3-17-2013

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International Journal of Pharmaceutical Applications
ISSN 0976-2639.Online ISSN 2278 – 6023
Vol 4, Issue 1, 2013, pp 11-18
http://www.bipublication.com

EFFECT OF ANTHRAQUINONE DYES ON THE CARBOHYDRATE, PROTEIN AND LIPID CONTENT IN THE MUSCLE OF *CHANNA PUNCTATUS* AND *CYPRINUS CARPIO*

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[Received-12/01/2013, Accepted-17/03/2013]

ABSTRACT

Channa punctatus and *Cyprinus carpio* was exposed to sub lethal concentrations viz., 6.15 and 6.60 mg/l of vat blue 4 and vat green 1 respectively for a period of thirty days. The cumulative effect of these two dyes on the total carbohydrate, protein and lipid levels in the muscle of both the fishes were assayed on 7^{th}, 18^{th} and 30^{th} day of exposure. The mean carbohydrate content of *C. punctatus* (both control and treated fish) collected on 18^{th} and 30^{th} day showed a significant difference ($P < 0.01$ to 0.04). In the case of control and treated *C. carpio* the data collected on 30^{th} day showed significant variation ($P < 0.04$) in the carbohydrate content of the muscle. Student's 't' test analysis conducted on the protein content of *C. punctatus* during different occasions registered a significant decrease; in case of lipid content, except between the 1^{st} and 30^{th} day in all other cases the 't' value is not significant. In case of *C. carp* protein and lipid content were observed to increase during the 7^{th} day and thereafter a marked decrease was recorded on the 18^{th} and 30^{th} day of exposure. When the data was subjected to student's 't' test, both in protein and lipid, significant difference could not be recorded.

Keywords: *Channa punctatus*, *Cyprinus carpio*, carbohydrate, protein, lipid, toxicity, anthraquione dyes

[I] INTRODUCTION

Water quality deteriorates mostly due to human activities. Those activities which lead to the aquatic pollution includes: industrialization, urbanization, mining, power stations, agriculture and transport [1]. Human destructive influence on the aquatic environment is in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life [2]. With regard to industries, waste waters from cotton textile industry are of highly polluting nature and affect the water quality in many ways [3]. The high alkalinity of the effluent causes an increase in pH value. Any increase in pH value of the receiving stream greater than 9.0 will have an adverse effect on aquatic life. The organic matters along with colours, dyes and oily

scum produce an unsightly appearance and the organic matter of textile waste like starch, dextrin and inorganic chemicals like sulphide, hydrosulphite and nitrite will exert immediate oxygen demand while dyes and colours will exert long term oxygen demand. Such changes in the oxygen balance of receiving streams will be deleterious to fish life and will also interfere with self-purification process. Chemicals that are released into the aquatic environment are transported and redistributed among the different compartments in the environment, with transport across membranes into organisms being one of the processes [4]. There are five possible routes for a toxicant to enter into a fish i.e., via contaminated food, non-food particles, gills, oral consumption of water and skin. Toxic chemicals like sulphide, chlorine, chromium and aniline dyes will also affect the aquatic life. First, effluents may cause physical or chemical changes in a receiving environment that directly affect fish (e.g., fish gill). Secondly, effluents may contain compounds that can cause biochemical responses in fish, and those biochemical changes can alter the growth, reproduction or survival of fish. Finally, effluents can affect the food of fish, and cause indirect effects on growth, reproduction, or survival. Stress response is characterized by physiological changes and the effect of pollutants on fish is assessed by acute and chronic toxicity tests [5]. Haematology, biochemical changes, growth rate and oxygen consumption of fish can be used in determining the toxicity of pollutants [6]. Though the research on the effect of various dyes on fishes is available, the information about the effect of anthraquinone vat dyes on fish is inadequate. Hence a preliminary study was conducted to determine the cumulative effect of two anthraquinone vat dyes viz., vat blue 4 and vat green 1 on the commercially important fish *Cyprinus carpio and a freshwater fish Channa punctatus.*

[II] MATERIALS AND METHODS

2.1. Collection of both native and culturable fishes

For ex-situ studies *C. punctatus* was collected from unpolluted Kadana river which is located about 10 km away from Alwarkurichi village, Tamil Nadu, South India. Samples of healthy *C. carpio* were collected from a fish farm which is situated at Kallidaikurichi, Tamil Nadu. The fishes used were having the average length of 12 to 15 cm and an average weight of 50 to 70 grams. After collection, the fish samples were immediately brought to the laboratory and were then acclimatised under laboratory conditions for a period of fifteen days in quality freshwater with adequate aeration using an aerator. The amount of water in the tank was one litre for every log of fish to avoid over crowding [7]. The fishes were fed twice (5% of body weight) a day with artificially prepared imported fish feed available in the market. The protein content of the feed was around 40%.

2.2. Toxicity determination of dyeing industry effluent

The effluents free of Vat Blue 4 and Vat Green 1 dyes were collected from the dyeing industrial complex. Both the dyes were added to that effluent in varying concentrations and then LC_0, LC_{100} and LC_{50} were enumerated. By adopting the probit analysis method of Finney [8], the Safe Application Factor Equation (SAFE) was calculated from the LC_0 and LC_{100} values. From the SAFE level and the LC_{50} values the Safe Application Range was calculated following the procedure of Ramadhas [9]. The mean values of the dyes coming between SAR and LC_0 were calculated and chosen for conducting laboratory bioassay (Table 1). Only at this concentration any toxicants is known to slowly and adversely affect the physiology of the fish and ultimately affect the various parts of the test fish [9]. The chosen mean concentration for Vat Blue 4 is 6. 15 mg/l and Vat Green 1 is 6.60 mg/l.

The fish samples were treated with sub lethal concentrations of the mixture of two dyes containing wastewater (6. 15 mg/l of Vat Blue 4 and 6.60 mg/l of Vat Green 1) for a period of thirty days and then the proximate composition i.e., carbohydrate, protein and lipid contents were estimated on 7^{th}, 18^{th} and 30^{th} day. The treated fish were sacrificed after treatment and the major metabolites were assessed following the standard procedures. Carbohydrate was estimated by following the standard procedure of Carrell *et al.* [10]. Protein and Lipid were estimated following the methods of Lowry [11] and Bragdon [12] respectively.

[III} RESULTS

3.1. Toxicity determination of dyeing industry effluent

The LC_0, LC_{50} and LC_{100} values reported for the dyes were given in Table 1. It is highly comparable to the previous bioassay report of Little and Lamb III [13].

The proximate composition (carbohydrate, protein and lipid) of the *C. punctatus* and *C. carpio* were analysed and the results are presented in Table 2. The mean carbohydrate content of the muscle of *C. punctatus* and *C. carpio* recorded during three different durations of exposure was subjected to student's 't' test analysis (Table 3). In the case of control and treated fishes the data collected on 30^{th} day showed significant variation ($P < 0.01$ to 0.04) in the carbohydrate content of the muscle of the two fish species.

When the student's 't' test analysis was conducted on the protein content of the muscle of both the fish species during different duration the protein content of the muscle of *C. punctatus* registered significant decrease and the contrary was true in the case of *C. carpio* (Table 4).

The results of the comparisons of the lipid content of the muscle of the two fish species during three different occasions are furnished in Table 5. Except between the lipid content of the muscle of *C. punctatus* between the 1^{st} and 30^{th} day in all other cases the 't' value is not significant.

[IV] DISCUSSION

In the present study the proximate composition i.e., protein, lipid and carbohydrate contents in the treated fish species generally exhibited a relative increase in protein and lipid contents during 7^{th} day with a marked decline on 18^{th} and 30^{th} day**.** Carbohydrate typically contributes to structural support, protection, and serves as nutrient and energy stores to be increased or decreased according to organismal need. The results obtained in the present study showed that the carbohydrate content decreased significantly in both the fish species exposed to the sublethal concentration of the dyes. The activity of the enzyme phosphorylase in the hepatopancreas and muscle has been found to reduce the carbohydrate level in the crab *Oziotelphusa senex senex* [14]. Reddy *et al*. [15] reported decreased carbohydrate level in the brain of the teleost fish *Channa punctatus* exposed to chlorocyclohexane stress. Karpagaganapathy *et al*. [16] reported that fishes exposed to sub lethal concentration of Benzene Hexa Chloride (BHC) showed a reduction in the liver glycogen level. Rajamanickam and Karpagaganapathy [17] reported marked decrease in protein content of the liver and muscles of *O. mossambicus* exposed to the sub lethal concentration of lindane. Somanath [18] reported reduction of carbohydrate level in the fish *Labeo rohita* due to the effect of sublethal concentration of tannic acid toxicity. Significant decrease in glucose and glycogen levels has been reported in the muscle of the cray fish *Porcamarus clarkia* due to the exposure to cadmium toxicity [19]. Haniffa and Murugesan [20] recorded a similar anamoly in *M. kelities*, in which the fish treated with textile industry effluent (sub lethal concentration) resulted in a corresponding decrease in carbohydrate content and an increase in the lipid content. Lorenzon *et al*., [21] reported

the changes in the haemolymph glucose level in the shrimp Palaemon elegans due to heavy metal toxicity. Further, the decrease in carbohydrate may also be due to hypoxia, since hypoxia increases carbohydrate consumption. Hypoxic condition of the fishes may be due to anaerobic breakdown of glucose, which is available to the cells by increased glycogenolysis. The depletion of liver and muscle glycogen is attributed to the stress of the effluent on the organism, which ultimately results in the extensive utilization of the energy stored through glycogenolysis to meet the extra demands of energy required for the quick and brisk movements [16]. The depletion of carbohydrate in both *C. punctatus* and *C. carpio* may be due to its rapid utilization to meet the energy demands under the impact of anthraquinone dyes and it indicated the possibility of active glycogenolysis.

Proteins play a crucial role in virtually all-biological processes. Under extreme stress conditions, proteins supply energy in metabolic pathways and biochemical reactions [22]. In our experiment the protein content of *C. punctatus* exhibited an increase till 18th day and thereafter showed a decrease while in *C. carpio* it exhibited a decrease from 7th day of exposure. Viswarajan *et al*. [23] studied the effect of tannic acid on the protein, carbohydrate and lipid levels in the fish *O. mossambicus* and reported that there was an elevation in the protein content of liver and muscle with the increasing concentration of tannic acid. They also suggested that this increase in the protein content could be attributed to the efforts of the fish to overcome the toxic effect by elevating protein synthesis. Bhagyalakshmi [24] reported an increase in protein level in all the tissues of crab, *Oziotelphusa sensex* after exposure to sumithion. Rajan [25] reported the effect of textile mill effluent on *Cyprinus carpio*. There was a significant decrease in protein content of muscle, liver and intestine. Govindan *et al* [26] observed the decrease protein in the muscle of *Gambusai affinis* exposed to Phosphoridon. Jone Nelson and Sunil kumar [27] reported the decrease level of protein in the muscle of *Etroplus maculates* after exposure of Ekalux. Geraldine *et al*., [28] reported protein depletion in the freshwater prawn *Macrobrachium malcolmsonii* in response to dichlorvos exposure. Susan *et al*., [29] also reported a significant decrease in protein content under sub lethal concentration of pyrethorid fenvalerate in the gill of the fish *Catla catla*. The decrease of protein content in the prawn *Penaeus indicus* post larvae after exposure to sublethal concentration of lead was recorded [30]. According to Sathyanarayana [31] the physiological status of animal is usually indicated by the metabolic status of proteins. Senthil kumaar *et al*. [32] observed decrease in protein content in all the experimental tissues of field crab *Spiralothelphusa hydrodroma* due to the effect of heavy metal copper. Naveed *et al*., [33] observed a decrease in the protein content of the *C. punctatus* exposed to lihocin. Randhir and Banerjee [34] reported that *Clarias batrachus* showed marked fluctuations in their protein (1.56 ± 0.79% in gills to 4.46 ± 1.54% in muscles). In the present investigation, reduction in total protein content was noted in the tissues of both *C. punctatus* and *C. carpio* due to the anthraquinone dyes. This was possibly due to the direct effect of the toxicant on protein metabolic demands following exposure to the toxic stress of the dyes. Reduction of protein content indicates the possibility of gluconeogenesis to meet the energy budget.

In the present study the lipid content of both the fish species exposed to the sub lethal concentration of the anthraquinone dyes exhibited a decrease from 18th day. The decline in lipid content was observed in *Macrobrachium idella* due to cadmium toxicity [35]. Tazeen *et al.,* [36] observed decline in the total lipid content when the catfish *Mystus vittatus* exposed to the

pesticide nuvan. Saravana Bhavan and Geraldine [37] reported the reduction in lipid content in the prawn *Macrobrachium malcolmsonii* when the prawn was exposed to chlorpyrifos and suggested that the accelerated hydrolysis of lipid might be to cope up with the increased energy demand occurring due to the pesticide toxicity. Randhir and Banerjee [34] reported that *Clarias batrachus* showed marked fluctuations in their lipid content (1.79 ± 0.89% in skins to 4.81 ± 1.15% in brain tissue). In stress condition induced by pesticide or heavy metal, the lipid content depleted to meet the energy demand. In the present investigation, stress imposed by sublethal doses of the anthraquninone dyes to *C. punctatus* and *C. carpio* resulted in a marked decrease in lipid content, there by indicating high-energy demand.

Thus, in the present study among the three metabolites, carbohydrate registered a steady decline in the muscle from the beginning to the termination of the experiment and this implied the fact that multiplication of microbes along with the stress imposed by the toxicant would have resulted in commendable consumption of carbohydrate from the muscle tissue. In case of protein and lipid up to 7th day there was a marked increment in the tissue and there upon these two metabolites decreased drastically confirming the onset of catabolism caused by both the enhanced population of bacteria and the sustained stress imparted by the pollutant. Thus after the 7th day all the three metabolites markedly decreased in the tissue affirming the onset of catabolism above anabolism. This observation confirmed that due to the diseased condition and the coupled effect of toxicant and microbes, all the three major metabolites got depleted drastically from the tissue. This onset of catabolism confirmed that the test animals would die soon due to the ensuing histopathological changes of the test fishes.

[V] CONCLUSION

When exposed to stress conditions animals alter their physiological status with the help of enzymes. In our present study to meet the energy demands both *C. punctatus* and *C. carpio* might have adopted glycogenolysis and gluconeogenesis. It is exhibited in the results as the protein and lipid content demonstrated a considerable decrease on exposure to sublethal concentrations of anthraquinone dyes for 30 days but the carbohydrate content showed a slight decrease on 7th day in both the fish species indicating more utilization of protein and lipids to meet the calorie demand. Dyes as an environmental toxicant affects the nutritive value of the fishes and the changes could also adversely affect the taste, texture and in turn the marketability of this edible fish species.

Moreover, chemical determination of any persistent toxicant concentration in water and sediment may not provide information on the severity of contamination, especially in the case of sublethal levels. Hence, biological monitoring using a series of assays in a ‘‘key species’’ has become inevitable as it could allow a sensitive approach to predict the potential risk of persistent contaminants, which is helpful in formulating the ‘‘safe levels’’ of such bioaccumulative chemicals having toxic potential. Acute toxicity and biochemical studies are the very first step in determining the water quality requirements and health of the fishes. These studies obviously reveal the toxicant concentrations (LC50) that cause fish mortality even at short exposure and also help in determining the biochemical changes that occurs in the fish due to the exposure to toxicants. Therefore, studies demonstrating the sensitivity of toxic effects of pollutants in aquatic organisms, particularly in fish are needed. Thus, it can be concluded from the present study that fish are highly sensitive to the anthraquinone dyes and their mortality rate is dose dependent.

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TABLES:

Name of the Dye	LC_0 (mg/l)	LC_{50} (mg/l)	LC_{100} (mg/l)	SAR (mg/l)	Chosen mean Concentration (mg/l)
Vat Blue 4	9.5	148	510	2.8	6.15
Vat Green 1	10	180	540	3.1	6.60

Table 1: Fixing of Sub lethal concentration of the dyes

Fish species	Source	Carbohydrate (mg/g)	Protein (mg/g)	Lipid (mg/g)
Control *C. punctatus*	Nil	13.00 ± 1.00	130.00 ± 2.00	14.33 ± 0.577
Treated C. punctatus	7th day	12.00 ± 1.00	140.00 ± 4.00	15.00 ± 1.50
	18th day	10.00 ± 1.00	147.00 ± 7.211	13.50 ± 1.50
	30th day	10.12 ± 0.12	146.66 ± 5.774	10.00 ± 1.00
Control *C. carpio*	Nil	11.50 ± 1.50	120.00 ± 20.00	13.00 ± 2.00
Treated C. carpio	7th day	10.80 ± 0.80	130.00 ± 10.00	15.00 ± 3.00
	18th day	9.30 ± 0.30	126.00 ± 8.00	14.50 ± 1.50
	30th day	8.40 ± 0.40	119.00 ± 7.00	12.00 ± 2.00

Table 2: Proximate composition of the whole body of control and treated fishes *C. punctatus* and *C. carpio*

***Fish* species**	**X 1**	**X 2**	**df**	**'t' value**	**Significance**
C. punctatus	Carbohydrate content of the muscle on the 1st day	Carbohydrate content of the muscle on the 7th day	4	1.225	NS
	Carbohydrate content of the muscle on the 1st day	Carbohydrate content of the muscle on the 18th day	4	3.674	P < 0.04
	Carbohydrate content of the muscle on the 1st day	Carbohydrate content of the muscle on the 30th day	4	4.953	P < 0.01
C. carpio	Carbohydrate content of the muscle on the 1st day	Carbohydrate content of the muscle on the 7th day	4	0.713	NS
	Carbohydrate content of the muscle on the 1st day	Carbohydrate content of the muscle on the 18th day	4	2.491	NS
	Carbohydrate content of the muscle on the 1st day	Carbohydrate content of the muscle on the 30th day	4	3.459	P < 0.04

Table 3: Student's 't' test analysis of the data collected on carbohydrate content of the muscle of *C. punctatus and C. carpio*

***Fish* species**	**X 1**	**X 2**	**df**	**'t' value**	**Significance**
C. punctatus	Protein content of the muscle on the 1st day	Protein content of the muscle on the 7th day	4	3.873	P < 0.02
	Protein content of the muscle on the 1st day	Protein content of the muscle on the 18th day	4	3.935	P < 0.02
	Protein content of the muscle on the 1st day	Protein content of the muscle on the 30th day	4	4.725	P < 0.01
C. carpio	Protein content of the muscle on the 1st day	Protein content of the muscle on the 7th day	4	0.774	NS
	Protein content of the muscle on the 1st day	Protein content of the muscle on the 18th day	4	0.482	NS
	Protein content of the muscle on the 1st day	Protein content of the muscle on the 30th day	4	0.0817	NS

Table 4: Student's 't' test analysis of the data collected on protein content of the muscle of *C. punctatus* and *C. carpio*

Fish species	**X 1**	**X 2**	**df**	**'t' value**	**Significance**
C. punctatus	Lipid content of the muscle on the 1st day	Lipid content of the muscle on the 7th day	4	0.718	NS
	Lipid content of the muscle on the 1st day	Lipid content of the muscle on the 18th day	4	0.898	NS
	Lipid content of the muscle on the 1st day	Lipid content of the muscle on the 30th day	4	6.50	P < 0.01
C. carpio	Lipid content of the muscle on the 1st day	Lipid content of the muscle on the 7th day	4	0.961	NS
	Lipid content of the muscle on the 1st day	Lipid content of the muscle on the 18th day	4	1.039	NS
	Lipid content of the muscle on the 1st day	Lipid content of the muscle on the 30th day	4	0.612	NS

Table 5: Student's 't' test analysis of the data collected on lipid content of the muscle of *C. punctatus* and *C. carpio*