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RESEARCH ARTICLE

Hepatoprotective activity of *Uncaria tomentosa* extract against sub-chronic exposure to fipronil in male rats

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Abstract

The effects of fipronil (FPN) on the liver of rats were studied. Rats ($n = 6$) were treated with 9.7 mg/kg (1/10 of FPN LD_{50}), and other rats ($n = 6$) received 120 mg/kg of 10% *Uncaria tomentosa* extract, while a mixture of 9.7 mg/kg FPN and 120 mg/kg of 10% *Uncaria tomentosa* extract were administered orally to the rats ($n = 6$) daily for 6 weeks. Body, hepatic weights, liver enzymes, and lipid profile were determined. Hepatic activities of MDA, TNO, TAC, TNF-α, and IL-6 in liver homogenate were measured. Immunohistochemistry of NF-kB and liver histopathology were performed. Fipronil-treated rats had a significant ($P = 0.02$) lower weight gain. Moreover, relative liver weight was significantly ($P = 0.003$) increased in FPN-treated rats. Rats administrated with FPN exhibited a significantly ($P = 0.02$) higher liver enzymes and promoted levels of MDA, TNO, TNF-α, and IL-6 ($P < 0.0001$) than that in the other groups. Immunostaining of NF-κB was increased ($P < 0.0001$) in FPN-treated rats. Interestingly, *Uncaria tomentosa* alone or with FPN decreased the liver immunostaining of NF-κB. In conclusion, FPN produced liver injury through lipid peroxidation and stimulation of NF-κB. However, *Uncaria tomentosa* combated the oxidative stress and liver damage induced by FPN via inhibition of NF-κB.

Keywords Rats · Fipronil · *Uncaria tomentosa* · MDA · NF-κB

Introduction

Pesticides were extensively used in modern agriculture, and their residues in food were directly dangerous to both environment and consumers (Amvrazi and Albanis 2009). Fipronil (FPN) [5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-fluoromethylsulfinyl pyrazole] is an insecticide from the second-generation phenylpyrazole and constitutes public health concerns since the mid of twentieth century. Public health concerns are increasing about the safety and exposure to phenylpyrazole pesticides due to their popular usage, discharges into the environment, and their toxic effects (Tingle et al. 2003).

Fipronil is used for controlling external parasites in domestic animals, like ticks and fleas. Those insects that resist different types of insecticides are sensitive to FPN and therefore it is being commonly used as an insecticide (Bobe et al. 1997). The wide variation of insecticides has encouraged the agricultural community to use them to control insects in different cereal crops, hoping to increase crop yield and decrease post-harvest losses. However, toxicity studies have been always debated in the scientific community (Eisenstein 2015).

The oral exposure of FPN to laboratory mammals was considered as a moderate toxic ($LD_{50} = 97$ mg/kg for rats; $LD_{50} = 91$ mg/kg for mice) (Tingle et al. 2003). Fipronil causes death via neural toxicity and paralysis by blocking the GABA-gated chloride channels in insect's central nervous system neurons (Hainzl et al. 1998). It is primarily

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metabolized by mammalian liver which is considered the central organ for metabolism connecting the digestive tract with the general circulation (de Medeiros et al. 2015).

It was recommended previously that pesticides modified both enzymatic and non-enzymatic antioxidants and induced oxidative stress that was attributed to pesticide toxicity in animals (Banerjee et al. 1999; Mohamed et al. 2004). The hepatotoxic effect of FPN was previously described (de Medeiros et al. 2015; Mossa et al. 2015; Silva 2008), although the actual mechanism of FPN hepatotoxicity has not been fully clarified. Administration of FPN at 1 and 10 mg/L for 45 days increased the activity of liver enzymes as well as induced different levels of hepatic histopathological changes ranged from mild to severe changes in male rats (Mossa et al. 2015).

Nuclear factor kappa B (NF-kB) is a transcription factor involved in the promotion of several genes. NF-kB is a heterodimeric complex which is impounded in the cytoplasm via interaction with the inhibitors of IkB family in dormant cells. Stimulation of these cells by other factors led to phosphorylation of IkB with further release of NF-kB, that directed into the nucleus where it dimerizes target genes' promoter region (Barnes and Karin 1997; May and Ghosh 1997). Several pro-inflammatory and cytotoxic cytokines genes were activated by NF-kB. Therefore, one possible mechanism by which oxidative stress may induce liver injury is through NF-kB activation (Baeuerle and Baltimore 1996; May and Ghosh 1997).

Uncaria tomentosa is a medicinal plant commonly known as cat claw and routinely used for curing several illnesses like arthritis by some Indians (Piscoya et al. 2001), cardiac disease, tumor (Cheng et al. 2007), and other inflammatory conditions (Heitzman et al. 2005). *Uncaria tomentosa* possess a medically important antioxidant (Gonçalves et al. 2005), antiviral, and anti-cancer properties (Reis et al. 2008). It has the ability to downregulate the production of pro-inflammatory cytokines and TNF-α (Allen-Hall et al. 2007).

In previous studies, several compounds were used to ameliorate the harmful effects of FPN on hepatorenal tissue. Abdel-Daim et al. (2018) found that both thymoquinone and diallyl sulfide relieved the FPN-induced oxidative damage, possibly by promoting antioxidant activities. In another study, oral gavage of rosuvastatin and vitamin E alone or in combination improved oxidative injury and apoptosis induced by FPN (Abdel-Daim and Abdeen 2018). Taurine and *N*-acetylcysteine alleviated the apoptotic effect of FPN on hepatorenal tissue (Abdel-Daim et al. 2019).

Although the use of FPN has been considerably increased over the last decade, the available information on its pernicious effects on animal and human health is scarce. Few studies were available on the effect of FPN on oxidative stress and hepatic biomarkers in male rats. Therefore, the current study was taken to investigate the effect of oral FPN administration on liver function, lipid peroxidation, and some hepatic oxidative stress biomarkers in adult male rats and to explore the prophylactic effect of *Uncaria tomentosa* extract as antioxidant and anti-inflammatory against sub-chronic FPN-induced hepatic injury and oxidative stress through the expression of NF-kB after 6 weeks of exposure.

Material and methods

Animals

Twenty-four Wister adult male rats were bought from the Laboratory of Animal House, Faculty of Veterinary Medicine, Suez Canal University, Egypt. The weight of the rats ranged from 200 to 220 g. Before the starting of the study, the rats were left for 2 weeks as a routine program to be adapted to the surrounding environment. Three rats per cage were kept in a room with saw dust covered floor and controlled temperature (25 ± 2 °C). The rats were permitted for unrestricted admission to standard diet and water. The procedures of this experiment were carried out under the approval (No. 2018063) and the guidelines of the committee of scientific research and biological ethics for animals used in laboratory experiments in the Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Plant material

Plant extract of (10:1) *Uncaria tomentosa* contained 1.5% oxindole alkaloids was obtained from Biotanica Co., New Zealand and used in the present study. A stock solution contained 10% from *Uncaria tomentosa* extract in distilled water (*w*/*v*) was prepared. The stock solution was used in a rate of 120 mg/kg bwt to supply the rats under the experiment.

Experimental design

Fipronil (Coash SC 20%), a preparation from Star Chem Company and manufactured by Zhejiang Yongnong Chem. Co., China, was used in the current experiment. The rats were assigned randomly into four groups, each group had six rats. Control rats received only distilled water. Rats were treated with 9.7 mg/kg FPN (1/10 of FPN LD_{50}). The dosage was chosen according to the available publications regarding oral LD_{50} of fipronil for rats (Tingle et al. 2003). Rats were given 120 mg/kg of 10% *Uncaria tomentosa* extract in distilled water and rats received both 9.7 mg/kg of FPN and 120 mg/kg of 10% *Uncaria tomentosa* extract in distilled water. The doses were given via gastric tube daily for consecutive 6 weeks. The body weight gain was checked weekly throughout the study.

Blood samples

Fasted overnight rats were anesthetized by diethyl ether at very low doses and decapitated at the end of the study. Blood samples were harvested in sterilized tubes. Serum was collected after centrifugation of blood at 3000 rpm for 20 min, then reserved at − 20 °C for the evaluation of liver enzymes and lipid parameters.

Liver enzymes

A colorimetric method of Diamond Diagnostic Kits (Lab Supply Co., Cairo, Egypt) was used to measure the levels of liver enzymes like alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in serum (Reitman and Frankel 1957).

Lipid profile

The levels of high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and triglycerides (TG) were estimated using enzymatic calorimetric kits (Cat. No. 0599, Stanbio Laboratory, USA), (Cat. No. 304710050, ELITech Diagnostic, France), and (Cat. No. 303113050, ELITech Diagnostic, France), respectively in serum of the rats according to Tietz (1990). Low-density lipoprotein cholesterol (LDL-C) was estimated as LDL cholesterol (mg/dL) = TC − HDL cholesterol − (triglycerides/5) based on the Friedewald equation (Davidson and Rosenson 2009).

Tissue samples

The liver of each experimental animal was excised immediately after scarification. In order to eliminate blood contamination, the liver was immersed in ice phosphate buffer saline (PBS) and dried by filter paper. Liver's weight was carried out and from which, the relative liver weight was estimated in relation to the body weight of the rats that measured before scarification. Several parts of the liver of each rat were frozen at − 80 °C until liver homogenate was prepared for measuring malondialdehyde (MDA), total nitric oxide content (TNO), total antioxidative capacity (TAC), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6) levels. Neutral buffered formalin (10%) was used to fix the remaining part of the liver for immunohistochemistry and histopathology examinations.

Tissue malondialdehyde (MDA)

The hepatic MDA contents, as an indicator of lipid peroxidation, were calorimetrically assayed using commercial kit (Cat No. K739-100, BioVision, USA) at wavelength ($\lambda = 532$ nm) according to Ohkawa et al. (1979). All steps were carried out according to the manufacturer's protocol.

Total nitric oxide assay (TNO)

Total nitric oxide activity in the liver homogenate was estimated by nitrate/nitrite commercial calorimetric kit (No. 780001, Cayman Chemical Co., USA) at absorbance 540 nm. The test procedures were performed according to the manufacturer's enclosed protocol.

Total antioxidative capacity (TAC)

Total antioxidative capacity in liver homogenate was assessed at absorbance 450 nm using calorimetrically commercial kit (Ref OX 20-4100, LDN, Germany) according to the manufacturer's instruction.

Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6)

Both TNF-α and IL-6 levels were assessed in the liver homogenate using ELISA commercial Kit (Cat. No. KT-19418, Kamiya Biomedical Co., USA and Code No. 27194, IBL Co., Japan, respectively) at absorbance 450 nm. The analysis was done according to the manufacturer's protocol.

Immunohistochemistry of nuclear factor kappa (NF-kB)

Slices of 5 μm mounted on positively charged slides for NF-kB immunohistochemistry were carried out on rat's liver embedded in paraffin, then deparaffinized in xylene (twice), and immersed in a series of descending concentrations of absolute, 95%, 70%, and 30% ethanol, followed by water. After blocking the endogenous peroxidase activity with 1% H_2O_2, microwave heating for 3 min in a 10-mM solution of sodium citrate was used to unmask the antigenicity. Moreover, to reduce the non-specific staining, a blocking solution of 2% dry milk in PBS (with 0.02% sodium azide) was used. Sections were incubated with primary rabbit polyclonal anti-NF-kB/p65 antibody (Cat. No. sc-109, Santa Cruz Biotechnology, USA) at 25 °C for 2 h at a dilution of 1:200 in a humidified chamber. After incubation, sections were washed three times for 3 min each with PBS and liver sections were co-incubated for 30 min with biotinylated polyvalent secondary antibody (Cat. No. 32230, Thermo Scientific Co., UK). After incubation, liver slides were washed three times for 3 min each with wash buffer and counterstained with suitable amount of Hematoxylin stain in order to cover the entire tissue surface. Quantitative analysis of the intensity of immuno-reactive area was carried out by an image analyzer (Image J program, Japan) after subtracting background noise according to Elgawish et al. (2015).

Histopathology

Liver sections were fixed in formalin (10%) and dehydrated in ascending concentrations of ethyl alcohol (70–100%) and then managed and stained with Hematoxylin and Eosin (H&E) for histopathology (Bancroft et al. 1996).

Statistical data analysis

Data were expressed as mean ± standard error of the mean. The differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test for inter-group comparisons using GraphPad Prism (Version 5.01, GraphPad Software, San Diego, USA). A $P < 0.05$ indicates a statistically significant difference between groups.

Results

Body weight gain and relative liver weight

At the end of the experiment, FPN-treated rats had a significantly ($P = 0.02$) lower body weight gain compared to that in *Uncaria tomentosa*-treated rats. Moreover, significantly ($P = 0.003$) higher relative liver weight was observed in rats given FPN compared to that in treated rats (Table 1).

Liver enzymes

Rats given FPN exhibited a significantly ($P = 0.02$) higher liver enzymes (ALP, AST, and ALT) than that in other rats. The administration of *Uncaria tomentosa* with FPN improved the liver enzymes nearly to the levels of control rats (Table 1).

Antioxidant biomarkers, TNF-α, and IL-6

Fipronil administration induced significantly ($P < 0.0001$) higher levels of MDA, TNO, TNF-α, and IL-6 compared with their levels in control and other treated rats. However, the TAC level was significantly ($P < 0.0001$) reduced after FPN administration compared to that in other groups (Table 2).

Lipid profiles

Fipronil induced significantly higher TC level in rats compared to the level of TC induced by *Uncaria tomentosa* ($P < 0.01$) and FPN with *Uncaria tomentosa* ($P < 0.05$). However, non-significant differences were observed in TG, HDL-C, and LDL-C levels between control and treated groups (Fig. 1).

Immunohistochemistry of NF-κB

There was no nuclear signal or immune-reactivity for NF-κB in the liver of control rats, while, the liver of rats treated with FPN showed significantly ($P < 0.001$) higher immunostaining of NF-κB, especially around portal area compared to that in other groups (Fig. 2). The immunostaining was presented in hepatocytes, as well as non-parenchymal cells. In contrary, the intensity of immunostaining for NF-κB was significantly ($P < 0.001$) decreased in the liver of *Uncaria tomentosa* alone or *Uncaria tomentosa* with FPN-treated rats (Fig. 2).

Histopathology

Control rats showed a well-preserved liver structure with normal central vein, hepatocytes, and sinusoidal spaces. However, inflammation and necrosis in the liver of rats treated with FPN were observed mainly in the portal region and hydropic degeneration was detected in hepatocytes. In addition to inflammation and necrosis, some livers had fatty infiltration, congestion of portal vein, and karyolysis of hepatocytes nuclei. The liver of *Uncaria tomentosa*-treated rats showed normal architecture with histological picture comparable to that of the control rats. *Uncaria tomentosa* administration with FPN improved the histological picture that appeared similar to that in the liver of control rats, although some hepatocytes showed minimal cytoplasmic areas of vacuolization and karyolised nuclei (Fig. 3).

Table 1 Body weight gain, relative liver weight, and liver enzymes (Mean ± SEM) after 6 weeks of experiment in control and treated rats

	Control	*Uncaria tomentosa*	Fipronil	Fipronil and *Uncaria tomentosa*
Body weight gain (g)	5.8 ± 1.5^{ab}	12.3 ± 2.8^{b}	-1.8 ± 3.4^{a}	3.5 ± 2.8^{ab}
*Relative liver weight (%)	3.0 ± 0.1^{a}	3.1 ± 0.2^{a}	4.2 ± 0.2^{b}	3.2 ± 0.3^{a}
ALP (U/L)	66.8 ± 1.0^{a}	66.2 ± 0.5^{a}	103.6 ± 4.2^{b}	81.2 ± 3.3^{c}
ALT (U/L)	25.1 ± 0.3^{a}	24.4 ± 0.2^{a}	66.3 ± 2.5^{b}	42.2 ± 1.0^{c}
AST (U/L)	46.7 ± 0.7^{a}	45.8 ± 0.2^{a}	109.9 ± 2.9^{b}	69.8 ± 1.9^{c}

Alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)

Significant differences are indicated by the subscripts being different within the same row

*Relative organ weight = [organ weight/body weight] × 100

Table 2 Antioxidant biomarkers, TNF-α, and IL-6 (Mean ± SEM) levels in liver of control and treated rats after 6 weeks of experiment

	Control	*Uncaria tomentosa*	Fipronil	Fipronil and *Uncaria tomentosa*
MDA (nmol/mg)	0.6 ± 0.01^{a}	0.5 ± 0.01^{a}	2.3 ± 0.09^{b}	1.3 ± 0.02^{c}
TNO (μM)	21.8 ± 0.2^{a}	21.1 ± 0.1^{a}	34.2 ± 0.7^{b}	27.2 ± 0.6^{c}
TAC (U/mL)	2.6 ± 0.02^{a}	2.8 ± 0.03^{c}	1.7 ± 0.05^{b}	2.2 ± 0.03^{d}
TNF-α (pg/ml)	20.3 ± 0.4^{a}	19.5 ± 0.3^{a}	66.8 ± 0.8^{b}	40.9 ± 1.3^{c}
IL-6 (pg/ml)	11.0 ± 0.3^{a}	10.4 ± 0.2^{a}	30.9 ± 0.8^{b}	21.3 ± 0.9^{c}

Malondialdehyde (MDA), total nitric oxide (TNO), total antioxidant capacity (TAC), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6)
Significant differences are indicated by the subscripts being different within the same row

Discussion

Fipronil is used in home and commercial applications; its utilization may have serious environmental and public health concerns (Tingle et al. 2003; Jennings et al. 2002). In the current study, rats given FPN showed a significant ($P = 0.02$) decline in body weight gain compared to that in *Uncaria tomentosa*-treated rats. This result was in accordance with Mossa et al. (2015) who found a body weight reduction in FPN-treated rats. In contrary, Badgujar et al. (2016) did not find significant variation in 28 days FPN-treated mice body weights when compared with those of control and vitamin-treated ones. The decrement in body weight may be attributed to toxic effect of FPN that induced oxidative stress that impaired normal homeostasis and weight gain (Mossa et al. 2015). The relative hepatic weight was significantly promoted in rats given FPN compared to that in other treated rats. In parallel to our findings, Badgujar et al. (2016) and Mossa et al. (2015) declared that the absolute weight of mice liver was significantly increased after FPN oral administration for both 28 and 45 days, respectively. As liver, is an important organ responsible for metabolism, increase in its weight is telltale of toxic nature of FPN that was augmented by the decrement in body weight gain. Moreover, the hypertrophy of FPN-treated liver may be attributed to the high numbers of rough endoplasmic reticulum and mitochondria. The most feasible reason for the promoted number of rough endoplasmic reticulum was believed to be their involvement in FPN metabolism therefore increasing the cytochrome P450 (CYP) and its isoforms producing ability (Ferreira et al. 2012).

In this study, a significant ($P = 0.02$) rise of liver enzymes (ALP, AST, and ALT) was observed in rats given FPN treatment. The administration of *Uncaria tomentosa* with FPN improved the liver enzymes nearly to the levels of control rats. In parallel to our results, dose-dependent significant rise in the hepatic enzymes and MDA were noted in mice administered different doses of FPN as compared with control (Badgujar et al. 2016; Mossa et al. 2015).

Lipid peroxidation has been widely applied as an oxidative stress biomarker that mainly generated due to lipid profile alterations (Kelany et al. 2017; Makni et al. 2008; Tsimikas and Miller 2011). The level of MDA was estimated with Thiobarbituric Acid Reactive Substances (TBARS) method; however, this method was considered non-specific. In spite of the commonly recognized limitations of the TBARS test and in particular its lack of specificity; it remains a representative method of MDA measurement in medical experiment (Karacay et al. 2010; Zortea et al. 2012). In the current experiment, FPN administration induced a significant high level of serum cholesterol and MDA in rats. The increment in hepatic TNO in FPN group is indicative for FPN-induced oxidative stress in rat liver. Consequently, hepatic oxidative stress led to hepatic lipid peroxidation that caused hepatic damage observed in this study. The hepatic damage was manifested by higher levels of serum level of ALP, AST, and ALT that are synthesized by hepatic cells where they exert their functions. Liberation of these enzymes into the blood by higher levels is indicative for hepatic cell membrane damage, alteration in hepatic cells permeability (Harper 1979), or necrosis of hepatocytes that led to leakage of such enzymes into circulation (Mansour and Mossa 2010). Treatment of rats with *Uncaria tomentosa* against FPN decreased serum cholesterol level, hepatic TNO, and MDA that suggests the hypolipidemic and antioxidant properties of *Uncaria tomentosa* as a protective agent. The antioxidant effect of phenolic compounds content in *Uncaria tomentosa* (Krishnaiah et al. 2011) prevented the free radicals induced DNA damage (Desmarchelier et al. 1997).

Hepatic TNO, TNF-α, and IL-6 suppression were observed after *Uncaria tomentosa* administration either alone or with combination with FPN. Both TNF-α and IL-6 are quiescent genes under normal circumstances; however, immune activation and inflammation induce numerous transcripts that give rise to the expression of these cytokines (Sandoval et al. 2002). Cat's claw downregulates the expression of inducible nitric oxide synthase, therefore reducing the production of nitric oxide thus negating cytotoxic effect and hepatic inflammatory pathway (Sandoval-Chacón et al. 1998). Furthermore, the inflammatory disorders related to TNF-α and IL-6 genes expression have been put a focus on NF-kB as the principle transcription factor in tissue damage and

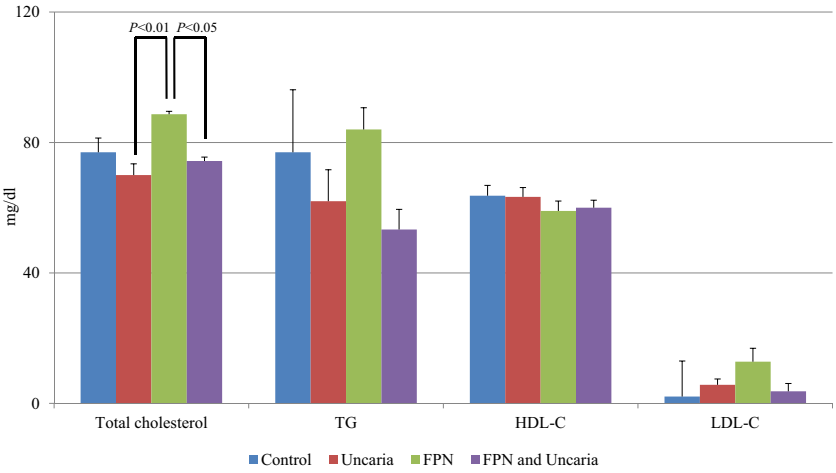

Fig. 1 Lipid profiles of control, *Uncaria* (120 mg/kg), FPN (9.7 mg/kg) and FPN and *Uncaria* treated rats for 6 weeks. Values represented as means ± SE. Total cholesterol was significantly elevated in FPN-treated rats compared to that in rats given *Uncaria* ($P < 0.01$) and FPN with *Uncaria* ($P < 0.05$)

inflammation (Jourd'heuil et al. 1997; Urdanibia et al. 2013). *Uncaria tomentosa* displayed excellent antioxidant properties and was capable to repress TNF-α production (Sandoval et al. 2002) and IL-6 (Urdanibia et al. 2013). Moreover, *Uncaria tomentosa* has the power to protect DNA against different oxidants like peroxynitrite (Sandoval-Chacón et al. 1998).

The liver of FPN-treated rats showed significantly higher expression of NF-κB. In contrary, the immunostaining for NF-κB was significantly decreased in liver of *Uncaria*

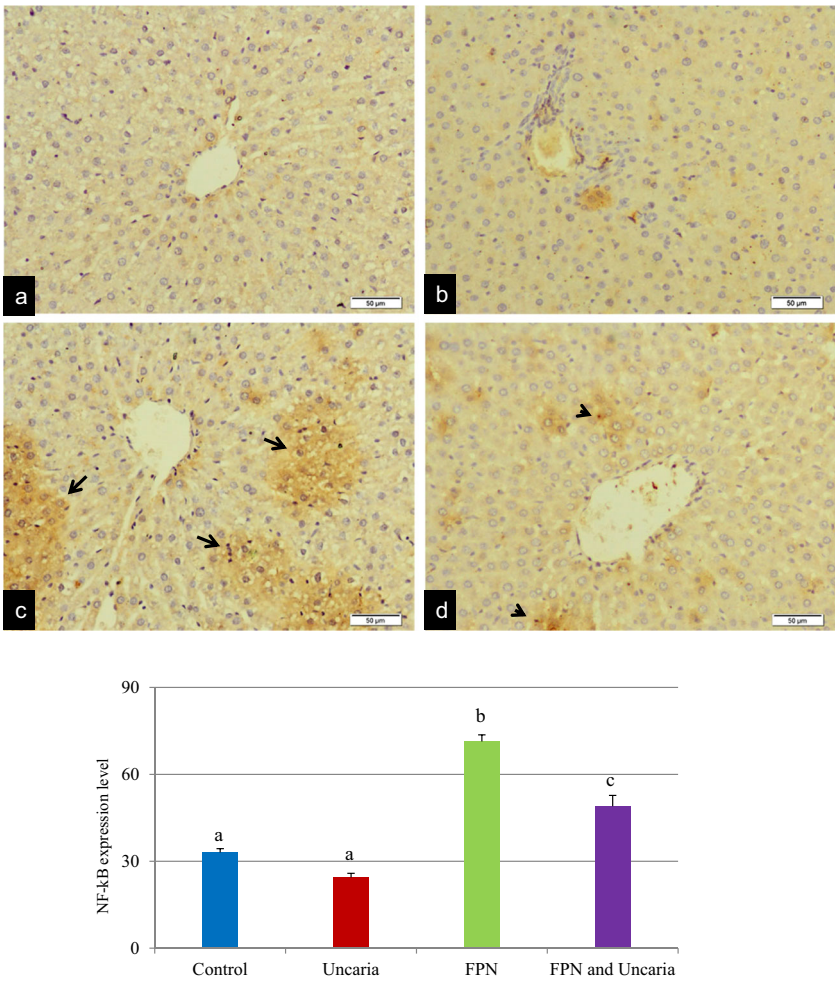

Fig. 2 Expression of NF-kB in rat liver. Liver from control (a) and *Uncaria* (b) showed nearly absent staining in hepatic tissue. The hepatic section from rats exposed to 9.7 mg/kg of FPN (c) showed strong staining (arrows). Section from the liver of 9.7-mg/kg FPN- and *Uncaria*-treated (120 mg/kg) rats shows minimal staining (head arrows). Bar = 50 μm. Positive proportions of NF-kB were increased in the liver of FPN-treated rats compared to that in other groups. Values represented as means ± SE. Means having the same letters are not significantly different from each other, $P < 0.05$

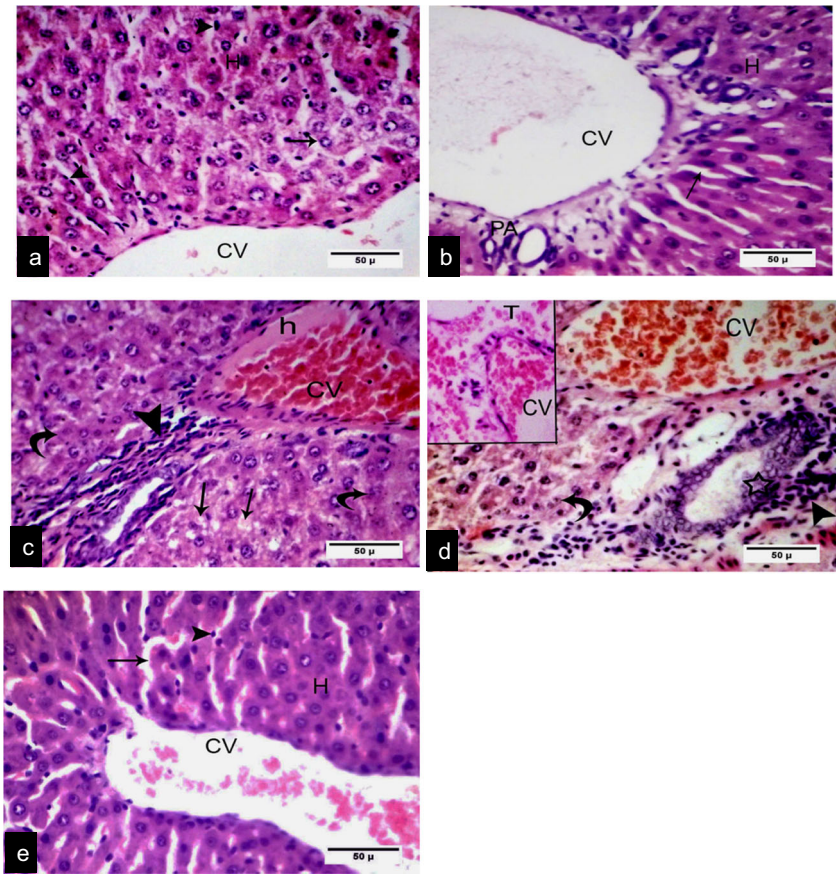

Fig. 3 Photomicrograph of liver sections stained by H&E for histopathological changes showing control (a) and 120 mg/kg of 10% *Uncaria* (b) with normal histological structure of hepatic lobule. Administration of 9.7 mg/kg of FPN (c) showing hyalinization (h) in the wall of the central vein which contained hemolyzed RBCs (CV), proliferation of the fibroblasts and angioblasts (head arrow) in the portal area, vacuolar degeneration in some hepatocytes (straight arrow), and some nuclei of the hepatocytes exhibited karyolysis (curved arrow). Additionally, 9.7 mg/kg of FPN (d) showing congested central vein (CV), hemorrhage (T), proliferation of the fibroblasts and angioblasts (head arrow) in the portal area, and proliferation in the epithelium of the bile duct (star). The FPN and *Uncaria* (e) group showing hepatocytes (H), blood sinusoid (straight arrow) contained Kupffer's cells (head arrow), central vein (CV), and portal area (PA), with remarkable improvement of hepatic tissue

tomentosa alone or *Uncaria tomentosa* with FPN-treated rats. Through the repression of NF-kB activation, *Uncaria tomentosa* could prevent many transcriptionally-regulated genes, particularly via NF-kB activation repression (Sandoval-Chacón et al. 1998). It is conceivable that the antioxidant criteria of *Uncaria tomentosa* play a role in this response (Desmarchelier et al. 1997; Sandoval et al. 2000) where NF-kB is considered as an oxidant-sensitive transcription factor (De Nigris et al. 2001). To the best of our knowledge, there were no available reports on the effect of FPN on NF-$_{\kappa}$B activity, although there were other studies discussing other hepatotoxic chemicals on the expression of NF-$_{\kappa}$B. Hepatic damage induced by CCl_4 in mice leads to oxidative stress that boosts NF-$_{\kappa}$B activity, and NF-kB activity has been shown to induce the cytotoxic cytokines expression. This injury was inhibited by α-tocopherol (Liu et al. 1995). Additionally, oligonol have anti-inflammatory and antioxidative effects in rats given CCl_4 by reducing oxidative load as well as NF-κB activation (Bak et al. 2016).

In the present study, inflammation in hepatocytes and karyolysis of some nuclei were observed in the liver of rats treated with FPN. These histopathological observations were affirmed by the significant promotion of the classical hepatic biomarkers denoting liver damage (ALP, AST, and ALT) as well as inflammatory biomarkers (TNF-α, IL-6, and NF-kB). Extensive degenerative and vacuolar changes in the hepatocytes with mononuclear cells infiltration as well as focal areas of necrosis sufficiently explained the FPN-induced hepatic damage (Badgujar et al. 2016). Similar histopathological alterations were observed in mice and rats after FPN administration (De Oliveira et al. 2012; Ferreira et al. 2012; Mossa et al. 2015). When *Uncaria tomentosa* was used with FPN, a remarkable improvement was observed in hepatic structure. Aguilar et al. (2002) found that Uncaria polyphenols and flavonoids ameliorated liver pathological effects through the reduction of hepatic damage biomarkers, lipid peroxidation, and inflammatory biomarkers (TNF-α, IL-6, and NF-kB).

Conclusion

This study indicated that the stimulation of NF-kB may explain the way by which FPN induced oxidative stress and promoted liver injury. Fipronil administration increased NF-kB activity, TNF-α, and IL-6 which may contribute in liver injury. The clarification of the issues that provide opportunities for understanding the exact role of *Uncaria tomentosa* in blockage of activation of NF-kB and reduction of cytokines production as well as alleviation of hepatic inflammation and necrosis still needed to be investigated.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

The procedures of this experiment were carried out under the approval (No. 2018063) and the guidelines of the committee of scientific research and biological ethics for animals used in laboratory experiments in the Faculty of Veterinary Medicine, Suez Canal University, Egypt.

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