Italian Journal of Food Science, 2025; 37 (2): 51–64

Please see corrigendum:

Protective effect of rosmarinic acid against 5-fluorouracil-induced cardiotoxicity in mice *via* modulation of inflammation, oxidative stress, and apoptosis and restoration of NRF2/HO-1

Afaf F. Almuqati

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hafr Al-Batin, Hafr Al-Batin, Saudi Arabia

Corresponding Author: Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hafr Al-Batin, Hafr Al-Batin, Saudi Arabia. Email: aalmaqati@uhb.edu.sa

Academic Editor: Prof. Tommaso Beccari—University of Perugia, Italy

Received: 22 November 2024; Accepted 7 January 2025; Published: 1 April 2025

PAPER

Abstract

Plant phytochemicals are bioactive substances that offer various biological and health benefits. The cardiotoxicity of 5-fluorouracil (5-FU) restricts its applicability in cancer treatment. The bioactive rosmarinic acid (RA) is an antioxidant and anti-inflammatory polyphenol. This study elucidated the protective efficacy of RA against 5-FU-associated cardiac injury in mice. Mice received RA (25 or 50 mg/kg, orally) for 10 days and then were treated with 5-FU (150 mg/kg) on the 8th day. 5-FU-intoxicated mice demonstrated higher lactate dehydrogenase, creatine kinase-MB, and troponin I levels with various cardiac histopathological alterations. 5-FU-injected mice showed a significant rise in cardiac protein carbonyl and malondialdehyde, associated with reduced myocardial glutathione content and catalase and superoxide dismutase activities. RA pretreatment of 5-FU-treated mice attenuated cardiac injury, decreased protein carbonyl and MDA levels, and promoted antioxidant defense mechanisms in the myocardium. Furthermore, RA significantly reduced cardiac inflammatory response by decreasing the expression of cardiac NF-κB p65 and proinflammatory cytokines in the heart. RA also mitigated 5-FU-induced cardiac apoptosis by attenuation of cardiac levels of Bcl-2, Bax, and caspase-3. It also restored the cardiac Nrf2/HO-1 signaling pathway. Collectively, RA exerts significant cardioprotective effects on 5-FU-induced cardiac injury, and therefore RA could be used as a potential effective adjuvant in alleviating myocardial injury associated with increased oxidative stress and inflammation.

Keywords: antioxidants; cardiotoxicity; inflammation; Nrf2; rosmarinic acid; 5-fluorouracil

Introduction

The 5-fluorouracil (5-FU) agent is widely applied in cancer chemotherapy (Hashem *et al.*, 2022; Potnuri *et al.*, 2018). However, the therapeutic benefits of 5-FU are overshadowed by its cardiotoxicity, which limits its clinical efficacy (González-Rodríguez *et al.*, 2020; Kenney *et al.*, 2001; Vassilakopoulou *et al.*, 2016). The 5-FU-associated cardiotoxicity manifests as sudden cardiac death, coronary thrombosis, angina, and arrhythmias (Afsar *et al.*, 2017). The 5-FU cardiotoxicity-related molecular mechanisms largely remain unexplained and might include higher ROS (reactive oxygen species) production, suppressed antioxidant defense, alleviated aerobic metabolism, and triggering of proinflammatory signaling pathways to cause cell death and direct cardiomyocyte injury (El-Agamy *et al.*, 2017; Ghobadi *et al.*, 2017; Shariatinia and Mazloom-Jalali, 2020).

A continuous high ROS generation coupled with compromised antioxidant defense mechanisms can lead to oxidative injury of nucleic acids, lipids, and proteins, and activation of NF-κB (nuclear factor kappa-B) proinflammatory transcription factor (Ekeleme-Egedigwe *et al.*, 2019; Khamis *et al.*, 2023). These phenomena contribute to cardiac injury. Therefore, the modulation of inflammation and oxidative stress is considered a novel therapeutic protective approach against 5-FU-associated cardiotoxicity.

Chemotherapy-linked tissue injury can be prevented by targeting the nuclear factor erythroid 2-related factor-2 (Nrf2) signaling pathway (Aladaileh *et al.*, 2019; Hamzeh *et al.*, 2019; Khamis et al., 2023; Kim and Choi, 2021; Sheweita *et al.*, 2016). The cytoprotective transcription factor Nrf2 controls the genes of cellular defense mechanisms to counter inflammation and oxidative tissue damage (Chen *et al.*, 2018; Ding *et al.*, 2021). This approach serves as an effective therapeutic tool against 5-FU-associated cardiotoxicity. Plant bioactive compounds positively influence human health (Aladaileh *et al.*, 2019; El-Tanbouly *et al.*, 2019; Khamis *et al.*, 2023). Particularly, the anti-inflammatory, antibacterial, anticancer, and antioxidant features of secondary plant metabolites (polyphenols and flavonoids) are beneficial for human health (Alaswad *et al.*, 2021; Roy *et al.*, 2022; Sedky *et al.*, 2017; Shamsudin *et al.*, 2022; Zhang *et al.*, 2022). Rosmarinic acid (RA; $C_{18}H_{16}O_8$) is a polyphenolic compound of rosemary, clary sage, lemon balm, and oregano that possesses cytoprotective, antiallergic, anti-inflammatory, antibacterial, and antioxidant properties (Al-khawaldeh *et al.*, 2024; Noor *et al.*, 2022). RA successfully protects the liver from acetaminophen side effects and ischemia or reperfusion injury in animal models by hindering inflammation (Ramalho *et al.*, 2014; Yu *et al.*, 2021). RA also improves cardiomyocyte energy metabolism, attenuates ROS overproduction, and alleviates cell apoptosis and hypoxia or reoxygenation damage in cardiomyocytes *via* p-Akt expression modulation (Li *et al.*, 2014b). It has significantly restricted the cobalt-related hepatocyte injury and lipopolysaccharide (LPS)-linked neuroinflammation in *in-vitro* models (Jeon YuJin *et al.*, 2014). Lu et al. (2022) reported RA-based attenuation of carbon chloride (CCl_4)-linked mice liver injury through activation of Nrf2. Nrf2 activation further mediated RA effects against cisplatin *via* inflammatory response and oxidative stress modulation in the liver (Xiang *et al.*, 2022). Despite pharmacological properties, RA protection against 5-FU cardiotoxicity demands further elaboration. Therefore, this study investigated RA's efficacy against 5-FU-linked cardiac damage through inflammation, oxidative stress, and apoptosis assessment. A deeper understanding of RA's protective role could help in devising novel treatments for 5-FU-associated cardiac injury.

Materials and Methods

Experimental design

Thirty Swiss albino mice (24–28 g) were kept under 12-hour alternating light cycles at 23–25°C and 50–60% humidity. The animals were fed on food and water *ad libitum*. The University of Hafr Al-Batin approved the animal protocols of this study. Five animal groups (6 mice/group) were acclimatized for 7 days before experiments. Group 1 (control) was orally treated with physiological saline for 10 days followed by a single intraperitoneal (i.p.) injection of physiological saline on the 8th day. Group 2 (RA) was orally administered with RA (50 mg/kg) (Biosynth Carbosynth, UK) that was dissolved in physiological saline. RA administration for 10 days was followed by a single physiological saline injection (i.p.) on the 8th day. Group 3 (5-FU) orally received physiological saline for 10 days followed by a single (i.p.) injection of 5-FU (150 mg/kg) (GenoChem World, Spain) on the 8th day. Group 4 (RA25+5-FU) and Group 5 (RA50+5-FU) were orally administered with physiological saline-dissolved RA (25 and 50 mg/kg; respectively) for 10 days followed by a single dose (i.p.) of 5-FU (150 mg/kg) on the 8th day. RA doses were based on a previous study, which reported its anti-inflammatory and antioxidant effects (Gautam *et al.*, 2019). The 5-FU dose was selected according to the findings of an earlier study (Hamzeh *et al.*, 2019).

Mice in all groups were anesthetized (ketamine–xylazine (100–10 mg/kg; i.p.]) after the experimental period, and blood samples were collected through cardiac puncture. Blood samples were allowed to clot, and serum was separated by centrifugation for biochemical analysis. Then, mice were dissected to quickly remove the hearts, which were rinsed in cold PBS buffer (phosphate-buffered saline, pH 7.0). Some heart portions were fixed in NBF (neutral buffered formalin, 10%) for histological studies, while the other sections were homogenized in cold PBS (10% w/v). The homogenates were subjected to centrifugation, and the supernatant was stored (–20°C) for further biochemical analysis.

Cardiac biomarkers in the serum

Cardiac troponin I (cTnI) serum levels were estimated using an enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, CA, USA). Creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) serum activities were measured using commercial Spectrum Diagnostics kits (Al-Qalyubia Governorate, Egypt). The assays were performed following the manufacturer's recommendations.

Antioxidants and oxidative stress levels in the heart

The cardiac malondialdehyde (MDA) contents, indicating ROS production and an end product of lipid peroxidation, were assessed by detecting thiobarbituric acid reactive substances (TBARS) (Ohkawa *et al.*, 1979). Briefly, sample MDA and TBA reaction in the acidic medium (95°C for 30 min) formed a pink product which was spectrophotometrically measured at 532 nm. The protein carbonyl level in heart homogenates was revealed by its reaction with 2,4-dinitrophenylhydrazine (DNPH) that produced dinitrophenyl (DNP) hydrazine (Levine *et al.*, 1990).

The reduced glutathione (GSH) cardiac contents were estimated by GSH-based reduction of 5, 5 dithiobis (2-nitrobenzoic acid) (DTNP) to yield 5-thio-2-nitrobenzoic acid (TNB) that was spectrophotometrically quantified at 412 nm (Griffith, 1980). SOD (superoxide dismutase) activity was spectrophotometrically (560 nm) quantified based on the inhibition of nitroblue tetrazolium dye reduction by NADH and phenazine methosulphate (Nishikimi *et al.*, 1972). The decomposition of hydrogen peroxide (H_2O_2, λ max = 240 nm) into oxygen and water demonstrated the CAT (catalase) activity (Aebi, 1984). ELISA kit (FineTest, China) was used to determine myocardial tissue's heme oxygenase 1 (HO-1) levels.

Proinflammatory cytokines level in the heart

ELISA kits (CUSABIO, TX, USA) were used to measure TNF-α (tumor necrosis factor-α) and interleukin-6 (IL-6) levels in the myocardial tissue.

Histopathological examination of heart sections

NBF (10%) fixed heart tissues were embedded in paraffin and cut into 5μm sections. Then, these sections were subjected to deparaffinization and rehydration followed by staining with H&E (hematoxylin and eosin) for cardiac injury's histological evaluation.

Immunohistochemistry

Immunohistochemistry (IHC) staining involved the dewaxing and immersion (citrate buffer [50 mM], pH 6.8) of heart sections to retrieve antigens. Then, heart sections were treated with H_2O_2 (0.3%) to inhibit endogenous peroxidase activity. Normal serum's addition for 20 min blocked the nonspecific binding. These treated sections were overnight incubated (4°C) with primary antibodies of the target proteins as follows: 1:100 dilution of NF-κB p65 (Santa Cruz Biotechnology, Dallas, TX, USA), 1:100 dilution of caspase-3 (Invitrogen, Waltham, MA, USA), and 1:100 dilution of Nrf2 (Invitrogen, Waltham, MA, USA). After washing, sections were incubated with secondary antibodies (EnVision+™ System Horseradish Peroxidase Labelled Polymer, Dako, Santa Clara, CA, USA), followed by color development with DAB substrate and counterstaining with Mayer's hematoxylin. The staining intensity was assessed by quantifying the positive expression area with ImageJ analysis software (NIH, Bethesda, MD, USA).

Statistical analysis

GraphPad Prism 8 (San Diego, USA) was used for the statistical analysis. Data were expressed as the mean ± SEM. Groups were differentiated through one-way ANOVA (analysis of variance) whereas means were compared with Tukey's *post-hoc* test at a significance level of $P < 0.05$.

Results

Rosmarinic acid pretreatment prevents 5-FU-associated cardiac injury

The CK-MB and LDH activities, cTnI serum levels, and cardiac tissue's histopathological changes demonstrated the cardiac injury level (Figure 1 and Figure 2). 5-FU administration significantly elevated the CK-MB and LDH activities and cTnI serum levels compared to the control group (Figure 1). However, RA (25 or 50 mg/kg) pretreatment of mice before 5-FU injection significantly improved the serum cTnI levels and CK-MB and LDH in a dose-dependent manner (Figure 1). Furthermore, H&E-stained cardiac sections from control and RA-treated mice demonstrated normal cardiac muscle fibers with normal sarcoplasm and a normal centrally located nucleus (Figure 2). H&E-stained cardiac sections from 5-FU-injected mice showed myocarditis features associated with congestion of blood capillaries, necrosis of the cardiac muscle cells, and infiltration of mononuclear inflammatory cells (Figure 2). Largely, pretreatment of 5-FU-injected mice with RA attenuated 5-FU-induced histopathological changes in the myocardium (Figure 2).

Rosmarinic acid pretreatment alleviates 5-FU-associated cardiac oxidative stress

A significant ($P < 0.05$) rise in MDA and protein carbonyl levels was noted after 5-FU-administration (Figure 3A and B). Simultaneously, GSH content and antioxidant

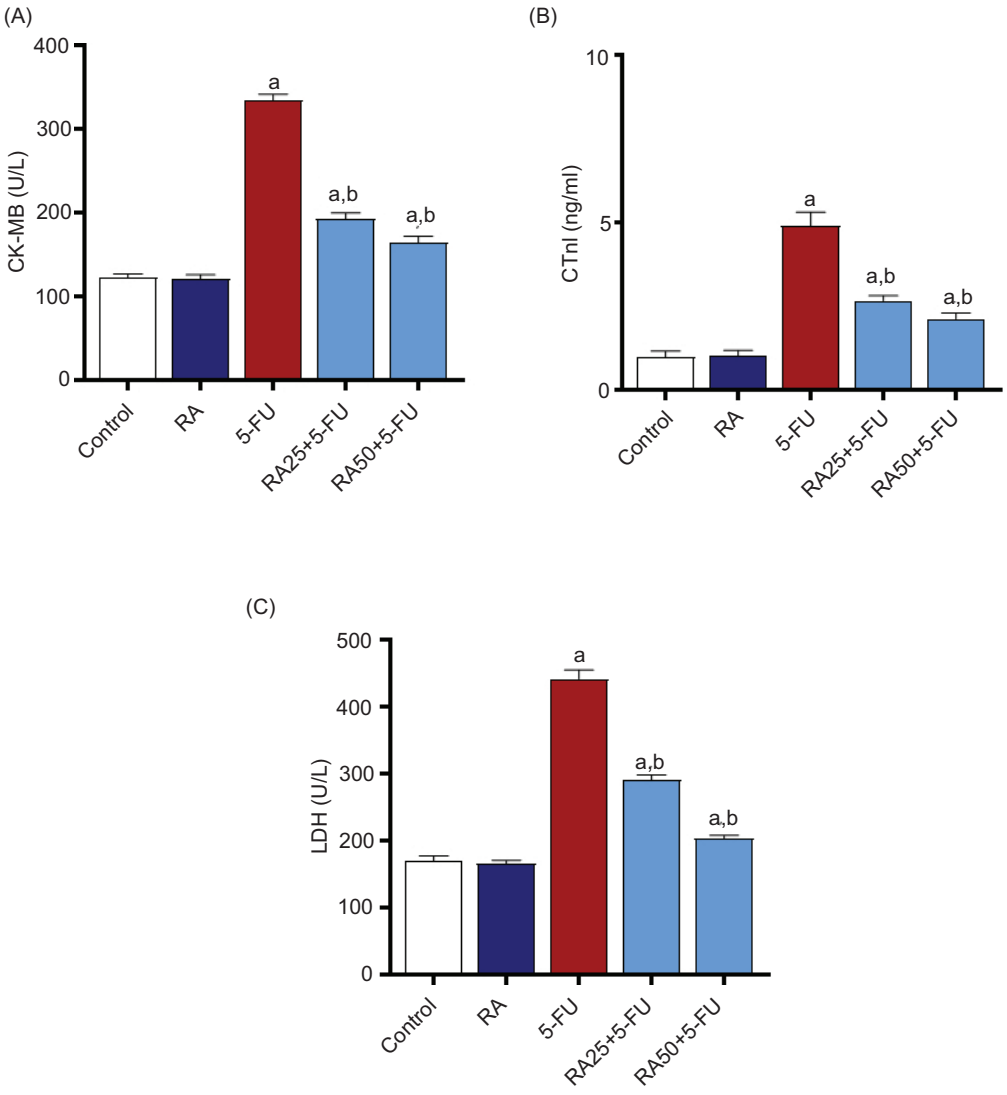

Figure 1. Rosmarinic acid-based prevention of 5-FU-induced myocardial injury. Cardiac injury assessment through serum analysis of (A) CK-MB, (B) cTnI levels, and (C) LDH in RA-treated and untreated mice. Data represent mean ± SEM (n = 6/group). a P < 0.0 vs control, and b P < 0.05 vs 5-FU-injected group.

(SOD and CAT) activities significantly ($P < 0.05$) declined (Figure 3C-E) in myocardial tissue as compared to the control group ($P < 0.05$). RA pretreatment significantly ($P < 0.05$) alleviated the impacts of 5-FU-administration.

Rosmarinic acid pretreatment suppresses 5-FU-associated cardiac inflammation

The inflammatory response is a key marker of 5-FU cardiotoxicity. IHC staining demonstrated significantly ($P < 0.05$) higher NF-κB p65 expressions in the cardiac tissue than in the control group (Figures 4A and 4B). TNF-α and IL-6 levels also significantly ($P < 0.05$) enhanced after 5-FU treatment as compared to the control group (Figures 4C and 4D). RA pretreatment considerably ($P < 0.05$) mitigated the effects of 5-FU administration (Figures 4A–4D).

Rosmarinic acid pretreatment mitigates 5-FU-associated cardiac apoptosis

RA protective effects on 5-FU-associated myocardial damage were further evaluated *via* ELISA-based measurement of apoptosis–regulating proteins (Bcl-2 and Bax) and IHC staining-based caspase-3 examination of the cardiac tissues. 5-FU-injected cardiac

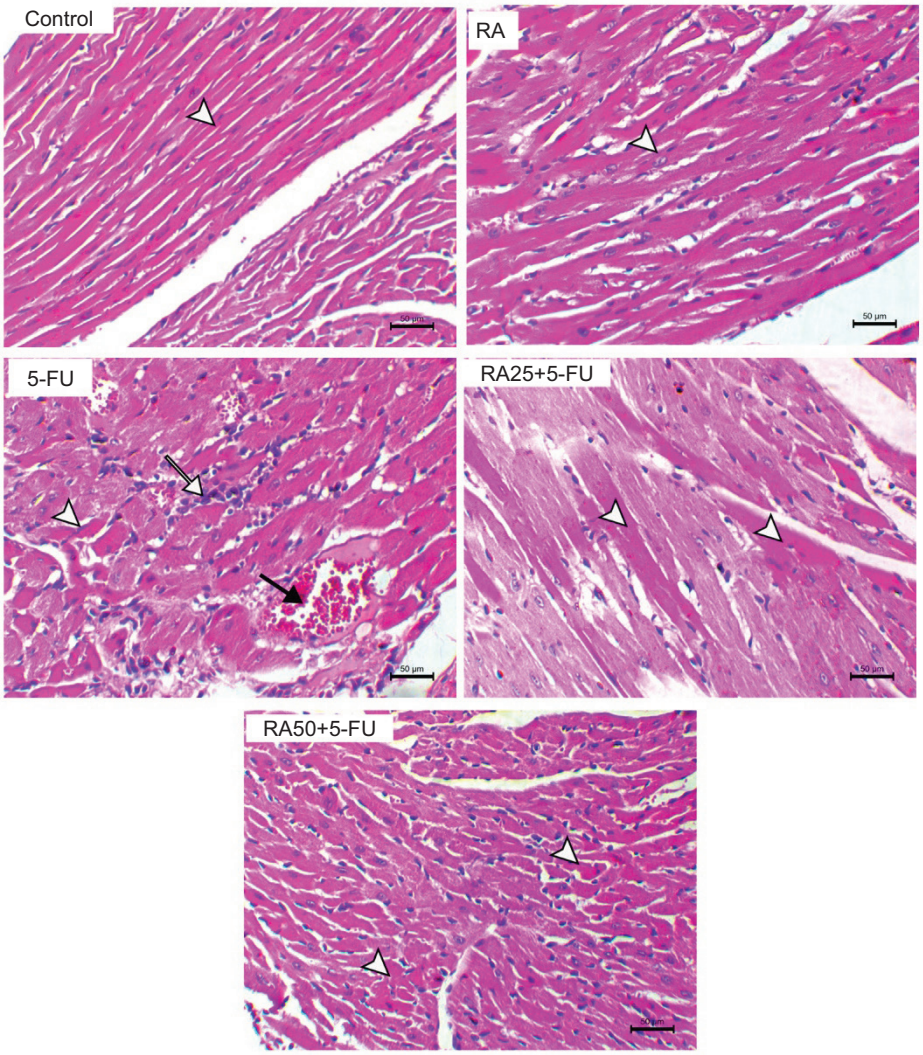

Figure 2. Rosmarinic acid-based prevention of 5-FU-induced histopathological changes in the heart. Representative images show H&E staining of all groups. (Control) H&E-stained cardiac sections from control mice demonstrated normal cardiac muscle fibers with normal sarcoplasm and a normal centrally located nucleus (arrowhead). (RA) H&E-stained cardiac sections from RA-injected mice showing normal cardiac muscle fibers (arrowhead). (5-FU) H&E-stained cardiac sections from 5-FU-injected mice showing myocarditis features associated with congestion of blood capillaries (black arrow), necrosis of the cardiac muscle cells (arrowhead), and infiltration of mononuclear inflammatory cells (white arrow). (RA25+5-FU) H&E-stained cardiac sections from 5-FU-injected mice treated with 25 mg/kg RA showing muscle broad fibers with myolysis and sarcoplasmic degenerative changes (arrowheads). (RA50+5-FU) H&E-stained cardiac sections from 5-FU-injected mice treated with 50 mg/kg RA showing marked improvement and demonstrating mild degree of sarcoplasmic degeneration of the cardiac muscle fibers (arrowheads). H&E; Scale bar = 50 µm.

tissues demonstrated significantly ($P < 0.05$) lower Bcl-2 expression (Figure 5A) whereas there was a significant ($P < 0.05$) rise in Bax (Figure 5B) and caspase-3 expressions (Figure 5C and D) in comparison to the control group. RA pretreatment resulted in significant ($P < 0.05$) alleviation of cardiac caspase-3, Bax, and Bcl-2 expressions (Figures 5A–5D).

Rosmarinic acid pretreatment modulates cardiac Nrf2/HO-1 signaling pathway

Since Nrf2/HO-1 pathway is known to modulate inflammation and oxidative injury in tissues, RA effects on Nrf2 expression and HO-1 contents were also assessed in myocardial tissues. 5-FU-administered mice presented

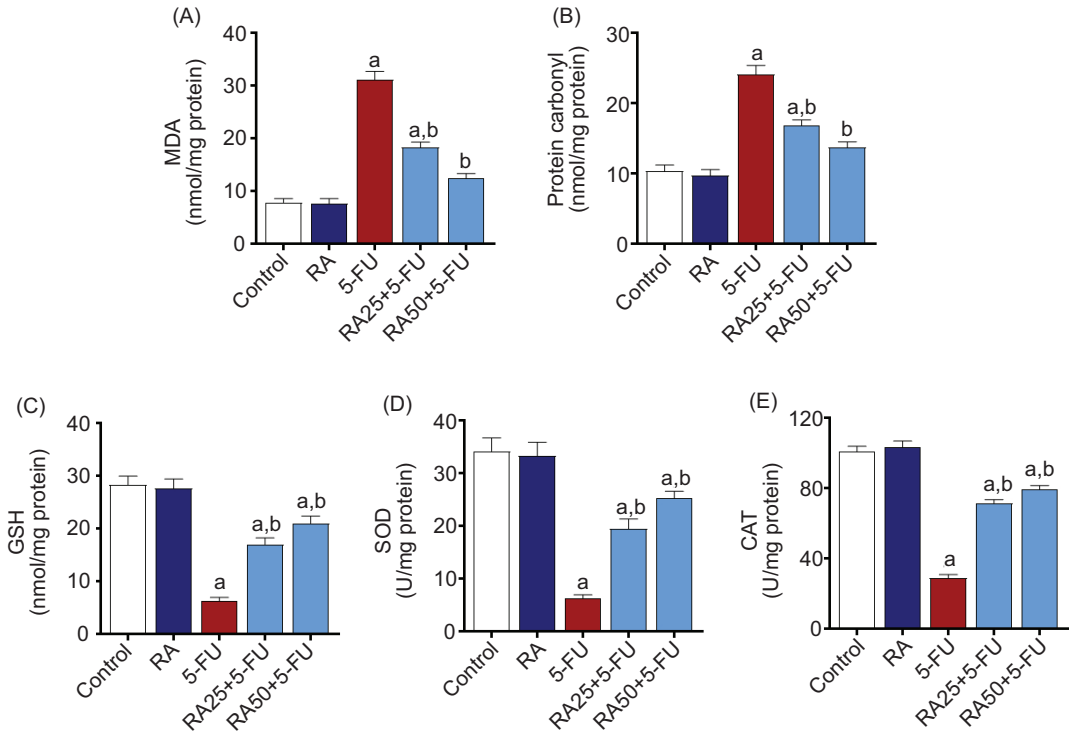

Figure 3. Rosmarinic acid-based attenuation of 5-FU-induced myocardial oxidative stress. RA attenuated (A) MDA and (B) protein carbonyl levels and reduced (C) GSH contents and (D) SOD and (E) CAT activities in the hearts of respective groups. Data represent mean ± SEM (n = 6/group). a P < 0.0 vs control, and b P < 0.05 vs 5-FU-injected group.

a significant ($P < 0.05$) reduction in myocardial Nrf2 expression and HO-1 levels than in the control group (Figure 6). RA pretreatment of 5-FU-administered mice restored ($P < 0.05$) Nrf2 expression and HO-1 levels in cardiac tissues (Figure 6).

Discussion

The 5-FU agent is extensively applied in cancer therapies (malignant and premalignant skin cancers). It is also administered to treat various noncancerous cutaneous conditions such as scarring (keloid and hypertrophic), pigmentary disorders (vitiligo and idiopathic guttate hypomelanosis), inflammatory dermatoses (sarcoidosis, Hailey-Hailey disease, and Darier's disease), cutaneous infections (viral warts and molluscum contagiosum), and cosmetic indications (filler nodules and photoaging) (Searle *et al.*, 2021). However, 5-FU is known to exert multiple types of toxicity including cardiotoxicity (Alter *et al.*, 2006; Anaka and Abdel-Rahman, 2023; Sorrentino *et al.*, 2012). 5-FU-linked tissue injury includes inflammation and oxidative stress that leads to cardiomyocyte damage and apoptosis (Focaccetti *et al.*, 2015; More *et al.*, 2021; Sravathi *et al.*, 2023). Therefore, novel strategies are mandatory to prevent/cure its cardiotoxicity. This study demonstrates RA-based prevention of 5-FU cardiotoxicity *via* modulation of cell apoptosis, oxidative injury in tissues, and inflammation. Moreover, it improved the Nrf2/HO-1 heart signaling as well.

5-FU-associated cardiotoxicities could manifest in multiple ways, and lead to heart failure or myocardial infarction (Jensen and Sørensen, 2006; Sorrentino *et al.*, 2012; Stewart *et al.*, 2010). During this study, 5-FU-linked cardiac injury appeared as elevated cTnI serum levels and higher LDH and CK-MB activities along with various histopathological heart changes, which is in line with the literature (Arafah *et al.*, 2022; Li *et al.*, 2023a; Safarpour *et al.*, 2022). Higher levels of these serum cardiac biomarkers indicate myocardial injury because of the affected integrity of the cardiomyocyte cell membrane. The situation ultimately results in necrosis and myocyte apoptosis with the infiltration of inflammatory cells (Bodor, 2016; Mishra *et al.*, 2019). However, RA treatment successfully prevented 5-FU-associated cardiac injury by decreasing cardiac biomarkers' serum levels and attenuating histological heart alterations in a dose-dependent manner. This cardioprotective efficacy of RA coincides with the previous *in-vitro* and *in-vivo* reports (Li *et al.*, 2014b; Rahbardar *et al.*, 2022; Zhang *et al.*, 2018).

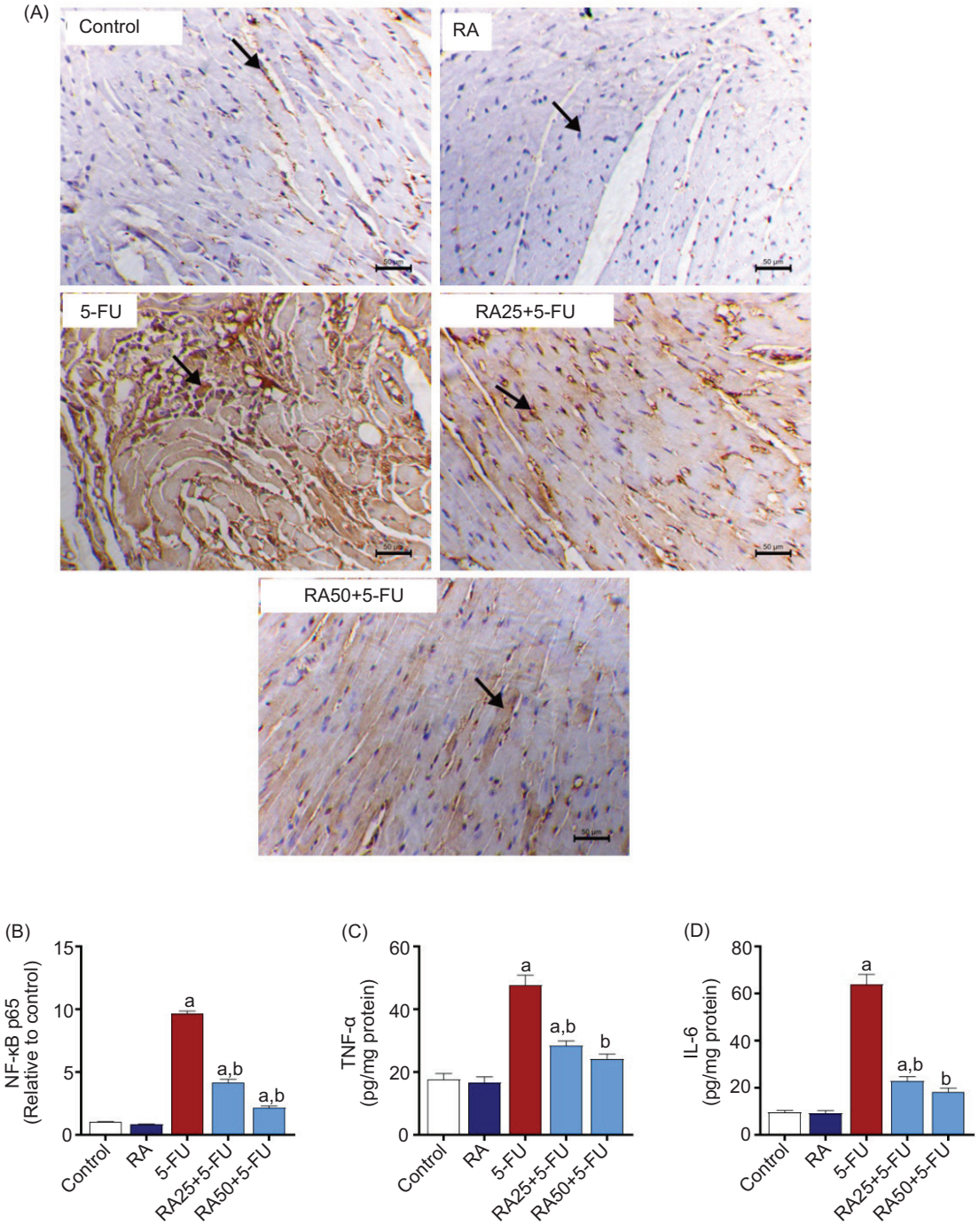

Figure 4. Rosmarinic acid-based mitigation of 5-FU-induced increased inflammatory response in mouse heart. (A) Representative photomicrographs represent NF-κB p65 immunostaining within all groups. Brown staining indicates a rise in NF-κB p65 staining intensity whereas arrowheads point towards cardiomyocytes (IHC; 50 µm scale bar). The photomicrograph of 5-FU represents a marked increase of cytoplasmic and nuclear immunoexpression of NFκB P65 antibody within the cardiomyocytes and interstitial inflammatory cells (arrow); while pretreatment of 5-FU-injected mice with RA markedly decreased the expression of NF-κB p65 immunostaining within the cardiomyocytes (arrow). (B) NF-κB p65 immunostaining quantification was calculated relative to the control. RA also significantly decreased cardiac levels of (C) Tumor necrosis factor-alpha (TNF-α) and (D) Interleukin-6 (IL-6) in 5-FU-injected mice. Data represent mean ± SEM (n = 6/group). a P < 0.0 vs control, and b P < 0.05 vs 5-FU-injected group.

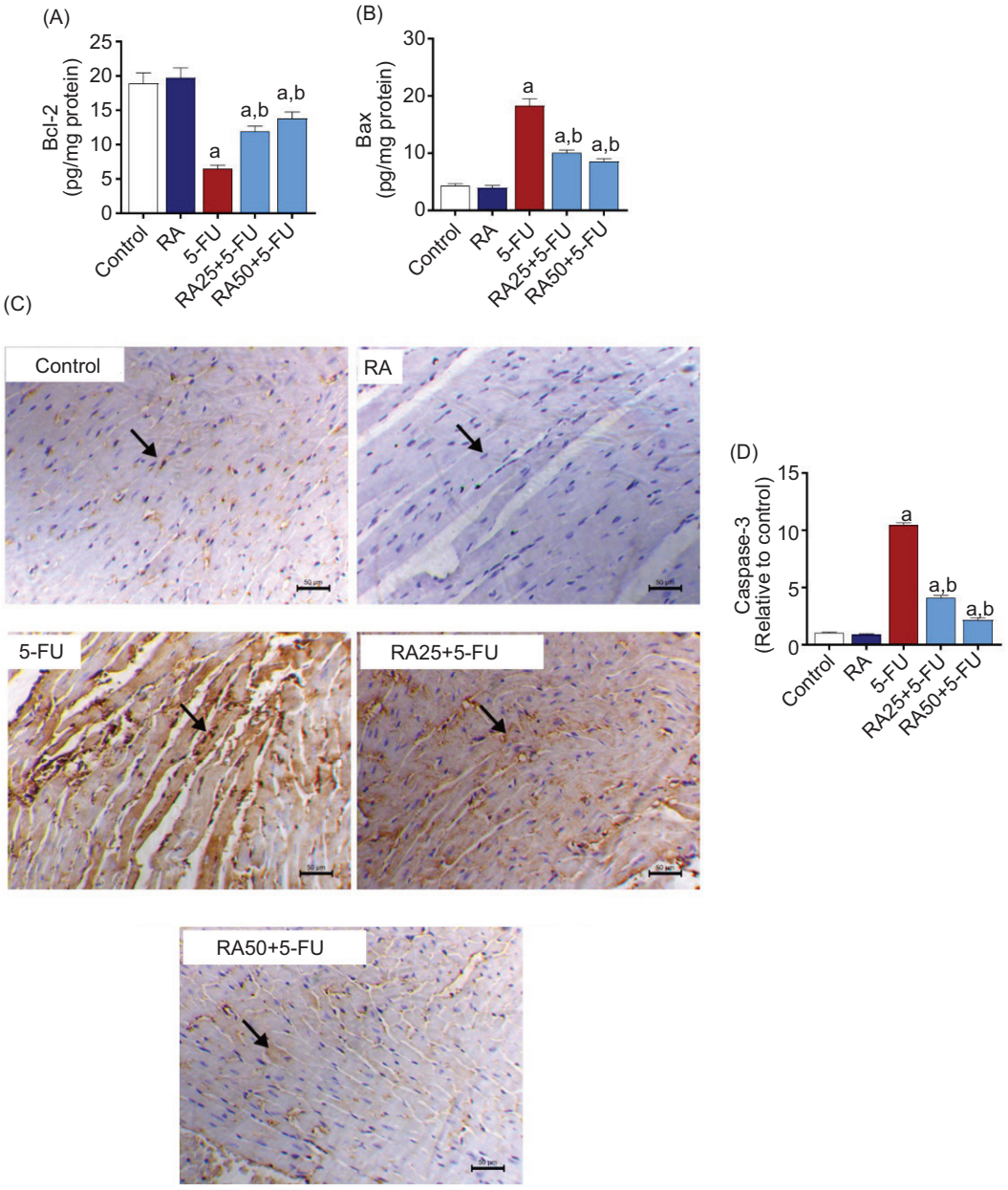

Figure 5. Rosmarinic acid-based suppression of 5-FU-induced myocardial apoptosis. RA ameliorated (A) Bcl-2 and (B) levels in the myocardial tissues of respective groups. (C) Representative photomicrographs represent caspase-3 immunostaining within all groups. Brown staining indicates a rise in caspase-3 staining intensity whereas arrowheads point towards cardiomyocytes (IHC; 50 µm scale bar). The photomicrograph of 5-FU represents a marked increase of both cytoplasmic and nuclear expression of caspase-3 within the cardiomyocytes (arrows); while pretreatment of 5-FU-injected mice with RA markedly decreased the expression of caspase-3 immunostaining within the cardiomyocytes (arrow). (D) Caspase-3 immunostaining quantification was calculated relative to the control. Data represent mean ± SEM (n = 6/group). a P < 0.0 vs control, and b P < 0.05 vs 5-FU-injected group.

5-FU cardiac injury-related molecular mechanisms remain unclear; however, myocardium's oxidative damage is primarily considered to cause pathologic changes (Alter *et al.*, 2006; Arafah *et al.*, 2022; Focaccetti *et al.*, 2015; Li *et al.*, 2023a; Sorrentino *et al.*, 2012). 5-FU-linked cardiac oxidative stress is known to elevate ROS levels. This phenomenon results in an affected antioxidant defense mechanism in cardiomyocytes, which leads to DNA damage, lipid peroxidation, and protein oxidation in the myocardium (Lamberti *et al.*, 2012; Matsubara *et al.*, 1980; Sara *et al.*, 2018). 5-FU-administered mice hearts demonstrated a significant rise in protein carbonyl

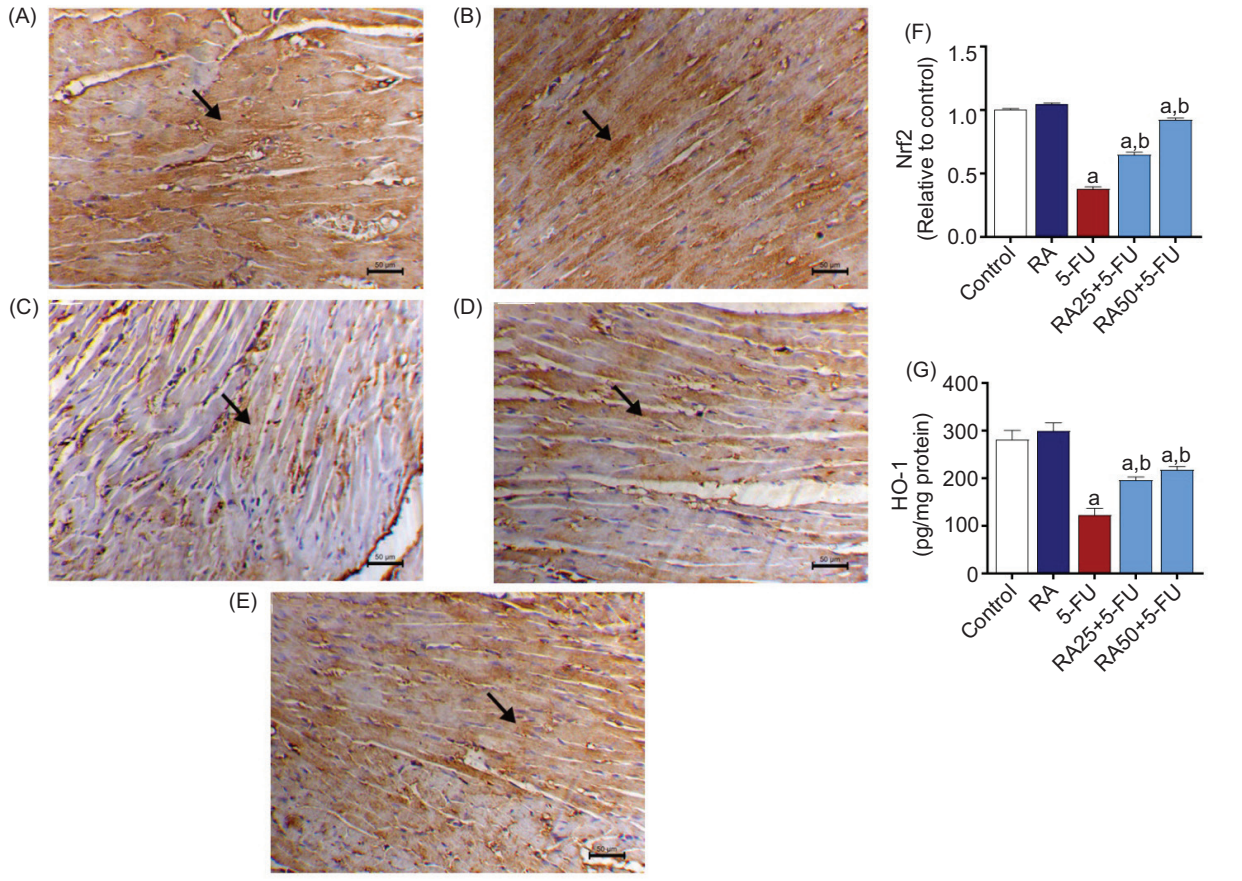

Figure 6. Rosmarinic acid-based restoration of Nrf2/HO-1 in heart of 5-FU-injected mice. (A) Nrf2 in the cardiac sections as determined by IHC staining and (B) its quantification in the respective groups. (C) shows the decreased HO-1 level in the heart upon 5-FU administration and its restoration by RA treatment. Data represent mean ± SEM (n = 6/group). a P < 0.0 vs control, and b P < 0.05 vs 5-FU-injected group.

and MDA levels along with reduced CAT and SOD activities and GSH content, which agrees with previous studies (Arafah *et al.*, 2022; Lokman *et al.*, 2023; Safarpour *et al.*, 2022). Lipid peroxidation modifies membrane fluidity, enhances tissue permeability, and inactivates membrane-bound enzymes and receptors resulting in membrane destruction and cell apoptosis (Kurutas, 2015). Moreover, proteins' oxidative alteration disrupts their structural conformation, increases protein fragmentation and degradation, and inhibits cellular enzymatic activity (Wang *et al.*, 2012).

Boosting antioxidant defenses and decreasing oxidative injury are the main therapeutic techniques to prevent 5-FU-associated cardiac injury. During the current study, RA pretreatment of 5-FU-administered mice considerably reduced protein carbonyl and MDA levels in addition to the restoration of CAT and SOD activities and GSH levels in myocardial tissues. An investigation has demonstrated RA–based protection against cardiac tissue's oxidative injury in diabetic (Type-2) female rats (Zych *et al.*, 2019). RA has also been reported to mitigate GSH and MDA contents during *in-vitro* and *in-vivo* experiments concerning doxorubicin-linked cardiotoxicity (Rahbardar *et al.*, 2022). RA alleviated the aconitase oxidative inactivation and ROS generation during myocardial ischemia or reperfusion injury in mouse models (Quan *et al.*, 2021). Moreover, RA is known to mitigate rodents' acetaminophen hepatotoxicity-linked oxidative damage (Yu *et al.*, 2021), CCl_4 and cisplatin-related liver damage (Lu *et al.*, 2022), chromium-associated DNA damage and hepatic oxidative stress (Khalaf *et al.*, 2020), and renal oxidative stress caused by chlorpyrifos (Abduh *et al.*, 2023) *via* reduction of lipid peroxidation. The antioxidant potential of RA depends on its free radical scavenging capability (Frezza *et al.*, 2019).

Higher oxidative stress after 5-FU exposure activates signaling pathways (stress and proinflammatory) to cause apoptosis of cardiomyocytes, which results in tissue

dysfunction and injury (Arafah *et al*., 2022; Focaccetti *et al*., 2015; Li *et al*., 2023a). 5-FU-administrated mice hearts had significantly lower Bcl-2 expression. Contrarily, NF-κB p65, caspase-3, Bax, TNF-α, and interleukins (IL-6 and IL-1β) levels were significantly increased. NF-κB activation contributes to fibrosis, endothelial dysfunction, apoptosis, and hypertrophy (Fiordelisi *et al.*, 2019). Proinflammatory cytokines are considered to disrupt myocardial hemodynamic loading conditions, trigger ROS yield, and alter myocyte viability and contractility leading to cellular dysfunction and necrosis (Thomas and Grisanti, 2020; Zhazykbayeva *et al.*, 2020). Moreover, continued ROS production in the heart after 5-FU administration might result in higher caspase-3-related apoptosis. The process involves the dissipation of mitochondrial membrane potential and the release of proapoptotic factors (cytochrome c) to trigger cell mortality (Lamberti *et al*., 2012; Muhammad *et al.*, 2020). Thus, attenuation of oxidative stress in myocardial tissues, inflammation, and cell apoptosis serves as a therapeutic target to prevent 5-FU cardiotoxicity.

RA-based suppression of NF-κB p65 and reduced TNF-α and interleukin (IL-6 and IL-1β) levels in 5-FU-administered mice heart indicate its anti-inflammatory properties. RA elevated Bcl-2 (antiapoptotic protein) expression and reduced caspase-3 and Bax (proapoptotic proteins) expressions in 5-FU-administered mice hearts. The RA-linked inflammatory response reduction has been reported in cardiac ischemia/reperfusion injury *via* alleviation of heart protein (p-NFκB and p-IκB-α) levels (Quan *et al*., 2021). Another study revealed RA-based prevention of cardiomyocyte apoptosis through the inhibition of FasL release in cardiac fibroblasts (Zhang *et al.*, 2019). Moreover, adriamycin-related reduced H9c2 cell viability and enhanced activation of caspase protease were ameliorated by RA treatment during a study (Kim *et al.*, 2005). RA is known to restrict LPS-linked inflammation, and attenuate cholestasis-related oxidative stress and inflammation by NF-κB downregulation in animals (Li *et al.*, 2017). During an investigation, RA inhibited TNF-α and NF-κB expressions and inhibited apoptosis in cisplatin-treated mice *via* caspase-3 and p53 suppression (Domitrović *et al.*, 2014). Another study reported the prevention of chlorpyrifos-associated apoptosis and inflammatory response in kidneys after RA treatment as it modulated apoptosis mediators and NF-κB p65 (Abduh *et al*., 2023).

This study further elucidated the protection mechanism of RA against 5-FU cardiac injury. Therefore, HO-1 levels and Nrf2 expressions were investigated in the hearts of all mice groups. These results depicted reduced HO-1 levels and Nrf2 expressions in RA-pretreated 5-FU-administered mice, which is in line with previous studies (Li *et al*., 2023a, 2023b; Lokman *et al*., 2023). The transcription factor Nrf2 is crucial for cellular protection against inflammation and oxidative stress in heart illnesses (Althunibat *et al.*, 2022; Li *et al.*, 2014a; Obeidat *et al.*, 2022; Satta *et al.*, 2017). Nrf2 suppresses the NF-κB-mediated inflammatory response through inhibition of oxidative stress-induced activation of NF-κB and blocking IκB-α proteasomal degradation (Saha *et al.*, 2020; Wardyn *et al.*, 2015). A study has highlighted the protective role of Nrf2 against chemotherapy-related cardiotoxicity. The study revealed that Nrf2 knockout enhanced myocardial necrosis, dysfunction, oxidative stress, and cardiac injury in mice exposed to doxorubicin (Li *et al*., 2014a). Thus, Nrf2 upregulation is a promising 5-FU cardiotoxicity prevention approach. RA pretreatment of 5-FU-injected mice significantly enhanced Nrf2 expression and HO-1 levels in myocardial tissues. These findings support previous reports regarding Nrf2 mediation in RA efficacy against CCl_4-related liver injury (Lu *et al*., 2022), cisplatin-associated kidney and liver damage (Xiang *et al*., 2022), and chlorpyrifos-linked kidney injury (Abduh *et al*., 2023) in rodents. Similarly, another study stated that Nrf2 deletion restricted RA-based ROS suppression in hepatic stellate cells (HSCs) (Lu *et al.*, 2017). Thus, RA-mediated Nrf2/HO-1 activation successfully contributed to its anti-inflammatory and antioxidant properties against 5-FU-induced mice cardiotoxicity.

Conclusions

The study demonstrates the ability of RA to attenuate 5-FU-induced cardiotoxicity in mice. RA modulated oxidative tissue injury, inflammation, and apoptosis to prevent 5-FU-induced cardiac injury. The results also revealed that positive RA impacts were linked to Nrf2/HO-1 restoration in myocardial tissues (Figure 7). Thus, it might have a protective role in the heart diseases associated with oxidative stress and inflammation. However, further investigations are necessary to unravel RA's protective mechanism against 5-FU cardiotoxicity.

Data Availability

All the data generated or analyzed during this study have been included in this manuscript.

Conflict of Interest

The author declares no conflict of interest.

Funding

None.

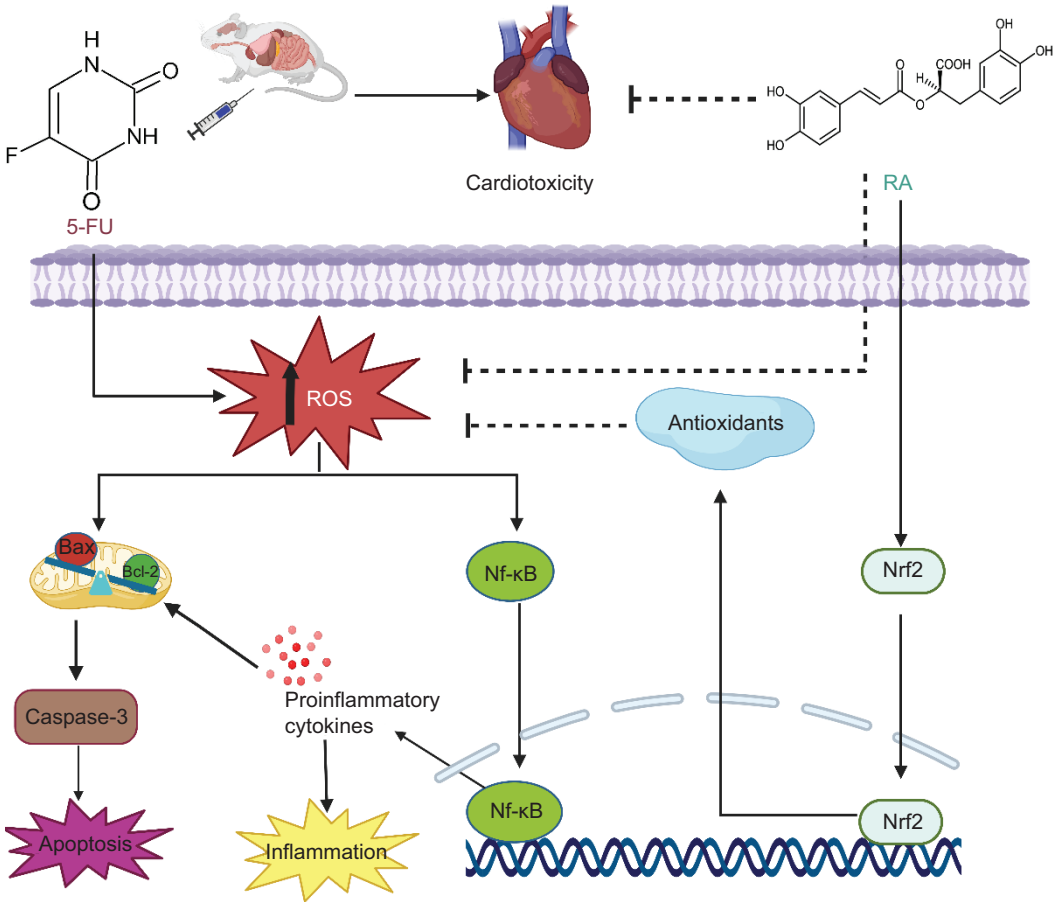

Figure 7. A schematic diagram of RA cardioprotection against 5-FU-induced cardiotoxicity in mice. RA ameliorated cellular redox levels, suppressed inflammatory response, mitigated apoptosis, and restored Nrf2/HO-1 pathway in mouse hearts.

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