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Research Article

TOXICITY OF COPPER OXYCHLORIDE (FUNGICIDE) IN *OREOCHROMIS MOSSAMBICUS* ON HAEMATO-IMMUNOLOGICAL AND BIOCHEMICAL ALTERATIONS AND RECOVERY ASSESSMENT BY MARINE ALGAE *CHAETOMORPHA AEREA*

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ABSTRACT

The effects of Copper oxychloride (Fungicide) on haematological, immunological, serum biochemical parameters were investigated in *Oreochromis mossambicus*. The 96-h LC_{50} value of copper oxychloride was found to be 30.53 mg l^{-1}. Examination of haematological, immunological parameters and biochemical parameters (was performed on *O. mossabicus* after 96 h of exposure of fungicide (30.53 mg l^{-1}). At end of the experiment, fish were exposure in 2.5 g/l^{-1} of *Chaetomorpha aerea* on 96 h of recovery assessment. Fish administered with 2.5 g of *C. aerea* neutralized the toxic effect of copper oxychloride as well as *C. aerea*, significantly lowered the hematological, immunological and biochemical response. Significant alterations in all the biochemical parameters were found to be dose dependent. Fish exposure fungicide, showed significantly ($p< 0.05$) enhanced TEC, TLC, total protein, NBT activity, serum lysozyme activity in fungicide treatment groups in comparison with control group. Similarly, SGOT, SGPT and blood glucose level were found to be significantly ($p< 0.05$) high but PCV and Hb did not differ significantly ($p>0.05$) in the treatment groups compared to control groups. The results showed improvement in samples treated with *C. aerea*. This study suggests that *C. aerea* can be effectively used to decrease the toxic effect of copper oxychloride on *O. mossambicus*.

Keywords: Acute toxicity, Fungicide, Copper oxychloride, *Oreochromis mossambicus*, $LC_{50.}$

INTRODUCTION

Today environmental pollution has become not only a national but also an international problem(Tamizhazhagan & Pugazhendy, 2015). Today, water quality management faces greater problems than at any time in its history. In addition to natural pollutants, varied contaminants exist in surface waters including multiple chemical compounds and different products of industrial and agricultural revolution. Pollution of the aquatic environment by toxic substances is a cause of growing concern throughout the world, especially in developing countries. Aquatic ecosystems that run through agricultural areas have high probability of being contaminated by runoff and ground water is reached by a variety of chemicals (Jayalakshmi *et al*., 2017). The immediate concern is human health and welfare, but the effect of pollution on aquatic organisms also has ecosystem wide consequences (Alam & Maughan, 1993). Many of the toxic substances are lipophilic and are not adversely affected by water. Heavy metals enter into aquatic habitats by a number of routes and cause hazardous effect on their morphology and physiology (Pichaimani *et al*., 2017a). These substances accumulate in fish fatty tissues or become protein bound, so it is of importance to know the critical concentration above which humans are affected and the commercial fish species become unsuitable as food (El Sayed and El Bahr, 2007). The weathering of rocks, soil

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forms and increased use of metal containing fertilizers in agriculture could lead acetate to a continued rise of the concentration of metal pollutants in freshwater reservoirs as a result of water runoff (Pichaimani *et al*., 2017b). Organophosphate pesticides constitute a large proportion of the total synthetic chemicals employed for the control of pests in the field of agriculture, veterinary practices and public health (Padmapriya *et al*., 2017). Fish are ecologically and economically important and widely used as animal models in toxicological research with goal of extrapolating the results to inform questions concerning the potential human health effects of chemical exposure (Di Giulio & Hinton, 2008). Fungicides are widely used in agriculture and industry because it is easy to apply, cost effective, and in some situations, it is only a practical method of control. However, benefits of fungicides are not derived without consequences Non-persistent nature. Recently studies have proved that extremely low quantities of pesticides which enter the aquatic environment can affect productivity of organisms to kill eggs and larvae (Padmapriya *et al*., 2017).

In fish, contaminants enter mainly through respiratory system, hence into blood and subsequently to different organs or systems. Blood is the most accessible component of the vertebrate body fluid system and consequences of direct and indirect damage to blood cells and their precursors are predictable and potentially life threatening. Therefore, haematological studies have been considered and used as diagnostic tool in order to investigate diseases and physiological and metabolic alterations. There are some different investigations reported about changes in serum protein (Das & Mukherjee, 2003), blood glucose level (Gimeno *et al*., 1995), total erythrocyte and leukocyte counts, and haemoglobin percentage (El-Sayed *et al*., 2007), because of different pesticides in various fish species. Blood parameters are increasingly used as indicators of the physiological or sublethal stress response in fish to endogenous or exogenous changes (Cataldi *et al*., 1998). Measurement of serum biochemical parameters can be especially useful to help identify target organs of toxicity as well as the general health status of animals, and is advocated to provide early warning of potentially damaging changes in stressed organisms (Folmar, 1993).

Biochemical and physiological indicators such as enzymes can be used to identify environmental contamination before the health of aquatic organism is seriously affected (Farrell, 2007). Changes in serum enzyme activities are related to the physiological and functional alterations in metal exposed fish. The industrial development and rapid urbanization have led to development of polluted zones discharging potentially toxic compounds in the environment (Jayakumar *et al*., 2018). Exposure of fish to heavy metals may result in variable degrees of ion regulatory disruption, and plasma ion levels may be employed for quantifying toxic effects of metal intoxication (Mayer *et al*., 2018). Shifts in the hydromineral balance may be a consequence of the action of pollutants on organs involved in osmoregulation, on the endocrine system, on metabolism, or on active transport processes (Martinez & Cólus, 2002).

Tilapia, considered to be future of aquaculture, is nicknamed ‘‘the aquatic chicken’’ due to its ability to grow quickly with poor-quality inputs. It is now-a-days co-cultured with shrimp in many parts of the world. Fish immune system, important for defense against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by the biochemical profile of the nervous system (Usha *et al*., 2017). Protein being the essential substance is desirable for growth and enlargement and also serves as energy source during the stress condition (Tamizhazhagan *et al*., 2017). As shrimp cultivation has been confronting difficulties because of white spot syndrome, tilapia culture could provide the relief. Besides, export of tilapia too holds great promise. *Oreochromis mossambicus* has been extensively used for studies on biochemical genetics, chromosome manipulations such as gynogenesis or ploidy level changes and transgenesis. Tilapia is a good biological model for toxicological and immunotoxicity studies (Solis *et al*., 2007). Environmental pollution occurs when the environmental degradation crosses the limit so that. It becomes lethal to living organisms (Usha *et al*., 2017). due to diverse characteristics, namely their high growth rates, efficiency in adapting to diverse diets, great resistance to diseases and handling practices, easy reproduction in captivity at prolific rate and finally, good tolerance to a wide range of environmental conditions (Figueiredo *et al*., 2006). The aim was to assess the effects of Copper oxychloride, recovery assessment by using *Chaetomorpha aerea* on immunological, haematological and biochemical responses of *Oreochromis mossambicus*.

MATERIALS AND METHODS

Experimental animals

Adult *O. mossambicus* (53.50±9.35 g and 15.33±0.89 cm), were obtained from local breeder and acclimated to laboratory conditions for four weeks. During acclimatization, fish were held in aerated free chlorine water (150 l aquariums) fed with a pelleted diet prepared in the laboratory (32.5% Crude Protein). Throughout the experimental period, the water quality was as follows: temperature 21.9 ±16.0, pH 8.5±0.32. Alkalinity 209±8.22 mg/l. $CaCO_3$, and hardness 245.43±15.3 mg/l, dissolved oxygen 6.9±0.3 mg/l, ammonia 0.03±0.1 mg/l. temperature, pH, dissolved oxygen, and ammonia were analyzed daily, and 12:12 photoperiod was used. Water was changed every 12 h.

Chaetomorpha aerea

The marine algae of *C. aerea* were obtained from Parangipettai, Tamilnadu, India. The identification of the species was carried out by following the authentic checklist of reference (Wynne, 2011). The algae were collected and washed in sterile water. They were shade dried and

converted into powder and stored in refrigeration at 4°C for further analysis.

Lethal concentration (LC_{50}) of Copper oxychloride

Stock solution of fungicide, (copper oxychloride) was prepared by dissolving analytical grade copper oxychloride ($Cu_2Cl(OH)_3$); from Merck) in distilled water. Required quantity of copper oxychloride was drawn from this stock solution for the further experiment. Range finding test was conducted using methodology as suggested by Das & Mukherjee, (2003). Experiment was carried out to determine the median lethal concentration (LC_{50}) of copper oxychloride in fish for 96 h by probit analysis method (Finney, 1971). Uniform sized rectangular glass aquaria (150 l capacity) were used for bioassay experiment. In each glass aquarium total volume of water was maintained at 100 l and was provided with round the clock aeration. Test organisms were exposed to a logarithmic increasing range of concentrations such as 0.01, 0.1, 0.25, 0.5, 1, 10.0 and 20.0 mg/l^{-1}. Static non-renewable bioassay was conducted with 10 test animals for each concentration. Percentage mortality was observed every 24 h interval at 24, 48, 72 and 96 h. In range finding test of copper oxychloride on *O. mossambicus*, it was found that mortality percentage of 0% and 100% lies in between 1.0 and 20.0 mg/l^{-1}. For definitive test eight copper oxychloride concentrations 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mg/l^{-1} concentration was selected and test was conducted in triplicates for each concentration containing five fishes in each replicate. Side by side one control was also kept slightly away from bioassay tank to avoid contamination. No feeding was done and static non-renewable method was applied. Percentage mortality was recorded at 24, 48, 72 and 96 h interval and dead fish were removed immediately. The data obtained from the experiment was processed by probit analysis using statistical software package SPSS (21.0). The 96 h LC_{50} value was recorded, which was found to be 30.53 mg l^{-1} with a 95% confidence interval of 25.46–45.78 mg l^{-1}.

Experimental design

For sub-lethal toxicity assay, *O. mossambicus* fingerlings were distributed into two groups one is control and the other is treatment with two duplicates for each group. Eight fishes were kept in each experimental tub. Uniform sized rectangular tubs (150 l capacity) were used as experimental units. The total volume of the water in each tub was maintained at 100 l throughout the experimental period. Round the clock aeration was provided. Fishes was exposed to 1/3rd of LC_{50} of copper oxychloride for 96 h, i.e. 30.53 mg l^{-1} (LC_{50} for 96 h is 30.53 mg l^{-1}) for assessment of its effect on different biochemical and immune-haematogical parameters of *O. mossambcius*. Daily water exchange was done with care to avoid stress to test organisms and test solution was renewed to provide constant effect of the chemical. Similar methodology was followed for sub-lethal toxicity studies by Das & Mukherjee, (2003); Vijayavel & Balasubramanian, (2009). The experiment was conducted for 96 hours.

Recovery assessment

After exposure to Treatments for a period of 96 h, fish were transferred to fungicide free water for recovery studies. All fish were exposure through water 0.25 g/l^{-1} of *C. aerea* after toxicity studies. At the end of 96 h suitable number of fish (8 fish from each aquarium) was collected for the abovementioned assays. The water quality parameters were monitored and maintained as that of the acclimation period.

Collection of blood

Blood was drawn from caudal vein of fish by using 1.0 ml hypo-dermal syringe and 24 gauge needles, which was rinsed with heparin (5,000 IU/ml) solution before use. The collected partially blood was immediately transferred to the test tube coated with thin layer of EDTA (as an anticoagulant) and shake well in order to prevent haemolysis and clotting blood. Serum was collected without using anticoagulant and was separated from remaining blood by keeping the tubes in slanting position for about 2 h and thereafter it was centrifuged at 3500 rpm for 15 min at 4° C followed by collection of straw coloured serum with micropipette and stored at -20° C for further analysis.

Haematological parameters

Freshly collected heparinized whole blood samples were used for estimation of hematological parameters like Haemoglobin was estimated by using Sahli's hemoglobinometer. The oxygen carrying capacity of the fish blood was calculated by multiplying the haemoglobin content by 1.25 oxygen combining power of Hb/h (Jacobson Kram & Keller, 2001). Erythrocyte count and leukocyte counts were studied by Neubauer's improved hemocytometer using Hayem's and Tuerk's solutions, respectively, as diluting fluids. packed cell volume or hematocrit value, were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuged for 5 min at 10,500 rpm in a microhematocrit centrifuge (Hettich, Germany) then measuring the packed cell volume; Hematocrit readings were performed with the aid of a microhematocrit reader. The hematological indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). The MCV was calculated as a quotient of hematocrit (Ht) and red blood cells (RBC). The value of MCV was expressed in femtolitres. The MCH expresses the average hemoglobin concentration in individual erythrocytes and is calculated as a quotient of Hb and RBC. The resultant value is given in picograms. The MCHC was calculated as a quotient of Hb and Ht and expressed in grams per deciliter. MCH, MCHC and MCV were calculated by the standard formulae of (Breatnach *et al*., 1981).

Serum biochemical parameters

The activity of glucose was determined by the method described Alkaline phosphatase (ALP) activity was assessed according to (Hillmann, 1971). The level of

glutamate–oxalacetate transaminase (GOT) and glutamate–pyruvate transaminase (GPT) were determined by (Finney, 1971) and lactate dehydrogenase (LDH) activity according to the method reported by Di Giulio & Hinton, (2008). Cholesterol level was determined by the method of (Folmar, 1993). Triglycerides were measured by the method of (Solis *et al*., 2007). Protein content was determined by the method of (Lowry *et al*., 1951). These parameters were measured with the help of Cintra 40 UV–visible spectrophotometer.

Nitroblue tetrazolium assay

The NBT assay was carried out following the protocol of (Anderson & Siwicki, 1995). Briefly, blood was collected from the heart of the fish and 0.1 ml of heparinized blood was taken in Eppendorf to which equal volume of 0.2% NBT (Sigma, St Louis, MO, USA) solution was added. The mixture was incubated for 30 min at 25°C. From the resultant suspension, 50 µl was taken, added to 1.0 ml N, N-dimethyl formamide (Qualigens, Mumbai, India) in a glass tube and centrifuged at 5,000 rpm for 5 min. The optical density (OD) of the supernatant was measured at 540 nm in the Systronics 117 model UV–VIS spectrophotometer.

Serum lysozyme assay

The lysozyme activity level was measured using the turbidimetric assay following the methods of (Sankaran & Gurnani, 1972) with partial modification using hen egg white lysozyme (Sigma) as standard. A solution of 20 mg of *Micrococcus lysodeikticus* suspension in 100 ml phosphate-citrate buffer (pH 5.8) was used. To a microplate, 15 µl of serum in 150 µl of the above suspension was mixed and initial OD was taken then incubated at 25°C for 1 h. The difference of 0.001 in Δ OD observed at 1 h is taken as the measure of enzyme activity.

Statistical analysis

All the data were analysed statistically at $P < 0.05$.To test their significance the *t* values were calculated between control and fungicide exposed group's value by Student's t-test.

RESULTS AND DISCUSSION

In the present study, the 96 h LC_{50} value (95% confidence limits) of fungicide to the fish *O. mossambicus* was found to be 30.53 mg l^{-1}. During the study period, behavioural changes such as profuse mucus production, erratic movement, wide open operculum, swimming in a slanting angle and jerking movement were observed in fungicide treated groups. There was significant difference ($P < 0.05$) in the survival percentage of fungicide exposed groups as compared to the control group (Figure 1). Fish are particularly sensitive to environmental contamination of water and therefore, pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes (Das & Mukherjee, 2003; Di Giulio & Hinton, 2008). Bioaccumulation of pesticides and heavy metals in tissues of aquatic animals (El Sayed *et al*., 2007) can be more harmful for human consumption. Pollution of the aquatic environments has become a more serious concern during the recent years. The loading of metals resulting from industrial and agricultural discharges into our environment creates water pollution problems due to their toxic effect on aquatic biota. Even though copper is an essential element serving many useful functions in fish, it is extremely toxic to fish when present at elevated levels (Solis *et al*., 2007). Tilapia (*Oreochromis mossambicus*) are among the easiest and most profitable fish candidates to aquafarm (Martinez & Cólus, 2002). The tropical climate as well as the adequate environmental conditions in tilapia aquaculture and fish farming has been an excellent factor for the constant abundance and economical value of the organism in the Asia. Hence, it is one of the most common fish in a aquaculture system due to its higher growth rate, marketability and price stability are considered the main factors for its worldwide distribution (Mayer *et al*., 2018). Effect of fungicide exposure on blood parameters of *O. mossambicus* like RBC, WBC, Hb, Hct, MCV, MCH, and MCHC are presented in Table 1. Effect of copper oxychloride exposure on blood parameters of *O. mossambicus* like RBC, WBC, Hb, Hct, MCV, MCH, MCHC, are presented in Table 1. Increased RBC, Hb, MCHC and level were significantly higher in fungicide exposed groups, whereas WBC, Hct, and MCV were significantly reduced in fungicide exposed groups as compared to the control group. The poisoning by pollutants through run-off water from agricultural fields has resulted in a series of toxicological and environmental problems (Pichaimani *et al*., 2017a; Tamizhazhagan & Pugazhendy, 2015). In toxicological assays, exposure of organisms to certain doses and periods at acute and sublethal concentrations provides better understanding of the dangerous levels of chemicals (Farrell, 2007). In the present investigation, the 96 h LC_{50} value of fungicide to the fish fingerlings *O. mossambicus was* found to be 30.53 mg L^{-1} indicating that fungicide is moderately toxic to the fish. The mortality of fish observed during LC_{50} determination may be due to inhibition of cholinesterase by the fungicide which may lead to respiratory paralysis, anoxia and finally death.

In toxicological research, biomarkers are widely used as early diagnostic tools of adverse effects caused by chemical aggression (Usha *et al*., 2017). Several studies have used haematology as a biomarker of pesticide exposure and also to monitor the interaction between a toxicant and biological system (Das & Mukherjee, 2003). Generally, a decrease in nonspecific immunity of the fish due to pesticide exposure leads to alterations in haematological parameters. Furthermore, the decrease in the haematological parameters might have resulted from disruptive action of the pesticides on the membranes and cell viability (Hillmann, 1971). Lysing or shrinkage of erythrocytes due to pesticide action on the erythropoietic

tissue may lead to a reduction in haemoglobin and haematocrit (Sankaran & Gurnani, 1972).The decrease in Hb and Hct value and RBC counts in both the treatments indicates a non-specific immunity of the fish to the insecticide furadan. In addition, the decrease in Hb and Hct value and RBC counts indicates defensive reaction against pesticide stress (Solis *et al*., 2007). WBCs act as an immune response of infections and chemical irritants in fish. The observed elevation of WBC count in treatments and recovery assessment indicates a protective mechanism against fungicide toxicity or the activation of immune system to manage the fish against the stress caused by copper oxychloride pointed out that an increase in antibody production may also lead to an elevation of WBC counts which helps in survival and recovery of fish exposed to pesticides.

Table 1. Effect of short of term exposure of copper oxychloride and recovery assessment of *C. aerea* on haematological parameters in *O. mossambicus.*

Parameters	Days	Control	Treatment
RBC ($x10^6$ cells/mm^3)	24	1.16±0.05	1.87±0.05*
	48	1.24±0.07	2.01±0.09**
	72	1.32±0.09	2.52±0.17
	96	1.19±0.10	2.06±0.22**
RA	96	1.31±0.27	3.32±0.76*
WBC (x 10^3 cells/mm^3)	24	180.60±1.16	145.26±5.24*
	48	210.10±2.72	138.60±4.27**
	72	245.29±3.14	120.12±3.28**
	96	210.29±4.17	112.60±3.94*
RA	96	295.12±3.22	232.67±2.04**
Hb (g/dl)	24	8.25±0.04	12.14±0.38
	48	8.36±0.05	13.16±0.15**
	72	8.40±0.01	12.45±0.26*
	96	8.31±0.11	10.37±0.37
RA	96	8.98±0.05	13.37±0.21*
PCV (%)	24	21.43±0.27	12.16±0.32**
	48	22.96±0.15	30.12±0.41*
	72	18.65±0.37	24.15±0.37*
	96	19.34±0.21	10.24±1.24*
RA	96	24.76±0.19	24.76±0.41**
MCV (fL)	24	184.74±1.19	65.02±2.47
	48	185.16±2.45	149.85±0.74*
	72	141.28±6.45	95.83±1.64**
	96	162.52±4.60	47.40±1.43*
RA	96	186.98±7.53	135.87±2.59*
MCH (pg)	24	71.12±0.10	64.91±1.24*
	48	67.41±1.02	65.47±2.35*
	72	63.63±0.98	49.40±0.92**
	96	69.83±0.19	48.00±0.95*
RA	96	72.78±0.05	69.65±0.34**
MCHC (g/dL)	24	38.49±1.10	99.83±0.95*
	48	36.41±2.72	43.69±1.89**
	72	45.04±3.47	51.55±2.79*
	96	42.96±1.42	101.26±0.53
RA	96	52.87±0.65	89.76±0.37*

Values are represented as (mean ± SE) of two replicates. Asterisks indicate values that are significantly different from control and copper oxychloride exposure for a period of 96 hours and recovery assessment of *C. aerea*. * $p < 0.05$. ** $p<0.01$, RA: Recovery assessment.

The observed increase in MCV and MCH in both the treatments indicates swelling of RBCs. Moreover, the release of large red blood cells into the circulation may also lead to an increase in MCV and MCH value (Wynne, 2011). However, the decrease in MCV and MCH value in Treatment I may be due to high percentage of immature RBCs in the circulation (Mayer *et al*., 2018). During recovery period Hb, Hct,WBC,MCV and MCH levelswere increased whereas RBC count was decreased when compared to control groups indicating impaired osmoregulation caused by the insecticide fungicide. The MCHC value was found to be more or less near to control groups. In contrast to the present study, a significant recovery of the haematological parameters was noted in fish *Labeo rohita* exposed to cypermethrin and carbofuran (Anderson & Siwicki, 1995; Tamizhazhagan *et al*., 2017) The authors also pointed out that the improvement in blood parameters of fish when transferred to pesticide-free freshwater indicates that pesticides entering into the system are slowly eliminated.

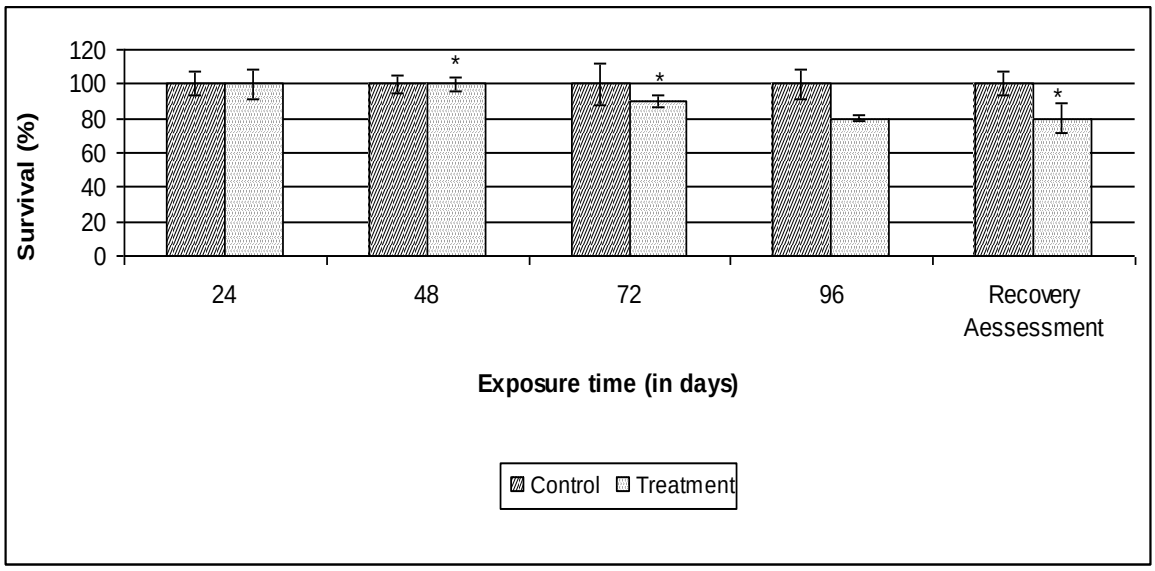

Figure 1. Changes in the survival in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

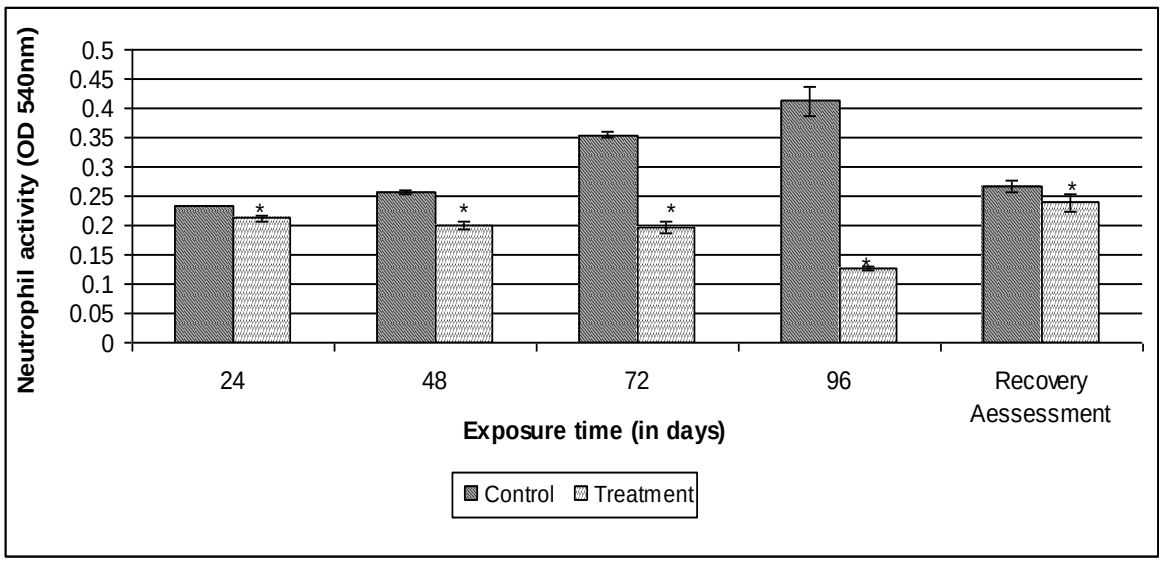

Figure 2. Changes in the neutrophil activity in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

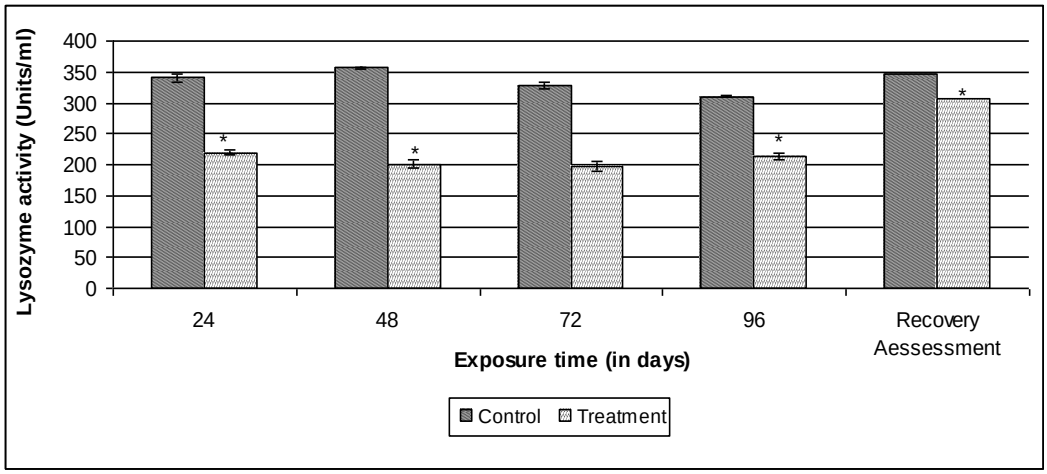

Figure 3. Changes in the lysozyme activity in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

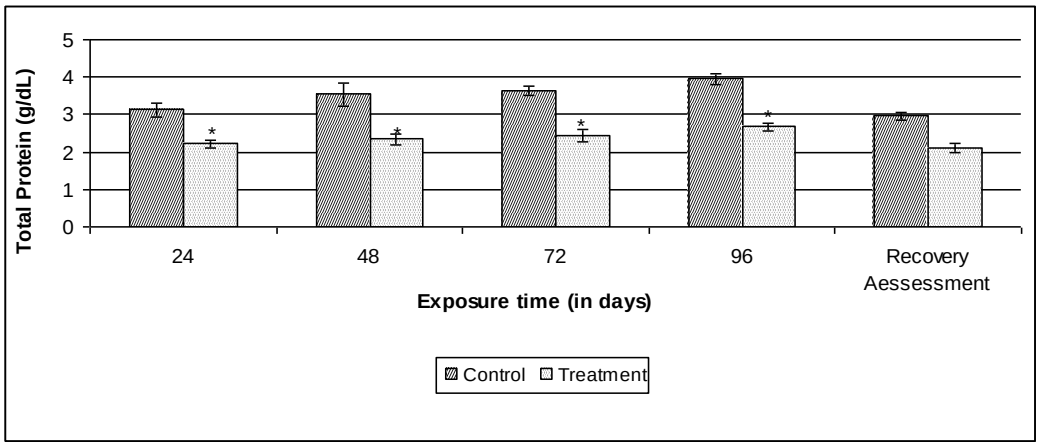

Figure 4. Changes in the total protein level in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

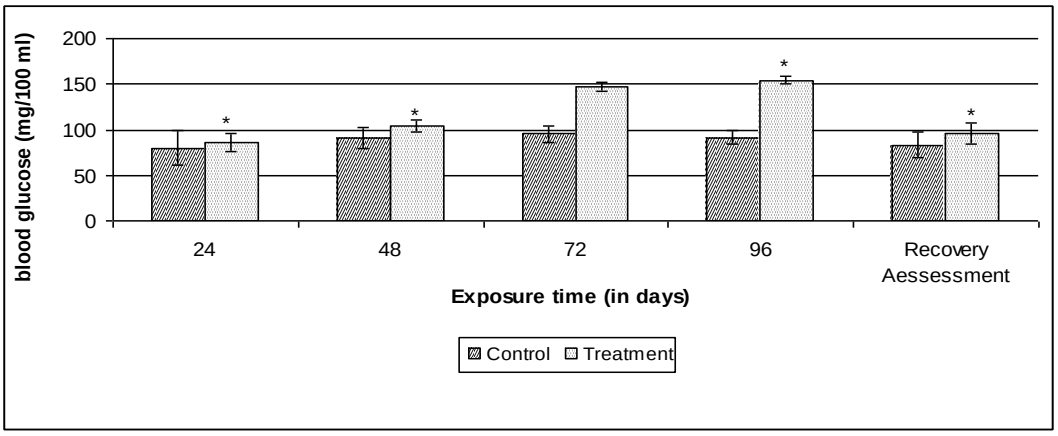

Figure 5. Changes in the blood glucose level in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

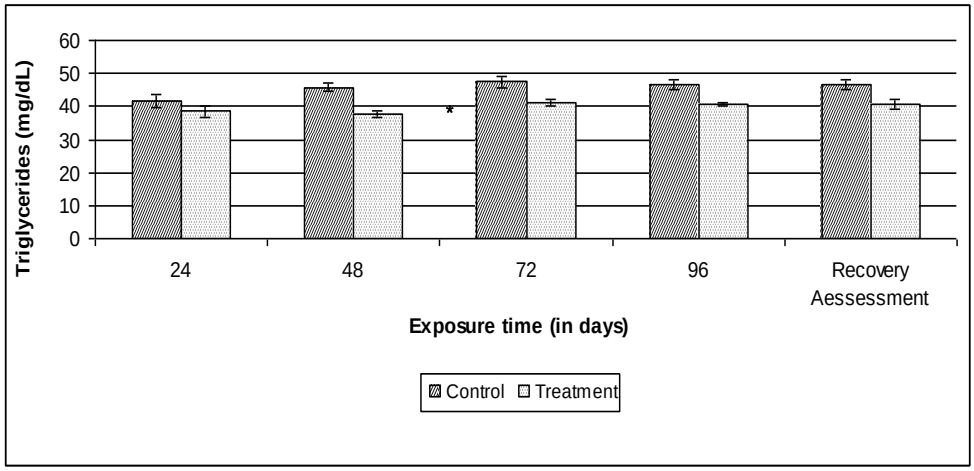

Figure 6. Changes in the triglycerides level in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

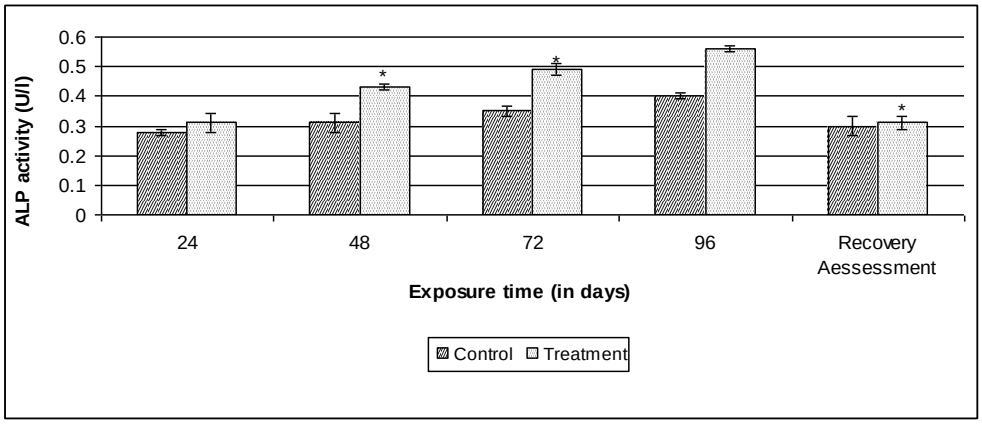

Figure 7. Changes in the ALP activity in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

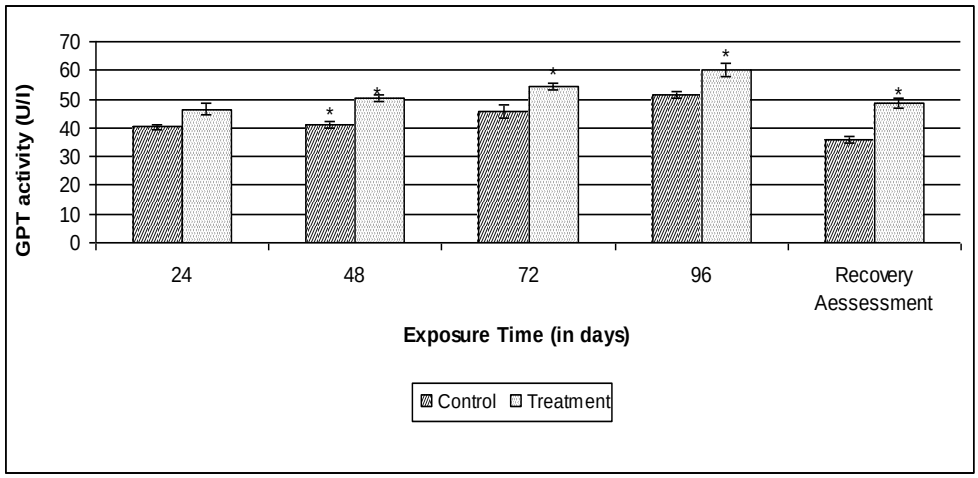

Figure 8. Changes in the GPT activity in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

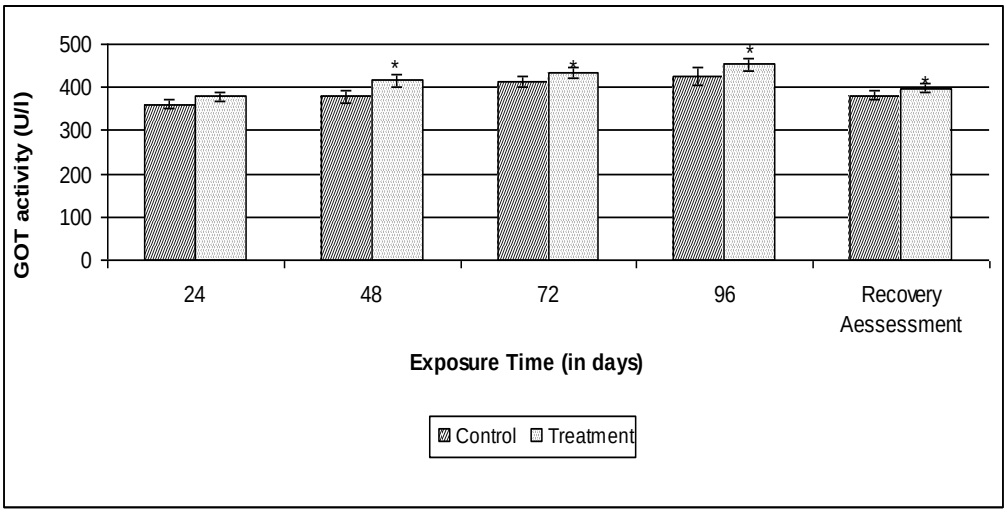

Figure 9. Changes in the GOT activity in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

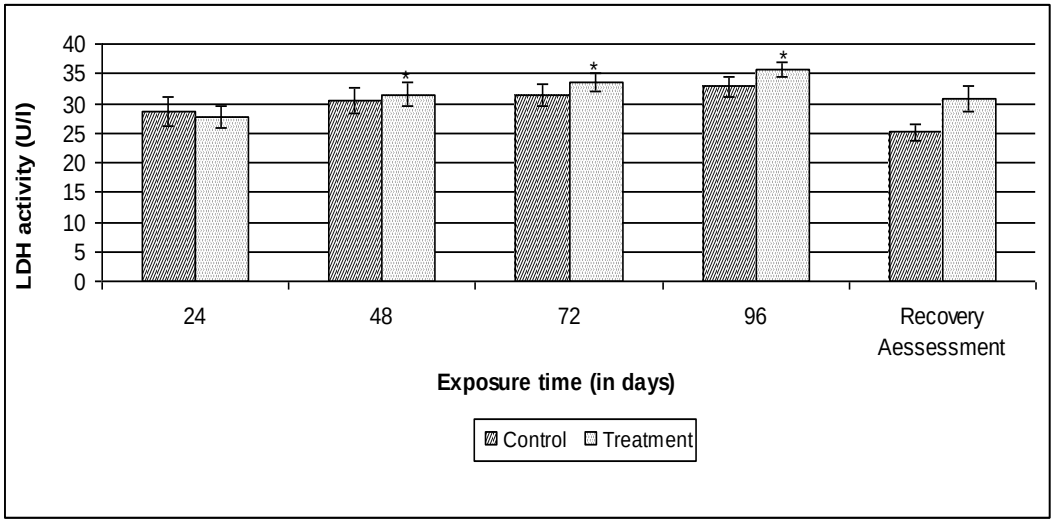

Figure 10. Changes in the LDH activity in the serum of *O. mossambicus* exposed to sublethal concentrations (30.53 mg l^{-1}) of oxychloride (fungicide) for 96 h and its recovery response after 96 h *C. aerea* in normal water. Data represent mean ± SE (n = 8). *Significant at 5% (based on t test).

The overall hamaetalogical parameters were significantly ($p<0.05$) increased in recovery assessment of *C. aerea* administered groups when compared to control. Effect of copper oxychloride exposure on blood parameters of *O. mossambicus* like Total protein, blood glucose, total glycerides, ALP, SGPT, SGOT, and LDH are presented in (Figure 4-10). The fungicide treated groups showed significantly ($p<0.05$) higher serum protein than the control group during the whole experiment groups; however, significantly lower ($p<0.05$) protein was recorded in *C. aerea* treated group at the end of experiment (Figure 4). There was no significant ($p>0.05$) effect on total glucose in fungicide treated groups. There was significantly lower ($p<0.05$) blood glucose level in *C. aerea* treated groups compared to control group during the whole experiment; there was no significant change in the blood glucose in the C2.0 and C.4.0 between 24 and 96 hrs (Figure 5). The fish in fungicide groups showed significantly higher ($p<0.05$) total triglycerides level than control group at the end of experiment (Figure 6). The ALP, GPT, GOT and LDH were significantly ($p<0.05$) increased when compared and control groups during the whole experiment and in contrast ALP, GPT, GOT and LDH were decreased on 96 h post recovery assessment. The NBT activity in all the experimental groups was significantly ($p < 0.05$) higher than the fungicide treated group, including *C. aerea* treated groups (Figure 2). The lysozyme activity was significantly ($p < 0.05$) lower than the fungicide treated groups, except *C. aerea* treated groups with compared to control (Figure 3). Enzymes such as GOT and GPT are considered as useful biomarkers to determine cellular impairment and cell rupture (Gimeno *et al*., 1995). In the present study the

decrease in GOT activity in gill, liver and kidney indicates disturbance in the structure and integrity of cell organelles. The alterations of these enzymatic parameters in organs/tissue during recovery period indicate that the recovery period (96 h) is not sufficient for the fish to restore the parameters at normal level. The moderate recovery of some haematological, biochemical and enzymological parameters during recovery period might have resulted from adaptation of fish to toxic stress. However, a longer recovery period was necessary to tolerate the stress and to maintain the normal physiology.

CONCLUSION

Results of the present investigation indicate that administration of sublethal concentration of fungicide is toxic to fish and caused alterations in the haematological, immunological and biochemical parameters of fish. The alterations of these parameters may provide an early warning signal for the determination of toxic level of pesticides and their effects in aquatic organisms. These alterations were reversed to a great extent with exposure of optimal dose of *C. aerea*. We recommended that cultured fish will be protected against any possible pesticide or fungicide stress using natural antioxidants such as *C. aerea* by through water.

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