



## COMPARATIVE STUDY OF HAEMOLYTIC ACTIVITY OF PHYTOTOXINS FROM *TEPHROSIA PURPUREA* IN SOME FRESHWATER FISHES

**R. G. Patil<sup>1</sup>, S.G. Nanaware<sup>2</sup> and M. P. Gujar<sup>3</sup>**

<sup>1</sup>Emeritus Fellow and Research Director, P.G. Department of Zoology,

Lal Bahadur Shastri College of Arts, Science and Commerce, Satara.

<sup>2</sup>Ex-Head Department of Zoology, Shivaji University, Kolhapur.

### ABSTRACT:

The plant *Tephrosia purpurea* shows piscicidal properties. The economically important freshwater fishes (*Labeo rohita*, *Catla catla* and *Cyprinus carpio*) were exposed to the sublethal concentration (115 ppm) of alcoholic extract of seeds of *Tephrosia purpurea* for 96 hrs. The toxic compounds in the seeds *Tephrosia purpurea* showed piscicidal properties and induced haemolysis and affected almost all haematological parameters. All parameters in the blood except E. S. R. were found to be decreased in all the fishes while E.S. R. was increased after 96 hrs of intoxication. Comparatively, haemolytic activity was lowest in *C. carpio* and highest in the *L. rohita*. The results were discussed in relation to mortality, metabolic activity and behaviour of these fishes.

**Keywords** - *Tephrosia purpurea*, Phytotoxin, Fish, Haematology

### INTRODUCTION

Blood shows immediate pathological changes before external signs of poisoning can be seen. The change in the haematological parameters give significance in assessing the physiological response of fishes (Joshi *et al.*, 1980). As in human medicine, the assessment has been done by many workers to diagnose the disease condition of the fish (McCay and Vars (1931), Hesser (1960) and Blaxhall (1973). Recently haematological experiments have done to measure the impact of various toxicants on fishes.

The data about the effect of some toxicants on fish blood are available from the studies of Panigrahi and Mishra (1978), Agrawal *et al.* (1979), Rai and Qayyum (1984) Thakur and Pandey (1990), Varadraj *et al.* (1993). However, the studies on the effects of piscicidal compounds of plants on haematological parameter in fish are scanty. Hence, the present paper reports the comparative study on the haemolytic activity of phytotoxin from *Tephrosia purpurea* in freshwater fishes *L. rohita*, *C. catla* and *C. carpio*.

### MATERIALS AND METHODS

The seeds of *Tephrosia purpurea* were collected, air dried and powdered mechanically. The ethanol extract of *T. purpurea* was dried in vacuum desiccators. The healthy adults of fish *L. rohita*, *C. catla* and *C. carpio* with average length 10 cm and weight 120 gm were collected from the local tanks. Fishes were kept in glass aquaria with continuous supply of tap water. They were acclimatized in laboratory conditions for a week.

Then fishes of each species were exposed for 96 hrs to the sublethal concentration (115ppm) of ethanol extract of seeds of *T. purpurea*. A control set was maintained. After intoxication for 96 hrs two fishes of each species were taken out and anaesthetized. Fishes were

wiped with blotting paper. Blood samples were collected in syringe rinsed with anticoagulant, by restoring to cardiac puncture and stored into glass tubes layered with anticoagulant (EDTA).

The total R.B.C. and W.B.C. counts were determined through improved Naebaur's haemocytometer using Hayem's diluting fluid for R.B.C. count containing 30% glacial acetic acid and methyl violet for W.B.C. count. Haemoglobin (Hb) concentration of blood was determined by Sahli's haemometer. Haematocrit values Packed cell volume (PCV) was determined by Wintrobe's haematocrit pipettes, for this purpose blood was centrifuged for 30 minutes at 4000 rpm. The mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by standard methods (Bauer, 1990). Erythrocyte sedimentation rate (ESR) was determined by Westergren method (Bauer, 1990).

### RESULTS AND DISCUSSION

Data on the effect on haematological parameters of blood of the fishes *L. rohita*, *C. catla* and *C. carpio* after exposure to alcoholic extract (115ppm) of seeds of *T. purpurea* for 96 hrs are given in Table 1.

In control set value of R.B.C. count, W.B.C. count, Hb percentage, PCV, MCV, MCH, MCHC and ESR after 96 hrs were observed as  $2.82 \times 10^6 / \text{mm}^3$ ,  $22.90 \times 10^3 / \text{mm}^3$ , 11.60 gm %, 28.85 dl,  $105.50 \text{ um}^3$ , 42.90 pg, 41.70 dl and 2.80 mm/hrs respectively in fish *L. rohita* while after intoxication for 96 hrs these values were found respectively as  $2.71 \times 10^6 / \text{mm}^3$ ,  $21.10 \times 10^3 / \text{mm}^3$ , 9.90 gm %, 26.90 dl,  $100.10 \text{ um}^3$ , 39.20 pg, 37.80 dl and 2.90 mm/hrs. This shows increase in all the parameters except ESR in fish *L. rohita*. Likewise, these values of different blood

parameters except ESR were increased in the other fishes also.

This fall in the data of R.B.C. count, W.B.C. count, Hb percentage, PCV, MCV, MCH is in agreement with Pandey (1976), Hooper and Sunderman (1978) and Bhatt and Farswan (1992) who have reported among malathion, low temperature, nickel, sulphide and phytotoxin treated fishes.

In this investigation when *L.rohita*, *C.catla* and *C.carpio* were treated with phytotoxin from *T. purpurea*, it enters into the body and then into the blood stream of fishes. The entry of phytotoxin in the blood affects erythrocytes and leucocytes. The injury of blood cells further results into decreased R.B.C.count, W.B.C. count, Hb percentage, MCV, MCH and MCHC. This type of fall in the haematological parameters in fishes was also observed by Kiptoom *et.al.*

(1982), Veena Gerg *et.al.* (1991) and Bhatt and Farswan (1992).

Increase in ESR was also observed in this experiment. This type of increase in ESR was observed by Bhat and Farswan, (1985) and he was of the opinion that decreased PCV and increased ESR is a effect of degradation of blood proteins in intoxicated fishes.

By the comparative study of the data of haematological parameters it is concluded that the phytotoxin from *T. purpurea* is comparatively more effective in *L. rohita* than the other two fishes. It can be also concluded that phytotoxin from *T. purpurea* induces haemolysis in the freshwater fishes *L.rohita*, *C.catla* and *C.carpio*, which affects oxygen carrying capacity of blood and it further affects respiratory metabolism. This effect on respiratory metabolism increases the rate of mortality in the fish.

**Table 1:** Phytotoxin from *T. purpurea* induced haematological values at 96 hrs of intoxication.

No.	Haematological Parameters	Name of the fish					
		<i>L. rohita</i>		<i>C. catla</i>		<i>C. carpio</i>	
		Control	Intoxicated	Control	Intoxicated	Control	Intoxicated
1.	R.B.C. count (X 10 <sup>6</sup> /mm <sup>3</sup> )	2.82	2.71	4.00	3.80	4.01	3.96
2.	W.B.C.count (X 10 <sup>3</sup> /mm <sup>3</sup> )	22.90	21.10	35.70	33.20	27.30	37.30
3.	Haemoglobin (Hb) (gm %)	11.60	9.90	10.20	9.20	12.20	10.90
4.	PCV (dl)	28.85	26.90	40.30	35.60	40.20	35.70
5.	MCV ( μm <sup>3</sup> )	105.50	100.10	98.70	95.20	98.85	94.20
6.	MCH (pg) (X 10 <sup>3</sup> /mm <sup>3</sup> )	42.90	39.20	23.20	21.60	34.30	30.90
7.	MCHC (dl)	41.70	37.80	25.30	24.25	32.20	31.20
8.	ESR (mm/hrs)	2.80	2.90	3.40	3.90	3.20	3.95

**ACKNOWLEDGEMENT**

The authors are thankful to Prin. Abhayakumar Salunkhe, Karyadhyaksha, Prin. Sou. Shubhangi Gavade, Secretary, Shri Swami Vivekanand Shikshan Sanstha, Kolhapur and Prin. Dr. R. V. Shejwal, Lal Bahadur Shastri College of Arts, Science and Commerce, Satara for providing facilities and continuous encouragement.

**REFERENCES**

Agrawal, S. J., A. K. Srivastava, and H. S. Chaudhry (1979) Haematological effects of nickel on a fresh water teleost *colisa fasciatus*. Acta Pharmacol. et Toxicol, 45, 2150217.

Bauer John D. (1990) Clinical Laboratory Methods (Page Nos. 178-191).

Blaxhall, P. C., and Daishley, K.W. (1973) Routine haematological methods for use with fish blod, j. Fish Biol 5, 7710782 (1973).

Bhatt, J. P. and H. R. Singh. (1985) Effects of Engelhardtia colebrookina (Lin.) on a fresh water teleost *barilius bendelisis* (Ham.) Science and culture 51 (4), 132-133 (1985).

Bhatt, J. P. and Faraswan Y. S. (1992) Haemolytic activity of piscicidal compounds of some plants to freshwater fish *B. bendelisia* (Ham.) J. Environ bio, 13 (4), 333-342.

Hesser, (1960) Methods for routine fish haematology, Progr. Fish-Cult 22, 164-171.

Hopper S. M. and F. W. Sunderman (1978) Magnese inhibition of nickel sulfide on erythrocytosis in rats. Res. Commun. Chem. Pathol. Pharmacol, 19, 337-345).

Joshi, B. D., Chaturvedi, L. D., and Daliral, R. (1980) Some haematological values of *Clarias batrachus* following its sudden transfer to varying ,temperatures Indian J. Exp. Biol., 18, 76-77.

Joshi B. D., L. D. Chaturvedi and R. Dabral (1979) Changes in blood cell components of *Heteropneustes fossilis* following transfer of polidol water at low temperature, Proc. Symp. Life and Toxic Environ, 86.

Kiptoon J. C., G. M. Mugeru and P. G. Waiyaki (1982) Haematological and biochemical changes in cattle poisoned by *Gnidia latifolia* Sin. *Lasiosiphon latifolius* (Thymelaescaeae).Toxicol, 25, 129-139.

McCay, C. M., and Vars, H. M. (1931) Studies upon fish blood and its relation to water pollution. N. Y. State Conserv. Dep. Ann. Rep. / 20, 230-233.

Panigrahi, A. K. & M<ishra B.N. (1978) Effect of mercury on the morphology of erythrocytes in *Anabas scandens*. Bull. Environ. Contam. Toxicol, 23, 784-787.

Pandey B.M., A. K. Chanchal, & M. P. Singh (1976) Effect of malthion on oxygen consumption and blood of *Channa punctatus*. Ind. J. Zootomy 17 (2), 95-100.

Rai, R. and M. A. Qayyum (1984) Haematological responses in a freshwater fish to experimental lead poisoning J. Environ, Biol, 5 (1), 53-56.

Thakur G. K. and Pandey P. K. (1990) BHC poisoning Effect on Leucocytes of an Airberathing. Fish, *Claris bacrachus* (linn). J. Environ, Biol, 11(3), 319-323.

Veena Garg, Tyagi S. D., Singh neera and Agrawal S. C. (1991) Thallium Nitrate induced haematobiochemical analysis of *Heterponeustes fossilis* blood J. Environ, Biol, 12 (3) 319-323.

Varadraj, G., Subramaniam M. A. and Nagarajan B. (1993) The effect of sublethal concentration of paper mill and pulp mill effluent on the haematological parameters of *O. mossambicus* (peters) J. environ. Biol. 14 (4), 321-325.