Title:

Modulation of NF-κB/NLRP3 inflammasome axis Nrf2/HO-1 signaling and attenuation of oxidative stress mediate the protective effect of ambroxol against cyclophosphamide cardiotoxicity

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Abstract:

Despite its potent chemotherapeutic efficacy, cyclophosphamide (CP) is associated with severe cardiac complications, limiting its clinical utility. Recent evidence suggests that the mucolytic agent ambroxol (ABX) exhibits antioxidant and anti-inflammatory properties, making it a candidate for mitigating CP cardiotoxicity. This study explores the protective effects of ABX against CP-mediated cardiotoxicity, with emphasis on oxidative stress, NF-κB/NLRP3 inflamamsome axis and Nrf2/HO-1 signaling. Rats were administered ABX (20 mg/kg) for 7 days and received a single injection of CP (100 mg/kg) on day 5, and blood and heart samples were collected for analyses. CP administration induced significant cardiac dysfunction, marked by elevated LDH, CK-MB, and troponin-I, alongside histopathological evidence of myocardial injury. ABX alleviated cardiac biomarkers, prevented histopathological alterations, reduced lipid peroxidation, and restored antioxidant defenses. CP upregulated NF-κB p65, NLRP3, ASC1, caspase-1, gasdermin D, and IL-1β, and suppressed Nrf2 and HO-1 in the heart of rats. ABX suppressed the NF-κB/NLRP3 inflamamsome axis mediators and upregulated Nrf2 and HO-1. *In silico* data revealed the binding affinity of ABX towards NF-κB p65 and NLRP3 and ASC1 PYD domains. In conclusion, ABX confers significant protection against CP-induced cardiotoxicity through multifaceted mechanisms, including attenuation of oxidative stress, inhibition of NF-κB/NLRP3 inflamamsome axis, and upregulation of Nrf2/HO-1 signaling. These findings suggest that ABX could serve as an effective adjunct therapy to improve the safety profile of CP in clinical oncology.

Keywords: Chemotherapy; Ambroxol; Cyclophosphamide; Cardiotoxicity; Oxidative stress; Inflammation.

1 Introduction

Drug-induced cardiotoxicity (DICT) represents a significant and growing health concern, particularly in the context of cancer therapy. In drug development, DICT is a major concern

and has been estimated to accounting for 10–14% of postmarket withdrawals [1]. Research indicates that cardiovascular safety issues were responsible for approximately 10% of drug withdrawals over the past 40 years, affecting well-known medications [2]. Additionally, cardiotoxicity ranks as the third most frequent cause of adverse drug reactions, linked to 14% of all withdrawals [3]. Chemotherapeutic agents, while effective in targeting malignant cells, often exert serious side effects on the cardiovascular system, leading to acute or chronic cardiac dysfunction. The adverse effects may lead to impaired heart function, forcing clinicians to alter, suspend, or withdraw essential treatments [4]. Therefore, DICT may severely affect well-being and survival of the patients, even when cancer itself is under control [4]. Cyclophosphamide (CP) is a widely used alkylating chemotherapeutic drug used in the treatment of various malignancies, including lymphomas, leukemias, and solid tumors [5, 6]. CP works by crosslinking DNA strands at the N-7 position of guanine, impairing replication and transcription, and ultimately inducing apoptosis in malignant cells [5, 6]. However, the clinical utility of CP is often limited by its severe off-target effects, including hemorrhagic cystitis, myelosuppression, hepatotoxicity, and gonadotoxicity; among these, cardiotoxicity represents a particularly severe and potentially life-threatening complication. The exact molecular mechanisms underlying CP-induced cardiotoxicity remain incompletely elucidated. Current evidence suggests that reactive CP metabolites mediate cardiac injury primarily through reactive oxygen species (ROS) generation and direct endothelial damage, leading to pathological extravasation of plasma proteins, erythrocytes, and cytotoxic compounds [7]. Subsequent endothelial cell disruption exacerbates myocardial and microvascular injury, culminating in characteristic histopathological findings, including tissue edema, interstitial hemorrhage, and microthrombosis [7, 8]. Recent advances in systemic cancer therapies have heightened the importance of managing treatment-associated toxicities to preserve therapeutic continuity and improve patient outcomes [9].

The pathogenesis of CP cardiotoxicity is closely linked to redox imbalance provoked by excessive ROS generation and oxidative stress. These detrimental effects are primarily mediated by its bioactive metabolites, acrolein and phosphoramide mustard generated via the enzymatic activity of hepatic microsomal cytochrome P-450 [10]. Acrolein possesses high reactivity and short biological half-life and is implicated in provoking ROS generation, oxidative stress, and vascular toxicity [11, 12]. Excess ROS along with acrolein can diminish cellular antioxidants and provoke lipid peroxidation (LPO), protein oxidation, and oxidative DNA damage [13, 14]. Additionally, excess ROS can trigger a cascade of inflammatory responses mediated by nuclear factor-kappaB (NF-κB) activation, upregulating numerous pro-inflammatory mediators [15, 16]. Besides the transcription of pro-inflammatory cytokines, NF-κB activation promotes the assembly of the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome, a multiprotein complex that amplifies inflammatory responses by facilitating the maturation and secretion of interleukin-1β (IL-1β) and IL-18 [17]. Following IL-1β secretion, transcriptional activation of genes associated with several disease processes occurs, inducing a pro-inflammatory endothelial phenotype that promotes leukocyte extravasation into injured tissues [18]. The NF-κB/NLRP3 inflammasome axis plays a pivotal role in mediating the inflammatory cascade associated with cardiovascular disease (CVD) and its activation has been observed as a potential driver of cardiotoxicity [19]. Persistent activation of this axis exacerbates myocardial damage, contributing to the development of cardiac dysfunction, atherosclerosis, myocardial infarction, heart failure and other CVD [20]. Therefore, targeting oxidative stress and the NF-κB/NLRP3 inflammasome axis represents a promising therapeutic approach to attenuate chemotherapy-induced cardiotoxicity.

Ambroxol (ABX), a mucolytic agent commonly used in the treatment of respiratory diseases, has recently gained attention for its pleiotropic pharmacological properties beyond its traditional clinical applications [21-24]. ABX showed beneficial effects in Gaucher disease,

Parkinson disease, pneumonia and others [22-24], and emerging evidence suggests its antioxidant and anti-inflammatory effects, making it a promising candidate for mitigating CP cardiotoxicity. ABX effectively mitigated LPO induced by doxorubicin (DOX) in murine heart [25], and protected against neurotoxicity [26], ischemic stroke [27], and kidney and liver ischemia-reperfusion (I/R) injury [28-30]; effects linked to suppression of pro-inflammatory mediators [28]. In a mouse model of CP-induced cystitis, ABX has shown protective effects mediated via suppression of inflammation and oxidative stress [31]. However, the cardioprotective effect of ABX against CP toxicity has not been explored. The aim of this study is to investigate the potential of ABX in mitigating CP-induced cardiotoxicity in a rat model, with a focus on its effects on redox homeostasis, NF-κB/NLRP3 inflammasome axis, and nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase 1 (HO-1) signaling. Nrf2 is a transcription factor that regulates the expression of antioxidant and cytoprotective genes, including HO-1, thereby mitigating oxidative stress and inflammation [32].

2. Materials and methods

2.1. Animals and experimental design

Male Wistar rats (180 ± 10 g) were maintained under standardized conditions (23 ± 1 °C, 50–60% humidity, and 12-h light/dark cycle) with *ad libitum* access to food and water. Twenty-four rats were randomly allocated into four groups (n = 6/group) to assess the cardioprotective potential of ABX against CP-induced cardiac injury. All procedures were approved by the Institutional Animal Ethics Committee of Al Azhar University, Egypt (Approval no. AZ-AS/PH-REC/6/25) and complied with institutional guidelines.

ABX (GNP, Egypt) and CP (Endoxan; Baxter Oncology, Germany) were suspended in 0.5% carboxymethyl cellulose (CMC; Sigma, USA) and physiological saline, respectively. The treatment groups were as follows:

Group I (Control): received 0.5% CMC orally.

Group II (ABX): administered 20 mg/kg ABX orally [29].

Group III (CP): injected intraperitoneally (i.p.) with 100 mg/kg CP [33].

Group IV (ABX + CP): received both CP (100 mg/kg) and ABX (20 mg/kg).

ABX was administered daily for 7 days, with CP delivered as a single dose on day 5. Groups I and II received a single i.p. injection of saline on day 5. On day 8, blood was collected via cardiac puncture under ketamine/xylazine (80/10 mg/kg) anesthesia for serum isolation. Rats were euthanized, and heart was rapidly excised. Sections were fixed in 10% neutral buffered formalin (NBF) for histology and other sample were homogenized in Tris-HCl buffer (pH 7.4) for biochemical assays.

2.2. Biochemical assays

Serum lactate dehydrogenase (LDH) and creatine kinase (CK)-MB were quantified using commercial kits (Spinreact, Spain, Cat. no. 41220 and 1001054, respectively). Heart homogenates were analyzed for oxidative stress markers (malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase) using Biodiagnostic kits (Egypt, Cat. no.: MD2528, SD2521, TA2511, and CA2517, respectively). Cardiac NF-κB p65 and IL-1β, and serum cardiac troponin I (cTnI) were determined using specific ELISA kits (Elabscience, China, Cat. no. E-EL-R0674, E-EL-R0012, and E-EL-R1253, respectively). HO-1 activity was assayed following the method of Abraham et al. [34].

2.3. Histopathological and immunohistochemical (IHC) assessment

Formalin-fixed heart tissues were paraffin-embedded, sectioned (5 μm), and stained with hematoxylin and eosin (H&E) for histopathological evaluation. Other sections were stained with Sirius red, periodic acid-Schiff (PAS) and Prussian blue. IHC analysis was

performed to assess the expression of NRLP3, ASC, caspase-1, gasdermin D (GSDMD), and Nrf2. Briefly, paraffin-embedded sections were treated with citrate buffer (50 mM, pH 6.8) for antigen retrieval and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide (H_2O_2), followed by incubation with a protein-blocking solution. Sections were incubated overnight at 4 °C with primary antibodies against NRLP3, ASC, caspase-1, GSDMD, and Nrf2 (Biospes, China, Cat. no. YPA1480, YPA1695, YPA2348, YPA2511, and YPA1865, respectively), washed, and then treated with a secondary antibody. Color development was achieved using DAB, and sections were counterstained with Mayer's hematoxylin. Staining intensity was quantified using ImageJ software (NIH, USA) by analyzing six randomly selected fields per sample.

2.4. Molecular Docking Studies

The interaction between ABX and NF-κB p65 (PDB: 5U01), NLRP3 PYD domain (PDB: 3QF2) and ASC PYD domain (PDB: 1UCP) was modeled using AutoDock Tools (v1.5.6), and virtual screening was performed using PyRx (v0.8) [35]. Visualization of binding interactions and modes was carried out using PyMOL (v2.3.2) and LigPlot+ (v2.2.8) [36].

2.5. Statistical Analysis

Data are expressed as mean ± standard deviation (SD). One-way ANOVA with Tukey's post hoc test using GraphPad Prism v8 were employed to determine significance. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. ABX attenuates CP-induced myocardial injury

The protective effect of ABX against CP-induced cardiotoxicity was assessed using biochemical markers (Fig. 1) and histopathological analysis (Fig. 2). CP administration resulted in significant increases in serum cTnI (Fig. 1A), CK-MB (Fig. 1B), and LDH (Fig. 1C)

compared to the control group ($p<0.001$). Treatment with ABX significantly alleviated these changes in myocardial injury biomarkers.

Histopathological examination of heart tissues revealed normal appearance of the myocardium as thin, branched, cylindrical muscle fibers with oval central nuclei, and normal blood vessels in both the control (Fig. 2A-B) and ABX-treated groups (Fig. 2C-D). In contrast, CP administration caused tissue damage manifested by congested and dilated blood vessels, hypertrophied muscle with enlarged nuclei, vacuolated cytoplasm, and hypereosinophilic myocytes with pyknotic nuclei (Fig. 2E-H). ABX markedly alleviated these pathological alterations and resulted in notable improvements in their myocardial blood vessels and cardiac muscle fibers (Fig. 2I-J). In addition to H&E, sections from the heart of all groups were stained with Sirius red, PAS, and Prussian blue (Fig. 3) to evaluate changes in collagen, mucopolysaccharides (MPS), and iron deposition, respectively. Control and ABX-treated rats revealed normal collagen, normal PAS stain intensity and distribution, and negative Prussian blue staining. CP increased collagen, MPS, and iron deposition in the heart of rats; effects that were prevented by ABX.

3.2. ABX mitigates CP-induced oxidative stress in rat heart

CP significantly increased MDA (Fig. 4A) and decreased GSH, SOD, and catalase in the heart of rats ($p<0.001$; Fig. 4B-D). Treatment with ABX significantly reduced cardiac MDA ($p<0.001$) while enhanced GSH ($p<0.01$) and antioxidant enzyme activities ($p<0.001$).

3.3. ABX suppresses NF-κB/NLRP3 inflammasome axis in CP-treated rats

CP administration significantly upregulated NF-κB p65 (Fig. 5A), NLRP3 (Fig. 5B-C), ASC1 (Fig. 5B,D), caspase-1 (Fig. 5B,E), GSDMD (Fig. 5B,F), and IL-1β (Fig. 5G) expression in the heart of rats as compared to the control ($p<0.001$). ABX effectively suppressed the expression of NF-κB p65, NLRP3, ASC1, caspase-1, GSDMD, and IL-1β in CP-administered rats. *In silico* data revealed the binding affinity of ABX with NF-κB p65 (Fig. 6A), NLRP3 PYD (Fig.

6B) and ASC PYD (Fig. 6C) with energies of -5.9, -5.3, and -5.4 kcal/mol, respectively. ABX showed hydrophobic interactions with 8, 6, and 7 amino acid residues, and hydrogen bonding with 2, 2, and 1 residues of NF-κB p65, NLRP3 PYD and ASC PYD, respectively.

3.4. ABX upregulated Nrf2/HO-1 signaling in CP-treated rats

CP administration significantly downregulated Nrf2 expression (Fig. 7A-B) and decreased HO-1 activity (Fig. 7C) in the heart of rats ($p<0.001$). Treatment with ABX upregulated Nrf2 and HO-1 in the heart of CP-exposed rats ($p<0.001$).

4. Discussion

The alkylating agent CP is widely used in the treatment of various malignancies. However, its clinical utility is significantly limited by its adverse effects, including cardiotoxicity, which manifests as myocardial damage, heart failure, and, in severe cases, fatal outcomes [7, 8]. Although the exact mechanism underling CP cardiotoxicity is not fully understood, redox imbalance and inflammation are implicated [8], representing a potential therapeutic target. This study aimed to investigate the protective effects of ABX against CP-induced cardiac injury, with a focus on its ability to modulate oxidative stress, NF-κB/NLRP3 inflammasome axis, and Nrf2/HO-1 signaling. The findings demonstrate that ABX exerts significant cardioprotective effects, as evidenced by improvements in cardiac function markers, histopathological alterations, and molecular signaling pathways. Furthermore, molecular docking studies provide insights into the binding affinity of ABX with key molecular targets, underscoring its potential as a therapeutic agent for mitigating CP cardiotoxicity.

The cardiotoxic effects of CP were evident in the significant elevation of cardiac function markers, including CK-MB, LDH, and cTnI, which are well-established indicators of myocardial injury [37]. These biomarkers reflect the extent of cardiomyocyte damage and membrane integrity loss, consistent with the histopathological findings in this study. Histopathological examination using H&E staining revealed congested and dilated blood

vessels, hypertrophied muscle with enlarged nuclei, vacuolated cytoplasm, and hypereosinophilic myocytes with pyknotic nuclei. Sirius red staining further demonstrated increased collagen deposition, indicative of fibrogenesis, while PAS staining highlighted elevated deposition of MPS. Cardiovascular pathology is associated with elevated disposition of MPS as shown in patients with mucopolysaccharidosis where the manifestations include cardiac hypertrophy and valvular defects [38]. In addition, Prussian blue staining confirmed iron accumulation, suggesting oxidative stress-mediated ferroptosis. Increased iron deposition in the heart leads to cardiomyopathy characterized by systolic or diastolic dysfunction [39]. Disrupted iron metabolism has been associated with the use of anthracycline and its involvement in cardiotoxicity of these drugs has been suggested [40], and CP increased iron content in the liver and spleen of mice [41]. The findings of our study collectively demonstrate that CP may induce significant metabolic and structural alterations in the heart and show new information on the involvement of MPS and iron deposition in CP cardiotoxicity. ABX markedly attenuated these pathological alterations, restoring cardiac function markers and improving heart architecture. The reduction in collagen, MPS and iron accumulation demonstrate the cardioprotective efficacy of ABX. The ability of ABX to protect the heart against chemotherapeutics has been scarcely studied. In DOX-challenged murine heart, ABX effectively mitigated LPO [25].

The efficacy of ABX to attenuate oxidative stress and inflammation has been demonstrated in pre-clinical models of I/R- and cisplatin-induced liver injury [29, 30, 42]. Owing to the involvement of oxidative stress and inflammation in CP cardiotoxicity [8], the cardioprotective efficacy of ABX could be ascribed to its ability to suppress these pathological processes. This study demonstrated a significant increase in LPO alongside a depletion of GSH and reduced activities of SOD and catalase, demonstrating oxidative stress. The metabolism of CP results in the generation of acrolein which elicits the production of ROS. Acrolein is a highly

electrophilic metabolite that depletes endogenous antioxidants, including GSH, and promotes LPO [11], as indicated by increased MDA levels in this study. The depletion of GSH, a pivotal regulator of cellular redox balance, compromises antioxidant defense mechanisms, thereby enhancing susceptibility to oxidative injury [43]. A concomitant decline in the antioxidant enzymes, SOD and catalase, leads to ROS accumulation that further aggravates oxidative stress [44]. CP-driven surplus ROS inflicts cellular injury through multiple pathways, including LPO, oxidative protein and DNA damage, and antioxidant depletion. LPO alters membrane integrity by disrupting lipid bilayer fluidity and permeability, while also inactivating integral membrane proteins, culminating in loss of membrane functionality [45]. Additionally, ROS induce post-translational modifications in structural proteins and oxidatively inactivate critical enzymes, perturbing cellular homeostasis and amplifying oxidative damage [45].

Besides oxidative damage, excess ROS can provoke inflammatory responses mediated by NF-κB activation that upregulates numerous pro-inflammatory mediators [15, 16] and promotes the assembly of the NLRP3 inflammasome [17]. NLRP3 inflammasome is a multiprotein complex that amplifies inflammatory responses by promoting the maturation and secretion of IL-1β and IL-18 [17]. Pro-inflammatory cytokines released following NF-κB activation promote inflammation and injury by recruiting immune cells, inducing further ROS production, and promoting fibrotic changes [46]. Together with ROS, the pro-inflammatory mediators provoke mitochondrial dysfunction via alteration of the mitochondrial membrane potential, resulting in enhanced permeability and release of cytochrome c into the cytosol. Cytochrome c interacts with caspase-9 and Apaf-1 to form the apoptosome complex, which subsequently activates caspase-3, initiating the execution phase of apoptosis [47]. In the current study, CP activated the NF-κB/NLRP3 inflammasome axis as demonstrated by upregulated NF-κB, NLRP3, ASC, caspase-1, IL-1β, and GSDMD in the heart of rats. ROS-mediated activation of NF-κB is closely associated with NLRP3 inflammasome induction across

multiple pathological conditions [17]. ROS further serve as direct activators of NLRP3, triggering a proinflammatory cascade characterized by increased IL-1β secretion [17]. This process upregulates disease-associated genes and promotes endothelial cell activation, which enhances immune cell recruitment to sites of tissue injury [18]. Structurally, NLRP3 comprises a NACHT domain with ATPase activity essential for oligomerization [48], an LRR domain, and a PYD domain. The PYD mediates inflammasome assembly by homotypic interactions with the adaptor protein ASC [49]. Subsequent recruitment and activation of caspase-1 catalyzes the proteolytic cleavage of pro-IL-1β into its bioactive form (IL-1β) and processes GSDMD [17]. GSDMD is a protein that plays a crucial role in pyroptosis, a type of programmed cell death, by forming pores in the plasma membrane, leading to cell lysis and the release of inflammatory cytokines [17].

ABX effectively mitigated these changes, reducing MDA levels and restoring GSH, SOD, and catalase activities. These findings suggest that ABX enhances the cellular antioxidant capacity, thereby counteracting CP-induced oxidative damage. The antioxidant properties of ABX are likely mediated through its ability to scavenge ROS and upregulate endogenous antioxidant defenses. This antioxidant efficacy has been reported in murine heart challenged with DOX where ABX attenuated LPO [25]. By attenuating LPO and enhancing antioxidant defenses, ABX mitigates oxidative stress and prevents subsequent cellular damage. Additionally, ABX suppressed the NF-κB/NLRP3 inflammasome axis evidenced by downregulation of NF-κB, ASC, NLRP3, IL-1β and GSDMD. This anti-inflammatory efficacy is likely mediated through the inhibition of ROS generation. The dual anti-inflammatory and antioxidant effect of ABX is supported by previous studies showing its ability to attenuate these processes in organs other than the heart. In rodent models of I/R-liver injury [29, 30] and cisplatin hepatotoxicity [42], ABX suppressed LPO and pro-inflammatory cytokines. Our study added support to the protective role of ABX against inflammation and oxidative stress by revealing its

cardioprotective efficacy against CP toxicity. To further explore the mechanism underlying the suppressive effects of ABX on NLRP3 inflammasome activation, we investigated its binding affinity to NLRP3 and ASC PYD domains. The results revealed that ABX forms stable interactions with the PYD domains of NLRP3 and ASC through hydrogen bonding and hydrophobic interactions, consistent with its ability to inhibit inflammasome assembly and activation. These findings highlight the anti-inflammatory properties of ABX, which likely contribute to its cardioprotective effects by attenuating the inflammatory cascade and preventing pyroptosis. Furthermore, the protective effect of ABX against CP cardiotoxicity involved upregulation of the Nrf2/HO-1 signaling pathway in the heart of rats. ABX upregulated Nrf2 expression and HO-1 activity, effects associated with attenuated myocardial oxidative stress and inflammation in CP-administered rats. Nrf2 is a master regulator of antioxidant gene expression. HO-1, a downstream target of Nrf2, is essential for mitigating OS and inflammation [32]. HO-1 catalyzes the degradation of heme into biliverdin and carbon monoxide, thereby exerting antioxidant and anti-inflammatory properties [50]. These findings suggest that ABX exerts its protective effects against CP cardiotoxicity, at least in part, through the modulation of Nrf2/HO-1 signaling. While this study provides compelling preclinical evidence for the cardioprotective effects of ABX against CP-induced toxicity, the preclinical nature of the study, the use of a single dose of ABX, the lack of data showing cytoplasmic and nuclear levels of Nrf2, and the lack of long-term outcome data could be considered as limitations.

5. Conclusion

This study demonstrates that ABX exerts significant cardioprotective effects against CP-induced cardiotoxicity by mitigating oxidative stress, suppressing NF-κB/NLRP3 inflammasome axis, and enhancing Nrf2/HO-1 signaling. The improvements in cardiac function markers, alleviation of histopathological alterations, and modulation of key signaling

pathways underscore the therapeutic potential of ABX in preventing CP cardiotoxicity. Molecular docking studies further elucidate the binding affinity of ABX with NLRP3 and ASC PYD domains, providing a mechanistic basis for its multi-faceted protective effects. These findings suggest that ABX could be repurposed as an adjunctive therapy to enhance the safety and efficacy of CP-based chemotherapy. Future studies are warranted to validate these findings in clinical settings and explore the translational potential of ABX in cardioprotection.

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Declaration of Competing Interest

All authors declare no conflict of interests in relation to the manuscript.

Availability of data and materials

The manuscript contains all data supporting the reported results.

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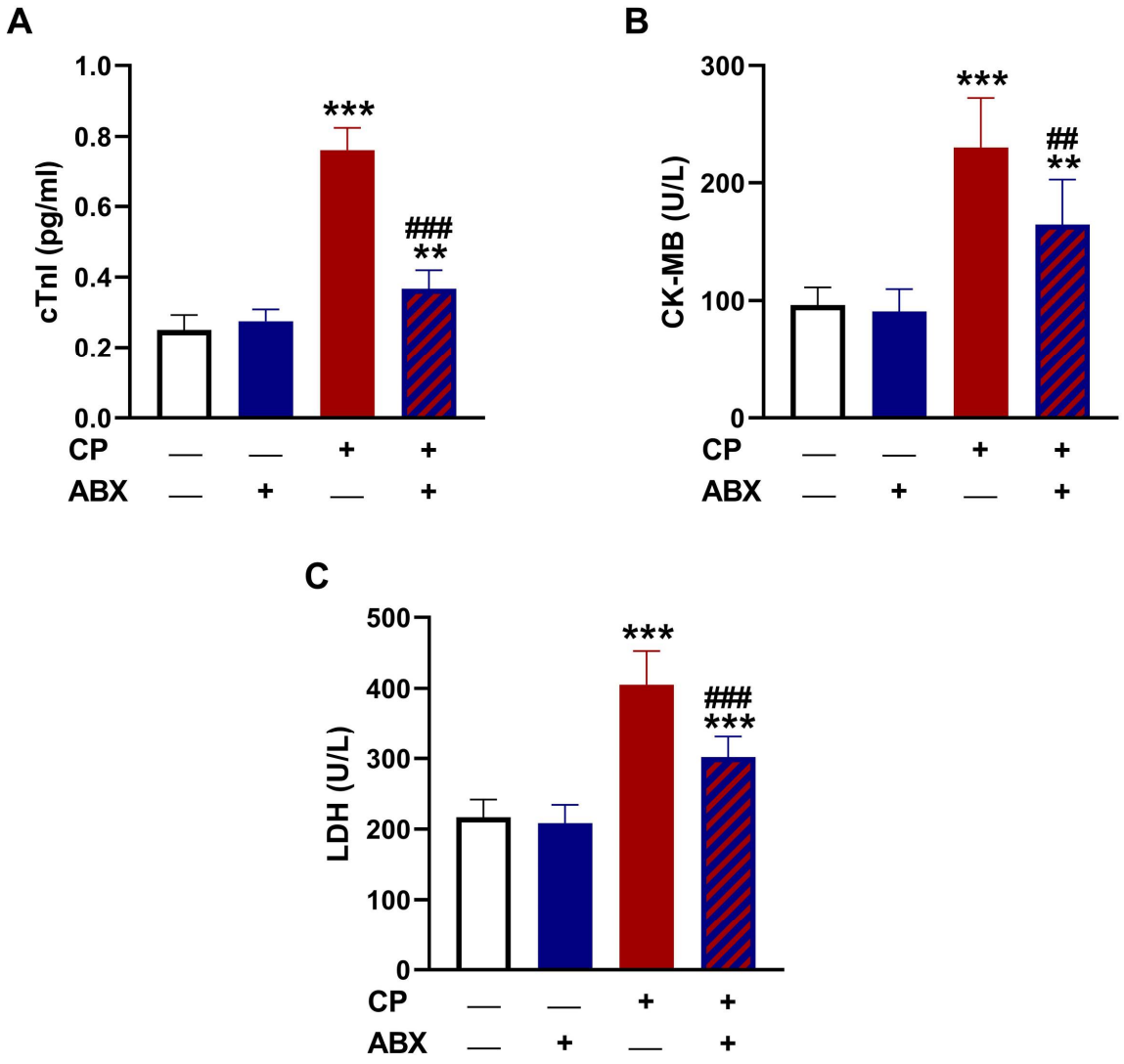
476 Figures:



477
478 Fig. 1. ABX ameliorated serum cTnI (A), CK-MB (B), and LDH (C) in CP-administered rats.
479 Data are mean ± SD, (*n*=6). $^{**}p<0.01$ and $^{***}p<0.001$ versus Control. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$
480 versus CP.

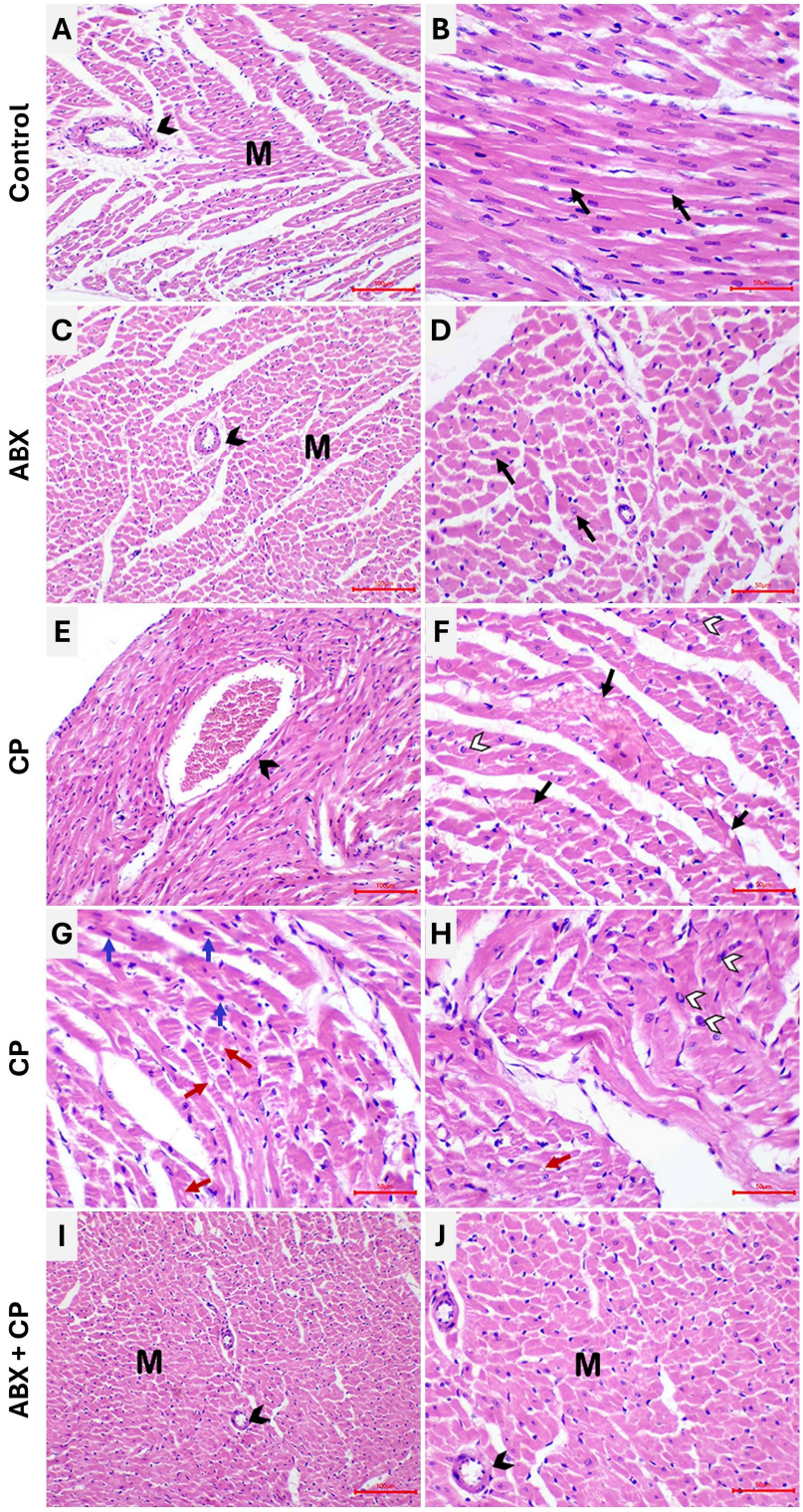


Fig. 2. ABX prevented cardiac injury in CP-administered rats. Photomicrographs of H&E-stained sections from the control (A-B) and ABX-supplemented rats (C-D) showing normal myocardium (M and arrows) and blood vessels (arrowheads); (E-H) CP-treated rats showing hypertrophied muscle with enlarged nuclei (white arrowheads), vacuolated cytoplasm (red arrows), congested and dilated blood vessels (black arrowheads and black arrows), and hypereosinophilic myocytes with pyknotic nuclei (blue arrows); and CP-administered rats treated with ABX showing notable improvements in blood vessels and cardiac muscle fibers (M and blue arrows) and relatively appear as the control group. (Scale bar = 100 µm (A, C, E, and I) and 50 µm (B, D, F, G, H and J)).

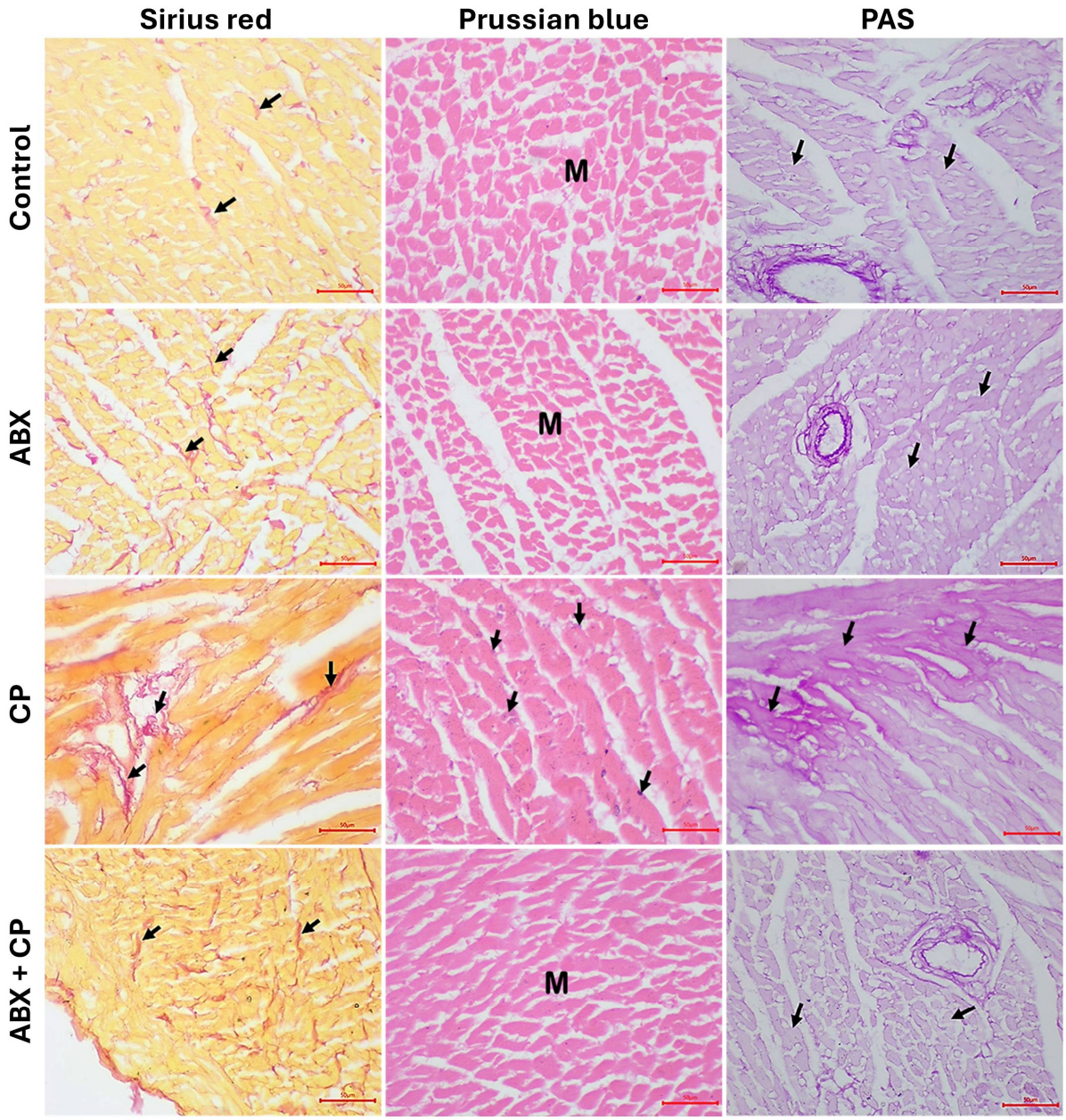


Fig. 3. Photomicrographs of Sirius red-, Prussian blue-, and PAS-stained heart sections. Sirius red staining shows normal distribution of a little amount of collagen fibers between the cardiac muscle fibers (arrows) in control and ABX-treated rats, increased collagen fibers (arrows) in CP-administered rats and normal collagen fiber (arrows) content in CP-administered rats treated with ABX. Control and ABX-supplemented rats show negative Prussian blue staining affinity, CP-administered rats show hemosiderin deposits (arrows), and CP-administered rats treated with ABX show no deposits. The heart of control and ABX-treated rats shows normal PAS stain intensity and distribution (arrows), whereas CP-administered rats show uneven distribution of PAS stain throughout the cardiac muscle fibers, with some muscle fibers displaying a highly intense reaction to the stain (arrows). ABX treatment alleviated PAS staining in CP-administered rats and the sections appear normal. (Scale bar = 50 µm).

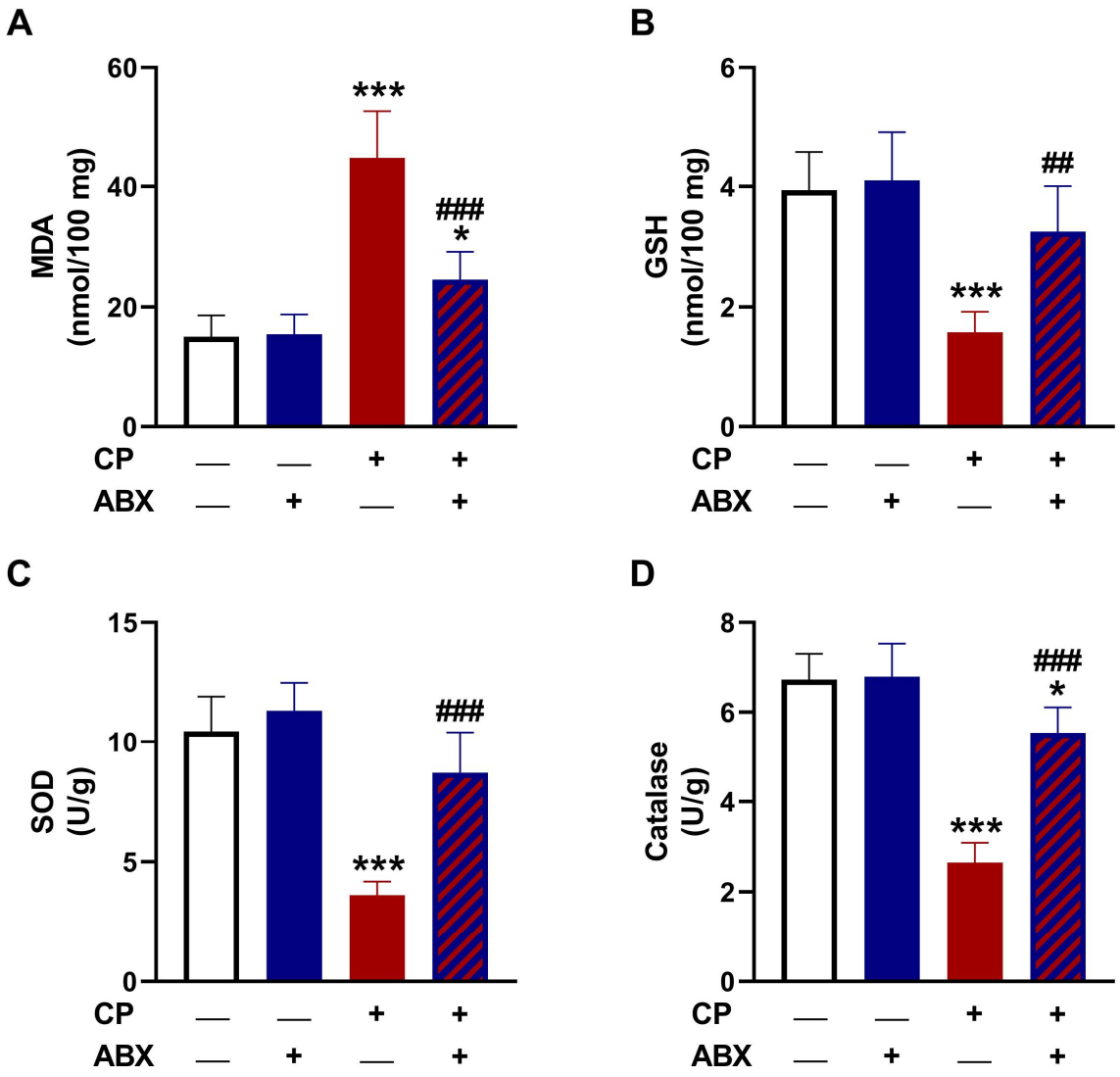


Fig. 4. ABX mitigated CP-induced oxidative stress. ABX decreased MDA (A), and increased GSH (B), SOD (C), and catalase (D) in the heart of CP-administered rats. Data are mean ± SD, (n=6). $^{*}p<0.05$ and $^{***}p<0.001$ versus Control. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ versus CP.

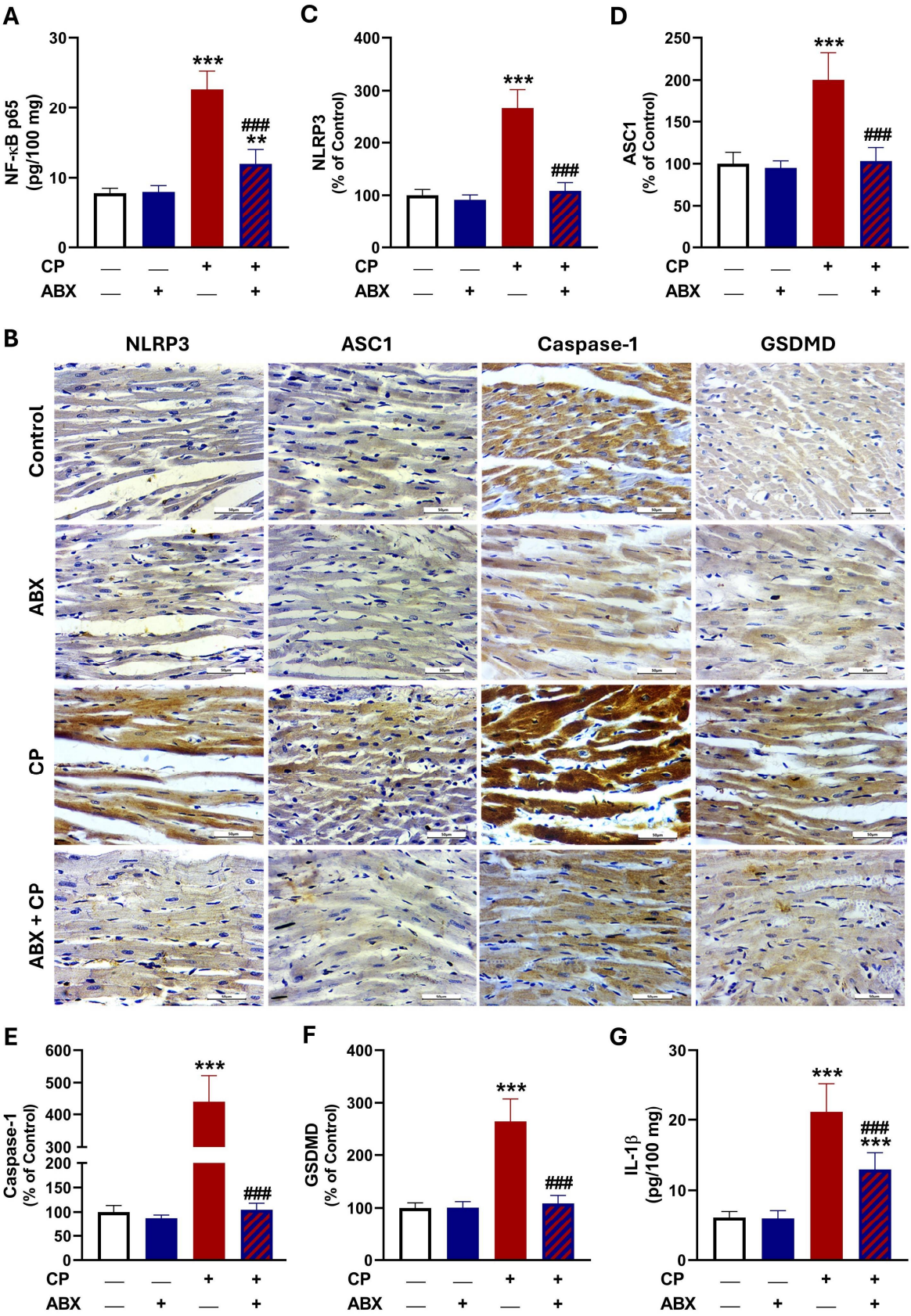


Fig. 5. ABX prevented NF-κB/NLRP3 inflammasome axis activation in CP-treated rats. ARB decreased NF-κB p65 (A), NLRP3 (B-C), ASC1 (B,D), caspase-1 (B,E), GSDMD (B,F), and IL-1β (G) in CP-administered rats. Data are mean ± SD, (n=6). $^{**}p<0.01$ and $^{***}p<0.001$ versus Control. $^{###}p<0.001$ versus CP.

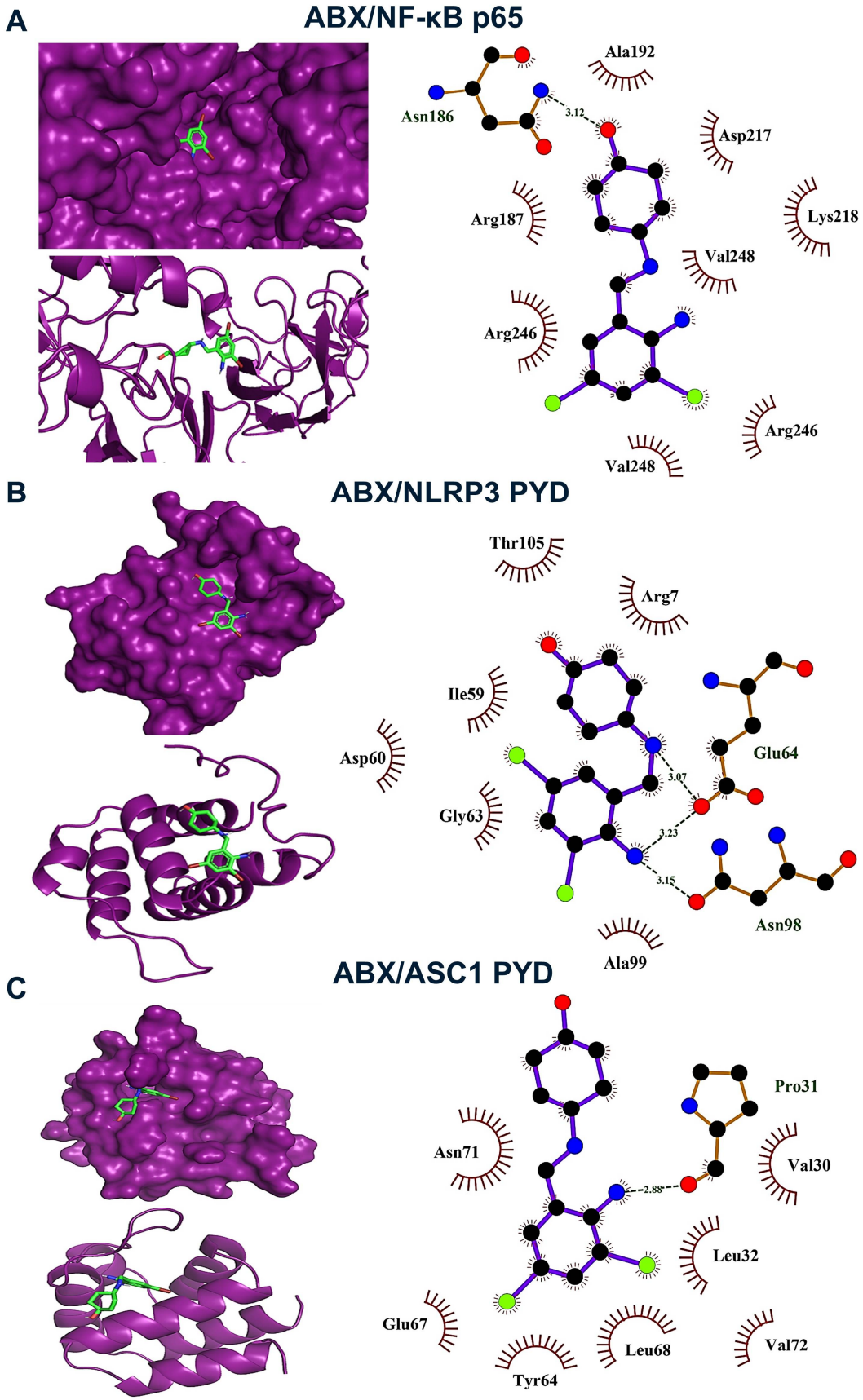


Fig. 6. Molecular docking showing the interaction between ABX and NF-κB p65 (A), NLRP3 PYD (B), and ASC1 PYD (C).

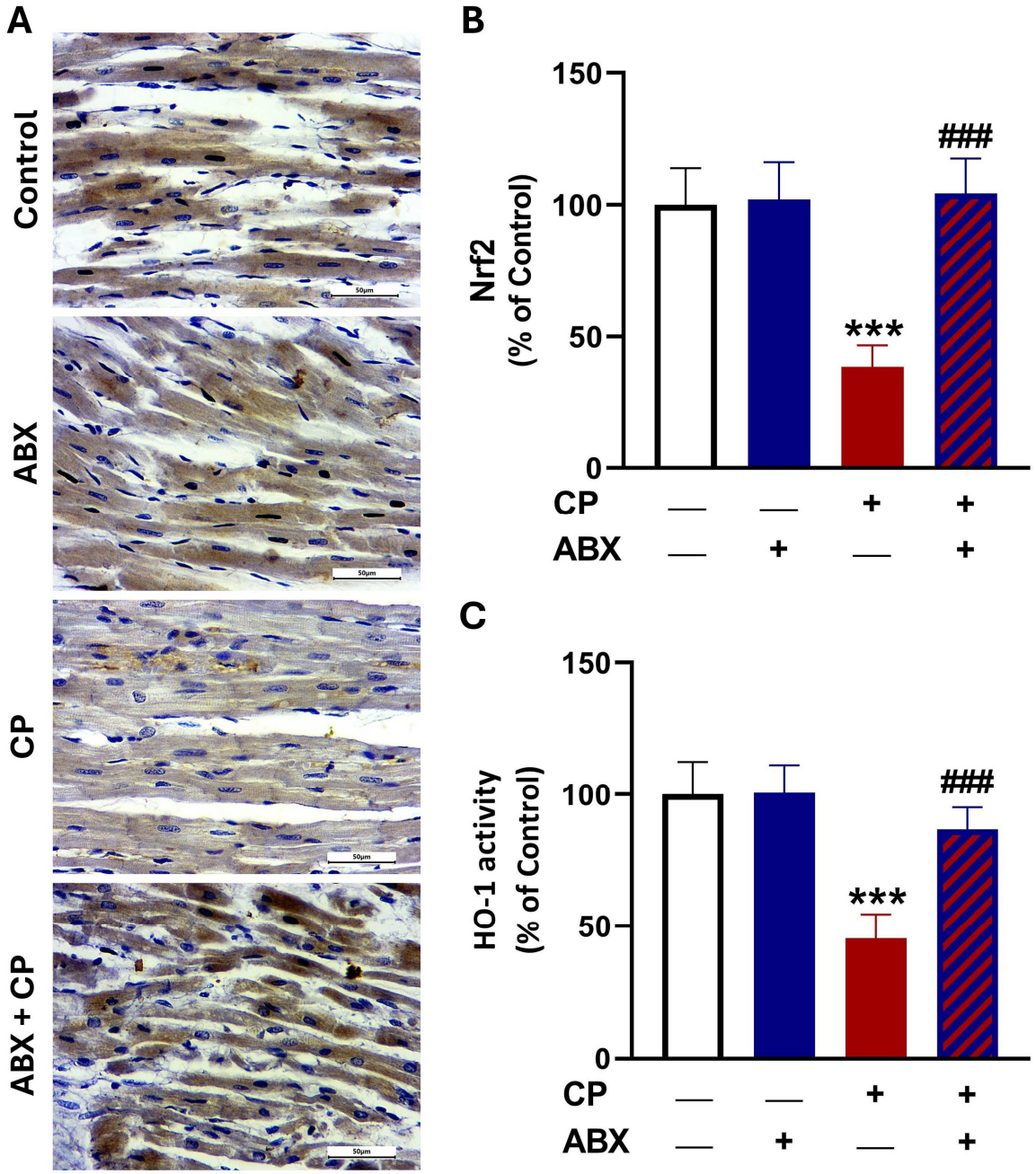


Fig. 7. ABX upregulated Nrf2/HO-1 signaling in the heart of CP-administered rats. ABX increased Nrf2 expression (A-B) and HO-1 activity (C) in CP-administered rats. Data are mean ± SD, (n=6). $^{***}p<0.001$ versus Control and $^{###}p<0.001$ versus CP.