Cerebellar Molecular and Cellular Characterization in Rat Models of Alzheimer's Disease: Neuroprotective Mechanisms of *Garcinia* Biflavonoid Complex

Olayemi Joseph Olajide^a Anita Temi Ugboanmi^a Bernard Ufuoma Enaibe^a
Kehinde Yomi Ogunrinola^a Susan Folashade Lewu^a
Nnaemeka Tobeckukwu Asogwa^b Tosan Akapa^b Aminu Imam^a
Abdulumumin Ibrahim^a Ismail Temitayo Gbadamosi^a
Emmanuel Olusola Yawson^a

^aDepartment of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, and

^bDepartment of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria

Keywords

Astrogliosis · Oxidative stress · Neurofilament degeneration · *Garcinia kola* · Glucose bioenergetics

Abstract

Background: Recent evidences suggest that cerebellar degeneration may be associated with the development of Alzheimer's disease (AD). However, previous reports were mainly observational, lacking substantial characterization of cellular and molecular cerebellar features during AD progression. **Purpose:** This study is aimed at characterizing the cerebellum in rat models of AD and assessing the corresponding neuroprotective mechanisms of *Garcinia* biflavonoid complex (GBc). **Methods:** Male Wistar rats were grouped and treated alone or in combination with PBS (ad libitum)/day, corn oil (CO; 2 mL/kgBw/day), GBc (200 mg/kgBw/day), sodium azide (NaN₃) (15 mg/kgBw/day) and aluminium chloride (AlCl₃) (100 mg/kgBw/day). Groups A and B received PBS and CO, respectively; C received GBc; D received NaN₃; E received AlCl₃; F received NaN₃ then GBc subsequently; G received AlCl₃ then GBc subsequently; H

received NaN₃ and GBc simultaneously while I received AlCl₃ and GBc simultaneously. Following treatments, cerebellar cortices were processed for histology, immunohistochemistry and colorimetric assays. **Results:** Our data revealed that cryptic granule neurons and pyknotic Purkinje cell bodies (characterized by short dendritic/axonal processes) correspond to indistinctly demarcated cerebellar layers in rats treated with AlCl₃ and NaN₃. These correlates, with observed hypertrophic astrogliosis, increased the neurofilament deposition, depleted the antioxidant system—shown by expressed superoxide dismutase and glutathione peroxidase, and cerebellar glucose bioenergetics dysfunction—exhibited in assayed lactate dehydrogenase and glucose-6-phosphate dehydrogenase. We further showed that GBc reverses cerebellar degeneration through modulation of neurochemical signaling pathways and stressor molecules that underlie AD pathogenesis. **Conclusion:** Cellular, molecular and metabolic neurodegeneration within the cerebellum is associated with AlCl₃ and NaN₃-induced AD while GBc significantly inhibits corresponding neurotoxicity and is more efficacious when pre-administered.

© 2017 S. Karger AG, Basel

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative condition which culminates in the loss of neurons and synapses in certain brain areas. Very little studies on AD brains paid thorough attention to the cerebellum, possibly due to the initial doctrine of being spared in the cellular and molecular manifestations of the disease. However, emerging facts suggest that degenerative changes within the cerebellum may be inclusive in both pathogenesis and symptomatic manifestation of AD [1–3]. Despite this interesting development, there is paucity of data to correlate the emerging hypotheses given that cellular and metabolic changes in AD cerebellar cortex are poorly characterized. Substantial characterization of the cerebellar cortex during the progression of AD may nonetheless expand our understanding of the overall molecular factors involved in the degenerative processes of the disease. Such understanding is also very necessary for screening potential protective agents against neuronal degeneration in AD and preventing resulting behavioral problems.

Although there exists competing hypotheses explaining the causes of AD, numerous studies have demonstrated elevated concentrations of aluminium (Al) within the brains of patients suffering from the disorder [4, 5]. It is established that Al crosses the blood–brain barrier through its specific high affinity for transferrin receptors [6]. Once in the brain, Al interferes with slow and fast axonal transports, alters calcium homeostasis, induces inflammatory responses, inhibits long-term potentiation, and causes synaptic structural abnormalities, leading to memory loss [7, 8]. Furthermore, Al induces protein misfolding and aggregation of highly phosphorylated cytoskeletal proteins, such as neurofilaments (NFs) and microtubule-associated proteins [9, 10]. Similarly, sodium azide (NaN_3) is a substance that is acutely neurotoxic [11]. Several studies conducted on its biological interaction with the central nervous system established it as a consistent and potent neurotoxin [12–14]. Selectively reduced complex IV activity is a pathogenic feature of post-mortem AD brains and the inhibition of this complex could be evoked by the chronic administration of NaN_3 in animals [14]. Partial inhibition of the mitochondrial respiratory chain produces free radicals, diminishes aerobic energy metabolism and causes excitotoxic damage that creates a deleterious spiral leading to neurodegeneration [15]. NaN_3 neurotoxicity has been explored in recapitulating behavioral and pathological features of AD [16, 17].

On the contrary, treatment and management of very complex diseases such as AD, which has no known cure yet, requires the support of certain plant derivatives with medicinal properties. In this connection, *Garcinia bflavonoid* complex (GBc) is a natural antioxidant and anti-inflammatory compound isolated from the seeds of *Garcinia kola* (bitter kola) [18, 19]. The plant is cultivated in West Africa and some parts of Asia where it is used as a treatment regimen for several infections and disease conditions owing to its ability as an antiviral, antibacterial and antifungal agent [20, 21]. In a previous study, we showed that GBc inhibits excess nitric oxide (NO) production, suppresses cytoplasmic expression of cell death regulatory proteins within neurons and inhibits neuronal cytoskeletal dysregulation [22]. However, the potentials of GBc in reversing cerebellar degeneration following Al and NaN_3 -induced AD are unknown. In line with this background, we aimed at investigating cellular, molecular and metabolic changes within the cerebellum in rat models of AD, while assessing the putative neuroprotective mechanisms of GBc on the corresponding pathobiology.

Methods

Male Wistar rats were bred at the animal holdings of the Faculty of Basic Medical Sciences, College of Medicine, University of Ilorin, Nigeria. The rats were kept in clean separate cages where they were served rat chow and water ad-libitum. Ethical approval was sought and received from the College of Medicine Ethical Committee, University of Ilorin, Nigeria. All protocols and treatment procedures complied strictly with the Institutional Animal Care and Use Committee guidelines. Four kilograms of *Garcinia kola* seeds were obtained from a single source at a market in Ilorin, Nigeria (April 2015). The seeds were then authenticated at the Department of Botany, Faculty of Physical Sciences, University of Ilorin, Nigeria. It was verified that the seeds were of uniform species, and the specimen voucher number in the herbarium is “UILH/001/1217”. Crystalline salts of aluminium chloride (AlCl_3) and NaN_3 were procured from Sigma-Aldrich (Germany). Phosphate-buffered saline (PBS; pH 8.0) was freshly prepared. Assay kits for superoxide dismutase (SOD), glutathione peroxidase (GPx), Glucose-6-phosphate dehydrogenase and lactate dehydrogenase (LDH) were acquired from Cell Signaling Technology, Danvers, USA. Antibodies (rat anti-glia fibrillary acidic protein [GFAP] and anti-NF) were also procured from Cell Signaling Technology, Massachusetts, USA. 3'3'-Diaminobenzidine tetrachloride (DAB; polymer) and methenamine silver intensification kits were procured from Sigma-Aldrich (Germany). Every other reagent used were sourced within our laboratories and verified before use.

Isolation and Purification of GBc from Garcinia Kola

Garcinia kola seeds were air dried at room temperature (28–30°C) for 3 weeks. Subsequently, GBc was isolated from the dry seeds of kola and characterized according to the method described

by Farombi et al. [50]. Briefly, the dried seeds were pulverized with an electric blender into fine powder. Powdered seeds were then extracted with light petroleum ether (boiling point [bp] 40–60°C) in a soxhlet extractor which was placed in an electric water bath for 24 h. After this, the defatted, dried marc was repacked and extracted with acetone (bp 56–60°C) in the water bath for 14 h. Subsequently, the resultant extract was concentrated and diluted twice its volume with distilled water and then extracted with ethyl acetate (7.250 mL). The concentrated ethyl acetate fraction gave a yellow solid which is referred to as GBc. The purity and identity of GBc was determined by subjecting it to thin layer chromatography using silica gel GF 254-coated plates and solvent mixture of methanol and chloroform in a ratio 1:4 v/v. The separation revealed the presence of 3 bands, which were viewed under UV light at a wavelength of 254 nm with RF values of 0.48, 0.71, and 0.76. The total yield of GBc was 7.3%, and it was kept at a temperature of 4°C before and after each use.

Preparation of Other Treatment Solutions

Salts of NaN_3 and AlCl_3 were dissolved in distilled water (20 mg/mL each) and adjusted to pH 7.4 with 0.1 M PBS. Both solutions were freshly prepared each morning of administration and kept at 4°C before use. GBc was dissolved (80 mg/mL) in corn oil (Carlini, ALDI Inc. Batavia), a solvent which totally dissolves it and allowed for oral administration. Administration of treatment solutions (corn oil, NaN_3 , AlCl_3 , and GBc) to rats across all groups was done orally using a modified oral cannula.

Animal Grouping and Treatments

For the purpose of this study, 54 male Wistar rats (average weight of 199 ± 6 g) were used. The rats were randomly assigned to 9 separate groups (A–I), each consisting of 6 rats. The groups were treated as follows: group A received PBS (ad libitum) daily for 28 days; group B received 2 mL of corn oil (CO) daily for 28 days (vehicle of GBc); group C received 200 mg/kgBw GBc only daily for 14 days; group D received 15 mg/kgBw NaN_3 (only) daily for 14 days; group E received 100 mg/kgBw AlCl_3 (only) daily for 14 days; group F received 15 mg/kgBw NaN_3 daily for 14 days followed by treatment with 200 mg/kgBw GBc for the subsequent 14 days; group G received 100 mg/kgBw AlCl_3 daily for 14 days followed by treatment with 200 mg/kgBw GBc for the subsequent 14 days; group H rats were treated simultaneously with 15 mg/kgBw NaN_3 and 200 mg/kgBw GBc daily for 14 days; and rats in group I received simultaneous administration of 100 mg/kgBw AlCl_3 and 200 mg/kgBw GBc daily for 14 days. Rats were weighed at 48-h intervals, beginning from day one of administration using Gallenkamp weighing balance. Treatment doses adopted in this study were previously reported by GBc [22]; AlCl_3 [23]; and NaN_3 [14].

Tissue Processing

Twelve hours after the last administration, rats for histology and immunohistochemistry were euthanized using 20 mg/kgBw of ketamine (intraperitoneal) and subjected to transcardial perfusion in which a flush of 50 mL of 0.1 M PBS (pH 7.4) was followed by 500 mL of 4% paraformaldehyde (PFA). The brain tissues were then excised, rinsed in 0.25 M sucrose 3 times for 5 min each and then post-fixed in 4% PFA for 24 h after which they were stored in 30% sucrose at 4°C until further processing. Rats processed for enzymatic studies were sacrificed by cervical dislocation (to eliminate ketamine interference with biochemical redox); brains were

then excised, rinsed in 0.25 M sucrose 3 times for 5 min each and placed in 30% sucrose at 4°C. Sagittal sections were made to expose the cerebellar cortices, following which they were processed routinely to obtain paraffin wax-embedded blocks for histology and antigen retrieval immunohistochemistry. Histological demonstration of cerebellar cytoarchitecture was carried out in paraffin wax-embedded sections, which were stained in haematoxylin and eosin (H&E) using the methods described by Kiernan et al. [24].

Immunohistochemical Studies

Serial sections (15 μm) were taken from paraffin blocks, with protein cross-linkages removed in the sections by applying 0.1% trypsin for 20 min at room temperature to activate the antigens. Hydrogen peroxide was used to block endogenous peroxidase, while 5% bovine serum albumin (BSA) was used to reduce non-specific protein reactions. Diluted primary antibody was added to each slide (500 μL) and incubated overnight at 4°C. Primary antibodies (anti-GFAP and anti-NF) dilution was done in blocking buffer (10% calf serum with 1% BSA and 0.1% Triton X-100 in 0.1 M PBS): both were diluted at 1:100. Following this, secondary biotinylated antibody were desalted and diluted in PBS (pH 8.0) prior to its application on tissue sections. Incubation with secondary antibody was done in the humidity chamber for 30 min at room temperature. Immunogenic reaction was developed using 3'3' DAB and intensified using methenamine silver kit (used according to manufacturer's instruction). The sections were counterstained in haematoxylin, and subsequently treated in 1% acid alcohol to reduce the counterstain intensity. Histology and IHC images were acquired using an Olympus binocular research microscope (Olympus, New Jersey, USA) connected to an Amscope Camera (5.0 MP).

Colorimetric Assay for Enzymatic Studies

Enzymatic assay for G6PDH, LDH, SOD, and GPx activities were carried out on carefully dissected cerebellar cortices of rats using spectrophotometric techniques. Equal weighing brain tissues (0.085 g) were homogenized in 0.25 M sucrose (Sigma) with an automated homogenizer at 4°C. The tissue homogenate was centrifuged for 10 min in a microfuge at 12,000 rpm to obtain the supernatant containing organelle fragments and synaptosomes. The supernatants were aspirated into plain labeled glass cuvette placed in ice. G6PDH, LDH, SOD, and GPx activities were assayed according to the manufacturer's instruction in the assay kit pack.

Data Analysis

All quantitative data were analyzed using the GraphPad Prism® software (version 6). G6PDH, LDH, SOD and GPx outcomes were plotted in ANOVA with Tukey's multiple comparisons test. Significance was set at $*p < 0.05$ (95% CI). The outcomes were represented in bar charts with error bars to show the mean and SEM, respectively.

Results

This study evaluated the cerebellum in response to NaN_3 and AlCl_3 -induced neurotoxicity in rats while assessing the corresponding palliative mechanisms of GBc. Basic histoarchitectural presentation of the cerebellum was studied in H&E, astrocytes and neuronal cytoskeletal

Fig. 1. Panoramic views of general histology in cerebellar cortices of rats, shown in hematoxylin and eosin staining (scale bars: 400 μ m). The molecular cell layer (M), Purkinje cell layer (P), granule cell layer (G) and the medullary layer of white matter (W) are demonstrated across study groups. Normal cerebellar histological features (blue arrows) are seen in rats treated with either PBS (**a**), CO (**b**), or GBc (**c**) which served as control. Treatment of rats with

NaN_3 (**d**) and AlCl_3 (**e**) resulted in fragmented granule cell layers and neuropil (red arrows) with comparatively increased cellular density. Also, rats given GBc after NaN_3 (**f**) and AlCl_3 (**g**) treatment have slight degenerative changes (red arrows), but shows normal-structured cerebellar layers. However, rats treated with GBc alongside NaN_3 (**h**) and AlCl_3 (**i**) appear normal, with distinctly demarcated cerebellar layers which is similar to those in control groups.

proteins were labeled immunohistochemically, and changes in cerebellar glucose bioenergetics and neural oxidative redox were examined. Data obtained in this experiment are discussed in line with findings that addressed similar objectives.

Cerebellar Neurodegeneration in AD with Protective Roles of GBc

Generally, cerebellar sections stained in H&E showed layer-specific micro-architectural disparities across the

experimental groups (Fig. 1). The well-arranged cerebellar layers and neuronal morphology in groups treated with PBS, CO, or GBc (Fig. 1a–c) suggests appropriate interconnectivity within the cerebellar cortex. In support of this, the transition areas between molecular layers and granule cell layers were interjected by large Purkinje neurons. Cellular densities within cerebellar layers of these groups were regular as well. Conversely, cerebellar sections from groups NaN_3 (D) and AlCl_3 (E) were characterized by fragmented granule cell layers with distorted

neuropil and associated increased cellular density. These findings correlate with the observed dendritic thickening, nerve cell loss, corkscrew-like dendrites and pyknotic nerve cells that are features associated with the pathogenesis of AD [14]. Furthermore, in both groups (D and E), perineural spaces can be seen surrounding clusters of granule cells. Such alterations in cellular morphology may lead to loss of signal processing, neuronal timing and synaptic efficacy in the cerebellum, and are often seen within cerebral cortices of demented patients [25]. Cholinergic and glutamatergic toxic activities of NaN_3 and AlCl_3 have been documented in neurons. It was reported that NaN_3 caused a selective reduction in choline acetyltransferase immunoreactivity and induced significant increase in vesicular acetylcholine transporter immunoreactive varicosities; while AlCl_3 disrupted the glutamate-NO-cyclic guanosine monophosphate signaling pathway, with both leading to apoptotic neuronal loss [4, 26]. These cytotoxic properties may account for the mechanisms through which NaN_3 and AlCl_3 initiated cerebellar degeneration in this study, and further suggests affection of the cerebellum during the progression of AD. Interestingly, treatment of rats with GBc following either NaN_3 (F) or AlCl_3 (G)-induced neurotoxicity partially restores the cerebellar morphology to normal. Few degenerative changes that are similar to those observed in groups D and E are present in groups F and G, although cerebellar layers are generally better structured and well delineated in the latter. Toxic insults including DNA damage, hypoxia, hypoglycemia, oxidative stress, viral infections and withdrawal of trophic support are known to damage neurons rapidly, making neural repair difficult. We suggest that the palliative/regenerative mechanisms shown by GBc may be through the inhibition of NaN_3 and AlCl_3 -activated neurotoxic cascades that trigger proteases, which destroy molecules that are required for neuronal survival and health. Cerebellar morphology in rats that received GBc simultaneously with either AlCl_3 (H) or NaN_3 (I) had normal cytoarchitecture with distinct structural layers similar to those in groups A, B, and C. GBc has been shown to inhibit lipid peroxidation and protect against hepatotoxicity and oxidative stress induced by several toxic agents, and also to exert protective effect against carcinogen-induced genotoxicity in human liver-derived HepG2 cells [27]. Therefore, prevention of peroxidation of neural cell membranes and possible antigenotoxic activities of GBc may explain its neuroprotective roles shown in this study.

Regarding cerebellar cellular disposition (Fig. 2), neither of PBS (A), CO (B), or GBc (C) treatment altered

cerebellar cellular morphology. The cellular arrangement in these 3 groups was characterized by large Purkinje cells with noticeable cell bodies and dendrites that project deeply into the molecular layers. Also, the granule cell layers in these groups consist of small granule neurons with regular clusters formed across the succinct architecture. These correspond with normal dendritic morphology that suggests appropriate and normal synapticity within the cerebellum, since in cooperation with the axons, the dendrites form the synapses, which serve as a channel for flow of signals [28, 29]. In support of this, the observed cellular relationships seen across cerebellar layers in these groups are such that Purkinje neurons have dendrites with numerous spiny branches that wound up into the molecular layers. Parallel fibers from the granule cells run through the many layers formed by spiny dendritic trees of the Purkinje neurons, where they normally would stimulate GABAergic responses with the neurons of the deep cerebellar and vestibular nuclei in the brainstem. However, the cerebellar granule cells of rats that were treated with NaN_3 (D) and AlCl_3 (E) were loosely arranged and have cryptic appearance. Purkinje cells in the groups have pyknotic cell bodies and damaged dendritic processes that are sparsely distributed around the indistinctly demarcated cerebellar layers. In addition to these degenerative changes, the neuropil appear bitty with irregularly shaped and sized neurons. Similarly, rats treated with GBc after exposure to NaN_3 (F) were characterized by Purkinje neurons with extramembranous spaces. It has been shown that outputs of the cerebellum stream towards many non-motor cerebral areas such as the prefrontal and posterior parietal cortices. Therefore, neuronal projections to different cortical areas originate from distinct output channels within the cerebellar nuclei [30]. Such cerebello-cortical connections influence the cerebellum with the structural architecture to affect the control of movement and cognition. Therefore, the loss of Purkinje neuronal projections as a result of NaN_3 and AlCl_3 toxicity may lead to motor and cognitive deficits seen in late stages of AD. These findings may validate the reports of pathogenic changes, including deposits of diffuse amyloid, ubiquitin-immunoreactive dystrophic neurites and increased microglia associated with AD cerebellum [31]. Furthermore, the cytoprotective properties of GBc were again demonstrated in the groups that were treated concurrently with GBc alongside either of NaN_3 (H) or AlCl_3 (I). Neuronal morphology of rats in both groups is largely characterized by normal neurons with perceptible axons and dendrites within the neuropil. Also, the transitional regions amid the cerebellar layers

Fig. 2. General neuronal morphology in the molecular (M), Purkinje (P) and granule cell (G) layers of the cerebellar cortex in rats, demonstrated in hematoxylin and eosin staining (scale bars: 25 μ m). Cerebellar neurons in control rats treated either of PBS (a), CO (b), or GBc (c) have Purkinje cells (blue arrows) with conspicuous cell bodies and dendrites, which are projecting deep into the molecular layers – with nuclei assuming the shape of a fan. Granule cell layers in the groups consist of small granule neurons (black arrows), which are in compact arrays. Rats treated with NaN_3 (d)

and AlCl_3 (e) however, shows loosely arranged and cryptic granule cells (red arrows), with degenerating Purkinje cells (dotted red circles) – which have pyknotic cell bodies and short dendritic processes. Perineural spaces also surround neurons within cortical layers of both groups f and g and the neuropil appear fragmented. Neuronal morphology and cerebellar layers in rats treated with GBc simultaneously with NaN_3 (h) and AlCl_3 (i) are generally characterized by neurons with normal cell bodies, appreciable axons and numerous dendritic processes within neuropil.

were better demarcated in groups G, H, and I (Fig. 2g–i), similar to those in groups A, B, and C. The neuroprotective properties shown by GBc against neuronal damage may be linked to its strong antioxidant and anti-inflammatory potentials. In support of this, the report by Farombi et al. [19] similarly showed that *Garcinia kola* extract significantly improves the antioxidant profile and abates the inflammatory responses evidenced by reduced proinflammatory cytokines and growth factors in diabetic rats.

GBc Prevents Astrogliosis and NF Pathology in Cerebellar Cortex

Astrocytes interact with neurons to provide structural, metabolic and trophic support, and are now emerging as key participants in many aspects of brain development, function and disease. Importantly, new evidence shows that astrocytes powerfully control the formation, maturation, function and elimination of synapses through various secreted and contact-mediated signals

Fig. 3. Immunohistochemical demonstration of astroglia using anti-rat-GFAP across the layers of the cerebellar cortex in rats (scale bars: 50 μ m). The molecular cell layer (M), Purkinje cell layer (P), and granule cell layer (G) are demonstrated across study groups. GFAP immunopositive cells (black arrows) in control rats treated with PBS (a), CO (b), and GBc (c) appear sparse around neurons and between layers, with regular processes, distribution and sizes within the neuropil. However, increased astrocytic densities and hypertrophic cells appear within the cerebellar layers in NaN_3 (d) and AlCl_3 (e) treated rats. Group D particularly presented with

reactive astroglia within granule cell layer while the molecular layer in group E appears to have the predominant hypertrophic astroglia. Hypertrophic astroglia deposited within the cerebellar sections of rats given GBc after NaN_3 (f) and AlCl_3 (g) treatment appear more than observed in control groups, but lesser than those expressed in photomicrographs D and E. Again, the expression of astroglia within the cerebellar cortex of rats treated with GBc simultaneously with NaN_3 (h) and AlCl_3 (i) bear close similarities to those in the A, B, and C groups. In both groups, astrocytic processes, cellular distribution and size are normal.

[32]. Studying astrocyte morphology remains crucial to the development of assay molecules to treat certain diseases [33]. Laying emphasis on all layers of the cerebellum (Fig. 3), there were no signs of degeneration in the astrocyte structure, processes and distribution in CO (A), PBS (B), and GBc (C) groups. In these groups, expressed astrocytes across the cerebellar layers were evenly dispersed across the neuropil, where they surround

neurons with normal sizes and processes. These findings suggest a balanced physiochemical microenvironment within cerebellar tissue in these groups, given that the progress and eventual manifestation of neurological diseases are dictated by the balance between destruction, neuroprotection and regeneration of astrocytes. Similarly, astrocytes are invariably involved in every kind of neuropathology which to a very large extent is shaped by

the astroglia performance [34]. On the contrary, increased deposition of hypertrophied astroglia was seen across cerebellar layers of rats treated with NaN_3 (D) and AlCl_3 (E). Labeled astrocytes in these groups were large and presented with neurodegeneration-related glia activation (astrogliosis). It has been shown that astrogliosis involves an increase in size and count of astrocytes and other glia cells in affected brain regions or inflamed tissue sites and can serve as an indicator of oxidative stress [35], which can be induced by superoxide anion [36]. GFAP immunohistochemistry in this study further showed that rats treated with NaN_3 have reactive astroglia peculiarly concentrated within the granule cell layer. The molecular layer of AlCl_3 -treated rats appears to have a predominant increase of astrocyte deposition. Similar to previous reports by Fischer et al. [37], it is suggested that the molecular basis of NaN_3 and AlCl_3 -induced astrogliosis was by the generation of ROS (particularly superoxide) via the inhibition of mitochondrial cytochromes oxidase which resulted in neuronal excitotoxicity, glia activation and neuronal death in cerebellar tissue. In a transgenic model of AD, hypertrophic astrocytes were seen around neuritic plaques, while astrocytes undergo atrophy throughout the brain parenchyma [38]. In this regard, hypertrophic astrogliosis within cerebellar cortices seen in experimental AD in this study may account for early changes in synaptic impairment processes that result in dementia. This provides proof that degeneration within the cerebellar cortex may contribute to the symptomatic manifestation of AD. GBc treatment after NaN_3 (F) and AlCl_3 (G)-induced neurotoxicity partially halted astrocyte degeneration, as only very few hypertrophied astrocytes are observable within the cerebellar layers of the groups. Another sign of GBc palliative effect in both groups is that the demonstrated astroglia morphology are better than those expressed in groups D and E. Surprisingly, astroglia expression and distribution in the cerebellar cortex of rats that received oral GBc simultaneously with NaN_3 (H) and AlCl_3 (H) were normal and similar to those expressed in the A, B and C groups. These findings suggest that one of the primary mechanisms underlying GBc neuroprotective properties is the inhibition of astrogliosis in the cerebellar cortex. The study by Farombi and Owoye [39] showed that GBc inhibited H_2O_2 more effectively than the standard antioxidants BHA and β -carotene and are equivalent in power to α -tocopherol; and that GBc significantly scavenged superoxide generated by phenazine methosulfate and as well scavenged hydroxyl radicals (which destroys cells by initiating DNA damage). We suggest from our findings

that the neuroprotective mechanisms of GBc may be through abolishing molecular inflammatory processes, via induction of detoxifying enzymes which then halted astrogliosis.

NFs aberration in neurodegenerative conditions is a hallmark of neuronal dysfunction, particularly marking axonal degeneration [40]. Abnormal accumulation of NF is primarily observed in many human neurodegenerative disorders including AD [41, 42]. While NF abnormalities in neurodegenerative disorders could simply reflect a pathological consequence of neuronal dysfunction, recent studies using transgenic mouse models suggested that NF disorganization itself can produce deleterious effects and therefore could contribute to the neurodegeneration process [43]. Focusing on cerebellar layers in this study (Fig. 4), immunohistochemical investigation of light chain NFs (the predominant cytoskeletal proteins in neurons and most affected in neurodegeneration) was carried out across experimental groups. Cerebellar sections of rats treated with PBS (A), CO (B), or GBc (C) showed quite low immunopositivity to NFL antibody. A few NFL were expressed within axons of neurons across cerebellar layers of these groups. Correspondingly, the layers of the cerebellar cortex in the aforementioned groups were relatively well arranged and succinctly delineated. On the contrary, cerebellar sections of rats treated with either NaN_3 (D) or AlCl_3 (E) presented with increased expression and assembly of NFL within the molecular, granular and Purkinje cell layers. Increased deposition of NF proteins due to phosphorylation has been shown as a central pathogenic process in AD and amyotrophic lateral sclerosis [40]. In the event of oxidative stress, NFs are phosphorylated, leading to the formation of protein aggregates. In AD brains, for example, there is an increased N-malondialdehyde formation which destroys NFL proteins [44]. Therefore, cerebellar degeneration associated with NaN_3 and AlCl_3 -induced AD involves the dysregulation of NFL and possibly other cytoskeletal proteins in the cerebellum of rats, because cytoskeletal components are interconnected through cross-linking proteins, and damage to one component affects the entire cytoskeletal network. Subsequent analysis showed that GBc treatment after NaN_3 (F) or AlCl_3 (G)-induced cerebellar toxicity did not totally halt hyperphosphorylation of NFL within cerebellar layers for the treatment duration. This is consistent with other notable neurodegenerative changes in the cerebellum of rats in corresponding groups, indicating a limited restorative role for GBc in neurotoxic processes. However, GBc exhibited pro-

Fig. 4. Immunohistochemical demonstration of light chain NFLs using anti-rat-NFL across the layers of the cerebellar cortex in rats (scale bars: 50 μ m). The molecular cell layer (M), Purkinje cell layer (P), and granule cell layer (G) are demonstrated across study groups. Cerebellar sections in control rats that received PBS (**a**), CO (**b**), and GBc (**c**) show low immunopositivity to NFL anti-body. Few NFL (red arrows) can be seen within the axons of neurons in these control groups. NaN_3 (**d**) and AlCl_3 (**e**) administration to rats, however, increased the NFL deposition and assembly within neurons, across

layers of the cerebellar cortex. On the contrary, NFL immunopositive cells within cerebellar cortices of rats given GBc after NaN_3 (**f**) and AlCl_3 (**g**) treatment appeared denser and aggregated within neurons compared with groups A, B, and C. Interestingly, both groups also showed normal general morphological presentation and distinct layering. NFL deposition within cerebellar cortex of rats treated with GBc simultaneously with NaN_3 (**h**) and AlCl_3 (**i**) is considerably normal, and is characterized by sparsely distributed sub-units which is very similar to those seen in the control rats.

nounced neuroprotective properties against NFL mutation within cerebellar neurons when administered to rats simultaneously with NaN_3 or AlCl_3 (H and I, respectively). The expressed NFL in both groups appeared normal and evenly distributed within neurons, similar to those expressed in groups A, B, and C. The general neuronal and neuroglia morphology also appeared normal in the groups. These findings support earlier observations of compact axonal and dendritic morphology in

the corresponding groups. It further reveals that the mechanisms of GBc neuroprotection against cerebellar damage in rats, may involve the inhibition of cytoskeletal dysregulation. We suggest that GBc prevented cerebellar NF degeneration by protecting the numerous phosphorylative sites of NFL from oxidative phosphorylation. This deduction is supported by reports of Kim et al. [45], which noted that oxidative phosphorylation of NFL mediated by H_2O_2 and copper (Cu^{2+}) resulted in

Fig. 5. Expression of antioxidant enzyme SOD as a measure of oxidative redox states in the cerebellar cortices of Wistar rats across treatment groups. Rats that received PBS (A), CO (B), and GBc (C) expressed a significantly higher cerebellar SOD level compared with the NaN₃ (D) and AlCl₃ (E) treated groups. Furthermore, rats that received GBc after treatment with either NaN₃ (F) or AlCl₃ (G) are characterized by low levels of SOD expression in cerebellar homogenates compared with groups A, B, and C; but these differences are not statistically significant. Antioxidant profiles in groups of rats that received GBc treatment concurrently with either NaN₃ (H) or AlCl₃ (I) was upregulated significantly, in comparison with groups D and E (** $p < 0.01$). Bars are mean $\pm$ SEM. * $p < 0.05$.

disrupted morphology of proteins in vitro, and this was significantly inhibited by radical scavenging and antioxidant molecules.

Impairment of Cerebellar Oxidative Redox and Free Radical Scavenging Properties of GBc

Impairments of neuronal oxidative redox (particularly those leading to the formation of reactive species) is central to many forms of NDDs. Disorders such as AD, Parkinson's disease, stroke and amyotrophic lateral sclerosis have been correlated with increased production of ROS which overwhelms endogenous antioxidant defenses, leading to subsequent oxidative damage and cell death [46, 47]. Emerging evidences suggests the formation of superoxide anion and expression/activity of its endogenous scavenger-SOD, as a common player in the pathogenic cascades of many NDDs like AD. To further evaluate the molecular mechanisms underlying cerebellar dam-

age in AD brain and GBc protective mechanisms, activities of SOD were assayed within cerebellar lysates in this study. Neural levels of GPx, which reduces lipid hydroperoxides to their corresponding alcohols (thereby preventing oxidative damage to cell membrane through lipid peroxidation) and reduces free hydrogen peroxide to water (further preventing oxidative toxicity) were assessed in this study. When compared with the control (PBS [A], CO [B], and GBc [C]) in Figure 5, rats treated with NaN₃ (D) and AlCl₃ (E) showed significantly depleted SOD levels in the cerebellar cortex. In normal physiochemical states, SOD catalyzes the neutralization of superoxide ($O_2^{\cdot-}$), a biologically toxic molecule that contributes to the pathogenesis of neurons via oxidative toxicity. Therefore, observed cerebellar degeneration in rats treated with NaN₃ and AlCl₃ may be due to increased neural $O_2^{\cdot-}$ shown by depleted SOD profiles. It has been documented that increased neuronal $O_2^{\cdot-}$ levels may result from impairment of complexes I and IV of the mitochondrial respiratory chain [48]. Oxidative impairment within the cerebellum may thus result from mitochondrial impairment as both NaN₃ and AlCl₃ selectively damage neuronal mitochondrial complexes [14, 31, 49]. Although not statistically significant ($p < 0.05$) compared to groups D and E, treatment of rats with GBc following NaN₃ (F) and AlCl₃ (G)-induced oxidative stress, improved the antioxidant status of the cerebellum seen by the expressed SOD profiles. Impressively, the antioxidant and free radical scavenging activities of the GBc was pronounced in groups treated with GBc alongside NaN₃ (H) and AlCl₃ (I). Both groups expressed significant increase ($p < 0.05$ and $p < 0.01$) in SOD within the cerebellar cortex when compared to those expressed in groups D and E, respectively. In support of GBc antioxidant potentials, reports by Farombi et al. [50] showed that the molecular mechanisms of the hepatoprotective effect of the *Garcinia kola* isolate were by abolishing the expression of COX-2 and iNOS proteins in dimethyl nitrosamine-treated rat liver, and they suggested that this compound may be important not only in alleviating liver inflammation, but also in the prevention of liver cancer. Similarly, abolishing the overexpressed SOD in the cerebellum of rats in this study is a hallmark for GBc neuroprotective mechanisms. Expressed levels of GPx within cerebellar homogenates (Fig. 6) from rats in all groups did not show significant differences. This result is quite surprising as GPx is one of the most effective antioxidant enzymes in neurons and its alteration has been associated with many neurodegenerative diseases [51], particularly those leading to neuronal cell death such as those observed in this study. Generally, however, insig-

Fig. 6. Expression of antioxidant enzyme GPx as a measure of oxidative redox states in the cerebellar cortices of Wistar rats across treatment groups. There are no significant differences between all study groups ($p < 0.05$). However, cerebellar sections of control rats treated with PBS (A), CO (B), or GBc (C) show fairly similar levels of GPx expression, which is fairly higher than in other groups. Similarly, expressed GPx in rats treated with NaN₃ (D), GBc after NaN₃ (F) and GBc with NaN₃ (H) shows notable but insignificant increases. On the contrary, almost all groups that received AlCl₃ solely or in combination with GBc (E, G, I) show reduced GPx expression in cerebellar homogenate. Bars are mean $\pm$ SEM.

nificant ($p < 0.05$) reduction in GPx activity is seen in all groups treated with NaN₃ and AlCl₃ in our study. Depleted neural GPx levels have been shown to result in increased tissue H₂O₂ content, hence severe cellular damage ensue [52]. Thus, we hold that cerebellar damage associated with experimental AD involves diminished activities of antioxidant enzymes; and that GBc, via its antioxidant properties, protects cerebellar degeneration.

Neuroprotective Effects of GBc against Glucose Bioenergetics Disruption Associated with Cerebellar AD Changes

Glucose metabolism provides the fuel for physiological brain function through the generation of ATP, the foundation for neuronal and non-neuronal cellular maintenance, as well as generation of neurotransmitters [53]. This is connected to cell death pathways by glucose-metabolizing enzymes with disruption of pathways of glucose delivery and metabolism leading to debilitating brain diseases [54]. We investigated the cerebellar levels of key enzymes of glucose metabolism to elucidate its involvement in cerebellar toxicity induced by NaN₃ and AlCl₃

Fig. 7. Expression of metabolic marker G-6-PDH as a measure of energy production in the pentose phosphate pathway of cerebellar cortices in Wistar rats across treatment groups. Compared with control rats treated with PBS (A), CO (B), and GBc (C), rats administered either NaN₃ (D) or AlCl₃ (E) show significant reduction in G-6-PDH expression within cerebellar homogenate; while GBc treatment normalized the depleted cerebellar G-6-PDH enzyme in rats that received it 14 days after NaN₃ (F) or AlCl₃ (G). Also, rats treated with GBc after AlCl₃ (H) treatment showed significant increase in G-6-PDH levels compared with groups D and E. Surprisingly, GBc treatment alongside AlCl₃ (I) administration did not restore neural lysate levels of G-6-PDH to normal (* $p < 0.05$; ** $p < 0.01$). Bars are mean $\pm$ SEM.

and the corresponding neuroprotective mechanisms of GBc protective roles. Determination of G6PDH activities in the cerebellar cortices was carried out as a measure of energy production in the pentose phosphate pathway, a metabolic pathway that supplies reduced energy to cells by maintaining the level of co-enzyme nicotinamide adenine dinucleotide phosphate. From Figure 7, neural levels of G6PDH was impaired in response to NaN₃ (D) and AlCl₃ (E) administration, as there was a significant reduction in its activities when compared to groups treated with PBS (A), CO (B), and GBc (C). In one study, reduction in neural levels of G6PDH reportedly led to decreased energy and ribose production through the pentose phosphate pathway, as seen in several neurological disorders [55]. It has also been shown that in advanced stages of AD, a reduction of cerebellar glucose metabolism is present [56], suggesting strongly that AD-related neurodegenerative changes may involve the cerebellum. One possible neuroprotective mechanism of GBc is through the prevention of dysfunctional cerebellar bioenergetics. This is shown by the normal G6PDH levels expressed in rats that received GBc after or simultaneously with NaN₃

Fig. 8. Expression of metabolic marker LDH for stress-related glucose metabolism in the cerebellar cortices of Wistar rats across treatment groups. Rats in the control groups which were treated with PBS (A), CO (B), or GBc (C) have a significantly lower cerebellar LDH levels, compared to both NaN₃ (D) and AlCl₃ (E) treated rats. Also, rats that received GBc after NaN₃ treatment (F) show overexpression of LDH enzyme similar to levels observed in groups D and E (but not significantly different from control), while rats that received GBc and NaN₃ (H) concomitantly expressed normal LDH levels. Also, the expression of LDH in cerebellar homogenate from rats treated with GBc before or after AlCl₃-induced metabolic stress (G, I), are similar to levels of the enzyme in groups A, B, and C (* $p < 0.05$; ** $p < 0.01$). Bars are mean $\pm$ SEM.

(F and H, respectively) treatments. Similarly, rats treated with GBc after AlCl₃ (G) administration showed significant increase in G6PDH levels compared with groups D and E. The comparatively higher G6PDH level in the cerebellum of rats in these groups is necessary to meet the energy requirement/demand, in a bid to synthesize proteins involved in endogenous modulating pathways to salvage neural cells from oxidative damage. It is, therefore, possible that GBc confers protective/regenerative functions on neural cells by upregulating the energy production, which is necessary for both complimentary trophic and metabolic support. Intriguingly, GBc treatment alongside AlCl₃ (I) administration did not significantly restore neural lysate levels of G6PDH to normal levels. The reason for this is unclear, especially given that it does not follow the trends of other findings.

Colorimetric assay for LDH levels in cerebellar homogenate further reinforces earlier findings (Fig. 8). Expressed LDH in cerebellar lysates of rats treated with PBS (A), CO (B), and GBc (C) are unaltered and similar. Increased expression of LDH was seen in both NaN₃ (D) and AlCl₃ (E) treated rats, indicating a shift to oxidative

stress-related glucose metabolism. LDH is released during tissue damage (especially those related to energy metabolic disorders) and is a marker of common injuries and diseases [57]. Ross et al. [57] suggested that LDH gene expression ratio is most altered in the brain (more than other metabolically active organs) in response to impaired oxidative phosphorylation. Similarly, LDH catalyzes the conversion of pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply [58]. AlCl₃ and NaN₃ have both been shown to cause chemical hypoxia and low energy production in the mitochondria with increased release of oxygen radicals [26, 59]. This may account for the significant upregulation of cerebellar LDH levels in groups D and E. The relative astrogliosis seen in both groups may contribute to neuronal bioenergetics deficit, since astrocytes store brain energy currency in the form of glycogen that can be mobilized to produce lactate for neuronal oxidative phosphorylation in response to glutamatergic neurotransmission. This synaptic-driven astrocytic coupling responds to glutamate, leading to the release of energy substrates back to neurons to match demand with supply [60]. Astrogliosis can therefore result in loss of such mechanisms, leading to unguided LDH synthesis. In line with results shown earlier, GBc administration in groups F and G did not restore LDH to levels in the control. This may partly explain its partial restorative role in inhibiting cerebellar degeneration in corresponding groups. Our findings further revealed that the administration of GBc alongside either NaN₃ (H) or AlCl₃ (I) treatment prevented LDH upsurge, such that cerebellar lysates in these groups have similar LDH expression as the control. Similar to these findings, study by Adedara and Farombi [61] showed the mediating properties of GBc on LDH activities disrupted by ethylene glycol monoethyl ether in the testes of rats. Being a hub for neurometabolic coupling, the role of GBc in preventing hypertrophic astrogliosis may also be a relevant mechanism necessary in the prevention of cerebellar bioenergetics dysfunction. This is a critical aspect of GBc's beneficial potentials, especially in considering the development of a broad range therapeutics for neurodegenerative processes characterized by chronic generalized metabolic impairment.

Discussion

Taken together, our study showed that NaN₃ and AlCl₃ interference with normal oxidative redox within the mitochondrial machinery of cerebellar cells resulted

in an increased production of free radicals, which overwhelmed the intrinsic antioxidant system. The highly reactive and unstable excess cytotoxic molecules then oxidized cellular components including DNA and lipids, thereby causing damage to the neuronal membrane. These degenerative changes compromised neuronal glucose bioenergetics and led to the aggregation of cytoskeletal proteins that subsequently initiated hypertrophy of neuroglia and neurons. We conclude that metabolic, molecular and structural degradation of the cerebellum occurs during AD progression and may be primarily involved in the symptomatic manifestation of the disease and related disorders. In addition, GBc exhibits plausible neuroprotective and inhibitory potentials in response to NaN_3 and AlCl_3 -induced cerebellar degeneration and should be explored further towards the development of assay molecules for neurodegeneration. It is important to note that GBc is more efficacious when given before than after cerebellar toxicity in rats.

Acknowledgements

We acknowledge Dr. Babatunde Oso of Central Research Laboratory, University Road Ilorin and Oyegbola Christiana (Department of Anatomy, University Ilorin) for their support during experimental procedures.

Author Contributions

O.J.O. and A.T.U. initiated the research. B.U.E. and S.F.L. participated in the design and implementation of the experiments. O.J.O., N.T.A., K.Y.O., A.I., T.A., I.T.G., and E.O.Y. participated in the implementation of the experiment, analysis of results and manuscript writing. O.J.O., B.U.E., and I.A. proof read the article for final corrections.

Disclosure Statement

The authors declare no conflict of interest. The manuscript is complied with International Committee of Medical Journal Editor's guidelines.

References

- Andersen K, Andersen BB, Pakkenberg B: Stereological quantification of the cerebellum in patients with Alzheimer's disease. *Neurobiol Aging* 2012;33:197.e11–e20.
- Thomann PA, Schäfer C, Seidl U, Santos VD, Essig M, Schröder J: The cerebellum in mild cognitive impairment and Alzheimer's disease – a structural MRI study. *J Psychiatr Res* 2008;42:1198–1202.
- Wu T, Hallett M: The cerebellum in Parkinson's disease. *Brain* 2013;136(pt 3):696–709.
- Mathiyazahan DB, Justin Thenmozhi A, Manivasagam T: Protective effect of black tea extract against aluminum chloride-induced Alzheimer's disease in rats: a behavioural, biochemical and molecular approach. *J Funct Foods* 2015;16:423–435.
- Exley C, Vickers T: Elevated brain aluminium and early onset Alzheimer's disease in an individual occupationally exposed to aluminium: a case report. *J Med Case Rep* 2014;8:41.
- Roskams AJ, Connor JR: Aluminum access to the brain: a role for transferrin and its receptor. *Proc Natl Acad Sci U S A* 1990;87:9024–9027.
- Campbell A: The potential role of aluminium in Alzheimer's disease. *Nephrol Dial Transplant* 2002;17(suppl 2):17–20.
- Rodella LF, Ricci F, Borsani E, Stacchiotti A, Foglio E, Favero G: Aluminium exposure induces Alzheimer's disease-like histopathological alterations in mouse brain. *Histol Histopathol* 2008;23:433–439.
- Mcdonald B, Esiri MM, Morris J: Aluminium and Alzheimer's disease. *Age Ageing* 1993;22:392–393.
- Crapper DR, Krishnan SS, Quittkat S: Aluminium, neurofibrillary degeneration and Alzheimer's disease. *Brain* 1976;99:67–80.
- Smith RP, Louis CA, Kruszyna R, Kruszyna H: Acute neurotoxicity of sodium azide and nitric oxide. *Fundam Appl Toxicol* 1991;17:120–127.
- Marino S, Marani L, Nazzaro C, Beani L, Siniscalchi A: Mechanisms of sodium azide-induced changes in intracellular calcium concentration in rat primary cortical neurons. *Neurotoxicology* 2007;28:622–629.
- Selvatici R, Previati M, Marino S, Marani L, Falzarano S, Lanzoni I, Siniscalchi A: Sodium azide induced neuronal damage in vitro: evidence for non-apoptotic cell death. *Neurochem Res* 2009;34:909–916.
- Szabados T, Dul C, Majtényi K, Hargitai J, Péntzes Z, Urbanics R: A chronic Alzheimer's model evoked by mitochondrial poison sodium azide for pharmacological investigations. *Behav Brain Res* 2004;154:31–40.
- Zhang L, Li L, Ban L, An W, Liu S, Li X, et al: [Effect of sodium azide on mitochondrial membrane potential in SH-SY5Y human neuroblastoma cells]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 2000;22:436–439.
- Albrekkan FM, Kelly-Worden M: Mitochondrial dysfunction and Alzheimer's disease. *Open J Endocr Metab Dis* 2013;3:14–19.
- Olajide OJ, Enaibe BU, Bankole OO, Akinola OB, Laoye BJ, Ogundele OM: Kolaviron was protective against sodium azide (NaN_3) induced oxidative stress in the prefrontal cortex. *Metab Brain Dis* 2016;31:25–35.
- Olaleye SB, Farombi EO: Attenuation of indomethacin- and HCl/ethanol-induced oxidative gastric mucosa damage in rats by kolaviron, a natural biflavonoid of *Garcinia kola* seed. *Phytother Res* 2006;20:14–20.
- Farombi EO, Adedara IA, Ajayi BO, Ayepola OR, Egbeme EE: Kolaviron, a natural antioxidant and anti-inflammatory phytochemical prevents dextran sulphate sodium-induced colitis in rats. *Basic Clin Pharmacol Toxicol* 2013;113:49–55.
- Farombi EO, Hansen M, Ravn-Haren G, Møller P, Dragsted LO: Commonly consumed and naturally occurring dietary substances affect biomarkers of oxidative stress and DNA damage in healthy rats. *Food Chem Toxicol* 2004;42:1315–1322.
- Iwu MM: Antihepatotoxic constituents of *Garcinia kola* seeds. *Experientia* 1985;41:699–700.
- Olajide OJ, Akinola BO, Ajao SM, Enaibe BU: Sodium azide-induced degenerative changes in the dorsolateral prefrontal cortex of rats?: attenuating mechanisms of kolaviron. *Eur J Anat* 2016;20:47–64.
- Kumar A, Prakash A, Dogra S: Neuroprotective effect of carvedilol against aluminium induced toxicity: possible behavioral and biochemical alterations in rats. *Pharmacol Rep* 2011;63:915–923.

- 24 Kiernan J, Lillie R, Pizzolato P, Donaldson P, Llewellyn B, Puchtler H: Haematoxylin eosin (H&E) staining. *Protocols Online* 2010;1.
- 25 Debanne D, Campanac E, Bialowas A, Carlier E, Alcaraz G: Axon physiology. *Physiol Rev* 2011;91:555–602.
- 26 Fujimura T, Kobayashi H, Suzuki T, Sato I, Hara S: [Neurotoxicity in sodium azide poisoning]. *Chudoku Kenkyu* 2002;15:281–288.
- 27 Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO: Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron – a Garcinia kola seed extract. *Food Chem Toxicol* 2000;38:535–541.
- 28 Coward LA: Neuron Physiology. Towards a theoretical neuroscience: from cell chemistry to cognition. *Theo Neurosci* 2013;33:53–95.
- 29 Major G, Larkum ME, Schiller J: Active properties of neocortical pyramidal neuron dendrites. *Annu Rev Neurosci* 2013;36:1–24.
- 30 Strick PL, Dum RP, Fiez JA: Cerebellum and nonmotor function. *Annu Rev Neurosci* 2009;32:413–434.
- 31 Larner AJ: The cerebellum in Alzheimer's disease. *Dement Geriatr Cogn Disord* 1997;8:203–209.
- 32 Clarke LE, Barres BA: Emerging roles of astrocytes in neural circuit development. *Nat Rev Neurosci* 2013;14:311–321.
- 33 Sofroniew MV: Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci* 2009;32:638–647.
- 34 Panicker KS, Norenberg MD: Astrocytes in cerebral ischemic injury: morphological and general considerations. *Glia* 2005;50:287–298.
- 35 GBD 2013 Mortality and Causes of Death Collaborators: Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015;385:117–171.
- 36 Quincozes-Santos A, Bobermin LD, Latini A, Wajner M, Souza DO, Gonçalves CA, et al: Resveratrol protects C6 astrocyte cell line against hydrogen peroxide-induced oxidative stress through heme oxygenase 1. *PLoS One* 2013;8:e64372.
- 37 Fischer AJ, Zelinka C, Gallina D, Scott MA, Todd L: Reactive microglia and macrophage facilitate the formation of Müller glia-derived retinal progenitors. *Glia* 2014;62:1608–1628.
- 38 Rodríguez JJ, Olabarria M, Chvatal A, Verkhratsky A: Astroglia in dementia and Alzheimer's disease. *Cell Death Differ* 2009;16:378–385.
- 39 Farombi EO, Owuoye O: Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. *Int J Environ Res Public Health* 2011;8:2533–2555.
- 40 Liu Q, Xie F, Alvarado-Diaz A, Smith MA, Moreira PI, Zhu X, Perry G: Neurofilamentopathy in neurodegenerative diseases. *Open Neurol J* 2011;5:58–62.
- 41 Al-Chalabi A, Miller CC: Neurofilaments and neurological disease. *Bioessays* 2003;4:346–355.
- 42 Liu Q, Xie F, Siedlak SL, Nunomura A, Honda K, Moreira PI, et al: Neurofilament proteins in neurodegenerative diseases. *Cell Mol Life Sci* 2004;61:3057–3075.
- 43 Perrot R, Eyer J: Neuronal intermediate filaments and neurodegenerative disorders. *Brain Res Bull* 2009;80:282–295.
- 44 Gardiner J, Overall R, Marc J: The nervous system cytoskeleton under oxidative stress. *Diseases* 2013;1:36–50.
- 45 Kim NH, Jeong MS, Choi SY, Hoon Kang J: Oxidative modification of neurofilament-L by the Cu,Zn-superoxide dismutase and hydrogen peroxide system. *Biochimie* 2004;86:553–559.
- 46 Kanthasamy A, Jin H, Anantharam V, Sondarva G, Rangasamy V, Rana A, et al: Emerging neurotoxic mechanisms in environmental factors-induced neurodegeneration. *Neurotoxicology* 2012;33:833–837.
- 47 Martin LJ: Mitochondrial and cell death mechanisms in neurodegenerative diseases. *Pharmaceuticals (Basel)* 2010;3:839–915.
- 48 Sayre LM, Perry G, Smith MA: Oxidative stress and neurotoxicity. *Chem Res Toxicol* 2008;21:172–188.
- 49 Cao LL, Du GH, Wang MW: [Effect of salidroside on mitochondria injury induced by sodium azide]. *Yao Xue Xue Bao* 2005;40:700–704.
- 50 Farombi EO, Shrotriya S, Surh YJ: Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappaB and AP-1. *Life Sci* 2009;84:149–155.
- 51 Miyamoto Y, Koh YH, Park YS, Fujiwara N, Sakiyama H, Misonou Y, et al: Oxidative stress caused by inactivation of glutathione peroxidase and adaptive responses. *Biol Chem* 2003;384:567–574.
- 52 Erdamar H, Demirci H, Yaman H, Erbil MK, Yakar T, Sancak B, et al: The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. *Clin Chem Lab Med* 2008;46:1004–1010.
- 53 Cholet N, Pellerin L, Welker E, Lacombe P, Seylaz J, Magistretti P, et al: Local injection of antisense oligonucleotides targeted to the glial glutamate transporter GLAST decreases the metabolic response to somatosensory activation. *J Cereb Blood Flow Metab* 2001;21:404–412.
- 54 Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R, et al: Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* 2007;55:1251–1262.
- 55 Olajide OJ, Adeyemi AP: Studies on effects of aqueous Garcinia kola extract on the lateral geniculate body and rostral colliculus of adult Wistar rats. *Med Pract Rev* 2011;2:23–28.
- 56 Ishii K, Sasaki M, Kitagaki H, Yamaji S, Sakamoto S, Matsuda K, et al: Reduction of cerebellar glucose metabolism in advanced Alzheimer's disease. *J Nucl Med* 1997;38:925–928.
- 57 Ross JM, Öberg J, Brené S, Coppotelli G, Terzioglu M, Pernold K, et al: High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. *Proc Natl Acad Sci U S A* 2010;107:20087–20092.
- 58 Howell BF, McCune S, Schaffer R: Lactate-to-pyruvate or pyruvate-to-lactate assay for lactate dehydrogenase: a re-examination. *Clin Chem* 1979;25:269–272.
- 59 Gupta VB, Anitha S, Hegde ML, Zecca L, Garruto RM, Ravid R, et al: Aluminium in Alzheimer's disease: are we still at a crossroad?. *Cell Mol Life Sci* 2005;62:143–158.
- 60 Newman LA, Korol DL, Gold PE: Lactate produced by glycogenolysis in astrocytes regulates memory processing. *PLoS One* 2011;6:e28427.
- 61 Adedara I, Farombi EO: Chemoprotection of ethylene glycol monoethyl ether-induced reproductive toxicity in male rats by kolaviron, isolated biflavonoid from Garcinia kola seed. *Hum Exp Toxicol* 2012;31:506–517.