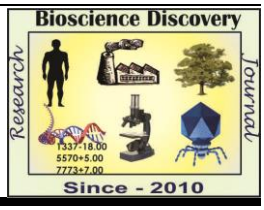
Bioscience Discovery, 8(2):198-201, April - 2017

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article



Haematotoxic potential of Acid Red 97 on *Catla catla* (Ham.)

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Article Info

Received: 03-03-2017,
Revised: 24-03-2017,
Accepted: 30-03-2017

Keywords:
Acid red 97, *Catla catla*, Haematotoxicity

Abstract

Acid Red 97 (AR 97) is famous world-wide and used extensively in the textile industry. This study was aimed to investigate the haematotoxic effects of azo dye AR 97 on *Catla catla*. Fingerlings were exposed to a sublethal concentration 8.5 mg/l (i.e $1/10^{th}$ of LC_{50} = 85mg/l) for (10, 20, 30) days. Some haematological parameters including Hb, RBC count and WBC count were evaluated. Result shows that AR 97 significantly decreases the Hb and RBC count where WBC count increases throughout the exposure. Hence, it was concluded that azo dye AR 97 has potential to cause haemotoxicity in fish.

INTRODUCTION

Although textile is one of the economic sectors in Surat, Gujarat, continuous discharge of textile wastewaters loaded with a variety of synthetic dyes and metals is considered as a huge threat to surrounding ecosystems (Tan *et al.*, 2000; Bhullar and Sud, 2012). Textile azo dyes are serious pollutants of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organism (Saratale *et al.*, 2011). The presence of contaminants or any disturbances in the aquatic environment is responsible for causing severe changes in fish blood (Fernades and Mazon, 2003). Haematological parameters are used as tools for assessing the health of fish (Sampath *et al.*, 1993). Erythrocytes, Hb content and their related indices could be used as sensitive indicators of changes in ecological conditions (Satheeshkumar *et al.*, 2012). Haematological parameters are patho-physiological reflectors of the whole fish body. Moreover, these blood parameters are too sensitive to any physicochemical, biological and/or environmental alterations. Hence, they are important markers to investigate the structural and functional status of fish exposed to various contaminants (Adhikari *et al.*, 2004).

This study was aimed to investigate the toxic effects of azo dye AR 97 on haematological parameters of *Catla catla* (Indian major carp) fingerlings. Several studies have investigated the alterations in the haematological profile of freshwater teleost as biomarker in pollution monitoring (Thangam *et al.*, 2014; Barot, 2015; Vaiyanan *et al.*, 2015; Deshmukh 2016).

MATERIALS AND METHODS

AR 97 was purchased from local market and used directly for the experiment. *Catla catla* fingerlings (13±1cm long and 25±2.5g weight) were procured from fish seed farm sivan, surat, Gujarat and acclimatized in the laboratory condition for 15 days according to APHA. After acclimatization, apparently healthy *catla* fingerlings were grouped in to 4 (10 in each) and used to carry out toxicity test. Among this one was considered as control and other 3 groups of *catla* fingerlings were exposed to sublethal concentration 8.5 mg/l (i.e $1/10^{th}$ of LC_{50} value 85mg/l) of AR 97 for 30 days in triplicates. At fixed interval (10, 20, 30 days) after the exposure blood samples were collected and were analysed. Blood was collected by direct puncturing heart and/or caudal vein using sterile syringe (2 ml) pre-rinsed with 2.7 % EDTA solution.

Collected blood samples were immediately transferred to vials (2 ml) coated with EDTA. Haemoglobin estimation was done by cyanmethemoglobin method (Dacie and Lewis, 1968). 20 µl of blood samples were taken in Thoma's pipettes. They were mixed with diluting fluids Turks' solution for WBC count and Haem solution for RBC count in the same pipettes. The mixtures were shaken well to suspend cells uniformly in solution. After 10 to 15 minutes, cells were counted using haemocytometer (Schalm *et al.*, 1975).

RESULTS AND DISCUSSION

Results obtained from this study show significantly decreased Hb content and RBC count as compared to control. On the other hand, WBC count increased in all AR 97 exposed fingerlings (Table-1). It clearly indicates the toxic potentials of AR 97 even at sublethal concentrations that affect the haemato-physiology of exposed fingerlings. All mentioned alterations indicate that exposed fingerlings suffered from anemia induced by dye. This is an indication of disruptive effects of azo dyes on erythropoietic tissues as well as cells viability (Sudakov, 1992). Our previous study also supports the results of the present study (Parmar and Barot, 2016).

Similar significant decrese in Hb and RBC was also reported by Barot and Bahadur (2015) in *Labeo rohita* exposed to dye. Significant decrease in haemoglobin has been reported in *Clarias batrachus* by Patnaik and Patra (2006) an exposed to sublethal concentration of propoxur and carbaryl. Similar decrease found in *Carassius auratus gibelio* when they were exposed to the toxic textile dye (Al-Sabti, 2000). Changes in haematological parameters of *Heteropneustes fossilis* has been supported our results when the exposed to galvanizing industry effluent (Majumdar *et al.*, 2010).

When fingerling *Catla catla* were exposed to dye AR 97 total count of RBC's reduced compare to control. Alterations in RBC count exposed to various toxicants have been reported by many researchers (Sampath *et al.*, 2003; Parma *et al.*, 2007). Significant reduction in RBC count was noted in cypermethrin treated *Labeo rohita* (Das and Mukherjee, 2003) and in freshwater *Cyprinus carpio* treated with diazinon (Svodova *et al.*, 2001). Decrease in the value of Hb, PCV, RBCs may results in hypochromic microcytic anemia which may be due to deficiency of iron and its decreased utility (Palanisamy *et al.*, 2011).

In the present study, WBC count increased following exposed dye AR 97. Significant increase was also observed in the total WBC count after exposure to malachite green and Pyceze (Srivastav and Roy, 2015). WBC plays a major role in the defense mechanism of fish. In other words, an immediate activation of the fish immune system is proved by increase in leucocytes (Dutta *et al.*, 1992). Gill and Pant, (1985) have reported that the stimulation of the immune system causes an increase in lymphocytes due to injury or tissue damage. Leucocytosis was also observed in other teleost fish after exposure to various toxicants (Srivastava *et al.*, 1996; Ramalingam, *et al.*, 2000; Gautam and Kumar, 2008). An initial leucocytosis, which may be directly proportional to the severity of the causative stress conditions, may be attributed to an increase in leucocyte mobilization (Van *et al.*, 1994). A rise in WBC count following exposure to insecticides has been also reported (Srivastava and Singh, 1994).

ACKNOWLEDGEMENT

Author, Avni Parmar is thankful to the UGC Rajiv Gandhi National Fellowship for financial support.

Table: 1 Effect of AR 97 on haematological parameters Hb, RBC and WBC counts

Concentration of AR 97	Exposure (Days)	Blood parameters		
		Hb (*g/dl*)	RBC count (10^6 cells/mm^3)	WBC count (10^6 cells/mm^3)
8.5 mg/l	Control	6.47±0.03	2.42±0.15	1.86±0.05
	10 days	5.84±0.08*	1.91±0.07*	3.17±0.13*
	20 days	5.29±0.16*	1.64±0.07*	3.57±0.07*
	30 days	5.04±0.09*	1.24±0.06*	3.96±0.08*

[Each value is mean ± SE of thirty individual observations; Asterisks (*) indicate significant ($p<0.05$) difference compared to control.]

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How to Cite this Article:

Parmar Avni and Barot Jagruti, 2017. Haematotoxic potential of Acid Red 97 on *Catla catla* (Ham.). *Bioscience Discovery*, **8**(2):198-201.