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Research Article

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Probiotic Effect on Zinc Sulphate Induced Haematological Toxicity in the Fish *Mystus montanus*

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ABSTRACT

Zinc is introduced into aquatic systems through an industrial process, such as smelting and the use of fertilizers in agriculture. At higher concentrations, Zn disrupts physiological and biochemical mechanisms causing both ionoregulatory disturbance and oxidative damage in fish. The present study was designed to evaluate the haematological parameters of the fish Mystus montanus intoxicated with $ZnSo_4$ and to analyse the protective effect of probiotic bacteria Lactobacillus rhamnosus on the fish for 60 days. Two sub-lethal concentrations of zinc (4.56 mg $ZnSo_4$/L (1/10th of LC_{50}) and 2.28 mg $ZnSo_4$/L (1/5th of LC_{50}) were prepared after determining the LC_{50} value (45.6 mg/L) and used for the toxicity study. All the haematological parameters were significantly decreased ($p<0.05$) from the control in the T3 (1/5th LC_{50} dose of $ZnSo_4$ and Basal diet) group of fish. However, the fish fed diet supplemented with probiotic bacteria in T4 (1/5th LC_{50} dose of $ZnSo_4$ and Probiotic diet) group showed a significant increase ($p<0.05$) from the antidote control group T3. This study suggests that dietary supplementation of probiotic bacteria can cope up with zinc pollutants in the aquatic medium.

Keywords: *Zinc sulphate, Mystus montanus, Lactobacillus rhamnosus, Haematology.*

INTRODUCTION

Surface water is a recipient of wastes resulting from the industrial and agricultural field which enter the nearby water bodies via runoff after heavy precipitation [1]. Increased discharge of heavy metals into natural aquatic ecosystems can expose aquatic organisms to unnaturally high levels of these metals [2]. Among aquatic organisms, fish cannot escape from the detrimental effects of these pollutants and are therefore generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems [3].

Patil and Hande [4] conducted a study on the toxic effect of Zn chloride on brain acetylcholinesterase of a marine teleost, *Arius nenga* and he was observed that Zn exerted the inhibitory effect on cytoplasmic and membrane-bound fractions of AchE. Acute zinc poisoning in fish is generally attributed to blockade of gas exchange across the gills, causing hypoxia at the tissue level.

Probiotics are commonly defined as mono or mixed cultures of live microbes that when applied to animals or humans, generate a beneficial effect on the health of the host. These beneficial effects include disease treatment and prevention as well as the improvement of digestion and absorption in the host [5]. The total erythrocyte count [6], total leucocyte count fed with *Saccharomyces cerviase* [7], haemoglobin, mean corpuscular volume, haemoglobin concentration and mean corpuscular haemoglobin concentrations, total protein, albumin, globulin, albumin-globulin ratio, alkaline phosphatase activity, alanine and aspartate aminotransferase activities, creatinine, sodium, cortisol, insulin and glucose were reported to increase in *Labeo rohita* provided with *Bacillus subtilis* as probiotics [8].

The specific objectives of this research were to determine the LC_{50} value of $ZnSO_4$ in *Mystus montanus* at different concentrations, to find out the effect of two sublethal concentrations on the hematological parameters of the fish and to observe the effect of Probiotic bacteria *Lactobacillus rhamnosus* on the zinc intoxicated fish.

MATERIALS AND METHODS

Experimental animal (Mystus montanus)

The present study was carried out in the Department of Zoology, A.P.C.Mahalaxmi College for Women, Thoothukudi. Healthy fishes of *Mystus montanus* with 8.9 ± 0.507 cm in length and weighing 3.74 ± 0.292 g were captured in Perunkulam pond, near Eral in Thoothukudi

District. They were acclimatized for 15 days in the laboratory conditions before starting the experiment. The physicochemical properties of the holding water during acclimation were determined by standard APHA methods [9].

Toxicant

Stock solution was prepared by using $ZnSo_4$. $7H_2O$ by dissolving 4.56 g in one litre of distilled water to get 1 ppt Zinc sulphate solution. From this stock solution various concentrations of Zinc solution were prepared and used for LC_{50} analysis and toxicity studies.

Determination of LC_{50} value

To find out the LC_{50} for $ZnSo_4$ a series of concentrations ranging from 20 mg/L to 80 mg/L were prepared separately from the stock toxicant. Well acclimatized 10 fishes of uniform size were selected and introduced into each toxicant concentration for 96 hours. Every day the fresh concentrations were prepared from the stock, to make the concentrations constant throughout the experimental study [10]. The experiment was repeated twice for various test concentrations and satisfactory reproducibility in the results was noted.

Probit/Log regression analysis

Probit analysis of log dose against response (mortality) was performed by adopting the standard protocol given by Finney [11]. The regression equation was calculated and 96 hrs LC_{50} values were derived from the equation. The LC_{50} value of Zinc sulphate to the fish for 96 hrs is 45.6 mg/l. Two experimental concentrations 4.56 mg $ZnSo_4$/L ($1/10^{th}$ of LC_{50}) and 2.28 mg $ZnSo_4$/L ($1/5^{th}$ of LC_{50}) were used for the present study.

Experimental design for $ZnSo_4$ toxicity analysis

The experimental design for the present experiment is shown in Table 1. Well acclimatized, healthy fishes of *Mystus montanus* were reared in 25 L capacity glass tanks. The ingredients used to prepare the feed is listed in Table 2.

Table 1: Study design.

Fish group	No. of fish	Treatment	Duration	Feeding %
Control	10	Basal diet	60 days	5% body mass
T1	10	$1/10^{th}$ LC_{50} dose of $ZnSo_4$ and Basal diet	60 days	5% body mass
T2	10	$1/10^{th}$ LC_{50} dose of $ZnSo_4$ and Probiotic diet	60 days	5% body mass
T3	10	$1/5^{th}$ LC_{50} dose of $ZnSo_4$ and Basal diet	60 days	5% body mass
T4	10	$1/5^{th}$ LC_{50} dose of $ZnSo_4$ and Probiotic diet	60 days	5% body mass

Table 2: Ingredients used for feed preparation.

S. No	Ingredients	Purpose of inclusion	Inclusion level (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
1	Fish meal	animal protein	16	62	5.8	3.7
2	Groundnut oil cake	plant protein	16	45.6	40.9	8.7
3	Soya flour	plant protein	16	71.6	10.7	9.8
4	Rice bran	carbohydrate	18	13.5	1.8	75.5
5	Tapioca flour	binder	18	5.8	12.5	76.3
6	Vitamins and minerals mix	vitamins and minerals	0.5	-	-	-
7	PrePro KID	*Lactobacillus rhamnosus*	106 CFU/g	-	-	-

Haematological procedures

Blood was collected through the caudal vein of the fish by hanging the fish in an upright position using a 2 ml sterile plastic syringe to prevent quick coagulation. All Haematological parameters were measured using SYSMAX XP 100 Auto Analyzer in the Laboratory of DCW Ltd, Sahupuram.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one-way Analysis of Variance (ANOVA), followed by multiple range tests using sigma plot for windows, version 14.0; Build 14.0.0.14. The experimental group of fish T1 and T2 were compared with the control. T2 was compared with its antidote control T1 and T4 was compared with its antidote control T3.

RESULTS

The LC50 value of Zinc sulphate to the fish *Mystus montanus* for 96 hrs is 45.6 mg/l. During the toxicant exposure period, the test fishes showed various behavioral responses like increased opercular movement, mucous secretion, and jerky movement, floating on the sides, hypersensitivity showing violent erratic and fast swimming. Clotting of blood on the gill surfaces was also observed from the dead fishes.

The knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological changes in the fishes [12]. The haematological parameters of the Zinc intoxicated fish and fish fed with probiotic diet are shown in Table 3.

Significant decrease of hematological parameters such as Hemoglobin, Packed Cell Volume, Red Blood Corpuscle (p<0.05), and ESR (p<0.001) were observed in the zinc intoxicated fish in T3 from the control group. However, all these parameters of the fish treated with probiotic diet in T4 showed significant improvement (p<0.05) of PCV, RBC and Hb and ESR (p<0.001) from the antidote control group of fish T3. The application of combined dosage of probiotics *L. sporogenes, L. acidophilus, B. subtilis, B. licheniformis, Saccharomyces cervisiae* in *Cirrihinus mrigal* [13] *B. Licheniforms* and *B. Subtilis* in *Rutilis frisii* kutum [14] *Bacillus cereus* in juvenile *Nile tilapia* [15], have been reported to increase the Haemoglobin levels. In these reports fishes fed probiotic-supplemented diets were indicated to have better health status compared to those fed control diets. These evidence support the fact that the probiotic bacterial growth in the gut prevents the absorption of Zinc sulphate in the gut, thus protecting the blood parameters.

Table 3: Haematological parameters.

Parameters	Control	T1 (1/10th of LC_{50})	T2 (1/10th of LC_{50} Zn and Probiotics)	T3 (1/5th of LC_{50})	T4 (1/5th of LC_{50} Zn and Probiotics)
Hb (g/dl)	8.45 ± 0.43	5.34 ± 0.72[b]	6.14 ± 0.32[c]	4.66 ± 0.12[a]	5.66 ± 0.14[b]
PCV (%)	13.22 ± 1.86	10.70 ± 2.1[a]	11.10 ± 1.1[a]	7.50 ± 1.18[a]	9.50 ± 1.48[a]
RBC × 10^6/mm^3	3.86 ± 0.17	2.20 ± 0.15[a]	3.20 ± 0.45[a]	1.95 ± 0.32[a]	2.95 ± 0.12[a]
ESR (mm)	1.15 ± 0.26	1.65 ± 0.35[a]	1.25 ± 0.65[a]	1.87 ± 0.20[b]	1.37 ± 0.40[b]
MCV (fl)	8.53 ± 0.61	6.53 ± 0.31[b]	7.74 ± 0.95[a]	5.74 ± 0.75[b]	6.86 ± 0.16[b]
MCH (pg)	30.56 ± 3.13	27.56 ± 1.43[a]	29.30 ± 2.28[a]	23.28 ± 2.68[a]	26.50 ± 1.48[a]
MCHC (%)	28.48 ± 2.32	23.48 ± 2.72[a]	26.57 ± 2.06[a]	22.67 ± 3.01[a]	21.95 ± 0.14[a]
HET (%)	43.00 ± 4.56	32.00 ± 3.32[a]	34.00 ± 2.32[a]	23.00 ± 2.54[b]	33.00 ± 1.54[b]
MON (%)	13 ± 3.67	10.45 ± 2.21[a]	11.45 ± 1.21[a]	7.87 ± 3.24[a]	9.87 ± 32.24[a]
EOS (%)	11.57 ± 2.23	9.43 ± 1.77[b]	10.43 ± 00.77[b]	8.60 ± 1.71[b]	8.20 ± 0.71[b]
BAS (%)	3.45 ± 1.76	2.70 ± 1.25[a]	3.10 ± .25[a]	1.63 ± 0.55[a]	2.23 ± 0.67[a]
LYM (%)	30.46 ± 2.40	25.70 ± 2.60[a]	27.70 ± 1.60[a]	25.36 ± 3.10[a]	28.36 ± 2.10[a]

Where Hb: Haemoglobin; PCV: Packed Cell Volume; RBC: Red Blood Cells; WBC: White Blood Cells; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; HET: Heterophil; MON: Monocyte; EOS: Eosinophil; BAS: Basophil; and LYM: Lymphocyte
In the table, a is Significant at 5%, b is Significant at 1% and c is Insignificant

The hematological indices such as MCH, MCHC (p<0.05), and MCV (p<0.001) decreased in the T3 fish groups from the control group of fish. In T4 group the same hematological indices (MCH, MCHC (p<0.05) and MCV (p<0.001) significantly increased from its antidote control T3. In the research reports, the application of a mixed probiotic species of *Lactococcus rhamnosus* and *Lactococcus lactis* in red seabream [16], *Bacillus sp.* in *Nile tilapia* [17] and *L. rhamnosus* on rainbow trout [18] have been reported to increase the Hct levels. It could be attributed to the fact that the probiotics increased the blood parameter from the antidote control values as a result of hematopoietic stimulation.

The leukocytes such as HET, BAS, MON, LYM (p<0.05), and EOS (p<0.001) decreased significantly in the T1 and T3 groups of fish. All these counts (HET, BAS, MON, LYM (p<0.05) and EOS (p<0.001) of the probiotic fed fish increased significantly in the T2 and T4 groups of fish. The present study confirms the role of probiotic bacteria *Lactobacillus rhamnosuson* for the improvement of differential leukocyte counts in the Zinc toxicated fish. The application of a mixed probiotic species of *B. subtilis* and *S. cerevisiae* in *C. mrigala* [19], *L. acidophilus* and *B. subtilis* in *Nile tilapia* [20] have been reported to increase the WBC levels. Munir et al. [21] reported that feeding fish with probiotic-supplemented diets enhanced immune defense This study confirms that *Lactobacillus rhamnosus* in the gut competitively excludes other bacteria and dominates probiotic bacteria in the gut and protect the fish from the toxic effect of Zinc sulphate.

CONCLUSION

The heavy metals and other pollutants present in the aquatic bodies affect the physio-chemical properties of the water. During drought periods when water in wetland areas is polluted the living ecosystem of the pond is imbalanced and mosquito populations thrive well. If the fish population in the pond is immunologically strengthened by administering probiotic bacteria it can able to withstand the extreme conditions and balance the ecosystem.

DECLARATION OF COMPETING INTEREST

There is no conflict of interest.

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