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Acute toxic effects of the textile dye, acid blue 113, on the biochemicals of teleost fish, *Tilapia mossambica* (Peteres) (Pisces: Teleostei, Cichlidae)

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ABSTRACT

Pollution problems due to textile dyeing effluent discharge in Coimbatore has been a serious issue from long time back. Several environmental problems such as destruction of vegetation, ruining of quality of water bodies, aesthetic degradation of water and massive death of fishes have been noticed. The main constituent imposing pollution in the dyeing effluent is the presence of various dyes. The individual effect of the most commonly used dye, Acid blue – 113 in edible fish, *Tilapia mossambica* has been investigated in the present study. Biochemical parameters such as carbohydrate, protein and lipid in the liver and muscle of fish the experimental fish decreased significantly ($P<0.05$) than that of control. Whereas enzymes such as GOT and GPT increased in both liver and muscle.

Key words: Textile dye – Acid Blue 113, *Tilapia mossambica,* Liver, Muscle, Biochemical Parameters

INTRODUCTION

Tilapia mossambica is a commercial teleost fish, which are widely cultured for human consumption[1]. Dyes are used in many industries to colour their products such as textile, rubber, paper, plastics, leather, cosmetics, food, paint and mineral processing industries. Coimbatore is an important industrial city of Southern part of India having more than 30,000 small, medium and large industries including textile dyeing units. The dyeing units discharge their effluents into prevailing drainage and ultimately reach lakes, wetlands and River Noyyal in the city[2]. Massive death of fish in Puttu vikki dam near dyeing units in Coimbatore was reported in the news paper, Dina Thanthi[3]. Dye bath effluent, in particular are not only aesthetic pollutants by nature of their colour, but also can interfere light penetration through water bodies[4] and thus affects the aquatic organisms.

Acid blue 113, a dark blue powder is extensively used as textile dye having diazo group within the molecular structure[5]. Azo dyes leads to formation of aromatic amines and these are known mutagens and carcinogens to human beings[6]. Dyes have been reported to precipitate in aquatic organisms leading to hematological and histopathological alterations in vital organs[7]. Biochemical changes in blood and tissue of fish under exposure to toxicant may be used to predict the toxic effects[8].

Various reports are available on the toxic effects of acid blue dyes such as acute toxicity bioassay in fish[9], allergen susceptibility and skin sensitization in fish, rat and rabbit[10,11], colour change in internal organs in fish and rat[12], changes in mineral constituents in fish liver and kidney[13]. The biochemical effects of acid blue -113 in various organs of fish need attention. In this context acute toxic effect of acid blue - 113 on the biochemical parameters of the fresh water fish *Tilapia mossambica* is attempted.

MATERIAL AND METHODS

Test animal

Tilapia mossambica of both sexes (with size i.e. length = 8 to 13cm and weight = 15 to 20g) were procured from Tamil Nadu Fisheries Department, Aliyar Dam hatchery at Coimbatore district, and brought to the laboratory in oxygen packs. Fish were then transferred into glass aquaria filled with aerated (chlorine free) tap water and stock fish were provided with commercial feed at the rate of 1% live weight per day. They were acclimatized for 15 days in the laboratory. Fish were not fed for 24 hours prior to testing, nor for the duration of the tests.

Test material

The textile dye, Acid blue 113 a diazo dye was purchased from the textile chemical shop in Coimbatore, Tamil Nadu.

Acute toxicity bioassay test (LC_{50} 96 hours)

Test was designed according to the static bioassay procedure of Standard Methods for the Examination of Water and Wastewater[14]. The quality of experimental dilution water was analysed according to Standard Methods[14]. Test concentrations were 5, 10, 20mg/l. Experimental troughs of 60 liters capacity was used for each exposure and 10 fish were introduced in each trough. Duration of all tests was 96 hours and fish were observed at 24 hour intervals to note the mortality in each trough. As a control, 10 fish were maintained throughout the test period under identical conditions. The LC_{50} value was derived by plotting the data on semi logarithmic coordinate paper.

Experimental design

There were five groups (4 troughs with sublethal doses and another trough with control) and each group has 10 fish. One tenth of lethal concentration was considered for 24, 48, 72 and 96 hours for test exposure.

Sampling

After the exposure periods, sublethal and control group fish were sacrificed for estimation of biochemical parameters. For the analysis of biochemical parameters, liver and muscle tissues were dissected out from the control and experimental fish. The tissues were weighed and centrifuged at 4,000 rpm using double distilled water. The supernatant was stored at 2^0C for analytical purpose. Colorimetric determination was performed using BTR – BIOTRONE 810(German) semi auto analyser.

Statistical analysis

All experiments were repeated for three times and values are expressed as mean ± Standard deviation. The individual values of all parameters were compared by Student's t – test (5% significance) and also percentage of decrease over control was noted.

RESULTS AND DISCUSSION

Biochemicals such as carbohydrate, protein and lipid and enzymes such as Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) were analysed in liver and muscle to determine the toxic effects of textile dye Acid blue – 113 in *Tilapia mossambica.* The biochemical aspects of liver are tabulated in Table 1 and muscle in Table 2. The study showed decrease in carbohydrate, protein and lipid content in both liver and muscle of dye exposed fish when compared to fish under control. The enzymatic aspects of liver are shown in Table 3 and for muscle in Table 4. GOT and GPT values in the liver and muscle are higher in experimental fish than in control.

Carbohydrates are essential components of all living organisms and are, in fact, the abundant class of biological molecules. In the present investigation, decline in the carbohydrate content of liver and muscle in all the experimental fish shows the primary metabolic symptom that can be noticed in organisms subjected to stressful situations. The significant decrease ($P<0.05$) of carbohydrates in the tissues indicated the excess utilization of glucose and the reserve polysaccharide glycogen to withstand the dye induced toxicities. In conformity with this finding, previous reports on *Tilapia zilii* to polluted water[15]; *Ophiocephalus* to dairy effluent[16]; *Catla catla* to cadmium chloride[17] and *Catla catla* to mercury chloride[18] are available.

Table 1: Biochemical parameters in liver of *tilapia mossambica* on exposure to acid blue - 113

Biochemical Parameters	Control Mean ±SD	Exposure Period – Hours			
		24 Mean ±SD	48 Mean ±SD	72 Mean ±SD	96 Mean ±SD
Protein(g/dl)	2.42±0.06	2.21±0.02-8.67	1.95±0.10*-19.42	1.46±0.05*-39.66	1.30±0.08*-46.28
Carbohydrate(mg/dl)	23.51±0.12	22.16±0.25-5.74	21.40±0.10-8.97	20.90±0.33*-11.1	20.82±0.16*-11.44
Lipid(mg/dl)	49.81±0.20	45.63±0.64-8.39	43.47±0.03*-12.72	43.19±0.39*-13.29	41.56±0.09*-16.56

*Significant at 5% level, SD = Standard Deviation, -indicates decrease over control.

Table 2: Biochemical parameters in muscle of *Tilapia mossambica* on exposure to acid blue - 113

Biochemical Parameters	Control Mean ±SD	Exposure Period – Hours			
		24 Mean ±SD	48 Mean ±SD	72 Mean ±SD	96 Mean ±SD
Protein(g/dl)	1.30±0.03	0.98±0.02*-24.61	0.97±0.01*-25.38	0.94±0.02*-27.69	0.95±0.00*-26.92
Carbohydrate(mg/dl)	16.90±0.13	13.9±0.17*-17.75	13.56±0.05*-19.76	13.31±0.15*-21.24	12.99±0.24*-23.13
Lipid(mg/dl)	20.85±0.19	20.02±0.07-3.98	18.65±0.10*-10.55	18.25±0.03*-12.47	17.43±0.05*-16.4

*Significant at 5% level, SD = Standard Deviation, -indicates decrease over control.

Table 3: Enzyme parameters in liver of *Tilapia mossambica* on exposure to acid blue - 113

Biochemical	Control	Exposure Period – Hours			
Parameters	**Mean ±SD**	**24** **Mean ±SD**	**48** **Mean ±SD**	**72** **Mean ±SD**	**96** **Mean ±SD**
GOT(IU/l)	8.58±1.21	14.85±0.14*+73.07	15.37±0.22*+79.13	13.92±0.31*+62.23	13.57±0.01*+61.65
GPT(IU/l)	9.26±1.06	19.87±0.37*+99.49	18.14±0.02*+82.12	14.85±0.18*+49.09	11.76±0.54*+18.07

*Significant at 5% level, SD = Standard Deviation, + indicates increase over control.

Table 4: Enzyme parameters in muscle of *Tilapia mossambica* on exposure to acid blue - 113

Biochemical	Control	Exposure Period – Hours			
Parameters	**Mean ±SD**	**24** **Mean ±SD**	**48** **Mean ±SD**	**72** **Mean ±SD**	**96** **Mean ±SD**
GOT(IU/l)	4.79±0.40	5.22±0.60+8.97	7.89±0.31*+64.7	6.48±0.80*+35.28	7.51±0.33*+56.78
GPT(IU/l)	5.67±0.03	8.14±0.07*+43.56	7.00±0.14*+23.45	7.09±0.18*+25.04	6.82±0.17*+20.28

*Significant at 5% level, SD = Standard Deviation, + indicates increase over control.

Proteins are highly sensitive to pollutants and are one of the earliest indicators of toxic effects[19]. It may be influenced by a large number of exogenous substances, mainly through a reduction of protein synthesizing capacity of endoplasmic reticulum in the cells. The results of the present study showed significant decrease ($P<0.05$) in protein content that might be attributed to diversification of energy to meet the impeding energy demands when the fish is under stress or altered enzyme activities. Decrease of protein in fishes due to toxicants was reported in Rainbow trout to malachite green[20]; in *Catla catla* to cadmium chloride[17]; in *Catla catla* to mercuric chloride[18].

Lipids are storage form of energy. The present study showed decreased level of lipid in muscle and liver of fish exposed to Acid blue – 113 when compared to control. The reduction in lipid was resulted due to their utilization under stress[16]. In conformity with the present findings, previous reports on *Cyprinus carpio* to tannery effluents[21]; *Ophiocephalus* to dairy effluents[16] and *Catla catla* to cadmium chloride[17] are available.

Decrease or increase in enzyme activity represents the response of organism to any kind of stress. GOT and GPT are two key enzymes known for their role in the utilization of protein and carbohydrates. Increased GOT and GPT activity in liver and muscle of the experimental fish exposed to Acid blue – 113 was noticed. Similar findings of increased transaminase activity on exposure to dye stuffs were reported in *Clarias lazera*[22]. Increased transaminase activity of the fish was due to stress caused by toxicity.

The results indicate that the dye induced, stressful environment to the fish. The organs considered for the study i.e., liver and muscle of fish showed remarkable changes to exposure of the dye. Further studies on the effects of chronic exposure of the dye on the other organs can reveal the basic understanding of the toxic effects.

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