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Hepatorenal protective effects of taurine and *N*-acetylcysteine against fipronil-induced injuries: The antioxidant status and apoptotic markers expression in rats

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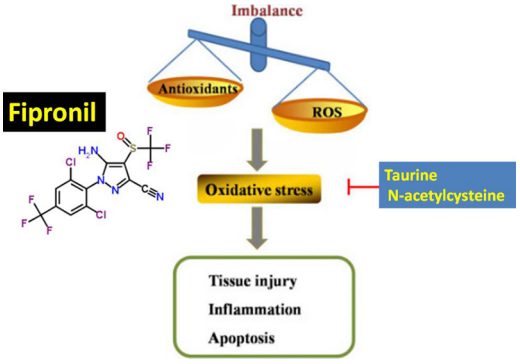
HIGHLIGHTS

- Fipronil induced oxidative hepatorenal injury in rats.
- Fipronil induced apoptosis in rats' liver and kidney.
- Taurine and *N*-acetylcysteine protect against fipronil-induced injuries.
- Taurine and *N*-acetylcysteine induced antioxidant and antiapoptotic effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Fipronil (FPN), a commonly used phenylpyrazole pesticide can induce oxidative tissue damage following hazard usage. Due to the extensive household and commercial usage of FPN, its toxic effects on mammals received considerable attention. Finding the proper antioxidant that can overcome FPN-induced damage is essential. Therefore, the present study aimed to assess the hepatorenal ameliorative outcomes of *N*-acetyl cysteine (NAC) and taurine (TAU) against hepatorenal damage induced by FPN in male Wistar rats. Compared to control rats, oral FPN (at a dose of 19.4 mg kg^{-1} BW for five successive days) significantly increased serum activities ($p \leq 0.05$) of alkaline phosphatase, lactate dehydrogenase and transaminases, in addition to total cholesterol, urea and creatinine levels. Moreover, FPN provoked oxidative damage indicated by increased malondialdehyde and nitric oxide formation and decreased glutathione concentration and activities of enzymatic antioxidants (superoxide dismutase, glutathione peroxidase and catalase) in the hepatic and renal tissues. Furthermore, FPN administration induced overexpression of the proapoptotic (Bax), while it downregulated the expression of the antiapoptotic (Bcl-2) protein. Interestingly, oral administration of TAU (50 mg Kg^{-1} BW) and NAC (50 mg Kg^{-1} BW), alone or in combination, five days prior to and five days along with FPN administration, significantly ameliorated ($p \leq 0.05$) and normalized the harmful effects of FPN on serum biomarkers of hepatorenal injury, lipid

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peroxidation and tissue antioxidants. In conclusion, TAU and NAC, alone or in combination, provided significant hepatorenal protection against oxidative stress and apoptosis induced by FPN.

1. Introduction

Fipronil (FPN), *N*-phenylpyrazole insecticide with broad-spectrum effect, used to control cockroaches, ants, fleas and other insects that resist organophosphates, carbamates and pyrethroids (Caceres et al., 2011). Therefore, FPN is widely-applied in agriculture, animal husbandry and public hygiene management (Tingle et al., 2003). Fipronil mechanism of action appears through antagonizing the gamma-amino butyric acid (GABA) chloride channels, resulting in selective neurotoxicity in insects rather than mammals (Cole et al., 1993). Nonetheless, like most insecticides affect any species if the dose exceeds the manufacturer's instructions or was applied inappropriately (Ahmed et al., 2017; Chodorowski and Sein Anand, 2004). Due to the extensive household and commercial usage of FPN, its toxic effects on mammals received considerable attention.

Fipronil can be metabolized in the environment into fipronil-desulfinyl metabolite, which is 9–10 times more toxic in mammals (Terçariol and Godinho, 2011; Tingle et al., 2003). Moreover, it can be metabolized into sulfone in the liver by virtue of cytochrome P450 (Roques et al., 2012), which remains in the adipose tissue for one week after exposure. The liver and kidneys are specifically susceptible to FPN oxidative injury because of their vital function in the biotransformation of insecticides (Mansour and Mossa, 2010; Refaie et al., 2014).

Previous studies reported that, FPN suppressed the cellular enzymatic and non-enzymatic antioxidants, giving rise to oxidative injuries (Abdel-Daim and Abdeen, 2018; Mossa et al., 2015). Therefore exogenous antioxidants administration may guard against oxidative damage caused by FPN. Sulfur-containing antioxidants (such as taurine, *N*-acetylcysteine (NAC), cysteine, methionine, glutathione, lipoic acid and α-mercaptopropionylglycine) are broadly diffused in the human tissues and essential for life (Atmaca, 2004).

Taurine (TAU; 2 amino ethane sulfonic acid) is a non-protein sulfur-containing essential amino acid, involved in several biological reactions (Huxtable, 1992). It is the end-product for L-cysteine metabolism which can enhance the intracellular levels of GSH (Das et al., 2008; Gürer et al., 2001). Taurine has been exhibited to scavenge the hydroxyl radicals and ameliorate the oxygen radical pathophysiology (Rashid et al., 2013; Ripps and Shen, 2012). Other authors showed its cytoprotective potential in the liver and kidneys against the toxicity, induced by potassium bromate, carbon tetrachloride, cadmium and alloxan-induced diabetes mellitus (Ahmad et al., 2013; Dinçer et al., 2002; Manna et al., 2008; Rashid et al., 2013).

N-acetylcysteine (NAC) is a pro-drug of L-cysteine, the precursor to glutathione (GSH). It is widely utilized as a safe antidote for paracetamol intoxication and mucolytic agent (Elbini Dhouib et al., 2016). Moreover, it can normalize the oxidant-antioxidant imbalance (Yalcin et al., 2008) by replenishing GSH level in the cell, inhibiting lipid peroxidation and scavenging reactive oxygen species (ROS) (Samuni et al., 2013). Former studies have presented NAC hepatorenal protective effects against several xenobiotics (Dobashi et al., 2002; Heidari et al., 2016; Modi et al., 2006; Wong and Ooi, 2003).

The hepatorenal toxicity of FPN has not been thoroughly examined. Therefore, we conducted this study for assessing the oxidative and apoptotic effects of FPN oral administration on rat liver and kidney, as well as the hepatorenal protective effects of TAU and/or NAC pre-administration in this regard.

2. Materials and methods

2.1. Chemicals

Fipronil (Fipromex 20% SC) (5-amino-1-(2,6-dichloro-α, α, α-trifluoro-p-tolyl)-4-[(trifluoro methyl) sulfinyl] pyrazole-3 carbonitrile) was manufactured by MAC-GmbH, Germany. TAU and NAC were bought from Sigma Chemicals (St Louis, MO, USA). The kits, used for biochemical analysis, were bought from Biodiagnostics Company (Dokki, Giza, Egypt). Other used reagents were of analytical degree.

2.2. Experimental animals

Fifty-six male Wistar rats, weighing between 140 and 160 g, were freely supplied with standard diet and water *ad libitum* and housed for one week to be acclimatized before starting the experiment under standardized circumstances (12 h light/dark period, temperature 22 ± 3 °C and humidity 50%). This study was executed according to the Institutional Animal Care and Use guidelines of the Faculty of Veterinary Medicine, Suez Canal University, Egypt. Animal handling and experimental protocol was approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (approval No. 2018055).

2.3. Experimental protocol

Rats were allocated into seven groups (8 rats/group): the 1st group served as a control, and received corn oil by gavage for 10 days. The 2nd group received oral TAU at 50 mg kg^{-1} daily for consecutive 10 days (Das et al., 2010). Rats in the 3rd group were given NAC at 50 mg kg^{-1} daily for consecutive 10 days (Sathish et al., 2011). The 4th group was supplied orally with FPN at 19.4 mg kg^{-1} (1/5 LD_{50}) (Tingle et al., 2003) for five days (from the 6th till the 10th day of the experiment). The 5th and 6th groups received TAU (50 mg kg^{-1} daily orally) and NAC (50 mg kg^{-1} daily orally), respectively (5 days before and 5 days along with FPN supplementation (19.4 mg kg^{-1}). The 7th group received TAU and NAC combination at the same doses of the 2nd and 3rd groups for 10 days with daily FPN supplementation in the last five days.

2.4. Blood and tissue samples

At the 11th day, blood samples were obtained from the retro-orbital venous plexus of the experimental rats, left to clot in plain dry tubes and centrifuged at 3000 rpm for 10 min. The obtained sera were kept at −20 °C for later biochemical assessments of liver function biomarkers (alanine transferase [ALT], lactate dehydrogenase [LDH], aspartate transferase [AST], alkaline phosphatase [ALP] and cholesterol) and renal function biomarkers (urea and creatinine). After blood sampling, rats were euthanized by cervical dislocation. The liver and kidneys were resected instantly and washed in saline. A small part of each organ was kept in 10% neutral buffered formalin for further histopathological and immunohistochemical examinations. The left parts of each organ were submerged in 10% ice-cold 100 mmol/L sodium phosphate buffered saline (pH 7.4), homogenized in 5–10 mL ice cold buffer/one gram tissue and centrifuged in cold centrifuge at 10000 rpm for