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Research Article

Effect of Parathion on Physio-Biological Aspects of Notopterus notopterus (Pallas, 1769)

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Abstract During the present experiment, *Notopterus notopterus* were exposed to lethal concentration (0.1 ppm) of parathion for a period of 60 min in triplicates. A marked reduction in the opercular beat frequency (90.0 to 30.0) and tail beat frequency (7.3 to 2.0) was observed at the end of 60 min. exposure time. The results on the hematological aspect of the experiment (5 replicas) revealed that significant (P < 0.05) increase in WBC (6.06 to 7.8), and decrease in Hb, RBC, PCV, MCV, MCH, MCHC and other non-specific defence cells. The increase in the WBC count is due to the non-specific immune response of the fish.

Keywords Parathion; Toxicity; Lethal Dose; Hematology

1. Introduction

The pesticide or pesticide residues are added ceaselessly to the aquatic environment, thereby polluting fish and water bodies. Fish and water bodies around the world offer not only food to people but also act as a means of generating employment and money for thousands. Unfortunately fish and water bodies are in grave danger because of bioaccumulation of pesticides. Pesticides suspend in water and bind with soil particles, thereby making the aquatic environment unfit for the aquatic inhabitants, which get badly affected.

Water is an important medium to affect the fish metabolism, as the epidermal cells have direct contact with the obnoxious materials carried by it, which in turn affect the fish. Fish being closely associated with the water, the bosom contact assuages the manoeuvre of chemicals into and through the mucous, skin and other external layers and becomes a pitfall to the aquatic inhabitants when nefarious chemicals, pollutants and contaminates enter the aquatic environment. The pollutants in turn disturb the physiological pathways of the fish, resulting in alteration of some important defence mechanisms including specific and non specific defence routes, hence making fish vulnerable to different stresses.

Blood scrutiny is indispensable in many fields of ichthyologic research, including toxicology, environmental monitoring, and fish farming, as blood is the only rightful indicator of the changes occurring in fish physiology (Adedeji *et al.*, 2000). Researchers who have appraised the consequence of various pesticides on the behaviors and hematological responses of different species of fish include (Anees, 1978; Benarji and Rajendranath, 1990; and Svoboda *et al.*, 2001). The authors found diverge riposte after exposing the fish to diverge sub lethal concentrations using the 96 h acute toxicity tests. The present paper on the effect of parathion on the blood profile of fish *Notopterus notopterus* Pallas" will be an engender in the field of fish toxicology and a value addition to the haematobiochemical profiles of fish exposed naturally and artificially to sub lethal/lethal concentrations of different pesticides.

2. Materials and Methods

Healthy *Notopterus notopterus* (Pallas) fish were purchased from local hatchery and accustomed for two weeks prior to the start of experiment. Balanced pelleted diet was given to fishes @ 2% body weight per day, containing 35% crude protein.

Parathion ($C_{10}H_{14}NO_5S$) is manufactured by Shivalic Agro Chemical Industries. The parathion is broad spectrum organophosphate pesticides used to control many insects pests. Parathion is used as non-systematic and as fumigant. For the present experimentation, 0.1 ppm of parathion was prepared. 30 L of the diluents water was used as control. 30 fishes were used for each treatment, kept in aquariums in triplicates. 0.1 ppm of the stock solution was introduced separately in each experimental tank. Mortality of the fish post-exposure was observed from 1-5 hours for any mortality.

In order to appraise the influence of pesticides on the physiological requirement of oxygen, opercular beat frequency (OBF) was computed by observing the opercular beats before and after the exposure. The OBF was enumerated using the stop watch, scrutinized for one minute after every 20 min post exposure. Tail beat frequency (TBF) is an inventory of enumerating the incidence of tail movements of the fish before and after the exposure to pesticides. Like OBF, TBF was calculated using stock watch and observing the change in movement of the fish after the exposure.

Blood samples from the confronted fishes were collected after every 10, 20 and 30 min. in fishes exposed to mixed solution of the pesticides. Blood samples were collected from the caudal fin with 21 gauge needles and 3 cc syringes before ventilatory riposte was noticeably disconsolate. PCV (%) was determined by centrifuging the blood for three minutes (3000 rpm). The hemoglobin content (Hb) of erythrocytes was determined by the haemoglobinimeter in g/100 ml. RBC value was determined by counting all the cells lying to the left and below the demarcation line of counting chamber. MCV, MCH and MCHC were calculated by the standard formulas (Blaxhall and Daisley, 2006).

The Neubauer counting chamber was used to count leucocyte, demarcated by triple lines (1 mm²). For differentiating small and large lymphocytes, Unna-Ziehl staining was used. Standard method of Romeis (1968) was used for differentiating neutrophils by using \in Granulation staining. ∂ -granulation staining was employed for differentiating monocytes and thrombocytes. The total serum protein was estimated by Gornall's biueret method (Ryan and Chopra, 1976).

3. Results and Discussion

Unpropitious effect of parathion on the physio-biological activities of *N. notopterus*, are depicted in the Tables 1 and 2. The results of the opercular beat frequency (OBF) for parathion are presented as mean±SE in Table 1. In case of 0.1 ppm parathion exposure to *N. notopterus*, the OBF decreased from 90.0±0.6 (0 min) to 58.3±2.9 (20 min post parathion exposure). The OBF showed further increase to 102.7±3.8 (40 minutes post parathion exposure) and again decreased to 30.0±0.5 (60 min

post parathion exposure). Same was the case with tail beat frequency (TBF). In case of 0.1 ppm parathion exposure to *N. notopterus*, the TBF reduced from 7.3 ± 0.6 (0 min) to 4.7 ± 0.6 (20 min post parathion exposure). The TBF showed further increase to 5.0 ± 2.0 (40 min post parathion exposure) and again decreased to 2.0 ± 0.1 (60 min post parathion exposure).

Pesticide	Exposure Duration									
	00 min	20 min	40 min	60 min						
OBF	90±0.5	58.3±2.9	102.7±3.8	30.0±0.5						
	00 min	20 min	40 min	60 min						
TBF	7.3±0.6	4.7±0.6	5.0±2.0	2.0±0.1						

Table 2: Mean Hematological Parameters of Notopterus notopterus Exposed to Five Trials of 0.1 ppm Parathion

Parameter	Control	20 min		40 min			60 min			
		Min.	Max	Mean±SE	Min.	Max	Mean±SE	Min.	Max	Mean±SE
PCV (%)	25.0±0.83	21.5	24.5	23.0±0.25 ^ª	18.8	23.2	21.0±2.11 ^b	16.8	19.2	18.0±0.98 ^ª
Hemoglobin (g/dL)	8.3±0.23	6.8	7.6	7.20±0.12 ^a	6.65	6.95	6.80±1.25 ^{ab}	5.8	6.8	6.3±0.25 ^a
RBC (Χ 10 ⁶ /μL)	2.61±0.06	2.15	2.45	2.30±0.03 ^a	1.75	2.25	2.00±0.92 ^a	1.5	1.7	1.6±0.69 ^ª
MCV (fL)	95.8±1.25	93.3	94.5	93.9±0.75 ^{ab}	76.4	85.4	80.9±0.45 ^a	86.3	90.5	88.4±0.45 ^a
MCH (pg)	31.8±0.92	28.3	29.3	28.8±1.20 ^{ab}	19.6	25.6	22.6±1.11 ^a	21.8	25.6	23.7±1.10 ^{ab}
MCHC (g/dL)	33.2±1.37	27.2	32	29.6±1.10 ^{ab}	26.5	29.3	27.9±0.19 ^a	25.3	28.3	26.8±1.21 ^b
WBC (X 10 ³ /µL)	6.06±0.24	6.13	6.85	6.49±0.12 ^b	6.99	7.45	7.22±0.05 ^b	7.3	8.3	7.8±0.10 ^b
Small lymphocytes (X 10 ³ /µL)	25.3±0.02	28.6	30.2	29.4±0.08 ^{ab}	29.7	32.1	30.9±0.02 ^b	34.7	37.3	36.0±0.08 ^b
Large lymphocytes (X 10 ³ /µL)	1.5±0.020	1.38	1.82	1.6±0.018 ^b	1.65	1.95	1.8±0.010 ^{ab}	2.1	2.5	2.3±0.018 ^b
Neutrophils (X 10 ³ /µL)	1.9±0.014	2.05	2.35	2.2±0.010 ^a	2.47	2.73	2.6±0.010 ^b	2.8	3.6	3.2±0.010 ^{ab}
Monocytes (X 10 ³ /µL)	1.65±0.02	1.8	2.2	2.0±0.020 ^a	2.67	2.93	2.8±0.20 ^b	3.2	3.8	3.5±0.010 ^a
Eosinophils (X 10³/µL)	0.5±0.020	0.7	0.9	0.8±0.01 ^a	0.88	1.02	0.95±0.02 ^b	0.8	1.2	1.0±0.001 ^{ab}
Thrombocyte like cells (X	1.8±0.021	1.24	1.56	1.4±0.014 ^a	1.79	2.21	2.0±0.020 ^{ab}	3	3.4	3.2±0.014 ^a
10 ³ /μL)										
Thrombocytes (X 10 ³ /µL)	34.9±0.02	28	32	30.0±0.04 ^{ab}	37.9	42.1	40.0±0.15 ^ª	45.1	46.9	46.0±0.01 ^a
Plasma protein (g/dL)	3.8±0.024	2.95	3.45	3.2±0.010 ^b	2.05	2.35	2.2±0.010 ^a	1.58	1.92	1.75±0.020 ^{ab}

Note: Results expressed as mean \pm SD of five replications (d.f. 5, 30).

*The values of the MCV, MCH and MCHC are calculated by the formulae, corresponding to the appropriate values of Hb, PCV and RBC.

Fifteen blood parameters were studied for appraisal into the effect of parathion on the hematological indices. The mean±SD value of normal PCV (%) was 25.0 ± 0.83 , which reduced after 60 min. of exposure, ranging from 16.8 - 19.2 with a mean±SD of 18.0 ± 0.98 , showing 'variance', 'regression equation' and 'correlation coefficient of 308.9, Y = -0.115X + 25.2 and 0.99 respectively. The normal haemoglobin (Hb) expressed in g/dL was 8.3 ± 0.23 , which decreased after 60 min. of exposure, ranging from 5.8 - 6.8 with a mean±SD of 6.3 ± 0.25 , with 'variance', 'regression equation' and 'correlation coefficient of 435.2, Y = -0.032X + 8.11 and 0.97 respectively.

The RBC count (x10⁶/µL) was 2.61±0.06, which decreased after 60 min. of exposure, ranging from 1.5 - 1.7 with a mean±SD of 1.6±0.69 showing 'variance', 'regression equation' and 'correlation coefficient of 507.7, Y = -0.016X + 2.627 and 0.99 respectively. Likewise MCV (fL) was 95.8±1.25, which showed a decrease after 60 min of exposure, ranging from 86.3 - 90.5 with a mean±SD of 88.4±0.45 showing 'variance', 'regression equation' and 'correlation coefficient' of 1324.4, Y = -0.176X + 95.03 and 0.68 respectively. The normal MCH (pg) was 31.8±0.92, which reduced after 60 min of exposure, ranging from 21.8 - 25.6 with a mean±SD of 23.7±1.10 with 'variance', 'regression

equation' and 'correlation coefficient' of 296.8, Y = -0.152X + 31.3 and 0.90 respectively. The MCHC (g/dL) was 33.2 ± 1.37 , which showed a decrease after 60 min of exposure, ranging from 25.3 - 28.3 with a mean±SD of 26.8±1.21 showing 'variance', 'regression equation' and 'correlation coefficient' of 289.18, Y = -0.104X + 32.51 and 0.96 respectively.

The normal WBC (x10³/µL) was 6.06±0.24 which showed an increase after 60 min. of exposure, ranging from 7.3 - 8.3 with a mean±SD of 7.8±0.10 with 'variance', 'regression equation' and 'correlation coefficient' of 437.79, Y = 0.030X + 6.023 and 0.98 respectively. The small lymphocytes count (x10³/µL) was 25.3±0.02, which showed an increase after 60 min. of exposure, ranging from 34.7 - 37.3 with a mean±SD of 36.0±0.08 with 'variance', 'regression equation' and 'correlation coefficient' of 294.13, Y = 0.168X + 25.36 and 0.98 respectively. The large lymphocyte count (x10³/µL) was 1.5±0.02, which later showed an increase after 60 min of exposure, ranging from 2.1 - 2.5 with a mean±SD of 2.3±0.018, with 'variance', 'regression equation' and 'correlation coefficient' of 512.9, Y = 0.013X + 1.41 and 0.94 respectively. The normal neutrophil count (x10³/µL) was 1.9±0.014 which showed an increase after 60 min. of exposure, ranging from 2.8 - 3.6 with a mean±SD of 3.2±0.010 with 'variance', 'regression equation' and 'correlation coefficient' of 502.3, Y = 0.021X + 1.83 and 0.98 respectively.

The monocytes count $(\times 10^{3}/\mu L)$ was 1.65±0.002 which later showed an increase after 60 min. of exposure, ranging from 3.2 - 3.8 with a mean±SD of 3.5±0.010 with 'variance', 'regression equation' and 'correlation coefficient' of 502.2, Y = 0.031X + 1.535 and 0.98 respectively. The eosinophils count (0.5 ± 0.02) showed an increase after 60 min. of exposure, ranging from 0.8 - 1.2 with a mean±SD of 1.0±0.001 with 'variance', 'regression equation' and 'correlation coefficient' of 529.13, Y = 0.008X + 0.565 and 0.94 respectively. The thrombolytic like cells (1.8±0.021) showed an increase after 60 min. of exposure, ranging from 3.0 - 3.4 with a mean±SD of 3.2±0.014 with 'variance', 'regression equation' and 'correlation coefficient' of 508.3, Y = 0.024X + 1.38 and 0.80 respectively. The thrombocytes (34.9±0.02) showed an increase after 60 min. of parathion exposure, ranging from 45.1 - 46.9 with a mean±SD of 46.0±0.01 with 'variance', 'regression equation' and 'correlation coefficient' of 322.9, Y = 0.216X + 31.23 and 0.81 respectively. The normal plasma protein content (g/dL) was 3.8±0.024, which showed a decrease after 60 min. of parathion exposure, ranging from 1.58 - 1.92 with a mean±SD of 1.75±0.020 with 'variance', 'regression equation' and 'correlation coefficient' of 98.4, Y = -0.035X + 3.81 and 0.98 respectively.

4. Discussion

The increase in OBF and TBF upon exposure to different pesticides either individually or in groups has earlier been reported by (Omoregie, 1995). The initial increases in OBF and TBF may be analogous with the response to shock. The change in behavioural response to different pesticides with prevalent change in the rate of OBF and TBF from control imputes an adjustment in physical fitness as a result of the stress condition (Leight and Van Dolah, 1999). (Grillitsch *et al.*, 1999) suggested that organisms unveil behavioural responses to chemical stress both at acute and sub lethal toxicity. This elicits the potency and sensitivity of the fish, *N. notopterus* to the test chemical, witnessed by the change in OBF and TBF.

During the present experiment the hematological parameters of *N. notopterus* were greatly disturbed on exposure to 0.1 ppm of parathion. Haemoglobin (g/dL) showed a decrease from 8.3 to 6.3; RBC ($\times 10^{6}/\mu$ L) from 2.61 to 1.6; PCV (%) from 25.0 to 18.0; MCV (fL) from 95.8 to 88.4; MCH (pg) from 31.8 to 23.7; MCHC (g/dL) from 33.2 to 26.8; and plasma proteins (g/dL) from 3.8 to 1.75. The work of (Murty *et al.*, 1984) on the toxicity of methyl parathion and fensulfothion to the fish *Mystus cavasius* reveals oxygen stressor in fishes subjected to the pesticides because of the decrease in number of RBC's and reduction in haemoglobin titer. (Prasada Rao and Ramana Rao, 1984) reported the inhibitory mechanism of acetylcholinesterase activity of parathion in the tissues of the teleost (*Tilapia mossambica*).

Calumpang *et al.*, 1997 reported significant (P < 0.5) decrease in the values of Hb and RBC after the exposure of fish and frogs to chlorpyrifos, fenubucarb, monocrotophos, and methyl parathion. The concept regarding the hematological changes and related metabolic dysfunctioning was assessed by (De La Vega Salazer *et al.*, 1997) who studied the bioaccumulation of methyl parathion and its toxicology in several species of the freshwater community in Ignacio Ramirez dam in Mexico. The study of (De La Vega *et al.* Salazer *et al.*, 1997) was further strengthened by the recommendation of (ATSDR, 2001) who investigated the complete toxicological profile for methyl parathion. Later on (Castillo *et al.*, 2002) studied the behavioural effects of exposure to endosulfan and methyl parathion in adult rats.

Extensive study on the effect of parathion on hematological parameters in the serum of male Bagrid fish (*Psudobagrus fulvidraco*) has been carried out by (Kyu-Seok Cho *et al.*, 2004). A significant (P < 0.1) decrease in RBC, Hb, PCV, MCV and MCH in the fish in their study. (Edwards & Tchounwou, 2005) further strengthened the study, who worked on environmental toxicology and health effects associated with methyl parathion exposure. The work of (Monteiro *et al.*, 2006) lend complete support to our findings, who worked on oxidative stress as biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). (Janice *et al.*, 2007) investigated parathion and methyl parathion toxicity to insecticide resistant and susceptible mosquitofish (*Gambusia affinis*) and observed that the resistant population demonstrates a 1.3 fold greater tolerance of methyl parathion than the susceptible population. This statement justifies the alteration in hematological parameters of the fish exposed to different pesticides.

A significant decrease was observed by (Bhat *et al.*, 2012) in values of hematological parameters like Hb, Hct, RBC and plasma protein throughout the exposure of methyl parathion. The authors reported an increase in leukocyte initially, which later recovered, showing a significant decrease at the end of the experiment. Same trend was observed in case of MCV and MCH, whereas MCHC value was more or less similar to control group up to the 21st day, and then a significant decrease was observed in the remaining study period. Plasma glucose values increased up to the 28th day (13.37 %) and then declined. The observations of the above also stand true for our results. The results are further strengthened by the work of (Xiang & He-Qing, 2012) who observed the alteration of the kidney membrane proteome of *Mizuhopecten yessoensis* induced by low-level methyl parathion.

5. Conclusion

The present investigation revealed a marked reduction in the opercular beat frequency (90.0 to 30.0) and tail beat frequency (7.3 to 2.0) in *N. Notopterus* exposed to parathion at the end of 60 min. exposure time. The results on the hematological aspect of the experiment (5 replicas) revealed that significant (P < 0.05) increase in WBC (6.06 to 7.8), and decrease in Hb, RBC, PCV, MCV, MCH, MCHC and other non-specific defence cells.

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