Mitochondrial bioenergetics and redox dysfunction in nephrotoxicity induced by pyrethroid permethrin are ameliorated by flavonoid-rich fraction

Nissaf Aoiadni (nissafaoiadni93@gmail.com)
Universite de Sfax Faculte des Sciences de Sfax https://orcid.org/0000-0003-2945-1668
Hajer Jdidi
Faculty of Sciences of Sfax: Universite de Sfax Faculte des Sciences de Sfax
Abdelfattah El Feki
Faculty of Sciences of Sfax: Universite de Sfax Faculte des Sciences de Sfax
Hamadi Fetoui
Faculty of Sciences of Sfax: Universite de Sfax Faculte des Sciences de Sfax
Fatma Ghorbel Koubaa
Faculty of Sciences of Sfax: Universite de Sfax Faculte des Sciences de Sfax

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Abstract

Recent studies suggest that mitochondrial bioenergetics and oxidative status perturbations may be common mechanisms involved in the progression of renal damage. The present study aimed to evaluate in vitro the potential anti-inflammatory using membrane stabilization and protein denaturation inhibition assays and in vivo protective effect of flavonoid- rich fraction from Fumaria officinalis (EAF) against permethrin (PER) induced nephrotoxicity in male rat. Animals were allocated into four groups: control; EAF (200 mg/Kg BW); PER (34.05 mg/Kg BW); and PER (34.05 mg/Kg BW) + EAF (200 mg/Kg BW) for 7 days. Our results suggest that EAF inhibited significantly protein denaturation and restored membrane stabilization. In vivo , permethrin-treated rats caused a substantial reduction of body weight gain and plasma calcium (Ca), phosphorus (P) and vitamin C levels as well as an increase of absolute and relative kidney weights and plasma lactate dehydrogenase (LDH) activity and kidney and mitochondria thiobarbituric acid reactive substance (TBARS), advanced oxidation protein product (AOPP) and protein carbonyl (PCO) levels. PER also caused renal and mitochondrial enzymatic and non-enzymatic antioxidant perturbation as well as mitochondrial NADH-ubiquinone reductase (complex I), ubiquinol cytochrome c reductase (complex III) and cytochrome c oxidase (complex IV) activities reduction associated with renal histopathological alterations. However, co-administration of EAF to the PER group restored oxidative status and mitochondrial bioenergetics. We suggest that EAF may be considered as a future therapeutic anti-inflammatory and may be used singly or as a co-therapeutic in the treatment of diseases associated with mitochondrial oxidative stress.

Introduction

At the largest metabolic and excretory organs in mammals, kidney play an important role in maintaining homeostatis (Maschmeyer et al. 215). Environmental toxicants can cause kidney injury, destroying physical functions and leading serious consequences of public health. Synthetic pyrethroid represent a potent group of pesticides with strong efficacy and insecticidal activity (Kumar et al. 2016). Permethrin (PER) is among the main pyrethroids (Type I) widely used in agriculture and horticulture (Saillenfait et al. 2018). The lipophilic nature of PER facilitates its diffusion into the cell membrane and stimulates excessive synthesis and ROS accumulation leads to oxidative stress (Wang et al. 2017). The latter provokes lipid peroxidation, protein oxidation and DNA fragmentation as well as pathway dysfunction of several cell injury (Georgiadis et al. 2018). Numerous experimental reports have considered PER to be cause severe damage, such as hepatotoxicity and nephrotoxicity causing tubular necrosis and mitochondria alteration (Jellali et al. 2018; Guvenc et al. 2013). This dysfunction is characterized by loss of the transmembrane electric gradient and therefore of the oxidative phosphorylation process as a consequence of Ca^{2+} accumulation (Gunter and Pfeiffer 1999). Given the limited efficacity of drugs in treating kidney injuries (Breyer and Susztak 2017). However, resort to herbal medicine to protect the emselves. In fact, natural substance-enrich plant and seafood are considered to low toxicity and positive biological properties (Fridlender et al. 2015). Flavonoids are low molecular weight compounds with a quinonoid-like chemical structure, which display significant ROS scavenging activities and other

antioxidant actions (Bors et al. 1994; Pannala et al. 1997; Heijnen et al. 2001). Because of ubiquinone radicals implication in mitochondrial ROS generation, respiratory chain substances can be seen as likely molecular targets for the cellular antioxidants effects of flavonoids (Nichlls and Budd 2014). In the present study, *Fumaria officinalis* (Fumariaceae), called also 'fumitory', has long been used as a dietary food and a traditional herbal medicine for the treatment of minor hepatobiliary dysfunction, gastrointestinal diseases, diuretic agents, cancer and skin disorders (Ahmed 2016). This fumitory contains polyphenolic compounds such as caffeic acid, rosmarinic acid, p-Coumaric acid, isoquercitrin, rutin, quercitrin, quercetin, kaempferol and apigenin, as well as alkaloids such as chalerythrine, sanquinarine, fumaritine and protopine (Stanojevic et al. 2018; Paltinean et al. 2017; Petruczynik et al. 2019). However, to the best of our knowledge, no observation and investigation has been proceeded to study the nephroprotective action of flavonoid-rich fraction (EAF) from *F. officinalis*. Therefore, the aim of this study was conducted to evaluate the possible protective effect of the EAF extracted from *F. officinalis* on permethrin (PER) induced nephrotoxicity in male wistar rat.

Materials And Methods

Chemicals and plant materials

The dry *Fumaria officinalis* aerial part was purchased from Parachimic Laboratory (Tunisia) (ref. HEMF001003) from flavonoids extraction. Permethrin (PER) was purchased from Protagri Company. Sfax. Tunisia. All products of analytical grade were purchased from Sigma Chemical Co. (St. Louis. MO. USA).

Preparation of flavonoid-rich fraction

Flavonoid-rich fraction from *F. officinalis* was extracted using the method described by Ćujić et al. (2016). Briefly, the powder was homogenized with ethanol/water ratio of 25 mL. g^{-1} and ethanol concentration at 60% for 75 min. The obtained homogenate was realized, and the solvent was removed by means of rotary evaporation at 50 °C. The total flavonoids were fractionated successively with petroleum ether to eliminate the lipophilic compounds and ethyl acetate. The latter's solution was concentrated using a rotary evaporation at 50 °C.

Experimental design *in vitro*

Mitochondrial suspension preparation

Rat kidney mitochondria were isolated from the differential centrifugation procedure according to Hoppel et al. (1979). In brief, homogenates were obtained by grinding a small piece using a Potter-Elvehjem homogenized with a loose-fitting Teflon pestle and centrifuged at 600 x g for 10 min at 4°C to remove nuclei and cell debris. Kidney mitochondria were obtained after supernatant centrifugation at 7000 x g for 10 min at 4°C, the pellet was resuspended in 1 mL medium containing 100 mM saccharose, 50 mM KCl, 50 mM Tris, and 5 mM EDTA (pH 7.4) and stored at – 20°C until analysis.

Lipid peroxidation inhibition assay

Lipid peroxidation inhibition was performed according to the method described by Rajneesh et al. (2008). In brief, 1 mL of each concentration (0 to 1000 µg. L^{-1}) was added to 100 µL of 15 mM $FeSO_4$ and 50 µL of 0.1 mmol/L ascorbic acid and shaken vigorously and incubated at 37°C for 1 h. Next, 1 mL of 15 % trichloroacetic acid and 1 mL of 0.67 % thiobarbituric acid were added the mixture. After boiling t 92 °C and cooling for 30, the mixture was centrifuged at 2200 x g for 20 min at 25 °C. Control's solution was contained 1 mL of water with the same mixture. Absorbance was measured spectrophotometrically at 532 nm and the results were expressed as % inhibition using the following equation:

$$\%\text{Inhibition} = \left[\frac{\text{Ab (control)} - \text{Ab(sample)}}{\text{Ab (control)}}\right] X100 \quad (\textbf{Eq. 1})$$

Determination of mitochondria swelling

Mitochondria swelling was determined using the method described by Cardoso et al. (2017). Briefly, isolated mitochondria (1 mg. mL^{-1}) were suspended in 2 mL of buffer D (5 µmol/L FeSO4 and 0.1 mmol. L^{-1} ascorbic acid) over 60 min at 37 °C and then centrifuged at 1000 x g for 10 min at 4°C. Control's solution was suspended in same mixture without FeSO4 while inducer's solution was suspended in same mixture with 1 mL of isolation buffer (100 mM saccharose, 50 mM KCl, 50 mM Tris, and 5 mM EDTA, pH 7.4). The absorbance at □=520 nm was immediately recorded with Shimadzu UV-61 double beam spectrophotometer.

Anti-inflammatory activity determination

Protein denaturation inhibition

Protein denaturation inhibition was performed by the method of Sakat et al. (2010) and *Mizushima and Kobayashi* (1968). 500 µL of 1% bovine serum albumin was added to 100 µL ethyl acetate fraction (EAF) for denaturation after keeping at 37°C in a water bath for 10 min, followed by 20 min heating at 51°C. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Diclofenac sodium was taken as a standard. The experiment was carried out in triplicates and Percent inhibition for denaturation was calculated from control using equation (1).

Membrane stabilization

Red blood cell (RBC) suspension preparation

10 mL of anticoagulated rat blood was centrifuged at 3000 x g for10 min and was washed three times with equal volume of 9 % NaCl. RBC layer was diluted to make 10 % v/v using phosphate buffer saline (PBS) (Sakat et al. 2010).

Heat induced hemolysis

100 µL of RBC (10%) was added to equal volume of the sample (1000 µg mL^{-1}) and the reaction was heated at 56°C for 30 min. Diclofenac sodium (1000 µg mL^{-1}) was used as standard. Then all were centrifuged at 2500 rpm for 10 minutes at room temperature. The absorbance of the supernatants was recorded at 560 nm (Sakat et al. 2010). The experiment was performed in triplicates for all the test. Membrane stabilization percentage was calculated as equation (1).

Experimental design *in vivo*

Animals

Males albino wistar rats (180 ± 5.02 g) were purchased form the Society of Pharmaceutical Industries of Tunisia (SIPHAT. Ben Arous. Tunisia). All animals were maintained under controlled conditions (25 ± 2°C with 12:12 h light/dark periods and minimum relative humidity (50 - 60%)). All rats's experiments were fed with a commercial feed (SICO. Sfax. Tunisia) and drinking water ad *libitum*. They were conducted using the Ethical Committee Guidelines for the care and use of Research and Animal Experimentation of the Faculty of Science. Sfax University. Tunisia (ethics approval number: 1204).

Experimental protocols

Male Wistar rats were randomly divided into the following 4 groups:

Group C (negative control group): rats (n=6) received corn oil by gavage at a dose of 2 mL /Kg BW for 7 days.

Group EAF (positive control group): rats received corn oil by gavage at a dose of 2 mL /Kg BW and ethyl acetate fraction from *F. officinalis* at a dose of 200 mg/Kg BW for 7 days (Verma 2011).

Group PER: rats (n=6) received permethrin by gavage at a dose of 34.05 mg/Kg BW (corresponding to 1:50 of DL_{50}) dissolved in corn oil for 7 days (Cantalamessa 1993).

Group PER+EAF: rats (n=6) received by gavage permethrin (the same dose of that of PER group) and ethyl acetate fraction (similar concentration to that of EAF group) for 7 days.

Wistar rats's experimental received pellet diet and drinking water ad libitum for 7 days. Body were monitored daily for 7 days. At the end of the experiment. all animals were sacrificed by cervical decapitation to avoid any stress.

Collection and kidney preparation

Blood samples were allowed into heparined tubes, centrifuged at 3000 x g for 15 min and kept at - 20 °C for biochemical analysis. Kidney tissues were excised, minced with ice cold saline and blotted of filter paper. 1 g of kidney slices was used for mitochondria isolation, 1 g was homogenized using an Ultra-

Turrax homogenizer (T25. Germany) with Tris-buffered saline solution (TBS, pH 7.4) for hepatic parameters analysis and other kidney sections were used for histological examination.

Mitochondrial fraction reparation

Kidney mitochondria was isolated form the differential centrifugation procedure according to Hoppel et al. (1979).

Biochemical markers in plasma

Calcium and phosphorus levels and lactate dehydrogenase (LDH) activity were determined spectrophotometrically using commercial kits according to manufacturer's recommendations (Abbott (Abbott Park. IL. USA) Architect/Aeroset. Ref 20051, 1001150, 20102 and 2P5621, respectively).

Oxidative stress markers determination

Protein quantification

Renal and mitochondrial protein contents were evaluated using Folin's reagent and bovine serum albumin (BSA) as standard following the method described by Lowry et al. (1951).

Thiobarbituric acid reactive substance determination

Renal and mitochondrial TBARS levels were measured calorimetrically at 532 nm using the method of Esterbauer and Cheeseman (1990). Data are expressed as nanomoles/milligram of protein.

Advanced oxidation protein product determination

Renal and mitochondrial AOPP levels were assayed calorimetrically at 340 nm according to the method of Witko et al. (1992). Data are expressed as micromoles/milligram of protein.

Protein carbonyl determination

PCO levels in kidney and mitochondria were measured spectrophotometrically at 370 nm according to Resnik and Packer's method (1994).

Vitamin C content determination

Kidney and mitochondria vitamin C rates were determined spectrophotometrically using the method described by Jagota and Dani (1982). Data are expressed as nanogram/milligram of protein.

Antioxidant enzyme activities in the kidney and mitochondria

Superoxide dismutase (SOD) activity was measured spectrophotometrically at 580 nm as described by Asada et al. (1974). The activity was expressed as units/milligram of protein.

Catalase (CAT) activity was assayed calorimetrically at 240 nm according to the method of *Aebi* (1984). Results were expressed as micromoles of H_2O_2 consumed/milligram of protein.

Glutathione peroxidase (GPx) activity was evaluated using Flohé and Gunzler's method (1984). Data were expressed as micromoles of reduced GSH/milligram of protein.

Renal and mitochondrial glutathione content determination

GSH content was determined spectrophotometrically at 412 nm according to the method of Ellamn (1959) modified by Jollow et al. (1974). The obtained results were expressed as micromoles/milligram of protein.

Renal mitochondrial enzymes activities determination

Complex I (NADH ubiquinone reductase) activity was evaluated calorimetrically at 37°C in renal mitochondria by monitoring the oxidation of NADH at 340 nm according to Malgat et al. method (1999).

Complex III (ubiquinol-cytochrome c reductase) activity was evaluated at 550 nm according to Malgat et al. (1999) by decreasing cytochrome c.

Complex IV (cytochrome c oxidase) activity was determined by monitoring the decrease of cytochrome c at 550 nm using the method described by Malgat et al. (1999).

Histology

After fixation into10 % formalin solution for 48 h. kidney tissue is embedded in the paraffin. Small sections of 5 µm were prepared and stained with hematoxylin and eosin (H&E) for microscopic examination (X 400 magnifications).

Statistical analysis

All data were repeated three times and results analysis were performed using SPSS 23.0 analysis software. Data are expressed as mean ± standard deviation (SD). $P < 0.05$ means statistical difference.

The parametric Pearson correlation coefficient test was determined to assess the bivariate correlation between SOD, CAT and GPx activities with TBARS levels, a probability level of 95 % was used.

Results

In vitro study

Protective effects of EAF against lipid peroxidation

As can be seen from Fig.1, flavonoid-rich fraction (EAF) decreased kidney and mitochondrial lipid peroxidation levels in a dose-dependent manner in comparison to control test. The percent inhibition at

the highest EAF concentration (1mg.mL^{-1}) was 76.74 % and 76.02%, respectively).

Protective effects of EAF against mitochondrial swelling

After the mitochondrial sample was added to the reaction buffer (pH 7.2), the mitochondrial absorbance at 520 nm increased, indicating mitochondrial swelling due to alteration in osmotic pressure (Fig.2). However, co-incubated of mitochondria with EAF was found to be significantly increased when compared to control and inducer tests. This indicates that EAF has the potential to improve the impaired mitochondrial function.

Mitochondrial swelling and lipid peroxidation showed a close and significant correlation (R^2= 0.966, $p < 0.001$) (Fig.3).

Protective effects of EAF against protein denaturation

As shown in Table 1, EAF showed a significant inhibition of protein denaturation in comparison to control. At 1 mg. mL-1, Diclofenac sodium stimulated the most denaturation inhibition that EAF at 64.74 and 60.06 %, respectively.

Protective effects of EAF on membrane stabilization

EAF inhibited the heat induced hemolysis of RBCs when compared to normal test (Table 1). The percent inhibition of EAF (1 mg. mL^{-1}) has the highest to those of Diclofenac sodium by 83.45 % and 77.62%, respectively.

In vivo study

Effect of PER on rat body and kidney weights

Rat body weight gain was significantly decreased associated with a significant increase of absolute and relative kidney weights in the PER-treated rats as compared to control group (Table 2). Co-administration of flavonoid-rich fraction from *Fumaria officinalis* in to the PER group restored approximately these variations in comparison to PER-treated rats.

Effect of PER on biochemical markers

The effects of PER and EAF and their combination on some biochemical parameters in rats are exhibited in Fig.4. Our results showed that permethrin caused a significant decrease of plasma calcium and phosphorus levels associated with an increase of plasma lactate dehydrogenase activity (LDH) in comparison to control group. Co-administration of EAF in to PER-treated rats improved significantly these parameters when compared to PER group.

Effect of PER on oxidative markers

Renal and mitochondrial TBARS, AOPP and PCO levels were statistically increased with a significant reduction of vitamin C content in PER-treated rats in comparison to controls values (Table.3). These alterations were eventually improved after co-supplementation of EAF in to PER-treated rats.

Effect of PER on antioxidant enzymes activities

After permethrin exposure, a significant increase of SOD and GPx activities in the kidney associated with a rise of CAT and GPx activities in mitochondria when compared to control group were found by our results (Fig.5). A significant reduction in renal CAT activity and mitochondrial SOD activity were also observed. There was no difference in these activities between the control group and the rat treated only with EAF. However, co-administration of EAF with PER-treated rats improved kidney antioxidant activity when compared to PER group.

Effect of PER on antioxidant non-enzyme activity

Renal and mitochondrial GSH contents were significantly increased in the PER-treated rats to those of the normal values (Fig.6). The supplementation of EAF in PER-treated rats restored this parameter in comparison to permethrin exposure.

Effect of PER on mitochondrial enzymes activities

NADH-ubiquinone reductase (complex I), ubiquinol cytochrome c reductase (complex III) and cytochrome c oxidase (complex IV) activities were significantly reduced in permethrin-treated rats (Fig.7) in comparison to those of control group. No significant perturbation was found in the EAF group. However, co-treatment of PER-treated rats with EAF improved these mitochondrial enzymes near to normal values.

Pearson correlation between SOD, CAT and GPx activities with TBARS levels

Pearson correlation analysis performed to better clarify the interaction between enzymatic antioxidant system with lipid peroxidation (TBARS) content in the kidney mitochondria ‘rat (Fig.8). Our results showed a non-significant strong positive correlation between SOD, CAT and GPx activities with TBARS levels by R^2= 0.5819, $p > 0.05$; R^2=0.6023, $p > 0.05$ and R^2 = 0.1858, $p > 0.05$, respectively.

Histopathological examination

Histological sections of the kidney showed that control rat shows normal architecture of glomerulus and tubules (Fig.9 (**C**)). kidney histological was normal structure in EAF group (Fig.9 (**EAF**)). Kidney of PER-treated rats shows multiple foci of leucocytes infiltration, hypertrophy of glomeruli cells showing reduction of Bowman’s space and cloudy swelling of tubules when compared to control (Fig.9 (**PER**)). However, co-administration of ethyl acetate fraction form *F. officinalis* in to the permethrin group remarkably reduced these pathological lesions (Fig.9 (**PER+EAF**)), which were in collaboration with our data of renal functional markers, lipid peroxidation and antioxidant status.

Discussion

Earlier reports support the influence of oxidative stress, inflammation, apoptosis and autophagy in the pathophysiology of pesticide-caused nephrotoxicity (Caglayan et al. 2018). One of the major and well documented cause of inflammation is protein denaturation and cell membrane injury. Hence, alternative approaches are explored using plant-based flavonoids to combat oxidative stress due to xenobiotic intoxication. Our data showed the ability of flavonoid-rich fraction from *F. officinalis* to inhibit effectively protein denaturation and heat-induced hemolysis with compared to standard drug. This evidence proves that membrane stabilization is a main mechanism of anti-inflammatory action of flavonoid-rich fraction. The latter might be stabilizing the RBC membrane by precluding the discharge of lytic enzymes and other active inflammatory mediators (Yesmin et al. 2020). A strong correlation ($R^2 > 0.760$) between the protein denaturation inhibition action and caffeic acid, ferulic acid and quercetin were found (Bouhlali et al. 2020). According to Tarahovsky et al. (2014) and Oteiza et al. (2005) reporting also that flavonoids may interact at the water lipid interface with the polar head of phospholipids by hydrogen bonding leading to increase the membrane rigidity and lipid bilayers stability as well as decrease its fluidity.

Permethrin (PER), which tends to accumulate in the food chain, is one of the greatest hazards to people health that causes all human organs, with known respiratory, neurologic, gastrointestinal and nephrotoxicity effects. Weight loss can be linked to excessive protein breakdown (Andallu and Varadacharyulu 2003). Our experimental data demonstrated that PER-treated rats caused a significant decrease of body and relative kidney weights as well an increase of absolute kidney weight to those of the control groups. Similar results were founded by Wang et al. (2017). Weight loss could be attributed to several causes including food consumption reduction, inadequate nutrients absorption and/or protein synthesis inhibition. A daily co-administration of EAF to the PER-treated rats improved the signs of metabolic syndrome including body and kidney weights with compared to PER group. This modulatory action could be due to its richness of ferulic acid (2021), which reverse almost all the deleterious morphological changes after 15 and 30 days of cadmium-treated rats, as reported by Sanjeev et al. (2019).

Calcium and phosphorus homeostasis are altered in chronic kidney disease (CKD) (Felsenfeld et al. 2015). Since the reabsorption of phosphorus is more in the proximal tubule and the reabsorption of Ca is in the proximal and distal tubule (Nogueira et al. 1978). Our experimental findings showed that PER-treated rats reduced the plasma P and Ca levels and increased plasma LDH activity as compared to normal values. In agreement with Ortega-Domínguez et al. (2017)

indicated that xenobiotic caused glomerular filtration reduction and proximal tubules damage. Co-administration of EAF to the PER-treated rats could exclusively restore renal damage in comparison to PER group. As reported by Orsolic et al. (2014) that flavonoids can prevent the calcium and phosphorus reduction in bones caused by retinoic acid.

As a result of stressful conditions, organic molecules are exposed to oxidizing species leading to chemical processes modification that in turn can lead to cells dysfunction. Proximal tubule damage and mitochondrial dysfunction are associated with permethrin-induced nephrotoxicity (Bashir et al. 2013). Our *in vivo* data showed an increase in renal and mitochondrial TBARS, AOPP and PCO levels in PER-treated rats when compared to normal values. It is reasonable to conclude that PER may act as an oxidant or a free radical in kidney tissue inducing oxidative stress. Co-administration of EAF to PER-treated rats improved eventually these parameters to those of control groups. This restoration could be explained by its richness of ferulic acid (FA) thereby minimizing the detrimental action of ROS. The antioxidant action of FA is attributed to its resonance stabilized phenoxy radical structure leading to quenching of free radicals (Srinivasan et al. 2007).

GSH acts both as non-enzymatic oxygen radical scavenger and as a substrate for various enzymes such as GSH-Px, participating in the ROS reduction (Waheed and Mohammed 2012). GSH content variation worsens renal function in mitochondria and renal ischemia (Baliga et al. 1999; Paller 1988) . Our results showed that PER-treated rats increased significantly renal and mitochondrial GSH content associated with a decrease of renal and mitochondrial vitamin C levels as compared to control groups. GSH rise could be due to its undergoes oxidation after being conjugated in redox process and is converted to GSH reduced form as an adaptive response (Kaur and Kaur 2017). Co-administration of EAF to PER-treated rats restored these levels when compared to PER group. This improvement could be due to its presence of ferulic acid which ameliorated LPS-induced acute kidney injury by enhancing antioxidant defenses as reported by Mir et al. (2018).

Antioxidant enzymes, mainly SOD, CAT and GPx are the first line of defense against ROS. In the present study, permethrin-treated rats caused a significant antioxidant system perturbation in the kidney and mitochondria when compared to control groups. This is in accordance with several studies that reported the significant changes in SOD and CAT activities in different mice/rat organs exposed to insecticides-like methomyl, cypermethrin and methiocarb (El-Demerdash et al. 2013; Sankar et al. 2012; Ozden et al. 2009). Our findings showed that a non-significant strong positive correlation between enzymatic antioxidant system and lipid peroxidation rate (R^2 = 0.5819, $p > 0.05$; R^2 = 0.6023, $p > 0.05$ and R^2 = 0.1858, $p > 0.05$, respectively) was observed. This is in agreement with the fact that during the dismutation of O_2 to H_2O_2, the mitochondrial SOD activity can be inactivated (Hink et al. 2002). However, supplementation of EAF to PER-treated rats alleviated the oxidative damage reaching normal values. Similar findings are in agreement with the study of Athira et al. (2016) reported that the emerging dietary flavonoids supplement against cisplatin-caused kidney damage. Hence, kaempferol, which is a bioactive compound in the EAF, demonstrated to function as a direct antioxidant that scavenges oxygen free radicals, hinder lipid peroxidation, prevents the increase in membrane permeability resulting from renal oxidative injury (Vijayaprakasha et al. 2013).

The release of $O_2^{\cdot -}$ across the inner mitochondrial membrane causes its depolarization and its dysfunction (Brady and Hamacher-Brady 2006; Ye et al. 2006). Loss in mitochondrial number leads to

ATP depletion which may cause interruption of apoptotic signaling as well as a secondary necrosis (Sverdlov et al. 2006). Our experimental data revealed that PER exposed in rats significantly decreased of complex I (NADH-ubiquinone reductase), complex III (ubiquino-cytochrome c reductase) and complex IV (cytochrome c oxidase) activities in the kidney mitochondria as compared to normal values. As reported by Guvenc et al. (2013) who showed that caspase-9-dependent and mitochondria-related apoptotic cell death could play a main role in permethrin-induced nephrotoxicity. Co-administration of flavonoid-rich fraction from *F. officinalis* to the PER-treated rats attenuated these mitochondrial activities; (1) decreased ROS level; (2) attenuated mitochondrial depolarization; (3) reduced mitochondrial swelling; and (4) increased ATP production reaching to normal values. This mito-protective action could be explained by its richness in naringenin which advocated its ability to rescue kidney mitochondrial function under oxidative stress conditions (Chandran et al. 2019).

Kidney of PER intoxication may affect acute tubular necrosis with extensive tubular degenerative variation, cytoplasmic vacuolation and biochemical markers (Bashir et al. 2013). Our histopathological examination showed that PER caused multiple foci of leucocytes infiltration, hypertrophy of glomeruli cells showing reduction of Bowman's space and cloudy swelling of tubules in comparison to control group. Similarly, Guvenc et al. (2013) reported that permethrin-exposed rats caused the epithelial cells degenerative and necrotic signaling of the renal proximal tubules. Both lysosomal overload and protein-bound toxic moieties may increase sensitivity to nephrotoxicity (Davis and Berndt 2001) . Besides, the co-treatment with EAF extract alleviated the kidney damage. This improvement may be due to the presence of ferulic acid (FA) in the EAF, suggesting its adjuvant therapy on apoptosis signaling by neither improving renal dysfunction and lipid peroxidation (Bami et al. 2017; Kelainy et al. 2019).

Denoting results of the current study, it is realistically presented for the first time that oral supplementation of EAF ameliorated renal and mitochondrial damage induced by PER administration. EAF shows this effect by reducing inflammation, balancing oxidant/ antioxidant system, inhibiting neutrophil infiltration and restoring mitochondrial function (Fig.10).

Conclusion

It has been concluded that permethrin administration leaded to renal and mitochondrial damage by depleting natural antioxidative status, mitochondrial bioenergetics dysfunction and renal morphology alteration. On contrary, co-administration of EAF in to PER-treated rats improved renal damage, indicating its nephroprotective action which could be due to its antioxidant and anti-inflammatory effects.

Declarations

Ethical Approval: 1204

Consent to Participate and Publish: Not applicable

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Author Contributions Statement:

Nissaf Aoiadni performed the *in vivo* pat, interpreted the results and the statistical analysis and drafted the work.

Hajer Jdidi effected the *in vitro* part experiment.

Abdelfattah El Feki corrected the manuscript

Hamadi Fetoui corrected the manuscript.

Fatma Ghorbel Koubaa designed the study and corrected the manuscript.

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Data availability: All the data is contained in the article.

Conflict of interest: The authors declare that they have no conflict of interest.

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Tables

Table 1 Effect of flavonoid-rich fraction on protein denaturation inhibition and membrane stabilization activity at room temperature.

	Dose ($\mu g.mL^{-1}$)	Protein denaturation		Membrane stabilization	
		Abs (nm)	% inhibition	Abs (nm)	% inhibition
Control		0.50 ± 0.01	-	0.506 ± 0.023	-
EAF	1000	0.20 ± 0.01	60.06 ± 8.25	0.17 ± 0.01	83.45 ± 4.25
Standard	1000	0.17 ± 0.05	64.74 ± 9.16	0.23 ± 0.04	77.62 ± 9.25

Table 2 Changes of rat body weight (%) and absolute (g) and relative (%) kidney weights at 7 days of treatment.

Treatment	C	EAF	PER	PER+EAF
% of weight gain	44.04 ± 2.03	34.29 ± 3.08	$-34.88 \pm 0.00^{***}$	$14.02 \pm 1.28^{**\#\#\#}$
Absolute kidney weight	0.82 ± 0.14	0.76 ± 0.04	$0.87 \pm 0.01^{*}$	$0.84 \pm 0.05^{*\#}$
Relative kidney weight	0.38 ± 0.02	0.38 ± 0.05	$0.53 \pm 0.05^{***}$	$0.46 \pm 0.03^{**\#}$

Values are mean ± SD, n = 6 determinants. PER and PER+EAF-treated groups vs. control group: *p < 0.05, **p < 0.01, and ***p < 0.001; PER+EAF group vs. PER group: # p < 0.05 and ### p < 0.001

Table 3 Thiobarbituric acid reactive substances (TBARSs), protein carbonyl (PCO), protein oxidation products (AOPPs) and vitamin C levels in the kidney and mitochondria in rats of different groups after 7 days of treatment.

Treatment	Test groups			
	C	EAF	PER	PER+EAF
Kidney				
TBARS	1.70 ± 0.03	1.52 ± 0.40	3.77 ± 0.01	2.81 ± 0.61
AOPP	5.07 ± 1.27	5.80 ± 1.45	9.21 ± 2.30	7.06 ± 1.76
PCO	0.065 ± 0.02	0.07 ± 0.01	0.17 ± 0.01	0.13 ± 0.01
Vitamin C	2.99 ± 0.13	3.06 ± 0.06	1.47 ± 0.03	2.29 ± 0.01
Mitochondria				
TBARS	0.38 ± 0.02	0.40 ± 0.06	0.62 ± 0.01	0.61 ± 0.04
AOPP	1.43 ± 0.36	1.41 ± 0.35	1.68 ± 0.42	1.44 ± 0.36
PCO	0.01 ± 0.00	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Vitamin C	1.27 ± 0.42	1.22 ± 0.41	0.62 ± 0.24	0.84 ± 0.28

Values are mean ± SD, n = 6 determinants. PER and PER+EAF-treated groups vs. control group: *p < 0.05, **p < 0.01, and ***p < 0.001; PER+EAF group vs. PER group: # p < 0.05 and ### p < 0.001

Figures

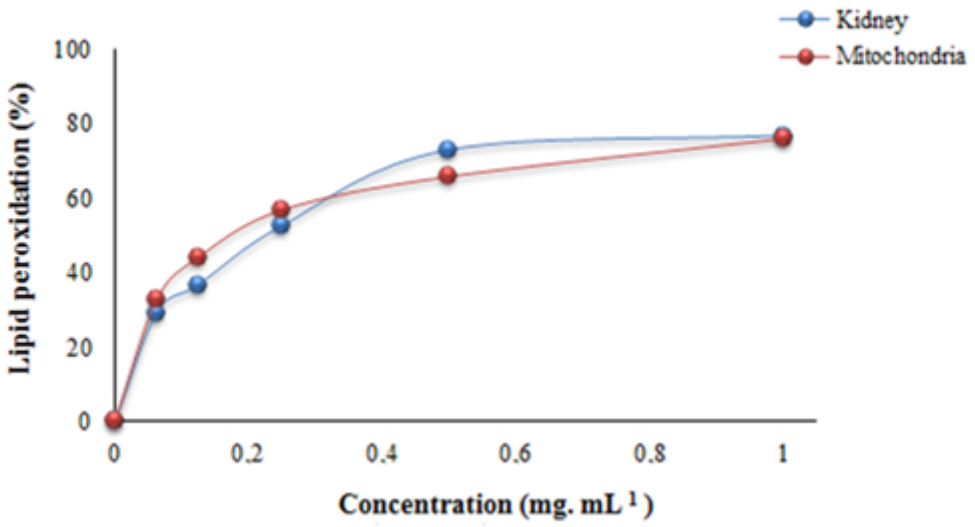

Figure 1

Effect of flavonoid-rich fraction against lipid peroxidation (% inhibition) in the kidney and mitochondria.

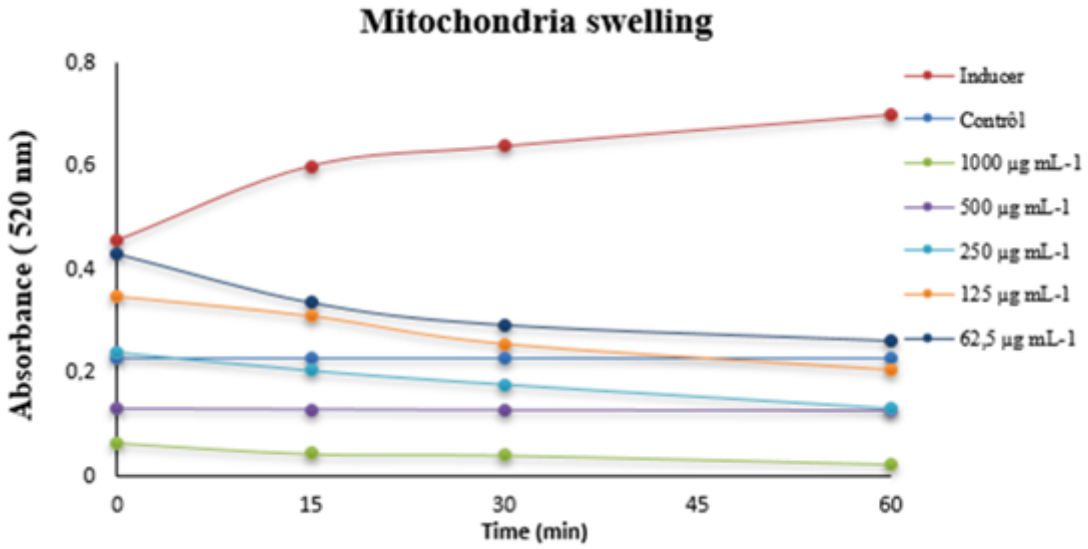

Figure 2

The normalized absorbance spectra of isolated mitochondria treated with flavonoid-rich fraction (EAF) for mitochondrial swelling at 520 nm over 60 min at room temperature.

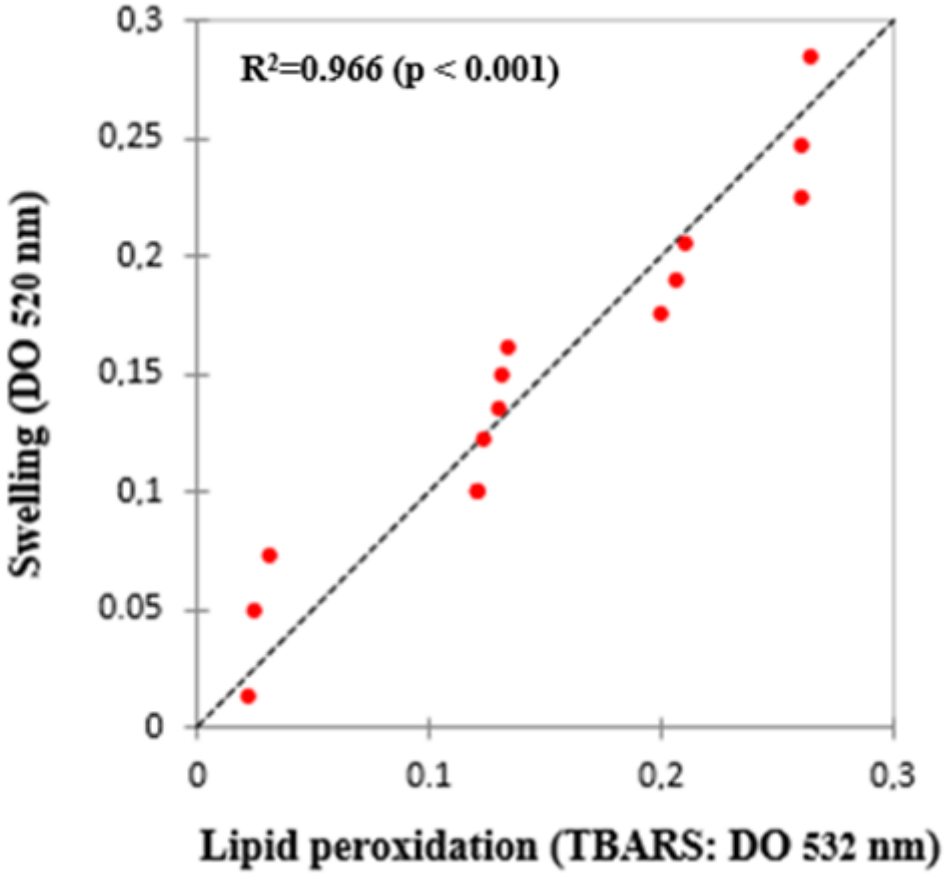

Figure 3

Relationship between mitochondrial swelling and lipid peroxidation in the rat kidney. n = 14.

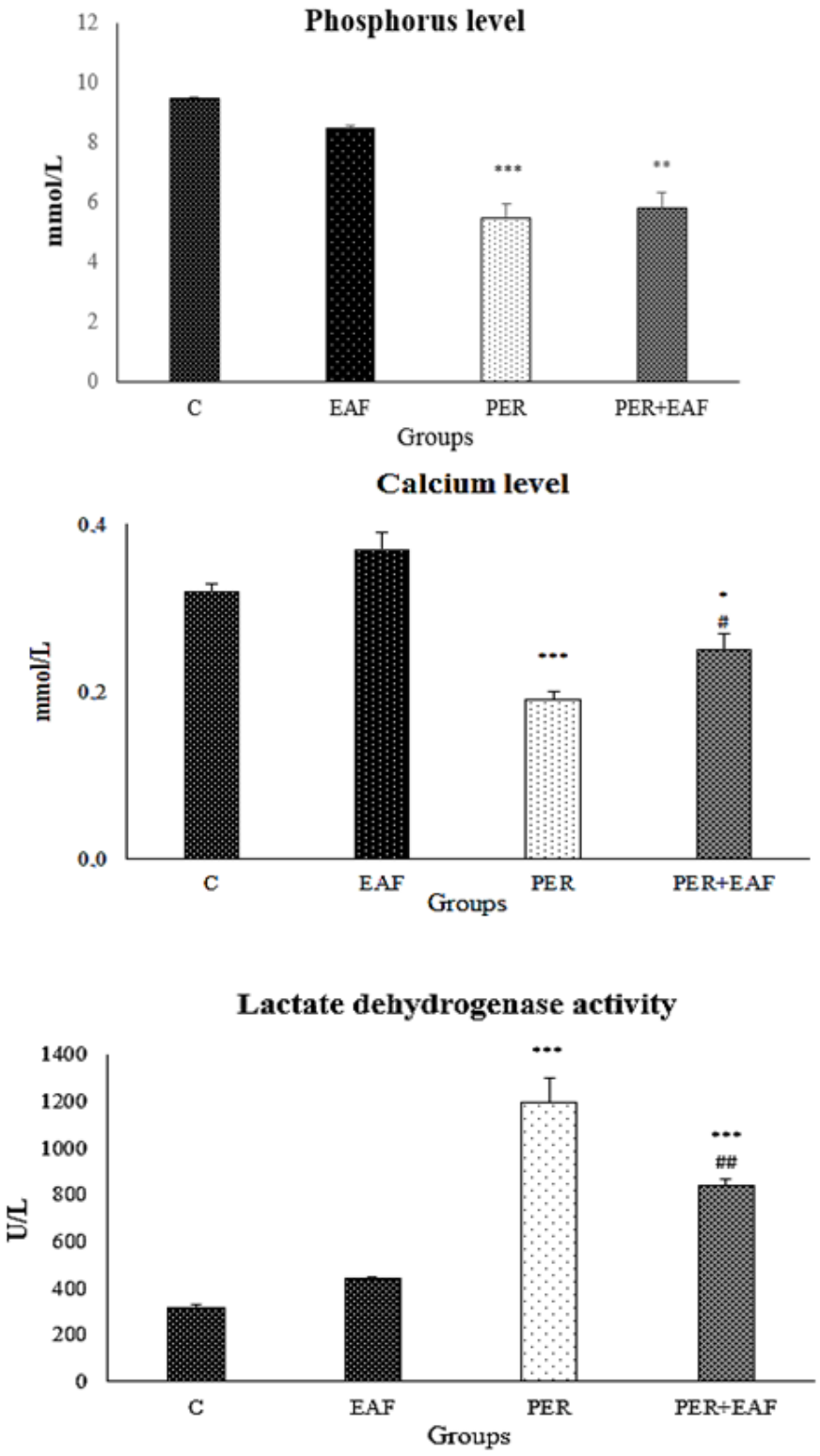

Figure 4

Plasma calcium (Ca) and phosphorus (P) levels and lactate dehydrogenase (LDH) activity variations of controls and treated rats during 7 days. Values are mean ± SD, n = 6 determinants. PER and PER+ EAF-treated group vs. control group: *p < 0.05, **p < 0.01, ***p < 0.001; PER+EAF group vs. PER group: #p < 0.05, ##p < 0.01.

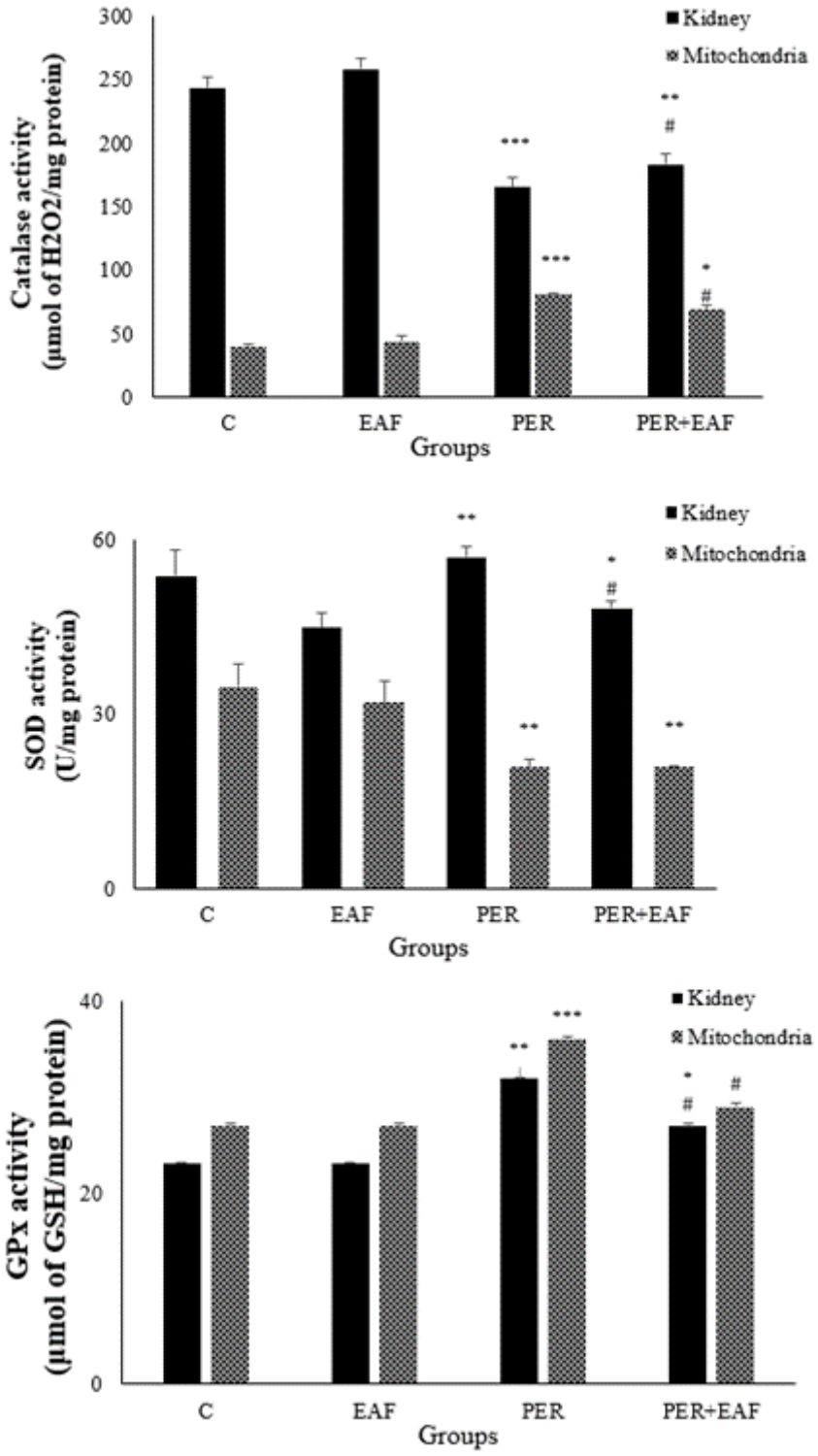

Figure 5

Catalase activity (CAT), superoxide dismutase activity (SOD), and Glutathione peroxidase activity (GPx) in kidney and mitochondria of controls and treated rats during 7 days. Values are mean ± SD, n = 6 determinants. PER- and PER+ EAF-treated group vs. control group: *p < 0.05, **p < 0.01, ***p < 0.001; PER+EAF group vs. PER group: #p < 0.05.

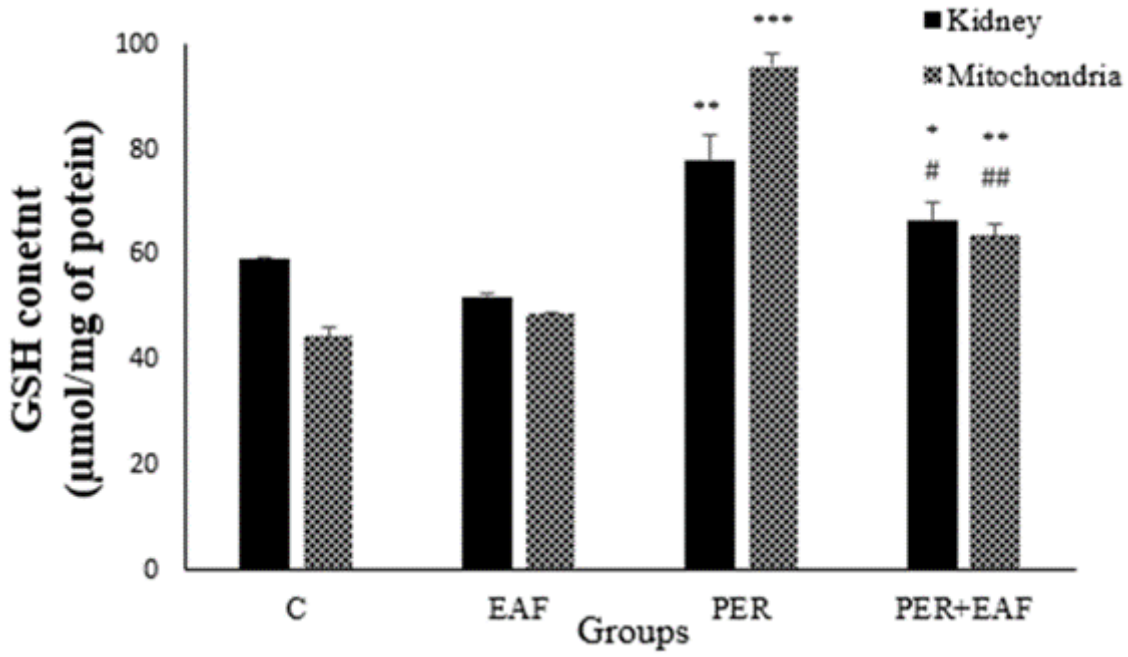

Figure 6

Glutathione (GSH) content in kidney and mitochondia of controls and treated rats during 7 days. Values are mean ± SD, n = 6 determinants. PER- and PER+ EAF-treated group vs. control group: *p < 0.05, **p < 0.01, ***p < 0.001; PER+EAF group vs. PER group: #p < 0.05, ##p < 0.01.

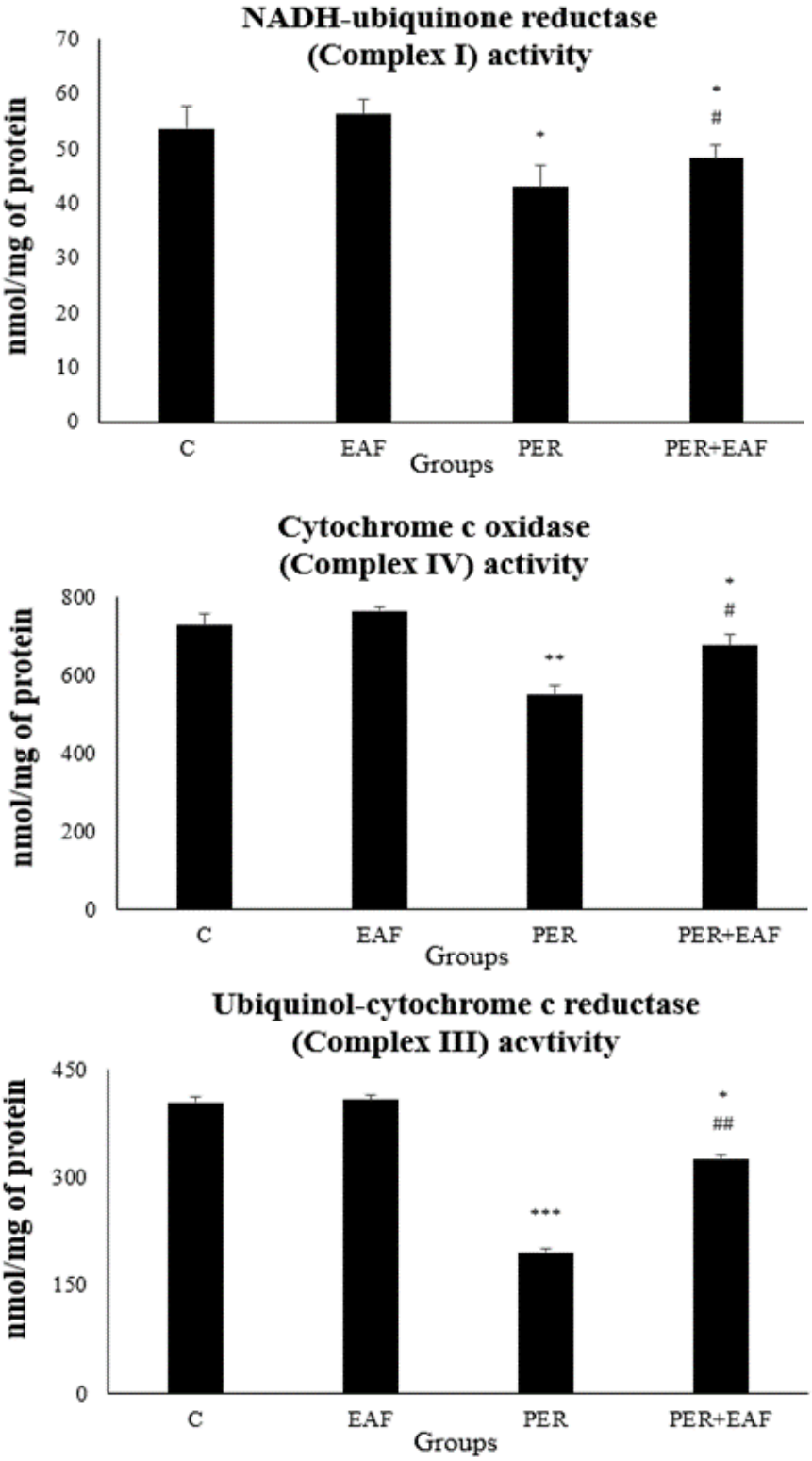

Figure 7

Hepatic mitochondrial enzyme activities in rats after 7 days of treatment. Values are mean ± SD, n = 6 determinants. PER- and PER+ EAF-treated group vs. control group: *p < 0.05, **p < 0.01, ***p < 0.001; PER+EAF group vs. PER group: #p < 0.05, ##p < 0.01.

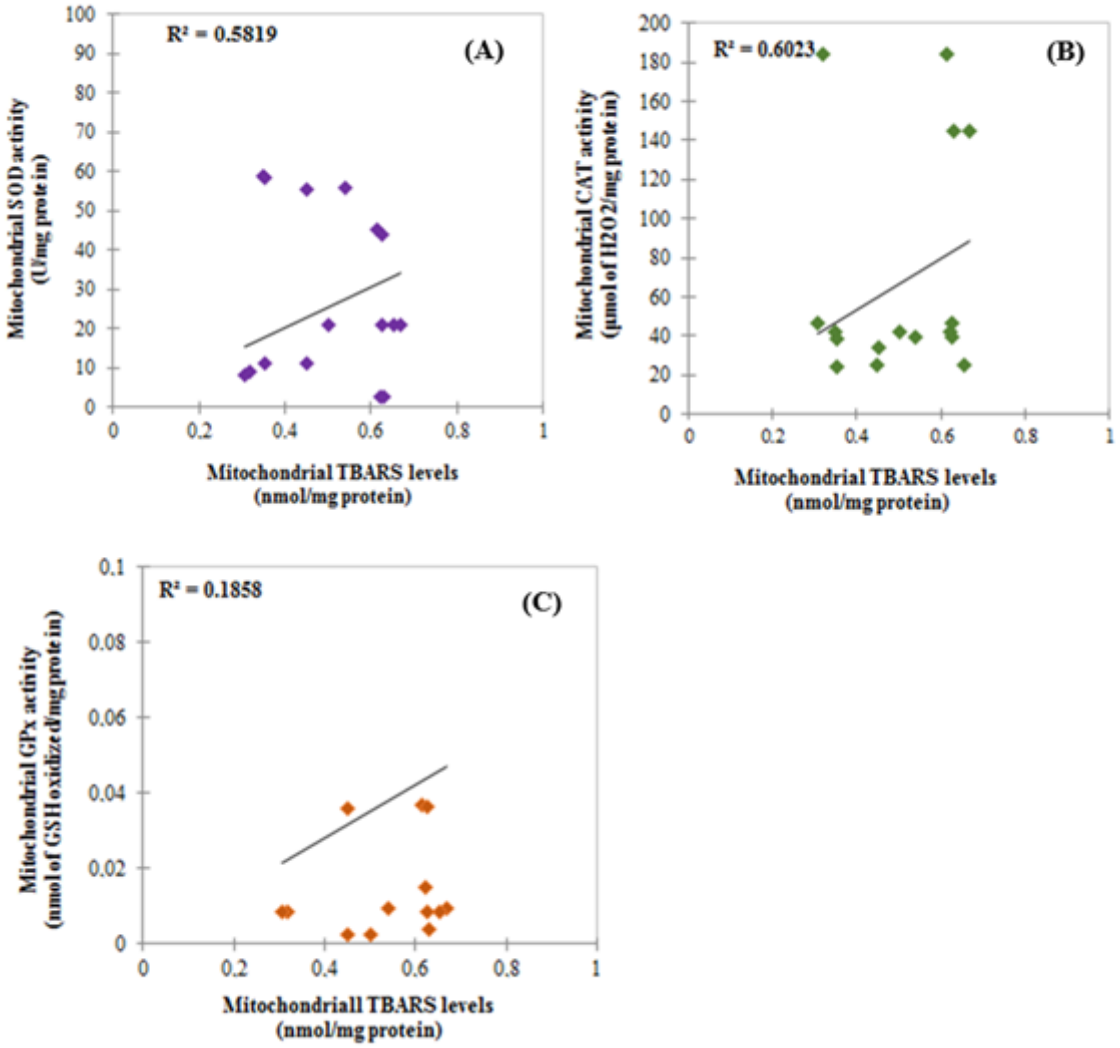

Figure 8

Correlation between (A): SOD activity and TBARS levels (R2 = 0.5819, p > 0.05, n= 16); (B) CAT activity and TBARS levels (R2 = 0.6023, p > 0.05, n=16) and (C): GPx activity andTBARS levels (R2 = 0.1858, p > 0.05, n=16) in the kidney mitochondria' rat.

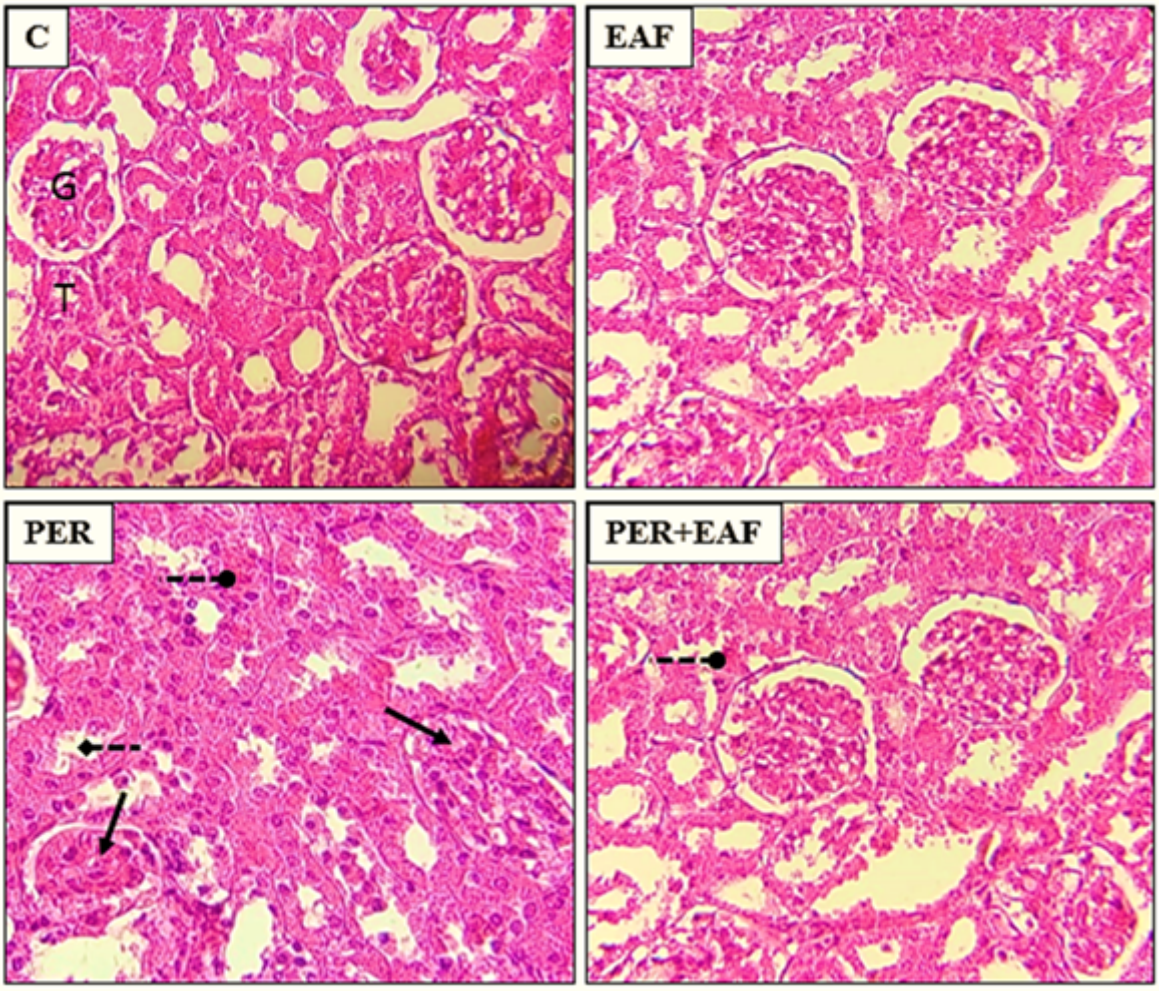

Figure 9

Light microscopic photographs of kidney' paraffin section stained with H&E taken at 400 x magnification. (C) Contol, (EAF) ethyl acetate fraction-treated rats, (PER) permethrin-treated rats and (PER+EAF) co-treatment of permehin with ethyl acetate fraction. The arows indicate: G: Glomerulus ; T : Tubule ; Arrow : Hyperthrophy of glomeruli cells; Dotted line : Tubular dilatation; Arrow with broken line : Leucocyte infiltration.

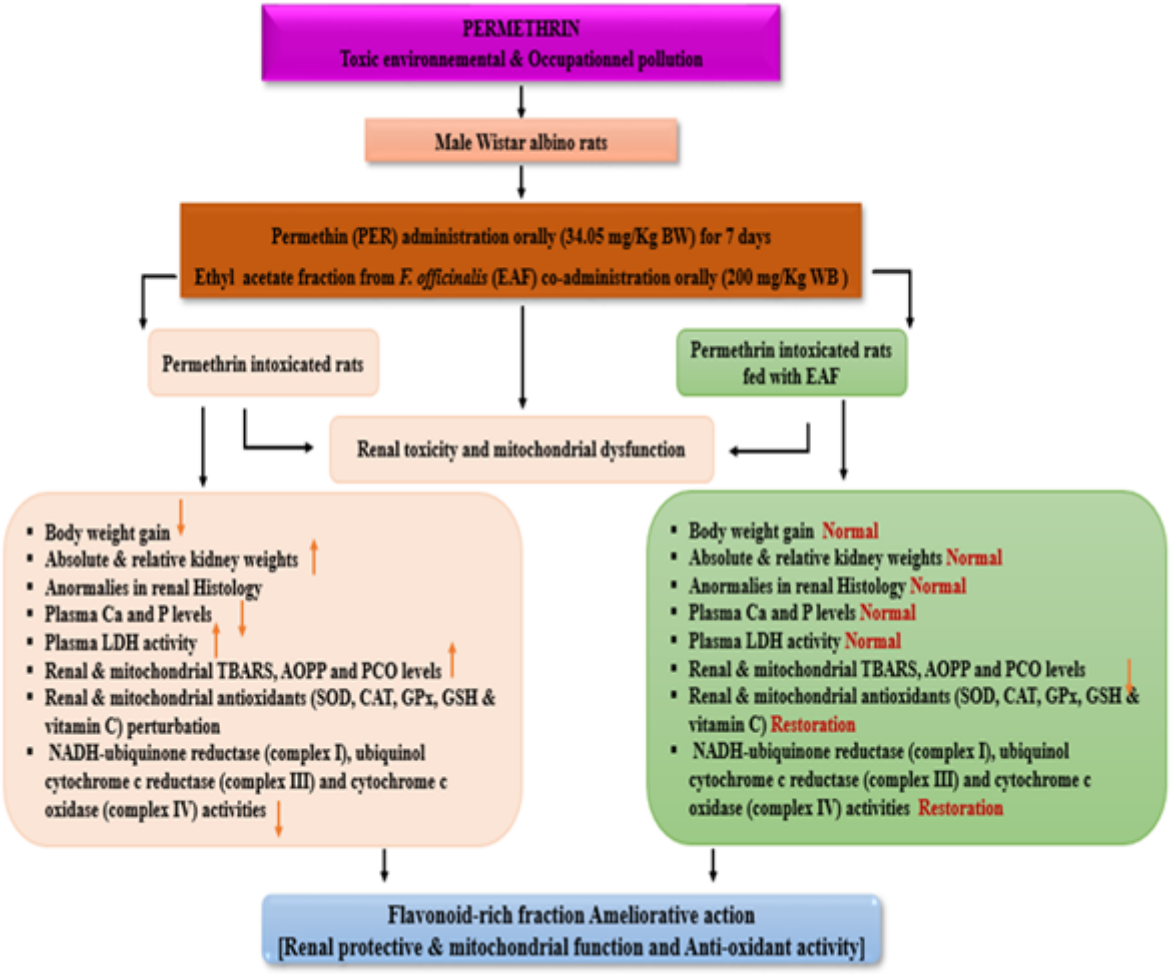

Figure 10

Action mechanism of flavonoid-rich fraction (EAF) on permethrin-induced renal toxicity and mitochondrial dysfunction in Wistar albino rats.