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Dimethoate Mediated Some Behavioural, Hematological and Histopathological Changes in Channa Punctatus

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Abstract: ***The present study investigated some behavioural, hematological and histopathological changes caused by sublethal concentration (LC_{50} value= 5.56 µg/l) of dimethoate in Channa punctatus. No adverse behavioral and physiological changes were observed in the control group, but the pesticide exposed group exhibited erratic swimming, rapid opercula movement, and release of bubbles, frequent surfacing, loss of equilibrium (somersaulting) and sudden jerk movement. The RBC reduced significantly (p<0.05). WBC significantly (p<0.05) increased by 4.72% and 9.23% in test Groups C and D, respectively. Contrariwise, mean hemoglobin (Hb) values reduced significantly (p<0.05) by 5.11%, 19.23% and 28.24% in Groups B, C and D, respectively compared to the control. Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean cell volume (MCV) followed the same pattern as Hb and PCV with the lowest values in the highest pesticide concentration. Exposure group showed a disorganization of intra-cardiac chamber structure, and saw-toothed intra-cardiac chamber muscles with loss of intra-cardiac muscle fiber. Damage to the cardiac muscles was worse in group D with focal loss of cardiac muscle and intra-cardiac hemorrhage. Spongiosis and infiltration of inflammatory cells (microglial cells) were observed in the brain of exposure group. In the gills, histological damages with distortion of gill epithelium, lifting of gill epithelium from the gill stock, disorganization and fragmentation of parts of the gill. Aggregation of inflammatory hepatocytes, and necrosis and distortion of hepatic architecture were observed in the pesticide exposed groups. The kidneys of the fish were exposed to different concentrations of the pesticide showed distortion of cell architecture, fragmentation, loss of renal tissues and intra-renal hemorrhages (bleeding in the renal cells). Histology of the fin of exposed fish showed fragmentation in the fin and separation from the attached muscles.***

Keywords: Dimethoate pesticide, *Channa punctatus*, Hematology, Histopathology

1. Introduction

Chemical contamination found everywhere in nature. Among these, pesticides are the most common type of aquatic ecosystem contaminants. Exposure of pesticide influences many behavioural and physiological functions of fish. It greatly affects fish production and human health too through ecological cycling and biomagnifications. Different researches of morphology, physiology and biochemistry of different species of fishes have shown that pesticides affect different physiological functions of fish depending species sensitivity concentration and exposure time. Fish assimilates pesticides through gills and contaminated food. Gills are the principal channels of pesticide penetration. Pesticide effects on fishes are numerous and varied. They cause mortality by starvation (indirectly by destroying organisms they feed on), effect hatching growth rate, can lead to malformation during embryogenesis, effect reproductive rate, modify enzyme activity and cause histopathological changes in various organs.

Dimethoate ($C_5H_{12}NO_3PS_2$) is a synthetic organo-triphosphate compound widely used in agriculture to control broad range of insects. The mode of action of the pesticide is phosphorylation of serine residues of the active site of acetylcholinesterase (AChE), which results to inhibition of AChE [1] and subsequent overstimulation of effecter organs by acetylcholine. Ultimately, nervous system failure, impairment of the respiratory myocardial and neuromuscular transmissions and death occur [2]. Acute exposure to pesticide resulted in reduced fish populations and increased mortality [3]. Chronic exposure to small amounts of pesticide increased the incidence of disease, stress, and behavioral disorders [4]. Pesticide bioaccumulation causes a major danger—bioaccumulation factor of cypermethrin in fish is 1200 times [5]. Despite being banned in India, these pesticides are frequently sprayed on rice paddies along river floodplains in Ganganagar, Rajasthan. Incidentally, these floodplains also serve as breeding sites for some economically important fishes such as *Channa punctatus*. Over 99% of applied pesticides remain in the ecosystem [6] and through run-offs and atmospheric dropout, these can reach surface waters close to agricultural lands where fish breed [7]. [8] documented that the pesticides used in aquaculture farms were accumulated in farm sediment during fish production period in Kolleru Lake. Reports have shown that pesticides affect fish at ecosystem, population, organismal and sub-organismal (system and organ functions) levels [9]. Government of India has banned its frequent use in agriculture in 2011. Therefore, this study aims at evaluating the effects of dimethoate pesticide on the behavior, hematology and histopathology of *Channa punctatus*.

2. Materials and Methods

(a) Experimental fish and chemicals

Juvenile *C. punctatus* (mean weight of 9.4±0.20 g and mean total length of 11.8±0.27 cm) procured from a fish farm were acclimated for two weeks in the laboratory before commencement of the experiment. During acclimation, the fish were fed daily with commercial fish food at 2% of their body weight. The pesticide used for the experiment is a

commercial formulation of dimethoate (250 g/L) with the trade name "Rogor" [Figure 1].

Figure 1: *Channa punctatus*

(b) Experimental design

After the acclimation period, series of tests to determine the range of toxicant concentrations that produced a targeted range of effects were conducted. Thereafter, a static lethal toxicity assay was conducted to determine the 96-hour LC_{50}. The choice of static non-renewal experimental design was based on the knowledge that the half-life of most synthetic pesticides is longer than 48 hours and the desire to accommodate time-dependent degradation of them. Four different concentrations of pesticides, No pesticide (Group A or control group) 0.75 µg/L pesticide (Group-B), 1.13 µg/L pesticide (Group-C) and 5.56 µg/L (Group D) of dimethoate pesticide were prepared. The experiment was conducted in triplicate glass tanks (60×30×30 cm) containing 10 fish each in 20 L of water for each group. At the end of the 96-hour experiment, the LC_{50} was computed and three different concentrations were used to test the sublethal effect of the toxicant.

Throughout the duration of the experiment, the fish were visually monitored for behavioral changes such as erratic swimming, standing erect, somersaulting, air gulping, sudden jerk movement, rapid opercula movement and release of bubbles. These were virtually assessed daily by subjective comparison between test groups and control.

(c) Hematological analysis

At the end of the 96-hour experiment, blood from five different fish from each tank was collected from the caudal fin using heparinized syringe and transferred to EDTA tubes. Hematological analysis of red blood cell (RBC), packed cell volume (PVC), hemoglobin (HB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean cell volume (MCV) was performed using hematology analyzer.

(d) Histopathological analysis

Fishes were euthanized and the liver, kidney, brain, gill and fin were collected for histological analysis.

- Fish organs were fixed in 10% normal saline and then dehydrated using alcohol series of 70%, 90% and 100% alcohol for ten minutes each.
- They were dried by immersion in three changes of xylene for 10 minutes each.
- They were impregnated in paraffin wax in a hot oven at a temperature of 60 °C.
- Thereafter, blocks were made, sectioned at 5 µm thickness using a rotary microtome, rehydrated in distilled water and stained with Hematoxylin-Eosin (H-E).
- The rehydrated sections were examined and micrographed under compound microscope.

(e) Statistical analysis

The statistical difference between test groups was estimated with analysis of variance (ANOVA) using Statistical Programme for Social Sciences (SPSS) software, version 23 in order to ascertain the level of significance.

3. Results

Table 1: Physico-chemical analysis of water as per standard method of APHA (2005) and Trivedy and Goyal (1986)

SN	Parameters of water	Amount figure
1	pH	7.40 ± 0.1
2	Temperature	23 ± 2 °C
3	TDS	378 ± 2.0 mg/L
4	Electrical Conductivity	755.0 ± 0.5 micro mho/cm
5	DO	8.0 ± 0.3 mg/L
6	Total hardness	320.0±0.7 mg/L
7	Turbidity	8 ± 0.1 ntu
8	Chloride	40 ± 0.1 mg/L
9	Nitrate	1.12 ± 0.1 mg/L
10	Sulphate	Trace

Acute toxicity: The percentage mortality in the exposed fish varied from 0 in the control group to 100 in Group IV. The 96-hour LC_{50} value derived from probit method was equivalent to 5.56 µg/L dimethoate.

Behavioral Results

No adverse behavioral change, discoloration and no mortality was observed in the control group. The fish in the different concentrations of the pesticides (Group B,C and D) exhibited erratic swimming, rapid opercula movement, release of bubbles, frequent surfacing (gulping of air), loss of equilibrium (somersaulting) and sudden jerk movement (attempts to jump out of the tanks). The severity of these pathological behavioral changes was concentration and time dependent.

Table 2: Behavioural changes in *Channa* exposed to different concentrations of dimethoate

Behavioural changes↓	Group A				Group B				Group C				Group D			
Hours →	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Erratic swimming	0	0	0	0	0	0	0	0	+	+	+	++	+	+	++	++
Rapid opercula movement	0	0	0	0	0	+	+	+	+	+	+	+	++	++	++	++
Release of bubbles	0	0	0	0	0	0	0	0	+	+	+	+	++	++	++	++
Gulping of air	0	0	0	0	+	+	+	+	+	+	0	0	+	+	++	++
Loss of equilibrium (somersaulting)	0	0	0	0	0	0	0	0	+	0	0	++	+	+	++	++
Sudden jerk movement	0	0	0	0	0	0	+	+	+	+	+	++	+	+	++	++

Denotes: 0=No effect, + =Low effect, ++ = High effect

Hematological Results

The red blood cells count (RBC) of the control group showed a mean value of 3.73×10^{6} mm^{-3}. The RBC in Groups B, C and D reduced significantly ($p<0.05$) by 0.93, 11.77 and 21.34%, respectively. A mean white blood cells count (WBC) of 5.53×10^{3} mm^{3} was observed for the control group, while comparatively significant ($p<0.05$) increases of 4.72% and 9.23% were observed in test Groups C and D, respectively. Contrariwise, mean hemoglobin (Hb) values reduced significantly ($p<0.05$) by 5.11%, 19.23% and 28.24% in Groups B, C and D, respectively compared to the control. Consistent with Hb, PCV decreased significantly ($p<0.05$) in a concentration dependent manner, with the lowest mean value (25.00±0.58%) recorded for the highest concentration (Group D). Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean cell volume (MCV) followed the same pattern as Hb and PCV with the lowest values in the highest pesticide concentration.

Table 3: Changes in hematological parameters in pesticide exposed group

SN	Parameters↓	Control group	Exposure groups		
		Group A	Group B	Group C	Group D
1	RBC (Per/mm^3)	3.84×10^{6}	3.45×10^{6}	3.379×10^{6}	2.764×10^{6}
2	WBC (Per/mm^3)	5.820×10^{3}	6.093×10^{3}	6.137×10^{3}	6.36×10^{3}
3	Hb (gm/100ml)	7.70±0.52	7.15±0.53	6.21±0.51	5.49±0.52
4	PCV (%)	32.5±0.5	32.3±0.5	31.8±0.5	30.9±0.5
5	MCHC (g/dl)	31.70 ±0.15	30.90 ±0.15	30.80±0.15	30.20±0.15
6	MCH (pg)	31.80 ±0.52	30.90 ±0.52	30.70±0.52	30.10±0.52
7	MCV (fl)	95.00±0.25	95.00±0.25	93.00±0.25	92.00±0.25

*P>0.05 means that no effect was observed in control group but value is statistically significant whereas in exposure group value of p<0.05 significantly different.

Histopathological Response

Histopathological examination showed normal cardiac muscles and cardiac chambers in the heart of Channa fish in the control group. Group B showed a disorganization of intra-cardiac chamber structure, while group C showed saw-toothed intra-cardiac chamber muscles with loss of intra-cardiac muscle fiber. Damage to the cardiac muscles was worse in group D with focal loss of cardiac muscle and intra-cardiac hemorrhage.

There was no evidence of damage in the brain of fish in the control group but spongiosis and infiltration of inflammatory cells (microglial cells) were observed in group B. Group C showed a focal area of liquefactive necrosis with infiltration of inflammatory cells. In group D, there was severe distortion of the brain architecture, focal area of liquefactive necrosis and loss of brain tissues in the area.

In the gills, histological damages were distortion of gill epithelium, lifting of gill epithelium from the gill stock, disorganization and fragmentation of parts of the gill. Normal hepatocytes were observed in the liver of the control group but aggregation of inflammatory cells, and necrosis and distortion of hepatic architecture were observed in the pesticide exposed groups. The kidney of *C. punctatus* exposed to different concentrations of the pesticide showed distortion of cell architecture, fragmentation, loss of renal tissues and intra-renal hemorrhages (bleeding in the renal cells). The severity increased as the concentration of the toxicant increased. Histology of the fin of fish from group B showed fragmentation in the fin and separation from the attached muscles, while the fin from group C shows focal destruction and loss of fin components.

Table 4: Histopathological responses in pesticide exposed group

SN	Parameters	Group A	Group B	Group C	Group D
1.	Heart	normal cardiac muscles and cardiac chambers in the heart	disorganization of intra-cardiac chamber structure	Saw-toothed intra-cardiac chamber muscles with loss of intra-cardiac muscle fiber	Damage to the cardiac muscles was worse with focal loss of cardiac muscle and intra-cardiac hemorrhage
2.	Brain	No evidence of damage	Spongiosis and infiltration of inflammatory cells (microglial cells) were observed	Focal area of liquefactive necrosis with infiltration of inflammatory cells	Severe distortion of the brain architecture, focal area of liquefactive necrosis and loss of brain tissues in the area
3.	Gills	Normal gills	Histological damages were distortion of gill epithelium,	Lifting of gill epithelium from the gill stock,	Disorganization and fragmentation of parts of the gill
4.	Liver	Normal hepatocytes	Aggregation of inflammatory	Necrosis and distortion of	Necrosis and distortion of hepatic

		were observed	liver cells	hepatic architecture were observed	architecture were observed
5.	Kidney	Normal	Distortions of cell architecture, fragmentation were observed.	Loss of renal tissues and intra-renal hemorrhages (bleeding in the renal cells).	The severity increased as the concentration of the toxicant increased
6.	Fins	Normal	Mild tissue damage	Focal destruction and loss of fin materials or components	Loss of fin component

4. Discussions

The LC_{50} of the pesticide is equivalent to 5.56 µg/L of dimethoate. These values are within the lower limits when compared to previous reports on the toxicity of pyrethroid compounds to different fish species. For instance, Saha and Kaviraj [12] reported 0.67 µg/L for *H. fossillis.* Velmurugan et al. [13] reported 100.4 µg/L for *C. gariepinus* for cypermethrin. Dogan and Can [14] reported an LC_{50} of 7.35 mg/L for dimethoate for *O. mykiss.* Similarly, Fai et al. [15] observed a higher mortality in *O. niloticus* exposed to a mixture of cypermethrin and dimethoate than in either chemical. The observed abnormal behaviors were similar to reported toxicity response of other fish species to either cypermethrin or dimethoate [16]. Behavioral changes were analogous to *Oncorhynchus mykiss* and *Heteropneustes fossilis*, respectively exposed to dimethoate and cypermethrin [17]. Ariful et al. [18] reported abnormal behaviors such as erratic jerky swimming, frequent surfacing movement with gulping of air, secretion of mucus on the body and gills were observed in response to the increasing exposure concentrations. Histopathological alterations of liver, gill and muscle tissues were demonstrated as vacuolization in hepatocytes, congestion of red blood cells (RBCs) in hepatic portal vein; deformed secondary lamellae and disintegrated myotomes with disintegrated epidermis in African cat fish (*C. gariepinus*). These are similar to hematological toxicity of endosulfan and phosphamidon on *Barbus conchonius* [19]. Narra [20] observed WBC increase in *C. butrachus* exposed to dimethoate. Narra suggested that in the presence of toxicants, leucopoiesis may increase thus elevating WBC. However, Velisek et al. [21] reported that a higher concentration of 3.4 µg/L of cypermethrin had no effect on the WBC of *O. mykiss*. Impliedly, the higher the level of intoxication, the higher the amount of WBC released, which is purely a defense mechanism against the toxicant. It is therefore clear that dimethoate exert toxic effect on the physiological functions of fish.

Histopathological damages to the fish exposed to dimethoate were disorganization of intra cardiac chamber structure, saw-toothed intra-cardiac muscle, loss of muscle fiber and cardiac hemorrhage. Such changes will impair the functions of the heart. Brain damages were exhibited as spongiosis, infiltration of inflammatory cells, focal area of liquefactive necrosis and distortion of brain architecture. Distortion of gill epithelium, lifting of gill epithelium from gill stock, disorganization and fragmentation (desquamation) were observed in the gills of the exposed fish. These damages though commonly associated with pesticide toxicity [22], appeared more severe. Histopathological damages would impair the normal functioning of the gill, including its capacity to extract oxygen from the aquatic environment and several metabolic activities, which could lead to respiratory failure and death. The liver is the primary organ of detoxification and elimination of xenobiotics, which predisposes it to the toxicity of these toxicants. Observed hepatotoxic effects are consistent with available reports on the toxicity of pesticides to fish [23]. Tubular necrosis, distortion of renal tissues and intra-renal hemorrhage were observed in the kidney of exposed fish, which are similar to the findings of [24]. In the present study, kidney and gill hemorrhages were probably due to damage to the blood capillaries. This pathological damage is commonly associated with pesticides [25]. In addition, the mobilization of inflammatory cells in most of the organs provides good explanation for the increase in WBC and such mobilization confirms that it is a response to the toxicity of the pesticide. Alaa et al [26] found significant decrease in WBC with increase doses of 4-NP on juvenile *C. gariepinus.*

5. Conclusion

The study revealed that juvenile *C. punctatus* exposed to sublethal concentrations of dimethoate suffered severe hematological and organ injuries. This is worrisome consequent of the dimethoate toxicity in the aquatic environment and some other pesticide formulations. There is urgent need to regulate the use of pesticides containing these chemicals, especially in floodplains that serve as breeding sites for commercially and ecologically important fish species.

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* LC_{50} is stands for lethal concentration, means an amount of a chemical in water or in the air that kills 50% of the test animals during the observation period.
**LD_{50} is stands for a lethal dose, an amount of chemical dose which can kill at once 50 % of test animals during observation period.