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Sublethal haematological and histopathological effects of lindane on the Indian freshwater cat fish, *Clarias batrachus* (Osteichthyes:Clariidae)

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Abstract

The acute toxicity of Lindane to Indian fresh water Catfish (Clarias batrachus) was assessed in a static renewal technique. Three graded concentrations of lindane were prepared as 0.40, 0.60 and, 0.80 mg/L and a control experiment (0 mg/L). The 96 h (LC50) value computed on logarithm was 0.36 mg/L. At various concentration of lindane, fish showed uncoordinated behaviour such as incessant gulping of air and increase in opercular ventilation. Mortality was recorded earliest in the highest concentrationsof 1.8 mg/L and with increasing period of exposure to lindane. Marked diffuse fatty degenerative hepatocytes and heterophilic infiltration and pyknosis and degenerative changes of liver were the major histopathological effects distinctively shown. The results show that lindane is highly toxic to Clarias batrachus.

Keywords: toxicity, lindane, hemoglobin,WBC, RBC, hepatocyte etc.

1. Introduction

Pesticides are potentially most harmful chemicals introduced in to the environment. The contamination of surface water by pesticides used in agriculture is a problem of world wide importance.(Hill,1985).The use of banned pesticides on a large scale in developing countries continue to pose severe health and environmental problems. (WHO,1989). Though they have contributed considerably for human welfare, their adverse effect on non target organisms are significant. The use of pesticides brings changes (biochemical, physiological and histological) as an indication of exposure and effect of genobiotic chemicals. (Stegeman, J.J, *et al.,* 1992 and Simon, L.M, *et al.*,1983). A survey of literature on pesticidal toxicity clearly shows that pesticides cause several haematological and biochemical disorders both in laboratory animals as well as on aquatic organism.

Present study reveals the toxicity of lindane (gamma-hexachlorocyclohexane, Y-HCH) an agro based insecticide which is used as an agricultural insecticide and as pharmaceutical treatment for lice and scabies. Lindane is considered as persistent organic pollutant (POP) because of its ability to resist degradation and to remain in the environment for years.

Since blood parameters are considered as pathophysiological indicators of the whole body and diagnostic tool for the structural and functional status of fish exposed to toxicants (Adhikari *et al.*, 2004).The present study investigates the impact of sub lethal concentration of lindane on hematological and histopathological changes on Indian fresh water cat fish, *Clarias batrachus*.

2. Materials and method

Alive, healthy and disease free fishes (*Clarias batraclus* weight 80-100gm and length 18-22cm) were collected from local fish market, Ambagan and brought

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to the laboratory. The fishes were kept in the glass aquarium to observe any visible pathological symptoms. Before introducing into the treatment tubs, fishes were treated with 0.1% $KMno_4$ solution to obviate any dermal infection. Then the fishes were acclimatized in laboratory condition for a period of one week. No mortality was recorded during this period. The fishes were fed chopped meat daily during acclimatization .Physico-chemical properties of water was followed after APHA (1989) method (temp.27±1^0 C, pH 6.8±0.05 at 27 C and DO 6.9-7.4 mg /L). The tub water was aerated continuously and food was provided in the form of chopped small fish, chopped chicken intestine *ad libitum* as in the controlled (Arockiaraj *et al.*, 2004).

Stock solution of lindane was prepared by dissolving analytical grade of lindane (Himedia) in double distilled water. Acute toxicity of lindane to *C. batrachus* was determined using a standard static renewal technique (APHA,1989).

After determining the Lc 96 hr value by following the method described by Finney, 1971, the required quantity of lindane to constitute Lc 50 dose for 24 hr,48hr and 72 hr exposure was found to be 0.80,0.60 and 0.40 mg /L respectively.The fishes were kept in a plastic tubs containing 6L tap water. Each tub contains three experimental animals. Fishes were exposed for 24 hr, 48 hr ad 72 hr exposure period to sub lethal concentration of lindane as stated above. Controlled animals were kept in similar conditions without any treatment. After 35 days the blood from the controlled and treated were obtained by severance of caudal peduncle and collected in Eppendorf tubes containing EDTA (Mgbenka *et al.*, 2003). These treated and controlled blood samples were used to estimate the hematological and cytological parameters.

For histological analysis, liver and testes of both controlled and treated are collected immediately after sacrifice, fixed in carnoy's fluid and taken to the laboratory.Tissues were washed in running tap water, dehydrated in alcohol and xylene, and embedded in paraffin wax. Sections (6-7 µm) were cut using a rotary microtome. Finally sections were rehydrated in distilled water followed by staining with Haema-toxylene –eosin and mounted in DPX for microscopic study.

2.1 Total RBC count

Total RBC count were done by using an improved Neubaur haemocytometer (Shah and Altindag, 2004). Blood was diluted 1:200 with hayem's diluting fluid (Mishra and *et al.*, 1973). Erythrocytes are counted in the loaded haemocytometer chamber and total no were reported as 10^6 /mm^3 (Wintrobe, 1967)

2.2 Total WBC count

Total WBC cell's are counted using an improved Neubaur Haemocytometer(Shah and Altindag, 2004). Blood was diluted to 1:20 with turks diluting fluid and placed in haemocytometer. The total no of WBC is reported as 10^3/mm^3(Wintrobe, 1967)

2.3 Estimation of hemoglobin

Amount of haemoglobin was determined Sahli's haemoglobinometer.Values of treated groups were compared statistically with controlled one by student's t-test. Significance was established at $P<0.05$ using the Microsoft Excel 2000 programme. Significance of data was further checked with the percent change (+ increase and – decrease) in blood parameters of *C. batrachus*.

3. Results

3.1 Total RBC count

The erythrocyte counts of healthy controlled showed a mean value of 3.39×10^6/mm^3. On the other hand fishes exposed to sub lethal concentrations of lindane showed mean values of RBC's as 2.78×10^6, 2.45×10^6 and 2.36×10^6/mm^3 for 0.40 mg/L, 0.60 mg/L and 0.80 mg /L of diferential concentrations of lindane respectively. The reduction was dose dependent. The treatment was found to inflict a drastic reduction in the total RBC count.

3.2 Total WBC count

The mean values of total WBC count in the blood of controlled fishes were found to be 10.6×10^3/mm^3. The fishes exposed to sub lethal concentration showed the mean values of WBC as11.6×10^3, 12.0×10^3 and 12.5×10^3/mm^3 for 0.40 mg/L, 0.60 mg/L and 0.80 mg/L of differential concentrations of lindane respectively. The values of treated has showed a significant increase as compared to controlled. In all cases, the differential leukocyte count was deviating significantly from normal values. The increase was observed in the number of lymphocytes and eosinophils while decrease was noticed in the number of monocytes and neutrophils.

3.3 Estimation of haemoglobin

The mean value of haemoglobin is found to be 14.5g/dL in the controlled fishes. But the fishes exposed to sub lethal concentration of lindane showed a significant decrease as compared to controlled. It was found to be 11.5g/dL, 10.1g/dL and 8.6g/dL haemoglobin at 0.4mg/L, 0.6mg/L and 0.8 mg/L of differential concentrations of lindane treatment respectively.

3.4 Histopathology of liver

Histopathological changes in the liver of fishes, exposed to sub lethal concentration of lindane are found to be dose dependant.The liver of control showed no significant changes ,having a homogenous mass of polygonal cells or hepatocytes with centrally located nuclei and granular cytoplasom. The hepatocytes enclose the bile canaliculi which open in to the hepatic ducts.In the lindane treated fish moderate fatty degeneration and infiltration in the portal areas of liver seen.With the increase dose of exposure results more degeneration in the portal areas seen.

4. Discussion

Blood is highly susceptible to internal and external environmental fluctuations as it is acted as vehicle for the transport of pollutant (Blaxhall,1972). The fish serves as bio indicator of water quality and the impact of pesticide can well be understood by analyzing either blood or serum. The toxic effect of pesticides to the blood of fishes has been studied by many researchers (Dawson,1935).

The result of the present study shows that the treatment of lindane inflicted a drastic reduction in the total RBC count and which was dose dependent (Panigrahi and Mishra,1978). They observed reduction in the Hb% and RBC count in the fish, *Anabus*, when treated with mercury.

Decrease in hemoglobin, RBC count was observed in the fish *Tinca tinca* exposed to mercuric chloride and lead (Shah and Altindag, 2004). Lowering of Hb percentage might cause anaemia. This may be due to the decreased rate of production of red blood cells or increased loss of these cells.

White blood cells play a major role in the bodily defense of fishes and consist of granulocyte, monocyte, lymphocyte and thrombocytes. Granulocyte and monocytes function as phagocytes to salvage the debris from injured tissues and lymphocytes produce antibodies (Wedemeyer and Mcleay, 1981).

An increase in WBC count may be compensatory response of lymphoid tissues to the destruction of circulating lymphocyte (Shah and Altindag,2005)

The study in the rivers, Gomti (Jaunpur) and Ganga(Varanasi) polluted with HCHs, DDTs are reported to affect the reproductive physiology of some edible catfishes and carps during the pre monsoon season by lowering the levels of estradiol-17 in plasma (Singh, P.B. and Singh, V., 2008)

The pesticides affect the survival, metabolism, growth rate fecundity and reproductive activity of fish (Hirose, 1975). Toxic substances even in very low concentration which is sub-lethal have been reported to interfere with basal metabolism and suppressed reproduction (Kondal *et al.,* 1989), steroidogenesis (Saxena *et al.,*1986; Wester and Vos, 1994; Singh and Canario, 2004), lipid metabolism (Sangalang *et al.,*1981; Lal *et al.,* 1987; Singh and Singh, 1992; Singh and Canario, 2004), degenerative changes in gonadotropin cells and reduction in interstitial cells size (Zutshi, 2005), gonadotropin levels (Van Der Kraak *et al.,* 1992; Singh *et al.,* 1994)act as reproductive biomarkers (Sepulveda *et al.,* 2004) and alsoas endocrine disruptors (Pawlowski *et al.,* 2004). Although the effectsof pesticides on fishes are studied extensively and also reviewed (Edwards, 1974; Holden, 1974; Gupta, 1977; Murty, 1986), there is need for more information on the toxicant effect.

In conclusion, present investigation reveals that the differential toxicity of lindane to *batrachus* can be attributed to the differences in susceptibility and tolerance resulted to its accumulation, biotransformation and excretion. Present results suggest that the pesticide may weaken the immune system resulting severe physiological problems and ultimately leading to the death of fishes. Long term exposure of organisms to pesticide means a continous health hazard for the population. Therefore the amount of such pesticides in the aquatic systems should be monitored to prevent the decreasing nutritive value of fish and fish mortality in particular and risk of human population in general. The pesticides affect the survival, metabolism, growth rate fecundity and reproductive activity of fish (Hirose, 1975).

The indiscriminate and extensive uses of pesticides have not only adversely affect organisms of aquatic ecosystem but also affect biological system according to their chemical properties which leads physiological stress and alteration in biochemical components among organisms. Among the inland fisheries the catfishes and other air breathing fishes are second most important group with regard to their commercial purpose. Catfishes or air breathing fishes as these grow in swamps and marshes can fill up the deficit. India has vast resources of swamps and marshes, which can be utilized for cultivating air-breathing fishes.

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