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RESEARCH PAPER

Neonicotinoids Toxicity in *Hypophthalmichthys nobilis* (Richardson, 1845): Clinico-haematological and Erythrocytic Morphological Alterations

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Abstract

Neonicotinoids or chloronicotinyl insecticides (neonics) are a new group of insecticides that are frequently used for the protection of agricultural crops. The Nitenpyram (NTP), a commonly used neonicotinoid insecticide on a global scale, has been recognized as a non target aquatic organism contaminant. This research has been done to explore the toxic effects of NTP on the freshwater carp (*Hypophthalmichthys nobilis*). Following a week of acclimatization period, a total of 108 healthy fish with average body weight 48±0.07 g were divided into three groups, with each group being replicated twice (12 fish per replicate). C0 was untreated group (control), while group C1 and C2 were exposed to different concentrations of NTP, 0.02 and 0.04 mg/L respectively for 14 days. NTP-intoxicated fish manifested severe behavoral changes include convulsions, jerking, exhaustion, increased opercular movement, body curvature, breathing problems, mouth redness, fin erosion, and gasping. There were significant changes in the hematological indices because of NTP exposure, e.g. Mean Corpuscular Volume (MCV), Neutrophils (N), Lymphocytes (L), Monocytes (M), and Leukocytes (W) were highly elevated, while Red Blood Cells (RBC), Hemoglobin (HGB), and Hematocrit (HCT) were considerably reduced in experimental groups C1 and C2 as compared to control group C0 ($P<0.05$). The percentage of erythrocytic cellular changes comprising, microcytes, acanthocytes, pear shaped erythrocytes, leptocytes and fused erythrocytes, along with erythrocytic nuclear abnormalities influencing micronucleus, kidney shaped nuclei, eccentric nuclei, blabbed nuclei, and bilobed nuclei were recognized. These results indicated that exposure to NTP caused significant adverse effects on fish blood health, suggesting that the ecosystem and non-target aquatic life is at danger from this widely used pesticide.

Introduction

Neonicotinoids (NEOs) are a class of pesticides that followed the carbamates, pyrethroids, and organophosphates, and are typically used in the pest control of agri-crops by insect removal (Bustamante et al., 2024). These pesticides are very strong and efficient in killing insects and safe for human beings (Ayilara et al., 2023). The NEOs insecticides have a unique action mode by which they interact with nicotinic acetylcholine receptors (nAChR) and bind; thus they cause neurological stimulation, paralysis, and ultimately death in their target animals (Tu et al., 2023). That's the reason behind, the NEOs have a lower fragility against the environment and non-target organisms in comparison with highly toxic Organophosphorus insecticides (Maloney et al., 2021). Another aspect of the problem is that fruit and vegetable residues of NEOs are the primary source of NEOs exposure to both humans and animals (Pan et al., 2022). Thus, the human exposure to NEOs has been reported in many studies. Martelli et al, (2020) found-human-nicomorphines in 49.1% of NEOs and their metabolites in 3038 urine samples from the United States and six major NEOs were detected in these samples. Therefore, the question of whether NEOs can damage the environment and human health is raised.

Nitenpyram (NTP) is the most used neonicotinoid insecticides after imidacloprid. In urban and forest environments, NTP is also sprayed on the leaf surface or injected into the tree trunk. However, It is also widely used in the agriculture field as a seed protective agent (Wei et al., 2025). The data reveals that it is a chemical that dissolves quickly in water and was found 89% to 100% of the water samples, particularly those taken during agriculture activities, according to worldwide surface water quality monitoring studies (Malhotra et al., 2021). The potential risks of NTP are uncertain, as only a few studies have been done yet, and some data suggest that the genotoxic potential of NTP towards non-target species should not be underestimated. It appears that the use of NTP in a innocuous concentration of 0.5 mg/l was still able to damage the liver DNA in juvenile Chinese rare minnows and induce oxidative stress (Tian et al., 2020). Through a treatment of rats with NIT via 900-7200 mg/g for 100 weeks, the incidence of esophageal papilloma at squamous type showed a significant increase in mammals of both sexes, which also indicated the potential carcinogenicity (Bhat et al., 2010). It should be noted that no tests with human cells that concerned the genotoxicity of NTP have been carried out except for the study of Liu et al, (2022). He investigated that NTP is capable of underlining mutations by causing oxidative stress and by suppressing PI3K/Akt, AMPK, and mTOR signaling pathways, which could also be associated with cancer propagation (Wei et al., 2020). However, acute NTP concentrations in the environment themselves are not too risky for human health, yet synergistic or joint effects to aquatic life with othe rpesticides should not be further neglected. The detrimental impacts and potential risks that NTP brings to aquatic ecosystems must be closely monitored because of its widespread usage and penetration into the aquatic environment (Alsafran et al., 2022).

Haematological analyses is often employed in scientific research to check the effects of environmental factors as well as to assess the well-being and health of fish kept in aquaculture (Fazio, 2019; Witeska et al., 2022). Blood indices are quick-reacting and sensitive indicators of a variety of environmental effects, such as hazardous agent-induced water contamination (Witeska et al., 2023). Blood parameters provide extensive data regarding an assortment of physiological activities and are reliable markers of an organism's performance, reflecting a wide range of physiological conditions, both adaptive and disruptive (Bojarski & Witeska, 2020). In contrast to other tissues that were collected from species that are alive, blood sampling is less intrusive and can be done in fields as well as in laboratories (Cestonaro et al., 2020). Important hematological parameters for fish include red blood cells (RBC), hemoglobin concentration (Hb), hematocrit, total white blood cells count (WBC), and erythrocyte indices, including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and evaluation of stained peripheral blood film (Alarape et al., 2024). Stained blood smears can be used for quantitative evaluation of the erythrocyte and leucocyte populations in order to ascertain the percentage of immature erythrocytes (erythroblasts), erythrocyte cellular and nuclear abnormalities, these parameters are helpful for assessing cytotoxic and genotoxic effects, erythropoietic activity, and immune system health (Qiu et al., 2020).

Aquatic life is the primary carrier of stress syndrome arising from toxic pollutants that stimulate the formation of the micronucleus and, thus, the growth of the erythrocyte (Butcherine et al., 2021). Fish health status is therefore often assessed in toxicological investigations employing erythrocytic morphological features and haematological parameters as potential diagnostic tools (Chen & Luo, 2023). *Hypophthalmichthys nobilis*, or Bighead carp with an annual global output of 3.18 million tons, is one of the most important freshwater farmed economic fish in Asia (Szabó et al., 2019). Because of its ease of catch, minimal disease burden, cheap culture costs, and high economic value, *H. Nobilis* has gained popularity as a freshwater food fish (Zhou et al., 2024). The bulk of research has examined the NTP manufacturing, analytical methods and effectiveness studies, alongwith its sudden intoxication of different species (Butcherine et al., 2021). However, prior only one study has investigated that NTP has caused apoptosis, genetic mutation, and the mechanism of liver's detoxifying enzymes of zebrafish (Cheghib et al., 2020). Although there is a lack of information on NTP toxicity in freshwater fish species, this study has evaluated the haematological parameters as well as the erythrocytes cellular and nuclear abnormalities in order to better understand the detrimental effects of NTP in *H. nobilis*. The current experiment aims to examine the nuclear and hematological alterations in fish erythrocytes caused by different exposure to NTP doses.

Materials and Methods

Fish Holding and Chemical

Nitenpyram (50%w/w) issued by instant super pesticide company was purchased from M/S Ali Akbar Enterprises, a commercial scientific shop located in Lodhran, Punajb, Pakistan. Healthy *H. nobilis* individuals were carried from a nearby fisheries farm in Bahawalnagar, Pakistan. Fish were transported to the testing lab in oxygen-filled polythene containers to guarantee their survival.

Ethical Approval

The current study was carried out at the Aquaculture, Genetic Toxicity, and Molecular Biology

Laboratory of the Department of Zoology at the Islamia University of Bahawalpur, Pakistan. The institutional Bioethics Committee (IBC) strictly followed its recommendations on animal handling ethics.

Acclimazation

Fish were housed in glass aquaria with a 150-liter water retention capacity and dimensions of 100 cm × 40 cm × 40 cm for a week during the acclimation period. The glass aquaria were filled with dechlorinated tap water, having optimal water conditions for fish; temperature 21-23°C, pH 7, and 100 percent dissolved oxygenation. During the whole experiment, the water quality parameters were monitored and maintained within the optimal range. Pre-calibrated Multi-parameter (Hanna HI 9147) was used to monitor temperature (28°C±0.02), DO (5.5 mg/L±0.01), pH (7.7±0.02), and ammonia (<0.037 mg/L±0.04) levels on a daily basis.

Experimental Design

After acclimatization period, eighty one *H. nobilis* (mean weight= 48±0.07 g) were allocated to 3 groups (C0, C1 & C2) with two replicates (12 fish per replicate). Fish of groups CI and C2 were exposed to different concentrations of NTP @0.02mg/L and 0.04mg/L respectively while group C0 was kept as control group for 14 days. The fish were fed with commercial feed that contained 30% of crude protein, used in the feeding trial, was formulated as: maize glutelin 1.5% (64%), broken rice (11%), wheat gruel (24%), maize glutelin 6.5% (31%), maize gruel (15%), ghar meal (3.5%), decorticated cottonseed meal (8%), limestone (7.8%), soybean meal (6.12%), fish meal (6.7%), rice polishings (12%), dicalcium phosphate (1.9%), a vitamin/mineral premix (0.8%), and molasses (3.8%), at the rate of 3% of body weight. Fish were inspected daily for signs of clinical illness and mortality. This study was divided into two dissections; the first was done on day 7 and the second on day 14.

Behavioral Observation

During the 14-day experiment, *H. nobilis* exhibited a variety of abnormal behaviors, including rapid gulping, jerking, convulsions, opercular movement, static movement, breathing difficulties, fin erosion, gasping, faintness, and mouth redness. Following exposure to NTP, these behaviors were closely observed after every 24 hours.

Haematological Parameters

On days 7 and 14, three fish from each group were caudally venipunctured using a disposable hypodermic needle measuring 21 gauge., yielding a blood sample of approximately 2.5 ml. Immediately after the procedure, to create thin blood smears for the micronuclei test, a few fresh blood droplets were placed on glass slides, and 0.5 ml of blood was extracted for hematological analysis into anticoagulant EDTA vials. Serum separation was achieved at 4°C by centrifuging 2 mL of blood for 5–8 minutes at 4000 x g. The values of neutrophils, lymphocytes, monocytes, hemoglobin, hematocrit, and total erythrocytes and leukocytes were analyzed using a hematology analyzer (Sysmex, Kx21). According to the approach used by Jain et al. (1993), the subsequent formulas were used to calculate the Packed Cell Volume (PCV=%Hb × 3), Mean Corpuscular Hemoglobin (MCH= (% Hb/RBC in millions) × 10), and Mean Corpuscular Volume (MCV= (PCV/RBC in millions) × 10).

Erythrocytic Alterations

By using a conventional procedure, (Islam et al., 2019) checked cellular and nuclear erthrocytic alterations in *H. nobilis*. Blood samples were spread out onto glass slides and left to air dry for 10 minutes as a heads-up. After Ten minutes of fixation in methanol, glass slides were cleaned using distilled water and stained with 5% Giemsa. They were mounted using DPX following an overnight air drying period. Two thousand cells with nuclear and cellular membranes were scanned from each of the three slides using a 100x magnification computer-aided light microscope (MICROS Austria, MCX100). The Features of both erythrocytic cellular and erythrocytic nuclear changes have been determined employing the practices outlined by (Sadiqul et al., 2016).

Statistical Analysis

Each group mean and standard error (SE) values for hematological parameters, as well as the frequency of nuclear and cellular erythrocytic changes, were computed using IBM® SPSS® Statistics 28.0.0.0 (190) (2021). For data sets meeting the requirements of homogeneity and normality, a one-way analysis of variance (ANOVA) was performed, following Tukey post hoc tests with significant level $P<0.05$. Graphs were prepared using Microsoft Graphpad Pism (Version 9)

Results

Clinical Signs and Behavioral Changes

The control group (C0) fish that were found to be healthy in our experiment showed no mortality or clinical symptoms. The most critical clinical signs of fish being exposed to NTP were found in group C2 at elevated concentration of 0.04 mg/L NTP during the experimental days. However, the NTP (0.02mg/L and 0.04mg/L) exposed fish showed abnormal clinical behavioral changes from light to severe like breathing difficulties, jerking, convulsions, fainting, surface breathing, surface running, bottom running, body unbalancing, operculum movement, tin tilting, and static lurization were observed. For 1-14 days normal clinical signs were observed in group C0 but groups C1 and C2 indicated severe clinical abnormalities (Table 1).

Hematological Responses

The response level of hematological parameters was the following: a linear concentration-proportional decline in PCV, Hb, hematocrit contents, and total RBC count, accompanied by a rise in MCV and WBC count (Figure 1). According to the data that has been statistically tested, RBC count and the concentration of the hemoglobin reached at the lower significance level (P<0.05) just for the fish in group C2 at day 7 and 14, after NTP exposure in comparison with C1 and C0 group. The fish of group C2 experienced an abrupt reduction of hematocrit content at day 14 of the treatment whereas the control group fish did not show such a sharp decrease. After 14 days NTP exposure, the hematocrit counts of fish belonging to group C1 were also reduced. During the experimental period, the PCV and the MCH concentration reduced considerably in experimental groups whereas, the MCV started to decrease in both treated groups. When compared to group C0, the leukocyte count in both NTP treated groups decrease considerably at day 14 whereas the numbers of monocytes and neutrophils drastically increased, in treated groups following NTP exposure.

Erthrocytic Cellular Alterations

NTP exposure caused cellular morphologic abnormalities characterized by microcytes, acanthocytes, pear-shaped erythrocytes, leptocytes, and fused erythrocytes in *H. nobilis* during the experimental period (Figure 2). The erythrocyte cellular alterations were increased during the experimental period in accordance with concentrations of NTP was directly proportional to the increase in the frequency of alterations (Table 2). At day 7 of NTP exposure, the largest frequency of cellular changes was seen in group C2 (0.04 mg/L), but group C1 did not exhibit any significant alterations (P>0.05). As NTP concentrations increased on day 14, 0.04 mg/L C2 group had the greatest frequencies in comparison to the untreated C0, while the C1 (0.02 mg/L) group shoed the significant rise in erythrocytes cellular abnormalities like; leptocyte, acanthocyte, and pear-shaped erythrocyte counts increased considerably (P< 0.05) (Table 2).

Erythrocytic Nuclear Alterations

Numerous nuclear alterations were detected in cells of *H. nobilis*, such as micronucleus, kidney-shaped nuclei, eccentrically placed nuclei, incompletely devoured and bilobed nuclei, (Figure 3). Throughout the experiment, it was discovered that higher NTP concentrations increased the frequency of erythrocyte nuclear changes. During the period of the experimental study, C2 showed a statistically significant (P<0.05) increase in erythrocyte nuclear abnormalities, including a considerably greater (P<0.05) number of blebbed and fused nuclei on 14-day. The frequency of fused erythrocytes and blebbed nuclei was likewise considerably (P<0.05) higher in group C1 (0.02 mg/L) at day 14 of NTP exposure. Group C2 (0.04 mg/L) had the greatest incidence of all anomalies across the whole exposure period (Table 3).

Discussion

The great number of neonicotinoid insecticides exposure to aquatic bodies as well as to the soil from where they have been in water has been reported as a more regular incidence in the terrestrial and aquatic ecosystems (Zhang & Lu, 2022). Several instances have been reported where the insecticides have ended up in some water bodies such as lakes, rivers, ground water, and streams. However, the chemical contamination of a system can alter the structure and function of the community (El-Garawani et al., 2021). Several experiments have examined the effects of NEOs on non-target species, including as humans, birds, insects, and mammals (Zhang et al., 2023). The acute toxicity of NTP was studied in many species like *Bombyx mori* (Sun et al., 2023) *Gobiocypris rarus* (Tian et al., 2018) and *Danio rerio* (Yan et al., 2015). The consumption of neonicotinoids by aquatic species has detrimental effects on the ingesting, motility, immunity, growth and

Table. 1. Clinical signs observed in *H.nobilis* for 1-14 days nitenpyram administration

Grasping	+	+	_
Jerking	+	+	+++
Convulsion	_	_	++
Surface breathing	+	+	++
Faintness	_	_	++
Surface Running	+	+	_
Bottom Running	+	+	++
Body unbalancing	_	_	_
Operculum movement	+	++	+++
Tilting of fin	+	+	++
Static position	+	+	+

Absent (-), Mild to Moderate (+), Severe (++), very severe (+++)

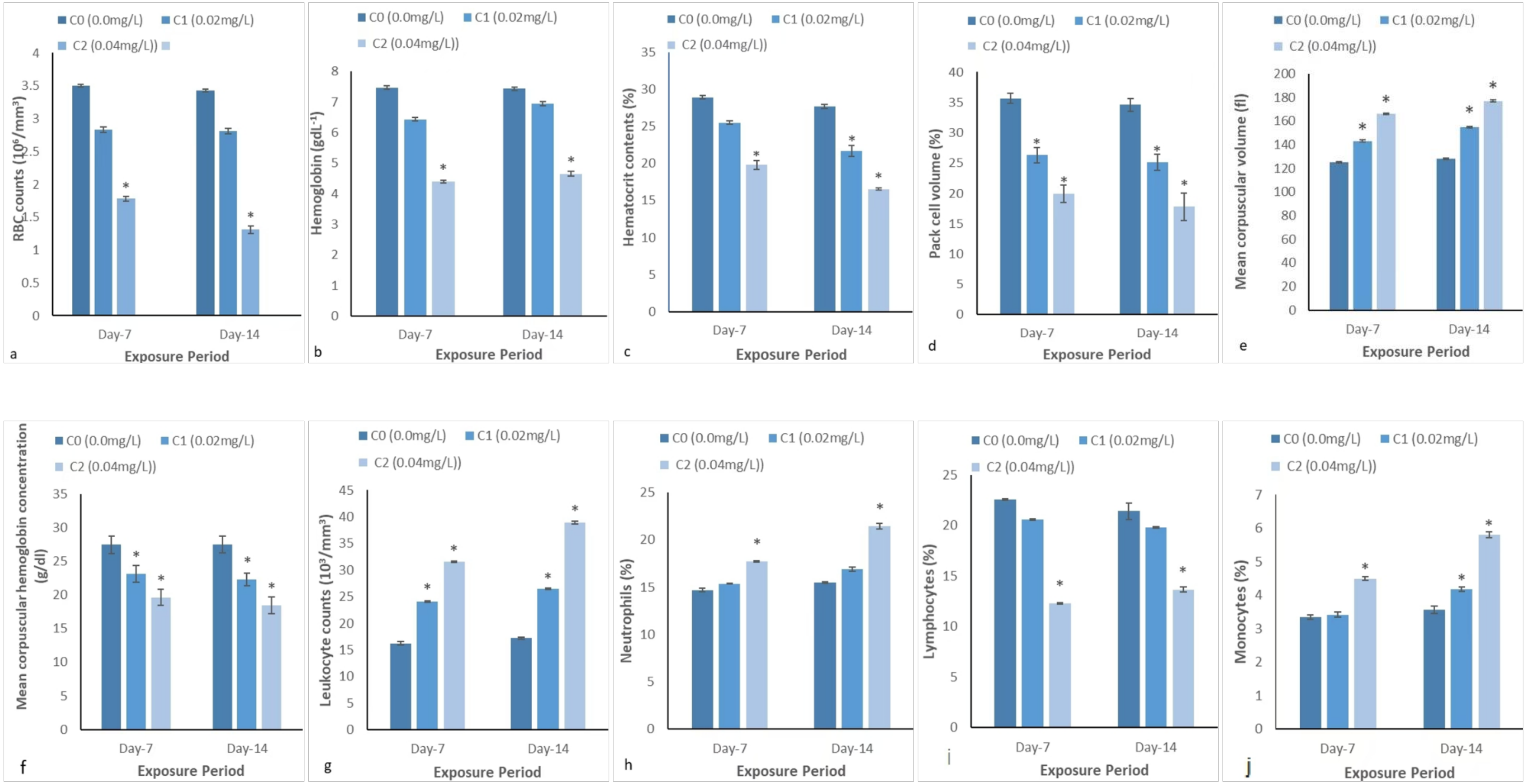

Figure 1. Graphs representing hematological parameters with symbol (*) denotes significant deviation (P<0.05) between the control and experimental groups of*H. nobilis*, (a) RBCs count (10^6/ mm³), (b) Hemoglobin (gd/l), (c) Hematocrit contents (%), (d) Pack cell volume (%), (e) Mean corpuscular volume (fl), (f) Mean corpuscular hemoglobin concentration (g/dl), (g) Leukocyte coun.

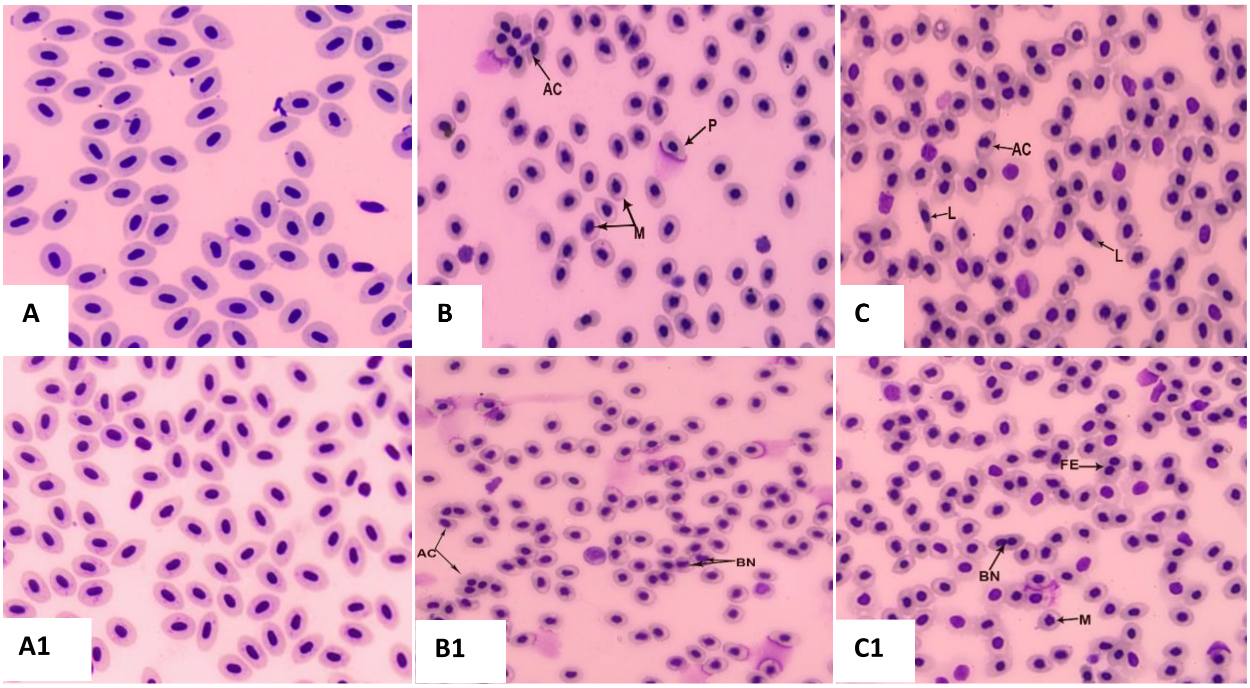

Figure 2. Blood smear of *H. nobilis* exposed to varying concentrations of nitenpyram showing different types of erythrocyte cellular changes: A, A1) regular morphology B, B1) AC; Acanthocytes, M; Microcytes, P; Pear shaped erythrocyte, C, C1) AC; Acanthocytes, L; Leptocytes, M; Microcytes FE; Fused Erythrocytes.

Table. 2. Various cellular alterations (%) observed in *H. nobilis* erythrocytes exposed to varying concentrations of nitenpyram

Parameters/Days	Exposed groups		
	C0 (0.0mg/L)	C2 (0.02mg/L)	C3 (0.04mg/L)
Microcytes			
7	0.11±0.01	0.14±0.01	0.20±0.02*
14	0.12±0.02	0.16±0.01	0.25±0.02*
Acanthocytes			
7	0.13±0.02	0.15±0.01	0.23±0.04*
14	0.14±0.04	0.19±0.02*	0.29±0.05*
Pear shaped Erythrocytes			
7	0.14±0.04	0.17±0.01	0.24±0.04*
14	0.15±0.05	0.18±0.03	0.27±0.05*
Leptocytes			
7	0.16±0.01	0.19±0.02	0.31±0.035*
14	0.17±0.02	0.27±0.03*	0.42±0.036*
Fused Erythrocytes			
7	0.18±0.02	0.26±0.04*	0.44±0.03*
14	0.19±0.04	0.30±0.03*	0.49±0.04*

Mean±SD values with asterisk in each line show significant difference to control group (one way ANOVA, Significant level P<0.05, Tukey multiple comparisons tests in SPSS–26.0.).

development of the organisms repoted in numerous studies (Shen et al., 2022). To our best knowledge, this is the first study to check the ntp toxicity in feshwater fish *H.nobilis*.

Although Nitenpyram (NTP) is widely applied to promote rural development, the resultant compounds are harmful to the agricultural and aquatic environment, and non-target species as well (Wang et al., 2024). High NTP toxicity to bees and high leaching potential, however, have set off a chain of environmental problems that might possibly impact the ecosystem, food chain, and even human health (He et al., 2024; Thompson et al., 2020). A recent report indicated that a collection of bee samples recorded a measure of 1341mg/kg of NTP contaminants (Song et al., 2018). It has also been shown that the level of toxicities that can be reached by NTP in bees and silkworms was such that it clearly represented that the non-target animals would be affected by NTP after only a short period of exposure (Wu-Smart & Spivak, 2016). NTP was found to be highly toxic for some of the aquatic non-target organisms (Malhotra et al., 2021). As studied by Yan et al, (2015), NTP exposure can affect the liver, DNA content, and of level of antioxidants in zebrafish.

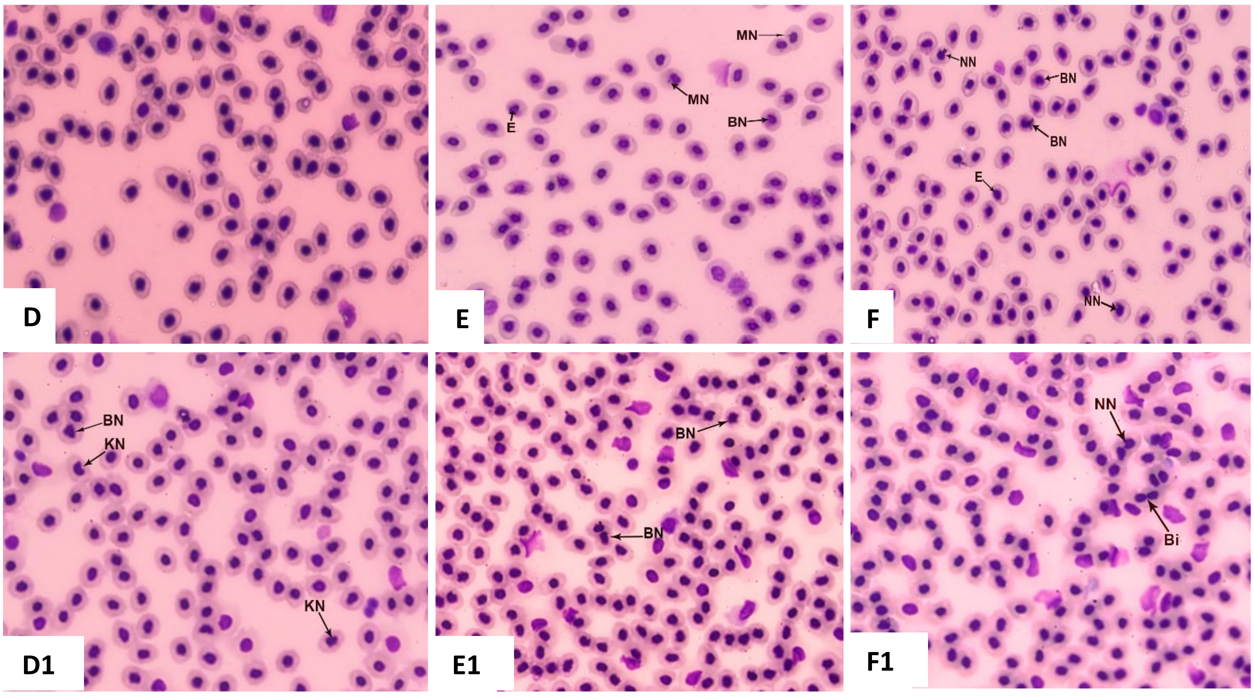

Figure 3. Blood smear of *H. nobilis* exposed to varying concentrations of nitenpyram showing different types of erythrocyte nuclear changes: D, D1) regular nuclear morphology; KN; Kidney shaped nuclei; BN: Blabbed Nuclei E, E1) MN; Micro Nucleus, E: Erythrocytes with eccentric nuclei, BN; Blabbed Nuclei F, F1) MN; Micro Nucleus, E: Erythrocytes with eccentric nuclei, BN; Blabbed Nuclei, Bi; Bilobed nuclei.

Table 3. Various nuclear alterations (%) observed in H. nobilis erythrocytes exposed to varying concentrations of nitenpyram

Parameters/days	Exposed groups		
	C0 (0.0mg/L)	C1 (0.02mg/L)	C2 (0.04mg/L)
Micronucleus			
7	0.12±0.02	0.15±0.01	0.23±0.01*
14	0.11±0.01	0.16±0.01	0.27±0.02*
Kidney shaped nuclei			
7	0.13±0.02	0.15±0.01	0.23±0.01*
14	0.14±0.01	0.17±0.02	0.29±0.02*
Bilobed nuclei			
7	0.15±0.01	0.17±0.01	0.24±0.02*
14	0.17±0.02	0.24±0.05*	0.33±0.02*
Blabbed nuclei			
7	0.14±0.031	0.23±0.046	0.31±0.035*
14	0.15±0.025	0.27±0.035*	0.44±0.056*
Erythrocytes with eccentric nuclei			
7	0.18±0.025	0.30±0.03*	0.42±0.045*
14	0.19±0.03	0.35±0.06*	0.53±0.050*

Mean±SD values with asterisk in each line show significant difference to control group (one way ANOVA, Significant level P<0.05, Tukey multiple comparisons tests in SPSS–26.0.).

Our study has also identified a number of behavioral changes such as: abnormal swimming, opercular movement, body convulsions, trembling, feeling faint, the body's lateral or rolling position, gasping, the fin being on the side, redness on the mouth, the absence of breathing. It could be that the experimental fish suffered from edema of their gill, which leads to a decrease in their overall body function. Later stages of exposure resulted in the treated fish lying on their sides on the aquarium bottom, making very little movement and remaining motionless until death. This was attributed to respiratory impairment and irritation caused by the toxicant in the water that affects the gills. Additionally, the observed difficulty in respiration primarily reflects a decreased respiratory capability due to the damage to the gills. The current abnormal swimming patterns and other clinical alterations in fish behaviour are identical with those reported by El-Bouhy et al, (2023) in the Grass carp being treated with the insecticide propenofos showed the semi-circular swimming patterns, increased opercular movement as well as knocking the walls of the aquariums. However, our results also show resemblance with Ghaffar et al, (2015), in terms of the effects on *Labeo rohita* being treated with butachlor (chloroacetanilide herbicide), where higher concentrations of this herbicide (0.75 and 1.00 mg/L) caused a variety of clinical symptoms, including rapid breathing, gasping at the water's surface, dizziness, opercular movement, loss of balance, and erratic swimming.

Haematological markers are significant for toxicological studies, they are among the bio-indicators carrying the animals physiological responses to stimulations, thereby giving the researchers a clear image of the study (Catana et al., 2022). However, our experiment is about how NTP has negative effects on the blood parameters of fish *H. nobilis.* Hemoglobin is a fish critical protein necessary to fulfill the function of oxygen transportation from the surroundings to the internal organs through the bloodstream, and hence the metabolic needs are covered accordingly (Manning & Manning, 2020). NTP treated *H. nobilis* showed a drop in hemoglobin levels in the blood, and they remain significantly low, indicating hypochromic microcytic anemia in the fish. Inhalation of fenitrothion by *H. fossilis* showed a smiliar decreasing trend for hemoglobin (Ritu et al., 2022). Fish red blood cells facilitate hemoglobin and also have a role in gaseous exchange, immunological responses, and control. This decrease in hemoglobin level and red blood cell count might be caused by deficiencies or disturbances in the fish erythrocytes that are produced by kidney and spleen, which are important hematopoietic organs (Nikinmaa et al., 2019). The reduction in erythrocytes in our study aligns with numerous prior data including, sub-lethal concentrations of lindane showed the significant (P<0.05) reduction in RBCs in *Labeo rohita* (Afzal et al., 2024). Using *C. gariepinus* and *O. niloticus* as model organisms, Kanu et al, (2023) investigated the red blood cells count decreased to pesticide pulse exposure. Naseer et al, (2024) reported the similar deceasing pattern in freshwater Bighead carp treated with sub-lethal mixture of two insecticides acetamiprid and pyrifroxyfen.

White blood cells, which are involved in defending the body against illness and external invaders, indicates the stronger antibody synthesis to help the body adapt to stressful situations (Shahjahan et al., 2020). Our current study results showed a considerable rise in white blood cells, while Sharafeldin et al, (2015) and Al-Emran et al, (2022) revealed that both acute and chronic pofenofos exposure significantly increased the number of WBCs in Nile tilapia. Following exposure to cadmium (5.7 mg/l), lead (7.6 mg/l), and mercury (0.6 mg/l), *C. idella* also showed a significant rise in WBC Saeed et al. (2024). The fish immune system was stimulated by this significant rise in WBC, which led to the release of lymphocytes from lymphomyeloid tissues. This enabled the organism recovery and servival from exposure to harmful contaminants (Xu et al., 2021). Fish leukocyte counts are a sensitive barometer of environmental and chemical stressors, and changes in these counts are named immuno-dev Bigger MCV as well as MCH values being an increase in RBC size indicates that the cells have in fact got growth which is very large and swol (Burgos-Aceves et al., 2019). The decrease in PCV in this work could have been caused by the NTP-induced Hb production in *H. nobilis*. Furthermore, such a decrease in the PCV of the fish eventually showed an increase in MCV, MCH, and reduction in PCV levels (Das et al., 2021). The lack of PCV was due to the substantial increase in the size and numbers of the mentioned cells. The change in MCH and MCV values along with decreased PCV values, RBC possess the ability of the cellular shapes and retain evidence even if they are extremely vulnerable to the environmental factors or the body metabolism (Farooq et al., 2023). The results of this investigation showed that decreased PCV could be caused by the NTP-induced Hb production in *H. nobilis.* Hypochromic microcytic anemia is indicated by a substantial rise in MCH and MCV levels. In *Labeo rohita*, Profenofos poisoning resulted in similar anemia (Bantu et al, (2017) likewise significant increase in MCV and MCH concentrations in *H. nobilis* treated with NTP, we have observed in our study.

Erythrocyte cells, which contain hemoglobin, are responsible for maintaining the hematopoietic system in fish. Certain external stimuli can cause erythrocytes to respond, and the most prevalent way that pesticides are present in water bodies is through molecular alterations in erythrocytes (Caramello et al., 2019). The erythrocyte cellular changes in *H. nobilis* that were examined in this study comprised microcytes, acanthocytes, pear shaped erythrocytes, leptocytes and after being exposed to NTP, fused erythrocytes have also been discovered. Moniruzzaman et al, (2024) examined the similar results like spindle, tear-drop and twin shaped cells in the Java barb (*Barbonymus gonionotus*) treated with Celcron (Cec), an organophosphate insecticide. Likewise Sharmin et al, (2021) reported the significantly higher frequency of erythrocytic cellular abnormalities across diffeent sumithion concentrations (0.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/L) compared to the control group. Erythrocytes become more prone to rupture or deformability as a result of the morphological changes caused by lipid peroxidation accessing the plasma membrane layer of tiny capillaries. The morphological development of erythrocytes may be impacted by the direct or indirect effects of hazardous chemicals on the ion permeability, metabolism, and cell membrane structure of erythrocytes (Putter & Seghatchian, 2017). The inhalation of arsenic in *Oreochromis mossambicus* (Ahmed et al., 2015), *Channa punctatus* (Patowary et al., 2012) and quinalphos pesticide toxicity in *Barbonynus gonionotus* (Sadiqul et al., 2016) showed a similar pattern of cellular deformity. Das et al, (2024) proposed that the alteration in erythrocytes in fish subjected to malathion-induced hypoxic stress was explained by adenosine triphosphate depression. According to Liang et al, (2019) the transformation of normal erythrocytes into different aberrant forms has been caused by apoptosis-causing factors, including ionizing radiation, reactive oxygen intermediates, exogenous oxidants, in vitro aging, and elevated cytosolic calcium.

The current study has reported numerous types of erythrocyte nuclear alterations that were identified in the blood smear of the NTP-treated fish and were ranked from the most to the least prevalent; Fused erythrocytes > blabbed nuclei > bilobed nuclei > erythrocytes with eccentric nuclei > micronucleus are the types of the nuclear alterations. The frequency rates shown in the current study vary to a certain degree from those represented in another study carried on Silver barb (*Barbonymus gonionotus*) treated with an insecticde convoy (Sadiqul et al., 2017). The results of this experiment demonstrated that the erythrocytes in the convoy-treated groups had nuclear changes such as binuclei (BN) and karyopyknosis (KP) blebbed (BL) notched nuclei (NT) (nuclear bridge (NB) and nuclear bud (NBd), while the erythrocytes in untreated group were oval in shape and had a regular condensed nucleus. The sequence of nuclear abnormalities in fish subjected to convoys was KP>BN>NT>BL>NB>NBd. The fact that aneuploidy can happen in *Channa punctata* was explicitly shown by treatment of this fish with agrochemicals, especially with atrazine. This aspect is found in the experimental embryonic stages of the pesticide malathion, in which atrazine treated, and symbiotic plants are also present (Nwani et al., 2014). It was found out later that the disappearance of the nuclear envelope is due to not from telophase but rather it is triggered with the onset of the nuclear mitosis from metaphase because camptothecin and leptomycin B at early mitotic stages stop it (Salmina et al., 2020). The lesser rates are found in 40–45% of the whole population, whereas the higher rates are shown in 98%. The majority of the increased counts of binucleated cells, nuclear bridge, and notched nucleus primarily appear after the splicing process of the erythrocytic nuclei and are characterized as genotoxic effects that change a stage from anempirical to toxicological (Ritu et al., 2022). Micronuclei, which are apparent as daughter nuclei in the plasma membrane of red blood cells along with to the principal nucleus, are the result of acentric chromosomes, which produce failed anaphasing towards the formation of daughter nuclei (Cherednichenko et al., 2024). Islam et al, (2019), reported that when striped catfish were exposed to sumithion concentrations of 3–6 mg/l, the frequency of micronuclei significantly increased (P<0.05). A higher frequency of micronuclei in erythrocytes signifies that NTP-induced chromosomal aberrations in *H. nobilis* have disrupted mitosis. DNA fragment mispairing after chemical exposure can also lead to an increase in micronuclei production (Fenech et al., 2011). A large number of micronuclei must increase the risk of chronic diseases and genetic abnormalities because of damaged DNA and impair chromosomes regardless of the region they are generated (Khatun et al., 2021).

However, for ecotoxicological analysis, the present research was conducted on bighead carp (*Hypophthalmichthys nobilis*), to investigate the changes in *H. nobilis* blood profiles under varying concentrations (0.02 mg/L and 0.04 mg/L) of NTP. Furthermore, no information about NTP's toxicity to freshwater carp fish species is known. Better knowledge of intra-individual factors is indicated by the current study's findings, which may also aid in freshwater organism health monitoring in the face of agricultural pesticides. With an emphasis on the genotoxic and mutagenic consequences of *H. nobilis*, this study recommends more investigation into chronic NTP exposure. Additionally, the results of this study are mainly useful for evaluating the dangers to human health that might result from consuming intoxicated fish.

Conclusion

The present study concludes that nitenpyram exposure induces changes in blood parameters of *H. nobilis*. The observed erythrocytic abnormalities, including indicators of oxidative stress, genotoxicity, and mutagenic effects, highlight the NTP detrimental impact on fish health. These hematological and cellular responses provide valuable insights into the physiological stress experienced by freshwater organisms under pesticide exposure and can serve as effective biomarkers for ecological monitoring. Furthermore, the continuous release of NTP into aquatic ecosystems raises serious environmental concerns, particularly for non-target aquatic organisms. This study underscores the need for stricter regulatory frameworks and consistent monitoring of insecticide residues in freshwater systems. It also reinforces the potential human health risk associated with the consumption of contaminated fish, emphasizing the broader implications for food safety and environmental conservation. As Pakistan continues to rely heavily on agricultural practices, the responsible management of insecticide usage is critical to mitigating ecological and public health hazards.

Ethical Statement

The ethical guidelines for animal research were strictly followed during all experimental procedures. Laborious measures were taken to reduce the animals' distress and external causes of stress, pain, and discomfort. The number of animals used in the current investigation is within the range required to provide valid scientific data. This article doesn't cite any human studies by the authors.

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Author Contribution

Adeeba Naseer & Noman Waheed: Conceptualization, Investigation; Formal Analysis, Writing -original draft, Methodology. Habiba Jamil: Visualization, Data Curation, Review and editing. Abdul Ghaffar: Supervisor, Review final draft; Ghulam Mustafa: Review final draft.

Conflict of Interest

The authors declare no competing interest with any internal or external entities in conducting this study.

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