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Fenpropathrin Induces Oxidative Stress, Inhibits Cholinesterase, and Causes Genotoxicity in *Pethia conchonius* (Hamilton, 1822)

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Abstract

Pesticide contamination in water bodies is a serious threat to aquatic organisms. Among the new generation pesticides, synthetic pyrethroids enter the aquatic environment from agricultural runoff and are more persistent in aquatic environment. In this study, we investigated the effect of fenpropathrin, a type II pyrethroid, on *Pethia conchonius*. The median lethal concentration for commercial formulation of fenpropathrin (Danitol®) was determined to be 2.43 µg/L. Based on the median lethal concentration, the fish were exposed to 1/5th (0.486 µg/L) and 1/10th (0.243 µg/L) of median lethal concentrations for 30 days. After the exposure period, antioxidant enzymes status (superoxide dismutase and catalase), oxidative stress parameters (lipid peroxidation and reduced glutathione) in brain, liver, and kidney, cholinesterase enzyme activity in brain and muscles, and incidences of micronucleus were evaluated. In the treatment groups, alteration in antioxidant enzyme levels were observed in brain, liver, and kidney. Lipid peroxidation, which is indicative of oxidative stress, was observed but did not show much variation. Reduced glutathione was also altered. Cholinesterase activity was significantly different in the brain tissues between control and treatment groups; however, no significant difference was observed between the cholinesterase activities of muscles in control and treatment groups. Micronucleus incidence in treatment groups was higher than that in the control. Our study indicates that fenpropathrin altered the antioxidative enzyme status, inhibited cholinesterase activity in brain, and exhibited potential genotoxic effects in the fish *Pethia conchonius*.

Keywords: Fenpropathrin, Genotoxicity, Oxidative Stress, *Pethia conchonius*, Pyrethroid Toxicity

1. Introduction

Insecticides are chemicals with diverse structures, and many of them are stable in environment[1]. The role of insecticides in providing crop protection is immense. Major insecticide groups include organochlorines, organophosphates, carbamates, and pyrethroids[2]. Owing to their property to persist in the environment, organochlorines and organophosphates have been replaced with new generation pesticides. One such insecticide class is pyrethroids, that have been introduced since 1980s as they exhibit high degree of effectiveness and low mammalian toxicity[3]. Pyrethroids are synthetic insecticides that are structurally similar to pyrethrins, which are derived from the plant *Chrysanthemum*[4]. Synthetic pyrethroids are more beneficial than their natural counterparts because of their photostability[5,6]. Based on the chemical structure, synthetic pyrethroids are categorized into Type I and Type II. Type II synthetic pyrethroids are more neurotoxic than Type I synthetic pyrethroids because they show affinity to voltage gated sodium channels due to the presence of a cyano group[7,8].

Fenpropathrin, a Type II synthetic pyrethroid, is used to control mites and a broad-spectrum insecticide[7]. Similar to other synthetic pyrethroids, fenpropathrin is less soluble in water but highly soluble in organic solvents and bioconcentrates in the tissues of organisms[7]. The half-life of fenpropathrin is 2.7 weeks in natural water

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bodies; its degradation in aqueous media is affected by factors such as pH and temperature[9]. Fenpropathrin is known to cause degeneration of dopaminergic neurons and induce parkinsonism in mice[10]. With fenpropathrin being detected in soil and water systems[11-13], studies on fenpropathrin affecting aquatic organisms remain highly limited.

Fish occur in diverse aquatic habitats, from freshwater to marine environments[14], and their biology can be influenced by human activities. Pollutants enter aquatic environment and can trigger oxidative stress in fish[14]. Any disturbance in the production of free radicals and antioxidant activity results in oxidative stress[1]. Superoxide Dismutase (SOD) and Catalase (CAT) are two key enzymes that are vital in neutralizing the dangers posed by the Reactive Oxygen Species (ROS), which are resultant of cellular respiration. SOD catalyzes the reaction wherein superoxide anion is dismutated to hydrogen peroxide and water, whereas CAT nullifies hydrogen peroxide to water and oxygen[1]. Lipids are membrane components, and oxidative stress results in Lipid Peroxidation (LPO), which damages the membrane[15]. Measurement of LPO by Thiobarbituric Acid Reactive Substances (TBARS) is a well-known oxidative stress marker[16]. The role of reduced Glutathione (GSH) is well known in combating ROS-mediated stress. GSH is a substrate for enzymes like glutathione peroxidase and glutathione-S-transferase[17]. GSH is converted into Glutathione disulfide (GSSG) during nullifying ROS[15]. Impairment or decline in GSH:GSSG ratio serves as a marker for oxidative stress[18]. Cholinesterase Enzyme (ChE) is a well-known biomarker of organophosphate and carbamate insecticide stress[19]. Unlike the molecular targets of organophosphates and carbamates, those of Type II synthetic pyrethroids are different; they mainly act on voltage gated sodium channels and delay their closure[8,20]. ChE assay serves as a useful biomarker in the toxicological studies involving Type II synthetic pyrethroids[21-24]. Fish are sensitive to various contaminants and act as sentinel organisms in assessing potential genotoxic compounds[25,26]. Micronucleus assay is useful to assess genotoxic damage[27]. Fish micronucleus assay is a time-efficient method in assessing compounds that exhibit genotoxicity. Fish erythrocytes are nucleated, and hence can be scored for micronucleus[25]. Fish have served as invaluable study models in evaluating toxicity of compounds. *Pethia conchonius* (Cypriniformes: Cyprinidae), popularly known as rosy barb among aquaculturists, is native to Afghanistan, Pakistan, India, Nepal, and Bangladesh[28]. Studies on rosy barb's early development[28] and gonadal differentiation[29,30] are well documented. Furthermore, the use of rosy barb as model animal in studies involving heavy metal and pesticide toxicity is well known[31-33]. Considering its potential as an experimental fish, we aimed at employing *Pethia conchonius* to assess the effects of fenpropathrin in the present study.

2. Materials and Methods

2.1 Animals

Healthy adult rosy barbs, mean standard length 3.78 ± 0.27 cm and mean weight 1.45 ± 0.3 g, were procured from a local aquarium (Fish Fair; Dharwad, India) and were transported to laboratory in aerated plastic bags. Fish were transferred to glass tanks (volume: 40 L, dimension: 60 cm × 30 cm × 30 cm) and allowed to acclimatize for 20 days. Water quality parameters such as dissolved oxygen (11.6 ± 0.536 mg/L), total hardness (225 ± 7.5 mg/L), photoperiod (12h light : 12hr dark), temperature 26 ± 0.5 °C, and pH (7.1-7.3) were regularly monitored.

2.2 Chemicals

Commercial formulation of fenpropathrin (Danitol® 10% EC; Sumitomo Chemical India Limited, India) was purchased from a local vendor.

2.3 Determination of LC50

LC50 was determined according to Organization for Economic Co-operation and Development[34]. In brief, after acclimation, the fish were randomly transferred to six different tanks, each tank having seven fish. One tank represented control, and the remaining five tanks were assigned to treatment groups i.e., 1.5 µg/L, 2.0 µg/L, 2.5 µg/L, 3.0 µg/L, and 3.5 µg/L. The test chemical was prepared in distilled water and diluted to desired concentration. The feeding of the fish was stopped 24h prior to the start of the experiment. The experiment was conducted thrice to check the consistence of results. The LC50 was calculated by probit analysis[35].

2.4 Sublethal Experiment

Fish (n = 7) were exposed to 0.486 µg/L and 0.243 µg/L representing 1/5th and 1/10th of median lethal concentration, respectively, for 30 days. During the

experiment, the fish were fed with pellets (Taiyo fish feed, Chennai, India), and the tanks were aerated for 30 min twice daily. The fish were monitored carefully, and no mortality was observed during the study period.

2.5 Total Protein

Total protein in the brain, liver, and kidney tissues of fish were determined[36].

2.6 Oxidative Stress and Antioxidant Parameters

At the end of the sublethal exposure period, fish were euthanized using 2-phenoxyethanol. The fish were dissected, and their brain, liver, and kidney were used to measure SOD, CAT, LPO, GSH. The tissues were weighed and homogenized in cold phosphate buffer (pH 7.4). The homogenate was centrifuged (MPW-350R, MPW Med. Instruments, Warsaw, Poland) at 10,000 rpm for 10 min at 4°C, and postmitochondrial supernatant was used for the estimation of total protein and TBARS. The supernatant was further centrifuged at 15,000 rpm for 1 h at 4°C, and this supernatant was used for the determination of SOD, CAT, and reduced GSH.

SOD activity was determined according to the previous described methods[37], which is based on the ability of SOD to inhibit the auto-oxidation of epinephrine to adenochrome at alkaline pH. Briefly, 25-µL supernatant from the homogenate was added to a mixture of 0.1mM epinephrine in carbonate buffer (pH 10.2) in a total volume of 1 mL, and the formation of adenochrome was measured at 295 nm using a spectrophotometer (UV-VIS Spectrophotometer AU 2702; Systronics, Ahmedabad, India). CAT was assayed according to the previous methods[38]. The assay mixture consisted of 1.95mL 0.05 M phosphate buffer (pH 7.0) and 1.0mL 0.019 M hydrogen peroxide to which 50 µL of homogenate (10% w/v) was added. Changes in the absorbance were recorded at 240 nm at 1 min intervals using a spectrophotometer (AU 2702) and was calculated as nM H_2O_2 consumed/min/mg protein. Levels of TBARS for LPO were evaluated according to previous methods, with modification[39] with modifications. A volume of 0.5 mL 10% homogenate containing 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 5 N HCl was incubated for 15 min. The mixture was cooled and centrifuged, and the absorbance was measured at 512 nm against blank using a spectrophotometer (AU 2702). The amount of LPO was expressed as nmoles TBARS/mg protein.

Reduced GSH was estimated accordingly[40]. Hundred microliters of homogenate or pure GSH was added to 1.0 mL 0.2 M Tris-EDTA buffer (pH 8.2) and 0.9 mL of 20 mM EDTA (pH 4.7) that was followed by addition of Ellman's reagent (10 mmol/L DTNB in methanol). The samples were incubated at room temperature for 30 min, and the absorbance was recorded at 412 nm using a spectrophotometer (AU 2702).

2.7 Cholinesterase Assay

ChE assay was performed[41]. In brief, brain and muscle tissues were dissected, weighed, and homogenized in Tris HCl buffer (pH 8). The homogenate was centrifuged (MPW 350R) at 10,000 rpm for 8min. The supernatant was used for determining the ChE activity. The reaction mixture contained 3 mL DTNB, 20 µL supernatant, and 100 µL acetylthiocholine iodide. The absorption of the reaction mixture was measured at 405 nm (AU 2702). The activity was expressed in µmol/min/g (wet weight).

2.8 Micronucleus Assay

The micronucleus assay was performed[42]. The caudal peduncle of euthanized fish was severed, and the blood was immediately smeared on clean glass microslides. The smear was air-dried, fixed in absolute ethanol for 10 min, and again air-dried. The slides were stained with Giemsa stain for 20 min. The slides were washed to remove excess stain and were again allowed to dry. The slides were cover slipped, and a total of 2000 erythrocytes were counted from each fish under a microscope (100x; CX 43, Olympus, Japan). The erythrocyte nucleus showing small spherical structure and sizing nearly one-third of nucleus was considered as micronucleus[27].

2.9 Statistical Analyses

The datasets were checked for normality. We used one-way ANOVA with Tukey's post hoc test for data that showed normal distribution, whereas the data deviated from normality were assessed using Kruskal–Wallis test with Dunn's test. A p-value < 0.05 was considered as statistically significant. All the statistical tests were performed in Paleontological Statistics program (PAST; version 4.05)[43].

3. Results

3.1 Acute Toxicity

The 96-h LC50 for fenpropathrin was 2.438 µg/L. Mortality was observed to be dose-dependent (Table 1). The control group and the 1.5 µg fenpropathrin/L treatment group showed no mortality throughout the 96h, whereas the 3.5 µg fenpropathrin/L treatment group showed 100% mortality by 48 h. During the 96-h exposure, the fish in the treatment groups started displaying behavioral changes. The behavioral changes included altered swimming (in circles), increased gill ventilation, and excess mucus secretion.

Table 1. Mortality data of *Pethia conchonius* exposed to various concentrations of fenpropathrin for 96 h

Fenpropathrin concentrations, µg/L	Cumulative mortality at different exposure period (n = 7)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
1.5	0	0	0	0
2.0	0	0	1	1
2.5	0	1	3	4
3.0	2	3	6	6
3.5	5	7	7	7

3.2 Total Protein

In all the three tissues, total protein in 0.243 µg fenpropathrin/L and 0.486 µg fenpropathrin/L was significantly ($p < 0.05$) lower than that of the control (Table 2).

Table 2. Levels of total protein (mg/mL) in different tissue of *Pethia conchonius* exposed to fenpropathrin

	Control	0.243 µg Fenpropathrin/L	0.486 µg Fenpropathrin/L
Brain	268.31 ± 6.27^{a}	176 ± 5.15^{b}	123.6 ± 4.09^{c}
Liver	373.18 ± 2.39^{a}	213.95 ± 1.12^{b}	221.96 ± 2^{c}
Kidney	242.41 ± 7.2^{a}	174.5 ± 3.18^{b}	213.95 ± 3.97^{c}

Groups not sharing the same superscript indicate significant differences ($p < 0.05$), groups sharing the same superscripts indicate insignificant difference ($p > 0.05$). Values are mean ± SE.

3.3 Oxidative Stress and Antioxidant Parameters

Tables 3, 4, and 5 show the results of SOD, CAT, TBARS, and GSH in brain, liver, and kidney tissues of rosy barb after fenpropathrin exposure. SOD was significantly decreased ($p < 0.05$) in liver tissues of 0.243 µg fenpropathrin/L and 0.486 µg fenpropathrin/L treatment groups compared with that in control. In kidney tissues, SOD activity was decreased significantly ($p < 0.05$) in 0.486 µg fenpropathrin/L treatment group compared with that of control, whereas in brain the SOD activity was significantly increased ($p < 0.05$) in 0.243 µg fenpropathrin/L and 0.486 µg fenpropathrin/L treatment groups compared with that of control. The CAT activity was elevated in all three tissues, wherein significant difference ($p < 0.05$) was observed between 0.486µg fenpropathrin/L treatment group and control in brain and between 0.243 µg fenpropathrin/L treatment group and control in kidney; however, no significant differences ($p > 0.05$) in CAT activity were observed in liver tissues. TBARS remained high in the three tissues of 0.243 µg fenpropathrin/L and 0.486 µg fenpropathrin/L treatment groups compared with that of control. There were significant differences ($p < 0.05$) between the TBARS of treatment groups and control in brain tissue. Significant difference ($p < 0.05$) in TBARS were observed in kidney tissue of 0.486 µg fenpropathrin/L and control, whereas no significant differences ($p > 0.05$) in TBARS levels were observed in liver. The levels of reduced GSH showed variation, wherein reduced GSH levels in brain were decreased in all treatment groups compared with that of control, and significant differences ($p < 0.05$) were observed between 0.243 µg fenpropathrin/L treatment group and control. In liver, there was a decrease in the levels of reduced GSH, and no significant differences ($p > 0.05$) were observed across all treatment groups. In kidney, the reduced GSH levels were elevated in 0.486 µg fenpropathrin/L treatment group, and the GSH level this treatment group showed significant difference ($p < 0.05$) with that of the control.

Table 3. Levels of antioxidant enzymes, and oxidative stress in the brain tissue of *Pethia conchonius* exposed to fenpropathrin

Parameter	Control	0.243 µg Fenpropathrin/L	0.486 µg Fenpropathrin/L
SOD, U/mg protein	88.5 ± 6.35[a]	134.64 ± 1.53[a]	168.2 ± 6.26[b]
CAT, U/mg protein	0.683 ± 0.015[a]	0.715 ± 0.011[a]	2.486 ± 0.0906[b]
TBARS, nmoles TBARS/mg protein	66.116 ± 1.299[a]	172.57 ± 6.664[b]	314.87 ± 8.051[c]
Reduced GSH, nmoles/mg protein	37.033 ± 0.328[a]	33.3 ± 0.776[b]	36.3 ± 1.216[a]

Groups not sharing the same superscript indicate significant differences ($p < 0.05$), groups sharing the same superscripts indicate insignificant difference ($p > 0.05$). Values are mean ± SE. SOD, superoxide dismutase; CAT, catalase; TBARS, Thiobarbituric acid reactive substances; GSH, glutathione.

Table 4. Levels of antioxidant enzymes, and oxidative stress in liver tissue of *Pethia conchonius* exposed to fenpropathrin

	Control	0.243 µg Fenpropathrin/L	0.486 µg Fenpropathrin/L
SOD, U/mg protein	265.3 ± 22.89[a]	81.6 ± 3.17[b]	157.69 ± 26.02[c]
CAT, U/mg protein	0.356 ± 0.013[a]	0.52 ± 0.077[a]	0.603 ± 0.024[a]
TBARS, nmoles TBARS/mg protein	122.5 ± 2.223[a]	225.3 ± 7.057[a]	224.76 ± 3.316[a]
Reduced GSH, nmoles/mg protein	59.36 ± 1.604[a]	26.56 ± 0.523[a]	32.7 ± 6.08[a]

Groups not sharing the same superscript indicate significant differences ($p < 0.05$), groups sharing the same superscripts indicate insignificant difference ($p > 0.05$). Values are mean ± SE. SOD, superoxide dismutase; CAT, catalase; TBARS, Thiobarbituric acid reactive substances; GSH, glutathione.

Table 5. Levels of antioxidant enzymes, and oxidative stress in kidney tissue of *Pethia conchonius* exposed to fenpropathrin

	Control	0.243 µg Fenpropathrin/L	0.486 µg Fenpropathrin/L
SOD, U/mg protein	166.066 ± 6.06[a]	83.38 ± 3.38[b]	134.41 ± 3.702[a]
CAT, U/mg protein	0.687 ± 0.048[a]	0.539 ± 0.015[b]	0.735 ± 0.048[a]
TBARS, nmoles TBARS/mg protein	25.88 ± 0.611[a]	51.83 ± 1.924[a]	76.46 ± 2.817[b]
Reduced GSH, nmoles/mg protein	19.71 ± 0.519[a]	19.46 ± 0.249[a]	27.22 ± 0.263[b]

Groups not sharing the same superscript indicate significant differences ($p < 0.05$), groups sharing the same superscripts indicate insignificant difference ($p > 0.05$). Values are mean ± SE. SOD, superoxide dismutase; CAT, catalase; TBARS, Thiobarbituric acid reactive substances; GSH, glutathione.

3.4 Cholinesterase Assay

The results of ChE assay in brain and muscle are shown in Figure 1. Significant differences were observed between

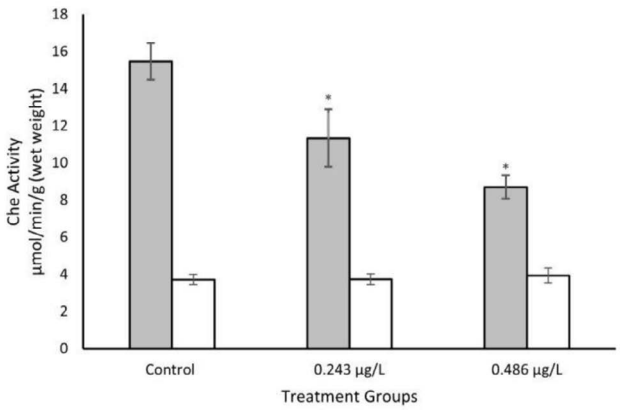

Figure 1. Cholinesterase activity in brains and muscles of rosy barbs treated with fenpropathrin. Grey and white columns indicate brain and muscle cholinesterase activities, respectively. * indicates significant difference between the control and treatment groups $p < 0.05$.

the enzyme activities in brains of control and treatment groups ($p < 0.05$). The treatment group, 0.486 µg fenpropathrin/L showed 43.73% less ChE activity than that of the control, whereas 27.8% of reduced enzyme activity was observed in 0.243 µg fenpropathrin/L treatment group. However, the ChE activity in the fish muscles of control and treatment groups did not vary significantly ($p > 0.05$); nevertheless, a slight increase in the activity was noticed in 0.486 µg fenpropathrin/L treatment group.

3.5 Micronucleus Assay

The frequency of micronucleus incidences is shown in Figure 2. There was a significant difference between micronucleus incidences in the fish of control and treatment groups ($p < 0.05$). Fish exposed to 0.486 µg fenpropathrin/L showed 5.65 times more micronucleus incidence compared to control, while 0.243 µg fenpropathrin/L showed 4.57 times more micronucleus occurrence per 2000 cells compared with that of control. The control group fish exhibited the lowest number of micronucleus.

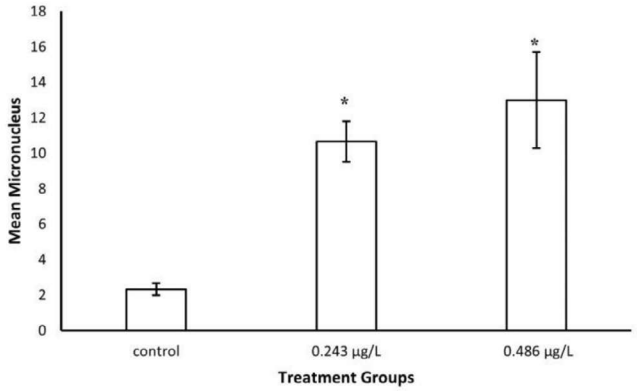

Figure 2. Mean micronucleus in control and fenpropathrin treatment groups. * indicates significant difference between the control and treatment groups $p < 0.05$.

4. Discussion

4.1 Acute Toxicity of Fenpropathrin

In the current study, the results show that fenpropathrin is toxic to the fish system. The LC50 of fenpropathrin to rosy barbs observed in our study is in the range similar to those observed in the previous studies on fishes. Acute toxicity of fenpropathrin for Chinese grass carp and zebrafish is 3.589 µg/L and 5.6 µg/L, respectively[44,45]. The 96-h LC50 of fenpropathrin in *Alburnus mosulensis* was found to be 121.38 ± 11.84 µg/L[46]. In a risk assessment study, 0.220 µg/L of fenproapthrin in surface waters of Lebanon[11]. Based on the observed environmental concentrations and laboratory acute toxicity study, presence of fenpropathrin in waterbodies will adversely affect the fish biology.

4.2 Total Protein

Proteins are important biomolecules as they are involved in regulating cell functions, such as structural components, a source of nitrogen metabolism and source of energy under chronic stress[47]. In our study, the total protein levels were reduced in brain, liver, and kidney tissues. Furthermore, reduction in the total protein levels was more pronounced in liver tissue, wherein the fenpropathrin treatment groups exhibited the lowest total protein levels. In a study involving cypermethrin-induced acute toxicity, total protein was reduced in brain, gills, liver, and muscle tissues of *Tor putitora*[47]. As proteins are major regulators of cell activity, their decline in cell can affect physiological processes, which was evident in the alteration in activity of antioxidant enzymes and ChE enzyme.

4.3 Oxidative Stress and Antioxidant Parameters

The primary role of antioxidant enzymes is to combat oxidative stress that arises when an organism's capacity to douse the ROS is overwhelmed[14]. Any imbalance between the oxidative stress and antioxidant defense can perturb fish physiology in their adaptation to aquatic environment; pyrethroids are known to cause oxidative stress toxicity in gills, liver, muscles, and kidney of fish[1]. In the present study, SOD activity was elevated in brain, whereas its activity was decreased in liver and kidney. The increased SOD activity in brain may be due to dismutation of oxygen radical[48], whereas the decline in SOD activity in liver and kidney is probably due to inhibition of SOD activity by excess production of ROS[49]. Furthermore, the antioxidant system's response to oxidative stress in different tissues varies owing to tissue-specific antioxidant potentials[50], and this can be attributed to the differences in the SOD activity in brain, liver, and kidney. Different tissues have different antioxidant capacities, distinct sensitivity in oxidative damage, and varied rates of free radical generation[51]. A study showed decreased SOD activity in the liver and kidney tissues of *Oreochromis niloticus* exposed to deltamethrin[52]. In another study, the

hepatic SOD levels decreased in common carp exposed to commercial formulation of fenpropathrin, Danitol®[53]. Our findings agree with those of previous studies. CAT defends against oxidative stress by eliminating hydrogen peroxide, which is produced in mitochondrial matrix[54], to water and oxygen[47]. In our study, fenpropathrin exposure caused elevated CAT activity in brain, liver, and kidney tissues of rosy barb. Such an increase in the CAT activity in fish exposed to xenobiotics has been reported in several studies[15,47,50,55]. The increase in CAT activity in the three tissues tested in our study could be due to higher oxyradical production[55] caused by increased oxygen consumption during the elimination of hydrogen peroxide[56]. In a study, increase in whole body CAT activity was observed in *Alburnus mossulensis* when exposed to fenpropathrin[46].

LPO has widely been used as an indicator of oxidative damage[49]. Estimation of TBARS formed in LPO is widely used as marker for oxidative stress[16]. In our study, TBARS levels were high in brain, liver, and kidney in all tested concentrations compared with that of control. Oxidation of oxygen to superoxide radicals may result in high LPO[57]. Cypermethrin, belonging to the same class as fenpropathrin, toxicity in fish results in LPO[47,48]. GSH donates an electron to ROS, thereby neutralizing and preventing risk posed by ROS, forming GSSH; thus, its activity in the cell is a well-established biomarker for assessing oxidative stress[15]. In a study, no significant alterations in the levels of hepatic reduced GSH upon exposure to cypermethrin in *Prochilodus lineatus*[17]. In the present study, reduced GSH levels were not significantly altered in the tissues of fenpropathrin exposed fish than that of the tissues of unexposed fish. However, studies on fenpropathrin-mediated oxidative stress on fish systems are limited; hence, further studies are required for more integrated understandings in its action.

4.4 Effect of Fenpropathrin on Cholinesterase Activity

ChE enzymes, which include acetylcholinesterase and butyrylcholinesterase, occur in the nervous systems of vertebrates and invertebrates[58]; its occurrence is primarily in neuromuscular junctions and erythrocyte membranes[19]. In the present study, we observed ChE inhibition in brain but not in muscles for the tested concentrations. Acetylcholine is released from the vesicles of presynaptic neuron into the synapse, binds to receptors of postsynaptic neuron, and stimulates it leading to the generation of nerve impulses. Acetylcholinesterase (AChE) regulates this critical step by hydrolysis of acetylcholine to acetic acid and choline[58], thus preventing prolonged binding of acetylcholine on postsynaptic nerve receptors. Inhibition of AChE leads to the accumulation of acetylcholine in synapse causing an influx of sodium ions. Additionally, Type II synthetic pyrethroids like fenpropathrin delay the closing of sodium ion channels, which leads to higher accumulation of sodium ions resulting in the generation of multiple nerve impulses, buildup of acetylcholine in nerve synapses, decreased cholinergic transmission, and neurotoxic effects[59]. Previous studies on the effect of fenpropathrin on the ChE activity in fishes are scarce. A reduction in acetylcholinesterase activity after 15 d upon exposure of fenpropathrin was observed in *Alburnus mosulensis*[46]. In an another study no inhibition in ChE activity in the muscles of catfish *Ancistrus multispinis* following a 96-h exposure to another type II insecticide deltamethrin[22]. Similarly, no apparent change in the acetylcholinesterase activity in the muscle and brain tissues of common carp after a 30-d exposure to deltamethrin[60]. In our study, there was no inhibition of ChE in the muscle tissues of rosy barb, but fenpropathrin inhibited ChE activity in brain tissue.

4.5 Fenpropathrin-Induced Micronucleus

Acentric chromosome fragments or whole chromosomes that fail to be included into the nucleus are termed micronucleus[25]. This failure is due to the absence of centromere or damage to mitotic spindle[61]. With respect to fenpropathrin, there are no studies reporting its micronucleus induction property in fish. However, few studies on fenpropathrin inducing micronucleus in mammalian systems are available. Intraperitoneal injection of doses 27, 53, and 105 mg fenpropathrin/kg body weight did not significantly alter micronuclei induction among mice bone marrow cells[62]. Fenpropathrin induced a significant increase in micronucleus and micronucleated binucleated cells of human whole blood cell cultures at 10 and 50 µg/mL dose; however, the formation of micronucleus and micronucleated binucleated cells was not dose dependent[63]. In our study, we noticed a significant increase in micronucleated cells with the increase in fenpropathrin concentration. Our study gives the first report of micronucleus induction by fenpropathrin in the fish systems.

In our study, we exposed sublethal concentrations of fenpropathrin to rosy barbs and studied its chronic effects on protein levels, oxidative stress, ChE activity, and genotoxicity. We found that fenpropathrin reduced the total protein levels, severely altered the antioxidant enzymes and induced oxidative stress, inhibited brain ChE activity, and induced nuclear abnormalities. Furthermore, in the present study, the tested sublethal concentration of fenpropathrin (0.243 µg fenpropathrin/L) was similar to the levels detetcted in the environment (0.22 µg fenpropathrin/L), the results show that fenpropathrin affected the antioxidant enzymes, affected ChE activity, and induced the micronuclear erythrocytes. Surface waters are frequently contaminated with pyrethroid pesticides. As observed in our study, severe alterations in the fish biochemistry could lead to physiological changes in the organisms. This would reflect in the overall health, leading to a decline in the fish population.

5. Conclusions

Our study shows that fenpropathrin is toxic to rosy barb at the concentrations tested. Based on acute toxicity, the commercial formulation of fenpropathrin, Danitol®, is highly toxic, induces oxidative stress, inhibits ChE activity in brain, and induces micronucleus in erythrocytes. As fenpropathrin is a new class of Type II synthetic pyrethroid, studies remain limited; further studies addressing susceptibility of fish at different life stages *viz.* juvenile, mature, and reproductive stage, and its possibility of being an endocrine disruptor are required.

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