

## EFFECT OF EKALUX TOXICITY ON SELECTED PHYSIOLOGICAL PARAMETERS IN *ANABAS TESTUDINEUS*

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**Abstract.** Impact of short-term exposure of sub lethal concentration of organophosphate pesticides Ekalux on physiology of sub adults of *Anabas testudineus* in the Kol paddy fields of Central Kerala was studied. Results of the present study indicated that even short-term, exposure to Ekalux is detrimental to *A. testudineus*. Among the parameters that showed significant changes were haematological parameters such as Hb, PCV, RBC count, and ESR. Cell morphology shifted from a normal elliptical shape after 24 hr to crenated or fringed and shrank appearance at 15<sup>th</sup> day of exposure. It was also seen that protein and amino acid concentration are clear indicators of immediate stress. Histopathological studies of liver showed hypertrophied hepatocytes with reduced number of normal cells after 15 days of exposure. The number of haematopoietic tissue was found to have declined in the kidney also in addition to increased number of melano-macrophage centres (MMC). However, once these fishes were reared in clear water, devoid of any pesticide for six months, values for Hb, PCV, protein, amino acid and liver and renal tissues showed signs of revival. The present study depicts that severity of Ekalux toxicity to sub adults of *Anabas testudineus* is both dose and time dependant.

**Keywords:** *Wetland, Ekalux, Anabas testudineus, Haematology, Histopathology*

### Introduction

Kole lands are water logged paddy fields spread over Thrissur and Malappuram districts in Central Kerala. These Kole lands promote sustainable aquaculture practices through the integration of agriculture and pisciculture. But the application of persistent and toxic insecticides to the rice, the fish cultured in these fields are increasing getting contaminated by a variety of pesticide residues. Ekalux is an organophosphorous pesticide (Quinalphos) which is widely used in paddy fields of Malappuram district. Till date no data is available on the deleterious impact of Ekalux on the ecology of Kole lands in general and fishes in particular. Bioaccumulation of fish leads to major structural changes. They include histological structure changes, hematological changes as well as physiological stress, which are witnessed in the form of changes in biochemical constituents in the body of target organism (Grant & Mehrle, 1973; Wedemeyer and Yasutake, 1977; Jagoe, 1996). Under the above background a study was carried out to determine the impact on the haematological, histological and biochemical constituents of freshwater fish *Anabas testudineus* when exposed to sub lethal concentration of Ekalux. This study warrants much attention to the fact the most of the paddy fields throughout the culture period act as sink for various pesticides. Fishes being inherent inhabitants of these ecosystems need to adapt to this rather hostile environments. Present study also investigates whether these negative effects of short-term exposure to Ekalux could be nullified if the fishes were re-exposed to normal clear water.

## Materials and Methods

Live and healthy sub adult specimens of freshwater fish *Anabas testudineus* (Bloch) with an average weight of  $20.25 \pm 0.29$  g and length of  $12.05 \pm 0.24$  cm were collected from a local pond. A 96 hr  $LC_{50}$  for Ekalux on sub adults of *Anabas testudineus* was estimated following Yasmineen *et al.* (1991). For bioassay studies 48 healthy fishes of uniform size were selected and reared in four separate FRP tanks of 200 L capacity. The first set of tanks was not dosed with pesticide hence they were maintained as control tank (T1). In all the remaining tanks Ekalux was added at a concentration of 0.05 ppm. In T2, fishes were sacrificed at the end of 24 hr period, while in T3 fishes were reared for 15 days before sacrificing for bioassay studies. In the last set of tanks (T4), after exposing the fishes to sub lethal concentrations for 15 days, fishes were transferred to freshwater devoid of any pesticide residue and reared for another 6 months. At the end of the clear water phase all the fishes were sacrificed for routine assessment. Throughout the experimental period the fishes fed with artificial feed twice daily. Tanks were aerated and water exchange was done every alternate day.

**Haematological assay:** Haematological parameters assessed for the study were Packed Cell Volume (Microhaematocrit method) and Total RBC (Blaxhall and Dansel, 1973), Haemoglobin following Sahli's Acid Haemoglobin method (Hesser, 1960), Erythrocyte Sedimentation Rate (Das and Mukherjee, 2002) and Cell size following Wright's Giemsa staining method of Hesser (1960).

**Biochemical studies:** Biochemical constituents analyzed during the present study were Total protein (Lowry *et al.*, 1951), Total amino acids by colorimetric method (Yemm and Cocking, 1955), DNA using diphenylamine method (Burton, 1956) and RNA following Orcinol reagent method (Hiroshi, 1962).

**Histopathological examinations:** Tissues examined during the present study included the liver and kidney. Tissues from fishes from four treatments were dissected out and preserved in 10% Neutral Buffered Formalin (NBF), embedded and sectioned at 6  $\mu$  thickness using microtome. The sections were then stained with Mayer's Hematoxylin and Eosin and mounted with DPX to be photographed at a magnification of 40X.

**Statistical Analysis:** All the parameters observed were statistically evaluated following Gomez and Gomez (1984). Statistical analysis conducted included Analysis of Variance (ANOVA) and Duncun's Multiple Range Test (DMRT) following SPSS ver. 17.0 for Windows.

## Result and discussion

### *Determination of $LC_{50}$*

The 96 h  $LC_{50}$  mean value of Ekalux for subadults of *Anabas testudineus* in the present study was found to be 1 ppm (*Table 1*). The results showed that Ekalux is highly toxic to *A. testiduneus* and highest mortality of specimens was recorded at 4 ppm concentration.

**Table 1.** Percentage mortality of *A. testudineus* exposed to Ekalux

Treatment	Concentration ppm	Fishes exposed	Percentage of survival			
			24 hr	48 hr	72 hr	96 hr
1	1	16	100	100	81	69
2	2	16	90	88	56	43
3	3	16	81	56	50	37
4	4	16	81	50	50	31

### Haematological studies

Result of the present study showed that there exists a discernable difference in all the haematological parameters studied among the treatments. However, these variations were mostly dependent on the exposure period of the Ekalux rather than the concentration of the pesticide itself. Results of one way analysis of variance (ANOVA) confirmed the above findings. Values of ANOVA showed a significant difference at less than 1% level ( $P < 0.01\%$ ) for all the parameters observed i.e, haemoglobin ( $F = 15.391$ ), Packed Cell Volume ( $F = 14.214$ ), RBC count ( $F = 204.11$ ) and Erythrocyte Sedimentation Rate ( $F = 39.111$ ). While analysing the performance of different haematological parameters following Duncan's Multiple Range Comparison Test (DMRT), it was observed that the value of haemoglobin were comparable in all the treatments except for T2 (24 hr exposure) (Table 2).

**Table 2.** Comparative evaluation of different haematological parameters of *Anabas testudineus* reared under different treatments

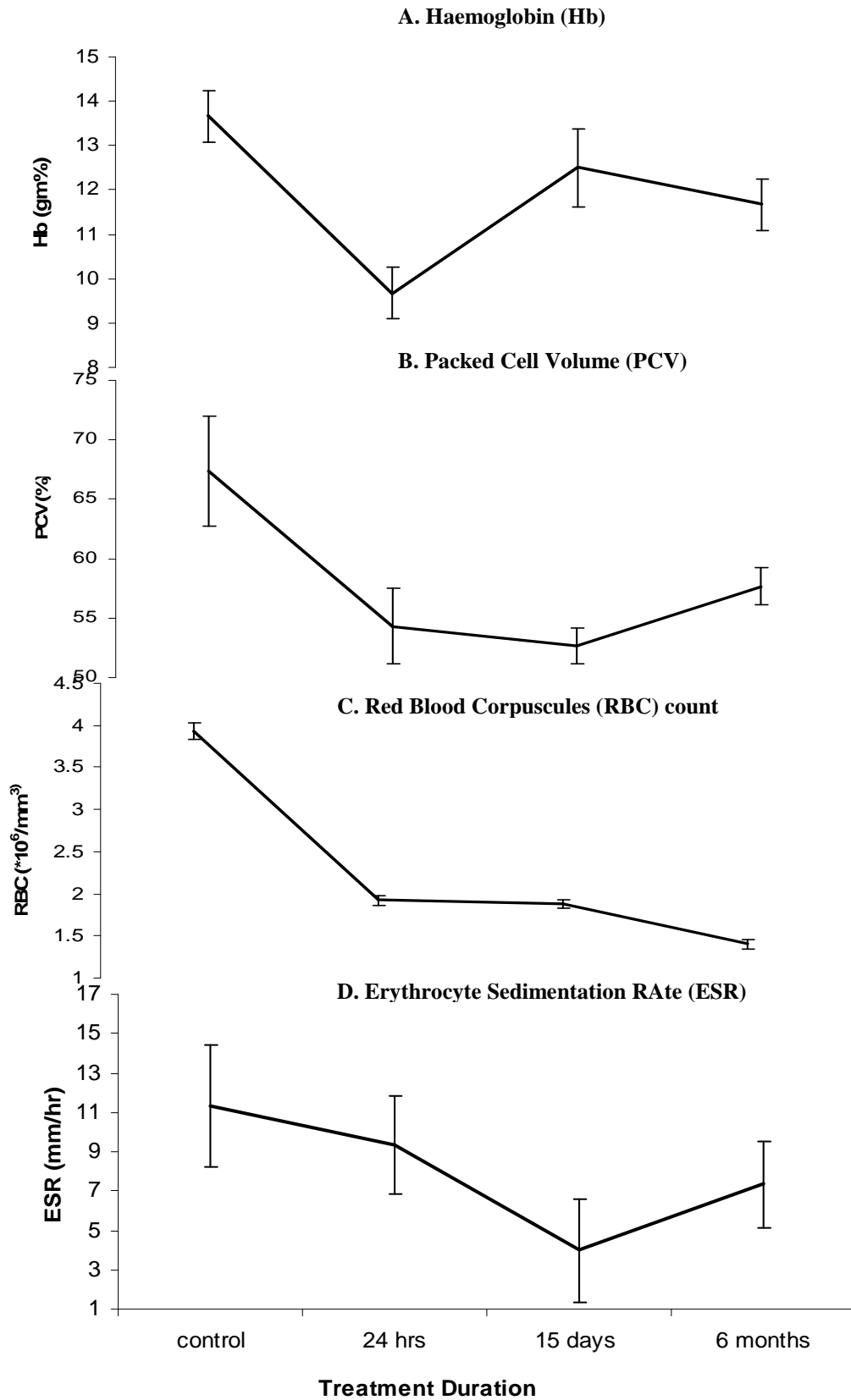
Variable	Source	df	Sum of Squares	Mean Square	F	Sig.
Haemoglobin (Hb)	Between Treatments	3	27.896	9.299	15.391	0.001
	Within Treatments	8	4.833	0.604		
	Total	11	32.729			
Packed Cell Volume (PCV)	Between Treatments	3	387.333	129.11	14.214	0.001
	Within Treatments	8	72.667	9.083		
	Total	11	460.00			
Red Blood Cells (RBC)	Between Treatments	3	28.962	9.654	204.11	0.000
	Within Treatments	8	4.552	0.569		
	Total	11	33.514			
Erythrocyte Sedimentation Rate (ESR)	Between Treatments	3	2.216	0.709	24.248	0.000
	Within Treatments	8	0.232	0.029		
	Total	11	2.358			

This means the impact of pesticide is immediately felt in the concentration of Hb, which later improves to a level similar to that of the control tanks (Fig 1A). However, the trend shown by PCV during such analysis was in total contrast to that of the Hb. Once exposed to sub lethal concentrations of Ekalux, the corresponding PCV values differed significantly from that of the control tank (Table 2). However after being reared in clear water there was a gradual improvement in the values of PCV after 6 months (Fig 1B). Similar results were seen for RBC count as a clear difference exists among the control and treated tanks (Table 2). However, a noteworthy observation was that even after reintroducing the fishes to pesticide free environment there wasn't any significant

improvement in the net RBC count (*Fig 1C*). ESR values of all the treatments differed significantly enabling them to be grouped under three different subsets (*Table 5*). The mean progression of ESR were in total contrast to the trend shown for PCV, whereby with time the ESR values increased but once the effect of pesticide was removed the values started to decline (*Fig 1D*).

Haemoglobin levels significantly decreased after 24 hour may be due to the disruption of the iron synthesizing machinery (Beena and Viswarajan, 1987). It may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production (Ahammed *et al.*, 1999). The significant decrease may also be due to either an increase in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis (Reddy and Bashamohideen, 1989). Musa and Omoregie's (1999) reported similar results when fishes were exposed to polluted environment under laboratory conditions. Reduction in the RBC count of *A. testudineus* noticed in the present study could be due to the haemodilution resulting from impaired osmoregulation across the gill epithelium. Reduction of TEC may be suggestive of an appreciable decline in the haematopoiesis leading to various type of anaemia. Similar reduction of RBC were observed in *Tilapia mossambica* exposed to Sumithion and Sevin (Koundinya *et al.*, 1979), *Cyprinus carpio* exposed to Cypermethrin (Reddy and Bashamohideen, 1989) and in *Anabas testudineus* exposed to Azodrin (Santhakumar *et al.*, 1999). Decrease in the PCV may be attributed to the reduction of TEC. Decrease in the PCV may show the extent of shrinking cell size due to the insecticide intoxication (Ahmad *et al.*, 1999). Naidu *et al* (1989) from a 24 hour short term study reported an increased in the Hb and PCV in *Cyprinus carpio* exposed to sub lethal concentration of Endosulfan. In the present investigation, even though, the RBC count decreased considerably initially, the fish tried to overcome the stressed condition by elevating the concentration of Hb.

Studies on the cell morphology of erythrocytes exposed to sub lethal levels of Ekalux also showed varying degree of changes in external cell structure (*Fig 3*). When the fishes were exposed to pesticide the cell wall of erythrocytes of such fishes were crenated and showed distortion. Rather than maintaining the usual disc-like shape which the blood cell normally has (*Fig 3A*), RBC of exposed fished fishes showed small extendings (*Fig 3B*). The central area in these cells were considerably reduced (*Fig 3C*). Even after a revival phase of 6 months, similar manifestations in the external morphology of RBC were witnessed in fishes exposed to pesticides (*Fig 3D*). Some of the cells after 24hr exposure to Ekalux became elliptical in shape. With the increase in exposure period (15 days), the disappearance of the erythrocyte membrane, shrinkage of red blood cells and reduction in the size of the nucleus and erythrocyte matrix, were observed in these fishes.



**Figure 1.** Variation in the Haematological parameters in *Anabas testudineus*

### Biochemical studies

The result of the present study clearly suggests that exposure of sublethal concentration of Ekalux in *Anabas testudineus* showed variations in protein, amino acid, DNA & RNA (Table 3). The difference was highly significant ( $P < 0.01$ ) level for protein ( $F = 18.560$ ) and amino acid ( $F = 8.111$ ). While the difference for DNA ( $F = 4.513$ ) & RNA ( $F = 4.919$ ) were significant at 5% level. Result of DMRT test (Table 4) showed that once the fishes were exposed to Ekalux there was a rapid shift in the concentration of protein from the normal levels. However, when re-exposed to clear water there was a revival in the value of protein after six months (Fig 2A).

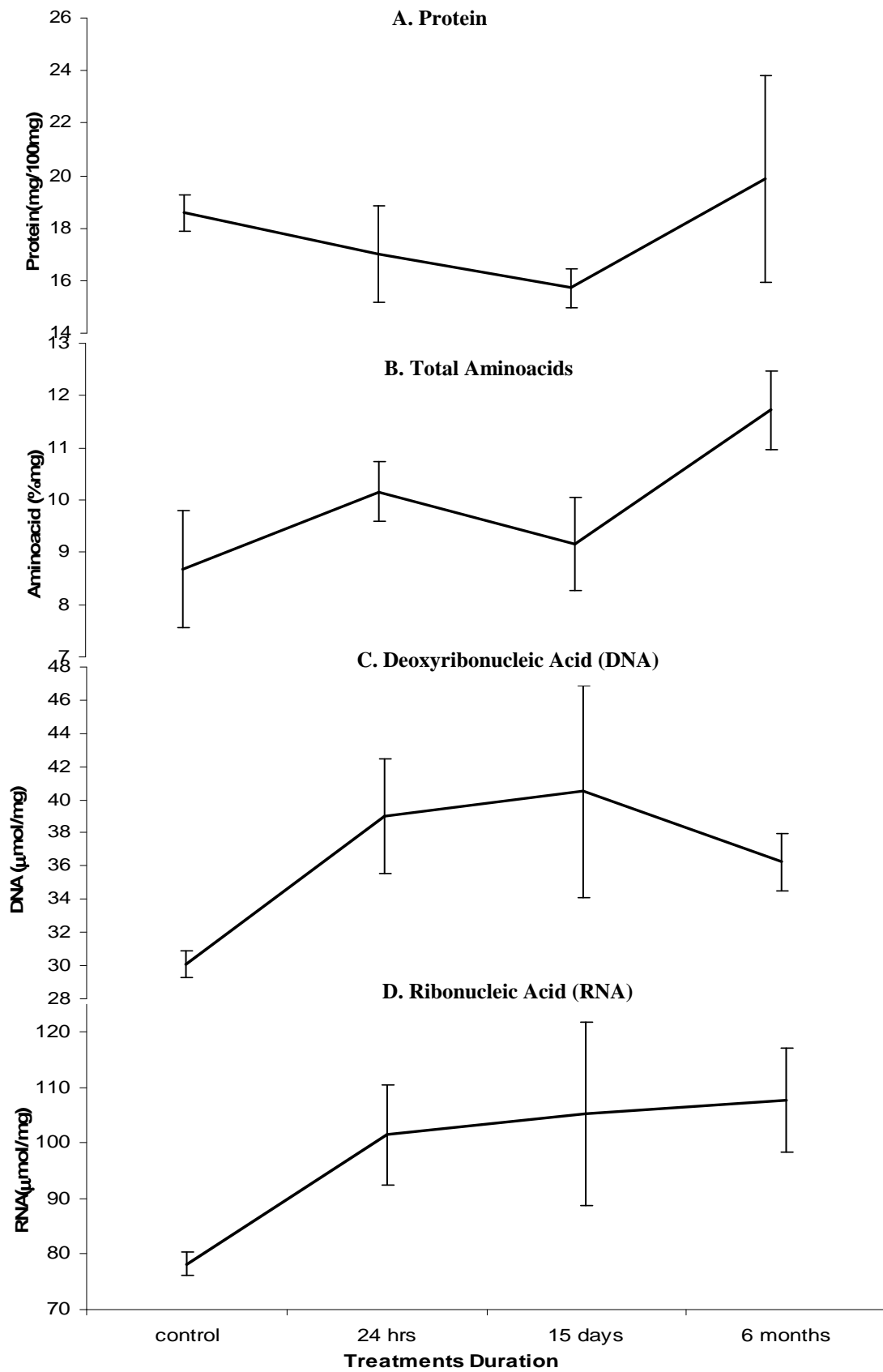
**Table 3.** Results of DMRT on difference in the concentration of Heamoglobin (Hb), Packed cell volume (PCV), RBC count and ESR rate

Treatment	Hb	PCV	RBC	ESR
T1	13.6667 <sup>b</sup>	67.3333 <sup>b</sup>	3.9300 <sup>a</sup>	2.1800 <sup>a</sup>
T2	9.6670 <sup>a</sup>	54.3333 <sup>a</sup>	1.9200 <sup>b</sup>	2.7833 <sup>b</sup>
T3	12.5000 <sup>b</sup>	52.6667 <sup>a</sup>	1.8800 <sup>b</sup>	3.2600 <sup>b</sup>
T4	13.0000 <sup>b</sup>	57.6667 <sup>a</sup>	1.4000 <sup>c</sup>	2.3367 <sup>c</sup>

Means for groups in homogeneous subsets are displayed.

Similarly for Amino acids also a sudden change in their concentration was discernible. However, the changes did not follow any particular trend (Fig 2B). While analysing the performance of DNA and RNA among the four treatments, it was observed that the impact of pesticide is more pronounced in these biochemical components. Interestingly, the concentration of both these parameters increased substantially as soon as the fishes came in contact with Ekalux. In the case of DNA (Table 4) the values were highest at 15 day interval following which they started to attain a normal level slowly when they were prolongingly exposed to normal conditions (6 months). This shift is more clearly illustrated in Fig 2C. However for RNA although the trend was similar to that of DNA (Fig 2D), results of DMRT suggest that once exposed to even sub lethal concentration of Ekalux, the values never revived to normal level (Table 5). Hence, RNA values can be considered as good indicators for assessing the impact of pesticide on the biochemical composition of fishes.

A fall in muscle protein is indicative of reduced protein synthesis and low assimilation of food and low amino acid uptake for protein synthesis. Similar results were documented by Murty and Devi (1982) and Narayan Ram and Sathyanesan (1986). The depletion in total protein content may be due to augmented proteolysis and possible utilization of their product for metabolic purposes as reported by (Ravinder and Suryanaraya, 1988). Decreased total protein level was observed in the muscle and liver tissue of the freshwater teleost fish *C. fasciatus* exposed to sublethal dose of Malathion are Carbaryl pesticides (Tripathi and Singh, 2003). The present study suggests that level of total amino acid after exposure to Ekalux was higher than compared with control group. Protein degradation, resulting in elevation of total amino acid level may be a reason as noted in *B. gueirini* exposed to Endosulfan by Reddy *et al.* (1991).



**Figure 2.** Variation in the Biochemical parameters in *Anabas testudineus*

**Table 4.** Comparative evaluation of different biochemical parameters of *Anabas testudineus* reared under different treatments

Variables	Source	df	Sum of squares	Mean squares	F	Sig.
Protein	Between Treatments	3	58.790	19.597	18.560	.001
	Within Treatments	8	8.447	1.056		
	Total	11	67.236			
Amino acid	Between Treatments	3	13.409	4.470	8.111	.008
	Within Treatments	8	4.408	0.551		
	Total	11	17.817			
DNA	Between Treatments	3	191.191	63.730	4.513	.039
	Within Treatments	8	112.980	14.122		
	Total	11	304.170			
RNA	Between Treatments	3	1658.937	552.979	4.919	.032
	Within Treatments	8	899.378	112.422		
	Total	11	2558.315			

**Table 5.** Results of DMRT on difference in the concentration of Protein, Total Amino acids, DNA and RNA

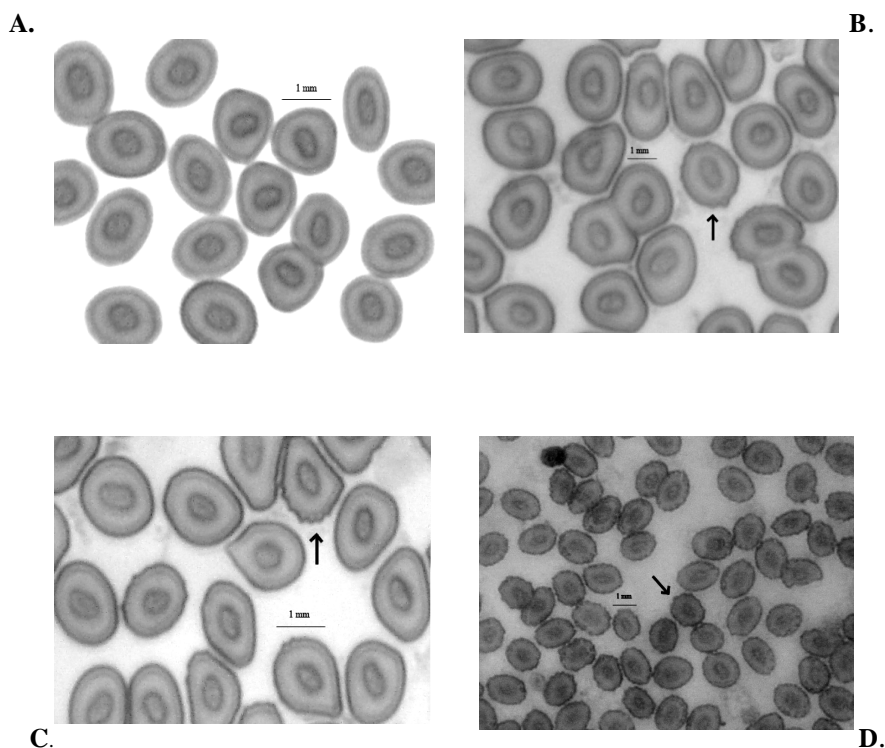
Treatment	Protein	Amino acid	DNA	RNA
T1	19.2600 <sup>b</sup>	10.3433 <sup>a</sup>	30.0667 <sup>a</sup>	78.1733 <sup>a</sup>
T2	16.3500 <sup>a</sup>	9.1600 <sup>a</sup>	39.0167 <sup>b</sup>	101.4467 <sup>b</sup>
T3	15.7200 <sup>a</sup>	9.1500 <sup>a</sup>	40.4933 <sup>b</sup>	105.2833 <sup>b</sup>
T4	21.1867 <sup>b</sup>	11.7200 <sup>b</sup>	36.2333 <sup>b</sup>	107.7500 <sup>b</sup>

Means for groups in homogeneous subsets are displayed.

### Histopathological studies

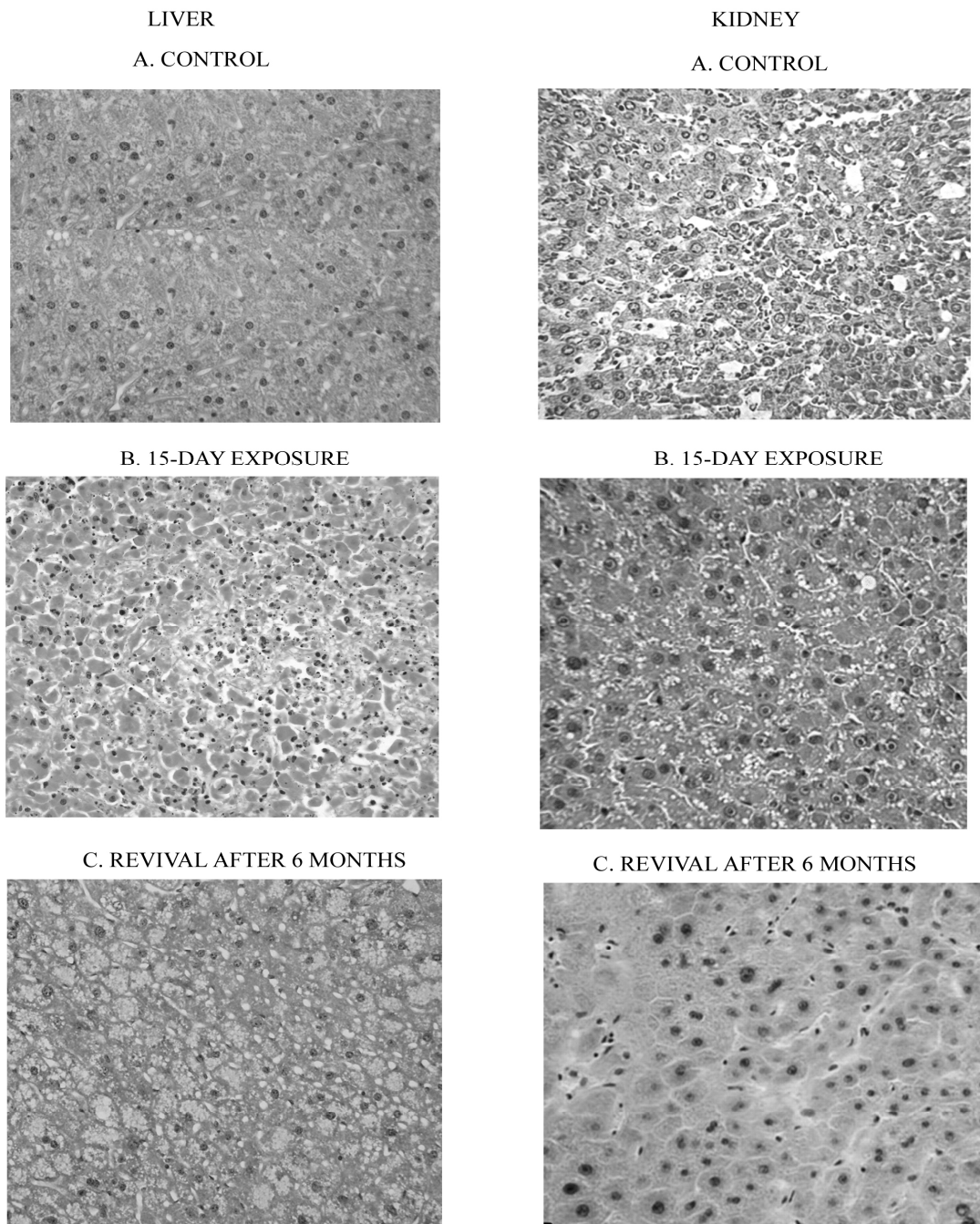
Histological differences in the liver and renal tissues of fishes exposed to sublethal concentration of Ekalux are depicted in *Fig. 4*. In the present study some of the significant lesions were observed in liver and kidney exposed to Ekalux only after 15 days (*Fig 4B*). The liver had a low number of hepatocytes and necrotic lesions over the tissue. At sublethal concentration, an increased number of melanomacrophage centres with decreased amount of haematopoietic tissue were observed in the kidney of *A. testudineus*. This is in total contrast to those observed in control groups where only loose mass of cells could be seen (*Fig 4A*). However, after reintroducing to normal environment for a period of 6 months, there was signs of tissue revival (*Fig 4C*). The hepatocytes appeared normal and could be compared to those of the control tanks. The haematopoietic tissue increased to normal levels in the kidneys of these fishes and the melanomacrophage centres decreased considerably, which are an indicator that at least 6 months of buffer period is required for tissue to regain its original form for a short-term exposure to Ekalux.





**Figure 3.** Morphological Changes of Blood Cells under Different Treatments (A. Control, B. 24 Hour, C. 15 Days, D. 6 Months)

In the present study, some of lesions found in liver and kidney related to exposure to Ekalux at sublethal concentration. The morphological lesions are mainly associated with hepatocytes. These lesions include hypertrophy of the hepatic cells and their reduced number. Sastry *et al.* (1979) stated that the sublethal concentrations of Endrin produced swollen hepatic cells, liver chord disarray, necrosis and vacuolation of cytoplasm in the liver of *Channa punctatus*. Hyperplasia and hypertrophy of the hepatic cells were the most notable features found by Abraham *et al.* (2003) in the liver of *Anabas testudineus* treated with Neemax. Hence the cells may become larger in size and to meet the requirement, cells proliferate much faster. Endosulfan exposure to rainbow trout revealed similar lesions, necrotic hepatocytes, vacuolar dystrophy and hypertrophy of hepatocytes and enlarged hepatic perisinusoidal areas (Altinok and Capkin, 2007). The results of histopathological investigations of renals tissues showed increased amount of melanomacrophages with reduced number of hematopoietic cells in kidney tissue similar to that reported by Capkin *et al.* (2006). Deteriorated levels of cell structure were prominent with greater exposure to pesticide in the present study also. Observations made herewith were closely related to marked pathological changes such as highly degenerative changes in hematopoietic tissue, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm as reported by Butchiram *et al.* (2009) in *C punctatus* when exposed to sublethal concentration of alachlor. In the present study the destruction of renal tissue was moderate however; there was a marked increase in the level of destruction on cell with increase in the period of exposure. The results of the study indicate that even sublethal concentration of Ekalux is enough to elicit significant histological alterations in liver and kidney of *A. testudineus*.



**Figure 4.** Histopathological investigation of Ekalux toxicity in Liver and Renal tissues of *Anabas testudineus*

## Conclusion

The results of the present study clearly indicated that pesticides have a direct impact on the biochemical, physiological and structural alterations in sub adults of *Anabas testudineus*. Ekalux is known to impair the metabolic and the physiological activities of the organism. The results emerging from the present study also points out to the fact

that through repeated exposure the pesticides tend to accumulate in tissues of animals even at sub lethal concentration. The alterations in the histology of liver and renal tissue seen in the present study is indicative of the fact that unlike bioaccumulation in terrestrial animals which is less relevant, in aquatic environment the impact is manifold. The study also affirms the view that histopathological analysis is a suitable method in toxicological studies to understand the pathological conditions of tissues under stress. Haematological and biochemical studies clearly indicates that through effective mitigation steps and proper management of our ecosystem the negative impact of pesticide toxicity on fishes could be reduced to certain levels. It is also suggestive that these type of toxicological studies are highly required to monitor the aquatic system and to assess the toxic effect of pesticides on aquatic organisms particularly fish.

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