

Vitamin C Attenuates Trichloroacetic Acid-Induced Cardiac Injury and Fibrotic Remodeling in Rats: Biochemical and Histopathological Evidence

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Abstract

Trichloroacetic acid (TCA), a widely used chemical in industrial, educational, and research settings, has been associated with various toxic effects, including inflammation-mediated tissue injury. This study investigated the protective effects of vitamin C against TCA-induced cardiac injury in rats. Twenty male Wistar rats were randomly assigned to four groups ($n = 5/\text{group}$): control, TCA, vitamin C, and TCA plus vitamin C. Treatments were administered orally by gavage for 21 consecutive days. At the end of the experiment, serum interleukin-1 β (IL-1 β) levels and creatine phosphokinase (CPK) activity were measured, and cardiac tissues were evaluated histopathologically for structural alterations and myocardial fibrosis. TCA administration significantly increased serum IL-1 β concentrations and CPK activity compared with the control group ($P < 0.0001$). Co-administration of vitamin C significantly reduced IL-1 β levels ($P = 0.0211$) and CPK activity ($P = 0.0182$) compared with the TCA-treated group. Histopathological examination revealed marked myocardial degeneration, structural disorganization, and increased cardiac fibrosis following TCA exposure ($P < 0.0001$), whereas these alterations were significantly attenuated by vitamin C treatment ($P < 0.0001$). These findings demonstrate that TCA induces cardiac injury, inflammation, and myocardial fibrosis, while vitamin C partially mitigates these adverse effects through improvements in both biochemical and histopathological parameters. Notably, despite its established role as a cofactor in collagen biosynthesis, vitamin C was associated with reduced myocardial fibrosis under pathological conditions, suggesting a potential cardioprotective and anti-fibrotic role against TCA-induced toxicity.

Introduction

Trichloroacetic acid (TCA) is a major oxidative metabolite of trichloroethylene, a widely used industrial solvent. Exposure to TCA has been associated with toxic effects in several organs, primarily through mechanisms involving oxidative stress and inflammation (Lash, Chiu, Guyton, & Rusyn, 2014; Yoo et al., 2015). While the hepatotoxic and nephrotoxic effects of TCA have been extensively investigated, its potential cardiotoxic effects remain insufficiently characterized (Arruda et al., 2024; Srivastava et al., 2016).

Cardiac injury induced by toxic chemicals is frequently accompanied by inflammatory responses, oxidative damage, and tissue remodeling (Liu et al., 2025). Interleukin-1 β (IL-1 β) is a key pro-inflammatory cytokine implicated in cardiovascular injury and adverse cardiac remodeling (Abbate et al., 2020; Bonaventura et al., 2023). In addition, persistent inflammation may contribute to the pathophysiology of heart failure and fibrosis, which can impair cardiac structure and function (Roman-Pepine, Serban, Capras, Cismaru, & Filip, 2025).

Vitamin C (ascorbic acid) is a water-soluble antioxidant with well-documented free radical scavenging and anti-inflammatory properties (Hemani & Sonawane, 2024). Previous studies have demonstrated the protective effects of vitamin C against toxicant-induced tissue injury, including cardiovascular damage

(Nsairat et al., 2025; Rozemeijer, Hemilä, van Baaren, & de Man, 2023; Tai et al., 2022; Xu, Zheng, Slabu, Liehn, & Rusu, 2025).

Although vitamin C is a cofactor for prolyl and lysyl hydroxylases involved in collagen synthesis and tissue repair (Piersma et al., 2017), Emerging evidence suggests that it may also attenuate pathological fibrosis through its antioxidant and anti-inflammatory effects (Rodrigues da Silva et al., 2018). Studies have shown that vitamin C can reduce pro-fibrotic mediators and extracellular matrix accumulation in experimental models of pulmonary and cardiac fibrosis (Fang et al., 2024). Despite the recognized systemic toxicity of TCA, the roles of inflammation and fibrosis in TCA-induced cardiac injury remain incompletely understood. Therefore, this study investigated the protective effects of vitamin C against TCA-induced cardiac injury in rats by evaluating IL-1 β , CPK activity, histopathological alterations, and myocardial fibrosis.

Materials and Methods

Animals and Experimental Design

Twenty healthy adult female Wistar rats (180 ± 10 g) were obtained from the animal facility of Babol University of Medical Sciences. All procedures were approved by the local Ethics Committee (IR.MUBABOL.AEC.1402.004) and conducted according to the NIH Guidelines for the Care and Use of Laboratory Animals (Clinical trial number: Not applicable). The rats were housed in standard polypropylene cages under controlled environmental conditions (temperature: $22 \pm 2^\circ\text{C}$, relative humidity: 50–60%, and 12-h light/dark cycle) with ad libitum access to food and water.

The animals were randomly divided into four experimental groups ($n = 5$ per group):

- Group 1 (Control): Received normal saline (1 mL/day, gavage).
- Group 2 (TCA): Received trichloroacetic acid (400 mg/kg/day) via oral gavage.
- Group 3 (Vitamin C): Received vitamin C (50 mg/kg/day) via oral gavage.
- Group 4 (TCA + Vitamin C): Received both TCA (400 mg/kg/day) and vitamin C (50 mg/kg/day) via oral gavage.

After 21 days of treatment, rats were anesthetized intraperitoneally with ketamine (80 mg/kg) and xylazine (5 mg/kg). Blood was collected via cardiac puncture into plain tubes, allowed to clot at room temperature, and centrifuged at 3000 rpm for 10 min; the separated serum was stored at -80°C until analysis. Following euthanasia, hearts were excised, rinsed in cold saline, and fixed in 10% neutral-buffered formalin for 24 h with one solution change to ensure complete fixation.

Biochemical Assay

Serum interleukin-1 beta (IL-1 β) levels were determined using a rat-specific ELISA kit (Carmania Parsgen Co., Iran) according to the manufacturer's instructions. The activity of creatine kinase (CPK) was

measured using a colorimetric assay kit (Pars Azmoon Co., Iran)

Tissue Processing and Sectioning

Cardiac tissues were fixed in 10% neutral-buffered formalin for 48 hours, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin using standard histological protocols. Paraffin blocks were sectioned at a thickness of 7 μm using a rotary microtome, and the sections were then mounted on glass slides for subsequent staining.

Hematoxylin and Eosin (H&E) Staining

Paraffin-embedded sections (7 μm) were deparaffinized in xylene, rehydrated through graded ethanol, and rinsed in tap water. Nuclei were stained with Mayer's hematoxylin (Asiapajouhesh, Iran; Cat. No. 0303633), differentiated in acid alcohol, blued, and counterstained with eosin (Asiapajouhesh, Iran; Cat. No. 0303633). Following dehydration and clearing, slides were mounted with synthetic medium. Stained sections were evaluated using an Olympus BX53 light microscope at 18 $\times$, 45 $\times$, and 180 $\times$ magnifications.

Masson's Trichrome Staining

Collagen deposition was evaluated using a commercial Masson's trichrome kit (Asiapajouhesh, Iran; Cat. No. 140011), applied according to the manufacturer's protocol. Briefly, sections were rehydrated and sequentially stained with Weigert's hematoxylin (10 min), Biebrich scarlet–acid fuchsin (2 min), phosphotungstic–phosphomolybdic acid (10 min), and aniline blue (2 min). After rinsing in 1% acetic acid, slides were dehydrated, cleared, and coverslipped.

Histopathological Evaluation

Sections were examined under an Olympus BX53 microscope (Olympus, Japan) equipped with an AmScope digital eyepiece camera (AmScope, USA; 4.5 $\times$ lens magnification; objectives: 4 $\times$, 10 $\times$, 40 $\times$). Images were captured and analyzed with Zistrad software (v97-1). Histopathological features—including sarcolemma disruption, cardiomyocyte necrosis, fiber separation, myofibrillar loss, lipofuscin accumulation, hyalinization, vascular congestion, and interstitial fibrosis—were graded on a semi-quantitative scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). All evaluations were performed blindly (Ahmed et al., 2021; Othmène, Hamdi, Amara, & Abid-Essefi, 2020; Rezaeyan, Haddadi, Hosseinzadeh, Moradi, & Najafi, 2016).

Quantification of cardiac fibrosis

Interstitial fibrosis in cardiac tissue was evaluated on Masson's trichrome–stained sections. Images were captured at 18 $\times$ magnification and analyzed with Fiji software (ImageJ, NIH, Bethesda, MD, USA). Collagen fibers were separated using color deconvolution with the Masson's trichrome vector, followed by application of a fixed threshold uniformly across all sections. For each animal, 4–5 randomly chosen

fields of the myocardium were analyzed, and the collagen-positive area fraction (% area) was calculated (see Figure S1).

Statistical Analysis

All statistical analyses were conducted using GraphPad Prism 10.5.0. IL-1 β and CPK analysis were performed using one-way analysis of variance (ANOVA), the Kruskal-Wallis test was used for histopathological evaluation, and Nested one-way ANOVA for Quantification of cardiac fibrosis. Results are expressed as mean $\pm$ standard error of the mean (SEM), and p-values < 0.05 were considered statistically significant. A p-value $\leq$ 0.05 was considered statistically.

Results

Serum IL-1 β Levels

Figure 1a shows significant differences in serum IL-1 β levels among the experimental groups. The TCA group exhibited the highest IL-1 β concentration (127.5 ± 35.76 pg/mL), whereas the control group showed the lowest level (6.125 ± 0.4748 pg/mL). IL-1 β levels were significantly increased in the TCA group compared with the other groups ($P < 0.0001$; Table S1). Co-administration of vitamin C significantly reduced IL-1 β levels in the TCA+VitC group (76.75 ± 19.07 pg/mL) compared with the TCA group ($P = 0.0211$) (Fig. 1a; Table S1).

CPK Activity

Serum CPK activity was significantly elevated in the TCA group (208.8 ± 17.27 U/L) compared with controls (78.50 ± 11.27 U/L). Vitamin C treatment significantly attenuated TCA-induced CPK elevation (175.5 ± 14.18 U/L; $P = 0.0182$ vs. TCA). No significant difference was observed between the control and vitamin C groups ($P = 0.0638$) (Fig. 1b; Table S1).

Histopathology of Heart Tissue

H&E staining revealed normal myocardial architecture in the control and vitamin C groups. In contrast, TCA-treated rats exhibited marked histopathological alterations, including disruption of myocardial fibers, sarcolemmal damage, widened intercellular spaces, and pyknotic nuclei. These changes were markedly attenuated in the TCA+VitC group, which showed substantial preservation of normal cardiac morphology (Figs. 2, S1, and S2).

Masson's trichrome staining demonstrated minimal collagen deposition in the control and vitamin C groups. In contrast, the TCA group showed extensive collagen accumulation and interstitial fibrosis in both the endomysium and perimysium. These fibrotic changes were markedly reduced following vitamin C treatment (Fig. 3).

Histopathological scoring confirmed these observations (Fig. 4; Table S2). No significant lesions were observed in the control group, while the vitamin C group showed only mild vascular congestion. TCA administration induced significant pathological alterations, including severe sarcolemmal damage, myofibrillar loss, vascular congestion, and fiber separation ($P < 0.0001$). Co-treatment with vitamin C significantly improved all evaluated histopathological parameters ($P < 0.0001$), although mild-to-moderate congestion and cardiomyocyte necrosis remained evident. Detailed statistical results are presented in Supplementary Tables S2 and S3.

Effects of TCA and vitamin C on cardiac fibrosis

Semi-quantitative fibrosis scoring demonstrated significantly greater myocardial fibrosis in the TCA group than in controls ($P < 0.0001$), whereas vitamin C treatment markedly reduced fibrosis severity ($P < 0.0001$ vs. TCA) (Fig. 5a).

Quantitative image analysis confirmed these findings (Fig. 5b; Table S5). Fibrotic area increased from $5.73 \pm 0.55\%$ in controls to $15.53 \pm 1.65\%$ following TCA exposure ($P < 0.0001$). Vitamin C significantly reduced fibrosis to $7.33 \pm 0.69\%$ ($P < 0.0001$ vs. TCA), with no significant difference between the VitC and TCA+VitC groups.

Discussion

Inflammation and ROS are increased in chronic heart failure. Oxidative stress also induces inflammation, tissue hyperemia, and myocardial damage, finally leading to the progression of heart failure. Notably, inflammatory factors such as IL-1 β and CPK substantially increased in chronic heart failure and hold clinical relevance. Consequently, anti-inflammatory and antioxidant drugs may be considered adjunctive therapies for individuals at risk of heart failure. However, it is important to emphasize that a restricted number of clinical trials investigating antioxidants—such as alamipertide, vitamin C, and coenzyme Q10—have produced encouraging outcomes (Aimo et al., 2020).

In this study, the maximal levels of IL-1 β and CPK have been marked within the TCA group and with statistically significant shifts against the other groups. Furthermore, this group demonstrated histopathological changes in the destruction of tissues that were represented by parameters of sarcolemma damage, cardiac fiber breakage, myofibril loss (with hypochromic cytoplasm), cardiac fiber separation, lipofuscin deposition, hyalinization, blood congestion, and fibrosis. Previous studies have pointed out that high oxidative stress, inflammation, and hypomethylation are the mechanisms implicated in tissue damage, cancer, and metastasis induced by TCA. This is attributed to its metabolism through the cytochrome P450 system, and TCA causes tissue damage through oxidative stress production (Alam et al., 2022; Larson & Bull, 1992). Moreover, IL-1 β is a pivotal cytokine mediating inflammation-induced cardiac dysfunction. Shang et al. demonstrated that transverse aortic constriction-induced heart failure correlates with elevated IL-1 β levels and increased pulmonary inflammation. Notably, IL-1 β signaling blockade using neutralizing antibodies improved left ventricular

systolic function in heart failure rat models, while concurrently reducing oxidative stress, inflammasome activation, fibrosis, right ventricular hypertrophy, and pulmonary T-cell activation (Shang et al., 2020). Further supporting this paradigm, Jia et al. reported that trichloroethylene (TCE) exposure significantly increased serum levels of IL-1 β , IL-6, IL-8, and TNF- α in hypersensitivity dermatitis patients compared to controls. Interestingly, in vitro comparison of trichloroethanol and TCA effects on HaCaT keratinocytes revealed that only trichloroethanol elevated IL-1 β and IL-6 levels in a dose-dependent manner (Jia et al., 2012). The pathogenic role of IL-1 β extends beyond cardiac tissue, as demonstrated by Ibrahim et al.'s work showing IL-1 β inhibitors exert therapeutic effects in osteoarthritis while exhibiting anticancer properties through apoptosis induction (Ibrahim et al., 2021). These collective findings establish a bidirectional relationship between IL-1 β -mediated inflammation and tissue injury, corroborating our results. However, unlike Jia's study, our investigation did not examine TCA dose-dependence.

Notably, the TCA+Vitamin C group showed significantly lower IL-1 β levels than TCA-only animals ($p < 0.05$), confirming vitamin C's anti-inflammatory efficacy. Furthermore, CPK levels showed a significant downward trend alongside improved cardiac morphology (a two-grade scoring reduction), which may be mediated by nitric oxide pathways., which may reflect vitamin C's modulation of inflammatory mediators and sarcolemmal repair mechanisms (Chong; Saetang et al., 2025). These findings corroborate prior evidence demonstrating vitamin C's immunomodulatory effects in elderly populations, where improved clinical outcomes correlated with sustained reductions in inflammatory markers (Carr & Maggini, 2017), and Elevated CPK levels directly indicate myocardial injury due to sarcolemmal rupture, releasing intracellular enzymes into the bloodstream (Jong, Pinney, & Packard, 2022).

Histopathological analysis revealed that vitamin C treatment ameliorated TCA-induced damage, showing mild vascular congestion and cardiac morphology, while significantly decreasing collagen deposition and fibrosis in both endomysial and perimysial, consistent with its established role in suppressing fibrotic pathways. This observation converges with Xu. et al noted that despite vitamin C's well-established role in collagen synthesis as a cofactor for prolyl and lysyl hydroxylases, its paradoxical anti-fibrotic effects in cardiovascular remodeling are highlighted (Xu et al., 2025). While vitamin C is essential for collagen maturation and ECM stability, it simultaneously suppresses TGF- β signaling, reduces ROS-driven fibrosis, and downregulates MMP activity, preventing excessive collagen deposition and maladaptive cardiac fibrosis. This dual regulation positions vitamin C as a unique therapeutic agent that balances ECM repair while inhibiting pathological fibrosis, particularly during fibrosis remodeling (Abbaszadeh, Khezri, & Ghasemnejad-Berenji, 2025; Li, Liu, Lee, & Li, 2021). However, contrasting reports exist, such as El-Shitany et al.'s observation that vitamin C failed to prevent azithromycin-induced myocardial atrophy and even exacerbated vascular congestion (El-Shitany & El-Desoky, 2016). These discrepancies likely reflect differences in experimental models, dosing protocols, or exposure durations, underscoring the need for further investigation into vitamin C's dose-response relationships in cardioprotection.

The vascular benefits observed in our TCA+vitamin C group (demonstrating a two-grade scoring reduction in congestion severity) may be mediated through nitric oxide pathways. This mechanism is

supported by Heller et al.'s demonstration that ascorbic acid enhances endothelial NO synthesis (Heller, Munscher-Paulig, Gräbner, & Till, 1999), and by Yan et al.'s findings that vitamin C combined with tetrahydrobiopterin improves vascular perfusion following ischemic injury through eNOS upregulation (Yan, Tie, & Messina, 2012).

Based on the present findings, a putative mechanistic pathway is proposed (Fig. 6), whereby TCA exposure promotes oxidative stress and inflammatory responses, leading to increased IL-1 β production, myocardial injury, CPK release, histopathological alterations, and ultimately myocardial fibrosis. Conversely, vitamin C appears to interrupt this cascade through its antioxidant and anti-inflammatory properties, as evidenced by the reduction in IL-1 β and CPK levels, preservation of cardiac architecture, and attenuation of fibrotic remodeling. Although the precise molecular pathways were not investigated in this study, the proposed mechanism provides a biologically plausible framework linking the biochemical and histopathological findings observed in the present experimental model.

Conclusion

TCA exposure induced cardiac injury and myocardial fibrosis, accompanied by increased IL-1 β and CPK levels. Co-administration of vitamin C significantly attenuated these alterations. The findings suggest that under pathological conditions, the antioxidant and anti-inflammatory properties of vitamin C may help protect against toxin-induced cardiac injury and fibrosis. Further studies are needed to elucidate the underlying molecular mechanisms.

Strengths and Limitations

A strength of this study is the demonstration that vitamin C reduced myocardial fibrosis and serum markers of inflammation and tissue injury in a model of TCA-induced cardiotoxicity. However, the underlying molecular pathways were not investigated. Future studies evaluating fibrogenic and inflammatory signaling pathways, including TGF- β -related mechanisms, are required to clarify the anti-fibrotic effects of vitamin C.

Declarations

Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the Ethics Committee of Babol University of Medical Sciences (Approval Code: IR.MUBABOL.AEC.1402.004) (Clinical trial number: Not applicable). Our study is a preclinical animal study and not a human clinical trial. Therefore, the registration of a clinical trial number does not apply to this work.

Conflict of interest

The authors declare that they have no conflict of interest.

Consent to publish

Not applicable.

Use of AI-Assisted Tools

ChatGPT-5 (OpenAI) was used only for language editing and improving manuscript readability. All scientific content and conclusions are the sole responsibility of the authors.

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Author Contribution

H.N. conceived and designed the study. A.I. was responsible for data acquisition. Z.B. performed histopathological analyses, while M.M. conducted the statistical analyses. The initial draft of the manuscript was prepared by Z.B. and subsequently revised by H.N. All authors reviewed and approved the final version of the manuscript.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon request.

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Figures

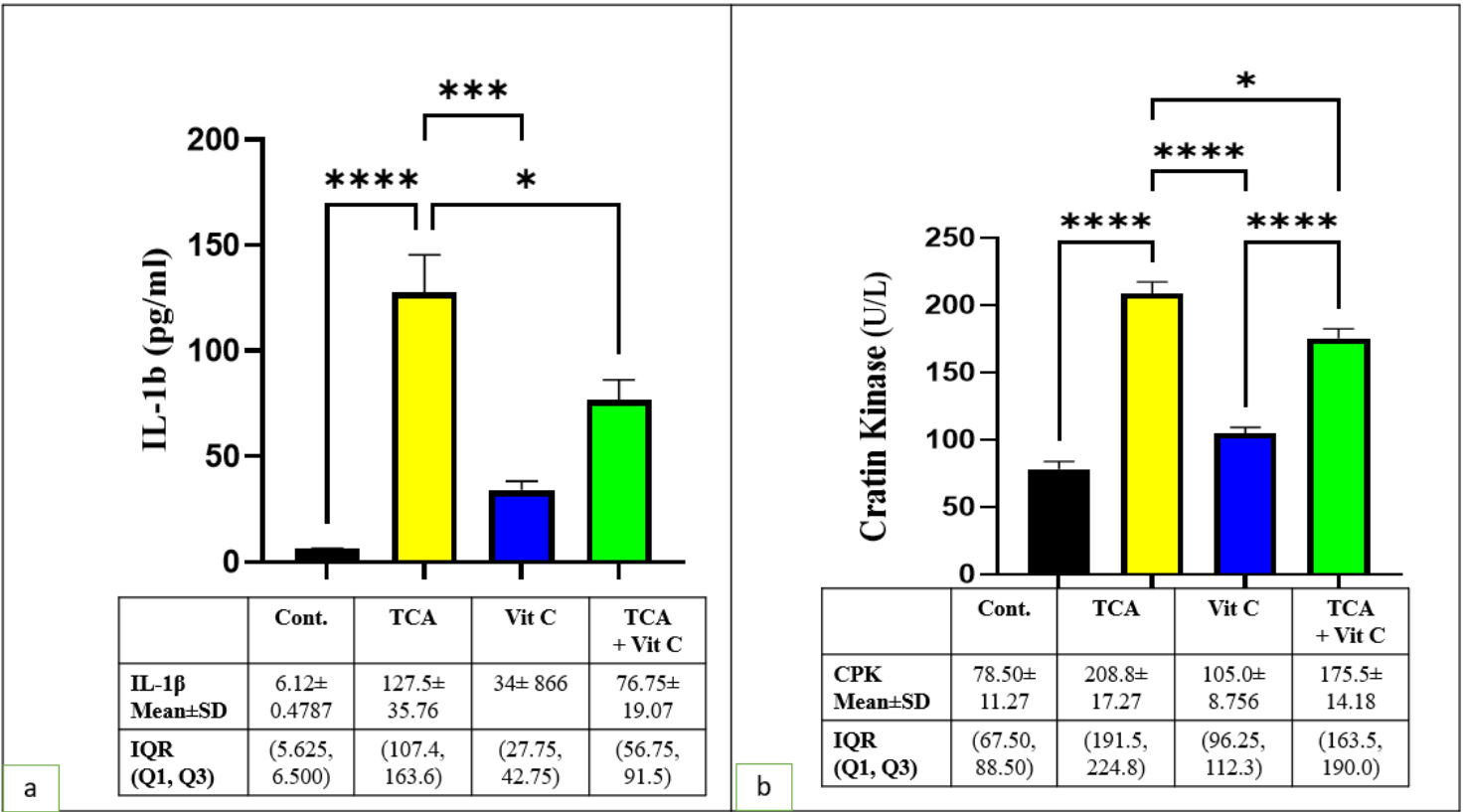


Figure 1

Mean±SD and IQR of IL-1β and CPK enzyme activity in different studied groups by the Ordinary One-Way ANOVA test.

Figure 1-a indicates a significant difference in IL-1β between the group receiving TCA and other groups.

Figure 1-b indicates a significant difference in CPK between the TCA group compared to control and Vit C groups. Comparison of the Vit C and treated group (TCA+VitC) shows a significant difference.

IQR (Q1, Q3): (25% Percentile, 75% Percentile)

****: $P \leq 0.0001$

***: $P \leq 0.001$

** : $P \leq 0.01$

*: $P \leq 0.05$

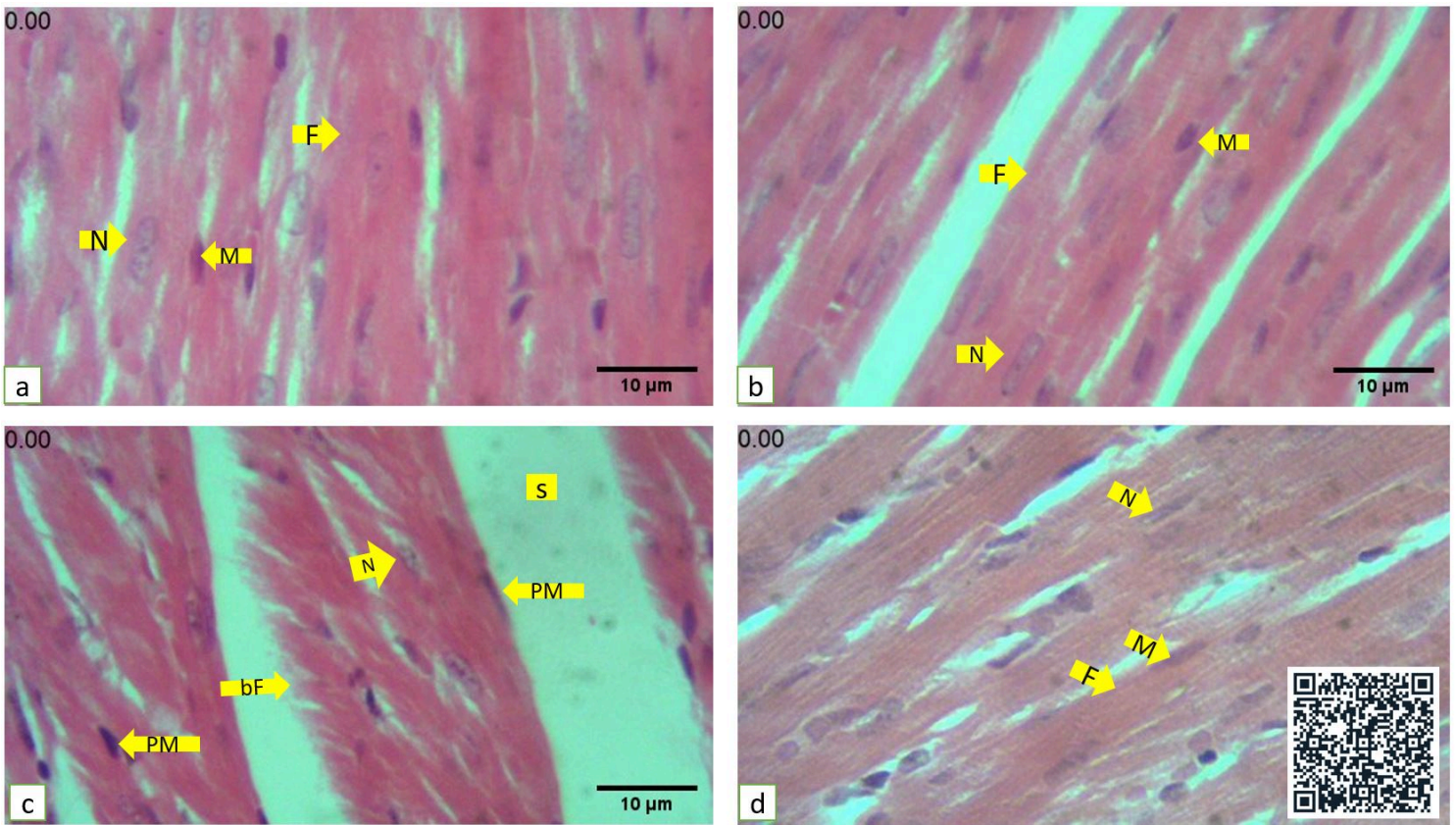


Figure 2

H&E staining of cardiac tissues in a longitudinal section. **a**: Control: cardiac cells (F), and the situation of the cardiac nuclei is in a normal state. The cardiac nucleus is euchromatin and located in central (N)

myocytes. Nuclei are normal and in normal situations. **b:** Vitamin C: Cardiac cells(F), cardiac nucleus (N), and myocytes (M) are in normal conditions. **c:**receiving TCA: Abnormal cardiac muscles, dentate muscle membrane (bf), myocytes nuclei are pyknosis and dark (PM), and the separation between muscles (S) can be seen in a large amount. **d:** TCA+ Vitamin C: cardiac cells(F) returned to a normal state, and the position of the muscle nucleus (N) and myocyte nucleus (M) is normal. a, b, c, and d magnification 180X. Scale bar=10μm

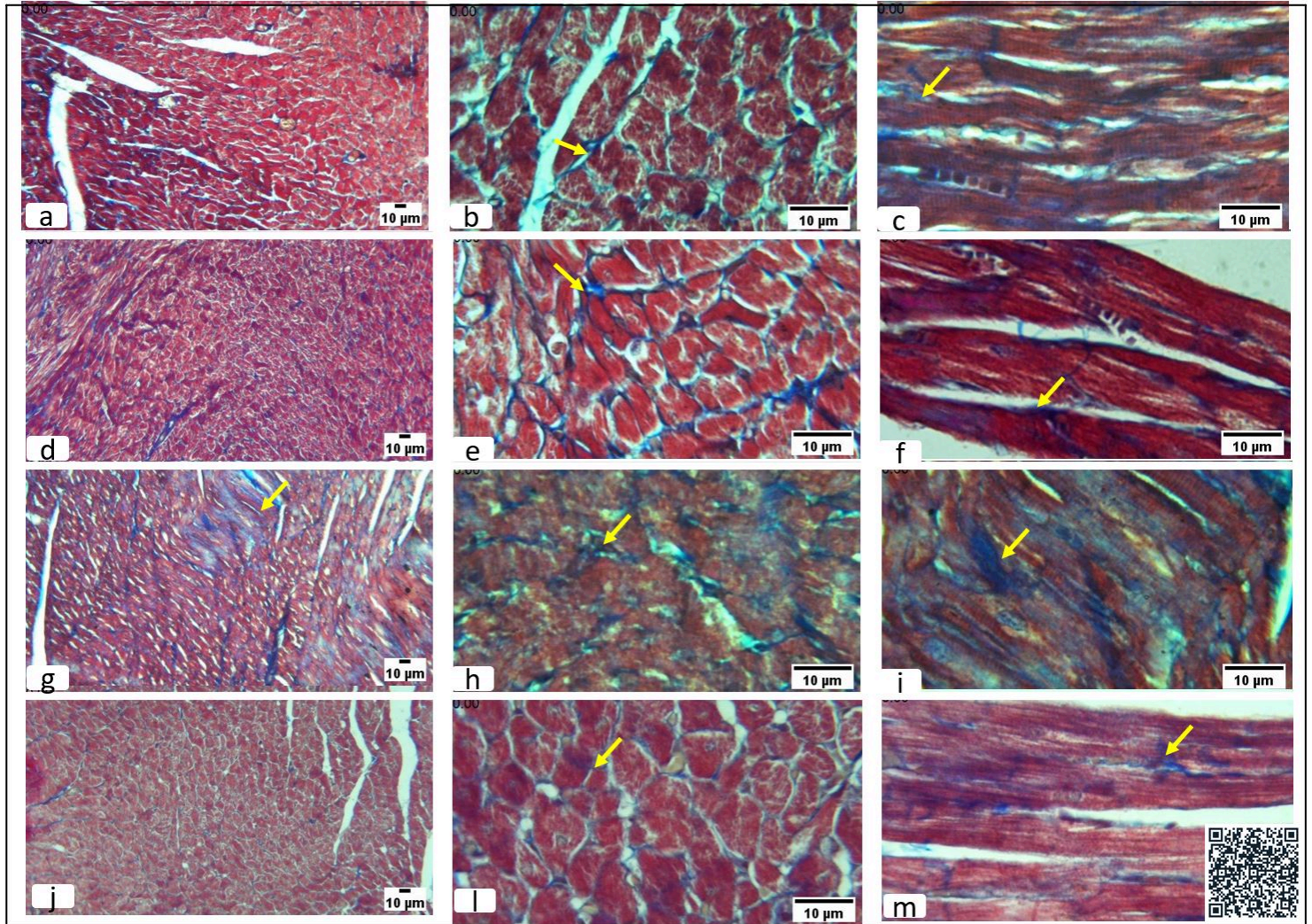


Figure 3

Masson's trichrome staining. **a,b,c:** Control: fibrosis is not observed between the cardiac cells (endomysium) and between the fascicles (perimysium), the yellow arrow. **d, e, f:** Vitamin C: collagen bundles in the endomysium and perimysium are normal (yellow arrow). **g, h, i:** receiving TCA: the density of collagen fibers in the endomysium and perimysium is significantly increased, and fibrosis is demonstrated (yellow arrowed). **j, l, m:** TCA + vit C: The density of collagen fibers and fibrosis in the endomysium and perimysium has decreased significantly compared to the TCA sample. (a, d, g, j =18X), (b, e, h, l=45X), (c, f, l, m=180X). Scale bar=10μm

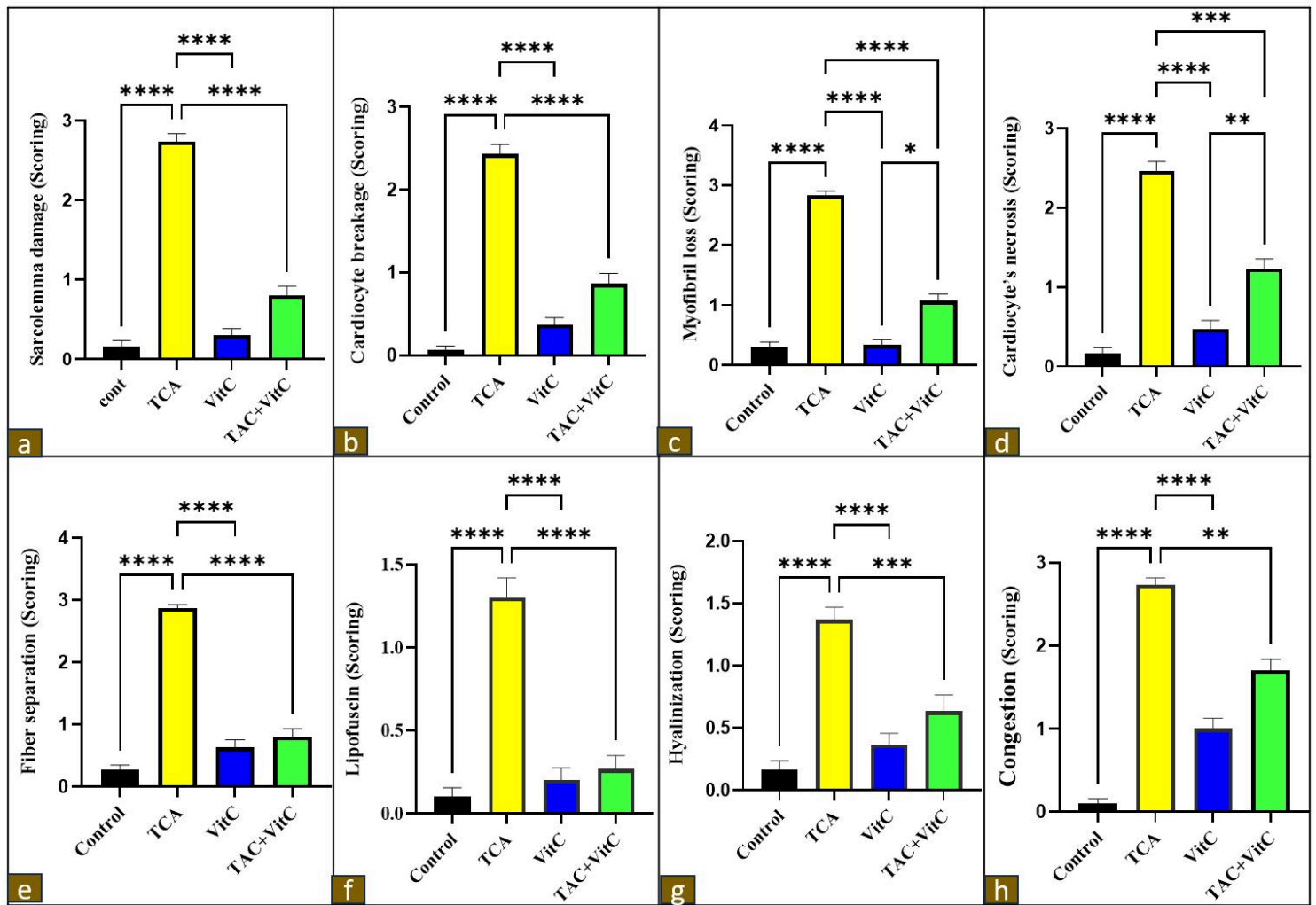


Figure 4

Comparison of the Mean±SE histopathological pattern in different groups.

Comparison of the mean ± SE histopathological pattern was performed using the Kruskal-Wallis test in the control, TCA, Vit C, and TCA+Vit C groups. (a) Sarcolemma damage (b) Cardiocyte breakage, (c) Myofibril loss, (d) Cardiocyte necrosis, (e) Fiber separation, (f) Lipofuscin accumulation, (g) Hyalinization, and (h) Vascular congestion.

IQR (Q1, Q3): (25% Percentile, 75% Percentile)

****: $P \leq 0.0001$

***: $P \leq 0.001$

** : $P \leq 0.01$

*: $P \leq 0.05$

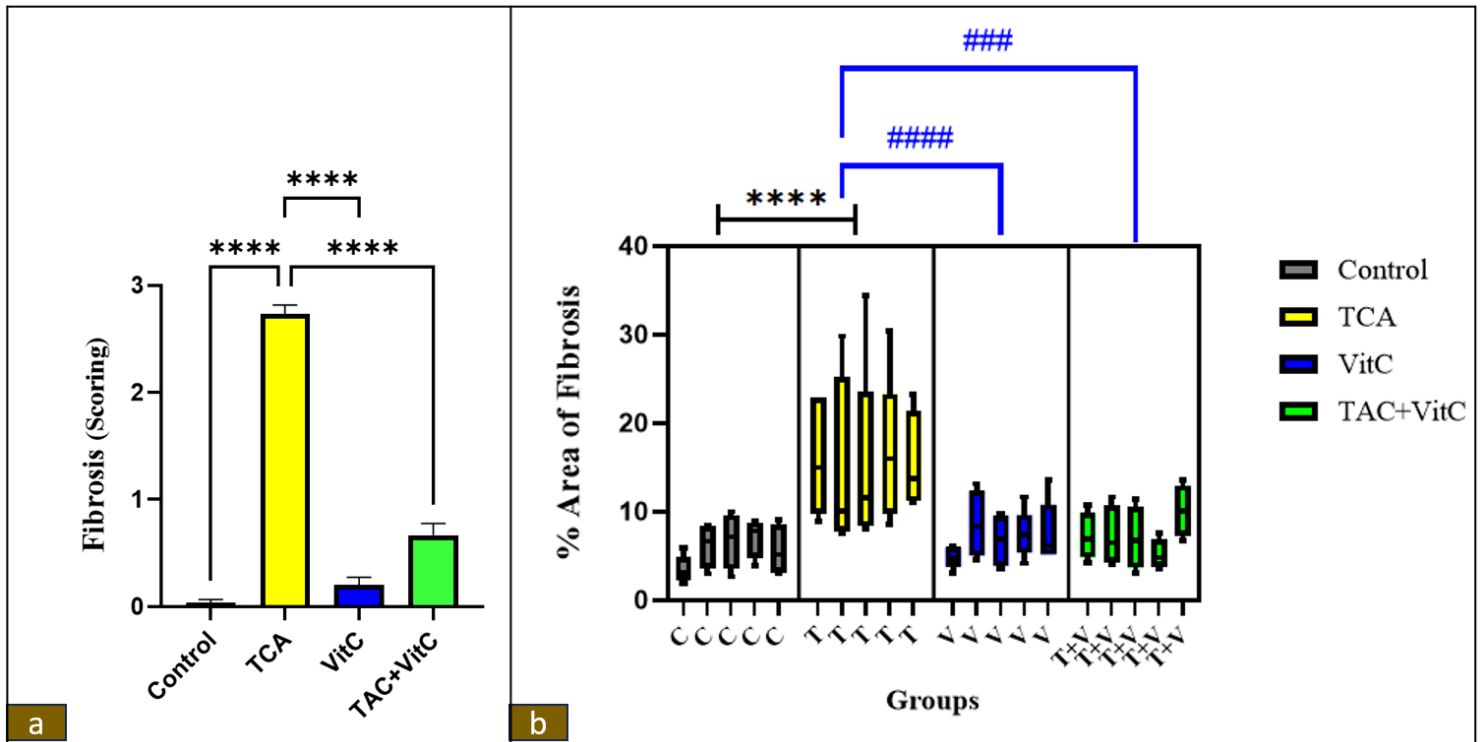


Figure 5

Protective effect of vitamin C against TCA-induced cardiac fibrosis.

(a) Semi-quantitative scoring of myocardial fibrosis shows a pronounced increase in fibrotic changes after TCA exposure compared with control, which was significantly attenuated by vitamin C treatment (**** $p < 0.0001$). (b) Quantitative assessment of fibrotic area (% fibrosis) indicates that vitamin C substantially reduced TCA-induced collagen deposition, and combined treatment with TCA+VitC also alleviated the fibrotic response. Data are expressed as mean $\pm$ SEM; **** $p < 0.0001$ vs. control, ### $p < 0.001$ vs. TCA.

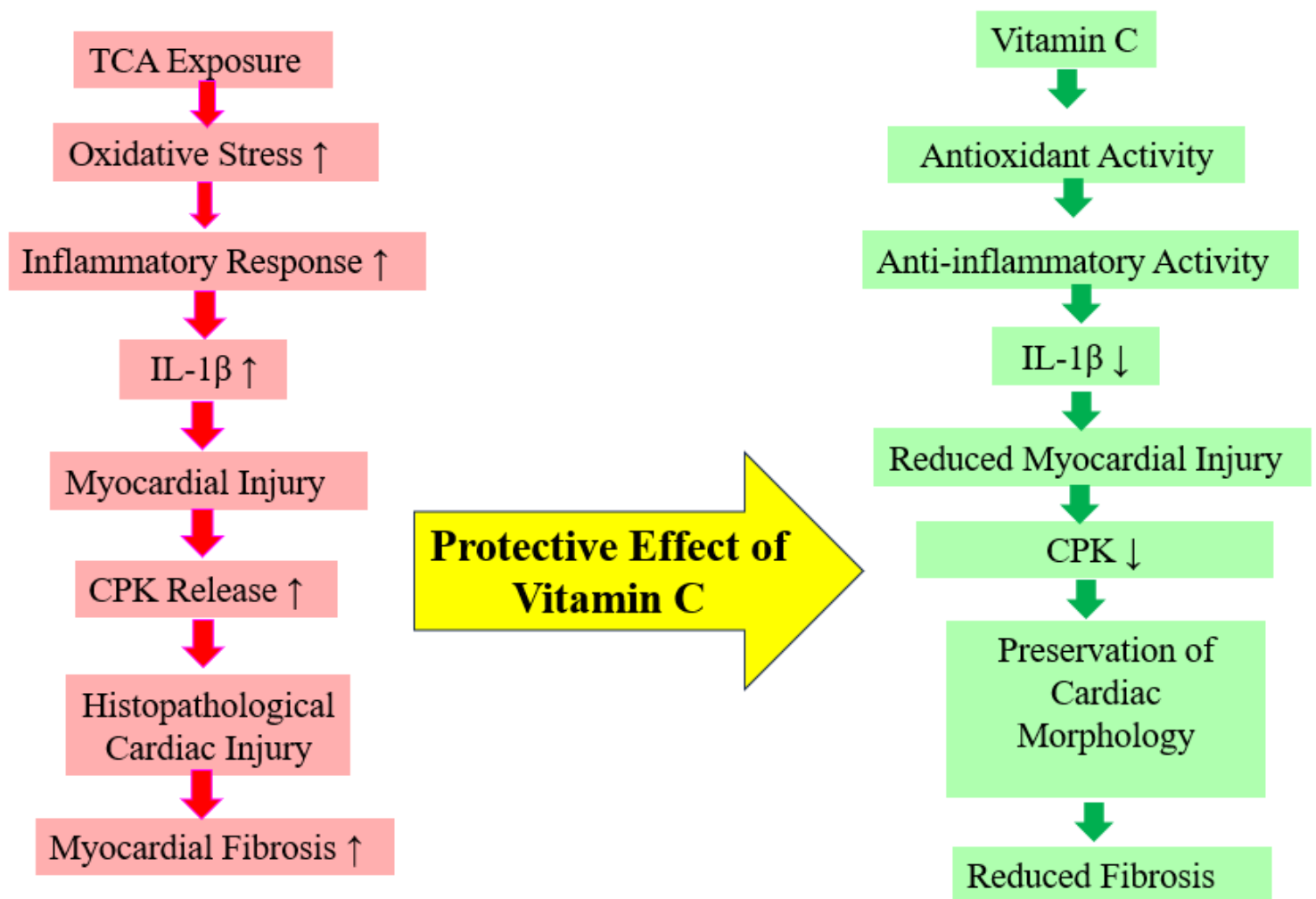


Figure 6

Putative mechanism of vitamin C-mediated protection against trichloroacetic acid-induced cardiac injury and fibrosis.

Trichloroacetic acid (TCA) exposure is proposed to induce oxidative stress and inflammatory responses, leading to increased IL-1 β levels, myocardial injury, elevated serum CPK activity, histopathological alterations, and myocardial fibrosis. Vitamin C may counteract these effects through its antioxidant and anti-inflammatory properties, resulting in reduced IL-1 β and CPK levels, preservation of cardiac morphology, and attenuation of fibrotic remodeling. The proposed pathway is based on the findings of the present study, together with evidence from previous literature.

Supplementary Files

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