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Potential Protective Effects of Boldine in Rat with an Experimental Myocardial Ischemia-Reperfusion Model

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Abstract

Objectives: Myocardial ischemia-reperfusion injury (MIRI) remains a major challenge in cardiovascular medicine due to its complex pathophysiology involving oxidative stress, inflammation, and cellular dysfunction. Boldine, a potent natural alkaloid with antioxidant and anti-inflammatory properties, has demonstrated protective effects in various pathological conditions. However, its potential cardioprotective effects in MIRI remain largely unexplored. This study aims to evaluate the protective effects of Boldine in a rat model of MIRI by assessing oxidative stress markers, histopathological changes, and inflammatory responses.

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Abstract

Materials and Methods: Male Albino Wistar rats were randomly assigned to four groups: Control, Boldine, myocardial ischemia-reperfusion (MIR), and myocardial ischemia-reperfusion + Boldine (MIR+B). Myocardial ischemia was induced by ligating the left anterior descending coronary artery for 30 minutes, followed by 120 minutes of reperfusion. Boldine (50 mg/kg) was administered intraperitoneally at the onset of reperfusion. Cardiac tissue samples were collected for histopathological evaluation and biochemical analysis, including total antioxidant status (TAS), total oxidant status (TOS), and Oxidative Stress index (OSI).

Results: Histopathological analysis revealed significant myocardial disorganization and inflammation in the MIR group compared to controls ($p=0.05$). Boldine treatment significantly reduced inflammation and myocardial disorganization in the MIR+B group ($p<0.05$), suggesting a protective effect. Biochemical analysis showed a marked decrease in TAS levels and an increase in TOS and OSI in the MIR group ($p<0.001$). However, Boldine administration significantly restored TAS levels and reduced TOS and OSI in the MIR+B group ($p< 0.001$), indicating attenuation of oxidative stress.

Conclusion: Boldine exhibits significant cardioprotective effects in a rat model of MIRI by reducing oxidative stress, mitigating myocardial disorganization, and alleviating inflammation. These findings suggest that Boldine may serve as a therapeutic agent in ischemic heart disease. Further research is warranted to elucidate its precise mechanisms of action and potential clinical applications.

Keywords: Myocard, ischemia-reperfusion, boldine, TAS, TOS, OSI, interstitial fibrosis, inflammation, myocardial disorganization

Introduction

Ischemic heart disease (IHD) remains one of the leading causes of morbidity and mortality world-wide, with cardiovascular diseases accounting for nearly half of all deaths annually in developed countries[1]. The most common form of IHD, coronary artery disease, is characterized by the progressive narrowing of coronary arteries due to atherosclerotic plaque formation, and, in some cases, vasospasm[2]. This reduction in myocardial blood supply often manifests clinically as angina, myocardial infarction, or chronic heart failure, placing a substantial burden on healthcare systems globally[3]. The pathophysiological hallmark of IHD is an imbalance between myocardial oxygen supply and demand, which leads to ischemic injury and subsequent complications[4]. As therapeutic interventions continue to advance, current research is increasingly focused on strategies to mitigate ischemic damage and promote myocardial recovery[5-7].

Myocardial ischemic injury results from a significant disruption in coronary blood flow, leading to a spectrum of clinical manifestations. Decades of research have provided insights into the complex responses of myocardial tissue to ischemia, uncovering a cascade of metabolic and structural changes that can progress to irreversible damage[2-4]. Ischemia is marked by oxygen deprivation due to insufficient blood supply, which causes cellular energy depletion and the accumulation of toxic metabolic byproducts[2,8,9]. Although the restoration of blood flow is essential for clearing metabolites and initiating tissue repair, reperfusion paradoxically exacerbates tissue damage through mechanisms such as oxidative stress, calcium overload, and inflammatory responses[2,10,11]. This dual-edged nature of ischemia-reperfusion (IR) injury highlights the intricate complexity of myocardial pathology.

Myocardial ischemia-reperfusion injury (MIRI) involves a cascade of metabolic, structural, and histopathological changes that begin during ischemia and are exacerbated upon reperfusion. During ischemia, the deprivation of oxygen and nutrients leads to a decline in

oxidative phosphorylation, resulting in a marked reduction in the synthesis of high-energy phosphates such as adenosine triphosphate (ATP) and phosphocreatine. This energy deficit impairs the Na^+/K^+-ATPase pump, leading to intracellular accumulation of sodium and calcium ions[12]. Elevated calcium levels activate proteases and phospholipases, compromising the integrity of cellular membranes and contractile proteins[2,12]. Anaerobic glycolysis becomes the primary energy source, producing lactate and hydrogen ions, which cause intracellular acidosis and disrupt ion transport systems. This acidosis promotes the production of pro-inflammatory cytokines and diminishes antioxidant enzyme activity, rendering cells highly vulnerable to oxidative stress upon reperfusion[2,4]. Histologically, ischemic cardiomyocytes exhibit early changes such as mitochondrial and sarcoplasmic reticulum swelling, cytoplasmic vacuolization, and chromatin clumping in the nucleus[2,12]. Prolonged ischemia leads to irreversible damage, including contraction band necrosis due to hypercontracted myofibrils and calcium phosphate deposition in mitochondria. These changes are accompanied by membrane defects and leakage of intracellular contents. Upon reperfusion, the rapid influx of oxygen exacerbates these injuries by generating excessive reactive oxygen species (ROS). ROS destabilize mitochondrial membranes, further activating proteases and phospholipases, and amplifying cellular damage[3,12]. Reperfusion also elicits significant inflammatory responses, marked by neutrophil infiltration. These inflammatory cells release proteolytic enzymes and ROS, compounding the damage. Histopathological findings include interstitial edema, hemorrhage, and microvascular obstruction commonly referred to as the "no-reflow" phenomenon. In infarcted zones, necrotic myocytes display cellular and organelle swelling, while apoptotic cells exhibit nuclear fragmentation and membrane blebbing. These injuries often extend beyond the infarcted region, contributing to adverse ventricular remodeling and long-term myocardial dysfunction[2-4]. This intricate interplay of metabolic and structural damage, coupled with the inflammatory response, underscores the dual challenge posed by IR injury and highlights the critical need for targeted therapeutic strategies to mitigate its effects.

Boldine, an alkaloid derived from the Chilean boldo tree (Peumus boldus), has gained recognition as a potent natural antioxidant with remarkable pharmacological properties[13,14]. Its antioxidant efficacy is primarily attributed to its ability to neutralize ROS, prevent lipid peroxidation (LPO), and enhance cellular antioxidant defenses[14,15]. Studies have demonstrated that Boldine effectively inhibits free radical-induced erythrocyte hemolysis and protects against LPO in human liver microsomes[13,16]. In diabetic animal models, Boldine has been shown to normalize elevated activities of manganese superoxide dismutase (SOD) and glutathione (GSH) peroxidase in pancreatic mitochondria, underscoring its role in mitigating oxidative stress-induced damage[14,17]. Furthermore, Boldine exhibits protective effects on the vascular system by reducing oxidative stress and improving endothelial function. Specifically, Boldine attenuates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated superoxide production, a mechanism implicated in endothelial dysfunction in conditions such as hypertension and diabetes. By decreasing superoxide levels, Boldine preserves nitric oxide (NO) bioavailability, a critical factor for maintaining vascular health[17]. Additionally, studies in animal models have reported that Boldine treatment reduces malondialdehyde levels, a marker of LPO, and enhances mitochondrial integrity in organs such as the liver and pancreas[18-21]. Boldine's neuroprotective properties have also been demonstrated under ischemic conditions. Konrath et al.[22] observed that Boldine significantly reduced lipoperoxidation and enhanced cellular viability in hippocampal slices subjected to oxygen and glucose deprivation, mimicking stroke-induced oxidative stress. Its dual antioxidant and anti-inflammatory properties extend to suppressing inflammatory cytokines, such as interleukin-6 (IL-6), and mitigating oxidative damage in inflammatory conditions.

Although Boldine has been extensively studied for its antioxidant and cytoprotective effects across various organ systems, research specifically addressing its impact on myocardial tissue remains limited. Cardiomyocytes are particularly vulnerable to oxidative damage, especially during IR injury, as the imbalance between ROS production and antioxidant defenses results in cellular dysfunction and necrosis. While Boldine's efficacy has been well-documented in neuroprotective and hepatoprotective models, its potential to safeguard myocardial tissue from IR injury has yet to be fully explored.

This study aims to evaluate the protective effects of Boldine in a rat model of experimental MIRI. By investigating its antioxidant properties and underlying mechanisms of action, we seek to determine whether Boldine can mitigate oxidative damage, preserve myocardial function, and reduce overall tissue injury during IR. This research aims to provide new insights into Boldine's therapeutic potential in cardiovascular medicine and explore its applicability as a cardioprotective agent in IHD.

Materials and Methods

Animals

This study was conducted using male Albino Wistar rats, aged 12 weeks and weighing between 270 and 320 grams. The animals were sourced and raised within a specialized experimental research facility in Ankara, Türkiye (approval no: E-66332047-604.01-1128916, date: 27.12.2024) and were performed in a dedicated laboratory for animal research. The rats were housed individually in a controlled environment, with the temperature maintained at 20-21 °C and humidity levels kept within a range of 45-55%. A 12-hour light/dark cycle was strictly observed, and the animals had unlimited access to standard laboratory chow and purified water.

Chemicals

Boldine was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). For treatment, a fresh solution was prepared by dissolving the powdered compound in distilled water, which was then administered via intra-abdominal injection at a dose of 50 mg/kg. The dosage used in this study was based on previously established reference data[23].

Experimental Protocol

The animals were randomly divided into four groups, each consisting of six rats: Control group (C), Boldine group (B), myocardial ischemia-reperfusion (MIR) group, and myocardial ischemia-reperfusion + Boldine group.

Anesthesia was induced using intraperitoneal injections of 50 mg/kg ketamine and 10 mg/kg xylazine, with the same dose of ketamine administered to all rats to standardize its effect on cardiac output[10]. The rats' trachea was cannulated for artificial ventilation, and the rats' chest was shaved before securing the rats in a supine position on the operating table. A left thoracotomy was performed approximately 2 mm left of the sternum, between the fourth and fifth intercostal spaces. After opening the pericardium, the left anterior descending (LAD) coronary artery was visualized, and an 8/0 silk suture was carefully placed around it using a 10-mm micropoint reverse-cutting needle. Ischemia was induced by tightening the suture with a plastic snare for 30 minutes. Reperfusion was initiated by releasing the tension and allowing unrestricted blood flow for 120 minutes. Boldine (50 mg/kg)[23] was administered intraperitoneally 30 minutes after LAD occlusion. This timing was chosen based on previous studies suggesting optimal absorption and bioavailability during ischemic stress, thereby enhancing its cardioprotective effects during subsequent reperfusion[6].

Throughout the procedure, anesthesia depth was monitored every 10 minutes by assessing reflex responses (intermittent tail pinch and corneal reflex). If a positive response was observed, additional doses of 20 mg/kg ketamine and 5 mg/kg xylazine were administered intraperitoneally[10]. At the end of the reperfusion period, all rats were deeply anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine; Then they were sacrificed via

aortic blood collection (5-10 mL)[6-10]. After the cessation of both heartbeat and respiration, death was confirmed by the absence of the corneal reflex and spontaneous breathing for an additional 2 minutes. Following 120 minutes of reperfusion, myocardial tissue samples were carefully excised for biochemical and histopathological analysis. The tissues were collected intact to prevent trauma and were immediately processed. Samples intended for histopathological examination were fixed in 10% formalin, while those for biochemical analysis were frozen in liquid nitrogen and stored at -80 °C.

Histopathological Analysis

For histological analysis, cardiac tissue samples were fixed in 10% neutral-buffered formalin for 48 hours and subsequently processed for paraffin embedding. The fixed tissues were dehydrated using a graded alcohol series (70%, 80%, 96%, 96%, 100%, 100%), cleared in xylene, and embedded in paraffin blocks. Five-micrometer-thick sections, parallel to the apex-to-base plane, were obtained from paraffin blocks using a microtome (Leica RM2245, Germany), and stained with hematoxylin (05-06004/L, BioOptica, Italy) and eosin (05-11007/L, BioOptica, Italy). Histopathological assessments focused on inflammation, myocardial disorganization, and interstitial fibrosis, which are key indicators of MIRI. Hematoxylin and eosin-stained sections were examined under 200× and 400× magnifications using a light microscope (Leica DM 4000B, Germany) equipped with a computer, and images were analyzed using Leica LAS V4.9 software (Germany). Inflammation was evaluated based on the presence of inflammatory cell infiltration (neutrophils and mononuclear cells) in the interstitial space. Myocardial disorganization was assessed by examining cardiomyocyte alignment, loss of striation, and nuclear changes, including pyknosis and karyolysis. Interstitial fibrosis was analyzed by assessing collagen deposition within the myocardial interstitium. Each parameter was scored using a semi-quantitative grading system, as previously described in the literature. A blinded histologist independently assessed and scored each tissue sample. Mean scores for inflammation, myocardial disorganization, and interstitial fibrosis were calculated for each group and statistically compared using ANOVA followed by post-hoc Tukey tests[5,6,10].

Biochemical Analysis

At the end of the study, serum and cardiac tissue samples were frozen in liquid nitrogen and stored at -80 °C until analysis. Total oxidative status (TOS), total antioxidant status (TAS), and Oxidative Stress index (OSI) were measured to evaluate oxidative stress and antioxidant capacity. Cardiac tissue samples were homogenized in phosphate-buffered saline (PBS, pH 7.4) using a mechanical homogenizer at 4 °C. The homogenates were centrifuged at 10,000 × g for 10 minutes at 4 °C, and the supernatant was collected for biochemical analysis. TAS reflects the total antioxidant capacity of the tissue, which counteracts oxidative stress. TOS quantifies the total oxidant load, representing the degree of oxidative damage. OSI, calculated as the ratio of TOS to TAS, provides a comprehensive measure of redox balance and oxidative stress intensity. TAS levels were assessed using a commercial TAS assay kit (RelAssay Diagnostic®, Türkiye), following the manufacturer's instructions. Briefly, 500 µL of reagent 1 (measurement buffer) was mixed with 30 µL of the tissue homogenate, and the initial absorbance (A1) was recorded at 660 nm using a spectrophotometer (NanoDrop® ND-1000, Thermo Scientific, USA). Subsequently, 75 µL of reagent 2, which contains 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, was added to the reaction mixture. The sample tubes were sealed with paraffin and incubated in a 37 °C water bath (ST 30, NUVE, Türkiye) for 5 minutes. Following incubation, the second absorbance (A2) was measured at 660 nm. For standard calibration, a 1 mmol/L Trolox equivalent (Trolox Eq) solution was used. All measurements (A1 and A2) were performed in triplicate, and the mean values were recorded. The change in absorbance ($^{\Delta}$Abs) was determined as A2-A1, and TAS levels were expressed as mmol Trolox Eq/L. TOS was determined using a commercial TOS assay kit (RelAssay

Diagnostic®, Türkiye) according to the manufacturer's protocol. 500 µL of reagent 1 (measurement buffer) was mixed with 75 µL of the cardiac tissue homogenate, and the initial absorbance (A1) was measured at 530 nm using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, USA). Next, 25 µL of reagent 2 (pro-chromogenic solution) was added to the reaction mixture. The tubes were sealed with paraffin and incubated in a 37 °C water bath (ST 30, NUVE, Türkiye) for 5 minutes. After incubation, the A2 was recorded at 530 nm. For standard calibration, a 10 µmol/L hydrogen peroxide (H_2O_2) equivalent solution was used. The change in absorbance ($^{\Delta}$Abs) was calculated as A2-A1, and TOS levels were expressed as mmol H_2O_2 Eq/L. The OSI was calculated as: OSI = (TOS (µmol H_2O_2 Eq/L) / TAS (µmol Trolox Eq/L) x 100

OSI values provided a quantitative measure of oxidative stress levels in cardiac tissue samples[5,24,25].

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed visually (histograms and probability plots) and analytically (Kolmogorov-Smirnov and Shapiro-Wilk tests). For parametric data, results were presented as mean ± standard error. For histopathological assessments and biochemical analysis, differences among groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons in normally distributed data. A type I error level of 5% ($p<0.05$) was considered statistically significant.

Results

Histopathological Findings

Histopathological evaluations revealed significant differences in inflammation and myocardial disorganization across experimental groups. Inflammation was significantly higher in the MIR group compared to the C and B-only groups (p=0.005 for both comparisons). However, the MIR+B group exhibited a significant reduction in inflammation compared to the IR group (p=0.048), as shown in Table 1 and Figures 1-4.

Similarly, myocardial disorganization was significantly more pronounced in the MIR group, compared to the C and B groups (p=0.003 for both). Notably, the MIR+B group demonstrated a significant reduction in myocardial disorganization compared to the MIR group (p=0.037), suggesting a protective effect of Boldine against structural disorganization (Table 1, Figures 1-4).

In contrast, interstitial fibrosis did not differ significantly among the groups (p=0.073), although the IR+B group exhibited a trend toward reduced fibrosis compared to the IR group (Table 1, Figures 1-4).

Biochemical Findings

Biochemical assessments of cardiac tissue revealed significant intergroup differences in oxidative stress parameters, including TAS, TOS, and OSI. TAS levels were significantly lower in the MIR group compared to the C and B groups ($p<0.001$ and p=0.012, respectively). However, TAS levels were significantly elevated in the MIR+B group compared to the MIR group (p=0.028),

Table 1. Histopathological scores of myocardial tissues (Mean ± SE)

	Control (C)	Boldine (B)	Myocardial ischemia-reperfusion (MIR)	Myocardial ischemia-reperfusion + Boldine (MIR+B)	p-value
Inflammation	0.17±0.17	0.17±0.17	1.17±0.31*;&	0.50±0.22+	0.015
Myocardial disorganization	0.33±0.21	0.33±0.21	1.33±0.33*;&	0.67±0.21+	0.010
Interstitial fibrosis	0.33±0.21	0.33±0.21	1.17±0.31	0.50±0.22	0.073

*p: Statistical significance level was determined using the ANOVA test, with p<0.05 considered significant. *p<0.05: indicates a significant difference compared to the Control group (C). & p<0.05: indicates a significant difference compared to the Boldine group (B). + p<0.05: indicates a significant difference compared to the myocardial ischemia-reperfusion group (MIR), SE: Standard error*

indicating an enhancement of antioxidant capacity with Boldine treatment (Table 2).

TOS levels were markedly higher in the MIR and MIR+B groups compared to the C and B groups ($p<0.001$ for both comparisons). Importantly, the MIR+B group demonstrated significantly reduced TOS levels compared to the MIR group ($p<0.001$), highlighting Boldine's potential to mitigate oxidative burden (Table 2).

OSI, which represents the balance between oxidants and antioxidants, was significantly elevated in the MIR and MIR+B groups relative to the C and B groups ($p<0.001$ for all comparisons). However, the MIR+B group exhibited significantly lower OSI values compared

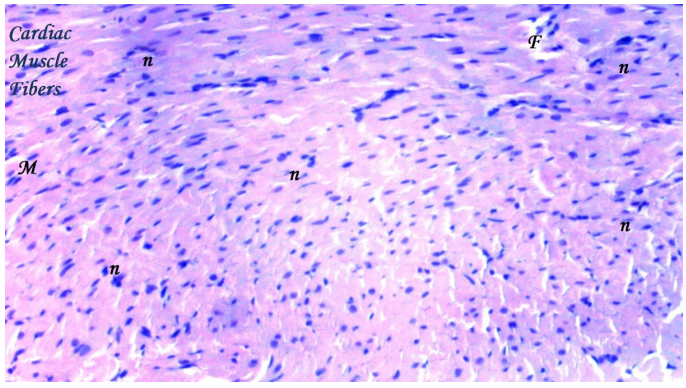

Figure 1. Group Control
M: Myocard, F: Fibrosis, n: Nucleus, H&Ex100

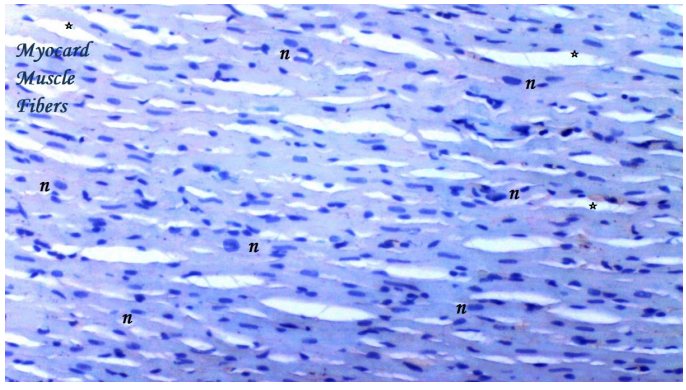

Figure 2. Group Boldine
n: Nucleus, H&Ex100

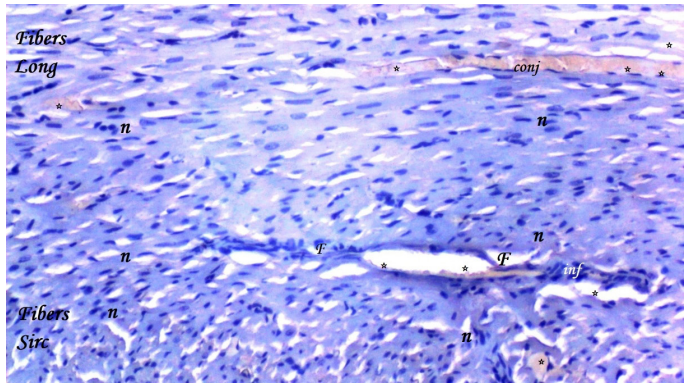

Figure 3. Group Ischemia-reperfusion
F: Fibrosis, n: Nucleus, conj: Congestion, inf: Inflammation, H&Ex100

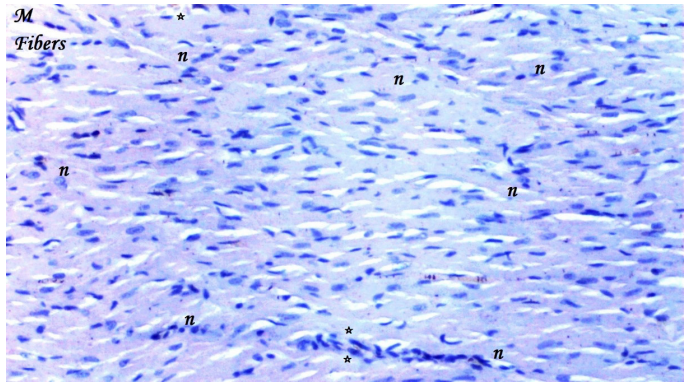

Figure 4. Group Ischemia-reperfusion + Boldine
M: Myocard, n: Nucleus, H&Ex100

Table 2. Oxidative stress parameters in myocardial tissue (Mean ± SE)

	Control (C)	Boldine (B)	Myocardial ischemia-reperfusion (MIR)	Myocardial ischemia-reperfusion + Boldine (MIR+B)	p-value
TAS (mmol/L)	2.47±0.22	2.13±0.15	1.57±0.09*;&	2.05±0.05+	0.003
TOS (µmol/L)	7.78±0.60	10.26±1.20	52.34±3.00*;&	32.25±1.33*;&;+	<0.001
OSI	0.33±0.04	0.51±0.08	3.40±0.27*;&	1.58±0.06*;&;+	<0.001

*p: The statistical significance level was calculated using the ANOVA test, where $p<0.05$ is considered significant. *$p<0.05$: indicates a significant difference compared to the Control group (C). & $p<0.05$: indicates a significant difference compared to the Boldine group (B). + $p<0.05$: Indicates a significant difference compared to the myocardial ischemia-reperfusion group (MIR), TAS: Total antioxidant status, TOS:* Total oxidant status, *OSI: Oxidative Stress index, SE: Standard error*

to the MIR group ($p<0.001$), reflecting the restoration of redox balance with Boldine administration (Table 2).

Discussion

MIRI occurs when blood flow is restored to previously ischemic myocardial tissue, paradoxically resulting in additional cardiac damage. This injury is mediated by several interrelated mechanisms, including oxidative stress, inflammation, calcium overload, mitochondrial dysfunction, and the activation of cell death pathways[12,26]. During the ischemic phase, the deprivation of oxygen and nutrients forces myocardial cells to rely on anaerobic glycolysis, leading to the accumulation of hydrogen ions and lactate, which disrupts cellular homeostasis[4]. Upon reperfusion, the rapid reintroduction of oxygen triggers a burst of ROS production, primarily from mitochondria and NADPH oxidase, resulting in oxidative stress[4,12]. This oxidative damage affects lipids, proteins, and DNA, further aggravating tissue injury and contributing to cardiomyocyte necrosis and apoptosis[3]. In addition, inflammation plays a pivotal role in the pathogenesis of MIRI, with the nuclear factor-kappa B (NF-κB) pathway serving as a central regulator of the inflammatory response[12,26]. NF-κB activation occurs during reperfusion in response to signals such as cytokines, Toll-like receptors, and hypoxia-inducible factors, leading to the release of pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α) and interleukins, which exacerbate myocardial damage. Collectively, these processes contribute to the complex pathophysiology of MIRI, which represents a significant contributor to myocardial infarction-related injury.

This study evaluated the potential protective effects of Boldine on myocardial tissue in a rat model of experimentally induced IR injury. To the best of our knowledge, this is the first comprehensive study investigating Boldine's role in mitigating oxidative stress and preserving myocardial structure and function during IR injury. The findings of this research highlight Boldine's ability to enhance antioxidant capacity, reduce oxidative burden, and attenuate inflammation and myocardial disorganization. These results provide a foundation for future advanced studies to explore Boldine's mechanisms of action, including its impact on molecular and signaling pathways involved in MIRI.

Boldine exerts its antioxidant and anti-inflammatory effects through various mechanisms, making it a promising therapeutic agent. As a natural alkaloid derived from the Peumus boldus tree, Boldine exhibits potent free radical scavenging properties, protecting cells from oxidative stress by neutralizing ROS and inhibiting LPO[14]. Although most studies have focused on systems other than the myocardium, their findings provide valuable insights relevant to our research. For instance, a study by Subramaniam et al.[27] demonstrated Boldine's ability to reduce oxidative stress and modulate tumor-related biomarkers in diethylnitrosamine-induced hepatocarcinogenesis in rats. The study observed improvements in oxidative stress markers, including SOD, catalase, and reduced GSH. Similarly, Calbiague et al.[28] investigated Boldine's antioxidant effects in protecting retinal cells against high glucose-induced oxidative damage in diabetic retinopathy. They found that Boldine decreased oxidant levels and increased antioxidant enzyme activity by modulating oxidative-nitrosative stress markers and mitochondrial function. Shuker et al.[18] also studied the antioxidative effects of Boldine in steroid-induced liver toxicity by assessing parameters such as LPO, GSH, GSH reductase, GSHPx, and SOD. Their findings highlighted Boldine's role in reducing oxidative damage by improving antioxidant enzyme activity. Moreover, Konrath et al.[22] evaluated Boldine's antioxidant and cytoprotective effects in hippocampal slices under ischemic stress. They measured ROS levels, lactate dehydrogenase release, and lipoperoxidation, concluding that Boldine effectively reduced oxidative damage at low doses, although potential pro-oxidant effects were observed at higher concentrations. Beyond animal models, Srivastava et al.[29] provided a detailed chemical and spectroscopic analysis of Boldine, emphasizing its antioxidant potential and the

structural properties contributing to its pharmacological effects. Using Raman and IR spectroscopy alongside computational modeling, the study demonstrated that Boldine's molecular structure enhances its radical-scavenging properties. In our study, TAS levels in the Boldine-treated MIR group were significantly higher than both the control and MIR-only groups. Although the exact mechanism underlying Boldine's antioxidant effects remains unclear. It can be inferred that Boldine enhances antioxidant enzyme activity in myocardial tissue. The observed reduction in TOS levels with Boldine treatment further supports its ROS-scavenging capability.

Boldine's role extends to reducing vascular oxidative stress, improving endothelial function, and preventing vascular damage associated with hypertension and diabetes[17]. Lau et al.[17] highlighted Boldine's therapeutic relevance in endothelial dysfunction by modulating NO bioavailability, decreasing ROS levels, and improving vascular relaxation. Shuker et al.[18] also examined NO levels as a parameter, reporting a significant increase in NO levels in the methylprednisolone (MPL)-treated group, which was linked to oxidative and nitrosative stress. This rise in NO was attributed to the activation of the NF-κB/inducible nitric oxide synthase (iNOS) pathway, a key mechanism in ROS/RNS-mediated oxidative damage. Co-administration of Boldine with MPL significantly reduced NO levels, suggesting that Boldine inhibits iNOS activation and mitigates nitrosative stress, thereby protecting tissues from NO-mediated injury. In the context of MIRI, excessive NO production contributes to tissue damage via peroxynitrite formation and endothelial dysfunction. Biochemical markers in our study indicate an overall reduction in oxidative stress, evidenced by improved TAS and reduced TOS and OSI. These findings suggest that Boldine likely mitigates oxidative and nitrosative stress in myocardial tissues by modulating the NF-κB/iNOS pathway and reducing NO levels. However, further studies focusing on specific molecules and signaling pathways are required to fully elucidate Boldine's antioxidative mechanisms.

In addition to its antioxidant properties, Boldine demonstrates significant anti-inflammatory effects across various tissues. For instance, Shuker et al.[18] reported that Boldine exhibited hepatoprotective effects in steroid-induced liver toxicity by attenuating inflammation and apoptosis. The study observed a reduction in IL-6 and TNF-α levels, along with histopathological evidence of decreased apoptosis and inflammation. Similarly, Pandurangan et al.[30] investigated Boldine's anti-inflammatory effects in dextran sulfate sodium-induced colitis. They found that Boldine reduced the expression of inflammatory cytokines, including TNF-α and IL-6, by inhibiting the NF-κB and IL-6/STAT3 signaling pathways. Histopathological analysis of colonic tissue further confirmed Boldine's anti-inflammatory effects, showing reduced inflammation and crypt damage. Another study on xylene-induced ear edema and carrageenan-induced paw edema in rodents highlighted Boldine's effectiveness in mitigating inflammation through the JAK2/STAT3 and NF-κB pathways[31]. This was supported by histological improvements in edema and reductions in TNF-α and IL-6 levels. Furthermore, Catalán et al.[32] explored Boldine's ability to prevent inflammation in cardiac fibroblasts. They demonstrated that Boldine inhibits the SGK1-NFκB signaling pathway, reducing the expression of pro-inflammatory cytokines such as IL-1 beta and TNF-α, as well as adhesion molecules like ıntercellular adhesion molecule 1 and vascular cell adhesion molecule 1, which are critical mediators of leukocyte recruitment to inflamed tissues. In our study, histopathological assessments were performed to evaluate inflammation, myocardial disorganization, and interstitial fibrosis. Scoring revealed a significant reduction in inflammation and myocardial disorganization in the MIR group treated with Boldine compared to the untreated MIR group. These findings align with existing literature, reinforcing Boldine's anti-inflammatory effects. However, no significant differences in interstitial fibrosis were observed across the groups. This lack of effect may be attributed to the limited time frame and the single-dose protocol employed in our study. While our histological findings regarding inflammation

and myocardial disorganization are consistent with previous studies, future investigations should include a broader range of doses and parameters to provide a more comprehensive understanding of Boldine's therapeutic potential.

Study Limitations

This study is subject to several limitations that should be acknowledged to contextualize the findings appropriately. First, the experimental design employed a single-dose regimen of Boldine, which may not adequately reflect the potential dose-response relationship. Future research should involve dose-escalation studies to identify the optimal therapeutic window and evaluate possible dose-dependent toxicities. Second, the relatively short duration of the experimental protocol restricts the ability to assess long-term outcomes, such as chronic myocardial remodeling, fibrosis progression, or recovery of cardiac function. Longitudinal studies with extended observation periods are necessary to determine whether the acute benefits of Boldine translate into sustained cardioprotection. Third, while the biochemical and histopathological assessments demonstrated significant reductions in oxidative stress and inflammation, the precise molecular mechanisms underlying Boldine's effects remain insufficiently characterized. Advanced molecular investigations, including transcriptomic, proteomic, and metabolomic analyses, are warranted to elucidate the signaling pathways and cellular processes modulated by Boldine during IR injury. Finally, the use of a preclinical rat model limits the direct applicability of these findings to human MIRI. Although the rat model provides valuable insights into pathophysiological mechanisms, species-specific differences in metabolism, pharmacokinetics, and cardiovascular physiology may affect the translational relevance of Boldine. Thus, additional studies involving larger animal models and, eventually, human clinical trials are essential to validate Boldine's therapeutic potential in clinical settings.

Conclusion

This study demonstrates the potential cardioprotective effects of Boldine in a rat model of MIRI. The findings indicate that Boldine significantly attenuates oxidative stress, reduces inflammation, and mitigates myocardial disorganization, highlighting its role as an effective antioxidant and anti-inflammatory agent. The observed improvements in oxidative stress markers, such as enhanced TAS and reduced TOS and OSI levels, as well as histopathological improvements, underscore Boldine's capacity to protect myocardial tissue during IR events. While these results provide a foundation for understanding Boldine's therapeutic potential in cardiovascular diseases, further investigations are required to elucidate its molecular mechanisms of action, dose-response relationships, and long-term effects. Translational studies and clinical trials are essential to determine its safety, efficacy, and applicability in human IHD.

Ethics

Ethics Committee Approval: The animals were sourced and raised within a specialized experimental research facility in Ankara, Türkiye (approval no: E-66332047-604.01-1128916, date: 27.12.2024) and were performed in a dedicated laboratory for animal research.

Informed Consent: This study was conducted using male Albino Wistar rats, aged 12 weeks and weighing between 270 and 320 grams.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Demirtaş H, Özer A, Yıldırım AK, Concept: Demirtaş H, Yıldırım AK, Özer A, Dursun AD, Sezen ŞC, Yığman Z, Küçük A, Arslan M, Design: Demirtaş H, Yıldırım AK, Özer A, Dursun AD, Sezen ŞC, Yığman Z, Küçük A, Arslan M, Data Collection and/or Processing: Demirtaş H, Yıldırım AK, Özer A, Dursun AD, Sezen ŞC, Yığman Z, Küçük A, Arslan M, Analysis and/or Interpretation: Demirtaş H, Yıldırım

AK, Özer A, Dursun AD, Sezen ŞC, Yığman Z, Küçük A, Arslan M, Literature Search: Demirtaş H, Arslan M, Writing: Demirtaş H, Arslan M. Yıldırım AK.

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