Modulations in haematological aspects of wistar rats exposed to sublethal doses of fipronil under subchronic duration

Kartheek RM[1], David M[2,*]

[1]PhD Student, [2]Professor & Chairman, Environmental Toxicology & Molecular Biology Laboratory, Dept. of PG Studies & Research in Zoology, Karnataka University, Dharwad, Karnataka

***Corresponding Author:**
Email: mdavid.kud@gmail.com

Abstract

Fipronil (FPN) a phenylpyrazole insecticide is one of the preferred agricultural chemical known for controlling insect pests. Assessment of its safe and toxic levels has become a matter of serious concern as it is occupationally exposed to the farming community. The current investigation was thus aimed to elucidate the changes in haematological indices of Wistar rats on exposure to FPN. Healthy and mature male Wistar rats were randomly assigned into four groups C, E1, E2 and E3 and received 0.0, 6.46, 12.12 and 32.33mg/kg body weight of FPN respectively for a duration of 90 days. The results obtained indicated significant (*$p<0.05$) changes in RBC, WBC, Hb, PCV, MCH and MCHC values unlike MCV as compared to control. The decline noticed in RBC suggested anaemic conditions of rats. Significant (*$p<0.05$) elevation in WBC at E1 and subsequent decline at E2 and E3 was also witnessed under current study. As the modulatory outcome of FPN is evident on haematological aspects, it is thereby suggested that FPN under selected doses should be avoided for application into agricultural fields unless precautionary measures are taken to avoid exposure of toxicant as it threatens the health of the mammalian class resulting in compromised haematological condition.

Keywords: Fipronil, Haematotoxicity, Immune system, Red Blood Corpuscles and Wistar rats.

Introduction

The widespread use of pesticides in agriculture can lead to soil, water and air contamination resulting in adverse effects on inhabiting non-target organisms.[(1)] Many chiral pesticides are introduced into the environment as racemates despite the fact that their activity is usually the result from a preferential reactivity of only one enantiomer.[(2)] The pesticides currently in use include a wide variety of chemicals with great differences in their mode of action, uptake by the body, metabolism and elimination from the body, and toxicity to non-target organisms including humans.[(3)] In the present era a large diverse group of insecticides are used extensively to control insect pests and to enhance agriculture yield eventually reach the aquatic ecosystem through agriculture runoff thereby entering the food chain in more adequate amounts.[(4)] After organochlorines,[(5)] carbamates,[(6)] synthetic pyrethroids[(7)] and organophosphates;[(8)] new class insecticides are being explored for their ability to selectively kill insect pests with lesser or no damage to non-target organisms. A greater degree of attention is being given to the mammalian toxicity studies as the adverse effects caused by new and conventional group of pesticides are innumerable.[(9)] Toxicity of pesticides on mammalian species include effect on biochemical, neurological, hepatic, renal and haematological faction.[(10,11)]

Haematological indices are the important indicators of changes in the internal and/or external environment of animals and variations in their indices within an individual can result in inadequate responses to chemical stressors; however, these variations are nonspecific to a wide range of substances.[(12)] Exposure to chemical pollutants especially pesticides is thought to alter the hematological levels[(13)] often affecting the survival of exposed animal. Hence, in the measurement of health conditions and toxicological symptoms of organisms, hematological and biochemical parameters are exercised as crucial indicators.[(14-16)] Even though broad attempts of studies have been performed to establish demarcate the haematotoxic potentials of conventional group of pesticides, the same for new class insecticides like phenylpyrazole have been understudied for their ability to cause disturbances in blood of an organism.

Fipronil (5- amino- 1- (2, 6-dichloro- α, α, α- trifluoro- p- tolyl)- 4-trifluoromethylsulfinylpyrazole-3-carbonitrile) (FPN) is broad spectrum, first N-phenylpyrazole insecticide introduced with an objective of controlling insect pest.[(17)] It belongs to the group of "new generation" insecticide as its mode of action is dissimilar to pyrethroids (sodium channel blockers), organophosphates, and carbamate (cholinesterase inhibitors) to which a variety of insect pests have gained resistance.[(18)] Nonetheless, the mechanism of action of FPN has been previously discussed by Cole et al.,[(19)] who found that the phenylpyrazole insecticide interferes with the γ-aminobutyric acid (GABA) gated channel thereby disrupting normal nerve influx transmission and at sufficient dose causes excessive neural excitation, severe paralysis and finally death.[(18,20,21)] Even though FPN demonstrates a selective toxicity by having a higher binding affinity toward the GABA-regulated chloride channels of insects (LC_{50} can be low as 24.8 nM or ~11.7 μg/L)

than for mammalian GABA receptors,[22] its ability to cause haematological alterations through other modes cannot be ignored. Hence, in the present study, an attempt has been made to evaluate the impact of FPN on blood indices and determine safe and toxic dose levels against wistar rats following exposure to subchronic duration.

Materials and Method

Animal procurement: Male Wistar albino rats (6 weeks) were obtained from animal house facility, Department of PG studies and Research in Zoology, Karnatak University, Dharwad, Karnataka, India. Rats were housed in the polypropylene cages with *ad libitum* access to standard pellet feed and drinking water. The room was maintained under a 12/12 h light– dark cycle, an ambient temperature of 23-30 °C with a relative humidity of 45 (±15)%.

Experimental design and test doses: For a 90-day study of oral toxicity, the 6 week old Wistar rats were randomly assigned into four groups (C, E1, E2 and E3) of six males each. Group C of untreated rats served as the control group. Rats in the group E1, E2 and E3 were exposed to of 0.0, 6.46, 12.12 and 32.33mg/kg body weight of FPN dose in drinking water.

Haematological indices: Blood was drawn by puncturing the retro-orbital plexus under diethyl ether anesthesia. Whole blood for haematogram was collected in bottles containing the anticoagulant, ethylene diamine tetra-acetic acid. Total erythrocytes (RBC), total leukocyte count (WBC), packed cell volume (PCV), Mean corpuscular volume (MCV), MCH and MCHC were estimated by using the standard procedure of Rodak,[23] Hemoglobin (Hb) by the method of Van Kampen and Zijlstra.[24]

Statistical analysis: The values for haematological indices are reported as the mean ± standard error of the mean (SEM) obtained from triplicates. The data were subjected to one-way analysis of variance and further subjected to Tukey's test for post hoc analysis by defining the significance level at $*p<0.05$.

Ethical committee: All rats were housed for a duration of 1 week for acclimatization before initiation of the experiments. The maintenance of experimental rats and all the procedures implemented are in accordance with standard guidelines issued by CPCSEA followed with approval of the Institutional Animal Ethics Committee (IAEC) of the institute.

Results and Discussion

Changes in haematological indices are considered to be one of the crucial biomarkers of physiological stress.[25] The present investigation indicated significant ($p<0.05$) variations in haematological indices of rats exposed to different sublethal doses of FPN for 90 days and have been presented in figure 1-7. No changes were observed in control rats which advertised normal count. The rats under FPN stress however advertised changes in RBC faction with percent decline of -19.57, -19.01 and -57.60% for E1, E2 and E3 respectively. Changes in RBC count which was found to be in an irregular trend, which suggests the involvement of FPN in either demolishing the RBC concentrates or intervening in erythropoietic mechanism as reported previously by Mehra et al.[26] Since the RBC's account for transportation of oxygen and deoxygenated blood, variation in this ratio within the individual is thought to play a crucial role in maintenance of balance between oxygen and carbon dioxide levels.[27] Our reports are in agreement with Barna-Lloyd et al.,[28] Goel et al.[29] and Ambali et al.[30] who stated that exposure to toxicants under long term durations have been known to cause anomalies including anemia in non-target organisms.[31]

Changes in WBC count was found to be in irregular pattern with an elevated cell count at E1 (41.57%) and E2 (28.09%) unlike for E3 wherein a decline of -57.30% was witnessed. The elevation in WBC may be due to the activation of the animal's defence mechanism against the toxic invasion by FPN concentrates. However, the E3 group demonstrated significant decline in WBC's which indicates the probabilities of systemic damage to the immune system due to persistence of high doses of FPN. Similar reports have been given by Lohner et al.[32] and Shahi and Singh,[33] who reported decline and increase in WBC's respectively under different conditions. Also Janeway et al.,[34] explained that decreased number of lymphocytes may be due to reduced production or rapid removal from circulation and subsequent destruction. The reduction of lymphocytes is indicative of immunosuppressive effects and has been previously reported for organophosphates. Moreover, the results obtained by Goel et al.[29] confirmed that, decrease in leucocyte counts following intoxication with another organophosphate, Chlorpyrifos, could be attributed either to the slower rate of production of leucocytes or due to their inhibited release into the blood circulation. It is to be noted that the report on WBC decline is reported for the first time against the FPN for the subchronic duration of 90 days. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates.[35]

Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals. Changes in were noted for Hb which advertised highest decline at E3 (-55.82) followed by E2 (-23.66) and E1 (-18.92), which further suggests lesser difference between E2 and E1. The variation in Hb and decline in RBC was found to be dependent on the dose level of FPN administered suggests that FPN induces toxicity against RBC's of rats resulting in increased rate of erythrocytes destruction which could be taking place in hemopoietic organs.

PCV under current investigation demonstrated highest damage under E3 group followed by E1 and E2. However, the change was found to be significant ($p<0.05$) under all the exposed group. Reports by Isaac et al.[36] have suggested that PCV is primarily associated in the transport of oxygen and absorbed nutrients. Since the present investigation was recorded for decreased PCV levels, the possibility of lack of oxygen and nutrient transportation cannot be overlooked. The reduction in both quality and quantity of PCV may be a consequence of severe haemorrhage and is often correlated with RBC count and Hb levels. This in turn results in the dilution of blood caused by the influx of cells and fluids from body stores.[37] In fact, Hb concentration and PCV values are directly correlating with RBC's count, this part of our study is in support of reports from El-Bakary et al.,[38] and the mechanism behind could be mainly due to the synergistic link among these blood parameters in all vertebrates. This close correlation between erythrocyte count, haemoglobin concentration and haematocrit value has been also reported for other vertebrates including man.[39]

Change in MCV under all the groups could be as a consequence of varying erythrocyte size that in turn may be due to the process of osmotic imbalance in fluid conditions. Our results are in agreement with reports of Al-Amoudi,[40] who reported similar trend in blood indices of rats after exposure to organophosphate. The data for MCV unlike for MCH and MCHC advertised insignificant variation. A slight decline was observed at E1 (-0.71%) and a trivial increase was noted for E2 (0.21%). However, E3 showed a significant elevation of 46.89%. Evaluation of MCV thus provides an information on the size and status of erythrocytes within the exposed organism.[41] Reports given by Anandkumar et al.[42] suggested increase in MCV that is caused due to endosmosis. Endosmosis leads to the passage of solvent from less concentrated solution to more concentrated one. This results in haemodilution, further increasing the MCV value. Previously Garg, *et al*.,[43] Dhanapakiam and Ramasamy,[44] reported increase in MCV within non-target organisms exposed to toxicants.

Under MCH, the values suggested significant variation ($p<0.05$) with percent change of 97.3, 84.5, 103.9% for E1, E2 and E3 respectively. The MCHC is a good indicator of red blood cell swelling.[45] The results from the present study speculates the possibility of FPN inducing physical change in rat erythrocyte morphology from the normal discoid shape to other forms including echinocytes, dacrocytes and schistocytes. Echinocytes transformation plays an anti-haemolytic role – the expansion of the plasma membrane increases the cell-volume ratio/membrane-area, thus allowing swelling of the cells before lysing.[46] The current investigation revealed MCHC values which were found to be significant ($p<0.05$) and elevated under the FPN intake resulting in a percent change of 98.86, 84.21 and 38.93% for E1, E2 and E3 respectively. As MCH and MCHC are derived from Hb and RBC, any sort of alteration in the levels of Hb and RBC would commonly result in the alteration of MCH and MCHC. Atamanalp, *et al*.[47] reported a marked rise in the values of MCHC, MCH and MCV in *O. mykiss* on exposure cypermethrin. Parma, *et al*.,[48] however, noticed minor alterations in the values of MCV, MCH, and MCHC concentrations in *Prochilodus lineatus* treated with 0.6 ppm of cypermethrin which indicates the haematotoxic of effect of pyrethroids. As reported previously, various environmental toxicants are known to induce changes in organs like brain,[49] liver,[50,51] kidney[52] and spleen[53] causing impairments, resulting in the persistence of FPN residues in blood thereby causing significant variations in its indices. The current work is viewed as a nutshell and further needs molecular investigations to elucidate the mechanism of FPN in demolition of haematological integrity of the exposed organism.

Conclusion

FPN exposure to rats was found to alter total erythrocyte and leucocyte counts suggesting anaemic condition and instigation of immune response respectively. The decline of Hb as observed under current investigation indicates the possibilities of hypoxic conditions of exposed animals. Significant change in PCV, MCH and MCHC indicates the direct impact on overall haematological aspects within the exposed animal. Thus based on the outcome, it could be ascertained that FPN is toxic at all the selected sublethal doses when exposed for a duration of 90 days. It is therefore concluded that care is to be taken when the toxicant is used or sprayed under mammalian vicinity.

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Conflict of Interest

Authors hereby declare no conflicts of interest with this article.

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