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Vertical Administration of Vanadium through Lactation Induces Behavioural and Neuromorphological Changes: Protective Role of Vitamin E

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Summary: The work investigated the protective role of vitamin E on vanadium induced neurotoxicity. Three adult female rats were divided into three groups, A-C with each dam and her pups forming a group. Group A served as control. The dam in Group B was given 3mg/kg b.w./day of vanadium from PND 1 while the Group C dam were given 3mg/kg b.w./day of vanadium, for 14 days and 500mg/kg b.w. of vitamin E 72 hourly in the same time frame. The results showed that pups from Group B, exhibited behavioural deficits in most tests, a significant reduction in body weight gain and absolute brain weight; in addition immunohistochemistry showed reactive astrogliosis induced by vanadium exposure. All these findings were however attenuated in pups whose dam was exposed to vanadium and vitamin E depicting the significant protective effects of this antioxidant against vanadium. This study is novel in that both vanadium and vitamin E were introduced through the lactation route. We conclude that though caution remains essential in the posology of vitamin E, the management of lactating mothers who have been exposed to vanadium occupationally, environmentally or therapeutically with supplementation of this antioxidant may be beneficial at least in the short term to both mother and offspring.

Keywords*:* Vanadium, Vitamin E, Vertical administration, Neuroprotection.

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INTRODUCTION

Vanadium is a transition metal widely distributed in nature and extensively used in modern industry (Ray et al., 2006); therefore, acute environmental and occupational exposure to vanadium is not uncommon (Hope, 1994; Shrivastava, 2007; Wenning and Kirsh, 1988).

Occupational exposure to Vanadium and its oxide occurs at production sites, during processing and refining of vanadium ores and sludges, during manufacturing of vanadium-containing products, in the course of combustion of vanadium-rich fuels, and by handling of catalysts in the chemical industry (Plunkett 1987). Environmental exposure occurs via inhalation in the vicinity of metallurgical plants or through consumption of contaminated foods (Barceloux 1999; IARC 2006) and recently from massive oil burning as seen in Arabian Gulf (Haider et al, 1998), the Niger-Delta region of Nigeria (Igado et al., 2008) and the Gulf of Mexico.

Vanadium causes a number of adverse effects depending on oxidation state and circulating vanadium levels, however, V^{5+} seems to be more toxic than V^{4+} and V^{3+} (Sanchez et al, 1998; Soares et al., 2008). Once vanadium has been incorporated into tissue cells, it is only very slowly eliminated from mammals, leading to progressive vanadium accumulation during the life span (Soares et al, 2008). Vanadium is a catalytic metal reported to induce reactive oxygen species (ROS) generation *in vitro* as well as lipid peroxidation and oxidative damage in a great variety of biological systems including rat brain (Garcia et al., 2004; Haider and el-Fakhri, 1991; Haider et al., 1998; Sasi et al., 1994). Rats exposed to vanadium fumes develop neuroinflammatory changes in the brain (Avila Costa et al., 2004; 2006) and in addition, vanadium crosses the blood brain barrier (Berman, 1980) and induces alterations in neurochemical substances of the brain (Witkowska and Brzezinski, 1979). Garcia et al. (2004) and Todorich et al. (2010) reported a significant decrease in grooming responses and locomotor activity respectively in rats treated intraperitoneally with sodium metavanadate; also, behavioural alterations were observed in lactating rat pups whose dams were exposed to sodium metavanadate (Soazo and Garcia, 2007).

Antioxidants provide protection against deleterious metal mediated free radical attacks. Vitamin E is an important element which prevents free radical generation, protecting biological membranes from damage in *in vitro* systems and in metal loaded animals (Ramanathan et al., 2003; Stefanidou et al., 2005). Vitamin E has the ability to stabilize membrane by interacting with unsaturated fatty acid chain (Shrivastava , 2007).

In this present work we investigated the protective role of vitamin E on vanadium induced neurotoxicity, focusing on the behavioural perturbations and immunohistochemical changes.

MATERIALS AND METHODS

Animals

Three nursing albino rats were used for this experiment. They were obtained and housed in the experimental animal house of the neuroscience unit of the Department of Veterinary Anatomy of the University of Ibadan, Ibadan, Nigeria. The animals were fed with rat pelleted feed and water *ad libitum*.

Treatment

The animals were divided into three groups, A-C with each dam and her pups forming a group. Vanadium (sodium metavanadate), dissolved in sterile water was administered at a dose rate of 3mg/kg body weight intraperitoneally (IP) while vitamin E was administered at a dose rate of 500mg/kg body weight per os to the dam.

The three groups were as follows:

Group A: Dam received sterile water (control)

Group B: Dam was given 3mg/kg b.w./day of vanadium, IP for 14 days from postnatal day 1

Group C: Dam was given 3mg/kg b.w./day of vanadium, IP for 14 days and 500mg/kg b.w. of Vit. E orally every 72 hours from postnatal day 1.

Brain and Body weights

The body weights of the pups were taken daily from post natal day 7-13 with a bench top balance.

On day 15, behavioural tests were carried out on the pups after which they were anesthetized with a combination of ketamine 100mg/kg and xylazine 10mg/kg and sacrificed by quick decapitation.

The brains were quickly removed over ice and weighed with a bench top balance before being processed for immunohistochemistry.

Behavioural Studies

The pups were tested on the open field, the hanging wire, and for negative geotaxis as described by Garcia et al., (2004) and Soazo and Garcia (2007).

***Open-field test*:** Each pup was placed in the center of a square cage *(120x120cm)*. The floor was divided into *20cm* squares drawn in black ink. The rats were allowed to move freely around the open field to explore the environment for 5 minutes. The following observations were manually recorded by the same set of observers:

- Locomotion that is, number of times pup crossed from one square to another entering at least its two front paws
- Number of rearings that is, number of times pup stood on its hind legs
- Number of grooms that is, sets of heterogeneous constituents comprising face washing, body licking, paw licking, head and body shaking, scratching and genital licking

The open field box was washed with 30% alcohol solution before placing the subsequent animals in it in order to avoid possible biasing effects due to odour clues left by previous rats.

Forelimb support (hanging wire): Each pup's forepaws were placed on a horizontally suspended wire *(1mm in diameter)*, placed *47cm* above a soft foam landing area. Pups were timed from the moment they were placed on the wire until they dropped from the wire. This reflects muscular strength in neonate rats.

Negative geotaxis: Each pup was placed in the middle of a slab, 30^{o} inclined to the surface plane, in a head down position and latency to turn 180^{o} to a head up position was measured. This reflects vestibular function, and motor development and activity.

Immunohistochemistry

The brains were processed for immunohistochemistry based on the methods described by Todorich et al (2010). Briefly, brain sections were immersed in 4% phosphate buffer formalin. Antigen retrieval was done by microwave heating in 10 mM citrate buffer for 25 minutes, with subsequent peroxidase quenching in 3% H_2O_2/methanol. All the sections were blocked in 2% skimmed milk overnight and probed with anti-GFAP mouse monoclonal antibody 1:400 (Sigma) for 16 hours at 4^{o}C. Detection of bound antibody was done using appropriate HRP-conjugated secondary antibodies in VECTASTAIN kit (Vector Labs) according to manufacturer's protocol. Reaction product was enhanced with DAB for 6-10 minutes, with subsequent dehydration in ethanol and mounting on salinized slides. The immunoreactive astrocytes were studied. Images were acquired with a Sony® digital camera.

Statistical Analysis

All data are presented as mean ± SD. The behavioural data was statistically evaluated by the Student's *t* test using the Graph pad Prism 5 System.

RESULTS

Behavioural tests show in most instances, a reduction in locomotor activity, forelimb support, and negative geotaxis in the vanadium exposed group compared to the control and a recovery of this reduced functions in the vanadium+vitamin E group although the differences were not statistically significant as shown in table 1.

There is a statistically significant reduction in the body weights as well as rate of weight gain in the pups exposed to vanadium when compared to both the controls and the vanadium+vit.E group The mean brain weight of the vanadium-only pups at PND 15 was significantly less than that of the controls and vanadium + vit.E – exposed group. Though the brain weight of the vanadium + vit.E –exposed group was slightly less than the controls, it was not statistically significant.

Table1.
Mean values for the behavioural tests on 15 day old rats

	Group A (Control)	**Group B (Vanadium only)**	**Group C (Vanadium + Vt E)**
Center time (secs.)	42±7.07	155±115.10	64.5±10.61
Rearings	2±0.00	1±0.00	N/A
Groomings	3±1.00	2±0.58	2±0.96
Transitions	12±6.51	7±3.51	8±4.95
Forelimb support (secs.)	6.53±6.02	3.81±2.59	4.94±3.25
Negative geotaxis (secs.)	2.66±0.76	4.06±2.28	3.15±0.90

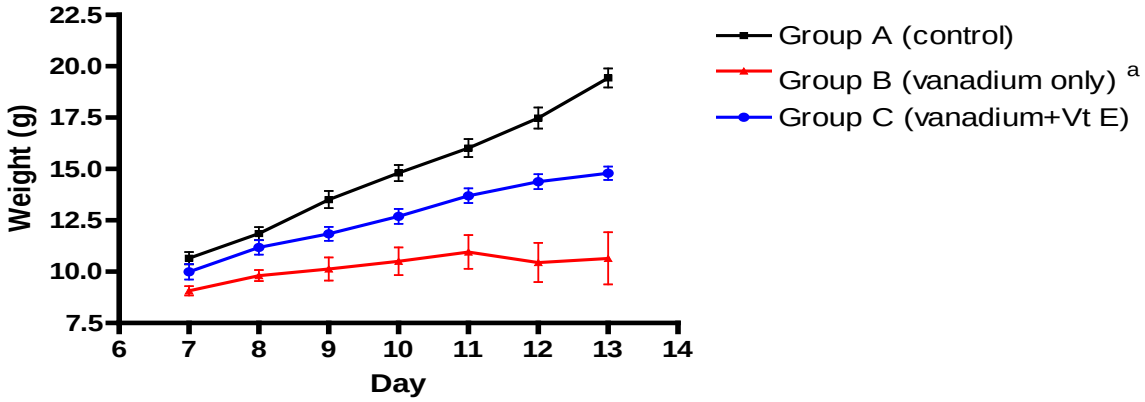

Figure 1. Mean values for pups' daily body weight from post natal day 7-13. The values are expressed as means±SEM. [a] indicates statistically significant difference compared to control and to the vanadium+vit E (Group A and C).

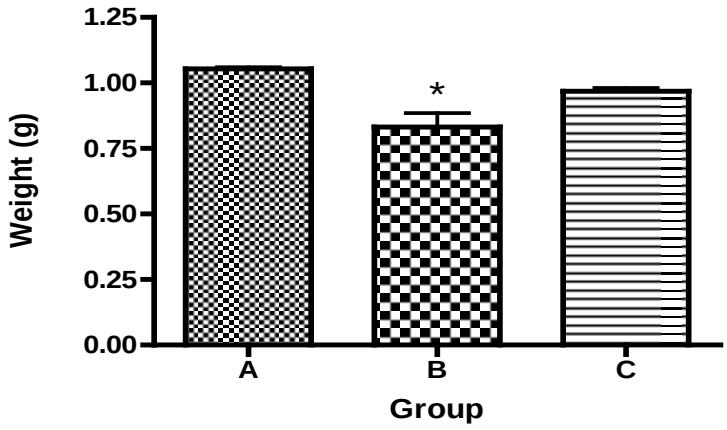

Figure 2. Mean brain weight values of rat pups at PND 15. * represents significant difference ($p<0.05$) in the group that received only vanadium (Group B) compared to control (Group A) and to the vanadium+vit E group (Group C).

Immunohistochemistry

Glial fibrillary acidic protein (GFAP) immune-histochemical staining showed a high astrocytic response in vanadium exposed group which was reduced in the vanadium+vitamin E group when compared to the control group.

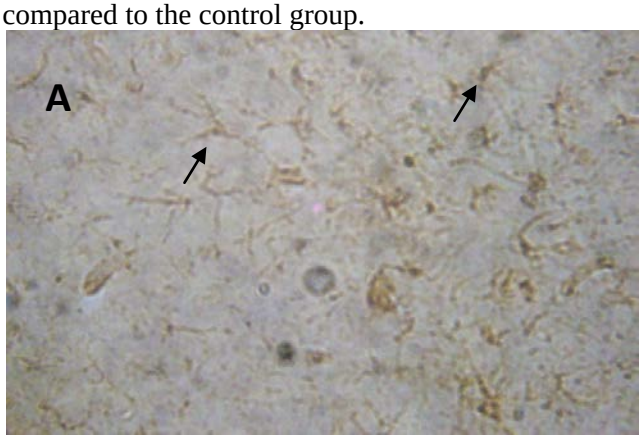

Figure 3A:
Group A control x400. GFAP-immunolabeled astrocytes (arrows) localized at the hippocampal region are elaborated.

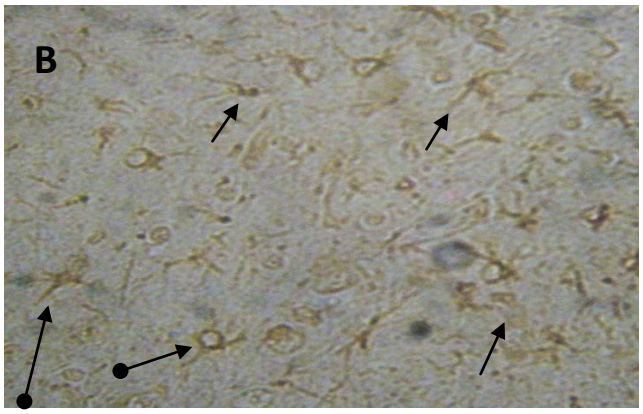

Figure 3B:
Group B vanadium only x400. GFAP-immunolabeled astrocytes (arrows) localized at the hippocampal region are markedly large and with more tortuous cytoplasmic processes (arrows with clob ends) than the control group.

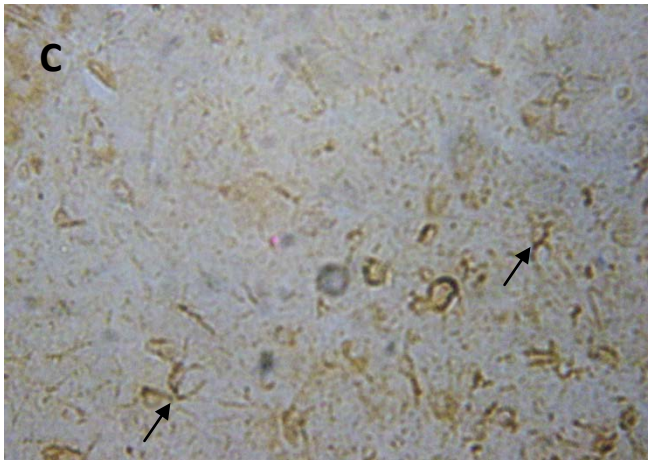

Figure 3C:
Group C vanadium + vit E x400. GFAP-immunolabeled astrocytes localized at the hippocampal region are reduced in size and with less tortuous cytoplasmic processes (arrows) than the vanadium exposed group B.

DISCUSSION

The performance of the rats on the open field, which provides an overall measurement of locomotor activity, reveals reduced horizontal and vertical movement in the V^{5+}exposed rats compared to controls; this was improved in the V^{5+} and vitamin E-exposed rats. This is likely to be due to muscular weakness since the forelimb support test, which measures muscular strength, also gave similar results. Negative geotaxis, a preweaning reflex test which reflects motor development and vestibular function revealed a longer latency in V^{5+}exposed rats suggesting that the V^{5+} affected their muscular development.

In this study, a significant reduction was seen in the body and brain weights of vanadium exposed animals when compared to controls. This is in contrast to the findings of Garcia et al. (2004; 2005) who reported that body and brain weight of V^{5+} exposed rats (at the same dose used in this study) did not differ from control rats, and physical conditions were almost normal. The most obvious reason for this is the fact that three month old rats were used for their studies while in this experiment; the animals were exposed at PND 1. Sanchez et al (1991), Altamirano et al., (1993) and Todorich et al (2010) all reported significant reduction in body weights and body weight gains of vanadium exposed fetuses and neonatal pups compared to controls. It appears that vanadium has more effect on growth when exposed to tissue and animals that are undergoing a high degree of cellular proliferation. Vanadium is a potent inhibitor of DNA and protein synthesis and affects several metabolic processes (Roldan and Altimirano, 1990; Leonardo and Geber, 1994). The reduced brain weight from vanadium exposure is thus most likely the product of neuronal loss in the brain (Avila-Costa et al., 2004, 2006).

Astrocytes are the first line of defense for the brain against oxidative and toxic insults. They also provide trophic support for other cells in the developing brain, including neurons and oligodendrocytes (Sortwell et al., 2000)and perform a central role in the regulation of the neuronal microenvironment, accomplishing a variety of metabolic and structural functions in the normal state or in response to injury and disease (Jensen, 1995). Reactive astrogliosis is a characteristic response of astrocytes to inflammation and trauma of the CNS (Balasingam et al., 1994) and is a condition characterized by an extensive hypertrophy of the cell body and cytoplasmic processes accompanied by rapid synthesis of GFAP intermediate filaments (Eng

and Ghirnikar, 1994). Neonatal astrocytes can become reactive if an adequate injury stimulus is presented with the release of immunoregulatory cytokines by cells around the lesion sites contributing to the production of gliosis (Balasingam et al., 1994). Vanadium induced astrogliosis has been reported after parenteral exposure in rats (Garcia et al., 2005; Todorich et al., 2010). This present study shows that development of reactive astrogliosis also occurs in animals exposed to V^{5+} through the lactation route.

Antioxidants have been known to prevent and reverse morphophysiological and behavioural deficits induced by vanadium as seen in the testes and liver (Uche et al., 2008); the adrenal gland and serum enzyme homeostasis (Chandra et al., 2007), kidney (Shrivastava et al., 2007), brain myelin profile (Aschner et al., 2010) and amelioration of behaviour (Sanchez et al., 1999). The development of antidotes to Vanadium induced neurotoxicity remains a novel and growing field (Olopade and Connor, 2010). In this study, vitamin E produced a reversal trend in most of the negative effects of behaviour, body and brain weight loss and astrogliosis induced by vanadium exposure. This study is however novel in that both vanadium and vitamin E were introduced through the lactation route. Vitamin E-induced counteraction of vanadium toxicity depicts the significant protective effects of this antioxidant against vanadium. Though caution remains essential in the posology of vitamin E (Gallo et al., 2010), it seems important to explore further, the management of lactating mothers who have been exposed to vanadium occupationally, environmentally or therapeutically with supplementation of this antioxidant as this may be beneficial at least in the short term to both mother and offspring.

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REFERENCES

Altamirano M, Alvarez L, Roldan E. (1993): Cytogenetic and teratogenic effects of vanadium pentoxide on mice. Med Sci Res. 21:71 l-713.

Aschner, M, LevinED, Suñol C, Olopade JO, Helmcke KJ, Avila DS, Sledge D, Ali RH, Upchurch L, Donerly S, Linney E, Forsby A, Ponnoru P and Connor JR. Gene-Environment Interactions: Neurodegeneration in Non-Mammals and Mammals. Neurotoxicology (In Press)

Avila-Costa MR, Montiel, Flores E, Colín-Barenque L, Ordoñez, JL, Gutiérrez, AL, Niño-Cabrera G, Mussali-Galante P and Fortoul TI (2004): Nigrostriatal Modifications After Vanadium Inhalation: An Immunocytochemical and Cytological Approach. Neurochem. Res. 29:1365-1369

Avila-Costa MR, Fortoul TI, Niρo-Cabrera G, Colvn-Barenque L, Bizarro-Nevares P, Gutiurrez-Valdez AL, *et al*. (2006): Hippocampal cell alterations induced by the inhalation of vanadium pentoxide (V9O) promote memory deterioration. Neurotoxicity. 27:1007-1012

Balasingam V, Tejada-Berges T, Wright E, Bouckova R, Yong VW (1994): Reactive astrogliosis in the neonatal mouse brain and its modulation by cytokines. J Neurosci 14:846–856.

Barceloux, DG (1999): Vanadium, J. Toxicol. Clin. Toxicol. 37:265–278

Berman E. (1980): Toxic Metals and Their Analysis, Heyden and Sons, London

Eng LF, Ghirnikar RS (1994): GFAP and astrogliosis. Brain Pathol. 4:229–237.

Gallo C, Renzi P, Loizzo S, Loizzo A, Piacente S, Festa M, Caputo M, Tecce MF, Capasso A (2010). Potential therapeutic effects of vitamin E and C on placental oxidative stress induced by nicotine: an in vitro evidence. Open Biochem J. 4:77-82.

Garcia GB, QA, Sturtz N, Martinez AI and Biancardi ME (2004): Morphological Alterations of Central Nervous System (CNS) Myelin in Vanadium (V)-Exposed Adult Rats, Drug and Chemical Toxicology 27(3): 281-293.

Garcia GB., Biancardi ME and Quiroga AD (2005): Vanadium (V)-Induced Neurotoxicity in The Rat Central Nervous System: A Histo-Immunohistochemical Study. Drug and Chemical Toxicology. 28(3):329 - 344 .

Haider SS, Abdel-Gayoum, AA, el-Fakhri M, and Ghwarsha KM (1998): Effect of Selenium on Vanadium Toxicity in Different Regions of Rat Brain, Hum. Exp. Toxicol. 17(1):23-8.

Haider SS, el-Fakhri M (1991): Action of alpha-tocopherol on vanadium-stimulated lipid peroxidation in rat brain. Neurotoxicology 12(1): 79-85

Hope BK (1994): A global biogeochemical budget for vanadium. Sci Total Environ 141(1–3):1–10

International Agency for Research on Cancer (IARC) (2006). Vanadium pentoxide. IARC Monogr Eval Carcinog Risks Hum. 86:227–292.

Igado OO, Olopade JO, Onwuka SK, Chukwudi AC, Daramola OA, Ajufo UE (2008): Evidence of environmental pollution in caprine brains obtained from a relatively unindustrialized area in Nigeria.

African Journal of Biochemical Research 11:305–309

Igado OO and Olopade JO (2009): Metals and the Brain. Archives of Ibadan Medicine.11 (1): 27-30.

Jensen KF (1995): Neuroanatomical techniques for labeling neurons and their utility in neurotoxicology. In: Neurotoxicology: Approaches and Methods. Chang LW, Slikker WJr eds. London, United Kingdom: Academic Press. pp 27–64.

Leonard A, Gerber GB (1994): Mutagenicity, carcinogenicity and teratogenicity of vanadium compounds. Mutat Res. 317:81-88

Olopade JO and Connor JR (2010): Vanadium and Neurotoxicity. A Review. Current Topics in Toxicology. 7:33-39

Plunkett ER (1987): Handbook of Industrial Toxicology. New York: Chemical Publishing Co, 563–664

Ramanathan K, Shila S, Kumaran S and Panneereselvam C (2003): Protective role of

ascorbic acid and alpha tocopherol on arsenic induced microsomal dysfunction. Human Exp. Toxicol. 22:129-136.

Ray RS, Rana B, Swami B, Venu Vand Chatterjee M (2006): Vanadium mediated apoptosis and cell cycle arrest in MCF7 cell line. Chem Biol Interact. 163(3):239–247

Roldan E, Altamirano M (1990): Chromosomal aberrations, sister chromatid exchanges, cell-cycle kinetics and satellite associations in human lymphocytes cultures exposed to vanadium pentoxide. Mutat Res. 245:61-65.

Sánchez DJ, Ortega A, Domingo JL and Corbella J (1991): Developmental toxicity evaluation of orthovanadate in the mouse. Biol Trace Elem Res. 30:219-226.

Sánchez DJ, Colomina MT and Domingo JL (1998): Effects of vanadium on activity and learning rats. Physiol. Behav. 63(3):345–350.

Sasi MM, Haider SS, el-Fakhri M and Ghwarsha KM (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. Neurotoxicology. 15(2): 413-420.

Shrivastava S, Jadon A and Shukla S (2007): Effect of Tiron and Its Combination with Nutritional Supplements Against Vanadium Intoxication in Female Albino Rats. J. Toxicol. Sci. 32(2):185-192.

Soazo M and Garcia GB (2007): Vanadium Exposure Through Lactation Produces Behavioural Alterations and CNS Myelin Deficit in Neonatal Rats. Neurotoxicol. Teratol. 29(4): 503-10.

Soares SS, Henao F, Aureliano M, Gutiérrez-Merino C (2008): Vanadate induces necrotic cell death in neonatal rat cardiomyocytes through mitochondrial membrane depolarization. Chem. Res. Toxicol. 21: 607-618.

Sortwell CE, Daley BF, Pitzer MR, McGuire SO, Sladek JR Jr, Collier TJ (2000): Oligodendrocyte-type 2 astrocyte-derived trophic factors increase survival of developing dopamine neurons through the inhibition of apoptotic cell death. J Comp Neurol. 426(1):143-153.

Stefanidou M, Maravelias C, Dona A and Spiliopoulou C (2005): Metals, toxicity and oxidative stress. Curr. Med.Chem. 12:1161-1208.

Todorich B, Olopade JO, Surguladze N, Zhang, X, Neely E and Connor JR (2010): The mechanism of vanadium-mediated developmental hypomyelination is related to destruction of oligodendrocyte progenitors through a relationship with ferritin and iron. Neurotoxicity Research(In Press)

Uche FI, Obianime AW and Gogo-Abite M(2008): Effects of Vanadium Pentoxide on the Histological and Sperm Parameters of Male Guinea Pigs. J. Appl. Sci. Environ. Manage. 12(3):107 – 115

Wenning, R., Kirsch, N. (1988). Vanadium. In: Handbook on Toxicity of Inorganic Compounds. Marcel Dekker, eds. New York: Seiler-Sigel-Sigel, pp. 749–765.

Witkowska D and Brzezinski J (1979): Alteration of brain noradrenaline, dopamine and 5-hydroxy-tryptamine levels during vanadium poisoning. Pol. J**.** Pharmacol. Pharm.31:393-398.