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HAEMATOLOGICAL AND BEHAVIOURAL STUDIES OF FRESH WATER FISH, *CHANNA ORIENTALIS* (SCH) EXPOSED TO NOVEL DERIVATIVE OF 1, 3, 5-TRIAZINE

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ABSTRACT
The fish *C. orientalis* was exposed to triazine derivative *i.e.*1-phenyl-2-thio (1H)-4-[(1-phenyl-2-thio (1H)-6-amino-1, 2-dihydro-1, 3, 5-triaz-4-yl)]-amino-6-phenylamino-1, 2-dihydro-1, 3, 5-triazine which was synthesized from 2-phenylimino-4[(2-imino-6-phenylimino)-1, 3, 5-thiadiaz-4-yl]-amino-6-phenylimino-1, 3, 5-thiadiazine. To study the effect of triazine, the experiments were conducted in two different phases. In first phase, lethal concentration of toxicant was determined and in the second phase, toxicity was studied with respect to selected lethal concentration of toxicant. The study revealed that the fishes were sensitive to the triazine derivative, high concentration of triazine was very toxic to aquatic organism. 1, 3, 5-triazine treatment inflicted a drastic reduction in the total count of RBCs and WBCs along with morphological changes in the fish.

INTRODUCTION

The fish has been widely used for bioassay than any other group aquatic organisms. It is mainly because of its availability and easy to maintain in the laboratory conditions. Many workers have documented acute toxicity effect of various heavy metal and their compounds on different species of Indian fishes. As most of the herbicides and pesticides contain triazine skeleton. These chemical compound enter into aquatic ecosystems can cause hazardous effects on marine and freshwater organisms (Russo *et al*., 2004). It was thought interesting to study the effect of triazine derivative on haematology and blood biochemistry in different fish species are of comparative physiological interest (Parma and Croux, 1994). It contributes to a greater understanding of habitat, food selection and mode of life of the species. Celik (2004) reported that fish blood is a suitable means of indicating and identifying the effects of stress, influence of environment, and health status of fish in a given area.

The extensive studies on the biology of teleose fish *Channa orientails* has been under taken with particulate reference to aquaculture. Peer and Kutly in (1981) studied the influence of ambient oxygen on random activity of fresh water fish while Sherekar and Kulkarni in (1989) studied the protein changes in the fish *Channa orientalis* exposed to triazine. It has been interesting to study the effect of triazines on aquatic life. The present work deals with the physiological responses of *Channa orientails* (SCH) exposed to 1, 3, 5-triazine.

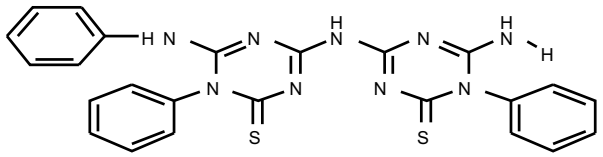
MATERIALS AND METHODS

The freshwater fish, *Channa orientalis (Sch)* has been selected for the present work as its availability and easy to maintain in the laboratory conditions. The fishes were obtained from fresh water Rishi Lake near Karanja (Lad) Dist-Washim, Maharashtra, India. Locally the fish is known as *Dok* and it is edible fish. The fishes were treated with 1% $KMnO_4$ solution for live minutes for dermal disinfection and acclimatized for a period of fortnight to laboratory conditions and were fed on small pieces of boiled eggs and fish food once in a day especially in the morning. Mortality was less than 5% before preceding the test. Then fishes were maintained in separate aquaria as a control and experimental groups. Throughout the experiment, the water used was aged tap water which was stored in overhead tank for about 10 days. The details of physiochemical characteristics of water used are studied in laboratory (Table 1).

Synthesis of derivative of 1, 3, 5- triazine

The most of the herbicides and pesticides contain triazine skeleton and many derivative of 1, 3, 5- triazine also use as herbicides and pesticides. This derivative synthesis is the novel attempt to study it's haematological and behavioral effect on

fishes. For synthesis, the 2-phenylimino-4 [(2-imino-6-phenylimino)-1,3,5-thiadiaz-4-yl] -amino-6-phenylimino-1, 3, 5-thiadiazine (0.05M) was suspended in 5% ethanolic sodium bicarbonate solution and refluxed for 30 minutes on water bath. The reactants went into solvent during heating. After distillation of excess solvent, pale brown crystals were isolated. It was recrystalised with glacial acetic acid to get 1-phenyl-2-thio (1H)-4-[(1-phenyl-2-thio (1H)-6-amino-1, 2-dihydro-1, 3, 5-triaz-4-yl)]-amino-6-phenylamino-1, 2-dihydro-1, 3, 5-triazine.



1-phenyl-2-thio (1H)-4-[(1-phenyl-2-thio (1H)-6-amino-1, 2-dihydro-1, 3, 5-triaz-4-yl)]-amino-6-phenylamino-1, 2-dihydro-1, 3, 5-triazine

Toxicity study

To study the toxicity effect of triazine, the experiment was conducted. For the determination of LC_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) is usually an initial step in the assessment and evaluation of the toxic characteristics of a derivative of 1, 3, 5-triazine. About 24 adult fishes of length (40 – 50 cm) and weight (28-37g) were selected for LC_{50} determination against derivative of 1, 3, 5- triazine. The average mortality in each concentration was taken to determine the LC_{50} by using the methodology of Omitoyin *et al*., 2006). Distilled water was used in the experiment as diluents. As toxicants were of unknown toxicity, first experiments were conducted to determine toxicity range Holden (1973) and Pragatheswaram *et al*. (1989). The animal divided into control group and three experimental groups of 6 animals each. Aqueous (CDW) solutions of toxicants ranging from 25 to 100 ppm were added into to glass aquaria (45X25X25 cm) containing 15 L of toxicant treated water. The toxicant solution was added drop by drop with constant stirring and then an acclimatized 18 experimental fishes were added into toxicant solution to study the toxicity of 1,3,5- triazine. The fishes were fed with small pieces of boiled eggs and fish food, twice in a day. Observations were made for 24, 48 and 96h from which the concentration was selected for LC_{50} and investigate the some morphological, physical and behavioural changes in altered environment of exposure to sublethal and chronic concentration of these pesticide was also studied with the criteria of Das and Konar (1974); Joshi and Rege (1980); Gowda *et al* .(1981); Narasimha and Murphy (1983).

Behavioural study

During bioassay tests, treatment of triazine is given on alternative days for 30 days and behavioural changes in the fish were observed by following the methods of Carpenter, (1927); Wedemeyer and Mcleay (1981); Pragatheswaram *et al*. (1989). The time at which the fish loses its sense of balance and floats its side or upside down movement, faster opercular activity, surfacing and gulping of air, erratic swimming with rapid jerky movements, hyper spiraling, convulsions and tendency of escaping from aquaria was noted. The test concentration for fish *Channa orientalis* (SCH) selected were 10 mg/L from the result of LC_{50}. In all cases retreatment was carried out for the period of 96h and every 24h, the solution of toxicant were replaced by freshly prepared solution of corresponding concentration. This helped in maintaining a uniform concentration throughout the period of experiment. Suitable mortal controls were also run along with experimental sets. Mortality was recorded after every 24h. Three replicate were run for each toxicant. Study of concentration of dissolved O_2 and CO_2 was carried out by using Winkler's method followed by measuring the pH value of water using for behavioural study (Table 2, 3).

Haematological study

The 5 fishes were taken and introduce at LC_{50} concentration *i.e.* at 100 ppm, because 50% mortality was observed at 100 ppm concentration. The treatment was carried out for the period of 96h and every 24h. A facility for oxygenation of the test solution provided and experiment was carried out for 30 days. After 30 days the blood from the control and 1, 3, 5-triazine treated fishes was obtained by severance of caudal peduncle and collected in Eppendorf tubes containing EDTA anticoagulant (Mgbenka and Oluah, 2003). These treated and control blood sample were used to estimate the haematological parameter.

Total count of RBC

Total red blood cells (RBCs) were counted using an improved Neubaur haemocytometer (Shah and Altindag, 2004). Blood was diluted 1:20 with Hayem's fluid (Mishra and Pandey, 1977). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as 10^3 mm^3 (Wintrobe, 1967).

Total count of WBC

Total white blood cell (WBCs) were counted using an improved Neubaur haemocytometer (Mgbenka and Oluah, 2003; Shah and Altindag, 2004). Blood was diluted 1:20 with Tunk's diluting fluid and placed in haemocytometer. Four large corner squares of the haemocytometer were counted under the microscope (Olympus) at 400 X. The total number of WBC was calculated in10^3 mm^3 (Wintrobe, 1967).

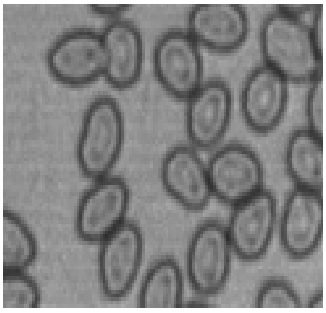
Statistical analysis

Data are expressed as mean $\pm$ SEM. Statistical analysis was done by using paired student´s t-test (Mahajan, 1997).

RESULTS AND DISCUSSION

The LC_{50} values were determined using different concentrations of derivative of 1, 3, 5-trizine for fishes for different time of exposure. The 24h at 25 ppm, LC_{50} of 1, 3, 5-trizine which shows the no mortality while for 48h at 50 ppm, one fish shows mortality and for 96h at 100 ppm, it was found that the three fishes shows mortality. Which is high when compared with the literature of Kartz (1961) for Coho Salmon (1.3 ppm), for brook trout (1007) and for rainbow trout (1.47). The value obtained was also higher than juveniles of *Clarius garipinius* (0.38 ppm) studied by Omitoyin *et al*. (2006). Vittozi and De Angelis (1991) summarized the 96h LC_{50} values of malathion 0.091 to 22.09 ppm for different species.

The difference in toxicity to the different species mentioned



Exposure time hours - 00

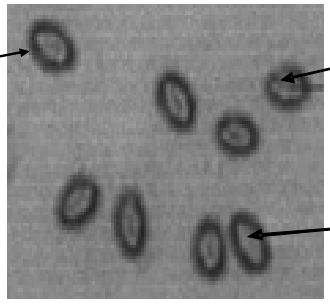


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Figure 1: Morphological changes in RBC count in *Channa orientalis* exposed to 1, 3, 5-triazine (Wright's stain x 100)

Table 1: Physiochemical characteristics of water used for experimental study

S. No.	Characteristics	Values
1.	Dissolved oxygen	10.2 mg/L
2.	pH	8.2 ± 0.2 s.u.
3.	Temperature	25± 2ºC
4.	Alkalinity	85 ppm
5.	Total Hardness	64.8 ppm
6.	Parmenant Hardness	43.2 ppm
7.	Temporary Hardness	21.6 ppm
8.	Salinity	0.01 ppm

Each value is average of five observations; All values are expressed in mg^{-1} except pH and temperature (ºC); Test Toxicant - 1, 3, 5-Triazine; The solution of 1, 3, 5-Triazine was prepared in 70% ethanol-water mixture.

Table 2: Dissolved oxygen and free CO_2 for control and experimental fish

Treatment group	Duration	O_2	CO_2
	2 h	09. 613	0.03
	1 day	4. 370	0.0216
Control fish	7 days	2.004	0.0391
	15 days	1.285	0.025
	2 h	4.8681	0.0104
	1 day	2.9834	0.0176
Experimental fish	7 days	1. 9685	0.0296
	15 days	1. 7267	0.0248

All Values in mg/L

Table 3: pH values of water from control and experimental fish aquaria

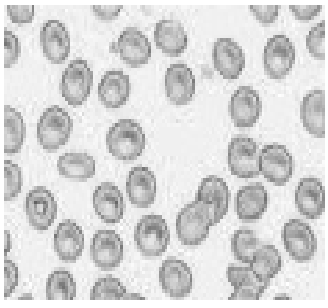
Duration	Control fish	Experimental fish
2 h	7.81	7.95
1 day	7.83	7.94
7 days	7.84	7.95
15 days	7.84	7.94

Table 4: Haematological changes in RBC count and WBC count in Channa orientalis exposed to 1, 3, 5-triazines

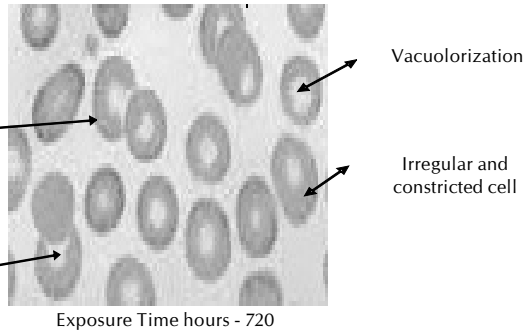
Exposure (Time h)	Erythrocyte ($\times 10^3 mm^3$)	Leucocyte ($\times 10^3 mm^3$)
00	3.21±0.09	13.4±0.28
24	2.95±0.23*	12.5±0.65 ns
48	2.74±0.47*	12.1±0.23**
72	2.23±0.04***	11.7±0.16*
96	2.11±0.07*	11.2±0.08*
240	1.76±0.12ns	10.5±0.06**
360	1.06±0.08**	10.1±0.18***
720	0.97±0.04*	9.2±0.54*

Values in Mean ± S.E. (Standard error), n = 5, *p < 0.05, **p < 0.01, ***p < 0.001, When compared between all group, ns = non-significant

above might be due to differences in Absorption of pesticide, their accumulation, biotransformation and excretion. Differences in metabolic pathways among species may result in different patterns of biotransformation leading to more or less toxic metabolites (Johnson and Taledo, 1993). The magnitude of toxic effects of pesticides also depends on length and weight, corporal surface to body weight ration and breathing rate (Singh and Narain, 1982; Alkahem *et al*., 1998). The physical, morphological and behavioural changes observed for their doses of LC_{50}. The experimental fish exhibited abnormal behavioural response. During the exposure time, triazine treated fish initially showed rapid movement, faster opercular activity, surfacing and gulping of air. Then fish showed erratic swimming with rapid jerky movements, hyper spiraling, convulsions and tendency of escaping from aquaria. It was also observed that with an increasing exposure time, these activities were relatively increased initially and subsequently reduced, expressing the sign of distress. Besides this remarkable colour change was observed and the loss of



Exposure Time hours - 00



Exposure Time hours - 720

Figure 2: Morphological changes in WBC count in *Channa orientalis* exposed to 1,3,5-triazine (Wright's stain x 100)

equilibrium along with profuse mucus secretions and its coagulation all over the body of fish in the dose at LC_{50} of 1, 3, 5-triazine. Later on fish struggled hard for aerial breathing with their restricted swimming movements and indicated poor response to external stimulants. This was followed by loss of equilibrium and fish slowly moved upward in a vertical direction. There after fish become progressively lethargic swimming with widely spread fins and were often found on surface of water. The increase in opercular movement and bottom to upward movement to overcome on hypoxic condition was seen in the 48h and 96h dose. The abnormal swimming behaviour and attired movements considered to be the result of excessive elimination of skeletal minerals (Pragatheswaram *et al*., 1989). While the heavy exculpation of mucus over the body and body dispigments are attributed to the dysfunction of endocrine, pituitary glands under chromatophore (Mishra and Pandey, 1977). Surfacing and erratic movements were observed in triazine treatment. These findings agreed with the studies of Waiwood and Johnson (1974); Alkahem *et al*. (1998); Omitoyin *et al*. (1999); Sambasiva and Rao (1999).

Carpenter (1927) first introduced the concept of coagulation anoxia in zinc poisoned fishing. She observed a veil like film of coagulated mucus all over the body of zinc exposed fish. This theory was supported by Ellis and Roberts (1978) who showed that many other chemicals induced similar precipitation of mucus which filled space between gill filament and gill lamella. Ultimately affecting gaseous exchange, leading to stasis of blood and death of fish. Later on numbers of workers Skidmore (1964) and Burtan, *et al*., (1972) have showed that death is related to tissue hypoxia because the gaseous exchange at the level of gills is no longer sufficient to supply the O_2 necessary for the survival of animals.

Damage of gills by different heavy metals has been reported by number of workers Ghate (1981) and Khangrarot (2003). Therefore anoxia may be an important factor causing death of fishes exposed to pollutants. Another factor causing much damage to survival of fish is subtle effect of pollutants on osmoregularity mechanism of the fishes. It is well known that fish gills are involved in ionic regulation Evans (1975) hence impairment of gills function may also affect osmoregulation. In the present work, abnormal behaviour of fishes may be due to unequal accumulation of ligand in various tissues and their translocation within the body from one tissue to other.

From (Table 3) due to increase in the pH value of toxicant solution probably extra cellular and intra cellular diffusion of oxygen may be affected resulting in the hypoxia condition and leading to the disturbances in many physiological processes such as osmoregulation, The increase in the pH cause change in the morphological and some physiological beheviour as given above. These findings agreed with the studies of Burtan *et al*. (1972). Thus it could be concluded from the present study that the fishes are highly sensitive to the triazine under study and the mortality rate is concentration dependent. Therefore, high concentration of triazine is very toxic to aquatic organisms.

In the present haematological investigation it was found that the 1, 3, 5- triazine treatment inflicted a drastic reduction in the total count of RBCs and WBCs. The reduction was dosage dependent (Table 4). In *C. orientalis* prominent destruction in cell membrane, nuclear contents, cells constriction, Vacuolorization, and hyperchromality were observed. Morphologically, in *C. orientalis* there is increase in cell surface area, destroyed cell membrane, sickle shape cells, few irregular and constricted cells of RBCs and WBCs were observed (Fig. 1, 2). Similar result were found to Maheswaram *et al*. (2008) while working on haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride.

CONCLUSION

The result of the present study indicates that derivative of 1, 3, 5-triazine exerts toxic effect on fish and show quick and lethal response to these toxicants. Thus the use of derivative of 1, 3, 5-triazine should be properly and strictly control and regulated by appropriate legislation in order to prevent its bioaccumulation in the environment, aquatic animals and ultimately to the human being.

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