

Research Article



Study on the Toxic Effects of Nano-Fipronil on the Physiological Aspects of Male Rabbits

QASSIM AMMAR AHMOOD AL-JANABI^{1*}, AHMED HADI ABDUL SAHEB², AQEEL KHALEEL IBRAHEEM³

¹Environmental Pollution Department, College of Environmental Sciences, Al-Qasim Green University, Babylon 51013, Iraq; ²Environment Department, College of Environmental Sciences, Al-Qasim Green University, Babylon 51013, Iraq; ³Ministry of Education, Education Babylon, Iraq.

Abstract | Fipronil is a broad-spectrum phenyl-pyrazole insecticide commonly used in agriculture. However, its residues in the environment pose a toxic threat to animals, bees, aquatic life, and humans, and it has been classified as a carcinogen. The present study aimed to investigate the adverse effects of sub-acute exposure to doses of 50 mg of fipronil (FPN) and 50 mg and 25 mg of nano-fipronil (N-FPN) on hematological parameters in male rabbits over a period of 45 days. The study involved twenty-four male rabbits, aged 11 months and weighing between 800–1200 g, which were randomly assigned to four equal groups. The results showed a significant reduction ($P < 0.05$) in red blood cell count (RBC: $8.667 \pm 0.59 \times 10^9/L$) and an increase ($P < 0.05$) in white blood cell count (WBC: $13.54 \pm 0.56 \times 10^9/L$) in the treated groups compared to the control. Additionally, liver and kidney enzymes were elevated ($P < 0.05$) due to pesticide exposure, with AST at 59.65 ± 1.19 U/L, ALT at 48.76 ± 3.12 U/L, and ALP at 43.89 ± 8.45 U/L. Uric acid levels (7.19 ± 0.10 mg/dL) and urea levels (60.14 ± 4.99 mg/dL) were also higher ($P < 0.05$) compared to the control. Oxidative stress was indicated by elevated ($P < 0.05$) MDA levels (3.89 ± 0.17 nmol/mL) and catalase (CAT: 2.52 ± 0.49 μ mol/mL) activity compared to the control group. These findings highlight the adverse effects of fipronil on rabbits and suggest the need for strategies to mitigate exposure to pesticide agents in the environment.

Keywords | Hematological, Physiological, Fipronil, Toxicological, Rabbi

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***Correspondence** | Qassim Ammar Ahmood AL-Janabi, Environmental Pollution Department, College of Environmental Sciences, Al-Qasim Green University, Babylon 51013, Iraq; Email: aalqassim@environ.uoqasim.edu.iq

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INTRODUCTION

Pesticides are essential tools for farmers to control agricultural pests and protect crops, as well as to eliminate insects that transmit diseases to humans or animals (Atiya and Abdulhay, 2022; Al Defferi *et al.*, 2019; AL-Janabi *et al.*, 2021). Despite farmers' awareness of the dangers associated with pesticide use and the negative effects they can have on human and animal health, as well as the environment (AL-Hedny *et al.*, 2018; AL-Janabi *et al.*, 2019), their widespread use continues to raise concerns about po-

tential adverse impacts on human health, animal welfare, and the environment.

Fipronil is a broad-spectrum insecticide belonging to the phenylpyrazole chemical family, commonly used in agriculture. However, its residues in the environment pose a toxic threat to animals, bees, aquatic life, and humans, and it has been classified as a carcinogen (Zhou *et al.*, 2021). While the future of nanotechnology in agricultural development looks promising, nano-pesticides, such as nano-fipronil, have emerged as potential alternatives. These nano-pes-

ticides are among the key methods used by farmers to control agricultural pests that threaten crops and boost production (AL-Janabi *et al.*, 2021). Nano-pesticides are engineered nano-materials designed for plant protection, aiming to minimize application losses, enhance leaf coverage, improve stability, and reduce the quantities of active ingredients in formulations (Khleif *et al.*, 2020; Casillas *et al.*, 2022). These formulations can include self-organized systems, such as liposomes, dendrimers, metallic and bimetallic nanoparticles, and active encapsulating ingredients like nanoemulsions, polymeric nanoparticles, lipid nanoparticles, and nanotubes. This study aims to investigate the toxic effects of nano-fipronil on the physiological aspects of male rabbits by examining biomarkers of liver function, kidney function, and oxidative stress. By understanding the potential risks associated with nano-fipronil, we can guide future research and develop strategies for its safe and sustainable use.

MATERIAL AND METHODS

MATERIAL

Fipronil (FPN), 97% pure, was obtained from ISAGRO Company, USA. The concentration used in the study was prepared in the Environmental Technologies Laboratory at the College of Environmental Sciences, Al Qasim Green University. Nano-Fipronil (N-FPN) and liposome preparation were carried out at the College of Environmental Sciences, Al Qasim Green University, following the method described by Nakhaei *et al.* (2021).

EXPERIMENT DESIGN

The experiment involved twenty-four male rabbits, aged 11 months and weighing between 800-1200 g, which were randomly divided into four equal groups for a 45-day testing period. Group (A) served as the control group, Group (B) was treated with 50 mg of Fipronil (FPN), Group (C) was treated with 50 mg of Nano-Fipronil (N-FPN), and Group (D) was treated with 25 mg of Nano-Fipronil (N-FPN) (AL-Janabi *et al.*, 2023). The study followed the guidelines of the Ethics Committee of Al-Qasim Green University, Ministry of Higher Education and Scientific Research, Iraq (no. 923A.E. in 14, 4, 2023).

LABORATORY ANALYSES

At the end of the study, fresh blood samples were collected from the rabbits for biochemical tests, including a complete blood count (CBC). The isolated serum was then analyzed for liver function (activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)), kidney function (urea, creatinine, and uric acid), and oxidative stress markers (malondialdehyde (MDA), glutathione (GSH), and catalase (CAT)) following the methods outlined by AL-Janabi *et al.* (2024).

STATISTICAL ANALYSIS

The SPSS program (2012) was used to analyze the effects of the different groups, and the Least Significant Difference (LSD) test was applied to compare the means. A difference of less than 5% between the means was considered statistically significant.

RESULTS AND DISCUSSION

Treating rabbits with different doses of N-FPN for 45 days did not result in any signs of death. Several studies in rabbits have suggested that FPN may cause specific neurotoxicity. Table 1 shows the effects of N-FPN on blood parameters in male rabbits over the 45-day period. An increase (P<0.05) in white blood cell (WBC) levels was observed compared to the control group, along with a decrease (P<0.05) in the number of red blood cells and hemoglobin levels (Wafa *et al.*, 2013). Since iron is essential for hemoglobin, a reduction in hemoglobin concentration could be due to either a decrease in hemoglobin synthesis or an increase in hemoglobin oxidation. The iron deficiency in rabbits could be attributed to reduced food intake or a lack of iron-rich supplements, which are necessary for hemoglobin synthesis. Additionally, a decrease in hemoglobin concentration can be associated with impaired red blood cell production or issues in heme synthesis within the bone marrow (Ali *et al.*, 2017).

Table 1: Effects of Fipronil and Nano-Fipronil on blood parameters in male rabbits.

Groups	Mean ± S.D			
	RBC 10 ⁹ /L	WBC 10 ⁹ /L	PLT(10 ⁹ /L)	H.b(g/dl)
A	9.982±0.54a	9.766±0.31c	891.9±11.32a	18.783±0.41a
B	9.781±0.56a	11.32±0.33c	824.7±12.83a	17.671±0.37a
C	8.989±0.57b	12.56±0.35b	785.8±13.45a	15.346±0.33a
D	8.667±0.59c	13.54±0.56a	714.7±15.78a	14.223±0.29a
LSD	0.46	0.62	31.19	1.51

RBC: Red blood cells; WBC: White blood cells; PLT: platelets; Hb: Hemoglobin; *(a, b, c, d) represent significant differences (P≤0.05) between the treated groups as compared to the control.

High WBC levels are indicative of an inflammatory response, which can also be triggered by certain parasitic infections. Defects in platelet structure, quantity, or stability can lead to bleeding or coagulopathy, which may ultimately result in death. An elevated platelet count can contribute to venous and arterial thrombosis (Mustafa *et al.*, 2019). These findings align with those of Jaber *et al.* (2020).

Table 2 shows the effect of FPN and N-FPN on liver function in male rabbits. It was observed that significant increase (P<0.05) in the level of ALT, AST and ALP was

recorded in the treated groups as compared to the control group. According to the results of the current study, compared to control rabbits, those treated with the pesticides experienced more significant liver and kidney damage, as indicated by elevated liver enzyme levels (Hajer and Al-Easawi, 2019). Liver function enzymes are commonly used to assess liver health, as they are crucial for detoxification, biochemical activities, and the production of energy components necessary for a variety of vital functions. They also serve as specific indicators of liver disease (Al-Hamawandy and Al-Bakri, 2020; Jaber *et al.*, 2020). The observed rise in these enzymes could be indicative of liver dysfunction, which may alter the permeability of the liver membrane and interfere with the synthesis of these enzymes (AL-Janabi *et al.*, 2024).

Table 2: Effects of Fipronil and Nano-Fipronil on liver function in male rabbits.

Groups	Mean ± S.D		
	AST(U/L)	ALT(U/L)	ALP(U/L)
A	42.22±1.08d	33.62±0.79d	25.17±2.67c
B	49.12±2.79c	38.14±1.71c	32.95±5.12b
C	53.76±3.19b	42.67±2.59b	37.59±6.23a
D	59.65±1.19a	48.76±3.12a	43.89±8.45a
LSD	2.62	3.82	7.18

AST: Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **ALP:** Alkaline phosphatase; *(a, b, c, d) represent significant differences(P≤0.05) in the treated groups as compared to the control.

Table 3: Effects of Fipronil and Nano-Fipronil on kidney function in male rabbits.

Groups	Mean ± S.D		
	Urea(mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
A	33.22±2.77d	5.25±0.03d	1.95±0.12a
B	42.81±3.09c	5.96±0.06c	2.09±0.23a
C	51.22±4.12b	6.65±0.08b	2.38±0.29a
D	60.14±4.99a	7.19±0.10a	3.46±0.37a
LSD	3.16	2.51	2.39

*(a, b, c, d) represent significant differences(P≤0.05) in the treated groups as compared to the control.

The impact of Fipronil (FPN) and Nano-Fipronil (N-FPN) on male rabbit renal enzymes is shown in Table 3. Elevated (P<0.05) uric acid concentrations in rabbits treated with FPN could result from the breakdown of purines and pyrimidines, as well as increased production or the inability to excrete uric acid. Additionally, elevated (P<0.05) creatinine levels indicate compromised renal function (AL-Janabi *et al.*, 2023). Previous studies have reported higher levels of

uric acid, creatinine, and urea in rabbits exposed to pesticides (AL-Janabi *et al.*, 2024).

Table 4: Effects of Fipronil and Nano-Fipronil on oxidative stress in male rabbits.

Groups	Mean ± S.D		
	MDA(nmol/ml)	CAT (µmol/ml)	GSH (µmol/ml)
A	2.21±0.12d	0.94±0.11d	63.92±4.34a
B	2.95±0.14c	1.26±0.28c	51.33±3.91a
C	3.11±0.15b	1.97±0.36b	47.21±3.01a
D	3.89±0.17a	2.52±0.49a	40.95±2.79a
LSD	0.76	0.59	2.39

MDA: Malondialdehyde; **GSH:** Glutathione; **CAT:** Catalase; *(a,b,c,d) represent significant differences(P≤0.05) in the treated groups as compared to the control.

Table 4 displays the effects of FPN and N-FPN on oxidative stress in male rabbits. MDA level and CAT activity was significantly (P<0.05) enhanced by the treatments of FPN and N-FPN as compared the control group. According to Wafa *et al.* (2013), these compounds increase oxidative stress (lipid peroxidation, LPO), which is linked to several liver and kidney diseases and damages the integrity of cellular membranes. Pesticides like fipronil have been shown to increase oxidative stress in animals, including effects on liver and kidney tissues (El-bendary *et al.*, 2021). FPN leads to the production of reactive oxygen species (ROS), resulting in increased oxidative stress and lipid peroxidation (Sharma *et al.*, 2018). Other studies suggest that FPN may increase oxidative stress in *Cyprinus carpio* (*C. carpio*), as evidenced by high lipid peroxidation levels and alterations in antioxidant enzymes (Sunaryani and Rosmalina, 2021; Sweeney *et al.*, 2021). Three oxidative stress-related enzymes—MDA, GSH, and CAT—are essential for the transfer of intracellular receptors and the detoxification of bioactive substances, such as pesticides (Crayton *et al.*, 2020). Particularly when GSH levels fall, these enzymes may produce reactive intermediates that could be harmful (Saheed *et al.*, 2019).

CONCLUSIONS AND RECOMMENDATIONS

All treatment groups showed a reduction in RBC levels compared to the control group, while WBC levels were increased in all treated groups. The results indicate that the adverse effects of Nano-Fipronil insecticide were more pronounced than those of Fipronil, leading to increased oxidative stress, as well as disruptions in liver and kidney functions.

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NOVELTY STATEMENTS

The authors encourage the use of nanotechnologies in various daily applications and bio-fertilizers are one of these applications

AUTHOR'S CONTRIBUTIONS

All contributed to the writing of the research, the method of collecting samples, and the statistical analysis of the results discussed.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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