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THE HAEMATOLOGICAL STUDY OF ZEBRAFISH *DANIO RERIO* ON THE EXPOSURE OF TRICLOSAN, WITH GARLIC EXTRACT AND VITAMIN C

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ABSTRACT The zebra fish (*Danio rerio*) has proven an excellent model for the study of vertebrate development and genetics. Mutagenesis studies have produced many blood mutants with defects ranging from haematopoisis to coagulation. The over helming majority of zebra fish studies have focused on development and mutation effects in embryos, whereas effects in mature zebra fish have gone largely unexplored. Zebra fish will prove a valuable model for studying of ageing and age related diseases and we have sought to characterize some of the basic features of mature zebra fish. Accordingly, blood was collected from adult zebra fish and was analyzed to determine the hematological parameters. Hematological parameters are important for toxicological research environmental monitoring and as indicators of disease and environmental stress). Blood is known to exhibit pathological changes before the onset of any external symptoms of toxicity Zebrafish have contributed to hematological research for more than 100 years. Interest in zebrafish embryology and they have long used for toxicology and other zoological research. We believe results such as these will help define normal adult zebra fish, which have a tremendous potential for use in the study of human disease, hematological disorders and aging.

KEYWORDS : Zebrafish, haematopoisis, toxicological, triclosan

INTRODUCTION

The hematologic values of zebra fish have demonstrated that, their suitability are proving the utility and have been supplied timely and novel discoveries and are poised for further significant contributions. Zebra fish have contributed to hematological research for more than 100 years. Interest in zebra fish embryology dates to the 1930s (Roosen- Runge.E., 1987) and they have long used for toxicology, and zoological research. The first description of zebra fish blood cell morphology appeared in the1970s (Rieb. J. P.la., 1973: Al- Adhami M.A., Kunz Y.W., 1979). As a model is that the species is readily amenable to large scale mutagenesis studies. Allowing the offspring one unique aspect of zebra fish among currently used vertebrate animals of mutagenized zebra fish to reach maturity could lead to the identification of phenotypes that model various human diseases and subsequently discovery of the genes responsible for these diseases. For example a mutant zebra fish line, Sauternes (sau) has been reported to model congenital sideroblastic anemia (CSA) in human and represent the first animal model of this disease (Al- Adhami M.A., Kunz Y.W., 1979; Brownie *et al*., 2000). To fully explode the potential of these unique animal models we have sought to characterize some of the basic features of mature zebra fish by examining hematological parameters. The values of hematological parameters depend on seasons and slow or active movement of fishes. (Wang *et al*., 2007; Haffter *et al.,* 1996; Driever *et al*., 1996) reported that the hematological parameters are influenced by microbial infection of fish and toxicants. Though numerous works are available on hematology of fishes the present study deals with the important of blood parameters of zebra fish (*Danio rerio*). The modern phase of zebra fish hematology research driven by genetic experimental approaches, started just over 10 years ages with the collection of zebra fish mutants with hematopoitic defects mostly recognized for anemia (Stainer *et al.*, 1996 ; Weinstein et al., 1996; Ransom *et al*., 1996).

MATERIALS AND METHODS

Mature zebra fish (approx one years old) were observed from scientific haturies. All fish were healthy and free from of signs of diseases. Our zebra fish supplier screens their facility for *capillaria*, and *mycobacterium spp, flavobacterium columnaue*, external skin and gill parasites (*Gyrodactyles, Oodinium,Ihcthyphthirius and Tricodirnaa*) *Aeromonas spp* and *Microspirida* and it has been free of these disease causing organisms. Fishes were housed in 10 gallon aquaria containing conditioned tap water at 28ºc in groups of approximately 25 animals / tanks, resulting in a starting density of 2.5 fish/ gal. The water was conditioned by mixing tap water (adjustable to 28ºc) water detoxifies that remove ammonia, chlorine and chloramines at a ratio of 1tsp Am Quel /10 gal of tap water. Water quality was assessed weekly by measuring P_H, ammonia, nitrite and nitrate values using aquarium pharmaceuticals, water test kids. Water P_H was maintained between 6.8 to 7.4. The light dark cycle was maintained at 14:10 fish were fed a 50:50 mixture of commercial flake food and freeze dried brine shrimp twice daily and fresh brain shrimp once daily.

Freshly peeled cloves of garlic (*Allium sativum*, purchased from local market) were sliced into small pieces and ground in a clean mortar using a mortar pestle to produce a fine paste. The working solution was then prepared by dissolving 5 g of the paste in 100 ml of distilled water, where 1 ml of the extract contains 50 mg of crude garlic. Fresh garlic extract was dissolved in the aquarium tank daily. A pure form of L-ascorbic acid was supplied as pure crystals by I.L.E.Co., Kattangulathur, Chennai. A freshly prepared aqueous solution of L-ascorbic acid (1g) was dissolved in aquarium tank containing 10 liters of water daily throughout the experiments.Technical grade triclosan $C_{12}H_7Cl_3O_2$ [5-chloro-2-(2, 4-dichlorophenoxy) phenol] was purchased from (The I.L.E.co., Chennai. India). The LC_{50} value of triclosan was determined in the laboratory. Three hundred fishes were randomly distributed into six aquarium tanks (100 L) filled with different concentration of triclosan (0. 20, 0.22, 0.24, 0.26, 0.28, 0.30 and 0.32 mg/L). The mortality was recorded for 96h. The LC_{50} of triclosan calculated with the help of probit analysis using SPSS software. The 96h concentration (0.32mg/L) of calculated LC_{50} value was selected.

Each fish was quickly euthanized by immersion in MS-222 (3g in 1000 ml ice water) blood was immediately collected from the dorsal fin Blood welling up from this incision was rapidly collected by use of a micro pipette. Blood yields from individual fish ranged from 1 to 10µl. smears were immediately prepared from fresh whole blood. For total erythrocyte counts the micro pipette tip was coated with EDTA prior to blood collection and whole blood from groups of five fishes was pooled in an EDTA coated with microtubes for serum acquisition, whole blood from groups of 10 zebra fish was pooled in a 0.5 µl micro centrifuge tube allowed to clot, and span for 10 minutes at 2500 rpm then the serum pipette off the top. The heparinized blood was carefully pipette into the RBC pipette till 0.5 mark without air bubble and immediately RBC diluting fluid was pipette up to 101 mark. The blood and diluting fluid were mixed thoroughly by gently rolling the pipette horizontally. The diluted blood was carefully layered on a neubauer chamber and the diluted blood was spread over the chamber by placing a cover slip. The cells were allowed to settle for 2-3 min and counted using light microscope.

RESULT

The hematologic values of zebra fish have demonstrated that, their suitability are proving the utility and have been supplied timely and novel discoveries and are poised for further significant contributions. As a model is that the species is readily amenable to large scale

mutagenesis studies. These studies have generally been used as effective and sensitive indicators to check physiological and pathological changes in fishes. The hematological parameters like RBC, WBC) were analyzed for 7 and 28 days exposure of triclosan. I was observed that the triclosan is moderately toxic to zebrafish (Braneydaniom rerio) and its 96h LC_{50} was recorded as 0.32 mg/ L. The supplementary feed of garlic extract (1ml/ L) and vitamin C (1g/ L). The red blood cell count white blood cell cdount, are significantly decresed in ($p<0.05$) in triclosan exposure in zebrafish than the control. heamotological parameters were significantly ($p<0.05$) increased in exposure of triclosan with Garlic extract (1ml /L) and vitamin C (1g/L) then the triclosan exposure and the control groups. Those parameters were significantly increased in Garlic extract (1ml/ L) and Vitamin C (1g/L) alone.

Fig:1 RBC levels (10^6/mm^3) in the Blood of Zebrafish exposed to Triclosan Supplementary feed of Garlic extract and Vitamin C for 7 and 28 days

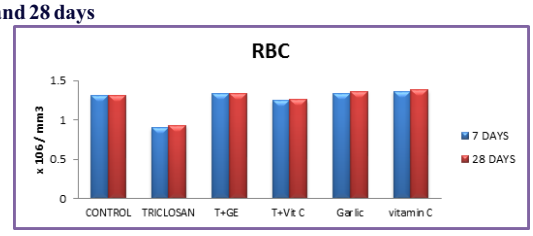
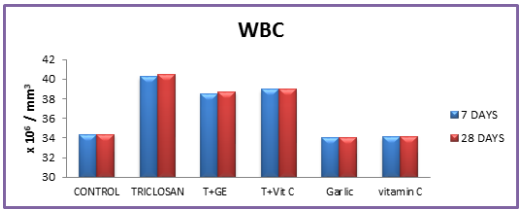


Fig:2 WBC levels (10^3/mm^3) in the Blood of Zebrafish exposed to Triclosan Supplementary feed of Garlic extract and Vitamin C for 7 and 28 days



DISCUSSION

We believe these values will be useful in future studies that examine various disease models in screens of mutagenic zebra fish lines. In the study reported that we were determined hematological parameters in zebra fish (*Danio rerio*). The Hematological parameters like RBC and WBC were easily observed (countable) and good indicator of any stress situation (Banerjee 1979; Basha Mohindeen and Sailabaala 1992 ; Sampash 1993). In fishes just like other vertebrates the hemoglobin content of whole fish blood varies with the number of RBC present thus RBC account for 99% of O_2 uptake (Lagler *et al*., 1977). In recent years there has been a tremendous increase in the number of zebra fish with genetic mutations. There is great potential for the use of these mutants as model of human diseases. Several anemic of mutant zebra fish with phenotypes that resemble human disorders have been described. These mutants have altered erythrocytes indices, compared with those of wild type zebra fish. For example zebra fish mutant sauternes (sau) has a microcytic, hypochromic anemia due to a mutation in the gene coding for the enzyme ∂- aminolevulinate synthase (ALAS 2) (Brownlie *et al*., 1996). Mutation in ALAS-2 is known to cause CSA in humans and the sau-mutant zebra fish represents the first animal model of this disease. Positional cloning also revealed has the gene responsible for the hypochromic anemia of the zebra fish of this gene ferroportin I, may be perturbed in mammalian disorder of iron deficiency or overload. In addition to hematological mutants such as these zebra fish also have been documented as potential models for the study of other human disease, such as haepato erythropoietic porphyria (HEP) (Wang *et al*., 1998). Huntingtons disease (Karlvich *et al*., 1998), Alzimers disease (Leimer *et al*., 1999) multiple endocrine neoplasia type I (MENI I) (Khodaei et al., 1999, Manicham *et al*., 2000) and congenital coarctation of the aorta (Towbin *et al*., 1995) to name a few. The field of transgenesis has also begun to emerge in the zebra fish community (Jowelt, T. 1999 ; Lin.S,. 2000). Although haematologic data have been reported for several species of fish the result vary considerably within and between species. The value reported here for the zebra fish are within the range reported here for the mammalion species and fish (Hrubee, T.C and Smith .S.A., 2000; Hrapkiewiz et al.,1998 ; Stoskopf.M.K., 1993; Eastman Kodak company 1993). Accurate analysis of many zebra fish mutant generated requires determination of the normal characteristics of zebra fish. We have described some of the basic hematological of mature zebra fish, which have a tremendous potential for use in the study of human disease and aging.

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