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EFFECT OF FIPRONIL ON THE PHYSIOLOGICAL STATUS OF FEMALE RATS

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ABSTRACT
Fipronil was an extensively used insecticide for crop protection in the world wide from the last decade due to its low soil persistence and insecticidal activity at low application. The present study was designed to investigate the adverse effects of sub-acute exposure to Fipronil on biochemical blood profile and histological parameters in male rats according to the permissible limits of acceptable daily intake (ADI). The results hematological parameters indicated that red blood cells (RBCs) and Hemoglobin (H.b) decreased in all treated groups compared with the control. While PLT and white blood cells (WBCs) was significantly increased in all treated groups compared with the control. The toxicity of effects of nano-fipronil pesticides leads to higher levels of liver function parameters (AST, ALT and ALP) and kidney function (Blood urea and Creatinine and Uric acid) results demonstrated highly significant differences ($P \leq 0.05$) in all groups treated compared with the control. Nano-fipronil caused histopathological alterations in liver and kidney tissues of male rats.

KEYWORDS: Chronic; Nano-Fipronil; Biochemical; Hematological; Rats.

INTRODUCTION
Increasing food production to satisfy growing demand is one of the most significant difficulties facing the agricultural sector, given the current trend towards precision farming and sustainable agricultural techniques [1]. Increasing crop yields to keep up with the world's population increase is a difficult undertaking that calls for thorough observation of interconnected environmental factors and the application of balanced, case-specific solutions with the least amount of direct control, the primary objective for attaining agricultural sustainability is to maximize crop yield while minimizing losses due to the fast population growth and growing demand for food under inadequately regulated production systems [2].
Farmers' decisions to apply pesticides have been less affected by the alarmingly high cost of these products. However, within the context of sustainable agricultural practices, pest control is still crucial for optimizing agricultural productivity by protecting preventive resources without adversely affecting the environment [3]. However, in order to accomplish basic objectives for the production of food and energy, chemical pesticide systems must be applied, However, the traditional use of synthetic pesticides contaminates groundwater, which worsens the environment and pollutes the surrounding ecosystem [4]. Due to exposure to various natural processes, such as biodegradation, photodegradation, and hydrodegradation, toxic chemicals released and/or broken down by pesticides are susceptible to environmental deterioration. Furthermore, rainfall-induced leaching and runoff exacerbate the environmental issues linked to pesticide use by facilitating the movement of these compounds and contaminating nearby areas because of these combined effects, only 1% or less of the applied pesticide really reaches the target areas, and the residues that remain interact negatively with non-target organisms, raising the dangers to the environment and human health [5].
Food quality and soil fertility have declined in tandem with the notable increase in food production rates. The growing detrimental effects of conventional farming methods on agricultural ecosystems are also indicated by studies that show a dramatic decrease in soil biodiversity and the emergence of resistance to diseases and agricultural pests [6].
An intelligent strategy must be considered in order to handle the possible effects of chemical pesticides on non-target creatures and the environment. It is necessary to implement a balanced agricultural strategy that has the least detrimental effects on the environment. It must also be very specialized to the target species and affordable for farmers [7]. Thus, a crucial prerequisite for sustainable agricultural practices is the creation of a novel targeted pesticide with minimal toxicity to non-target species and low pesticide residues because of its special qualities—such as their small size, high surface area to volume ratio, enhanced permeability, thermal stability, solubility, and biodegradability—engineered nanomaterials (ENMs) have drawn a lot of attention for usage in the agricultural industry [8].
These characteristics have improved the possibility of using nanomaterials in contemporary agricultural applications in an efficient manner, because of their unique characteristics, nanoparticles can be utilized to encapsulate agricultural chemicals in safer and more stable ways, improving crop yield and efficiency within the context of sustainable agriculture [9]. Making the best use of these particles may also help lessen possible harm

to human health and the environment [10]. The current study aims to determine the effect of fipronil on the physiological status of female rats.

MATERIALS AND METHODS

Fipronil was obtained from ISAGRO and the concentration used in the study was prepared in the Environmental Technologies Laboratory at the Faculty of Environmental Sciences, Qassim University [11].

Experimental animals

The current study included 30 adult female Albino rats obtained from the animal house at the Faculty of Science, University of Qadisiyah. The rats ranged in age from 16 to 14 weeks and weighed between 300 and 200 g. The rats were placed in plastic boxes (ten rats per box) under special living conditions. The rats were acclimatised for one week before the start of the experiment and then randomly divided into four equal experimental groups, with ten rats in each group. The first group was named the negative control group (NCG), the second group (1F) was treated with 20 mg/kg/ body weight of Fipronil for 30 days, the third group (2F) was treated with 10 mg/kg/ body weight of Fipronil for 30 days, and the fourth group (3F) was treated with 5 mg/kg/ body weight of Fipronil for 30 days. All experiment rats were kept according to the national and global enactments and research protocols placed down by the Ethics Committee about the animal well-being as well as the strategies was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" of Al-Qasim Green University, College of Environmental Science, Department of Environmental [12].

Sample collection and analyses

At the end of the trial period, blood samples were collected in tubes containing EDTA anticoagulant used in complete blood counts (CBC) by a Coulter device, an automatic digital counter manufactured by Coulter-Beckman (United States the CBC included the total number of red blood cells (RBC), hemoglobin (Hb), white blood cells (WBC), platelets (PLT), and the total white blood cell count (WBC) [13]. Liver function biomarkers: This test includes measuring the following enzymes (ALT, AST, ALP) details are provided in the section on commercially available assays (Linear, Spain), Biomarkers of Oxidative Stress This test includes measuring serum biomarkers (MDA, GSH, and CAT) according to the details provided in the following commercially available kit (Linear, Spain).

Statistical analysis

Using statistical analysis software (SPSS -2012) to detect the effect of groups of differences in study transactions. Use (LSD) of the least significant difference test for meaningful comparison between methods in this study. p-value less than 0.05 is considered significant. The data is presented as a standardized mean (E.S)

RESULTS AND DISCUSSION

Treatment of rats with various doses of FPN for 30 days did not show any signs of death. Numerous investigations conducted on mice, rats and humans have indicated that FPN may be capable of cause certain neurotoxicity [14]. Fibronil and nanofibronil were administered for 30 days, causing blood changes. Mice treated with FPN-IMI mixture had significantly lower levels of white blood cells and platelets compared to the control group, but the number of red blood cells and hemoglobin levels decreased (P 0.05).

An increase in the rate of hemoglobin oxidation or a decrease in the rate of hemoglobin synthesis may be the cause of the decrease in hemoglobin concentration. Iron is essential for this synthesis and is standard in relation to vegetable sources and ferritin stores [15]. It is likely that the reduced total food intake of exploratory rats and the lack of supplementary iron is the cause of iron deficiency in rats, which is necessary for hemoglobin synthesis. The lower H.b concentration may also be related to smaller red blood cells or a barrier to haem synthesis in the bone marrow [16].

Although it can also occur after certain parasitic infections or bone tumours, an elevated white blood cell count is usually an indication of an inflammatory response, often caused by infection. Any defects in the structure, quantity, or stability of platelets can lead to bleeding or clotting disorders, ultimately leading to death. A high platelet count can lead to venous and arterial thrombosis. Signs and symptoms vary depending on the location of the clot [17].

Table 1: Effect of Nano-Fipronil on hematological parameters of female rats for 30 days

Groups	Mean ±SD			
	RBC 10^9/L	WBC 10^9/L	PLT 10^9/L	H.b g/dl
(NCG): negative control group	8.622±0.41 A	8.541±0.22 C	972.9±11.32 A	16.653±0.56 A
(F1): 20 mg/kg/ b. w. of Fipronil	8.181±0.42 A	9.351±0.23 C	920.7±12.83 A	15.301±0.49 A
(F2): 10 mg/kg/ b. w. of Nano-Fipronil	7.839±0.43B	10.089±0.2 B	811.8±13.45 A	14.886±0.43 A
(F3): 20 mg/kg/ b. w. of Nano-Fipronil	7.767±0.45C	10.728±0.2 A	747±15.78 A	13.732±0.35 A
LSD	0.436	0.541	21.981	1.279

Each value is a mean ±SE; n=28; Statistical difference from the control: *significant at P≤ 0.05

According to the results of the current study, sub-chronic exposure to FPN and nano-FPN damages the liver and kidneys in treated animals more than in control mice, as evidenced by chemically elevated levels of serum enzymes AST, ALT, and ALP, as well as creatinine, uric acid, and total protein. Enzymes including AST, ALT, and ALP are primarily used to assess liver function. In the process of metabolism metabolism and amino acid synthesis, aspartate aminotransferases (AST and ALT) are essential. They perform detoxification, biochemistry, and energy component production activities for several vital purposes and serve as specific markers of liver disease [18].

The reason for the elevation of these enzymes may be a functional imbalance in the liver, which may lead to disruption of their synthesis and alteration of liver membrane permeability [19]. Several investigations conducted on various pesticides provide support for the findings [20]. Mice treated with FPN had significantly higher serum levels of uric acid and creatinine [21]. High concentrations of uric acid may result from the breakdown of purines and pyrimidines or from increased production or inability to excrete, and therefore elevated levels of creatinine levels are an indicator of impaired kidney function [22]. Further research has shown that mice exposed to multiple pesticides had increased concentrations of urea, creatinine, and uric acid in their blood [23].

Table 2: Effect of Nano-Fipronil on liver function parameters of male rats for 30 days

Groups	Mean ±SD		
	AST U/L	ALT U/L	ALP U/L
(NCG): negative control group	31.59±1.08D	27.108±0.79D	16.947±1.45C
(F1): 20 mg/kg/ b. w. of Fipronil	37.242±2.79C	32.094±1.71C	22.95±4.77B
(F2): 10 mg/kg/ b. w. of Nano-Fipronil	44.064±3.19B	35.883±2.59B	27.849±7.99A
(F3): 20 mg/kg/ b. w. of Nano-Fipronil	48.339±1.19A	39.249±3.12A	29.526±9.19A
LSD	3.801	2.313	6.885

Each value is a mean ±SE; n=28; Statistical difference from the control: *significant at P≤ 0.05

Table 3: Effect of Nano-Fipronil on kidney function parameters of male rats for 30 days

Groups	Mean ±SD		
	Urea mg/dl	Uric acid (mg/dl)	Creatinine mg/dl
(NCG): negative control group	29.861±1.89D	4.905±0.02D	0.585±0.01A
(F1): 20 mg/kg/ b. w. of Fipronil	40.743±2.29C	5.346±0.05C	1.179±0.03A
(F2): 10 mg/kg/ b. w. of Nano-Fipronil	53.154±2.62B	6.075±0.07B	1.278±0.04A
(F3): 20 mg/kg/ b. w. of Nano-Fipronil	58.644±2.87A	6.192±0.08A	1.566±0.07A
LSD	2.436	1.098	1.567

Each value is a mean ±SE; n=28; Statistical difference from the control: *significant at P≤ 0.05

The reduction in oxidative stress (MDA, GSH, and CAT) in this study is considered the first line of defence against lipid peroxidation of large cell molecules. This is because MDA accelerates the decomposition of superoxide anion into a less reactive molecule reactive (H2O2), which GSH and CAT use to rapidly generate water and oxygen [40,41]. The excess production of O2•, which is rapidly converted to H2O2 by SOD and to water by CAT, may be the reason for the reduced oxidative stress activity in the kidneys and liver of mice exposed to FPN. According to previous research, oxidative stress in the liver and kidneys of animals is reduced by insecticides such as fipronil [24].

Greater amounts of lipid peroxides and oxidative stress result from the production of reactive oxygen species (ROS) as a result of FPN [48]. According to reports, FPN causes oxidative stress in C. carpio, which manifested through changes in antioxidant enzymes and high levels of lipid peroxides [25]. MDA and GSH and CAT, three enzymes associated with oxidative stress, are essential for detoxifying organisms from substances such as pesticides and transporting metabolites within cells these enzymes have been linked to the potential production of reactive intermediates, particularly when intracellular GSH levels are low, which may have toxic effects [26]. These enzymes have been linked to the potential production of reactive intermediates, especially when GSH levels in cells are low, which may have toxic effects [27]. In the present investigation, animals exposed to FPN had significantly lower GST activity and GSH levels in the liver and kidneys. Due to cell damage and the death of healthy cells that can respond to oxidative stress, the concentrations of antioxidant enzymes in the liver and kidney organs decreased [28]. Furthermore, this could be the result of damage from reactive oxygen species and the insufficient detoxification capacity of FPN [29].

Table 4: Effect of Nano-Fipronil on Oxidative stress parameters of male rats for 30 days

Groups	Mean ±SD		
	MDA nmol/ml	CAT µmol/ml	GSH µmol/ml
(NCG): negative control group	1.206±0.09D	0.675±0.0D	59.171±3.11C
(F1): 20 mg/kg/ b. w. of Fipronil	1.485±0.11C	0.756±0.04C	46.225±2.89C
(F2): 10 mg/kg/ b. w. of Nano-Fipronil	1.719±0.12B	0.819±0.03B	38.306±2.41C
(F3): 20 mg/kg/ b. w. of Nano-Fipronil	1.881±0.14A	0.882±0.02A	31.378±2.09C

LSD	0.107	0.014	1.048

Each value is a mean ±SE; n=28; Statistical difference from the control: *significant at P≤ 0.05

CONCLUSIONS

The pesticide caused changes in the physiological behavior of post-dose mice, such as hematological factors, liver and kidney enzyme function, oxidative stress, hormonal alterations, and lipid characteristics, when compared to control mice.

Recommendations

Using pesticides in nano-forms results in better efficiency and greater effectiveness at a lower concentration compared to conventional pesticide concentrations.

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