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Protective effects of vanillic acid against isoprenaline-induced heart failure in Wistar rats: A biochemical and histological investigation

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Abstract

BACKGROUND: Heart failure (HF) continues to pose a major health concern worldwide, frequently arising from ongoing myocardial damage driven by oxidative stress, inflammatory processes, and programmed cell death. While synthetic medications are widely used, natural compounds with multi-targeted actions are gaining attention. Vanillic acid (VA), a plant-derived phenolic compound, is suggested to possess cardioprotective properties; yet, its effects in experimental HF models remain underexplored.

METHODS: This study evaluated the impact of VA pretreatment on isoprenaline (ISO)-induced HF in male Wistar rats. A total of forty animals were randomly allocated into four distinct groups: sham, VA, ISO, and ISO + VA. ISO was administered subcutaneously to induce cardiac injury, while VA was given orally for 10 days prior to ISO exposure. Biochemical markers of myocardial damage, oxidative stress, inflammatory cytokines, infarct size, and apoptotic gene expression (Bax, Bcl-2) were assessed through ELISA, histological staining, and real-time PCR.

RESULTS: ISO administration caused significant elevation in cardiac enzymes (LDH, CK-MB, cTn-I), pro-inflammatory markers (IL-1β, TNF-α), lipid peroxidation (MDA), and Bax expression, along with reduced antioxidant enzyme activity (CAT, SOD, GPx), IL-10, and Bcl-2 levels. VA pretreatment mitigated these alterations, demonstrating a notable decrease in infarct size, oxidative damage, inflammation, and apoptosis. No adverse effects were observed in the VA-only group.

CONCLUSION: VA exhibited substantial cardioprotective effects against ISO-induced myocardial injury, likely through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. The results indicate that VA could be a valuable natural adjunct in the management of HF.

Keywords: Vanillic Acid; Isoprenaline-Induced Heart Failure; Oxidative Stress: Inflammation; Cardioprotection

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Introduction

Heart failure (HF) continues to be a critical worldwide health issue and a primary contributor to morbidity and mortality[1]. It is defined by the heart's reduced capacity to pump blood effectively, leading to inadequate delivery of oxygen and nutrients to peripheral tissues. A variety of molecular and cellular mechanisms contribute to the pathogenesis of HF, including chronic inflammation, oxidative stress, apoptosis, and structural remodeling of cardiac tissue[2]. Gaining insight into these fundamental mechanisms is crucial for creating novel therapeutic approaches that can slow down or potentially reverse the advancement of HF[3]. One of the drugs used to induce HF in laboratory animals is isoprenaline (ISO). ISO, a synthetic β-adrenergic agonist, is commonly used to induce experimental HF in animal models. When administered at high doses, ISO mimics sympathetic overstimulation, leading to myocardial injury, oxidative stress, inflammatory responses, and apoptosis in cardiac tissue[4]. The ISO-induced HF model in rats is widely used due to its reproducibility and ability to simulate many pathological features of human HF[5]. Over the past few years, interest has increased in investigating naturally derived compounds that possess cardioprotective effects. Among them, vanillic acid (VA; $C_8H_8O_4$), a naturally occurring phenolic compound found in many edible plants, has attracted attention due to its potent antioxidant, anti-inflammatory, and anti-apoptotic effects[6,7]. Previous research has demonstrated that VA significantly lowers lipid peroxidation, as indicated by reduced malondialdehyde (MDA) levels, while also boosting the activity of the heart's natural antioxidant defense mechanisms in isolated rat hearts exposed to ischemia-reperfusion (I/R) injury[8]. Similarly, some past studies have confirmed the protective roles of VA in various models of oxidative damage and metabolic dysfunction[7]; however, its specific role in the context of HF has not been fully elucidated[9].

This study is designed to explore whether pretreatment with VA can protect against cardiac inflammation, oxidative stress, apoptosis, and histopathological changes in a heart failure model induced by ISO in male Wistar rats. By assessing biochemical indicators and examining tissue structure, the research aims to deepen our understanding of VA's therapeutic potential in cardiovascular diseases. The results could pave the way for new complementary treatments based on natural compounds to help manage heart failure and associated cardiac conditions.

Materials and Methods

Materials

A 2% solution of xylazine and a 10% HCl-ketamine preparation were obtained from Alfasan (Netherlands) for use in animal anesthesia[10]. The biochemical assay kits used to determine creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) activities were provided by ZellBio (Germany). Levels of cardiac troponin-I (cTn-I) were determined using a specific assay kit from Monobind Inc. (Lake Forest, California, USA). The catalase (CAT) enzyme kit was also obtained from ZellBio (Germany), while kits for superoxide dismutase (SOD) and glutathione peroxidase (GPx) came from Randox (UK). Interleukin-1β (IL-1β) was measured with a kit from Diaclone (France), and interleukin-10 (IL-10) kits were provided by ZellBio (Germany). All chemicals, including VA, ISO, and 2,3,5-triphenyltetrazolium chloride (TTC), were sourced from Sigma-Aldrich (USA).

Animals

This study utilized 40 adult male Wistar rats, weighing between 250 and 300 grams, obtained from the animal facility at Lorestan University of Medical Sciences. One week before the experiment began, the animals were housed in an environment with controlled temperature (22 ± 2 °C) and a consistent light/dark cycle of 12 hours each. They had unrestricted access to both food and water. This acclimation period allowed the animals to adjust to their new environment before any experimental procedures started. All protocols were reviewed and approved by the Research Ethics Committee of Lorestan

University of Medical Sciences (Approval ID: IR.LUMS.REC.1403.405; Date: April 28, 2025). The rats were then randomly divided into four groups, with 10 animals in each group. Based on previous studies, a group size of 8 to 10 rats is typically sufficient to achieve statistical significance. However, due to the potential for mortality following surgical procedures in this model, a group size of 10 was selected to ensure adequate power[11].

Experimental Groups

The study consisted of four groups:

1. **Sham Group (Sham):** Administered normal saline orally once daily for 10 days[12].
2. **VA Group (VA):** Given VA dissolved in saline at a dose of 20 mg/kg per day by oral gavage for 10 days straight[12].
3. **ISO Group (ISO):** Received normal saline orally for 10 days, followed by subcutaneous injections of ISO at 100 mg/kg on days 11 and 12[13].
4. **ISO + VA Group (ISO + VA):** Treated with VA (20 mg/kg/day) via oral gavage for 10 days, combined with subcutaneous ISO injections (100 mg/kg/day) on days 11 and 12[13].

Inclusion and Exclusion Criteria

Only healthy male rats within the specified weight range, displaying calm and normal behavior, were included. Animals showing signs of skin or respiratory diseases, aggression, or any other pathological conditions were excluded from the study.

Induction of Cardiac Injury

For two days in a row, with a 24-hour gap between doses, the animals were given ISO (100 mg/kg) dissolved in saline through subcutaneous injection[13]. On the final day of the study, one day after the second ISO dose (day 13), all rats were first anesthetized with ketamine (60 mg/kg) and then humanely sacrificed. Following euthanasia, both blood and cardiac tissue samples were collected for subsequent biochemical and histological evaluations[11].

Evaluation of Myocardial Injury

On the 13th day of the experiment, to evaluate cardiac injury, blood was drawn directly from the heart. Using an ELISA reader and following the protocol included with each assay kit, we measured the levels of LDH, cTn-I, and CK-MB activity.

Infarct Size Measurement

Upon conclusion of the experimental protocol, the subjects were humanely sacrificed and their hearts were carefully extracted. Each heart was wrapped in foil and placed in a −20 °C freezer for 2 hours. The tissue was then cut into 2-mm-thick sections, which were stained with TTC solution at 37 °C for 20 minutes to visually distinguish healthy tissue from damaged areas. After staining, the sections were preserved in 10% formalin for 24 hours. Digital analysis using ImageJ software (NIH, Bethesda, MD, USA) was employed to measure the infarcted areas, with results calculated as the percentage of affected tissue compared to the total area of each section, evaluated from both surfaces[14].

Assessment of Inflammatory Markers

The concentrations of IL-1β, TNF-α, and IL-10 in heart tissue were assessed to evaluate pro-inflammatory and anti-inflammatory responses[11]. After the experimental period, heart tissues were homogenized and centrifuged, and the levels of these cytokines were measured in the supernatant using ELISA kits according to the manufacturers' instructions. Absorbance values were then recorded with an ELISA reader (Biotek, USA).

Measurement of Antioxidant Enzyme Activity

The activities of CAT, SOD, and GPx in cardiac tissue were evaluated to assess antioxidant defense[14]. After the experimental period, heart tissues were homogenized and centrifuged, and the enzyme activities were measured in the supernatant using ELISA kits, following the manufacturers' instructions.

Lipid Peroxidation Assessment

To evaluate lipid peroxidation, MDA levels were measured in cardiac tissue[14]. The hearts were promptly excised and snap-frozen in liquid nitrogen before being stored at −80 °C until further analysis. Prior to testing, the frozen samples were homogenized in ice-cold 50 mM phosphate-buffered saline (10% w/v) and then centrifuged to separate the components. The resulting supernatants were collected and analyzed using a commercial MDA assay kit (ZellBio, Germany), with absorbance measured at 532 nm.

Real-Time Polymerase Chain Reaction (RT-PCR)

For gene expression analysis, total RNA was extracted from frozen heart samples using an RNA purification kit (Ampliqon, Denmark) to study the expression patterns of Bax, Bcl-2, and the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). RNA quality and quantity were assessed by spectrophotometric analysis at 260 nm and 280 nm (Eppendorf, NanoDrop Thermo Scientific, S.N: D015). cDNA was synthesized following the manufacturer's guidelines using a cDNA synthesis kit (Ampliqon, Denmark). Quantitative PCR was performed on a RunMei Q200 system (China) to quantify Bax and Bcl-2 mRNA levels in cardiac tissue. Specific primers for each target gene were obtained from Bioneer (Daejeon, South Korea), with sequences listed in Table 1. Relative gene expression was calculated using the comparative Ct method, and fold changes were determined by the ΔΔCt method, with data analysis performed using RunMei QC 3.2 software[10].

Table 1. Primer sequences used in RT-PCR

Gene	Specific primer sequence
Bax	F: 5- CTGGGATGGCCTCCTTTCC-3 R: 5- CTTTCCCCGTTCCCCATTCA-3
Bcl-2	F: 5- TCTCATGCCAAGGGGGAAAC-3 R: 5- CGGTAGCGACGAGAGAAGTC-3
GAPDH	F: 5- GCAGAGATATGCCCATGTGGT-3 R: 5- GGTGAGCTGACAGTGGACAT-3

Statistical Analysis

All statistical procedures were implemented through SPSS version 22 software. Quantitative results are displayed throughout as mean ± SD values. Prior to analysis, we confirmed data normality using the Kolmogorov-Smirnov test. For between-group comparisons, we employed one-way ANOVA with Tukey's post hoc correction when appropriate. Throughout our analyses, we considered $P < 0.05$ to indicate statistically significant differences.

Results

Evaluation of cardiac markers

Following the second isoproterenol administration, LDH activity showed a significant increase at 24 hours post-injection when compared to the sham-treated controls ($P < 0.05$). Notably, pretreatment with VA in the VA + ISO group resulted in a substantial reduction in LDH levels compared to the ISO-only treated group ($P < 0.05$). The data further revealed that LDH activity in the VA-treated group did not significantly differ from the sham group, as illustrated in Fig. 1A.

Twenty-four hours following the second ISO injection, marked elevations in CK-MB were apparent compared to the sham group ($P < 0.05$). VA pretreatment effectively mitigated this response, with the VA + ISO group showing lower CK-MB levels than the ISO-treated group ($P < 0.05$). It is noteworthy that VA administration alone did not cause a change in CK-MB levels and was indistinguishable from the sham group (Fig. 1B).

The data presented in Fig. 1C reveal a substantial elevation in cTn-I concentrations 24 hours following the second isoproterenol administration when compared to the sham group ($P < 0.01$). This ISO-induced increase in the cardiac injury marker was significantly attenuated by VA pretreatment in the VA + ISO group compared to ISO-treated animals ($P < 0.01$). Notably, VA administration alone maintained cTn-I levels at baseline, showing no statistically significant difference from the sham group.

Infarct Size Measurement

The results of TTC staining, as depicted in Fig. 2, demonstrate a substantial enlargement of infarct size in the ISO group at the experimental endpoint when compared to the sham group ($P < 0.001$). This ISO-induced myocardial damage was significantly mitigated by VA pretreatment in the VA + ISO group compared to the ISO-only group ($P < 0.05$). Notably, no infarct areas were detectable in either the VA or sham animals.

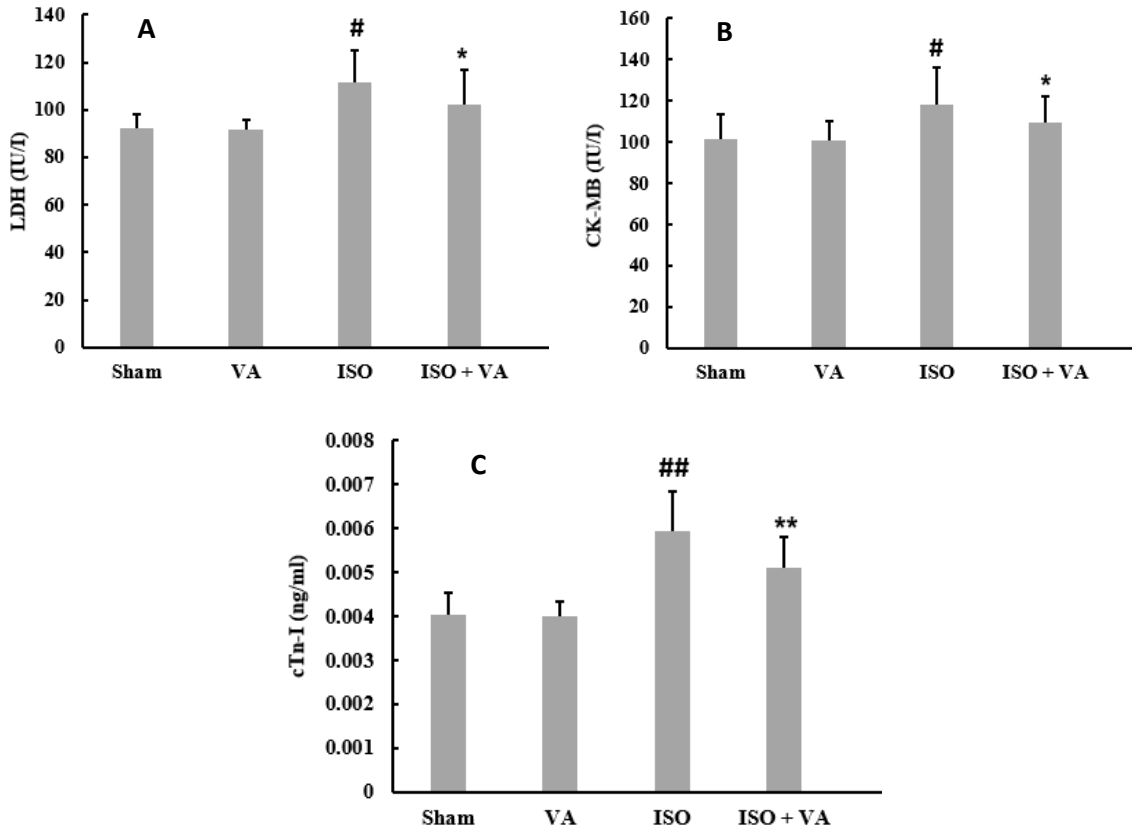


Figure 1. Effects of vanillic acid on cardiac enzyme levels in isoprenaline-induced cardiac injury
(A) LDH (lactate dehydrogenase), (B) CK-MB (creatine kinase-MB), and (C) cTn-I (cardiac troponin-I) levels were evaluated following treatment with vanillic acid (20 mg/kg/day, gavage). VA: vanillic acid; ISO: isoprenaline.
#p < 0.05 and ##p < 0.01 vs. sham group; *p < 0.05 and **p < 0.01 vs. ISO group.

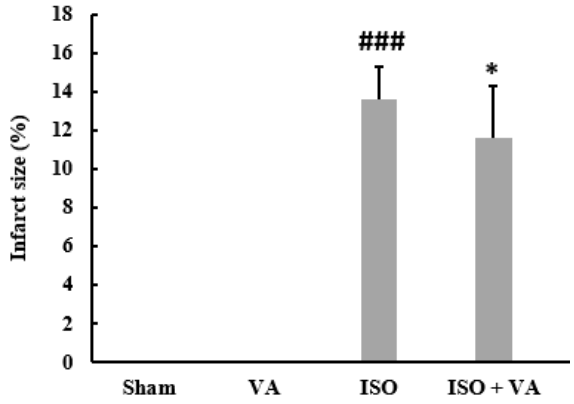


Figure 2. Effects of vanillic acid on myocardial infarct size determined by TTC staining
Myocardial infarct size was evaluated following treatment with vanillic acid (20 mg/kg/day, gavage). VA: vanillic acid; ISO: isoprenaline; TTC: 2,3,5-triphenyltetrazolium chloride.
###p < 0.001 vs. sham group; *p < 0.05 vs. ISO group.

Assessment of Inflammatory Cytokines

By the end of the experiment, the ISO group showed a significant decrease in the anti-inflammatory cytokine IL-10 levels compared to the sham group ($P < 0.05$). Pretreatment with VA in the VA + ISO group, however, significantly elevated IL-10 levels relative to the ISO group ($P < 0.05$). Importantly, no change in IL-10 levels was detectable in either the VA or sham animals (see Fig. 3A).

As demonstrated in Fig. 3B, ISO administration resulted in a significant upregulation of the key inflammatory marker IL-1β when compared to the sham group ($P < 0.01$). This pro-inflammatory response was effectively attenuated by VA pretreatment in the VA + ISO group compared to ISO-treated animals ($P < 0.01$). Importantly, VA treatment alone maintained IL-1β concentrations at baseline levels, showing no statistically significant difference from the sham group.

The data presented in Fig. 3C reveal a substantial increase in TNF-α expression in the ISO-treated group when compared to the sham group ($P < 0.01$). This inflammatory response was significantly attenuated by VA pretreatment in the VA + ISO group compared to ISO-treated animals ($P < 0.05$). Notably, VA administration alone maintained TNF-α at baseline levels, showing no significant difference from the sham group.

Measurement of Antioxidant Enzyme Activity

Fig. 4A illustrates the antioxidant defense response, demonstrating a marked reduction in CAT enzyme activity in ISO animals compared to sham animals ($P < 0.01$). This antioxidant marker was significantly restored by VA pretreatment in the VA + ISO group relative to the ISO group ($P < 0.01$). Notably, VA treatment alone showed no statistically significant difference from the sham group.

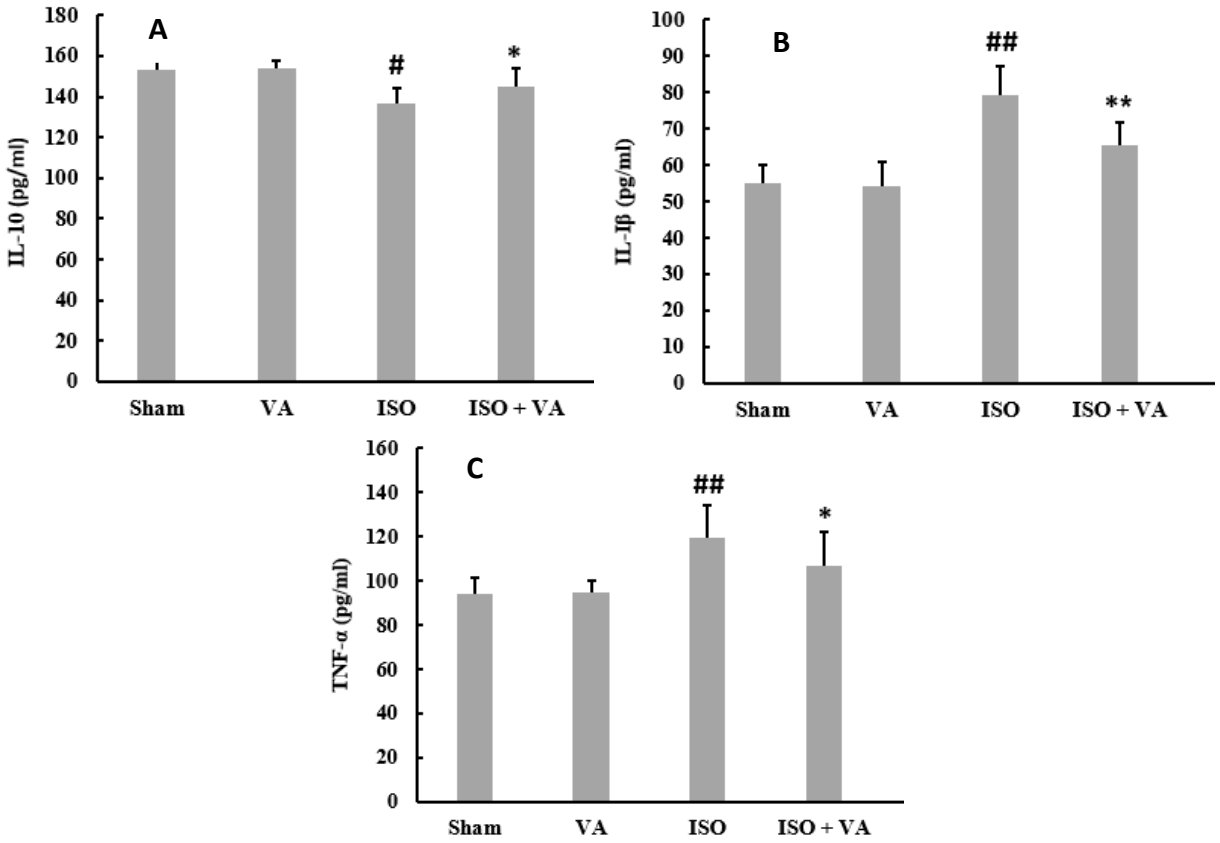


Figure 3. Effects of vanillic acid on inflammatory cytokine levels in isoprenaline-induced cardiac injury
(A) IL-10 (interleukin-10), (B) IL-1β (interleukin-1β), and (C) TNF-α (tumor necrosis factor-α) levels were evaluated following treatment with vanillic acid (20 mg/kg/day, gavage). VA: vanillic acid; ISO: isoprenaline.
#$p < 0.05$ and ##$p < 0.01$ vs. sham group; *$p < 0.05$ and **$p < 0.01$ vs. ISO group.

The results depicted in Fig. 4B show a significant decrease in SOD activity in the ISO group when compared to the sham group ($P < 0.05$). This reduction in antioxidant defense was effectively counteracted by VA pretreatment in the VA + ISO group compared to ISO-treated animals ($P < 0.05$). Notably, VA administration alone maintained SOD levels comparable to those observed in the sham group, with no significant differences detected.

The data presented in Fig. 4C reveal a substantial decline in GPx activity in ISO animals compared to the sham group ($P < 0.01$). This reduction in antioxidant capacity was significantly ameliorated by VA pretreatment in the VA + ISO group relative to ISO-treated animals ($P < 0.05$). Importantly, VA administration alone maintained GPx activity at baseline levels, showing no statistically significant difference from the sham group.

Assessment of MDA Content

The lipid peroxidation analysis presented in Fig. 5 demonstrates a significant elevation in MDA content in ISO animals compared to the sham group ($P < 0.01$). This marker of lipid peroxidation was significantly reduced by VA pretreatment in the VA + ISO group relative to ISO-injected animals ($P < 0.01$). Remarkably, VA administration alone maintained MDA concentrations at baseline levels, with no significant difference observed from the sham group.

Bax Gene Expression Analysis

The protein expression analysis shown in Fig. 6A indicates that ISO administration significantly increased Bax levels compared to the sham group ($P < 0.001$). This pro-apoptotic effect was substantially mitigated by VA pretreatment in

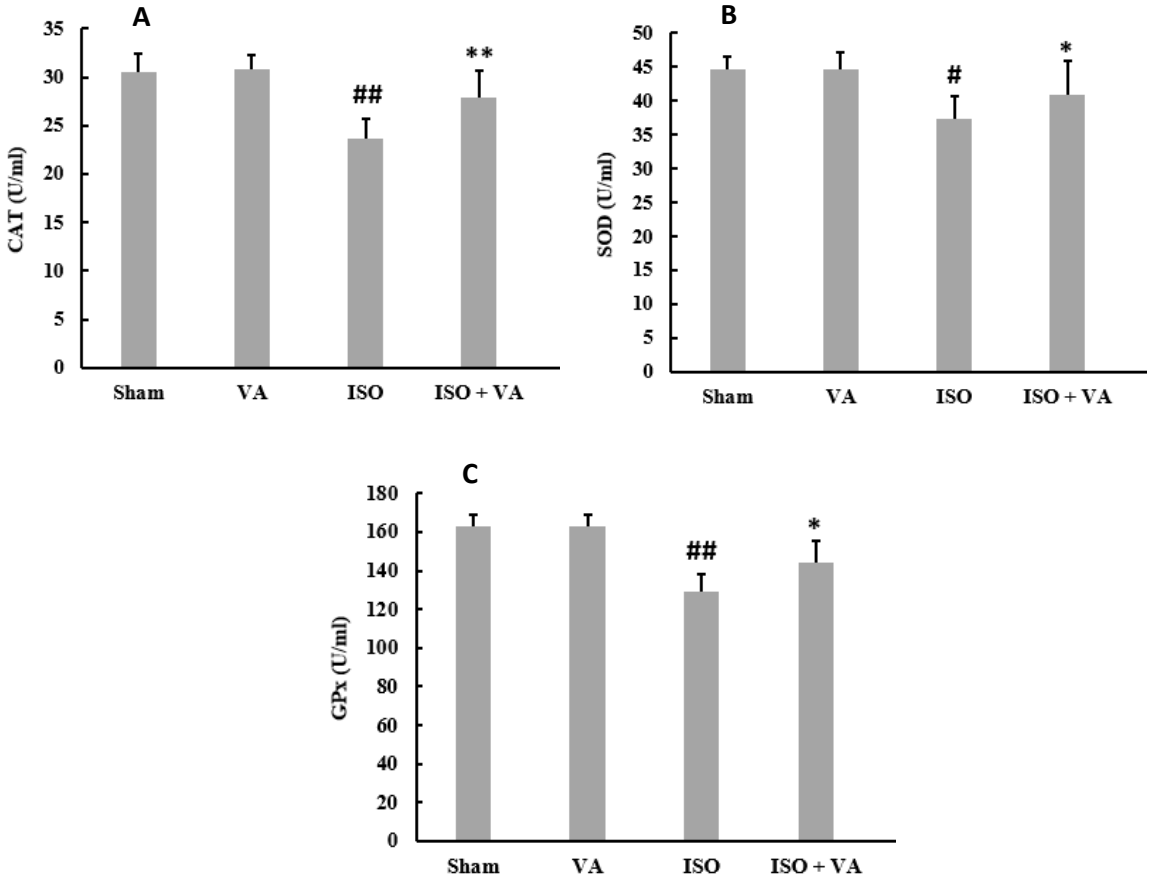


Figure 4. Effects of vanillic acid on antioxidant enzyme activities in isoprenaline-induced cardiac injury
(A) CAT (catalase), (B) SOD (superoxide dismutase), and (C) GPx (glutathione peroxidase) activities were evaluated following treatment with vanillic acid (20 mg/kg/day, gavage). VA: vanillic acid; ISO: isoprenaline.
#p < 0.05 and ##p < 0.01 vs. sham group; *p < 0.05 and **p < 0.01 vs. ISO group.

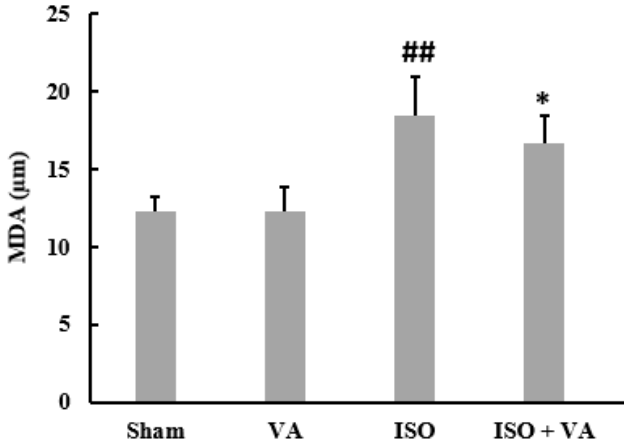


Figure 5. Effects of vanillic acid on lipid peroxidation in isoprenaline-induced cardiac injury
Lipid peroxidation was assessed by measuring MDA (malondialdehyde) levels following treatment with vanillic acid (20 mg/kg/day, gavage). VA: vanillic acid; ISO: isoprenaline.
##p < 0.01 vs. sham group; *p < 0.05 vs. ISO group.

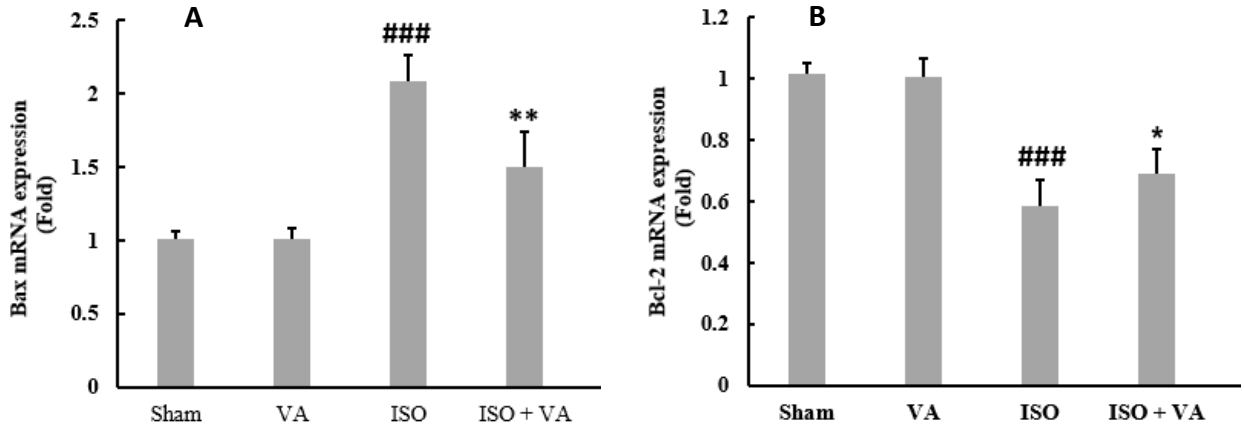


Figure 6. Effects of vanillic acid on apoptotic gene expression in isoprenaline-induced cardiac injury
(A) Bax and (B) Bcl-2 activities were evaluated following treatment with vanillic acid (20 mg/kg/day, gavage). VA: vanillic acid; ISO: isoprenaline.
###p < 0.001 vs. sham group; *p < 0.05 and **p < 0.01 vs. ISO group.

the VA + ISO group versus ISO-injected animals (P < 0.01). VA treatment alone maintained Bax expression at levels comparable to the sham group, with no statistically significant difference observed.

Bcl-2 Gene Expression Analysis

The anti-apoptotic gene Bcl-2 showed a pronounced decrease in expression within the ISO-treated group when compared to the sham group (P < 0.001). However, administration of VA (in the VA + ISO group) significantly restored Bcl-2 expression levels compared to the ISO-only group (P < 0.05). VA treatment alone maintained Bcl-2 expression at levels comparable to the sham group, with no statistically significant difference observed (Fig. 6B).

Discussion

The findings **of** this study collectively demonstrate that VA pretreatment significantly attenuates ISO-induced myocardial injury, suggesting its strong cardioprotective potential through multiple mechanisms, including the modulation of oxidative stress, inflammation, apoptosis, and infarct formation. One of the

primary indicators of myocardial damage is the elevation of cardiac enzymes such as LDH, CK-MB, and cTn-I, which are released into circulation following myocardial cell injury or necrosis[15]. In this study, ISO administration led to a marked increase in the serum levels of these markers, reflecting significant myocardial damage. This observation aligns with previous research reporting elevated cardiac biomarkers following β-adrenergic overstimulation by ISO, which induces myocardial necrosis and membrane leakage[4,16]. Notably, VA pretreatment significantly reduced these enzyme levels, suggesting its ability to stabilize cardiomyocyte membranes and prevent leakage of intracellular contents. These results are in agreement with the findings of Prince et al., who demonstrated that VA mitigates ISO-induced cardiotoxicity and enzyme leakage by reinforcing myocardial membrane integrity[17]. On the other hand, the reduction in infarct size observed in the VA + ISO group, as demonstrated by TTC staining, further confirms the protective role of VA against myocardial ischemic injury. ISO is known to cause regional myocardial necrosis and simulate pathological features of infarction due to excessive β-adrenergic receptor stimulation[18]. VA's ability to significantly reduce infarct size in this model indicates its potential in preserving viable myocardium under stress conditions. This is likely attributable to its antioxidant properties, as previously reported in ischemia-reperfusion models[9,19].

Inflammation is a key contributor to the development and progression of HF[20]. The pro-inflammatory cytokines IL-1β and TNF-α play critical roles in mediating cardiac inflammation, apoptosis, and remodeling[21]. In this study, ISO administration significantly elevated IL-1β and TNF-α levels in cardiac tissue, consistent with reports highlighting the pro-inflammatory effects of catecholamine-induced cardiotoxicity[22]. The findings of this research showed that VA pretreatment significantly suppressed the levels of these cytokines, indicating its anti-inflammatory potential. This is consistent with previous studies demonstrating that VA inhibits pro-inflammatory cytokine production in cardiovascular and metabolic disease models[8,23]. Additionally, IL-10, a key anti-inflammatory cytokine[24], was significantly reduced in the ISO group but restored by VA, suggesting that VA not only suppresses pro-inflammatory responses but also enhances anti-inflammatory signaling to maintain immune homeostasis.

Cardiac injury induced by ISO is strongly linked to oxidative stress, which arises when the production of reactive oxygen species (ROS) overwhelms the body's antioxidant defense mechanisms. This imbalance in oxidative homeostasis contributes significantly to myocardial damage[25]. ISO generates excess ROS through auto-oxidation and metabolic pathways, leading to lipid peroxidation, protein damage, and mitochondrial dysfunction[25,26]. In this study, ISO significantly elevated MDA levels, a primary indicator of lipid peroxidation, while simultaneously decreasing the activities of antioxidant enzymes such as CAT, SOD, and GPx. These findings are in agreement with prior reports that ISO depletes endogenous antioxidants, thereby exacerbating oxidative damage in the myocardium[27,28]. VA pretreatment significantly restored the activities of these enzymes and reduced MDA content, suggesting its potent antioxidant capacity. This antioxidant effect may be attributed to the phenolic structure of VA, which enables free radical scavenging and metal ion chelation, thereby mitigating oxidative injury[7,29].

Apoptosis is another major pathological mechanism in HF, contributing to the loss of functional cardiomyocytes and progression of cardiac dysfunction[30]. The balance between pro-apoptotic (like Bax) and anti-apoptotic (like Bcl-2) proteins governs cell survival in cardiac tissue[31]. In the current study, ISO significantly upregulated Bax and downregulated Bcl-2 expression, indicating activation of apoptotic pathways. These results are consistent with previous findings showing that ISO promotes mitochondrial-dependent apoptosis in cardiac cells[32]. Interestingly, VA pretreatment reversed these changes by downregulating Bax and

upregulating Bcl-2, highlighting its anti-apoptotic effects. These observations are in line with prior reports where VA was shown to inhibit apoptosis in various tissues by modulating Bax/Bcl-2 expression and preserving mitochondrial integrity[33–35].

Collectively, these findings suggest that VA exerts a multifaceted cardioprotective effect in the setting of ISO-induced HF. Its efficacy appears to be mediated by a combination of antioxidant, anti-inflammatory, and anti-apoptotic actions, which together help preserve myocardial structure and function. The absence of significant differences between the VA and sham groups across most parameters indicates that VA alone does not negatively affect cardiac homeostasis and is well tolerated at the administered dose. From a translational perspective, the data support the potential of VA as a natural therapeutic agent or adjunct treatment in cardiovascular diseases characterized by oxidative stress, inflammation, and apoptosis. Given its presence in various dietary sources and its relatively low toxicity profile, VA could be considered for further development into functional supplements or pharmacological formulations[36,37]. However, it is essential to recognize the limitations of this study. First, the current findings are based on an acute model of HF in animals, which may not fully replicate the complex and chronic nature of human HF. Second, the study did not explore long-term effects or the molecular signaling pathways in detail. Future investigations should consider exploring the chronic administration of VA, its interaction with standard HF therapies, and its underlying mechanisms at the molecular and genomic levels. Moreover, evaluating the pharmacokinetics and bioavailability of VA in vivo, along with its efficacy in larger animal models or clinical trials, would be critical steps toward its therapeutic translation. Further studies may also examine the potential synergistic effects of VA when combined with other natural compounds or conventional drugs, particularly in multifactorial conditions like HF.

Conclusion

In conclusion, the present study provides strong experimental evidence supporting the cardioprotective role of VA in ISO-induced HF in rats. Through the modulation of oxidative stress, inflammation, and apoptosis, VA significantly improved biochemical, molecular, and histopathological outcomes. These findings highlight VA's promise as a candidate for future cardioprotective strategies and lay the groundwork for subsequent preclinical and clinical research.

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Conflict of interests

The authors declare no conflict of interest.

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Author's Contributions

Study Conception or Design: NP; FS
Data Acquisition: NP; AN; RA; FS
Data Analysis or Interpretation: MK; AN; RA; FS
Manuscript Drafting: NP; MK; AN; RA; FS
Critical Manuscript Revision: NP; MK; AN; RA; FS

All authors have approved the final manuscript and are responsible for all aspects of the work.

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