Anti-oxidative and anti-inflammatory role of naringin against vanadium-induced neurotoxicity in adult Wistar rats

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SUMMARY

Vanadium is a trace element that can induce oxidative damage in the brain due to excess accumulation, which leads to programmed neuronal cell death. Naringin as a natural flavonoid has been reported to have a broad range of pharmaceutical bioactivities. We aimed to explore the therapeutic effects of naringin against oxidative stress and inflammation induced by vanadium exposure. Forty adults male Wistar rats were indiscriminately distributed into four (4) groups (n = 10). The groups received the followinh treatments: 5 ml/kg double distilled water (control), Naringin (Intraperitoneally, 30mg/kg BW), Vanadium & Naringin (Vanadium at 10mg/kg & Naringin at 30mg/kg respectively), Vanadium (Intraperitoneally, 10mg/kg BW). The result of vanadium administration showed an increase in oxidative stress, as seen in the reduction of glutathione peroxidase and catalase level of the brain (hippocampus), a decrease in numbers of viable cells and significant increase in inflamed cells. A decrease in memory function following vanadium administration was also observed. Therapeutic administration with naringin following vanadium exposure showed an elevation of glutathione peroxidase levels and catalase level of the hippocampus, a significant decrease in the number of inflamed cell and an improvement in memory function. This study is a proof that naringin can serve as a neuroprotective agent against oxidative stress and inflammation following vanadium toxicity in the brain.

Key words: Naringin – Vanadium – Antioxidant – Inflammation – Neurotoxicity – Glutathione peroxidase

INTRODUCTION

Dietary and occupational exposure is a public health hazard that has been linked to oxidative damage in the liver, kidney, and neurological damage (Xiong et al., 2021; Adekeye et al., 2020). Vanadium exposure can be through infected food and water; it can also be through air, or typical factory systems. Recent studies on health risk assessment of heavy metals have explore cognition, memory and the motor system following exposure to this chemical metal known as vanadium (Adekeye et al., 2020).

There have been recent reports of global environment problems on contamination of water with heavy metal, owing to the advancement of economy and development of industry. One of the contaminants that research has reported to pose

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a potential threat to animals and human beings is vanadium (Eze et al., 2022). This has become a major concern because increasing mining, smelting, weathering of rocks and sediments rich in vanadium have resulted in a high concentration of vanadium in the groundwater (Usende et al., 2017). Ways by which the concentration of vanadium in soil and natural water have been increased are through the uses of pesticides and fertilizers, as well as other human activities such as industrial production, mining, fossil fuel combustion and recycling of household waste. Vanadium can enter the body through either the lungs or the stomach and can be uptaken via the blood stream, cross the blood brain barrier and deposit in the brain. Its accumulation in the brain might be linked with the pathological processes of some nervous system disorders. After a long-term exposure, its deposition can result in more severe pathologies (Adekeye et al., 2020).

Vanadium is a trace element (atomic number 23), and is the 21st most abundant element in the outer regions of our planet. Vanadium is a special element that can interrelate with diverse physiological substrates that are otherwise put into work by phosphate as the tetrahedral anion vanadate (V) (Rehder, 2015). It can switch between the oxidation states +2, +3, and +4 in a physiological environment and can efficiently increase its sphere beyond tetrahedral coordination (Rehder, 2015; Olaolorun et al., 2021). The most stable oxidation state is the quadrivalent salts (VO^{2+}, vanadyl) (Barceloux and Barceloux, 1999). Vanadium is a heavy metal and is specially stored in certain organs, mainly in the bones, the kidneys, and the liver (Cortizo et al., 2000). The daily uptake of vanadium compounds via breathing air, drinking water and nutrients in nonpolluted areas varies between 10 µg and 2 mg (Rehder, 2013). The absorption of its compounds hinges on their solubility as well as the route of entry (Barceloux and Barceloux, 1999). Upon uptake, vanadium compounds undergo speciation by redox interaction and ligand removal/exchange with ingredients of body fluids in the various body compartments (Rehder, 2016). Dietary vanadium occurs either as vanadyl or as vanadate, with the latter being absorbed about 3 times more adequately by the digestive tract than the former (Barceloux and Barceloux, 1999). Critical exposure to vanadium compounds is confined to inhalation of vanadium oxides, and are readily absorbed in the lungs. It easily enters the respiratory tree deep into the airways, crossing the blood–gas barrier and reaching the bloodstream (Ghosh et al., 2014). Once in the bloodstream, high-molecular mass transporters such as transferrin (plasma) serum albumin (Ab) and immunoglobulin G (Ig) form binary complexes and ternary complexes with vanadium (Rehder, 2013). Further distribution from blood to the inner compartments (heart, liver and kidneys), the outer compartments (tissue and brain) and the bone structure occurs. Ultimately, vanadium is excreted via feces and urine (Ghosh et al., 2014). At nutritional concentrations, intoxication with vanadium is unlikely (Rehder, 2016). Vanadium compounds have the ability to interchange into different species constantly within the cell in the presence of ROS, which makes it a powerful inducer of cell death (necrosis) (Rojas-Lemus et al., 2020). Neurotoxic metals such as vanadium cause blood brain barrier disruption, neuropathology, and neuronal damage, which can trigger CNS alterations. The hippocampus, being a part of the limbic system, performs several brain functions such as learning, memory, and spatial coding

The hippocampus is vulnerable to oxidative stress, especially the subfields CA1 and CA3, and granule cells of DG are highly influenced by oxidative damage (Avila-Costa et al., 2006).

Thus, oxidative damage to this brain area can cause impairment in multiple brain functions. The management of hippocampal neurons' normal redox state is essential in the management and prevention of cognitive decline (Marosi et al., 2012). The living framework is known to have a natural antioxidant defense framework to neutralize the ROS produced within the metabolic handle (Viswanatha et al., 2017). The antioxidant defense is constituted by the antioxidant enzymatic actions. When this natural antioxidant defense falls brief, the antioxidants ought to be supplemented from an outside source by implies of supplements (Marosi et al., 2012). Flavonoids such as naringin possess antioxidant, anti-inflammatory, anti-apoptotic, anti-ulcer, anti-oste-

oporotic and anti-carcinogenic properties (Chen et al., 2016). Naringin is one natural flavanone, and reports have shown that it has a broad range of pharmaceutical bioactivities against oxidative stress, hyperlipidemia, and neurodegeneration (Chen et al., 2018). Orally administered naringin is deglycosylated into naringenin by hydrolytic enzymes of the epithelium of the oral cavity, or epithelial cells of the small intestine and by the intestinal microflora (Zou et al., 2015; Memariani et al., 2020). Research have shown that naringenin is not properly absorbed in the human digestive track, with merely 15% oral bioavailability (Josh et al., 2018). NGE is partially absorbed and then metabolized to glucuronide and sulfate metabolites through stage I and stage II metabolic reactions (Memariani et al., 2020). After absorption, Flavanones are bound to the albumin in the blood and immediately circulated to the thoroughly perfused organs like liver, kidney, heart, spleen, and cerebellum. Peng et al., 1998, reported the apparent permeability of Naringenin to be between 250-350 nm/s. Subsequently, Naringenin have high porousness across in situ BBB

models as well as in vitro examinations. Naringin has proven to be effective in the reduction of expression of signaling factors associated with the inflammatory response, such as, interleukin-6 (IL-6), interleukin-8 (IL-8), inducible nitric oxide synthase (Inos), nuclear factor erythroid 2-related factor 2 and tumour necrosis factor alpha (Chen et al.,2016). Protective genes against oxidative stress, inflammation, and accumulation of toxic metabolites contain a promoter element known as antioxidant response element (ARE). Nuclear factor erythroid 2- related factor-2 (Nrf2) binds to antioxidant response element (ARE). This action leads to the induction of cytoprotective genes, which reports have shown to play a protective role in central nervous system diseases (Gopinath et al., 2012). Continuous exposure to vanadium and its accumulation in the brain can result in a series of health conditions, and this might be linked with the pathogenesis of some specific neurological disorders. The consequence of the long- period exposure can even be more severe pathologically, and this in-turn may affect day-to-day activities and safety at workplace (Adekeye et al., 2020). This study gives us a chance to see the antioxidant effect of naringin on neurotoxicity caused by oxidative stress from vanadium oxides.

MATERIALS AND METHODS

Ethical approval and experimental design

All the procedures performed during this experiment are in accordance with the National institute of Health Guide for care and use of Laboratory Animals (NIH, 1985) and the Department of Human Anatomy, College of Medical and Health Sciences, Afe Babalola University Ado Ekiti, Nigeria (AB/EC/22/01/95). We endeavor to minimize the number of animals used for this experiment, as well as their suffering. Forty healthy male Wistar rats weighing 150-180 g were purchased from the Animal Holdings of the Department of Anatomy, Afe Babalola University, Ado-Ekiti. The rats were placed in a standard laboratory rat cage. They were given access to water and standard rat chow ad libitum. After a two-week acclimatization period, animals were randomly divided into four (4) groups with n = 10 per group. This included: Control as group CTL (received H_2O), Naringin as group NAR (received 30 mg/kg bw of naringin orally for 14 days), Vanadium + Naringin as group NAR+VAN (received 10 mg/kg of vanadium intraperitoneally for 7 days and 30 mg/kg bw of naringin orally for 14 days), and Vanadium as group VAN (received 10 mg/kg bw of vanadium Intraperitoneally for 7 days).

The oral administrations were carried out with an oral cannula and the Vanadium was administered intraperitoneally. The duration of the experiment was 21 days (Fafure et al., 2020; Yang et al., 2021).

Chemicals and reagents

RBFOX3 polyclonal antibody (Elabscience China, Cat No- E-AB-70267, 1:150), NLRP3 inflammasome polyclonal antibody (Elabscience China, Cat No- E-AB-65952, 1:200), 2- step plus poly-HRP Anti-mouse/rabbit IgG Detection system, DAB solution (Elabscience China, Cat No- E-IR-R217), Triton-X (Elabscience China, Cat No- E-IR-R122), phosphate buffered solution (Elabscience China, Cat No- EP-CM-L0007), Naringin (Sigma-Aldrich,

St. Louis, MO, USA; Cat No-N1376), Vanadium (Chembid, Germany, Cat No- 13718-26-8).

Neurobehavioral study

Memory index were assessed in the animals using novel object recognition (NOR) (IITC Life Science, Woodland Hills, CA, USA), and Y-maze test. These tests carried out in a sound- controlled behavior analysis room with a proper illumination as previously described by (Lueptow, 2017; Kraeuter et al., 2019). A practice session was conducted at the beginning of the experiment, and the final test for both NOR and Y-maze was done at the end of the treatment.

Animal sacrifice and sample preparation

The animals were anesthetized with 50 mg/kg bw of sodium pentobarbital (intraperitoneally) 48 hours after the last administration, followed by intra-cardiac perfusion fixation with normal saline and 4% paraformaldehyde respectively. The animals meant for biochemical analysis were perfused intracardially with phosphate buffered solution. The brain was removed, and the region of the hippocampus was homogenized in phosphate buffer solution and centrifuged at 10,000 rpm for 10 min at 4°C (Olaniyi and Areloegbe, 2022). The supernatant was collected and frozen until it was needed for the biochemical assay (Glutathione Peroxidase and Catalase). The brain meant for histological analysis was post-fixed in 4% paraformaldehyde, In addition, the region of the hippocampus (interaural 3.72 mm: 5.28 mm away from the Bregma) was grossed using rat brain atlas (Paxinos and Watson, 2017), and passed through routine tissue processing. Sections from the embedded blocks were obtained for immunohistochemical analysis.

Immunohistochemical procedure

For immunohistochemistry studies, Sections from 5 animals were routinely prepared for immunohistochemistry and quantified using the cell counter plug-in of ImageJ (Version 1.53). Sections of the CA1 field of the hippocampus (5 µm thick) were coronally cut with a microtome. Antigen retrieval with citrate buffer was done using the microwave (15 min) immediately after hydrating the sections, after which endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide solution for 10 min. Sections were washed with phosphate buffered solution for 2 min, 3 times. Normal goat serum was used to block the nonspecific binding sites with incubation time of 20 min. Sections were immunolabelled with primary antibodies directed against RBFOX3 (for neuronal cell distribution) and NLRP3 (for inflammation) polyclonal antibodies (NLRP3 1:200; RBFOX3 1:150, Elabscience, China), with incubation time of 2 hours at a room temperature or 37°C. Sections were incubated in Polyperoxidase-anti-Mouse/Rabbit IgG (E-IR-R217) secondary antibody for 20 min (Fafure et al., 2022). Sections were washed with phosphate buffered solution for 2 min, 3 times. A fresh prepared DAB working solution was added to the section, rinsed after obtaining the desired coloration, followed by counterstaining in Hematoxylin solution, dehydrating, transparentizing and sealing were carried out. Sections were photographed using an OPTU-EDU light microscope.

Data and statistical analysis

Image J was used to count the number of neurons present in each group after the experiment using a grid of 20000-60000 depending on the size of neurons. Statistical group analysis was performed using the Graphpad prism 8.01. Data are presented as means ± SD. One-way ANOVA was used to compare the mean values of variables and post hoc analysis was performed with post hoc Turkey test. Statistically significance was considered at $p < 0.05$.

RESULTS

Effects of Naringin on memory alteration following vanadium induction

The NOR test was used to assess non-spatial short-term working memory, while Y-maze test is used to evaluate short-term spatial working memory in the animals. The analysis showed that exposure to vanadium decreased memory index as seen in the vanadium (VAN) group and treatment with Naringin was effective in improving memory impairment as seen in the naringin and vanadi-

um (NAR+VAN) group. A decrease in memory index after exposure to vanadium can be seen in the vanadium (VAN) group and treatment with Naringin was effective in ameliorating these changes, as seen in the naringin and vanadium (NAR+VAN) group.

Effect of Naringin on Oxidative stress changes following vanadium induction

Glutathione peroxidase (GPX) and Catalase (CAT) levels were assessed in a section of hippocampus of rat following the administration of vanadium to induce neurotoxicity and treatment with naringin. Significant decreased in the glutathione peroxidase levels upon exposure to vanadium was seen in the vanadium (VAN) group. Treatment with Naringin showed a significant increase in GPX levels (GPX) levels as seen in the naringin and vanadium (NAR+VAN) group. Also, a reduction in the catalase activity upon exposure to vanadium was noticed in the vanadium (VAN) group. An improvement in catalase levels was noticed after treatment with Naringin as seen in the naringin and vanadium (NAR+VAN) group. Catalase levels in NAR+VAN group when compared to VAN group. There is also an increase in the hippocampal catalase level, when exposed to just Naringin in the NAR group when compared to VAN group.

Immunohistochemistry demonstration of Inflammasome and neuronal cells in the CA1 field of the hippocampus

RBFOX3 antibodies are used to identify mature neurons in cell cultures and tissue sections. This stain was used to identify viable neuronal cell bodies in the CA1 field of the hippocampus proper of the rats. The VAN group showed very little number of viable neuronal cells, while the CTL and NAR group revealed a lot of viable neuronal cells, as shown in the photomicrograph. In the NAR+VAN group also showed a lesser number of viable cell bodies when compared to the CTL and NAR groups, but showed a higher number of viable cell bodies when compared to the VAN group.

NLRP3 inflammasome is used as a marker for inflamed or damaged cell in the CA1 field of the hippocampus. The CTL and NAR groups showed very little NLRP3immuno-positive cells, whereas the VAN group showed the highest number of NLRP3 immuno-positive cells as seen in the photomicrograph below. Also, the NAR + VAN group showed expression of NLRP3 immuno-positive cells.

DISCUSSION

Metals are the most distributed pollutants globally with a propensity to amass in some human tissues and with huge toxic potentials at relatively low concentration. Vanadium is a heavy metal and is specially stored in certain organs, mainly in bone, kidney, and liver (Cortizo et al., 2000). Vanadium compounds have the ability to interchange into different species constantly within the cell in the presence of ROS, which makes it a powerful inducer of cell death (necrosis) (Rojas-Lemus et al., 2020). Neurotoxic metals such as vanadium cause blood brain barrier disruption, neuropathology and neuronal damage, which can trigger CNS alterations.

The hippocampus, being a part of the limbic system, performs several brain functions such as learning, memory, and spatial coding (Avila-Costa et al., 2006). The hippocampus is vulnerable to oxidative stress and oxidative damage (Salim et al., 2016). Thus, oxidative damage to this brain area can disrupt normal synaptic neurotransmission, cause impairment in memory function, cell proliferation and degeneration; it can alter structural changes, and distort neurogenesis. Therefore, the maintenance of a normal redox state in hippocampal neurons is crucial in the impediment of memory decline (Marosi et al., 2012).

Oxidative stress is a result of a mismatch between the generation of faulty reactive oxygen species (ROS) and the organism's capacity to moderate their harmful effects (Viswanatha et al., 2017). The antioxidant defense is made up by the antioxidant enzymatic actions.

Superoxide radicals are converted to hydrogen peroxide (free radical), and the hydrogen peroxide is eliminated by glutathione peroxidase (GPx) and/or catalase (CAT). These enzymes are responsible for the destruction of excess hydrogen peroxide formed in the nervous tissue. However, when

this intrinsic antioxidant defense is low, supplementation from an external source is required to strengthen the antioxidant (Marosi et al., 2012).

Naringin possesses various pharmacological properties such as antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, anti-diabetic, anti-atherosclerotic, anti-hyperlipidemic, reno- protective, cardio-protective properties, and so on (Viswanatha et al., 2017). Several published papers have reported the protective role of naringin through modulations of antioxidant in numerous parts of the brain (Viswanatha et al., 2017).

Behavioral analysis result, using the Novel Object Recognition (NOR) test and the Y maze test to assess spatial memory. The results from the Y maze analysis shows a significant decrease in the percentage of alteration in the vanadium-only (VAN) group as compared to the other groups, as shown in Fig. 1A. This result is in correspondence with the study by Franklin et al. (2021) on the ameliorative role of Khaya anthotheca in vanadium-induced cognitive dysfunction in mice. Vanadium elicits its harmful reactions through the formation of free radicals, when it crosses the blood brain barrier and in turns leads to lipid peroxidation and a damage in the antioxidant defense of the tissue. In the study by Franklin et al. (2021), it was reported that the vanadium-induced animals had the least number of arms entries in the Y maze. The Novel Object Recognition test (NOR) is also widely used to evaluate recognition memory in mice. In Fig. 1B, it can be noted that there was a steady decrease in percentage of memory index, with the least value being the vanadium only (VAN) group. This has been proven in the study by Khadija et al. (2018) on how short-term exposure to titanium, aluminum, and vanadium (Ti 6Al 4V) alloy powder extremely affects behavioral and antioxidant metabolites in male albino mice. The study reported significant decrease and compromise in the animals' novel object recognition capability. In this current study, it is noted that the decline in memory index between all four groups were progressive, as seen in Fig. 1B.

In the results of the biochemical analysis, it was noted that a decrease in glutathione peroxidase and catalase (Cat) level was observed in the vanadium-only (VAN) group, as seen in Fig. 2A. This was in accordance with Folarin et al. (2017a), who studied changes in the central nervous system antioxidant level following chronic vanadium

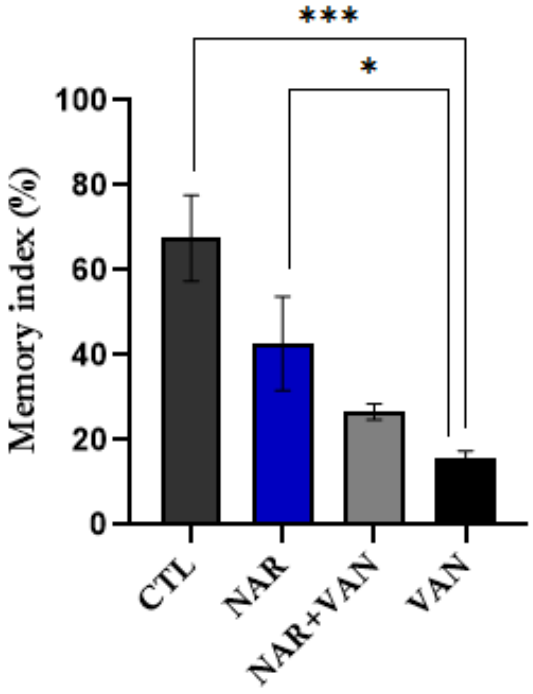

Fig. 1A.- Bar chart representation showing changes in non-spatial memory function from the NOR test following exposure to VAN and treatment with Naringin, comparison between groups by one-way ANOVA followed by Tukey's multiple comparison test shows significant decrease in memory index in VAN group (***p< 0.0001) when compared with CTL group. Administration of naringin only showed an increase in memory index of the NAR only group when compared to VAN. Legend: CTL = control group; NAR = naringin group; NAR+VAN= Naringin + Vanadium group; VAN = vanadium group.

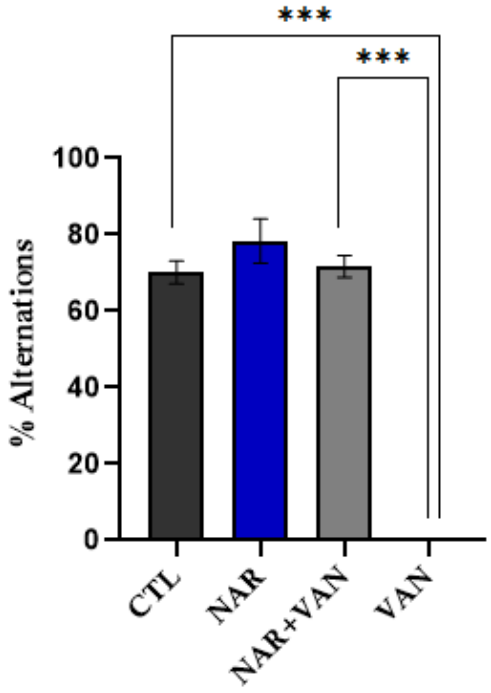

Fig. 1B.- Bar chart representation showing changes in short term spatial memory following exposure to VAN and treatment with Naringin. Comparison between groups by one-way ANOVA followed by Tukey's multiple comparison test shows significant decrease in memory index in VAN group (***p<0.0001) when compared with CTL group. Treatment with Naringin significantly increased memory index in NAR+VAN group (***p<0.0001) when compared to CTL. Legend: CTL = control group; NAR = naringin group; NAR+VAN= Naringin + Vanadium group; VAN = vanadium group.

exposure in mice. It was reported that chronic vanadium administration led to oxidative damage shown by significant increase nitric oxide, Malondialdehyde, and hydrogen peroxide production, associated with a reduction in the activities of intrinsic antioxidant system, superoxide dismutase (SOD), Glutathione peroxidase (GPX), and Glutathione (GSH) in the brain. An increase in glutathione peroxidase and catalase (Cat) level was observed in the naringin + vanadium (NAR + VAN) group after treatment with naringin following exposure to vanadium as seen in Fig. 2B. This is in correspondence with the research by Kumar et al. (2010) in the study of Naringin's attenuation of memory impairment, and oxidative stress induced by d-galactose in mice. Results from the immunohistochemistry analysis showed changes in numbers of viable cells and numbers of inflamed cells. The RBFOX3 marker was used to evaluate the number of viable neuronal cells in the CA1 region of the hippocampus. From Fig. 3A, the vanadium-only (VAN) group shows a decrease in number of viable cells. In accordance with Folarin et al. (2017b) study on neuro-inflammatory profiles and metal distribution after chronic vanadium administration and withdrawal in mice, where he revealed that damaged pyramidal cells, with morphological alterations including cell clustering, loss of layering pattern and cytoplasmic vacuolation in the vanadium-exposed brains. The naringin + vanadium (NAR + VAN) group showed an increase in number of viable cells after administration of naringin following vanadium exposure. The NLRP3 Inflammasome marker used to detect NLRP3 positive cell. Fig. 3B showed that the vanadium-only (VAN) group had the largest amount of NLRP3 positive cell. Inflammation is a protective response of an organism to the biologically, chemically, or physically mediated injury. The process is initiated by inflammatory mediators, including cytokines and chemokines that are released by injured tissue, which attract leukocytes to the damaged site (Zwolak, 2013). Therapeutic treatment with naringin following vanadium administration as seen in the naringin + vanadium (NAR+VAN) group showed a lower amount of NLRP3 positive cells. The anti-inflammatory effect of Naringenin was linked to the activation of transcription factor Nrf2, which functions as an agonist of aryl-hydrocarbon receptors, and subsequently reduces the formation of ROS and inflammatory mediators in the cells (Joshi et al., 2018).

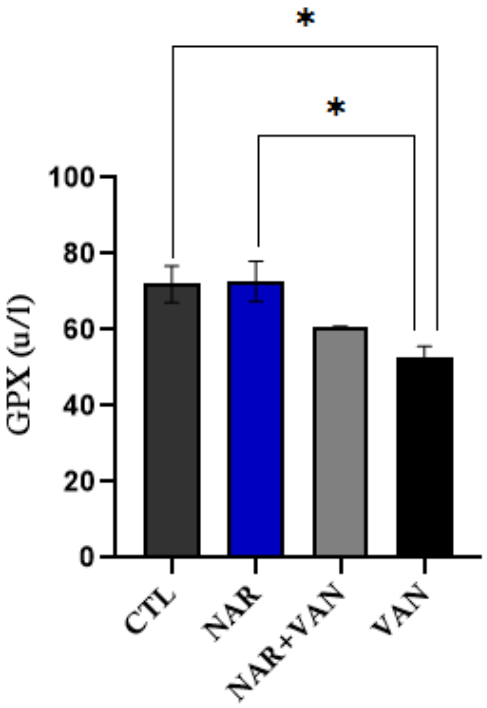

Fig. 2A.- Bar chart representation showing changes in glutathione peroxidase level in the hippocampus across all the groups of rats used in the experiment. The groups were compared by one-way ANOVA followed by Tukey's multiple comparison test. A decrease in the hippocampal glutathione peroxidase level in (VAN) group when compared with control (CTL) group (*p<0.01). Treatment of the (VAN) group with Naringin show an increase in the glutathione peroxidase levels as seen in NAR+VAN group when compared to VAN group. There is an increase in the hippocampal glutathione level in rat exposed to just Naringin in the NAR group when compared to VAN group (*p<0.02).

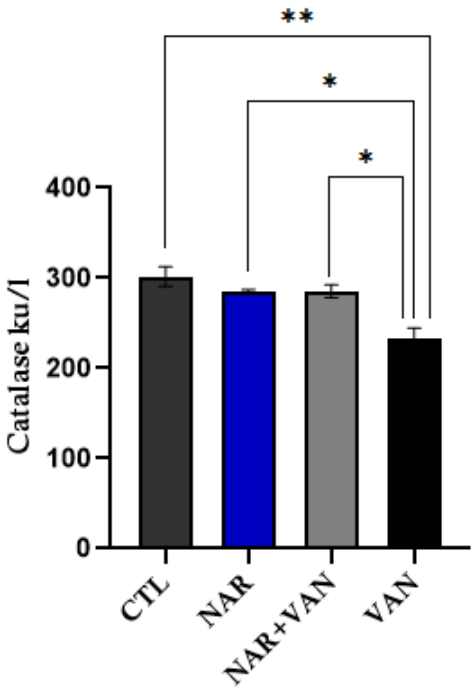

Fig. 2B.- Bar chart showing changes in hippocampal catalase level using one-way ANOVA followed by Tukey's multiple comparison test to compare between the group. The result of the analysis showed a decrease in the hippocampal catalase level in VAN group when compared with CTL group(**p<0.05). Upon treatment with Naringin, there is an increase in hippocampal catalase levels in NAR+VAN group when compared to VAN group. There is also an increase in the hippocampal catalase level, when exposed to just Naringin in the NAR group when compared to VAN group.

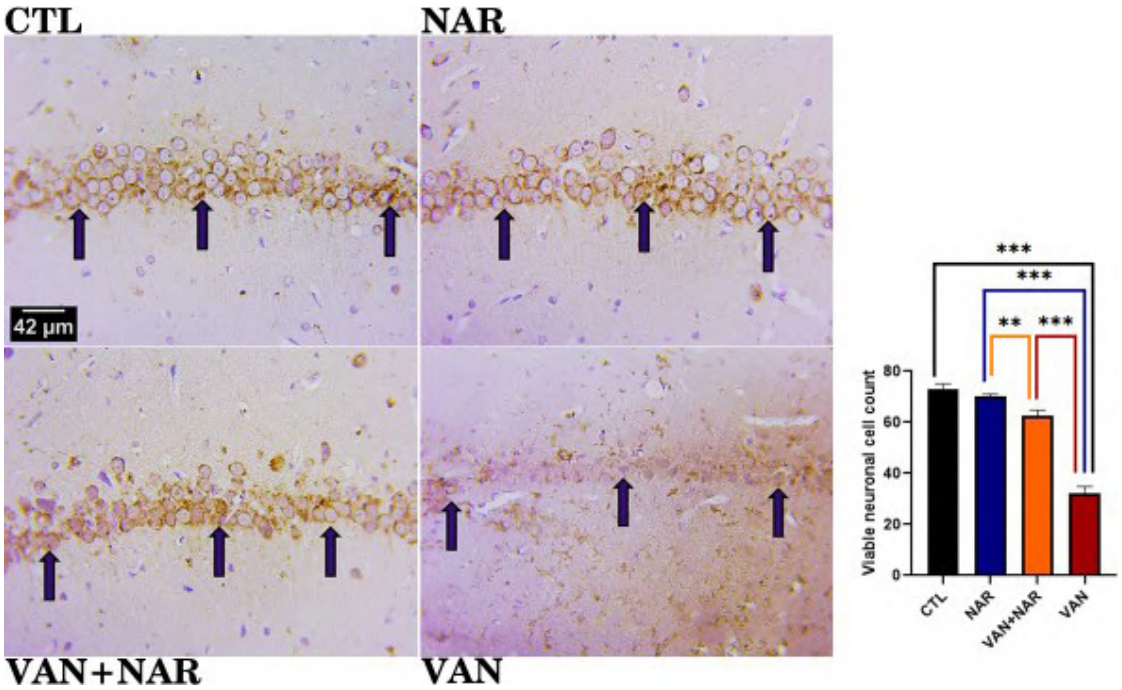

Fig. 3A.- Photomicrograph showing RBFOX3 viable cell bodies in the CA1 region. Dark brownish round cells indicate the viable nerve cell bodies. Bar chart representation showing viable neuronal cell count following exposure to VAN and treatment with naringin. Comparison by one-way ANOVA followed by Tukey's multiple comparison test showed a significant decrease in viable neuronal cells in VAN group when compared with CTL group(***p<0.0001). Upon treatment with Naringin, an increase in the number of viable neuronal cell count in NAR+VAN group was observed when compared to VAN group (***p<0.0001). There is also an increase in the viable neuronal cell count in rat the NAR group when compared to VAN group (***p<0.0001). x1200 (Scale bar: 42µm).

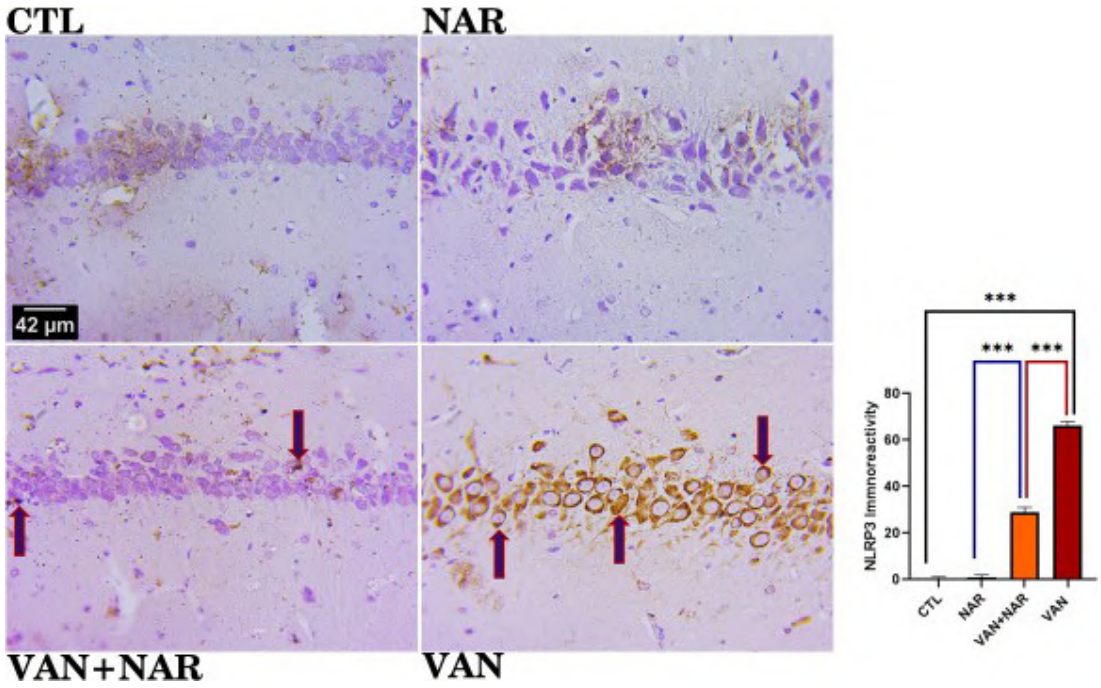

Fig. 3B.- Photomicrograph showing NLRP3 immuno-positive cells in the CA1 region. Dark brownish round cells indicate the cells that expressed inflammasome. Bar chart representation showing the cell count following exposure to VAN and treatment with naringin. Comparison between groups by one-way ANOVA followed by Tukey's multiple comparison test shows a significant increase in inflammasome in VAN group when compared with CTL group (***p<0.001). Treatment with Naringin reduced the immunopositive cell count in NAR+VAN group when compared to VAN group (***p<0.001). There is equally a statistically significant reduction in the immunopositive cell count in rat exposed to just Naringin in the NAR group when compared to VAN group (***p<0.001). x1200 (Scale bar: 42µm).

CONCLUSION

This study has proven the adverse effects of chronic vanadium exposure on the body. It has also shown the therapeutic effects of naringin as an antioxidant and anti-inflammatory agent in mitigating the neurotoxic of oxidative stress by heavy metals, especially vanadium.

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