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Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice

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ABSTRACT

Imidacloprid (IC) is a systemic insecticide related to the tobacco toxin nicotine. IC is a toxic substance frequently used into combat insects, rodents and plants pests and other creatures that can pose problems for agriculture. We, therefore, planned this study to assess risk factors, biochemical and histological alterations associated with hepatotoxicity and nephrotoxicity. Forty-eight adult male albino mice were divided into four groups of 12 animals each. All the animals were given standard synthetic pellet diet. One group served as control, and the other three were served as experimental groups. Decrease in the body weight of the high dose group was observed at 15 mg/kg/day, and no mortality occurred during the treatment period. High dose of imidacloprid caused a significant elevation of serum clinical chemistry parameters, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate kinase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TBIL). Histology of liver and kidney indicates hepatotoxicity and nephrotoxicity at a high dose of imidacloprid. Based on the morphological, biochemical and histopathological analysis, it is evident that imidacloprid induced toxicological effects at 15 mg/kg/day to mice. The results of the present study demonstrate that IC had significant effects on body weight, liver functions and kidney ($p < 0.05$) at a dose of 15 mg/kg body weight. IC treatment 5 and 10 mg/kg/day may be considered as no observed adverse effect level (NOAEL) for mice. It was concluded that IC can cause hepatotoxicity and nephrotoxicity at a dose much lower than the LD_{50} (131 mg/kg body weight) in mice.

1. Introduction

Fruits and vegetables are an essential part of a nutritious and healthy diet; however, the health benefits are compromised by consistent contamination with pesticide residues. In our previous work, pesticide was detected in a range of fresh vegetables [1]. These highly stable compounds can last for years or decades before breaking down. They

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circulate globally and persistent pesticides released in one part of the world can, through a repeated process of evaporation and deposit, be transported through the atmosphere to the areas far away from the source [2]. Human health risks vary with the type of the pesticides and also with the extent of vulnerability. Immediate human health hazards from pesticides include mild headaches, flu, skin rashes, blurred vision and other neurological disorders, and rarely, paralysis, blindness, and even death. Long run health impacts include cancer, infertility, miscarriage, male sterility, birth defects, and effects on the nervous system [3]. Pesticides can also interfere with drug-metabolizing enzymes, especially Cytochrome P450 leading to drug interactions [4].

The liver cytochrome P450 (CYPs) is the major enzymes involved in drug metabolism, accounting for ~75% of the total metabolism [5] and activation or detoxification of neonicotinoids [6]. Studies of the metabolites of neonicotinoids have shown that they can be bioactive and act as nAChR agonists or cause secondary toxicity in mammals [7]. Nicotinoids can be formed as metabolites of neonicotinoids with greater selectivity for vertebrate nAChRs than to insect nAChRs [6,7].

Nephrotoxicity of pesticides has been reported in mice [8] and in rats [9]. Imidacloprid, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, a chloronicotyl is an extensively used insecticide for crop protection in the world wide for the last decade due to its low soil persistence and high insecticidal activity at low application rate [10]. Imidacloprid, nitenpyram, acetamiprid, thiacloprid, thiamethoxam, and others act as agonists at the insect nicotinic acetylcholine receptor (nAChR) [6]. It is the fastest growing in sales because of its high selectivity for insect nicotinic acetylcholine receptors [11]. IC is most widely used neonicotinoids insecticide in agriculture can exaggerate the toxic properties and adverse effects of insecticide and can be fatal for human as well as animal health. Buckingham et al. showed that imidacloprid affects both AChRs sensitive to -BTX and -BTX-insensitive nicotinic acetylcholine receptors (nAChRs) can act on pharmacologically diverse nAChR subtypes [12]. Oral LD50 of imidacloprid is 131 mg/kg in mice. It is rapidly absorbed from the gastrointestinal tract and eliminated via urine and feces [13].

In the present study, we examined possible effects of imidacloprid on hepatotoxicity and nephrotoxicity by using biochemical and histological techniques in mice.

2. Materials and methods

Forty-eight adult male albino mice weighing 25 to 30 g were obtained for this study. The mice were obtained from the Experiment Animal Center of the Fourth Military Medical University (Xi'an, Shaanxi, China) and housed six per cage in our lab for 15 days before the experiment. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. All experimental procedures followed the principle of laboratory animal care and were carried out according to a protocol approved by the local animal ethics committee. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.1. Experimental design

The adult male mice were randomly divided into a total of four groups 12 in each group. One group served as control and the other three groups were served as experimental groups, given 5, 10 and 15 mg/kg body weight imidacloprid by an oral gavage method for 15 days. The animals were maintained under conditions of controlled temperature (22 ± 3) and humidity (30–70%) with 12 h light and dark cycle. The animals were given standard synthetic pellet diet.

2.2. Chemicals

Commercial Imidacloprid 20% EC formulation, with the name Confidor was obtained from Bayer. Kits for SGOT, SGPT, ALP and TBIL were purchased from Human, Germany.

2.3. Sign of toxicity and mortality

Signs of toxicity such as salivation, lacrimation, diarrhea, tremor, convulsion, paralysis and death were observed once daily throughout the period of exposure. After 15 days of dosing mice, blood was collected in non-oxalate tubes for the separation of serum.

2.4. Urine examination

Urine of the individual animals was collected and arranged group wise, initially (day 0) before exposure, at day 5, 10 and finally at 15 days of treatment for the qualitative analysis of pH, specific gravity, presence of blood, glucose, bilirubin and protein by the help of the automatic urine analyzer (Urometer 600, Japan). Urine was collected by using metabolic cages in animal house.

2.5. Biochemical analysis

Blood samples were collected from all mice of the control and experimental group. The blood samples were centrifuged at $1500 \times g$ for 10 min after standing for 2 h. The serum was separated and immediately frozen to $-80\,^{\circ}C$ until analysis. Serum GOT, GPT, ALP and TBIL (biochemical parameters for LFTs) were measured through fully automated biochemical analyzer using standard kits (Human, Germany).

2.5.1. Serum creatinine and blood urea nitrogen (BUN) analysis

For creatinine, and blood urea nitrogen (BUN) plasma obtained in the above step was used. Creatinine, and blood urea nitrogen (BUN) concentrations were determined in plasma. Plasma creatinine was measured by used high-performance liquid chromatography (HPLC) methods in mice [14]. Samples were additionally spiked with 10 μl of a creatinine standard stock solution in 0.2 N HCl (Sigma, Munich, Germany) or 10 μl 0.2 N HCl to controls. Renal function is assessed by serum creatinine (SCR)