Effect of Zinc Oxide Toxicity on African Cat Fish *Clarias gariepinus* Present in the River Nile (Hawamdya)

Mona, S. Zaki[*1], Nabila, Elbattrawy[2] Nagwa, S. Ata[3] and Shalaby S. I[4]

[1]Department of Hydrobiology, National Research Centre, Cairo, Egypt
[2]Department of Microbiology, Reproductive institute, Cairo, Egypt
[3]Department of Microbiology, National Research Centre, Cairo, Egypt
[4]Deptartment of Animal Reproduction, National Research Center, Giza, Egypt
[*]dr_mona_zaki@yahoo.co.uk

Abstract: The effect of dietary carbohydrates and mercuric oxide on haematalogical profile, blood chemistry and hormonal level was studied in African cat fish (*Clarias gariepinus)*. Thirty Fish were divided into 3 equal groups, exposed to different doses of zinc oxide and carbohydrate. Group (1) was served as control. Group (2) was fed with carbohydrate and mercuric oxide (10 mg Kg^{-1} diet ration). Group (3) was fed with carbohydrate and zinc oxide (1 5 rng Kg^{-1} diet ration). There is a significant decrease in hemoglobin and P .C.V in group (3). There is a significant increase in serum corlisol, cholesterol, AST, ALT, urea, creatinine and alkaline phosphorous in group (3). Also there is a significant decrease in serum phosphorous, sodium and potassium in treated fish. There is a significant high level of zinc content in kidney, muscles, heart and spleen in group (3) suggesting toxic effects of zinc oxide on African cat fish (*Clarias gariepinus)*. The total viable count of bacteria identified higher in fish fed on carbohydrate zinc and the predominate bacteria were identified as, *E. coli* and Pseudomonas, fluorscences. We emphasize the finding that an increase carbohydrate concentration causes harmful pathological effect which reduces humoral immune responses and enhances dietary zinc toxicity.

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1. Introduction:

Fish plays an important role, not only in human food diets but also in animal and poultry rations. It is a palatable and easily digested food which is rich in vitamins, calcium, phosphorous and iodine. In Egypt, fish is considered as a cheap food article if compared with other foods of animal origin. The flesh of healthy fish is considered as a marker for the natural aquatic environment. Because of the relatively high solubility of zinc compounds, this metal is widely detected in freshwater. Indeed, it is important that it should do so because it is an essential element for aquatic life: for example, it occurs in the enzyme carbonic anhydrase, catalyses the formation of carbonic acid from carbon dioxide in the blood. Small amount in the water or in the diet are therefore essential. It is also follows that the organisms will have an internal mechanism to transport zinc around the body in order to manufacture such vital enzymes. When the zinc, in the water rises to a level where the amount entering the organism through the gills exceeds the requirement for this metal, the surplus has to be excreted and this will require a certain amount of energy. At higher levels this detoxification mechanism may be insufficient to copy with the influx and the zinc will then exert a direct action. It was originally thought that the direct toxic action of zinc on fish was to precipitate the layer of mucus on the surface of the gill, causing suffocation. While this may be true for those species which produce a copious supply of mucus, the white precipitate observed on the gills of say rainbow trout is mainly composed of disintegrating epithelial cells which may be associated with the onset of mortality. However, zinc may also cause a certain amount of tissue damage by reacting with protein and this could effected the respiratory efficiency as well as the osmoregulatory of function of the gills**.** The major environmental factor which affects the toxicity of zinc to fish is the calcium concentration of the water. Calcium, like zinc and zinc is a divalent ion and both complete for binding sites on protein molecules. Although some completion may occur on the gill surface, the main site of action may be inside the epithelial cell where the calcium concentration is in equilibrium with that in the surrounding water. Therefore, if fish are removed from hard calcium-rich, water to a soft water, they will slowly lose their resistance to zinc toxicity as their body calcium is reduced to a lower equilibrium level. The relationship between the concentration of zinc acutely toxic to rainbow trout and hardness of the water. Similar data exist for other species of fish, although in some cases in inadequate acclimation period to different water

harnesses may affect the extent of the different obtained. [1-8]

2. Materials and Methods

Experimental design:

Thirty African cat Clarias *gariepinis* were used to assess the effects of zinc oxide. Fish weighting from 180-250g were obtained from Nile river then they were kept in glass aquaria supplied with dechlorinate tap water at rate of one litter for each cm of fish's body. Fish were acclimated to the laboratory conditions for two weeks before the beginning of the experiment, they were fed a commercial fish diet [9]. The composition of diet is illustrate in table (1). The experiment was determined after 4 weeks. Fish were divided into three groups (n=10) and exposed to different doses of mercuric oxide and carbohydrate. Group (1) was served as control, group (2) was fed with carbohydrate and mercuric oxide (10 mg kg-1 diet rations) and group (3) was fed with carbohydrate and zinc oxide (15 mg/kg^{-1} diet ration).

Mean of the initial body, weight of the each examined fish at the beginning of the experiment then after 2-4weeks of exposure were determined.

Blood samples:

Blood samples were collected from the caudal vein after 4 weeks of exposure. Each sample was divided into two parts the first one was heparinized for haematological investigations, while the second was centrifuged at 3000 rpm for 5 minutes to obtain serum for biochemical studies.

Some Hematological Analysis:

Haematological studies were performed according to Sandnes *et al.* [10], where blood haemologlobin (Hb) and haematocrit (Ht) values were evaluated.

Biochemical Analysis:

The activities of alkaline phosphatase, aspartic aminotransferase (AST) and alanine aminotrarsferase (ALT) as well as cholesterol urea and creatinine level were determined according to the method of Varley *et al*. [11] by using commercial kits (Bio Merieus, France)

Total serum protein was estimated according to Drupt [12]. Serum cortisol was analyzed by a Gamma counter using 125 I cortisol radioimmunassay Kit) Baxter Health Care Corporation USA) according to the method described by Pickering and Pottinger [13]. Potassium, Sodium and phosphorous concentrations were determined by atomic absorption spectrophotometer [11].

Tissue analysis:

Liver, kidney and spleen samples were washed with distilled water then were dried in hot air oven. Sulphuric acid and hydrogen peroxide were added on samples then were heated until the mixture became transparent after performing a wet ash digestion according to the method of Issac and Kerber [14].

Identification ion of bacteria:

The liver, kidney, spleen, muscle, stomach and gill from each examined fish were diluted immediately after sampling in sterile 0.9% saline and 0.1 ml volumes of appropriate dilutions and were spread over the surface of the typtic soy agar (oxide).The plates were incubate at 22^{O}C and inspected daily for up to 4 weeks.

The isolates were classified and identified according to Steverson [15] and Quirm e*t al.* [16].

The data were evaluated statistically according to Gad-Weil [17].

Water samples:

Two water of samples were collected from River Nile (Hawamdya) as well as two water samples from any heavy metal pollution El-Kasr El-Eini (control) were analyzed for mercuric concentration by atomic absorption spectrophotometer.

3. Results and Discussion:

Data in Table (1) showed that, the mercuric oxide level in Hawamdya region was clearly higher than the maximum allowable concentration for human consumption as recommended internationally according to WHO (World Health Organization). Nadal *et al*. [2] concluded that the occurrence of mercuric in nature and its use in various industrial processes has increased its inputs in the environment. From the present study it is clear that the low mercuric levels were reported in water samples collected from areas far from industrial discharges, while high zinc levels in the present study may be due to the collection of samples from areas subjected to industrial pollution.

In Table (3) there is a significant decrease in body weight in group 3 (fish fed 15 mg/kg diet zinc oxide for 4 weeks) than in group 1 (control) and group 2 (fish fed l0 mg mercuric), this results agree with that reported by Khalaf-Allah [18].

The results present in Table (5) showed the cholesterol levels between different groups. The level was significantly increased in group 3 (fish fed on l5 mg vanadium) than in group 1(control). Hypercirolestremia might be due to necrotic changes occurring in liver with liberation of cholesterol as a byproduct of cell destruction. The present data suggest that impaired liver function lead to increased

serum levels of alkaline phosphat, AST and ALT among group 3 (fish fed on l5 mg mercuric) and among group 2 (fish fed 10 mg mercuric) compared with group 1(control). In this concern Khalaf-Allah [8] concluded that ALT and AST enzymes are good indices for the health status of liver parenchymatous, tissue necrosis is considered as the main source of AST and its increase in the serum of African cat fish *(Clarias Gariepinus)* and declared these necrotic changes [18]. In addition, exposure of fish to environmental pollutants might result in stimulation or depression of the enzyme activity depending on the concentration of pollutant and the duration of exposure [19, 20].

Regarding the effect of zinc oxide on serum cortisol level in African cat fish (*Claias gariepinus)* highest level was obtained in group 3 (fish fed on 15 mg mercuric) then in group 2 (fish fed on 10 mg vanadium) as compared to that obtained in group 1 (control). The significant increase of cortisol level is probably due to the activation of hypothalamus pituitary internal axis [21].

From the data present in Table (5), it is clear that elevation of zinc oxide level in the diets fed to *(Clarias gariepinus)* was positively correlated to hemoglobin (Hb) levels and haematocrit (Ht). A marked decrease in the IIb and Ht was recorded after feeding diet containing 15 mg and 10 mg mercuric , respectively. Reduced Hb reflects metabolic adjustment according to reduced need for oxygen by change in blood PH.

Moyle and Ceeh, Hall and Cliffs recorded actived acetchlinesterase of erthrocytes [22, 23] Further more Pickeringand Dusten [24] concluded that a consistent effect of cortisol was the reduction in the hemoglobin and iron levels as a result of decrease in appetite in rainbow trout fish or more likely to be the direct-result of catabolic effect of cortisol in the fish tissues [24].

The mean phosphorus, sodium and potassium values in the serum of fish of group 3 (fish fed 15 mg zinc oxide) were significantly increased respectively than those recorded in the group 1 (control). This retention maybe attributed to kidney dysfunction, whereas, the kidney is the normal pass for sodium and potassium.

Kidney dysfunction may also explain the increase in serurn urea and creatinine especially in group3, but little known about the mechanisms involved in this association.

The results displayed also in Table (5) showed that there was general decrease in the mean total protein value in serum samples collected from the fish of group 3 and 2, respectively. The mean value of these parameters was lower than in group 1. Jagadeesh *et al*. [25] estimated marked decrease in glycogen in tissues of fresh water fish after exposure to vanadium [25].

This experiment showed that the body weight of the examined fish was significantly decreased than the initial body weight after 4 weeks of exposure to 15mg zinc oxide. Also, Hilton and Better [25] recorded a significantly reduced growth and increased mortality among feeding diets of mercuric (0, 10, 100, 1000, 10000mg Kg-') [26]. The increase in muscles and tissue lactic acid (2 fold) in association with decrease in pyruvic acid (72 in muscles +26% in liver) reflect a shift towards an anaerobic metabolism of fish following long term exposure to zinc [26]

Table (6) showed that, the bacterial isolates and counts were increased by feeding the fish with CHO and zinc. The carbohydrates affect immunity and resistance to infection as recorded by Waagbo et al. [19] Utility of vanadate, zinc protein phosphate inhibitors to protect fish from microorganism [27]. The increase of bacterial count among the fish fed on mercuric may be related to the increased level of corlisol which decreases the host immunity.

In the course of experiment, a high concentration or zinc levels has been found in kidney, liver, spleen, heart and muscles of cat African catfish *(Clarias gariepinus)* fed 15 mg mercuric (Table 4). This suggests that these organs could be useful as a marker for vanadium in the aquatic environment. In this concern Ray et al. recorded a high concentration of mercuric in kidney liver and other organs of African cat fish as the concentration of mercuric in the tissues increased with its concentration in the aquatic environment and exposure time[28].After exposure of fish to increased doses for 4 days, the mercuric content in the muscle then increased in all tissues [20, 25, 26] The capability of zinc to be present in fish muscle is of particular interest in assessing the exposure of man to environmental mercuric as ingested by food.

Clinicopathological observations:

Abnormal swimming darkling of the skin, scale loss and haemorrhasges, water seen on the external body surface. In addition to congestion of gills, eyes mouth, liver, kidney, spleen, and intestine. This was notice in fish exposed to mercuric oxide 15mg (group 3) but not in fish exposed to zinc oxide 10 mg (group 2).

In conclusion: we emphasize that, the reported finding increase of carbohydrate concentrations causes harmful physiological effects, reduces hormonal immune response and enhances dietary toxicity.

Table 1: Ingredients and Proximate composition of diets used in the experiments with zinc oxide

Ingredients	Control	Group 2	Group 3
Fish meal	25	25	30
Meat and bone meal	5	5	10
Wheat bran	20	20	20
Skimmed milk	12	12	7
Yeast	10	10	15
Starch	-	10	15
Cod liver oil	2	2	2
Vitamin premix	1	1	1
Mercuric oxide	-	10	15
Crude protein %	40.35	35.95	38.89
Metabolizable energy k cal/kg]	2205.4	2551.78	2315.4
Ether extract%	4.29	4.21	2.86
Crude fiber %	4.46	3.73	4.27
Ash%	5.56	6.26	10.25
Lysine%	2.13	1.88	2.29
Methionine %	0.62	0.55	0.613

Mineral and vitamin premix per/kg of pellet food Vit A, 8000 g/u, vit D 900 g/u vit E/u, vit k 4mg, vit B2 3.6 niacin 20mg, pyridoxine 0.2mg Vit B1 25, Mn 70mg, Se 60mg

Table 2: zinc oxide concentration in water samples collected from two areas in Egypt.

Areas	Sampe No.	Concentration of zinc p.p.m
Hawamdya	1	0.05
	2	1.10
Ak-Kasr El-Aini	3	0.128
	4	0.152

Table 3: Changes in body weight in African cat fish (*Clarias gariepinis)* fed on different levels of dietery carbohydrate s in addition to zinc oxide.

Weight / Group	Group 1	Group 2	Group 3
Initial body weight (g)	71±0.15	84±0.17	93±0.48
After 2 weeks (g)	101±0.43	103±0.24	92±0.68
After 4 weeks (g)	154±0.25	123±0.64	92±0.61*

Table 4: The mean zinc concentration in the organs of fish mg/g net weight

Groups	Muscles	Spleen	Heart	Kidney	Liver
Group1	0.34±0.12	0.68±0.68	0.84±0.39	2.12±0.1	3.11±0.80
Group2	0.48±0.48	0.61±0.82	0.78±0.81	3.00±0.73	4.11±0.90
Group3	0.65±0.36	0.94±0.21	0.98±0.32	6.72±0.72	5.13±0.09

Table 5: Some haematological, biochemical parameters in African cat fish *Clarias gariepinis* on different levels of dietery carbohydrates in addition to zinc oxide

Parameters/Group	Group 1	Group 2	Group 3
Hemoglobin g/dl	38±0.28	39±0.13	40.2±0.52*
HCT %	0.93±0.34	0.96±0.13	1.62±0.73*
Cartisl ng/dl	8.4±0.52	96±0.36	82±0.81*
Phosphorous mg/dl	136±1.26	131±0.67	104±0.23*
Sodium M.EQ	7.1±0.23	7.2±0.23	68±0.71*
Potassium M. EQ	21.42 ±6.2	26±0.68	25±0.12*
Alkphosphatase U/L	136±0.21	140±0.42	142±0.66*
AST U/L	26±0.18	28±0.21	31±0.62*
Cholestrol mg	142±0.32	150±0.23	168±0.43*
Total protein g/dl	9.0±0.72	9.5±0.95	10.5±0.72*
Urea mg/dl	3.3±0.68	3.5±0.86	5.6±0.33*
Creatinine mg/dl	0.95±0.64	0.94±0.88	0.99±0.76*

Table 6: Bacterial isolates recorded from the examined fish

Groups	Bacterial isolates	Site of isolation	Bacterial count
Group 3 (n= 10)	-E. Coli	-Muscles	$2X10^6$
	-Streptococcus	-External surface, Stomach	$3X10^6$
	-E. Coli	Gills	----
	-Aeromonas	Gills, Stomach	$5.6X10^6$
Group 2 (n= 10)	-Enterbacter	Liver, Kidney	$1X10^6$
	-Pseudomonas flurose	-Spleen, Muscles	$3X10^6$
	-Fluroscences	-Stomach	$3X10^8$
	-Lactobacillus	-Gills	----

Corresponding author
Mona, S. Zaki
Department of Hydrobiology, National Research Centre, Cairo, Egypt
dr_mona_zaki@yahoo.co.uk

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