Study on the effect of Urea on blood parameters of an air breathing fish,

Anabas testudineus (BLOCH)

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Abstract:

Fresh water climbing perch *Anabas testudineus*, locally called "Kabai" is a common air-breathing fish of wetlands of North Bihar. The present study attempts to define acute (96 h) and chronic effects of certain chemical Urea on the important air breathing fish, *Anabas testudineus*. The 96 h LC 50 value is 12.6 mgl^{-1}. Both acute and chronic exposures resulted in the reduction of blood characteristics viz. erythrocyte count, haemoglobin content and haematocrit value. The highest values (12.0 g% in male and 10.1 g% in female) were observed in the month of November fall till the month of February (in males) and March (in females).The observed similar Hb concentration in the same sex between two fish is expected. Levels of Hb found in both sexes of *Anabas testudineus.* (Bloch). Levels of Hb found in both sexes of *Anabas testudineus.* (Bloch). Examination of erythrocyte profile was carried out on 6 control and 9 experimental female specimens at the end of 96 hr toxicity test with Sevin in concentration of 12.6 mgl^{-1} and 14.6 mgl^{-1} and the control group was examined for haematological indices.

Keywords: *Anabas testudineus*, Urea, Blood parameters, LC 50 value, Haemoglobin, Erythrocyte, and Haematocrit.

INTRODUCTION:

Fresh water climbing perch *Anabas testudineus*, locally called "Kabai" is a common air-breathing fish of wetlands of North Bihar The climbing perch, *Anabas testudineus* (Bloch), also popularly known as *'Koi'* is a well known air breathing edible fish, inhabiting fresh waters and brackish waters. The common name, climbing perch, originated from the Asian legend that Anabas climbs palm trees to suck juice. Probably the origin of this myth is that birds pick Anabas when they travel overland and place it on palm trees (Norman, 1975).

Aquatic pollution induced by the indiscriminate discharge of fertilizers and allied wastes has drawn the attention of environmentalists because of their hazardous actions at various levels. Extensive programme of analysis of water of polluted fed fisheries has been undertaken now a days to explain the abnormalities in the fish growth and frequent fish mortality (Ganpati and Chacko, 1970).

The blood constituents in fishes are influenced by factors like temperature ecological habitat, food selection and mode of life. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations (Mulcahy, 1975). Alteration in physiological parameters of toxicant treated fish has recently emerged as an important tool for water quality assessment in the field of environment toxicology. This is because blood in the gill has direct contact with the water medium and any unfavourable change in the

water could be reflected in the circulatory system. These studies could be used to indicate the health status of fish as well as quality. Blood chemistry has long been a helpful diagnostic tool in pathological, toxicological and general clinical tests. Blood being the medium of intercellular and intracellular transport, which comes in direct contact with various organs and tissues of the body, the physiological state of an animal at a particular time is reflected in its blood. *Aanabas testudineus* maintain their normal physiological process and their body fluid homeostasis with the help of ion/osmoregulatory processes (Hwang and Lee, 2007). Typically haematological parameters are non-specific in their responses towards chemical stressors; nevertheless, they may provide important studies for e.g. by providing and to the general physiology and health status of the organism under investigation (Beyer, 1996).Haematological parameters can provide satisfactory information of on the physiological response of fish to environmental stressors for two major reasons, namely, the close association of the circulatory system with the external environment and the case of availability of fish blood (Houston, 1997; Lohner *et al*., 2001; Cazenave *at el*., 2005). The use of primary haematological indices such as red blood cell count (RBC count) in assessing sub-lethal concentrations of two different carbonic compounds such as Urea and D.A.P. are considered. Changes in the erythrocyte count or in haemoglobin values following chronic stress are useful as indicators of blood volume changes (haemodilution or haemoconcentration) that have occurred. Haemolysis of red blood cells provides simple and rapid way of studying the effect of pollutants on biological membranes (*Harington et al., 1971*) numerous investigations have considered membrane model a measure of pollutant's cytotoxicity (*Allison et al., 1966)*. The red blood cells membrane haemolysis has proved to be a simple and rapid way of attempting to find the possible correlation between toxicity and haemolytic activity *(Macnab and Harington, 1967).* In the present study a clear trend was observed linking latex and plant extract concentration with membrane damage. The impact was the most severe on fishes exposed to the highest of the three sub-lethal concentrations of Urea of *Anabas testudineus*. In this concentration some changing also observed regarding its physical activities.

Ammonia eliminated by fish is frequently oxidized on nitrite and nitrate through the nitrification of Nitrosomonas and Nitrobacter (Sharma and Alert, 1977). In grow-out ponds ammonia and nitrite increase exponentially over time and may have detrimental effects on fish and their larvae (Hilmy et al., 1987). Inorganic nitrite is a strong methaemoglobin former both in *vivo* and *vitro* (Bodansky, 1951). Since methaemoglobin is unable to carry oxygen; heavy nitrite loads producing high methaemoglobin contents may cause death as a result of hypoxia. Therefore, the accumulation of nitrite and their removal from a closed culture system are important concerns in fish culture. Information is scarce on the toxic effects of nitrite on prolonged exposure, Moreover; very few reports exist on the effect on urea on any parameter in the blood other than methaemoglobin (Hilmy et al., 1987). Keeping all these facts in mind, this study was designed to collect acute and chronic toxicity data for juvenile *Anabas testudineus and* to find if exposure to urea causes alteration in blood characteristics.

MATERIAL AND METHOD:

Static renewal acute toxicity bioassays were conducted in conformity with the guidelines established by APHA (`1996) and Parrish (1985) for the determination of LC 50 value. Based on exploratory tests the toxic range was determined, subsequently a series of seven different concentrations of sodium nitrite solutions were prepared by adding calculated volume of the stock to each test container containing 50 litre dechlorinated tap water. Ten healthy fish were transferred to each of the test containers. The test solutions were renewed at 24h intervals to avoid the possibility of reduction in urea content with time. For everyexperimental group a control unit was also maintained. During the entire duration of the experimental period all tanks were aerated to maintain the dissolved oxygen content at saturation. Physico-chemical qualities in the water were monitored throughout the experiment. Mortalities were assessed over period of 24, 48, 76 and 96h and dead fishes were discarded after each observation. The 96h LC 50 value was estimated by using a computerised programme of Finney (1971).

To find out alterations in blood characteristics, acute and chronic toxicity tests were conducted. The tests of acute toxicity began by transferring 30 samples fish to each of tanks, one test and one control. In test tank fish were exposed to concentration if 96h LC 50 value of urea. Fishes were drawn from test and control tanks during the intervals of 24, 48, 76 and 96h for sampling. The experimental system of chromic toxicity consisted of two tanks with dechlorinated tap water, one test and one control. In the test tank 2.7 mgl^{-1} of urea (1/10 the 96h LC50 value) was added. Fishes were sampled on 30, 60, 90 and 120 days.Blood samples were collected from the haemal arch in the caudal peduncle into heparinised and non heparinised vials for both the acute and chronic tests. Blood in the heparinised tubes were used immediately for the determination of various blood characteristics such as erythrocyte count, haemoglobin content, haematocrit value and metaemoglobin concentrations. Blood samples in non-heparinised vials were allowed to clot and then centrifuged at 8000 r.p.m. for 5 min to obtain serum. Total haemoglobin was measured by the cyanomthaemoglobin method (Mattonheimer, 1970). Erythrocyte counts were made adopting the techniques a drastic increase in the methaemoglobin suggested by Hesser (1990). Micro-centrifugation method of Larsen and Snieszko (1961) was used for the determination of haematocrit. The method of Brnet and Youden (1970) was used for the quantification of total protein in serum.Statistical analysis of the data was made using Student's t-test and a probability level of $P < 0.005$ was accepted as statistically significant.

RESULT AND DISCUSSION:

As the fish came in contact with water of Urea, restlessness was observed and supplemented by sudden rapid movement in circles. A neural paralytic effect was observed in fish Poisoned with Stevin. The fish tried hard to swim and swam in half circles Haematological parameter: Significant reduction in erythrocytes and haemoglobin was observed at sub lethal concentrations. The erythrocytes (RBC) values for treated fish indicated decline when compared with the reference fish (Table 1). Decrease in haemoglobin in experimental animals might be due to destruction of (decrease in haemoglobin has been reported by Bhaktavasalam(1991). Erythrocytes and inhibition of erythropoiesis, which is confirmed by

decreased MCHC Values. Lowering of Erythrocyte values has been reported by Svoboda et al (2001) in *Anabas testudineus* due to the effect of carbonic compound, Prabha et al (1999) have reported the Effect of paper and pulp mill effluent on haemopoietic alterations in *Anabas testudineus*. Effect of sub lethal concentration of propoxur has been reported by Singh et al (1991) with significant decrease in haematocrit value and haemoglobin concentration in air breathing fish and decrease in haemoglobin has been reported by Bhaktavasalam (1991) in *Anabas testudineus*. Fall in the number of red blood Cells followed by PCV (Packed cell volume) confirms anemia in *Clarias batrachus.* MCV and MCH values also reduced after exposure to various concentrations. Some of the blood cells became elliptocyte i.e. more cigar like shape and some cells showed variation in size which confirms anisocytosis due to carbaryl effect on blood cells.(Fig 1)

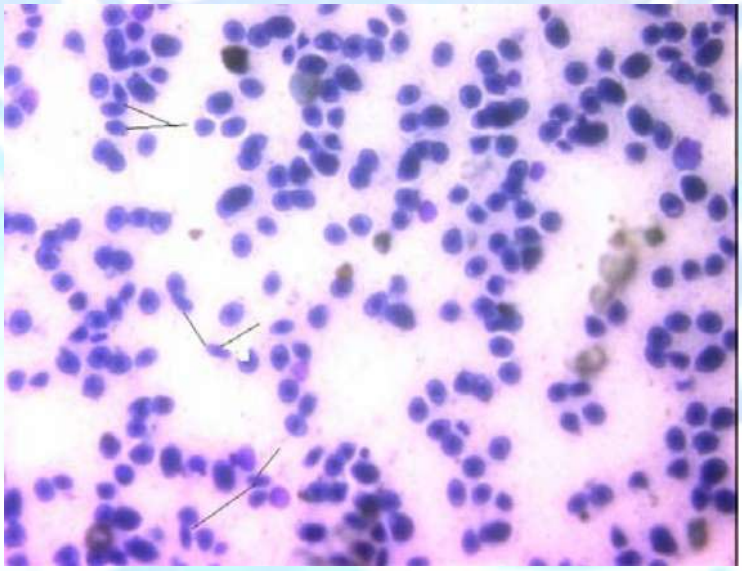

Fig .1 Change in the shape of bloodcells (Anisocytosis) at 12.6mg/l

During the course of 96 hr toxicity test of Sevin based carbamate on *Anabas testudineus* there was no death of fish in control tank. The main haematological response on exposure to Sevin was significant decrease of haemoglobin content, haematocrit value and erythrocyte count. The shape of blood cells also showed changes at concentration of 12.9 mg/l and formed chain like structure at concentration of 14.9 mg/l as is evident from Fig.1 Decrease in erythrocytes has been reported in Tilapia zilii due to water Pollution Abdelmeguid et al (2002). Changes in blood cell profile have been reported in air breathing fish due to the effect of Urea by Svoboda *et al* (2001). Organophosphate effect on haematological indices has been reported by Chindah et al (2004) in *Tilapia guineensis*. Decrease in various indices of blood after exposure to Sevin at different Concentrations indicated that *Anabas testudineus* became anemic (Table 1). The same has been reported in *Tilapia guineensis* after exposure to chloropyrifos by Chindah et al., (2004). Anisocytosis and crenation of erythrocyte membrane has been reported by Birendra et al., (1991).

The blood volume of fishes varies between 2 and 8% of their body volume. One third to one half of the total blood volume in fish consists of blood cells, the rest is fluid plasma. In spite of

systematic diversity all fishes possess two main types of blood cells erythrocytes (red cells) and leucocytes (white cells). The average total erythrocyte counts were found to vary from 1.04 to 2.40 x 10^6/mm^3 in male and 0.75 to 1.85 x 10^6 mm3 in female (table). Female showed counts than males in all the months. The highest values (2.40 x 10^6 mm3 in male and 1.85 x 10^6 mm^3 in female) were obtained in November (figure – 1). This peak was followed by a continuous fall until February (1.04 x 10^6/mm^3 in male and 0.87 x 10^6 mm^3 in female), and again in May and June in both the sexes of *Anabas testudineus.* Unlike the RBC counts, the Hb content also shows seasonal fluctuation in both males females. (6.4 to 12.0 in males and 6.3 to 10.1 in females) (Table 2). The highest values (12.0 g% in male and 10.1 g% in female) were observed in the month of November fall till the month of February (in males) and March (in females).The observed similar Hb concentration in the same sex between two fish is expected(8). Levels of Hb found in both sexes of *Anabas testudineus.* (Bloch). Examination of erythrocyte profile was carried out on 6 control and 9 experimental female specimens at the end of 96 hr toxicity test with Sevin in concentration of 12.6mgl^{-1} and 14.6 mgl^{-1} and the control group was examined for haematological indices.

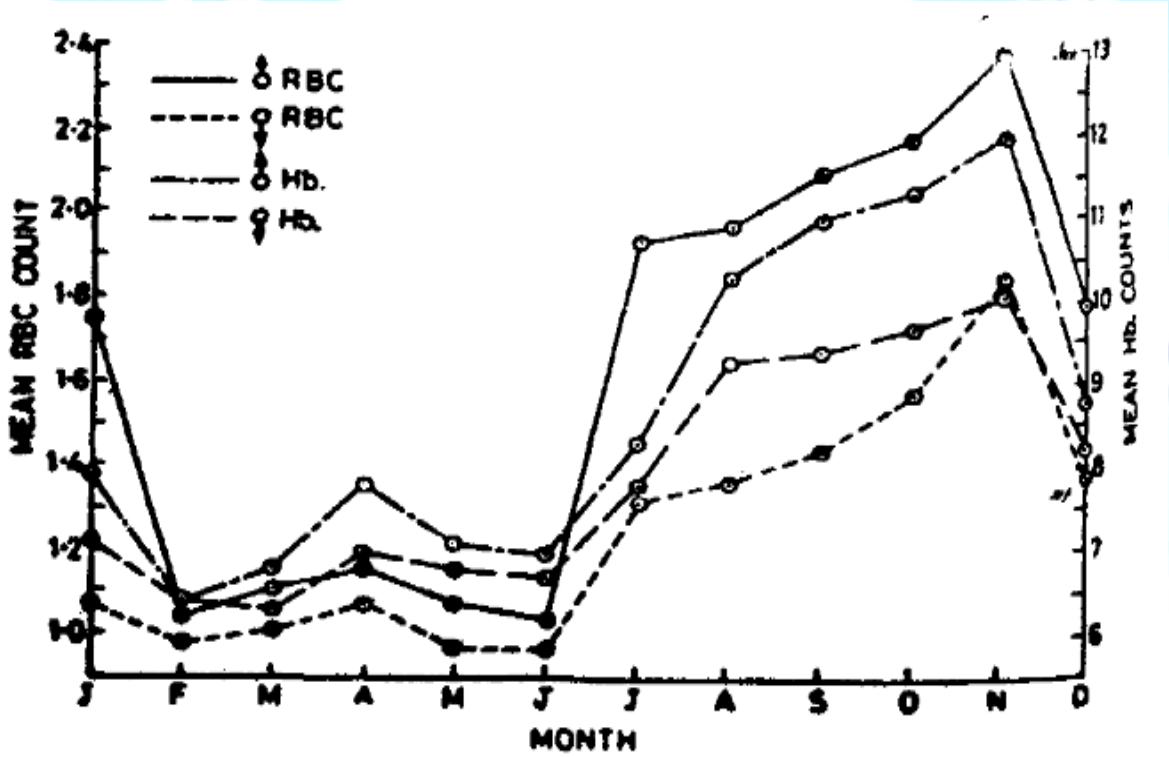

Fig. : Seasonal variations in the erythrocyte counts and haemoglobin content of *Anabas testudineus.*

The morphological and physiological characters of fish blood have also taxonomic significance (Gulliver 1875). The fish haematology has attracted less attention of the workers especially in freshwater fishes. Perusal of the available literature shows that little work has been done on seasonal variations in the fish blood. However, Joshi and Tandon (1977) in *Heteropneustes fossilis*, Khan (1977), in *Clarias batrachus* and The principal cellular component of the blood of

air breathing fish(*O.striatus, O.puntatus, C. mangur* and *H. fossilis* consists of erythrocytes, thrombocytes lymphocytes eosinophils and neutrophils. Not a single basophil was found in the whole study, and also no attention was paid to trace the presence of immature cells in the circulating blood of the above four species.

Total number of erythrocytes ranged from 2.73 – 4.16 millions in *O. struatus,* 2.20 – 3.02 millians in *O. punctatus,* 1.90 – 3.47 millions in *C. mangur* and 2.58 – 2.85 millians in *H. fossolis,* and the mean blood cell counts were found to be 3.24 , 2.59, 2.57 and 2.73 millions cell/cu.mm. in the four species respectively. Maximum numbers of erythrocyte were found in (table – 1). The mean values of the three species are almost the same.

TABLE – 1

Measurement and erythrocyte counts of species of freshwater air – breathing fishes

Species	Number of indivi-duals	Total RBC (millions cell/cu.mm.)		Size of RBC (Millions)	
		Range	Mean	Range	Mean
Anabas testudineus . .	16	2.78 - 4.16	3.24	10.41 x 7.48-11.43 x 8.20	10.95 x 7.84
Ophicephalus puntatus	7	2.20-3.02	2.59	10.50 x 7.60-11.22 x 3.08	10.95 x 7.79
Clarias mangur . .	16	1.90-3.47	2.57	11.41 x 8.87-11.63 x 10.20	10.97 x 9.56
Heteropneustes fossilis	4	2.58-2.85	2.73	10.98 x 9.27-11.45 x 8.82	11.13 x 8.98

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