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Title:

Attenuation of chlorpyrifos-induced liver injury, oxidative stress and inflammation by selenium nanoparticles via SIRT1/FXR/Nrf2 signaling pathway modulation

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Abstract

The pesticide chlorpyrifos (CPF) poses significant environmental and health risks due to its toxicity. Selenium nanoparticles (Se NPs) exhibit promising therapeutic properties. This study evaluated the effects of Se NPs against CPF hepatotoxicity, focusing on oxidative and inflammatory responses, and the SIRT1/FXR/Nrf2 pathway. Rats were exposed to CPF (5.4 mg/kg body weight), with or without Se NPs (0.5 mg/kg body weight), for 28 days, followed by biochemical, histopathological, and molecular analyses. CPF administration significantly increased serum ALT and AST, reduced albumin, and induced histopathological alterations. Se NPs effectively ameliorated liver function biomarkers and mitigated histopathological changes. CPF also elevated malondialdehyde and nitric oxide, and depleted enzymatic antioxidants and GSH, which were mitigated by Se NPs. CPF upregulated NF-κB p65, TNF-α, IL-6, iNOS, Bax and caspase-3, and downregulated Bcl-2. Se NPs suppressed inflammation and apoptosis by downregulating NF-κB p65, pro-inflammatory cytokines and pro-apoptosis markers. These effects were linked to upregulation of SIRT1, FXR, Nrf2 and HO-1 and suppression of Keap1. In conclusion, Se NPs protect against CPF-induced liver injury by attenuating OS, inflammation, and apoptosis, and by upregulating SIRT1//FXR/Nrf2 signaling. These findings highlight the therapeutic potential of Se NPs in mitigating hepatotoxicity induced by exposure to CPF.

Keywords: Pesticides; Selenium; Hepatotoxicity; Inflammation; Oxidative stress.

1. Introduction

The global reliance on pesticides to enhance agricultural productivity has come at a significant cost to human health and the environment [1]. Among these chemicals, chlorpyrifos (CPF), a widely used organophosphate insecticide, has been particularly concerning due to its persistence in ecosystems and its potential to cause harm to non-target organisms, including

humans [2]. Chronic exposure to CPF is linked to a range of toxic effects, including neurotoxicity, developmental disorders, and damage to liver, kidney, and other vital organs [3-6]. These organs are especially vulnerable to pesticide-induced injury because of their central roles in detoxification and metabolic regulation. CPF exerts its insecticidal effect through the inhibition of acetylcholinesterase, which disrupts nervous system function [2]. However, its toxicity is non-selective and extends beyond its primary mechanism of action. CPF toxicity extends to non-target organisms, including fish, mammals, and aquatic invertebrates, thereby posing ecological and human health risks [2]. Detectable residues of CPF above acute reference thresholds have been found on vegetables, grains, and fruits and therefore human exposure to CPF may occur through multiple routes [7, 8].

The induction of oxidative stress (OS) and inflammation has been suggested to mediate CPF toxicity [1, 9]. OS, characterized by an overproduction of reactive oxygen species (ROS) and a depletion of antioxidants, is a key driver of CPF-induced hepato-, nephro-, and neurotoxicity [3-6]. Excessive ROS generation promotes lipid peroxidation (LPO), and protein and DNA damage, ultimately compromising cellular integrity and function [10, 11]. Concurrently, CPF exposure triggers inflammatory responses by upregulating pro-inflammatory cytokines, further exacerbating tissue injury [10, 11]. The interplay between OS and inflammation provokes organ damage [12], highlighting the need for therapeutic interventions that target these interconnected pathways. Understanding the mechanisms underlying CPF-induced toxicity and identifying effective protective strategies are critical for mitigating its adverse health impacts.

The Sirtuin 1 (SIRT1)/farnesoid X receptor (FXR)/nuclear factor erythroid 2-related factor 2 (Nrf2) signaling has emerged as a critical regulator of cell defensive mechanisms against OS and inflammation [13-16]. SIRT1 is a NAD+-dependent deacetylase which is central in modulating cellular metabolism, stress responses, and longevity [15]. It exerts anti-inflammatory and antioxidant effects by deacetylating and activating transcription factors,

including nuclear factor-kappaB (NF-κB), FXR and Nrf2 [17]. In macrophages, SIRT1 inhibited the activation of NF-κB signaling cascade and suppressed mitogen-activated protein kinases, thereby promoting the reprogramming of cholesterol homeostasis and attenuating M1 polarization of macrophages [18]. Activation of SIRT1 resulted in protective effects in experimental models of acute liver failure [19] and cholestatic liver injury [20]. Studies revealed that SIRT1 regulates FXR which is a transcription factor (TF) that can mitigate OS [21][22-24]. Nrf2, in turn, serves as a key regulator of the antioxidant response, promoting the expression of genes encoding protective enzymes, including heme-oxygenase-1 (HO-1) [14]. Enhancement of Nrf2/HO-1 signaling mitigated inflammation and oxidative injury in different liver disorders [25]. Therefore, the SIRT1/FXR/Nrf2 signaling represents a promising therapeutic target for mitigating CPF hepatotoxicity.

Nanotechnology can offer novel approaches to combating OS and inflammation. In this context, selenium nanoparticles (Se NPs) have gained significant attention due to their unique physicochemical properties and potent biological activities, such as biocompatibility and bioavailability [26]. Se is an essential micronutrient required by all living organisms and ubiquitously present in various dietary sources, including grains, seafood, dairy products, and vegetables [27]. As an essential component of selenoproteins, Se plays a critical role in the cellular antioxidant defense mechanisms, contributing to the maintenance of redox homeostasis [28]. Se demonstrated anti-inflammatory properties in lipopolysaccharide (LPS)-treated macrophages [29], and mitigated LPS-induced inflammatory responses and regulate the expression of the selenogenome in porcine and murine models [30, 31]. Despite Se is an essential element and possesses several beneficial effects, its use in conventional form is limited by its narrow therapeutic window and potential toxicity at high doses [26]. Se NPs have been demonstrated to exhibit significantly reduced acute and sub-chronic toxicities compared to other Se compounds, thereby offering a safer alternative for applications [32]. Given the

growing body of evidence supporting the beneficial effects of Se NPs, this study investigated their protective role on liver injury induced by CPF, with a focus on the involvement of the SIRT1/FXR/Nrf2 signaling.

2. Materials and methods

2.1. Synthesis and characterization of Se NPs

Se NPs were synthesized via a high-energy ball-milling technique, adapted from established protocols [33]. Briefly, Se powder was subjected to mechanical milling in a vertical planetary ball mill, employing a ball-to-powder mass ratio of 10:1. The milling process was carried out at a rotational speed of 200 rpm for a total duration of 20 h. Post-milling, the resultant powder was dried at 80°C for 24 h to remove residual moisture. The structural and morphological properties of the synthesized NPs were assessed using SEM (Fig. 1), XRD, and dynamic light scattering (DLS) (Suppl. Fig. I).

2.2. Animals and treatments

Male Wistar rats weighing 180 ± 10 g were acclimatized under controlled temperature (23 ± 2 °C), relative humidity (50–60%), and a 12-h light/dark cycle. Animals were provided *ad libitum* access to a standard rodent diet and water. A total of 24 rats were randomly allocated into four experimental groups (n = 6 per group) to investigate the hepatoprotective efficacy of Se NPs against CPF-induced toxicity. 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 experimental protocol was approved by the Institutional Animal Ethics Committee of Beni-Suef University (Egypt) (Approval no. 021-130).

Se NPs and CPF (Agro Chem, Egypt) were suspended in 0.5% carboxymethyl cellulose (CMC) (Sigma, USA) 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 [34].

Group III (CPF): received 5.4 mg/kg CPF (1/25 of the LD_{50}) [35].

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 isolated for subsequent biochemical analyses. Liver was rapidly excised post-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.

2.3. Biochemical assays

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin were quantified using commercially available kits (Spinreact, Spain). ELISA were employed to measure tumor necrosis factor (TNF)-α, interleukin (IL)-6 (Elabscience, China; Cat. no. E-EL-R2856 and E-EL-R0015, respectively), and caspase-3 (Cusabio, China; Cat. no. CSB-E08857r). Malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase were assessed in liver homogenate using Biodiagnostic (Egypt) kits. HO-1 activity was determined according to the method described by Abraham et al. [36].

2.4. Histopathological and immunohistochemical (IHC) evaluations

Liver 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 SIRT1, Nrf2, HO-1, and NF-κB p65 [24].

Briefly, paraffin-embedded sections were dewaxed, rehydrated, and subjected to antigen retrieval using citrate buffer (50 mM, pH 6.8). Endogenous peroxidase 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 against SIRT1, Nrf2, HO-1, and NF-κB p65 (Biospes, China), washed, and then treated with secondary antibodies (Biospes, China). Color development was achieved using 3,3′-diaminobenzidine in H_2O_2, and sections were counterstained with Mayer's hematoxylin. Quantitative analysis of staining intensity was performed using ImageJ software (NIH, USA).

2.5. qRT-PCR

Total RNA was extracted from liver 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 primers (Table 1). The mRNA expression levels of *KEAP1*, *NRF2*, *SIRT1*, *FXR*, *NOS2*, *BAX*, *BCL2*, and *CASP3* were normalized to β-actin (*ACTB*), and relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method [37].

2.6. 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). Significance was considered at a P-value of < 0.05.

3. Results

3.1. Se NPs attenuate CPF-induced liver injury

The hepatoprotective effect of Se NPs against CPF-induced toxicity was evaluated using biochemical markers and histopathological analysis (Fig. 2). CPF provoked significant

elevations in serum ALT (Fig. 2A) and AST (Fig. 2B), alongside a marked reduction in albumin levels (Fig. 2C) compared to the control group ($P<0.001$). Se NPs significantly attenuated these alterations in hepatic biomarkers ($P<0.001$). Notably, no significant differences in body weight were observed among the experimental groups.

Histopathological examination of liver tissue revealed normal hepatic architecture, including intact hepatocytes, sinusoids, and central veins, in both the control and Se NPs-treated groups (Fig. 2D). In contrast, CPF administration induced severe hepatic damage, characterized by dilated congested portal vein, degenerative changes, and scattered apoptotic hepatocytes. Treatment with Se NPs markedly ameliorated these pathological alterations, with only mild degenerative changes observed in hepatocytes (Fig. 2D).

3.2. Se NPs attenuate liver OS in CPF-exposed rats

CPF exposure significantly increased liver MDA (Fig. 3A) and NO (Fig. 3B) ($P<0.001$). Concurrently, CPF administration led to a significant depletion of GSH, SOD, and catalase in the liver (Fig. 3C-E). Se NPs significantly reduced MDA and NO while restoring GSH and antioxidant enzymes ($P<0.001$).

3.3. Anti-inflammatory and anti-apoptotic effects of Se NPs

CPF significantly upregulated NF-κB p65 in the liver of rats (Fig. 4A-B) ($P<0.001$). Similarly, CPF exposure increased hepatic TNF-α (Fig. 6C) and IL-6 (Fig 4D), and iNOS mRNA levels (Fig. 4E) ($P<0.001$). Supplementation with Se NPs effectively suppressed hepatic NF-κB p65, TNF-α, IL-6 and iNOS levels in CPF-administered rats. In addition to inflammation, CPF exposure triggered apoptotic pathways, as shown by significant downregulation of Bcl-2 (Fig. 5A) and upregulation of Bax (Fig. 5B) and caspase-3 mRNA (Fig. 5C) and protein (Fig. 5D) in the liver ($P<0.001$). Se NPs co-administration significantly mitigated apoptosis, as indicated by increased Bcl-2 expression and reduced Bax and caspase-3 ($P<0.001$).

3.4. Modulation of SIRT1/FXR/Nrf2/HO-1 signaling by Se NPs

CPF exposure resulted in a significant downregulation of SIRT1 at both the mRNA (Fig. 6A) and protein levels (Fig. 6B-C), along with a marked reduction in FXR mRNA expression (Fig. 6D). CPF also disrupted the antioxidant defense pathway, evidenced by an increase in Keap1 mRNA expression (Fig. 7A), and a decrease in both Nrf2 mRNA (Fig. 7B) and protein levels (Fig. 7C-D), as well as a decline in HO-1 expression (Fig. 7C and 7E) in the liver ($P<0.001$). Treatment with Se NPs significantly reversed these alterations, restoring SIRT1, FXR, Nrf2, and HO-1 expression, while reducing Keap1 expression ($P<0.001$), indicating a protective role of Se NPs in preserving the integrity of this signaling axis.

4. Discussion

The extensive use of CPF to enhance crop yields by controlling pests has raised significant concerns due to its detrimental effects on human health and the environment [1, 2]. OS and inflammatory responses have been suggested to mediate CPF organ toxicity, including hepatotoxicity [5, 38]. Therefore, there is a need for therapeutic strategies that target these mechanisms to mitigate CPF-induced liver damage. Se NPs have emerged as a promising candidate due to their beneficial properties against OS and inflammation in different disorders [39]. This study demonstrates the protective effects of Se NPs against CPF-induced hepatic injury, highlighting the involvement of the SIRT1/FXR/Nrf2 signaling pathway in mediating these effects.

The liver is a vital organ that serves as the primary site for drug metabolism, plays a central role in a wide array of metabolic processes, and is integral to the detoxification of xenobiotics. Liver damage can significantly compromise its detoxification capacity, leading to the accumulation of harmful metabolites and systemic toxicity [40]. Such impairment can disrupt hepatic functions and maintaining liver health is therefore critical for ensuring efficient

metabolic and detoxification processes [40]. In this study, CPF administration induced significant hepatotoxicity, as evidenced by elevated serum markers of liver dysfunction, alongside reduced serum albumin and histopathological alterations. These findings are consistent with previous reports demonstrating the hepatotoxic role of CPF and its impact on cellular integrity and function [5, 38, 41-43]. Hepatocyte injury could be attributed to the lipophilic nature of CPF as an organic molecule, enabling its accumulation in the liver and subsequent penetration of cellular membranes [38]. High residual levels of CPF can alter membrane permeability [38], resulting in hepatocyte injury and leakage of aminotransferases to the circulation. In rats exposed to CPF, ALT and AST were elevated and hepatocyte degeneration, necrosis, blood vessel congestion, and dilation of the sinusoids were observed in the liver tissue [5]. More recently, the study of Fu et al [38] revealed altered liver function and tissue architecture following exposure of rats to CPF. The same study demonstrated a positive correlation between the administered CPF concentrations and the residual levels in rat liver, pinpointing the hepatic metabolism and accumulation of CPF following oral ingestion in rats [38]. In the present study, Se NPs effectively attenuated these toxic effects, restoring biochemical markers and preserving liver tissue architecture. The ability of Se NPs to maintain hepatocyte integrity suggests their potential as a therapeutic agent against CPF-induced hepatotoxicity. The hepatoprotective role of Se NPs has been investigated in previous studies. In a WRL68 fatty liver cell model, Se NPs attenuated lipid accumulation in hepatocytes *in vitro* [44]. In rats intoxicated with acetaminophen (APAP) overdose, intraperitoneal administration of Se NPs resulted in ameliorated circulating transaminases and histopathological alterations. In addition, Se NPs exerted protective effects against CCl_4-induced liver damage in mice [45] and toxicity induced by the toxic fungal metabolite patulin [46]. Our findings added support to the hepatoprotective efficacy of Se NPs by showing their ability to attenuate CPF hepatotoxicity.

Owing to the growing evidence of the role of OS and inflammation in mediating the toxic effects of CPF and numerous environmental pollutants [3-6, 47], the protective effects of Se NPs against CPF-induced toxicity are likely mediated through their antioxidant and anti-inflammatory properties. CPF exposure significantly increased hepatic OS markers, including MDA and NO, while depleting key antioxidants, GSH, SOD, and catalase. These data align with the well-established role of CPF in generating ROS and disrupting antioxidant defense systems [38, 41, 48]. Excessive production of ROS induces oxidative damage to key cell lipids, proteins, and DNA. This manifests as LPO, protein oxidation, and DNA strand breaks or modifications, which collectively disrupt cellular integrity and function. Such oxidative damage can overwhelm cellular antioxidant and repair mechanisms, leading to irreversible cell injury and ultimately culminating in cell death [10, 11]. Exposure to CPF has been consistently linked to the induction of OS through the excessive generation of ROS in a variety of experimental systems. This phenomenon has been documented in Neuro-2a cells, where CPF exposure disrupts redox balance and promotes oxidative damage [49], as well as in *in vitro* models utilizing microglial cells, which exhibit heightened ROS production and subsequent inflammatory responses [50]. Additionally, studies in rat renal tissues have demonstrated that CPF exposure leads to the accumulation of ROS, resulting in oxidative injury and impaired renal function [6]. Similar results were recently reported in rats subjected to different concentrations of CPF where a positive correlation was observed between the concentrations and OS parameters [38]. These findings along with results of the present study collectively underscore the role of CPF as a potent inducer of OS, highlighting its potential to disrupt cellular redox homeostasis and contribute to tissue damage. Additionally, CPF-induced ROS activate NF-κB, a central regulator of inflammation. In this study, CPF administration upregulated NF-κB p65, iNOS, IL-6, and TNF-α in the liver, indicating a pro-inflammatory response. The activation of NF-κB promotes pro-inflammatory mediators, including TNF-α,

IL-6, and iNOS, which exacerbate tissue injury [51]. Recent findings have demonstrated liver inflammatory response following exposure of rats to CPF. This included the activation of NF-κB and increased iNOS, IL-6, TNF-α, and other proteins involved in inflammation [38]. In conjunction with ROS, pro-inflammatory mediators exacerbates cellular dysfunction and injury by inducing mitochondrial dysfunction and triggering apoptotic pathways [12]. This process involves the disruption of the mitochondrial membrane potential, leading to the release of cytochrome *c* into the cytoplasm and subsequent activation of caspase-3 which initiates the execution phase of the apoptotic cascade, culminating in programmed cell death [52]. CPF-induced mitochondrial dysfunction and caspase-3 activation were evidenced by increased expression of pro-apoptotic markers (Bax and caspase-3) and decreased levels of the anti-apoptotic protein Bcl-2.

Se NPs effectively mitigated these effects by suppressing MDA, NO, NF-κB p65, iNOS, IL-6, and TNF-α, enhancement of GSH and antioxidant enzymes, and prevention of apoptosis. These findings highlight the dual antioxidant and anti-inflammatory roles of Se NPs in protecting against CPF-induced liver damage. Accordingly, Se NPs suppressed LPO and enhanced catalase, SOD and GPx in a murine model of carbon tetrachloride-induced liver damage [45]. These effects were associated with amelioration of liver dysfunction and injury [45]. In rats challenged with high APAP doses, Se NPs mitigated OS as shown by decreased liver MDA and enhanced antioxidant enzymes [53]. In cells exposed to patulin toxicity, Se NPs were effective in suppressing ROS and MDA and enhance antioxidant defenses [46]. Additionally, probiotics enriched with Se NPs showed anti-inflammatory, anti-apoptotic, and antioxidant properties mediated via suppression of NF-κB and Bax, and enhancement of Bcl-2 and catalase in cadmium-administered rats [54]. To further elucidate the mechanisms underlying the protective effects of Se NPs, we investigated the SIRT1/FXR/Nrf2 signaling. CPF exposure significantly downregulated liver SIRT1, FXR, Nrf2, and HO-1 and upregulated Keap1. SIRT1 plays a

295 critical role in regulating cellular stress responses by deacetylating NF-κB and Nrf2, thereby
296 exerting anti-inflammatory and antioxidant effects [17]. The TF FXR dimerizes with retinoid
297 X receptor (RXR) to modulate numerous genes critical for metabolic homeostasis.
298 Dysregulation of FXR has been linked to inflammatory conditions and various pathologies,
299 such as diabetes and cholestasis [24, 55, 56]. Recent studies indicate that FXR activation
300 markedly decreases LPO by enhancing the expression of key ferroptosis inhibitors, such as
301 GPX4 and PPARα [57]. Nrf2 and its downstream HO-1 are essential for mitigating oxidative
302 and inflammatory damage [14]. Under normal conditions, Nrf2 is sequestered in the cytosol by
303 Keap1 and this binding is disrupted upon exposure to ROS followed by nuclear translocation
304 of Nrf2 and promotion of cytodefensive and antioxidant enzymes [14]. Therefore, the
305 suppression of SIRT1/FXR/Nrf2 signaling by CPF exacerbates cellular vulnerability to
306 oxidative and inflammatory injury. Previous studies have demonstrated downregulation of
307 these signaling molecules under conditions of prolonged ROS generation [58-61]. Consistent
308 effects of CPF on Nrf2 have been observed across diverse experimental models, including
309 human neuroblastoma cells, fruit flies, and rodent liver [48, 62, 63]. Se NPs enhanced SIRT1,
310 Nrf2, and HO-1, and suppressed Keap1 in CPF-exposed rats, suggesting that their protective
311 effects are mediated, at least in part, through the activation of this pathway. The upregulation
312 of HO-1 is particularly noteworthy, as it catalyzes the degradation of heme into biliverdin and
313 carbon monoxide, both of which possess antioxidant and anti-inflammatory properties [64].
314 These findings are consistent with previous reports demonstrating that Se NPs enhance Nrf2
315 expression and HO-1 activity in broilers exposed to heat stress-induced oxidative damage to
316 organs [65]. Our study introduced new information on the hepatoprotective efficacy of Se NPs
317 and the involvement of SIRT1/FXR/Nrf2 signaling (Fig. 8). However, the use of a single dose
318 and duration for both CPF and Se NPs, the lack of investigation into additional pathways and
319 molecular targets beyond the SIRT1/FXR/Nrf2 signaling, and the absence of complementary

in vitro models or mechanistic inhibitors represent limitations of the current study. Nonetheless, these limitations do not detract from the validity of the core findings, as the observed hepatoprotective effects of Se NPs were consistently supported by multiple lines of biochemical, histological, and molecular evidence.

5. Conclusion

This study demonstrates that Se NPs effectively counteract CPF-induced hepatotoxicity by targeting multiple pathological pathways. Se NPs attenuated CPF-induced OS by mitigating LPO while restoring the activity of key antioxidants. Se NPs exhibited potent cytoprotective properties, as evidenced by the downregulation of NF-κB. pro-inflammatory mediators, Bax and caspase-3, with significant upregulation of Bcl-2. A key finding of this study is the ability of Se NPs to enhance the SIRT1/FXR/Nrf2 signaling pathway, which plays a pivotal role in cellular defense mechanisms against oxidative damage and inflammation. By upregulating this pathway, Se NPs enhanced the endogenous antioxidant response and mitigated CPF-induced tissue injury. These results underscore the multifaceted therapeutic potential of Se NPs with the ability to modulate critical cellular signaling pathways. Further studies are warranted to optimize dosing regimens and evaluate the long-term safety of Se NPs in clinical applications.

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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.A.A.M., and 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. The authors confirm that no paper mill and artificial intelligence was used.

Declarations

Ethical Approval

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

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554 Tables:

555 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
FXR	CATTAACAACGCGCTCACCTG	TTCCTTAGCCGGCAATCCTG
NOS2	ATTCCCAGCCCAACAACACA	GCAGCTTGTCCAGGGATTCT
SIRT1	TCTCCCAGATCCTCAAGCCAT	TTCCACTGCACAGGCACATA
ACTB	AGGAGTACGATGAGTCCGGC	CGCAGCTCAGTAACAGTCCG

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Figures:

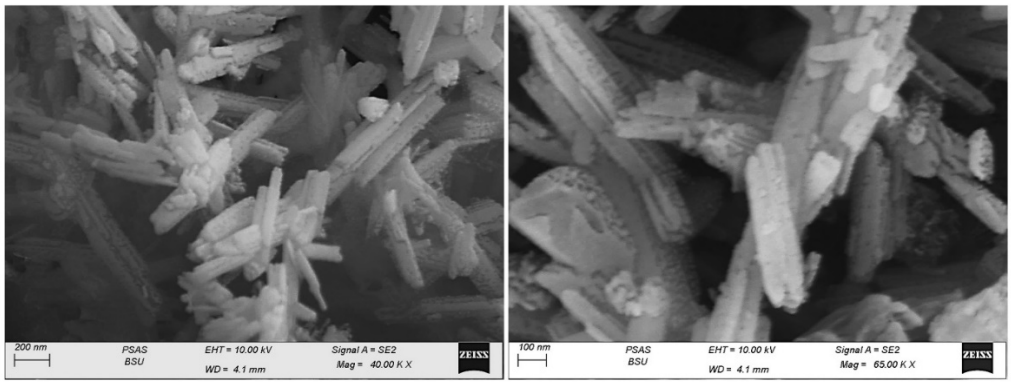


Figure 1. SEM of Se NPs.

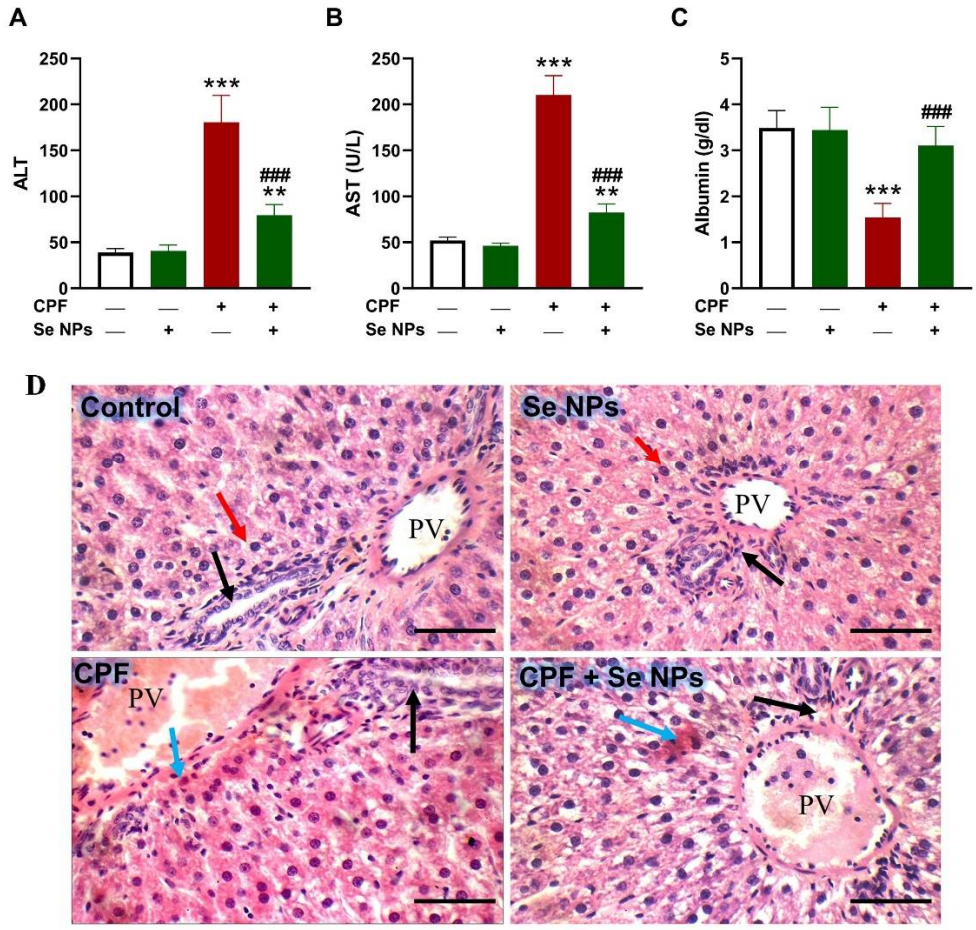
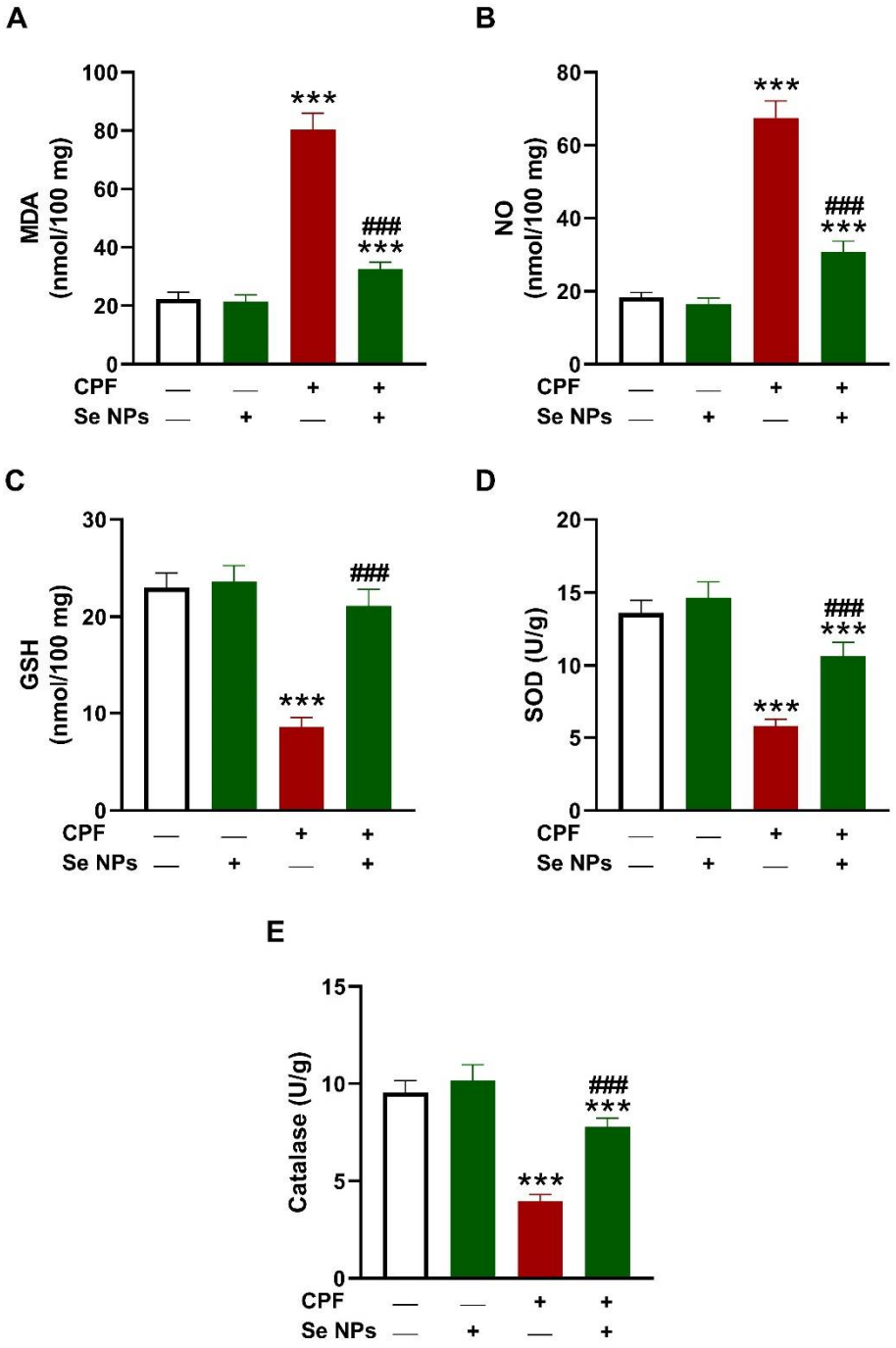


Figure 2. Se NPs mitigated CPF-induced liver injury. Se NPs ameliorated serum ALT (A), AST (B), and albumin (C) in CPF-administered rats. Data are mean ± SEM, (n = 6). **P<0.01 and ***P<0.001 vs Control, and ###P<0.001 vs CPF. (D) Photomicrographs of sections in liver of control and Se NPs-supplemented rats showing average portal tract with average portal vein (PV), average bile ducts (black arrow), and average hepatocytes in peri-portal area (red arrow), CPF-administered rats showing portal tract with mildly dilated congested portal vein (PV), average bile ducts (blue arrow) and scattered apoptotic hepatocytes in peri-portal area(red arrow), and CPF-induced rats treated with Se NPs showing showing average portal tract (black arrow) with mildly dilated congested portal vein (PV), and scattered apoptotic hepatocytes in peri-portal area (blue arrow). (H&E – X400 – Scale bar = 50 µm).



572
573 Figure 3. Se NPs mitigated hepatic OS in CPF-intoxicated rats. Se NPs decreased liver MDA
574 (A) and NO (B), and increased GSH (C), SOD (D) and catalase (E) in CPF-administered rats.
575 Data are mean ± SEM, (n = 6). ***P<0.001 vs Control, and ###P<0.001 vs CPF.

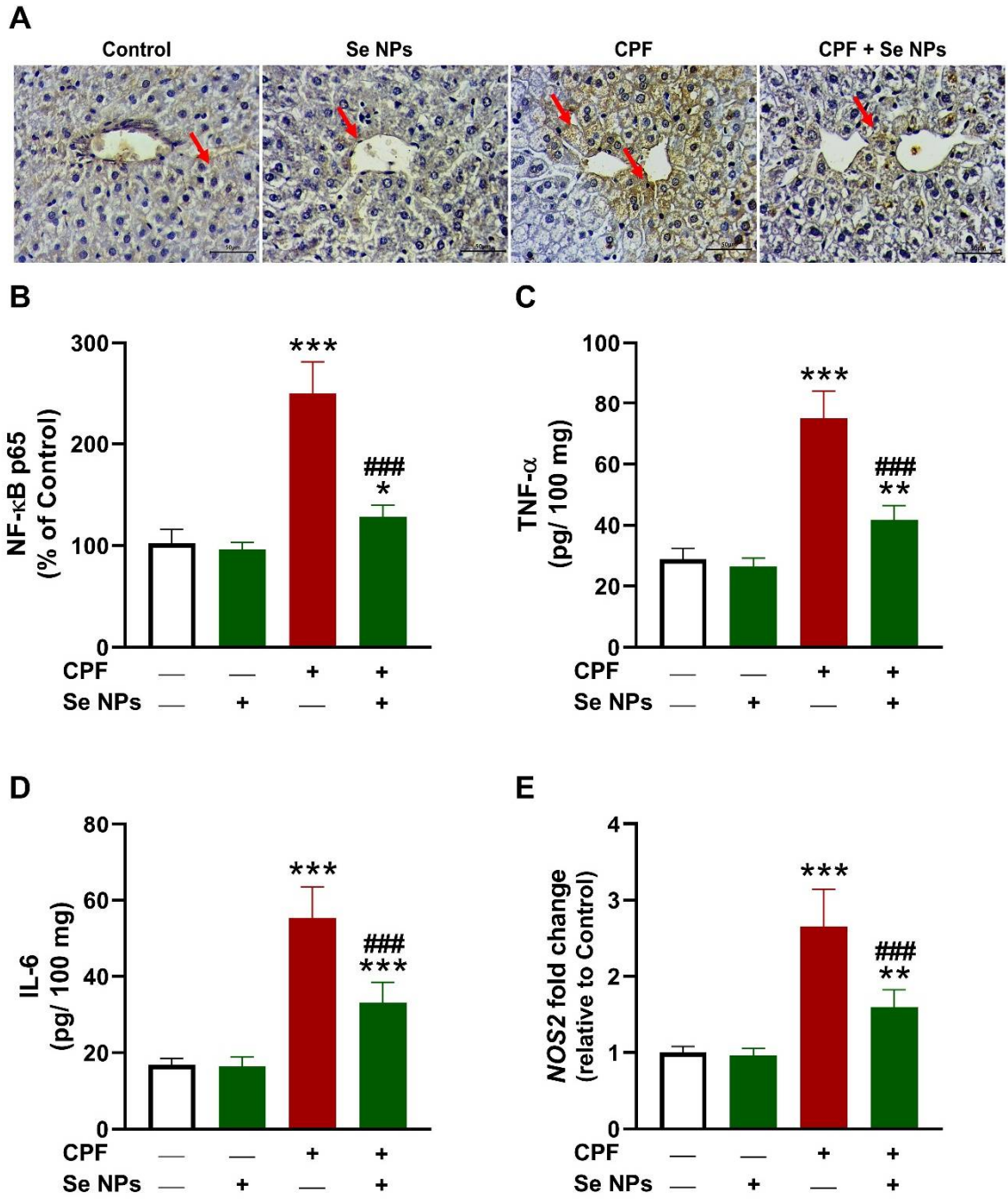


Figure 4. Se NPs attenuated hepatic inflammation in CPF-intoxicated rats. Se NPs decreased liver NF-κB p65 (A-B) and TNF-α (C), IL-6 (D) and iNOS (E) in CPF-administered rats. Data are mean ± SEM, (*n* = 6). *P<0.05, **P<0.01, and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

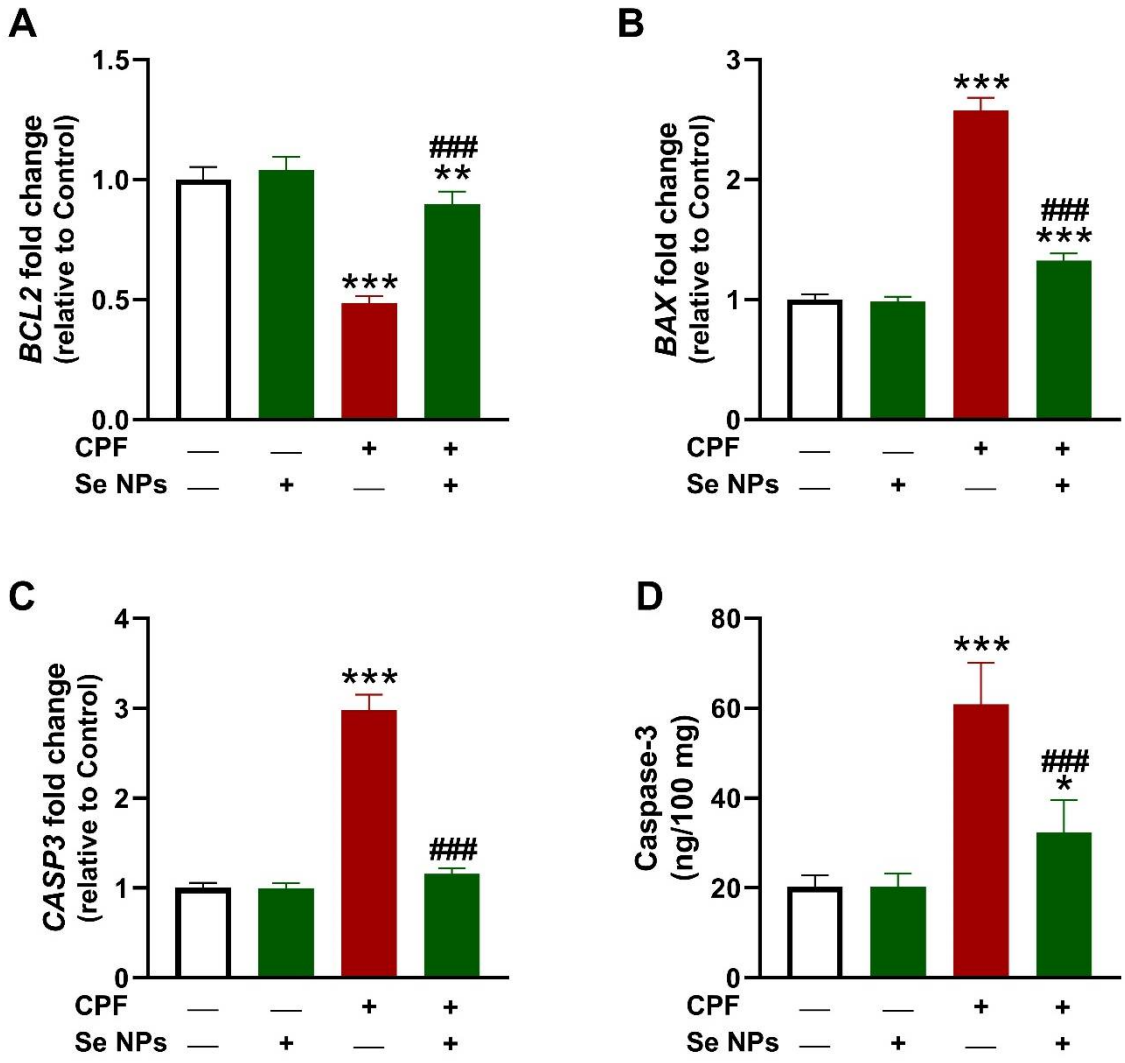


Figure 5. Se NPs prevented apoptosis in CPF-intoxicated rats. Se NPs upregulated liver Bcl-2 mRNA (A) and decreased Bax (B), and caspase-3 mRNA (C), and caspase-3 protein (D) in CPF-administered rats. Data are mean ± SEM, (*n* = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

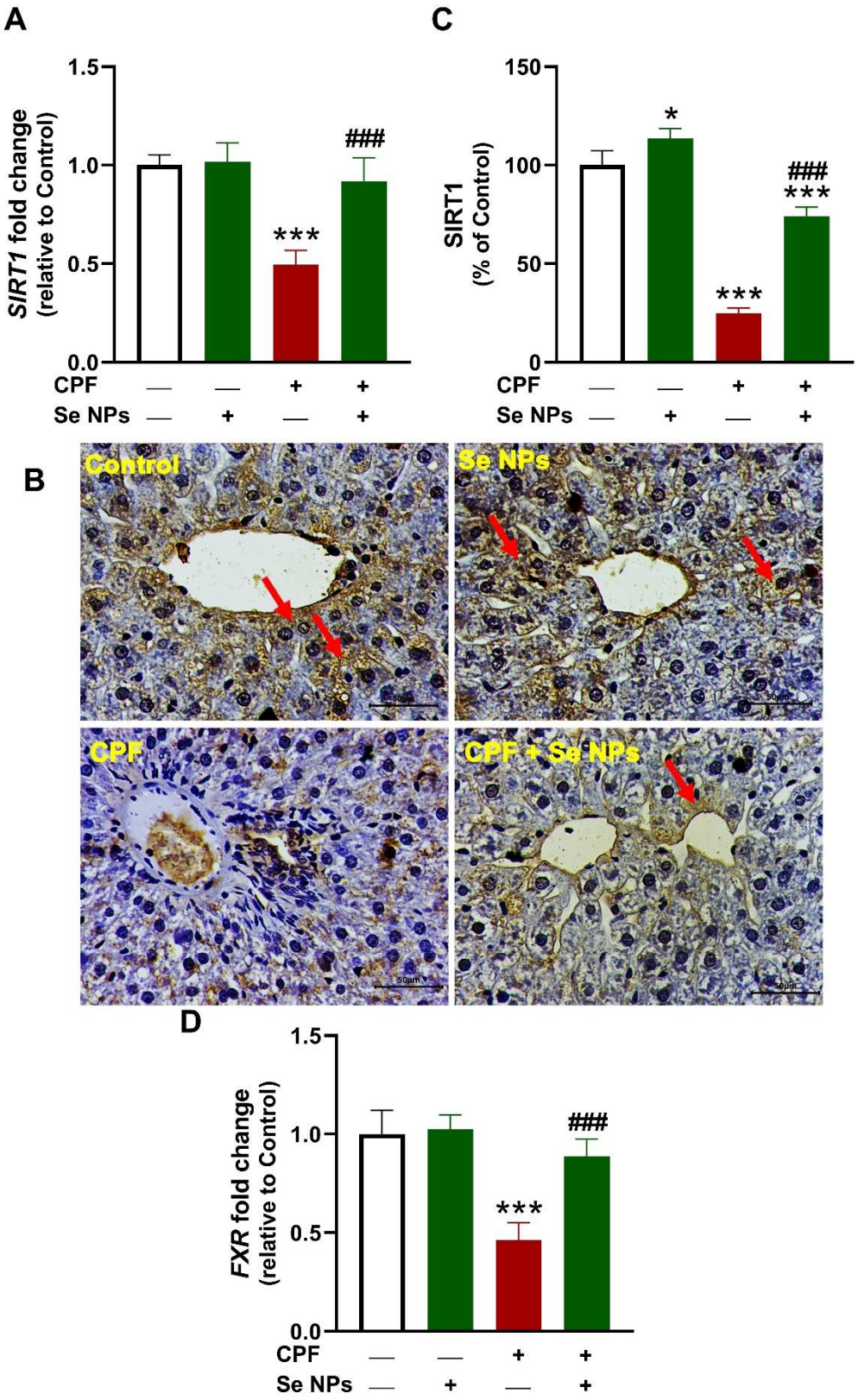


Figure 6. Se NPs upregulated SIRT1 mRNA (A) and protein (B-C), and FXR mRNA (D) in liver of CPF-intoxicated rats. Data are mean ± SEM, (n = 6). ***P<0.001 vs Control, and ###P<0.001 vs CPF.

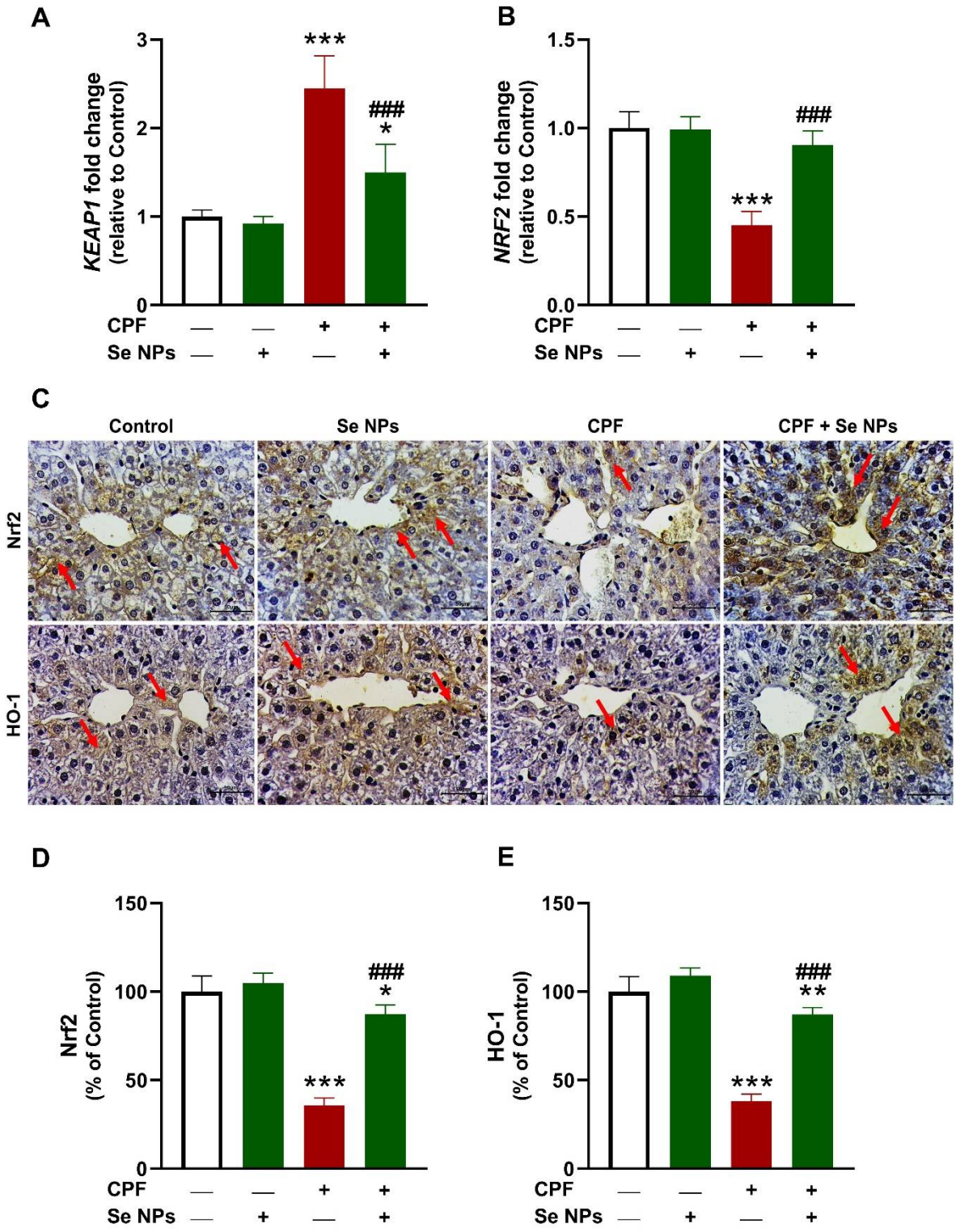


Figure 7. Se NPs enhanced Nrf2/HO-1 signaling in liver of CPF-intoxicated rats. Se NPs downregulated Keap1 mRNA (A), upregulated Nrf2 mRNA (B) and protein (C-D) and HO-1 protein expression (C,E) in CPF-administered rats Data are mean ± SEM, (n = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control, and ###P<0.001 vs CPF.

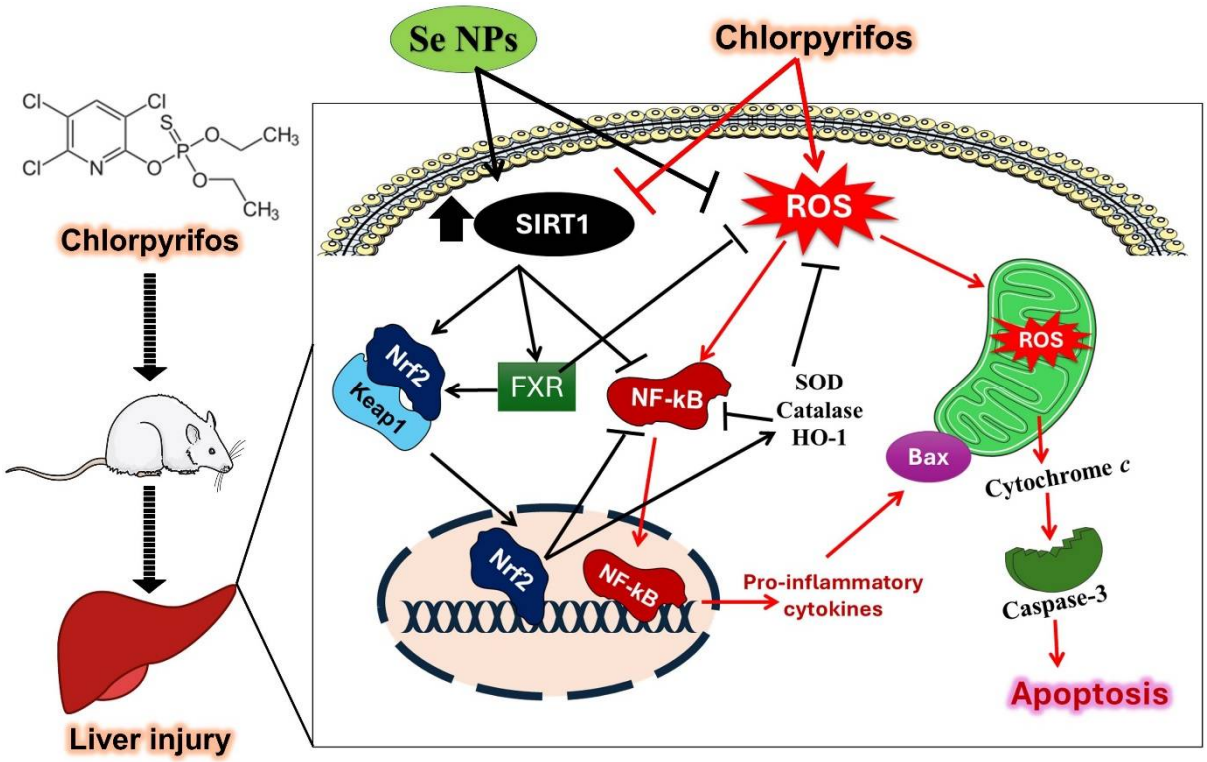


Figure 8. Mechanistic illustration of the protective effects of Se NPs against CPF-induced liver injury. CPF exposure increases ROS, provokes, oxidative stress, inflammation, and apoptosis, and suppresses SIRT1, FXR, and Nrf2/HO-1 signaling. Se NPs counteract CPF toxicity by enhancing SIRT1, which upregulates FXR and Nrf2 signaling, inhibits NF-κB, reduces ROS, suppresses inflammation and apoptosis, and restores antioxidant enzymes, collectively mitigating hepatocellular damage.