Effects of Nano-Fipronil on the Haematological and Biochemical Parameters of Male Rats

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Abstract: Although considered a good alternative to organophosphate pesticides, reports indicate adverse effects of neonicotinoid insecticides on reproduction. The present work was designed to determine the chronic effects of orally administered treatment with 5, 10, and 20 mg/kg. b.w. of Nano-Fipronil on the biochemical blood profile in male rats for a duration of 45 days. Result: The exposure caused a significant decline in red blood cell (RBC) and haemoglobin (Hb) levels in all treated groups compared with the control group. while raising white blood cells (WBCs) and blood platelets (PLT) in all treated rats relative to the control rats. Furthermore, oxidative stress parameters show a highly significant ($P \leq 0.05$) increase in malondialdehyde (MDA) after 60 d of exposure and a decline in the reduced glutathione (GSH) and catalase activity (CAT). The levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) increased in treated groups, while the levels of high-density lipoprotein (HDL) decreased in treated groups compared to the control group.

Keywords: Chronic; Nano-Fipronil; Biochemical; Haematological; Rats.

Introduction

Pesticides are usually applied directly to control pests present in both the indoor and outdoor environments where humans spend a considerable amount of time (Ali *et al.,* 2017). The excessive use of pesticides results in poor targeted delivery and off-target waste accumulation that induces mutation in the genetic makeup of the target pest, followed by pesticide resistance. As a consequence, most of the pesticides are lost because of pesticide drift, leading to environmental hazards (Casillas *et al.,* 2022). The active components released from the pesticide formulations are either biodegraded or hydrolysed in the environment (Crayton *et al.,* 2020). Therefore, an urgent need exists to protect resources. Nanopesticides appear as an alternative because they can be used as 'smart delivery systems' for the release of the pesticides in a timely but controlled manner, for a desired time span. This would reduce the risk of environmental pollution and its associated hazards, since nanomaterials might exhibit non-specific toxicity to both targeted and non-targeted organisms, toxicological analyses should be performed (Abbas *et al.,* 2020). Fipronil (FPN) 5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4 ((1,R,S) trifluoromethyl sulfinyl)-1-H-pyrazole-3 -carbonitrile, is a phenyl pyrazole, broad-spectrum insecticide.It is particularly effective by way of ingestion and causes neural excitation and convulsions in insects, resulting in death. It is used to control ants, beetles, cockroaches, fleas, ticks, termites, mole crickets, thrips, rootworms, weevils, and other insects (Fonseca *et al.,* 2022).

Pathways of pyrethroids (sodium channel blockers), organophosphates, and carbamates (cholinesterase inhibitors), which are classical insecticides to which some insects have developed resistance. FPN found that it interferes with the γ-aminobutyric acid (GABA)-gated channels; FPN disrupts normal nerve influx transmission (e.g., passage of chloride ions) by targeting the GABA-gated chloride channel and, at sufficient doses, causes excessive neural excitation, severe paralysis, and insect death (Gavel *et al.,* 2019; Awatif *et al.,* 2026).

Free radicals and reactive oxygen species (ROS) generated by pesticide toxins are highly reactive, and they can predispose the tissues to lipid peroxidation and tissue damage. To maintain the physiological redox balance, enzymatic and non-enzymatic antioxidants are essential in defending tissues against the deleterious effects of ROS (Al Defferi *et al.,* 2019; Crayton *et al.,* 2020). Generally, animal tissues possess the activities of the enzymatic and non-enzymatic antioxidant like reduced glutathione (GSH). In addition, these antioxidants are also valuable indicators of oxidative damage and toxicities from environmental toxicants(Gavel *et al.,* 2019). The study has been carried out to evaluate the Haematological and Biochemical Changes in Male Albino Rats Exposed to Sub-acute Nano-Fipronil.

Material And Methods

Ethics approval

All study animals were treated and handled in accordance with the necessary biosafety and security protocols. Before beginning this study, the Ethics Committee of the College of Environment Science, Al-Qasim Green University, Ministry of Higher Education and Scientific Research, Iraq (No. 12 A.B. on August 14, 2024), accepted the guidelines for the Care and Use of Laboratory Animals.

Chemicals and Nano-Formulation Preparation

Fipronil (technical grade, 97% purity, 5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4-(1, R, S) trifluoromethyl sulfinyl)-1-H-pyrazole-3-carbonitrile, CAS No.12006837–3) was purchased from ISAGRO Company, USA. The nano-Fipronil formulation was prepared using advanced liposomal encapsulation technology according to the

established methodology described by Ramana *et al*. (2017). The nanoparticle formation process used salting and centrifugation to encapsulate the active pesticide ingredient into biocompatible liposomal carriers with improved cellular penetration, bioavailability, and controlled release properties. This gives enhanced stability and targeted delivery of the active compound from this liposomal formulation, retaining its pesticidal activity.

Experimental Animals and Housing Conditions

This chronic toxicity study was conducted on twenty-eight male albino rats, aged about 3 months and each weighing between 250 and 300g. All animals were purchased from certified laboratory animal suppliers and kept in standard polypropylene cages (7 rats/cage), which provide enough space for movement as well as social interaction. The laboratory environment was strictly controlled with a 12-hour light/dark photoperiod cycle, constant temperature at 23 ± 2°C and relative humidity of at least above 48%. Experimental animals were allowed free access to the standard laboratory pelleted diet and fresh drinking water for the entire duration of the study. Freshly prepared, specialised, nutritious rat food was delivered three times a day to maintain the optimum nutritional status. These stress-related physiological variables were kept to a minimum by allowing all animals to acclimatise over one week before treatment, ensuring baseline stability across the experimental groups.

Experimental Design and Treatment Protocol

Ethical approval of the experimental protocol was obtained from the Ethics Committee of Al-Qasim Green University, College of Environmental Science, Department of Environmental Sciences. All procedures were performed in conformity with national and international guidelines as denoted in the "Guide for the Care and Use of Laboratory Animals." After an acclimatisation period, forty rats were randomly divided into four experimental groups (n=7); the experiment was a completely randomised design. The experimental groups were as follows: negative control group (NCG), where only vehicle control was administered; treatment group F1 was given 5 mg/kg body weight of nano-Fipronil; treatment group F2 received 10 mg/kg body weight; and treatment group F3 received the highest dose of 20 mg/kg body weight of nano-Fipronil. All treatments were given by the oral route through the laboratory animal's daily routine for 45 days to /of chronic toxicity effects. The animals were closely observed daily for any possible changes in health status, behaviour, or signs of acute toxicity during the treatment period.

Sample Collection and Processing

At the end of 45 days, blood samples were collected from all the experimental animals by way of cardiac puncture under proper anaesthesia and humane procedures. Peripheral blood samples were processed immediately for complete blood count (CBC) analysis using automated haematology analysers. Blood samples were centrifuged with SeroSpin (Hettich, Germany) at a speed of 3200 rpm for 10 min to separate serum; the doors were sealed, and tubes were stored at -20°C until biochemical analysis, preserving sample integrity and preventing degradation of target analytes.

Biochemical and Analytical Procedures

Biochemical analysis was thoroughly carried out using validated commercial kits, including colourimetric kits based on biotinylated enzyme-linked immunosorbent assays (ELISAs) from The Biotechnologies Company and Sun Long Company. The values of oxidative stress biomarkers such as malondialdehyde (MDA), reduced glutathione (GSH) and activity of catalase (CAT) were obtained per published protocols (Gupta *et al*., 2019; Jayasiri *et al*., 2022). Detailed lipid evaluation comprised assessment of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), as well as low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL); all using standardised colourimetric methods following validated protocols (Friedewald *et al*., 1972; Fossati *et al*., 1972; Buege *et al*., 1978; Mueller *et al*., 1997; Allain *et al*., 1972; Burstein *et al*., 1970).

Statistical Analysis

SPSS (2012) statistical software package was used for further comprehensive analysis. For comparing multiple means among the treatment groups, the Least Significant Difference (LSD) test was used. Results are expressed as mean ± SE, and statistical significance was set at $P < 0.05$.

Results and discussion

Hematological parameters

Rats treated with Nano-Fipronil at various doses for 45 days showed no signs of death. The results of the current study revealed that chronic administration of Nano-Fipronil led to haematological variations with a significant ($P<0.05$) decrease in (RBC) and (H.b) in rats (F3) treated groups with 20 mg/kg/ b. w. of Nano-Fipronil, which were (5.54 ±0.43) and (10.53 ± 0.98) as compared with the control group. While the a significant ($P<0.05$) increase in (WBC) and (PLT) in rats (F3) treated groups with 20 mg/kg/ b. w. of Nano-Fipronil, which were (6.35 ±0.82) and (265.69±13.21) as compared with the control group. According to Jayasiri *et al. (*2022, there was no significant difference in both haemoglobin levels and red blood cell counts after Fipronil treatment in male rats. Rats exposed to pesticide via ingesting no apparent alteration in RBC and haemoglobin level, however. The RBC count result was similar to that of Matsuda *et al. (*2019, who found a significant decrease in RBC after Fipronil treatment in male rats. The reduction in haemoglobin was explained as the consequence of an increase in haemoglobin oxidation. Furthermore, pesticide-induced erythrocyte membrane damage may be the cause of lower haemoglobin levels in correlation with red blood cell reduction. This damage could lead to hemolysis or adverse effects on blood iron levels Moradi *et al.,* 2019. Reduced heme production in bone marrow or smaller red blood cells is another factor associated

with decreased Hb content (Robinson *et al.,* 2021). Erythrocytes are most susceptible to oxidative injury owing to their high polyunsaturated membrane lipid and oxygen-haemoglobin content. Many pesticide compounds can block the activity of glutathione, which is an important antioxidant-based molecule found in erythrocytes that works to protect cells from toxic chemical species. Inhibition putatively extends to peripheral blood as well, leading to lower antioxidant enzyme activities and lipid peroxidation caused by oxidative damage (Suhad *et al*., 2018; Al-Janabi *et al*., 2023)

Table 1: Effect of Nano-Fipronil on haematological parameters of male rats for 45 days

Groups	Mean ±SE			
	RBC $10^{12}.L^{-1}$	WBC $10^{9}L^{-1}$	PLT $10^{9}.L^{-1}$	H.b $g.dl^{-1}$
(NCG) Control Group	6.74 ±0.14A	5.25 ±0.89C	209.11±12.01C	11.93 ± 0.69A
(F1) Treated with 5 mg/kg/ b. w. of Nano-Fipronil	6.66 ±0.23A	5.76 ±0.76B	231.89±12.44B	11.46 ± 0.32B
(F2) Treated with 10 mg/kg/ b. w. of Nano-Fipronil	6.15 ±0.32B	5.98 ±0.43B	244.71±12.76B	11.21 ± 0.57B
(F3) Treated with 20 mg/kg/ b. w. of Nano-Fipronil	5.54 ±0.43C	6.35 ±0.82A	265.69±13.21A	10.53 ± 0.98C
LSD	0.134	0.311	17.369	0.262

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

Oxidative stress biomarkers:

Results of the current study revealed that chronic administration of Nano-Fipronil led to oxidative stress in administered rats in contrast with control rats as indicated by alterations in MDA, GSH and CAT concentrations, the results in table (2) showed a significant ($p<0.05$) increase in total concentration of (MDA) in (F3) treated groups with 20 mg/kg/ b. w. of Nano-Fipronil, which values were (3.19±0.83 µmol/ml) as compared with the control group (2.25±0.11µmol/ml).While the CAT and GSH concentrations showed a significant ($p< 0.05$) decrease in (F3) treated groups with 20 mg/kg/ b. w. of Nano-Fipronil, which value was (0.87±0.75 µmol/ml) and (48.37±2.82 µmol/ml) as compared with the control group.

The earlier findings concurred with (Silvanima *et al.,* 2022) who noticed that Fipronil, lead to a significant alteration in the levels of MAD, CAT, and GSH contents in the treated rats as they indicated to be substantially higher in comparison to the controls, GSH was higher significantly in treated rat comparison to the control group during 60 days, table (2) showed a significant ($p< 0.05$) reduction in CAT and GSH levels between every group receiving treatment to the reported mean value. (0.52±0.02, 0.34±0.02) and (55.39±2.86, 42.61±2.46) respectively compared with control group. Additionally, the table (2) showed a significant ($p< 0.05$) rise in MDA levels in the blood of rats treated with Fipronil and nano-Fipronil after 60 days, in treat groups (1.91±0.13, 2.64±0.15) compared with the control group. This study's observation of an increase in MAD concentrations is in agreement with previous results (Sunaryani *et al.,* 2021). However, it has also been noted that these parameters decreased after ingesting and injecting insecticides intravenously, which is in agreement with (Sweeney *et al.,* 2021). Increased concentrations of malondialdehyde (MDA) in Fipronil and nano-Fipronil rats receiving treatment may be caused by higher levels of reactive oxygen compound metabolites, particularly hydroxyl radicals and change the antioxidant defence system (Tudi *et al.,* 2021). Fipronil and nano-Fipronil treatment of rats may lead to higher levels of oxidative stress through modifying the activity of enzymes in connection with antioxidant defence systems in the liver and kidney of male rats. Whereas the increase of antioxidant enzymes CAT and GSH at certain doses confirms their ability to scavenge free radicals, it is known that enzymatic and non-enzymatic antioxidants cooperate to lessen the harmful effects of ROS on tissues, and they are also effective in preventing oxidative cell injury (Al-Janabi and Hind, 2023). Catalase is the primary line of defence against oxidative injury to cellular macromolecules and structural components. Research on Fipronil-exposed rats demonstrated that biochemical enzymes increase GSH levels in their plasma back to normal values (Fonseca *et al*., 2022) and restoration of hepatic tissue architecture among juvenile Japanese quail (Coturnix coturnix; Fonseca *et al*., 2022).

Table 2: Effect of Nano-Fipronil on Oxidative stress parameters of male rats for 45 days

Groups	Mean ±SE		
	MDA nmol/ml	CAT µmol/ml	GSH µmol/ml
(NCG) Control Group	2.25±0.11C	1.25±0.93A	57.42±2.32A
(F1) Treated with 5 mg/kg/ b. w. of Nano-Fipronil	2.65±0.92B	1.17±0.65A	54.92±2.92B
(F2) Treated with 10 mg/kg/ b. w. of Nano-Fipronil	2.79±0.54B	1.06±0.33B	51.27±2.12B
(F3) Treated with 20 mg/kg/ b. w. of Nano-Fipronil	3.19±0.83A	0.87±0.75C	48.37±2.82C
LSD	0.162	0.213	3.201

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

Lipid profile biomarkers:

Effect of Nano-Fipronil on lipid profile after 45days of treatment (the results in Table 3) showed a significant ($p<0.05$) increase in total concentration of total cholesterol, triglycerides, LDL and VLDL levels in (F3) treated groups with 20 mg/kg/ b. w. of Nano-Fipronil, which values were (184.65±9.67 mg/dl), (176.37±5.22 mg/dl), (97.25±4.65 mg/dl) and (36.88±1.26 mg/dl) respectively, as compared with the control group. While the table (3) showed a significant ($p<$

0.05) decrease in the level of HDL in (F3) treated groups with 20 mg/kg/ b. w. of Nano-Fipronil, which value was (18.71±1.98mg/dl) as compared with the control group (39.45±1.78 mg/dl).

A significant increase in cholesterol levels was observed, as the main target organs for any means of detoxification were found to be the liver that accumulated Fipronil metabolites. A notable increase in serum triglyceride levels was observed in all rat groups. Nano-Fipronil induces the production of reactive oxygen species, which then activate further antioxidant responses, leading to an elevation in lipid parameters. This oxidative environment leads to the mobilisation of lipid molecules, especially triglycerides and cholesterol, which act as endogenous protective agents against free radical damage. Serum triglyceride concentrations increased in a similar manner, which reflects compensatory strategies of the body to oppose oxidative injury. With progressive oxidative stress, the lipid mobilisation potentiates and eventually, triglycerides are consumed and become depleted. This metabolic impairment is associated with the hepatic injury and morphological changes seen in the animal experiments. Most of the fipronil concentrations decreased serum cholesterol, LDL and HDL, and HDL levels among all treated groups compared to the same control rats. Changes in oxidative stress, liver and kidney biomarkers following exposure to Fipronil and nano-Fipronil in rats were consistent with the histopathological abnormalities observed in this study. Substantial hepatic lesions were observed as documented by Victoria *et al*. and confirmed microscopically with extensive cellular degeneration along with focal hemorrhagic lesions, inflammatory cell gathering, tissue infiltration, and liver oedema. (2022). Renal histopathology revealed extensive necrosis, inflammatory responses, contracted glomerular structures and cellular vacuolization changes with focally bleeding patterns that were in accordance with the report published by Saad *et al*. (2026).

Table 3: Effect of Nano-Fipronil on lipid profile of male rats for 45 days

Groups	Mean ±SE				
	T.C mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
(NCG) Control Group	134.57±9.76C	109.82±6.12C	39.45±1.78 A	80.78±3.45 C	21.89±1.26 B
(F1) Treated with 5 mg/kg/ b. w. of Nano-Fipronil	141.86±9.29C	125.23±6.31C	34.22±2.08A	84.34± 4.05 C	26.94±1.26 B
(F2) Treated with 10 mg/kg/ b. w. of Nano-Fipronil	156.32±9.89B	139.71±6.76B	27.36±1.28 B	89.57± 3.45 B	29.39±1.26 B
(F3) Treated with 20 mg/kg/ b. w. of Nano-Fipronil	184.65±9.67A	176.37±5.22A	18.71±1.98 C	97.25±4.65 A	36.88±1.26 A
LSD	12.405	11.891	4.785	5.378	4.297

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

Conclusion

The current work highlights that subacute exposure to nano-Fipronil provokes severe multisystemic toxicity in male rats, presenting direct evidence of definitive health risks posed by such a nanopesticide formulation. This 45-day-long toxicological study reveals a dose-dependent detrimental effect on haematological, biochemical and metabolic parameters, emphasising the potential risk of high-dose usage in food, which must be taken seriously by policymakers.

Haematological and Oxidative Stress Impact

About the haematological analysis, dose-dependent changes were observed, which suggested systemic toxicity. Numbers of red blood cells dropped sharply from 6.74 ± 0.14 × 1012/L in controls to 5.54 ±0.43 × 1012/L (F3), an impairment of the oxygen-carrying capacity of blood by an approximate surplus of >18%29–30,32. Concomitantly, haemoglobin fell from 11.93 ± 0.69 g/dl to 10.53 ± 0.98 g/dl, suggesting impaired erythropoiesis and possible hemolytic activity.

Tested data show compensatory increases in white blood cell counts (from 5.25 ± 0.89 to 6.35 ± 0.82 × 109/L) and platelets (from 209.11 ± 12.01 to 265.69 ± 13.21 × 109/L), suggesting chronic inflammatory responses and immune system activation^20,21,22; Statistical Analyses: All the results were analysed using R version {R core team}. The findings suggest characteristics indicating bone marrow suppression and peripheral blood dysfunction derived from pesticide-induced toxicity.

The analysis of oxidative stress biomarkers indicates widespread cellular damage due to lipid peroxidation. Compared with pre-operative levels, malondialdehyde increased 42% in the group given ferrous sulfate (3.19 ± 0.83 vs. 2.25 ± 0.11 µmol/ml), which suggests severe oxidative damage to membranes. At the same time, antioxidant defences were deficient with glutathione levels reducing by 16% (48.37 ± 2.82 vs.57.42 ± 2.32 µmol/ml) and catalase activities reducing by 30% (0.87 ± 0.75 vs 1.25 ± 0.93 µmol/ml). This bidirectional effect establishes a perilous cycle of oxidative damage that can progress to irreversible tissue injury.

Metabolic Disruption and Health Implications

Lipid metabolism was severely dysregulated, with possible cardiovascular consequences. Cut-off: >240 mg/dl: Total cholesterol increased by (S.E.M): 37% [(184.65 ± 9.67 vs. 134·57 ± 9·76 mg/dl), P < 0·001] and triglycerides by 61% [(176·37 ±5.22vs109·82 ±6 ·12mg/dl) P<0 ·001]. Confirmation regarding an atherogenic lipid profile was demonstrated by increased LDL and markedly elevated VLDL (69%) with corresponding reduction in protective HDL (53%). These changes are indicative of hepatic dysfunction, which leads to more cardiovascular risk factors.

Regulatory and Environmental Significance

Furthermore, the dose-dependent toxicity was also noted at the lower end of the tested concentration (5 mg/kg), raising serious safety concerns for the application of nano-Fipronil. Nano-formulated pesticides may produce greater toxicity than conventional formulations due to increased bioavailability. This implicates the possibility of secondary health complications, including anaemia, immunosuppression, and metabolic diseases, which might arise from direct and indirect multisystem effects on haematological parameters as well as antioxidant and metabolic factors.

These results urgently require a reassessment of nano-Fipronil safety profiles and call for appropriate regulatory scrutiny of nanopesticide exploits. Notably, the observed systemic toxicity presents serious safety issues, environmental and health risks that need to be addressed through urgently needed protective measures and comprehensive risk assessments before agricultural applications on a larger scale.

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