Title:

Farnesol prevents chlorpyrifos nephrotoxicity by modulating inflammatory mediators, Nrf2 and FXR and attenuating oxidative stress

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Abstract:

Chlorpyrifos (CPF) is a broad-spectrum insecticide widely employed in agricultural field for pest control. Exposure to CPF is associated with serious effects to the main organs, including kidneys. Significant evidence denotes that oxidative stress (OS) and inflammation are implicated in CPF toxicity. This study aimed to evaluate the potential of farnesol (FAR) to modulate inflammatory mediators and farnesoid-X-receptor (FXR) and Nrf2 in a rat model of CPF nephrotoxicity. CPF and FAR were orally supplemented for 28 days and blood and kidney samples were collected for investigations. CPF administration elevated blood creatinine and urea, kidney MDA and NO, and upregulated NF-κB p65, IL-1β, TNF-α, iNOS, and caspase-3. In addition, CPF upregulated kidney Keap1, and decreased GSH, antioxidant enzymes, and Nrf2, FXR, HO-1 and NQO-1. FAR ameliorated creatinine and urea, prevented histopathological alterations, decreased MDA and NO, and enhanced antioxidants in CPF-administered rats. FAR modulated NF-κB p65, iNOS, TNF-α, IL-1β, caspase-3, Keap1, HO-1, NQO-1, Nrf2 and FXR. *In silico* investigations revealed the binding affinity of FAR towards Keap1 and FXR, as well as NF-κB, caspase-3, iNOS, and HO-1. In conclusion, FAR prevents CPF-induced kidney injury by attenuating OS, inflammation, and apoptosis, effects associated with modulation of FXR, Nrf2/HO-1 signaling and antioxidants.

Keywords: Pesticides; Oxidative stress; Inflammation; Sesquiterpene; Nephrotoxicity.

1. Introduction

Organophosphorus (OP) pesticides are primarily used in agriculture for pest control and crop yield improvement. OP pesticides are also employed in various domestic and veterinary settings, leading to a rise in human exposure to these toxic substances [1]. The excessive use of OP and other chemical pesticides represent a major source of environmental pollution and poses health risk for both animals and human [1]. Owing to the increased and widespread use

of OP pesticides and human exposure during mixing, application, or cleaning processes, high rates of illness and mortality, as well as acute and chronic health issues have been reported [2]. Among OP compounds, chlorpyrifos (CPF) is a pesticide widely used against various pests attacking crops, fruits, and vegetables, making it popular for indoor and agricultural applications [3]. It has a broad spectrum and its primary mechanism of pesticidal and insecticidal actions involves the inhibition of acetylcholinesterase, resulting in disrupted peripheral and central nervous system functions. This crucial enzyme functions at the neuromuscular junctions and cholinergic synapses and its inhibition leads to death of the affected insects [4]. However, this non-selective toxic effect may affect the nervous system of other organisms, resulting in pathological or biochemical disorders [5]. Accordingly, the toxicity of CPF has been linked with several neurological, endocrine, hematological, and reproductive disorders [3, 6, 7]. Human exposure to CPF can occur on daily basis through direct contact, skin absorption, or inhalation, as this compound is frequently used in households to control flies, cockroaches, and ants [8]. Besides direct exposure, CPF can reach humans and animals through the consumption of contaminated food and its residues were detected on the surface of agricultural products in amounts above the acute reference range [3, 9-11].

Exposure to CPF through different routes has been linked to toxicity of the main organs, including kidney, liver, nervous system, and reproductive organs [3, 12, 13]. Although the exact mechanisms are not fully understood, CPF toxicity and that of its byproducts are linked to increased reactive oxygen species (ROS) as reported in neuronal and microglial cells *in vitro* [14, 15] as well as in rat kidney [16]. Excessive ROS provokes oxidative stress (OS) and inflammation, resulting in cellular damage and apoptosis [17, 18]. ROS can harm cells by impairing DNA, lipids, and proteins, activating inflammatory responses and NF-κB, and ultimately affecting cell survival. Previous studies have shown OS in various cell types exposed to CPF, including the kidney, along with elevated biomarkers of inflammation [18-20].

Therefore, kidney damage is one of the deleterious effects of CPF, with OS and inflammation playing a significant role [18, 21]. Therefore, strategies that focus on reducing OS and inflammation have been shown to protect against CPF-induced kidney toxicity [13, 16]. Activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream genes, including heme oxygenase 1 (HO-1), along with farnesoid X receptor (FXR) serves as an effective defense against kidney OS and inflammation induced by chemical exposure, medications, and metabolic imbalances [22-24]. Nrf2, a transcription factor (TF) that regulates multiple antioxidant and protective genes, is a viable target for multiple disorders associated with OS [25]. FXR, a TF widely expressed in the kidney, has been demonstrated to protect kidney tissues against OS triggered by ischemic events, metabolic changes, and other stressors [22, 26]. Moreover, the dual upregulation of Nrf2 and FXR was effective against ischemia- and hyperglycemia-induced kidney injury [22, 26], demonstrating their crucial role in protecting the kidney against various insults and maintaining its proper function.

The discovery of natural plant compounds with anti-inflammatory and antioxidant properties and elucidating their mechanism of action could greatly contribute to advancing methods to ameliorate nephrotoxicity. Several phytochemicals, including farnesol (FAR), have been found to scavenge and neutralize free radicals, thereby alleviating OS, inflammatory response, and cellular damage [27, 28]. FAR is a sesquiterpene found in the essential oils of ambrette seeds and citronella, and present in many aromatic plants. It possesses antioxidant, anti-cancer, and anti-inflammatory properties *in vivo* and *in vitro* [27-31]. In addition, it inhibits the formation of biofilms from different microbial species [32], and has been used as a flavoring agent in food industry [33]. While the protective effects of FAR in various conditions have been well-documented, its potential in protecting the kidney against CPF-induced toxicity has not yet been explored. Therefore, in this study, we used a rat model of CPF nephrotoxicity to address

the hypothesis that FAR exhibits nephroprotective properties against OS, inflammation, and apoptosis, with a focus on the role of Nrf2 and FXR.

2. Materials and Methods

2.1. Animals and treatments

Male Wistar rats (180-200 g) were housed under standard conditions of temperature and humidity on a 12h dark/light cycle with free access to food and water. The animal study protocol was approved by the ethics committee of Al-Azhar University (Assiut, Egypt) (AZ-AS/PH-REC/36/24). After acclimatization for a week, the rats were allocated into four groups (n = 6). CPF (Agro Chem, Egypt) and FAR (Purity 95%; Sigma, USA) were dissolved in corn oil and 0.5% carboxymethyl cellulose (CMC), respectively, and orally administered for 28 days. Group I received the vehicles and Group II and IV received 10 mg/kg FAR [28] and corn oil. Group III and V received 10 mg/kg CPF [34] and 0.5% CMC (Group III), and 10 mg/kg FAR [28] (Group V). At the end of the treatment period, blood was collected under anesthesia using ketamine/xylazine and serum was prepared. After scarification, the kidneys were excised, and samples were collected on 10% neutral buffered formalin (NBF) and others on RNALater. Other samples were homogenized in cold Tris-HCl buffer (pH=7.4), centrifuged and the supernatant was collected for biochemical analysis.

2.2. Biochemical assays

Serum creatinine and urea were determined using Biodignostic (Egypt) kits. NF-κB p65, TNF-α, IL-1β, and caspase-3 in the kidney homogenate were measured using Cusabio (China) ELISA kits. Malondialdehyde (MDA), GSH, nitric oxide (NO), SOD, GPx, and catalase were assayed using Biodiagnostic (Egypt) kits. All assays were conducted as instructed by the manufacturers. The activity of HO-1 was assayed following the method of Abraham et al [35].

2.3. qRT-PCR

The changes in mRNA levels of NF-κB p65, IL-1β, TNF-α, iNOS, HO-1, NQO-1, caspase-3, Keap1, FXR, and Nrf2 were evaluated using qRT-PCR. Total RNA was isolated from the frozen samples using RNA Purification Kit (Thermo Scientific, USA), quantified using a nanodrop and RNA with OD260/OD280 ≥ 1.8 was reverse transcribed into cDNA. SYBR Green master mix (Thermo Scientific, USA) and the set of primers listed in Suppl. Table I were used to amplify cDNA. The obtained Ct values were analyzed via the $2^{-\Delta\Delta Ct}$ method [36] using β-actin as a housekeeping gene.

2.4. Histopathological and immunohistochemical (IHC) examinations

Tissue samples were fixed in 10% NBF for 24 h and then dehydrated, cleared, and embedded in paraffin wax. Five-μm sections were cut and stained with hematoxylin/eosin (H&E). Other sections were processed to evaluate changes in cleaved caspase-3 using IHC staining as we recently reported [22]. In brief, the tissue sections were deparaffinized and treated with citrate buffer (50 mM, pH 6.8), 0.3% H_2O_2 and then protein block. Primary anti-body (Biospes, China) was added, and the sections were incubated overnight at 4°C. After washing and incubation with the secondary antibody, the color was developed using DAB followed by Mayer's hematoxylin counterstaining. The color intensity was measured (6 images/rat) using ImageJ (NIH, USA).

2.5. Molecular docking

The affinity of FAR towards NF-κB (PDB ID: 5U01), FXR (PDB ID: 7D42), iNOS (PDB ID: 3EAI), Keap1 (PDB ID: 5CGJ), caspase-3 (PDB ID: 1NME), and HO-1 (PDB ID: 1DVE) was explored using PyRx virtual screening software (version 0.8) [37]. The protein targets were prepared using Autodock Tools (ADT; v1.5.6). The binding affinities of FAR, RA839 (selective activator of Nrf2 signaling), and tropifexor (FXR agonist) were investigated, PyMOL (v2.3.2) was used for molecular visualization and binding mode inspection, and LigPlot (v2.2.8) [38] was used to obtain protein-ligand interactions.

2.6. Statistical analysis

Results are expressed as means ± SD. Statistical significance of the data was calculated by one-way ANOVA followed by Tukey's post-hoc test using GraphPad Prism 7 software. $P < 0.05$ was considered significant.

3. Results

3.1. FAR ameliorated CPF-induced kidney injury

CPF caused injury to the kidney as assessed through biochemical and microscopic investigations (Fig. 1). Circulating creatinine (Fig. 1A) and urea (Fig. 1B) levels increased markedly in CPF-administered rats ($P<0.001$). Tissue sections in CPF-administered rat kidney showed glomerular damage, hemorrhage, tubular degeneration, and other manifestations (Fig. 1C). FAR ameliorated circulating creatinine and urea, and tissue damage in CPF-intoxicated rats.

3.2. FAR attenuates CPF-induced OS in rat kidney

Kidney MDA levels increased significantly in rats that received CPF as compared to the control animals (Fig. 2A; $P<0.001$). Kidney GSH content and activities of SOD, CAT and GPx were inversely changed in CPF (Fig. 2B-E). FAR effectively decreased kidney MDA and boosted antioxidants in CPF-administered rats ($P<0.001$).

3.3. FAR suppresses kidney inflammation in CPF-administered rats

Changes in the expression of NF-κB p65 and inflammatory mediators were determined to evaluate the impact of FAR on CPF-induced inflammation. Additionally, molecular docking was employed to investigate the binding affinity of FAR towards NF-κB p65 and iNOS. Data shown in Figure 3A and 3B revealed upregulated kidney NF-κB p65 mRNA and protein levels, respectively, following CPF administration ($P<0.001$). FAR suppressed NF-κB p65 expression in CPF-administered rat kidney. *In silico* data revealed the affinity of FAR to bind 10 amino acid residues of NF-κB RelA dimer with 2 polar bonding and 8 hydrophobic interactions (Fig.

3C & Table 1). TNF-α and IL-1β mRNA levels were upregulated in rats administered with CPF (Fig. 4A-B). Similarly, the protein levels of both cytokines (Fig. 4C-D) were remarkably ($P<0.001$) increased in CPF-administered rat kidney. Kidney iNOS mRNA was also upregulated (Fig. 5A) and NO levels increased (Fig. 5B) in CPF-administered rats ($P<0.001$). FAR decreased TNF-α, IL-1β, and iNOS expression and NO production in the kidney of CPF-administered rats ($P<0.001$). FAR exhibited in silico affinity towards iNOS with 2 polar bonds and 10 hydrophobic interactions (Fig. 5C & Table 1).

3.4. FAR prevents apoptosis in CPF-administered rats

Caspase-3 mRNA (Fig. 6A) and cleaved caspase-3 (Fig. 6B) were upregulated in CPF-treated rat kidney ($P<0.001$). FAR showed a suppressive effect on kidney caspase-3 in rats that received CPF. In silico, FAR exhibited 4 polar bonding and 5 hydrophobic interactions with 9 amino acid residues of caspase-3 (Fig. 6D & Table 1).

3.5. FAR modulates kidney Keap1/Nrf2/HO-1 signaling and FXR in CPF-administered rats

CPF increased kidney Keap-1 (Fig. 7A), while decreased Nrf2 (Fig. 7B), HO-1 (Fig. 7C), NQO-1 (Fig. 7D), and FXR (Fig. 7F) mRNA significantly ($P<0.001$). HO-1 activity was remarkable declined in rats that received CPF ($P<0.001$; Fig. 7E). FAR effectively decreased Keap-1, and enhanced Nrf2, HO-1, NQO-1, and FXR in CPF-administered rat kidney. Of note, the effect of FAR on FXR mRNA in the kidney of normal rats was significant ($P<0.05$).

In silico investigations of the affinity of FAR towards Keap1 showed its ability to form polar bonding with 2 amino acid residues and hydrophobic interactions with 14 residues (Fig. 8A & Table 1). Twelve of the amino acid residues were observed in the binding mode of the selective Nrf2 signaling activator RA839 (Suppl. Fig. I & Table 1). With HO-1, FAR exhibited 2 polar bonding and 9 hydrophobic interactions with amino acid residues (Fig. 8B & Table 1). The binding mode of FAR with FXR revealed binding with 13 amino acid residues (Fig. 9 & Table

1), of which 11 were observed in the binding mode of the FXR agonist tropifexor (Suppl. Fig. II & Table 1).

4. Discussion

The extensive use of pesticides, including CPF to control pests in households and agricultural settings may lead to increased risks of toxic effects on animals and humans [3]. OS and inflammation are centrally implicated in the reported toxic effects of CPF [14-16]. Kidney injury associated with elevated ROS and inflammatory mediators is among the deleterious effects of exposure to CPF [16]. Therefore, attenuation of these processes could be effective in mitigating CPF nephrotoxicity. FAR is an isoprenoid alcohol with demonstrated efficacy against OS in rodent models of hypercholesterolemia [28], ferric nitrilotriacetate (Fe-NTA) nephrotoxicity [29], and cigarette smoke extract (CSE)-induced lung injury [30]. Through *in silico* and *in vivo* investigations, this study evaluated the role of FAR against CPF nephrotoxicity, OS, inflammation, and apoptosis, pointing to the possible role of Nrf2 and FXR.

We used a model of CPF-induced nephrotoxicity in rats to explore the potential of FAR in ameliorating kidney injury. CPF intoxication resulted in a remarkable kidney injury indicated by a significant elevation of creatinine and urea, which is in parallel with other investigators' findings [16, 18, 39]. In addition to these essential biomarkers, our previous study revealed increased circulating levels of the transmembrane protein kidney injury molecule-1 in rats exposed to CPF. Indeed, Kim-1 is not present in normal physiological conditions, and it is overexpressed in proximal tubule apical membrane during kidney injury to control cellular injury and improve tubular reepithelization [40]. Besides biochemical findings of this study, histopathological investigation revealed remarkable degenerative glomeruli, tubular damage, hemorrhage, and other structural changes in CPF-treated rats. Interestingly, FAR demonstrated nephroprotective potential against the toxic effects of CPF by significantly reducing the serum

levels of creatinine and urea, in addition to the remarkable attenuation of histopathological changes indicated by the improvement of kidney structure. These findings are in line with the previously reported protective effect of FAR against Fe-NTA-mediated renal injury [29]. The neuroprotective effect of FAR was linked to attenuation of OS [29], and in view of the role of OS and inflammation in CPF toxicity, the beneficial effect of FAR could be directly connected to its antioxidant and anti-inflammatory properties. Here, CPF increased MDA and decreased renal GSH, SOD, catalase, and GPx, demonstrating OS. Neuronal and microglial cells challenged with CPF in vitro exhibited surplus levels of ROS [14, 15], and effect that has been demonstrated in rat kidney [16, 18]. There are increasing number of investigations on the ability of CPF to promote inflammatory response linked to surplus ROS production [14]. Excess ROS can activate various signaling molecules associated with inflammation and cell dysfunction, including NF-κB [14, 17]. In the current study, CPF increased the mRNA and protein expression levels of NF-κB p65, TNF-α, IL-1β, and iNOS as well as NO levels in rat kidney. These findings denoted the provoked inflammatory response in rat kidney. Accordingly, CPF upregulated iNOS in liver [41] and kidney of rats [16]. This explained the reported increase in NO that reacts with superoxide and the produced peroxynitrite can break DNA and causes cell death [42]. Collectively, our findings suggest the toxic effects and the ability of CPF to initiate inflammatory responses in renal tissues. The produced cytokines and the generated free radicals by CPF have a detrimental effect on the permeability of mitochondria, leading to cytochrome *c* release into the cytoplasm and the activation of caspase-3 which initiates apoptosis. We found that CPF increased cleaved caspase-3, an executioner responsible for triggering cell death by provoking breakdown of DNA and the cytoskeleton [43, 44].

FAR effectively mitigated kidney OS, inflammatory response and apoptosis induced by CPF, demonstrating a potent neuroprotective efficacy. Treatment with FRA resulted in remarkable

restoration of renal antioxidants as well as a reduction of renal MDA. Additionally, FAR was able to counteract the deleterious effects of CPF on renal tissues by mitigating the inflammatory responses through inhibition of NF-κB and downregulation of the release of pro-inflammatory mediators. Attenuation of OS and inflammation mediated the anti-apoptotic role of FAR in rats that received CPF. These findings supported studies showing that FAR exhibited potent anti-inflammatory and antioxidant properties [28, 30, 45, 46]. In rats with hypercholesterolemia, FAR inhibited OS and inflammation and enhances antioxidant defenses in the liver [28]. Ku and Lin demonstrated the ability of FAR to suppress inflammation by downregulating TNF-α in the lung of asthmatic mice [45]. In rats with cigarette smoke extract-induced lung injury [30], FAR ameliorated MDA, GPx and catalase, and suppressed inflammation. FAR supplementation provided potent anti-inflammatory properties by reducing IL-6, TNF-α, and iNOS levels associated with gliosis in mice [47], and prevented OS and early tumorigenesis in a rat model of Fe-NTA nephrotoxicity [29]. In primary human renal epithelial cells, FAR suppressed inflammatory gene expression as reported recently by Müller et al [48]. The ability of FAR to suppress caspase-3 was demonstrated in rats with colonic damage [46]. These studies along with our findings demonstrated the antioxidant, anti-inflammatory, and cytoprotective efficacies of FAR. Furthermore, we employed molecular docking to further explore these beneficial effects of FAR. Our investigation revealed the affinity of FAR with NF-κB p65, iNOS, and caspase-3. The binding of FAR with these protein targets involved numerous polar bonds and hydrophobic interactions, reflecting the stability of the formed complexes. The bindings between FAR and NF-κB, iNOS, and caspase-3 might be involved in mitigating inflammation and apoptosis by preventing the nuclear translocation and binding of the p65 subunit to DNA.

To further explore the nephroprotective mechanism of FAR, in particular suppression of OS and inflammation, we evaluated changes in FXR and Nrf2/HO-1 signaling in rat kidney. CPF

upregulation kidney Keap-1, downregulated Nrf2, HO-1, NQO-1, and FXR mRNA, and decreased HO-1 activity. These data demonstrated a suppression of FXR and Nrf2/HO-1 signaling which coincided with the surplus levels of ROS and declined antioxidants. Nrf2 plays a crucial role in regulating the balance of redox within the cell, and it is usually held in place by the endogenous inhibitor Keap-1 in the cytoplasm. However, upon exposure to electrophiles or increased ROS, Nrf2 detaches from Keap-1 and translocates into the nucleus to promote the expression of antioxidant and cytodefensive genes [25]. A lack of Nrf2 is associated with kidney injury [49], and CPF has been shown to hinder Nrf2 signaling in many organisms and different tissue types [41, 50, 51]. We also reported in this study that administration of CPF resulted in a significant downregulation of renal FXR. FXR belongs to the nuclear receptor superfamily of ligand-regulated transcription factors that pairs with RXR to regulate transcription of many target genes, including NF-κB [52]. Moreover, FXR is highly expressed in different organs, including the kidney, and it is involved in several pathophysiological conditions such as inflammation and immune response [52, 53]. FXR deficiency was associated with accelerated progression of diabetic kidney disease [54], whereas its activation significantly reduced LPO by upregulating ferroptosis gatekeepers [55], inhibited mitochondrial dysfunction and excess ROS in ischemic kidney [26], and attenuated OS and inflammation in diabetic kidney [56]. Recently, we have demonstrated in rats that upregulation of Nrf2 and FXR by simvastatin mitigated diabetic nephropathy and attenuated OS and inflammation [22]. In the current study, FAR significantly downregulated renal Keap-1 expression, while upregulated Nrf2, HO-1, NQO-1, and FXR expression. These findings suggested the nephroprotective properties of FAR and its ability to activate Nrf2 and FXR to scavenge ROS and attenuate the inflammatory response associated with cell death. In support of our results, FAR activated Nrf2 and protected the hippocampus of mice against cognitive impairment as recently reported by [57]. This study introduced novel information that FXR

and Nrf2 upregulation contributed to the protective effect of FAR against CPF nephrotoxicity. However, the lack of protein expression levels of FXR could be considered a limitation. The in silico findings introduced interesting information about the binding affinity of FAR with Keap-1 and FXR. FAR exhibited high binding affinity with Keap-1 and FXR comparable to the specific Nrf2 signaling activator RA839 and tropifexor, respectively. In addition, FAR exhibited interaction potency with HO-1. The observed relatively low binding energies and the multiple hydrophobic interactions and polar bonding proposed the potency of FAR to modulate Keap-1, HO-1, and FXR.

5. Conclusions

This study is the first to demonstrate the protective role of FAR against OS and inflammation in rat kidney induced by CPF, and the implication of Nrf2/HO-1 and FXR in its nephroprotection. FAR inhibited kidney injury, attenuated OS, and mitigated inflammatory responses in the kidney of CPF-administered rats. In addition, FAR upregulated the expression of Nrf2/HO-1 and FXR and improved the cellular defense system by upregulating renal antioxidants. *In silico* investigations showed the binding affinity of FAR towards NF-κB, iNOS, caspase-3, HO-1, Keap-1 and FXR. This study demonstrated that FAR has a potential against renal injury induced by exposure to the toxic pesticide CPF. Nevertheless, further investigations and elucidation of underlying mechanisms and clinical trials are highly recommended.

Declaration of competing interest

No conflict of interest is to be declared.

Data availability

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

Acknowledgment

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References:

[1] S. Suratman, J.W. Edwards, K. Babina, Organophosphate pesticides exposure among farmworkers: pathways and risk of adverse health effects, Reviews on environmental health 30(1) (2015) 65-79.
[2] J.V. Peter, T.I. Sudarsan, J.L. Moran, Clinical features of organophosphate poisoning: A review of different classification systems and approaches, Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine 18(11) (2014) 735-45.
[3] H.U. ur Rahman, W. Asghar, W. Nazir, M.A. Sandhu, A. Ahmed, N. Khalid, A comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: Evidence of mechanisms, exposures and mitigation strategies, Science of The Total Environment 755 (2021) 142649.
[4] R.D. Burke, S.W. Todd, E. Lumsden, R.J. Mullins, J. Mamczarz, W.P. Fawcett, R.P. Gullapalli, W.R. Randall, E.F.R. Pereira, E.X. Albuquerque, Developmental neurotoxicity of the organophosphorus insecticide chlorpyrifos: from clinical findings to preclinical models and potential mechanisms, J Neurochem 142 Suppl 2(Suppl 2) (2017) 162-177.
[5] J. Perry, J. Cotton, M.A. Rahman, S.A. Brumby, Organophosphate exposure and the chronic effects on farmers: a narrative review, Rural and Remote Health 20(1) (2020) 206-222.
[6] J. Li, F. Ren, Y. Li, J. Luo, G. Pang, Chlorpyrifos induces metabolic disruption by altering levels of reproductive hormones, Journal of agricultural and food chemistry 67(38) (2019) 10553-10562.
[7] A. Mehta, R.S. Verma, N. Srivastava, Chlorpyrifos-induced DNA damage in rat liver and brain, Environmental and molecular mutagenesis 49(6) (2008) 426-433.
[8] J.E. Casida, G.B. Quistad, Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets, Chemical research in toxicology 17(8) (2004) 983-998.
[9] C. Uchendu, S.F. Ambali, J.O. Ayo, The organophosphate, chlorpyrifos, oxidative stress and the role of some antioxidants: a review, (2012).
[10] M. Alamgir Zaman Chowdhury, A.N.M. Fakhruddin, M. Nazrul Islam, M. Moniruzzaman, S.H. Gan, M. Khorshed Alam, Detection of the residues of nineteen pesticides in fresh vegetable samples using gas chromatography–mass spectrometry, Food Control 34(2) (2013) 457-465.
[11] E.F.S. Authority, P. Medina-Pastor, G. Triacchini, The 2018 European Union report on pesticide residues in food, EFSA Journal 18(4) (2020).
[12] S. Sharma, P. Chadha, Induction of neurotoxicity by organophosphate pesticide chlorpyrifos and modulating role of cow urine, SpringerPlus 5(1) (2016) 1-7.
[13] M. Saoudi, R. Badraoui, F. Rahmouni, K. Jamoussi, A. El Feki, Antioxidant and protective effects of Artemisia campestris essential oil against chlorpyrifos-induced kidney and liver injuries in rats, Frontiers in Physiology 12 (2021) 618582.
[14] J.-W. Lin, S.-C. Fu, J.-M. Liu, S.-H. Liu, K.-I. Lee, K.-M. Fang, R.-J. Hsu, C.-F. Huang, K.-M. Liu, K.-C. Chang, C.-C. Su, Y.-W. Chen, Chlorpyrifos induces neuronal cell death via both oxidative stress and Akt activation downstream-regulated CHOP-triggered apoptotic pathways, Toxicology in Vitro 86 (2023) 105483.
[15] G.C.C. Weis, C.E. Assmann, V.B. Mostardeiro, A.O. Alves, J.R. da Rosa, M.M. Pillat, C.M. de Andrade, M.R.C. Schetinger, V.M.M. Morsch, I.B.M. da Cruz, I.H. Costabeber, Chlorpyrifos

pesticide promotes oxidative stress and increases inflammatory states in BV-2 microglial cells: A role in neuroinflammation, Chemosphere 278 (2021) 130417.
[16] M.S. Abduh, R.S. Alruhaimi, H.A. Alqhtani, O.E. Hussein, M.H. Abukhalil, E.M. Kamel, A.M. Mahmoud, Rosmarinic acid mitigates chlorpyrifos-induced oxidative stress, inflammation, and kidney injury in rats by modulating SIRT1 and Nrf2/HO-1 signaling, Life Sci 313 (2023) 121281.
[17] N. Kopjar, S. Žunec, G. Mendaš, V. Micek, V. Kašuba, A. Mikolić, B.T. Lovaković, M. Milić, I. Pavičić, A.M.M. Čermak, A. Pizent, A. Lucić Vrdoljak, D. Želježić, Evaluation of chlorpyrifos toxicity through a 28-day study: Cholinesterase activity, oxidative stress responses, parent compound/metabolite levels, and primary DNA damage in blood and brain tissue of adult male Wistar rats, Chemico-Biological Interactions 279 (2018) 51-63.
[18] S. Küçükler, S. Çomaklı, S. Özdemir, C. Çağlayan, F.M. Kandemir, Hesperidin protects against the chlorpyrifos-induced chronic hepato-renal toxicity in rats associated with oxidative stress, inflammation, apoptosis, autophagy, and up-regulation of PARP-1/VEGF, Environmental toxicology 36(8) (2021) 1600-1617.
[19] S. Ghahremani, M. Soodi, A. Atashi, Quercetin ameliorates chlorpyrifos-induced oxidative stress in the rat brain: Possible involvment of PON2 pathway, Journal of food biochemistry 42(3) (2018) e12530.
[20] B. Mesbahzadeh, H. Salarjavan, S. Samarghandian, T. Farkhondeh, Chlorpyrifos with age-dependent effects in cardiac tissue of male rats, Current Molecular Pharmacology 14(5) (2021) 894-899.
[21] P. Ma, Y. Wu, Q. Zeng, Y. Gan, J. Chen, X. Ye, X. Yang, Oxidative damage induced by chlorpyrifos in the hepatic and renal tissue of Kunming mice and the antioxidant role of vitamin E, Food and chemical toxicology 58 (2013) 177-183.
[22] I.H. Hasan, S.Y. Shaheen, A.M. Alhusaini, A.M. Mahmoud, Simvastatin mitigates diabetic nephropathy by upregulating farnesoid X receptor and Nrf2/HO-1 signaling and attenuating oxidative stress and inflammation in rats, Life Sciences (2024) 122445.
[23] A.M. Mahmoud, O.E. Hussein, S.M. Abd El-Twab, W.G. Hozayen, Ferulic acid protects against methotrexate nephrotoxicity via activation of Nrf2/ARE/HO-1 signaling and PPARγ, and suppression of NF-κB/NLRP3 inflammasome axis, Food & function 10(8) (2019) 4593-4607.
[24] S.M. Abd El-Twab, O.E. Hussein, W.G. Hozayen, M. Bin-Jumah, A.M. Mahmoud, Chicoric acid prevents methotrexate-induced kidney injury by suppressing NF-κB/NLRP3 inflammasome activation and up-regulating Nrf2/ARE/HO-1 signaling, Inflammation research : official journal of the European Histamine Research Society ... [et al.] 68(6) (2019) 511-523.
[25] S. Satta, A.M. Mahmoud, F.L. Wilkinson, M. Yvonne Alexander, S.J. White, The Role of Nrf2 in Cardiovascular Function and Disease, Oxidative Medicine and Cellular Longevity 2017 (2017) 9237263.
[26] Z. Gai, L. Chu, Z. Xu, X. Song, D. Sun, G.A. Kullak-Ublick, Farnesoid X receptor activation protects the kidney from ischemia-reperfusion damage, Scientific Reports 7(1) (2017) 9815.
[27] R. Santhanasabapathy, G. Sudhandiran, Farnesol attenuates lipopolysaccharide-induced neurodegeneration in Swiss albino mice by regulating intrinsic apoptotic cascade, Brain Research 1620 (2015) 42-56.
[28] M.H. Abukhalil, O.E. Hussein, M. Bin-Jumah, S.A.M. Saghir, M.O. Germoush, H.A. Elgebaly, N.M. Mosa, I. Hamad, M.M. Qarmush, E.M. Hassanein, E.M. Kamel, R. Hernandez-Bautista, A.M. Mahmoud, Farnesol attenuates oxidative stress and liver injury and modulates fatty acid synthase and acetyl-CoA carboxylase in high cholesterol-fed rats, Environmental Science and Pollution Research 27(24) (2020) 30118-30132.
[29] T. Jahangir, T.H. Khan, L. Prasad, S. Sultana, Farnesol prevents Fe-NTA-mediated renal oxidative stress and early tumour promotion markers in rats, Human & experimental toxicology 25(5) (2006) 235-242.

[30] W. Qamar, S. Sultana, Farnesol ameliorates massive inflammation, oxidative stress and lung injury induced by intratracheal instillation of cigarette smoke extract in rats: an initial step in lung chemoprevention, Chemico-biological interactions 176(2-3) (2008) 79-87.
[31] Y.Y. Jung, S.T. Hwang, G. Sethi, L. Fan, F. Arfuso, K.S. Ahn, Potential anti-inflammatory and anti-cancer properties of farnesol, Molecules 23(11) (2018) 2827.
[32] A.F. Costa, L.D.C. Silva, A.C. Amaral, Farnesol: An approach on biofilms and nanotechnology, Med Mycol 59(10) (2021) 958-969.
[33] G. de Araújo Delmondes, D.S. Bezerra, D. de Queiroz Dias, A. de Souza Borges, I.M. Araújo, G. Lins da Cunha, P.F.R. Bandeira, R. Barbosa, H.D. Melo Coutinho, C.F.B. Felipe, J.M. Barbosa-Filho, I.R. Alencar de Menezes, M.R. Kerntopf, Toxicological and pharmacologic effects of farnesol (C15H26O): A descriptive systematic review, Food and Chemical Toxicology 129 (2019) 169-200.
[34] B. Wielgomas, J. Krechniak, Effect of α-Cypermethrin and Chlorpyrifos in a 28-Day Study on Free Radical Parameters and Cholinesterase Activity in Wistar Rats, Polish Journal of Environmental Studies 16(1) (2007).
[35] N.G. Abraham, J.D. Lutton, R.D. Levere, Heme metabolism and erythropoiesis in abnormal iron states: role of delta-aminolevulinic acid synthase and heme oxygenase, Experimental hematology 13(8) (1985) 838-843.
[36] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods. 2001 Dec;25(4):402-8. (2011).
[37] S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, Methods Mol Biol 1263 (2015) 243-50.
[38] A.C. Wallace, R.A. Laskowski, J.M. Thornton, LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, Protein Eng 8(2) (1995) 127-34.
[39] S.E. Owumi, U.J. Dim, Manganese suppresses oxidative stress, inflammation and caspase-3 activation in rats exposed to chlorpyrifos, Toxicology Reports 6 (2019) 202-209.
[40] J.V. Bonventre, Kidney Injury Molecule-1 (KIM-1): a specific and sensitive biomarker of kidney injury, Scandinavian journal of clinical and laboratory investigation. Supplementum 241 (2008) 78-83.
[41] G. Albasher, R. Almeer, F.O. Al-Otibi, N. Al-Kubaisi, A.M. Mahmoud, Ameliorative Effect of Beta vulgaris Root Extract on Chlorpyrifos-Induced Oxidative Stress, Inflammation and Liver Injury in Rats, Biomolecules 9(7) (2019).
[42] P. Pacher, J.S. Beckman, L. Liaudet, Nitric Oxide and Peroxynitrite in Health and Disease, Physiol Rev. 87(1) (2007) 315-424.
[43] D.R. Green, Apoptotic pathways: the roads to ruin, Cell 94(6) (1998) 695-8.
[44] M. Redza-Dutordoir, D.A. Averill-Bates, Activation of apoptosis signalling pathways by reactive oxygen species, Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1863(12) (2016) 2977-2992.
[45] C.-M. Ku, J.-Y. Lin, Farnesol, a sesquiterpene alcohol in herbal plants, exerts anti-inflammatory and antiallergic effects on ovalbumin-sensitized and-challenged asthmatic mice, Evidence-Based Complementary and Alternative Medicine 2015 (2015).
[46] R. Khan, S. Sultana, Farnesol attenuates 1, 2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic responses in the colon of Wistar rats, Chemico-biological interactions 192(3) (2011) 193-200.
[47] R. Santhanasabapathy, S. Vasudevan, K. Anupriya, R. Pabitha, G. Sudhandiran, Farnesol quells oxidative stress, reactive gliosis and inflammation during acrylamide-induced neurotoxicity: Behavioral and biochemical evidence, Neuroscience 308 (2015) 212-227.
[48] A. Müller, M. Lozoya, X. Chen, V. Weissig, M. Nourbakhsh, Farnesol Inhibits PI3 Kinase Signaling and Inflammatory Gene Expression in Primary Human Renal Epithelial Cells, Biomedicines 11(12) (2023).

[49] M. Liu, D.N. Grigoryev, M.T. Crow, M. Haas, M. Yamamoto, S.P. Reddy, H. Rabb, Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice, Kidney international 76(3) (2009) 277-285.
[50] N.R. Rodrigues, J.E.d.S. Batista, L.R. de Souza, I.K. Martins, G.E. Macedo, L.C. da Cruz, D.G. da Costa Silva, A.I. Pinho, H.D.M. Coutinho, G.L. Wallau, T. Posser, J.L. Franco, Activation of p38MAPK and NRF2 signaling pathways in the toxicity induced by chlorpyrifos in Drosophila melanogaster: Protective effects of Psidium guajava pomífera L. (Myrtaceae) hydroalcoholic extract, Arabian Journal of Chemistry 12(8) (2019) 3490-3502.
[51] M.W. Zhao, P. Yang, L.L. Zhao, Chlorpyrifos activates cell pyroptosis and increases susceptibility on oxidative stress-induced toxicity by miR-181/SIRT1/PGC-1α/Nrf2 signaling pathway in human neuroblastoma SH-SY5Y cells, Environmental toxicology 34(6) (2019) 699-707.
[52] Y.D. Wang, W.D. Chen, M. Wang, D. Yu, B.M. Forman, W. Huang, Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response, Hepatology 48(5) (2008) 1632-43.
[53] Y. Zhang, H.R. Kast-Woelbern, P.A. Edwards, Natural structural variants of the nuclear receptor farnesoid X receptor affect transcriptional activation, Journal of Biological Chemistry 278(1) (2003) 104-110.
[54] X.X. Wang, T. Jiang, Y. Shen, Y. Caldas, S. Miyazaki-Anzai, H. Santamaria, C. Urbanek, N. Solis, P. Scherzer, L. Lewis, F.J. Gonzalez, L. Adorini, M. Pruzanski, J.B. Kopp, J.W. Verlander, M. Levi, Diabetic nephropathy is accelerated by farnesoid X receptor deficiency and inhibited by farnesoid X receptor activation in a type 1 diabetes model, Diabetes 59(11) (2010) 2916-27.
[55] J. Tschuck, L. Theilacker, I. Rothenaigner, S.A.I. Weiß, B. Akdogan, V.T. Lam, C. Müller, R. Graf, S. Brandner, C. Pütz, T. Rieder, P. Schmitt-Kopplin, M. Vincendeau, H. Zischka, K. Schorpp, K. Hadian, Farnesoid X receptor activation by bile acids suppresses lipid peroxidation and ferroptosis, Nat Commun 14(1) (2023) 6908.
[56] T. Jiang, X.X. Wang, P. Scherzer, P. Wilson, J. Tallman, H. Takahashi, J. Li, M. Iwahashi, E. Sutherland, L. Arend, M. Levi, Farnesoid X receptor modulates renal lipid metabolism, fibrosis, and diabetic nephropathy, Diabetes 56(10) (2007) 2485-93.
[57] Y. Li, Z. Xie, X. Luo, X. Wang, Y. Wang, M. Guo, Z. Zhou, R. Sun, D. Hua, A. Luo, S. Li, Farnesol Exerts Protective Effects against Chronic Sleep Deprivation-Induced Cognitive Impairment via Activation SIRT1/Nrf2 Pathway in the Hippocampi of Adult Mice, Mol Nutr Food Res 67(11) (2023) e2200735.

Tables:

Table 1. Binding affinities of FAR towards NF-κB, iNOS, caspase-3, HO-1, Keap1, and FXR.

	Lowest binding energy (kcal/mol)	**Polar interacting residues**	**Hydrophobic interacting residues**
NF-κB/FAR	-5.9	Asp217, Asn186	Gln247, Lys218, Val248, Val248, Arg246, Arg246, Lys218, Arg187
iNOS/FAR	-7.8	Leu119, Tyr483	Trp188, Cys194, Arg193, Tyr484, Phe363, Pro192, Ala191, Met349, Phe482, Tyr485
Caspase-3/FAR	-5.2	Ala227, Asp228, Lys224, Leu223,	Leu81, Thr77, Ala221, Asn80, Gln225
HO-1/FAR	-6.4	Arg136, Thr135	Phe167, Leu54, Asn210, Met51 ,Val50, Phe37, Leu147, Gly139, Gly144
Keap1/FAR	-5.7	Val604, Leu365	Val463, Ile416, Val465, Ala366, Gly367, Val606, Ile559, Gly364, Gly464, Gly417, Leu557, Gly603, Val418, Gly605
Keap1/RA839	-9.5	Val604, Val418, Leu557	Val463, Ile416, Val465, Ala366, Gly367, Val606, Ile559, Ala510, Cys513, Val512, Gly419, Val420, Gly605, Leu365, Thr560, Ala607, Val608
FXR/FAR	-6.9	Tyr369, Ser332	Leu287, Trp454, Phe461, Phe443, Ile352, Val325, Phe329, Met328, His447, Met365, Trp469
FXR/Tropifexor	-10.9	Arg331	Leu287, Trp454, Phe461, Phe443, Ile352, Val325, Phe329, Met328, His447, Ser332, Ile357, Phe284, Thr288, Ala291, Tyr369, Ile335, Leu348, His294, Met265

Figures:

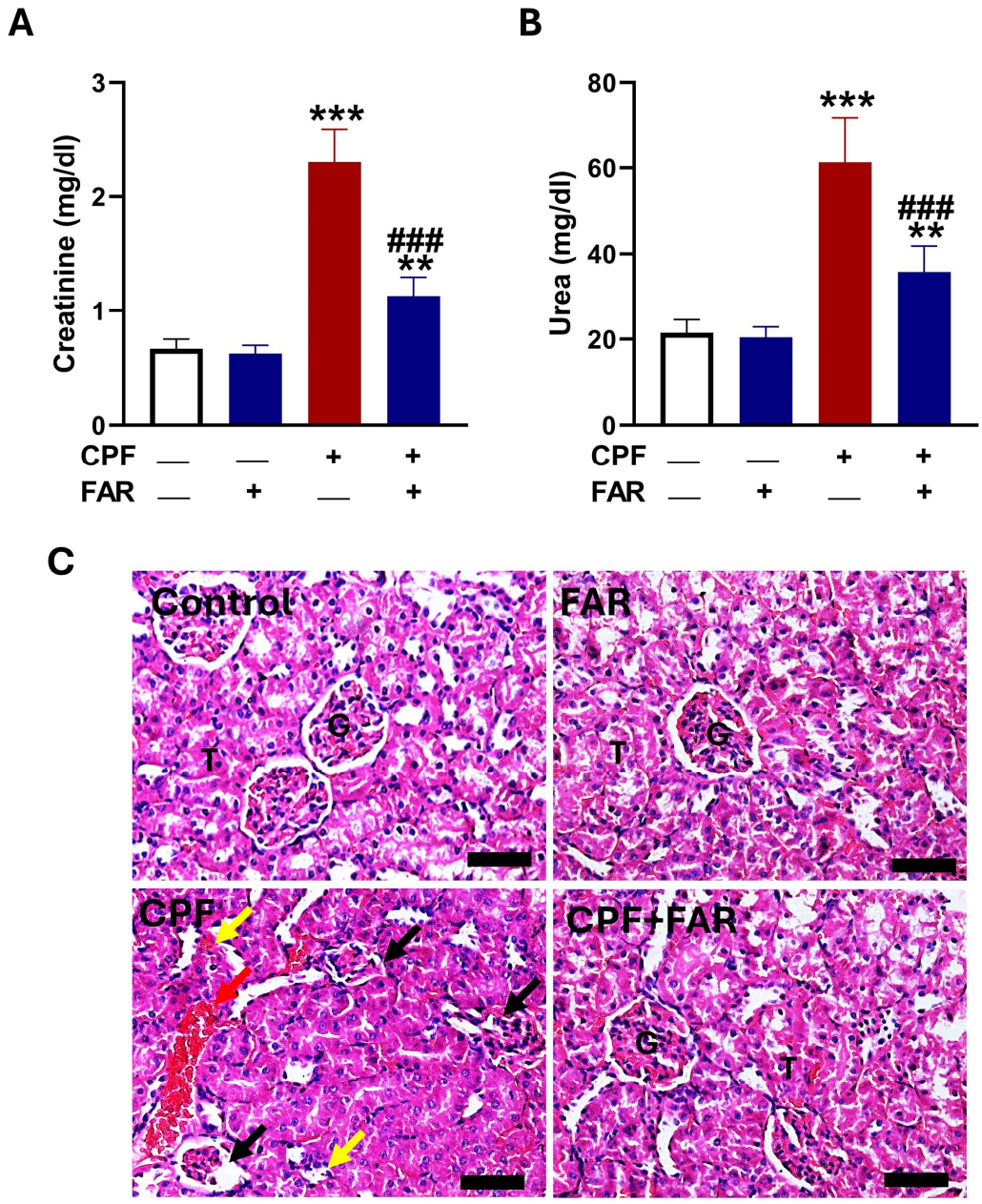

Fig. 1. FAR prevented CPF-induced kidney injury. (A-B) FAR ameliorated serum creatinine (A) and urea (B). Data are mean ± SD, (n = 6). **P<0.01 and ***P<0.001 vs Control, and ###P<0.001 vs CPF. (D) Photomicrographs of H&E-stained sections in kidney of control and FAR-treated rats showing normal glomeruli (G) and tubules (T), CPF-administered rats showing altered glomeruli (black arrow), degenerative changes in renal tubules (yellow arrow) and hemorrhage (red arrow), and CPF-administered rats treated with FAR showing improvement in glomeruli (G) and renal tubules (T). Scale bar = 50 µm.

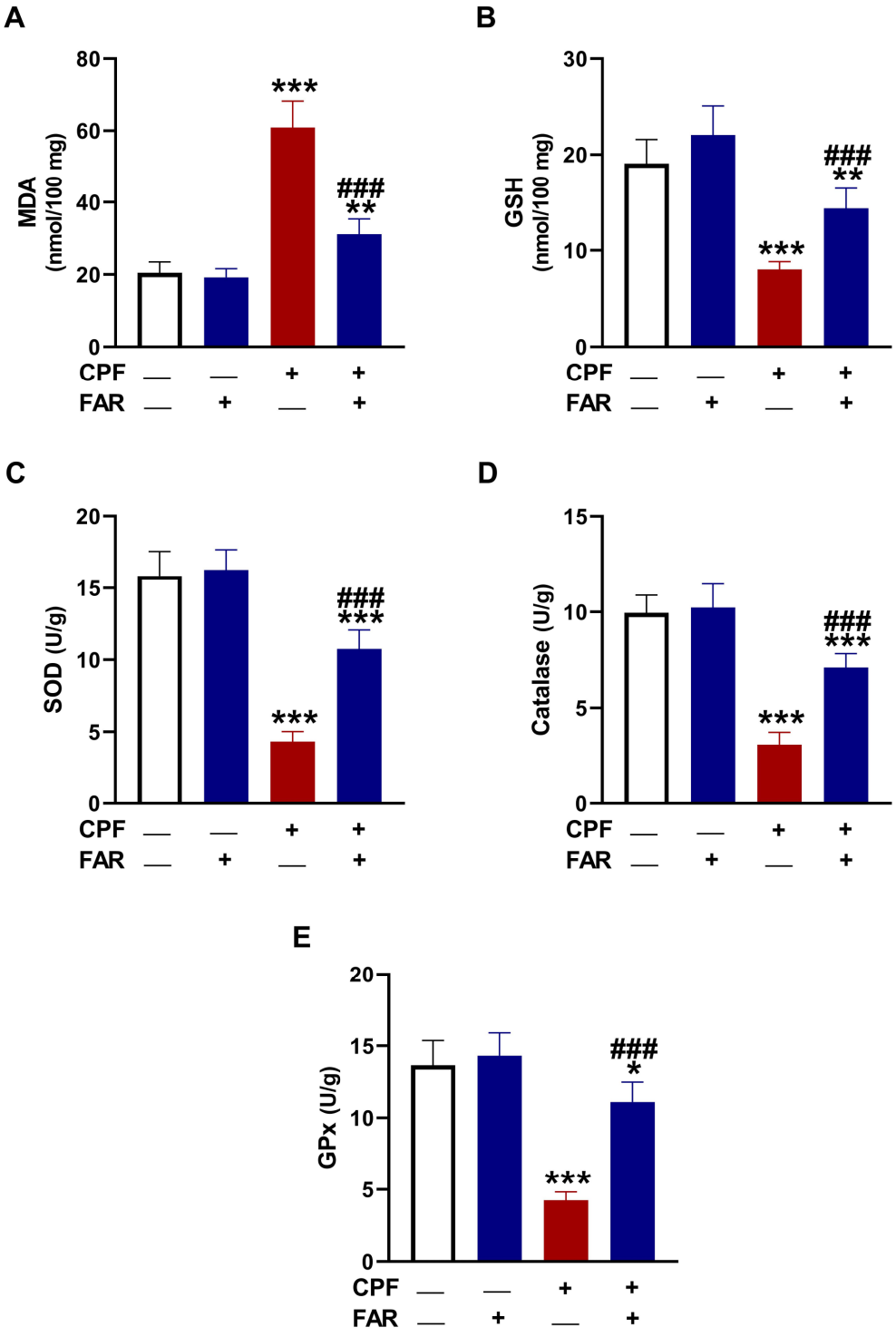

Fig. 2. FAR prevented CPF-induced oxidative stress in rat kidney. FAR decreased MDA (A) and increased GSH (B), SOD (C), catalase (D), and GPx (E) in CPF-administered rats. Data are mean ± SD, (n = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs CPF.

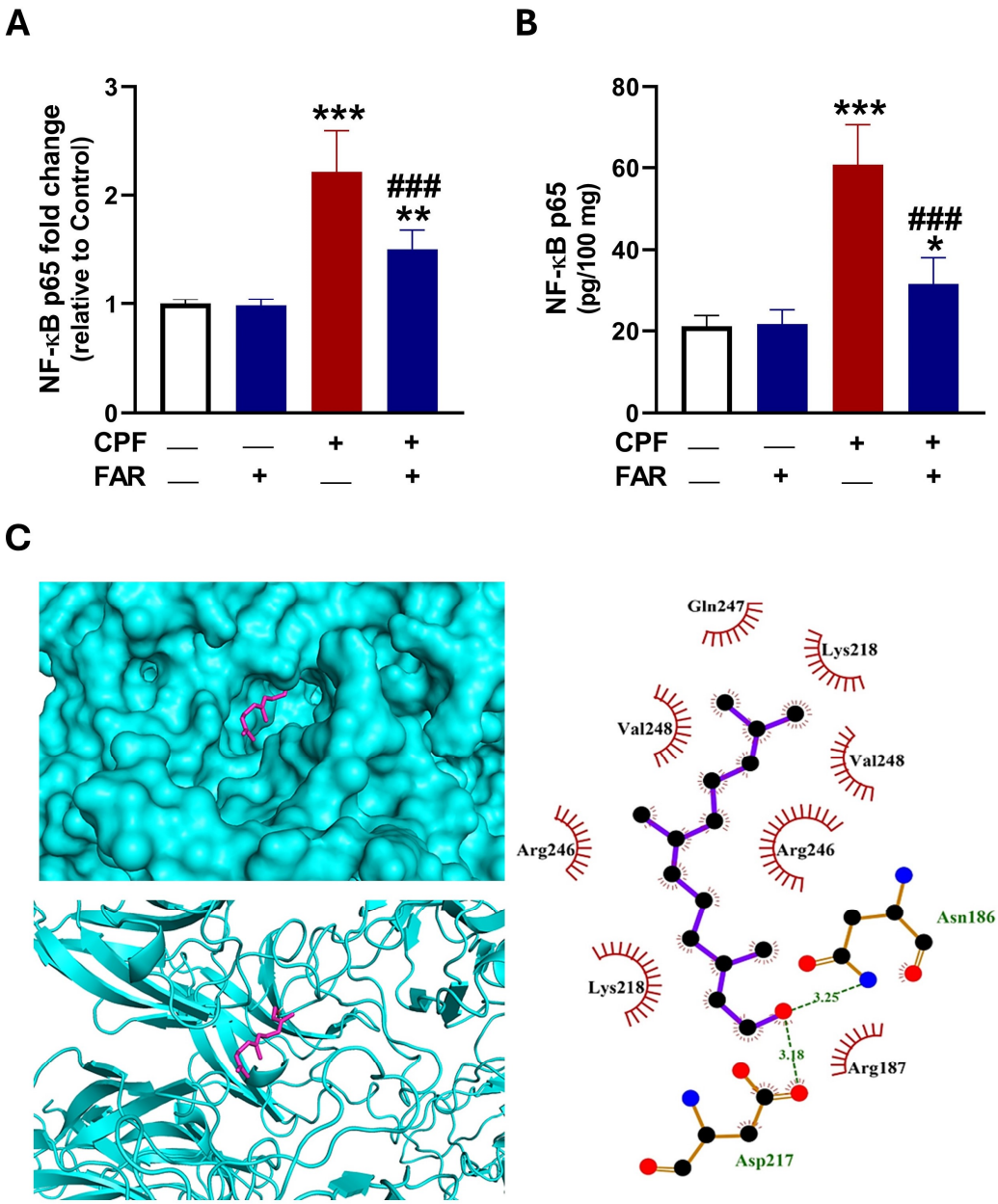

Fig. 3. FAR downregulated kidney NF-κB p65 mRNA (A) and protein (B) in CPF-administered rats. Data are mean ± SD, (*n* = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs CPF. (C) Molecular docking of FAR with NF-κB showing the crystal structure and amino acid residues involved in polar bonding and hydrophobic interactions.

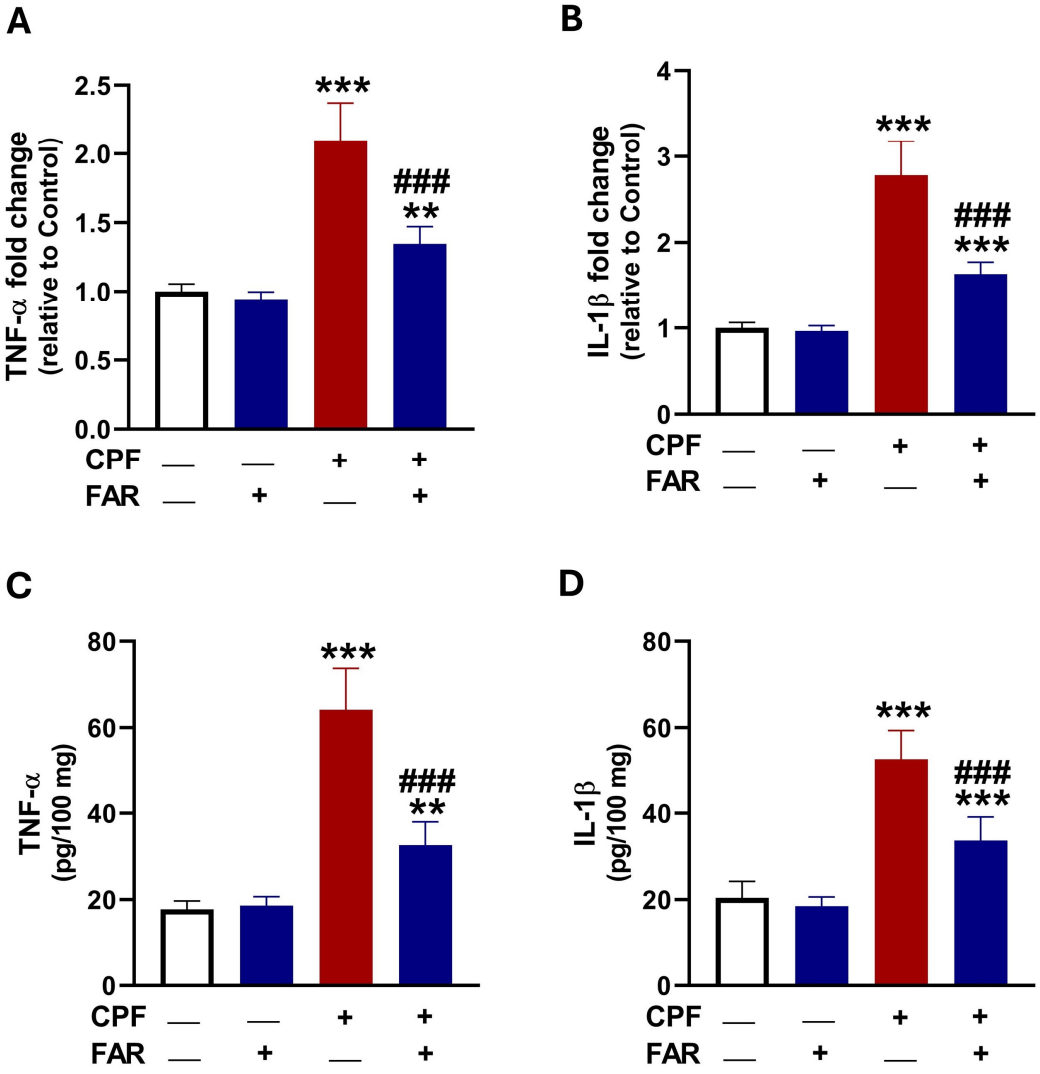

Fig. 4. FAR downregulated kidney TNF-α (A) and IL-1β (B) mRNA, and TNF-α (C) and IL-1β (D) protein levels in CPF-induced rats. Data are mean ± SD, (n = 6). **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs CPF.

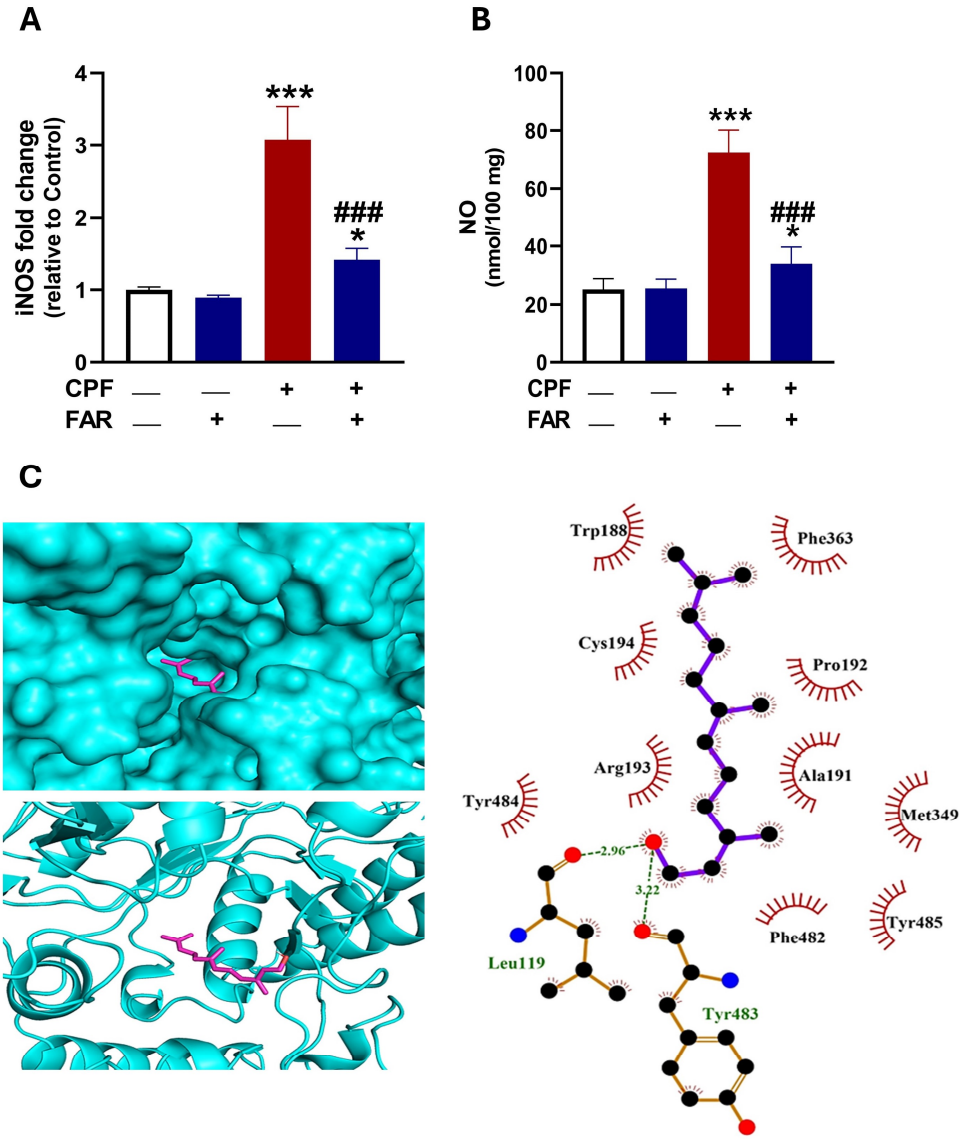

Fig. 5. FAR downregulated kidney iNOS mRNA (A) and NO (B) levels in CPF-administered rats. Data are mean ± SD, (n = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs CPF. (C) Molecular docking of FAR with iNOS showing the crystal structure and amino acid residues involved in polar bonding and hydrophobic interactions.

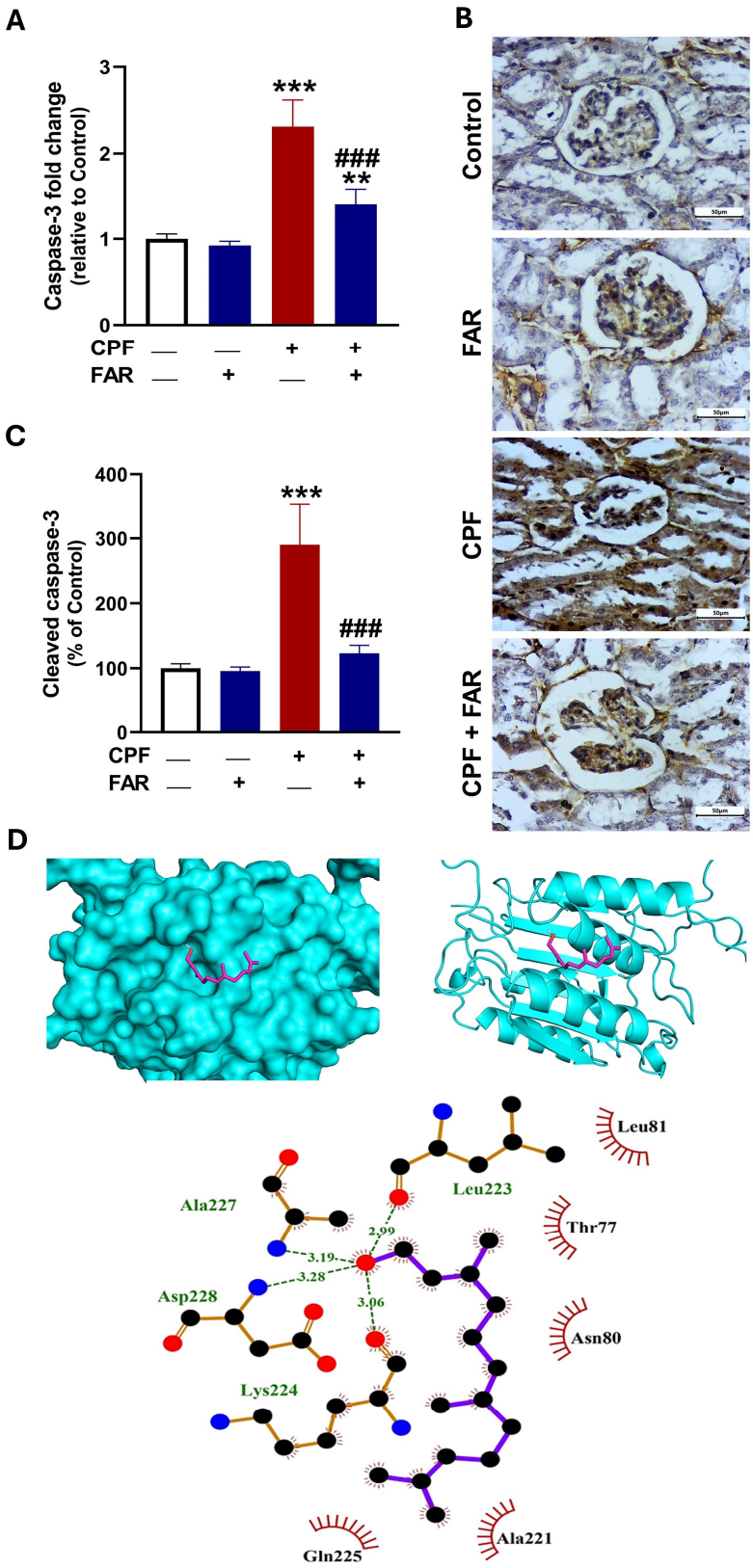

Fig. 6. FAR downregulated kidney caspase-3 mRNA (A) and protein (B-C) levels in CPF-administered rats. Data are mean ± SD, (n = 6). **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs CPF. (C) Molecular docking of FAR with caspase-3 showing the crystal structure and amino acid residues involved in polar bonding and hydrophobic interactions.

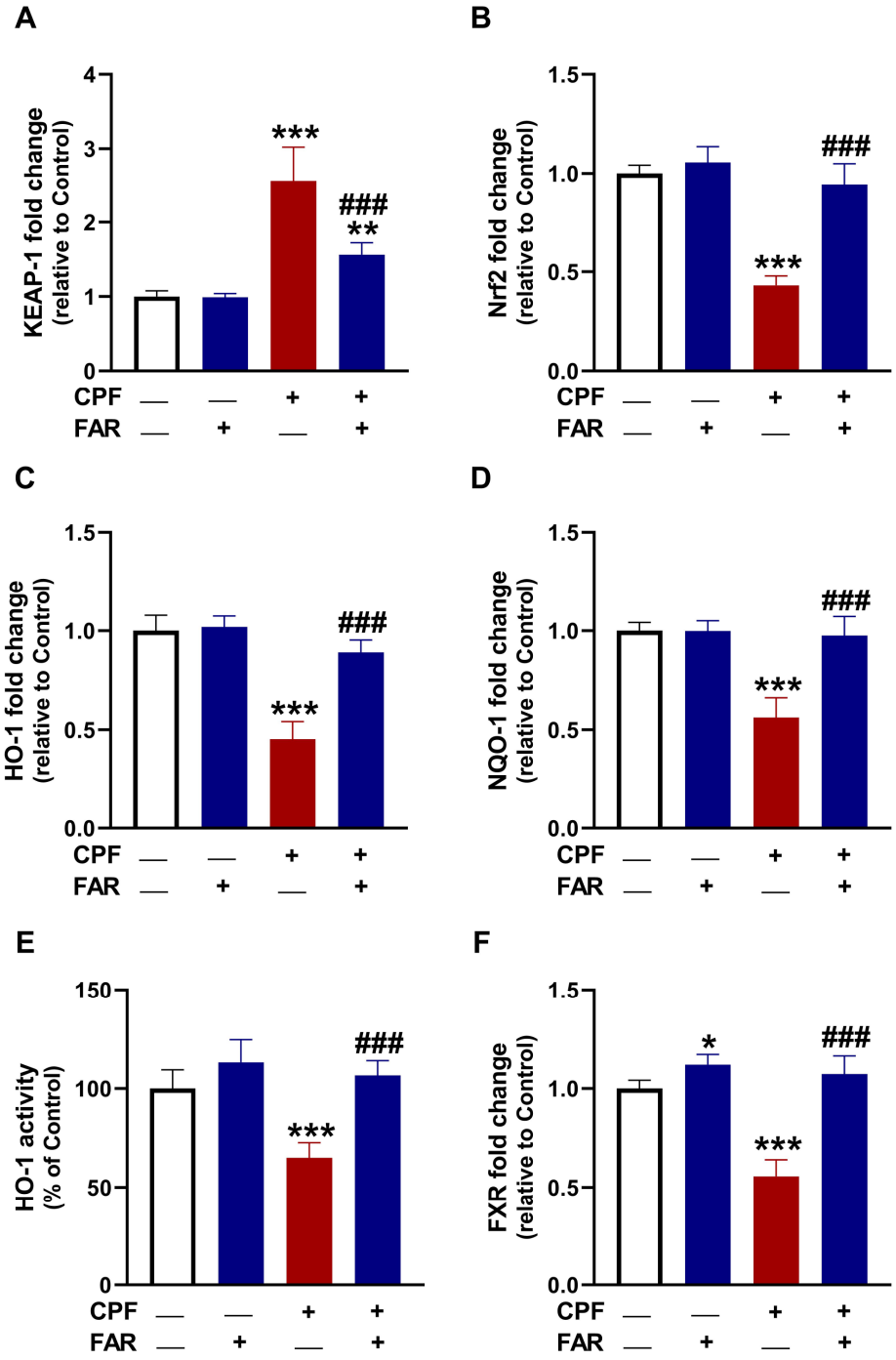

Fig. 7. FAR downregulated Keap-1 mRNA (A) and increased Nrf2 (B), HO-1 (C), NQO-1 (D) and FXR (E) mRNA and HO-1 activity (E). Data are mean ± SD, (*n* = 6). *P<0.05, **P<0.01 and ***P<0.001 vs Control. ###P<0.001 vs CPF.

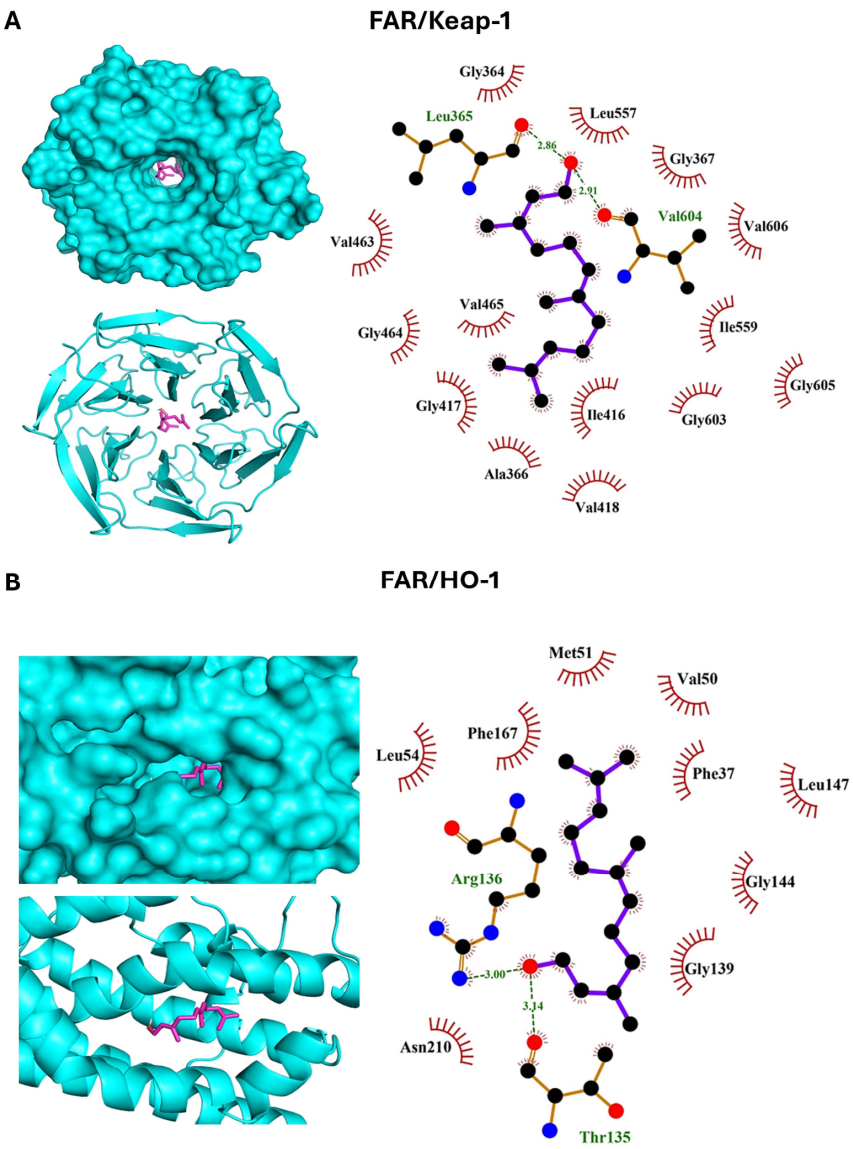

Fig. 8. Molecular docking of FAR with Keap-1 (A) and HO-1 (B) showing the crystal structure and amino acid residues involved in polar bonding and hydrophobic interactions.

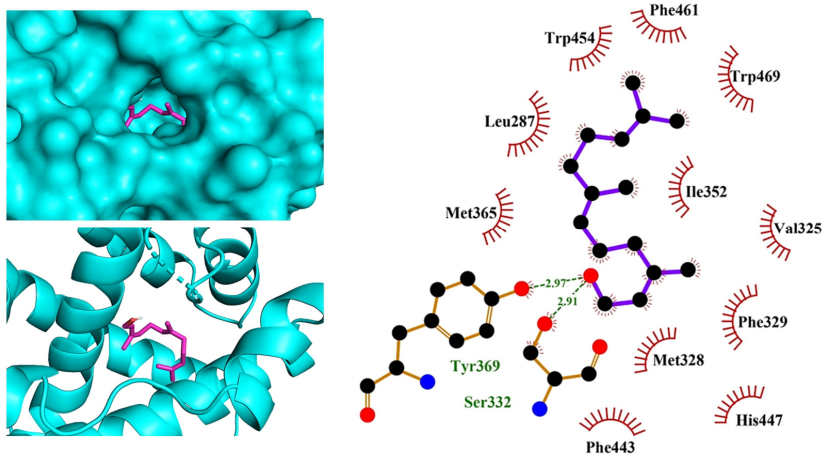

Fig. 9. Molecular docking of FAR with FXR showing the crystal structure and amino acid residues involved in polar bonding and hydrophobic interactions.