Title:

Selenium nanoparticles mitigate chlorpyrifos-induced nephrotoxicity by modulating oxidative stress, inflammation, and the SIRT1/Nrf2/HO-1 signaling pathway

Authors and affiliations:

Alaa A. A. Mahmoud[1], Ayman M. Mahmoud[1,2]*, Adel Abdel-Moneim[1], Sulaiman M. Alnasser[3], Reem S. Alruhaimi[4], Emad H.M. Hassanein[5], Sanaa M. Abd El-Twab[1]*

[1]Molecular Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef 62514, Egypt.

[2]Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK.

[3]Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah, 52571, Saudi Arabia.

[4]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia.

[5]Department of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University-Assiut Branch, Egypt.

*Corresponding authors:

Sanaa M. Abd El-Twab

Molecular Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef 62514, Egypt. E-mail: thanaa.mahmoud@science.bsu.edu.eg

Ayman M. Mahmoud

Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK. E-mail: a.mahmoud@mmu.ac.uk

Abstract

Chlorpyrifos (CPF), a widely used pesticide, is associated with significant renal toxicity, raising concerns about its impact on kidney health. Selenium nanoparticles (Se NPs) have emerged as a potential therapeutic agent due to their beneficial properties. This study evaluated the effects of Se NPs against CPF-induced nephrotoxicity, focusing on oxidative stress, inflammation, and the SIRT1/Nrf2/HO-1 signaling pathway. Rats were administered CPF, with or without Se NPs, for 28 days. Renal function was assessed through biochemical markers, histopathological examination, and molecular analyses. CPF exposure significantly elevated serum creatinine, urea, and Kim-1 levels, accompanied by histopathological damage in renal tissues. Se NPs treatment effectively restored renal function and attenuated structural abnormalities. CPF-induced oxidative stress was evident through increased lipid peroxidation and suppressed antioxidant enzymes and reduced glutathione (GSH), which were counteracted by Se NPs. Furthermore, CPF upregulated pro-inflammatory and apoptosis mediators (NF-κB, TNF-α, IL-6, iNOS, Bax, and caspase-3), while downregulating anti-apoptotic Bcl-2. Se NPs mitigated these effects by suppressing inflammatory and apoptotic pathways, effects associated with decreased Keap1 and enhanced SIRT1, Nrf2, and HO-1. In conclusion, Se NPs confer protection against CPF-induced kidney injury by alleviating oxidative stress, inflammation, and apoptosis, and by modulating the SIRT1/Nrf2/HO-1 signaling. These findings underscore the potential of Se NPs as a therapeutic intervention for CPF-associated nephrotoxicity.

Keywords: Chlorpyrifos; Selenium; Nephrotoxicity; SITR1/Nrf2 signaling; Inflammation; Oxidative stress.

Introduction:

The extensive use of pesticides in agriculture has been instrumental in enhancing crop yields and controlling pests, but it has also raised significant concerns regarding their impact on human health and the environment [1]. Among the various classes of pesticides,

organophosphorus (OP) compounds, particularly chlorpyrifos (CPF), have gained considerable attention due to their widespread applications and potential toxicity to non-target organisms [2, 3]. CPF is a broad-spectrum insecticide that effectively targets a range of pests, making it widely applicable in both agricultural and domestic settings [4]. However, its persistence in ecosystems and non-selective toxicity pose substantial risks to non-target organisms, including humans [1, 3]. Accordingly, detectable residues of CPF above acute reference thresholds have been found on grains, vegetables, and fruits, and therefore human exposure may occur through several routes [5, 6]. Chronic exposure to CPF has been linked to a spectrum of adverse health effects, including neurotoxicity, hepatotoxicity, and nephrotoxicity [4, 7-9], underscoring the need for a deeper understanding of its toxicological mechanisms.

The insecticidal mechanism of CPF involves the inhibition of acetylcholinesterase (AChE), resulting in the disruption of neurotransmission in target pests [3]. However, its detrimental effects extend beyond AChE inhibition, as CPF and its metabolites are known to induce oxidative stress (OS) and inflammation, which are central to its toxicity in non-target organisms [10]. OS arises from an imbalance between the release of reactive oxygen species (ROS) and the cellular antioxidant defenses, resulting in oxidative damage to lipids, proteins, and DNA [11, 12]. This oxidative damage disrupts cellular homeostasis and contributes to the dysfunction of vital organs, such as the liver and kidneys, which are particularly susceptible due to their roles in detoxification and metabolic regulation [11, 12]. Concurrently, CPF exposure activates inflammatory pathways, including the upregulation of pro-inflammatory cytokines and transcription factors such as nuclear factor-kappaB (NF-κB), further exacerbating tissue injury [11, 12]. The interplay between OS and inflammation provokes cellular damage and death via apoptosis [13], highlighting the need for therapeutic strategies that target these interconnected pathways.

Recent research has identified the Sirtuin 1 (SIRT1)/nuclear factor erythroid 2-related factor 2 (Nrf2) signaling axis as a critical regulator of cellular defenses against OS and inflammation [14-16]. SIRT1, a NAD+-dependent deacetylase, plays a pivotal role in modulating cellular metabolism, stress responses, and longevity [16]. It exerts its protective effects by deacetylating and activating transcription factors such as Nrf2 and suppressing pro-inflammatory signaling pathways, including NF-κB [17]. Nrf2, in turn, serves as a master regulator of the antioxidant response, promoting the transcription of genes encoding cytoprotective enzymes such as heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and catalase [15]. Activation of the SIRT1/Nrf2 pathway has been shown to mitigate oxidative injury and inflammation in various experimental models of organ damage, making it a promising target for therapeutic interventions [18-20]. Given the critical role of the SIRT1/Nrf2 axis in cellular defense [18-20], targeting this pathway may offer a novel approach to mitigating CPF-induced toxicity. By enhancing antioxidant capacity and suppressing inflammatory responses, activation of SIRT1 and Nrf2 could potentially counteract the detrimental effects of CPF exposure.

Recent advancements in nanotechnology have opened new avenues for addressing OS and inflammation, with selenium nanoparticles (Se NPs) emerging as promising therapeutic agent due to their unique properties and biological efficacy [21]. Se, an essential trace element, is a vital component of selenoproteins, which play a crucial role in maintaining cellular redox homeostasis and defense mechanisms [22]. This essential element is important for cellular functions in all organisms and occurs naturally in dietary sources such as seafood, grains, and dairy products [23]. However, the therapeutic use of conventional Se compounds is constrained by their narrow safety margin and potential toxicity at elevated doses [21]. In contrast, Se NPs exhibit enhanced biocompatibility, reduced toxicity, and improved bioavailability, making them a safer and more effective alternative for biomedical applications [24]. Studies have revealed the anti-inflammatory and antioxidant potential of Se NPs, including their ability to

modulate inflammatory responses in models of lipopolysaccharide (LPS)-induced inflammation and regulate selenoprotein expression in both porcine and murine systems [25, 26]. This study explored the efficacy of Se NPs against CPF nephrotoxicity, with a focus on OS and inflammation, and evaluates their therapeutic potential of modulating the SIRT1/Nrf2 signaling.

Materials and methods:

Se NPs synthesis and characterization:

Se NPs were synthesized using a high-energy ball-milling technique, following previously established protocols [27]. Briefly, elemental Se powder was mechanically milled in a vertical planetary ball mill with a ball-to-powder mass ratio of 10:1. The milling process was performed at a rotational speed of 200 rpm for 20 h. After milling, the resulting powder was dried at 80°C for 24 h to remove residual moisture. The structural and morphological characteristics of the synthesized nanoparticles were evaluated using scanning electron microscopy (SEM) (Fig. 1), X-ray diffraction (XRD), and dynamic light scattering (DLS) (Suppl. Fig. I).

Animals and treatments:

Male Wistar rats weighing 180 ± 10 g were acclimatized under controlled environmental conditions, including a temperature of 23 ± 2°C, relative humidity of 50–60%, and a 12-h light/dark cycle. The animals were given free access to food and water. A total of 24 rats were randomly divided into four experimental groups (n = 6 per group) to investigate the nephroprotective effects of Se NPs against CPF-induced toxicity.

Se NPs and CPF (Agro Chem, Egypt) were suspended in 0.5% carboxymethyl cellulose (CMC) and corn oil, respectively. Both compounds were administered orally once daily for 28 consecutive days. The experimental groups were designed as follows:

Group I (Control): Received 0.5% CMC and corn oil.

Group II (Se NPs): Administered 0.5 mg/kg Se NPs [28].

Group III (CPF): Treated with 5.4 mg/kg CPF (1/25 of the LD_{50}) [29].

Group IV (CPF + Se NPs): Co-administered 5.4 mg/kg CPF and 0.5 mg/kg Se NPs.

Twenty-four h after the final treatment, blood samples were collected under ketamine/xylazine-induced anesthesia. Serum was separated for subsequent biochemical analyses. The kidneys were rapidly excised following euthanasia. Portions of the tissue were fixed in 10% neutral-buffered formalin (NBF), while others were preserved in RNALater at -80°C for molecular studies. Additional tissue samples were homogenized in Tris-HCl buffer (pH 7.4), and the resulting homogenates were centrifuged to obtain supernatants for biochemical assays.

Biochemical assays:

Serum levels of creatinine and blood urea nitrogen (BUN) were quantified using commercially available kits (Spinreact, Spain). ELISA was used to measure the levels of kidney injury molecule-1 (Kim-1), tumor necrosis factor (TNF)-α, interleukin (IL)-6, and caspase-3 (Elabscience, China). In kidney homogenates, malondialdehyde (MDA), nitric oxide (NO), SOD, GSH, and catalase were assessed using Biodiagnostic (Egypt) kits. HO-1 activity was determined according to the method described by Abraham et al. [30].

Histopathological and immunohistochemical (IHC) evaluations:

Kidney tissues fixed in 10% NBF were processed for paraffin embedding. Tissue sections (5 µm thick) were stained with hematoxylin and eosin (H&E) for histopathological examination. IHC analysis was performed to evaluate the expression of Nrf2, NF-κB p65, and SIRT1, [31]. Briefly, paraffin-embedded sections were processed through deparaffinization and rehydration followed by antigen retrieval using citrate buffer (50 mM, pH 6.8). Endogenous peroxidase activity was inhibited with 0.3% hydrogen peroxide (H_2O_2), followed by blocking with a protein-blocking solution. Sections were incubated overnight at 4°C with primary antibodies (Biospes, China), washed, and then treated with a secondary antibody (Biospes, China). Color development was achieved using 3,3'-diaminobenzidine, and sections were counterstained with

Mayer's hematoxylin. Quantitative analysis of staining intensity was performed using ImageJ software (NIH, USA) by evaluating six randomly selected fields per sample.

qRT-PCR:

Total RNA was extracted from kidney tissues using an RNA purification kit (Thermo Scientific, USA). RNA purity was confirmed by assessing the A260/A280 ratio (≥ 1.8). cDNA was synthesized using a reverse transcription kit. Quantitative PCR amplification was performed using SYBR Green master mix and gene-specific primers (Table 1). The mRNA expression levels of Kelch-like ECH-associated protein 1 (*KEAP1*), *HO1*, *SIRT1*, inducible NO synthase (iNOS/*NOS2*), B-cell lymphoma 2 (*BCL2*), BCL2 associated X (*BAX*), and *CASP3* were calculated using the $2^{-\Delta\Delta Ct}$ method [32] and β-actin as a housekeeping gene.

Statistical analysis:

All data are expressed as mean ± standard error of the mean (SEM). Intergroup comparisons were conducted using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism (version 8). A p-value of < 0.05 was considered statistically significant.

Results:

Se NPs attenuate CPF-induced kidney injury:

The nephroprotective effects of Se NPs against CPF-induced toxicity were assessed using biochemical markers (Fig. 2) and microscopic analysis (Fig. 3). To evaluate renal function, serum levels of creatinine, BUN, and Kim-1 were measured. CPF exposure significantly elevated serum creatinine (+167.2%), BUN (+163.3%), and Kim-1 levels (+311.2%) compared to controls ($P < 0.001$), indicating pronounced renal dysfunction (Fig. 2A-C). Co-treatment with Se NPs significantly decreased creatinine (-52.1%), BUN (-54.4%), and Kim-1 levels (-60.2%) in serum of CPF-administered rats ($P < 0.001$). The decrease in serum creatinine and Kim-1 in CPF-administered rats was significant compared to the controls ($P < 0.05$ and $P <$

0.01, respectively). Supplementation of Se NPs has no effect on these renal biomarkers in normal rats ($P > 0.05$).

Histopathological examination of kidney tissue revealed normal renal architecture, including intact glomeruli, tubules, and interstitial spaces, in both the control (Fig. 3A) and Se NPs-treated (Fig. 3B) groups. In contrast, CPF administration induced severe renal damage, characterized by hypercellular glomeruli with very narrow Bowman's spaces, proximal tubules with scattered apoptotic lining, and mildly dilated congested blood vessels (Fig. 3C). These lesions were markedly reduced in rats co-treated with Se NPs, which showed improved tissue architecture and reduced pathological alterations (Fig. 3D).

Se NPs suppress kidney oxidative stress in CPF-exposed rats:

To investigate oxidative stress, levels of MDA and NO were measured as indicators of LPO and nitrosative stress, respectively. CPF exposure significantly increased kidney MDA (+253.9%) and NO (+323.2%) compared to the control group ($P < 0.001$; Fig. 3A-B). Additionally, CPF significantly ($P < 0.001$) decreased antioxidant defenses, as shown by reduced levels of GSH (+59.8%), SOD (-68.2%), and catalase (-59.4%), as depicted in Figures 3C-E. Treatment with Se NPs not only reversed the increase in MDA (-59.5%) and NO (-69.8%) but also significantly restored GSH (+155.1%), SOD (+174.6%), and catalase (+132.8%) in CPF-administered rats ($P < 0.001$). The decrease in MDA and NO along with the increase in SOD activity in the kidney of CPF-administered rats were significant compared to the control group. Normal rats that received Se NPs showed non-significant ($P > 0.05$) changes in MDA, NO and antioxidants.

Anti-inflammatory and anti-apoptotic effects of Se NPs:

CPF significantly upregulated NF-κB p65 (+134.2%) in the kidney of rats (Fig. 4A-B) ($P < 0.001$). Similarly, CPF exposure increased renal levels of TNF-α (+231.9%), IL-6 (+187.1%), and iNOS mRNA (+130.2%) significantly ($P < 0.001$; Fig. 4C-E). In addition, CPF exposure

triggered apoptotic pathways, as evidenced by significant downregulation of Bcl-2 (-59.7%) and upregulation of Bax (+111.4%) and caspase-3 mRNA (+203.6%) and protein (+124.9%) in the kidney ($P < 0.001$; Fig. 5A-D). Se NPs effectively suppressed renal NF-κB p65 (-38.8%), TNF-α (-49.3%), IL-6 (-47.7%), iNOS (-35.9%), Bax (-42.2%), and caspase-3 mRNA (-61.4%) and protein (-40.3%), and increased Bcl-2 (+93.8%) in CPF-administered rats ($P < 0.001$). The changes in NF-κB p65, TNF-α, and iNOS, Bax, Bcl-2, and caspase-3 mRNA in the kidney of CPF-exposed rats treated with Se NPs were significant compared to the control rats. Of not, Se NPs exerted non-significant effects on both inflammation and apoptosis markers in normal rats.

Modulation of SIRT1/Nrf2/HO-1 signaling by Se NPs:

CPF significantly downregulated the levels of SIRT1 mRNA (-53.2%; Fig. 6A) and protein (-58.5%; Fig. 6B–C), upregulated Keap1 mRNA (+139.7%; Fig. 7A), and downregulated Nrf2 mRNA (-50.6%; Fig. 7B) and protein (-66.3%; Fig. 7C–D), as well as HO-1 expression (-59.2%; Fig. 7C, E) and activity (-51.8%; Fig. 7F) in the kidney as compared to the control group ($P < 0.001$). Se NPs upregulated SIRT1 mRNA (+106.1%) and protein (+95.9%), Nrf2 mRNA (+92.0%) and protein (+146.1%), and HO-1 (+103.4%) while downregulating Keap1 (-45.3%) in the kidney of CPF-exposed rats ($P < 0.001$). Additionally, HO-1 enzymatic activity, which was reduced by CPF, was significantly elevated upon Se NPs treatment (+98.6%; $P < 0.001$). The effect of Se NPs on SIRT1, Nrf2 and HO-1 protein levels in CPF-administered rats was significant when compared to the control rats. No significant changes in SIRT1, Keap1, Nrf2, and HO-1 were observed in the kidney of normal rats that received Se NPs.

Discussion:

Organophosphorus pesticides, such as CPF, are extensively used in agriculture to control pests and enhance crop yields. However, their widespread application has raised significant concerns due to their detrimental effects on human health and the environment [1, 3]. Chronic exposure to CPF has been linked to nephrotoxicity, primarily mediated by OS and inflammation [8-10].

These mechanisms highlight the importance of developing therapeutic strategies that target these pathways to mitigate CPF-induced kidney injury. Se NPs have emerged as a promising candidate due to their potent antioxidant and anti-inflammatory properties reported in different experimental models [33]. This study explored the protective effects of Se NPs against CPF-induced kidney injury, emphasizing the role of the SIRT1/Nrf2/HO-1 signaling pathway in mediating these effects.

The kidney is a vital organ responsible for maintaining homeostasis, regulating electrolyte balance, and excreting metabolic waste products. Its high metabolic activity and role in detoxification make it particularly susceptible to xenobiotic-induced damage [34]. CPF-induced nephrotoxicity is characterized by impaired renal function, structural damage, and disruption of cellular integrity, as evidenced by elevated serum markers of kidney dysfunction, including creatinine, BUN, and Kim-1, alongside histopathological alterations. These findings align with previous reports on the nephrotoxic effects of CPF, including tubular epithelial damage, glomerular atrophy, and inflammatory cell infiltration [8-10, 35]. The lipophilic nature of CPF facilitates its accumulation in body organs, where it disrupts cellular membranes and induces oxidative and inflammatory damage [36]. In the present study, Se NPs effectively attenuated CPF-induced nephrotoxicity, restoring biochemical markers and preserving renal tissue architecture. The ability of Se NPs to maintain renal tubular integrity suggests their potential as a therapeutic agent against CPF-induced kidney injury. These data supported the nephroprotective efficacy of Se NPs reported in previous studies. For instance, Se NPs protected HK-2 cells against acute injury induced by hypoxia/reoxygenation and the kidney of mice against acute injury provoked by ischemia/reperfusion (I/R) [37, 38]. The protective effect of Se NPs was linked to downregulation of Kim-1 in the kidney of mice challenged with I/R [37]. In canines, Se NPs showed protective effect against acute renal failure induced by adenine evidenced by alleviated BUN, creatinine, and histopathological alterations [39].

The protective effects of Se NPs against CPF-induced nephrotoxicity are likely mediated through their antioxidant and anti-inflammatory properties. CPF exposure significantly increased renal OS markers, while depleting the key antioxidants GSH, SOD, and catalase. In accordance, the role of CPF in generating ROS and disrupting antioxidant defense systems in different body organs is well-documented [9, 35, 40, 41]. Exposure to CPF provoked the generation of excessive ROS across various experimental models, including Neuro-2a cells [42], microglial cells [43], and renal tissues in rats [9], highlighting the role of CPF in inducing OS, which disrupts redox homeostasis and promotes tissue damage. Recently, rats administered different concentrations of CPF exhibited a positive correlation was observed between CPF concentrations and OS parameters [36]. Excessive ROS production induces oxidative damage to cellular lipids, proteins, and DNA, leading to LPO, protein oxidation, and DNA strand breaks. This oxidative damage overwhelms cellular repair mechanisms, resulting in irreversible cell injury and death [11, 12]. CPF-induced ROS also activate NF-κB, a central regulator of inflammation. In this study, CPF administration upregulated NF-κB p65, TNF-α, IL-6, and iNOS in the kidney, indicating a pro-inflammatory response. The activation of NF-κB promotes pro-inflammatory mediators, which exacerbate tissue injury and contribute to renal dysfunction [44]. The association between OS and inflammation provoked by CPF has been recently demonstrated in experimental animals. An inflammatory response characterized by activation of NF-κB and upregulated iNOS, IL-6, TNF-α, and other proteins involved in inflammation was reported in liver of rats that received CPF [36]. Upregulated NF-κB and its subsequent inflammatory response mediated via pro-inflammatory cytokines was also reported in the kidney of rats exposed to CPF [9, 10]. ROS work alongside pro-inflammatory mediators to elicit cellular damage and dysfunction by impairing mitochondrial activity and activating apoptotic mechanisms [13]. This cascade includes the alteration of the mitochondrial membrane potential, which results in cytochrome c being released into the cytoplasm. This, in

turn, activates caspase-3, a key enzyme that drives the final stages of apoptosis, leading to programmed cell death [13]. The current findings show that CPF exposure triggers mitochondrial dysfunction and caspase-3 activation, as indicated by elevated levels of pro-apoptotic markers such as Bax and caspase-3, alongside a reduction in Bcl-2.

Se NPs effectively mitigated these alterations by suppressing MDA, NO, NF-κB, iNOS, IL-6, and TNF-α, while enhancing GSH and antioxidant enzyme activities. These data highlight the dual antioxidant and anti-inflammatory role of Se NPs in protecting the kidney against CPF toxicity. The ability of Se NPs to scavenge ROS and stabilize cellular membranes is critical for restoring redox homeostasis and preventing oxidative damage. The restoration of GSH levels, in particular, is noteworthy, as GSH serves as a primary intracellular antioxidant and detoxifying agent [45]. Se NPs revealed effective antioxidant efficacy manifested by reduced MDA and boosted antioxidant enzymes in the kidney of canines that received adenine [39]. Se NPs have also shown potent anti-inflammatory efficacy mediated via inhibition of NLRP3 inflammasome in a murine model of kidney I/R injury [37]. Additionally, Se NPs prevented CPF-induced apoptosis, as evidenced by increased Bcl-2 and suppressed Bax and caspase-3. These findings suggest that Se NPs protect against CPF-induced renal injury by modulating OS and inflammation. In accordance, Se NPs suppressed OS, inflammation and cell injury in the liver of rats challenged with high APAP doses [46] and patulin-treated hepatocytes [47]. In these studies, Se NPs decreased ROS and MDA and enhanced antioxidant enzymes [46, 47]. Furthermore, Se NPs-enriched probiotics demonstrated significant anti-inflammatory, anti-apoptotic, and antioxidant effects, which were mechanistically mediated through the suppression of NF-κB and Bax pathways, alongside the upregulation of Bcl-2 and catalase activity in a rat model of cadmium hepatotoxicity [48].

To further elucidate the protective mechanism(s) of Se NPs, we investigated the SIRT1/Nrf2/HO-1 signaling pathway. CPF exposure significantly downregulated renal SIRT1,

Nrf2, and HO-1, while upregulating Keap1. SIRT1 is critical in regulating cellular stress responses by deacetylating NF-κB and Nrf2, thereby exerting anti-inflammatory and antioxidant effects [16]. Nrf2, a master regulator of the antioxidant response, promotes the transcription of genes encoding cytoprotective enzymes, including HO-1, SOD, and catalase [15]. Under normal conditions, Nrf2 is sequestered in the cytosol by Keap1, but ROS exposure disrupts this interaction, allowing Nrf2 to translocate to the nucleus and activate antioxidant gene expression [15]. The suppression of Nrf2/HO-1 signaling by CPF exacerbates cellular vulnerability to oxidative and inflammatory damage, as demonstrated in previous studies [49-52], and the negative impact of CPF on Nrf2 has been reported across diverse experimental models, including human neuroblastoma cells, fruit flies, and rodent liver [41, 53, 54]. Se NPs significantly upregulated SIRT1, Nrf2, and HO-1, while downregulating Keap1 in CPF-exposed rats, suggesting that their protective effects are mediated, at least in part, through the activation of this pathway. The upregulation of HO-1 is particularly noteworthy, as it catalyzes the degradation of heme into biliverdin and carbon monoxide, both of which possess antioxidant and anti-inflammatory properties [55]. These findings align with studies revealed that Se NPs enhance Nrf2 expression and HO-1 activity in experimental models of oxidative damage induced by heat stress in in broilers [56], and adenine-induced renal failure in canines [39]. The activation of SIRT1/Nrf2/HO-1 signaling by Se NPs likely contributes to their ability to restore redox balance, suppress inflammation, and prevent apoptosis in CPF-induced kidney injury. Therefore, this study demonstrates the nephroprotective effects of Se NPs against CPF-induced kidney injury, mediated through the modulation of OS, inflammation, and apoptosis. The activation of the SIRT1/Nrf2/HO-1 signaling contributes to these protective effects, highlighting the therapeutic potential of Se NPs in mitigating CPF-induced nephrotoxicity. These findings provide new insights into the mechanisms underlying CPF toxicity and the

protective efficacy of Se NPs, offering a promising avenue for further research and therapeutic development.

Conclusion:

This study demonstrates that Se NPs effectively mitigate CPF nephrotoxicity by attenuating OS, inflammation, and apoptosis. Se NPs suppressed LPO, boosted GSH, SOD. and catalase, and downregulated NF-κB, pro-inflammatory mediators, and pro-apoptotic markers, alongside the upregulation of Bcl-2. A key finding of this study is the ability of Se NPs to enhance the SIRT1/Nrf2/HO-1 signaling, which plays a central role in cellular defense mechanisms against oxidative damage and inflammation. By upregulating SIRT1, Nrf2, and HO-1, Se NPs enhanced the endogenous antioxidant response, suppressed inflammatory signaling, and mitigated CPF-induced renal tissue injury. These results highlight the multifaceted therapeutic potential of Se NPs, emphasizing their ability to modulate key cellular signaling pathways involved in OS and inflammation. The findings of this study provide valuable insights into the mechanisms underlying CPF-induced nephrotoxicity and the protective efficacy of Se NPs. However, further research is needed to optimize dosing regimens, evaluate long-term safety, and explore the translational potential of Se NPs in clinical applications. Overall, this study underscores the promise of Se NPs as a therapeutic agent for mitigating CPF-induced kidney damage and opens new avenues for future investigations.

Acknowledgment:

Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2025R381), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Authors' contributions:

Conceptualization: A.M.M., and S.M.A-E.; Methodology: A.M.M., S.M.A-E., A.A., A.A.A.M., E.H.M.H., R.S.A., and S.M.A.; Investigation: A.M.M., S.M.A-E., E.H.M.H., and A.A.A.M.; Data curation: A.M.M., S.M.A-E., A.A., and A.A.A.M.; Formal analysis: A.M.M.;

Resources: S.M.A-E., A.A., R.S.A., and S.M.A.; Supervision: A.M.M., S.M.A-E., and A.A.; Writing-Original draft: A.M.M.; Writing-review and editing: A.M.M. All authors have read and approved the manuscript for publication.

Declarations

Ethical Approval

The experimental protocol was approved by the Institutional Animal Ethics Committee of Beni-Suef University (Egypt) (Approval no. 021-130).

Funding:

Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2025R381), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Declaration of competing interests:

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

Availability of data and materials:

The manuscript and supplementary material contain all data supporting the reported results.

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Tables:

Table 1. Primers used for qRT-PCR.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
BCL-2	ACTCTTCAGGGATGGGGTGA	TGACATCTCCCTGTTGACGC
BAX	AGGACGCATCCACCAAGAAG	CAGTTGAAGTTGCCGTCTGC
CASP3	GGAGCTTGGAACGCGAAGAA	ACACAAGCCCATTTCAGGGT
KEAP1	TCAGCTAGAGGCGTACTGGA	TTCGGTTACCATCCTGCGAG
NRF2	TTGTAGATGACCATGAGTCGC	TGTCCTGCTGTATGCTGCTT
NOS2	ATTCCCAGCCCAACAACACA	GCAGCTTGTCCAGGGATTCT
SIRT1	TCTCCCAGATCCTCAAGCCAT	TTCCACTGCACAGGCACATA
ACTB	AGGAGTACGATGAGTCCGGC	CGCAGCTCAGTAACAGTCCG

Figures:

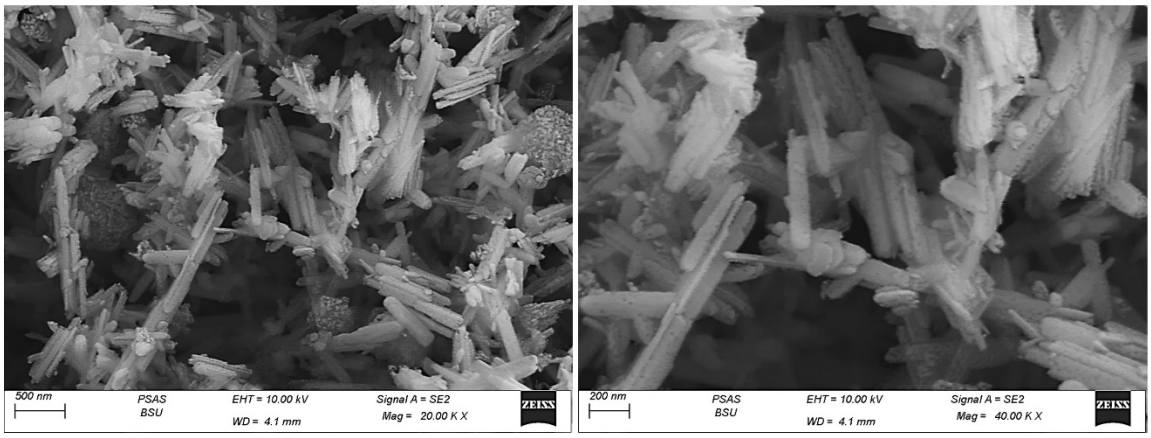


Figure 1. Photomicrographs of SEM of Se NPs.

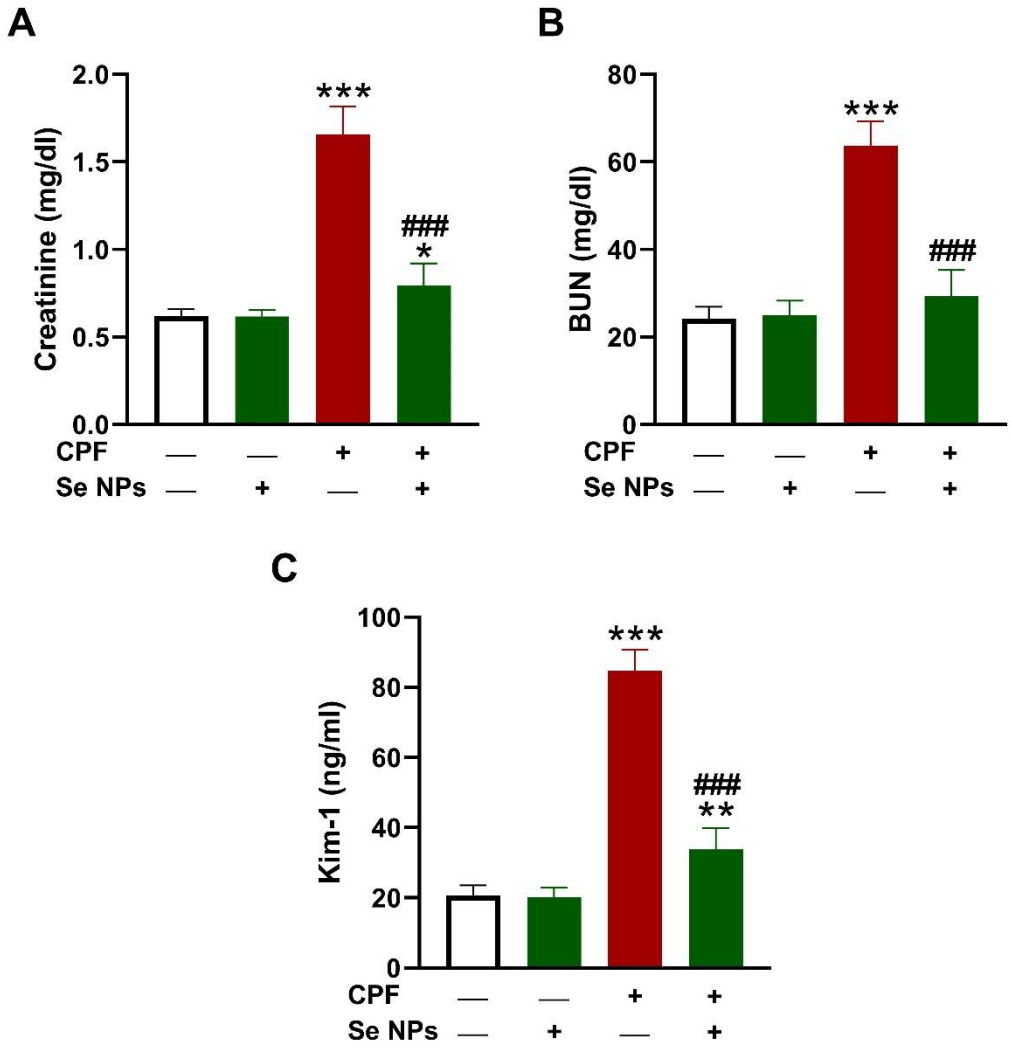


Figure 2. Se NPs mitigated CPF-induced kidney injury. Se NPs ameliorated serum creatinine (**A**), BUN (**B**), and Kim-1 (**C**) in CPF-administered rats. Data are mean ± SEM, (n = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

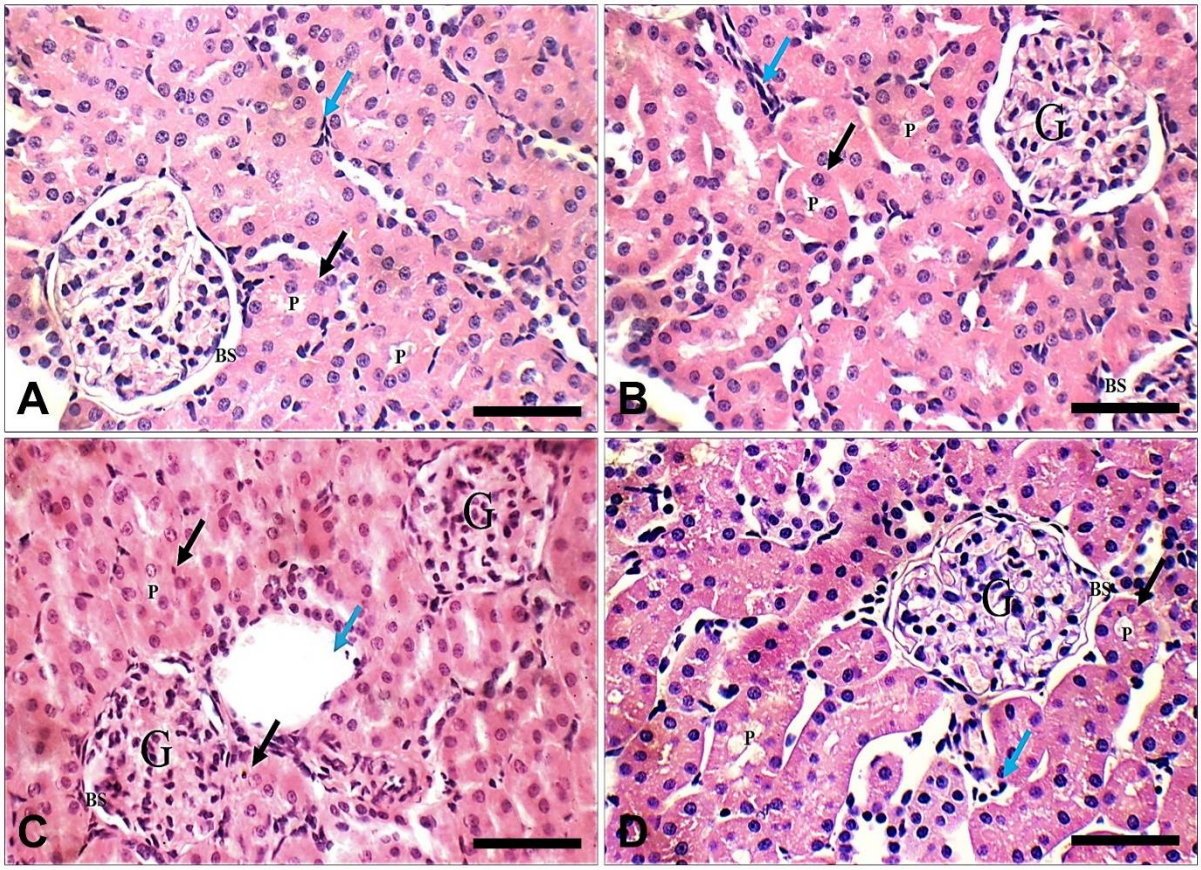


Figure 3. Se NPs prevented CPF-induced kidney tissue injury. Photomicrographs of sections in kidney of control (**A**) and Se NPs-supplemented rats (**B**) showing average glomeruli (G) with average Bowman's spaces (BS), proximal tubules (P) with average lining (black arrow), and average interstitium (blue arrow), CPF-administered rats (**C**) showing hypercellular glomeruli (G) with very narrow Bowman's spaces (BS), proximal tubules (P) with scattered apoptotic lining (black arrow), and mildly dilated congested blood vessels (blue arrow), and CPF-induced rats treated with Se NPs (**D**) showing glomeruli (G) with average Bowman's spaces (BS), proximal tubules (P) with average lining (black arrow), and average interstitium (blue arrow). (H&E – X400 – Scale bar = 50 µm).

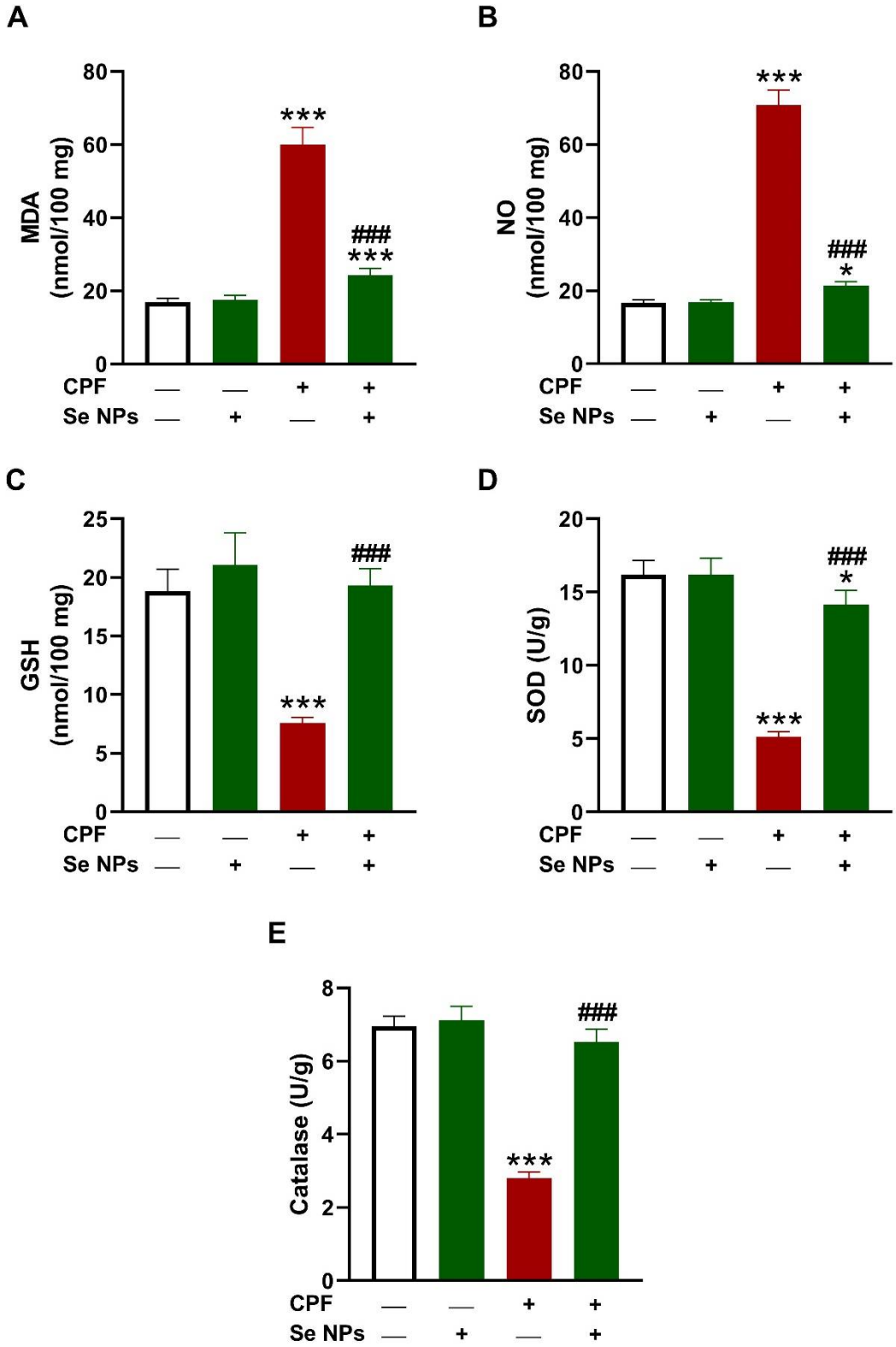


Figure 4. Se NPs attenuated CPF-induced OS in rat kidney. Se NPs decreased liver MDA (**A**) and NO (**B**), and increased GSH (**C**), SOD (**D**) and catalase (**E**) in CPF-administered rats. Data are mean ± SEM, (*n* = 6). *P<0.05 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

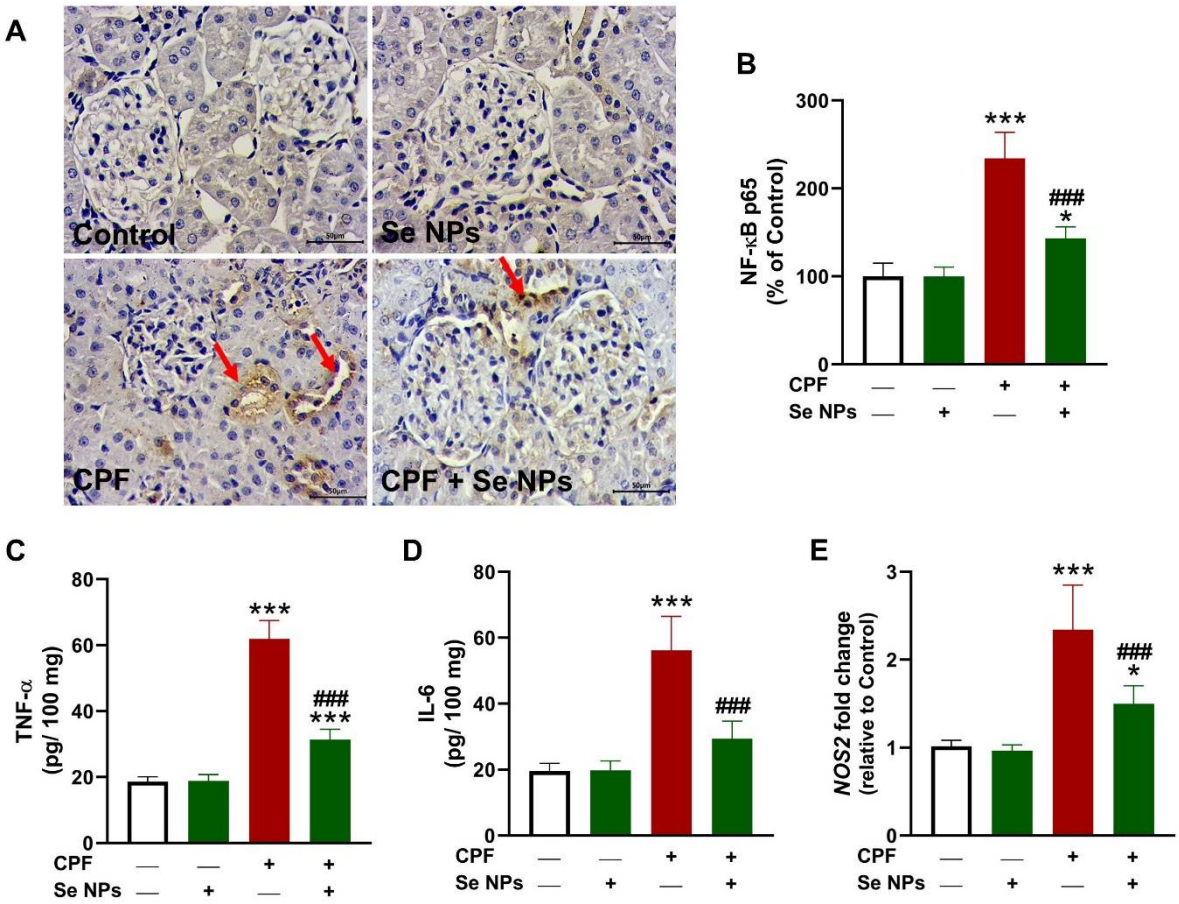


Figure 5. Se NPs suppressed kidney inflammation in CPF-intoxicated rats. Se NPs downregulated NF-κB p65 (A-B) and TNF-α (C), IL-6 (D) and iNOS (E) in CPF-administered rats. Data are mean ± SEM, (n = 6). *P<0.05 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

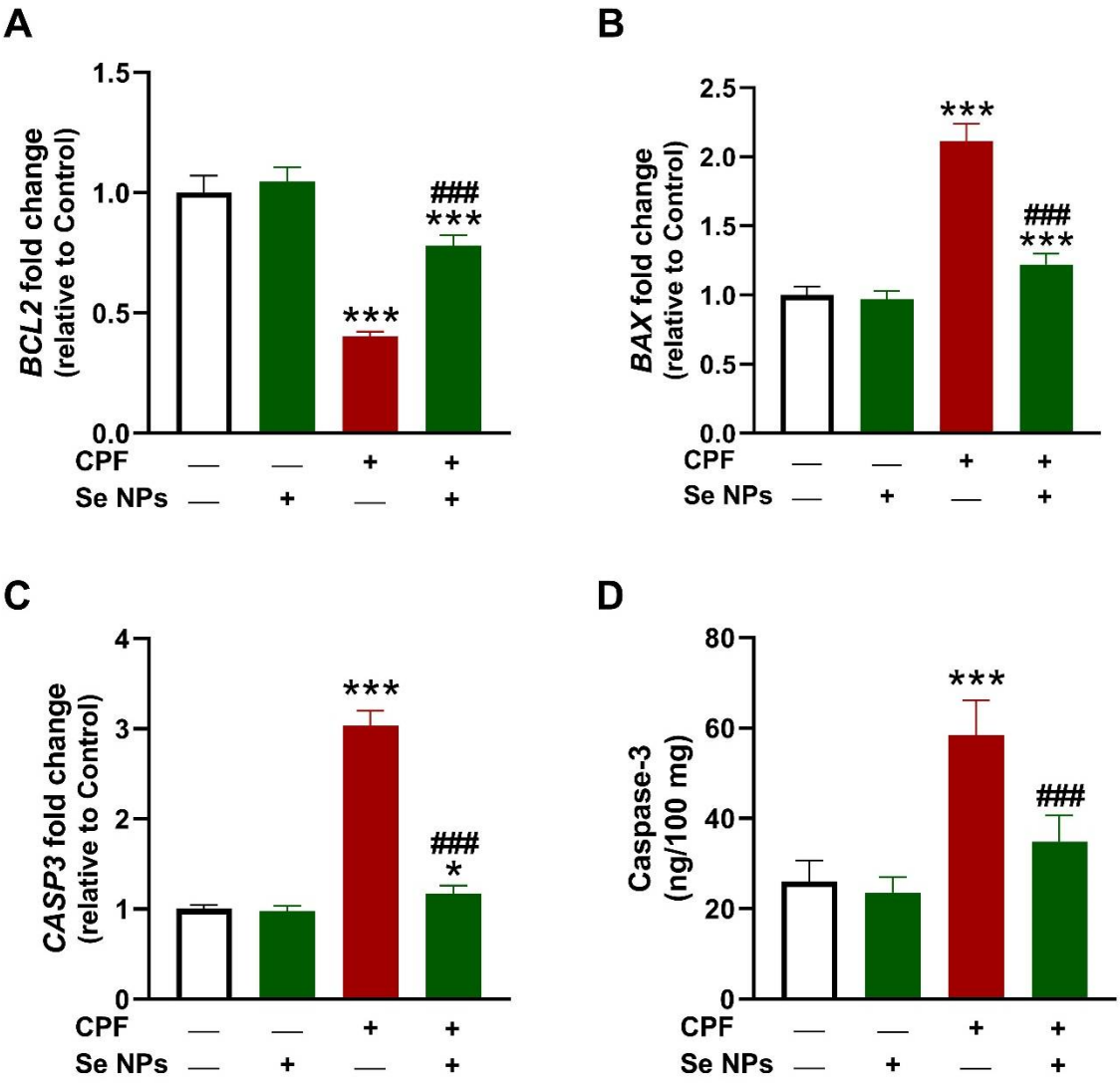


Figure 6. Se NPs mitigated apoptosis in CPF-intoxicated rats. Se NPs upregulated kidney Bcl-2 mRNA (A) and decreased Bax (B), and caspase-3 mRNA (C), and caspase-3 protein (D) in CPF-administered rats. Data are mean ± SEM, (n = 6). *P<0.05 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

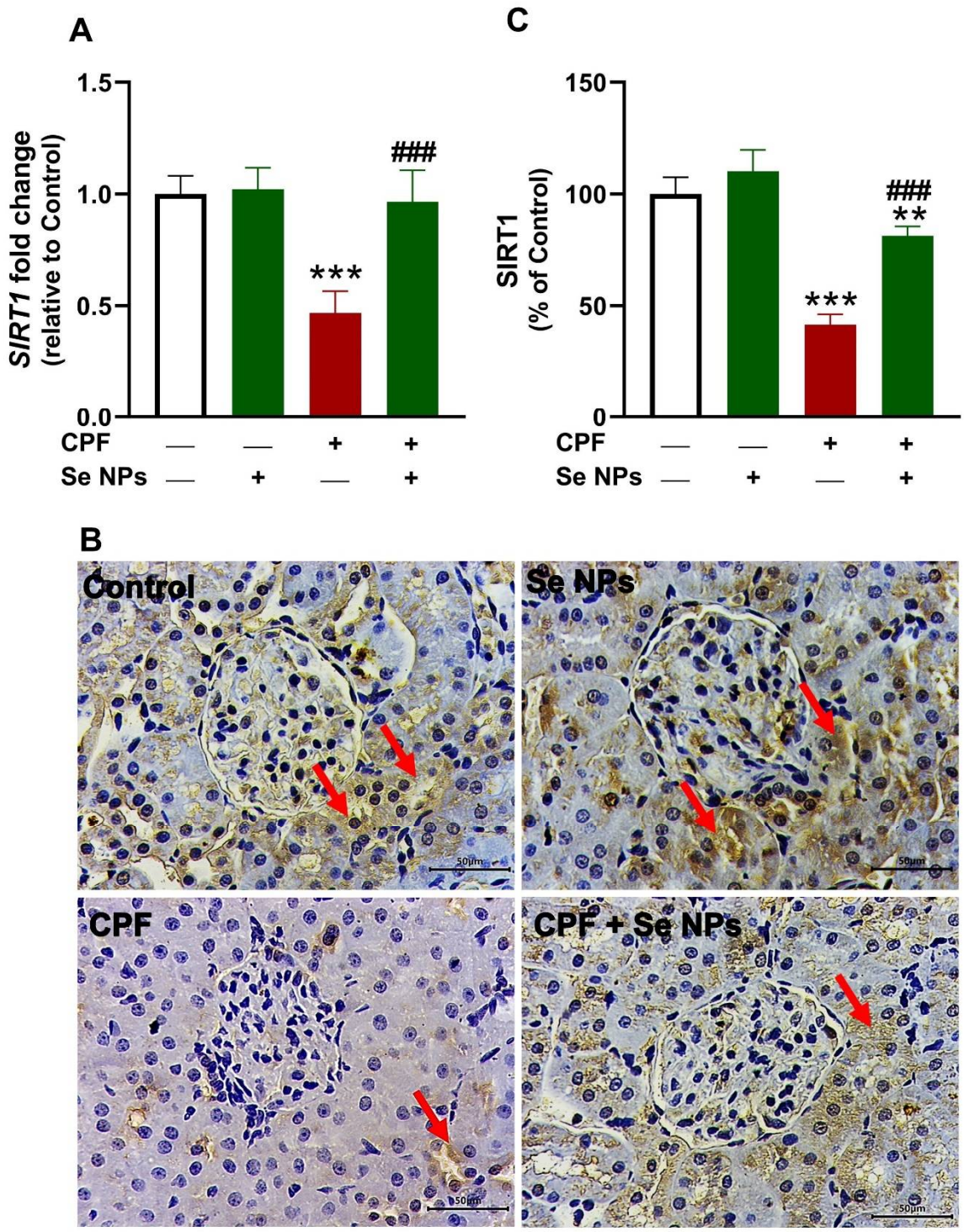


Figure 7. Se NPs upregulated SIRT1 mRNA (A) and protein (B-C) in CPF-intoxicated rats. Data are mean ± SEM, (n = 6). **P<0.01 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

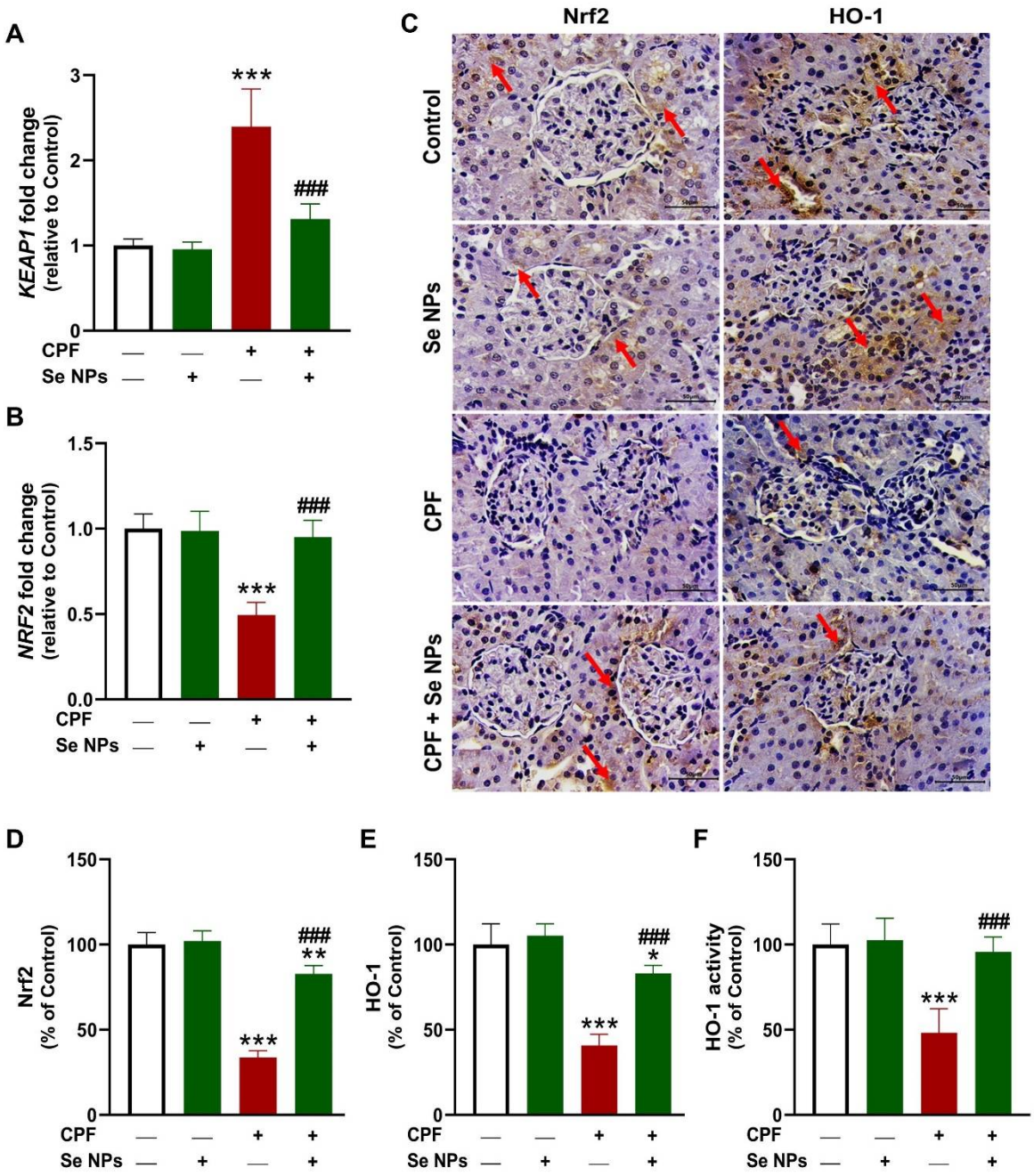
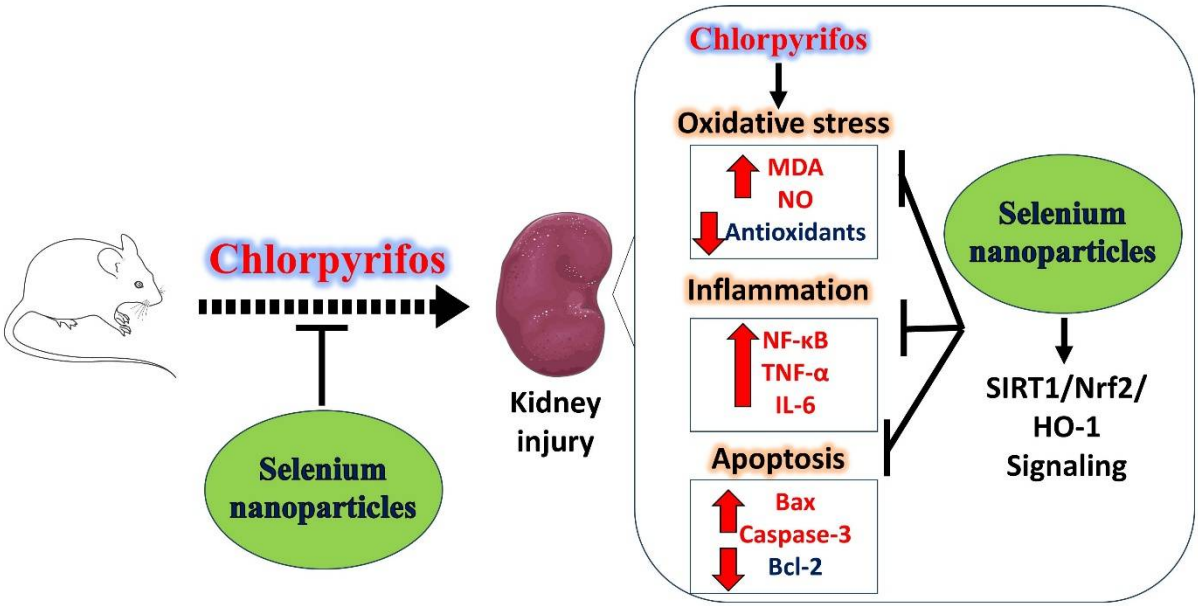


Figure 8. Se NPs enhanced Nrf2/HO-1 signaling in CPF-intoxicated rats. Se NPs decreased Keap1 mRNA (A), upregulated Nrf2 mRNA (B) and protein (C-D), HO-1 protein expression (C,E), and HO-1 activity in CPF-administered rats Data are mean ± SEM, (*n* = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

578 Graphical abstract



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