

**Effect of Mercuric Oxide Toxicity on some Biochemical Parameters on African Cat Fish *Clarias gariepinus* Present in the River Nile**

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**Abstract:** The effect of dietary carbohydrates and mercuric oxide on haematological profile, blood chemistry and hormonal level was studied in African cat fish (*Clarias gariepinus*). Fish were divided into 3 groups (n=10), 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<sup>-1</sup> diet ration). Group (3) was fed with carbohydrate and mercuric oxide (15 mg Kg<sup>-1</sup> diet ration). There is a significant decrease in hemoglobin and P.C.V in group (3). There is a significant increase in serum cortisol, 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 mercuric content in kidney muscles, heart and spleen in group (3) suggesting toxic effects of mercuric oxide on African cat fish (*Clarias gariepinus*). The total viable count of bacteria identified higher in fish fed on carbohydrate mercuric. Predominate bacteria were identified as, E. coli, Streptococcus, Pseudomonas, and Fluorococcus. We emphasize the finding that an increase carbohydrate concentration causes harmful pathological effect which reduces humoral immune responses and enhances dietary mercuric toxicity.

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**Keywords:** *Clarias gariepinus*, mercuric pollution, haematological, biochemical, clinicopathological, Bacteria account

## 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.

In animals, mercuric oxides cause inhibition of certain enzymes, which has several neurological effects. Next to the neurological effects vanadium can cause breathing disorders, paralysis and negative effects on the liver and kidneys. Laboratory tests with test animals have shown that mercuric and vanadium can cause harm to the reproductive system of male animals and rat it accumulates in the female placenta. Vanadium can be found in fishes and many other species. In mussels and crabs mercuric and vanadium strongly bioaccumulate, which can lead to concentrations of about 10<sup>5</sup> to 10<sup>6</sup> times greater than the concentrations that are found in seawater [1-8]

In recent years, much attention had been paid to the possible danger of metals poisoning in human as a result of consumption of contaminated

fishes. So, the present study was carried out to elucidate the impact of mercuric oxide on African cat fish (*Clarias gariepinus*). Its haematological, biochemical and hormonal parameters were studied as well as the bacteriological and clinicopathological investigation.

## 2. Material and methods

### Experimental design:

Thirty African cat *Clarias gariepinus* were used to assess the effects of mercuric oxide. Fish weighting from 180-250 were obtained from Nile river and were kept in glass aquaria supplied with dechlorinated tap water at rate of one liter 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 illustrated 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<sup>-1</sup> diet rations), group (3) was fed with carbohydrate and mercuric oxide (15 mg/kg<sup>-1</sup> diet ration).

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

#### **Hematological Analysis:**

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

#### **Biochemical Analysis:**

The activities of alkaline phosphatase, aspartic aminotransferase (AST) and alanine aminotransferase (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 Merieux, France)

Total serum protein was estimated according to Drupt [12]. Serum cortisol was analyzed by a Gamma counter using <sup>125</sup>I cortisol radioimmunoassay 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 dried in hot air oven. Sulphuric acid and hydrogen peroxide were added on samples then heated until the mixture became transparent after performing a wet ash digestion according to the method of Issac and Kerber [14].

#### **Identification 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 tryptic soy agar (oxide). The plates were incubated at 22°C and inspected daily for up to 4 weeks.

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

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

#### **Water samples:**

Two water samples were collected from River Nile (Hawamdy) 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 Hehvan 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 mercuric 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 mercuric oxide for 4 weeks) than in group 1 (control) and group 2 (fish fed 10 mg mercuric), this result agrees 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 15 mg vanadium) than in group 1 (control). Hypercholesterolemia 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 leads to increased serum levels of alkaline phosphatase, AST and ALT among group 3 (fish fed on 15 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 mercuric oxide on serum cortisol level in African cat fish (*Clarias 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 mercuric oxide level in the diets fed to (*Clarias gariepinus*) was positively correlated to hemoglobin (Hb) levels and haematocrit (Ht). A marked decrease in the Hb 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 activated acetylcholinesterase of erythrocytes [22, 23]. Further more Pickering and 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 mercuric oxide) were significantly increased respectively than those recorded in the group 1 (control). This retention may be attributed to kidney dysfunction, whereas, the kidney is the normal pass for sodium and potassium.

Kidney dysfunction may also explain the increase in serum urea and creatinine especially in group 3, 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 mercuric 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<sup>-1</sup>) [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 mercuric [26]

Table (6) showed that, the bacterial isolates and counts were increased by feeding the fish with CHO and mercuric. The carbohydrates affect immunity and resistance to infection as recorded by Waagbo *et al.* [19]. Utility of vanadate, mimetic 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 cortisol which decreases the host immunity.

In the course of experiment, a high concentration of mercuric 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 mercuric 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 lighting of the skin, scale loss and haemorrhages, 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 mercuric 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 mercuric oxide**

Ingredients	Diet Control	Diet 2	Diet 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: Mercuric oxide concentration in water samples collected from two areas in Egypt.**

Areas	SampeNo.	Concentration of mercuric p.p.m
Hawamdya	1	1.05
	2	1.28
Ak-Kasr El-Aini	3	0.156
	4	0.164

**Table 3: Changes in body weight in African cat fish (*Clarias gariepinis*) fed on different levels of dietary carbohydrates in addition to mercuric oxide.**

Weight / Group	Group 1	Group 2	Group 3
Initial body weight (g)	70±0.15	85±0.17	94±0.20
After 2 weeks (g)	100±0.45	102±0.24	97±0.62
After 4 weeks (g)	155±0.26	124±0.64	95±0.60*

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

Groups	Muscles	Spleen	Heart	Kidney	Liver
Group1	0.24±0.14	0.52±0.83	0.64±0.49	3.15±0.72	2.11±0.69
Group2	0.43±0.25	0.51±0.71	0.70±0.41	4.00±0.83	3.11±0.70
Group3	0.55±26	0.82±0.41	0.83±0.24	6.74±0.74	6.13±0.05

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

Parameters/Group	Group 1	Group 2	Group 3
Hemoglobin g/dl	36.20±0.26	36.2±0.28	31.5±0.25*
HCT %	0.82±0.22	0.95±0.11	1.40±0.68*
Cortisol ng/dl	9.6±0.62	92±0.26	81±0.77*
Phosphorous mg/dl	122±1.24	111±0.75	103±0.14*
Sodium M.EQ	6.23±0.82	6.02±0.44	61±0.74*
Potassium M. EQ	20.42 ±3.2	21±0.73	26±0.72*
Alkphosphatase U/L	135±0.41	134±0.88	140±0.23*
AST U/L	23±0.17	25±0.74	36±0.28*
Cholesterol mg	140±0.25	145±0.13	161±0.54*
Total protein g/dl	8.2±0.76	8.02±0.81	7.02±0.72*
Urea mg/dl	2.1±0.78	3.4±0.76	4.8±0.23*
Creatinine mg/dl	0.75±0.23	0.72±0.76	0.90±0.52*

**Table 6: Bacterial isolates recorded from the examined fish**

Groups	Bacterial isolates	Site of isolation	Bacterial count
<b>Group 3 (n= 10)</b>	-E. Coli	-Muscles	----
	-Streptococcus	-External surface, Stomach	2X10 <sup>3</sup>
	-E. Coli	Gills	----
	-Aeromonas	Gills, Stomach	6.6X10 <sup>4</sup>
<b>Group 2 (n= 10)</b>	-Enterobacter	Liver, Kidney	1X10 <sup>3</sup>
	-Pseudomonas	-Spleen, Muscles	4X10 <sup>3</sup>
	-Fluorescences	-Stomach	2X10 <sup>6</sup>
	-Lactobacillus	-Gills	----

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