Effect of Titanium oxide toxicity on Biochemical, Haematological and clinicopathological Changes in *Clarias lazera* Present in the River Nile

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Abstract: The effect of dietary carbohydrates and titanium oxide on haematalogical profile, blood chemistry and hormonal level was studied in cat fish *Clarias lazera*. Fish were divided into 3 groups (n=10) ant exposedto different doses of titanium oxide and carbohydrate. Groupi was served as control. Group 2 was fed with carbohydrate and titanium oxide (10 mg Kg^{-1} diet ration), group 3 was fed with carbohydrate and titanium 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 r group (3), also there is a significant decrease in serum phosphorous, sodium and potassium in treated fish. There is a significant high level of titanium content in kidney muscles, heart and spleen in group (3) suggesting toxic effects of titanium on cat fish *Clarias lazera*. The total viable count of bacteria identified higher in fish fed on carbohydrate titanium. Predominate bacteria were identified as Aeromona, E. coli, Streptococcous, Pseudomonas, Fluorscences and Lacto bacilus species. We emphasize the finding that increase carbohydrate concentration causes harmful pathological effects which reduces humoral immure responses and enhances dietary titanium 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 palatabie and easily digested food which is rich in vitamins, calcium, phosphon.rs 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.

Titanium is a rare, element found combined in certain minerals and used mainly to produce certain alloys. Most of the vanadium (about 80%) produced is used as ferrovanadium or as a steel additive. Mixed with aluminiurn in titanium alloys is used in jet engines and high speed air-frames and steel alloys are used in axles, crankshafts, gears and other critical components. Titanium oxide (Ti_2O_5) is used as a catalyst in manufacturing sulfuric acid and in making ceramics. It is added to glass to produce green or blue tint [1]

Titanium oxide is never found unbound in nature. Titanium oxide occurs in carbon containing deposits such as crude oil, coal, oil shale and tar sands. Titanium is abundant in most soils, in variable amounts, especially in areas where chemicals or petrochemicals complex were located, where these areas showed a significant increase in its concentration [2].

Humans may be exposed to excessive vanadium in several situations for example, overconscription of vanadium-rich foods (e.g. seafood) [3], ingestion of certain dietary regimens specially that of body building, or inhalation of vanadium-rich environmental pollutants in certain occupations including boilermakers and power plant workers, who are often exposed to high levels of vanadium-rich compounds at work.

Titanium was first discovered in 1971 as a trace element that is essential for normal growth. Since then, Titanium has been found to regulate the activity of various enzymes that induce pronounced changes in metabolic function.

Because titanium is vasoactive, individuals expose to excessive titanium may develop adverse vascular effects [4] especially pulmonary vascular diseases [5] as well as nonoparticulate of titanium oxide potential titanium toxicity in human cells [6] and Niclel and titanium rich pollutant dust could be responsible for therespiratory problems reported [7].

Titanium is one of the eight most abundant in the earth's crust and consequently enters the food chain to some degree. Human are estimated to consume approximately 300g/titanium/day in (Dundord et al., 1997). Moreover, TiO2 accounts forabout 70% of the total volume of pigment production world wide (Bann et al., 2006).

The Federal Regulations of US Government limit usage of TiO2 In food products to 1% by weight (Ghoropade et al., 1995 and Wang et al., 2007). Oral routs is a potential exposure route for general population due to TiO2 used as white pigment on tooth paste, drug capsule, in tableted drug products (Ghoropade et al., 1995), in dairy based products as a whitener in manufacture of different types of cheese (Leone, 1973), dairy base drinks, chocolate, milk, coca, soybean products, milk powder, margarine, processed meat, table and soda water, sausage casing in bread flour and in the confectionary. Also, TiO2 therapeutically used in sunscreens and cosmetic creams. There have been a relatively few systematic studies that have employed pigmentary TiO2. Wang et el. (2007) reported that, until now, most studies on TiO2 toxicity in mammals were focused on the pulmonary impact of inhaled. Mahrousa (2004) reported that 4mg/kg body weight of TiO2 for 90 days in rates resulted in non significant change in DNA, and RNA content in liver and testis

Chronic exposure to titanium oxide dust and fumes may cause severe irritation of the eye, skin, upper respiratory tract, persistent inflammations of the trachea and bronchi, pulmonary edema and systemic poisoning. Signs and symptoms of overexposure include; conjunctivitis, nasopharyngitis, cough, labored breathing, rapid heart beat, lung changes, chronic bronchitis skin pallor, greenish-black tongue and an allergic skin rash[1,7].

In animals, titanium oxides cause inhibition of certain enzymes, which has several neurological effects. Next to the neurological effects vanadium can cause breathing disorders, paralyses and negative effects on the liver and kidneys. Laboratory tests with test animals have shown that 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 vanadium strongly bioaccumulates, which can lead to concentrations of about 10^5 to 10^6 times greater than the concentrations that are found in seawater [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 titanium on catfish *Clarias lazera*. It's haematological, biochemical and hormonal parameters were studied as well as the bacteriological and clinopathological investigation.

2. Materials and methods

Experimental design:

Thirty catfish *Clarias lazera* were used to assess the effects of titanium oxide. Fish weighting from 180-250 were obtained from Nile revier and 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 with a commercial fish diet[9], the experiment was determined after 4 weeks. Fish were divided into three groups (n=10) and exposed to different doses of titanium oxide and carbohydrate. Group 1 was served as control, group2 was fed with carbohydrate and titanium oxide (10 mg kg-1 diet rations), group 3 was fed with carbohydrate and titanium 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.

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 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 scrum 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 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 typtic soy agar (oxide).The plates were incubate at22^{O}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 f samples were collected from River Nile (Hehvan) as well as two water samples from any heavy metal pollution El-Kasr El-Eini (control) were analyzed for titanium concentration byatomic absorption spectrophotometer.

3. Results:

Data in Table I showed that, the titanium 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 titanium in nature and its use in various industrial processes has increased its inputs in the environmental. From the present study it is clear that the low titanium levels were reported in water samples collected from areas far from industrial discharges, while high titanium 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 1.5 mg titanium for 4 weeks) than in group 1 (control) and group 2 (fish fed l0 mg titanium), this results agree with that reported by Khalaf-Allah [18].

The results present in Table 6 showed the comparison of cholesterol levels between 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 titanium) and among group 2 (fish fed 10 mg titanium) compared to 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 catfish *Clarias Lazera* declared these necrotic changes [18]. In addition, exposure of fish to environmental pollutants might result h stimulation or depression of the enzyme activity depending on the concentration of pollutant and the duration of exposure [19, 20].

Regarding the effect of titanium on serum cortisol level in cat fish *Claias lazera* highest level was obtained in group 3 (fish fed on 15 mg titanium) 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 6, it is clear that elevation of titanium level in the diets fed to *Clarias lazera* 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 titanium, respectively. Reduced Hb reflect 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 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 titanium) were significantly increased respectively than those recorded in the group 1 (control). This retention maybe attributing to kidney dysfunction, whereas, the kidney is the normal pass for sodium and potassium.

This 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 6 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. 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 titanium. Also, Hilton and Better recorded a significantly reduced growth and increased mortality among feeding diets of titanium (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 titanium [26]

Table 4 showed that, the bacterial isolates and counts were increased by feeding the fish with CHO and titanium. 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 titanium may be related to the increased level of corlisol which decreases the host immunity.

In the course of experiment, a high concentration or titanium levels has been found in kidney, liver, spleen, heart and muscles of catfish *Clarias lazera* fed 15 mg titanium (Table 5). 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 titanium in kidney liver and other organs of catfish as the concentration of titanium 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 titanium content in the muscle then increased in all tissues [20, 25, 26] The capability of titanium to be present in fish muscle is of particular interest in assessing the exposure of man to environmental titanium as ingested by food.

Clinicopathological observations:

Abnormal swimming darking 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 titanium oxide 15mg (group 3) but not in fish exposed to titanium 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: titanium concentration in water samples collected from two areas in Egypt.

Areas	No	Concentration of titanium p.p.m
Helwan	1	1.05
	2	1.28
Ak-Kasr El-Aini	3	0.156
	4	0.164

Table 2: Ingredients and Proximate composition of diets used in the experiments with titanium supplementation

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
Titanium Mg	-	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, Sn 60mg

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

Group	Group 1	Group 2	Group 3
Initial body weight g	74±0.15	86±0.16	93±0.23
After 2 weeks g	109±0.45	101±0.23	96±0.67
After 4 weeks g	150±0.27	123±0.63	94±0.63*

Table 4: Bacterial isolates recorded from the examined fish

No of examined fish 10/group	Bacterial isolates	Site of isolation	Bacterial count
Group 3	-Aeromonas	Kideny, spleen, muscle	4X104
	-E. Coli	-Muscles	-----
	-Streptococcus	-External surface, Stomach	3X103
	-E. Coli	Gills	----
	-Aeromonas	Gills, Stomach	5.6X104
	-Lactobacillus	Gills	
Group 2	-Enterbacter	Liver, Kidney	4X103
	-Pseudomonas	-Spleen, Muscles	5X103
	-Fluroscences	-Stomach	3X106
	-Lactobacillus	-Gills	----

Table 5: The mean titanium concentration in the organs of fish mg/g net weight

Groups	Muscles	Spleen	Heart	Kidney	Liver
Group1	0.28±0.14	0.51±0.83	0.68±0.49	3.25±0.72	2.21±0.69
Group2	0.47±0.25	0.61±0.71	0.73±0.41	4.20±0.83	3.21±0.70
Group3	0.58±26	0.92±0.41	0.87±0.24	7.74±0.74	6.23±0.05

Table 6: Some haematological, biochemical parameters in catfish *Clarias lazera* on different levels of dietery carbohydrates in addition to titanium oxide

Group	Group 1	Group 2	Group 3
Parameters	6.5±0.23	6.5±0.23	6.52±0.12*
Hemoglobin g/dl	37.2±0.27	37.2±0.27	32.5±0.24*
HCT %	0.83±0.21	0.94±0.10	1.50±0.67*
Cartisol ng/dl	9.7±0.64	9.3±0.27	82±0.67*
Phosphorous mg/dl	123±1.24	112±0.75	102±0.14*
Sodium M.EQ	7.23±0.82	7.02±0.44	62±0.74*
Potassium M. EQ	21.42 ±3.2	22±0.73	27±0.72*
Alkphosphatase U/L	134±0.41	132±0.88	143±0.23*
AST U/L	22±0.17	24±0.74	37±0.28*
Cholestrol mg	143±0.25	148±0.13	171±0.54*
Total protein g/dl	9.2±0.76	9.02±0.81	8.02±0.72*
Urea mg/dl	3.1±0.78	34±0.76	4.7±0.23*
Creatinine mg/dl	0.77±0.23	0.73±0.76	0.93±0.52*

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