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Research Article

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Alterations in Some Bio-Parameters of Adult Male *Clarias gariepinus* (Burchell, 1822) Experimentally Exposed to Vanadium Pentoxide

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Abstract The effect of vanadium toxicity on organosomatic indices, haematological profile, plasma biochemistry and histomorphology on adult male *Clarias gariepinus*. Adult fish were divided into 3 groups (n=12) and exposed to different doses of vanadium pentoxide (V_2O_5). Group 1 served as control, group 2 (Tx 1) was fed with Vanadium pentoxide(V_2O_5) (0.4mg/ml at 10 mg/ Kg IP), group 3(Tx 2) was fed with Vanadium pentoxide (V_2O_5) (4.0mg/ml at 10 mg/Kg IP). Organosomatic indices indicated a significant increase in testes and vesicular gland in Tx 1 and Tx 2 in kidney, in spleen, also gill apparatus in (3). Also, there were dramatic changes in testicular histo architecture in the treatment groups, the testes reveal severe degeneration of germinal epithelium and sertoli cells in the vanadium treated groups. The liver also showed severe glycogenic vacuolation in Tx 1 and 2, with portal congestion in Tx 1 but more severe histopathology was seen in Tx 2. Absolute monocyte values decreased significantly in TX 1 and 2 as well as absolute eosinophil values in Tx 1, there is a significant increase in heterophils in Tx 2 as well as basophils in Tx 1 and 2. There is a significant increase in albumin-globulin ratio in Tx 3, also there is a significant decrease in Alkaline phosphatase in Tx 3. Vanadium Pentoxide administration has seemingly unrelated pathological alterations in biochemical, hematological, the liver and testis and tissues of *Clarias gariepinus*.

Keywords *Clarias gariepinus*, Organosomatic index, Haematology, Biochemical, Histopathology, Vanadium

Introduction

Several investigators both in the developed and developing countries of the world have become interested in the level of heavy metal contamination of the aquatic system over the past few decades [1]. The rate at which trace metals have been on the increase in the Nigerian environment has been of great concern especially regarding their roles in the environment. Part of this concern arises from the impact of these metals on aquatic life and indirectly to man. Some of these potentially toxic metals include lead, zinc, nickel, chromium, arsenic, selenium, vanadium, beryllium and barium [2]. These metals have the ability to accumulate in the environment and cannot be destroyed through biological degradation making these toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic products as sources of food. Aquatic animals can bioaccumulate heavy metals in their tissues and such tissue concentrations of heavy metals can be of public health concern to both animals and humans [1].

As an important component in modern steel production, vanadium, with atomic number 23, is a rare element that was first discovered in 1971 as a trace metal that is essential for normal growth. Widely distributed and never found unbound in nature, vanadium occurs in carbon containing deposits such as crude oil, coal, oil shale and tar sands and

is abundant in most soils and areas where petrochemical complexes are located have showed a significant increase in its concentration. It has been found to regulate the activity of various enzymes that induce pronounced changes in metabolic functions [3].

Vanadium is mainly used to produce certain alloys. For example, it is mixed with aluminium to produce titanium alloy used in jet engines and high speed air-frames, as well as in steel alloys that are used in critical components of automobiles. Vanadium pentoxide (V_2O_5) is used as a catalyst in manufacturing sulfuric acid, added to glass to produce green or blue tint and in making ceramics [3].

Vanadium compounds exert various toxic effects, which are dependent on the oxidation state and circulating levels of vanadium although it has been shown that V5+ seems to be more toxic than $V4^+$ and $V3^+$ [4]. Other factors that affect toxicity include the nature of species, dose, route and duration of administration. Animal studies have revealed various toxic effects induced by vanadium compounds with the most affected organs, as indicated by histopathological alterations, been the liver and kidney [5]. In addition, intraperitoneal injections of rats with orthovanadate induced nephrotoxicity [6]. The reproductive and developmental functions of rats have also been well established to be affected by vanadium. Signs of acute vanadium toxicity include dehydration, reduction of body weight, loss of appetite, renal tubular necrosis and pulmonary haemorrhage. Vanadium participates in reactions involving formation of reactive oxygen species and free radicals [7].

Vanadium can be found in fry fishes and many other species. In mussels and crabs, vanadium strongly bioaccumulates to concentrations of about 105 to 106 times greater than the concentrations that are found in seawater [8]. The net accumulation of a substance from water into an aquatic organism resulting from the simultaneous uptake and elimination of the substance is known as bioaccumulation. Aquatic organisms accumulate metals to concentrations many times higher than it is actually present in the water.

African catfish (*Clarias gariepinus*) is the most common, commercially accepted, fresh water fish in Nigeria [1]. Consumption of African catfish is high in Nigeria and the awareness by both farmers and consumers is rising. This resultant increase in local production of Catfish using commercial earthen ponds can attract increased contamination by surface run-off of vanadium contaminated soil. Vanadium pentaoxide from crude oil deposits and some industrial waste can also contaminate water bodies.

This study was carried out to evaluate the effect of vanadium on the brain and selected organs of adult male African catfish (Clarias gariepinus).

Material and Methods

Experimental Design

Twelve adult male African catfish (*Clarias gariepinus*) were used in this study. Adult ACF weighing from 250-400g were obtained from local farmers and kept in an experimental plastic aquaria supplied with dechlorinated water at 30 liters per tank for each group. Adult fish were acclimated to the laboratory conditions for 8 days before the beginning of the experiment and were fed with commercial pellets. The fish were divided into 3 groups with four (4) catfish per group. Group1 served as control (Cx) and was dosed group 2 (Tx1) was dosed with vanadium pentoxide (V2O5) at 0.4 mg/ml at 10 mg/ Kg intraperitoneally and group 3 (Tx2) was dosed (V_2O_5) at 4.0 mg/ml at 10 mg/Kg intraperitoneally. All groups were dosed daily for seven (7) days. Individual body weights of each fish was taken at the beginning of the experiment and daily until the end of the experiment.

Gross Examination and Organosomatic (OS) Indices

The fish and the organs were closely observed for any gross alterations. The gross weight of each fish at sacrifice were determined using a digital balance. After sacrifice by cervical dislocation, the testes, brain, liver, heart, kidneys, spleen, skin sections, pseudo branchial organ and gill apparatus were removed from each fish, wiped dry and weighed on the digital balance and the organosomatic index (relative organ- carcass weight) determined using the formula: OS Index =(weight of organ/weight of carcass x 100)%.

Histomorphology

The testes, brain, liver, heart, kidneys, spleen, skin sections, pseudobranchial organ and gill apparatus obtained from each fish were fixed in Bouin's fluid for 24hrs and then transferred to 70% ethanol. They were routinely processed, using paraffin-wax embedding method, and stained with Haematoxylin and Eosin (H&E).

Hematology and Biochemistry

Blood samples were collected from the caudal vein after 7 days of exposure. Each sample was divided into two parts the first one was heparinized for haematological investigation, while the second was centrifuged at 3000 rpm for 5 minutes to obtain plasma for biochemical studies. Haematological studies were performed according to Sandnes *et al* 1988 [9], where a complete haematological profile was obtained. The activities of alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), urea and creatinine level were determined according to the method of Varley *et al*. [10] by using commercial kits(Bio Merieus, France). Total plasma protein was estimated according to Drupt [11].

Statistical Analysis

All data obtained were subjected to one-way analysis of Variance (ANOVA) at a P-value <0.05 using the Graph Pad Prism 4 software.

Results

The organosomatic indices increased significantly across group (2) in the kidney (Renosomatic index), Spleen (Splenosomatic index), Gill apparatus (gill apparatus-carcass index). The organosomatic indices of the testes & vesicular gland that had a significant increase across group (2) and (3) at p<0.05. While the organosomatic indices of the other organs: heart, liver, brain and the pseudo branchial organ remained insignificant at p>0.05

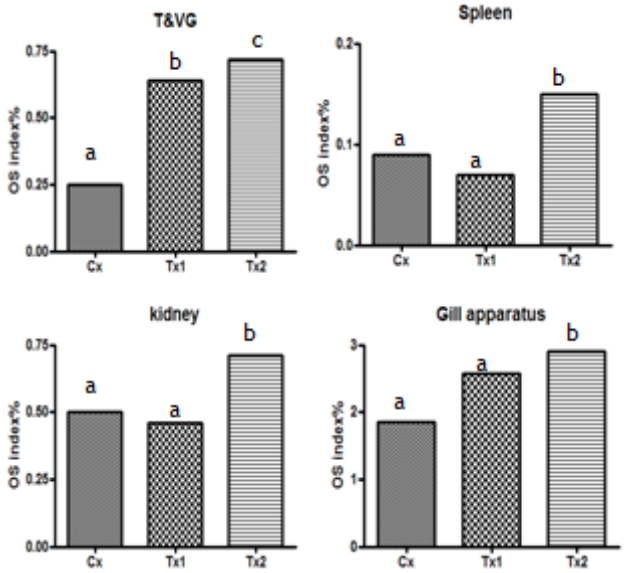

Figure 1: Organosomatic indices (%) of Testes &Vesicular Gland, splenosomatic, renosomatic, gill apparatus of Vanadium-treated male AfricanCatFish Values with different superscripts are significantly different at p < 0.05 Cx: untreated (control) .Tx1: Vanadium-treated.(0.4 mg/ml) Tx2: Vanadium treated (4 mg/ml)

Histomorphology

At gross observation, the carcasses were in good body condition. No apparent alteration in colour or consistency of organs across all the groups. At histology here was a severe disruption of sertoli cell and germinal epithelium in Tx1 and Tx2 (Figure 2) There were also severe glycogenic vacuolation observed in the liver (Figure 3) though this changes did not follow a dose dependent manner in the two affected organs of both treated groups. No visible lesions were seen in other organs.

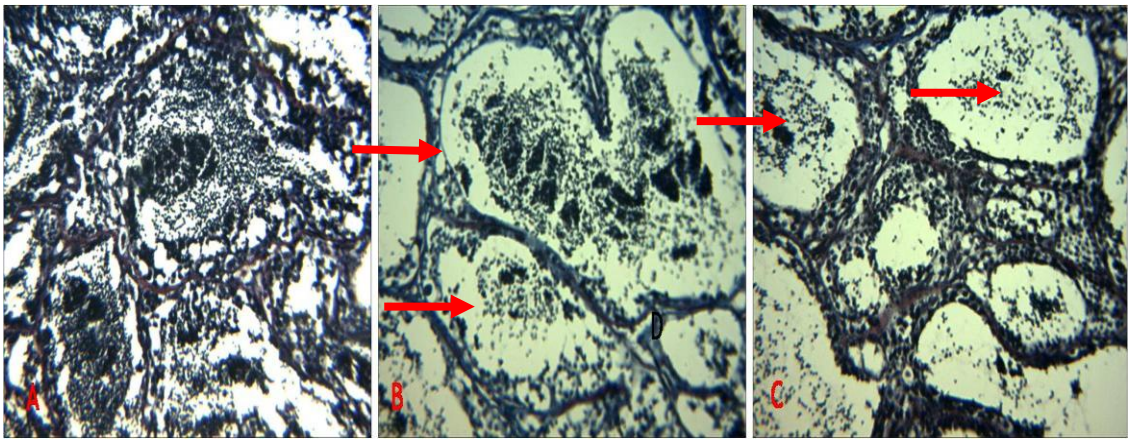

Figure 2: Light micrograph of Testes of vanadium-treated African Catfish A: Cx showing no visible lesions, B: Tx 1(0.4mg/ml) and C: Tx 2 (4mg/ml) showing severe disruption of sertoli cell and germinal epithelium (arrows) (MT) M X100

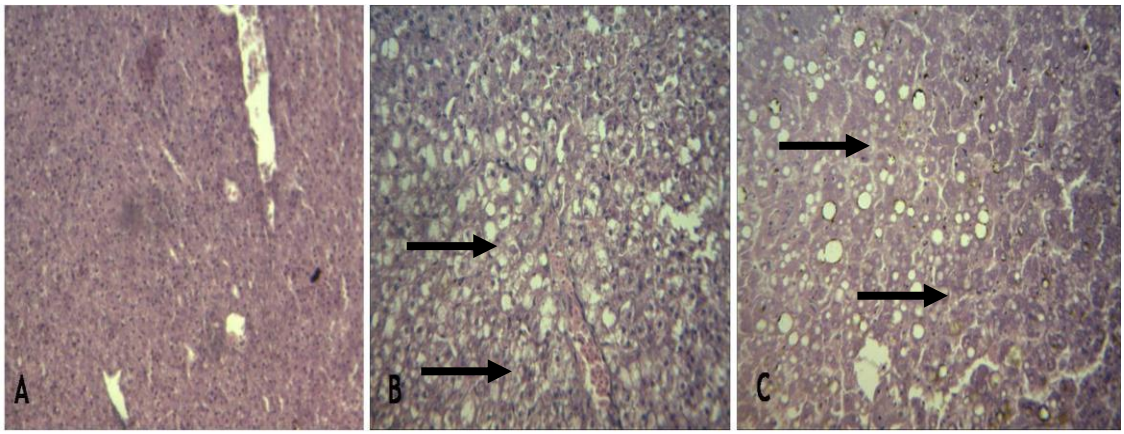

Figure 3: *Light micrograph of the Liver of vanadium-treated African Catfish A: Cx untreated showing no visible lesions,* ***B****: Tx 1(0.4mg/ml) and C: Tx 2 (4mg/ml) showing severe glycogenic vacuolation of thehepatocytes(arrows) (H&E) M X100*

Haematology

The overall effect of vanadium on the haematological profile did not present a direction. Changes were significant in a few parameters, like the monocyte that there was a significant reduction in its value at $p<0.05$ across groups Tx 1 and Tx 2 when compared with control group. There were also increased heterophil values for the 4mg/kg vanadium traeted group. The basophil values of both treated groups were significantly higher than control untreated, the eosinophil value at 0.4mg/kg was significantly lower compared to control untreated.

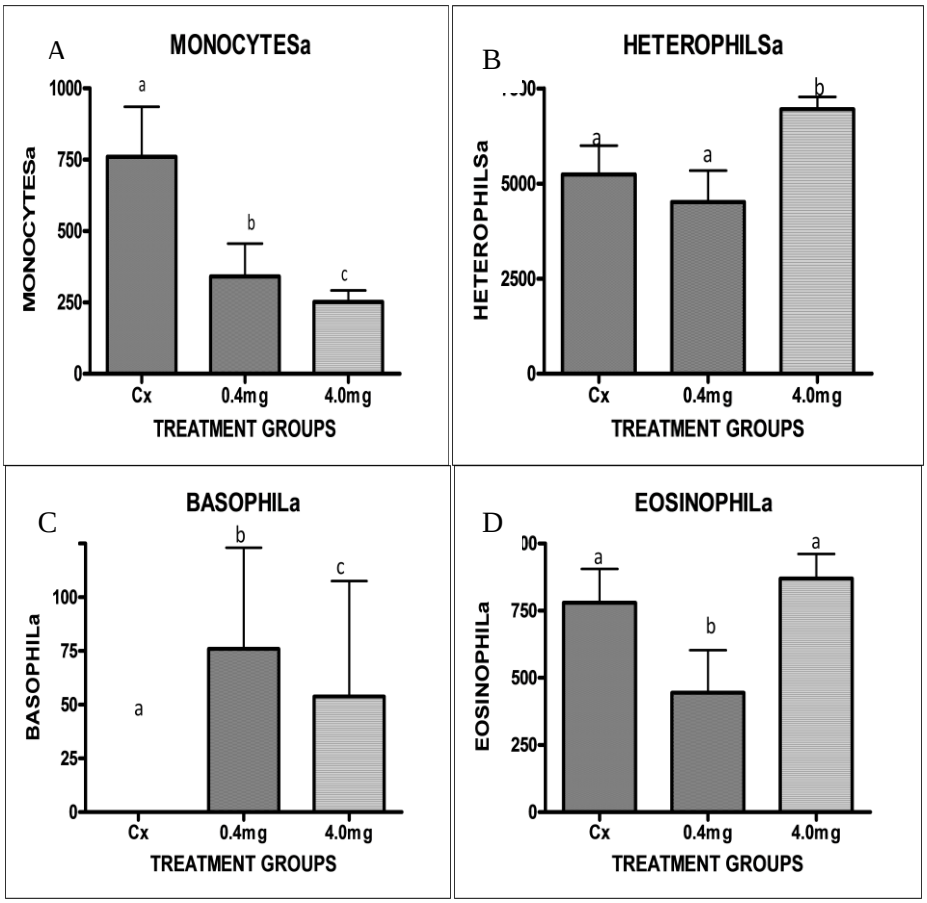

Figure 4: Hematology of African Catfish treated with 0.4 mg/kg and 4mg/kg Vanadium showing (A) significant reduction in absolute monocyte values across the treatment gradient (B) significant increase in absolute heterophil values at treatment 4.0mg/ml as compared to the control untreated group; (C) Significant increased in eosinophil values of the treated groups compared to control untreated; (D) significant reduction in the eosinophil value at 0.4mg/kg compared to control

Plasma Biochemistry

The plasma biochemistry revealed a significant decrease in plasma enzyme ALP. Albumin Globulin ratio was significantly increased at group (Tx 2).

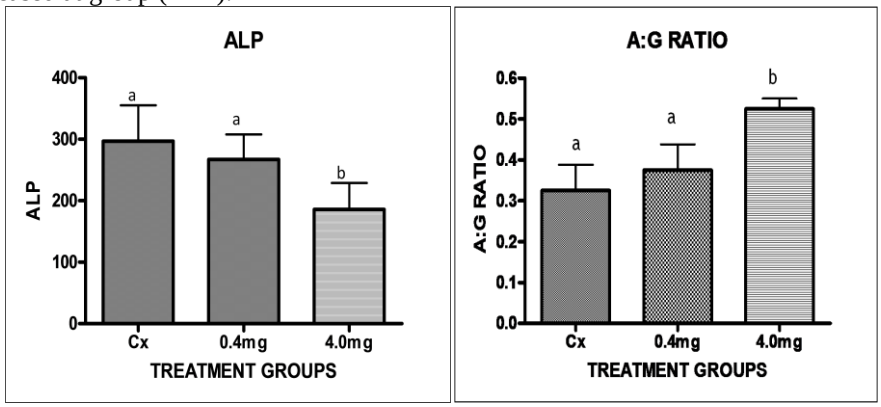

Figure 5: Haematology of African Catfish treated with 0.4 mg/kg and 4mg/kg Vanadium showing (A) a significant decrease in ALP in 4.0mg treatment group as compared to the control and Tx 1 (0.4mg/kg group. (B) Change in Albumin: Globulin ratio was significant at 4.0mg treatment group. Values with different superscript are significantly different at P<0.05

Discussion

Reduced ALP enzyme levels, with heightened AG ratio does not present a pattern of liver disease, but the results show that there is alteration in liver function in the 4.0mg/kg group. Though absolute values of some cells significantly changed, the general RBC and WBC values were not deviant with the control. The other non dramatic alterations in the haematology and plasma biochemistry profile of the vanandium treated African Catfish compared with the results seen in *Clarias lazera* gives a hint to the possible resilience of African catfish *Clarias gariepinus* as against *Clarias lazera* with respect to individual responses vanadium toxicity [12]

The drastic testicular changes observed can be associated with reproductive disturbances or infertility in the male. The non uniform glycogenic vacuolation observed in the liver is indicative of a compromise in the efficiency of fat mobilisation by the treated liver. The significant changes in normal structures that were recorded in the vital organs is likely related to pathology in animals and humans exposed to vanadium but may be at a different level of exposure. Hence, the effect of exposure may be multi organ dysfunctions as partly seen in this experiment. These effects however will be seen in long term exposures to low doses of vanadium. It is expedient to understand that the most significant effect in the various vital organs of the exposure of vanadium occurs after a long time.

In the course of experiment, significant pathology was recorded in the liver and testes, of African cat fish *Clarias gariepinus* injected with 4.0mg/ml of vanadium. 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 fry fish as the concentration of vanadium in the tissues increased with its concentration in the aquatic environment and exposure time [13].

This research has shown that continued exposure of the African cat fish *Clarias gariepinus* to high concentration of vanadium can cause a number of pathology to the health and performance of the fishes. Also, because it is a well accepted protein source for local consumers in developing countries like Nigeria and its enormous commercial potential, consumption of fishes exposed to vanadium in human may be harmful, may probably have the same effects as in the fishes. Commercial farmers may however run at a loss if vanadium has so much effect on the testes of the male African catfish in terms of reproduction.

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