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Haematological, Enzymological and Biochemical effects of antibiotic Oxytetracycline in a fresh water fish *Labeo rohita*

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Abstract

Oxytetracyclin (OTC), an antibacterial agent, is extensively used in aquaculture practices all over the world. Despite its use, the toxicity of OTC to fresh water fish has been scarcely investigated. In this study *Labeo rohita,* were exposed to OTC. Fish were exposed to the 1000mg concentration for a period of 21 days, during which fish were killed at the end of every 7 days to analyse certain haematological, enzymological and biochemical parameters. During the exposure period, haemoglobin, haemacrit, red blood cell and white blood cell increased, while erythrocyte rate decreased. Enzymatic levels of acid phosphatase and alkaline phosphatase were increased in the vital organs (gill and muscle tissue) of fish. The alterations of these parameters lead to the conclusion that these parameters maybe used as biomarkers in monitoring OTC toxicity in aquaculture and fisheries farm.

Introduction

The water pollution is shown to affect feeding, oxygen consumption, metabolic turnover, muscular action, endocrine co-ordination and enzyme action as well as reproduction of aquatic organisms. Even at sub lethal concentrations, the pollutants affects the life of aquatic fauna, which are manifested as changes in physiology, biochemistry, activity levels of many enzymes and genetic makeup of organism (Mishra, 2003).

Most substances from pharmaceuticals used in human medicine enter the environment through waste water from sewage treatment plants, the use of sludge in agriculture and leakages from waste disposal sites. Veterinary pharmaceuticals may reach the environment through liquid and solid manure or they can be directly applied in the environment (Ambili, 2008).

There are many reports on potential risks of pharmaceuticals as toxic contaminants in aquatic environments. The specificity of pharmaceuticals is designated to cause a biological effects to human and animals. The most common antibiotics in the environment are Erythromycin, Ofloxacin, Chlortetracycline, Oxytetracycline, Streptomycin, Flumequine, Ciprofloxacin, Trimetoprim, Lincomycin, Spiramycin, etc.

Antibiotics are used largely as growth promoters and therapeutic

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treatment in livestock and microbial control in aquaculture. Some of the antibiotics widely used include erythromycin, nitrofuras, Oxytetracycline, Sulphaono methoxine (Carlsson *et al.,* 2006). In intensive fish farming, the antibiotics are mostly given as medicated feed pellets and calculations have indicated that 70-80 % of these compounds end up in the environment. Antibiotics are suspicious environment contaminants as they are biologically active, which obviously is a part of their nature. Antibacterial agents are usually very soluble. In order to be as effective as possible they often have a low biodegradability. Antibiotics present in the environment can produce resistance in microbial assemblages, which can have potentially drastic effects upon human health. (Alder *et al*., 2001).

Fishes are approximate as environmental toxicity bio-indicator organism, both due to their role in the aquatic chain and because of their sensitivity to low concentrations of toxic substances, characteristic of polluted aquatic environments (Cavas and Gozeekara, 2005). Haematological parameters may be altered due to environmental contaminant which has been used as an indicator of environmental stress by many workers. Blood is a patho-physiological reflector of the whole body, so, blood parameters are important in diagnosing the structural and functional status of the animal exposed to the toxicant. Fish respond to environmental pollutant by altering their metabolic functions. The alterations in the level of the activities of non-enzymatic antioxidants reflects the differential effects of pollution stress, which can be considered as biomarkers of exposure and subsequently as tools for bio monitoring in the assessment of environmental pollution (Christensen *et al.,* 1997).

Oxytetracycline is an extensively used veterinary antibiotic in aquaculture. The drug was found in concentrations capable of causing anti-microbial effects up to 12 weeks after administration. The persistence of oxytetracycline in bottom deposits from fish farms is also investigated. This pharmaceutical may cause deleterious effects on wild aquatic organisms accidentally exposed to them. Environmental contamination by pharmaceuticals needs to be monitored for several reasons including reliable assessment of risks for the environment and through the food chain for man

Materials and Methods

The experimental animal used was *Labeo rohita.* They are fresh water forms. In the present study, the fish *Labeo rohita* was used based on easy availability, feeding flexibility, easy acclimatization to the laboratory conditions, economic value etc. Fish were acclimatized to laboratory condition for about 10 days before the commencement of the experiment. Aquarium was cleaned frequently to avoid fungal growth and contamination by metabolites. The feeding was with held for 24 hours before the commencement of the experiment to keep the experimental animals more or less in the same metabolite state. From the stock fish with an average length of 15cm and weight 20g were segregated and transferred to clean rectangular glass aquarium tanks of 15L water capacity.

Stock solution of Oxytetracycline was prepared by dissolving 1g (1000mg) of Oxytetracycline in 1litre of distilled water. For the sublethal toxicity studies a glass aquaria was taken and filled with 15 Litre of water.1g of Oxytetracyclin was added to the tank. Subsequently, healthy fish were introduced into the tank. A glass tank of toxicant free water was maintained as control. Water was changed daily in order to avoid accumulation of faecal matter and excess feed and renewed with the toxicant. Water was changed daily. Experiments were conducted for a period of 21 days. Mortality was observed during the experimental period.

Fishes were sacrificed without anesthetizing for further analysis. The blood samples of control and test fishes were collected from the caudal region and heart in asceptic condition by means of a standard syringe. Blood was transferred into small vials, which is previously rinsed with heparin. The RBC's and WBC's were counted using haemocytometer and the whole blood was used for the estimation of haemoglobin and haematocrit of standard method by Cyanmet haemoglobin method (Nelson and morris 1989). The gill and muscle tissues were isolated from the control and experimental fish, each tissue were homogenized with 5ml of sucrose solution in ice cold condition (Amitha, 2001). The homogenates were centrifuged for 15 minutes at 6000 rpm. The clear supernatant fluid was taken for various estimations such as Protein, glucose and enzyme assay (Remya,2008)

Results and Discussion

In the present investigation when fishes were exposed to different concentrations of antibiotic namely oxytetracycline marked behavioral changes were noticed. The fish showed erratic jumping movements, irregular swimming activity, floating upside down, with abdomen directed upwards, spreading of excess mucus all over the surface of the body, rapid opercular movements, gulping of air and asphyxiation occurred. After prolonged exposure decoloration of gill and liver were noticed. The above said behavioural changes were minimum during initial days, however after prolonged exposure the said behavioural changes were maximum and finally mortality of the fish were noticed.

The changes in the haemoglobin content in the blood of *Labeo rohita* exposed to oxytetracycline for 21 days is presented in Table 1. During the study period haemoglobin value was increased in the drug exposed fish. Table 2 represent the data on changes in haematocrit value of the fish *Labeo rohita* exposed to oxytetracycline for 21 days. The haematocrit value was increased gradually as the exposure period.

Changes in the red blood cell count of the blood of *Labeo rohita* exposed to oxytetracycline for 21 days are presented in Table 3. During the above treatment period RBC count was decreased throughout the study period. Table 4 shows the changes in the Leucocytes count of the blood of *Labeo rohita* exposed to oxytetracycline for 21 days. The WBC count was increased in the drug treated fish showing a direct relationship with the exposure period, showing a maximum level at the end of 21st day.

Table 5 & 6 shows the changes in the Alkaline phosphatase level of both muscle tissue and gills of *Labeo rohita* exposed to oxytetracycline for 21 days. Alkaline phosphatase level in the muscle tissue was increased in the drug treated fish. Alkaline phosphatase value was increased gradually as the exposure period. Table 7&8 represents the data on changes in the Acid phosphatase level in both muscle tissue and gills of the fish Labeo rohita exposed to oxytetracycline for 21 days.

During the treatment period, Acid phosphatase in the gill was increased throughout the study period. Changes in the protein level of *Labeo rohita* exposed to oxytetracycline for 21 days are presented in table 9&10. Protein level was increased in both gills and muscle tissue of *Labeo rohita* .Changes in the glucose level of *Labeo rohita* exposed to oxytetracycline for 21 days are presented in table 11&12.Glucose level was increased in both gills and muscle tissue of *Labeo rohita.*

Fig:1 The experimental animal ***Labeo rohita***

Table 1: Changes in the Haemoglobin level in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	**Haemoglobin (mg/dl)**	
	Control	**Test**
7	4.55	4.55
14	4.45	7.64
21	4.41	8.60

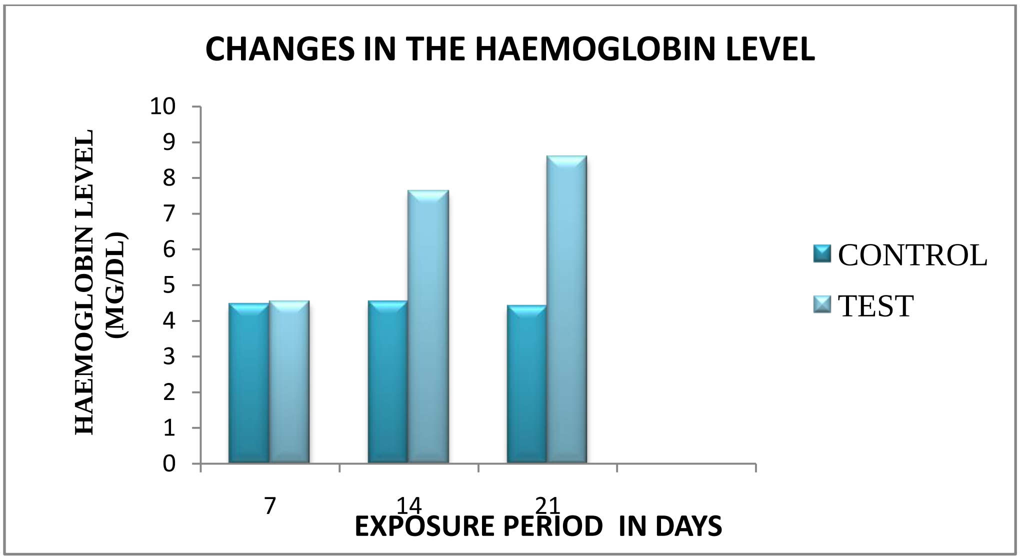

Fig:2 Changes in the Haemoglobin level in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 2: Changes in the Haematocrit level in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	**Haematocrit (%)**	
	Control	**Test**
7	15.32	14.28
14	15.15	18.10
21	14.95	20.12

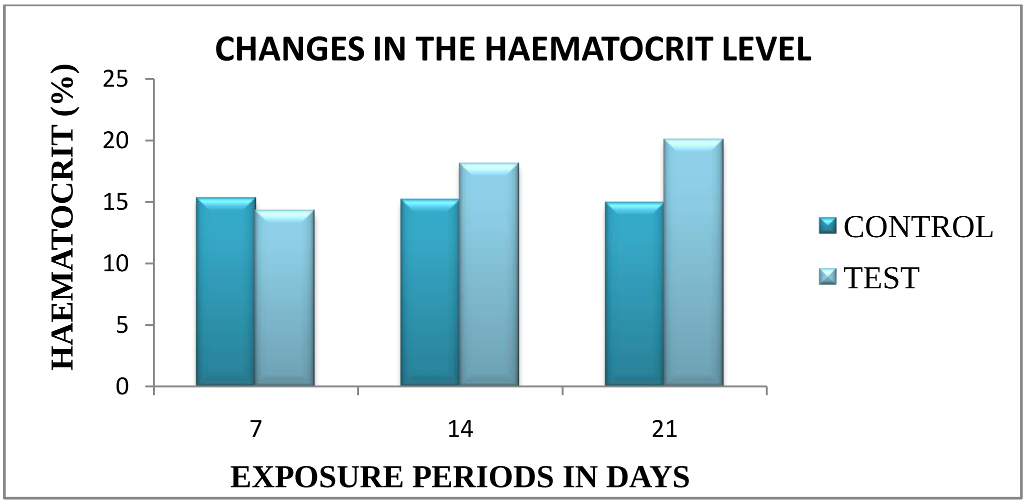

Fig: 3 Changes in the Haematocrit level in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table3: Changes in the Erythrocyte count in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Erythrocyte (million/cu.mm.)	
	Control	**Test**
7	$3.39*10^6$	$3.46*10^6$
14	$3.45*10^6$	$2.50*10^6$
21	$3.41*10^6$	$2.41*10^6$

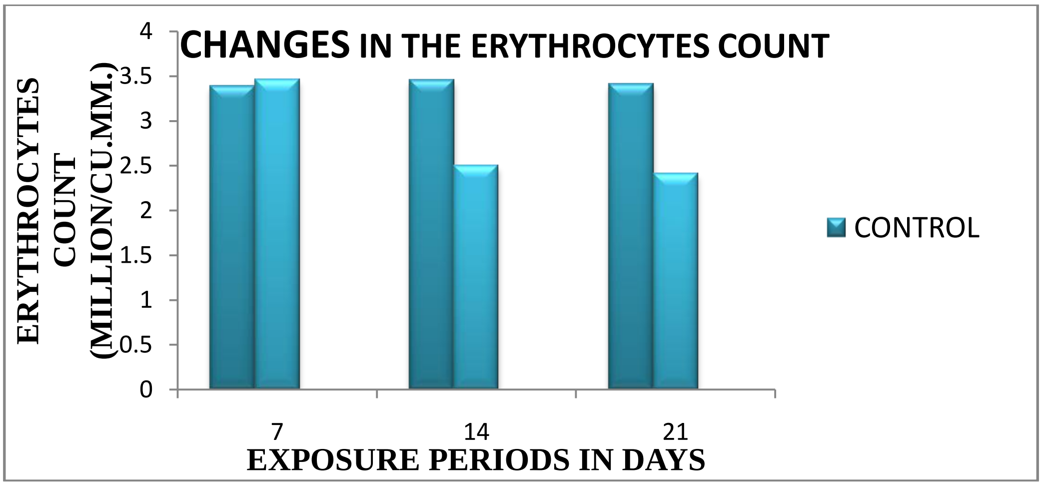

Fig: 4 Changes in the Erythrocyte count in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 4: Changes in the Leucocyte count in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Leucocyte (1000/cu.mm.)	
	Control	Test
7	$4.3*10^3$	$4.6*10^3$
14	$4.6*10^3$	$5.8*10^3$
21	$4.1*10^3$	$7.3*10^3$

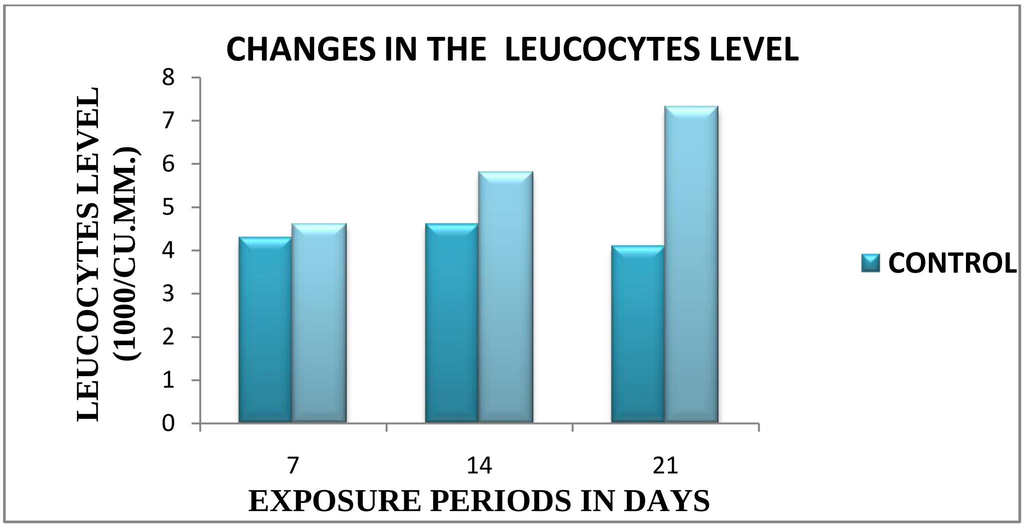

Fig: 5 Changes in the Leucocyte count in a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 5: Changes in the Alkaline phosphatase value in the muscle tissue of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Alkaline phosphatase (IU/L)	
	Control	Test
7	42.38	66.66
14	46.56	68.75
21	41.48	73.25

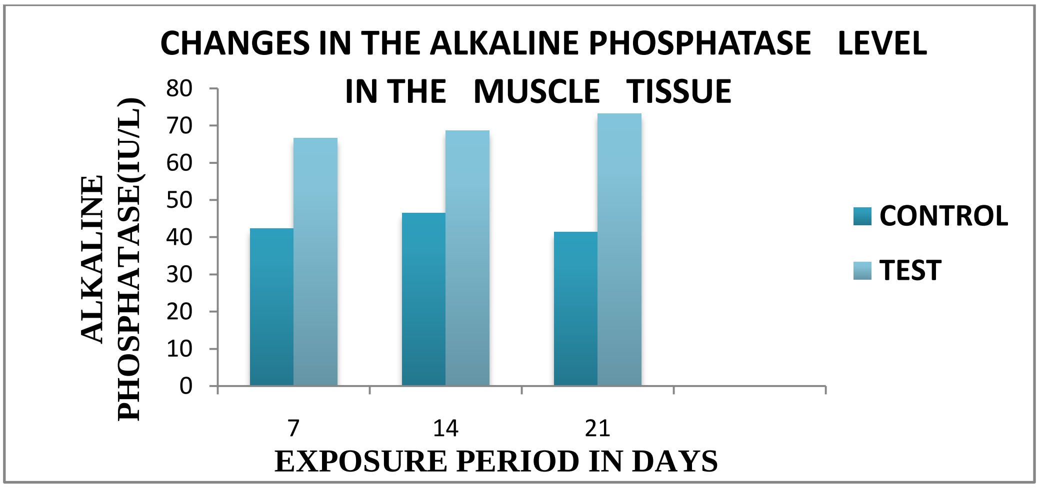

Fig: 6 Changes in the Alkaline phosphatase value in the muscle tissue of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 6: Changes in the Alkaline phosphatase value in the gill of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Alkaline phosphatase (IU/L)	
	Control	Test
7	43.26	56.32
14	42.11	61.50
21	40.95	65.80

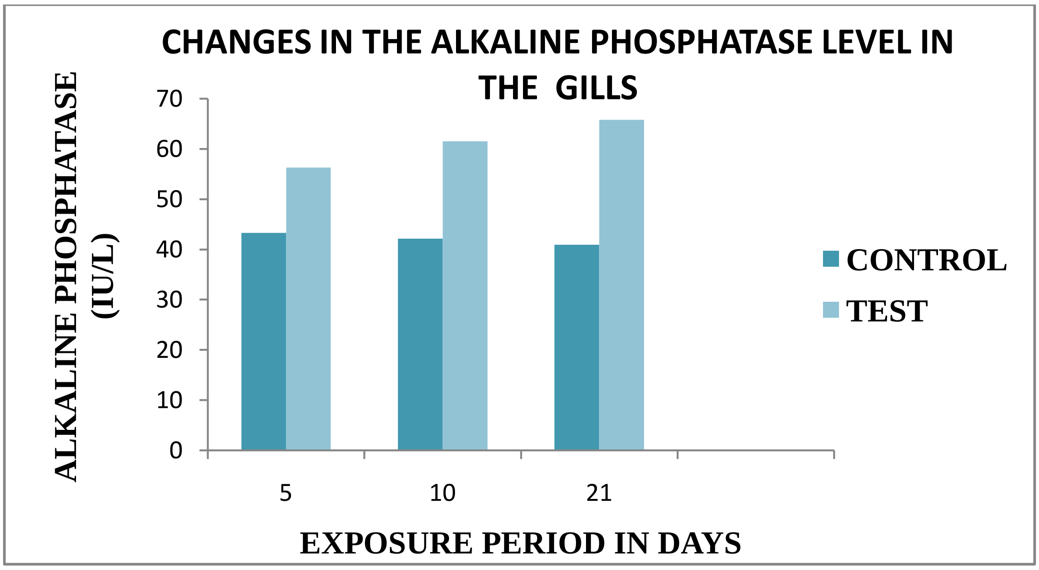

Fig: 7 Changes in the Alkaline phosphatase value in the gill of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 7: Changes in the Acid phosphatase value in the muscle tissue of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Acid phosphatase (IU/L)	
	Control	Test
7	25.45	26.50
14	27.45	25.63
21	26.33	28.85

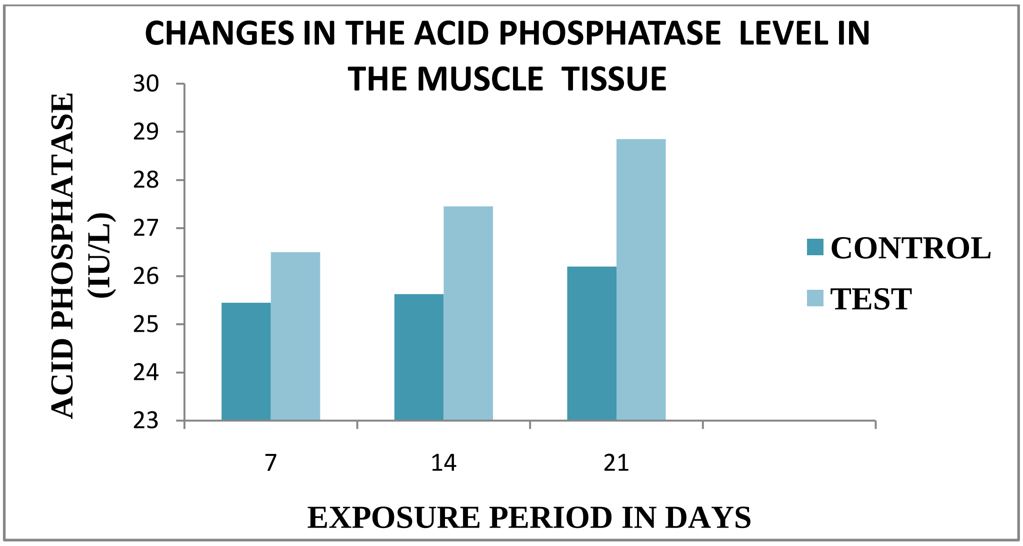

Fig: 8 Changes in the Acid phosphatase value in the muscle of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table: 8 Changes in the Acid phosphatase value in the gills of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days

Exposure period(In days)	Acid phosphatase (IU/L)	
	Control	Test
7	16.10	15.25
14	15.55	18.50
21	15.59	20.15

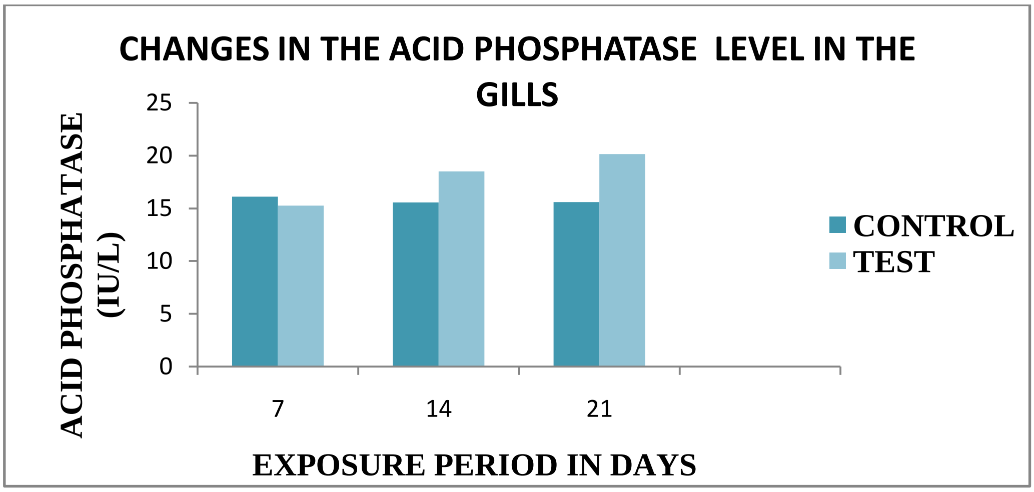

Fig: 9 Changes in the Acid phosphatase value in the gills of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 9: Changes in the Protein level in the muscle tissue of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Protein level (g/dl)	
	Control	Test
7	4.44	4.90
14	4.35	5.15
21	4.50	5.65

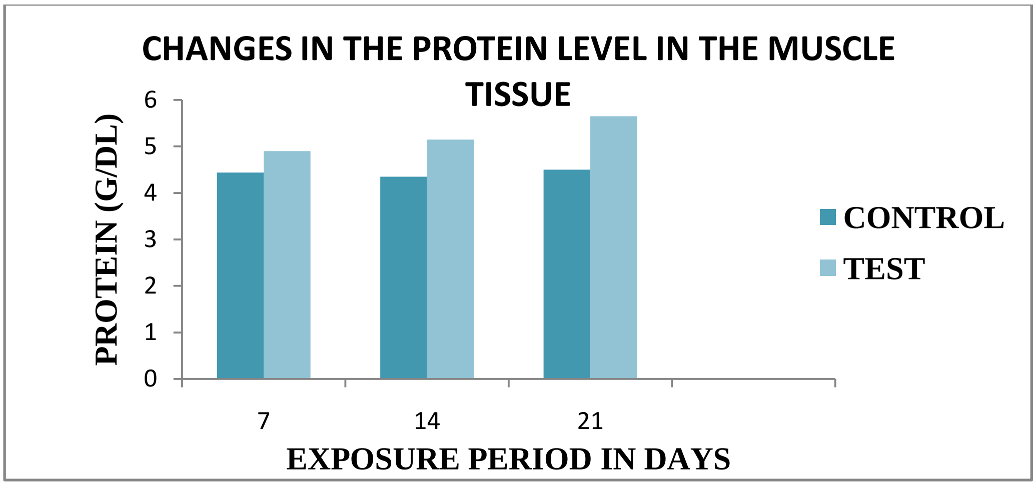

Fig:10 Changes in the Protein level in the muscle tissue of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 10 : Changes in the Protein level in the gills of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Protein level (g/dl)	
	Control	Test
7	3.82	3.95
14	3.79	3.81
21	3.85	4.50

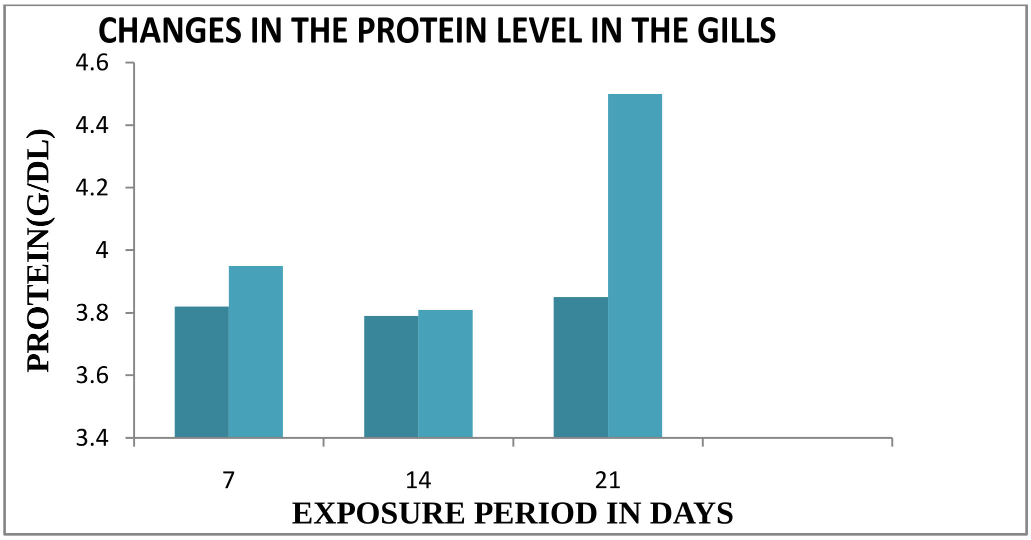

Fig: 11 Changes in the Protein level in the gills of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 11 : Changes in the glucose level in the muscle tissue of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Glucose level (mg/dl)	
	Control	Test
7	66.50	67.48
14	65.74	70.23
21	65.95	71.33

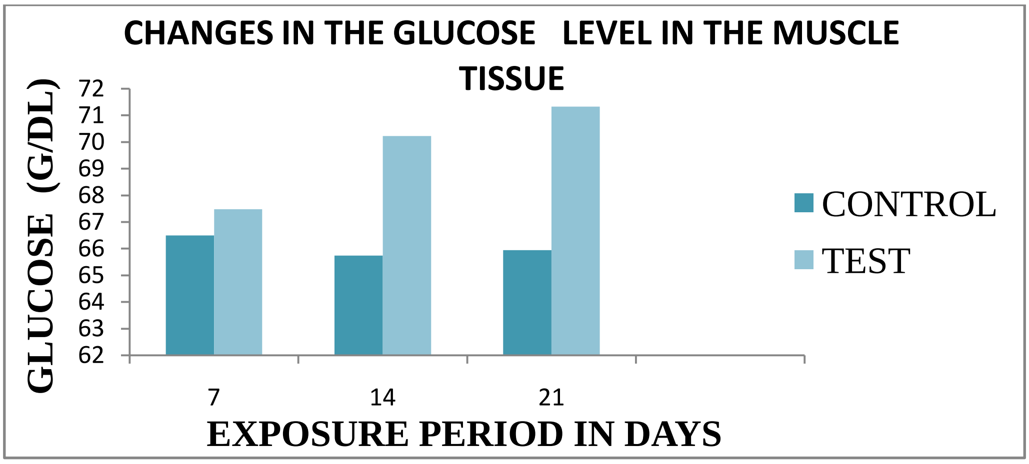

Fig: 12 Changes in the glucose level in the muscle tissue of a fresh water fish *Labeorohita* exposed to an antibiotic Oxytetracycline for 21 days.

Table 12 : Changes in the glucose level in the gills of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Exposure period(In days)	Glucose level (mg/dl)	
	Control	Test
7	53.55	55.60
14	55.45	68.10
21	56.20	75.55

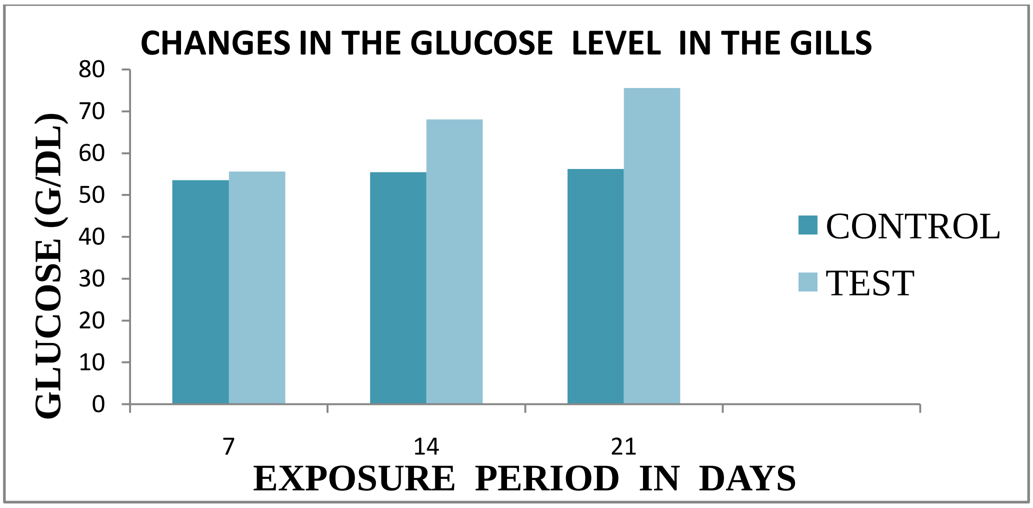

Fig: 13 Changes in the glucose level in the gills of a fresh water fish *Labeo rohita* exposed to an antibiotic Oxytetracycline for 21 days.

Babin *et al.,* (2005) reported that human pharmaceuticals are mostly released to municipal effluents, and through the waste water treatment plants into fresh water and marine system. The number of pharmaceuticals detected in natural water is continuously increasing, but the availability of ecotoxicological data is limited. The need for assessing the environmental risk of veterinary and human pharmaceuticals has become evident. The specificity of pharmaceuticals is designed to cause a biological effect to human and animals. Therefore it implies that they often have similar types of physiochemical behaviors which are characterized by xenobiotics (Barton, 1997).

Several authors have observed an increase in the haemoglobin content in fish when exposed to oxytetracycline. The hyper activity may be due to hypoxia faced by the fish due to gill damage by the irritants and increased haemoglobin content may be a response to compensate impared respiratory efficiency (Ambili, 2008). And also replacement of oxidized denatured haemoglobin and additional oxygen supply to fish tissue under OTC stressed condition (Sharma, 1999). Addition of oxygen increases, then haemoglobin iron rich protein in blood also increases. Increases in haematocrit values were associated with osmotic shifts; as the PH of the blood decreased, erythrocytes swelled and plasma volume decreased (Amit, 2000**).** Erythrocyte level was found to be depressed in fishes subjected to stressful conditions (Kumar and Barthwal, 1991). The reduction in number, increase in surface area and distorted shape of red blood cells(RBCs) in ammonia exposed fish Cirrhinus mrigala may have resulted from the hypoxia condition (Ash, 2002).

In the present study, the decrease in RBC count during chronic exposure may be due to severe anemia state or hemolysing power of the antibiotic particularly on the red cell membrane. The anemia with erythropenia has also been reported, reduction in RBC value indicated erythropoiesis, haemosynthesis and osmoregulatory dysfunction. earlier in fishes after exposure to toxicant like Oxytetracycline (Ahmed and Munshi, 1989). So due to exposure of antibiotic will decreases RBC which affected anaemia and impaired osmoregulation. Macrocytic anaemia is the deficiency of vitamine by folic acid which affect the life span of RBC. OTC affects the erythropoietic tissue, so decreases RBC count.

Increase in WBC count in fishes exposed to chronic and lethal doses indicates leucocytosis (Amemiya, 1986). Increasing WBC means the protective response of fish to antibiotic OTC. It is a defensive mechanisam. Leucocytosis is an adaptation, commonly observed in fish to meet stressful condition. Stimulation of the immune system as a response to OTC toxicity may be another reason to increase WBC (Goel and gupta 1985). Antibody productions which help in survival and recovery of the fish exposed to OTC. Leucocytosis observed by the toxic effects of manganese in *Heteropneustes fossilis* is an adaptation to meet the stress condition in fish as reported by Bala and Sinha, (1995). Increases WBC in the form of leucocytosis with heteropilia and lymphopenia which are characteristic leucocytic response in animals exhibiting stress. Increases in WBC count can be correlated with an increase in antibody production. Which helps in survival and recovery of the fish exposed to oxytetracycline (Joshy *et al*.,2002). The present study indicates that the significant increases in WBC count indicate hypersensitivity of leucocytes to oxytetracycline and these changes may due to immunological reaction to produce antibodies to cope up with stress induced by oxytetracycline.

Acid phosphatase level increases in muscle and gills were lead to its death and they can be used as indicators and increases the acid phosphatase level in muscles and gills may be due to increased lysosomal activity. Acid phosphatas as marker enzyme for the detection of lysosome in cell fractions and can be altered by the presence of oxytetracycline (Alder, 2001) and leakage of the enzyme from cytosol across the damaged plasma membrane in to the general blood circulation. Increases the enzyme (Alkaline phosphatase & acid phosphatase) which lead to release of cellular enzymes and depletion of intercellular nucleotides and changes in the membrane potential cellular damage which lead to tissue lyses (Sharma, 1999).

Fishes respond to environmental pollutant by altering their metabolic function. Increase in the protein and glucose leads to the levels of serum ALT, AST, LDH creatine and cortisol production were significantly increased. Increase in glucose level due to the hyperglycemia. Gills and muscle tissues were sensitive indicators of aquatic pollutants. The increase of glucose in muscle and gills noted that cortisol has shown to promote catabolism of peripheral tissues and increased gluconeogenesis leading to hyperglycemia. Gluconeogenesis is to provide energy for the increased metabolic demands by the stress oxytetracycline. Increased protein content in muscles and gills may be due to protein synthesis and possible utilization of their product for metabolic purposes (Campell, 1981**).** Transaminase plays an important role in protein and glucose metabolisam.Glutamate oxalocetate transaminase and glutamic pyruvate transaminase enzymes will increases for the activity of transaminase. Increased protein content may be related to food intake, increased energy homeostasis, not tissue repair and do not used protein for detoxification mechanism during stress (Remya, 2008).

Pharmaceuticals, especially antibiotics have an important role in the existence of life of both human and other animals. The present study has given a small picturisation about the toxic effect of the antibiotic oxytetracycline. Therefore, this serious issue on health disaster has to be dealt with care and efficient alternative should be encouraged. Aquaculture systems are a potentially significant source of antibacterial agents to the aquatic environment. Since the antibiotic oxytetracycline (OTC) have been widely used in aquaculture this may cause deleterious effects on wild aquatic organisms accidentally exposed to them. Oxytetracycline has a good sedimentation property than other antibiotics. Accumulation of antibiotics will lead to the most dangerous bacterial resistance in the environment.

Conclusion

Oxytetracyclin (OTC) is an extensively used veterinary antibiotic in aquaculture. In the present study, the level of haemoglobin, haemacrit and white blood cell increased and the erythrocyte rate decreased. Enzymatic levels of acid phosphatase and alkaline phosphatase increased in the vital organs (gill and muscle tissue) of fish. The level of protein and glucose also increased in the gills and muscle tissues of fish. The alterations of these parameters lead to the conclusion that these parameters maybe used as biomarkers in monitoring OTC toxicity in aquaculture and fisheries farm.

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