Investigations on the effects of lead, mercury and cadmium on the immune response of *Oreochromis niloticus*

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Abstract : Evaluation of the effects of lead, mercury and cadmium on both humoral and cellular immune response of *Oreochromis niloticus* "Tilapia nilotica" fish was challenged with an important fish pathogenic bacteria "*Pseudomonas flourscens"*. The effects on cell mediated immune response was determined by using the phagocytic assay "phag. index". The results revealed that, lead, mercury and cadmium have inhibitory effect on phagcytic acgivity of fish macrophages and so having an inhibitory effect on cell mediated immune response. The results also revealed that. The inhibitory effect of lead, mercury was of the same level along the time of exposure while in cdmium the inhibitory effect was high in the first weet of exposure then the percentage of phagocytosis re-increased after 3 weeks and re-increased again after 6 weeks . The effect of these metals on humoral immune response revealed also that these metals having inhibitory effect on humoral immune functions which is manifested by low levels of antibodies and high mortality rates in fish exposed to these metals than in the control fish after experimental infection by *Pseudomonas flourscens* . Immune response by these metals provides opportunities for the entry of pathogens and developing of many diseases in fish.
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Key words : lead – mercury – cadmium - tilapia nilotica - phagocytic assay - antibody titer - pseudomonas flourscens

Introduction

For the first time in his entire cultural history, man is facing one of the most horrible ecological crisis which is pollution of his environment which in the past was pure, virgin, undisturbed and uncontaminated and basically quite hospital to him.(**Katyal and Stack, 1993**).

Water pollution referred to the addition to the water of an excess of material that is harmful to humans, animals and fishes (**Vesilland *et al,* 1990**). The materials found in water and considered toxic to fishes in one way or another can be categorized into (oxygen debilitating materials, toxic materials, toxic gases, toxic organic compounds and pesticides (**Post, 1989**).

Heavy metals are surrounded with great care and special importance due to their highly toxic effects on fish as they affect survivability, growth and reproduction(**Gill and Pant 1985, Sorenson 1991 and Thuvander 1998)**

No doubting all living creatures, immune system and immune response come about as a protective mechanism and although fishes are the most primitive vertebrates, but they too had to develop an immune system proficient enough to react and protect them from attack by various microorganisms and parasites.(**Vorkamp *et al* 2004 ; Andreji *et al* 2005**)

Suppression of immune system and immune response may results from the action of several pollutants including heavy metals which provide opportunities for entering of many pathogens, but till now the effect of heavy metals on the immune system and immune response is not fully understood (**Compagno 2001;Storelli *et al* 2002;Liu and Kuch2005**).

The aim of this study was evaluation of the effects of lead, mercury and cadmium on humoral and cellular immune response of *Oreochromis niloticus* which is the most popular fish in Egypt.

Material and Methods

1-Fish for experimental work: one hundered and eight *Oreochromis niloticus* with a range of weight (140-160g) and a range length(20-22cm) were used. The fish were obtained from an intensive fish farm in kaliubia governorate. Fish acclimiated and kept under observation for 2 weeks before starting of the experiments and were fed once daily on artificial dry pillets according to (De selva 1991).

2- chemicals:

Heavy metals used in the experiment were :

-Lead (pb): as lead acetate salt c4h6o4pb.3h2o, (riedel dehaen, Germany.)

-Mercury (Hg): as mercuric chloride salts (Hgcl2), rhone poulenc, France.

-Cadmium (cd): as cadmium chloride. 1 hydrate cdcl2, rhone poulenc, France.

3. Bacterial strain used for the challenge experiment:

Pseudomonas flourscens was isolated from diseased *Oreochromis niloticus* showding signs of septicaemia. The organism was identified using microscopical examination, culture and biochemical characters according to **Austin and Austin (1989)** .

4-Evaluation of phagocytic activity in fish exposed to 20% of LC50\96 hrs of lead acetate, mercuric ,chloride and cadmium chloride separately :

The phagocytic activity was carried out according to Mathews *et al* (1990). Forty eight *Oreochromis niloticus* were divided into four equal groups, the first group was exposed to lead acetate at dose 20% of LC50\96 hrs, the second group to mercuric chloride at the same dose, the third group was exposed to 20%of LC50\96 hrs of cadmium chloride while the fourth group was left as a control group.

Before the beginning of the experiment, 3 fish were taken from each group in order to determine their phagocytic activity in comparison with the non exposed control group.

5-Evaluation of humoral immunity in fish exposed to 20% of the LC50\96 hrs of lead acetate, mercuric and cdmium chloride after challenge by *Pseudomonas flouresens*:

Sixty *Oreochromis niloticus* fish were used in this experiment classified into 5 equal groups each of 12 fish. The first, second and third groups were exposed to lead,mercury and cadmium at concentration 20\ 100 of their LC50\96 hrs. while the 4^{th} and 5^{th} groups were left as a control non exposed groups. After 2 months of exposure to metals, the fishes were challenged with pseudomonas flourescens given by injection I/m with a dose of 0.2ml/fish of 2x108 bacterial cell/ml., also the group 4 was injected with the same dose.(control) .

Mortality rate was recorded and serum samples were collected after one, 3 and 6 weeks post infection in order to determine the level of immunoglobulines in the serum to evaluate the humoral immune response using microagglutination test.

Results and Discussion

Table(1) Showing the percentages of phagocytosis in different groups before and after 1,3 1nd 6 weeks of exposure to metals.

Group / Time	Mean ± SD of phagocytosis percentages			
	Lead acetate group	Mercuric chloride group	Cadmium cloride group	Control group
One week before exposure	64.0 ± 2.0	66.6 ± 1.15	62.0 ± 2.0	62.3 ± 7.51
After one week of exposure	18.3 ± 1.53	35.3 ± 1.53	22.3 ± 2.52	61.6 ± 7.64
After 3 weeks of exposure	18.3 ± 1.53	32.7 ± 2.52	43.0 ± 2.65	63.0 ± 9.53
After 6 weeks of exposure	17.7 ±2.08	31.3 ± 2.31	46.7 ± 2.52	64.3 ± 6.03

(1)- Phagocytic activity:

The obtained results as recorded in table (1) showed that the percentage of phagocytosis one week before exposure to metals were 64% ±0.2, 66.6% ± 1.15, 62%± 0.2 and 62.3% ± 7.51 for lead, mercury, cadmium and control group respectively.

After one week of exposure, the mean values of percentage of phagocytosis were 18.3%± 1.53, 32.7% ± 2.52, 43.0 ±2.65 and 63.0 ± 9.53 for lead, mercury, cadmium and control groups respectively.

After 3 weeks of exposure the mean values of percentages of phagoctosis were 18.3% ± 1.53, 32.7%± , 2.52, 43.0 ± 2.65 and 63% ± 9.53 for lead, mercury, cadmium and control group respectively. After 6 weeks of exposure the mean values of percentage of phagocytosis were 17.7 ± 2.08, 31.3% ± 2.31, 46.7% ± 2.52 while in the control group were 64.3% ± 6.03.

Table (2): Mortality rate and antibody titer in infected *Oreochromis niloticus* exposed to lead acetate, mercuric chloride and cadmium chloride.

Group	Titer of antibodies			Mortality %
	One week of infection	3 weeks post infection	6 weeks post infection	
Lead acetate exposed group	No titer	1/16	1/16	66.6
Mercuric chloride exposed gp.	¼	1/512	1/128	50.0
Cadmium chloride exposed gp.	¼	1/128	1/64	41.6
Infected non exposed control gp.	1/32	1/2048	1/512	25.0
non infected non exposed control	Zero	Zero	Zero	Zero

Table (2) showed that the mortality rate and antibody titer in Oreochromis niloticus fish exposed to lead acetate, mercuric chloride and cadmium chloride after experimental infection by *Pseudomonas flourscens* bacteria. The mrtolity rate was 66.6%,50%,41%, and 25% for lead acetate,mercuric chloride, cadmium chloride and the infected non exposed control group, respectively . the antibody titer one week after challenging was zero, ¼, ¼ and 1/32 for lead acetate, mercuric chloride, cadmium chloride and the control group, respectively. After three weeks of experimental infection, the antibody titer was 1/16, 1/512, 1/128, and 1/2048 for lead acetate, mercuric chloride, cadmium chloride and the control group, respectively. While the antibody titer six weeks after expremintal infection was 1/16, 1/128, 1/64, and 1/512 for lead acetate, mercuric chloride, cadmium chloride and the control group, respectively. The non infected non metal exposed control group showing no titre 1,3 and 6 weeks during the expermient while no mortality could be detected .

The obtained results as recorded in table 1 revealed that there were no significant differernce between the different groups one week before exposure to metals, while after one week of exposure to metals (table 1) there were a highly significant difference between all metal exposed groups and the control group. ($p < 0.001$) (**Freiras and Rochas 2000:Hung et al 2004**) i.e in lead acetate, mercuric chloride and cadmium chloride the percentage of phagocytosis were significantly decreased than the control group ($p < 0.001$) (**Ward and Neumann 1999; Anderson et al1999;Canli and Atli 2003**). But there were no significant difference between lead acetate group and cadmium chloride group which indicated that both have approximately the same inhibitory effect after one week of exposure. While the inhibitory effect of mercuric chloride is of less evident.(**Ward and Neumann1999;Watanab et al 2003).**

The results after 3 weeks of exposure revealed that in all metal exposed groups, the percentage of phagocytosis was significantly decreased than the control group ($p < 0.05$)which indicated that the 3 metals have a supprisive effect on cellular immune functions ,this result nearly agree with **Mormede and Davis (2001) & Watanab *et al* (2003).**

Also there was a significant difference ($p < 0.001$) between the exposed groups, explaining that lead acetate have inhibitory effect on phagocyic activity of fish macrophage more than mercuric chloride and cadmium chloride. It is clear also that mercuric chloride has inhibitory effect more than cadmium chloride after 3 weeks of exposure.

The results after 6 weeks of exposure revealed that in lead acetate, mercuric chloride and cadmium chloride groups, the percentage of phagocytosis was significantly decreased in comparison with the control group. The differences were also significant between the exposed groups ($p < 0.001$), as lead acetate has inhibitory effect more than mercuric chloride And mercuric chloride more than cadmium chloride after 6 weeks of exposure, i.e. in all exposed groups, there were a highly significant difference before and after exposure to metals ($p < 0.001$) which revealed that the 3 metals have inhibitory effect on phagocytic activity of fish macrophages which means that they have an inhibitory effect on cell mediated immunity.

Also along the time of exposure, there were no significant differences at different time of exposure to lead acetate and mercuric chloride b (the inhibitory effect is of the same level) but in case of cadmium chloride, the inhibitory effect was temporary in the first week of exposure then the percentage of phagocytosis reincreased after 3 weeks and also reincraesed again

after 6 weeks. The obtained results agree with **Moszoznski** and **Moszoznski (1988),Bozelka** and **Burkholder (1982),Sjobeck** et al**.(1984),Hurtenbach et al.(1988),and Thuvander (1989) .**

The sunppresive effect observed by lead acetate, mercuric chloride and cadmium chloride may resulted from the effect of these metals on the haematopoietic tissues mostly in the anterior kidney and spleen which are the sources sites of formation of macrophages.

Regarding cadmium exposed group the difference among the recorded literature and also with other results could be attributed to variation in dose of cadmium, route of administration, duration of exposure which could be modulate the immune response .

Evaluation of humoral immune response toward pseudomonas floureseens in fish exposed to lead acetate, mereuric chloride and cadmium chloride seperately at concentration of 20% of their LC 50/96 hrs in comparison with a control group.

Our results showed that the antibody titer after infection by *Pseudomonas flourscences* in case of lead acetate group were zero, 1/16 and 1/16 after 1,3, and 6 weeks post- challenging respectively while in mercuric chloride exposed group were ¼, 1/512, and 1/128 and in cadmium chloride exposed group were ¼, 1/128 and 1/64 respectively. While the antibody titre in the infected non exposed control group was 1/32, 1/2048 and 1/512 after 1,3, and 6 weeks respectively .

Analysis of these results revealed that, lead, mercury and cadmium having inhibitary effect on humoral immune function which is manifested by the low levels of antibodies in comparison with the infected non exposed fish than the control group 66.6% mortality in lead acetate group, 50% in Mercuric chloride and 41.6% in cadmium chloride group while in the control group was 25%. On comparing the results with the previous recorded data we can found the following :

The obtained results agree with those recorded by **Moszcynski and Moszcynski (1988), O'Neill (1981), Kawamura et al. (1983) and Robohm (1986)** but our results not agree with **Thuvander (1989)** who reported that humoral antibody production was enhanced in cadmium exposed group. This variation may be due to species difference or due to variation in the dose or time of exposure. All these factors could modulate the effect of cadmium on humoral immunity response .

The inhibitory effect of these metals on humoral immune response and antibody formation may resulting from the damaging effects of these metals on haematopioetic tissues in liver, spleen and kidney .

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1/20/2011

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