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Haematological alterations induced by lindane in a fish, *Aspidoparia morar*

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Abstract

Lindane, an organochlorine pesticide, has been widely used in public health and agriculture in several countries including India. Lindane has been associated with pollution due to its prolonged persistence and quick accumulation in blood as well as tissues. It is considered a possible carcinogen, mutagen, teratogen, immunotoxin, and neurotoxin. The present study thus aims to review the works of toxic effects of lindane on haematological indices of fish, *A. morar* for the experimental period of 60days. LC_{50} value of lindane for *A. morar* comes out to be 1mg/l. The studies revealed that Total erythrocyte count (TEC), haemoglobin (Hb), haematocrit (Hct), and Total leucocyte count (TLC) exhibited marked decline while calculated values viz., Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) showed fluctuating pattern. The observed alterations were ultimately become the causative for affecting the general health status of the fish.

Keywords: *Organochlorine, lindane, toxic, A. morar.*

Introduction

Aquatic environment is plagued with different kinds of aquatic pollutants (Devi *et al*., 2008, Sachar and Raina, 2014). Insecticides are one such category of organic pollutants which play an important role in controlling different types of insect/ pests that cause damage to crop plants. Unfortunately, most of the insecticides are not biodegradable and tend to persist for years together in soil and water (Gaafar *et al*., 2010). Lindane (an organochlorine insecticide) is a broad spectrum insecticide used to control insect pests of rice, cotton, soyabean etc. These insecticides by their easy entry into the aquatic ecosystem (as runoff) may also result in damage of non-target organism particularly fishes. Determination of extent of damage to different body systems viz., respiration, feeding, osmoregulation and reproduction including blood (Du Preez and Van Vuren, 1992) exposed to different xenobiotics therefore become very important. Among different systems haematology act as an essential index of the general health status of the fish (Larsson *et al*., 1985). Lindane is commonly used insecticide in crop field and its bioaccumulation is known to cause impairment in various physiological processes under the conditions of long term exposure.

Therefore, presently an attempt has been made to study the effect of lindane on haematological parameters (TEC, Hb, Hct, TLC, MCV, MCH and MCHC) of fish, *Aspidoparia morar* for the experimental period of 60days.

Materials and Methods

Adult specimens were collected with the help of cast net from Nikowal stream of River Tawi from R.S. Pura area, J&K, India. The fishes were acclimatized for about 15 days. LC_{50} value of lindane for *A. morar* after 96hrs. found to be 1mg/l. Three sublethal concentrations viz., 10% (0.1mg/l), 20% (0.2mg/l) and 30% (0.3mg/l) were employed for the experimental duration of 60 days. 0.5ml of blood was taken directly by cardiac puncture with the help of heparinized needles using EDTA as an anticoagulant.

Among blood parameters TEC and TLC were counted with the help of improved Neubauer cytometer (Shaw, 1930). Hb% was determined by using Sahli's haemoglobinometer (Dethloff *etal*., 1999), Hct ws determined by centrifugation method (Wintrobe, 1967). MCH, MCV and MCHC were calculated by using formulae:

MCV= Hct × 10/ RBC Count Its unit is fentolitre (fl)
MCH = Hb% × 10/ RBC counted per mm3
MCHC = Haemoglobin in g/100ml × 100/ Vol. of packed RBCs in 100ml. It represented as percentage (%).

Identification of cellular components: Identification of various blood cells (differential leucocyte count) was done by methodology as adopted by Anderson (2003).

Microphotography: Slides of blood smears were scanned and photographed with Sony SSC-DC378P-Semi-Digital camera attached with Olympus CH20i Research microscope.

Experimental data and those of control were statistically analyzed by means of analysis of variance (ANOVA). Significance was set at P=0.01. All analysis were performed using SPSS software.

Results and Discussion

Total Erythrocyte count

Changes in total erythrocyte count of control and fishes treated with lindane are depicted in Table 1. Comparison of data of control with that of the treated groups very clearly indicates that there is a marked decline in TEC of the fishes in all the treated groups following subjection to three sublethal concentrations of lindane (viz., 10%, 20% and 30% of LC50 value of lindane) during the experimental period of 60 days. One way ANOVA results reveal that the changes in TEC were highly significant ($p<0.01$) at all intervals of lindane exposure in all the treated groups. Similar decline has also earlier been reported in the fishes by Das and Mukherjee (2000b), Verma (2007), Raina (2012) following exposure to different xenobiotics.

Decline in TEC present author feels seemingly appears to be due to combined effect of haemolysis of RBCs and malfunctioning of haemopoietic organs. Decline in TEC also appears to be the outcome of i) an increase in the rate of erythrocyte destruction due to their lysis and ii) reduced surface area of RBC due to their abnormal shapes. The microscopic examination of smear preparations of blood of *A. morar* indicates the distorted shape of erythrocytes (Fig. 3), which present author feels, may cause an imbalance in the respiratory physiology of the fish by reducing the surface area of haemoglobin and its access to oxygen. It can, therefore, be very safely inferred that lindane has induced conspicuous alterations (both qualitatively and quantitavely) in TEC of *A. morar*.

Similar to TEC, Hb and Hct (Table 1) also exhibited similar decline. Present author proposes that decline in Hb seemingly appear to cause rapid oxidation of haemoglobin to methaemoglobin and or release of oxygen free radical. Free radical of oxygen by causing hemolysis may lead to reduced oxygen carrying capacity of blood. Prolonged reduction in Hb content may be deleterious to oxygen transport.

The haematocrit is a measure of how much space red blood cells occupy in blood. It finds significant utility in evaluation of whether the organism is suffering from anemia or not. Decline in Hct content of *A. morar* present author infers can seemingly be attributed to the release of erythroblasts (immature erythrocytes) and lysis of erythrocytes in the general circulation which become apparent from day 1 of the experiment (Fig. 5). The prevalence of erythroblasts and the extent of damage to RBC by lysis gets aggrevated as the chronicity of lindane progresses. In this context, observations of Srivastava and Mishra (1979) who behold hemolytic anemia due to lysis of erythrocytes in *Colisa fasciatus* after exposure to lead (Pb) very emphatically support the presently held viewpoint. From the above discussion and observations, it can be safely stated that the insecticide lindane by interfering with the normal physiology of RBC possibly result in shrinkage of cell size of RBC which ultimately affect the Hct/ PCV of the fish.

Calculated values

Mean corpuscular volume (MCV) (the size of RBC), Mean corpuscular haemoglobin (MCH) (average Hb content of single RBC) and Mean corpuscular haemoglobin concentration (the average Hb concentration in 100 ml. of blood) are the calculated values of RBC, Hb and Hct which usually are evaluated to ascertain the health status of the fish like any other organism including human beings. Any deviation of these calculated values could, therefore, become indicator/ diagnostic of health of any organism. These calculated values are actually the reflection of status of RBC, Hb and Hct. Changes in calculated values of MCV, MCH and MCHC are given in Table 1. It is evidently clear from the data that MCV exhibit a significant increase ($p<0.01$) in all the treated groups. Compared to controls MCH on the other hand exhibited fluctuating pattern and MCHC a significant decline in their values. Such variant perturbances of calculated indices observed during the present studies simply appear to be a defensive response against the toxic effect of lindane. While increase in MCV values may be taken as an index of cell swelling or macrocytosis of the treated fishes (Fig. 5), fluctuation in MCH values clearly indicate that the concentration of Hb in the RBCs was much lower in the exposed fishes than in the control and is clearcut indication of anemic condition. MCHC, the third calculated indices which has been observed to exhibit significant decrease in *A. morar* seemingly appears to be marker of red blood cell destruction and or decrease in Hb syntheisis. Presently too, on the basis of decline in RBC, Hb and Hct on one hand and increase in MCV of the fishes on the other, two type of anemia's have been reported:

i) Hypochromic microcytic anemia: It finds association with decline in RBC, Hb and Hct of the fishes which in turn is related to either iron deficiency or its decrease utilization during Hb synthesis

ii) Macrocytic anemia: This type of anemia is characterized by decrease in Hb (ultimately affecting the oxygen transport) but increase in MCV which result in cell swelling due to increased concentration of carbondioxide in the swelled erythrocytes.

Total leucocyte count

The results on effect of lindane at different time intervals during 60 days experimental period on total leucocyte count of the fish is given in Table 1 and 2, Fig. 3. Results clearly indicate that leucocyte count decline (leucopenia) in all the treated fishes. Present results are in conformity to the results of Elasaesser and Clem (1986), Chindah *et al.* (2004) and Adedeji *et al*. (2009). The microscopic examination of smear of blood of *A. morar* reveals that among leucocytes while lymphocytes, neutrophils as well as monocytes obseved numerical thining (Figs. 3 and 4). Eosinophils and basophils, however, did not record any significant changes ($p<0.01$) in their numerical values (Tables 2 and Fig. 4) compared to controls ones (Table 2 and Fig. 2). Gradual thinning of TLC has been found to go hand in hand with the gradual decline of lymphocytes and hence indicate lymphocytopenia. So, lymphocytopenia appear to be the major contributor for the decline of TLC. Possibly the release of hormone cortisol (although not measured presently) in response to stress of lindane reduces the life span of lymphocytes and even may also be causative of their elimination from blood. Other than lymphocytes, monocytes and neutrophils too observed decline in their numbers. Both of these leucocytes play an important role in immune functions. While monocytes can move quickly to the sites of infection in the tissues and divide or differentiate into macrophages to elicit response, Neutrophils act as shock troops and are attracted to the infected area by chaemotaxis as reported by Arora and Sabharwal (2007). Decline in numerical count of both monocytes and neutrophils in the general circulation in presently studied fish, *A. morar* clearly reflects that fishes become immunologically very weak under the continuous stress of lindane and rather become unable to withstand severity of its stress. Their decline also makes the fish highly susceptible to various infections. Similar observations were also made by Thakur and Pandey (1990) in *C. batrachus* upon BHC intoxication. During present studies observations clearly reveals that eosinophils and basophils did not undergo any alterations in the numerical count of these cell types in any set of experimental fishes (Table 2). But as the experiment advances, it has been observed that RBC besides decline lymphocytes and neutrophils become abnormal and also undergo degenerative changes. The elimination of debris produced by decayed RBC need to be rid off from the body and this seemingly appears to be achieved by these eosinophils and basophils. This process of phagocytosis present author proposes is also additionally aided by macrophages which have been witnessed to make their appearance from day 10 onwards (Fig. 6). The fact that macrophages increase numerically as toxicity of lindane increase with the advancement of experimental period simply give added support to viewpoint that macrophages now help in the process of phagocytosis also. The prevalence of these macrophages in all of treated group of fishes reflects that their appearance in blood help to combat the stress caused by lindane toxicity.

Thrombocytes constitute yet another component of blood cells involved in blood coagulation besides being phagocytic in nature (Anderson, 2003). Presently, marked increase in their number has been observed in all the treated fishes (Table 2 and Fig. 3). Increased number of thrombocytes in the treated groups (Table 2 and Fig. 3) compared to control fishes (Table 2) and (Fig. 3) present author purposes, may plausibly be to arrest the internal bleeding that might have occurred in stressed fish. Besides playing role in blood clotting their numerical increase appears to strengthen the already operational phagocytic machinery of the fishes and thus indirectly may help to counter the stress caused by lindane toxicity. Similar viewpoint has also been putforth by Srivastava (1969a), Eaton (1974) and Anderson (2003).

Conclusion

Haematological parameters related to oxygen transport (RBC, Hb and Hct), defense mechanisms (WBC) and the calculated indices (MCV, MCH and MCHC) all exhibited marked differences between control and experimental groups in response to insecticide lindane in presently studied fish, *A. morar*. Present studies thus confirm that haematological parameters are very sensitive indicators in fishes under toxicity of chemicals (presently lindane). The alterations in the haematological parameters ultimately become the causative for affecting the general health status of the fish.

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Annexure

Table 1: Haematological Parameters of *A. morar* (Mean ±S.D.) for various Concentrations of Lindane

Parameters	Control	10%	20%	30%
TEC($\times 10^6$cm/mm^3)	2.05±0.05	1.86±0.77	1.71±0.05	1.20±0.01
Haemoglobin(gm/dl)	5.5±0.62	4.77±0.11	4.13±0.91	3.65±0.23
Haematocrit (%)	25.2±0.26	24.14±0.39	23.19±0.14	21.95±0.47
MCV(fl)	122.93±0.77	129.96±0.89	136.54±0.31	209.51±0.18
MCH(pg)	26.93±0.44	25.58±0.24	23.85±0.19	32.50±0.47
MCHC(%)	21.82±0.80	19.73±0.80	17.66±0.50	16.42±0.66
TLC($\times 10^6$cm/mm^3)	7.50±0.5	5.35±0.10	4.69±0.38	4.15±0.86

Table 2: Differential leucocyte count of *A. morar* (Mean±S.D.) for various Concentrations of Lindane

Parameters	Control	10%	20%	30%
Lymphocytes(%)	24.09±0.l8	13.69±0.81	11.53±0.79	8.30±0.37
Monocytes(%)	15.66±0.99	9.46±0.66	7.82±0.44	6.54±0.49
Neutrophils(%)	21.69±0.80	14.90±0.72	12.60±1.46	8.91±0.68
Eosinophils(%)	6.03±0.11	6.03±0.37	6.02±0.19	6.02±0.39
Basophils(%)	6.02±0.80	6.03±0.15	6.02±0.84	6.02±0.55
Thrombocytes(%)	26.51±0.22	49.87±0.77	56.0±0.92	64.20±0.81

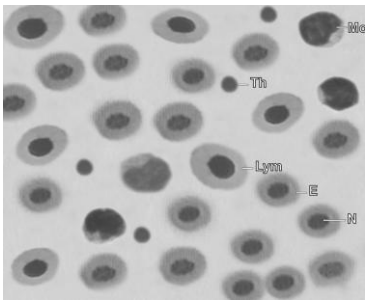

Fig. 1 Microphotograph of blood smear from control showing Normal Erythrocytes (E) with Nucleus (N),Lymphocytes (Lym), Monocytes (Mo) and Thrombocytes (Th) (H&E×1000)

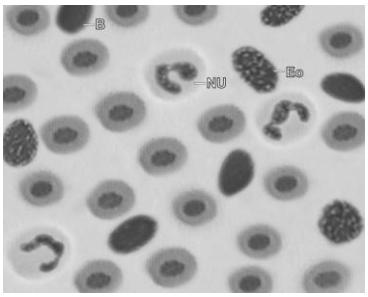

Fig. 2 Microphotograph of blood smear from control showing Neutrophils (Nu), Eosinophils (Eo) and Baasophils (B) (H&E×1000)

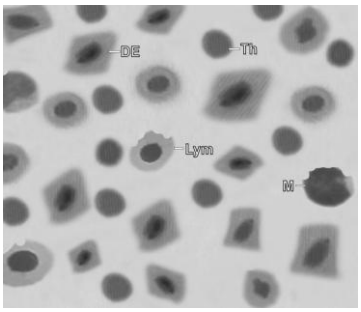

Fig. 3 Microphotograph of blood smear from lindane treated fish showing Distorted erythrocytes (DE), decline in Lymphocytes (Lym) and Monocytes (Mo) with increase thrombocyte count after 1 day of the experiment (H&E×1000)

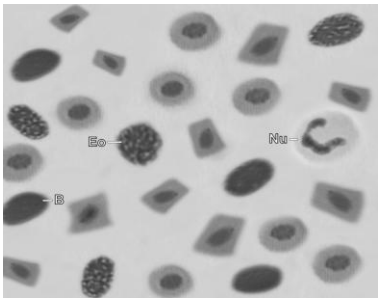

Fig. 4 Microphotograph of blood smear from lindane treated fish showing decline in Neutrophils (Nu), Eosinophils (Eo) and Basophils (B) after 1 day of the experiment (H&E×1000)

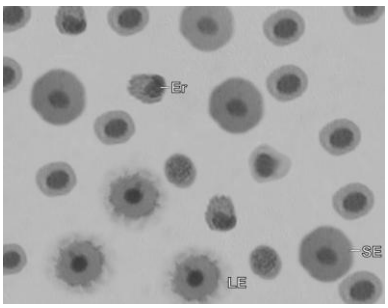

Fig. 5 Microphotograph of blood smear from lindane treated fish showing Swelled Erythrocytes (SE), Lysed Erythrocytes (LE) and appearance of Erythroblasts (Er) after 1 day of the experiment (H&E×1000)

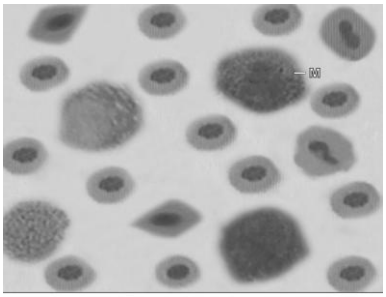

Fig. 6 Microphotograph of blood smear from lindane treated fish showing increase in Macrophages (M) after 10 days of the experiment (H&E×1000)