Reproductive Hormones Imbalance, Germ Cell Apoptosis, Abnormal Sperm Morphophenotypes and Ultrastructural Changes in Testis of African Giant Rats (Cricetomys gambianus, Waterhouse, 1840) Exposed to Sodium Metavanadate Intoxication

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Abstract

Environmental exposure to vanadium has been on the increase in recent time. This metal is a known toxicant. The current study was conducted to investigate the reproductive toxicity of sodium metavanadate (SMV) in male African giant rats. Administration of SMV was done intraperitoneally daily for 14 consecutive days at a dosage of 3mg/kg body weight. Sterile water was administered to the control group. We analyzed serum reproductive hormones, sperm reserve and quality as well as testicular ultrastructural changes following SMV treatment. Our results showed SMV exposed AGR group had statistically increased progesterone but decreased testosterone, FSH and LH concentrations. Also, SMV treated group had statistically decreased sperm motility and mass activity with increased percentage of abnormal morphophenotypes of spermatozoa and upregulation of \( P^{53} \) immunopositive cell. Ultrastructural study revealed vacuolation of germ and Sertoli cells, cytoplasmic and nucleus; and mitochondrial swelling and vacuolations were also observed. There was severe disintegration of the seminiferous tubules, atrophy and degeneration of myeloid cells and apoptosis of the Leydig, Sertoli and germ cells. In conclusion, intraperitoneal SMV exposure exerts severe adverse effects on some serum reproductive hormones, reduction of sperm reserve and quality, apoptosis and degenerative changes of the Leydig, Sertoli and germ cells which can lead to infertility.

Introduction

Environmental pollution in third world countries has been on the increase for the past five decades due to increased exploration of minerals (Calderon-Garciduenas et al., 2003; Olopade et al., 2005; Usende et al., 2016). Metals such as vanadium are emitted as a result of this constant increase of environmental pollution mainly due to combustion of fossil fuel (Calderon-Garciduenas et al., 2003; Igado et al., 2008; Usende et al., 2016, 2017).

Vanadium, a metal in the first transition series is widely distributed in nature and a potent environmental toxicant (Usende et al., 2017, 2018a, b). Although, some derivatives of vanadium have been found useful in medicine and industry, acute environmental and / or occupational exposure to this metal is of serious health concern to both humans and animals (Ray et al., 2007; Shrivastava et al., 2007; Tracey et al., 2007; Li et al., 2020). Vanadium compounds have been used pharmacologically as antidiabetic drug (Tsiani and Fantus 1997; Sanchez et al., 1996); as blood tonic, antiseptics, as well as antituberculotic agents (Shrivastava et al., 2007; Morinville et al., 1998). Despite these medicinal and industrial usefulness, the major problem with vanadium has been its potential toxicity (Sanchez et al., 1996).

Decreased food intake, diarrhoea and weight loss (Usende et al., 2018b), changes in biochemical and hematological parameters, hepatotoxicity and nephrotoxicity amongst others have been found in healthy rats and mice after vanadium intoxication (Wilk et al., 2017; Usende et al., 2018b). In humans, vanadium intoxication is common and occurs due to occupational processes, vanadium ore processing and purification, fossil fuels combustion, and in the production of vanadium containing chemicals (Ehrlich et al., 2008). Humans occupationally exposed to vanadium intoxication manifests clinical disorders...
including anaemia, nasal epithelium irritations, dry throat and eyes, and chest pain (Kim and Ferry, 2004; Sughis et al., 2012). These clinicopathological events are time dependent (Usende et al., 2018a, b) confirming earlier reports of Agramunt et al., (2003) of significantly increased urine vanadium concentration corresponding with the period of occupational exposure in human subjects. Of recent, focus is on the toxic effect of vanadium on the reproductive system (Aragon et al., 2005), and the use of wildlife rodents as toxicological models (Usende et al., 2017, 2018a, b).

Vanadium toxicity of the male reproductive system is of special interest due to their role in the formation of the male sex gamete necessary for continuity of life (Aragon and Altamirano-Lozano 2001; Aragon et al., 2005). Information on the toxic effects of vanadium on the male reproductive system has been controversial and restricted to testicles and sperm cells with less emphasis on the male reproductive hormones (Aragon et al., 2005). Whereas Aragon and Altamirano-Lozano (2001) reported no difference in sperm count between control and vanadium treated rat groups, Uche et al. (2008) reported that spermatozoa counts were significantly diminished after vanadium exposure. On the other hand, African giant rats as experimental animal model for ecotoxicological studies have received recent attention in recent time since these wildlife rodents are exposed to various metals especially vanadium in oil producing areas of most poor resource setting (Usende et al., 2017, 2018a, b, 2020). African giant rats (AGR) (Cricetomys gambianus, Waterhouse, 1840) are also known as pouched rat and belong to the family Muridae and order Rodentia (Ibe et al., 2014). They are found in Central and West African countries and have been suggested to be experimental model for ecotoxicological research due to their nocturnal and ubiquitous nature (Usende et al., 2017; 2018a, b). Little effort has been made in using species domiciled in a particular polluted area to investigate alterations in toxicological responses and more specifically in relation to environmental toxins (Usende et al., 2018b). Therefore, the aim of this study is to investigate the reproductive toxicity of sodium metavanadate (SMV) in male African giant rat model.

**Materials And Methods**

**Reagent**

Sodium metavanadate (Sigma, Milano MI, Italy) used for this study was obtained as powder and diluted with sterile injection water to a stock solution of 2.5mM. Aliquots were taken and kept at -20°C and were used as needed. Reagents for transmission electron microscopy study were purchased from Electron Microscopy Sciences (Giza 12111, Egypt). Testosterone, progesterone, 17β estradiol, follicle stimulating (FSH) and luteinizing (LH) hormonal kits and all other chemicals, were of analytical grade and were purchased from Bristol Scientific Company, (Sigma-Aldrich, Lagos Nigeria), unless otherwise specified.

**Animals and treatment**

Young adult AGR weighing 875±30g were used. The AGR were obtained from Ibadan, Nigeria and were kept singly in metal cages in a pathogen-free, well-ventilated Animal Core facility of the Neuroscience Unit, University of Ibadan, under controlled light conditions (12 h light/12 h dark) with free access to
water and food (standard commercial pelletized rat feed, fresh groundnut and yam). The AGR are known to have preference for nuts and tubers in their natural habitat (Usende et al., 2018a). The animals were acclimatized to this environment to avoid transitory increase in abnormal sperm seen at onset of spermatogenesis (Bakare et al., 2005). The animal experimental protocol received approval by the University of Abuja Ethics Committee for Animal Use (UAECAU/2017/007) and in accordance with ethical standard of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Vanadium Administration

Twelve (12) AGR were used and were randomly divided into two groups of six animals each. Group 1 served as control and were injected sterile water intraperitoneally (i.p) for 14 days. Group 2 served as the SMV treated group and were administered SMV at 3mg/kg body weight once daily for 14 days. This dose has been shown to induce hypomyelination and neurobehavioural deficits in Sprague-Dawley rats and oxidative stress, hepatorenal and cytogenotoxicity in AGR (Usende et al., 2016, 2018a, b; 2020). Intraperitoneal route of injection was utilized because this can be carefully controlled and also because the SMV administered in this route can be rapidly absorbed from the peritoneum into the blood stream causing various pathologies (Usende et al., 2018b). Twenty-four (24) hours after the last injection, five millilitres (5ml) venous blood was collected at room temperature from the retro-orbital sinus plexus from all AGR of both groups into plain sterile tubes. The blood was centrifuged at 4000 rpm for 15 minutes to obtain a clear serum. The clear serum obtained was separated with Pasteur pipettes into another plain tube and stored at -20°C until the time of hormonal assays.

Reproductive hormones assay

Serum collected and stored from all AGR of SMV treated and control groups were used for reproductive hormones assays. Serum testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), 17β estradiol and progesterone concentrations from all two groups were assayed in duplicate using commercially available radioimmunoassay kit (Bristol Scientific Company, Sigma-Aldrich, Lagos Nigeria) following manufacturer’s instruction. The results are presented as ng/ml (mean ± standard error of mean (SEM)).

Assay of sperm motility and viability/abnormalities

The AGR from SMV group and the control groups after collection of blood were deeply anaesthetized using xylazine and ketamine (90/10mg/kg). The test for sperm cells motility and abnormality was carried out according to modified method described by Bakare et al. (2005) and Mofio et al., (2020) with some modifications. The right caudal epididymides of both groups were surgically exteriorized, blotted free of blood and thereafter punctured with a 21-gauge sterile needle. Sperm smears were prepared from the epididymides as previously described (Mofio et al., 2020). From each animal, two slides were prepared.
For each AGR 600 sperm cells, 300 from each slide were assessed for motility, mass activity, viability/vitality and morphological abnormalities of the sperm cell according to modified criteria of Bakare et al. (2005).

**Determination of testicular (gonadal) and epididymal (extra-gonadal) sperm reserves**

Determination of testicular and epididymal sperm reserves was done following the method described by Ladipo, (2015) and Mofio et al., (2020) with some modifications. Briefly, the left testis and its caudal-epididymis of both SMV-treated and control groups were carefully dissected, trimmed of extraneous tissues and weighed separately using bench-top sensitive electronic balance (LP 502A, China) with sensitivity of 0.1 to 5kg. The testes and caudal-epididymides collected from the rats were macerated separately in a ceramic mortar and pestle and 10 ml of normal saline was used in homogenizing the macerated testes and caudal-epididymides. The suspensions were mixed and sieved through a double layer of sterile wire gauze into clean glass test tubes after which 1 ml of the filtrate were aspirated out using sterile syringes and were made up to 10ml using 9 ml of white blood cell (WBC) diluting fluid. Gonadal and epididymal sperm concentrations were determined by direct haemocytometric count. The sperm cells in the four large corner squares (measuring 1 mm$^2$ each) were counted and the total multiplied by a correction factor of 250,000 to give the number of sperm cells per ml of gonadal and epididymal sperm reserve.

**Perfusion fixation**

After the collection of left testis and left and right caudal epididymides, all AGR from both groups were perfused transcardially; first with normal saline to wash out the blood cells and then with freshly prepared 4% paraformaldehyde (PFA) in 0.1M phosphate buffer till well fixed using stiffness of muscles and pallor of the liver as indicator for good fixation. Following perfusion, the right testis was quickly dissected out and bisected into two halves. One half was excised and used for immunohistochemistry while the other half was excised and used for transmission electron microscopy.

**Immunohistochemistry**

The half testis for immunohistochemistry was fixed for 4 hours in the same 4% PFA solution used for perfusion. Samples were then rinsed and transferred into 0.1% sodium azide in 0.1M phosphate buffered saline (PBS) and stored at 4°C until sectioning. About 4-6 mm of testis tissue was prepared for immunohistochemistry as described by Usende et al. (2016). Briefly, the prepared testis slides were air dried and labelled with pencil. Slides were baked for 30 mins at 60°C to dewax, then deparaffinized in two changes of xylene and hydrated in decreasing percentage of ethanol. Antigen retrieval was done in 10 mM citrate buffer (pH=6.0) for 25 mins, with subsequent peroxidase quenching in 3% H$_2$O$_2$/methanol for 20 mins. Slides were then washed in PBS and sections circled with PAP pen. Samples were then blocked in 2% PBS milk for 1hr in humidity chamber (200 μl/slide). All sections were probed with monoclonal antibody against p53 (PAb240) diluted in 1% PBS milk overnight in humidity chamber at 4°C. 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 DAB (1:25 dilution) for 5-10 mins, with subsequent dehydration in ethanol. The slides were mounted with permount, coverslipped and allowed to dry. Images were captured using a light microscope (Olympus CX31 light microscope) connected to a laptop computer (Hp, China) with digital camera.

Transmission Electron Microscopy

Protocol for TEM was as described by Hoeich et al. (2002) with minor modifications. Briefly, perfused half testis samples were quickly immersion fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer. Testes were then sectioned into small blocks of about 1-2mm³. They were post fixed in 1% osmium tetroxide, buffered to pH 7.4, dehydrated in graded ethanol concentrations, and embedded in Epon. Semi-thin sections were obtained with a LKB (8800 ultrotome) microtome, stained with toluidin blue for examination of areas of interest using Olympus CX31 light microscope. Thereafter, thin sections (60-80nm) were obtained with Reichert-ultracut S (Leica) microtome according to areas of interest, placed on copper grids, and stained with uranyl acetate and lead citrate. The sections were analyzed in a JEOL (JEM-100CXII) transmission electron microscope and photographed with a CCD digital camera (model XR-41M).

Data analysis

Numerical data obtained are presented as mean ± SEM. The SMV-treated group was compared to control group by Students’ t-test. Analysis was done on a PC with graph-pad prism 7.0 software and A P value of < 0.05 was accepted as statistically significant.

Table 1: Effects of intraperitoneal exposure of AGR to 3mg/kg body Sodium metavanadate for 14 days on some sperm parameters

| Parameter/ Group                  | CONTROL          | SMV (3mg/kg)         |
|-----------------------------------|------------------|----------------------|
| Testicular count (x10⁶/ml)        | 636.5 ± 17.31    | 175.4 ± 7.12a        |
| Epididymal count(x10⁶/ml)         | 147.2 ± 2.34     | 33.75 ± 3.96a        |
| Total abnormal sperm cell count   | 255 ± 7.93       | 2106 ± 202.0b        |
| % abnormal sperm cells            | 8.5 ± 0.26       | 70.4 ± 2.81b         |
| % Headless                        | 3.4 ± 0.24       | 34.4 ± 0.51b         |
| % Hook head                       | 1.6 ± 0.24       | 13.6 ± 0.75b         |
| % Bent mid piece                  | 2.2 ± 0.66       | 14 ± 0.63b           |
| % Bent tail                       | 1.2 ± 0.58       | 8.4 ± 0.68b          |
Mean ± SEM within same horizontal row that has superscript are statistically different (a, b=P < 0.001) from the ones that do not have superscript (a = decrease and b = increase)

Results

The effects of SMV on serum reproductive hormones after 14 days i.p exposure are presented in figure 1. Serum testosterone, FSH and LH concentrations of SMV exposed AGR were significantly lower (P<0.01; P<0.001; P<0.0001 respectively) compared to the serum concentrations of these hormones in control AGR (Fig. 1a-c). On the other hand, serum progesterone and 17β estradiol concentrations of AGR exposed to i.p SMV were higher than control group (Fig. 1d and e). While it was statistically significant for progesterone (P<0.0001) (Fig. 1d); no statistical difference was seen in 17β estradiol concentration of SMV exposed group compare to control (Fig. 1e).

The gonadal and extra-gonadal sperm reserves of AGR treated for 14 days with 3 mg/kg body weight SMV and the control match are presented in table 1. Both the gonadal and extra-gonadal sperm reserve of AGR were significantly reduced (P<0.0001) by SMV. Concerning the sperm quality, SMV exposed AGR group had significant decreased percentage sperm motility (P<0.0001), mass activity (P<0.0001) and viability/vitality (P<0.0001) when compared to control (Fig. 2a-c).

Furthermore, exposure of AGR to 3mg/kg body weight SMV intraperitoneally induced significant increased abnormal sperm count (2106±202.0) compared to control (255±7.93) (Table 1; Fig 3). When expressed in percentage, SMV induced 70.4±2.81% abnormal sperm cells. This was statistically significant compared to control (Table 1). To investigate the different abnormal morphological phenotypes (Fig 3) induced by exposure to 3 mg/kg body weight SMV in AGR, we classified them in percentages (Table 1). Headless sperm cells (Fig. 3b) were the highest abnormal morphological phenotypes induced. Next was bent mid piece and hook head (Fig. 3c and d). Bent tail (Fig. 3e) was seen as the least abnormal morphological phenotype (Table 1). These abnormal morphological phenotypes were statistically significant (P<0.0001) when the SMV exposed group was compared to control.

The data above suggested that SMV was cytotoxic to sperm cells and induced various morphological phenotypes. To begin to examine if changes will lead to death through the apoptotic mechanism, we stained the testes tissue with anti-P53 antibody, a protein known to be activated by cellular stress signals, and when activated triggers cell cycle arrest or apoptosis. Upon immunohistochemical examination, we observed that SMV exposure (Fig. 4b) was associated with increased expression of P53 immunostain compared to control (Fig. 4a).

Exposure of AGR to 3mg/kg body weight of SMV caused severe degeneration and necrosis of spermatogonia, primary spermatocytes and secondary spermatocytes within the seminiferous tubules with marked oedema of the interstitial spaces between the seminiferous tubules (Fig 5). The necrosis observed led to thinning of the stratified epithelium of the tubules and cellular debris within the lumen of the tubules. Ultrastructural examination of the seminiferous tubules and its surrounding basal lamina of basement membrane of SMV exposed AGR showed oedematous, disintegrated and several irregular thin
membrane, wavy in appearance and indented (Fig. 6b). The peritubular myeloid cells, Leydig, Sertoli and germ cells of the control group had apparently typical healthy normal cells population (Fig. 6a). In the SMV exposed group, the myeloid cells were atrophied and degenerated and in some cases, totally destroyed (Fig. 6b). Examination of the interstitial tissue from SMV treated AGR testis showed distortion, disintegration with dispersion of fibrous tissue, oedema and few lipid droplets. Leydig cells were necrotic and some, apoptotic with fragmented nuclei and deformed mitochondria (Fig. 6b). Also, Sertoli cells of SMV treated AGR were shrunken in size with electron dense swollen and cup shaped mitochondria. The nuclei were vacuolated, irregular in shape, indented and dislocated from the basal portion creating abnormal space with the myeloid cells (Fig. 6b). There were also numerous cytoplasmic vacuoles, lipid droplets and oedema seen in the Sertoli cells of SMV exposed AGR (Fig. 6c). Concerning the spermatogonic series, the spermatogonia in SMV exposed AGR lost their normal architecture becoming shrunken and irregular in shape with pyknotic nuclei. The spermatocytes decreased in size revealing condensed and clumped chromatin materials at the periphery and sometimes evenly distributed in their nucleus and at several points severe fragmentation (Fig. 6c and d). Intranuclear vacuolations were also seen. In the cytoplasm were swollen mitochondria that have lost their cristae and numerous vacuolations (Fig. 6f). Also, spermatocytes with rupture plasma membrane at some positions were also encountered. Round spermatids with vacuolated cytoplasm, electron dense and swollen mitochondria and misshaped acrosomal cap were frequently notice in SMV exposed AGR (Fig. 6e and f). Their nucleoli were disappearing reflecting early stage of karyolysis. Elongated spermatids were not seen. Numerous deeply stained and misshaped sperm heads without tail and neck piece and phagocytised by Sertoli cell processes were frequently seen in SMV treated group (Fig 6e).

**Discussion**

As a transition metal, vanadium causes a wide adverse effects which is of biological, industrial, occupational and environmental concern (Rodriguez-Mercado *et al.* 2002). Vanadium has been shown to induce irreversible adverse effects to DNA damage and chromosomal mal-segregation secondary to free radical generation (Usende *et al.*, 2018b; Wang *et al.*, 2018). The male reproductive organ is of special interest in this study due to its role in the formation of gamete necessary for fertilisation of the female ova resulting in progeny and continuity of life (Aragon *et al.*, 2005; Chandra *et al.*, 2007). Any adverse effect in the testes is translated and therefore can affect next generations which if not controlled can lead to extinction. Many wildlife rodents in highly polluted poor resource settling and oil producing and industrial areas are constantly exposed to several environmental toxins including vanadium and may go into extinction (Usende *et al.*, 2017). African giant rat is a good example and have been proposed to be a laudable experimental animal model for ecotoxicological and toxicological studies (Usende *et al.*, 2017, 2018a, b, 2020).

We report herein significant reduction of spermatozoa due possibly to disturbances of the process of spermatogenesis, a cyclical phenomenon of cell proliferation and or germ cell death. Similar findings have been reported by Chandra *et al.*, (2010). It has been reported that the production of appropriate
numbers of spermatozoa depends upon the stimulation of the testes by FSH and LH, and that in response to LH, testosterone which is necessary for maintenance of spermatogenesis is produced (Aragon et al., 2005; Chandra et al., 2007; 2010). Contrary to the work of Aragon et al. (2005) who do not observe any modifications in testosterone concentration of vanadium tetraoxide exposed mice and concluded that Leydig cells are not target for vanadium toxicity, we showed a significant reduction of this hormone as well as FSH and LH in serum of AGR exposed to SMV for 14 days intraperitoneally and thus the quantitative low sperm count observed herein. Testosterone, estradiol and FSH are required for initiation of spermatogenesis and its significant reduction explains the inability of testosterone to sustain spermatogenesis and prevent germ cell loss (Allen et al., 2010; Wang et al., 2018). On the other hand, acute testosterone reduction as seen in the present study can lead to depletion of germ cells of the testes; especially spermatocytes and spermatids through changes involving the disassociation of the spermatid to Sertoli cell cytoskeleton (O'Donnell et al., 2009) as observed in our ultrastructural studies, and induction of increased germ cell apoptosis (Tapanainen et al., 1993; Billig et al., 1995; Marathe et al., 1995; Sinha-Hikim and Swerdloff, 1995) seen in our report as increased P53 expression. It therefore implies that testosterone and FSH functions as cell survival factor, protecting germ cells from apoptosis (Zirkin 1998; Aragon et al., 2005; Chandra et al., 2007; 2010).

Furthermore, we demonstrated elevated serum concentration of progesterone and 17β estradiol in SMV exposed AGR. This is not surprising as it has been reported that plasma concentration of LH were suppressed after single injection and subcutaneous implants in ram of progesterone (Turner et al., 2001). The elevation of progesterone likely led to reduced concentration of LH and testosterone seen in this study suggesting a physiological role of negative feedback by progesterone on LH and testosterone following SMV exposure in this animal model. However, the site at which progesterone acts to regulate the secretion of LH is still unknown (Turner et al., 2001). Simply put, we demonstrated that elevation of progesterone act to suppress LH secretion. It has been reported that the presence of progesterone enhances negative feedback action of testosterone (Turner et al., 2001). In this study, testosterone may have acted directly or followed aromatisation pathway to be converted to 17β estradiol hence its decrease serum concentration and increased serum concentration of 17β estradiol. Similar observation had been made by Turner et al. (2001).

The results observed herein also showed overt reproductive toxicity after intraperitoneal SMV exposure to AGR. The spermatozoa counts were significantly diminished in SMV treated AGR group. Similar findings have been reported by Uche et al. (2008) and Chandra et al., (2010). However, Aragon et al. (2005) reported no difference in sperm count between control and vanadium treated rat groups. Spermatogenesis and maturation depends on genetically normal and adequate process of cell division, multiplication and specialisation as well as optimal environmental condition including hormonal regulations (Altamirano-Lozano et al., 1996) which were altered in this study. A significant decrease in sperm motility, mass activity and viability was also reported in this study in SMV exposed AGR group. Similar findings have been reported by Chandra et al., (2010) following exposure of three months old male albino rats of Sprague Dawley strain to vanadium but contrast the work of Llobet et al. (1993) after
SMV exposure. The reason for the difference seen in this work compared to the works of Llobet et al. (1993) could be attributed to the animal model used. While they (Llobet et al., 1993) used mice, we used African giant rat and different strains of rodents can react to xenobiotics differently. It has been reported that sperm motility and viability depend on factor such as functional mitochondria acting during sperm production and its transition through the epididymis (Altamirano-Lozano et al., 1996).

Some of the major pathways by which vanadate induce its damage is by generation of reactive oxygen species (Usende et al., 2016, 2018a; Todorich et al., 2011), decreasing ATP concentration (De Lamirande and Gagnon, 1992), and lipid peroxidation (Todorich et al., 2011). In the testis, vanadium increases lipid peroxidation and reduce glutathione concentration (Usende et al., 2018a) explaining the mechanism for the decreased motility, mass activity and viability seen in this study. Earlier, Altamirano-Lozano et al. (1996) attributed alteration of mitochondrial energy production and defect in chemomechanical energy transduction in the dynein/microtubule sliding mechanism to cause decrease sperm motility after vanadium pentoxide exposure. Severe mitochondria defects are reported herein. These alterations will ultimately lead to infertility as non-motile abnormal spermatooza cannot swim to locate the oocyte for fertilisation to take place. In this study, SMV induced abnormal sperm morphology ranging from headless to bent mid piece and bent tail sperm cells, indicative of its effects on spermatogenic process. Similar findings have been reported by Aragon et al. (2005). The mechanism for this type of defects could be the ability of vanadium to inhibit microtubule polymerization (Hantson et al., 1996; Ramirez et al., 1997) and induction of 2'deoxyguanosine hydroxylation and to cause DNA breaks by means of free radical mediated reactions as well as single-stranded breaks (Shi et al., 1996; Usende et al., 2018b). This is further explained by the increased expression of anti-P53 immunostain observed in SMV treated group. P53 protein plays key role in response to genotoxic stress (Allemand 1999) as reported in previous findings (Usende et al., 2018a, b). Although maintained at very low basal levels in normal adult tissue, this protein can be activated and accumulated following DNA damage due to cell cycle arrest or apoptosis (Allemand 1999). This is consistent with the apoptotic changes such as shrunken spermatogonia, separation of spermatogonial cells from each other and from the basal lamina as well as mitochondrial vacuolations seen in our ultrastructural findings.

Ultrastructural examination of SMV treated AGR group revealed various alterations of the testicular tissue. The basal lamina of the tubular basement membrane was disintegrated and oedamtous affecting both the structural and functional integrity of the tissue. The disruption seen can affect oxygen transport, nutrition, metabolite and hormonal integrity of the testis (Zheng et al., 2008). This is true as the basal lamina have been shown to play a very vital role in maintaining substance transportation between the spermatogenic epithelium and the interstitial tissue (Richardson et al., 1998). Altered testicular tubular basement membrane has been associated with many testicular disorder and functional impairment of the testis (Elshennawy and Elwafa 2011) similar to our findings herein. We also showed that SMV exposure led to atrophy, degeneration and destruction of the peritubular myoid cells. These myoid cells are known to interact with Sertoli, Leydig and germ cells, communicating through the extracellular matrix of the basal membrane for spermatogenesis to get its complete course (Elshennawy and Elwafa
Due to their abundant actin filaments, these myoid cells which also contain androgen receptors, and are involved in retinol processing, are very contractile and transport spermatozoa and testicular fluid in the tubule (Maekawa et al., 1996). Although several factors such as prostaglandin and oxytocin levels have been suggested to affect these cells (Maekawa et al., 1996), there have been no reports on the effects of vanadium on these cells. Their destruction as reported herein may have led to the loss of functional and structural integrity of the tubules. The present study for the first time, revealed hallmark destruction of myoid cells following vanadium exposure to AGR. The mechanism by which vanadium induced this damage however, remains to be elucidated. We hypothesise that vanadium induced low testosterone level and damaging of seminiferous tubular epithelium leading to destructions of the peritubular myeloid cells. We also showed that SMV exert an effect on the Leydig cells resulting to their death. The deteriorations seen in the interstitial space and the degeneration, necrosis and apoptosis of the Leydig cells were consistent in all members of the SMV treated group and would have resulted to the decrease testosterone level reported and similar to the findings of Khaki et al. (2009). These findings contrast the work of Aragon et al. (2005) who argue that Leydig cells are not target for vanadium. Our finding is true as there exists a close relationship between Leydig cells and blood vessels suggesting their high risk of exogenous toxins (Elshennawy and Elwafa 2011). Although Aragon et al. (2005) do not report Sertoli cell death, these are known to be target for various toxicants (Krishnamoorthy et al., 2005). In the present work, damage of Sertoli cells following SMV exposure was evident. Key amongst the functions of Sertoli cells is to provide physical support, form sites for attachment (Richburg, 2000; Sawada and Esaki 2003), and foster the development and maintenance of viable germ cells by secreting hormones and nutritive factors into the specialised compartment formed by tight junctions between the adjacent Sertoli cells and the germ cells (Elshennawy and Elwafa 2011). It therefore implies that injury to the Sertoli cells is injury to spermatogenic series as reported in this present work. The spermatogenic cells showed severe defects following intraperitoneal exposure to SMV ranging from loss of shape to severe oedema, mitochondrial swelling, extensive cytoplasmic and nuclear vacuolations. The vacuolations seen may be attributed to ionic and osmotic imbalance caused by SMV on these cells leading to inhibition of water, and cell degeneration, dilatation of smooth endoplasmic reticulum leading to permeability changes similar to the report of El-Gerbed (2013). The mitochondria on the other hand are key organelle representing cellular damage (Kalender et al., 2005) and vanadium derived mitochondrial pathologies is yet to be fully elucidated. Report had shown that spermatogonia are particularly vulnerable to toxicant (De Rooij and Russell 2000), including vanadium due to their mitotic activities. This will lead to their destruction hindering their basic roles of initiating spermatogenesis and regulation of final germ cell population necessary for fertility (Elshennawy and Elwafa 2011). The different abnormal morphological phenotypes noticed in the spermatocytes and spermatid of SMV exposed AGR testis reflect the disturbances in the microenvironment of the Sertoli cells that affect the protein synthesis machinery necessary for proper germ cell differentiation. Similar findings have been reported by Elshennawy and Elwafa (2011). These proteins are secreted at their peak during spermatid elongation and spermiation (Manivannan et al., 2009). Elongated spermatid where not seen in testis of SMV exposed AGR and this may justify those abnormal phenotypes seen in the sperm cells.
Data from the present study have demonstrated the detrimental effects of vanadium, an environmental pollutant to adult males. We conclude that intraperitoneal SMV exposure to AGR for 14 days exerts severe adverse effects on reproductive hormones, depletion of gonadal and extragonadal sperm reserve and quality, apoptosis and degenerative changes of the Leydig, Sertoli and germ cells which if prolonged can lead to total infertility. The translational implications of these findings are highly relevant for the human population living in these environmental polluted areas, not only in Nigeria but elsewhere in the world.

**Declarations**

**Ethical approval**

The animal experimental protocol received ethical approval by the University of Abuja Ethics Committee for Animal Use (UAECAU/2017/007)

**Consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and materials**

Note applicable

**Conflict of interest**

The authors declare no conflict of interest

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**Author contributions**

ILU was involved in Conceptualization, Formal analysis, Investigation, Methodology, and Writing - original draft & editing. FOO was involved in Investigation, Methodology and Writing - review & editing. AOA was involved in Investigation, Methodology and Writing - review & editing. BOE was involved in Conceptualization, Writing - review & editing, Supervision. AMN was involved in Investigation, Writing - review & editing. JOO was involved in Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration.

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**Figures**

![Graph A: Mean Testosterone](image1)

![Graph B: Mean FSH](image2)

![Graph C: Mean LH](image3)

![Graph D: Mean Progesterone](image4)

![Graph E: Mean 17β estradiol](image5)
Figure 1

Bar graph showing the effects of exposure of 3mg/kg body weight SMV Testosterone (A), FSH (B), LH (C), progesterone (D) and 17β estradiol (E) concentrations in male AGR for 14 days compared to control match. Values are presented as mean±SE (*p<0.05; **p<0.001; ***p<0.0001; NS, not significant)

Figure 2

Bar graph showing the effects of exposure of 3mg/kg body weight SMV on Percentage sperm motility (A), mass activity (B), and viability and vitality (C) in male AGR after 14 days in comparison to controls. Values are presented as mean±SE (***p <0.0001)
Figure 3

Micrograph of various abnormal sperm morphological phenotypes (B-E) observed in epididymal smear of 14 days SMV intoxicated AGR group in comparison to control (A). A: Normal morphology from control, B: Bent midpiece; C: Ascending hook shape; D: Hook head; E: headless

Figure 4

Micrographs showing that in vivo SMV intoxication for 14 days resulted in upregulation of p53 positive immunostaining (white arrow heads) (B) in the testis of AGR when compared with control (A).

Figure 5

Micrographs showing that in vivo SMV intoxication for 14 days resulted in marked degeneration and necrosis of spermatocytes (black arrows) with severe interstitial tubular oedema (X) and cellular debris within the lumen of the tubules (star) (B and C) when compared with control (A) with normal spermatogonia (red arrow), primary spermatocyte (single arrow head) and secondary spermatocytes (double arrow head). Note the thinning of stratified epithelium of the tubules in the SMV intoxicated group (B and C).
Figure 6

Transmission electron micrographs of testis of control (A) compared to 14days SMV intoxicated (B-F) AGR groups. Note the disintegrated wall of the seminiferous tubules (red arrow head), severe oedema (E), intranuclear vacuolation (black arrow head) of Sertoli cell and fat droplet (white arrow head), apoptotic Leydig cell (aLc), and also severe oedema of the spermatogonic series, Sertoli cells and interstitial space (E), intracytoplasmic (yellow arrow head) and intranuclear (blue arrow heads) vacuolation of Sertoli cell,
fragmentation of spermatocytes (fSpc), clumping of chromatin at the periphery, ruptured spermatocytes (rSpc), with light electron dense proteinous materials (p) as well as swollen and cup shaped mitochondria (blue arrow), detached spermatozoa heads (orange arrow) and deformed spermatid (green arrow) in Fig. 5B-F