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ORIGINAL ARTICLE

EFFECTS OF INDUSTRIAL EFFLUENTS ON THE ACTIVITIES OF GLUTAMATE OXALOACETATE TRANSAMINASE AND GLUTAMATE PYRUVATE TRANSAMINASE OF FISH *Arius maculatus* FROM UPPANAR ESTUARY, CUDDALORE DISTRICT, TAMILNADU

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ABSTRACT

The present study is aimed to analyse the activities of glutamate oxaloacetate transaminase (GOT) and Glutamate Pyruvate Transaminase(GPT) in muslce tissues of *Arius maculatus* exposed to sub lethal concentration industrial effluents collected from Uppanar estuary, Cuddalore, District,Tamilnadu. The activities of glutamate oxaloacetate transaminase (GOT) and Glutamate Pyruvate Transaminase(GPT) were incresead in muslce tissues of *Arius* maculatus exposed to sub lethal concentration industrial effluents. The present study concludes that the effect of industrial effluents changes the activities of glutamate oxaloacetate transaminase (GOT) and Glutamate Pyruvate Transaminase(GPT) in *Arius maculatus*.

Keywords: Industrial effluents, GDT,DPT,Musle, *Arius maculatus*

1.INTRODUCTION

Water pollution occurs due to the presence of dissolved inorganic and organic materials such as proteins, fats, carbohydrates and other substances found in domestic and industrial water and physical factors such as turbidity, colour, temperature, associated radioactivity etc. Both metals and potential pollutants are affecting the *ichthyofauna* either directly or indirectly (Kumar and Pant, 1981). The effluents released by various industries are causing a lot of problems and their disposal involves a complicated task. Many industries do not have proper facilities to treat the effluents and about 68.5 cubic million litres of industrial effluents are discharged as such into the environment (Natarajan, 1984). Alterations in the chemical composition of the aquatic environment usually affect behavioural and physiological activities of the inhabitants, particularly the fish population (O' Brien, 1967). The alarming increase in the volume of pollutants affect the living organisms and in particular pose a health hazard in humans. Toxic substances pollute the fishery reservoirs, sometimes causing heavy mortality of the fish population and their food organisms. Pandey *et al*.(1979) have reported that the biocides contain toxic substances alter the physiological conditions of the aquatic environment and destroy the utility and quality of the freshwater. The industrial effluents are constantly polluting the natural waters and their effects are manifold on living organisms including economically important fishes. They are also responsible for various disturbances in physiological and biochemical parameters of fish (Shaffi, 1979; Koundinya and Ramamurthi, 1978, 1979). Industrial effluents containing different toxic substances also affect the aquatic system by interfering with their respiratory metabolism (Haniffa and porchelvi, 1985; Jeyachandran and Chockalingam, 1987; Vaidyanathan *et al.*, 1995).

The wastes of industries are either released into the atmosphere or mixed with large bodies of water. Many industrial and agricultural processes have contributed to the contamination of fresh water system thereby causing adverse effects on aquatic biota and human health (Dautremepuits *et al.*, 2004). It is therefore, necessary to identify and manage these pollution sources, and to maintain their effects on the health of aquatic ecosystems.

The fishes constitute one of the major sources of cheap nutritions and also economically important components of freshwater ecosystem. Fish is an important source of protein rich food for humans and has become a popular component of human diet during the last few decades in entire Indian subcontinent. In the world, India ranks ninth place in fish production. Fishes are relatively sensitive to changes in their surrounding environment. Fish health may thus reflect, and give a good indication of the health status of a specific

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aquatic ecosystem. Early toxic effects of pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Natural water reservoirs are traditionally being used for aquaculture and they contribute significantly to total fish production across the globe. Unfortunately, these natural resources are getting polluted with environmental pollutants and contaminants (Kumar et al., 2007;Burger, 2008). Fishes are sensitive to a wide variety of toxicants in water and are used as pollution indicators in water quality management. As fish is an important source of protein in a nation's diet, a thorough understanding of effluent effects on fishes and their safe permissible concentration in the aquatic environment would be more rewarding for fish conservation and fisheries development (Holden,1972). In view of this, the need for toxicological testing has increased from 1970 and a standardization of this technique provides the usefulness, allowing the study of various aspects of acute toxicity (Stephen, 1982). The evaluation of acute toxicity test is worthwhile to know the levels, below which it may be considered safe for a particular toxicant in the aquatic environment.

Fishes are relatively sensitive to changes in their surrounding environment. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Natural water reservoirs are traditionally being used for aquaculture and they contribute significantly to total fish production across the globe. Unfortunately, these natural resources are getting polluted with environmental pollutants and contaminants (Kumar *et al.*, 2007; Kumar *et al.*, 2008; Burger, 2008). From the experimental station of Uppanar estuary, fish were caught either using cast net or hand lines. Cast net are made of nylon. The mesh varies from 10 to 15 mm. Hand lines with mono filament and hooks were used with prawn or mussel as bait for the capture of *Arius maculatus*. The fish were transported to the laboratory and maintained in the same way as the control fish collected from Perumal Lake. The water was renewed once in two days.

2.MATERIALS AND METHODS

2.3. Procurement of fish

Live specimens of *Arius maculatus* with an average length of 8.5 ± 0.50 cm and weight of 15.0 ± 0.5 g were collected from Uppanar brackish water by operating cast net. The fish were acclimatised in the aquaria of 120 litres capacity containing well aerated sea water (salinity 28 ‰; pH 7.69; oxygen content 4.32 mg/l) and water temperature (32.6°C) for a period of one week prior to experiment. During acclimatization, the fish were fed withchopped prawn and clams. Food was withheld one day before the' commencement of the experiment. The water was changed along with waste feed and faecal matter every 24 hours. Fish collected from Perumal lake (Plate 1A) were used as control and Uppanar brackishwater area was selected as experimental site (Plate 1B).

Acute Toxicity Test

The raw and partially treated effluent was collected from the discharging point of industries surrounding the Uppanar eaturry for acute toxicity test. In the acute toxicity bioassay, mortality could be observed within a short period. LC_{50} was calculated by the following method of Finney (1978) to observe mortality and behavioural response of the test fish, *Arius maculatus* on exposure to effluents of different concentrations.

Static acute toxicity was employed to evaluate the adverse effects of industrial effluents surrounding the Uppanar estuary on the fish, *Arius maculatus* under standardised laboratory conditions.

Food was withheld one day before the toxicity test with a view to avoid the possible change in the toxicity of the pollutants after addition of the effluent into the test tank with 100 litres of sea water having 10 fishes. Mortality was recorded after 24, 48, 72 and 96 hr and five replicates were maintained simultaneously for the purpose. Fishes showing respiratory and lack of response to tactile stimuli were considered, nearing dead and removed immediately. Percentage mortality was calculated and the values were subjected to Probit analysis (Reddy *et al.*, 1992). Confidential limits (upper and lower) of the Regression coefficient with Chi - square test were calculated.

Design of sublethal toxic study

Sublethal studies are helpful to assess the response of the test organism to stress caused by the effluents. Based on acute toxicity test two sublethal concentrations (2% and 6%) on *Arius maculatus* were derived and used as the experimental concentrations. Sublethal of safe level concentration were derived from 96 hr LC_{50} value.

In the present study 2% (1/50) and 6% (1/30) dilution (in *Arius maculatus*) of the 96 hr LC_{50} were selected as sublethal concentrations. The experimental fish were exposed in each concentration for a period of 7, 15 and 30 days. A control batch corresponding to each test group was maintained simultaneously.

Estimation of Glutamate Oxaloacetate Transaminase (GOT) Activity

It was assayed following the procedure of Reitman and Frankel (1957).

100 mg of wet tissue (gill, muscle and liver) was homogenised using 2 ml of ice cold distilled water. The homogenised mixture was centrifuged for five minutes at 3000 rpm and 0.05 ml of supernatant was taken for the. estimation of GOT activity. Six test tubes each with 0.25 ml of the a - ketoglutarate substrate solution (buffered aspartate) of pH 7.4 were incubated in a water bath maintained at 37°C for 5 minutes. 0.05 ml of tissue extract was added to each. The test tubes with the extract were mixed well and kept in the water bath maintained at 37°C for 1 hr. 0.25 ml of 2, 4 - dinitrophenol hydrazine were added to each tube and shaken well and allowed to stand at room temperature, for 20 minutes. The reaction was arrested in the extract mixture by adding 2.5 ml of 4 N sodium hydroxide (NaOH) and the mixture was mixed well and allowed to stand at room temperature for 10 minutes and the brown colour formed at the end of the reaction was read at 505 nm in Bausch and Lomb Spectronic 21 Spectrophotometer and the results were expressed in Units/l00 mg of wet tissue.

Estimation of Glutamate Pyruvate Transaminase (GPT) Activity

It was assayed following the procedure of Reitman and Frankel (1957).

100 mg of wet tissues (gill, muscle and liver) was homogenized using 2 ml of ice cold distilled water. The homogenised mixture was centrifuged for five minutes at 3000 rpm and 0.05 ml of the supernatant was taken for estimation of GPT activity. Six test tubes each with 0.25 ml of the Cl-ketoglutarate substratesolution (buffered alanine) of pH 7.4 were taken and incubated in a water bath maintained at 37°C for 5 minutes. 0.05 ml of tissue extract was added to each. The test tubes with extract were mixed well and kept in a water bath and maintained at 37°C for 1 hr. 0.25 ml of 2, 4 - dinitrophenol hydrazine were added to each tube. The test tubes were then shaken well and allowed to stand at room temperature for 20 minutes. The reaction was arrested in the extract mixture by adding 2.5 ml of 4 N sodium hydroxide (NaOH) and allowed to stand at room temperature for 10 minutes and the brown colour formed at the end of the reaction was read at 505 nm in Spectronic 21 Spectrophotometer. The results were expressed in Units/l00 mg of wet tissue.

Statistical Analysis

Data from the present studies were subjected to Standard deviation and the significance of difference obtained was assessed by two way ANOVA for the study between the various periods of exposure as well as concentrations. The significant difference within the groups of exposure periods and within the concentrations were separately assessed by one way ANOVA and by the Turkey - HSD test (Multiple range test). The 't' test was assessed to study the significant difference between the control group and the test samples of field study using SPSS package (Statistical package for social science). Acute toxicity study was analysed using Probit analysis and Chi - square test.

3.RESULTS

Activity of Glutamate Oxaloacetate Transaminase (GOT) in muscle tissue

The activity of Glutamate oxaloacetate transaminase in the muscle of the control sample revealed a mean value of 47.655, 49.213 and 45.527 Units/100 mg of wet tissue respectively (Table 3.60). But the activity in the muscles at 2% sublethal concentration revealed an increase in the mean value of 91.705 (for 7 days), 89.638 (for 15 days) and 113.060 Units/100 mg of wet tissue (for 30 days). But at 6%, the activity for 7, 15 and 30 days further increased its value to 117.030, 119.235 and 129.235 Units/100 mg of wet tissue respectively (Table 1).

The Two way ANOVA for the activity of GOT in the tissues showed a significant value at 1% level between the two sublethal concentrations and the exposure periods. Furthermore, the muscle showed infinite F-value and hence, it is highly significant (Table 3.61). In all the tissues a significant value was observed between the control and sublethal concentrations of the effluent in the One way ANOVA (Table 3.62). The gill and muscle showed an insignificant difference between the various periods of exposure. The Multiple range test showed a significant value in the liver at 5% level for 7 and 15 days of exposures (Table 3.63). A significant value in the Glutamate oxaloacetate transaminase activity at 1% level in the field samples was observed between the control and test samples (Table 1).

Table 1Activity of glutamate oxaloacetate transaminase activity [GOT (units/100 mg tissues)] in the tissues of *Arius maculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	2%	6%
7 Days	Muscle	47.655 ± 0.013	91.705 ± 0.024	117.030 ± 0.018
15 Days	Muscle	49.213 ± 0.022	89.638 ± 0.017	119.235 ± 0.031
30 Days	Muscle	45.527 ± 0.022	113.060 ± 0.037	129.235 ± 0.031

Values are mean of five replications ± SD

Activity of Glutamate Pyruvate Transaminase (GPT) in muscle tissue

The activity of Glutamate pyruvate transaminase in the muscle of the control sample disclosed a mean value of 179.325, 169.415, 171.630 Units/l00 mg of wet tissue respectively (Table 3.65). Glutamate pyruvate transaminase activity in the muscle at 2% sublethal concentration revealed an enhanced mean value of 209.613 (for 7 days), 204.813 (for 15 days), 199.530 Units/l00 mg wet tissue (for 30 days). Besides at 6%, the activity of the enzyme during the exposure of 7, 15 and 30 days increased its level as 215.153, 209.660 and 204.845 Units/l00 mg of wet tissue respectively (Table 3.65 and Graph 34).

The Two way ANOVA for the activity of GPT showed a significant value at 1% level (P = 0.01) in the tissues between the two sublethal concentrations and the exposure periods. Nevertheless, the liver offered an infinite F-value hence; it was highly significant (Table 3.66). A significant difference at 1% level was observed between the control and the two sublethal concentrations of the effluent by the One way ANOVA in all the tissues. The Multiple range test for gill showed a significant value at 5% level particularly between the control and 6% concentration (Table 3.67). The muscle and liver showed an insignificant value for the various periods of exposure. The gill showed a significant value at 5% level particularly for 15 and 30 days of exposures (Table 3.68). A significant value in the Glutamate pyruvate transaminase at 1% level in the field was observed between the control and test samples (Table 2).

Table 2 Analysis of glutamate pyruvate transaminase activity (GPT (units/100 mg wet tissue)) in the tissues of *Arius maculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	2%	6%
7 Days	Muscle	179.325 ± 0.171	209.630 ± 0.030	215.153 ± 0.061
15 Days	Muscle	169.415 ± 0.013	204.813 ± 0.022	209.660 ± 0.018
30 Days	Muscle	171.630 ± 0.026	199.530 ± 0.014	204.845 ± 0.013

Values are mean of five replications ± SD

4.DISCUSSION

Transaminases are enzymes, which play vital role in the metabolism of non-essential amino acids. These enzymes are commonly employed as diagnostic tools in the assessement of liver damage in clinical practice (Goetz, 1980) and cellular damage of vital organs when treated with toxicants (Moss *et al.,* 1986; Sankarsamipillai and Jagadeesan, 2005). During cellular damage three enzymes are leaked into the serum and hence elevation of the activities of these enzymes in serum is considered as a sensitive indicator of even minor cell damage because the levels of these enzymes exceed those of extra cellular fluid by more than threefold increase (Moss *et al.,* 1986).

In the present study the GPT activity showed a significant increase in the muscle exposed to the effluent. The elevated transaminase activities may also be attributed as a response to provide energy to the aminase in the event of depressed oxidative metabolism. The increase in the GPT may also indicate the mobility of amino acids towards the formation of pyruvic acid as documented by Reddy *et al.* (1983) in the crab muscles due to chronic sumithion toxicity.Similar results made reported by Hwang and Wang (2001). They reported that the level of AST and ALT activities are increase due to heavy metals in chronic liver damage. The activity of AST and ALT can be used to indicate the tissue damage of liver and kidney (Begum, 2008). Hori *et al.,* (2006) have observed the level of AST in the liver tissue of *Brycon cephalus* exposed with phenol. Alteration in the activity of AST and ALT will be reflected nitrogen metabolism on the energy yielding TCA cycle (Beyer *et al.,* 1996).

Transaminase enzymes are indicators of environmental stress as they undergo changes in their activity and serve as markers towards the manifestation of pathology. Gupta and Paul,(1978) and Palanivelu,(2005) reported that the Increases in GOT and GPT levels after 15 and 60 days of exposure to monocrotophos also indicate liver damage, since increases in the activities of blood transaminase have been attributed to tissue damage, particularly the liver of *O.mossambicus*. Sastry and Sharma (1980) observed an increase in GOT and GPT in the blood of *C. punctatus* following the treatment of mercuric chloride. Liver damage has also been observed in *Clarias batrachus* following chronic exposure of carbofuran. The GOT showed a significant increase in the activity, particularly in the tissues of gill, muscle and liver of the fish caught from the polluted rivers (Wieser and Hinterleitner, 1980). Since the transminases activity requires pyridoxal phosphate for the conversion of aminoacids into ketoacids, on enhancement of GOT activity might be probable in the tissues which exhibited limited ammonia excretion (Giri *et al.,* 1997). It is generally accepted that an increase of these enzyme activities in the extracellular fluid or plasma is a sensitive indicator of even minor cellular damage (Palanivelu, 2005). Agrahari and Gopal (2007) reported that the measurement of transaminase and phosphatases activities in fish of *Channa punctatus*. Vutukuru *et al.* (2007) reported that significant increase in transaminases (AST and ALT) activity in fish exposed to arsenic could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver in *Labeo rohita*. Chen and Cheng (2003) demonstrated an increased activity of ALT and AST and hepatocyte ultra structure of common carp, *Cyprinus carpio* after gallium exposure.

Several investigators also reported that heavy metal intoxication showed a significant increase in AST and ALT activities in the liver tissue of animals (Rana *et al.,* 1996; Khandelwel *et al.,* 2002). The hepatocellular necrosis is generally associated with alterations in the liver tissue and serum (Zimmerman, 1978). The elevated level of AST and ALT indicate stopped up transmutation where feeding of amino acids into the TCA cycle occurs in order to cope up the energy crisis during cypermethrin toxicity (Philip *et al.,* 1995). The significant increase of these enzymes in the tissues seems to indicate possible dysfunction, taking place in the tissues of animals (Casilla *et al.,* 1983). Sharma (1999) has reported that similar pattern of increase in AST and ALT in the liver tissue of *Channa punctatus* exposed to pesticides. Chandravathy and Reddy (1991) have reported that the elevation of AST and ALT in the gill and brain tissues of *Anabas scandens* exposed to lead nitrate. Usha and Raj (1993) have reported the increase in the lever of AST and ALT in the animals exposed to vanadium. Mukhopadhyay *et al.* (1982) have observed that an increase level of AST and ALT activities in the liver tissue of *Clarius batrachus* exposed to carbofuron. Similar results observed by Ganguli *et al.* (1997). They reported that level of this enzyme increase in the gill, liver and kidney tissues of *Anabas testudineus* exposed to lindene, and furandan.

Enhanced transaminase activity is also suggestive of the increased catabolism of protein. It can be concluded that in fish farming, an elevation in the bold enzymes is indicative of acute intoxication (Shakoori *et al.,* 1994). In fish, the increase in the activity of serum enzyme after intoxication appears to be more rapid than mammals (Heath, 1987).

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