

Effect of Vanadium Toxicity on Biochemical, Haematological and Clinicopathological Changes in *Clarias lazera* Present in the River Nile

¹Mona S. Zaki, ²Nevin E. Sharaf and ³Mostafa H. Osfor

¹Department of Hydrobiology, National Research Center, Cairo, Egypt

²Department of Environmental and Occupational Medicine, National, Research Center, Cairo, Egypt

³Department of Nutrition, National, Research Center, Cairo, Egypt

Abstract: The effect of dietary carbohydrates and vanadium toxicity on haematological profile, blood chemistry and hormonal level was studied in cat fish *Clarias Lazera*. Fish were divided into 3 groups (n=10) and exposed to different doses of vanadium sulfated and carbohydrate. Group1 was served as control, group 2 was fed with carbohydrate and vanadium sulfate (10 mg Kg⁻¹ diet ration), group 3 was fed with carbohydrate and vanadium sulfate (15 mg 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 cortisol, cholesterol, AST, ALT, urea, creatinine and alkaline phosphatase 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 vanadium content in kidney muscles, heart and spleen in group (3) suggesting toxic effects of vanadium on cat fish *Clariious Lazara*. The total viable count of bacteria identified higher in fish fed on carbohydrate vandium. Predominate bacteria were identified as *Aeromonas*, *E. coli*, *Staph aureus*, *Pseudomonas*, *Fluorscences* and *Lacto bacilus* species. We emphasize the finding that increase in carbohydrate concentration causes harmful pathological effects which reduces humoral immune responses and enhances dietary vanadium toxicity.

Key words: *Clarias Lazera* • Vanadium Pollution • Haematological • Biochemical • Clinicopathological • Bacterial count

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, phosphorus 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.

Vanadium 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 aluminium 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. Vanadium oxide (V₂O₅) 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].

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

Vanadium is never found unbound in nature, Vanadium occurs in carbon containing deposits such as crude oil, coal, oil shale and tar sands. Vanadium 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, overconsumption 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.

Because vanadium is vasoactive, individuals exposed to excessive vanadium may develop adverse vascular effects [4] specially pulmonary vascular diseases [5] as well as nanoparticulate of vanadium oxide potentiated vanadium toxicity in human lung cells [6] and Nickel and vanadium rich pollutant dust could be responsible for the respiratory problems reported [7].

Chronic exposure to vanadium pentoxide dust and fumes may cause severe irritation of the eyes, 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, vanadium causes the 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 that 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 vanadium on cat fish *Clarias Lazera*. Its haematological, biochemical and hormonal parameters were studied as well as the bacteriological and clinicopathological investigations.

MATERIALS AND METHODS

Experimental design: Thirty cat fish *Clarius Lazera* were used to assess the effect of vanadium sulfate. Fish weighing from 180-250g 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 with a commercial fish diet [9], the experiment was determined after 4 weeks. Fish were divided into 3 groups (n=10) and exposed to different doses of vanadium sulfated and carbohydrate.

Group1 was served as control, group 2 was fed with carbohydrate and vanadium sulfate (10 mg Kg⁻¹ diet ration), group 3 was fed with carbohydrate and vanadium sulfate (15 mg Kg⁻¹ diet ration).

Mean of the initial body weight of each examined fish was at the beginning of the experiment then after 2-4 weeks 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 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 125 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 spectrophotometry [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 (oxid). The plates were incubated at 22°C and inspected daily for up to 4 weeks.

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

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

Water samples: Two water samples were collected from River Nile (Helwan) as well as two water samples free from any heavy metal pollution El-Kasr El-Eini (control) were analyzed for vanadium concentration by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Data in Table 1 showed that, the vanadium level in Helwan 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 vanadium 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 vanadium levels were reported in water samples collected from areas far from industrial discharges, while high vanadium 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 vanadium for 4 weeks) than in group 1 (control) and group 2 (fish fed 10 mg vanadium), 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 15 mg vanadium) than in group 1(control). Hypercholesteremia might be due to necrotic changes occurring in liver with liberation of cholesterol as a by-product of cell destruction. The present data suggest that impaired liver function lead to increased serum levels of alkaline phosphate, AST and ALT among group 3 (fish fed on 15 mg vanadium) and among group 2 (fish fed 10 mg vanadium) compared to group 1 (control). In this concern Khalaf-Allah [18] 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 cat fish *Clarias Lazera* 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].

Table 1: Vanadium concentration in water samples collected from two areas in Egypt

Areas	No	Concentration of Vanadium p.p.m.
Helwan	1	1.04
	2	1.27
Al- Kasr El- Aini	3	0.154
	4	0.163

Table 2: Ingredients and Proximate composition of diets used in the experiment with vanadium supplementation

Ingredient%	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
Vanadium 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 %	5.65	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 2/u, vit K 4 mg, vit B2 3.6, niacin 20 mg., pyridoxine 0.2 mg, vit B125, Mn 70 mg, Sn 60 mg.

Table 3: Changes in body weight in cat fish (*Clarias Lazera*) fed on different levels of dietary carbohydrates in addition to vanadium sulphate

Groups	Group 1	Group 2	Group 3
Initial body weight g	75±0.15	80±0.16	82±0.23
After 2 weeks g	100±0.45	100±0.23	97±67
After 4 weeks g	140±0.27	120±0.63	91±0.63*

p<0.01

Regarding the effect of vanadium on serum cortisol level in cat fish *Clarias Lazera*, highest level was obtained in group 3 (fish fed on 15 mg vanadium) 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 vanadium level in the diets fed to *Clarias Lazera* 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

Table 4: Bacterial isolates recovered from the examined fish

No of examined fish 10 /group	Bacterial isolates	Site of isolation	Bacterial count
Group 3	-Aeromonas sp	-Kidney, spleen, muscles	3 x10 ⁴
	- E. coli	-Muscles	-----
	- Staph Aureus	- External surface, Stomach	2x10 ³
	- E. coli	- Gills	-----
	- Aeromonas	- Gills, stomach	6.6x10 ⁴
Group 2	- lactobacillus	- Gills	-----
	-Enterbacter sp.	-Liver, kidney	3x10 ³
	-Pseudomonas	-Spleen, muscles	4x10 ³
	-Fluroscences	-Stomach	2x10 ⁶
	-Lactobacillus	-Gills	-----

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

groups	muscles	spleen	Heart	kidneys	Liver
Group 1	0.28±0.13	0.51±0.82	0.68±0.48	3.25±0.72	2.27±0.69
Group 2	0.47±0.24	0.61±0.70	0.73±0.40	4.20±0.83	3.20±0.70
Group 3	0.58±0.27*	0.92± 0.40*	0.87±0.23*	7.74± 0.74*	6.24±0.05

*p<0.01 * Significant

and 10 mg vanadium, respectively. Reduced Hb may reflect metabolic adjustment according to reduced need for oxygen by change in blood pH.

Moyle and Ceeh, Hall and Cliffs recorded, active acetylcholinesterase of erythrocytes [22, 23]. Further more, Pickering and 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 vanadium) were significantly increased respectively than those recorded in the group 1 (control). This retention may be attributing to kidney dysfunction, whereas, the kidney is the normal pass for sodium and potassium. This 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 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].

Table 6: Some haematological, biochemical and hormonal parameters in cat fish *Clarias Lazera* fed on different levels of dietary carbohydrates in addition to vanadium sulphate

Groups	Group 1	Group 2	Group 3
Parameters			
Hemoglobin g/dl	7.5±0.23	7.52±0.14	6.52±0.12*
H.CT %	38.2±0.27	38.4±0.14	31.5±0.24*
Cortisol ng/ml	0.82±0.21	0.93±0.10	1.40±0.67*
Phosphorous mg/dl	9.8±0.64	9.1±0.27	8.01±0.67*
Sodium M.E.Q	121±1.24	114±0.75	101±0.14*
Potassium M.E.Q	7.22±0.82	7.01±0.44	6.1±0.74*
AlkPhosphatase U/L	21.42±3.2	22±0.73	27±0.72*
AST U/L	124±L41	131±0.88	144±0.23*
ALT U/L	22±0.17	24±0.74	37±0.28*
Cholesterol mg	144±0.25	149±0.13	170±0.54*
Total protein g/dl	9.23±0.76	9.01±0.81	8.01±0.72*
Urea mg/dl	3.2±0.78	3.3±0.76	4.8±0.23*
Creatinine mg/dl	0.78±0.23	0.74±0.76	0.97±0.52*

p < 0.01 * Significant n = 30 Fish 10 / group

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 15 mg Vanadium. Also, Hilton and Better recorded a significantly reduced growth and increased mortality among the fish feeding diets of Vanadium (0, 10, 100, 1000 or 10000 mg Kg⁻¹) [26]. The increase in muscles and tissue lactic acid (2-12 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 vanadium [26].

Table 4 showed that, the bacterial isolates and counts were increased by feeding the fish with CHO and vanadium. The carbohydrates affect immunity and resistance to infection as recorded by Waagbo *et al.* [9]. Utility of vanadate, mimetic protein phosphates inhibitors to protect fish from microorganism [27]. The increase of bacterial count among the fish fed on vanadium may be related to the increased level of cortisol which decreases the host immunity.

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

Clinicopathological observations: Abnormal swimming, lighting of the skin, scale loss and haemorrhages, were seen on the external body surface. In addition to congestion of gills, eyes, mouth, liver, kidney, spleen and intestine. This was noticed in fish exposed to vanadium sulphate 15 mg (group 3) but not in fish exposed to vanadium sulphate 10 mg (group 2).

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

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