3-Indolepropionic Acid Prevented Chlorpyrifos-Induced Hepatorenal Toxicities in Rats By Improving Anti-Inflammatory, Antioxidant And Apoptotic Responses and Abating DNA Damage

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Abstract

We examined the individual and combined effect of 3-Indolepropionic acid (IPA) and Chlorpyrifos (CPF) on rat hepatorenal function. The experimental cohorts (n=6) were treated *per os* for 14 consecutive days as follows: Control (Corn oil 2 mL/kg body weight), CPF alone (5 mg/kg), IPA alone (50 mg/kg) and the co-treated cohorts (CPF: 5 mg/kg + IPA: 25 or 50 mg/kg). Biomarkers of hepatorenal damage, antioxidant and myeloperoxidase (MPO) activities, the levels of nitric oxide (NO), lipid peroxidation (LPO) and reactive oxygen and nitrogen (RONS) species were spectrophotometrically evaluated. Besides, the concentration of tumour necrosis factor-alpha (TNF- α), interleukin-1 β (IL-1β) and caspase-3 activity and 8-hydroxy-2'-deoxyguanosine adducts (8-OHdG) was also assessed by Enzyme-Linked Immunosorbent Assay. Treatment with CPF alone increased biomarkers of hepatorenal toxicity was significantly (*p<0.05*) alleviated in rats co-exposed to CPF and IPA. Moreover, the decrease in antioxidant status as antioxidant elevation in RONS and LPO were lessened (*p<0.05*) in rats co-treated with CPF and IPA. CPF mediated increases in TNF-α, IL-1β, NO, MPO and caspase-3 activity were reduced (*p<0.05*) in the liver and kidney of rats co-exposed to CPF and IPA. In addition, 8-OHdG adducts formation were reduced in rats treated with 3-IPA dose-dependently. Light microscopic examination showed that histopathological lesions severity induced by CPF were alleviated in rats co-exposed to IPA and CPF. In conclusion, the results demonstrated that rats co-exposed to IPA and CPF exhibited reduced CPF-induced oxidative stress, inflammation, DNA damage and caspase-3 activation of the liver and kidney.

Introduction

Chlorpyrifos (CPF) is a broad-spectrum organophosphate compound mainly used as a pesticide to control soil-borne insects and mites and as a herbicide to control weeds and foliage (Pal, Kokushi, Koyama, Uno, & Ghosh, 2012). CPF exposure occurs primarily through consuming contaminated foods, inhalation, and absorption through the skin during preparation and application (Albasher, Almeer, Alarifi, et al., 2019). As a result, the risk of exposure and uncontrolled use of organophosphate (OP) insecticides such as CPF has increased worldwide, especially in developing countries (Kokushi, Uno, Pal, & Koyama, 2015). CPF principally acts as an acetylcholinesterase (AChE) inhibitor, thus inducing neurotoxicity (Mahmoud, Abdel Moneim, Qayed, & El-Yamany, 2019; Nasr, El-Demerdash, & El-Nagar, 2016). Exposure to CPF causes several pathological conditions, including hepatotoxicity, nephrotoxicity, endocrine disturbance, reproductive toxicity, genotoxicity and immunological perturbations, in both animals and humans (Albasher, Almeer, Alarifi, et al., 2019). CPF toxicity is induced via several biochemical mechanisms that include oxidative stress, generation of reactive oxygen species, apoptosis and production of pro-inflammatory cytokines in target tissues (Verma, Mehta, & Srivastava, 2007; Zhao, Yang, & Zhao, 2019).

Indole-3-propionic acid (IPA) is a metabolite derived from the metabolism of tryptophan by gut bacteria alongside other products, including indole-3-acetic acid (I3A), indole-3-lactic acid, (I3L), indole-3-carboxylic acid (I3C), indole and its liver metabolite indoxyl sulphate (IS) (Huc, Nowinski, Drapala, Konopelski, & Ufnal, 2018; Konopelski & Ufnal, 2018; Michael, Drummond, Doeden, Anderson, & Good,

1964; Yu, Kim, & Kang, 2011). Over the years, IPA has been established as a unique potent antioxidant devoid of pro-oxidant activity (Bendheim et al., 2002). Contrary to other antioxidants, IPA is not converted to reactive intermediates with pro-oxidant activity and does not undergo autoxidation in the presence of transition (Bendheim et al., 2002). As a result, IPA has been shown to mitigate lipid peroxidation, carcinogenesis, accumulation of 8-OH-dG and subsequent DNA damage induced by several compounds such as chromium, 17β-Estradiol (E_2), Iron due to its hydroxyl radical scavenging ability (Aust & Eveleigh, 1999; M. Karbownik, Garcia, Lewinski, & Reiter, 2001; M. Karbownik, Gitto, Lewinski, & Reiter, 2001; Małgorzata Karbownik, Reiter, Cabrera, & Garcia, 2001; Qi et al., 2000).

To our knowledge, no such study is currently present in the scientific literature. We treated rats with CPF and IPA for four consecutive weeks and evaluated hepatic and renal toxic responses to achieve these goals. Specifically, we assessed serum level of hepatorenal toxicity, biomarkers of inflammatory responses, oxidative stress, apoptosis besides a biomarker of DNA damage and histopathological changes in experimental rats hepatorenal system. The data we report here present more insight into IPA supplementation's beneficial effect and the underlying mechanisms of IPA protective activity. We conclude that 3-IPA dose-dependently augmented the antioxidative capacity of rats, decreased oxidative stress, apoptosis, and inflammatory responses. IPA also prevented histological damages in the liver and kidney of rats resulting from CPF toxicity.

Materials And Methods

Chemicals, Reagents and Kits

Chlorpyrifos, **3-Indolepropionic acid (IPA)**, thiobarbituric acid (TBA), 5', 5'-dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), epinephrine, hydrogen peroxide (H_2O_2), and glutathione (GSH) were purchased from Sigma Chemical Co. (St Louis, MO, USA). In addition, enzyme-Linked Immunosorbent Assay (ELISA) kits for 8-hydroxy-2’-deoxyguanosine interleukin-1β (IL-1β), interleukin-10 (IL-10) and caspase -3 and 9 estimations were purchased from Elabscience Biotechnology Company (Wuhan, China). All other reagents and chemicals used were obtained commercially and of analytical grade.

Animal care and experimental design

For the avoidance of type I and II errors, sample size computation was done using the G* Power software version 3.1.9.4 (Faul, Erdfelder, Lang, & Buchner, 2007). The effect size of 0.40 (Larger effect) at .05 alpha error of probability for one way analysis of variance (ANOVA) was adopted in the study, and this concurs with the Cohen’s guideline (Cohen, 1992). Based on this, a total sample size of 125 was obtained at 95% power. To adhere to the 3R guidelines for the welfare and use of experimental animals (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010; Workman et al., 2010), the computed sample size was minimized to 30 animals. Specifically, 30 (consisting of n=6 rats, i =5) male Wistar rats, approximately 150 g body weights, were procured from the Central Animal House, College of Medicine, University of Ibadan, Nigeria,

for this study. The experimental rats were acclimatised for seven days before they were subjected to different treatments. The rats were fed with standard rat pellets (Ladokun Feeds Limited) and water *ad libitum* and subjected to natural photoperiod of about 12 hours of light/darkness cycle daily. The present study consisted of five experimental groups, with each group containing six rats each. They were treated for 14 consecutive days. Group I (**Control**) rats received normal drinking water, Group II (**CPF alone**) rats orally received 5 mg/kg body weight, Group III (**1PA alone**) rats received IPA alone in 50 mg/kg. In contrast, Group IV (**CPF + IPA**) rats received CPF and IPA1 25 mg/ kg and Group V received CPF and IPA2 50 mg/ kg. Separate stock solutions (5 mg/kg) of CPF and IPA were prepared freshly every other day. The doses of CPF (5 mg/kg) and IPA (50 mg/kg) used in the current study are selected based on previously published data (Owumi & Dim, 2019; Wikoff et al., 2009; Z. H. Zhao et al., 2019).

Termination of the experiment and excision of tissues

Twenty-four hours after the last treatment, blood was collected into non-heparinised tubes and the rats sacrificed by cervical dislocation after carbon dioxide (CO_2) asphyxiation (AVMA, 2001; Hawkins et al., 2016; Owumi, Nwozo, Arunsi, Oyelere, & Odunola, 2021). Subsequently, the serum was prepared by centrifuging the clotted blood at 4,000g for 10 minutes using a centrifuge. The liver and kidney were excised, rinsed in ice-cold 1.15% KCl solution, blotted with filter paper and weighed. The organs were sectioned for histological examination, and the remaining portion homogenized in 0.1M of phosphate buffer (pH 7.4) using a Teflon homogeniser. The resulting homogenates were centrifuged at 12,000 rpm for 15 minutes using a cold centrifuge to obtain the post mitochondria fraction. Finally, the supernatant was collected and used for biochemical analyses.

Evaluation of liver and kidney function indices

Liver function indices, namely the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),gamma-glutamyl transferase (□-GT), were evaluated using available commercial kits from Randox^{TM} Laboratories Limited, UK. Moreover, kidney functions indices, namely serum urea and creatinine concentrations, were determined using Randox^{TM} Laboratories Limited (Crumlin, UK).

Assessment of oxidative stress indices in the liver and kidney of experimental rats

Hepatic and renal protein concentration was determined following Lowry et al.'s method, and as previously reported (Lowry, Rosebrough, Farr, & Randall, 1951). The activity of superoxide dismutase -SOD- was determined by the previously reported method of Misra and Fridovich (Adefisan, Madu, Owumi, & Adaramoye, 2020; Misra & Fridovich, 1972). The evaluation of catalase -CAT- activity was according to the reported method of Clairborne (Clairborne, 1995; Owumi, Nwozo, Effiong, & Najophe, 2020). Total sulfhydryl -TSH- group was measured by Ellman's method (Arowoogun et al., 2020; Ellman, 1959); reduced GSH was determined using the method described by Jollow *et al.*, and as previously reported (Jollow, Mitchell, Zampaglione, & Gillette, 1974; Owumi, Aliyu-Banjo, & Danso, 2019); GST was assayed (monitored at 340 nm) using Habig's method (Habig, Pabst, & Jakoby, 1974); GPx activity was

determined according to the process of Rotruck *et al*., and as previously reported (Owumi, Danso, & Nwozo, 2020; Rotruck et al., 1973); xanthine oxidase was quantified by the method of Bergmeyer *et al*. and reported recently (Buege & Aust, 1978; Owumi, Elebiyo, & Oladimeji, 2020) and lipid peroxidation marker was quantified as malondialdehyde (MDA) according to the method described by Ohkawa and as previously reported (Folayan et al., 2020; Ohkawa, 1979) and expressed as µmol MDA/mg protein. Additionally, TRX and TRXR levels were evaluated using commercially available ELISA Kits (Elabscience Biotechnology Company, Beijing, China) with the aid of a SpectraMax plate reader (Molecular Devices, CA, USA) as stated in the manufacturer's manual.

Assessment of reactive oxygen/nitrogen species (RONS) level

The quantification of the RONS level was done according to an established protocol based on the RONS-dependent oxidation of 2',7'-dichlorodihydrofluorescin diacetate (DCFH-DA) to DCF (Owumi & Dim, 2019). Succinctly, the reaction mixture comprised of 10 µL of the liver or kidney homogenate, 35 µL of distilled water, 150 µL of 0.1M potassium phosphate buffer (pH 7.4), and 5 µL of DCFH-DA. The fluorescence emission of DCF as a result of DCFHDA oxidation was monitored for 10 min (30s intervals) at 488 nm excitation and 525 nm emission wavelengths using a SpectraMax plate reader (Molecular Devices, CA, USA). The rate of DCF formation was expressed in the percentages relative to the control group.

Evaluation of inflammatory biomarkers

Myeloperoxidase (MPO) activity was determined by the modification of the method described by Granell et al. (2003) (Granell et al., 2003). Nitric oxide (NO) level was determined according to Green et al. (1982) (Green et al., 1982) by measuring the tissue nitrites content, the stable end-products of nitric oxide. The nitrites content was obtained by extrapolation using a standard sodium nitrite curve, and the result expressed as µM of nitrites/mg protein. The activity of xanthine oxidase (XO) was determined in line with the standard procedure of *Bergmeyer et al.,* sample (0.10 mL), xanthine solution (1.00 mL), and phosphate buffer (1.90 mL) pipetted into cuvettes were mixed quickly by inversion and the readings (absorbance at 290 nm) monitored for 3 minutes every other minute. A blank reference reading was taken by substituting samples (0.10 mL) with distilled deionized water. Furthermore, hepatic and renal concentrations of IL-10 and IL-1β were evaluated using ELISA Kits from E-labscience (Beijing, China) following the manufacturer's protocol. All reading was obtained using an M384 SpectraMax^{TM} Multi-modal plate reader (Molecular Devices, San Jose, CA, USA).

Assay of apoptotic and DNA damage biomarkers

Caspase-3 and caspase-9 activities and 8-OHdG level were evaluated using commercially available ELISA Kits (Elabscience Biotechnology Company, Beijing, China) with a SpectraMax plate reader (Molecular Devices, CA, USA) as stated in the manufacturer's manual.

Histological evaluation of the liver and kidney tissues

Liver and kidney samples that were earlier fixed in phosphate-buffered formalin (10%; for three days) were randomly selected for histological analysis. The samples were embedded in paraffin following sequential dehydration processes. Subsequently, microtome sliced tissue-liver and kidney- (4-5µm) were fixed on charged microscopic glass slides and stained with hematoxylin and eosin (H & E) following established protocol (Bancroft & Gamble, 2008; Owumi, Olusola, Arunsi, & Oyelere, 2021). Histopathologic examination of hepatic and renal tissue was conducted using a Carl Zeiss Axio-Scope A1 light microscope (G ttingen, Germany) to identify pathological abnormalities. The slides holding the tissue were scored by a pathologist oblivious of the experimental groups. Representative images were captured from the slides using a Carl Zeiss Axiocam 105 digital colour Camera (G ttingen, Germany) attached to the microscope.

Statistical Analysis of Results

The analysis of the data generated from this study was performed by one-way analysis of variance (ANOVA) followed by a post-hoc test (Bonferroni) using GraphPad Prism version 8.3.0 for Mac (www.graphpad.com; GraphPad, CA, USA,). Statistically significant differences were set at values of $p<0.05$. The results are expressed as the mean ± SD of replicates.

Results

Effects of CPF and IPA on organ weight and markers of hepatorenal injury in rats

There is a significant ($p < 0.05$) reduction in the liver of rats treated with CPF alone compared to control by 7.37% Table 1. The kidney of animals treated with CPF alone showed no difference in weight relative to control. The kidney of rats co-treated with CPF and IPA (25 and 50 mg/kg) increased by 2.76%, while the liver of rats co-treated with CPF and IPA (25 and 50 mg/kg) increased by 10.29% and decreased by 5.98% compared to CPF alone treated rats. The effect of IPA on CPF-induced hepatic and renal toxicities was evaluated from serum levels of hepatic transaminases and creatinine and urea, respectively Fig. 1. Exposure to CPF (5mg/kg) increased ($p < 0.05$) biomarkers of hepatic and renal injury in rat serum compared to the control rats. The activities of AST, ALT, ALP, and GGT and creatinine and urea levels increased by 13.28%, 12.57%, 82%, 86.18%, 60.41%, and 76.49%, respectively when compared to the control group. In rats co-treated with IPA (25 and 50 mg/kg) and CPF, IPA decreased CPF-mediated increases by (76.53%, 72.70%) in the level of urea. Rats co-treated with IPA and CPF (25 mg/kg) showed no decrease in creatinine level. However, rats co-treated with IPA and CPF (50 mg/kg) showed a significant reduction in creatinine level by 6.2% compared to CPF alone treated animals. Furthermore, a significant decrease in the serum activity of ALT by (29.82% and 1.67%), AST by (99.43% and 83.56%), ALP by (24.66% and 4.04%) and GGT by (122.64% and 124.17%) in rats co-treated with 3-IPA (25 and 50 mg/kg) compared to rats treated with CPF alone.

Table 1
Effects of CPF alone, and IPA on experimental rat organ and body weight

	Control	CPF	IPA	CPF + IPA_1	CPF + IPA_2
Initial body weight (g)	162.70 ± 14.6	169.70 ± 7.2	161.7 ± 1.37	170.5 ± 5.4	174.50 ± 5.9
Final body weight (g)	187.20 ± 16.9	199.30 ± 7.5	199.80 ± 15.2	203.80 ± 8.3	195.70 ± 8.3
Liver weight (g)	6.75 ± 0.94	6.27 ± 0.68	6.72 ± 0.62	6.95 ± 0.87	5.98 ± 0.49
Kidney weight (g)	1.07 ± 0.14	1.07 ± 0.12	1.05 ± 0.11	1.10 ± 0.17	1.1 ± 0.11
Relative Liver weight (%)	3.64	3.15	3.94	3.42	3.06
Relative Kidney weight (%)	0.57	0.54	0.53	0.54	0.56

CPF (5 mg/kg); IPA_1 and IPA_2 (25 and 50 mg/kg respectively) body weight; n = 6. Data are expressed as mean ± SD. *: $p < 0.05$ versus control; *: $p < 0.05$ versus CPF alone. CPF: Chlorpyrifos; IPA: 3-indolepropionic acid.

Effects of CPF and IPA on hepatic and renal antioxidant enzymes activities, and GSH, in rats

The effect of IPA on the oxidative stress indices in CPF-treated rats was evaluated and the results presented in Figs. 2 and 3. Results showed a decrease ($p < 0.05$) in the activities of SOD and CAT in the liver of CPF treated rats by 7.62% and 21.87%, respectively and in the kidney by 47.93% 17.35%, respectively compared to the control Fig. 2. A decrease ($p < 0.05$) in GST and GPx in the liver of CPF alone treated rats by 37.14% and 21.75% and in the kidney of rats treated with CPF alone by 50.37% and 6.29%, respectively compared to the control Fig. 3. Co-treatment with 3-IPA at 25 and 50 mg/kg abated ($p < 0.05$) reduction in hepatic SOD by 7.28% and 25.85%, CAT by 46.41% and 33.68%, GST by 50.93% and 56.46% and GPx by 21.12% and 3.79% respectively. Increases ($p < 0.05$) in SOD (33.28% and 42.90%), CAT (64.50% and 80.22%), GST (92.26% and 102.12%) and GPx (19.45% and 29.05%) occurred dose-dependently (3-IPA at 25 and 50 mg/kg) in rat kidney co-treated with IPA and CPF. Also, exposure to CPF alone markedly decreased GSH in the kidney and liver of the treated rats when compared to the control group Fig. 3. The level of GSH reduced significantly by 61.9% in the liver and 61.54% in the kidney of rats treated with CPF alone compared to the control group. The level of GSH in the co-treated groups increased by 49.35% (IPA1) and 79.17% (IPA2) in the liver and by 56% (IPA1) and 28.57% (IPA2) in the kidney when compared to rats treated with CPF alone.

Effect of CPF and IPA on hepatic and renal RONS generation and induction of LPO and total thiol levels in rats

The effect of IPA on RONS generation and subsequent LPO-induction in CPF treated rats were evaluated, and the results are shown in Fig. 4. Exposure to CPF alone evidently increased ($p < 0.05$) RONS levels in the kidney by 107.5% and liver by 141.5% compared with the control group. LPO levels in the kidney and

liver of the CPF treated rats also significantly increased by 80% and 92.71%, respectively, compared with the control group. Co-treatment with IPA protected against renal and hepatic damage evidenced by decreases ($p < 0.05$) in RONS and LPO levels than rats treated with CPF alone. There was a reduction in RONS levels in groups co-treated with CPF + IPA1 and CPF + IPA2 by 59.7% and 108.6%, respectively, compared to CPF alone in the liver 0.87% and 36.17% respectively compared to CPF alone group in the kidney. LPO in groups co-treated with CPF + IPA1 and CPF + IPA2 was reduced ($p < 0.05$) by 95.33% and 132.87% compared to CPF alone treated groups in the liver and by 50.82% and 49.45% in the kidney when compared to CPF alone treated rats. Also, TSH levels decreased ($p < 0.05$) in the liver and kidney of rats treated with CPF alone by 58.21% and 71%, respectively, compared to the control group. TSH levels in the co-treated groups increased 56.25% (IPA1) and 79.37% (IPA2) in the liver, and 76.09% (IPA1) and 98.22% (IPA2) in the kidney compared to rats treated with CPF alone.

Effects of CPF and IPA treatments on biomarkers of inflammation in rats

The influence of IPA on the biomarkers of inflammation was assessed in the liver and kidney of CPF-treated rats. Administration of CPF increased ($p < 0.05$) the hepatic and renal MPO, XO activity, and level of NO compared to the control group Fig. 5. In the liver, the activities of MPO and XO in CPF alone treated rats increased by 7.74% and 158.85%, respectively, compared to the control group; MPO and XO levels in the kidney also increased 58.58% and 90.32% compared to the control. Results also show an increase in the kidney and liver levels of NO by 47.16% and 3.77%, respectively, compared to control groups. However, administration of 3-IPA abrogated CPF-induced increase in these biomarkers of inflammation in the liver and kidney of rats compared with rats administered CPF alone. MPO activities in groups co-treated with CPF + 3-IPA1 and CPF + 3-IPA2 were reduced ($p < 0.05$) by 49.53% and 34.29% compared to CPF alone treated groups liver by 37.22% and 3.43% in the kidney when compared to CPF alone treated rats. There was a reduction in XO activities in groups co-treated with CPF + IPA1 and CPF + IPA2 by 90.70% and 168.55%, respectively, compared to CPF alone in the liver and 121.43% and 47.46% respectively compared to CPF alone group in the kidney. There was also a reduction in the levels of NO in groups co-treated with CPF + IPA1 and CPF + IPA2 by 12.59% and 43.58%, respectively, compared to CPF alone the liver and 1.12% and 27.87% respectively compared to CPF alone group in the kidney.

Effects of CPF and IPA on pro-inflammatory and anti-inflammatory cytokines in rats

IL-1β and IL-10 levels in rats treated with CPF alone significantly increased (62.9%) and reduced (73.5%), respectively, in the liver and by 78.8% (IL-1β) and 105.6% (IL-10) in the kidney compared to the control Fig. 6. In the co-treated groups, liver IL-1β levels decreased by 15.9% (IPA1) and 38.3% (IPA2), and in the kidney, 34.9% (IPA1) and 67% (IPA2) compared to CPF alone treated rats. Conversely, IL-10 level increased ($p < 0.05$) in the co-treated rat liver by 36% (IPA1) and 77.5% (IPA2) and the kidney by 120.9% (IPA1) and 148% (IPA2), compared to rats treated with CPF.

Effects of CPF and IPA on biomarkers of apoptosis in rats

Caspase − 3 and − 9 activities increased ($p < 0.05$) in the liver by 116.8% and 133.3% and in the kidney by 92.7% and 97.7%, respectively, compared to the control group Fig. 7. Co-treatment with IPA protected the liver and kidney from excessive apoptosis in a dose-dependent manner, as seen in the significant decreases ($p < 0.05$) in caspase-3 and caspase-9 activities compared with rats treated with CPF alone. There was a reduction in caspase 3 activity in groups co-treated with CPF + IPA1 (69%; 34.8%) and CPF + IPA2 (90.2%; 64.2%) in the liver and kidney (respectively) compared to CPF alone treated animals. Caspase-9 activity in groups co-treated with CPF + IPA1 and CPF + IPA2 was also reduced ($p < 0.05$) by 66.3% and 110.1% (liver) and by 28.7% and 43.8% (kidney) compared to CPF alone treated rats.

Effects of CPF and IPA on Thioredoxin (Trx), Thioredoxin reductase (Trx-R) and 8-hydroxy-2' - deoxyguanosine (8-OHdG) in rats.

The effect of IPA on the biomarkers of oxidative stress and DNA damage was assessed in the liver and kidney of CPF-treated rats. CPF Administration caused a significant decrease in Trx and Trx-R and increased 8-OHdG levels in the liver and kidney of treated rats relative to the control group Figs. 8 and 9. In the liver, Trx, Trx-R levels in CPF alone treated rats decreased by 27.6% and 90.7%, whereas the 8-OHdG level increased by 58.7% compared to the control group. Contrary, in groups co-treated with CPF + IPA1 and CPF + IPA2, our data show a dose-dependent significant increase in Trx (24.3% and 36.5%) and Trx-R (90.3% and 106.6%) levels compared to CPF alone treated rats. There was a significant decrease in the 8-OHdG level in the co-treated groups by 13.2% (IPA1) and 79.5% (IPA2) compared to CPF alone group in the liver.

Treatment with CPF significantly increased the level of 8-OHdG (68.1%) and decreased Trx (27%) and Trx-R (70.9%) levels in the kidney compared to the control. However, co-treatment with CPF + IPA1 and CPF + IPA2 decreased 8-OHdG by 16.2% and 55.6%, respectively, compared to CPF alone treated rats. In addition, a dose-dependent significant increase was observed in the levels of Trx (20.3% and 44%) and Trx-R (89.5% and 124.3%) in CPF + IPA1 and CPF + IPA2, respectively, relative to CPF alone group.

Effects of CPF and IPA on liver and kidney histology of rats

Control: There is no visible lesion. CPF alone: there is centrilobular hepatocellular degeneration and necrosis with Kupffer cell hyperplasia, multifocal necrotizing hepatitis. IPA alone: There is no visible lesion. CPF + IPA1 (25mg/Kg): There is centrilobular hepatocellular degeneration, necrosis and inflammation. CPF + IPA2: There is no observable lesion Fig. 10. Control: there is no noticeable lesion. CPF alone: there is atrophy of the glomerular tuft, with proliferative glomerulonephritis epithelial coagulation and necrosis. 3-IPA alone: there is no visible lesion. CPF + IPA1 (25mg/Kg): there is distension between Bowman's space and cast in the tubular lumen. CPF + IPA2: there is moderate glomerular atrophy with improvement in kidney cyto-architecture Fig. 11.

Discussion

Chlorpyrifos (CPF) is an organophosphate pesticide commonly used to control mosquitoes, flies, and other household and agricultural pests (Albasher, Almeer, Al-Otibi, Al-Kubaisi, & Mahmoud, 2019; Spodniewska, Barski, & Gizejewska, 2015). Despite the United States Environmental Protection Agency's (US EPA) ban on domestic and agricultural uses of CPF in 2000, it is still one of the most commonly used OP insecticides worldwide (Bjorling-Poulsen, Andersen, & Grandjean, 2008; Maroni, Colosio, Ferioli, & Fait, 2000). As a result of its widespread use, acute and chronic exposure to CPF has been associated with a variety of abnormalities which include hepatotoxicity (Deng, Zhang, Lu, Zhao, & Ren, 2016; Goel, Dani, & Dhawan, 2006; Owumi & Dim, 2019), nephrotoxicity (Deng et al., 2016; Owumi & Dim, 2019), neurotoxicity (Shou et al., 2019), haematotoxicity (Aroonvilairat et al., 2018), reproductive toxicity (Adedara et al., 2018) and cardiotoxicity (Zafiropoulos et al., 2014). The current study thus assessed the mitigating effect of 3-IPA in CPF induced hepatorenal damage.

In the current study, the body weight gain of CPF-treated rats increased ($p < 0.05$) compared with the control. Our results are in accordance with other studies, revealing an increase in body weight gain in rats mediated by CPF (Ambali, Ayo, Ojo, & Esievo, 2011; Ezzi et al., 2016; Meggs & Brewer, 2007). The weight increase represents an increase in fatty tissues. OP insecticides such as CPF may induce accelerated differentiation of immature adipocytes into mature fat cells (Meggs & Brewer, 2007). However, other studies recorded a marked reduction in net body weight gain in CPF-exposed rats (Heikal & H. Mossa, 2012; Tanvir et al., 2016).

The increase in the body weight gain in the co-treated group occurred at the higher dose of IPA (50mg/ kg) instead of the lower dose of IPA (25mg/ kg). Our results are in tandem with previous findings, which reported reduced body weight (Ayaso, Ghattas, Abiad, & Obeid, 2014; Konopelski et al., 2019). In the present study, oral administration of CPF resulted in a significant reduction in the relative liver weight. However, there was no substantial change in the relative kidney weight in CPF treated rats compared with the control. The decrease in relative liver weight may be an indication of liver toxicity. Our results are consistent with results from previous studies, which also reported a reduction in organ weight (Ezzi et al., 2016; Joshi, Mathur, & Gulati, 2007). Co-administration of IPA improved relative liver and kidney weights in co-treated groups compared to CPF alone treated rats.

The present investigation assessed the levels of serum marker enzymes. These enzymes include AST, ALT, ALP and GGT and are found in the liver under normal physiological conditions. However, cellular stress and tissue injury induced by various toxins could release these enzymes into the bloodstream, indicating liver injury (Kaplan, 1993). In the present study, CPF administration resulted in a statistically significant increase in ALP and GGT, indicating hepatic toxicity, resulting in liver necrosis and inflammations (Kuzu et al., 2007). There was, however, a non-significant increase in the activities of AST and ALT. Thus, increased activities of hepatic transaminases in serum correlates with hepatocyte injury in CPF treated rats. These findings are consistent with earlier CPF in experimental rodents (S. A. Mansour & Mossa, 2009; Tanvir et al., 2016). Also, in this study, increases in activities of serum hepatic transaminases observed in CPF treated rats were dose-dependent reduced upon co-treatment with IPA, reflecting the protective potential of IPA against CPF hepatoxicity.

The kidneys play an essential role in the excretion of waste products and toxins such as urea and creatinine. Urea is a by-product of protein degradation, whereas muscles produce creatinine as a by-product of creatinine breakdown (Dorgalaleh et al., 2013). Both are excreted from the body by the kidney in urine and serve as indicators of renal function. They are also helpful in detecting nephrotoxicity caused by exogenous compounds in living organisms (Sandilands, Dhaun, Dear, & Webb, 2013). This study found a significant increase in serum urea level and a non-significant increase in serum creatinine level in CPF-treated rats than controls, indicating that CPF caused kidney damage due to various factors, including oxidative stress (S. G. Mansour et al., 2017). The reduced serum urea and creatinine levels in rats co-administered IPA and CPF imply that IPA alleviated CPF-mediated renal dysfunction in the treated rats.

Antioxidants, both endogenous and exogenous, and enzymatic and non-enzymatic, are essential in mitigating oxidative stress. The enzymatic antioxidants (SOD, CAT, GPx, and GST) strongly defend against liver injury and other disorders; thus, they receive a lot of attention (Zhou, Zhang, Yin, Jia, & Shan, 2015). Furthermore, these antioxidant enzymes limit the effects of oxidants in cells and tissues and act in the defence against oxidative cell injury scavenging free radicals (Halliwell & Gutteridge, 2015). Our data indicated a decrease in SOD, CAT, GST and GPx in the liver and kidney of rats treated with CPF alone compared to the control in the current study. The reduction in these antioxidant enzymes may be related to decreases in the synthesis of these enzymes or inactivation/inhibition induced by exposure to CPF (Heikal & H. Mossa, 2012; Owumi & Dim, 2019). This could result in the accumulation of free radicals in the liver and kidney cells of rats treated with CPF alone. Interestingly, our results show an increase in the activities of these antioxidant enzymes in rats co-exposed to CPF and IPA, revealing the antioxidant effects of IPA against CPF mediated oxidative stress in the treated rats.

Reduced glutathione is an endogenous non-enzymatic antioxidant that operates in association with other endogenous antioxidants in preventing oxidative stress. Our results show decreases in the levels of GSH in the liver and kidney of animals treated with CPF alone compared to the control, indicating over-utilisation in the detoxification process to shield the system from oxidative stress. Consequently, exposure to CPF predisposes the liver and kidney to free radical-mediated cellular damage in rats exposed to CPF. Co-administration with IPA and CPF brought about a significant increase in hepatic and renal GSH levels compared to animals treated with CPF alone. IPA has been proven to effectively scavenge hydroxyl radicals effectively and work in tandem with glutathione to minimize oxidative stress (M. Karbownik, J. J. Garcia, et al., 2001). Besides scavenging the hydroxyl radical, IPA has also been shown to scavenge the superoxide anion radical, which aligns with our result as co-administration with IPA and CPF brought about a significant increase in hepatorenal GSH levels compared to animals treated with CPF alone. The present study revealed a significant decrease in TSH level in the liver and kidney of rats treated with CPF alone compared to the control group, signifying a reduction in antioxidant status and an increased accumulation of free radicals. However, there was a significant increase in TSH level in the liver and kidney of rats co-treated with CPF and IPA in a dose-dependent manner compared to the CPF alone treated group, thus showing the antioxidant ability of IPA.

Both ROS and RNS, when present in a typical physiologically system, play essential roles in normal cellular functions such as fighting against infection, regulating different intercellular signalling pathways (Nunes Silva, 2015). However, when present in high concentrations, they overwhelm the innate antioxidant defence system, resulting in oxidative stress, leading to cellular dysfunction via lipid peroxidation, protein, and DNA damage (Martindale & Holbrook, 2002). In this study, rats treated with CPF alone revealed a marked increase in RONS levels compared to the control, implying that CPF activated the formation of free radicals in liver and kidney tissues. A significant increase in hepatic and renal LPO levels was also increased in rats treated with CPF alone. The increase in LPO levels can be attributed to the rise in RONS levels due to CPF treatment. Our findings are in tandem with previous observations (Heikal & H. Mossa, 2012; S. A. Mansour & Mossa, 2009, 2010). The current study revealed that IPA reversed the elevation of RONS and lipid peroxidation in liver and kidney tissues of co-treated rats. This may be attributed to the ability of IPA to scavenge free radicals capable of inducing lipid peroxidation and further oxidative stress.

The induction of inflammatory biomarkers (NO, MPO, XO) and cytokines (IL-1β, IL-10) could be stimulated by exogenous toxicants such as CYP (Gangemi et al., 2016). These biomarkers are often triggered alongside increased oxidative stress and induce inflammatory responses via activation of redox-sensitive transcription factors, such as NF-κB (Forrester, Kikuchi, Hernandes, Xu, & Griendling, 2018). In the current study, CPF administration in rats resulted in increased NO levels, MPO and XO activities, as well as IL-1β levels. It decreased IL-10 levels in the liver and kidney of rats treated with CPF alone compared to the control rats. The increased levels of these inflammatory biomarkers and cytokines could be attributed to the ROS-mediated activation of NF-κB induced by CPF (Wang et al., 2018). CPF-induced oxidative stress has also been reported previously to trigger the inflammasome and subsequent innate immune response (Jang et al., 2015), thus causing inflammation in cells and tissues. As a result of the inflammation being associated with increased oxidative stress, ROS scavengers can mitigate the inflammatory responses induced by CPF. Co-treatment with IPA significantly increased IL-10 and decreased IL-1β levels, reduced MPO and XO activities, and NO level in the liver and kidney of rats relative to the CPF alone group.

RONS generation resulting from CPF exposure upregulates pro-apoptotic proteins, including caspase 3 and caspase 9 and downregulate anti-apoptotic proteins such as Bcl-2 (Fu, Li, Song, Zhang, & Xie, 2019). The induction of apoptosis by CPF is characterized by NF-κB activation and loss of mitochondrial membrane integrity (Lee, Lim, Park, Park, & Koh, 2014). Our results show that CPF caused a significant increase in 8-OHdG adduct, caspase 3, caspase 9 activity, and a considerable decrease in Trx level and Trx-R activity in the experimental rats liver and kidney. Besides, increases in RONS result in lipid peroxidation, loss of membrane integrity, increased production of pro-apoptotic proteins, DNA fragmentation and damage (Wakf et al., 2018). Furthermore, an increased level of 8-OHdG in rats liver and kidney treated with CPF alone demonstrates the genotoxic effect of CPF by inducing DNA damage (Alavanja, Ross, & Bonner, 2013; Ethikic et al., 2012). In the current study, co-treatment with IPA dose-dependently attenuated the expression of caspase-3 and caspase 9, increased Trx and Trx-R and decreased 8-OHdG level in the liver and kidney of rats compared to CPF alone treated rats. These

observations further demonstrate the protective effect of IPA against CPF-mediated oxidative damage and induction of apoptosis.

Conclusion

Exposure to CPF can cause hepatorenal toxicity by increased oxidative-inflammatory responses, apoptotic mediators and DNA damage. Co-treatment with IPA reduced hepatorenal toxicities brought about by CPF exposure by decreasing oxido-inflammation stress and apoptosis as depicted in our proposed mechanism of action for CPF toxicity and IPA mitigating roles in Fig. 12. IPA could be used as a potential intervention to minimise hepatorenal toxicities caused by CPF and other organophosphate insecticides acting by these mechanisms.

Declarations

Ethical Approval

The experiment was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC) UI-ACUREC/033-0521/7 and following the United States National Academy of Sciences guidelines.

Consent to Publish

All the authors listed that participated in the study agreed to the content of the manuscript and the publication thereof.

Authors Contribution

Solomon Owumi: Conceptualization. Sarah Najophe, Moses Otunla**:** Data curation, analysis, investigation. Project administration Solomon **Owumi,** Sarah Najophe, Moses Otunla**,** Validation. **Solomon Owumi,** Sarah Najophe, Moses Otunla**,** Writing, review, and editing: Solomon **Owumi,** Sarah Najophe.

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Competing Interest

The authors (Dr Solomon E. Owumi, Sarah Najophe, and Moses Otunla) declare no conflicts of interest.

Availability of Data and materials

The data from this study can be obtained on request from the corresponding author.

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Figures

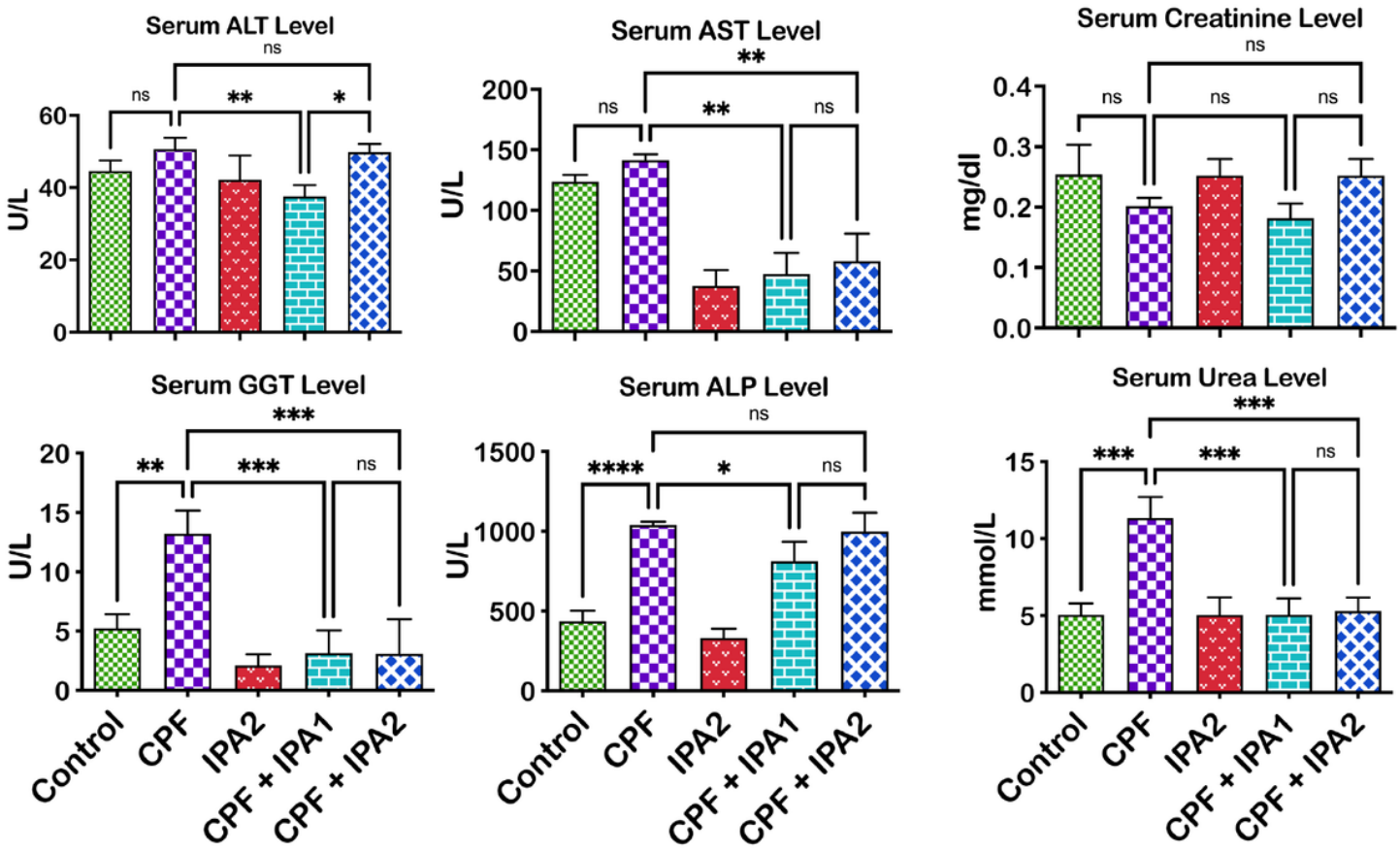

Figure 1

Influence of CPF alone, IPA alone, and their combination on markers of hepatorenal injury in rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: p<0.05 versus control; *: p<0.05 versus CPF alone; ns=not significant. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase.

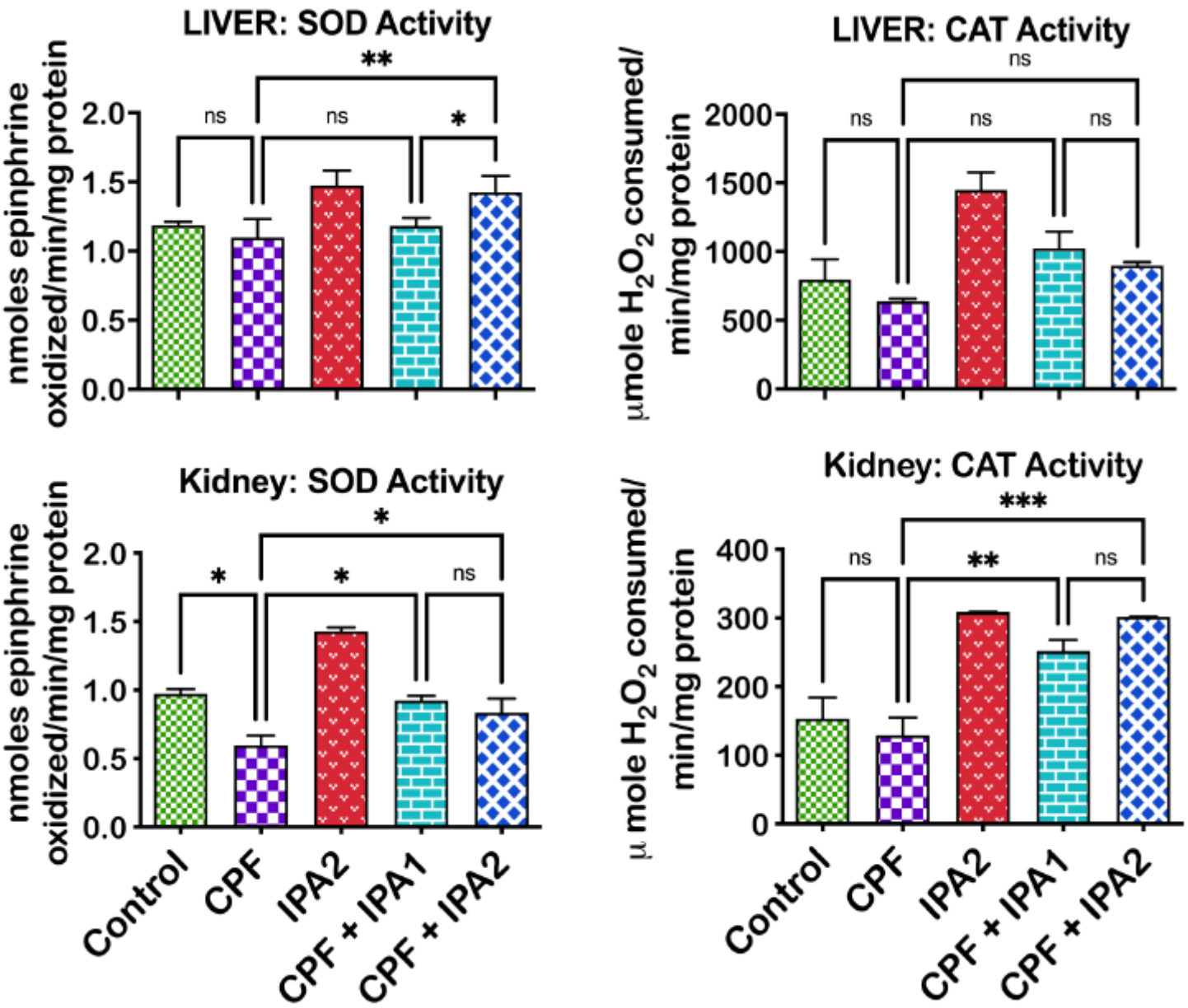

Figure 2

Influence of CPF alone, IPA alone and their combination on SOD and CAT activities in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: p<0.05 versus control; *: p<0.05 versus CPF alone; ns=not significant. SOD: superoxide dismutase; CAT: catalase.

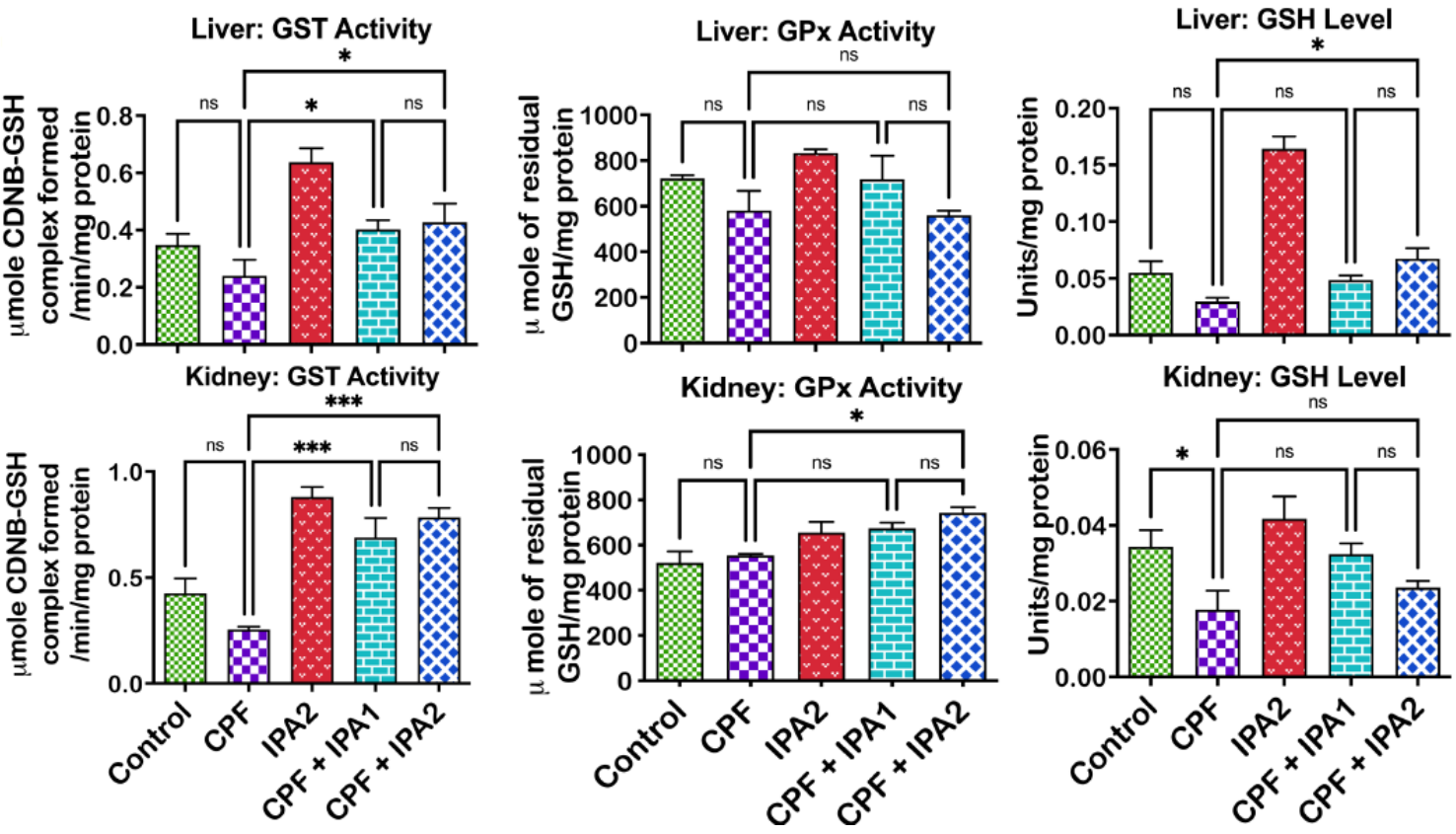

Figure 3

Influence of CPF alone, IPA alone and their combination on GSH level and activities of GSH-dependent enzymes -GST and GPx- in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: $p<0.05$ versus control; *: $p<0.05$ versus CPF alone; ns=not significant. GST: glutathione-S-transferase; GPx: glutathione peroxidase; GSH: glutathione.

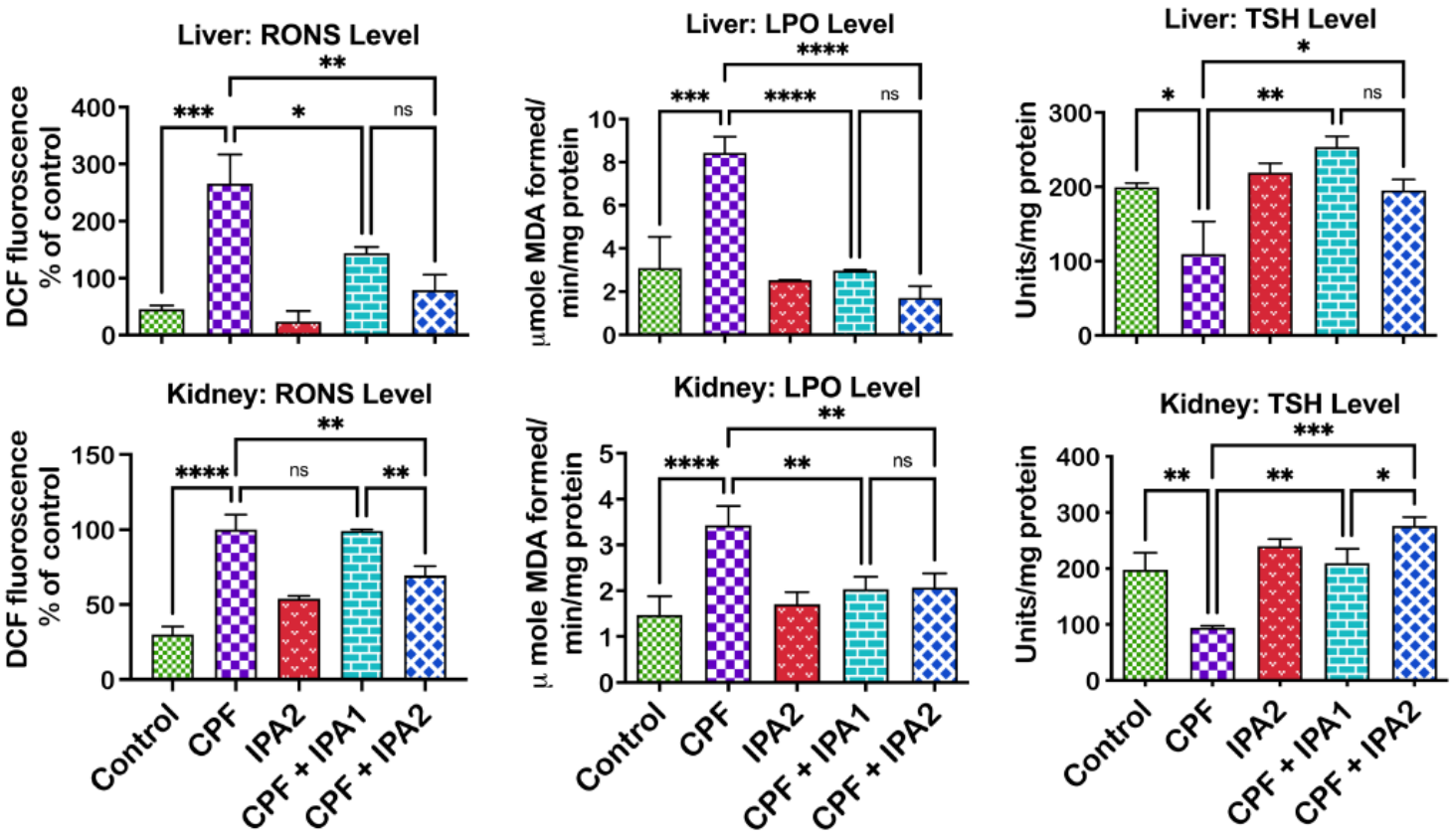

Figure 4

Influence of CPF alone, IPA alone and their combination on LPO, RONS and TSH levels in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: $p<0.05$ versus control; *: $p<0.05$ versus CPF alone; ns=not significant. RONS: reactive oxygen and nitrogen species; LPO: lipid peroxidation; TSH: total thiol.

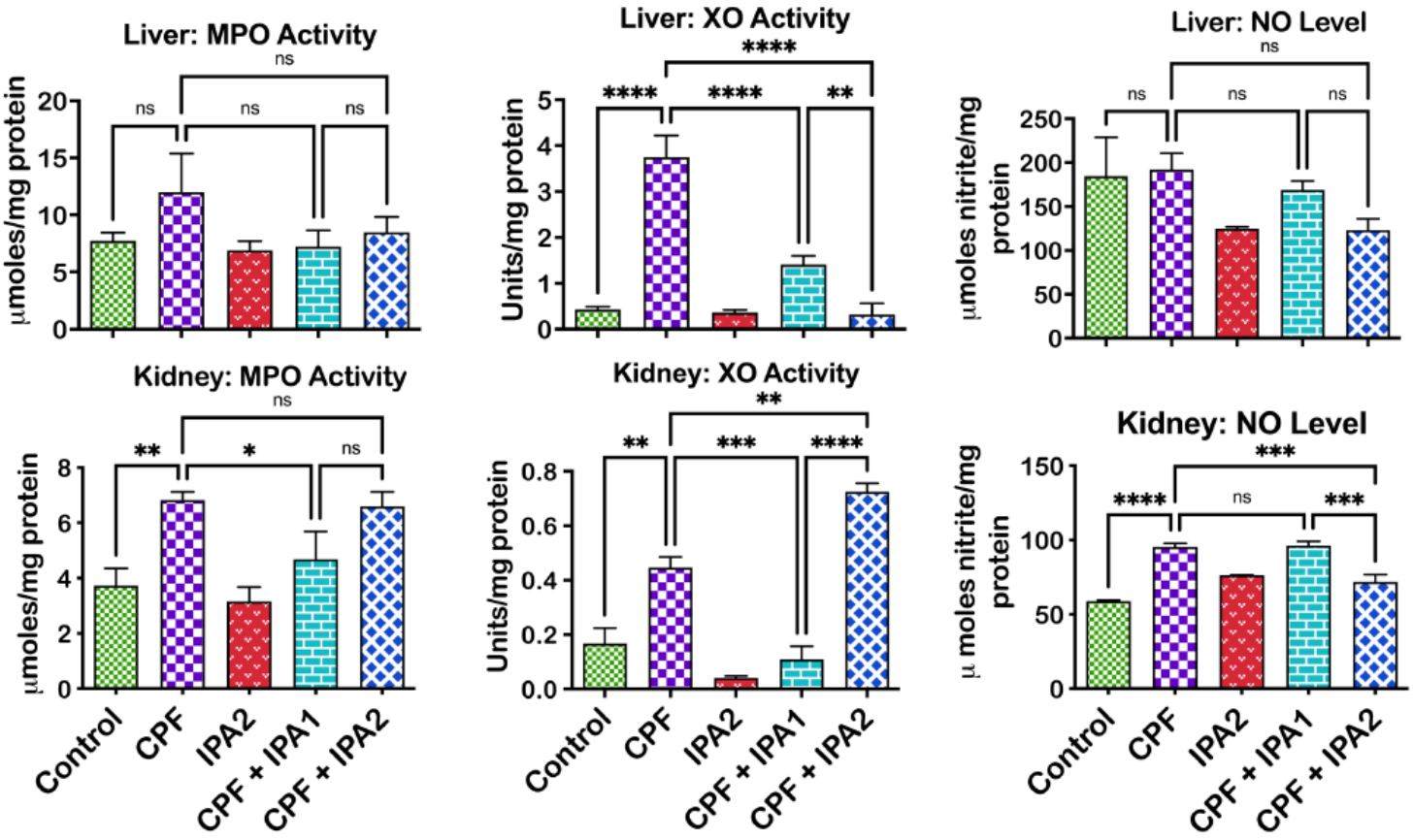

Figure 5

Influence of CPF alone, IPA alone and their combination on MPO, XO activity and NO level in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: p<0.05 versus control; *: p<0.05 versus CPF alone; ns=not significant. MPO: myeloperoxidase; XO: xanthine oxidase; NO: nitric oxide.

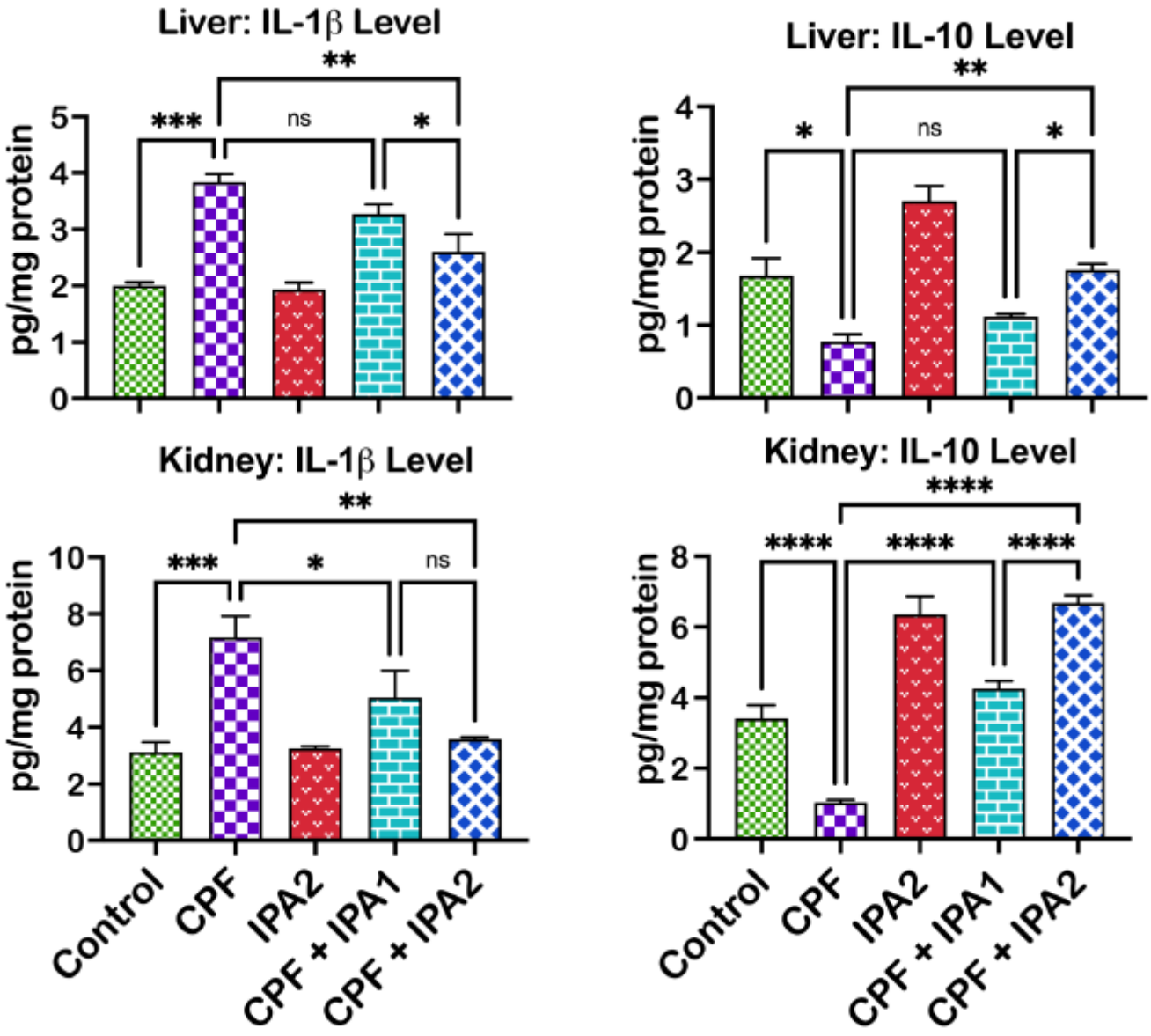

Figure 6

Influence of CPF alone, IPA alone and their combination on IL-1β and IL-10 levels in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: $p<0.05$ versus control; *: $p<0.05$ versus CPF alone; ns=not significant. IL-1β: interleukin-1beta; and IL-10: interleukin 10.

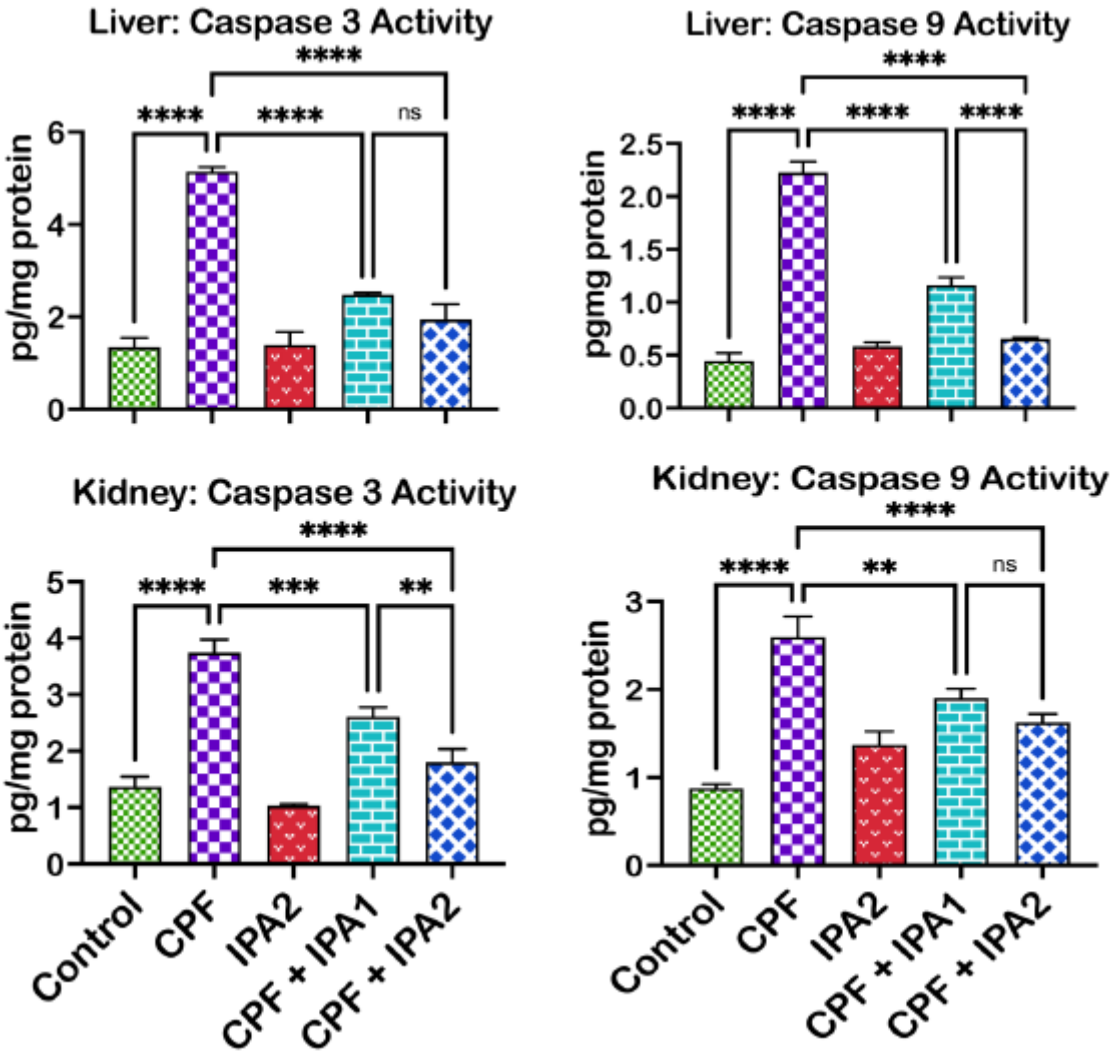

Figure 7

Influence of CPF alone, IPA alone and their combination on caspase-3 and caspase-9 activities in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: $p<0.05$ versus control; *: $p<0.05$ versus CPF alone; ns=not significant.

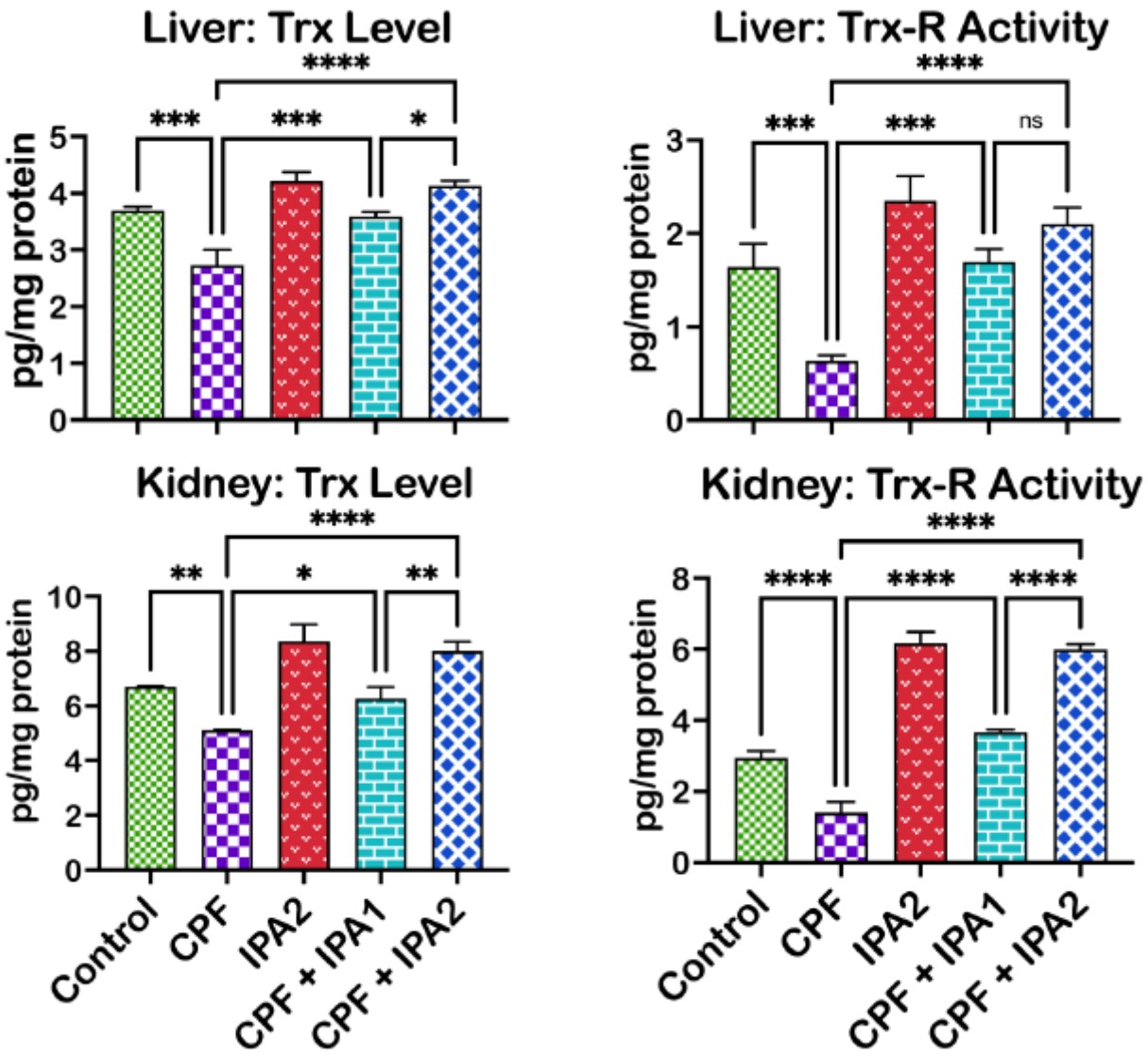

Figure 8

Influence of CPF alone, IPA alone and their combination on Trx level and Trx-R activity and 8-OHdG level in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: $p<0.05$ versus control; *: $p<0.05$ versus CPF alone; ns=not significant. Trx: thioredoxin; Trx-R: thioredoxin reductase.

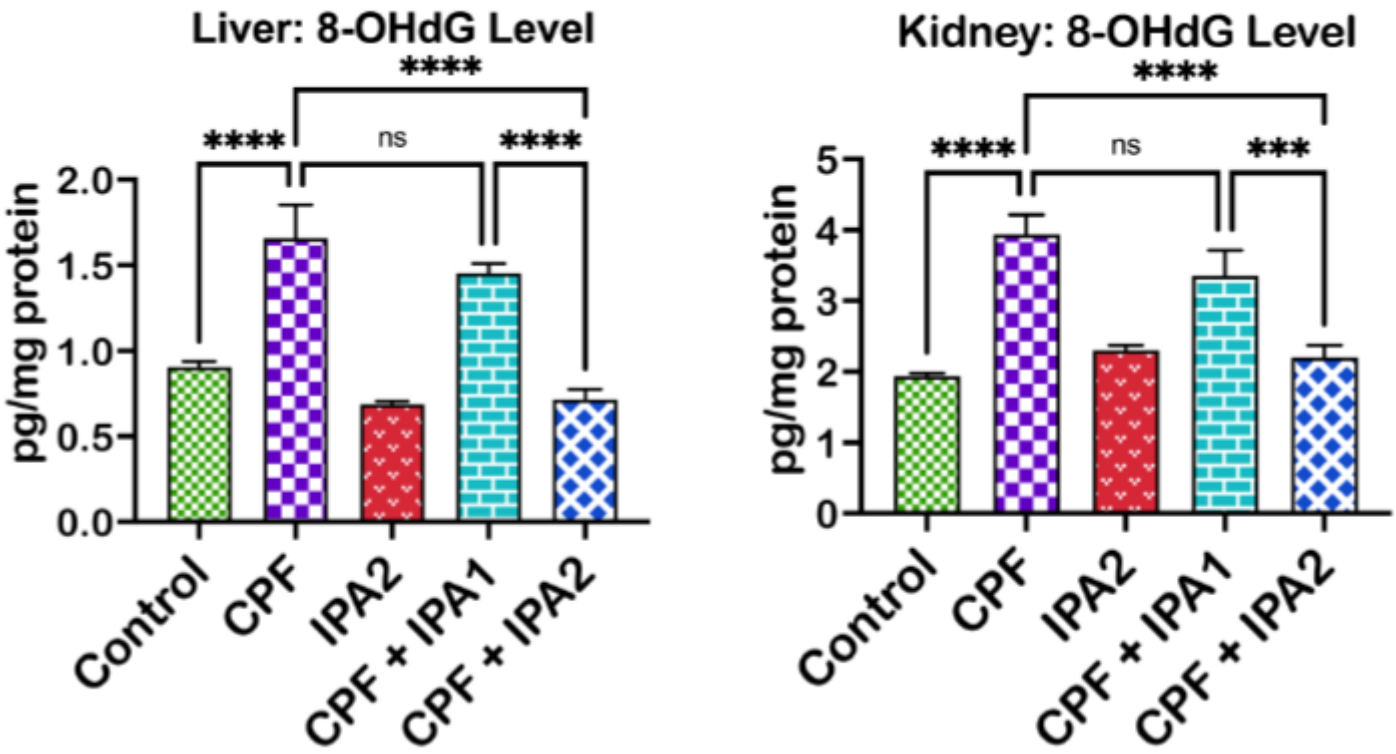

Figure 9

Influence of CPF alone, IPA alone and their combination on the 8-OHdG level in liver and kidney of rats. CPF: Chlorpyrifos (5 mg/kg); IPA: 3-Indolepropionic acid -IPA1 and IPA2 (25 and 50 mg/kg respectively). Each bar represents the mean ± SD of 6 rats. *: $p<0.05$ versus control; *: $p<0.05$ versus CPF alone; ns=not significant. 8-OHdG: 8-hydroxydeguanosine.

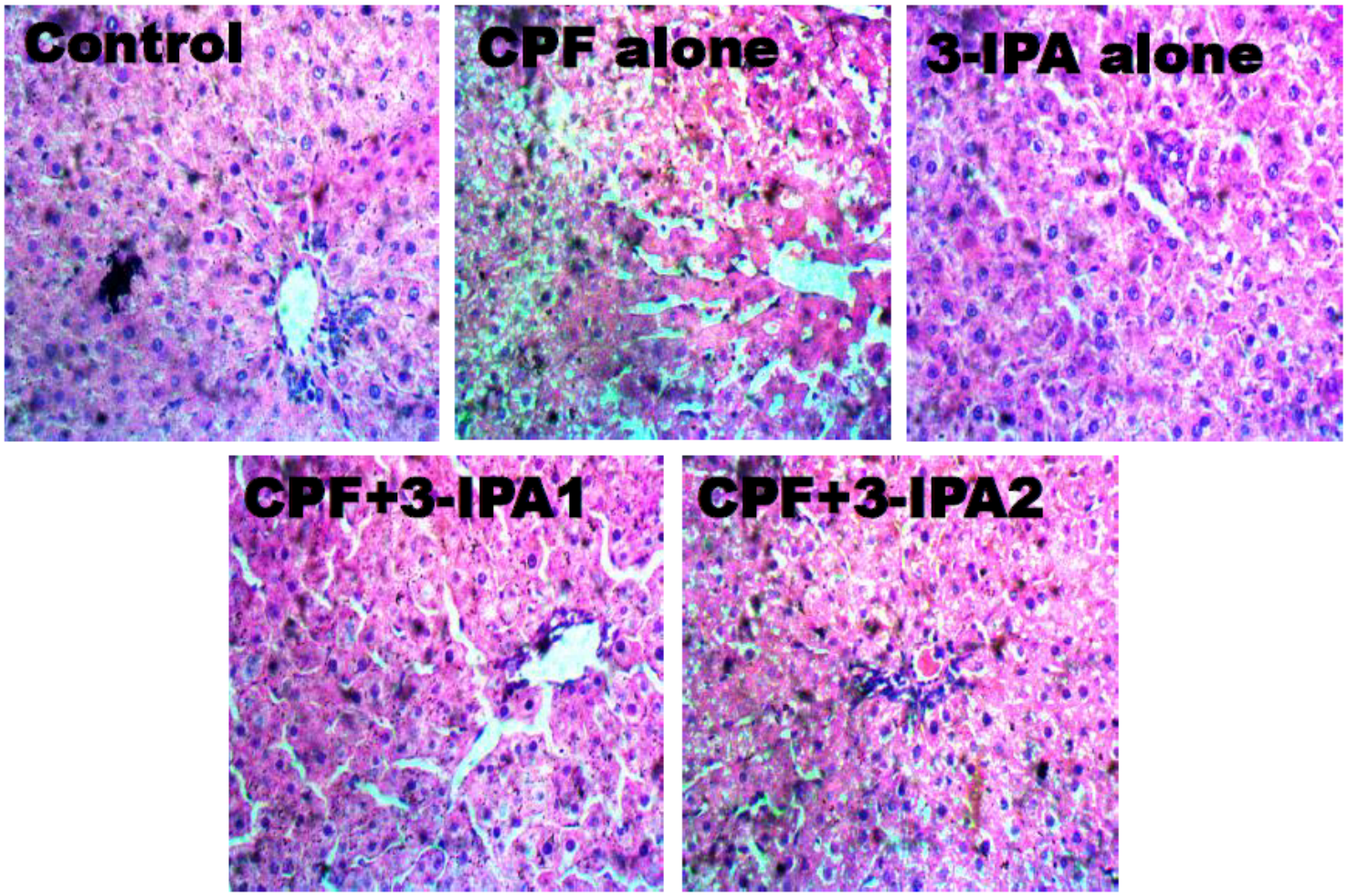

Figure 10

Representative photomicrographs of liver from control, CPF alone, IPA alone and co-exposure group. Control: There is no visible lesion. CPF alone: there is centrilobular hepatocellular degeneration and necrosis with Kupffer cell hyperplasia, multifocal necrotizing hepatitis. 3-IPA alone: There is no visible lesion. CPF+IPA1 (25mg/Kg): There is centrilobular hepatocellular degeneration, necrosis and inflammation. CPF+IPA2: There is no visible lesion. CPF: chlorpyrifos (5mg/Kg); IPA: 3-indolepropionic acid alone (50mg/Kg); IPA1 (25mg/Kg); IPA2 (50mg/Kg). H&E; Magnification x400.

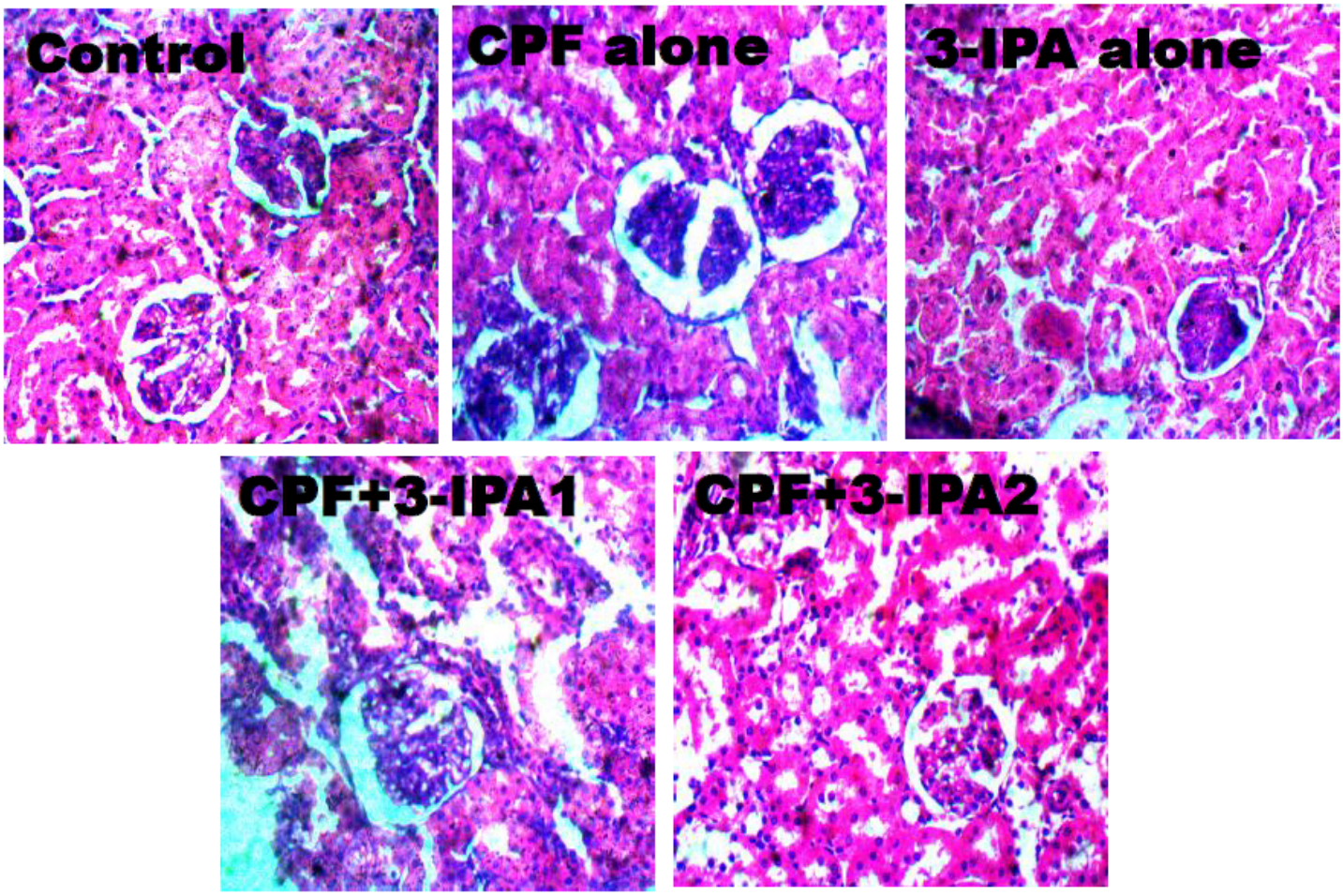

Figure 11

Representative photomicrographs of kidney from control, CPF alone, IPA alone and co-exposure group. Control: there is no visible lesion. CPF alone: there is atrophy of the glomerular tuft, with proliferative glomerulonephritis epithelial coagulation and necrosis. IPA alone: there is no visible lesion. CPF+IPA1 (25mg/Kg): there is distension between Bowman's space and cast in the tubular lumen. CPF+IPA2: there is moderate glomerular atrophy with improvement in kidney cyto-architecture. CPF: chlorpyrifos (5mg/Kg); IPA: 3-indole propionic acid alone (50mg/Kg); IPA1 (25mg/Kg); IPA2 (50mg/Kg). H&E; Magnification x400.

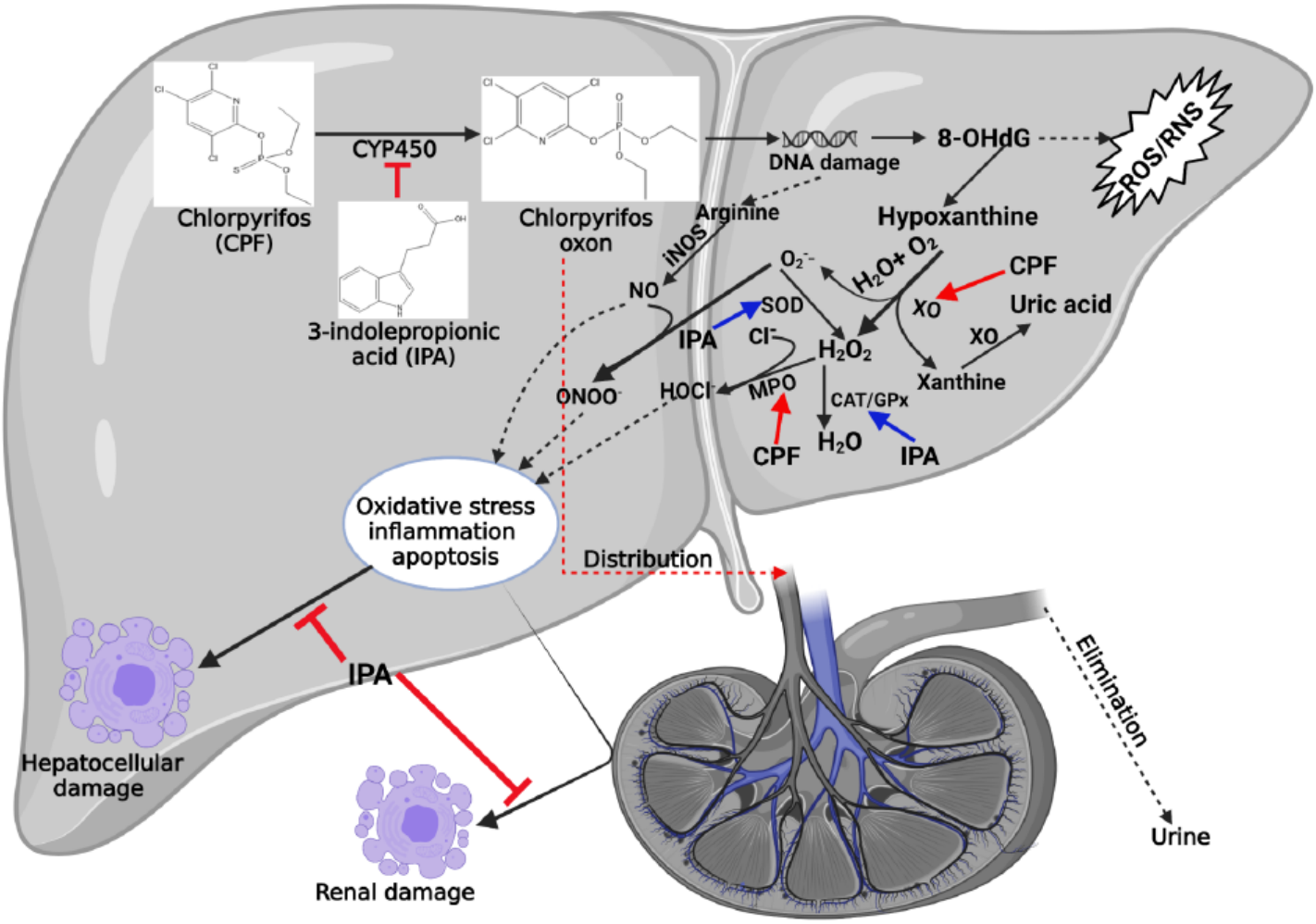

Figure 12

A proposed mechanism of the ameliorative effect of IPA against CPF-induced oxidative stress, inflammation, and apoptosis in male Wistar rats. Administration of CPF by gavage to rats results in the expression of cytochrome P450 (CYP) enzymes in the liver. CYP mediates the bioactivation of CPF into chlorpyrifos-oxon (CPFO), the most toxic intermediate. CPFO then binds to the DNA to induce oxidative DNA damage, forming 8-hydroxydeoxyguanine (8-OHdG). 8-OHdG is further biodegraded to hypoxanthine, which is acted upon by xanthine oxidase (XO) in the presence of metabolic H2O and O2 into xanthine and subsequently uric acid with the simultaneous release of abundant reactive oxygen species such as hydrogen peroxide (H2O2) and superoxide anion radical (O2.-). Superoxide dismutase (SOD) mediates the dismutation of O2.- into H2O2. Excess H2O2 is converted to molecular water by catalase (CAT) and glutathione peroxide (GPx). In the presence of chloride ion (Cl-), H2O2 is further converted to hypochloride (HOCl) by myeloperoxidase (MPO). Additionally, the accumulation of CFP in the liver activates the expression of inducible nitric oxide synthase (iNOS). iNOS deplete arginine, forming excess nitric oxide (NO). NO then combines with O2.- to create peroxyl nitrite. Accumulated levels of reactive oxygen species and reactive nitrogen species (ROS/RNS) induce oxidative stress, inflammation, and apoptosis, leading to hepatic and renal damages. IPA abrogates CPF-induced oxido-inflammatory and apoptotic changes by

scavenging excess ROS/RNS, suppressing DNA damage, resolving inflammation, and inhibiting apoptosis. CPF: Chlorpyrifos, IAP: 3-indolepropionic acid, CYP: Cytochrome P450, CPFO: Chlorpyrifos-oxon 8-OHdG: 8-hydroxydeoxyguanine, XO: xanthine oxidase H2O2: hydrogen peroxide, O2.-: superoxide anion radical, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxide, HOCl-: hypochloride, MPO: myeloperoxidase, iNOS: inducible nitric oxide synthase (iNOS), NO: nitric oxide ONOO-: Peroxyl nitrite, Reactive oxygen species (ROS), Reactive nitrogen species (RNS).