ORIGINAL ARTICLE

Official Journal of the Neuroscience Society of Nigeria (NSN)

https://doi.org/10.47081/njn2022.13.4/002
ISSN 1116-4182

Effects of *in-vivo* Vanadium-Induced Neurotoxicity and Withdrawal on Three Consecutive Generations of Mice Brains: A Neurobehavioural and Histo-Immunohistochemical Study

Ayodeji D. Lijoka[1], Olumayowa O. Igado[1], Funmilayo E. Olopade[2], James O. Olopade[1]

[1]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria

Received: ……. December 2022
Accepted: ……. January 2023

ABSTRACT

Heavy metals exposure causes multi-systemic pathologies in biological systems. Due to their genotoxic effects, some of these anomalies have been reported to transcend to unexposed generations. Vanadium, a transition metal, crosses the blood brain barrier, causing neuroinflammatory and demyelinating lesions with neurobehavioural inadequacies in exposed subjects. There are however scarce scientific information on vanadium neurotoxicity over generations of exposed subjects. This study explored the effects of vanadium exposure on the body weight, neurobehavioural (open field and negative geotaxis tests) and neurohistological changes (Purkinje cell, astroglial and myelin histoarchitecture) in three consecutive generations – two exposed generations (G1 and G2) and one withdrawal generation (G3), of mice. Neonatal pups of BALB/c mice in G1 and G2, were treated with sodium metavanadate every 48 h, first via lactation from post natal day (PND) 1-14, and later intraperitoneally (PND 15-21) for males; PND 15-45 for female pups till they were mated with unexposed males. The G3 pups were not treated with sodium metavanadate. The vanadium treated pups had irregular body weight gain. In all the studied generations (G1-3), there were reduced locomotor and exploratory activities, and diminished motor and vestibular function compared to the controls. Furthermore, there was multilayering and pyknosis of the cerebellar Purkinje cells, moderate to marked astrogliosis and generalized demyelination in the corpus callosum, hippocampus (CA1-4 and the dentate gyrus), cerebral cortex, thalamus and cerebellum. This study revealed that effects of vanadium exposure including neurobehavioural anomalies, astrogliosis, demyelination and cerebellar Purkinje cell pathology can persist across multiple generations, including generation not initially exposed.

Keywords: *Astrogliosis; Cognitive deficits; Demyelination; Generation; Vanadium neurotoxicity; Withdrawal*

INTRODUCTION

Environmental pollution by heavy metals like lead, cadmium, mercury, vanadium, iron, zinc, manganese, aluminum, nickel, and cobalt, has been on the rise since the advent of industrialization (Briffa et al. 2020). The multi-systemic toxicopathology and genotoxic effects of these heavy metals have been proven via experimental and clinical studies in exposed subjects (Ciranni et al. 1995; Martinez-Zamudio and Ha 2011; Timothy and Williams 2019; Kocadal et al. 2020). Of interest among these heavy metals studies is vanadium. Vanadium is a ubiquitous transition metal that is of great industrial and biphasic biological importance (Rehder 2016). About 80% of the world produced vanadium is used in the steel industry, production of fertilizer, pesticide, glass, ceramic, pigment, redox battery, as well as in nuclear applications (Emsley 2011; Imtiaz et al. 2015). Biologically, vanadium compounds have dual effects depending on the quantity of exposure. It is essential

Correspondence: Olumayowa O. Igado, PhD; Department of Veterinary Anatomy, University of Ibadan, Ibadan, Nigeria. Email: oo.igado@mail.ui.edu.ng; Phone: +2348035790102; ORCID: 0000-0001-5062-854X

in trace amounts (0.05 µM) with broad pharmacological activities (Preet et al. 2005) and toxic in excess (>10µM) (Das et al. 2012).

Vanadium compounds are naturally released into the environment through volcanoes, continental dusts, sea salts spray, and forest fires (Nriagu 1990). In addition to these natural sources, anthropogenic activities especially burning of fossil fuels, spillage of crude oil, and industrial processes have largely contributed to the release of vanadium in levels that are toxic to biological systems (Duce and Hoffman 1976; Igado et al. 2008; Emsley 2011). Humans and animals alike are therefore at risk of exposure to toxic levels of vanadium compounds via inhalation, ingestion or skin absorption since they are present in and can accumulate in foods (Habib and Ibrahim 2011), water (Rehder 2012), soil (Khan et al. 2011) and air (Imtiaz et al. 2015). Transplacental (Underwood 1971; Li et al. 2019) and lactational (Soazo and Garcia 2007; Olopade et al. 2011) exposure of vanadium compounds to embryos and neonates, respectively have been reported in developmental neurotoxicity chiefly characterized by neurobehavioural deficits, astrogliosis, and demyelination (Olopade et al. 2011; Mustapha et al., 2014; Azeez et al. 2016). In the last two decades, restive communities like the Arabian Gulf and the Niger Delta region of Nigeria have witnessed crude oil burning (a potential source of atmospheric vanadium) on an increasingly large scale (Haider et al. 1998: Todorich et al. 2011), thereby putting the population at risk of vanadium inhalation and toxicity.

Although the transgenerational effects of intoxication by other heavy metals have been studied extensively in *Caenorhabditis elegans* (Yu et al. 2013; Zhang and Yu 2020; Wei et al. 2020; Zhang et al. 2021) and zebra fish (Carvan et al. 2017; Chen et al. 2017; Tian et al. 2021), there however remains very little to no scientific information on transgenerational effects of vanadium toxicity in the mammalian brain. Therefore, this present study investigated the neurotoxic effects of vanadium exposure via lactation and intraperitoneal injection over two consecutive generations of mice, and its subsequent withdrawal in the third. The effect of vanadium on body weight, neurobehaviour, Purkinje cell, astroglial, and myelin histoarchitecture were determined across the three generations of study, up to the early postnatal period (PND 21) of the mice..

MATERIALS AND METHODS

Experimental Animal Handling

Twenty pregnant BALB/c mice were obtained from the mice colony established in the animal holding facility of the Neuroscience Unit, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. They were housed individually in plastic cages with wood shavings as beddings in the same facility under natural environmental parameters of temperature (27-30℃), humidity (81-89%) and a 12 h light/dark cycle. They were fed pelleted rat feed and water was provided *ad libitum*. Animals were humanely handled, as not to cause undue pain.

Ethical Approval

This study was performed in line with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH 1996). All experiments were approved and carried out in accordance with the guidelines of the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan, and the ethical approval code number for this experiment being UI-ACUREC/20/040.

Experimental Design

The nursing mice (n=10) with their pups (7 ± 2 pups per dam) were randomly assigned into two groups: control and vanadium (treated) (n=10 nursing dams). These groups were maintained for three generations (G1-3) with G1 being the offspring of the initial pregnant dams. The experimental regimen described below and depicted in Figure 1 was followed for the two groups in the first two generations (G1 and G2)

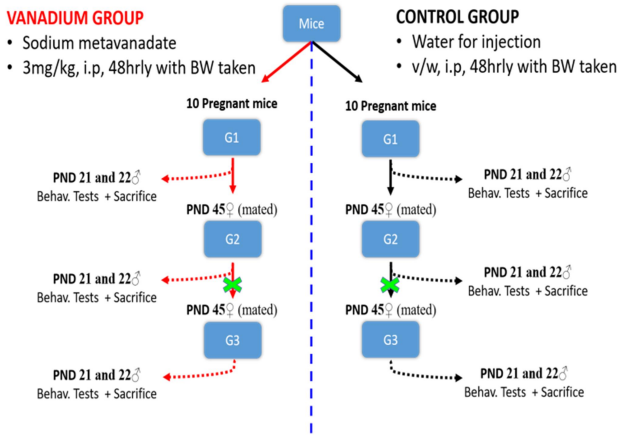

Fig. 1: Schematics outlining the materials and methods. The green "X": Cessation of vanadium treatment for the third generation; BW: Body weight; V/W: Volume per weight; ♂: Male; ♀: Female

while the two groups in the third (G3 – withdrawal group) were not treated.

Thus, in groups 1 (Control) and 2 (vanadium), the nursing mice were respectively, administered sterile water (at volumes corresponding to the dose rate of sodium metavanadate) and sodium metavanadate (Santa Cruz Biotechnology, Inc. USA, 3 mg/kg in sterile water), every 48 h intraperitoneally (i.p), from PND 1-14. After day 14, their pups were subsequently treated in the same manner as their dams (PND 15-21 for male and 15-44 for female pups).

The female pups in the experimental groups of preceding generations were allowed to breed on PND 45 with adult males selected from the pool of breeders in the animal holding facility to give rise to successive generations. Each time gap between generations was 21 ± 2 days. The G3 female offspring were not bred because the experiment terminated with the third generation. Treatment for the G1 and G2 females in each group was paused during mating and gestation period for 21 ± 2 days until the first day after parturition (PND 1).

All the pups in the two groups across the three generations (G1-3) were weighed using a pocket-sized electronic weighing balance (0.01 g precision) (Bijoux Sterling Electronic Scales, China) every 48 h, from PND 1-21.

The male pups, in each group across the three generations, were subjected to neurobehavioural tests (open field test and negative geotaxis) on PND 21 and were sacrificed on PND 22.

Neurobehavioural Tests

The 21-day old male pups (n = 8 pups per group) in the three generations (G1-3), were subjected to neurobehavioural tests: Open field test (OFT) and negative geotaxis (NG) before sacrifice on PND 22. The two tests were administered as described by Olopade et al. (2011).

Open Field Test: Each pup was placed in the centre of a white square wooden box (120 × 120 cm^2). The floor was divided into 20 cm^2 squares drawn in black ink, with a 20 cm^2 square in the centre. The mice were allowed to move freely around the open field to explore the environment for 5 min. In between tests, the box was thoroughly dry cleaned with 70% ethanol to remove odour cues that might affect mice behaviour in subsequent tests. The mice movements were recorded using a phone camera attached to a makeshift stand and sets of observations as described by Brown et al. (1999), and were later manually analysed. The following parameters were recorded: Line crossing, centre square entries, centre square duration, rearing, grooming, freezing and defecation.

Negative Geotaxis: Each mouse was placed in the middle of a wooden slab (30 cm × 45 cm), inclined 45° to the surface plane, in a head down position and the latency to turn 180° to a head up position was measured. Three replicates were performed for each animal, with successive trials done after all the animals had completed previous trials, so that enough rest was ensured. The wooden slab was cleaned with 70% ethanol after each test. This test reflects vestibular function, and motor development and activity.

Tissue Collection and Processing

The male pups, at PND 22, were anaesthetized by intraperitoneal injection of ketamine hydrochloride (50 mg/kg). Quick cervical decapitation was done once there was non-response to pedal stimulation. The brains of the animals were quickly removed over dry ice as described by Olopade et al. (2011), and fixed in 10% neutral buffered formalin for 48 h, after which they were transferred into 0.1% sodium azide in phosphate buffered saline (PBS) and stored at 4℃. Coronal and mid-sagittal sections of the brains were dehydrated in increasing grades of alcohol, cleared in xylene, embedded in paraffin and 5 µm thick sections were cut using a rotatory microtome (HM 330, Microme, Germany).

Haematoxylin and Eosin (H & E) Stain

Sections were prepared for H & E stain as previously described by Igado et al. (2020). Brain sections mounted on slides were dewaxed in two changes of xylene, thereafter rehydrated in decreasing grades of alcohol (100%, 90%, 80%, 70% and 50%) then rinsed in distilled water. The sections were immersed in haematoxylin for 25 min, rinsed in tap water until water was clear, and were transferred to eosin stain for 2 min. Sections were rinsed, and the slides were dehydrated in ascending grades of alcohol (50 %, 70 %, 80 %, 95 %, and 100 %). Sections were then cleared in xylene, mounted with dibutylphthalate polystyrene xylene (DPX) (Atom Scientific, UK) and cover-slipped with glass slips.

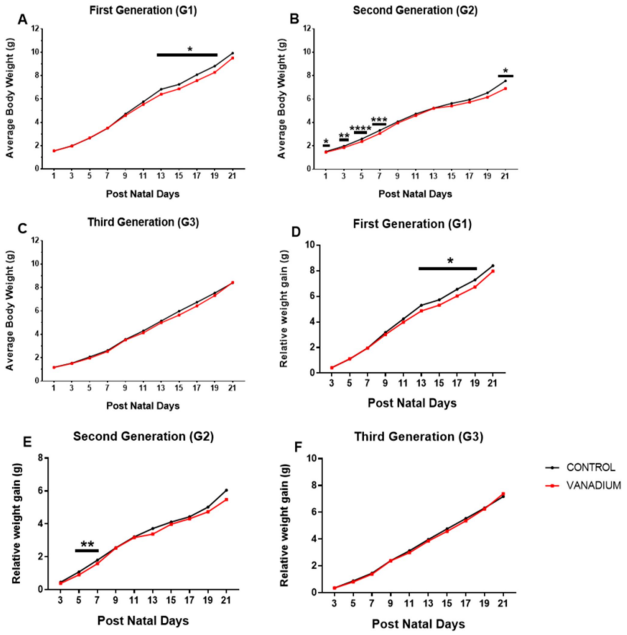

Fig. 2A–F: The average body weight (A – C) and relative body weight gain (D – F) in pups by generations. Body weight (A – C) was recorded from PND 1 – 21, while relative body weight gain (D – F) was calculated from PND 3 – 21, in vanadium and control groups of G1, G2 and G3 (n = 50 – 80 pups per group per generation). Statistically significant differences between control and vanadium groups for G1 and G2 are indicated on the graphs as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. There was no observable difference in body weight and weight gain in G3.

Immunohistochemistry

The brains were processed for immunohistochemistry as described by Usende et al. (2016). Briefly, the cut sections were placed in the oven for 10 min at 60℃ to soften the wax, then dewaxed in two changes of xylene and rehydrated in decreasing grades of ethanol. Antigen retrieval was done in 10mM citrate buffer (pH 6.0) for 25 min, at 98℃, with subsequent peroxidase quenching in 3% H_2O_2/methanol for 20 min. Slides were washed in 1% PBS and blocked in 2% PBS milk for an hour in a humidity chamber. All sections were probed with anti-myelin basic protein (MBP) mouse monoclonal antibody for myelinating oligodendrocytes (1:500, Abcam) and anti-GFAP rabbit polyclonal antibody for astrocytic morphology (1:1000, Abcam) (diluted in 1% PBS milk) overnight at 4℃. Detection of bound antibody was done using appropriate HRP-conjugated secondary antibodies in Vecta-stain kit (Vector Labs) according to manufacturer's protocol. Reaction product was enhanced with 3,3'-diaminobenzidine (DAB, 1:25 dilution) for 2-5 min, with subsequent dehydration and clearing in ethanol and xylene respectively. The slides were mounted with DPX, cover-slipped and allowed to dry on the bench.

Microscopy

Brain sections were viewed under Leica DM 500 light microscope (Leica Microsystems, Wetzlar, Germany). Images were captured under the same lighting conditions using an in-built camera system (Leica ICC50 E) linked to a laptop computer.

Data Analysis and Statistics

The body weight and behavioural tests' data were analysed using the Student's t test on GraphPad Prism® 7 software for Windows. Myelin basic protein (MBP) intensity was analysed using the free software, ImageJ. Values obtained were subjected to a two-way ANOVA. All data were presented as mean ± SEM and $p < 0.05$ was considered statistically significant.

RESULTS

Body Weight

In the first generation (G1), no difference was observed in the body weight of the pups during the early lactation period (PND 1-11) in the control and vanadium groups. However, at the early onset of postnatal period when the pups started receiving sodium metavanadate intraperitoneally, there was a statistically significant reduction ($p < 0.05$) in both average body weight (Fig. 2A) and relative weight gain (Fig. 2D) from PND 13–19, compared to the control pups.

In the second generation (G2), there was statistically significant reduction ($p < 0.05$) in both average body weight (Fig. 2B) and relative weight gain (Fig. 2E) of the pups in the vanadium group during the first week of life (PND 1–7), followed by a period of no difference (PND 9–19) until PND 21 when there was a significant reduction in average body weight relative to the controls.

In the third (withdrawal) generation (G3), there was neither significant difference between the average body weights (Fig. 2C) nor the relative weight gain (Fig. 2F) of the untreated vanadium and control groups in all the studied post-natal timelines.

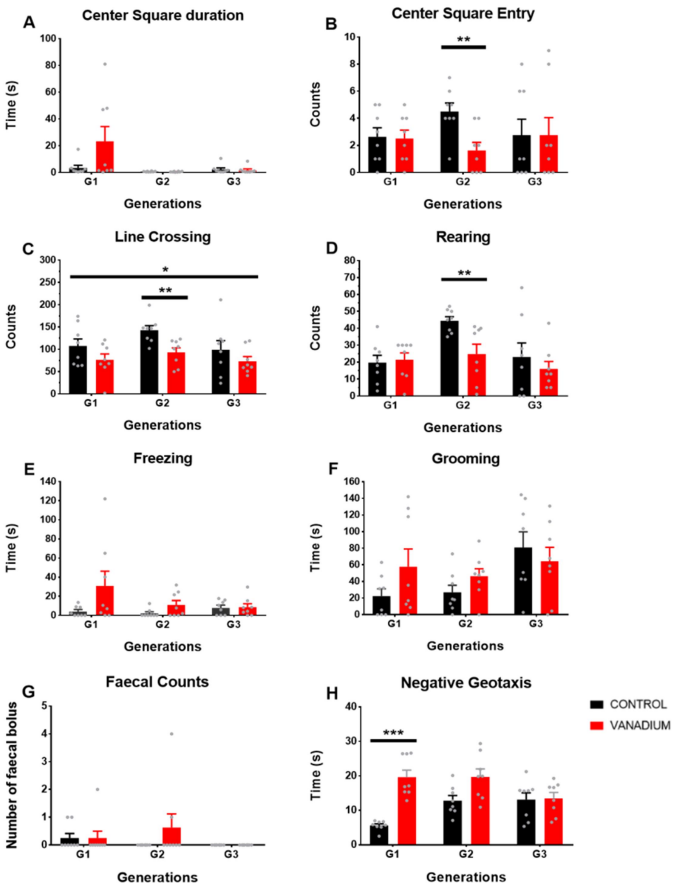

Fig. 3A–H: The open field test and negative geotaxis parameters analyzed across G1, G2 and G3. Vanadium groups in all the three generations had reduced locomotion, exploration and increased anxiety compared to controls. Vanadium groups except the third generation took more time to turn against gravity compared to controls (n = 8 pups per group per generation). * $p < 0.05$, ** $p < 0.01$

Behavioural Tests

Open Field Test: In the OFT, there were overall decreased locomotor activities across all the three generations of vanadium (treated and withdrawal) groups compared to the controls. This was shown by significant reduction ($p < 0.05$) in number of lines crossed through all the generations (G1-3) of vanadium group compared to their respective controls (Fig.

3C). Furthermore, statistical comparison (using unpaired Student's t test) between the vanadium and control groups within each generation, showed only the G2 vanadium treated group having significant difference, with reduction ($p < 0.01$) in counts of centre square entry (Fig. 3B), line crossing (Fig. 3C) and rearing (Fig. 3D). Other parameters did not show any statistically significant difference ($p>0.05$) in the G2 and G3.

In addition, an observable increase in freezing time was noticed for vanadium treated groups in G1 and G2 compared to their controls, while there was no observable difference in the withdrawal G3 groups (Fig. 3E). There was also an observable increase in the number of faecal bolus voided from G1 to G2 vanadium groups compared to their respective controls. Howbeit, no faecal bolus was voided in G3 groups (Fig. 3G).

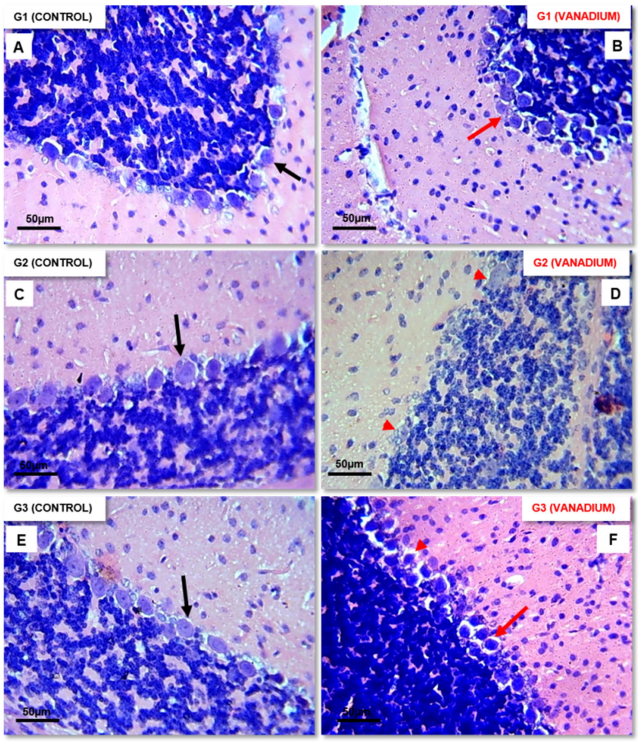

Fig. 4: The cerebella sections showing the Purkinje cell layer. There is multilayering or stratification (red arrow) and pyknosis (red arrowhead) in the Purkinje cell layer of vanadium groups compared to the single layered, healthy Purkinje cell layer (black arrow) in control groups. H&E staining

Negative geotaxis: In G1, there was a statistically significant increase ($p < 0.001$) in the time taken (longer latency) to exhibit righting reflex against gravity in the vanadium group compared to control. There was also an observable (but not statistically significant) increase in time taken to turn against gravity in G2 vanadium group relative to the control, but there was almost equal latency between the G3 (withdrawal) mice and the control (Fig. 3H).

Haematoxylin and Eosin (H & E) Stain

In comparison to their respective controls, G1 vanadium group showed multilayering of the Purkinje cells, in multiple foci (Fig. 4B). G2 vanadium group showed pyknotic Purkinje cells and extended areas of Purkinje cell layer loss (Fig. 4D). In the G3 withdrawal group, the Purkinje cells, although generally appearing normal, had an area of bi-stratification and very few regions of loss, relative to G2 vanadium group (Fig. 4F).

Immunohistochemistry

Glial Fibrillary Acid Protein (GFAP): GFAP immunostaining showed moderate to marked astrocytic activation or reactivity (astrogliosis) in the hippocampal CA1 region (Fig. 5A–D) and in the polymorphic layer of the dentate gyrus (Fig. 6A–D) of the G1 and G2 vanadium treated groups respectively compared to the resting astrocytes in controls. Moreover, the astroglial reactive changes observed in the G3 vanadium withdrawal group waned, compared to the marked reactivity observed in the G2 vanadium group. Despite this, moderate astroglial reactivity was observed in the hippocampal CA1 region (Fig. 5F) and in the polymorphic layer of the dentate gyrus

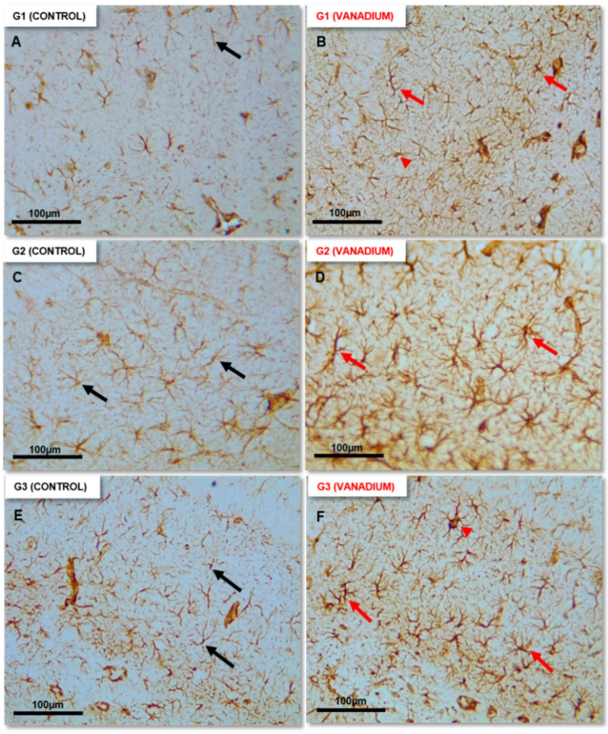

Fig. 5: The sections of hippocampal CA1 region. Reactive astrocytes (red arrows) with reactive cell bodies (red arrow heads) were observed in the stratum radiatum of the CA1 region in all vanadium groups across the three generations compared to resting/unreactive astrocytes (black arrows) in respective controls. Note the increased number of astrocytes in all the vanadium groups. GFAP immunohistochemistry

(Fig. 6F) of the G3 withdrawal group compared to the control (Fig. 5E and 6E).

Characterizing the observed astrogliosis in all the vanadium (treated and withdrawal) groups showed increased expression of astrocytic population with thick, intensely stained astrocytic processes and reactive cell bodies. In addition, thick astroglial processes were observed to span across the granular layer of the dentate gyrus in the vanadium groups compared to the thin and faintly stained processes in their respective controls (Fig. 6A–F).

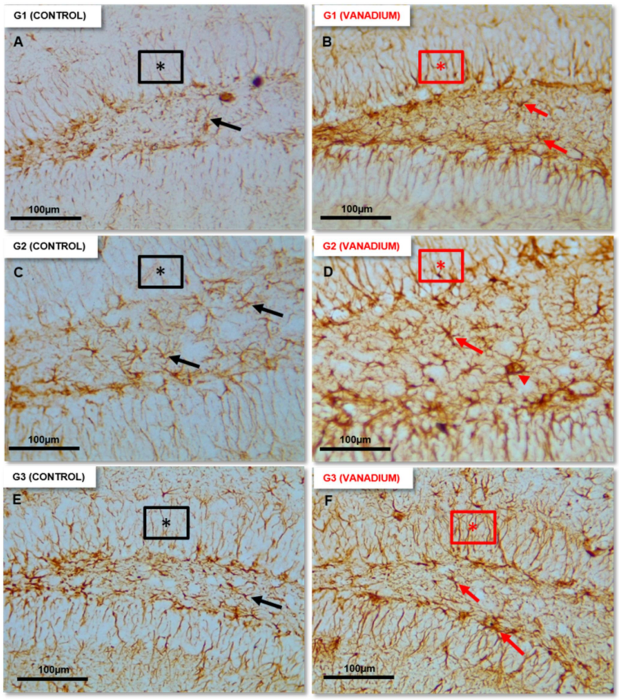

Fig. 6: The dentate gyrus sections. Reactive astrocytes (red arrows) with reactive cell bodies (red arrowheads) were observed in the polymorphic layer of the DG vanadium groups in all the three generations compared to resting/unreactive astrocytes (black arrows) in controls. Notice the thick astrocytic processes in the granular layer (asterisks in boxes) of vanadium groups relative to the thin and less stained processes in controls. GFAP immunohistochemistry

Myelin Basic Protein (MBP): Myelin basic protein immunohistochemistry revealed demyelination in the portions of the corpus callosum adjacent to the hippocampal CA1 region of the vanadium groups (treated and withdrawal) in all the three generations (G1-3), relative to the rich myelin staining in their respective controls. Similarly, there was generalized demyelination in the granular layer of the dentate gyrus, the pyramidal layer of the hippocampal CA1-3 regions, the cerebral cortex and in the thalamus (Fig. 7A–F). G3 vanadium withdrawal group was observed to be most affected (compared to other vanadium groups) by the demyelination effects of vanadium in the same brain regions as above (Fig. 7F). Statistical analysis (quantitative analysis) of ImageJ results of intensity showed statistically significant difference between the control and vanadium groups of G1 and G2 (Fig. 7G).

Demyelination was also observed in the cerebellar white matter tract (arbor vitae) of the G1 and G2 vanadium treated groups compared to relatively normal controls (Fig. 8A-D). There was however, no observable difference in the intensity of myelin staining of the cerebellar white matter in the G3 vanadium withdrawal and control groups (Fig. 8E and F). Quantitative analysis of intensity with ImageJ did not show any statistically significant difference between the control and vanadium groups of G1–3, in spite of the obvious pallor observed visually in the G2 vanadium-treated group (Fig. 8G).

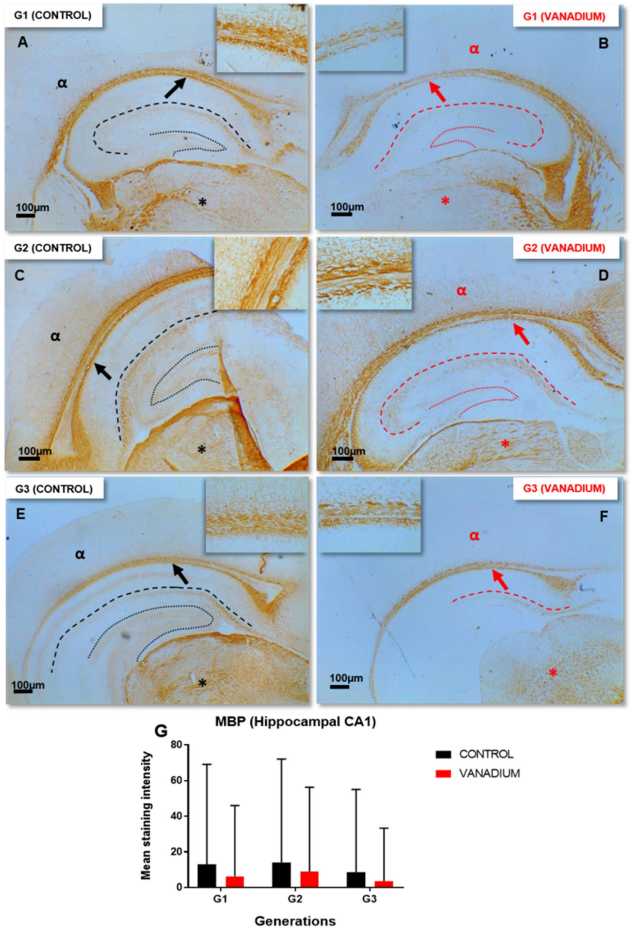

Fig. 7: The areas around hippocampal formation. Regions of demyelination in the corpus callosum (red arrows) of vanadium groups are compared with similar regions of rich myelin staining (black arrows) in controls. These regions are shown in insets. Notice the myelin pallor in the hippocampal formation (dotted lines), thalamic region (asterisk), and in the cerebral cortex (α). G is the myelin quantifications of the CA1 region. There was observable but not statistically significant difference in the mean staining intensity between the vanadium treated and control groups across the three generations). MBP immunohistochemistry

DISCUSSION

The dosage of vanadium (3 mg/kg) used in this study has been proven by previous studies to result in neurobehavioural, neurohistological and neurochemical changes (García et al. 2004; Igado et al. 2012, 2020). Consistent with previous reports on vanadium neurotoxicity were cognitive deficits and histoarchitectural changes; although variations in body weight changes have also been reported.

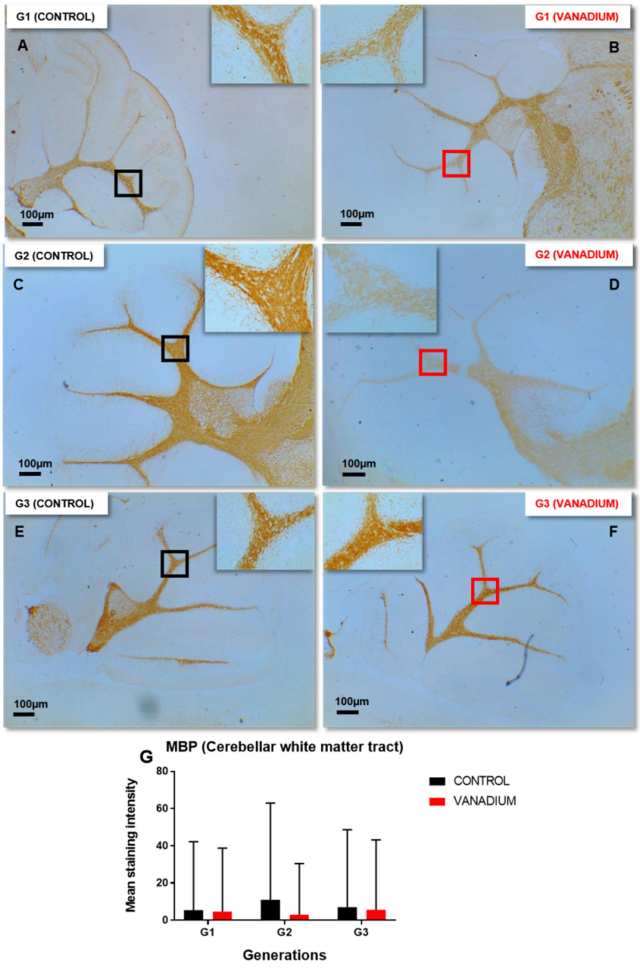

Fig. 8: The cerebellar white matter tract (arbor vitae). A–F show regions of the arbor vitae in the vanadium groups (red box) compared with almost corresponding regions in the control (black box). Notice the myelin pallor in the insets of G1 and G2 vanadium groups compared to those of the control. There was no observable difference in MBP staining intensity between the G3 vanadium group and its control. G is the myelin quantifications of the cerebellar tracts. There was no observable difference in the mean staining intensity between the vanadium treated and control groups in the first and third generations while the difference between the groups in the second generation was not statistically significant). groups relative to the thin and less stained processes in controls. MBP immunohistochemistry

Vanadium altered body weight negatively in exposed generations. In the first generation, there were significant reductions in body weights from PND 13–19 unlike in PND 1–11. These results may be ascribed to the reduced toxic dose of vanadium through lactation. It may also be due to the buffering effect the mother's milk might have against the adverse catabolic activities of vanadium, before they were directly exposed to the vanadium on PND 14–21. Vanadium-induced weight loss observed between PND 13–19 has been reported to be caused by vanadium-induced anorexia (Wang et al. 2001) and the ability of vanadium to alter multiple metabolic processes (Roldán and Altamirano 1990; Léonard and Gerber 1994), including anabolic protein synthesis in exposed subjects. The findings in the present study are similar to the reports of Mustapha et al. (2014) and Azeez et al. (2016) who respectively, exposed mice pups to 3 mg/kg of vanadium on PND 1–21 (via lactation every 24 h) and PND 1–90 (via lactation every 24 h for the first 21 days, then i.p thrice a week for 3 months). In these two studies, it is note-worthy that vanadium caused no effect on the body weight of pups in the first two postnatal weeks of exposure (PND 1–14). Howbeit, significant reduction in body weights relative to the control was observed between PND 15 and 21 (Mustapha et al. 2014), and in both studies, the reduction spanned to PND 45, and in the third month of experimentation (PND 65–86) as reported by Azeez et al. (2016). These reports are at variance to the reports of no significant difference in the body weight after exposing 14-day old mice to 3 mg/kg vanadium for 14 days. This reported variance might be due to the age of commencement of exposure, duration of exposure and rate of exposure as suggested by Igado et al. (2020).

The second-generation vanadium pups had significantly lower body weights in the first week of life (PND 1–7) and in PND 21 compared to their controls. In between these period of low body weights, was a period of no significant difference, spanning from PND 9–19. The period of no significance could be attributed either to the attenuating effect of the dams' milk on weight loss or the pups' resistance to vanadium effects in possible low dose exposure in milk via hermetic response. Hermetic, a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces an adaptive beneficial effect on the cell or organism with initial homeostatic disruption (Mattson 2008). The reduction in body weight of the G2 vanadium pups observed in the first week of life suggests vanadium accumulation in the tissues, including the liver, kidney, spleen, muscles, brain, lungs and bones (Rehder 2013) of the dams: Possibly by *in utero* transfer of vanadium to pups and concurrent weight loss effect on the offspring. This is because the G2 dams were exposed to vanadium while they were pups.

The withdrawal vanadium group of the third generation had no significant reduction in body weight throughout the study timeline in comparison with matched controls. This could be explained as a consequence of withdrawal of vanadium and its weight-loss effects from the system of the G3 pups.

Vanadium caused neurobehavioural deficits across multiple generations. There was decreased locomotion and exploration in the OFT; a common measure of exploratory behaviour and general activity in rodents (Gould et al. 2009). Reduced line crosses, rearing, grooming time, centre square entry and duration indicate reduced locomotion, exploration and increased anxiety (Mustapha et al., 2014). Furthermore, high counts of faecal bolus and increased freezing time suggests increased anxiety. Although not statistically significant ($p>0.05$), the higher value for freezing time in G1 vanadium groups relative to the G2 and G3 may indicate adaptation and recovery respectively.

In the present study, there was decreased locomotor and exploratory activity in the two vanadium and vanadium withdrawal groups (G1-3) compared to the controls. This was typified by a statistically significant reduction in line crosses and rearing through all the generations. The reduced line crosses and rearing counts in the vanadium groups, including the withdrawal G3 group, may be attributed to vanadium-induced muscular weakness (Olopade et al. 2011). In addition, the neurobehavioural deficits in unexposed G3 vanadium group suggests either a lingering presence of vanadium in their system with attendant neurobehavioural deficits or vanadium-induced genotoxicity (Owusu-Yaw et al. 1990) to critical genes required for neuromuscular functions. The present findings, especially for G1 and G2 vanadium treated groups, are in line with the reports of Soazo and Garcia (2007), Olopade et al. (2011), Mustapha et al. (2014), Azeez et al. (2016) and Igado et al. (2020) on the functional deficits in vanadium treated animals. Similar to the findings in the withdrawal G3 group, heavy metals (cadmium, copper, lead, zinc, mercury) toxicity in *C. elegans* and zebrafish (*Danio rerio*) caused transgenerational neurobehavioural effects in unexposed progenies (Yu et al. 2013; Carvan et al. 2017). In another study on the zebrafish, cadmium chloride caused locomotor deficits, disruption of neurotransmitters and alteration of expression of genes responsible for nervous functions in progenies of exposed parents (Tian et al. 2021).

There was alteration of motor function in negative geotaxis, a reflex test that assesses motor development and vestibular function by measuring the latency for an animal to reposition itself when placed head down on a plane inclined at 45˚ (Olopade et al. 2011). In this study, the latency to turn against gravity was observed to reduce down the generation with G1 vanadium treated group having the longest latency and G3 vanadium withdrawal group having the shortest latency. The G1 and G2 vanadium treated groups had longer latency relative to their controls, while the latency for the G3 vanadium withdrawal group was observed to be almost equal with that of control.

The longer latencies in the G1 and G2 might be due to vanadium-induced muscle weakness (Olopade et al. 2011) in exposed animals as the pups displayed a reluctance to turn against gravity when placed head down on the inclined plane. Also, demyelination of cerebellar white matter and possible demyelination of vestibulocochlear nerve and projection pathways (like corticospinal, tectospinal tracts), all of which are responsible for vestibular function and visuospatial balance, might be responsible for the longer latency observed. These findings in the G1 and G2 vanadium group is congruent with the reports of Olopade et al. (2011) and Mustapha et al. (2014) for neonatal murine models. The neurobehavioural variation observed in the G3 suggests either a hormetic response to vanadium toxicity as observed in *C. elegans*' gst-4 induced resistance to increasing or accumulating doses of mercury at toxic levels (Helmcke and Aschner 2010), or withdrawal of vanadium and its neurobehavioural effects.

Vanadium caused histopathological changes in cerebellar Purkinje cells. Purkinje cells are large GABAnergic inhibitory neurons and are the only output neurons from the cerebellar cortex (Fonnum and Lock 2000). The Purkinje cells are formed from the ventricular zone (of the fourth ventricle) during embryonic development, and they migrate into the developing cerebellum to form a stratified layer of about six cells thick after which they are transformed into a single layer by PND 3–4 (Fonnum and Lock 2000; Sergaki and Ibáñez 2017).

Histopathological changes, including stratification and pyknosis of the Purkinje cell layer were observed in the vanadium exposed groups (G1 and G2) and withdrawal group (G3). These abnormal findings has been previously reported in vanadium-exposed neonatal mice (Igado et al. 2020), and have been attributed to vanadium-altered cell migration and vanadium-induced neuronal cell death (Igado et al. 2012; Igado et al. 2020). These Purkinje cell pathologies corroborate the locomotor and neurobehavioural deficits observed in the neurobehavioural tests as cerebellum is key for muscular functions in movement and coordination (Schmahmann 2019). Purkinje cell death with resultant reduction in its population has been reported in offspring of non-human primates administered ethanol during pregnancy (Bonthius et al. 1996). Stratification of Purkinje cell layer has also been reported in lead poisonings in mice (Jaarsma et al. 2014) and in goats (Jubril et al. 2019).

Vanadium caused reactive changes in the glial cells across multiple generations, resulting in astrogial reactivity in all the generations. Astrocytes, being one of the first responders in central nervous system

insults (Olopade et al. 2011; Folarin et al. 2017a), is triggered into a reactive state by reactive oxygen species (Sofroniew 2009; Sofroniew and Vinters 2010), which vanadium generates intracellularly (Sasi et al. 1994; Haider et al. 1998; Garcia et al. 2005; Ścibior and Kurus 2019).

In the present study, vanadium exposed groups (G1 and G2) had moderate to marked astroglial reactivity respectively in the CA1 region of their hippocampi and in the polymorphic layer of their dentate gyri. Moreover, fibres of reactive astrocytes were more obvious in the granular layer of the dentate gyrus than in the controls. The marked reactivity in G2 might imply an accumulative effect of vanadium exposure from the previous generation (G1). It is however interesting to note the sharp reduction in astrocytic reactivity in the untreated G3 vanadium group compared to that observed in the G2, which supports the gradual withdrawal of vanadium and its subsequent toxic effects from the system in the withdrawal group (G3). It also suggests that intense astrogliosis might be short-lived in unexposed offspring of individuals previously exposed to the metal. Astroglial reactions observed in G1 and G2 vanadium group is similar to the ones reported by Olopade et al. (2011); Mustapha et al. (2014); Azeez et al. (2016) and Igado et al. (2020) in neonatal mice irrespective of the age of commencement of vanadium exposure, even though these studies did not report any form of vanadium accumulation over generations. Reduction in intensity of astroglial reactivity, as observed in the unexposed G3 vanadium group, has been reported in cases of vanadium withdrawal (Folarin et al. 2017a).

There was generalized demyelination in all the generations. The brain is susceptible to lipid peroxidation because it contains a high amount of polyunsaturated fatty acids, high aerobic metabolism and relatively low level of antioxidant enzymes (Wang and Michaelis 2010). Vanadium causes membrane lipid depletion by inducing oxidative stress (Folarin et al. 2017b), and by activating the calcium-dependent phospholipase A2 (Korbecki et al. 2015). Neonate murine brains are particularly more susceptible to the effects of vanadium on myelin because the proliferation of oligodendrocytes is completed 1–2 weeks after birth. Running concurrently with this proliferative phase is a rapid myelination phase (6-30 days after birth) (Simone et al. 2010). It was therefore no coincidence in the present study that MBP immunohistochemistry revealed generalized demyelination in the region of the corpus callosum around the CA1 hippocampal area, the hippocampal formations, the cerebral cortices and in the thalami of the vanadium treated and withdrawal groups (G1-3) relative to the rich myelin staining in the matched controls. The lack of recovery in the withdrawal group might be due to damage to genes responsible for oligodendrocyte development and function. The hippocampal formation has been reported to be one of the sites of neurogenesis (Fares et al. 2019; Abdissa et al. 2020) and it plays a major role in memory and learning (Dhikav and Anand 2012). Generalized demyelination of the granular neurons of the dentate gyri, the pyramidal neurons of the hippocampus, and the cerebral cortices of the pups in all the vanadium treated groups (G1 and G2), most especially in the withdrawal group (G3) suggests higher chances of memory and learning impairments with attendant reduction in the rate of neurogenesis relative to the controls. In the same vein, demyelination of the thalamus across all the vanadium groups and withdrawal group might affect the relaying of neural information, and could further explain the neurobehavioural deficits observed in them.

Moreover, there was moderate to marked demyelination, with reduced branching of the white matter in the cerebellum of the G1 and G2 vanadium groups respectively. The marked demyelination reaction in the cerebellum of the G2 vanadium group corroborates the possibility of the effects of compounded vanadium exposure from the previous G1 generation. Further, corroborating these neurohistological findings in the vanadium treated groups are the reduced locomotion activity in the OFT, and the longer latency in the negative geotaxis. These results substantiate the hypothesis of vanadium-induced demyelination made for neurobehavioural deficits (Mustapha et al. 2014), possibly, of nerves responsible for toning of skeletal muscle and spatial balance for movement. In contrast to the pallor observed in the cerebellar white matter of G1 and G2 vanadium treated groups, G3 withdrawal group had myelin staining intensity close to that of their control counterparts. These results further buttress the negative geotaxis observations for G3 and the aforementioned hormetic effect. Surprisingly in G3 vanadium withdrawal group, the rate of demyelination around the hippocampal areas were worse compared to the cerebellar arbo vitae. This difference in the rate of demyelination might be due to either an earlier recovery of the arbo vitae from the demyelinating effect of vanadium or the vanadium-induced damage of myelinated neurons in the hippocampal formation and surrounding regions. It is therefore suggested that unexposed offspring from parents exposed to vanadium might not show obvious locomotor neurobehavioural changes compared to unexposed population but might have retarded cognitive functions.

The regions of hypomyelination were observed to coincide with areas of astrocytic activation just as reported by Azeez et al. (2016) in mice. These findings are similar to those reported by Soazo and Garcia (2007), Olopade et al. (2011), Todorich et al. (2011), Mustapha et al. (2014) and Igado et al. (2020) in vanadium treated murine models. Tian et al. (2021) reported alterations of genes responsible for nervous functions in unexposed progenies of

zebrafish parents exposed to cadmium chloride, a compound of heavy metal.
The MBP immunohistochemistry is a basic test to assess demyelination. The visual evidence of severe demyelination in G2 cannot be denied in spite of the statistically insignificant results. More sensitive tests shedding light on the effects of the study on myelinated axons, oligodendrocytes and oligodendrocyte precursor cells, amongst other tests, may give a more wholistic result. Further experiments intend to shed light on the questions raised in this current study.

Conclusion

In conclusion, this study showed that astrogliosis and generalized demyelination in key brain regions occurred in unexposed offspring of exposed maternal generations with accompanying neurobehavioural deficits and possible memory and learning perturbations. This is of crucial health importance in the susceptible and vulnerable populace (like those living in the Niger Delta region of Nigeria, or the Arabian Gulf), whose offspring might show phenotypic signs of vanadium toxicity even in the absence of exposure. Based on these, it can be hypothesized that longer exposures to higher doses of vanadium over more than two generations might either result in more adverse effects down the generations or lead to resistance through a hormetic response. Either way, there exists an open window for incidences of neurodegenerative diseases through damage of the genes critical for nervous development. This is of great importance in younger and newer generations as damaged genes can be inherited. There is therefore a need to further investigate the molecular mechanism(s) of generational vanadium toxicity and the effects of transplacental vanadium transfer to foetus during gestation in vanadium exposed dams.

Grants and Financial Support
This work was supported partly by Tertiary Education Trust Fund (TETFUND) (TETF/DR&D/CE/NRF/STI /15. VOL. 1) received by J.O. Olopade.

Conflict of Interest
None declared.

Acknowledgements
This study was partly funded by the TETFUND granted to Olopade, J.O. The authors gratefully acknowledge the technical assistance of Dr. Olubusayo Folarin, of IAMRAT, College of Medicine, University of Ibadan, Nigeria.

Authors' Contribution
Conceptualization – JOO; animal experiments, neurobehavioural tests, histopathology – ADL, OOI, FEO; data analysis and interpretation – all authors; initial manuscript draft – ADL & OOI; manuscript editing – JOO, OOI and FEO. All authors read and approved of the final manuscript.

Data Availability
The datasets generated and analysed during the course of this study are not publicly accessible due to a probable absence of a public repository for the type of data generated and analysed in this study. However, the data are available from the corresponding author on request.

REFERENCES

Abdissa, D., Hamba, N. and Gerbi, A. (2020) Review article on adult neurogenesis in humans. Transl Res Anat 20: 100074. https://doi.org/10.1016/j.tria.2020.100074

Azeez, I.A., Olopade, F., Laperchia, C., Andrioli, A., Scambi, I., Onwuka, S.K., et al. (2016) Regional myelin and axon damage and neuroinflammation in the adult mouse brain after long-term postnatal vanadium exposure. J Neuropathol Exp Neurol. 75(9):843–854. https://doi.org/10.1093/jnen/nlw058

Bonthius, D.J., Bonthius, N.E., Napper, R.M.A., Astley, S.J., Clarren, S.K. and West, J.R. (1996) Purkinje cell deficits in nonhuman primates following weekly exposure to ethanol during gestation. Teratology. 53(4):230–236. https://doi.org/10.1002/(SICI)1096-9926(199604)53:4<230::AID-TERA5>3.0.CO;2-6

Briffa, J., Sinagra, E. and Blundell, R. (2020) Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon. 6(9):e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Brown, R.E., Corey, S.C. and Moore, A.K. (1999) Differences in measures of exploration and fear in MHC-Congenic C57BL/6J and B6-H-2K Mice. Behav Genetics. 29(4):263–271. https://doi.org/https://doi.org/10.1023/A:1021694307672

Carvan, M.J., Kalluvila, T.A., Klingler, R.H., Larson, J.K., Pickens, M., Mora-Zamorano, F.X., et al. (2017) Mercury-induced epigenetic transgenerational inheritance of abnormal neurobehavior is correlated with sperm epimutations in zebrafish. PLOS ONE. 12(5):e0176155. https://doi.org/10.1371/journal.pone.0176155

Chen, L., Wang, X., Zhang, X., Lam, P.K.S., Guo, Y., Lam, J.C.W., et al. (2017) Transgenerational endocrine disruption and neurotoxicity in zebrafish larvae after parental exposure to binary mixtures of decabromodiphenyl ether (BDE-209) and lead. Environ Pollut. 230:96–106. https://doi.org/10.1016/j.envpol.2017.06.053

Ciranni, R., Antonetti, M. and Migliore, L. (1995) Vanadium salts induce cytogenetic effects in in vivo treated mice. Mutation Research/Genetic Toxicology, 343(1):53–60. https://doi.org/10.1016/0165-1218(95)90061-6

Das, S., Chatterjee, M., Janarthan, M., Ramachandran, H. and Chatterjee, M. (2012) Vanadium in Cancer Prevention. Netherlands: Springer. https://doi.org/10.1007/978-94-007-0913-3_8

Dhikav, V. and Anand, K. (2012) Hippocampus in health and disease: An overview. Ann Indian Acad Neurol. 15(4):239. https://doi.org/10.4103/0972-2327.104323

Duce, R.A. and Hoffman, G.L. (1976) Atmospheric vanadium transport to the ocean. Atmospheric Environ. 10(11):989–996. https://doi.org/10.1016/0004-6981(76)90207-9

Emsley. J. (2011) Nature's Building Blocks: An A-Z Guide to the Elements (2nd Edition). New York: Oxford University Press.

Fares, J., Bou Diab, Z., Nabha, S. and Fares, Y. (2019) Neurogenesis in the adult hippocampus: history, regulation, and prospective roles. Int J Neurosci. 129(6):598–611. https://doi.org/10.1080/00207454.2018.1545771

Folarin, O.R., Adaramoye, O.A., Akanni, O.O. and Olopade, J.O. (2017b) Changes in the brain antioxidant profile after chronic vanadium administration in mice. Metab Brain Dis. 33(2):377–385. https://doi.org/10.1007/s11011-017-0070-9

Folarin, O.R., Snyder, A.M., Peters, D.G., Olopade, F., Connor, J.R. and Olopade, J.O. (2017a) Brain metal distribution and neuro-inflammatory profiles after chronic vanadium administration and withdrawal in mice. Front Neuroanat. 11:00058. https://doi.org/10.3389/fnana.2017.00058

Fonnum, F. and Lock, E.A. (2000) Cerebellum as a target for toxic substances. Toxicol Lett. 112–113:9–16. https://doi.org/10.1016/S0378-4274(99)00246-5

Garcia, G.B., Biancardi, M.E. and Quiroga, A.D. (2005) Vanadium (V)-induced neurotoxicity in the rat central nervous system: A histo-immunohistochemical study. Drug Chem Toxicol. 28(3):329–344. https://doi.org/10.1081/DCT-200064496

García, G.B., Quiroga, A.D., Stürtz, N., Martinez, A.I. and Biancardi, M.E. (2004) Morphological alterations of central nervous system (CNS) myelin in vanadium (v)-exposed adult rats. Drug Chem Toxicol. 27(3):281–293. https://doi.org/10.1081/DCT-120037747

Gould, T.D., Dao, D.T. and Kovacsics, C.E. (2009) The Open Field Test. New Jersey: Humana Press. https://doi.org/10.1007/978-1-60761-303-9_1

Habib, H.M. and Ibrahim, W.H. (2011) Nutritional quality of 18 date fruit varieties. Int J Food Sci Nutrition. 62(5):544–551. https://doi.org/10.3109/09637486.2011.558073

Haider, S.S., Abdel-Gayoum, A.A., El-Fakhri, M. and Ghwarsha, K.M. (1998) Effect of selenium on vanadium toxicity in different regions of rat brain. Hum Exp Toxicol, 17(1):23–28. https://doi.org/10.1177/096032719801700104

Helmcke, K.J. and Aschner, M. (2010) Hormetic effect of methylmercury on Caenorhabditis elegans. Toxicol Appl Pharmacol., 248(2):156–164. https://doi.org/10.1016/j.taap.2010.07.023

Igado, O.O., Olopade, J.O., Onwuka, S.K., Chukwudi, A.C., Daramola, O.A. and Ajufo, U.E. (2008) Evidence of environmental pollution in caprine brains obtained from a relatively unindustrialized area in Nigeria. Afr J Biomed Res. 11(3):305–309. https://doi.org/10.4314/ajbr.v11i3.50744

Igado, O.O., Andrioli, A., Azeez, I.A., Girolamo, F., Errede, M., Aina, O.O. et al. (2020) The ameliorative effects of a phenolic derivative of Moringa oleifera leave against vanadium-induced neurotoxicity in mice. IBRO Reports. 9:164–182. https://doi.org/10.1016/j.ibror.2020.07.004

Igado, O.O., Olopade, J.O., Adesida, A., Aina, O.O. and Farombi, E.O. (2012) Morphological and biochemical investigation into the possible neuroprotective effects of kolaviron (Garcinia kola bioflavonoid) on the brains of rats exposed to vanadium. Drug Chem Toxicol. 35(4):371–380. https://doi.org/10.3109/01480545.2011.630005

Imtiaz, M., Rizwan, M.S., Xiong, S., Li, H., Ashraf, M., Shahzad, S.M. et al. (2015) Vanadium, recent advancements and research prospects: A review. Environ Int. 80: 79–88. https://doi.org/10.1016/j.envint.2015.03.018

Jaarsma, D., Van den Berg, R., Wulf, P.S., Van Erp, S., Keijzer, N., Schlager, M.A., et al. (2014) A role for Bicaudal-D2 in radial cerebellar granule cell migration. Nature Comm. 5(1):3411. https://doi.org/10.1038/ncomms4411

Jubril, A.J., Obasa, A.A., Mohammed, S.A., Olopade, J.O. and Taiwo, V.O. (2019) Neuropathological lesions in the brains of goats in North-Western Nigeria: possible impact of artisanal mining. Environ Sci Pollut Res. 26(36):36589–36597. https://doi.org/10.1007/s11356-019-06611-y

Khan, S., Kazi, T.G., Kolachi, N.F., Baig, J.A., Afridi, H.I., Shah, A.Q., et al. (2011) Hazardous impact and translocation of vanadium (V) species from soil to different vegetables and grasses grown in the vicinity of thermal power plant. J Hazard Mater. 190(1–3):738–743. https://doi.org/10.1016/j.jhazmat.2011.03.105

Kocadal, K., Alkas, F., Battal, D. and Saygi, S. (2020) Cellular pathologies and genotoxic effects arising secondary to heavy metal exposure: A review. Hum Exp Toxicol. 39(1):3–13. https://doi.org/10.1177/0960327119874439

Korbecki, J., Baranowska-Bosiacka, I., Gutowska, I. and Chlubek, D. (2015) Vanadium compounds as pro-inflammatory agents: Effects on cyclooxygenases. Int J Mol Sci. 16(12):12648–12668. https://doi.org/10.3390/ijms160612648

Léonard, A. and Gerber, G.B. (1994) Mutagenicity, carcinogenicity and teratogenicity of vanadium compounds. Mutat Res Genet Toxicol. 317(1):81–88. https://doi.org/10.1016/0165-1110(94)90013-2

Li, A., Zhuang, T., Shi, J., Liang, Y. and Song, M. (2019) Heavy metals in maternal and cord blood in

Beijing and their efficiency of placental transfer. J Environ Sci. 80: 99–106. https://doi.org/10.1016/j.jes.2018.11.004

Martinez-Zamudio, R. and Ha, H.C. (2011) Environmental epigenetics in metal exposure. Epigenetics. 6(7):820–827. https://doi.org/10.4161/epi.6.7.16250.

Mattson, M.P. (2008) Hormesis defined. Ageing Res Rev. 7(1):1–7. https://doi.org/10.1016/j.arr.2007.08.007

Mustapha, O., Oke, B., Offen, N., Sirén, A.L. and Olopade, J. (2014) Neurobehavioral and cytotoxic effects of vanadium during oligodendrocyte maturation: A protective role for erythropoietin. Environ Toxicol Pharmacol. 38(1):98–111. https://doi.org/10.1016/j.etap.2014.05.001

NIH (1996) National Institute of Health Guide for the Care and Use of Laboratory Animals (1996). NIH Publications No. 80-23, revised 1996.

Nriagu, J.O. (1990) Global metal pollution: poisoning the biosphere? Environment: Science and Policy for Sustainable Development. 32(7):7–33. https://doi.org/10.1080/00139157.1990.9929037

Olopade, J.O., Fatola, I.O. and Olopade, F.E. (2011) Vertical administration of vanadium through lactation induces behavioural and neuromorphological changes: Protective role of vitamin E. Niger J Physiol Sci. 26(1):55–60.

Owusu-Yaw, J., Cohen, M.D., Fernando, S.Y. and Wei, C.I. (1990) An assessment of the genotoxicity of vanadium. Toxicol Lett. 50(2–3):327–336. https://doi.org/10.1016/0378-4274(90)90026-I

Preet, A., Gupta, B.L., Siddiqui, M.R., Yadava, P.K. and Baquer, N.Z. (2005) Restoration of ultrastructural and biochemical changes in alloxan-induced diabetic rat sciatic nerve on treatment with Na_3VO_4 and Trigonella—a promising antidiabetic agent. Mol Cell Biochem. 278(1–2):21–31. https://doi.org/10.1007/s11010-005-7815-1

Rehder, D. (2012) The potentiality of vanadium in medicinal applications. Future Med Chem. 4(14):1823–1837. https://doi.org/10.4155/fmc.12.103

Rehder, D. (2013) Vanadium: Its Role for Humans. Springer, Netherlands, pp. 139–169. https://doi.org/10.1007/978-94-007-7500-8_5

Rehder, D. (2016) Perspectives for vanadium in health issues. Future Med Chem. 8(3):325–338. https://doi.org/10.4155/fmc.15.187.

Roldán, R.E. and Altamirano, L.M.A. (1990) Chromosomal aberrations, sister-chromatid exchanges, cell-cycle kinetics and satellite associations in human lymphocyte cultures exposed to vanadium pentoxide. Mutat Res Lett. 245(2):61–65. https://doi.org/10.1016/0165-7992(90)90001-Z

Sasi, M.M., Haider, S.S., El-Fakhri, M. and Ghwarsha, K.M. (1994) Microchromatographic analysis of lipids, protein, and occurrence of lipid peroxidation in various brain areas of vanadium exposed rats: a possible mechanism of vanadium neurotoxicity. Neurotoxicol. 15(2):413–420

Schmahmann, J.D. (2019) The cerebellum and cognition. Neurosci Lett. 688:62–75. https://doi.org/10.1016/j.neulet.2018.07.005

Ścibior, A. and Kurus, J. (2019) Vanadium and oxidative stress markers - in vivo model: A review. Curr Med Chem. 26(29):5456–5500. https://doi.org/10.2174/0929867326666190108112255

Sergaki, M.C. and Ibáñez, C.F. (2017) GFRα1 regulates purkinje cell migration by counteracting NCAM function. Cell Reports. 18(2):367–379. https://doi.org/10.1016/j.celrep.2016.12.039

Simone, P., Cristiane, L., Eluza, S.C., Silvane, R.S. and Cristina, N.W. (2010) Diphenyl ditelluride induces neurotoxicity and impairment of developmental behavioral in rat pups. J Braz Chem Soc. 21 (11):2130–2137. https://doi.org/10.1590/S0103-50532010001100015

Soazo, M. and Garcia, G.B. (2007) Vanadium exposure through lactation produces behavioral alterations and CNS myelin deficit in neonatal rats. Neurotoxicol Teratol. 29(4):503–510. https://doi.org/10.1016/j.ntt.2007.03.001

Sofroniew, M.V. (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 32(12):638–647. https://doi.org/10.1016/j.tins.2009.08.002

Sofroniew, M.V. and Vinters, H.V. (2010) Astrocytes: biology and pathology. Acta Neuropathologica. 119 (1):7–35. https://doi.org/10.1007/s00401-009-0619-8.

Tian, J., Hu, J., Liu, D., Yin, J., Chen, M., Zhou, L., et al. (2021) Cadmium chloride-induced transgenerational neurotoxicity in zebrafish development. Environ Toxicol Pharmacol. 81:103545. https://doi.org/10.1016/j.etap.2020.103545

Timothy, N. and Williams, E.T. (2019) Environmental pollution by heavy metal: an overview. International IJEC. 3(2):72. https://doi.org/10.11648/j.ijec.20190302.14

Todorich, B., Olopade, J.O., Surguladze, N., Zhang, X., Neely, E. and Connor, J.R. (2011) The mechanism of vanadium-mediated developmental hypomyelination is related to destruction of oligodendrocyte progenitors through a relationship with ferritin and iron. Neurotox Res. 19(3):361–373. https://doi.org/10.1007/s12640-010-9167-1

Underwood, E.J. (1971) Trace elements in human and animal nutrition (3rd Edition). New York : Academic Press.

Usende, I.L., Leitner, D.F., Neely, E., Connor, J.R. and Olopade, J.O. (2016) The deterioration seen in myelin related morphophysiology in vanadium exposed rats is partially protected by concurrent iron deficiency. Niger J Physiol Sci. 31 (1):11–22.

Wang, J., Yuen, V.G. and McNeill, J.H. (2001. Effect of vanadium on insulin sensitivity and appetite. Metabolism. 50(6):667–673. https://doi.org/10.1053/meta.2001.23294

Wang, X. and Michaelis, E.K. (2010) Selective neuronal vulnerability to oxidative stress in the brain.

Front Aging Neurosci. 2(12):1–13. https://doi.org/10.3389/fnagi.2010.00012

Wei, C.C., Yen, P.L., Chaikritsadakarn, A., Huang, C.W., Chang. C.H. and Liao, V.H.C. (2020) Parental CuO nanoparticles exposure results in transgenerational toxicity in Caenorhabditis elegans associated with possible epigenetic regulation. Ecotoxicol Environ Saf. 203:111001. https://doi.org/10.1016/j.ecoenv.2020.111001

Yu, Z., Chen, X., Zhang, J., Wang, R. and Yin, D. (2013) Transgenerational effects of heavy metals on L3 larva of Caenorhabditis elegans with greater behavior and growth inhibitions in the progeny. Ecotoxicol Environn Saf. 88:178–184. https://doi.org/10.1016/j.ecoenv.2012.11.012

Zhang, J. and Yu, Z. (2020) Transgenerational effects of different sequential exposure to 2,2′,4,4′-tetra-brominated diphenyl ether (BDE47) and lead (Pb) on Caenorhabditis elegans. Environ Sci Eur. 32 (1):44. https://doi.org/10.1186/s12302-020-00318-5

Zhang, Y., Zhao, C., Zhang, H., Lu, Q., Zhou, J., Liu, R., et al. (2021) Trans-generational effects of copper on nerve damage in Caenorhabditis elegans. Chemosphere. 284:131324. https://doi.org/10.1016/j.chemosphere.2021.131324

Cite as: Lijoka A.D., Igado, O.O., Olopade, F.E. and Olopade, O.O. (2022). Effects of in-vivo vanadium-induced neurotoxicity and withdrawal on three consecutive generations of mice brains: A neurobehavioural and histo-immunohistochemical study. Nig. J. Neurosci. 13(4): 119-131. https://doi.org/10.47081/njn2022.13.4/002

© Copyright Nigerian Journal of Neuroscience. All rights reserved.