Title:

Attenuation of NF-κB/NLRP3 inflammasome axis and oxidative stress, and upregulation of Nrf2/HO-1 signaling mediate the protective effect of S-carboxymethylcysteine against cyclophosphamide-induced cardiotoxicity

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Abstract:

Cyclophosphamide (CP) is a potent chemotherapeutic and immunosuppressant agent used in the management of lymphoproliferative disorders and solid tumors. However, it induces cardiotoxicity and other severe adverse effects, thereby limiting its clinical application, highlighting the need for safe and effective cardioprotective agents. This study investigates the cardioprotective potential of carbocysteine (S-carboxymethylcysteine (SCMC)), a mucolytic agent with emerging pleiotropic properties, against CP-induced toxicity. The study explores the effect of SCMC on oxidative stress, NF-κB/NLRP3 inflammasome axis and Nrf2/HO-1 signaling. Rats received SCMC for 7 days and a single CP dose on day 5. CP provoked severe cardiac injury, evidenced by increased CK-MB, LDH, and troponin-I, alongside histopathological alterations, including vascular congestion, cytoplasmic vacuolization, hypertrophy, and nuclear pyknosis. SCMC significantly alleviated cardiac biomarkers and mitigated tissue damage in CP-treated rats. CP increased MDA, decreased antioxidants, increased cardiac NF-κB, IL-1β, and gasdermin D, upregulated NLRP3, ASC1, and caspase-1, and diminished Nrf2 and HO-1. SCMC reduced MDA, enhanced antioxidant defenses, and downregulated NF-κB, NLRP3, ASC, caspase-1, gasdermin D, and IL-1β in CP-administered rats. In addition, SCMC enhanced the expression of Nrf2 and activity of HO-1 in the heart of CP-administered rats. In conclusion, these findings demonstrate that SCMC mitigates CP-induced cardiotoxicity by targeting oxidative injury and inflammatory signaling. Its cardioprotective mechanism includes mitigation of oxidative stress and NF-κB/NLRP3 inflammasome axis, and upregulation of Nrf2/HO-1 pathway. Given its established clinical safety, SCMC may represent a translatable adjunctive therapy to protect against CP-induced cardiotoxicity. However, further studies and clinical trials are warranted to confirm these findings.

Keywords: Chemotherapy; Cardiotoxicity; Carbocysteine; Oxidative stress; Inflammation.

1. Introduction

Drug-induced cardiotoxicity (DICT) poses a significant public health challenge, as it can lead to severe cardiovascular manifestations, including arrhythmias and heart failure, often necessitating long-term monitoring and intervention. DICT remains a critical challenge in clinical oncology and can compromise the therapeutic efficacy of life-saving chemotherapeutics [1]. Cardiovascular complications account for a substantial proportion of postmarket drug withdrawals, with estimates suggesting that 10–14% of discontinued medications are attributed to adverse cardiac effects [1]. In this context, cardiovascular safety concerns have precipitated in the withdrawal of nearly 10% of pharmaceuticals over four decades, including several widely prescribed agents [1-3]. Despite their potent antitumor activity, chemotherapeutic agents frequently induce acute or chronic cardiovascular dysfunction, necessitating dose modifications or therapy cessation, thereby jeopardizing patient outcomes [4]. Consequently, DICT not only undermines treatment efficacy but also significantly impacts long-term survival, even in patients with controlled malignancies [4]. The alkylating agent cyclophosphamide (CP) is effective against lymphoproliferative disorders and solid tumors [5-7]. The mechanism of action of CP involves DNA crosslinking, resulting in the disruption of replication and transcription in rapidly proliferating cells [5, 6]. The severe effects associated with the use of CP limit its clinical application. These severe effects include hepatotoxicity, nephrotoxicity, hemorrhagic cystitis, and cardiotoxicity [8-10]. Although the exact mechanisms underlying CP severe effects and toxicity are not fully understood, the role of CP metabolites and reactive oxygen and nitrogen species (ROS and RNS) has been suggested [10, 11].

Redox imbalance and inflammation have been reported in CP-induced cardiomyopathy [12-14]. The CP metabolites phosphoramide mustard and acrolein, produced through hepatic cytochrome P-450 metabolism, provoke ROS overproduction, depleting endogenous

antioxidants and inducing lipid peroxidation (LPO), protein denaturation, and DNA damage [15-18]. The metabolites and ROS mediate cardiac injury primarily through direct endothelial damage and abnormal leakage of plasma proteins, red blood cells, and cytotoxic substances [11]. Endothelial injury intensifies both myocardial and microvascular injuries, resulting in characteristic histopathological features, such as interstitial hemorrhage, edema, and the formation of microthrombi [11, 19]. Excess ROS and oxidative stress are associated with inflammatory responses mediated via activation of several molecules, including nuclear factor-kappaB (NF-κB) [20]. ROS-driven activation of NF-κB initiates a pro-inflammatory cascade, upregulating cytokines and facilitating NLRP3 inflammasome assembly, which exacerbates tissue damage via interleukin-1β (IL-1β) [21], a cytokine promoting a pro-inflammatory state in endothelial cells and facilitate the migration of leukocytes into damaged tissues [22]. Sustained inflammasome activation perpetuates endothelial dysfunction, leukocyte infiltration, and microvascular injury, culminating in myocardial hemorrhage, edema, and fibrosis [23, 24]. Given the central role of oxidative and inflammatory pathways in CP-induced cardiotoxicity, therapeutic strategies targeting these mechanisms hold significant promise.

Carbocysteine (S-carboxymethylcysteine (SCMC)), a mucoactive agent clinically employed in chronic respiratory diseases, has recently emerged as a modulator of oxidative and inflammatory pathways, independent of its mucolytic properties [25, 26]. In addition to its well-established mucolytic activity, SCMC has demonstrated anti-inflammatory and antioxidant properties [25]. Experimental evidence indicates that SCMC effectively suppresses hydrogen peroxide (H_2O_2)-mediated oxidative stress in tracheal epithelial cells, thereby inhibiting apoptotic cell death [27]. Furthermore, preclinical studies in cigarette smoke-exposed rats revealed that SCMC preserves pulmonary and systemic antioxidant defenses, exerting significant cytoprotective effects [28]. Given these mechanisms, SCMC may offer therapeutic potential in mitigating CP-induced myocardial oxidative damage and associated

tissue damage. However, the protective efficacy of SCMC against CP-mediated myocardial injury remains unexplored in existing literature. This study investigates the efficacy of SCMC in attenuating CP-induced cardiotoxicity, with a focus on its modulation of oxidative stress, NF-κB/NLRP3 inflammasome axis, and the nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 regulates gens of antioxidant defenses, such as heme oxygenase-1 (HO-1), which counteracts oxidative damage and inflammation [29]. By elucidating these mechanisms, we aim to establish SCMC as a viable adjunctive therapy to mitigate CP-associated cardiovascular complications.

2. Materials and Methods

2.1. Animal experiments and treatment protocol

Twenty-four adult male Wistar rats (190 ± 10 g) were housed under controlled environment of temperature and humidity and 12-h light/dark cycle with unrestricted access to food and water. All animal experiments comply with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8523, revised 1996). The study was approved by the ethics committee of Al-Azhar University (AZ-AS/PH-REC/05/25). Following acclimatization, animals were divided into four experimental groups (n = 6): Group I (Control) received 0.5% carboxymethyl cellulose (CMC); Group II (SCMC) received 250 mg/kg SCMC (Amriya Pharmaceutical Industries, Egypt); Group III (CP) received 100 mg/kg CP (Endoxan®, Baxter) [30]; Group IV (SCMC + CP) received SCMC (250 mg/kg/day orally) [28] and CP (100 mg/kg i.p.). SCMC and CMC were administered orally for 7 consecutive days and CP was administered via intraperitoneal (i.p.) injection on day 5. A single i.p. injection of physiological saline was given to rats of Groups I and II on day 5.

On day 8, blood samples were collected under deep ketamine/xylazine anesthesia for serum separation. Rats were subsequently euthanized by cervical dislocation, and cardiac tissues were rapidly excised. For histopathological analysis sections of the left ventricle were fixed in 10%

neutral buffered formalin (NBF) for 48 h. The remaining myocardial tissue was homogenized in ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a Polytron homogenizer, with aliquots stored at -80°C.

2.2. Biochemical analyses

Serum creatine kinase (CK)-MB and lactate dehydrogenase (LDH) were determined using Spinreact kits (Spain; Cat. no. 41220 and 1001054, respectively) according to manufacturer's protocols. Cardiac malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase were assayed using Biodiagnostic kits (Egypt; Cat. no.: MD2528, TA2511, SD2521, and CA2517, respectively). Cardiac troponin I (cTnI), IL-1β, and NF-κB p65 were determined using specific ELISA kits (Elabscience, China; Cat. no. E-EL-R1253, E-EL-R0012, and E-EL-R0674, respectively), while HO-1 activity was determined via NADPH-dependent biliverdin reduction as previously described [31].

2.3. Histopathological and immunohistochemical (IHC) evaluation

Formalin-fixed heart samples were processed through graded ethanol series, embedded in paraffin, and sectioned at 5 μm thickness. Sections were stained with hematoxylin & eosin (H&E) for general histoarchitecture, Sirius red, Prussian blue, and PAS. The histopathological lesions were graded on a four point score from 0 to 4 according to the presence of congested blood vessels, myocyte hypertrophy, degenerative changes, and inflammatory cell infiltration (0 = normal, 1 = mild lesion, 2 = moderate lesions; 3 = severe focal lesions; and 4 = severe diffuse lesions). The diameter of cardiomyocytes and their nuclei was measured using ImageJ (NIH). For IHC, antigen retrieval was performed using citrate buffer (50 mM, pH 6.8) followed by blocking of endogenous peroxidase with 0.3% H_2O_2. Primary antibodies for NLRP3, Nrf2, caspase-1, ASC, and gasdermin D (GSDMD) (Biospes, China; Cat. no. YPA1480, YPA1865, YPA2348, YPA1695, YPA2511, and respectively) were applied overnight at 4°C and the sections were washed. HRP-conjugated secondary antibodies (Biospes, China) were applied,

and DAB was employed for color development. Following counterstaining with Mayer's hematoxylin, images were captured, and image analysis was conducted using ImageJ software (NIH) with six random fields quantified per sample.

2.4. Statistical Analysis

All data are presented as mean ± standard deviation (SD). Data normality was assessed using the Shapiro–Wilk test. Intergroup comparisons were performed using one-way ANOVA followed by Tukey's post hoc test (GraphPad Prism v8.0). Statistical significance was set at $p < 0.05$ for all analyses.

3. Results

3.1. SCMC mitigates myocardial injury in CP-administered rats

The cardioprotective effect of SCMC was evaluated through serum biomarkers and histopathological examination. CP induced a significant elevation in serum cTnI (Fig. 1A), CK-MB (Fig. 1B), and LDH (Fig. 1C) compared to the control group ($P<0.001$), indicating severe cardiac damage. SCMC remarkably ameliorated these alterations, restoring cardiac injury markers toward normal values ($P<0.001$).

Histopathological examination of H&E-stained sections revealed preserved myocardial architecture in control (Fig. 2A-B) and SCMC-only (Fig. 2C-D) groups, showing regular cardiomyocyte arrangement with intact nuclei and normal vascularity. In contrast, CP-treated rats (Fig. 2E-H) exhibited myocardial damage, including vascular congestion, cytoplasmic vacuolization, hypertrophy, enlarged nuclei, elongated nuclei, and nuclear pyknosis. SCMC markedly improved cardiac histoarchitecture, with near-normal myofibrillar organization and reduced vascular abnormalities, but some myocytes showed hypertrophy (Fig. 2I-J). Histopathological damage scoring and histomorphometry revealed significant tissue damage (Fig. 3A), and increased diameter of myocytes (Fig. 3B) and their nuclei (Fig. 3C) in CP-

administered rats. SCMC significantly prevented tissue damage and myocardial hypertrophy (Fig. 3A-C).

Additional staining with Sirius red, PAS, and Prussian blue demonstrated minimal collagen deposition, normal mucopolysaccharide (MPS) distribution, and absence of iron accumulation in control and SCMC groups (Fig. 4). CP intoxication caused pronounced interstitial fibrosis, increased MPS, and focal iron deposition. SCMC pretreatment significantly attenuated these pathological changes, reducing fibrosis, restoring MPS content, and preventing iron overload (Fig. 4).

3.2. SCMC attenuates CP-induced cardiac oxidative stress

CP intoxication resulted in significant oxidative damage, evidenced by elevated MDA levels ($P<0.001$; Fig. 5A) and depleted antioxidant defenses (GSH, SOD, catalase; $P<0.001$) (Fig. 5B-D). SCMC effectively reduced LPO while enhancing cellular antioxidants ($P<0.001$).

3.3. SCMC downregulates NF-κB/NLRP3 Inflammasome axis activation in CP-administered rats

CP triggered significant upregulation of NF-κB p65, NLRP3, ASC1, caspase-1 (Fig. 6A- E), GSDMD (Fig. 7A,B), and IL-1β (Fig. 7C) ($P<0.001$) in the heart of rats. SCMC effectively inhibited this response as indicated by suppressed NF-κB p65, NLRP3, ASC1, caspase-1, GSDMD, and IL-1β in CP-induced rats.

3.4. SCMC upregulates myocardial Nrf2/HO-1 pathway in CP-administered rats

CP intoxication suppressed Nrf2 ($P<0.001$; Fig. 8A-B) and HO-1 activity ($P<0.001$; Fig. 8C). SCMC increased Nrf2 expression and HO-1 activity in the myocardium of CP-administered rats ($P<0.001$).

4. Discussion

The clinical utility of the alkylating chemotherapeutic agent CP remains significantly constrained by its dose-dependent cardiotoxicity, which manifests as acute myocardial injury,

chronic cardiomyopathy, and potentially fatal outcomes in severe cases [11, 19]. Accumulating evidence implicates oxidative stress and inflammatory cascades as central mediators of CP cardiotoxicity [11, 19]. The present study investigated the potential of SCMC to mitigate CP-induced cardiotoxicity, with particular emphasis on its modulatory role on redox balance, NF-κB/NLRP3 inflammasome axis, and Nrf2/HO-1 pathway. Our results indicate that SCMC confers substantial protection against CP-induced myocardial injury via multimodal mechanisms encompassing antioxidant and anti-inflammatory properties.

The cardiotoxic manifestations of CP were unequivocally established in our model through the remarkable increase in serum cardiac injury biomarkers and characteristic histopathological alterations. The elevated biochemical markers, CK-MB, LDH, and cTnI, indicate membrane integrity loss and tissue damage, consistent with previous clinical observations linking CP administration with acute cardiac events [32]. Histopathological evaluation revealed profound structural disruptions, including hypertrophied muscle, vascular congestion, vacuolated cytoplasm, and nuclear pyknosis, findings that correlate with the biochemical data. In addition to H&E staining, Sirius red revealed an increase in collagen, indicative of fibrosis, while PAS revealed elevated MPS. Our study provides novel insights into the metabolic perturbations associated with CP cardiotoxicity, demonstrating the concurrent accumulation of MPS and iron deposits in cardiac tissue. The observed MPS deposition bears particular pathophysiological relevance, as excessive glycosaminoglycan accumulation in mucopolysaccharidoses is well-established to cause progressive valvulopathy and cardiomyopathy [33]. Similarly, the detected iron overload aligns with emerging evidence implicating ferroptosis, an iron-dependent form of regulated cell death, in chemotherapy-induced cardiotoxicity [34]. This phenomenon has been extensively characterized in anthracycline cardiotoxicity [35], and our findings suggest a parallel mechanism may contribute to CP-induced myocardial injury, supported by prior reports of CP-induced hepatic and splenic iron accumulation [36]. The present findings reveal

that CP can trigger both metabolic and structural changes in cardiac tissue and provide novel insights into the implication of MPS and iron accumulation in CP-induced cardiotoxicity. SCMC treatment effectively mitigated these changes, demonstrating significant reductions in serum CK-MB, LDH, and cTnI, and attenuation of histopathological alterations, collagen deposition, MPS accumulation, and iron overload, effects that collectively underscore its multimodal cardioprotective activity.

Considering the roles of oxidative stress and inflammation in CP-induced cardiotoxicity [19], the cardioprotective effects of SCMC may be attributed to its capacity to mitigate these detrimental pathways. CP intoxication provoked severe oxidative stress, as evidenced by marked LPO and depletion of endogenous antioxidants (GSH, SOD, and catalase). These observations align with the established paradigm of acrolein-mediated toxicity, wherein this reactive CP metabolite depletes cellular thiol reserves and generates cytotoxic ROS through multiple pathways [16]. The reduction in GSH, a key modulator of intracellular redox equilibrium, impairs cellular antioxidant capacity, and the simultaneous decrease in the activity of antioxidant enzymes exacerbate ROS accumulation and increase vulnerability to oxidative damage [37, 38]. Excessive ROS generation induced by CP triggers cellular injury via different mechanisms, such as LPO, oxidative modifications of proteins and DNA, and depletion of antioxidants. LPO compromises membrane stability by altering permeability and impairing membrane proteins, ultimately leading to impaired membrane function [39]. Furthermore, ROS-mediated post-translational alterations of structural proteins and oxidative inactivation of essential enzymes disrupt metabolic equilibrium, exacerbating oxidative injury [39]. The consequent redox imbalance initiates a vicious cycle of oxidative damage, compromising membrane integrity through LPO, inducing deleterious protein modifications, and causing oxidative DNA lesions [39].

Beyond direct macromolecular damage, ROS serve as critical second messengers in pro-inflammatory signaling, particularly through activation of the redox-sensitive transcription factor NF-κB [20, 40]. Our data demonstrate that CP-induced oxidative stress triggers NF-κB activation, which in turn orchestrates upregulation of the NLRP3 inflammasome complex, a finding consistent with recent reports linking this pathway to chemotherapy-induced cardiotoxicity [23] and cardiovascular diseases [24]. The NLRP3 inflammasome, upon assembly, facilitates caspase-1-mediated maturation of IL-1β and IL-18 while cleaving GSDMD to execute pyroptotic cell death [21]. The structural basis of inflammasome activation involves critical interactions between the PYD domains of NLRP3 and ASC, which serve as molecular scaffolds for caspase-1 recruitment [41, 42]. This inflammatory cascade assumes particular significance in myocardial injury, as IL-1β promotes leukocyte recruitment, enhances vascular permeability, and stimulates cardiac fibrosis, processes that collectively exacerbate tissue damage [43]. Pro-inflammatory mediators, in conjunction with ROS, disrupt mitochondrial function, increase membrane permeability and facilitate the translocation of cytochrome c into the cytosol, which in turn triggers caspase-3 activation, executing the apoptotic cascade [44]. Additionally, GSDMD serves as a key effector of pyroptosis, a lytic form of programmed cell death. Upon activation, GSDMD oligomerizes to generate plasma membrane pores, promoting osmotic destabilization, cellular rupture, and the extracellular release of pro-inflammatory cytokines [21].

SCMC effectively suppressed LPO while concurrently restoring GSH, SOD, and catalase, underscoring its potent antioxidant capabilities. Through the inhibition of LPO and the reinforcement of antioxidant system, SCMC effectively counteracts oxidative stress, thereby protecting cells from subsequent injury. Furthermore, SCMC downregulated NF-κB, NLRP3, ASC1, Caspase-1, leading to a marked decrease in IL-1β and GSDMD. The antioxidant and suppression of the NF-κB/NLRP3 inflammasome axis are likely mediated via the efficacy of

SCMC to inhibit ROS generation. Histopathological assessments further corroborated these findings, revealing diminished fibrotic alterations and iron deposition, thereby reinforcing the anti-inflammatory properties of SCMC. By suppressing redox imbalance and NF-κB/NLRP3 inflammasome axis, SCMC attenuates myocardial inflammation and prevents tissue damage. The dual antioxidant and anti-inflammatory benefits of SCMC observed in this investigation align with and expand upon prior research. For instance, in oxaliplatin-treated L02 hepatocytes, SCMC effectively diminished ROS accumulation and mitigated apoptosis [45]. Similarly, in H_2O_2-exposed tracheal epithelial cells, SCMC alleviated oxidative stress and conferred substantial protection against apoptotic cell death [27]. In a rodent model of cigarette smoke exposure, SCMC preserved pulmonary and systemic antioxidant defenses, demonstrating strong cytoprotective effects against smoke-induced injury [27]. Additionally, SCMC displayed potent free radical-neutralizing properties, efficiently normalizing ROS levels and preventing the excessive depletion of GSH following exposure to hydroxyl radicals [46]. Collectively, these findings highlight the therapeutic promise of SCMC in mitigating oxidative stress and cell injury in the myocardium. Our study introduces novel evidence supporting the cardioprotective role of SCMC, demonstrating its efficacy in preventing CP-induced cardiotoxicity. The cardioprotective effect of SCMC involved upregulation of the Nrf2/HO-1 signaling. SCMC upregulated Nrf2 expression and HO-1 activity, effects associated with attenuation of the CP-induced myocardial oxidative stress and inflammation. Nrf2 is a master regulator of cellular stress responses. Nrf2 orchestrates the transcription of over 200 cytoprotective genes, including those encoding phase II detoxification enzymes and antioxidant proteins [29]. HO-1, a downstream effector of Nrf2, plays a critical role in counteracting oxidative stress and inflammation [29]. The observed upregulation of HO-1 - which catalyzes heme degradation into biliverdin and carbon monoxide (CO) likely contributed to the cardioprotective effects of SCMC. Biliverdin is subsequently converted to bilirubin, a

potent endogenous antioxidant, and CO exerts anti-inflammatory and vasodilatory effects [47]. This coordinated induction of endogenous defense systems distinguishes SCMC from conventional antioxidants and may underlie its superior efficacy in mitigating CP-induced cardiotoxicity. Although this study provides compelling evidence of the cardioprotective potential of SCMC, further mechanistic investigations, such as pathway-specific inhibition or gene knockdown, are warranted to establish causal relationships. The study focused on a single dose of SCMC and therefore dose-response relationships remain unexplored.

5. Conclusion

This study provides compelling evidence that SCMC mitigates CP-induced cardiotoxicity by attenuating oxidative stress, suppressing NF-κB/NLRP3 inflammasome axis activation, and potentiating Nrf2/HO-1 cytoprotective signaling. The cardioprotective efficacy of SCMC was demonstrated by its ability to alleviate serum cardiac biomarkers, preserve myocardial architecture, and mitigate histopathological alterations, including fibrosis and iron deposition. These findings were further corroborated by the capacity of SCMC to restore redox balance, inhibit pro-inflammatory signaling, and enhance endogenous antioxidant defenses. The translational implications of these findings are substantial. Given the established safety profile of SCMC in clinical use for respiratory conditions, it represents a promising candidate for repurposing as an adjunctive therapy in CP-based chemotherapy regimens. Future directions should focus on validating the findings of this study in clinical trials, particularly in oncology patients receiving CP. Additional preclinical studies could explore dose-response relationships, long-term cardioprotective outcomes, and potential synergies with other cardioprotective agents.

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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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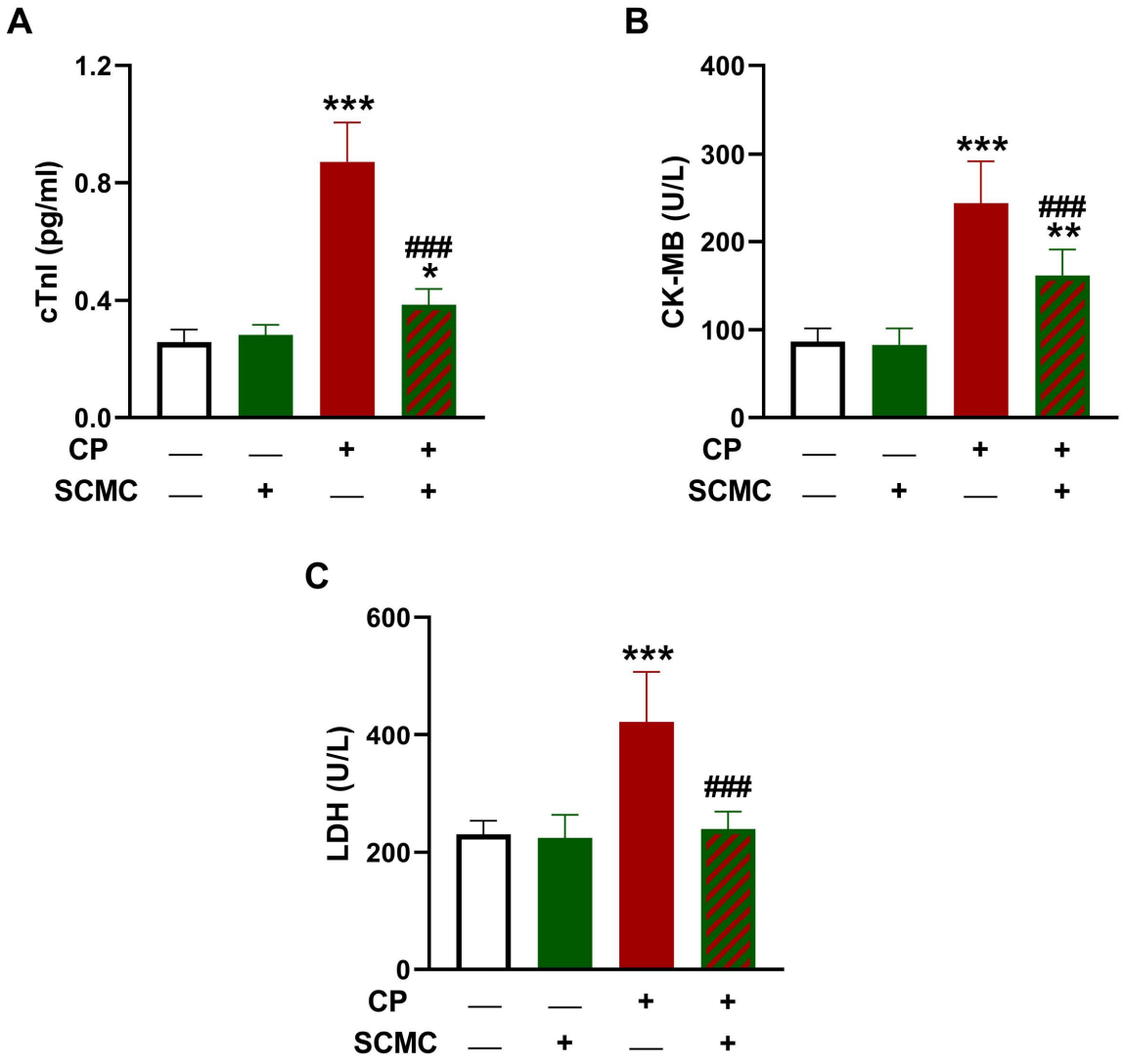
459 Figures:



460
461 Fig. 1. SCMC alleviated serum cTnI (A), CK-MB (B), and LDH (C) in CP-administered rats.
462 Data are mean ± SD, (*n*=6). *P<0.05, **P<0.01 and ***P<0.001 versus Control. $^{###}$P<0.001
463 versus CP.

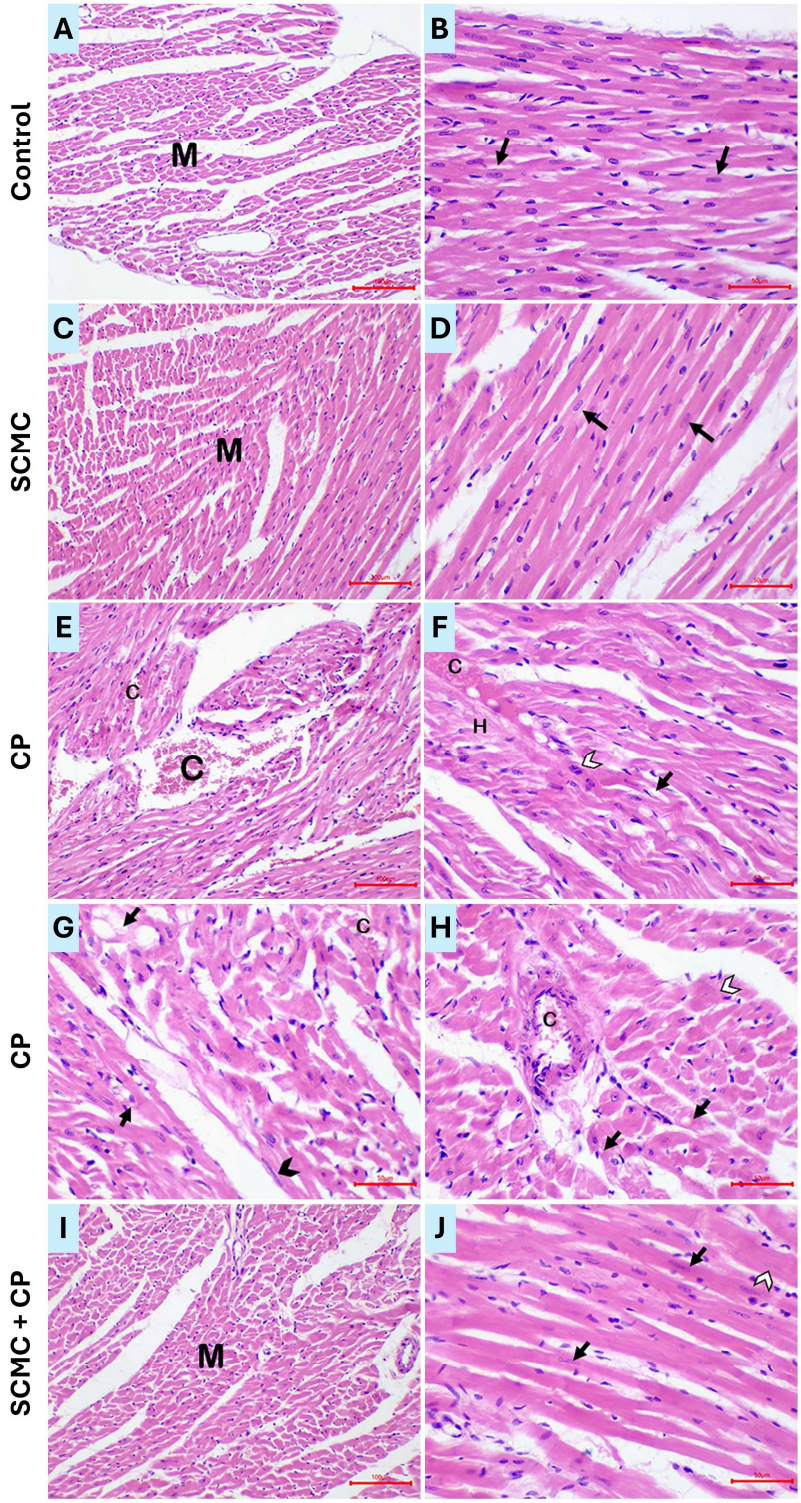


Fig. 2. SCMC prevented myocardial injury in CP-administered rats. Photomicrographs of H&E-stained sections from the control (A-B) and SCMC-supplemented rats (C-D) showing normal intact thin elongated branched cardiac muscle fibers with oval central nuclei (M and arrows); (E-H) CP-treated rats showing congested and dilated myocardial blood vessels (C). Furthermore, the cardiac muscle fibers displayed vacuolation (arrows), hyalinization (H), myocyte hypertrophy, enlarged nuclei (white arrowheads), and elongated nuclei (black arrowheads); and CP-administered rats treated with SCMC showing notable improvements in blood vessels and cardiac muscle fibers (M and blue arrows) , but some myocytes showed hypertrophy (arrowhead).

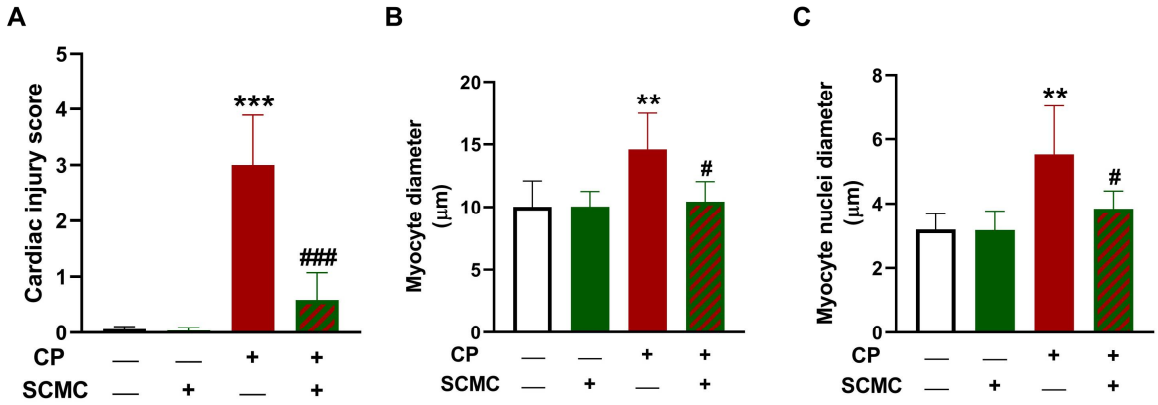


Fig. 3. SCMC mitigated CP-induced tissue injury (A) and alleviated the diameter of cardiomyocytes (B) and their nuclei (C). Data are mean ± SD, (n=6). **P<0.01 and ***P<0.001 versus Control. $^{\#}$P<0.05 and $^{\#\#\#}$P<0.001 versus CP.

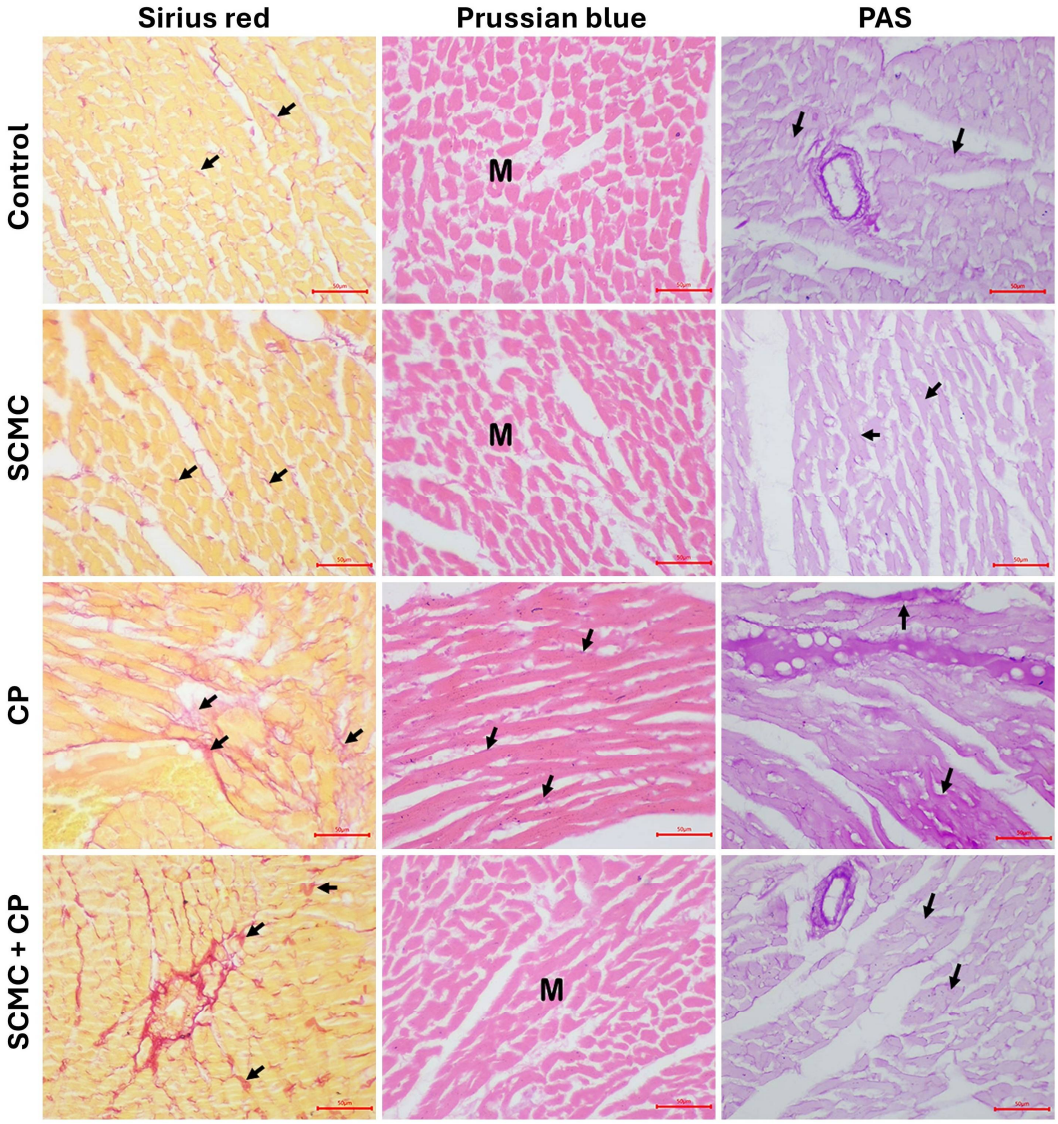


Fig. 4. Photomicrographs of heart sections stained with Sirius red, Prussian blue, and PAS. Sirius red staining shows a little amount of collagen fibers (arrows) in control and SCMC-treated rats, increased collagen fibers (arrows) in CP-administered rats and normal collagen

fiber (arrows) content in CP-administered rats treated with SCMC. Control and SCMC-administered rats show negative Prussian blue staining affinity, CP-administered rats show hemosiderin deposits (arrows), and CP-administered rats treated with SCMC show no deposits. Control and SCMC-treated rats show normal PAS stain intensity and distribution (arrows), whereas CP-administered rats show increased and uneven distribution of PAS with some muscle fibers displaying a highly intense reaction to the stain (arrows). SCMC alleviated PAS staining in CP-administered rats and the sections appear normal.

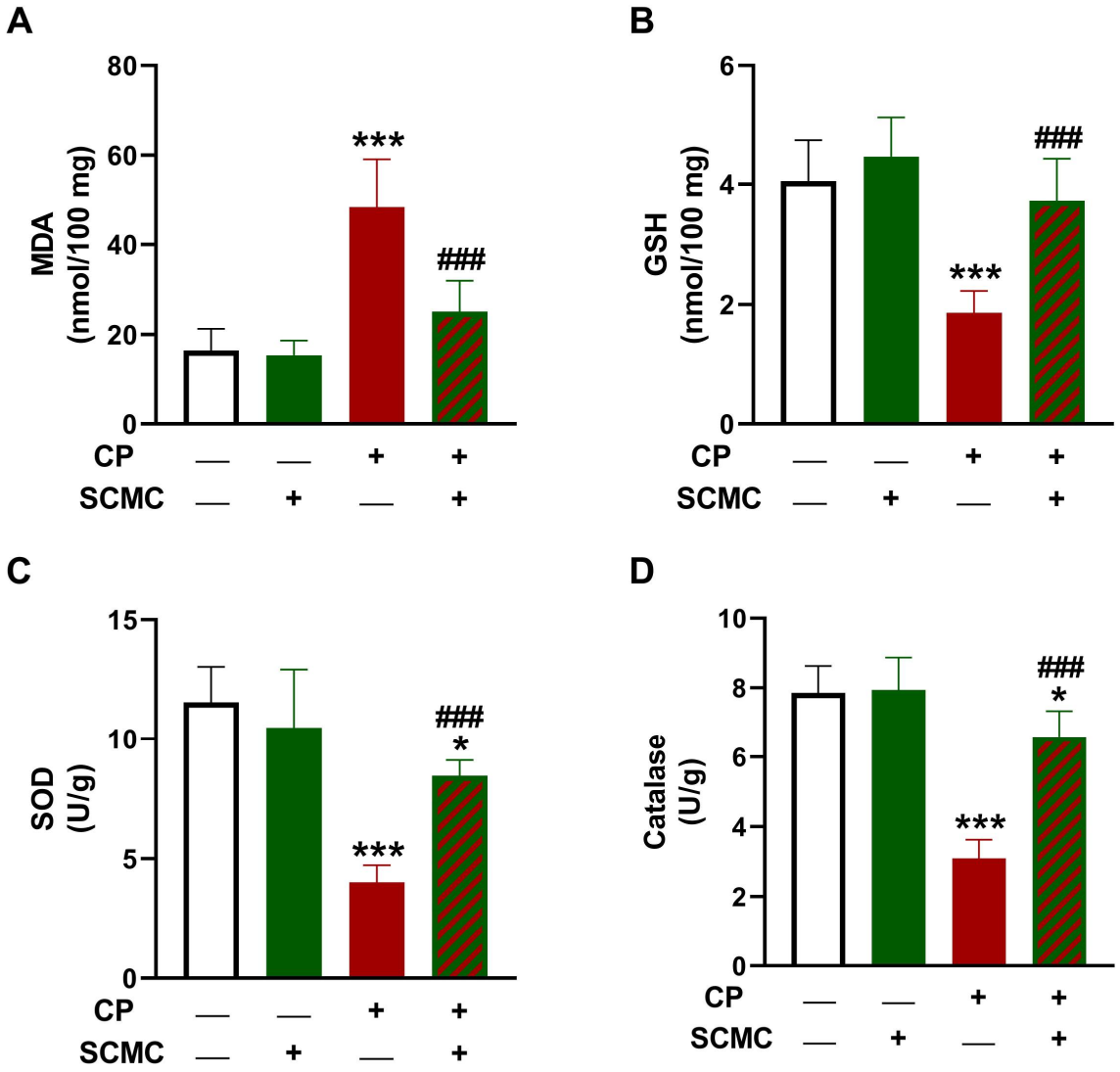


Fig. 5. SCMC attenuated CP-induced oxidative stress. SCMC decreased MDA (A), and increased GSH (B), SOD (C), and catalase (D) in the heart of CP-administered rats. Data are mean ± SD, (n=6). *P<0.05 and ***P<0.001 versus Control. $^{###}$P<0.001 versus CP.

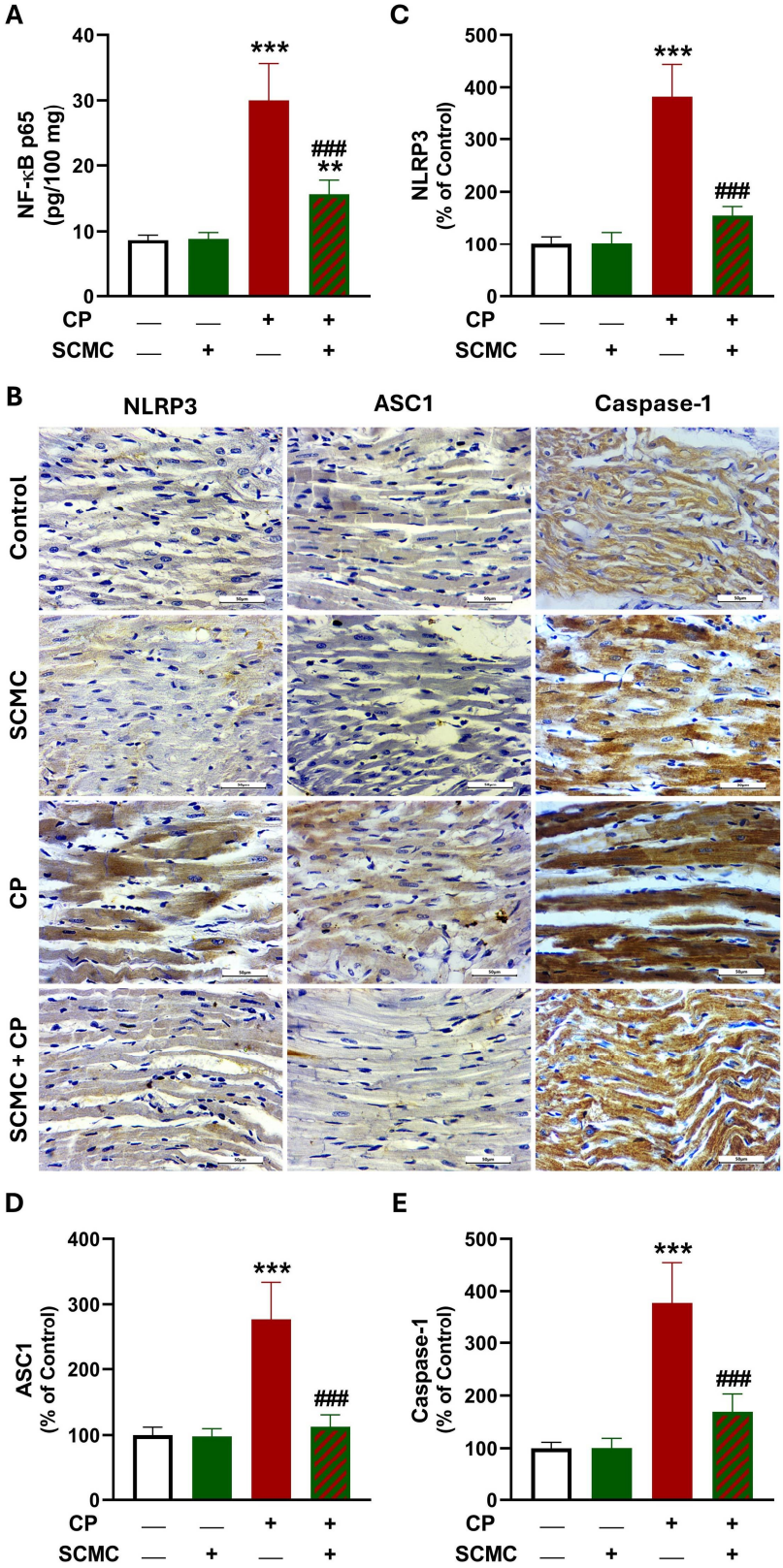


Fig. 6. SCMC suppressed NF-κB/NLRP3 inflammasome axis in CP-treated rats. SCMC decreased NF-κB p65 (A), NLRP3 (B-C), ASC1 (B,D), and caspase-1 (B,E) in CP-administered rats. Data are mean ± SD, (n=6). **P<0.01 and ***P<0.001 versus Control. $^{###}$P<0.001 versus CP.

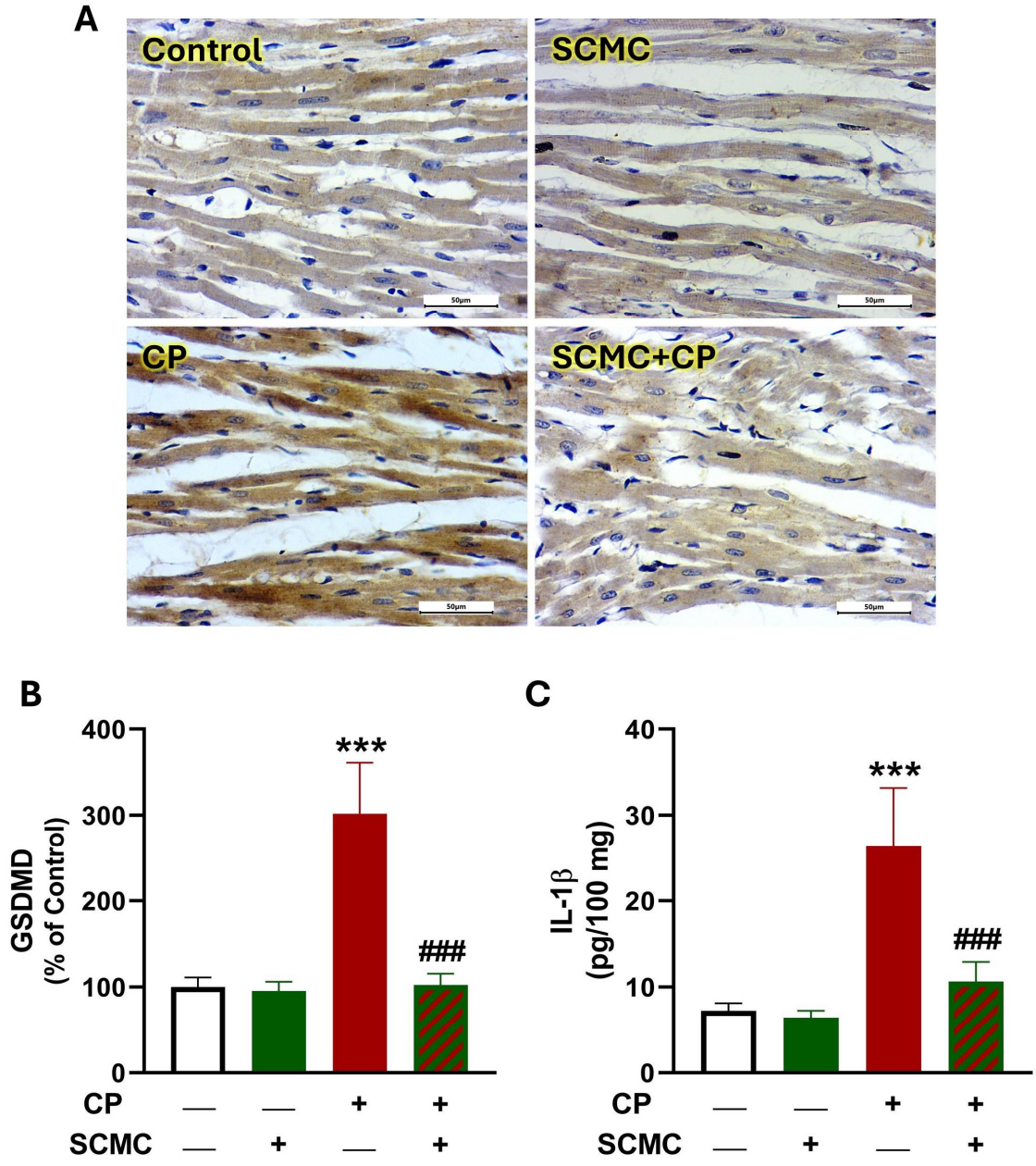


Fig. 7. SCMC decreased GSDMD (A,B), and IL-1β (C) in CP-administered rats. Data are mean ± SD, (*n*=6). ***P<0.001 versus Control and $^{###}$P<0.001 versus CP.

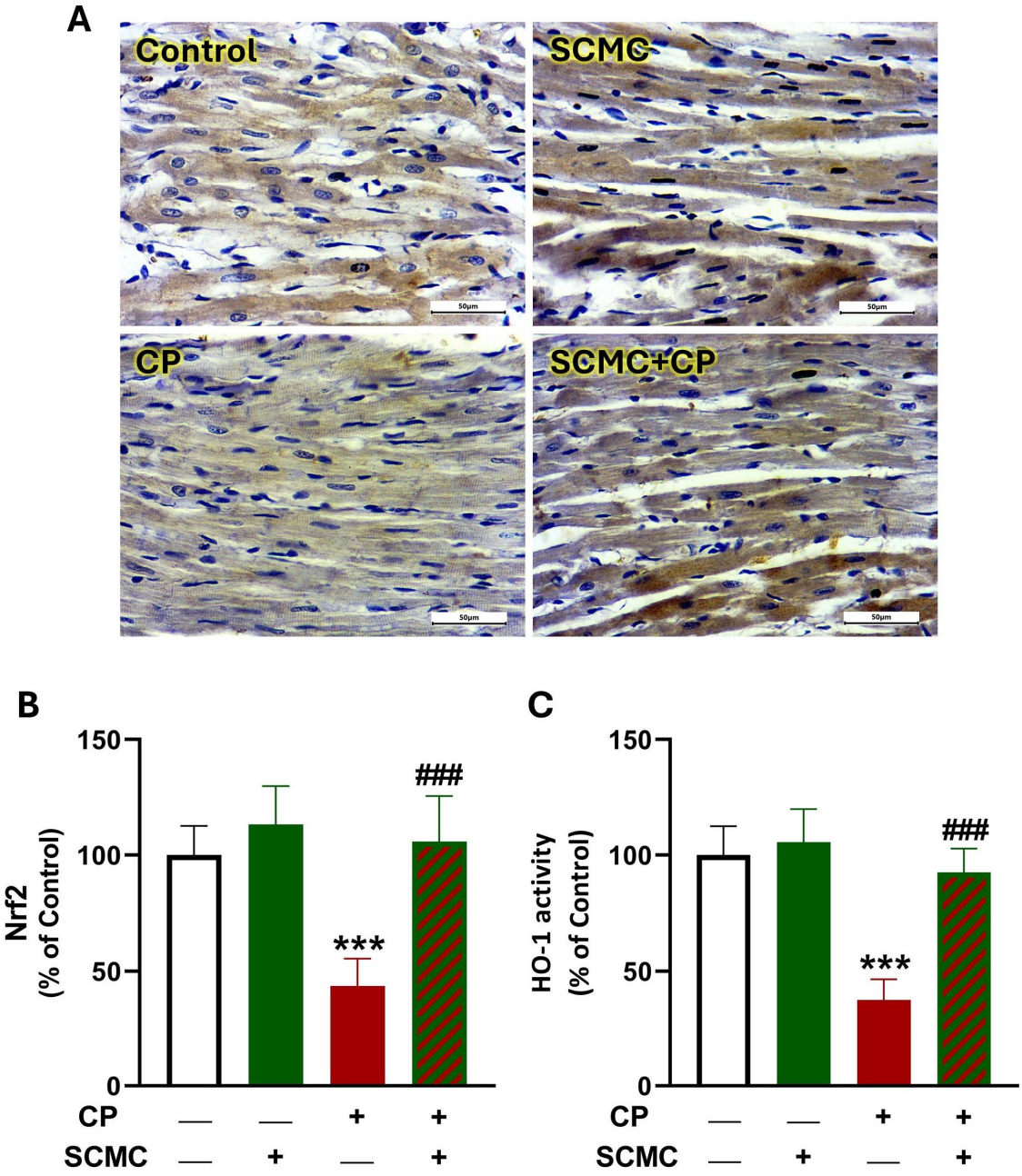


Fig. 8. SCMC increased Nrf2 expression (A-B) and HO-1 activity (C) in CP-administered rats. Data are mean ± SD, (*n*=6). ***P<0.001 versus Control and $^{###}$P<0.001 versus CP.