Cerebellar reduction in rats by gamma-irradiation is mitigated by pretreatment with methanolic extract of *Vernonia amygdalina* and alpha-tocopherol

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Summary

This study evaluated the radioprotective effect of a methanolic extract of *Vernonia amygdalina* (MEVA), as compared with alpha-tocopherol (TOCO), on the effect of gamma-irradiation on the histomorphometry of the cerebellum of rats.

Rats were administered with MEVA at 250, and 500 mg/kg/day, and TOCO at 500 mg/kg/day for 14 days, gamma-irradiated with 2.0 Gy on 15th day, and euthanized on the 16th day. Rat cerebella were processed for 5-6 µm paraffin-embedded sections and stained with Haematoxylin and Eosin and evaluated under the light microscope. The dimensions of the (i) the molecular layer (ML), (ii) the Purkinje layer (PL), (iii) the granular layer (GL), (iv) the density of Purkinje cell (DPc), and (v) the widest diameter of Purkinje cells (WDPc), were obtained using a microscope with a graticule. Data were analyzed using ANOVA.

Gamma radiation caused a statistically significant reduction in the means of the PL ($p<0.05$), ML, GL, WDPc ($p<0.01$), and the DPc ($p<0.001$). Pretreatment with both 250 and 500 mg/kg doses of MEVA elicited a significant elevation of the means of these parameters when compared with the irradiation only groups as follows: PL ($p<0.05$), ML, GL, WDPc ($p<0.01$), and DPc ($p<0.001$). Similarly, pretreatment with TOCO elicited a significant elevation of the means of the same parameters thus: ML and GL ($p<0.01$), DPc and WDPc ($p<0.001$). Relatively, the 500 mg/kg dose of MEVA was more potent than the 250 mg/kg dose, and TOCO pretreatment was more potent than MEVA.

The study demonstrates that pretreatment with MEVA and TOCO before exposure to 2 Gy of gamma rays significantly improves the radiation-induced changes in the cerebellum of Wistar rats.

Key words: Central nervous system – Cerebellum – Irradiation – *Vernonia amygdalina*

Introduction

Morbidity and mortality have been attributed to cancer globally. Cancer treatment includes chemotherapy, radiotherapy and surgery (Aunapuu et al., 2003). Radiotherapy uses high-energy rays (ionizing radiation), to kill/damage cancer cells, thereby stopping them from further growth and cell division,

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and also to shrink tumours (Wikipedia Cancer, 2009). Although oncologists try to protect adjacent normal tissue by limiting the radiation dosage and spreading treatment out over time, radiation may still affect normal tissue adjacent to the irradiated cancer, thereby causing radiation side effects (Meschan, 1973; Belka et al., 2001). Irradiation of tissues near the central nervous system (CNS) for example, neoplastic lesions of the nasopharynx, hypopharynx, and cervical lymph nodes, could have adverse effects on nearby normal nervous tissues. This may lead to neural injuries such as radiation encephalopathy, radiation myelopathy, or severe neuronal loss in the rat cerebellum (Bowen et al., 1996; Sert et al., 2000; Schmitz et al., 2005).

Radiation damage is caused by a photon, electron, proton, neutron, or ion beam directly or indirectly ionizing the atoms which make up the DNA chain of the biological molecule via free radical generation (Aruoma, 1998; Lee et al., 2006). Indirect ionization occurs as a result of the ionization of water, forming free radicals, notably hydroxyl radicals, which then damage the DNA (Riley, 1969; Naik et al., 2005). An overabundance of free radicals in cells may lead to uncontrolled chain reactions with polyunsaturated fatty acids (PUFA) in cell membranes which ultimately leads to lipid peroxidation (Adaramoye and Adeyemi, 2005; Farombi et al., 2008), and neural tissues are noted for their high content of PUFA. Radiation damage causes structural changes in nervous tissue, including: demyelination, endothelial loss, degenerative glial reactions, arterial vessel wall thickening, thrombosis and occasional telangiectasis (O'Connor and Mayberg, 2000; Belka et al., 2001).

The use of antioxidants would appear to be a profitable approach for reducing radiation side effects. Plant-based antioxidants have been found to be potent, yet relatively free of side effects in comparison with synthetics, fostering the search for such agents as radioprotectors. Some plants reported to possess radioprotective capabilities due to their antioxidative properties include *Aegle marmelos, Terminalia chebula, Phyllanthus emblica,* and *Terminalia bellerica* commonly called 'Triphala', and *Amaranthus paniculatus* (Jagetia et al., 2004; Naik et al., 2005; Maharwal et al., 2005). Most reports on plant-based radioprotectors come from studies of the gastrointestinal tract (and its ancillary organs) of experimental animals. There is a paucity of literature on the effect of these plant-based radioprotectors on the nervous tissue. *Vernonia amygdalina* leaves have been reported to contain antioxidant compounds (Igile et al., 1994). Considering the important function of the nervous system, and using the cerebellum as a model, this study was designed to answer the research question: can *Vernonia amygdalina* leaf extract mitigate the histomorphometric changes induced by gamma irradiation in the cerebellum of Wistar rats?

Materials and Methods

Experimental animals

Forty two adult male Wistar rats, aged 12-16 weeks, initial body weight between 200-240g were selected from a colony at the Central Animal House of the College of Medicine, University of Ibadan, and were fed with commercial mouse cubes (Ladokun Feeds Nig. Ltd, Mokola, Ibadan) and water *ad libitum.* They were housed in transparent plastic cages with wood shavings in a fly-proof, freely ventilated and naturally illuminated animal room. All procedures on animal handling conformed to acceptable guidelines on the ethical use of animals in research (Clarke et al., 1996).

There were seven groups of six animals per treatment group, as detailed in Table 1.

Table 1. Grouping and treatment of experimental animals.

Group	Treatment
Control	1ml d.w/rat/oral daily x 14 days
M 250	250 mg/kg/day/oral of MEVA x 14 days
M 500	500 mg/kg/day/oral of MEVA x 14 days
R	2.0 Gy gamma-radiation treatment as a single dose on Day 15
R + M 250	250 mg/kg/day/oral of MEVA x 14 days + 2.0 Gy gamma-radiation treatment as a single dose on Day 15
R + M 500	500 mg/kg/day/oral of MEVA x 14 days + 2.0 Gy gamma-radiation treatment as a single dose on Day 15
R + TOCO	500 mg/kg/day/oral of α -Tocopherol x 14 days + 2.0 Gy gamma-radiation treatment as a single dose on Day 15

MEVA= methanol extract of *V. amygdalina.*
d.w= distilled water.
Gy= Gray unit, (1Gy=100 rads)
R= Radiation
TOCO= alpha-tocopherol

Plant Material

The leaves of *Vernonia amygdalina* commonly called 'bitter leaf' belonging to the Asteraceae family were harvested at a farm in Ibadan, South-West Nigeria, in May, 2006. Botanical identification and authentication was done at the Forest Research Institute of Nigeria, Ibadan, Nigeria, where the voucher sample number FHI 107408 was deposited for reference. The leaves were rendered pest-free by treatment at the Plant Quarantine Service of the Federal Department of Agriculture, Ibadan, Nigeria. Thereafter, 4.55 kg of the air-dried and pulverized leaves were packed into white polythene bags with the open end sealed.

Chemicals

Chemicals and reagents were purchased from Sigma Chemical Co. USA. Methanol (MeOH) and other materials were of the highest analytical grade.

Extraction procedures, preparation and administration of MEVA

The leaves (4.55kg) were cold-extracted with MeOH (3 x 17 L) at room temperature over a period of three weeks. The cumulated solvent was evaporated with a Rotary Vacuum Evaporator [Eyela N.21, Tokyo] to afford a methanolic extract of *V. amygdalina* (MEVA) weighing 700 g, a yield of 15.4%. From MEVA, stock solutions of two different concentrations were prepared: namely the 250 mg/kg and 500mg/kg concentrations. 1mL of the prepared stock solution containing 250 mg/kg/day or 500 mg/kg/day, depending on the rat treatment group, was withdrawn with new 2 mL hypodermic syringes (Becton Dickinson S. A., Spain), after stirring with a clean glass rod. MEVA was administered to each of the experimental animals orally, employing a clean intra-gastric gavage daily for 14 days. All rats, irrespective of grouping, received 1 mL distilled water daily throughout the duration of the experiment because it was the vehicle for MEVA. The rats were weighed at the start of the experiment, on the day of irradiation and on the day of sacrifice.

Preparation and administration of α-tocopherol (Vitamin E)

Each soft gelatin capsule containing 100 mg of dl-α-tocopheryl acetate as 100 mg vitamin E acetate (G.A. Pharmaceuticals, Athens, Greece) was punctured with a new 21G needle (Hypojet, Spain) attached to a 1 mL hypodermic syringe (Becton Dickinson, La Port-de-Clair, France). The oily formulation of vitamin E was then neatly and completely aspirated out with the syringe. Each aspirate measured approximately 0.2 mL, containing 100 mg of dl-α-tocopherol. The insulin syringe was thereafter attached to an intra-gastric gavage through which each rat was administered the measured dose of 500 mg/kg/orally/per day for 14 days.

Animal irradiation procedures

Each experimental rat was sedated and immobilized with Ketamine hydrochloride injection (Rotex medica, Trittau, Germany, batch 40092) at 10 mg/kg body weight and Diazepam injection (Roche, Switzerland) at 3 mg/kg body weight, ip. Rats were strapped down in the prone position in the cardboard box specially designed for this purpose using cotton strapping and then whole-body irradiated with a single fraction of 2.0 Gy (200 rads) of gamma-rays, at a dose rate of 105.477 cGy/min, for 2.22 minutes, obtained from a Cobalt-60 source. The radiation was delivered by an AECL Theratron 780C Teletherapy machine at 1.25 MeV, at a source to surface distance of 72 cm, at a depth of 4 cm, and a field size of 18 cm by 18 cm, with an equivalent square area of 18 cm^2; the percentage depth dose was 85.32%. Animals were kept in cages in a warm room post-irradiation. The dosimetry and irradiation procedures were carried out at the Radiotherapy Department of the University College Hospital, Ibadan, Nigeria.

Sample collection

At the end of the experiments on day 16, all the control and MEVA-only treatment groups, plus all animals that received radiation doses, were weighed on a Swiss Microwa balance type 7720. Thereafter, Ketamine anaesthesia was administered at 10 mg/kg i.p to each rat. Each rat was positioned on the dissection table and the head was severed at the cervico-medullary junction for uniformity and then fixed in labeled bottles containing 10% formol saline. The brain remained in situ for seven days before its removal from the skull. Each head and its cranium were carefully dissected to avoid pressure that might deform the underlying brain. The brain and cerebellum were extracted and then processed by the routine method for paraffin wax-embedded sec-

tions. A rotary microtome was used to cut and collect five- to six-micrometer thick transverse sections, with three to five sections per slide. These slides were stained with haematoxylin and eosin (H&E), and examined under the light microscope (Olympus CH Japan).

Histomorphometry

The dimensions of the (i) molecular layer, (ii) Purkinje layer, (iii) granular layer, (iv) Purkinje cell density, and (v) maximum diameter of Purkinje cells were obtained by the use of a microscope with a graticule (micrometer embedded in the eye piece objective) at different magnifications. The micrometer was calibrated using a stage micrometer slide with a customized 2 mm ruler engraved on the cover slip (Leitz Wetzlar, Germany). The density of Purkinje cells was measured by counting the number of Purkinje cells that had nuclei observed within a given square area in a section (Sugihara et al., 2000). This was done by using the eyepiece of an Olympus CH (Japan) binocular microscope at x 40 magnification. The radius of the eye piece at x 40 was calibrated with a graticule to be 0.19 mm, and the area of the view at x40 magnifications was thus estimated as 0.11 mm^2 as described by Osuagwu (2007). Measurements were made on each section from all the experimental and control groups, and for each section ten observations were made from twenty adjacent but different high-power fields. The means of each of the dimension obtained were then calculated.

Statistical analysis

The data were analysed with one-way-Anova, and a Turkey post-test was performed for multiple comparisons using Graph Pad Prism version 4.0 (2003) for Windows GraphPad Software, San Diego, California, USA, www.graphpad.com. The level of significance was fixed at less than 5% probability for the null hypothesis being true by chance.

Results

This study evaluated the effect of gamma radiation on the nervous tissue in a mammalian model using the Wistar rat cerebellum. The possible ameliorative effect of the antioxidant property of the flavonoids in the leaf extract of *Vernonia amygdalina* (MEVA) on rat brain was studied using alpha-tocopherol (TOCO), an established antioxidant known for its potency in fatty tissue as a reference compound. The treatment groups are as detailed in Table 1.

General

The administration of M 250, M 500, and R + TOCO did not induce any mortality during the whole observation period. However, one each of the R, R + M 250 treatment groups died either during irradiation procedure or did not recover from the anesthesia.

Microscopic anatomy

(i) *Control group*

Figure 1A shows a photomicrograph of the cerebellum of the brain of a representative of the control rats showing a normal histological outline. The cortical layers, namely the molecular, Purkinje, and granular layers were observed to be normal. The Purkinje cells formed a monolayer, each having a large roundish soma closely and evenly arranged, with the nuclei visible within the perikarya. The white matter consisted of glial cells as well as neurons of the deep cerebellar nuclei, showing large somata, as shown in Fig. 2A.

(ii) *M 250 and M 500 treatment groups*

The histology of the representative of this group as shown in Figs. 1B and 2B reveals features similar to those of the control rats in Figs. 1A and 2A.

(iii) *R group*

The histology of the irradiated animals in Figs. 1C and 2C show an apparent reduction in the size of the molecular and granular layers, in addition to the paucity, wide separation, and apparent decrease in the size of the bodies of the Purkinje cells in the Purkinje layer of the cerebellum, as shown in Fig. 1A. There appeared to be a shrinking of the somata of the neurons of the white matter, as shown in Fig. 2C.

(iv) *R + M 250 and R +M 500 treatment groups*

Figures 1D and 2D show that the microscopic features of layers and cells of the cerebellum of the rats in the groups that received 250 mg/kg MEVA and 500 mg/kg MEVA for fourteen days before exposure to gamma radiation, were similar to those of the cerebella of the control rats shown in Figs. 1A and 2A.

(v) *R + TOCO treatment group*

The layers and the cells of the white matter of the cerebellum were similar to the histology of the control rats as shown in Figs. 1E and 2E.

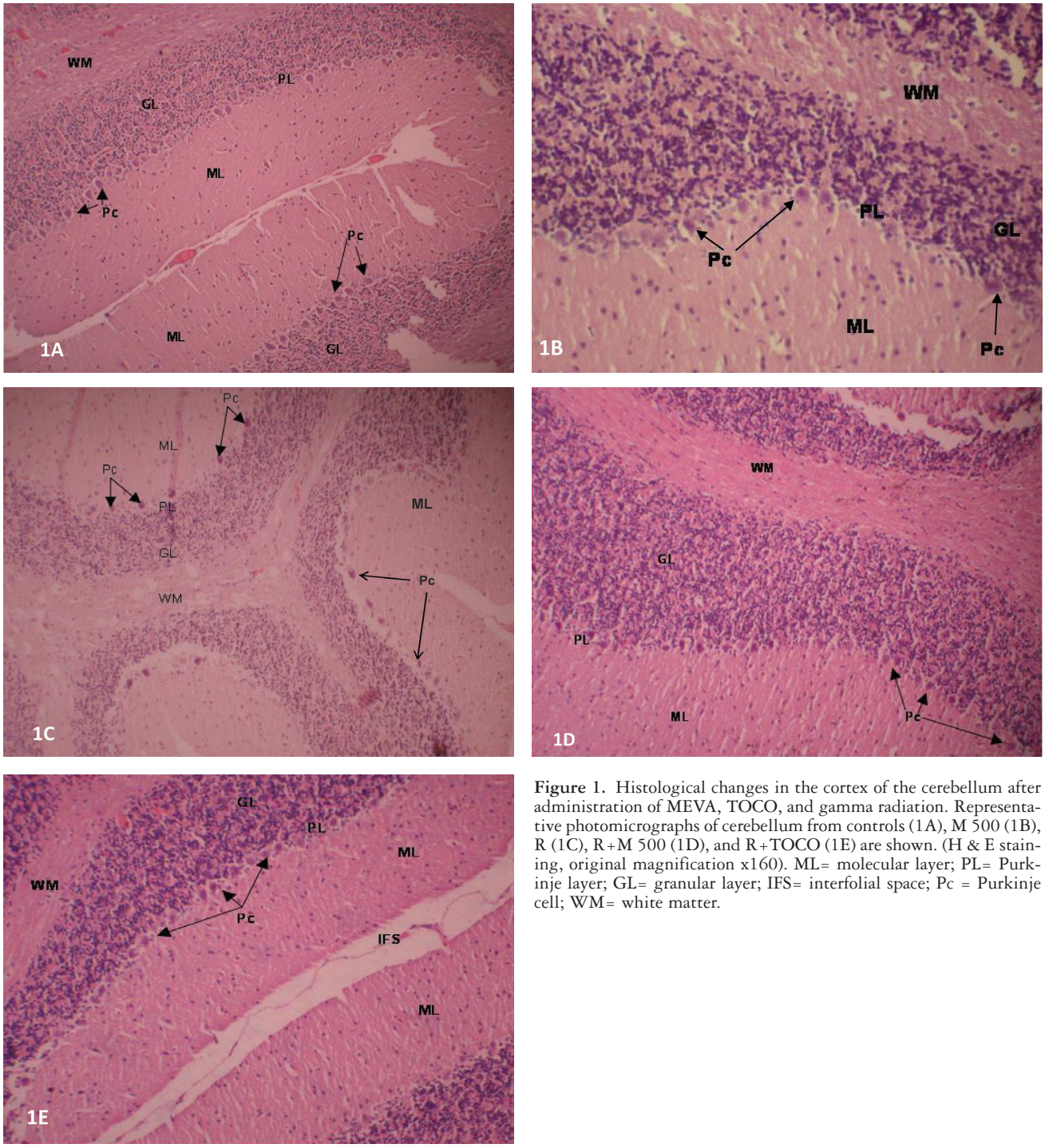

Figure 1. Histological changes in the cortex of the cerebellum after administration of MEVA, TOCO, and gamma radiation. Representative photomicrographs of cerebellum from controls (1A), M 500 (1B), R (1C), R+M 500 (1D), and R+TOCO (1E) are shown. (H & E staining, original magnification x160). ML= molecular layer; PL= Purkinje layer; GL= granular layer; IFS= interfolial space; Pc = Purkinje cell; WM= white matter.

Histomorphometry

(i) *Effect of treatments on the molecular layer (ML) of the cerebellum*

There was no significant alteration when the mean of the molecular layer in the control rats was compared with that of the radiation only group. Gamma radiation caused a 46.4% reduction in the size of the mean of the molecular layer (106.4 ± 18.6 µm) when compared with the control (198.4 ± 18.6 µm), as shown Table 2, which was statistically significant ($p<0.01$). This layer was, however, increased in a significant manner by pretreatment with both doses of MEVA (24.7%), as well as TOCO (42.6%) before irradiation when compared with the cerebellum of rats treated with radiation only ($p<0.01$).

(ii) *Effect of treatments on the Purkinje layer (PL) of the cerebellum*

MEVA treatment alone did not have any significant effect on the Purkinje layer of the cerebellum. However, treatment with gamma radiation caused a 15.7% reduction in the

Table 2. Effect of MEVA, α-tocopherol and γ-irradiation on the histological layers of the cerebellum of Wistar rat.

Group	Control	M250	M500	R	R+250	R+M500	R+TOCO
Molecular layer (μm)	198.4 ± 20.3	195.2 ± 10.6	195.0 ± 13.9	106.4 ± 18.6 #**	141.2 ± 8.1**	141.2 ± 5.1**	185.4 ± 7.6**
Purkinje layer (μm)	26.8 ± 1.6	25.2 ± 1.0	25.2 ± 1.0	22.6 ± 2.4*	24.8 ± 1.0	25.2 ± 1.0	24.5 ± 2.0
Granular layer (μm)	145.8 ± 14.3	134.2 ± 14.3	145.4 ± 6.0	97.8 ± 11.3 #**	105.0 ± 8.5**	106.8 ± 9.7**	145.2 ± 8.8**

Values are mean ± S.D of 5 animals per treatment. * Significantly different from control group ($p<0.05$); #** Significantly different from control group ($p<0.01$); ** Significantly different from the radiation only group ($p<0.01$). MEVA= methanolic extract of *V. amygdalina*; R = gamma radiation. M 250 = 250 mg/kg of MEVA. M 500 = 500 mg/kg of MEVA. TOCO = 500 mg/kg of α-Tocopherol.

Table 3. Effect of MEVA, α-tocopherol and γ-irradiation on the density and widest diameter of Purkinje cells of the cerebellum of Wistar rats.

Group	Control	M250	M500	R	R+250	R+M500	R+TOCO
Density (no/0.11mm^2)	9.54 ± 0.2	9.22 ± 0.6	9.46 ± 0.1	5.94 ± 0.5 #***	9.38 ± 0.24***	9.6 ± 0.2***	10.28 ± 0.9***
Widest Diameter (μm)	13.3± 1.5	12.3 ± 0.5	12.3 ± 0.5	8.54 ± 0.3**	10.94 ± 0.3**	10.88 ± 0.8**	13.64 ± 0.9***

Values are mean ± S.D of 5 animals per treatment. ** Significantly different from control group ($p<0.01$); #*** Significantly different from control group ($p<0.001$); ** Significantly different from radiation only group ($p<0.01$); *** Significantly different from the radiation-only group ($p<0.001$). MEVA=methanolic extract of *V. amygdalina*; R = gamma radiation. M 250 = 250 mg/kg of MEVA. M 500 = 500 mg/kg of MEVA. TOCO = 500 mg/kg of α-Tocopherol.

means of the Purkinje layer (22.6 ± 4.45 μm) when compared with the control (26.8 ± 1.6 μm, Table 2), and this was statistically significant ($p<0.05$). Although the means of the Purkinje cell layer in the R + M 250 and R + M 500 treatment groups showed slight increases in size when compared with those of R, these were not significant ($p>0.05$). Similarly, there was a non-significant increase in the means of the group pretreated with TOCO.

(iii) *Effect of treatments on the granular layer (GL) of the cerebellum*

The slight alteration in the means of the granular layer of the cerebellum in the MEVA treatment groups were compared with the control animals and observed to be insignificant ($p>0.05$). There was a 32.9% reduction in the means of the granular layer of the cerebellum of rats that were given gamma radiation treatment when compared with the control animals, and this was statistically significant ($p<0.01$, Table 2). However, there was a significant increase in the means of this layer of the cerebellum of rats that received MEVA and TOCO pretreatment before exposure to gamma radiation when compared with the radiation-only group ($p<0.01$, Table 2).

(iv) *Effect of treatments on the density of Purkinje cells (DPc) of the cerebellum*

Table 3 shows that gamma radiation caused a 37.7% reduction in the mean of the density of Purkinje cells (5.94 ± 0.54 μm) as compared with the control group (9.54 ± 0.19 μm), which was found to be significant ($p<0.001$). The figure also shows that pretreatment with both doses of MEVA and TOCO caused a significant increase ($p<0.001$) in the means of the density of Purkinje cells to 36.7-38.1% and 42.2% respectively, as compared with the irradiated rats.

(v) *Effect of treatments on the widest diameter of the Purkinje cells (WDPc) of the cerebellum*

Gamma radiation caused a statistically significant reduction ($p<0.01$) in the means of the widest diameter of the Purkinje cells in the irradiation-only rats (8.54 ± 0.34 μm) when compared with the control (13.3 ± 1.94 μm); this decrease was 35.8%, as shown in Table 3. However, there was a significant increase in this parameter in rats pretreated with both doses of MEVA ($p<0.01$) and also with TOCO ($p<0.001$) as compared with the irradiation-only treatment group, while it was increased in the MEVA groups by between 9.2-22%. TOCO pretreatment increased it by 37.4%.

(vi) *Effects of treatments with both 250 and 500mg/kg MEVA doses*

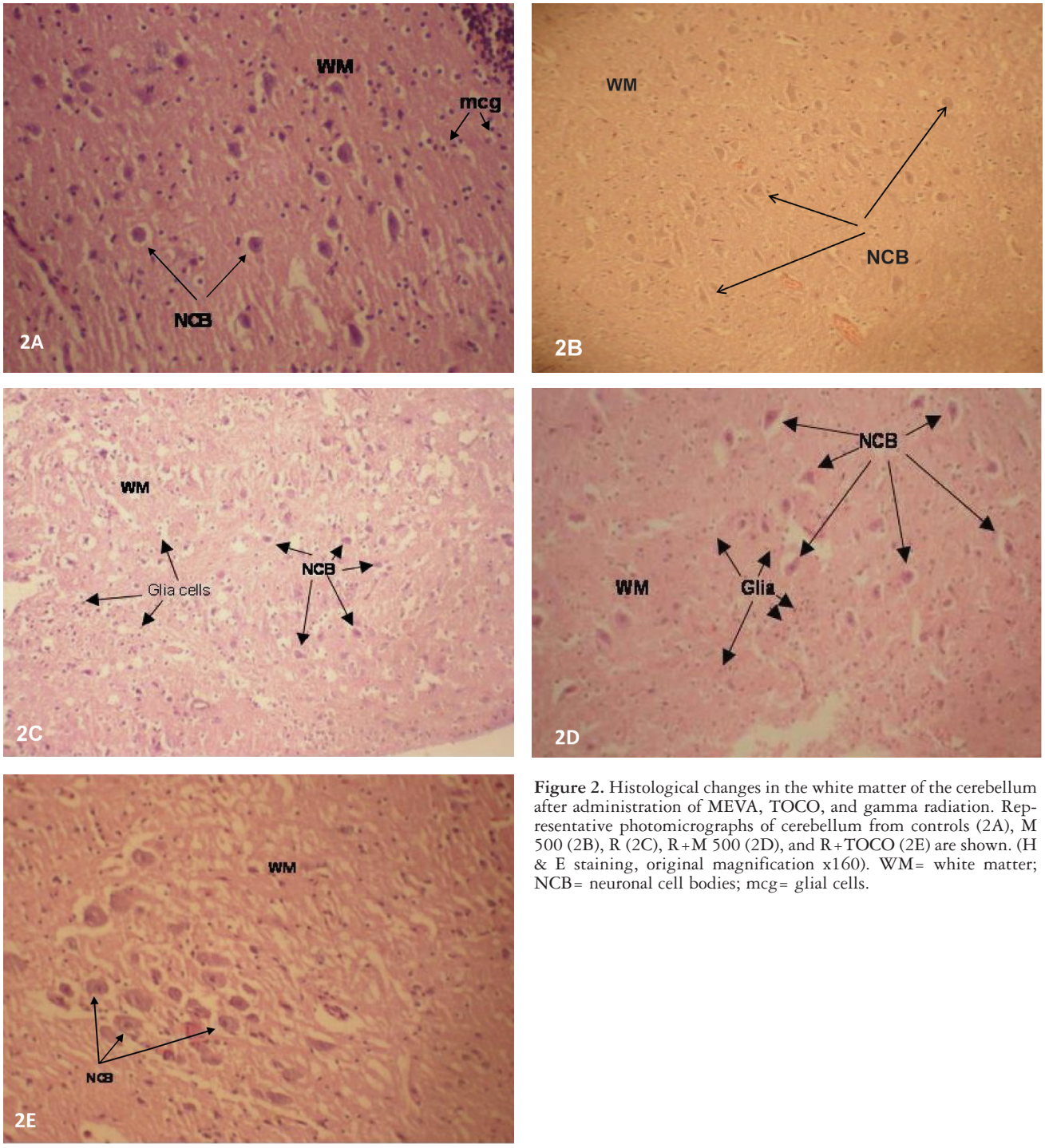

Figure 2. Histological changes in the white matter of the cerebellum after administration of MEVA, TOCO, and gamma radiation. Representative photomicrographs of cerebellum from controls (2A), M 500 (2B), R (2C), R+M 500 (2D), and R+TOCO (2E) are shown. (H & E staining, original magnification x160). WM= white matter; NCB= neuronal cell bodies; mcg= glial cells.

In the histomorphometric parameters considered, the 500 mg/kg dose of MEVA was relatively more potent than the 250 mg/kg dose.

(vii) *Comparison of effectiveness of treatments with MEVA and TOCO*

Comparatively, TOCO pretreatment before irradiation was more potent than the responses elicited by both doses of MEVA in the histomorphometric parameters obtained.

Discussion

We observed that gamma radiation caused a significant reduction in the means of the molecular layer (ML), Purkinje layer (PL), and granular layer (GL), as well as in the density of the Purkinje cells (DPc) and the widest diameter of the Purkinje cells (WDPc) of the cerebellum when compared with the controls. Equally significant was our observation that pretreatment of rats for 14 days with either MEVA or TOCO before their exposure to

gamma rays elicited an increase in these parameters when compared with rats that received gamma rays only. Sugihara et al., (2000) recorded an 83.3 to 90 per cent reduction in the ML of the cerebellum of rats that were irradiated using 5Gy of X-rays as compared to the 46.4 percent recorded here. However, both X-rays and gamma rays are known to be ionizing radiations having short wavelengths and high frequencies. The difference in our values in this study may be due to the fact that Sugihara and his colleagues irradiated rats on the fifth post-natal day, and concentrated the radiation upon the cerebellum and sub-adjacent brainstem, with lead protection of other body parts, whereas in this study a whole-body irradiation was given, concentrating the rays on the neural axis of twelve to sixteen week old rats.

The implication of a reduction in the molecular layer of the cerebellum due to the toxicity of irradiation is the effect this might have on the vestibular information carried by the parallel fibres that are the terminal bifurcation of the axons of granule cells in the molecular layer. Since parallel fibres synapse on the distal dendrites of Purkinje cells in the molecular layer (Singh, 2007), and since impulses are transmitted by the stellate and basket cells to the Purkinje cells in this same layer, the outward projection of the Purkinje cells, which are the principal neurons of the cerebellum to the deep cerebellar nuclei, might be affected. This may result in an alteration of the final projection of the deep cerebellar nuclei to cerebellar targets (Afifi and Bergman, 2005).

The implication of the significant reduction in the granular layer of the cerebellum due to radiation toxicity may be the effect on the glomeruli in this layer, since the numerous glomeruli contain the termination of the Mossy fibres bringing different sensory information to the cerebellum (vestibular, proprioception, and inputs from the cerebral cortices via the brachium pontis). Also located in the granular layer are climbing fibres, which bring visual information from the olivary nuclear complex on their way to synapse on the proximal dendrites of Purkinje cells (West, 1995; Afifi and Bergman, 2005). Again, this internal cerebellar circuitry may be affected, leading to a possible disruption of its smooth functioning.

Taken together, the implications of a significantly reduced Purkinje cell layer, reduced Purkinje cell density, and the widest diameter of Purkinje cells, are the possibility of a reduction in the effectiveness of the projections of Purkinje cells from the different cerebellar zones namely: from the vermal zone to the fastigial nucleus; the paravermal zone to the nucleus interpositus (emboliformis and globose), and the lateral zone to the nucleus dentatus. The effect of the toxicity is finally transferred from these deep cerebellar nuclei to the various cerebellar targets, namely the spinal cord, vestibular nuclei, reticular formation, and nucleus rubra in the midbrain, and nucleus ventrolateralis of dorsal thalamus (Afifi and Bergman, 2005). This effect might cause poor control of balance, poor coordination of eye movements with movements of the head, poor control of posture and muscle tone, and synergy during stereotyped movements, such as walking/running, and poor coordination of the planning of movements and the muscle tone required for accurate non-stereotyped (learned) movements (West, 1995). The importance of the Purkinje cell is that it is the focal neuron of the cerebellar cortex, because all afferent pathways ultimately converge on it, while its axons constitute the major exit of the cerebellar cortex, after synapsing on the deep cerebellar nuclei (Ellis, 2006).

The reduction in these parameters in the irradiated rats might have been due to the effect of the toxicity of gamma radiation on the neurons and tissues of the cerebellum. Radiation therapy is known to work by damaging the deoxyribose nucleic acid (DNA) of cells, the damage being caused by an ion beam directly or indirectly ionizing the atoms that make up the DNA chain of the biological molecule. Indirect ionization occurs as a result of the ionization of water, forming free radicals, notably hydroxyl radicals, which then damage the DNA. In the most common forms of radiation therapy, most of the effect of radiation is through free radicals (Aruoma, 1998; Lee et al., 2006; Wikipedia Radiation, 2009). Apart from DNA damage, the free radicals and reactive oxygen species (ROS) released generally have the capacity to damage the lipid membranes of cells, and this is very feasible in the nervous tissue, as exemplified by the cerebellum of rats used in this study, because it is rich in polyunsaturated fatty acids (Cook and Samman, 1996; Aruoma, 1998). The mammalian brain is known to be readily plundered by ROS because of its high oxygen utilization, oxygen being a ready

source of ROS (Reiter et al., 2001). The neuronal cell death may also have been due to secondary factors such as thrombosis, vascular luminal occlusion, demyelination, apoptosis or necrosis, among other factors (Li et al., 2002; Schmitz et al., 2005). The summed effects of any or all of the above factors would be to induce cell death, which may also lead to a reduction in brain size, since gamma radiation was given in excess of an acute radiation dose (Ferrer, 1996; Hyodo-Taguch et al., 1998).

The significant increase in the means of the ML, PL, GL, DPc, and WDPc by pretreatment with MEVA and the reference antioxidant, alpha-tocopherol, may have been due to the reported antioxidant capacity of both substances. Since oxidative damage through free radical release has been implicated in neuronal damage, this implies that a possible neutralization of such free radicals by the antioxidant content of both MEVA and TOCO may have played an ameliorative role. While alpha-tocopherol is a known antioxidant that is especially active in lipid-based tissues (Pauling, 2008), evidence for the antioxidant activity of the leaf extract of *Vernonia amygdalina* plant has been documented (Iwalewa et al., 2005; Adaramoye et al., 2008). The activity of this plant's antioxidant activity has previously been reported to be due to the presence of flavonoids such as luteolin, luteolin 7-0, β-glucuronide and β-glucoside by Igile (1994), and flavonoids are known to have antioxidant activity (Markham and Bloor, 1998; Farombi et al., 2002; Prabhakar et al., 2006). The improvement of radiation damage to the cerebellum would allow it to perform its essential function as the integration center for the coordination of voluntary muscular movements and posture (Hendelmann, 2000; Afifi and Bergman, 2005), and also enable it to coordinate saccadic and slow eye movements with the movements of the neck (West, 1995).

In conclusion, the present study has demonstrated that treatment with MEVA and TOCO for 14 days before exposure to 2 Gy of gamma rays significantly mitigated the radiation-induced histomorphometric changes in the cerebellum of Wistar rats, suggesting the radioprotective potential of both of them. The findings also suggest a need for further research concerning the potency of *Vernonia amygdalina* leaves as a radioprotector in other areas of the mammalian central nervous system.

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