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Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats

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ABSTRACT

Fipronil (FPN) is a broad-spectrum *N*-phenylpyrazole insecticide and has been used in agriculture and public health since the mid-1990s. The present study was designed to investigate the adverse effects of sub-chronic exposure to the FPN on the liver and kidney of male rats at three concentrations 0.1, 1 and 10 mg/L in drinking water for 45 days. Serum aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activity and levels of uric acid, creatinine and total protein were significantly increased in FPN-treated rats. Oxidative stress biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reduced (GSH) were significantly decreased, while lipid peroxidation (LPO) was significantly increased in treating rats in a concentration dependent manner. FPN caused histopathological alterations in liver and kidney of male rats. From our results, it can be concluded that FPN induced lipid peroxidation, oxidative stress, liver, and kidney injury in rats. These pathophysiological changes in liver and kidney tissues could be due to the toxic effect of FPN that associated with a generation of free radicals.

1. Introduction

Fipronil (FPN, 5-amino-1-(2,6-dichloro-4-(trifluoro-methylphenyl)-4-(trifluoro-methylsulfinyl) pyrazole-3-carbonitrile) is a phenylpyrazole insecticide that is extensively used to control insects in different cereal crops and in public health management [1]. It is more effective than organophosphate, carbamate and pyrethroids insecticides against several species of Lepidoptera, Orthoptera and Coleopteran [2,3]. Currently, exposure to phenylpyrazole pesticides is a global public health issue and concerns are increased regarding the relative safety of these pesticide groups because of widespread use, their toxicity, and releases into the environment.

FPN is neurotoxic to insects and the primary mechanism of action refers to blocks ion tropic gamma-amino butyric acid receptor (GABAR) of the central nervous system that causes hyper-excitation at low doses and convulsions leading to insect death at high doses [4]. FPN is more toxic to insects than mammals [5] and has moderately acute oral toxicity LD_{50}'s ranging from 40 to 100 mg/kg body weight in rats and mice [3,6]. Therefore, complete selectivity of pesticides is difficult and most of the pesticides are toxic to non-target organisms, including humans [7].

FPN is a strong uncoupler of oxidative phosphorylation at relatively low concentrations in SH-SY5Y human

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neuroblastoma cells in vitro and induced neuronal apoptosis, mediated by increased generation of reactive oxygen species (ROS) [8]. Both HepG2 cells and primary human hepatocytes are sensitive to the cytotoxic effects of FPN [9]. FPN and its metabolites induced cytotoxicity in epithelial model Caco-2 cells at micromolar concentration exposure [8]. FPN inhibits DNA and protein synthesis in rat neuronotypic pheochromocytoma PC12 cells and induced oxidative stress more than chlorpyrifos [10]. FPN causes endocrine disruption and adverse reproductive effects in female rats [11], elevation in lipid peroxidation (LPO) and decrease in the levels of glutathione (GSH) at the dosage of 0.5 mg/kg/day for 98 days to buffalo calves [12] and altered SOD and CAT activities in the liver of *Cyprinus carpio* [13]. It decreased total thyroxine (T4), increased hepatic enzymes in plasma of female rat [14], and caused acute human poisoning [15,16].

Liver and kidney are the most sensitive and main target organs of pesticide toxicity and damage [17], they play a major role in the biotransformation of pesticides. The sensitivity of these tissues to this stress to pesticides is a function of the disturbed balance between the degree of oxidative stress and the antioxidant capability [17,18]. Previous studies show that pesticides alter enzymatic and non-enzymatic antioxidant and induced oxidative stress in animals that was investigated as a potential mechanism of pesticide toxicity [16,18,19]. It has been reported that prolonged exposure to low doses of fipronil leads to oxidative stress in serum of pregnant rats and their offspring [20].

Pesticide formulations are complex mixtures that contain, besides the active ingredient(s), several other components, such as solvent, wetting, emulsifying agents, and additives; therefore the toxicity information on active ingredients alone is not sufficient to evaluate the adverse health effects of commercial pesticides. Therefore, the WHO emphasized the necessity of evaluating toxic hazard of the formulated pesticides [21]. Over the last decade, the usage of FPN has increased considerably and information on adverse health effects is very limited. To the best of our knowledge, there are no published studies that have examined the effect of formulated FPN on oxidant/antioxidant status and the liver and kidney function biomarkers in male rats. Therefore, this study aimed to evaluate the adverse effects of sub-chronic exposure to formulated FPN on oxidant/antioxidant status and liver and kidney biomarkers of male rats.

2. Materials and methods

2.1. Animals and management

Male albino rats weighing 105 ± 5 g were procured from the Animal Breeding House of the National Research Centre (NRC), Dokki, Giza, Egypt. Rats were housed in polypropylene cages (six rats in each), with free access standard pellet diet, water ad libitum, under standardized housing conditions (12 h light/dark cycle, temperature (23 ± 2 °C) and a minimum relative humidity of 48% in the laboratory. The rats were acclimatized for 1 week before the start of the experiment. All the rats were kept according to the guidelines and welfare regarding animal protection approved by NRC Local Ethical Review Committee and was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals".

2.2. Chemicals and reagents

Fipronil (Insecto SC 5%) is a product of BASF Company and manufactured by, Sinochem Group Ningbo Technical Co., Ltd., China. The assay kits used for biochemical measurements of aspartate aminotransferases (AST, EC 2.6.1.1.), alanine aminotransferases (ALT, EC 2.6.1.2), alkaline phosphatase (ALP, EC 3.1.3.1), lactate dehydrogenase (LDH, EC 1.1.1.27), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPx, EC 1.11.1.9), glutathione-s-transferase (GST, EC 2.5.1.13), glutathione reduced (GSH), lipid peroxidation (LPO), albumin, uric acid and creatinine were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. All other chemicals were of reagent grades and were obtained from reputed companies.

2.3. Experimental design

Rats were randomly divided into four experimental groups, six rats. Group I, received water and served as a control. The remaining three groups (II, III and IV) received FPN in drinking water at concentrations 0.1, 1.0 and 10 mg/L for 45 consecutive days. The concentrations of FPN were calculated depending on the percentage of active ingredients of commercial formulation of FPN. Concentrations of FPN were freshly prepared and body weights were monitored weekly during the experimental period. All rats were observed for signs of toxicity and mortality daily for 45 days.

The concentrations used in this study represent 2.0, 0.2 and 0.02 mg/kg b.wt. of FPN, based on average water consumptions and body weights of treated rats. The lower concentration of FPN represents the dose of no observable adverse effect level (NOAEL) of human [22] with descending concentration levels by 10-fold interval, i.e., 1 and 10 mg/L.

2.4. Blood and tissue samples

At the end of the experimental period, rats were fasted overnight and blood samples were collected by puncturing the retero-orbital venous plexus of the animals with a fine sterilized glass capillary, then rats were sacrificed by cervical dislocation. Blood samples were left to clot in clean dry tubes and centrifuged at 3000 rpm (600 x *g*) for 10 min at 4 °C using Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany) to obtain the sera. Serum samples were stored at −20 °C for further biochemical analysis, such as AST, ALT, ALP and LDH.

Liver and kidney were excised immediately after sacrificed, cleaned in saline and weighed. Small pieces of each liver and kidney were cut and kept in 10% natural formalin for histopathological study. The other portions of liver and kidney were homogenized in 10% (w/v) ice cold 100 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm (2000 × *g*) for 15 min at 4 °C, and then the supernatant was