

Full length Research Article

Neurobehavioural and Histological Study of the Effects of Low-Dose and High-Dose Vanadium in Brain, Liver and Kidney of Mice

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Summary: Vanadium is a ubiquitous transition metal that has been generating contrasting research interest. Therapeutically, vanadium possess antidiabetic, antitumor, antiparasitic and even neuroprotective activities. On the flip side, vanadium has been reported to cause multisystemic toxicities with a strong predilection for the nervous system. Despite several reports on potential benefits of low-dose vanadium (LDV) and toxic effects of high-dose vanadium (HDV), there are no comparative studies done thus far. This study therefore explored the comparative effects of LDV and HDV exposure in mice during postnatal development. A total of nine (9) nursing mice were used in this study; with three nursing mice and their pups (n = 12 pups per group) randomly assigned to each of the three test groups. The nursing dam were given intraperitoneal (i.p) injection of vanadium at 0.15mg/kg and 3mg/kg for LDV and HDV respectively, and subsequently to the pups from postnatal day (PND) 15 till sacrifice on PND 90. We discovered that neurodevelopmental motor function test of mice-pups exposed to LDV here showed improved motor development, muscular strength and memory capacities whereas HDV led to motor function impairment, reduced muscular strength and memory capacities. LDV-exposed mice showed mild histological lesions in cerebral cortex whereas high-dose showed distinct histological lesions in different parts of the brain ranging from cerebellar Purkinje neuronal pathology (central chromatolysis), pyramidal neuronal loss in CA1 region, architectural distortion as well as fewer neurons in olfactory bulb. We saw mild lesions with LDV in both liver and kidney, however, with HDV exposure, there was diffuse hepatocellular vacuolar degeneration and congestion of blood vessels in liver, shrinkage of renal glomerulus and degenerated epithelial cells of kidney. Conclusively, beneficial effect of vanadium is proven as it facilitated body weight gain which translate in organ weight at low-dose, while high-dose caused decreased neurobehaviour and histological lesions.

Keywords: Histological study, Neurobehavioral tests, High-dose vanadium, Low-dose vanadium

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INTRODUCTION

Vanadium is classed as a transition metal that is present in nearby environmental sources like water, soil, air and also in the biological system of living organism (Pessoa *et al.*, 2015). In the commercial world, vanadium is used in manufacturing and processing of pesticides, sulphuric acid, hardening of steel and as a catalyst in the production of many materials (Fatola *et al.*, 2019). Exposure of vanadium happens from various sources such as by-products of burning fuel-oils laden with vanadium (Amorim *et al.*, 2007) and mining of heavy metals (Moskalyk and Alfantazi, 2003). Small amounts of vanadium are beneficial to the growth and development of animals and its deficiency in mammals inhibits growth, impairs the generative functions,

thyroid metabolism and bone mineralization, and disturbs the lipid and carbohydrate balance (Nilsen and Uthus, 1990 and Facchini *et al.*, 2006). Therefore, vanadium is a necessary ingredient of the daily diet (French and Jones 1993; Rojas *et al.*, 1999; Moskalyk and Alfantazi 2003). The following properties: antibacterial, anti-carcinogenic, spermicidal, anti-viral, anti-parasitic, anti-HIV, antituberculosis, anti-atherosclerotic, anti-hypertensive, as well as anti-thrombotic have been further documented as uses of vanadium compounds (Omayone *et al.*, 2020). However, the major focus in their use is for anti-diabetic drugs because they are known to exhibit hypoglycaemic property (Huang *et al.*, 2014; Novotny and Kombian, 2014; León *et al.*, 2014; Rozzo *et al.*, 2017; Jaiswal and Kale, 2019; Treviño *et al.*, 2019).

On the other hand, exposure to larger dose of vanadium poses toxic risk to health as there have been reports of the toxicity in both humans (Rehder, 2013) and animal models (Olopade *et al.*, 2011, Folarin *et al.*, 2017, Igado *et al.*, 2020). Higher dose of vanadium causes irritation of the eyes and mucous membranes of the upper respiratory duct, coughing, fatigue and depression (Goc, 2006). Also, neurobehavioral deficits such as impaired short-term memory, reduced reaction speed and loss of coordination were reported in workers exposed to vanadium in a steel factory in China (Li *et al.*, 2013). In animal studies, vanadium has been shown to have toxic effects on various organ systems including the nervous, reproductive, gastrointestinal and urinary systems as reviewed (Wilk *et al.*, 2017). Exposure period, concentration and means of administration influence the outcome of vanadium exposure. To date, there is scant information on the neurobehavioral effects and histological study of low-dose exposure to vanadium. This study aimed to compare low-dose (therapeutic dose) and high-dose (toxic dose) vanadium oral ingestion effects on neurobehaviour, body and brain weights and assess effects on cellular architectures of the brain, liver and kidney in the mice after exposure to both high-dose and low-dose vanadium for 90 days (PND 1 – PND 90).

MATERIALS AND METHODS

Animals and Treatments: Pups from nine (9) nursing mice were treated with vanadium for three months (PND 90). They were separated into three groups. We secured all animals from the Animal House, Department of Veterinary Anatomy, University of Ibadan, Nigeria. Vanadium as sodium metavanadate ($\text{Na}_2\text{O}_3\text{V}$) was a product of Santa Cruz Biotechnology, Inc., Dallas. Schematic of the experimental setups is represented in Figure 1.

Control: The nursing dams were given daily intraperitoneal (i.p.) injection of sterile water for duration of two weeks (days 14). The pups at PND 15 started receiving sterile water daily up to PND 30. Afterwards, the pups received i.p. injection of sterile water every 72 hours (2 days interval) till sacrifice on PND 90.

Low-dose: The nursing dams were given daily intraperitoneal (i.p.) injection of vanadium 0.15mg/kg for duration of two weeks (14 days). The pups at PND 15 started receiving vanadium daily up to PND 30. Subsequently, the pups received intraperitoneal (i.p.) injection of vanadium every 72 hours until sacrifice on PND 90.

High-dose: The nursing dam were given daily intraperitoneal (i.p.) injection of vanadium 3 mg/kg for duration of two weeks (14 days). The pups at PND 15 started receiving vanadium daily up to PND 30. Afterwards, the pups received i.p. injection of vanadium every 72 hours until sacrifice on PND 90.

Body and Organ Weight: Every day we documented each pup's body weight from PND 1 – PND 30 and thereafter every 72 hours until they reached PND 90. The relative brain weights of all the three groups during sacrifice at PND 90 were harvested and weighed immediately, and measured based on the method described by Bailey *et al.*, (2004) and Igado *et al.*, (2020). The relative brain weights were later calculated as given below and then expressed in percentage.

$$\frac{\text{Brain Weight}}{\text{Body Weight}} \times 100$$

Cliff Aversion Test: Assessment of locomotor prowess as well as body strength of PND 2 and PND 7 mice was carried out using cliff aversion examination (Feather-Schussler and Ferguson, 2016). The mice pups were placed at the brim of a box without the nostrils or forearms touching but using just the elbows to hang. The time it takes each pup to face away from the box brim was documented. This assessment is repeated should a pup slip off the brim but interrupted if a pup does nothing in 30 seconds. The interruption was necessary to prevent the pups from getting accustomed to the test so as not to hamper analysis and the affected pup said to have failed the assessment.

Negative Geotaxis Test: Mice pups were evaluated at PND 2 and PND 7 using a plane, 45° inclined to the surface. We set the pups to face downward on the plane. After holding for five seconds, the time taken by the pup to face up the inclination (full 180° turn) was recorded. 30 seconds maximum period was allowed. This latency to turn is a natural reaction by the pup against gravitational pull.

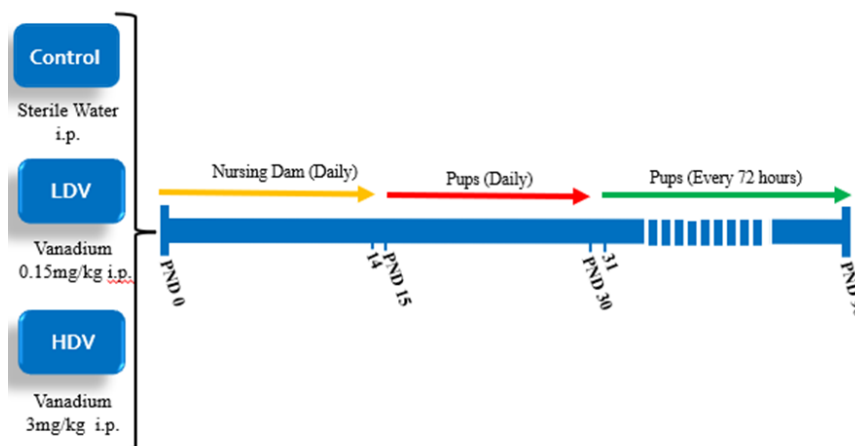


Figure 1:
Experimental setups

Forelimb Grasping Test: Evaluation of muscle strength and endurance involved subjecting the pups to forelimb grasping test at PND 14 and PND 21. A one-millimeter (1 mm) diameter wire was suspended horizontally while the pups were made to grip it with their forelimbs. The wire was positioned 50 cm above a soft-landing surface, and the time until losing grasp of the wire (Latency to drop) was noted (in seconds); a maximum cut-off time of 120 seconds was set.

Morris Water Maze: The Morris water maze is an evaluation tool designed to assess spatial learning and memory (Folarin *et al.*, 2016). The test set up is a black circular tank measuring 110cm in diameter and 30cm in height. The tank was topped with water up to the 30cm level and we maintained a room temperature of 26°C. A hidden platform measuring 10 cm in diameter was utilized as the escape target. Eagerness of mice getting away from a water-lodged area using the shortest course forms the basis of this test. The tank tagged South (S), West (W), North (N) and East (E) had a platform concealed at a definite location. The period taken by a mouse, when placed in the tank of water, to locate such concealed platform was noted. Inability to discover platform in a 60-second period resulted in such mouse being led to locate and linger fifteen seconds on the platform. Every animal went through a first phase of training trial as well as a second phase of test (probe) in the Morris water maze test. The mice capability in learning the concealed platform's spatial location was assessed in the first phase for 3days in succession. Concealed platform being taken away in the second phase (on day 4), the period taken by the mice to remain within that quadrant of the platform was noted in evaluating the spatial memory.

Hanging Wire Test: A measure for forearm strength is done with the test. 2 beams set vertically carrying a pole placed at a 60cm-distance from a padded floor comprise the set up. We made PND 90 mice to grip the pole at the centre with the forearms. The time (in seconds) it took the mice to drop off the pole was documented. Failure to drop off after 120 seconds would result in the mice being released from the pole. Every mouse underwent this test twice with the mean time documented for eventual assessment.

Organ Sample Collection: Sacrifice of 5 mice in each group by post-natal day 90, was done after behavioural assessment. Every mouse was euthanized with ketamine (100mg/kg b.w.) and perfused transcardially using 10% Neutral buffered formalin (NBF) and brains harvested according to Olopade *et al.*, (2011) method.

Tissue Processing for Microscopic Study: The brain samples went through paraffin-embedding routine process. 5µm-thick sections were produced using a microtome (Microm GmbH, D-6900 Heidelberg, West Germany) and stained with Haematoxylin and Eosin stain to evaluate general histology (Gilbert *et al.*, 2020). Cresyl violet was used to stain the Nissl granules in the brain according to Folarin *et al.*, (2017). Every stained slide was visualized using microscope (Leica Microsystems, Wetzlar, Germany).

Statistical analysis: We fixed the significance level of this study to 95%. and presented data as means \pm SEM. Gradual alterations within various groups were assessed using one-way/two-way analysis of variance. At p -value <0.05 , group variations were regarded significant. Every evaluation was carried out with GraphPad Prism (GraphPad Software, San Diego, version 5.0).

RESULTS

Body and Organ Weights: The effect of low-dose vanadium as compared to high-dose vanadium in mice exposed from postnatal day (PND) one for ninety days was assessed. At sacrifice (PND 90), the body weight of the mice exposed to low-dose was significantly higher than that of controls. Evaluation of relative body weight gain showed no statistical differences during the initial two weeks of postnatal development (from PND 1 – PND 13) across all the three groups. Significant differences in body weight occurred subsequently after two weeks both between LDV and HDV groups and between LDV and control groups (Figure 2).

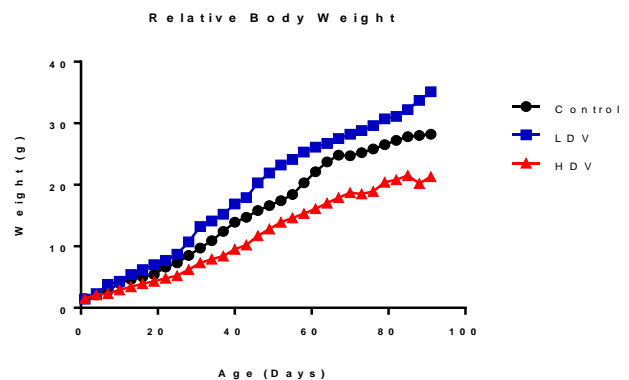


Figure 2:

Line graph showing the average body weight measurements of the mice pups from PND 1 – PND 90 for Control, LDV and HDV groups (n = 12 mice per group). * $p<0.05$, ** $p<0.01$ statistically significant.

We observed across groups the same similarity in the weight of brain upon sacrifice of PND 90 mice. In all the three groups, the brain of the LDV group had a slightly higher weight than control but they are not statistically different. Both LDV and control groups had brain weight significantly higher than HDV group. But there was no statistical difference between LDV and control groups even though LDV had brain weight slightly higher than control (Figure 3).

Hanging Wire Test: The assessment of neurobehavior revealed alteration in locomotor ability in high-dose treated group relative to low-dose exposed group and control at both developmental stage and subsequently at adult stage. In the hanging wire test, the mice in the LDV group had improved muscle endurance while the HDV had decreased muscle strength leading to significantly shortened latency to fall at 3 months of age (PND 90). Low-dose vanadium group performed better than control but it was not statistically significant. Both control and low-dose vanadium treated

group had a statistically significant increase in performance when compared to high-dose exposed group (Figure 4).

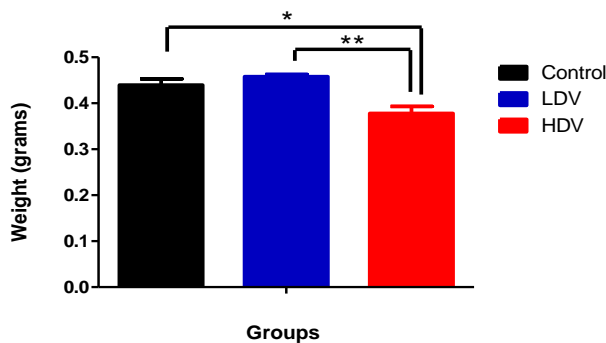


Figure 3: Bar chart graph showing relative brain weight. LDV had a relative brain weight that is higher than Control, however, not statistically significant. The relative brain weight of LDV was statistically significantly (** $p < 0.01$) higher than HDV. HDV group was higher than control group and it was statistically significant (* $p < 0.05$). Columns represent mean \pm SEM.

Morris water Maze test: The Morris assessment test for all the three groups exhibit no statistical differences in learning capacities except for day 3 trial where low-dose vanadium and control groups had a significant learning ability than high-dose vanadium group (Figure 5A). Subsequently, there was a statistically significant memory impairment after

exactly three months of high dose vanadium exposure when compared to control and low-dose vanadium treated groups. Furthermore, there was no statistical differences between control group and low-dose exposed group. However, low-dose treated mice shows slightly enhanced memory ability than control group (Figure 5B, 5C).

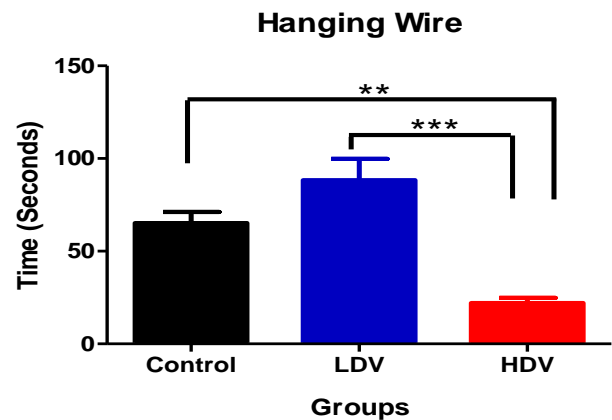


Figure 4: Muscle strength assessment using wire hanging test among mice. Hanging time (s) was measured on PND 90. The low dose vanadium exposure improved muscle endurance in mice whereby high dose exposure decrease muscle strength. LDV performed better than control but it was not statistically significant. Both control and LDV had a statistically significant increase in performance when compared to HDV. Columns represent mean \pm SEM.

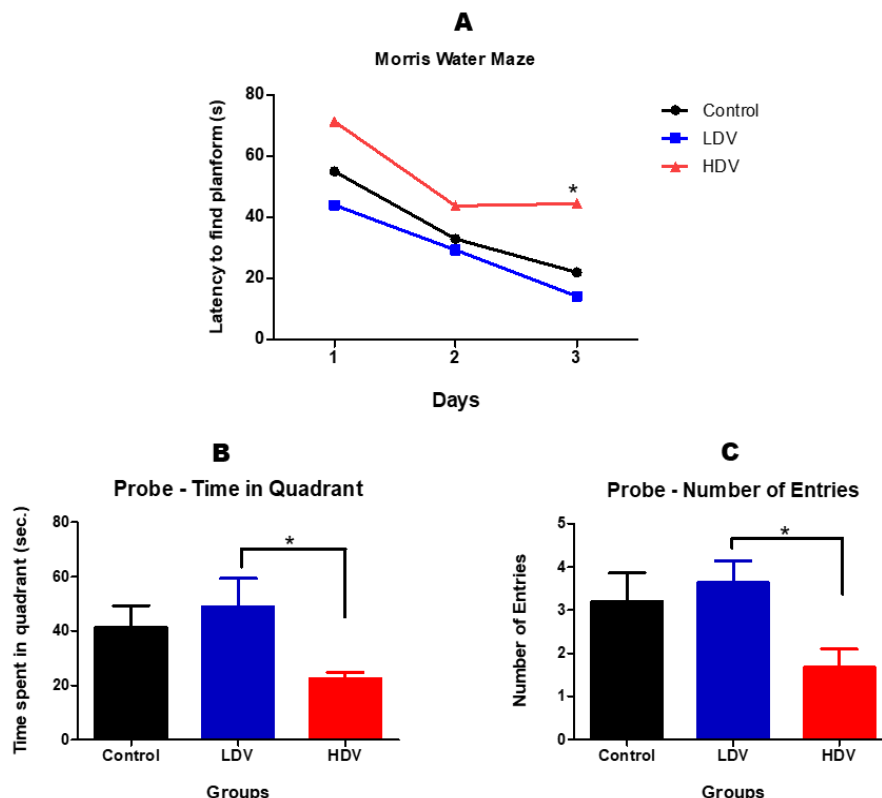
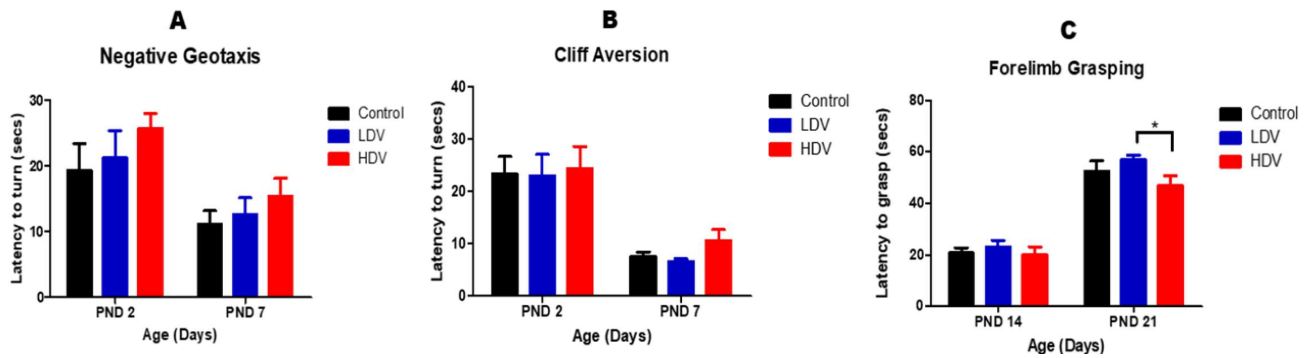
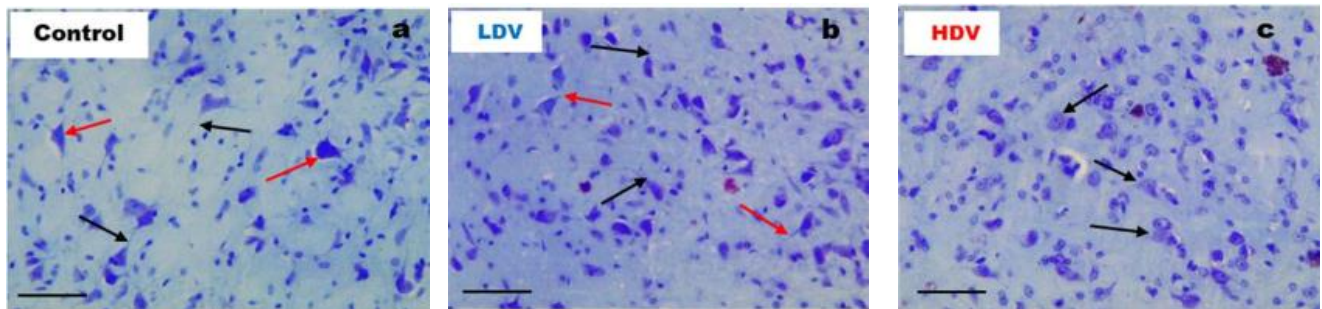


Figure 5: Effect of low-dose and high-dose vanadium treatment for three months on learning and memory in mice. **A.** The ability to learn the location of the hidden platform was improved in all the groups as they all gradually spent shorter times to locate the hidden platform with subsequent trainings. However, there was a significant reduction in the rate of learning in the HDV-exposed mice compared to the LDV-exposed mice. **B.** During probe trial, the HDV-exposed mice spent a significantly shorter time in the target quadrant than the LDV-exposed mice (* $P < 0.05$). Each point is the mean \pm SEM. **C.** The number of entries of the LDV-exposed mice into the target quadrant was significantly higher than the HDV group (* $P < 0.05$). Each bar is the mean \pm SEM.

**Figure 6:**

Neurodevelopmental motor function test of mice pups exposed to low dose and high dose vanadium from PND 1. **A.** Exposure to LDV and HDV did not affect the latency to turn at PND 2. However, at PND 7, control group tended to perform better than LDV group but it is not statistically significant. **B.** LDV improved the ability of the mice to turn and avoid cliff than LDV group because they use shorter time in turning away. **C.** LDV group exposure to low dose vanadium significantly improved latency to fall in the forelimb grasping test for pups at PND 21. Columns represent mean \pm SEM. * $P < 0.05$.

**Plate 1:**

Photomicrograph of cerebral cortex showing the pyramidal cell in mice. **a.** The control group showing normal pyramidal cells (red arrow) with dendritic extensions (black arrow). **b.** LDV group also showing relatively normal pyramidal cells (red arrow) that are still retaining some dendritic extensions (black arrow). **c.** The cerebral cortex of HDV showing multiple deformed pyramidal neurons (black arrow) lacking dendritic extensions. Cresyl Violet staining. $\times 40$ Magnification. Scale bar = 100 μ m.

Developmental Test: Negative Geotaxis, Forelimb Grasping and Cliff Aversion: Neurodevelopmental motor function test of mice pups exposed to low-dose vanadium right from PND 1 showed improved motor development and muscular strength whereas high-dose vanadium led to motor function impairment and reduced muscular strength. In negative geotaxis test, the high-dose vanadium exposed group had a longer latency to turn away from gravitation when compared to control and low-dose vanadium treated groups which was statistically significant on both PND 2 and PND 7. However, there was no statistically significant difference between control and low-dose vanadium treated groups (Figure 6A). In the Cliff aversion test, high-dose vanadium exposed mice had significantly longer latency to turn with head and arm compared to control and low-dose vanadium exposed mice pups, while there was no significant difference between low-dose vanadium exposed mice and control (Figure 6B). The forelimb grasping abilities was significantly reduced for high-dose vanadium treated mice compared to the low-dose vanadium group. However, there was no significant difference between low-dose vanadium and control (Figure 6C).

Histological Examinations: The cerebral cortex in the control group had normal pyramidal cells with intact dendrites, the same was also seen in the low-dose vanadium exposed group. However, in the high-dose vanadium group, multiple deformed pyramidal neurons in the cerebral cortex

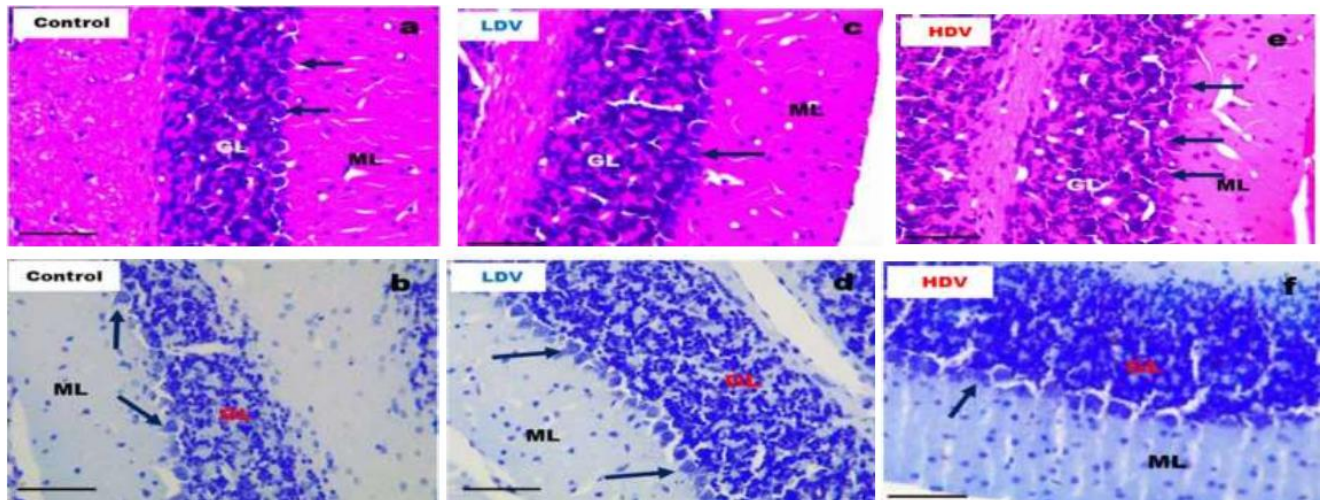
which are lacking in dendritic extensions were observed (Plate 1).

The cerebellum in the control and low-dose vanadium groups had normal architectural arrangement of the molecular, granular and Purkinje cell layers. However, in the high-dose vanadium group, we observed degenerated Purkinje neuron that are pyknotic and are devoid of dendritic arborization. We also observed loss of Purkinje cells (Plate 2).

The pyramidal cells in the cornus ammonis 1 (CA 1) region were normal in shape and arrangement in the control and low dose vanadium groups. However, in the high-dose vanadium group, the cells appeared pyknotic, with distorted neuronal morphology and reduced layers of neuronal cells in this hippocampal region (Plate 3).

The control group showed normal cellular architecture of dentate gyrus. LDV group also showed normal dentate gyrus, however, there is a relative mild vacuolation of dentate gyrus. The dentate gyrus in the HDV presented multiple deformed neurons and severe vacuolation as compared to control and LDV groups.

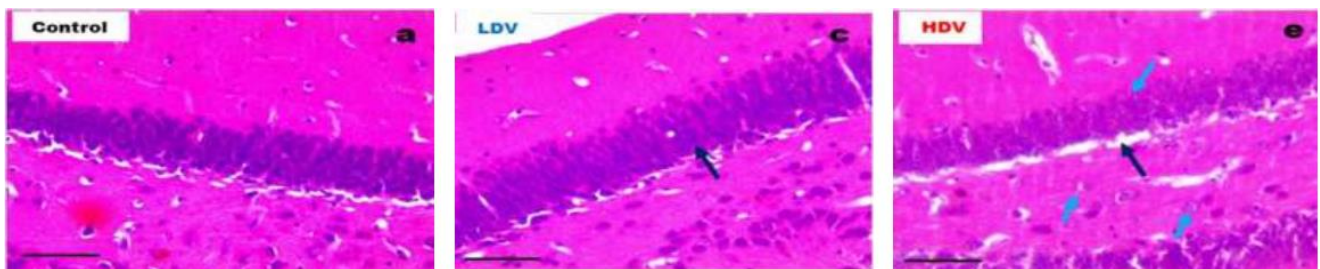
The olfactory bulb in mice in the control and low-dose exposed vanadium had normal olfactory glomerular cells and normal architecture of mitral cells and granular cells. However, in the high-dose vanadium group, we observed some olfactory glomerular cells undergoing degeneration and reduced number of glomerular cells. Furthermore, the mitral cell layer exhibited distorted granular cells with fewer mitral cells (Plate 4).

**Plate 2:**

Photomicrograph of cerebellum from mice. **a-b.** Control group showing normal molecular layer (ML), granular layer (GL) and Purkinje cells (black arrows) **c-d.** LDV group showing apparently normal molecular layer (ML), granular layer (GL) and Purkinje cells (black arrows). **e-f.** Mouse cerebellum from HDV group exhibiting Purkinje cells with pyknotic appearance, loss of Purkinje cells and also loss of dendritic arborization. **a,c,e** H&E staining while **b,d,f** Cresyl Violet staining. x40 Magnification. Scale bar = 100 μ m

**Plate 3:**

Photomicrograph of hippocampus showing the *cornu ammonis* 1 (CA1) in mice. **a.** The control group showing normal CA1 cells with dendritic extension. **b.** LDV group also showing normal pyramidal cells. **c.** The CA1 showing multiple apoptotic death neurons and the neuronal morphology appear distorted with reduced neuronal cells. H&E staining. x40 Magnification. Scale bar = 100 μ m

**Plate 4:**

Photomicrograph of hippocampus showing the dentate gyrus in mice. **a.** The control group showing normal dentate gyrus cellular architecture (H&E staining). **b.** The control group showing normal dentate gyrus cellular architecture (Cresyl violet staining). **c.** LDV group also showing normal dentate gyrus (H&E staining). **d.** LDV group also showing relatively dentate gyrus with mild vacuolation (Cresyl violet staining) **e.** The dentate gyrus in the HDV exhibiting multiple deformed neurons (blue arrow) and vacuolation (black arrow) (H&E staining). **f.** In HDV group, we saw also deformed neurons and vacuolation (black arrow). (Cresyl violet staining). x40 Magnification. Scale bar = 100 μ m.

Examination of the kidneys in the control group mice revealed normal glomeruli, distal, proximal tubules in the cortex and the medulla. The kidney in LDV mice also had normal glomeruli, but mild sloughing off of nephron tubules and normal medulla. The group exposed to HDV exhibited renal glomerular shrinkage and some of the renal tubules had abnormally larger lumen compared to the control and

LDV groups. There was also the presence of degenerated epithelial cells in the renal medulla of mice exposed to HDV

Mice in the control group presented normal hepatic cells, arranged in distinct sinusoids. The LDV group also had normal hepatic cells, but with mild sinusoidal dilatation. Furthermore, there was moderate congestion in the blood vessels of the liver and the hepatocytes showed diffused vacuolar degeneration in the HDV group.

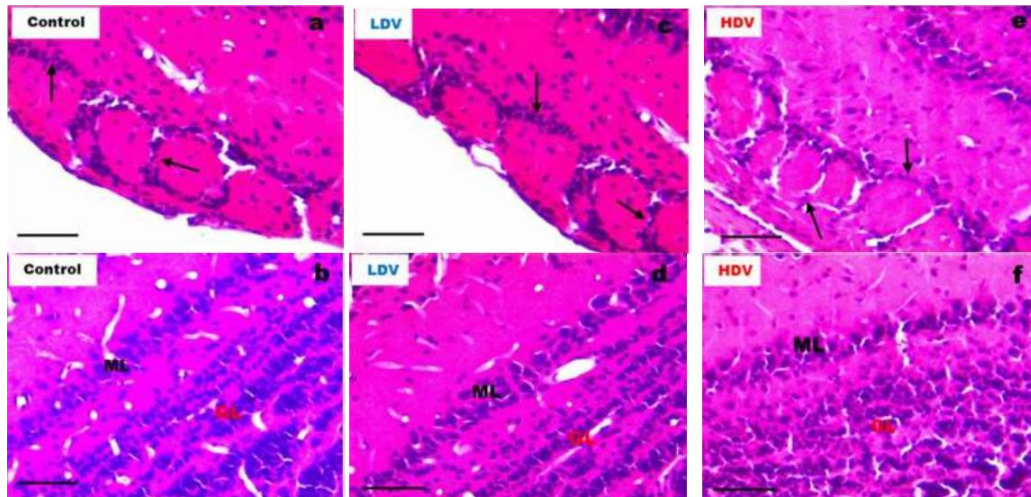


Plate 5:

Photomicrograph of the olfactory bulb in mice. a. Showing normal olfactory glomerular cells b. Control group also showing normal mitral cells and granular cell layer of olfactory bulb. c. The LDV group showing normal olfactory glomerulus. d. The LDV group showing normal mitral layer and normal granular layer. e. The HDV showing olfactory glomerular cells which are undergoing apoptosis and the glomerular cells are fewer in number. f. The mitral cell layer showing fewer cells and are undergoing apoptosis in HDV group with distorted granular cells layer. H&E staining. x40 Magnification. Scale bar = 100 μ m.

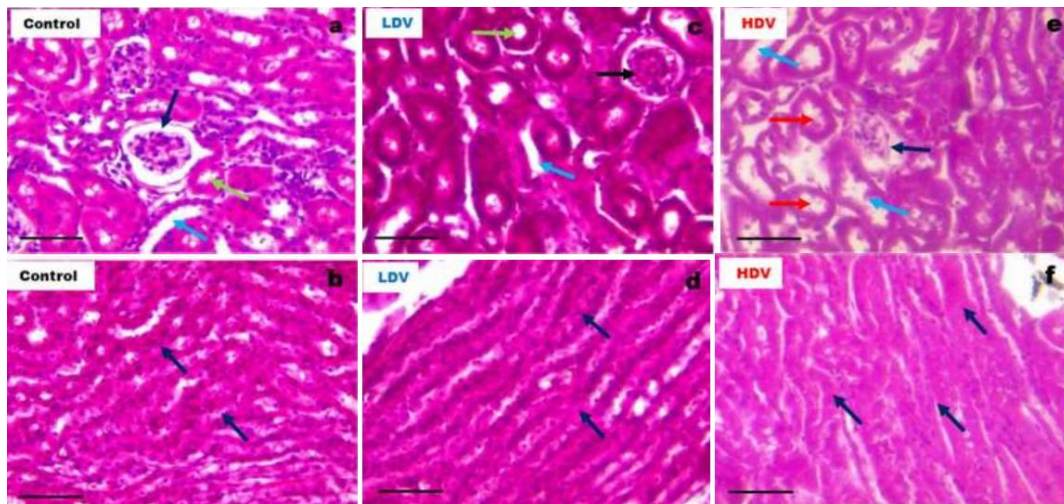


Plate 6:

Photomicrographs showing the effects of low dose and high dose vanadium exposure of on kidney of mice. a. Renal cortex of control group showing normal glomerulus distal (black arrow), proximal (red arrow) tubular and glomerulus (white arrow) structures are seen in the medulla of control kidney. b. The control group showing normal renal medulla c. LDV group also showing normal glomerulus (black arrow) and mild sloughing off of nephron tubules (black arrow). d. The LDV group showing normal renal medulla e. HDV group showing shrinkage of renal glomerulus (black arrow); some of the renal tubules with larger lumen (blue and red arrows). f. Renal medulla of HDV group showing degenerated epithelial cells (black arrow). H&E stain. x40 Magnification. Scale bar = 100 μ m

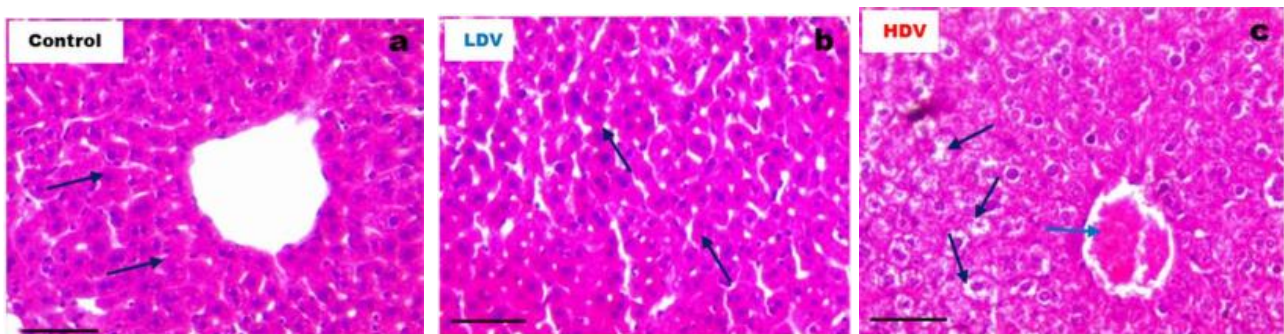


Plate 7:

Photomicrographs of the Liver of mice exposed to low dose and high dose vanadium. a. Control group showing normal hepatic cells. b. LDV group also having normal hepatic cells with mild sinusoidal dilatation. c. Showing diffuse hepatocellular vacuolar degeneration (black arrows) and moderate congestion of blood vessels (blue arrow). H&E staining. x40 Magnification. Scale bar = 100 μ m.

DISCUSSION

In this study, we observed that there was significant increase in average body weight of low-dose vanadium exposed mice while average body weight decrease was noted in high-dose vanadium exposed animals in comparison to the control group. This finding, especially for the low-dose vanadium group is similar to the work of Omayone *et al.*, (2020). Omayone and his team observed that exposure to low-dose vanadium after 10 weeks in neonatal rat led to significant increase in body weight. Likewise, in a recent report by Dyer and De Butte (2022), they saw significant increase in body weight of low-dose vanadium exposed mice over controls. In addition, Usende *et al.*, (2016) reported astrogliosis including oligodendrocytes (immature and mature) proliferation with low-dose vanadium. This demonstrates vanadium's capacity to regulate signal transduction pathways facilitated by growth factors, enhancing transformational changes in cells (Stern *et al.*, 1993). On the other hand, Olopade *et al.*, (2011), Azeez *et al.*, (2016), Usende *et al.*, (2016) and Audu *et al.*, (2020), have also reported a marked reduction of body weight in neonatal murine species treated with high-dose vanadium as compared to the control and low-dose vanadium groups. Contrary to the average body weight findings obtained for high-dose vanadium group, García *et al.*, (2004, 2005), Igado *et al.*, (2020) and Folarin *et al.*, (2017), exposed adult murine species to high-dose vanadium and they observed no statistical differences in body weight compared to their respective control groups. Thus, we can predict the reason why the current work showed differences as the period for which the animals are exposed to vanadium and variation in age of the rats utilized in the investigations. In their work, they used older murine but as early as postnatal day one our animals were being treated, and we observed variations in the weight as the mice developed. Therefore, both low-dose and high-dose vanadium strongly affect postnatal development.

In this study low-dose vanadium group had a slight observable increase in the relative brain weight while there was a statistically significant decrease in relative brain weight for the high-dose vanadium group compared to the control group. Although, decrease in relative brain weight has been reported by Olopade *et al.*, (2011), and Usende *et al.*, (2016) in neonatal murine species exposed to high-dose vanadium, there is a scarcity of reports on this same parameter for low-dose vanadium. The increased relative brain weight for low-dose vanadium in relation to control for this study could therefore be regarded the first report of low-dose vanadium positively affecting the brain.

Our study shows improvement in learning and motor functions for low-dose vanadium group. Low-dose vanadium mice had improved hanging strength than control and high-dose vanadium group. This is possible considering insulinomimetic properties of vanadium reported by Semiz and McNeill (2002), which cause increased glycogen storage in skeletal muscle increasing muscle endurance. Therefore, the improved performance of the low-dose vanadium group in the forelimb grasping in pups and hanging wire test in adult mice is likely to be as result of vanadium-induced increase in glycogen content of the skeletal muscle. Dyer and De Butte (2022), similarly observed improvement in motor activities of chronic low dose vanadium exposed rats.

We have also shown that mice with high-dose vanadium exposure had a significant reduction in latency to fall compared to controls. This is similar to report by Folarin *et al.*, 2017, who reported reduction in gripping strength of vanadium-treated animals relative to control, however, it was statistically insignificant. Additionally, administration of high dose vanadium has previously been reported to result in muscular weakness in mice as reported by Mustapha *et al.*, (2014), Azeez *et al.*, (2016) and Audu *et al.*, (2020).

The Morris water maze measures the hippocampal-dependent spatial navigation and learning which is assessed via multiple trials as well as memory ability to determine the platform area when the platform is removed, usually referred to as probe trial (Morris, 1993). In our study, we observed that mice with LDV-exposure had significantly improved memory capacities than the HDV-exposed mice, but comparable with the controls. Dyer and De Butte (2022), however reported that control rat performed better than rats with chronic low dose vanadium administration (0.05mg of vanadium powder/1000 ml of food mash). Long term high-dose vanadium administration in adult mice has also been previously reported to result in significant memory deficits compared to controls when tested using the Morris water maze (Folarin *et al.*, 2016).

Our study observed that the high-dose vanadium group take longer time to reorient themselves against gravity when compared to control and low-dose vanadium exposed mice, however, it is not statistically significant. This finding agrees with Usende *et al.*, (2016) who reported that high-dose vanadium treated groups could not make an 1800 turn in a shorter period after placement on inclined platform at PND 15 as well as PND 21 and this was statistically significant. Despite the longer grasping capacity of low-dose treated mice pups compared to control, there was no significant differences. Contrary to our finding in vanadium, exposure of neonatal rats to low concentration (50ppm) of lead over three months resulted in impaired motor functions (Mameli *et al.*, 2001). This finding is similar to our observation in high dose vanadium-exposed mice in this study, although, our study showed that high-dose vanadium exposure significantly affect forelimb grasping capacity in the mice pups.

The main organs susceptible to toxicity of vanadium are liver and kidney. These organs are reportedly the principal reservoirs for vanadium to pile up following absorption. (Sabbioni *et al.*, 1978; Ramanadham *et al.*, 1991; Sanchez *et al.*, 1998, Omayone *et al.*, 2020). Histological studies in the kidney revealed severe shrinkage of renal glomerulus in the high-dose exposed vanadium. Furthermore, some of the renal tubules have larger lumens while the renal medulla present degenerated epithelial cells in high-dose vanadium exposed mice; in the liver, there was diffuse hepatocellular vacuolar degeneration and moderate congestion of blood vessels among the high-dose exposed mice.

In conclusion, vanadium in low-dose is shown to facilitate increase in body weight which translated also into organ weight, while causing neurodegenerating changes at high-dose. We also discovered that low-dose vanadium exposure improved neurobehavioral/ neurodevelopmental performances whereas high-dose vanadium led to decreased neurobehavioural performances. Furthermore, our results demonstrated that low-dose exposure to vanadium caused

mild histological lesions in some parts of the brain, liver and kidney whereas high-dose showed distinct histological lesions in different parts of the brain, liver and kidney. Additionally, because only basic histological staining was included in our study, future research should include different additional tools such as immunohistochemistry and electron microscopy in order to determine further differences in how both low -dose and high-dose vanadium affects living system.

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