemin pretreatment significantly reduced these enzyme levels, suggesting protection against membrane rupture and cellular leakage. Activation of PI3K signalling is known to enhance the expression of anti-apoptotic proteins such as Bcl-2 and suppress pro-apoptotic signals line Bax and caspase-3, thereby preventing the progression from early stress responses to necrotic or apoptotic cell death [29]. The observed biochemical protection is therefore likely mediated, at least in part, by PI3K driven enhancement of cellular survival pathways that fortify membrane integrity. Oxidative stress plays a central role in isoproterenol-induced myocardial damage, and this was reflected in our study by the increase in MDA levels and the concurrent suppression of SOD and catalase activity in the disease control group. Elevated MDA level confirm the occurrence of lipid peroxidation, a direct result of free radical build up during the course of MI [30]. The ability of Hemin to reduce MDA levels and restore the antioxidant enzyme emphasizes HO-1's regulatory role in redox homeostasis, which has been reported to occur via both its enzymatic degradation products and its indirect antioxidant signalling [31]. Mechanistically, PI3K activation exerts a profound antioxidant effect. The PI3K pathway promotes the nuclear translocation of Nrf2, a master transcription factor that governs antioxidant gene expression [32]. This Nrf2/PI3K feedback loop fosters a cellular environment resilient to oxidative stress by enhancing enzymatic detoxification and scavenging of reactive oxygen species (ROS). Thus, the observed normalisation of MDA, SOD, and CAT levels in the Hemin group could be attributed to this coordinated protective mechanism, where both

HO-1 upregulation and PI3K activation work synergistically to neutralize oxidative insults. A pivotal observation in this study was the significant upregulation of HO-1 in the hemin-treated group, as quantified by ELISA. HO-1, an inducible stress-response enzyme, degrades pro-oxidant heme into carbon monoxide, biliverdin, and free iron – each having distinct cytoprotective functions [33]. Bilirubin serves as a potent lipid antioxidant, while carbon monoxide modulates inflammatory pathways and anti-apoptotic signalling, both of which could contribute to the observed reduction in myocardial injury. HO-1 induction by hemin was accompanied by the restoration of PI3K activity, which was markedly suppressed in the disease control group. The PI3k signalling pathway is widely recognized for its role in cellular survival, metabolism, and angiogenesis, and its activation represents a critical component of the heart's adaptive response to ischemic injury [34]. The reduction of PI3K activity in the disease control group likely reflects the inhibitory effects of excessive oxidative stress on survival pathways, as previously reported in models of myocardial infarction and oxidative injury [35]. The observed restoration of PI3K activity following hemin administration suggests that HO-1 mediated cardio protection may be driven through the PI3K signalling. Histopathological examination provided direct structural confirmation of myocardial protection. Isoproterenol exposure resulted in widespread myocyte necrosis, inflammatory infiltration, and interstitial edema, hallmarks of infarct pathology [36]. Conversely, Hemin treated hearts displayed remarkably preserved myocardial architecture, with reduced signs of necrosis and inflammation. Activation of the PI3K pathway exerts cardioprotective effects at the histological level by enhancing cardiomyocyte survival, reducing oxidative stress and inflammation, promoting angiogenesis, and stabilizing mitochondrial function [37]. Collectively, these observations support a functional model wherein Hemin-induced HO-1 induction triggers downstream PI3K pathway activation, conferring multifaceted card protection. The restoration of electrical stability, membrane integrity, oxidative balance, and structural preservation converge on the shared mechanism of enhanced PI3K signalling, positioning this pathway as a critical mediator of HO-1 driven cardiac resilience.

Conclusion

The present study demonstrates that pharmacological induction of heme oxygenase-1 by hemin offers significant protection against isoproterenol-induced myocardial infarction in rats. Hemin treatment effectively reduced myocardial injury markers, oxidative stress, and ECG abnormalities, while improving antioxidant enzyme activity and preserving cardiac tissue architecture. Moreover, the observed restoration of PI3K activity suggests that the cardioprotective effects of HO-1 induction may be mediated through the activation of the PI3K survival pathway. These findings not only highlight the therapeutic potential of HO-1 modulation in cardiac injury but also emphasize the importance of targeting both oxidative stress and survival signalling for comprehensive myocardial protection. Further studies incorporating targeted inhibitors and gene-silencing approaches will be essential to validate and expand upon these mechanistic insights.

Abbreviations

Animal Research: Reporting of In Vivo Experiments (ARRIVE), Beats per minute (BPM), Biliverdin (BV), Biliverdin reductase (BVR), Carbon monoxide (CO), Cardiovascular diseases (CVD), Catalase

(CAT), Committee for Control and Supervision of Experiments on Animals (CCSEA), Creatine kinase-myocardial band (CK-MB), Dimethyl Sulfoxide (DMSO), Electrocardiogram (ECG), Endothelial Nitric Oxide Synthase (eNOS), Enzyme-linked immunosorbent assay (ELISA), Ferrous iron (Fe^{2+}), Forkhead Box O (FOXO), Glycogen Synthase Kinase-3 Beta (GSK-3 β), Heme oxygenase-1 (HO-1), Institutional Animal Ethics Committee (IAEC), Intraperitoneal (IP), Ischemic Heart diseases (IHD), Isoproterenol (ISO), Lactate dehydrogenase (LDH), Malondialdehyde (MDA), Myocardial infarction (MI), Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2), Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF κ B), Phosphate-buffered saline (PBS), Phosphoinositide 3-kinase (PI3K), Subcutaneous (SC), Superoxide dismutase (SOD).

Author Contribution Statement

HK conceptualized the research idea, designed the experiments, performed experimental work including data collection and analysis, and prepared the initial manuscript draft. AM provided guidance on

study design, supervised the research process, reviewed and edited the manuscript, and contributed to data interpretation. Both authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest with regard to this study.

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