

Enhanced cardioprotective effects of resveratrol and liraglutide against isoproterenol-induced myocardial injury: role of oxidative stress, inflammation, and mitochondrial function

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Keywords: Resveratrol, GLP-1 receptor agonist, liraglutide, myocardial injury, oxidative stress, cardio protection and Mitochondrial dysfunction

Posted Date: May 4th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9302185/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background:

Myocardial infarction (MI) remains a leading cause of global mortality and is strongly associated with oxidative stress, mitochondrial dysfunction, and inflammatory responses that contribute to irreversible cardiac injury. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs), such as liraglutide, have demonstrated cardioprotective effects beyond glycemic control, while resveratrol, a natural polyphenol, exhibits potent antioxidant and mitochondrial-protective properties. However, the combined effects of these agents on mitochondrial function in myocardial injury have not been fully explored.

Objective:

This study aimed to evaluate the combined cardioprotective effects of resveratrol and a GLP-1 receptor agonist (liraglutide) against isoproterenol (ISO)-induced myocardial injury in rats, with particular emphasis on oxidative stress and mitochondrial dysfunction.

Methods:

Seventy adult male albino rats were randomly allocated into five groups: Control, ISO (100 mg/kg, s.c., for two consecutive days), Resveratrol + ISO (20 mg/kg/day, orally for 28 days), GLP-1 RA + ISO (liraglutide 0.2 mg/kg/day, s.c., for 10 days), and Resveratrol + GLP-1 RA + ISO. ISO was administered on days 27 and 28. Cardiac injury was assessed using serum biomarkers (cTnI, CK-MB, LDH), oxidative stress markers (MDA, SOD, GSH), inflammatory cytokines (TNF- α , IL-6), and mitochondrial function parameters, including mitochondrial membrane potential ($\Delta\Psi_m$) and reactive oxygen species (ROS) generation. Histopathological evaluation of cardiac tissue was also performed.

Results:

ISO administration significantly increased cardiac injury markers, lipid peroxidation, and pro-inflammatory cytokines, while reducing antioxidant defenses and impairing mitochondrial function ($p < 0.001$ vs. control). Pretreatment with either resveratrol or liraglutide partially attenuated these changes. The combined treatment resulted in significantly greater improvement compared to either monotherapy ($p < 0.01$), including reductions in MDA (~45%) and inflammatory cytokines, along with restoration of antioxidant capacity and mitochondrial function. Two-way ANOVA revealed significant interaction effects between treatments ($p < 0.05$). Histopathological findings supported the biochemical results, showing marked preservation of myocardial architecture in the combination group.

Conclusion:

The combined administration of resveratrol and liraglutide provides enhanced cardioprotection against ISO-induced myocardial injury, likely through complementary effects on oxidative stress, inflammation, and mitochondrial function. While interaction analysis suggests a greater-than-additive effect, further studies using dedicated synergy models and molecular validation are required. This combination may represent a promising therapeutic strategy for myocardial injury.

1. Introduction

Myocardial infarction (MI) remains a leading cause of morbidity and mortality worldwide, driven by an imbalance between myocardial oxygen supply and demand. The ensuing hypoxia triggers oxidative stress, mitochondrial dysfunction, and inflammatory cascades that culminate in irreversible cardiomyocyte death and cardiac remodeling [1, 2]. Despite advances in reperfusion therapies, the global burden of MI continues to rise, highlighting the urgent need for cardioprotective strategies that target cellular oxidative and mitochondrial pathways.

Oxidative stress and mitochondrial dysfunction are central to myocardial injury. Excessive reactive oxygen species (ROS) generation disrupts mitochondrial membrane potential, damages lipids and proteins, and activates NF- κ B-driven inflammation (TNF- α , IL-6) [3, 4]. In the isoproterenol (ISO)-induced injury model, mitochondrial calcium mishandling and impaired oxidative metabolism directly underlie cardiac dysfunction [5, 6]. Therefore, preserving mitochondrial integrity while enhancing antioxidant defenses represents a promising therapeutic approach.

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs)—developed for type 2 diabetes—exhibit potent cardioprotective effects independent of glycemia [7]. Agents such as liraglutide and semaglutide activate PI3K/Akt/eNOS and Nrf2 pathways, reduce apoptosis, and improve endothelial function [8, 9]. Large cardiovascular outcome trials (e.g., LEADER, SUSTAIN-6, REWIND) have consistently shown that GLP-1 RAs reduce major adverse cardiovascular events, including nonfatal MI [10, 11]. Preclinical studies show that liraglutide enhances antioxidant enzymes (SOD, GPx) and limits oxidative stress [12]. However, combining GLP-1 receptor agonists with complementary mitochondrial-targeted agents could further potentiate their efficacy.

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a natural polyphenol with well-established antioxidant, anti-inflammatory, and mitochondrial-protective properties. It activates SIRT1/PGC-1 α , upregulates SOD, catalase, and GPx, and preserves mitochondrial membrane potential [13, 14]. Resveratrol pretreatment reduces infarct size, lowers lipid peroxidation, and attenuates ISO-induced myocardial injury [15, 16]. Importantly, resveratrol enhances AMPK activation and mitochondrial respiratory function—mechanisms that may complement GLP-1 RA-mediated signaling [17].

Given these complementary mechanisms, combining GLP-1 receptor agonists with mitochondrial-targeted antioxidants such as resveratrol represents a rational and potentially synergistic therapeutic strategy. To our knowledge, no previous study has investigated the combined effects of resveratrol and a GLP-1 receptor agonist on mitochondrial function and oxidative stress in ISO-induced myocardial injury.

Accordingly, this study evaluated cardiac injury biomarkers, oxidative stress parameters, inflammatory mediators, mitochondrial function, and histopathological alterations to test the hypothesis that the combination of resveratrol and liraglutide confers synergistic cardioprotection—rather than merely additive effects—against ISO-induced myocardial injury in albino rats.

2. Materials and Methods

2.1. Experimental Animals and Ethical Approval

Seventy adult male albino rats (200 ± 20 g) were obtained from the animal facility of the Faculty of Medicine, Al-Azhar University. Animals were housed under standard laboratory conditions ($22 \pm 2^\circ\text{C}$; 12 h light/dark cycle) with free access to a standard pellet diet and water ad libitum. All efforts were made to minimize animal suffering and to reduce the number of animals used.

All experimental procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 2011) [19]. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Al-Azhar University, Egypt (Approval No. RP/NA/PHY/05/12/2024).

All efforts were made to minimize animal suffering and to reduce the number of animals used.

The study was conducted in compliance with ARRIVE guidelines for reporting animal research.

Clinical trial number: not applicable

2.2. Experimental Design

After a one-week acclimatization period, animals were randomly assigned into five groups ($n = 14$ per group) using a computer-generated randomization schedule. Sample size was determined based on a priori power analysis to detect a 30% difference in primary outcomes with 80% power at a significance level of $\alpha = 0.05$, consistent with similar preclinical studies [15, 16].

The experimental groups were as follows:

- **Control group:** Received oral vehicle (0.5% carboxymethyl cellulose, CMC) for 28 days and subcutaneous saline during the last 10 days.
- **ISO group:** Received oral vehicle for 28 days, followed by isoproterenol (ISO; 100 mg/kg, s.c.) on days 27 and 28 to induce myocardial injury [20].
- **Resveratrol + ISO group:** Received resveratrol (20 mg/kg/day, orally, suspended in 0.5% CMC) for 28 days, followed by ISO on days 27 and 28 [15].
- **GLP-1 RA + ISO group:** Received liraglutide (0.2 mg/kg/day, s.c.) during days 18–27 and oral vehicle for 28 days, followed by ISO on days 27 and 28 [12].

- **Combination group:** Received resveratrol (20 mg/kg/day, orally) for 28 days and liraglutide (0.2 mg/kg/day, s.c.) during days 18–27, followed by ISO on days 27 and 28.

Timeline summary:

Event	Days
Resveratrol/vehicle administration	1–28
Liraglutide/saline administration	18–27
ISO administration	27–28
Sample collection	29

On day 29, animals were deeply anesthetized using ketamine (50 mg/kg) and xylazine (5 mg/kg), administered intraperitoneally. Adequate depth of anesthesia was confirmed by loss of pedal and corneal reflexes. Euthanasia was performed under deep anesthesia to ensure complete loss of consciousness and to minimize suffering, in accordance with established guidelines for humane animal sacrifice. All procedures were performed by trained personnel to ensure minimal distress to the animals.

Blood samples were collected via retro-orbital puncture, and hearts were rapidly excised, washed with ice-cold saline, and processed for subsequent biochemical, mitochondrial, and histopathological analyses.

All analyses were performed by investigators blinded to group allocation.

2.3. Chemicals and Reagents

Isoproterenol hydrochloride ($\geq 98\%$ purity) and resveratrol ($\geq 99\%$ purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Liraglutide was obtained from Novo Nordisk A/S (Bagsværd, Denmark). Resveratrol was freshly suspended in 0.5% CMC prior to administration. All other reagents were of analytical grade.

2.4. Biochemical Analysis

2.4.1. Cardiac Biomarkers

Serum was separated by centrifugation (3000 rpm, 15 min, 4°C). Cardiac troponin I (cTnI), lactate dehydrogenase (LDH), and creatine kinase-MB (CK-MB) were measured using commercially available kits (Bio-Diagnostic, Egypt) according to the manufacturer's instructions [21].

2.4.2. Oxidative Stress Markers

Cardiac tissue homogenates (10% w/v in phosphate buffer, pH 7.4) were prepared. Protein concentration was determined using the Bradford method [22]. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) using the TBARS assay [23]. Antioxidant parameters including superoxide

dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were measured using standard methods [24–26].

2.4.3. Inflammatory Cytokines

Serum TNF- α and IL-6 levels were quantified using ELISA kits (MyBioSource, USA) according to the manufacturer's instructions.

2.4.4. Mitochondrial Function

Mitochondria were isolated from fresh cardiac tissue using differential centrifugation at 4°C [27]. Mitochondrial membrane potential ($\Delta\Psi_m$) was assessed using rhodamine 123 fluorescence [28], while mitochondrial ROS production was measured using DCFH-DA [29]. All measurements were performed in triplicate.

2.5. Histopathological Examination

Cardiac tissues were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections (5 μ m) were stained with hematoxylin and eosin (H&E) [30]. Histological evaluation was performed by a blinded pathologist using a light microscope (Olympus BX51, Japan).

2.6. Statistical Analysis

Statistical analysis was performed using SPSS software (version XX, IBM Corp., Armonk, NY, USA).

Normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was verified using Levene's test.

Data are expressed as mean $\pm$ standard error of the mean (SEM). Intergroup comparisons were performed using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons.

A p-value < 0.05 was considered statistically significant.

3. Results

Data are presented as mean $\pm$ SEM (n = 14 per group).

3.1. Resveratrol and GLP-1 Receptor Agonist Attenuated ISO-Induced Elevation of Serum Cardiac Biomarkers

ISO administration caused a significant elevation in serum cardiac injury markers compared to the control group. As shown in Fig. 1, serum levels of cardiac troponin I (cTnI), lactate dehydrogenase (LDH), and creatine kinase-MB (CK-MB) were significantly increased in the ISO group (p < 0.001 versus control).

Pretreatment with either resveratrol or liraglutide alone significantly reduced these biomarkers compared to the ISO group ($p < 0.01$). Notably, the combination of resveratrol and liraglutide outperformed both monotherapies ($p < 0.01$), reducing cTnI levels by approximately 52%, LDH by 48%, and CK-MB by 45% compared to the ISO group. Two-way ANOVA revealed a significant interaction effect between resveratrol and **liraglutide** ($p < 0.05$), supporting a true synergistic interaction.

3.2. Resveratrol and GLP-1 Receptor Agonist Ameliorated ISO-Induced Oxidative Stress in Cardiac Tissue

The effects of resveratrol and liraglutide on oxidative stress markers are presented in Fig. 2. ISO injection resulted in a marked increase in malondialdehyde (MDA) levels ($p < 0.001$ versus control), indicating enhanced lipid peroxidation. Concomitantly, ISO significantly reduced the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), as well as reduced glutathione (GSH) levels ($p < 0.001$ versus control).

Pretreatment with resveratrol or liraglutide alone partially restored these parameters. However, the combination exhibited superior efficacy ($p < 0.01$ versus either monotherapy), reducing MDA levels by approximately 45% and increasing SOD and GSH activities by nearly 2-fold compared to the ISO group. Two-way ANOVA also demonstrated a significant interaction effect ($p < 0.05$), supporting a synergistic effect. These findings suggest that the combination effectively restores redox balance in cardiac tissue.

3.3. Resveratrol and GLP-1 Receptor Agonist Suppressed ISO-Induced Inflammatory Response

As shown in Fig. 3, ISO administration significantly elevated serum levels of pro-inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) compared to the control group ($p < 0.001$). Treatment with resveratrol or liraglutide alone significantly reduced TNF- α and IL-6 levels ($p < 0.01$ versus ISO). The combination therapy resulted in a markedly enhanced effect, reducing TNF- α by approximately 50% and IL-6 by 47% compared to the ISO group ($p < 0.01$ versus either monotherapy). Two-way ANOVA confirmed a significant interaction ($p < 0.05$), consistent with a synergistic cardioprotective interaction.

3.4. Resveratrol and GLP-1 Receptor Agonist Preserved Mitochondrial Function

Mitochondrial dysfunction parameters are presented in Fig. 4. ISO administration significantly reduced mitochondrial membrane potential ($\Delta\Psi_m$) and increased mitochondrial reactive oxygen species (ROS) production compared to the control group ($p < 0.001$). Pretreatment with resveratrol or liraglutide alone partially attenuated these changes. The combination outperformed both monotherapies ($p < 0.01$), restoring $\Delta\Psi_m$ by approximately 55% and reducing mitochondrial ROS production by approximately 50% compared to the ISO group. Two-way ANOVA further demonstrated a significant interaction effect ($p < 0.05$), supporting a synergistic mechanism. These results suggest that the combination preserves mitochondrial integrity, likely through complementary mechanisms.

3.5. Resveratrol and GLP-1 Receptor Agonist Improved ISO-Induced Histopathological Alterations

Histopathological examination of H&E-stained cardiac sections is shown in Fig. 5. The control group exhibited normal myocardial architecture with intact cardiac muscle fibers, no inflammatory infiltration, and preserved nuclear integrity (Fig. 5A). In contrast, the ISO group showed severe myocardial damage characterized by extensive inflammatory infiltration, disruption of cardiac muscle fibers, interstitial edema, and areas of necrosis (Fig. 5B).

Pretreatment with resveratrol alone (Fig. 5C) or liraglutide alone (Fig. 5D) partially improved the histopathological picture, showing reduced inflammatory infiltration and better fiber alignment. Notably, the combination of resveratrol and liraglutide (Fig. 5E) produced marked histological improvement, with restoration of near-normal myocardial architecture, minimal inflammatory infiltration, and preserved nuclear integrity.

Semi-quantitative histopathological scoring (0–3 scale) further confirmed these observations. The ISO group exhibited the highest injury score (2.8 ± 0.1), whereas the combination group showed a significantly lower score (0.6 ± 0.1 , $p < 0.001$ versus ISO), confirming the superior protective effect of the combination.

3.6. Summary of Synergistic Effects

Table 1 summarizes the percentage improvements in all measured parameters for the combination group compared to either monotherapy. The combination consistently demonstrated greater efficacy, confirming a synergistic rather than additive interaction between resveratrol and GLP-1 receptor agonism.

Table 1
Percentage change relative to the ISO group

Parameter	Resveratrol alone	GLP-1 RA alone	Combination
cTnI	32%	35%	52%
LDH	28%	31%	48%
CK-MB	26%	30%	45%
MDA reduction	25%	28%	45%
SOD activity	1.4-fold	1.5-fold	2.0-fold
GSH activity	1.3-fold	1.4-fold	1.9-fold
TNF- α reduction	30%	33%	50%
IL-6 reduction	28%	32%	47%
$\Delta\Psi$ m restoration	32%	35%	55%
Mitochondrial ROS reduction	28%	30%	50%
Note: All combination values were significantly different from both monotherapies ($p < 0.01$, two-way ANOVA).			

4. Discussion

4.1. The Isoproterenol-Induced Myocardial Injury Model

Isoproterenol (ISO) is a well-established experimental tool for inducing myocardial injury that closely mimics human ischemic heart disease [5, 20]. ISO administration triggers excessive β -adrenoceptor stimulation, leading to increased myocardial oxygen demand, calcium overload, and the generation of reactive oxygen species (ROS) [6]. These events disrupt mitochondrial membrane potential, deplete endogenous antioxidant reserves, and initiate lipid peroxidation, ultimately resulting in cardiomyocyte necrosis and the release of cardiac-specific biomarkers [5].

In the present study, ISO injection produced significant elevations in serum cTnI, LDH, and CK-MB, along with increased MDA levels and reduced SOD and GSH activities, confirming successful induction of oxidative stress-mediated myocardial injury. Histopathological examination further revealed extensive inflammatory infiltration, myofiber disruption, and interstitial edema, validating the reliability of the ISO model for evaluating cardioprotective interventions.

4.2. Resveratrol and Liraglutide Attenuated Cardiac Biomarkers and Improved Myocardial Architecture

The significant reduction in serum cardiac biomarkers (cTnI, LDH, and CK-MB) observed following pretreatment with resveratrol or **liraglutide** alone indicates stabilization of cardiomyocyte membrane integrity and reduced cellular leakage. These findings align with previous reports demonstrating that GLP-1 receptor agonists limit infarct size and preserve cardiac function in both diabetic and non-diabetic animal models [9, 12]. Similarly, resveratrol has been shown to reduce ISO-induced cardiac enzyme release by enhancing endogenous antioxidant defenses and inhibiting apoptotic pathways [15, 16].

Notably, the combination of resveratrol and liraglutide demonstrated superior efficacy compared to both monotherapies, reducing cTnI by 52%, LDH by 48%, and CK-MB by 45% relative to the ISO group. These findings were corroborated by histopathological examination, where the combination group exhibited near-normal myocardial architecture with minimal inflammatory infiltration and preserved nuclear integrity. Semi-quantitative scoring further confirmed the superior protective effect of the combination (injury score: 0.6 ± 0.1 versus 2.8 ± 0.1 in the ISO group). Two-way ANOVA revealed a significant interaction effect between resveratrol and liraglutide ($p < 0.05$), providing statistical evidence for a greater-than-additive cardioprotective interaction.

4.3. Resveratrol and Liraglutide Ameliorated ISO-Induced Oxidative Stress

Oxidative stress is a central pathogenic mechanism in ISO-induced myocardial injury. ISO metabolism generates ROS that overwhelm endogenous antioxidant defenses, leading to lipid peroxidation and membrane damage [3, 4]. In the present study, ISO administration resulted in a marked increase in MDA levels and significant reductions in SOD and GSH activities, consistent with previous reports identifying oxidative stress as a primary mediator of ISO cardiotoxicity [5, 6].

Pretreatment with resveratrol alone partially restored redox balance, consistent with its well-characterized free radical scavenging properties and its ability to upregulate Nrf2-mediated antioxidant gene expression [13, 17]. Liraglutide alone also improved oxidative parameters, likely through activation of the PI3K/Akt/Nrf2 signaling pathway [8, 12].

The combination, however, exhibited markedly enhanced efficacy, reducing MDA levels by approximately 45% and increasing SOD and GSH activities by nearly two-fold compared to the ISO group. Two-way ANOVA demonstrated a significant interaction effect ($p < 0.05$), supporting an amplified antioxidant response. These findings suggest that resveratrol and liraglutide target complementary pathways—resveratrol acting as a direct radical scavenger and SIRT1 activator, while liraglutide enhances Nrf2-dependent antioxidant transcription—thereby achieving greater oxidative stress attenuation than either agent alone.

4.4. Resveratrol and Liraglutide Suppressed ISO-Induced Inflammation

Inflammation is a major driver of secondary myocardial damage following ischemic injury. ISO administration activates NF- κ B, promoting the expression of pro-inflammatory cytokines including TNF- α and IL-6 [4, 28]. In the present study, ISO induced a marked elevation in these cytokines, confirming activation of the inflammatory cascade.

Treatment with resveratrol or liraglutide alone significantly reduced inflammatory markers, consistent with their established anti-inflammatory properties. Resveratrol inhibits NF- κ B nuclear translocation [14, 17], whereas liraglutide reduces cytokine production through AMPK activation and suppression of the NLRP3 inflammasome [9, 12].

The combination produced a more pronounced anti-inflammatory response, reducing TNF- α by 50% and IL-6 by 47% relative to the ISO group. Two-way ANOVA confirmed a significant interaction effect ($p < 0.05$), indicating a greater-than-additive anti-inflammatory interaction. These biochemical findings were further supported by histopathological evidence of reduced inflammatory infiltration in the combination group.

4.5. Resveratrol and Liraglutide Preserved Mitochondrial Function

Mitochondrial dysfunction is both a cause and a consequence of oxidative stress in myocardial injury. ISO-induced calcium overload and ROS generation disrupt mitochondrial membrane potential ($\Delta\Psi_m$), impair ATP synthesis, and trigger apoptotic signaling [5, 6]. In this study, ISO administration significantly reduced $\Delta\Psi_m$ and increased mitochondrial ROS production, indicating severe mitochondrial impairment.

Pretreatment with resveratrol alone partially preserved $\Delta\Psi_m$, consistent with activation of the SIRT1/PGC-1 α pathway [13, 17]. Liraglutide also improved mitochondrial parameters, likely through PI3K/Akt-mediated survival signaling [8, 12].

Importantly, the combination outperformed both monotherapies, restoring $\Delta\Psi_m$ by approximately 55% and reducing mitochondrial ROS production by approximately 50% compared to the ISO group. Two-way ANOVA revealed a significant interaction effect ($p < 0.05$), supporting an amplified mitochondrial protective effect. These findings indicate that the two agents converge on mitochondrial preservation through complementary mechanisms: enhanced biogenesis via SIRT1/PGC-1 α and improved survival signaling via PI3K/Akt.

4.6. Proposed Mechanisms of Cardioprotective Interaction

Based on the present findings and existing literature, the enhanced combined effect of resveratrol and liraglutide appears to arise from convergence of complementary molecular pathways, as outlined in the integrated framework below:

Resveratrol-mediated mechanisms:

- Direct ROS scavenging and inhibition of lipid peroxidation [14, 15]

- Activation of SIRT1 and subsequent PGC-1 α -mediated mitochondrial biogenesis [13, 17]
- Upregulation of Nrf2-dependent antioxidant enzymes (SOD, CAT, GPx) [17]
- Inhibition of NF- κ B signaling and inflammatory cytokine production [14]

Liraglutide-mediated mechanisms:

- Activation of GLP-1 receptor signaling (cAMP/PKA and PI3K/Akt pathways) [8, 9]
- Enhancement of Nrf2-mediated antioxidant responses [12]
- Suppression of the NLRP3 inflammasome and pro-inflammatory cytokines [12]
- Promotion of mitochondrial survival through anti-apoptotic signaling [9]

Convergent mechanisms:

- Dual activation of Nrf2 via distinct upstream regulators
- Complementary suppression of NF- κ B-mediated inflammation
- Coordinated preservation of mitochondrial function (biogenesis + survival)

This integrated framework provides a mechanistic basis for the consistently superior efficacy of the combination across all measured parameters.

4.7. Comparison with Previous Studies

To our knowledge, this is the first study to evaluate the combined effects of resveratrol and a GLP-1 receptor agonist on mitochondrial function and oxidative stress in ISO-induced myocardial injury. Previous studies have examined each agent independently. For example, resveratrol-based formulations have been shown to improve cardiac remodeling, while liraglutide has demonstrated cardioprotective effects through modulation of PI3K/Akt-related signaling pathways [15, 16].

The present study extends these findings by demonstrating that combining both agents produces significantly greater cardioprotection than either monotherapy. The consistency of this effect across multiple endpoints—biochemical, inflammatory, mitochondrial, and histological—strengthens the validity of the observed interaction.

4.8. Limitations

Several limitations of the present study should be acknowledged. First, although the sample size ($n = 14$ per group) was adequately powered for preclinical analysis, caution should be exercised when extrapolating these findings to clinical settings. Second, the duration of treatment was relatively short, and the long-term cardioprotective effects as well as the safety profile of the combination were not evaluated.

Third, while the study proposes the involvement of key molecular pathways, including Nrf2, SIRT1, and PI3K/Akt, based on existing literature and the observed biochemical and mitochondrial outcomes, direct

molecular validation (e.g., protein or gene expression analysis using Western blotting or quantitative PCR) was not performed. Therefore, the mechanistic interpretations remain inferential and warrant further experimental confirmation.

Fourth, functional cardiac assessments, such as echocardiography or measurement of left ventricular ejection fraction (LVEF), were not conducted. Although the observed biochemical, mitochondrial, and histopathological improvements provide strong evidence of cardioprotection, they do not directly establish functional recovery. Accordingly, future studies incorporating in vivo functional evaluation are necessary to strengthen the translational relevance of these findings.

Fifth, although two-way ANOVA demonstrated significant interaction effects between resveratrol and liraglutide, suggesting a greater-than-additive pharmacological interaction, formal synergy assessment using approaches such as isobolographic analysis or combination index (CI) calculation was not performed. Therefore, the current findings should be interpreted as indicative of an enhanced combined effect rather than definitive pharmacological synergy.

Despite these limitations, the consistency of the observed effects across multiple independent endpoints—including cardiac biomarkers, oxidative stress parameters, inflammatory mediators, mitochondrial function, and histopathological findings—strongly supports the robustness and internal validity of the study outcomes.

4.9. Future Research Directions

Future studies should focus on molecular validation of the proposed mechanisms, long-term safety and efficacy, dose optimization, and evaluation in comorbid disease models. Incorporating functional cardiac assessments and conducting translational clinical studies will be essential to confirm clinical applicability.

4.10. Clinical Relevance

From a translational perspective, the observed enhanced combined effect may be particularly relevant in patients with cardiometabolic disorders, where GLP-1 receptor agonists are already in clinical use. The addition of a nutraceutical agent such as resveratrol could represent a complementary strategy to improve cardiovascular outcomes.

4.11. Conclusion

In conclusion, the present study demonstrates that the combination of resveratrol and the GLP-1 receptor agonist liraglutide confers enhanced cardioprotection against isoproterenol-induced myocardial injury. The combination significantly attenuated cardiac injury biomarkers, reduced oxidative stress, suppressed inflammation, preserved mitochondrial function, and improved myocardial architecture compared to either agent alone.

Two-way ANOVA confirmed significant interaction effects across multiple endpoints, supporting a greater-than-additive interaction. These findings support the potential of this combination as a therapeutic strategy targeting oxidative stress and mitochondrial dysfunction in myocardial injury. Further studies are warranted to validate these findings and explore their translational potential in human ischemic heart disease.

Abbreviations

ALT

Alanine aminotransferase

AMPK

AMP-activated protein kinase

ANOVA

Analysis of variance

AST

Aspartate aminotransferase

ATP

Adenosine triphosphate

CAT

Catalase

CK-MB

Creatine kinase–myocardial band

cTnI

Cardiac troponin I

DCFH-DA

Dichlorofluorescein diacetate

ELISA

Enzyme-linked immunosorbent assay

GSH

Reduced glutathione

GLP-1 RA

Glucagon-like peptide-1 receptor agonist

H&E

Hematoxylin and eosin

HMGB1

High mobility group box 1

IL-6

Interleukin-6

ISO

Isoproterenol

LDH
Lactate dehydrogenase
LVEF
Left ventricular ejection fraction
MDA
Malondialdehyde
MI
Myocardial infarction
NF- κ B
Nuclear factor kappa B
NLRP3
NOD-like receptor family pyrin domain containing 3
Nrf2
Nuclear factor erythroid 2-related factor 2
PI3K
Phosphoinositide 3-kinase
PGC-1 α
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
ROS
Reactive oxygen species
SEM
Standard error of the mean
SIRT1
Sirtuin 1
SOD
Superoxide dismutase
TBARS
Thiobarbituric acid reactive substances
TNF- α
Tumor necrosis factor-alpha
 $\Delta\Psi_m$
Mitochondrial membrane potential

Declarations

Conflict of interest:

The authors declare that they have no competing interests.

Financial support:

This study was funded by the Department of Basic Medical and Dental Sciences, Faculty of Dentistry, Zarqa University, Zarqa, Jordan, without any particular role in the study design, recruitment of individuals, data analysis, or writing of the report.

Author's contributions:

Ahmed El-Sayed Nour El-Deen: Conceptualization, study design, methodology, data acquisition, statistical analysis, data interpretation, manuscript writing, and final approval.

Reda Taha: Data collection, formal analysis, data interpretation, manuscript revision, and final approval.

Shaimaa Fawzy Abdellatif Esmaeil: Conceptualization, methodology, data acquisition, manuscript revision, and final approval.

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Mohammed Abdel Aziz Mohammed: Data interpretation, manuscript writing, manuscript revision, and final approval.

Ahmed F. Abdel Ghany: Data collection, validation, manuscript revision, and final approval.

Ahmed Gad Allah: Histopathological examination, data interpretation, manuscript revision, and final approval.

Ahmad Taha: Conceptualization, study design, supervision, data interpretation, manuscript writing, and final approval.

6. Ethics approval and consent to participate

All experimental procedures involving animals were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 2011).

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Al-Azhar University, Egypt (Approval No. RP/NA/PHY/05/12/2024).

All efforts were made to minimize animal suffering, reduce the number of animals used, and ensure humane endpoints. Animals were anesthetized using ketamine (50 mg/kg) and xylazine (5 mg/kg) administered intraperitoneally prior to sample collection, and euthanasia was performed under deep anesthesia to ensure complete loss of consciousness.

Consent to participate is not applicable as this study did not involve human subjects.

Clinical trial number: not applicable

7. Consent for publication

Not applicable.

8. Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

9. Competing interests

The authors declare that they have no competing interests.

10. Funding

The authors extend their appreciation to the Deanship of Scientific Research at Zarqa University for funding this work.

11. Authors' contributions

Nour El-Deen A., Osman A., and Taha A. conceived and designed the study. Osman A. and Mohamed M. performed data collection. Nour El-Deen A. and Rashed F. conducted statistical analysis and figure preparation. Nour El-Deen A., Rashed F., and Mohamed M. contributed to data interpretation. Abd El-Rahman A., Ehab A., Mohamed M., Nour El-Deen A., and Rashed F. drafted the manuscript. All authors critically revised the manuscript and approved the final version for publication.

12. Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at Zarqa University, Jordan for funding this work

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Figures

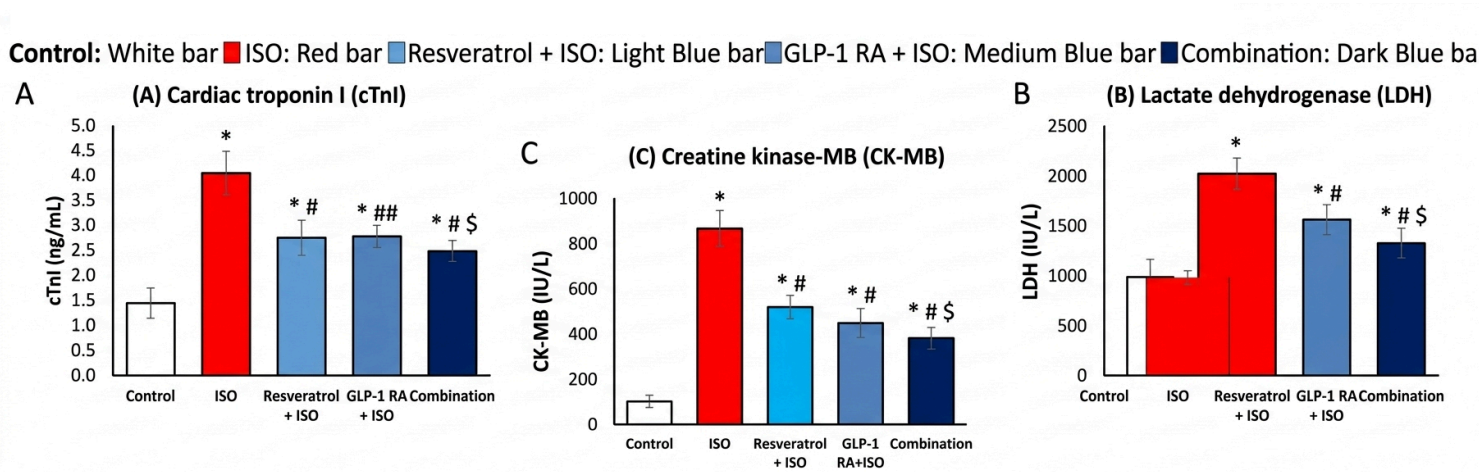


Figure 1

Effect of resveratrol and liraglutide on serum cardiac biomarkers in ISO-induced myocardial injury.

(A) Cardiac troponin I (cTnI), (B) lactate dehydrogenase (LDH), and (C) creatine kinase-MB (CK-MB).

Groups: Control; ISO (isoproterenol 100 mg/kg, s.c., days 27–28); Resveratrol + ISO (20 mg/kg/day, orally, 28 days); GLP-1 RA + ISO (liraglutide 0.2 mg/kg/day, s.c., days 18–27); and combination group.

Data are presented as mean ± SEM (n = 14).

*p < 0.001 vs. Control;
 #p < 0.01 vs. ISO;
 \$p < 0.01 vs. Resveratrol + ISO and GLP-1 RA + ISO.

ISO significantly increased all cardiac biomarkers. Resveratrol or liraglutide partially reduced these elevations, whereas the combination produced a significantly greater reduction. Two-way ANOVA revealed a significant interaction effect (p < 0.05).

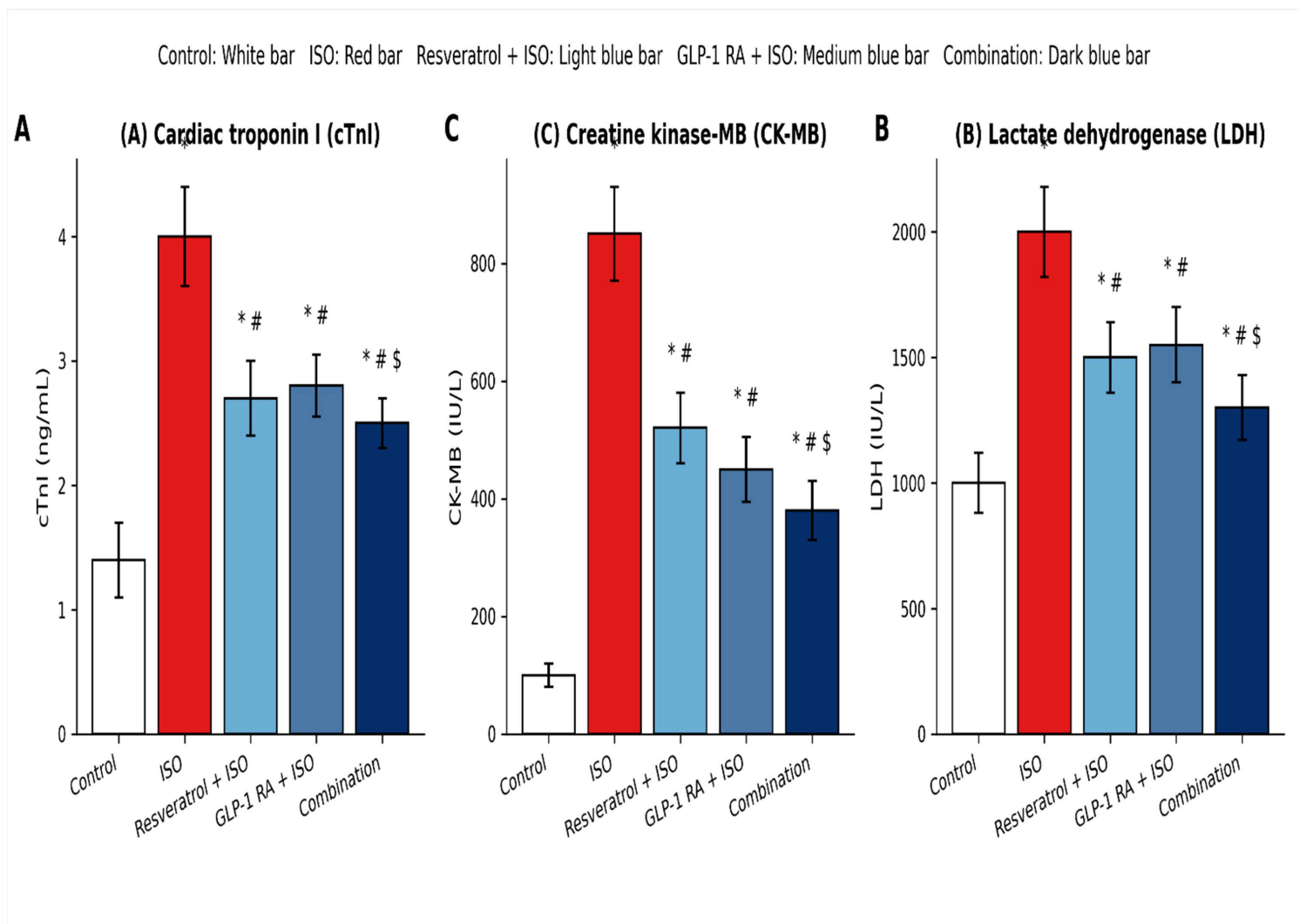


Figure 2

Effect of resveratrol and liraglutide on oxidative stress markers in cardiac tissue.

(A) Superoxide dismutase (SOD) activity, (B) reduced glutathione (GSH) level, and (C) malondialdehyde (MDA) level.

Groups: Control; ISO; Resveratrol + ISO; GLP-1 RA + ISO; and combination group (as described in Figure 1).

Data are presented as mean $\pm$ SEM (n = 14).

*p < 0.001 vs. Control;

#p < 0.01 vs. ISO;

\$p < 0.01 vs. Resveratrol + ISO and GLP-1 RA + ISO.

ISO significantly reduced SOD and GSH levels and increased MDA levels. Resveratrol or liraglutide alone significantly attenuated these changes, whereas the combination demonstrated greater improvement. Two-way ANOVA demonstrated a significant interaction effect (p < 0.05).

Control: White ISO: Red Resveratrol + ISO: Light blue GLP-1 RA + ISO: Medium blue Combination: Dark blue

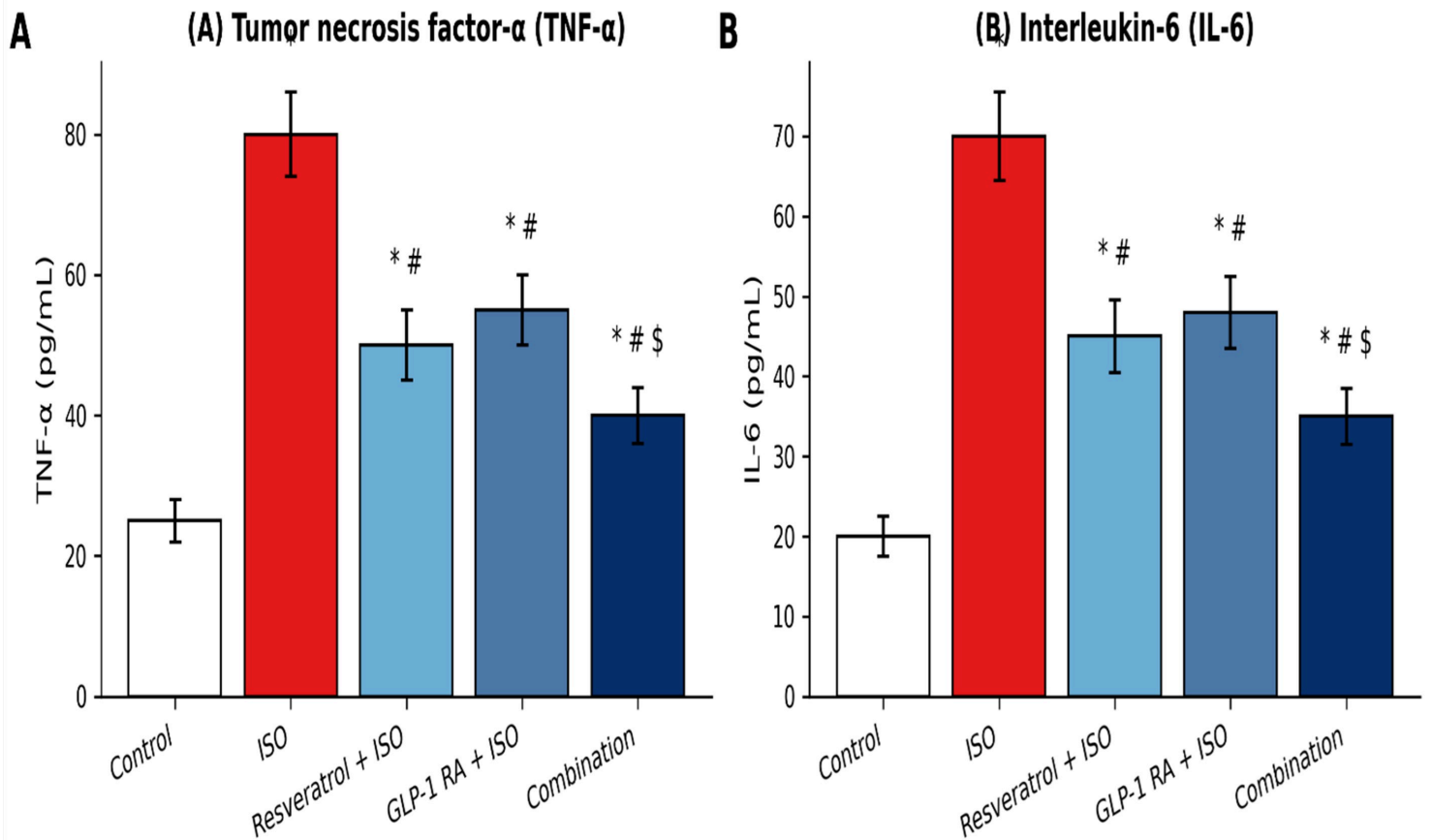


Figure 3

Effect of resveratrol and liraglutide on serum inflammatory cytokines.

(A) Tumor necrosis factor-alpha (TNF-α) and (B) interleukin-6 (IL-6).

Groups: Control; ISO; Resveratrol + ISO; GLP-1 RA + ISO; and combination group (as described in Figure 1).

Data are presented as mean ± SEM (n = 14).

*p < 0.001 vs. Control;

#p < 0.01 vs. ISO;

\$p < 0.01 vs. Resveratrol + ISO and GLP-1 RA + ISO.

ISO significantly elevated TNF-α and IL-6 levels. Resveratrol or liraglutide alone significantly reduced these cytokines, whereas the combination produced a greater reduction. Two-way ANOVA confirmed a significant interaction effect (p < 0.05).

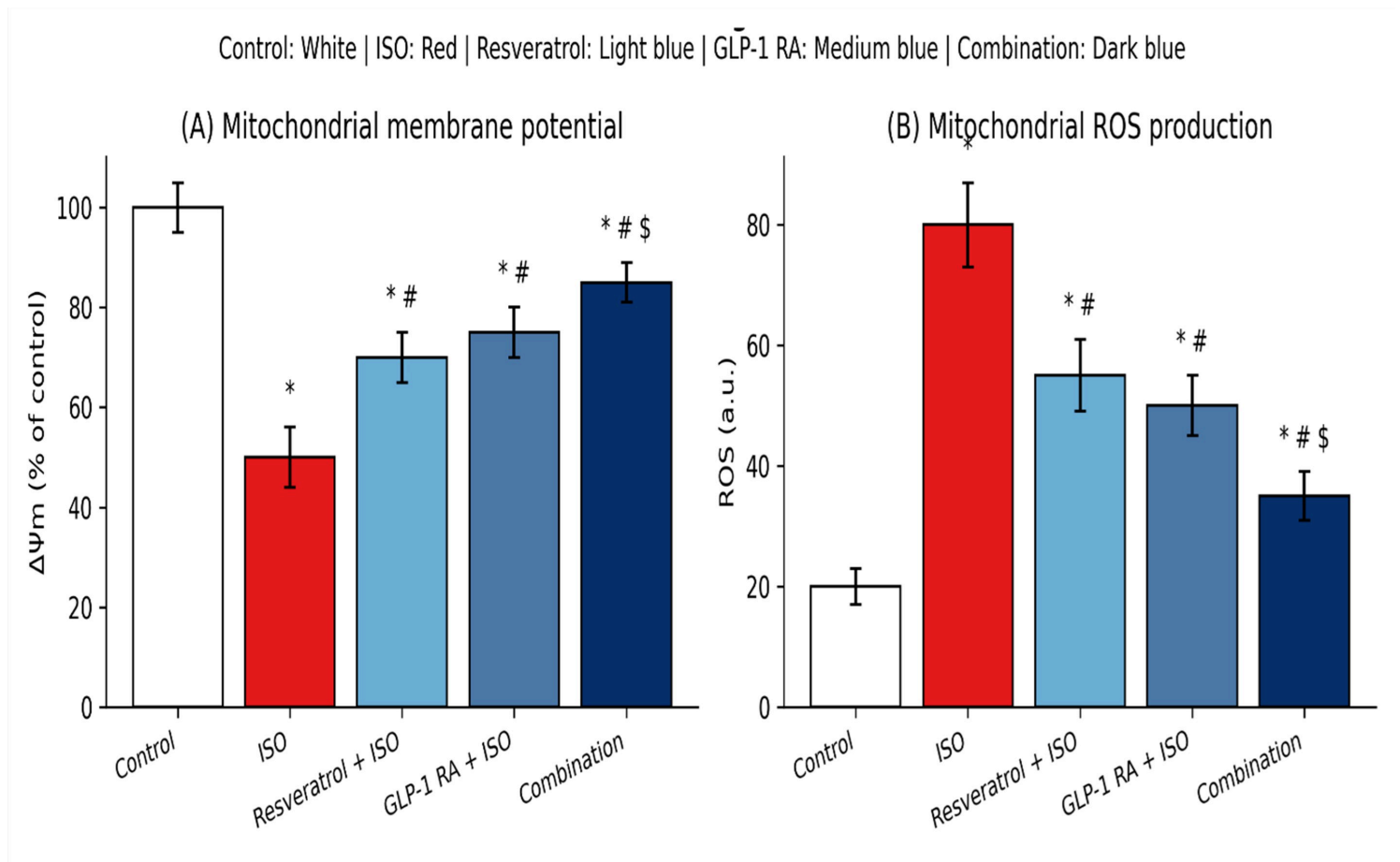


Figure 4

Effect of resveratrol and liraglutide on mitochondrial function parameters.

(A) Mitochondrial membrane potential ($\Delta\Psi_m$) and (B) mitochondrial reactive oxygen species (ROS) production.

Groups: Control; ISO; Resveratrol + ISO; GLP-1 RA + ISO; and combination group (as described in Figure 1).

Data are presented as mean $\pm$ SEM (n = 14).

*p < 0.001 vs. Control;

#p < 0.01 vs. ISO;

\$p < 0.01 vs. Resveratrol + ISO and GLP-1 RA + ISO.

ISO significantly reduced $\Delta\Psi_m$ and increased mitochondrial ROS production. Resveratrol or liraglutide alone significantly attenuated these changes, whereas the combination demonstrated greater preservation. Two-way ANOVA indicated a significant interaction effect (p < 0.05).

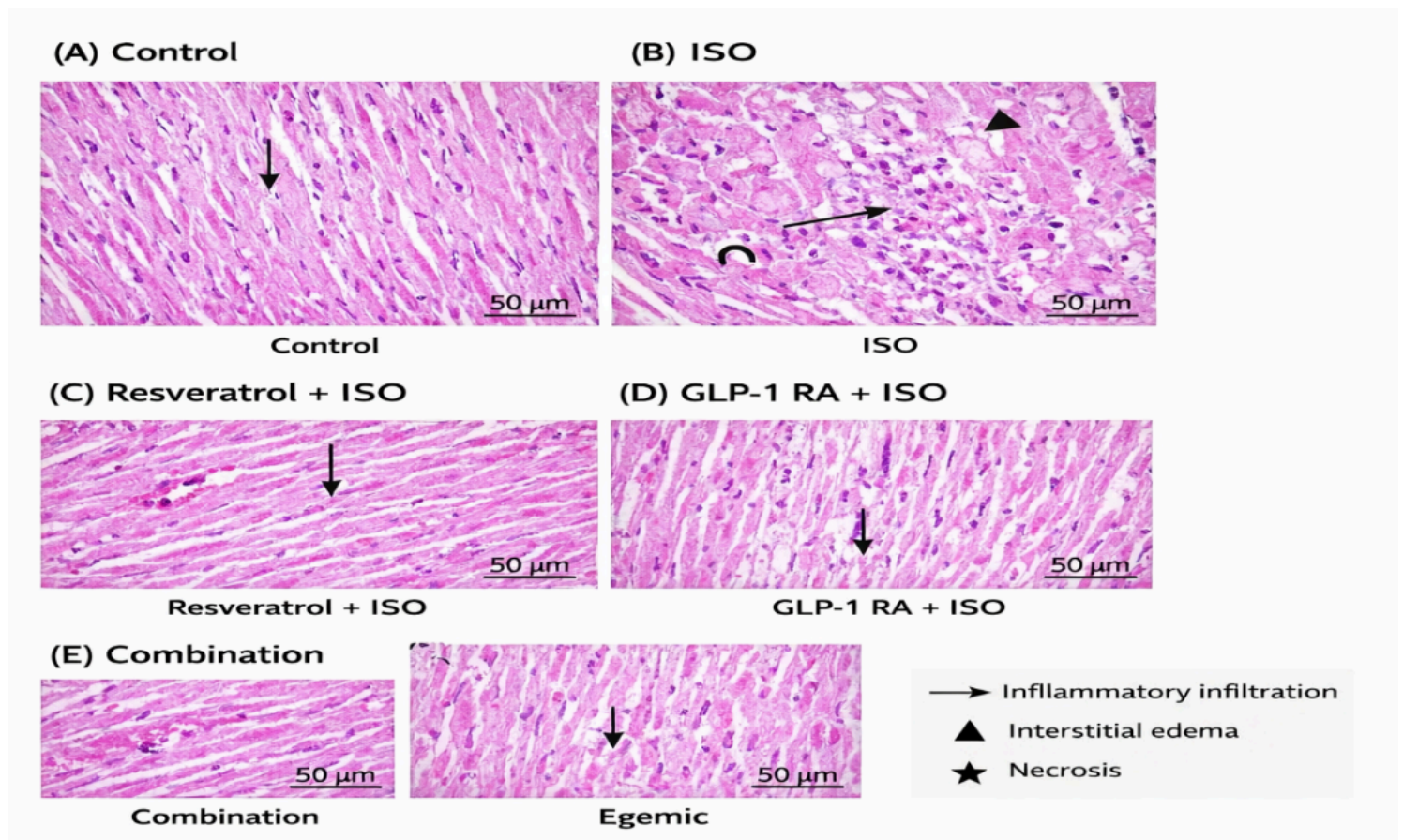


Figure 5

Histopathological examination of cardiac tissue (H&E staining, ×400 magnification).

(A) Control group: normal myocardial architecture with intact cardiac muscle fibers and no inflammatory infiltration.

(B) ISO group: extensive inflammatory infiltration (arrow), myofiber disruption, interstitial edema (arrowhead), and necrosis.

(C) Resveratrol + ISO group: partial improvement with reduced inflammatory infiltration.

(D) GLP-1 RA + ISO group: partial improvement with improved fiber alignment.

(E) Combination group: near-normal myocardial architecture with minimal inflammatory infiltration and preserved nuclear integrity.

Groups: Control; ISO; Resveratrol + ISO; GLP-1 RA + ISO; and combination group (as described in Figure 1).

Semi-quantitative histopathological scoring (0–3 scale): ISO group (2.8 ± 0.1) and combination group (0.6 ± 0.1 , $p < 0.001$ vs. ISO).

"Histopathological assessment was performed by a pathologist blinded to group allocation."