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Protective effects of rosuvastatin and vitamin E against fipronil-mediated oxidative damage and apoptosis in rat liver and kidney

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ABSTRACT

Fipronil (FPN) is a phenylpyrazole insecticide that is extensively used in agriculture and veterinary applications. However, FPN is also a potent environmental toxicant to animals and humans. Therefore, the current study aimed to investigate the protective role of rosuvastatin (ROSU) and vitamin E (Vit E) against FPN-induced hepatorenal toxicity in albino rats. Seven groups with eight rats each were used for this purpose; these groups included the control vehicle group that received corn oil, the Vit E group (1000 mg/kg, orally), the ROSU group (10 mg/kg, orally), the FPN group (20 mg/kg, orally), the FPN-ROSU group, the FPN-Vit E group, and the FPN-Vit E-ROSU group. The results revealed that FPN significantly increased serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, cholesterol, urea, and creatinine. In addition, there were substantial increases in the liver and kidney contents of malondialdehyde and nitric oxide, along with significant decreases in glutathione, superoxide dismutase, catalase, and glutathione peroxidase. FPN also caused histological changes and increased the expression of caspase-3 in the liver and kidney tissues. However, administration of ROSU and Vit E alone or in combination ameliorated the FPN-induced oxidative damage and apoptosis, possibly through their antioxidant properties.

1. Introduction

Fipronil (FPN) is a systemic insecticide belonging to the phenylpyrazole family. FPN has attracted attention for its potent effects against insects that show resistance to organophosphates, pyrethroids, and carbamate insecticides. This chemical has a long half-life and can persist in the environment for months or longer. FPN also has broad-spectrum activity against a wide range of insects (Chagnon et al., 2015; Lilia, 2003). Therefore, FPN is extensively used in the agricultural, veterinary, and home applications to control ants, wasps, flies, beetles, cockroaches, termites, thrips, rootworms, weevils, fleas, ticks, and others (Badgujar et al., 2015; de Oliveira et al., 2008; Oliveira et al., 2009; Simon-Delso et al., 2015; Slotkin and Seidler, 2010; Wu et al., 2014). The available literature shows that FPN is a potent toxicant to different animal species and humans (Lilia, 2003; Overmyer et al., 2007). The extensive use and high persistence of FPN in the environment result in high concentrations in the soil and water, offering multiple avenues of exposure of animals, fish, and humans to this hazardous environmental pollutant. Moreover, insecticide contamination of aquaculture hampers the expansion of global fisheries (Chagnon et al., 2015). Several studies have reported FPN poisoning in animals and humans by ingestion, accidently, or by misuse (Gill and Vinod, 2013; Lee et al., 2010). Previous publications have shown that FPN could cause injury in human hepatocytes (Das et al., 2006), thyroid dysfunction in rats (Leghait et al., 2009), oxidative damage in SH-SY5Y cells (Lee et al., 2011; Zhang et al., 2015), and neurotoxicity in zebrafish embryos (Stehr et al., 2006).

Cumulative evidence has shown that pesticide poisoning disrupts redox homeostasis along with induction of oxidative damage. Several reports have suggested that the disruption of redox homeostasis during FPN poisoning is due to the increased production of reactive oxygen species (ROS) (Abdel-Daim, 2016; Abdel-Daim et al., 2015b; Ortiz-Ortiz et al., 2009; Romero et al., 2016; Vidau et al., 2011). The endogenous antioxidant defense system (glutathione, GSH; glutathione peroxidase, GPx; superoxide dismutase, SOD; and catalase, CAT) plays a crucial role in scavenging the generated ROS. Therefore, when there is an imbalance between pro-oxidants and antioxidants, the cell becomes susceptible to the oxidative stress indicated by lipid peroxidation, mitochondrial dysfunction, DNA damage, and caspase-3 activation (Badgujar et al., 2015; de Medeiros et al., 2015; Lee et al., 2011; Romero et al., 2016; Slotkin and Seidler, 2010; Zhang et al., 2015).

Vitamin E (Vit E) is a non-enzymatic antioxidant naturally present

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in biological systems. This molecule protects the cell membrane from lipid peroxidation, which is induced by overproduction of ROS and reactive nitrogen species (RNS). Vit E has shown protective effects against different pesticides in vivo and in vitro (Kammon, 2012; Magdy et al., 2016; Niki, 2013; Sargazi et al., 2016; Saxena et al., 2011; Zingg, 2015). Badgujar et al. (2015) demonstrated that Vit E could decrease the lipid peroxidation and restore the antioxidant enzyme activities in mouse kidney and brain tissues after subacute exposure to FPN (at a dose of 10 mg/kg). However, although many published studies have described the protective role of Vit E against pesticide-induced oxidative damage, there are few in vivo studies of FPN.

Rosuvastatin (ROSU) is a member of the statin family, which is comprised of *anti*-hyperlipidemic agents. ROSU inhibits 3-hydroxy-3-methyglutaryl coenzyme reductase (Leite et al., 2017). Independent of its lipid-lowering effects, ROSU also has also anti-inflammatory and antioxidant properties (Maheshwari et al., 2015; Selim et al., 2017). Recently, ROSU was reported to provide protection against drug-induced nephrotoxicity (Selim et al., 2017) and ischemia-reperfusion injury in the heart, intestine, and spinal cord (Die et al., 2010; Maheshwari et al., 2015) through reduction of free radicals and up-regulation of antioxidant enzymes. However, to date, the protective effects of ROSU against insecticide-induced oxidative damages have not been studied. Therefore, this study was designed to investigate the potential effects of ROSU and Vit E alone or in combination as protective agents against FPN-induced hepatorenal toxicity. Another aim was to explore the underlying mechanisms of FPN-mediated oxidative damage independent of its primary target, gamma-aminobutyric acid (GABA) receptors. Serum biochemical parameters, oxidative stress markers, and caspase-3 expression were evaluated.

2. Materials and methods

2.1. Chemicals

Fipronil, FPN (BARS®, 10 mg/mL) was purchased from AVZ, Ltd. (Moscow, Russia) as a commercial product formulated for veterinary use. ROSU was obtained from AstraZeneca Company (Giza, Egypt) and Vit E was purchased from Pharco. Pharmaceuticals Industries (Alexandria, Egypt). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine, blood urea nitrogen, malondialdehyde (MDA), nitric oxide (NO), reduced GSH, GPx, SOD, and CAT kits were purchased from Biodiagnostics (Giza, Egypt). *Anti*-caspase-3 antibody was obtained from Dako Corporation (Life Trade, Egypt).

2.2. Animals and experimental design

A total of 56 male Wister Albino rats with an average body weight of 150 g were obtained from the Egyptian Organization for Biological Products and Vaccines. Then, all animals were kept at a temperature of 25 ± 2 °C and maintained under the 12:12 h light/dark cycle with free access to water and a normal pellet diet. All animals were treated in accordance with the guide for the care and use of laboratory animals approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

All rats were acclimatized under the abovementioned conditions for one week before starting the experiment. Next, rats were randomly divided into seven groups (8 rats each) and treated for 15 days. The first group served as a control and was given corn oil only once daily (vehicle control for Vit E and FPN); the second group of rats received Vit E at a dose of 1000 mg/kg orally once daily (Selim et al., 2017); the third group received ROSU at a dose of 10 mg/kg orally once daily (Selim et al., 2017); the fourth group received FPN at a dose of 20 mg/kg, 1/5 the LD_{50}, orally once daily during the last 5 days only (Caballero et al., 2015); the fifth group received FPN-Vit E; the sixth group received FPN-ROSU; and the seventh group received FPN-Vit E-ROSU. Vit E and ROSU were administered daily during the course of the experiment, while FPN was administered once daily during the last 5 days.

2.3. Blood collection, serum, and tissue preparations

At the end of the experiment (on day 15), blood samples were collected from the retro-orbital plexus, and then, serum was separated by centrifugation at 1200 g for 15 min. Serum was then collected and kept at −20 °C for further biochemical analyses. The rats were sacrificed by cervical decapitation. Liver and kidney were rapidly excised and washed with a normal saline solution (0.9% NaCl in distilled water) and perfused with ice-cold 50 mmol/L sodium phosphate buffered saline (100 mmol/L Na_2HPO_4/NaH_2PO_4, pH 7.4) containing 0.1 mmol/L EDTA to wash away the red blood cells and clots. Tissue samples were homogenized in 5–10 mL of ice-cold buffer/1 g tissue. Then, the homogenate was centrifuged for 30 min at 3000 g. The obtained supernatant was stored at −80 °C for analysis of MDA, NO, GSH, GPx, SOD, and CAT.

2.4. Serum biochemical analyses

The collected sera were used for assessing the liver function parameters, including AST, ALT, ALP, LDH, and cholesterol using commercial kits, the procedures were performed according to the manufacturer's instruction. Kidney function tests were also performed according to previously reported methods for urea and creatinine analysis (Al-Sayed et al., 2015).

2.5. Evaluation of oxidative stress markers

Lipid peroxidation was measured by determination of MDA content in liver and kidney homogenates. Additionally, NO and enzymatic and non-enzymatic antioxidants, including GSH, SOD, CAT, and GPx, were measured. All parameters were assessed according to our previous report (Abdel-Daim et al., 2015b).

2.6. Histopathology and immunohistochemistry

Liver and kidney specimens were fixed in 10% formalin for at least 24 h. Then, the samples were washed under running tap water followed by immersion in serial dilutions of ethyl alcohol. The specimens were then embedded in paraffin. Sections of 4 μm thickness were cut and stained with hematoxylin and eosin for histopathological examination under a light microscope. For immunostaining, liver and kidney sections were deparaffinized and dehydrated sequentially in graded ethyl alcohol. Next, the antigen retrieval was achieved by heating the slide in distilled water by autoclaving at 121 °C for 5 min. After the endogenous peroxidase had been inactivated by immersing the slides in 3% H_2O_2 and washed 3 times in PBS, the slide was blocked in 5% bovine serum albumin blocking reagent for 20 min to reduce nonspecific reactions. Then, the slide was incubated with *anti*-caspase 3 primary monoclonal antibody (1:100 dilution) at 37 °C for 1 h followed by an incubation with avidin-biotin complex (ABC kit, Vector Laboratories) at 37 °C for 45 min. The reaction product was visualized by treatment with 3,3-diaminobenzidine tetrahydrochloride (DAB), and the slide was counterstained with Mayer's hematoxylin.

2.7. Statistical analysis

Data are represented as the mean ± SE. Data were analyzed by one-way ANOVA using the statistical software package SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA), followed by Duncan's post hoc test for multiple group comparison. Statistical significance was accepted at $P < 0.05$.

Table 1
Effect of oral administration of fipronil, vitamin E and rosuvastatin and their combinations on serum biochemical parameters. Data are expressed as the mean ± SE (n = 8). Different superscript letters in the same row indicate statistical significance at P ≤ 0.05.

Parameters	Experimental groups						
	Control	ROSU	Vit E	FPN	FPN-ROSU	FPN-Vit E	FPN-Vit E-ROSU
AST (U/L)	56.84 ± 2.83^{a}	55.29 ± 2.36^{a}	54.48 ± 2.65^{a}	128.34 ± 5.70^{d}	81.80 ± 2.64^{c}	71.11 ± 2.08^{b}	61.36 ± 3.15^{a}
ALT (U/L)	26.57 ± 0.77^{a}	24.73 ± 1.37^{a}	24.45 ± 0.93^{a}	58.20 ± 3.36^{d}	40.46 ± 1.74^{b}	36.56 ± 1.41^{b}	28.14 ± 1.61^{a}
ALP (U/L)	61.19 ± 3.14^{a}	58.73 ± 1.80^{a}	57.22 ± 2.87^{a}	167.50 ± 10.32^{d}	87.93 ± 2.64^{b}	80.59 ± 1.73^{b}	66.77 ± 1.39^{a}
LDH (U/L)	228.68 ± 10.81^{ab}	226.54 ± 4.48^{ab}	206.88 ± 9.03^{a}	409.02 ± 10.07^{d}	335.29 ± 9.46^{c}	313.42 ± 11.14^{c}	247.89 ± 9.22^{b}
Cholesterol (mmol/L)	84.78 ± 4.17^{a}	80.25 ± 3.35^{a}	77.31 ± 3.48^{a}	153.29 ± 5.04^{d}	116.62 ± 4.07^{b}	107.91 ± 4.04^{b}	86.57 ± 2.89^{a}
Urea (mg/dL)	24.25 ± 1.16^{a}	23.13 ± 0.84^{a}	22.24 ± 0.91^{a}	68.49 ± 3.70^{d}	45.11 ± 1.94^{b}	41.00 ± 1.72^{b}	27.76 ± 1.83^{a}
Creatinine (mg/dL)	0.41 ± 0.07^{a}	0.37 ± 0.06^{a}	0.38 ± 0.06^{a}	3.15 ± 0.32^{d}	1.64 ± 0.16^{b}	1.39 ± 0.12^{b}	0.53 ± 0.09^{a}

Fipronil (FPN) at a dose of 20 mg/kg (1/5 LD_{50}); rosuvastatin (ROSU) at a dose of 10 mg/kg; vitamin E (Vit E) at a dose of 1000 mg/kg; aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP); lactate dehydrogenase (LDH).

3. Results

3.1. Serum biochemical analysis

As shown in Table 1, FPN induced hepatoxicity and nephrotoxicity as demonstrated by the elevation of serum liver and kidney biomarkers. The AST, ALT, ALP, LDH, urea, creatinine, and cholesterol levels were substantially increased (P ≤ 0.05) in response to FPN treatment compared to those of control rats. In contrast, these parameters were significantly reduced (P ≤ 0.05) when FPN treated-rats were administered ROSU, Vit E, or their combination compared to the FPN group. Notably, when a combined treatment with ROSU and Vit E was applied in FPN-intoxicated animals a significant decrease of the value of these parameters was observed (P ≤ 0.05) compared to rats administered with ROSU or Vit E alone. The decline of these values was non-significantly lower compared to the controls. These data suggested that when Vit E and ROSU were used in combination, they provided better protection against FPN-induced hepatorenal damage than either one alone.

3.2. Hepatic oxidative damage parameters

The effects of FPN intoxication and treatment with ROSU, Vit E, and their combination on lipid peroxidation and liver oxidative parameters are shown in Table 2. FPN-intoxicated rats showed significant increases (P ≤ 0.05) in MDA and NO levels along with significant decreases (P ≤ 0.05) in the GSH, GPx, SOD, and CAT levels in liver tissues compared to those of control rats. However, the toxic effects of FPN on hepatic MDA, NO, GSH, GPx, SOD, and CAT were significantly (P ≤ 0.05) reduced by administration of ROSU or Vit E alone, but these values were still significantly different (P ≤ 0.05) than the control values. Interestingly, these parameters were not significantly (P ≥ 0.05) different from the control values when the combination of ROSU and Vit E was administered, indicating a synergistic effect between Vit E and ROSU in the alleviation of FPN-induced oxidative damage in the liver.

3.3. Renal oxidative damage parameters

The markers for FPN-induced oxidative damage in renal tissue are shown in Table 3. FPN intoxication dramatically increased (P ≤ 0.05) the concentrations of MDA and NO and decreased (P ≤ 0.05) the concentrations of GSH and GPx and the SOD and CAT activities in the renal tissues compared to control values. However, the FPN-ROSU and FPN-Vit E groups showed significant (P ≤ 0.05) improvements in these parameters compared to controls. Along with these data, the FPN-Vit E-ROSU group showed (P ≤ 0.05) improvement in the FPN-induced oxidative damage in kidney tissues compared to the FPN-ROSU and FPN-Vit E groups.

3.4. Histopathological findings

Liver sections of control corn oil-treated rats had uniform polyhedral hepatocytes with normal sinusoids and central veins. In contrast, we observed portal vein congestion, severe hydropic degeneration (signet ring cells), necrosis, nuclear condensation, and lymphocytic infiltration around the portal vein in FPN-treated rats. ROSU, Vit E, and their combination notably restored the normal hepatic architecture (Fig. 1**)**.

As shown in Fig. 2, control rats had normal glomeruli and renal tubular epithelia. In contrast, FPN-intoxicated rats showed a severe loss of the brush border, tubular necrosis, and tubular vacuolization. In addition, FPN-treated rats exhibited moderate tubular dilatation and inflammatory cell infiltration. Treatment with ROSU, Vit E, or their combination caused a notable recovery of the histopathological appearance after FPN-induced renal injury.

Table 2
Effect of oral administration of fipronil, vitamin E and rosuvastatin and their combinations on oxidative stress markers in liver tissue.

Parameters	Experimental groups						
	Control	ROSU	Vit E	FPN	FPN-ROSU	FPN-Vit E	FPN-Vit E-ROSU
MDA (nmol/g)	52.03 ± 3.75^{a}	51.36 ± 3.72^{a}	50.79 ± 2.97^{a}	91.81 ± 4.72^{d}	78.85 ± 2.71c	69.40 ± 2.30^{b}	54.65 ± 2.23^{a}
NO (μmol/g)	71.95 ± 3.03^{ab}	68.17 ± 3.03^{a}	64.96 ± 3.30^{a}	117.30 ± 4.08^{d}	87.63 ± 2.99^{c}	80.93 ± 3.32^{bc}	73.36 ± 3.58^{ab}
GSH (mg/g)	60.42 ± 2.74^{cd}	62.29 ± 3.56^{cd}	66.61 ± 3.66^{c}	35.58 ± 1.80^{a}	41.83 ± 1.48^{ab}	47.91 ± 1.95^{b}	56.85 ± 1.93^{c}
GPx (mol/g)	101.46 ± 6.34^{c}	112.91 ± 5.46^{d}	118.21 ± 2.93^{d}	41.33 ± 3.00^{a}	50.94 ± 3.22^{a}	63.21 ± 2.93^{b}	95.97 ± 2.44^{c}
SOD (U/g)	19.47 ± 0.49^{c}	22.16 ± 1.00^{d}	23.48 ± 1.13^{d}	9.10 ± 0.35^{a}	12.91 ± 0.54^{b}	14.24 ± 0.77^{b}	17.41 ± 0.76^{c}
CAT (U/g)	2.13 ± 0.04^{d}	2.34 ± 0.16^{de}	2.41 ± 0.07^{e}	0.68 ± 0.07^{a}	1.37 ± 0.08^{b}	1.45 ± 0.05^{b}	1.80 ± 0.07^{c}

Data are expressed as the mean ± SE (n = 8).
Fipronil (FPN) at a dose of 20 mg/kg (1/5 LD_{50}); rosuvastatin (ROSU) at a dose of 10 mg/kg; vitamin E (Vit E) at a dose of 1000 mg/kg; malondialdehyde (MDA); nitric oxide (NO); reduced glutathione (GSH); glutathione peroxidase (GPx); superoxide dismutase (SOD); catalase (CAT).
Different superscript letters in the same row indicate statistical significance at P ≤ 0.05.

Table 3
Effect of oral administration of fipronil, vitamin E and rosuvastatin and their combinations on oxidative stress markers in kidney tissue.

Parameters	Experimental groups						
	Control	ROSU	Vit E	FPN	FPN-ROSU	FPN-Vit E	FPN-Vit E-ROSU
MDA (nmol/g)	104.83 ± 4.13^{a}	103.39 ± 2.83^{a}	99.03 ± 2.96^{a}	195.66 ± 10.88^{c}	156.34 ± 10.88^{b}	137.02 ± 10.88^{b}	110.20 ± 6.90^{a}
NO (μmol/g)	109.24 ± 2.82^{a}	106.93 ± 2.64^{a}	101.58 ± 2.92^{a}	170.54 ± 9.59^{d}	139.47 ± 9.46^{c}	127.15 ± 5.34^{bc}	112.33 ± 3.13^{ab}
GSH (mg/g)	114.38 ± 5.48^{d}	116.34 ± 4.26^{de}	121.74 ± 4.15^{e}	69.00 ± 3.71^{a}	85.19 ± 3.05^{b}	98.46 ± 1.93^{c}	107.76 ± 2.19^{cd}
GPx (mol/g)	66.11 ± 3.55^{cd}	69.16 ± 2.12^{d}	71.57 ± 3.13^{d}	28.70 ± 2.20^{a}	40.14 ± 2.19^{b}	45.43 ± 3.22^{b}	60.23 ± 3.62^{c}
SOD (U/g)	22.98 ± 1.01^{cd}	24.58 ± 1.44^{de}	25.94 ± 1.44^{e}	9.61 ± 0.39^{a}	15.63 ± 0.70^{b}	16.42 ± 0.63^{b}	20.48 ± 0.56^{c}
CAT (U/g)	2.41 ± 0.15^{d}	2.51 ± 0.10^{d}	2.55 ± 0.11^{d}	0.79 ± 0.05^{a}	1.48 ± 0.15^{b}	1.64 ± 0.12^{b}	2.05 ± 0.11^{c}

Data are expressed as the mean ± SE (n = 8).
Fipronil (FPN) at a dose of 20 mg/kg (1/5 LD_{50}); rosuvastatin (ROSU) at a dose of 10 mg/kg; vitamin E (Vit E) at a dose of 1000 mg/kg; malondialdehyde (MDA); nitric oxide (NO); reduced glutathione (GSH); glutathione peroxidase (GPx); superoxide dismutase (SOD); catalase (CAT).
Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

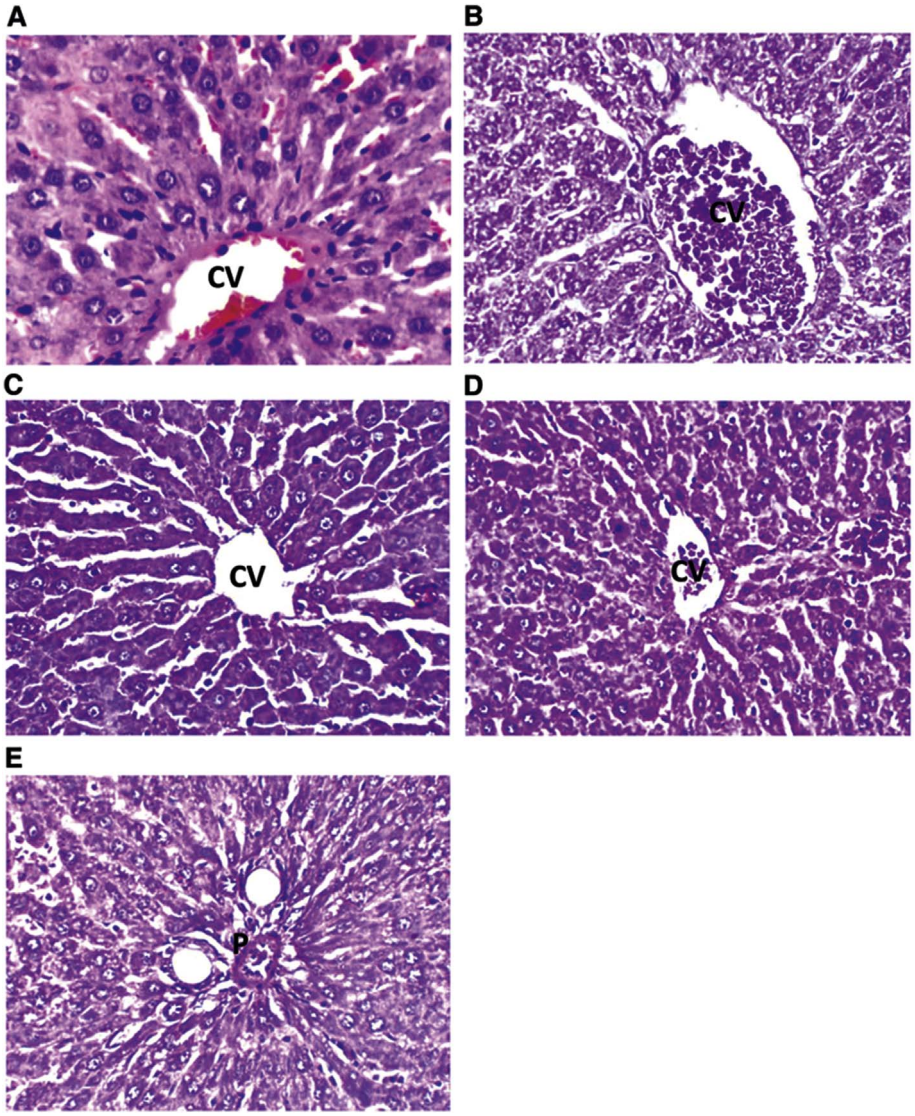

Fig. 1. Histopathological changes in liver sections after treatment with fipronil, rosuvastatin, and vitamin E. A: Liver sections from a control rat show uniform polyhedral hepatocytes with normal sinusoids. **B:** An FPN-intoxicated rat shows portal vein congestion, severe hydropic degeneration (signet ring cells), necrosis, nuclear condensation, and lymphocytic infiltration. **C** and **D:** The FPN-ROSU and FPN-Vit E groups, respectively, show mild hydropic degeneration, portal vein congestion, and few pyknotic nuclei. **E:** The FPN-Vit E-ROSU group shows substantial improvements in liver histology compared to FPN-intoxicated rats. (CV, central vein; P, portal area; H&E X400).

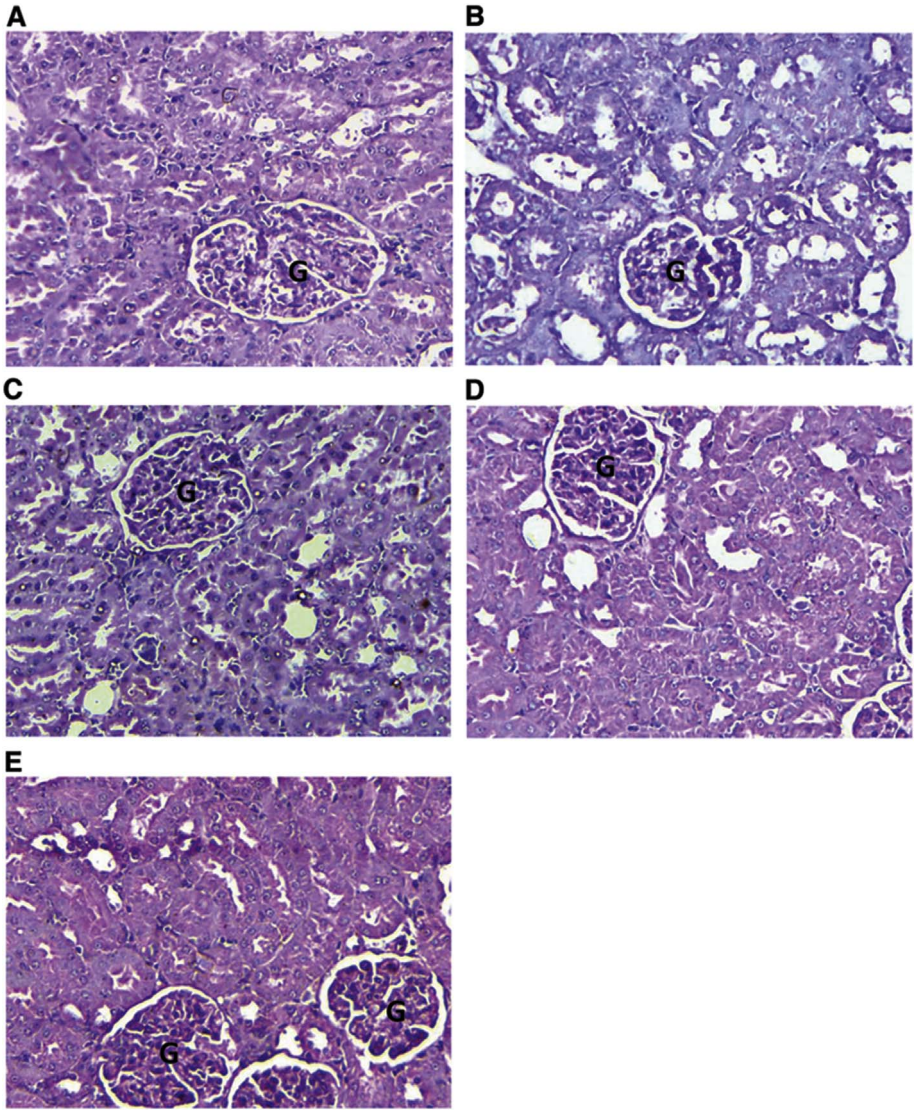

Fig. 2. Histopathological changes in kidney sections after treatment with fipronil, rosuvastatin, and vitamin E. A: Kidney section from a control rat shows normal architecture of glomeruli and renal tubules. **B:** An FPN-intoxicated rat shows a severe loss of brush border, cloudy swelling, vacuolar degeneration, and tubular dilatation. **C** and **D:** The FPN-ROSU and FPN-Vit E groups, respectively, show mild loss of the brush border and degenerative changes. **E:** The FPN-Vit E-ROSU group shows substantial improvements in kidney histology compared to FPN-intoxicated rats. (G: glomerulus; H&E X200).

3.5. Immunohistochemical study

Fipronil dramatically up-regulated the caspase-3 expression in liver (Fig. 3**)** and kidney (Fig. 4**)** tissues. In the FPN-ROSU and FPN-Vit E groups, there was slight up-regulation of caspase-3 expression compared to the control group. Combined treatment of ROSU and Vit E sharply reduced the FPN-induced caspase-3 up-regulation.

4. Discussion

Fipronil is a broad-spectrum insecticide widely used in agriculture and veterinary applications. However, it is a potent environmental toxicant to animals and humans. Multiple in vitro studies have shown the cytotoxic effects of FPN in different types of cell lines, but there are few in vivo studies (Badgujar et al., 2015; Das et al., 2006; Lee et al., 2011; Romero et al., 2016; Roques et al., 2012; Vidau et al., 2011). All of these studies are consistent with our findings, including the involvement of oxidative stress and apoptotic mechanisms in the FPN-induced hepatorenal damage in rat and the potential use of ROSU and Vit E as protective agents against FPN insult.

ROS are naturally generated in all mammalian cells during normal cellular respiration. The major ROS are superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) (Le Bras et al., 2005; Nordberg and Arnér, 2001; Small et al., 2012). Since ROS are cytotoxic molecules even when produced during normal respiration, for cell survival, they are naturally neutralized by the endogenous antioxidant defense system, primarily GSH, SOD, CAT, and GPx (Avery, 2011; Le Bras et al., 2005; Nordberg and Arnér, 2001; Small et al., 2012). When there is an imbalance between ROS production and antioxidants, the cell becomes vulnerable to severe oxidative stress-induced damage. ROS can attack cell membranes and other cellular molecules, causing lipid peroxidation, protein oxidation, and DNA damage, which results in cell disruption and loss of function and can lead to diseases such as cancers, atherosclerosis, diabetes, and renal failure (Abdel-Daim et al., 2015a; Klaunig et al., 2010; Small et al., 2012).

In the present study, there were substantial increases in MDA and NO levels along with dramatic decreases in GSH, SOD, CAT, and GPx in the liver and kidney tissues of FPN-intoxicated rats, indicating the presence of oxidative stress. Our results confirm the findings of previous in vitro studies, which have investigated the toxicity of FPN in hepatocytes, enterocytes, and neuronal cells (Das et al., 2006; Guelfi et al., 2015; Lee et al., 2011; Romero et al., 2016; Vidau et al., 2011). GABA

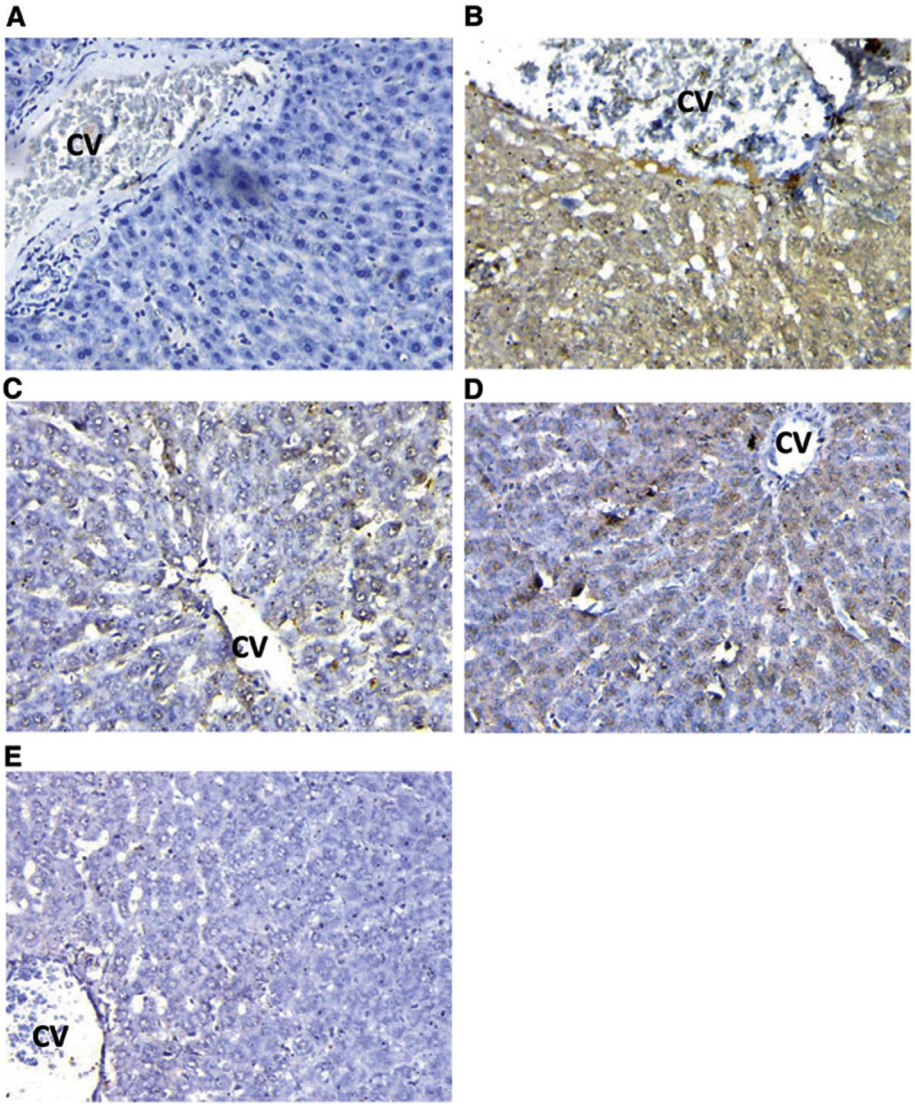

Fig. 3. Changes in hepatic caspase-3 expression after treatment with fipronil, rosuvastatin, and vitamin E. A: Control group; **B:** FPN-intoxicated rat; **C:** FPN-ROSU group; **D:** FPN-Vit E group; and **E:** FPN-Vit E-ROSU group. Immunostaining was performed using a specific antibody against caspase-3 and developed with 3,3-diaminobenzidine tetrahydrochloride (DAB). The positive staining of caspase-3 is presented as brown hepatocytes. (CV, central vein; X200). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

receptors in neurons are the primary targets of FPN (Wu et al., 2014). Recently, it was reported that FPN, independent to GABA receptors, can also attack the mitochondria and cause uncoupling of oxidative phosphorylation. These changes inhibit the electron flow through the electron transport chain and lead to severe ATP depletion with over accumulation of $O_2^{\bullet -}$ at levels that overwhelm the cell (Vidau et al., 2011). Concurrent with the decreases in cellular antioxidants, including GSH, SOD, CAT, and GPx (Tables 2 and 3), the cells fail to scavenge $O_2^{\bullet -}$ (Le Bras et al., 2005). H_2O_2 is then generated from $O_2^{\bullet -}$and further undergoes the Fenton reaction in the presence of transition metals (Fe^{2+}) to produce $OH^{\bullet}$, the strongest reactive radical among the ROS (Avery, 2011; Nordberg and Arnér, 2001). In the current study, MDA, a marker of lipid peroxidation, was drastically increased in FPN-intoxicated animals. This finding indicates cell membrane damage in hepatic and renal cells, which is attributed to the increased production of $OH^{\bullet}$. These data are consistent with results from the previous studies (Badgujar et al., 2015; Guelfi et al., 2015; Lee et al., 2011; Romero et al., 2016; Slotkin and Seidler, 2010; Vidau et al., 2011). Due to membrane damage, hepatic enzymes are released into the bloodstream, leading to elevation of serum AST, ALT, and ALP levels as shown in Table 1 (Ohta et al., 2009). Sun et al. (2016) has reported that FPN enhances adipogenesis *via* down-regulation of AMPKα in 3T3- L1 adipocytes disrupting the lipid metabolism, which may explain the increased level of serum cholesterol. The histopathological analysis also indicated a severe loss of the brush border in renal tubular epithelia, which may be due to the FPN-induced lipid peroxidation. When there is a reduction in ATP production by oxidative phosphorylation, the glucose metabolism shifts from aerobic glycolysis to anaerobic processes, which could explain the significant increase in serum LDH as a response to FPN (Table 1). This result is consistent with that obtained by previous in vitro studies (de Medeiros et al., 2015; Lee et al., 2011; Romero et al., 2016; Vidau et al., 2011).

Moreover, $OH^{\bullet}$ and MDA directly reacted with DNA, causing DNA adducts and nuclear condensation (Fig. 1), which, together with the induced mitochondrial dysfunction, promoted apoptosis via cytochrome *c* release and further caspase-3 activation as detected by immunostaining (Figs. 3 and 4**).** In this study, we therefore report that FPN induced cell death via apoptotic mechanisms, confirming the observations obtained by Lee et al. and Vidau et al. (2011) on SH-SY5Y cells, Lassiter et al. (2009) on PC12 cells, and Das et al. (2006) on HepG2 cells. Furthermore, our results indicated that the increased NO levels possibly react with $O_2^{\bullet -}$ through nitric oxide synthase and were

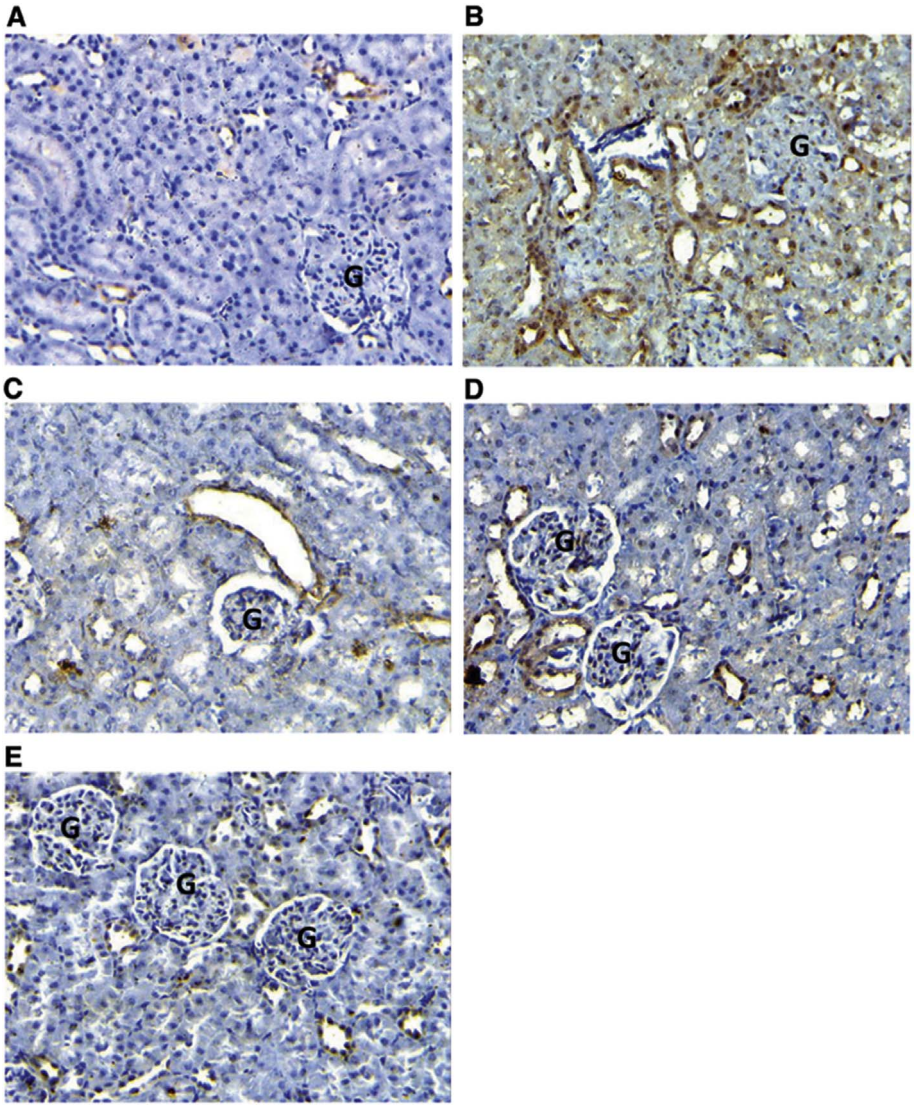

Fig. 4. Changes in renal caspase-3 expression after treatment with fipronil, rosuvastatin, and vitamin E. A: Control group; **B:** FPN-intoxicated rat group; **C:** FPN-ROSU group; **D:** FPN-Vit E group; and **E:** FPN-Vit E-ROSU group. Immunostaining was performed using a specific antibody against caspase-3 and developed with DAB. The positive staining of caspase-3 is presented as brown renal cells. (G: glomerulus; X200). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

converted into a very powerful RNS known as peroxynitrite ($ONOO^-$) (Nordberg and Arnér, 2001). $ONOO^-$ causes protein nitration and accumulation of damaged proteins, which may affect the structure and function of cellular antioxidant enzymes.

Kidney requires high energy levels for active transport of various molecules through the nephrons, particularly in the cortical segments, where mitochondria are abundant (Small et al., 2012). Interestingly, in this study, the kidney histopathological and caspase-3 expression data revealed that injury was observed in the cortical region rather than other kidney regions (Figs. 2B and 4B). These findings support the hypothesis that mitochondria have a central role in FPN-induced oxidative damage and apoptotic mechanisms independent of GABA receptors. Based on these observations, we hypothesized that FPN-induced histological alterations in kidney tissue with changes in kidney function tests, including creatinine and urea, were mediated by oxidative damage.

ROSU is an *anti*-hyperlipidemic agent. Recently, it was shown to have antioxidant properties. Previous studies have reported that the antioxidant activity of ROSU may be mediated by up-regulating antioxidant enzymes and reducing NADPH-dependent production of ROS (Grosser et al., 2004; Miersch et al., 2007; Selim et al., 2017). Vit E is a lipid-soluble vitamin that is incorporated in the lipid bilayer of the cell membrane. It has a hydroxyl group that acts as a hydrogen atom donor for $OH^•$, yielding a non-radical product, and prevents FPN-induced lipid peroxidation, as indicated by a significant decrease in MDA levels (Niki, 2013; Nordberg and Arnér, 2001; Small et al., 2012). Since it has been reported that $OH^•$ reacts with Vit E and GSH at the same constant rate ($10^{10}\,M^{-1}s^{-1}$) (Simic et al., 1992), supplementation with Vit E may increase GSH in the cytoplasm, which is required in ROS scavenging and GPx recycling. In the current study, pretreatment with ROSU or Vit E conferred protection against FPN hepatorenal toxicity, as shown by the improved histopathology, biochemical parameters, oxidative stress markers, and caspase-3 expression, but did not restore these parameters to normal levels. These data are consistent with previous investigations showing that ROSU and Vit E protect against spinal cord ischemia and gentamicin nephrotoxicity, respectively (Die et al., 2010; Kadkhodaee et al., 2005).

Interestingly, a combination of ROSU and Vit E could restore all parameters to normal, suggesting a synergistic antioxidant effect of both agents against FPN-induced hepatorenal injury. These observations support the data obtained by Selim et al. (2017), who investigated the protective effect of ROSU and Vit E together against amikacin nephrotoxicity.

Fig. 5 shows a scheme of the proposed protective pathways of ROSU

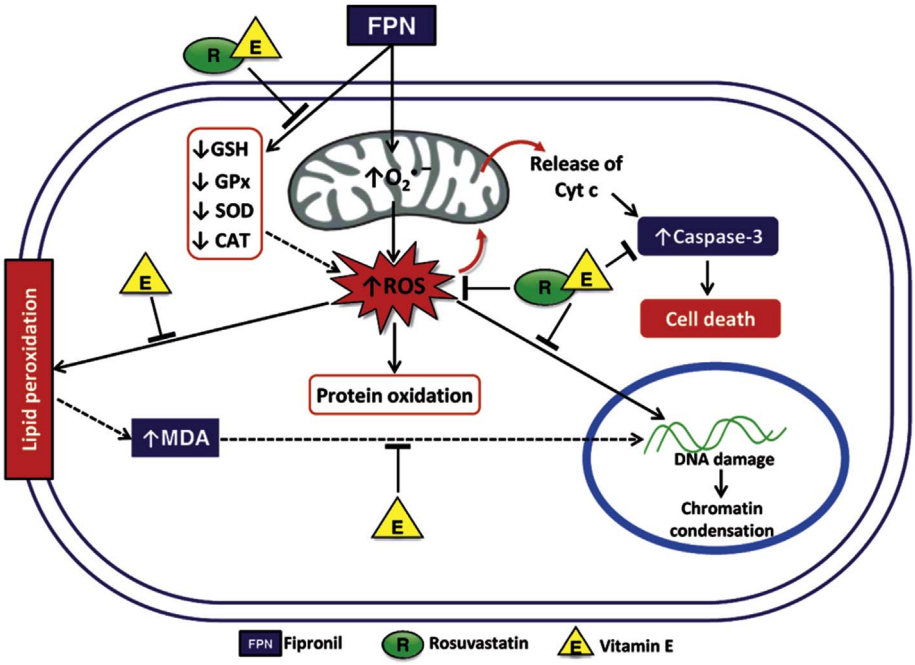

Fig. 5. Scheme of the proposed antioxidant mechanisms of rosuvastatin and vitamin E against fipronil-induced oxidative stress and apoptosis. FPN, fipronil; ROS, reactive oxygen species; MDA, malondialdehyde.

and Vit E against FPN-induced oxidative damage and apoptosis hepatic and renal cells. FPN induced production of ROS in significant amounts through disrupting the respiratory chain and inhibition of the antioxidant system leading to cascade of cellular injuries including lipid peroxidation, protein oxidation, and DNA damage followed by a release of Cyt *c* and activation of caspase-3 ended by apoptosis. On the other hand, ROSU and Vit E could alleviate FPN-induced damage through neutralization of ROS and up-regulation of antioxidant enzymes protecting the cell from lipid peroxidation, DNA damage, and from apoptosis as well (Fig. 5).

5. Conclusion

The overall data indicated that FPN could induce severe tissue damage in the liver and kidney mediated by oxidative stress and apoptotic mechanisms. Our findings also support the hypothesis that mitochondria play a crucial role in FPN-induced oxidative damage independent of GABA receptors. ROSU and Vit E in combination could be used as potential antioxidants against FPN-mediated oxidative damage in the liver and kidney.

Conflict of interest

This research received no specific grant from any funding agency. The authors declare that there are no conflicts of interest.

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