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RESEARCH ARTICLE

Morphological and biochemical investigation into the possible neuroprotective effects of kolaviron (*Garcinia* kola bioflavonoid) on the brains of rats exposed to vanadium

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Abstract

In this study, the morphological and biochemical susceptibility of the rat brain to vanadium, in the form of sodium metavanadate, and the comparative ameliorative effect of *Garcinia kola* and kolaviron (*G. kola* extract), was examined. Brain regions examined were the cerebrum, cerebellum, hippocampus and the olfactory bulb. We showed that vanadium administration caused cellular vacuolation, congestion, and Purkinje cell degeneration and a marked reduction in myelin tracts. Biochemical tests revealed increased lipid peroxidation induced by vanadium, which was ameliorated with the administration of *G. kola* and kolaviron. Vanadium administration caused an increase in thiobarbituric acid–reactive substances (TBARS) in the cerebrum and hippocampus, whereas the administration of kolaviron resulted in a reduction of the TBARS level by 65.7 and 80%, respectively, in the regions aforementioned. Also, the administration of kolaviron resulted in an increased activity of superoxide dismutase (61.24%) in all brain regions assessed, when compared with the group administered vanadium alone. Results obtained from this study led to the conclusion that kolaviron reduces vanadium-induced oxidative stress in the brain.

Keywords: Vanadium, sodium metavanadate, kolaviron, neurotoxicity, lipid peroxidation

Introduction

The increase in environmental pollution caused by the recent sustained, increased exploitation of minerals (Olopade et al., 2005) has suggested a strong link between environmental pollution and the incidence of neuropathologies of the brain (Calderon-Garciduenas et al., 2002; Igado et al., 2008).

Vanadium (atomic number 23) is a transition metal widely distributed in nature and extensively used in modern industry (e.g., hardening of steel, manufacture of pigments, in photography, in insecticides, and as a catalyst in the production of several materials) (Wenning and Kirsch, 1988; Hope, 1994). It is a major trace metal in fossil fuels, and combustion of these materials provides a significant environmental source of this element. Petroleum hydrocarbons from refineries and gas flaring are common occurrences in the oil-producing areas of Nigeria (Osuji and Avwiri, 2005), and this can result in the increased level of vanadium in the atmosphere (Bycakowski and Kulkarni, 1996). Vanadium in particulate emissions has been estimated to comprise approximately 53% of the total atmospheric vanadium (Hope, 1994).

In the brain, myelin accounts for high lipid content. The high metabolic activity and high concentration of polyunsaturated fatty acids of the brain (myelin) increases the susceptibility of the nervous tissue to peroxidative damage. Graded doses of vanadium, in the form of sodium metavanadate, when administered to rats, have been shown to cause lipid peroxidation (LPO), leading to alteration of the lipid metabolism and protein concentrations

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of different regions of the brain (Sasi et al., 1994). LPO is particularly dangerous, because lipoperoxy radicals are capable of reacting with many cellular components, initiating oxidative chain reactions. The fact that myelin as a membrane has a high relative content of phospholipids makes it a potential target for membrane oxidative damage caused by vanadium (García et al., 2004).

Garcinia kola, also known as "bitter kola," of the family *Guttiferae,* is found in moist forests throughout West and Central Africa. The constituents include biflavonoids, xanthones, and benzophenones (Iwu, 1993). Kolaviron, an extract from *G. kola* and a biflavonoid, has also been shown to exhibit antioxidant activity (Farombi et al., 2002). Kolaviron contains biflavonoids GB1 and GB2 and kolaflavanone and has been reported to significantly prevent drug-induced tissue damage in various experimental animal models (Farombi and Nwaokeafor, 2005).

The ameliorating effects of some antioxidants, such as selenium (Haider et al., 1998) and vitamin E (Sasi et al., 1994), have been examined in the brain after vanadium treatment. We assume that the properties of kolaviron may be important in the protection against vanadium effects, and this study is part of an ongoing effort of finding effective antidotes to vanadium-induced neurotoxicity (Olopade and Connor, 2011).

Methods

The study was conducted in two phases: first, a pilot study (experiment 1) using vanadium and the crude *G. kola*, then an experiment assessing the effect of kolaviron on brains of rats exposed to vanadium. All animal experiments were done in accord with the rules of the Faculty of Veterinary Medicine, University of Ibadan Animal Ethical Committee (Ibadan, Nigeria).

Experiment 1

Animals and treatments

Twenty-five Wistar albino rats were used for this experiment and were obtained from the Department of Veterinary Anatomy Animal House and housed in the Experimental Animal Unit Housing Facility of the Faculty of Veterinary Medicine, University of Ibadan. Rats were all males, aged 6 weeks at the time of purchase. Animals were stabilized for 2 weeks and were fed with rat pelleted feed and water *ad libitum*.

Animals were divided into five groups of 5 rats each. *G. kola* was administered at a dose of 100 mg/kg body weight (b.w.) orally (based on previous reports by Akpantah et al., 2003), whereas vanadium (sodium metavanadate) was administered at doses of 1.25 or 1.5 mg/kg intraperitoneally (i.p).

The groups were divided as follows: group A: 1.25 mg/kg b.w. of vanadium daily (i.p.) for 5 days; group B: 1.5 mg/kg b.w of vanadium/day (i.p.) for 5 days; group C: *G. kola* (GC) alone for 5 days; group D: *G. kola* (orally) for 10 days; vanadium 1.5 mg/kg b.w./day (i.p.) was started on day 6 until day 10; and group E (control): injected with normal saline (i.p.) for 5 days.

Rats were sacrificed on day 6, except for group D, which was sacrificed on day 11. Rats were sedated with chloroform in a gas chamber and euthanized. The brains were removed according to the method described by Olopade et al. (2005). One half of the brain, divided longitudinally, was preserved for hematoxylin and eosin (H&E) staining, whereas the other half was preserved for biochemical tests. Samples were collected from the cerebrum, cerebellum, olfactory bulb, and the hippocampus for biochemical and histological analyses. Samples for biochemical analysis were maintained at 4oC until analyzed, whereas samples for histology (H&E staining) were fixed in 10% phosphate-buffered formalin (PBF) for 3 days to allow for proper fixing and also to reduce the occurrence of artefacts (the PBF was changed daily).

Experimental test 1

Preparation of G. kola

G. kola seeds were purchased from a local market in Ibadan, Nigeria. The seeds were peeled, sliced, pulverized with an electric blender, and dried at 40°C in a Gallenkamp (London, UK) drying oven. The dried seeds were further pulverized with the electric blender and sieved with a fine sieve to give a uniform powder. A known quantity was diluted with drinking water, which was administered to rats at a dose rate of 100 mg/kg. The dose of 100 mg/kg for *G. kola* seeds was based on previous reports by Akpantah et al. (2003).

LPO

LPO was assessed by measuring the thiobarbituric acid–reactive substances (TBARS) present in the tissue samples using the procedure of Varshney and Kale (1990) and Farombi et al. (2000). Values obtained were expressed as micromolar of malondialdehyde (MDA)/g tissue. Briefly, the brain was homogenized and the homogenate was incubated for 6 hours at 37°C in a shaking water bath. Then, 0.01 mL of 5% (w/v) butylated hydroxytoluene (BHT) was added to a 0.5-mL incubation mixture, followed by the addition of 0.5 mL of 10% trichloroacetic acid (TCA) and 0.5 mL of 0.75% 2-thiobarbituric acid (TBA) in 0.1 mol/L of HCl. The mixture was heated at 90–95^{o}C for 20 minutes and, after cooling to room temperature, centrifuged for 10 minutes at 780×*g*. The supernatant was transferred into acid-resistant tubes and centrifuged at 32,000×*g* for 10 minutes. The absorbance of the resulting clear solution was determined at 532 nm using a Jenway 615 UV/visible spectrophotometer (Essex, UK). MDA was quantified by using the following equation: $\Sigma = 1.56 \times 10^5$L/mol per cm. Where Σ is the extinction coefficient. Value is expressed in nmol/mg protein.

Histology

Samples for histology (H&E staining) were prepared according to a modification of the method described by Clayden (1962). Briefly, brain tissues were fixed in

PBF for 3 days, and the PBF solution was changed daily to minimize shrinkage and prevent the occurrence of artifacts. Tissues were thereafter dehydrated by transferring to graded concentrations of alcohol (30, 50, 70, and, finally, 100%) and were then cleared using xylene and, after, embedded in paraffin wax. Embedded tissue was sectioned into ribbons using a microtome. Sectioned ribbons were placed on slides, stained with H&E, and mounted with DPX (a mountant).

Experiment 2

Animals and treatments

Twenty male (10-week-old) Wistar albino rats were obtained from the Department of Veterinary Anatomy Animal House. They were fed standard rat pellets and water *ad libitum* and were stabilized for 2 weeks before the start of the experiment. Rats were divided into four experimental groups of 5 rats each as follows: group A: normal saline (i.p.) daily for 7 days; group B: vanadium 1.5 mg/kg daily (i.p.) for 7 days (days 12–19); group C: kolaviron (100 mg/kg orally) and vanadium (1.5 mg/kg i.p.) daily for 12 and 7 days, respectively. Kolaviron was administered from days 1 to 12, whereas vanadium was administered from days 6 to 12; and group D: corn oil (diluent for the kolaviron administration) was given for 7 days orally. At the end of the experiments, rats were sacrificed with a xylazine-ketamine combination at a dilution factor of 1:9 and a dose rate of 0.003 mL/g b.w.

Corn oil served as the diluent for kolaviron, whereas normal saline was used for vanadium.

The kolaviron-treated group was omitted, because our pilot as preliminary investigations demonstrated that response obtained with the kolaviron-alone–treated animals was not different from that of the controls.

Preparation of brain samples

Brains were removed according to the method of Olopade et al. (2005). One half of the brain, divided longitudinally, was preserved for immunohistochemical staining, whereas the other half was preserved for biochemical tests.

Brains meant for immunohistochemical staining were fixed in 10% PBF for 3 days to allow for proper fixing and also to reduce the occurrence of artefacts (the PBF was changed daily). Brain tissues were dehydrated in graded concentrations of alcohol (30, 50, 70, and, finally, 100%), cleared in xylene, and embedded in paraffin wax, according to the method of Clayden (1962), as earlier described. The embedded tissue was sectioned into ribbons using a microtome. The ribbons were placed on individual salinized slides, deparaffinized in xylene, and then boiled in 10 mM of citrate buffer (pH = 6.0) for 25 minutes for antigen retrieval.

Brain tissue intended for biochemical tests were immediately stored at 4°C until analyzed, when they were first homogenized.

Experimental test 2

Myelin basic protein (MBP) immunohistochemical staining

Immunohistochemistry was based on works of Todorich et al. (2011). Briefly, brain sections were deparaffinized, then boiled in 10 mM of citrate buffer (pH = 6.0) for 25 minutes for antigen retrieval. Slides were then put in 3% H_2O_2/methanol for quenching of subsequent peroxidise activities. All sections were blocked in 2% milk overnight and treated with anti-MBP rabbit polyclonal antibody (1:1000; Sigma-Aldrich, St. Louis, Missouri, USA) diluted in 2% milk for 16 hours at 4°C. Conjugated antibody was done with appropriate horseradish peroxidise secondary antibodies in a VECTASTAIN kit (Vector Labs, Burlingame, California, USA), according to the manufacturer's protocol. The antibody was developed in rabbits and used against rats. Visualization of the reaction product was enhanced with diaminobenzidine (DAB) (1:25 dilution) for 6–10 minutes, with subsequent dehydration in ethanol. Slides were mounted with Gel Mount (Sigma-Aldrich), coverslipped, and dried. Images were acquired on a bright-field microscope equipped with a digital camera.

Biochemical assays

Chemicals

TBA, TCA, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), trolox, butylated hydroxyanisole, BHT, and all other reagents were of analytical grade and were purchased from the British Drug Houses (Poole and Dorset, UK). Sodium metavanadate was purchased from Sigma-Aldrich.

Extraction of kolaviron

The method of kolaviron extraction was carried out according to the method of Farombi and Nwaokeafor (2005). Briefly, *G. kola* peeled seeds (5 kg) were sliced, pulverized with an electric blender, and dried at 40°C in a Gallenkamp drying oven. The powdered seeds were then extracted with light petroleum ether (boiling point, 40–60°C) in a soxhlet for 24 hours. The defatted dried marc was repacked and extracted with acetone. The extract was diluted twice its volume with water and extracted with ethylacetate (6 × 300 mL). The concentrated ethylacetate yielded kolaviron as a golden yellow solid. Purity of extracted kolaviron was 96%, and no known contaminants were present. Kolaviron was identified by direct comparison of the ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and electron ionization mass spectral results with previously published data (Iwu, 1985).

Determination of LPO: as previously described for experiment 1

Determination of reduced glutathione (GSH) level

GSH level was determined according to the methods of Jollow et al. (1974). The principle is based on the fact that the reduced form of GSH, in most instances, comprises the bulk of cellular nonprotein sulfhydryl groups; thus,

the method is based on the development of a relatively stable (yellow) color when 5′,5′-dithiobis-(2-nitrobenzoic acid) (i.e., Ellman's reagent, DTNB) is added to sulfhydryl compounds. The chromophobic product resulting from the reaction of Ellman's reagent with the GSH, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm.

Reduced GSH was determined in the 10,000×*g* supernatant fraction. Briefly, an aliquot of brain homogenate was deproteinized by the addition of an equal volume of 4% sulfosalicylic acid, and the resulting solution was centrifuged at 10,000×*g* for 15 minutes at 4°C. The supernatant (0.5 mL) was then added to 4.5 mL of DTNB. Reduced GSH was proportional to the absorbance at 412 nm. Values are expressed in μg/mg protein.

Determination of superoxide dismutase (SOD) activity

SOD activity was determined according to the method of Misra and Fridovich (1972). The principle for this method is based on the ability of SOD to inhibit the auto-oxidation of epinephrine at pH 10.2. Superoxide (O_2^-) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adenochrome, and the yield of adenochrome produced per O_2^- introduced increased with increasing pH and also increased with increasing concentrations of epinephrine. SOD activity was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30°C. Briefly, brain sample (1 mL) was diluted in 9 mL of distilled water to make a 1 in 10 dilution. An aliquot (0.2 mL) of the diluted sample was added to 2.5 mL of 0.05 M of carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer, and the reaction was started by the addition of 0.3 mL of freshly prepared 0.3 mM of adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contained 2.5 mL of buffer, 0.3 mL of substrate (i.e., adrenaline), and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. Values are expressed in U/mg protein.

Determination of glutathione S-transferase (GST) activity

GST activity was determined according to the method of Habig et al. (1974), based on the fact that all known GSTs demonstrate a relatively high activity with 1-chloro-2,4,-dinitrobenzene (CDNB) as the second substrate; consequently, the conventional assay for GST activity utilizes CDNB as a substrate. When this substance is conjugated with reduced GSH, its maximum absorption shifts to a longer wavelength. The absorption increase at the new wavelength of 340 nm provides a direct measurement of the enzymatic reaction. GST activity was determined by using CDNB as a substrate. The reaction mixture (3 mL) contained 1.7 mL of 100 mmol/L of phosphate buffer (pH 6.5) and 0.1 mL of 30 mmol/L of CDNB. After preincubating the reaction mixture at 371°C for 5 minutes, the reaction was started by the addition of 0.1 mL of diluted cytosol and the absorbance was followed for 5 minutes at 340 nm. Reaction mixture without the enzyme was used as a blank. The specific activity of GST is expressed as nmoles of GSH-CDNB conjugate formed/min per mg protein using an extinction coefficient of 9.6×10^3 L/mmol/cm. Values are expressed in nmol/mg protein.

Determination of catalase (CAT) activity

The method of Sinha (1972) was used, based on the fact that the dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced was measured colorimetrically at 570–610 nm. Because dichromate has no absorbency in this region, the presence of the compound in the assay mixture did not interfere at all with the colorimetric determination of chromic acetate. The CAT preparation was allowed to split H_2O_2 for different periods. The reaction was stopped at a particular time by the addition of the dichromate/acetic acid mixture, and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Varying amounts of H_2O_2 (10–100 μmoles) were taken in small test tubes, and 2 mL of dichromate/acetic acid was added to each and subsequently heated for 10 minutes in a boiling water bath. The solution was cooled at room temperature, and the volume of the reaction mixture changed to 3 mL. Absorbance was measured with a spectrophotometer at 570 nm. The concentrations of the standard were plotted against absorbance. Values are expressed as μmole H_2O_2 consumed/min.

Statistical analysis

All data are presented as mean ± standard deviation. Data were analyzed using analysis of variance (ANOVA) across groups with GraphPad prism software (version 4; GraphPad Software, La Jolla, California, USA).

Results

Results are presented in Figures 1–10.

Experiment 1

In the *G. kola*+vanadium group, the cerebrum showed vacuolated, swollen (Figure 1C), and necrotized (Figure 1D and E) cells at the granular layer, whereas the hippocampus showed no visible lesions. In the group receiving vanadium (1.25 mg/kg), no visible histological lesions were observed in the cerebrum and hippocampus, whereas the Purkinje cell layer of the cerebellum showed degenerated and hypochromic nuclei (Figure 2A). The meningeal blood vessels of the 1.5-mg/kg vanadium group cerebellum were congested (Figure 2C). No visible lesions were observed in the hippocampus (Figure 3). No visible lesions were observed in the group receiving *G. kola* alone.

LPO was most pronounced in the 1.5-mg vanadium group olfactory bulb (1.16 ± 0.06 μmol/g). Values for the hippocampus were also high in groups 1.25 mg vanadium and *G. kola* alone, being 0.97 and 1.05

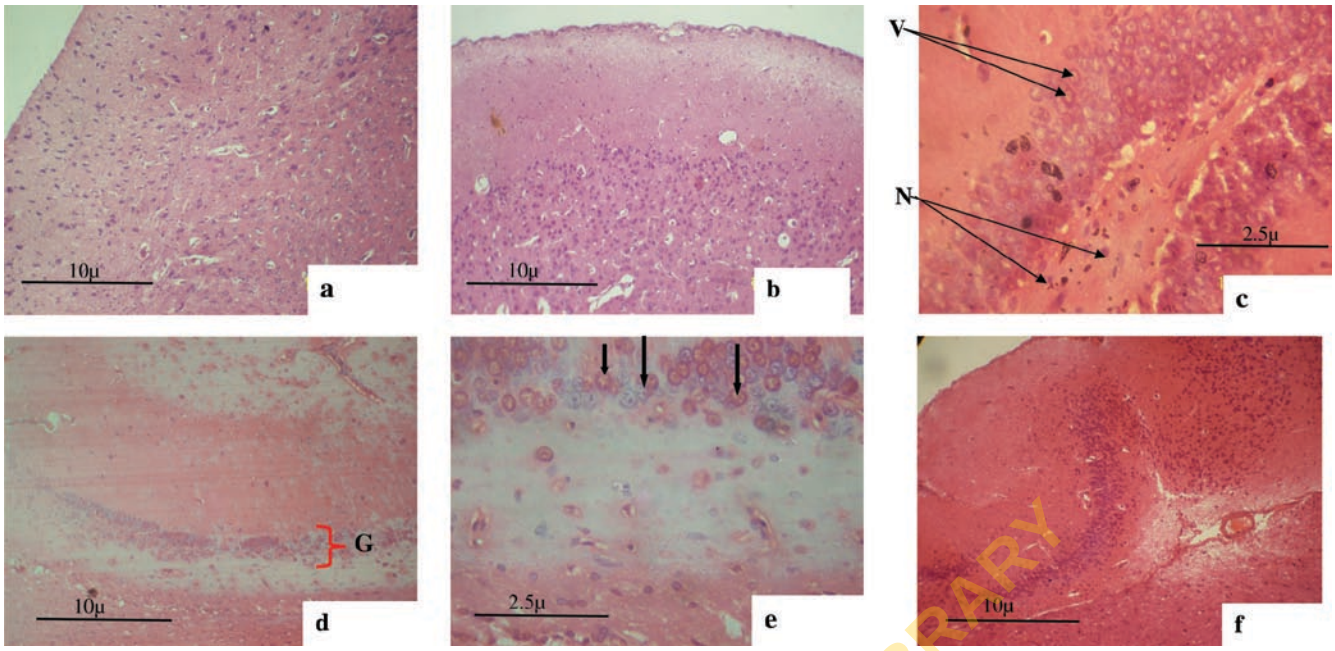

Figure 1. Cerebrum, using H&E stain. (**A**) Vanadium 1.5 mg/kg (×100). No visible lesions were observed. (**B**) *G. kola* alone. No visible lesions (×100). (**C**) *G. kola*+vanadium. Notice the swollen and vacuolated cells (V), compared with normal cells (N) (×400). (**D**) *G. kola*+vanadium. Notice the necrotized cells at the granular layer (G) (×100). (**E**) *G. kola*+vanadium. Magnification of the granular layer G in (**D**) above. Notice the necrotized cells (arrow) (×400). (**F**) Control group. No visible lesions (×100).

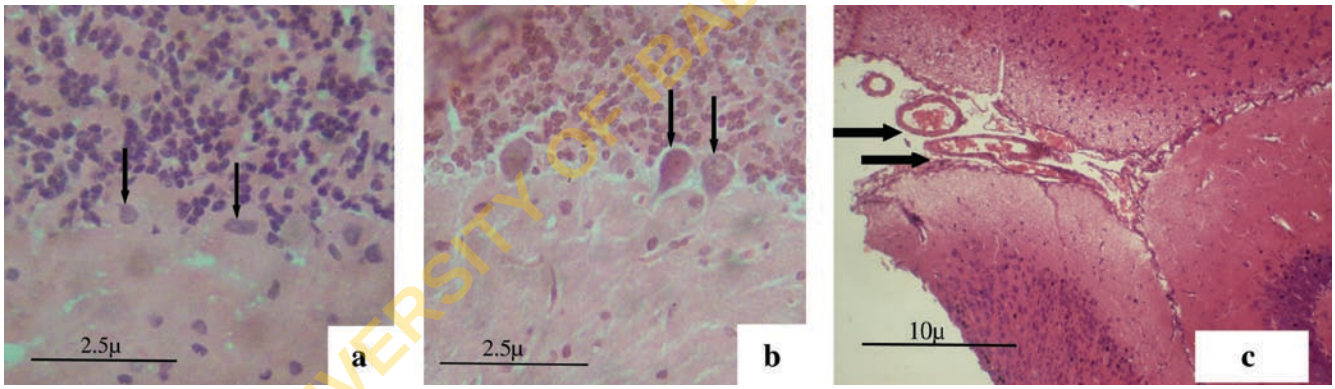

Figure 2. Cerebellum, using H&E stain. (**A**) Vanadium 1.25 mg/kg. Arrows indicate hypochromic and degenerated Purkinje cell-layer nuclei (×400). (**B**) Control group. Note the normal Purkinje cell-layer nuclei (arrows) (×400). (**C**) Vanadium 1.5 mg/kg. Arrows indicate congested meningeal blood vessels (×100).

µmol/g, respectively, but considerably low in the *G. kola*+vanadium group (0.006 µmol/g). Values obtained are expressed in µmol/g tissue (×10^7) (Figure 4).

Experiment 2

Effect of treatments on MBP

MBP immunohistochemical stainings showed a decreased myelin fiber density in the cerebellum of rats exposed to vanadium, relative to the groups exposed to normal saline and kolaviron+vanadium (Figure 5A–D).

Effect of treatments on LPO

The level of MDA (an empirical index of LPO) is presented in Figure 6. A significant increase in TBARS was noted in the cerebellum of the group exposed to vanadium, when compared with the normal saline-treated group ($P < 0.05$).

Kolaviron administration blunted the enhanced LPO level caused by vanadium exposure in the cerebrum, cerebellum, and hippocampus (65.7, 64.3, and 80%, respectively) ($P < 0.05$). In the cerebellum, the normal saline-treated groups were statistically significantly different from groups treated with vanadium ($P < 0.05$). Values obtained are expressed in µmol/g tissue (×10^7).

Effect of treatments on GSH

In the hippocampus, vanadium treatment elicited a statistically significant increase in GSH level, when compared with the normal saline-treated group ($P < 0.05$). In both the cerebellum and hippocampus, the administration of

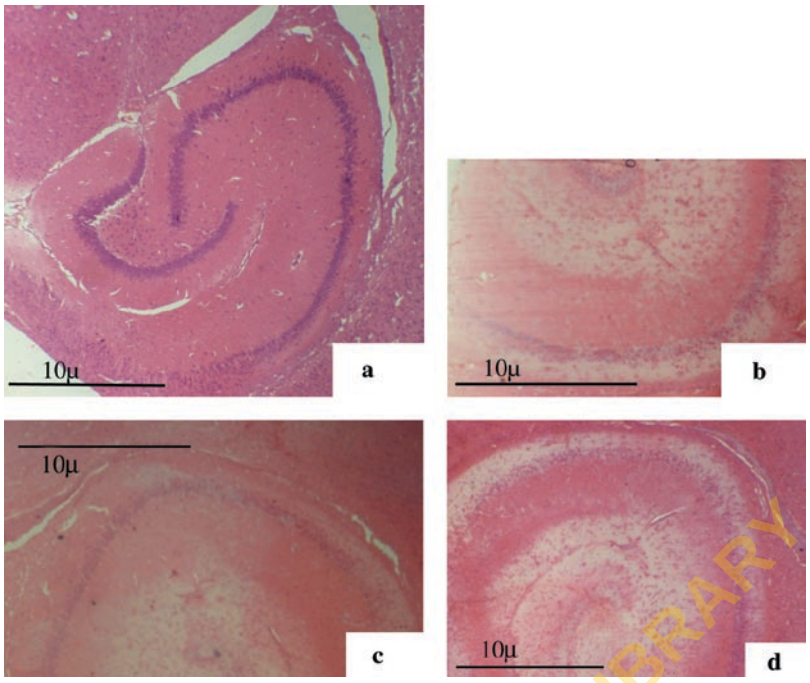

Figure 3. Hippocampus, using H&E stain. (**A**) Vanadium 1.5 mg/kg (×100). (**B**) *G. kola*+vanadium (×100). (**C**) Vanadium 1.25 mg/kg (×100). (**D**) *G. kola* (×100). No lesions were observed in all experimental groups. (**A**) CA1, CA2, CA3, and CA4. (**B–D**) CA1, CA2, and some parts of CA4.

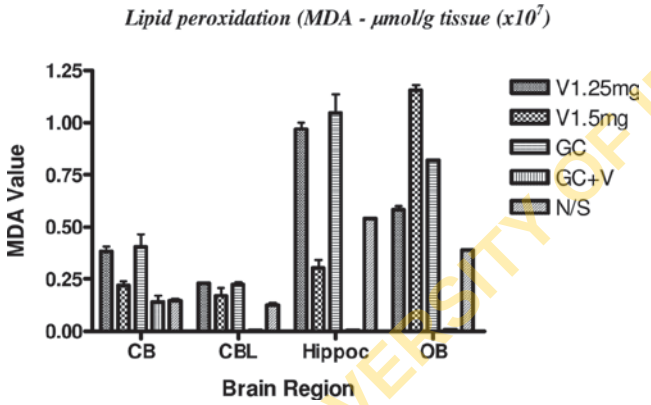

Figure 4. Mean values for MDA. Data represent means ± standard error of the mean of group averages ($n = 5$ per group), where CB is cerebrum, CBL is cerebellum, Hippoc is hippocampus, and OB is olfactory bulb.

kolaviron resulted in a decrease in GSH, when compared with the vanadium group, although a statistically significant difference was only observed in the hippocampus ($P < 0.01$). The normal saline-treated groups were not statistically different from groups treated with corn oil (Figure 7).

Effect of treatments on SOD

In the hippocampus, the administration of kolaviron with vanadium decreased the activity of SOD by 61.24%. Also, values obtained for the normal saline group was statistically significantly lower than those obtained for the vanadium and kolaviron+vanadium groups ($P < 0.001$ and $P < 0.05$, respectively). In the cerebrum and cerebellum, the result showed that on vanadium administration, there was a decrease ($P < 0.05$) in SOD activity of approximately 60.7% in the cerebrum and 33.21% in the cerebellum over values obtained from groups treated with normal saline (Figure 8). This response was diminished by 63.4% in the cerebrum and 42.3% in the cerebellum by kolaviron ($P < 0.05$) (Figure 8).

Effect of treatments on GST

The administration of vanadium caused neither a significant decrease nor increase in GST level in the brain regions examined relative to the control ($P > 0.05$) (Figure 9), whereas treatment with vanadium and kolaviron combination caused an increase in GST activity in all the brain regions studied ($P < 0.05$).

Effect of treatments on CAT

Vanadium treatment caused a statistically significant inhibition of 50% in the activity of CAT over the control value in the cerebrum (Figure 10). There was also a significant effect by vanadium on CAT activity in the cerebellum and, in addition, a 3-fold increase in CAT activity in the hippocampus ($P > 0.05$). Activity of CAT was increased both in the cerebrum and cerebellum with kolaviron treatment, when compared with groups treated with vanadium alone ($P < 0.05$).

Discussion

In experimental test 1, the groups exposed to vanadium alone showed more histopathologies than the group given vanadium with *G. kola*. The cerebellar and cerebral

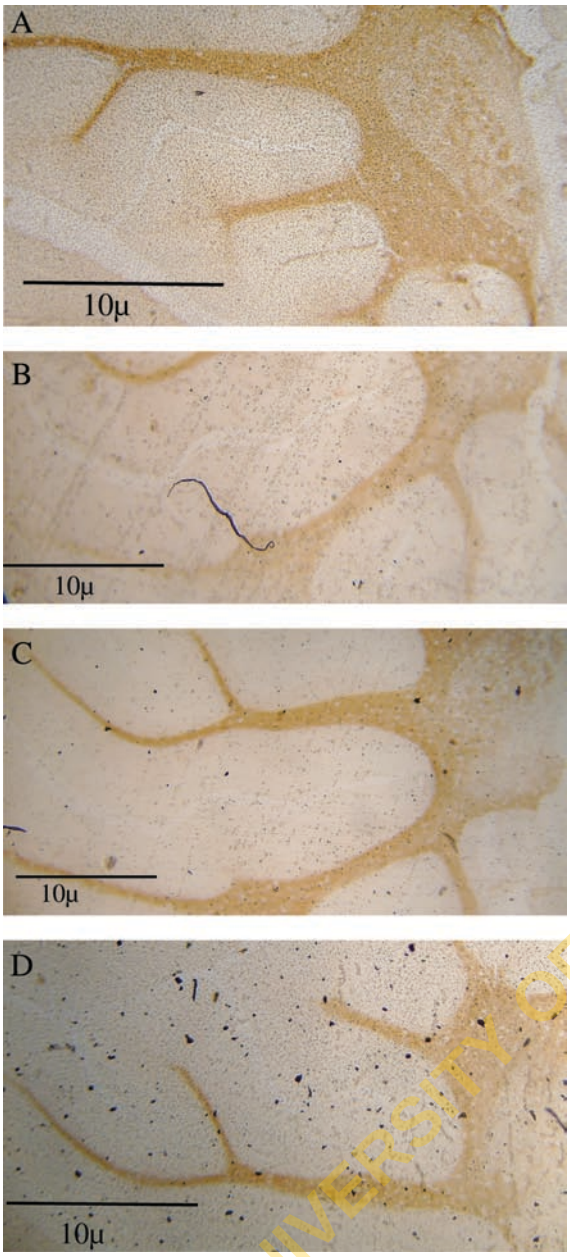

Figure 5. Light microphotographs ofanti-MBP immunostained sections showing myelin tracts in the cerebellum of control/normal saline (**A**), vanadium (1.5 mg/kg)-treated rats (**B**), kolaviron (100 mg/kg)+vanadium (1.5 mg/kg)-treated rats (**C**), and corn-oil–treated rats (**D**). Note the decreased immunolabeled fiber density in the vanadium-treated group (**B**) relative to the kolaviron+vanadium group (**C**) (×400).

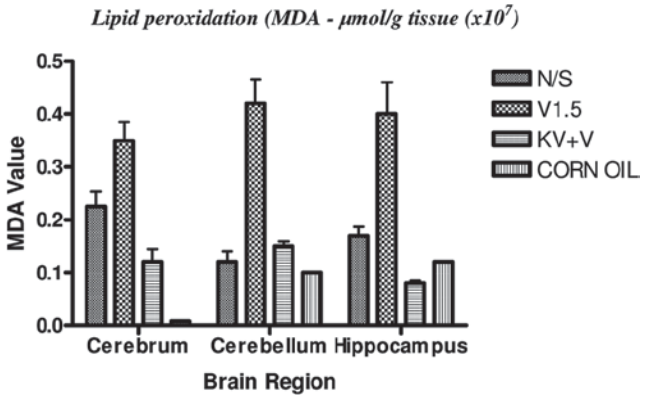

Figure 6. Mean values for MDA. Values represent mean ± standard error of the mean.

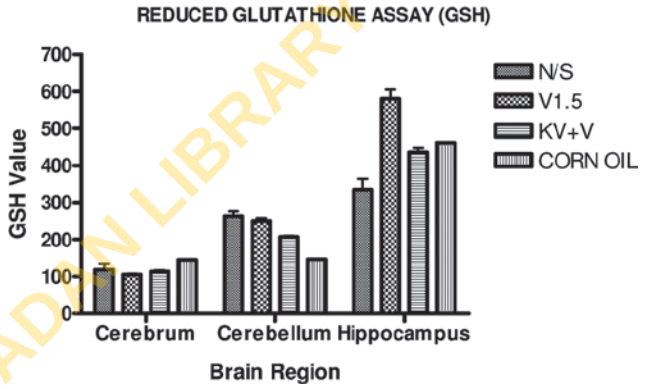

Figure 7. Mean values for GSH. Values represent mean ± standard error of the mean.

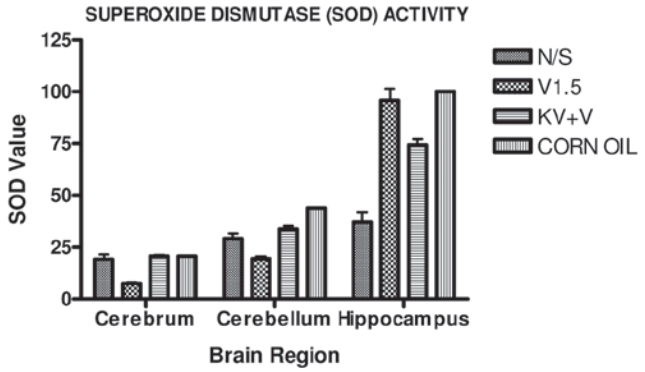

Figure 8. Mean values for SOD. Values represent mean standard ± error of the mean.

lesions observed in the groups taking 1.25 and 1.5 mg of vanadium alone imply that cognitive function, muscular movement, and coordination can be impaired. This supports the work of Barth et al. (2002), who reported reduced cognitive abilities in humans chronically exposed to vanadium. Of interest are the cerebral lesions found in group D (1.5 mg of vanadium+*G. kola*), in spite of the *G. kola* administered. This may imply that *G. kola* could not give complete protection against neuronal pathologies.

In the MDA assay, the values of the *G. kola*+vanadium group being lower than that of the control group showed that it was the least susceptible to LPO, despite the relatively high dose of vanadium. This may suggest that *G. kola* may be used as an effective antioxidant against LPO, especially in subjects exposed to vanadium toxicity. This administration of *G. kola*, however, may be in doses lower than 100 mg/kg, as administered in this study. Further research is required to establish the ideal dose of this potential antioxidant. The fact that the values for the *G. kola* alone group were not as low as could

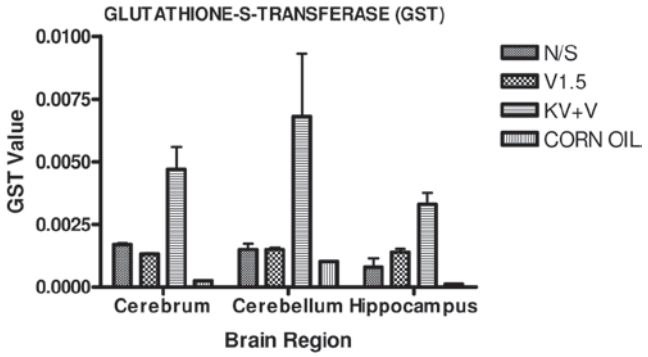

Figure 9. Mean values for GST. Values represent mean ± standard error of the mean. No statistically significant difference was observed across the groups ($P > 0.05$) for all the regions.

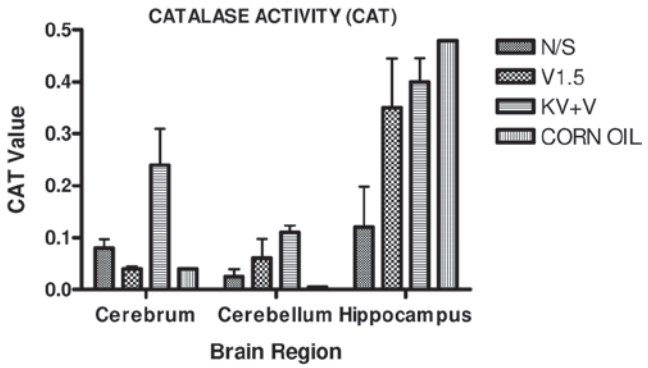

Figure 10. Mean values for CAT test. Values represent mean ± standard error of the mean.

be expected, and being higher than the GC+vanadium group and control could imply that G. kola is more effective as an antidote to a toxicant and might not yield as good a result when administered alone or when given in high doses, similar to α-tocopherol, where in vitro studies showed that administration of high doses resulted in pro-oxidative effects (Abudu et al., 2004). In experiment 1, the effect of vanadium on the brain regions using MDA was most pronounced in the hippocampus, except in the vanadium 1.5-mg group, where it was most pronounced in the olfactory bulb. This is interesting, because no histopathological lesions were observed in the hippocampus; this may suggest that higher oxidative stress by vanadium in the hippocampus does not confer direct histomorphological damage, even though molecular alterations may be evident. The increased LPO observed in the vanadium 1.5-mg group may also indicate that vanadium can affect the olfactory bulb, though the route of exposure is not through the nasal route. Vanadium exposure may thus lead to a decreased sense of smell, which could be particularly important to the mammalian ecosystem exposed to the burning of vanadium-containing fuel. The administration of *G. kola* and vanadium alone resulted in a significantly higher LPO, when compared with the administration of *G. kola* with vanadium, implying that the crude form of the *G. kola* seed has neurotoxic effects at the dose administered (100 mg/kg). This prompted the investigators to carry out another experiment using kolaviron to assess its possible neuroprotective effects from vanadium-induced neurotoxicity.

Considering the experimental test with kolaviron, the MBP stain showed a reduced density of myelin fibers in the group administered vanadium relative to the vanadium+kolaviron group. This is in consonance with the report of García et al. (2004), who observed a markedly reduced density of myelin tracts in rats administered vanadium at a dose rate of 1.5 mg/kg. Kolaviron appeared to have an ameliorating effect on vanadium, as was evidenced in the immunostaining. This was believed to be the result of the ability of kolaviron to chelate metal ions and inhibit LPO (Farombi and Nwaokeafor, 2005). The decrease in GSH, increase in TBARS, as well as decrease in activities of CAT, SOD, and GST observed in the cerebrum suggest a correlation between loss of antioxidant response and an increase in oxidative stress caused by exposure to vanadium. SOD, GST, and CAT constitute the main components of the antioxidant defense system, and modification in their expressions reflects a potential oxidative stress (Monleau et al., 2006). GSH plays an important role in the detoxification of reactive oxygen species ROS in brain. Haider and Kashyap (1988) have also shown that exposure to elemental vanadium inhibited GSH in various rat brain regions. In the cerebrum and cerebellum, there were decreases in SOD activities in these discrete brain regions. In the cerebrum, this suggests the accumulation of superoxide anion radical, which might be responsible for increased LPO observed in this brain region. Likewise, a greater production of H_2O_2 and an increase in TBARS and oxidized GSH (GSSG) contents could be expected (Latchoumycandane et al., 2002). Various studies (Cortizo et al., 2000; Younes and Strubelt, 1991) suggested that a strong correlation exists between vanadate-induced toxicity and the induction of LPO. In the present study, we observed the same result.

We observed a decrease in the activity of cerebral CAT in vanadium-intoxicated animals. These data suggest that vanadium treatment may result in the increased formation of oxygen-free radicals (Shi and Dalal, 1993), leading to oxidative stress. The superoxide radical could also inhibit the activity of CAT (Kono and Fridovich, 1982), and CAT-deficient cells may accumulate more H_2O_2 after oxidative stress (Vuillaume et al., 1992). The observed decline in the activity of GST in vanadium-treated rats in the cerebrum may partly be the result of the lack of its substrate, GSH, and also oxidative modification of its protein structure (Selvakumar et al., 2004). The increased level of GST in response to kolaviron treatment is indicative of enhanced detoxification of H_2O_2 and lipid hydroperoxides. Increasing the intracellular capacity for *de novo* synthesis of GSH could presumably effect GST, which uses GSH as a substrate. The protective role of kolaviron in this study may have been the result of its free-radical–scavenging capacity (Robak and

Gryglewsk, 1988) and the presence of the 3,4-diphenolic group in its structure (Middleton, 1984). Also, kolaviron prevents the reduction of SOD and CAT activities in the cerebrum and cerebellum of vanadium-exposed rats. In addition, that kolaviron inhibits hydroxy and superoxide anion radicals, which are known to play an important role in the process of LPO, has also been demonstrated (Farombi et al., 2002). In the hippocampus, administration of vanadium induced an increase in the activities of SOD, CAT, and GST, with a rise in GSH level and an increase in LPO. These increases may be an initial response that constitutes an adaptive effect to oxidative stress induced by vanadium exposure, which can result in toxicity. Occurrence of LPO was retarded in groups that received kolaviron with vanadium, in comparison with vanadium-treated groups. The combination of kolaviron and vanadium was observed to display different trends across the brain regions for the biochemical tests analyzed. The reasons for this would need further research. Also, the effect of kolaviron and vanadium on the olfactory bulb will need further investigation.

Conclusion

We suggest that the sensitivity in the cerebrum and hippocampus regions toward vanadium-induced oxidative stress could be higher than in the cerebellum, whereas administration of kolaviron prevents the oxidative stress induced by vanadium, especially in the cerebrum and hippocampus, and may be capable of sustaining global antioxidants in brain tissues.

Declaration of interest

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