



Protective role of *Telfairia occidentalis* in irradiation-induced oxidative stress in rat brain

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ABSTRACT

This study aimed at evaluating the protective role of *Telfairia occidentalis* extract (TOE) irradiation-induced oxidative stress in rat brain. Aqueous TOE was administered orally to adult rats for 30 days at doses of 400 mg/kg body weight, 800 mg/kg body weight and 1600 mg/kg body weight, and a corresponding group of rats were treated with 50 mg/kg body weight vitamin E (VE), a standard antioxidant before irradiation at a dose of 2 Gy of gamma rays. The control rats received distilled water only. The rats were observed and sacrificed at 24 hours, 15 and 30 days post-irradiation. The results demonstrated a significant increase in levels of malondialdehyde (MDA), an end product of lipid peroxidation (LPO) and hydrogen peroxide (H₂O₂) generation with a concomitant decrease in the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), a phase two xenobiotic metabolizing enzyme, and a corresponding decrease in the level of reduced glutathione (GSH) after twenty-four hours, 15 and 30 days post-irradiation compared with the control. Treatments with TOE and VE significantly reversed oxidative stress of irradiated rats when compared with the control rats. In conclusion, supplementation with TOE could reduce radiation-induced biochemical disorders in brain tissues.

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INTRODUCTION

Ionizing radiation are electromagnetic radiation (such as X or gamma rays) or particulate radiation that causes direct and indirect cellular damage through reactions with free radicals and reactive oxygen species (ROS) produced primarily from radiolysis of

water and this results in a chemical alteration in macromolecules that are critical for biological function (Jones et al., 1997). Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals and ROS formed.

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Research has shown that radiation elevates ROS in irradiated cells *in vitro* (Rugo et al., 2002) and that one of the mechanisms of radiation-induced genomic instability might be the prolonged elevation of ROS *in vivo* in various tissues studied after radiation exposure. ROS are constantly generated in cells and injure deoxyribonucleic acid (DNA) in the nucleus as well as deoxynucleotide triphosphate in its cellular pool following exposure to radiation (Gen et al., 2004). Thus, an increase in the production of ROS may cause genomic instability and facilitate the process of various pathological conditions since some enzyme activities in the system could wane.

Increasing experimental evidences have indicated that radiation causes damage to different organs, including the testes, liver, kidney and brain by induction of oxidative stress (Adaramoye et al., 2010; Xu et al., 2012; Bahattin et al., 2012). The brain is particularly vulnerable to oxidative damage because of its high oxygen utilization, its high content of oxidative polyunsaturated fatty acids (PUFAs), and the presence of redox-active metals. Hence, oxidative stress characterized by an excess of ROS is well recognized to be associated with neurodegenerative disorders such as ageing, cardiovascular diseases, cancer, Alzheimer's disease, stroke, Parkinson's disease and other neurodegenerative diseases (Chatterjee et al., 2007; Valko et al., 2007; Ebokaiwe et al., 2013).

Natural products (herbal preparations) from plants, fruits and leaves, have received much attention in the last decade and appear to be favourable in many respects as compared to chemical radioprotectors (Jagetia, 2007). These include lower toxicity in human beings (as many of these are used in alternative medicine in various countries for

centuries), are easily available, inexpensive and have shown good radioprotection in preclinical studies (Jagetia, 2007).

The radioprotective efficacy of plant extracts is as a result of their containing a large number of active constituents, such as antioxidants, immunostimulants and compounds with antimicrobial activity (Weiss and Landauer, 2003; Jagetia et al., 2007). Reduction of oxidative damage by natural antioxidants in diets provides a degree of protection against ionizing radiation injury (Weiss and Landauer, 2003). Vitamin E (alpha-tocopherol) and Vitamin C and related analogues are food nutrients that can scavenge singlet oxygen and superoxide- anion radicals (Sert et al., 2000).

Telfairia occidentalis leaf is rich in minerals (iron, potassium, sodium, phosphorus, calcium and magnesium), antioxidants and vitamins such as thiamine, riboflavin, nicotinamide and ascorbic acid, phytochemicals such as phenols (Kayode et al., 2009). The diet preparation of the air-dried leaves of the plant significantly increased red blood cell count, white blood cell count, packed cell volume and haemoglobin concentration in rats (Alada, 2000).

In the face of the above, the potential radio-protective effect of *Telfairia occidentalis* is widely acceptable in Nigerian folklore medicine but has not been investigated. This study is therefore aimed at investigating the protective role of *Telfairia occidentalis* extracts in irradiation-induced oxidative stress in rat brain.

MATERIALS AND METHODS

Plant materials

The leaves of *Telfairia occidentalis* used in this study were harvested from Precious Agro Farm[®] Limited, Pakiti Village, Olorunda Abaa, Ibadan, Oyo State in January,

2011. The plant taxonomical identification and authentication of these leaves was done in the Department of Botany, University of Ibadan, with a voucher number UIH- 22339. The leaves were dried in an oven (Gallenham, England) and then pulverized into powder with a milling machine (Fataroy, Nigeria) in the Department of Pharmacognosy, University of Ibadan. Altogether, 5 kg of the dry and pulverized leaves were packed into white polythene bag.

Extraction procedure of aqueous extract of *Telfairia occidentalis*

Two thousand five hundred grammes (2500 g) of dried leaves of *Telfairia occidentalis* was weighed, granulated into powder, soaked in 12.5 litres of distilled water for 48 hours and mixed thoroughly. The mixture was filtered using Teflon[®] filter paper. The solvent in the filtrate was removed by using Rotatory evaporator (Bibby Sterling[®], Germany) leaving only the soluble organic matter. The percentage yield of the aqueous extract was 18.00% (450 g).

Phytochemical screening of the constituent of the leaf extracts of *Telfairia occidentalis*

Phytochemical screening of the leaves of *Telfairia occidentalis* was done in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan. The following compounds as described by Trease and Evans (2002) were screened for; alkaloids, cardinolides, anthraquinone, saponins, tannins and flavonoids.

Animal care and handling

Ninety healthy adult rats of Wistar strain (weighing between 180 and 250 g) obtained from the Experimental Animal Unit of the Faculty of Basic Medical Sciences, University of Ibadan were used for the study.

The animals were maintained in wire mesh cages, under hygienic, freely ventilated and naturally illuminated animal house of the Department of Biochemistry, University of Ibadan. All animals received human care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals (prepared by the National Academy of Science and published by the National Institutes of Health).

Animal irradiation procedures

The irradiation procedures were carried out at the Radiotherapy Department of the Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria, as described by Owoeye et al. (2010). The rats were placed in a cardboard box. Each experimental animal then received whole body irradiation as a single fraction of 2.0 Gy of gamma rays at a dose rate of 1.081 Gy/unit. The radiation was delivered by a Linear accelerator teletherapy machine with energy of 6.0 Mev, at source to surface distance of 400 mm, at depth of 14 mm, and a field size of 400 mm, with an equivalent square area of 185 mm², the percentage depth dose was 1 mm.

Chemicals

Nicotinamide adenine dinucleotide phosphate, epinephrine, glutathione, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), hydrogen peroxide, thiobarbituric acid (TBA), and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA). All other reagents were of analytic grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Grouping of animals

Group I: Control rats (n = 15) received distilled water orally for 30 days.

Group II: Irradiated rats (n = 15) exposed to 2 Gy dose of gamma radiation on day 30.

Group III: Rats (n=15), received 50 mg/kg body weight vitamin E for 30 days + 2 Gy dose of gamma radiation on day 30.

Group IV: Rats (n=15), received 400 mg/kg body weight TOE for 30 days + 2 Gy dose of gamma radiation on day 30.

Group V: Rats (n=15), received 800 mg/kg body weight TOE for 30 days + 2 Gy dose of gamma radiation on day 30.

Group VI: Rats (n=15), received 1600 mg/kg body weight TOE for 30 days + 2 Gy dose of gamma radiation on day 30.

Five rats from each group were sacrificed at 24 hours, days 15 and 30 post radiation for the evaluation of oxidative stress parameters in the brain.

Biochemical analysis

The brains of the control and experimental rats were homogenized in eight volumes of 50 mM of Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate was centrifuged at 10,000 ×g for 15 minutes at 4 °C. The supernatant was collected for biochemical assays.

Determination of lipid peroxidation (LPO)

Lipid peroxidation was quantified as malondialdehyde (MDA) employing the method described by Farombi *et al.*, (2000) and expressed as micromoles MDA/mg tissue. The assay was based on the reaction of chromogenic reagent (2-Thiobarbituric acid) with, malondialdehyde (an end product of LPO) produced from the peroxidation of fatty acid membranes and food products under acidic condition to yield a stable pink chromophore with maximum absorption at 532 nm. The pink chromophore is readily extractable into organic solvents like butanol.

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at 30 °C and a pH of 10.2 by the method of Misra and Fridovich (1972). SOD activity was determined by measuring the rate of adrenochrome formation, observed at 480 nm, in a reaction medium containing glycine-NaOH (50 mmol/L, pH 10) and epinephrine (1 mmol/L). The result was expressed in Unit/mg protein.

Hydrogen peroxide generation assay

Hydrogen peroxide generation was assessed by the method of Wolff (1994), based on that the ferrus oxidation with xylenol orange (Fox-1) reagent and the color development were virtually read spectrophotometrically at 560 nm.

Determination of glutathione-S-transferase (GST) activity

Glutathione S-transferase (GST) activity was determined by the method of Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

Determination of reduced glutathione (GSH)

Reduced GSH was determined according to Jollow *et al.* (1974). The method is based on the development of a relatively stable light yellow colour when Ellman's reagent [5¹⁵1 - dithiobis - (2 - benzoic acid, DTNB)] is added to a sulfhydryl compound. The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione, 2 - nitro - 5 - thiobenzoic acid possesses a molar absorption at 412 nm. Reduced GSH is proportional to the absorbance at 412 nm.

Determination of glutathione peroxidase activity

The activity of glutathione peroxidase (GPx) was determined by the method of Rotruck et al. (1973). The reaction mixture contained 2.0 ml of 0.4M Tris- HCl buffer, pH 7.0, 0.01 ml of 10mM sodium azide, 0.2 ml of enzyme. 0.2 ml of 10 mM glutathione and 0.5 ml of 0.2 mM. H₂O₂. The contents were incubated at 37 °C for 10 minutes followed by the termination of the reaction by the addition of 0.4 ml 10% (v/v) TCA, centrifuged at 5000 rpm for 5 minutes. The absorbance of the product was read at 430 nm and expressed as nmol/mg protein.

Statistical Analysis

The data obtained were further subjected to statistical analyses using the standard student ‘t’ test of the graph pad prism software package. The data were expressed as mean ± SEM and the level of significance at 95% Confidence Interval calculated (p< 0.05).

RESULTS

The basic phytochemical constituent present in the leaf extracts of *Telfairia*

occidentalis include cardenolides, saponins, tannins and flavonoids (Table 1).

Antioxidant status of rat brain homogenate following treatment with TOE and irradiation

The antioxidant status in the brains of control and experimental animals is presented in Figures 1–6. Our data indicated that exposure to gamma radiation caused a significant (P < 0.05) decrease in activities of antioxidant enzymes (SOD, GST and GSH-Px (Figures 2, 4, and 6) and non enzymatic antioxidant GSH (Figure 5) at 24 hours, days 15 and 30. Levels of H₂O₂ and MDA, an index of lipid peroxidation, were significantly elevated in the brains of irradiated rats (Figures 1 and 3) at 24 hours, days 15 and 30. Pretreatment with vitamin E (50 mg/kg body weight) and TOE at doses of 400 and 800 mg/kg body weight reversed the various parameters to normal but not at 1600 mg/kg body weight when compared with the control group. Pretreatment with TOE at a dose of 1600 mg/kg body weight potentiated the radiation induced oxidative stress in the brain of rats (Figures 1- 6) at 24 hours, days 15 and 30.

Table 1: Phytochemical constituent of *Telfairia occidentalis* leaf extracts.

Phytochemicals	Composition
Alkaloids	-
Cardenolides	+
Anthraquinone	-
Saponins	+
Tannins	+
Flavonoids	+++

- Means absent, + means present in small quantity and +++ means present in large quantity.

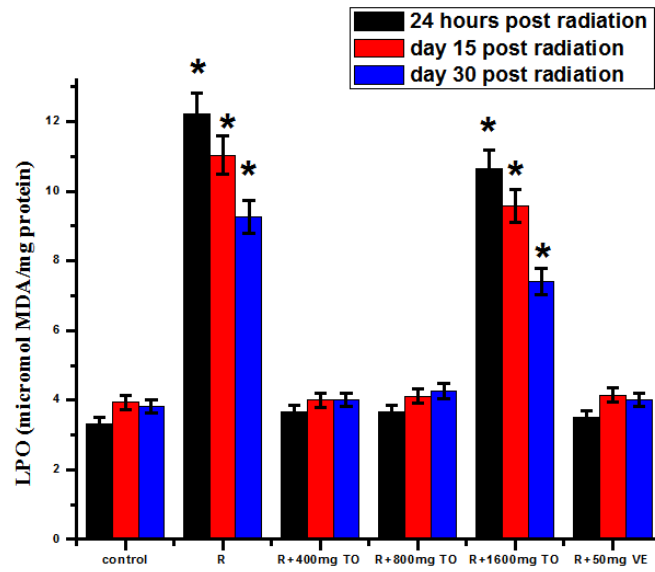


Figure1: Effects of *Telfairia occidentalis* on gamma radiation induced increase in levels of LPO in brain of male Wistar rats. N=5, + = significant difference at p<0.05.

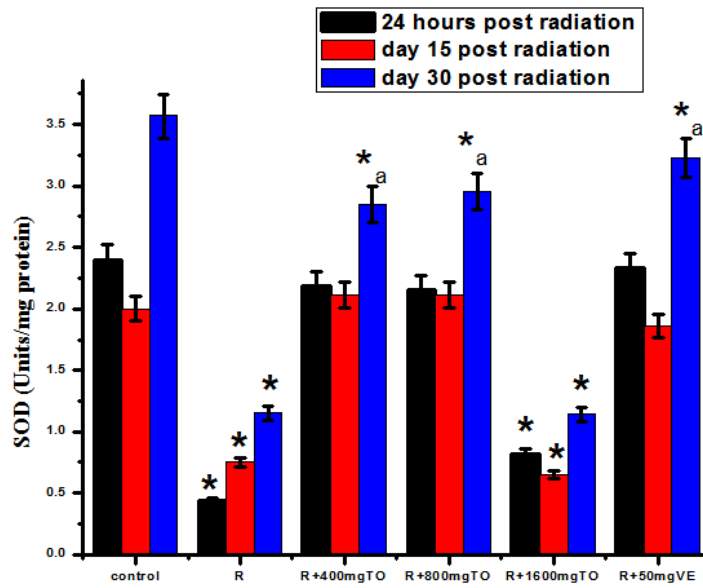


Figure 2: Effects of *Telfairia occidentalis* on gamma radiation induced decrease in activities of SOD in brain of male Wistar rats. N= 5, * = significant difference at p < 0.05.

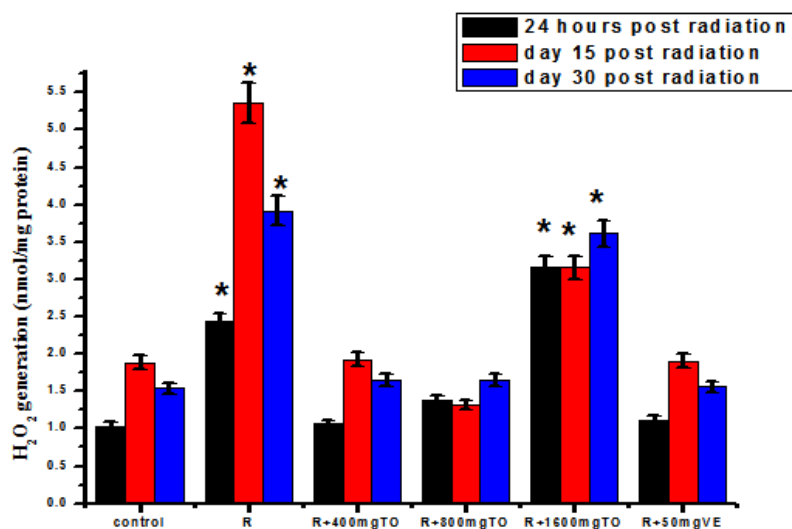


Figure 3: Effects of *Telfaria occidentalis* on gamma radiation induced increase in levels of H₂O₂ generation in brain of male Wistar rats. N= 5, * = significant difference at p < 0.05.

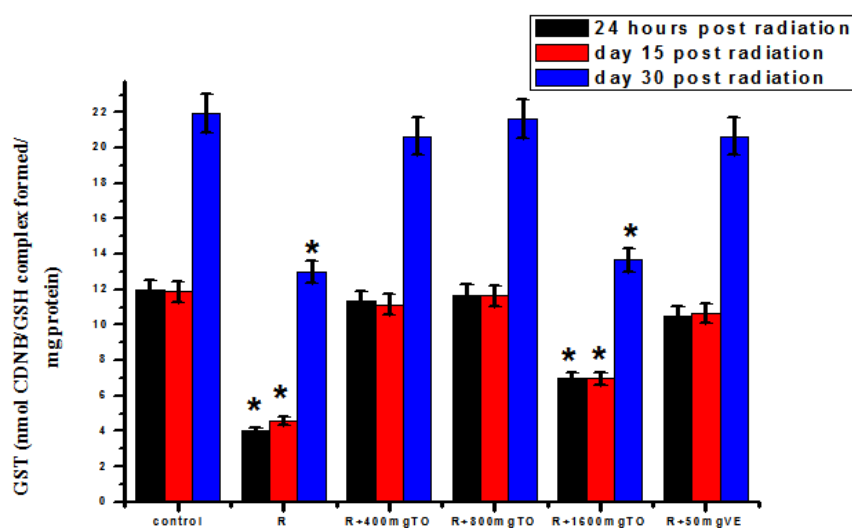


Figure 4: Effects of *Telfaria occidentalis* on gamma radiation induced decrease in activities of GST in brain of male wistar rats. N= 5, * = significant difference at p < 0.05.

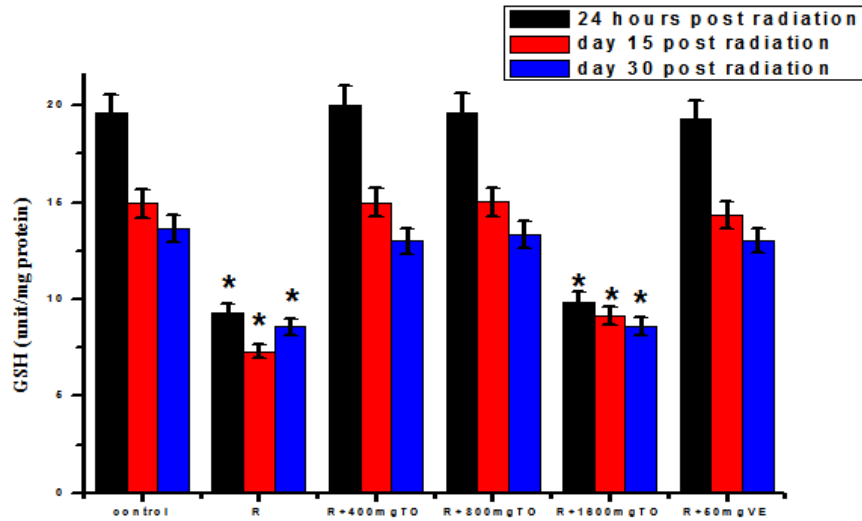


Figure 5: Effects of *Telfaria occidentalis* on gamma radiation induced decrease in levels of GSH generation in brain of male Wistar rats. N= 5, * = significant difference at p < 0.05.

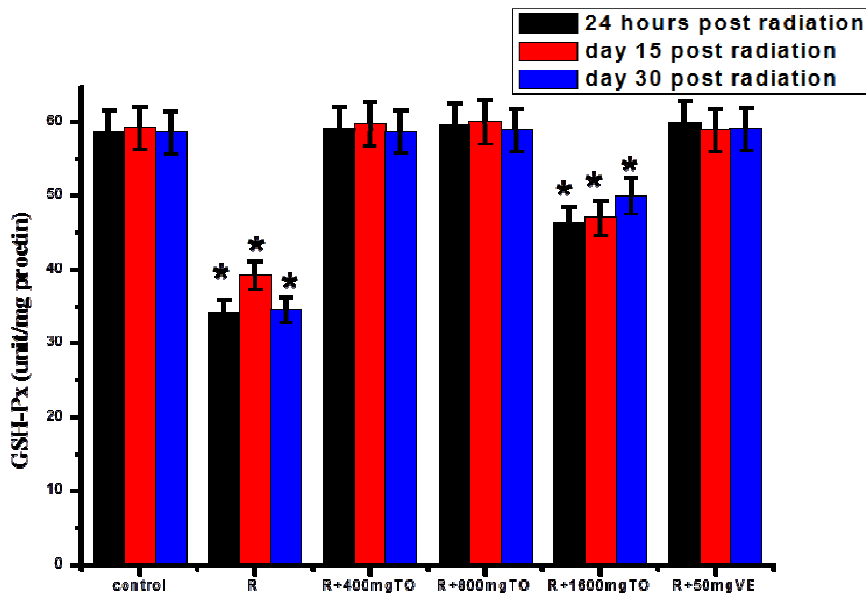


Figure 6: Effects of *Telfaria occidentalis* on gamma radiation induced decrease in activities of GSH-Px in brain of male Wistar rats. N= 5, * = significant difference at p < 0.05.

DISCUSSION

Gamma radiation exposure though a major therapeutic agent for cancer therapy constitutes a great threat to the environment. Radiation induces toxicity by generation of free radicals which can oxidize molecular targets such as deoxyribonucleic acid (DNA), protein and lipid in a process called oxidative stress resulting in cellular dysfunction (Zablocka and Janusz, 2008). Researchers have been snared into the search for safe agents that can mitigate this post radiation biological damage. The present study demonstrated that exposure to gamma radiation induced oxidative stress in brains of rats, as indicated by the concomitant increase in level of LPO, with increasing levels of H₂O₂ generation. Many environmental insults are known to cause oxidative stress, as indicated by LPO and H₂O₂ accumulation in cells (Schutzendubel *et al.*, 2001; Farombi *et al.*, 2010; Ebokaiwe *et al.*, 2013). Free radicals react with lipids and cause peroxidative changes, resulting in enhanced H₂O₂-generation and lipid peroxidation (Adedara *et al.*, 2012), which eventually was also accompanied by decreased activity of antioxidant enzymes in brain of irradiated rats in this study.

Superoxide dismutase (SOD) plays a key role in the detoxification of superoxide radical, thereby protecting cells from damage induced by free radicals (Fridovich, 1995). The observed decrease in SOD activity following gamma radiation exposure suggests increased production of superoxide radicals. Farombi *et al.* (2010) suggested that superoxide radicals by themselves, or after their formation to H₂O₂, caused oxidation of CAT and GSH-Px enzymes and thus decrease SOD activity. In this study, the reduction in GSH-Px activity may indicate inability of the brain cells to get rid of H₂O₂ generated due to exposure to gamma radiation. It may also imply enzyme inactivation caused by excess ROS formation in brain. The brain contains

less CAT levels, and hence GSH-Px has a major role in quenching H₂O₂ and other peroxides, which otherwise would lead to the production of hydroxyl and peroxy radicals in the presence of insult (Bast and Barr, 1997; Ebokaiwe *et al.*, 2013).

Glutathione-S-transferase (GST) is directly responsible for the elimination of electrophilic oxidants at the expense of GSH and has long been suspected to be important in protecting cells from oxidative stress by detoxifying some of the secondary ROS produced when ROS react with cellular constituents (Hubatsch *et al.*, 1998). The decrease in GST activity observed in gamma-radiation exposed brains may be the result of the decrease in the availability of substrate (GSH) and also alterations in its protein structure under oxidative conditions. Our observations corroborated the report of Ebokaiwe *et al.* (2013) on neurotoxicity of Nigerian Bonny light crude oil in male rats.

Interestingly, this alteration in antioxidant status of the brain of rats exposed to gamma radiation was observed as early as 24 hour post irradiation and did not reverse after day 30. It implies that the altered antioxidant defense system of brain following gamma radiation has a long term effect. However, supplementation with TOE or Vitamin E (VE) before radiation ameliorated radiation-induced neuronal toxicity in rats. This improvement could be attributed to the flavonoids content of TOE and its free radical scavenging properties which has enhanced the neuronal functions of antioxidant system of the rats.

However, pretreatment with TOE at a dose of 1600 mg/kg body weight aggravated the radiation toxicity as evident from our study from 24 hours to day 30 post radiation. This observation on TOE- induction of neuronal pathology at high doses agreed with the observations of Adedapo *et al.* (2008).

In conclusion, the above results suggested that TOE at lower doses could

significantly improve oxidative stress related pathological changes in brain of irradiated rats, and that the protective potential of *Telfairia occidentalis* on gamma radiation-induced neuronal oxidative toxicity in Wistar rats was noted. To our knowledge, this is the first report on the protective potential of TOE as a pretreatment for the management of neuronal antioxidant dysfunction following radiation exposure.

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