Effects of Mercuric Chloride on the Immunological, Hematological, Biochemical Parameters and Diseases Resistance of Nile Tilapia Challenged with *Aeromnas hydrophila*

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Abstract: Mercuric and its compounds have been parts of widespread pollutant of water environment. In view of the possible hazards of mercuric salts, the present study was designed to assess the effect of mercuric on immunomodulation system, as well as hematological and serum biochemical changes, in Nile tilapia (*Oreochromis niloticus*). The experimental fish were randomly divided into eight equal groups, each containing 80 fish. The first group (Gp.1) acted as a control. Gps.(2-4) were subjected throughout the experiment , 21 days to sublethal concentration of mercuric chloride 0.01, 0.05 and 0.1 ppm. Throughout the experiment, daily water mercuric concentration was estimated. At the end of experiment the non-specific defense mechanisms, cellular and humoral immunity, beside the total and differential leukocytic count were determined. Some selective biochemical parameters were estmiated (ALT, AST, creatinine, urea, uric acid, total protein, albumine and glucose). Also fish were challenged with *A. hydrophila* (0.4 X 10^8 cells ml^{-1}) via intra-peritoneal injection and the mortality rate was recorded up to 10 day post-challenge. Lymphocyte transformation index, phagocytic activity percent, phagocytic index, total lymphocyte count, serum bactericidal activity and nitric oxide were significantly decreased after 21 day in all mercuric treatment groups when compared with control. Normocytic normochromic anemia and significant decreases of total plasma protein and albumin in dose depended manner in mercuric chloride exposed group. On the other hand, the mortality rate, total leukocytes and neutrophils count, liver transaminase enzymes, crearinine and uric acid, were significantly increased. Serum lysozyme and neutrophils adhesion cells were significantly decrease in higher dose exposed group (Gp.4) when compared with control group. It could be concluded that from this study the water born mercury pollution is highly toxic to Nile tilapia (*Oreochromis niloticus*) as well as has immune suppression and subsequently decrease diseases resistance in fresh water fishes.
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Introduction

Dissolved metals occur naturally in minute amounts in the aquatic environment; however, through industry they may be transported, concentrated, changed into other forms and are reintroduced into the aquatic system as contaminations. Consequently, fishes in contaminated areas are often exposed too much higher concentrations or to chemical forms different than those that are normally in environment (**Pandey et al., 2005** & **Patrick et al., 1993**).

Mercury (Hg) is a naturally occurring metal due to erosion from earth crusts and volcanoes, but anthropogenic sources have increased the exposure in recent time. The most common forms of Hg in the environment are elemental Hg (Hg0), inorganic Hg (Hg+ and Hg2+), and organic compounds such as methyl mercury (MeHg), while ethyl mercury (EtHg) is more uncommon (**Clarkson, 2002).** Mercuric is black list element by environmentalists and is released into the environment by several sources mining, sewage disposal research laboratories, agriculture, fungicides and industrial operation where mercury is found in electrical equipment, paints, and disinfects **(Khangrot, 2003 & Dieter et al., 1992).**

Mercury has no biological function, potentially toxic and causes serious impairment in the metabolic and physiological function of the body. The toxic effects of mercuric in fish has been documented and this effects include, immunotoxicological effects which is likely to suppress fish immuncompetence **(Pandey et al., 2005 and Bly et al., 1997).**

The toxicity of fish by mercuric pollution at different lakes in Egypt at the level of cyto-genetic mechanism was described by **Siegel et al., (1994), Adel (2003)** & **Ahmed et al., (2008**). Some studies were done on the immunosuppressive effects of mercuric chloride on the non-specific and specific immune system of fishes, (*Oncorhynchus mykiss, Trichogaster trichopterus* and *Salvelinus namaycush*) **Isabelle et al., (1994) , Low and Sin (1998) and Miller et al., (2002)** respectively.

The aims of the present study were to asses immunotoxicological effect of mercuric chloride on immune response, beside investigation the alteration

on some selective hematological and biochemical parameters as well as diseases resistance against *Aeromnous hyydrophila* in Nile tilapia (*Oreochromis niloticus*).

2. Materials and Methods

2.1Fish

Nile tilapia (*Oreochromis niloticus*) fish weighing 100-120 gm were obtained from local, fish farm, Sharkia, Egypt. Fish were acclimated in fiberglass tank for two weeks filled with dechlorinated tap-water supplied with continuous aeration (pH 7.2 & total hardness 0.98 mM) and with a 12 h dark/12 h light photoperiod. The fish were randomly stocked at a rate of 10 fish per 120-L aquarium. The temperature was kept at 25- 27 ^{o}C throughout the experiment. Fecal matters were siphoned out once daily. The biomass of fish in each aquarium was measured at the beginning of experiment and after each sampling; thereby the daily ration was adjusted. Fish were fed twice daily with standard commercially prepared pellets at 2% of the body weight allover the experiment of period.

2.2. Preparation of mercuric chloride stock solution:

Stock solution has Cppm (concentration in part per million) which is prepared from standard mercuric chloride according to Nambiar et al., (1998). Mercuric was determined daily allover the experiment period according to APHA (1985) method using Shimadzu atomic absorption/flame spectrometer model (AA-630–01).The lower limit of detection for Hg using this method was 0.001 ppm.

2.3. Pathogen

Aeromonas hydrophila was previously isolated from naturally infected (*Oreochromis niloticus*) fish, identified according to standard bacteriological tests, it was cultured in nutrient broth (Oxoid) for 24 h at 37 0C. The broth culture was centrifuged at 3000 g for10 min. The supernatants were discarded and the pellets were re-suspended in phosphate-buffered saline (PBS 7.4), and the OD of the solution was adjusted to 0.5 at 456 nm, which corresponded to 1 X10^{7} cells/ ml (Sahu et al., 2007). This bacterial suspension was serially diluted using standard dilution technique with PBS and used for the challenge experiment and bactericidal activity.

2.4. Experimental Design

The experimental fish were randomly divided into eight equal groups, each containing 80 fish. The first group gp. (1) acted as a control. Gps.(2-4) were subjected throughout the experiment , 21 days to sublethal concentration of mercuric chloride 0.01, 0.05 and 0.1 ppm. Through out the experiment, daily water mercuric concentration was estimated. At the end of experiment challenge infection with *A. hydrophila* was performed for all groups.

2.5. Sample Collection

Thirty (*Oreochromis niloticus*) fish from each group (10 fish/ replicate) were randomly sampled from each group on 21st days of mercuric exposure. Blood samples were collected from the caudal vein of fish. Heparinized blood sample were collected to study the non-specific defense mechanism (neutrophils adhesion test, lymphocyte transformation test, total and differential leukocytes count). The remaining blood was centrifuged at 3000 rpm for 5 minutes for serum preparation to be used for serum lysozyme assay, nitric oxide and bactericidal activity.

2.6 Immunological Studies

2.6.1 Cellular immunological studies

2.6.1.1Neutrophils glass- adhesion

Neutrophils glass- adherent, using nitroblue tetrazolium assay, was determined according to **Anderson et al., (1992**). Briefly, within 15 minutes after blood samples were collected, one drop of blood using heparinized capillary hematocrit tubes was placed onto a 22-mm square cover-slip. The cover-slips were placed individually in Petri-dishes humid chambers and incubated for 30 minutes in room temperature (25^{o}C) to allow the neutrophils to stick to the glass. After incubation, the cover-slips were gently washed with PBS (pH 7.4) and the cells were transferred upside down to a microscope slide containing a 50 µl drop of 0.2% filtrated NBT solution. After other 30 minutes of incubation, the positive, dark-blue stained cells were counted under the microscope. Two cover-slips were examined for each fish. Three random fields were counted on each slide. The six fields were averaged. The mean and standard error of the mean of the fish lots were calculated.

2.6.1.2 Macrophages head kidney isolation

After blood sampling, macrophages head kidney isolation techniques were performed as described by **Chung, and Secombes (1998).** Briefly, the head kidney was removed from the fish, two head kidney were pooled macerated and homogenized passed through a 100-µm-pore-size mesh with Leibovitz-15 (L-15) cell culture medium (Mediatech Inc., Herndon, USA) supplemented with heparin (10 IU/ml) and 2% FCS (both from Sigma Diagnostics, St. Louis, Missouri USA). The cells were collected by centrifugation at 170 × *g* for 15 min at -4 ^{0}C and re-suspended in 1 ml L-15 with 2% FCS. Macrophage cells were then checked for viability and counted

using trypan blue exclusion test. The cells were again centrifuged at 170 × g for 15 min at -4 ^{0}C and re-suspended in 1 ml L-15 with 0.1% FCS

2.6.1.3 Macrophages oxidative burst

The macrophages oxidative burst was assayed according to **Rice et al., (1995).** Briefly, macrophages were suspended at 1X 10^7 Hanke's balance salt solution (HBSS) containing 2mM calcium chloride. The assay was conducted in flat bottom 96 well microtiter plates. Thirty µl of fetal bovine serum (FBS), 140 µl of 179 µg/ml nitroblue tetrazolium (NBT) in HBSS with 2 mM Ca^{++} and 100 µl of cell suspension were added to each well. The reaction was started by adding 30 µl of 10^{-5} M Phorbol Myristate Acetate (PMA) (Sigma Diagnostics) as a stimulant control. Both stimulated and un-stimulated cells were evaluated in triplicate for each fish. The plates were incubated for 45 minutes at 27 ^{o}C and 5% CO_2. Each well was aspirated to remove overlying media and 140µl of 2mM KOH and 120 µl dimethyl sulphoxide (DMSO) were added and mixed by automatic pipette. The plates were read in a computerized automated microplate ELISA reader (Bio TEC, ELX800G, USA) and the optical density for each well at 620 nm was recorded. The data were expressed as stimulation indices and calculated as the ratio of stimulated to un-stimulated O.D. readings.

2.6.1.4 Lymphocytes transformation index

Lymphocytes transformation index was determined according to **Barta (1984).** Briefly, equal whole blood RPMI mixture for lymphocytes isolated on cell separating medium Histopaque (Sigma Diagnostics). RPMI (Sigma Diagnostics) mixed with 40% bovine fetal serum. The harvesting cell washed 3 times with balanced Hank's salt solution without calcium and magnesium. Absence of calcium prevents clotting of lymphocytes isolated from heparinized blood. Standardizing the lymphocytes concentration was around 2X10^6 /ml. Flat-bottom microtiter plates were set up with 200µ/l (100µ/l RPMI serum mixture and 100 µ/l lymphocyte cell suspension). Five µ/l of mitogen Phytohemagglutinin (PHA) 1mg/ml (in sterile PBS) was added to each well. Non stimulated cultures were prepared in the same manner except without added mitogen. All assays were performed in triplicate for each sample. The microtiter plates were incubated at 37°C in a humid atmosphere containing 5% CO2 for 48 hours. Each culture medium was transferred in a micro-tube and centrifuged at 400g for 10 minutes. The supernatant was collected and glucose concentration was determined with semi-automatic spectrophotometer (BM-Germany 5010) using a standard (100 mg/dl) glucose solution **(Werner et al., 1970).** The blast transformation index (TI) was calculated as follows: TI%=[(MG–SG)/MG]X100, where MG=glucose concentration in the non stimulated culture medium and SG=glucose concentration in the sample after incubation **(Khokhlova et al., 2004)**.

2.6.1.5 Head kidney macrophage phagocytic activity

Phagocytic activity of the head kidney macrophage was carried out as described by Crosbie and Nowak (2004). Phagocytic activity was evaluated by estimating the mean percentage of phagocytes containing at least one yeast cell, in a random count of 100 phagocytes performed in duplicate. Phagocytic index (PI) was determined by estimating the average number of yeasts within counted phagocytes (Ainsworth et al., 1991).

2.6.2 Humeral immunological studies

2.7.2.1 Bactericidal activity

A. Bactericidal activity was determined as described by **Kampen et al., (2005)** with modifications for plasma according to **Welker et al. (2007).** 20 µl of sample plasma or Hank's Balanced Salt Solution for positive controls was added to duplicate wells of a round-bottom 96-well microtiter plate and incubated for 2.5 h with aliquots of a 24 h culture of *A. hydrophila* . To each well, 25 µl of 3-(4, 5 dimethyl thiazolyl-2)- 2, 5-diphenyl tetrazolium bromide (MTT; 2.5 mg/ml) (Sigma) was added and incubated for 10 min to allow the formation of formazan. Plates were again centrifuged at 2000 ×g for 10 min, the supernatant discarded, and the precipitate dissolved in 200 µl of dimethyl sulfoxide (DMSO). The absorbance of the dissolved formazan was read at 560 nm. Bactericidal activity was calculated as the decrease in the number of viable *A. hydrophila* cells by subtracting the absorbance of samples from that of controls and reported as absorbance units.

2.6.2.2 Serum lysozyme

Serum lysozyme was determined turbidometric assay by the method of **Parry et al., (1965).** Briefly, the lysozyme substrate was 0.75 mg/ml of gram positive bacterium Micrococcus lysodeikticus lyophilized cells (Sigma, St. Louis, MO) was suspended in 0.1 M sodium phosphate/citric acid buffer, pH 5.8. Serum (25 µl) was placed in triplicate into a microtiter plate and 175 µl of substrate solution was added to each well at 25 ^{0}C and reduction in absorbance at 450 nm read after 0 and 20 minutes using microplate ELISA reader (Bio TEC, ELX800G, USA). The unit of lysozyme presents in serum or mucous (µg/ml) was obtained from stander curve

made with lyophilized hen egg white lysozyme (Sigma).

2.6.2.3. *Nitric oxide:*

Serum nitric oxide was assayed spectrophotometric (5010, Photometer, BM Co. Germany) following commercial test kits (Bio-Chain, Inc. USA).

2.7. Total and differential leukocytes count determination:

Total and differential leukocytes count was performed in duplicate for each sample according to **Stoskoph (1993).**

2.8. Erythrogram parameters

Erythrogr am parameters, erythrocytes count, hemoglobin determination, PCV value, blood indices were estimated according t**o Stoskoph (1993).**

2.9. Some selective biochemical parameters:

Liver transaaminase enzymes, (ALT&AST), creatinine, uric acid, urea, total proteins, albumin and glucose were determined spectrophotometer, using commercial kites (Spean react, Spinach, & Randox UK) according to enclosed pamphlet.

2.10. Challenge of fish:

The challenge test was done in 3 replicates where 30 fish from each group (10 fish/ replicate) were transferred to glass aquaria and then i/p inoculated with pathogenic *A. hydrophila* (0.4 X 10^7 cells /ml) that had been previously isolated from moribund fish and studied for their pathogenicity. The challenged fish from each aquarium were observed for 10 days in order to record the daily mortality.

2.11. Statistical Analysis

Mean and standard error for each variable were calculated. Data were analyzed by analysis of variance (ANOVA) to indicate the groups, which were significantly different at ($P< 0.05$) by one way ANOVA with post-hoc LSD multiple comparison test using SPSS software statistical program (SPSS for windows ver.15.00, USA).

3. Result

The present study demonstrated that the mercuric chloride caused great disturbance in both cellular and humeral immune responses in Nile tilapia.

Table 1: The immunological parameters, macrophages oxidative burst; phagocytic activity percent and lymphocyte transformation index are significantly decreased in mercuric treated group when compared with control. Neutrophils adhesion function is significantly decreased in mercuric exposed GP.4 only in compared with control. Moreover macrophages oxidative burst and phagocytic activity percent are significantly decreased in higher treatment mercuric group (Gp.4) when compared with other mercuric exposed group (Gp. 2&3)

Table.2: The humeral immunological parameters, serum bactericidal activity and nitric oxide are significantly decreased in mercuric chloride treated groups (Gp.2, Gp.3 &Gp.4) in compare with the control group, while bactericidal activity is significantly decreased when compared with other mercuric exposed group (Gp.2 &3). Serum lysozyme is significant decreased in group (Gp.4) only when compared with the other groups.

Table.3 &4: Total and differential leukocytic count in Nile tilapia *Orechromois niloticus* exposed to different doses of mercuric chloride for 21 days. Leukocytosis, neutrophilia lymphopenia have been observed in all mercuric exposed groups when compared with control. While lymphopenia is significantly higher in mercuric treatment group (Gp.4) when compared with other, mercuric exposed group (Gp.2&3).

Normocytic normochromic anemia was also observed in mercuric exposed group (Gp.3&4).

Table.5: Creatinine, uric acid and urea are significantly increased in Gp. 4 in compare with the other group, while liver transaminas enzymes (ALT&AST) and glucose increased in GP.3&4 in compare with GP.1&2. Total plasma proteins and albumin are significant decreased in Gp.4 when compared with the other groups.

At the end of experiment the experiment fish were challenged with *Aeromonas hydrophil*. The mortality rate is 65 ±3.5, 71±2.45, 78±3.4 and 85 ±3.01 in groups (Gp.1, 2, 3 & 4) respectively.

4. Discussion

Regarding immunosuppression in fish, numerous studies, using diverse fish species demonstrated impaired immunity following environmental chemical exposure (**Gogal et al., 1999).** The mercuric compounds were found to cause serious impairment in immune and physiologic functions and suppressive non-specific and specific immune system of fishes (**Low and Sin 1998** & **Amélia et al., 2004).**

The non-specific immune parameters are useful to determine the health status of fish and to evaluate the immunomodulatory substances for fish farming as markers for pollution and diseases resistances **(Sahoo et al., 2005**). In the present study, the immunotoxicological effects of mercury on immune system were documented by evidence of impairment in neutrophil adhesion. Neutrophils are considered an important constituent of host defense so the evaluation

of the neutrophil function is valuable for the assessment of the fish health status (**Palic et al., 2005).** However, there is a wide inter-individual variation within fish species in the majority of immune parameters, including the neutrophil activity **(Sahoo et al., 2005).** It is known that the stress factors lead to immunosuppression in fish and act as inhibitors of neutrophil functions as phagocytosis, migration and oxidative burst (**Palic et al., 2005). Moszczyński (1997)** reported that mercuric has adverse effect on neutrophils function in mammals.

Table (1): Some cellular immunological parameters (mean values ± SE) in Nile tilapia treated with mercuric chloride for 21days.

Immunological Parameters	Gp.1	Gp.2	Gp.3	Gp.4
Neutrophils adhesion cells /HPF	12.2^{a}±0.81	12.1^{a}±0.61	11.8 ^{a}±0.52	9.1^{b}±0.41
Macrophage oxidative burst index	5.01^{a}±0.14	4.21^{b}±0.20	4.05^{b}±0.15	3.18 ^{c}±0.21
Lymphocyte transformation index	1. 15^{a}±0.11	0.94 ^{b}± 0.09	0.91^{b}± 0. 10	0.72 ^{c}±0. 12
Phagocytic activity %	16.4^{a}±1.1	13.6b±0.82	10.1^{c}±0.75	9.82 ^{c}±0.85
Phagocytic index	1. 65 ^{a}±0.12	1.31^{b}±0.52	1.01^{c}±0.41	0.98 ^{c}±0.45

Gp.1=control, Gp.2, Gp.3 and GP.4 exposed to mercuric chloride 0.01, 0.05 and 0.1 respectively for 21 days. The rows with the same superscript are not statistically significant at P<0.005. (Values are mean ± SE) (n = 10)

Table (2): Some humoral immunological parameters (mean values ± SE) in Nile tilapia treated with mercuric chloride for 21days.

Immunological Parameters	Gp.1	Gp.2	Gp.3	Gp.4
Bactericidal activity unit	1.12 ^{a}±0.08	0.81^{b}±0.06	0.75 ^{b}±0.07	0.55 ^{c}±0.05
Serum lysozyme (µg mL^{-1})	8.55^{a}±0.45	8.42^{a}±0.41	8.12 ^{a}±0.51	6.32 ^{b}±0.48
Serum nitric oxide (Mmol L^{-1})	0.81^{a}±0.10	0.59 ^{b}±0.08	0.46^{b}±0.07	0.41^{b}±0.09

Table (3): Leukograme values (mean values ± SE) in Nile tilapia treated with mercuric chloride for 21days.

Leukogram	Gp.1	Gp.2	Gp.3	Gp.4
Total Leukocytic Count (Thousand µL^{-1})	27.02^{a}± 1.10	30.41^{b}± 1.15	30.72 ^{b}± 1.31	29.96 ^{b}± 1.01
Neutrophils (Thousand µL^{-1})	9.12 ^{a}± 0.82	15.25^{b}± 1.48	15.95^{b}± 1.24	17.71^{b}± 1.65
Esinophils (Thousand µL^{-1})	0.41^{a}± 0.05	0.38 ^{a}± 0.04	0.34 ^{a}± 0.03	0.32 ^{a}± 0.04
Basophils (Thousand µL^{-1})	0	0	0	0
Lymphocytes (Thousand µL^{-1})	16.15 ^{a}± 1.54	13.41^{b}± 1.12	13.01 ^{b}± 1.02	10.41^{c}± 0.92
Monocytes (Thousand µL^{-1})	1.34 ^{a}± 0.16	1.38 ^{a}± 0.15	1.42 ^{a}± 0.14	1.52^{a}± 0.21

Table (4): Erythrograme (values mean values ± SE) in Nile tilapia treated with mercuric chloride for 21days.

Erythrogram	Gp.1	Gp.2	Gp.3	Gp.4
RBCs (Million µL^{-1})	2.14± 0.14^{a}	1.95± 0.15^{a}	1.61± 0.12^{b}	1.52± 0.10^{b}
Hemoglobin (gm dL^{-1})	8.31± 0.32 ^{a}	8.01± 0.46 ^{a}	6.71± 0.35 ^{b}	6.01± 0.29^{b}
PCV (%)	24.12± 1.15 ^{a}	22.49± 1.12 ^{a}	18.84± 1.01 ^{b}	17.02± 112 ^{b}
MCV (FL)	112.5± 8.15 ^{a}	115.1± 7.19 ^{a}	117.2± 9.10 ^{a}	112.3± 9.85 ^{a}
MCH (Pg)	38.6± 2.15 ^{a}	39.7± 2.95 ^{a}	413.± 3.45 ^{a}	39.2± 3.68 ^{a}
MCHC (%)	34.2±0.49 ^{a}	35.4± 0.45 ^{a}	35.7± 0.42 ^{a}	35.2± 0.31 ^{a}

Table (5): Serum biochemical values (mean values ± SE) in Nile tilapia treated with mercuric chloride for 21days.

Groups	ALT U/L	AST U/L	Creat mg/dl	UA mg/dl	Urea mg/dl	T. P g/dl	Album g/dl	Glucose mg/dl
GP1.	26.8 ^{c} ± 1.6	37.9 ^{c} ± 3.82	0.25 ^{b} ± 0.05	0.95 ^{b} ± 0.07	15.9 ^{c} ± 1.02	3.12 ^{a} ± 0.19	1.48 ^{a} ± 0.10	58.25 ^{b} ± 5.45
GP2	29.9 ^{c} ± 1.9	40.9 ^{c} ± 3.68	0.29 ^{b} ± 0.06	0.91 ^{b} ± 0.08	21.9 ^{b} ± 1.15	3.14 ^{a} ± 0.17	1.41 ^{a} 0.15	61.10 ^{b} ± 6.70
GP3	42.21 ^{b} ±2.98	58.12 ^{b} ± 4.95	0.31 ^{b} ± 0.08	1.05 ^{b} ± 0.10	22.8 ^{b} ± 1.32	2.89 ^{a} ± 0.34	1.12 ^{b} ± 0.09	81.20 ^{a} ± 5.62
GP4	61.95 ^{a} ± 3.51	78.05 ^{a} ± 6.40	0.42 ^{a} ± 0.06	1.38 ^{a} ± 0.09	29.1 ^{a} ± 1.20	2.54 ^{b} ± 0.21	1.08 ^{b} ± 0.07	85.15 ^{a} ± 6.20

Gp.1=control, Gp.2, Gp.3 and GP.4 exposed to mercuric chloride 0.01, 0.05 and 0.1 respectively for 21 days. The rows with the same superscript are not statistically significant at P<0.005. (Values are mean ± SE) (n = 10).

Our study show suppression in macrophages function (macrophages oxidative burst, phagocytic activity and pahgocytic index). The non-specific defense of fish consists of phagocytic cells (neutrophils and macrophages) and a range of antimicrobial proteins or glycoprotein in tissues and

body fluids (**Robertsen et al., 1994**). The macrophages play a central role in the cellular non-specific defense (**Dalmo and Seljelid 1995). Sarmento et al., (2004)** recorded impairment of head kidney macrophages integrity (apoptosis and necrosis) and functions (respiratory burst capacity and phagocytic activity) in sea bass exposed to mercuric chloride. The author attributed these effects to decrease in the activity or failure in activation by macrophage activating factor.

Lymphocytes are responsible for the activation and maintenance of customized immune responses and mediate both cellular and humoral immunity (**Netea et al., 2005**). **Sarmento et al., (2004)** concluded that exposure to mercury is inhibit lymphoblastogenesis, antibody titer against *Aeromonas hydrophila* in blue gourami fish. **MacDougal et al., (1996)** observed suppress DNA synthesis in fish leukocytes incubated with mercuric chloride. **Moszczyński (1997**) reported mercuric inhibited human lymphocyte functions including proliferation.

The current investigation reported significant decreased bactericidal activity, lysozyme and nitric oxide serum level in mercuric exposed fish. **Sarmento et al., (2004)** reported decrease in ROS production of head kidney macrophages of sea bass (*Dicentrarchus labrax*) incubated with mercuric chloride as compared to medium incubated cells**. MacDougal et al., (1996)** recorded mercury exposure was enhancing tissue lysozyme activity in blue gourami. The immune response of fish is differing according to mercury exposure dose. In rainbow trout, there is a great variability between individuals in the effects of mercuric chloride doses and incubation time on the immune responses (**Voccia et al., 1994**).Treatment with methyl mercury was found to inhibit the production of induced nitric oxide and nitric oxide synthase from alveolar macrophages in mammals (**Kuo 2008**).

Our result shows leukocytosis, neutrophilia and lymphopenia in merecuric treatment groups. There is an elevated total leukocytic count in fresh-water-fish (*Clarias batrachus, Oreochromis aureus* and *Tinca tinca L*) exposed to mercuric chloride (**Maheswaran et al., 2008, Shah and Altimdag 2005 & Allen, 1994**). Meanwhile, the small lymphocytic count was decreased in different types of fish exposed to mercury polluted water (**Dhanekar et al., 1985**). **Roales and Perlmutter** (**1980**) regard the lymphopenia and immunosuppressive effects of mercury in fish to preventing the proliferation of the white pulp of the spleen. **Sweet and Zelikoff (2001) and Bly et al., (1997)** recorded toxicological effects of mercuric on hematopoietic tissues in fishes**.** Exposure to inorganic mercury reduced total leukocytic count in tilapia and several other fish species **(Dhanekar et al., 1985)**. The explanation of our result could be to the higher toxicity of mercuric chloride and/or the increased susceptibility of cell type (**InSug et al., 1997**).

Non regenerative normocytic normochromic anemia reported in mercuric exposure fish. A reduction in RBC count might be attributed to inhibition of erythropoiesis as a result of the marked necrosis and degeneration of hematopoietic cells in the kidney of experimented catfish induced by the action of accumulated heavy metals **(Singal and Jain 1997).** The anemic effect of heavy metals were documented in Catfish *Clarisa gariepinus* **Mansour et al., (2001),** *Oreochromis niloticus* **El- Naggar et al., (1998)** *Ictalarus punctatus* **El-Boushy (1998)** and in *puntius conchonius* **Gill and pant (1981**). On the other hand **Cyriac et al., (1989)** recorded an increase in blood hemoglobin content of *Oreochromis mossambicus* fish after 168-h post exposure to mercury and could be attributed to as a result of stimulation erythropoiesis.

Elevated levels of ALT and AST were observed in mercuric exposure groups. This elevation might be due to the liver damage. These results agree with **Mansour et al., (2001) and El- Boushy (1998),** who reported increase liver transaminase activities in different fishes exposed to heavy metals. Hypoproteinemia and hypoalbuminemia in mercuric exposed fish may be attributed to excess renal excretion and or impaired protein synthesis by the liver **(Stoskoph 1993).** Several authors also recorded decrease in plasma protein level after exposure of fresh water fish to heavy metals **(Sing and reddy 1990** and **Van Vuren et al., 1994).** On contrast elevation in plasma protein level has been reported by **Mansour et al., (2001), Haggag et al., (1999) , Salah El-Deen et al., (1996) and Gazaly & Said (1995)** in fish collected from heavy metals polluted locations. The rise level of uric acid and creatinine in higher mercuric exposure groups revealed marked nephrotoxic effect of mercuric **(Stoskoph 1993).** Similarly effects have been reported by **Sastry and Sharma (1980)** and **Sharama et al., (1982)** in freshwater fish *Heteropneustes fossilis and Oreochromis mossambicus* respectively. Urea in fish is synthesis by the liver and excreted primarily by the gills rather more the kidney .The elevation of urea in our work may be attributed to gill dysfunction **Stoskoph (1993).** Elevated levels of blood glucose in mercuric exposure fish could be attributed to stress response and stimulation of gluconeogenesis **(Stoskoph 1993).** The hyperglycemia in fish exposed to heavy metals pollution has been reported by **Nath and Kumar (1987)** and **Health (1987).**

Mortality rate in mercuric exposed group

challenged with *Aeromonas hydrophila* is dose dependent. Increase mortality rate could be attributed to immunosuppressive effect of mercuric on Nile tilapia (*Oreochromis niloticus*). **Pandey et al., (2005)** concluded that dose- and dose-time-dependent increases the mortality rate in response to mercuric toxicity in fish. Similarly **Dalmo et al., (1998)** and **Wang and Wang (1997)** recorded decrease in the disease resistance in Atlantic salmon and Nile tilapia challenged with Aeromonas salmonicida and Aeromonas hydrophila respectively.

We could be concluded that from this study the water born mercury pollution is highly toxic to Nile tilapia (*Oreochromis niloticus*) as well as has immune suppression and decrease diseases resistance.

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