

British Journal of Medicine & Medical Research 4(12): 2431-2445, 2014



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Radiation-induced Testicular Injury and Its Amelioration by *Telfairia occidentalis*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAA designed the study, wrote the protocol and performed the statistical analysis. Authors JOO and APE managed the analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Author OOA read the histological slides. Author AA facilitated the radiation of experimental animals. Author EOF provided the reagents used for antioxidant assays. All authors read and approved the final manuscript.

Original Research Article

Received 6th December 2013 Accepted 18th January 2014 Published 7th February 2014

ABSTRACT

Aims: The radioprotective effect of *Telfairia occidentalis* aqueous extract (TOE) against gamma irradiation induced oxidative damage and altered sperm parameters in rats. **Study Design:** Randomized controlled experiment

Place and Duration of Study: Experimental Animal Unit and Department of Anatomy, University of Ibadan between January and April, 2012.

Methodology: Male Wistar strain rats were divided into six groups and pretreated with TOE (400, 800 and 1600 mg/kg of b.wt.) and vitamin E (VE) 50 mg/kg of b.wt prior to whole body gamma irradiation exposure of 2Gy. Sections of the testes were taken for the assessments of biochemical markers of oxidative stress: Lipid peroxidation (LPO), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) and histopathological variations.

Results: Exposure of animals to 2Gy gamma radiation resulted in decreased sperm

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characteristics and increased sperm morphological abnormalities; significant elevation in lipid peroxidation (LPO) and hydrogen peroxides, and decreased activities of superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GSH-Px) and levels of reduced glutathione (GSH) in testes; distorted architecture of seminiferous tubules from day 1 to 30 post-irradiation. The rats pretreated with TOE showed normal sperm parameters, normal testicular histo-architecture and normal antioxidant enzymatic activities.

Conclusion: Aqueous extract of Telfairia occidentalis has radioprotective activities.

Keywords: Telfairia occidentalis; testes; gamma irradiation; radioprotection; antioxidant.

1. INTRODUCTION

Plants have evolved the ability to produce chemical compounds that help them to protect against predators such as insects, fungi and herbivorous mammals. Some of these compounds whilst being toxic to plant predators turn out to have beneficial effects when used to treat human diseases; these secondary metabolites have antioxidants, immunostimulants, cell proliferation stimulators, anti-inflammatory and microbial agents that can protect against radiation-induced reactive oxygen species (ROS)-mediated damage [1]. They have been exploited successfully for the treatment of free radical-mediated diseases in humans such as rheumatoid arthritis, atherosclerosis, cancer, Alzheimer's disease, aging and several other conditions [2,3]. *Telfairia occidentalis* (family Cucurbitacea) is a tropical vine grown in West Africa and highly reputed in traditional medicine [4]. The leaves are rich in essential and non-essential amino acids, flavonoids and other antioxidants, vitamins and minerals. 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 [5].

The interaction of ionizing radiation with the biological system results in the generation of reactive oxygen species (ROS) like superoxide anion (O2⁻), hydroxyl radical (OH), hydrogen peroxide (H₂O₂), etc, which are highly reactive, short lived and known to cause damage to cellular and molecular components including lipid, protein, carbohydrate and DNA, which may lead to mutations and chromosomal aberrations [6]. Radiation induced free radicals and increase in membrane lipid peroxidation (LPO), impairs the antioxidative defense mechanism, resulting in many pathological states such as ageing, cancer, cardiovascular diseases, diabetes, inflammation and neurodegenerative diseases [7,8] and induction of oxidative stress impairs reproductive function [9]. Some damages are expressed early, while other may be expressed over a period of time depending upon the cell kinetics and radiation tolerance of the tissues [10]. In order to reduce or protect from damaging effect of oxidative stress, cells have evolved an endogenous antioxidant defence mechanism, which includes non-enzymatic entities such as glutathione, ascorbic acid, uric acid and enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px), etc. However, in the event of increased production of ROS, the host antioxidant defence mechanisms are overwhelmed resulting in the oxidative damage of cellular constituents [11].

Reproductive disorders and hazards to the health of reproductive system have become prominent issues in several reports of adverse effects of ionizing radiation on reproductive functions [12]. Oxidative stress has been a major factor in the etiology of male infertility.

Since the cells of spermatogenic lineage are especially vulnerable to radiation-induced reactive oxygen species (ROS) because they are constantly under mitosis or meiosis. Interestingly, under normal conditions, the testis is afforded with antioxidant protection as an elaborate array of antioxidant enzymes, free radical scavengers and low oxygen tension in order to support the dual action of this organ as germ cell spermatogenic [13] as well as leydig cells steroidogenic function [14]. This lends testes credence of being a highly radiosensitive organ with wide range of radiosensitive germ cells [15].

In recent times, knowledge of a wide range of natural and herbal compounds in the environment serving a radioprotective influence is of public interest. However, the use of available synthetic radioprotective agents has been limited due to their toxicity at their therapeutically effective concentrations. Therefore, a need exists for non toxic and inexpensive environmental natural products for clinical radiation protection. As radioprotection is a phenomenon, which is mediated by number of mechanisms such as scavenging of free radicals and inhibition of LPO etc., therefore use of single compounds to minimize radiation induced injuries may not yield the desired outcome [10].

Therefore, the present study was designed to determine the radioprotective effect of *Telfairia occidentalis* extract (TOE) against gamma irradiation induced oxidative stress in testes of rat.

2. MATERIALS AND METHODS

2.1 Chemicals

The following components were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA): nicotinamide adenine dinucleotide phosphate (NADP); glucose-6-phosphate; I-γ-glutamyl-3-carboxyl-4-nitronilide; glycylglycine; epinephrine; glutathione (GSH); 5, 50-dithio-bis-2-nitrobenzoic acid; hydrogen peroxide; thiobarbituric acid and 1-chloro-2, 4-dinitrobenzene. All other reagents were of analytic grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

2.2 Animal Protocol

Ninety healthy adult male Wistar rats weighing approximately 240±10 g, obtained from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, were randomly assigned to 6 groups of 15 animals each. They were housed in suspended plastic cages placed in a well-ventilated rat house, provided with rat pellets and water ad libitum and subjected to a 12:12 light-to-dark photoperiod. All animals received humane 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. Ethic regulations were followed in accordance with National and Institutional Guidelines for the Protection of Animal Welfare during the experiments (PHS 1996).

2.3 Animal Irradiation Procedures

The rats were anaesthetisized with intraperitoneal (i/p) administration of xylazine and ketamine combination at the dosage of 10mg/kg and 100mg/kg body weight respectively and then placed in a cardboard box. Each experimental animal then received 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 (Siemens one, Georgia, United States of America) 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 1mm. The entire parts of the rats' body were exposed, after radiation; the rats were placed in their cages. The dosimetry and irradiation procedures were carried out at the Radiotherapy Department of the Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria.

2.4 Experimental Design

Group I (control): Rats in this group were given distilled water for 30 days (n = 15). Group II (irradiated only): Rats in this group were exposed to 2 Gy dose of gamma radiation only (n = 15) on day 30.

Group III (radiation + vitamin E): Rats in this group were given Vitamin E (50 mg/kg/ b.wt.) for 30 days and then exposed to 2Gy dose of gamma radiation (n = 15).

Group IV (radiation + 400 mg/kg of b.wt TOE): Rats in this group were given 400 mg/kg of b.wt TOE for 30 days and then exposed to 2 Gy dose of gamma radiation (n = 15).

Group V (radiation + 800 mg/kg of b.wt TOE): Rats in this group were given 800 mg/kg of b.wt TOE for 30 days and then exposed to 2 Gy dose of gamma radiation (n =15).

Group VI (radiation + 1600 mg/kg of b.wt TOE): Rats in this group were given 1600 mg/kg of b.wt TOE for 30 days and then exposed to 2 Gy dose of gamma radiation (n = 15).

Five rats from each group were sacrificed at 24 hours, 15 and 30 days post radiation for the evaluation of biochemical, sperm parameters and histological variations in testes.

2.5 Biochemical Assays

Five right testes were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate was centrifuged at 10,000g for 15 min at 4°C. The supernatant was collected for the estimation of Superoxide dismutase (SOD) by the method described by Misra and Fridovich [16]. Glutathione-Stransferase (GST) was assayed by the method of Habig [17]. The activity of glutathione peroxidase (GSH-Px) was determined by the method of Rotruck [18]. GSH was determined at 412 nm using the method described by Jollow [19]. Hydrogen peroxide generation was assessed by the method of Wolff [20]. Lipid peroxidation was quantified as MDA according to the method described by Farombi [21] and expressed as μ m MDA/g tissue.

2.6 Sperm Motility Assay

Sperm motility was assessed by the method described by Zemjanis [22]. The motility of epidiymal sperm was evaluated microscopically within 2–4 min of their isolation from the cauda epididymis, and data were expressed as percentages.

2.7 Sperm Count

Epididymal sperm count was obtained by mincing the 5 pairs of caudal epididymis in distilled water and filtering through a nylon mesh. The spermatozoa were counted by haemocytometer using the improved Neubauer (Deep 1/10 m; LABART, Munich, Germany) chamber as described by Pant and Srivastava [23].

2.8 Live/Dead Ratio

The live-dead staining principle is based upon the observation that eosin B penetrate and stain the dead sperm cells whereas the viable cells repel this stain. In order to benefit from the live-dead method, the staining was done without delay.

2.9 Morphologic Abnormalities and Percentage Viability Assay

A portion of the sperm suspension placed on a glass slide was smeared out with another slide and stained with Wells and Awa's stain (0.2 g eosin and 0.6 g fast green dissolved in distilled water and ethanol in a 2:1 ratio). A total of 400 spermatozoa from each rat were used for morphologic examination. Also, sperm smeared slides were stained with 1% eosin and 5% nigrosine in 3% sodium citrate dehydrate solution for live-to-dead ratio assessment using the method described by Wells and Awa [24].

2.10 Histopathology

Samples from testes were fixed with Bouin's solution, sectioned, and stained routinely with haematoxylin and eosin for microscopy. All slides were coded before examination using a light microscope (Olympus, USA) by investigators who were blinded to the control and treatment groups.

2.11 Statistical Analysis

Statistical analysis were carried out using one-way analysis of variance, followed by Student's *t*-test, to compare experimental groups; p<0.05 was considered statistically significant.

3. RESULTS

3.1 Biochemical Assays

The effects of pretreatment with TOE against gamma irradiation on antioxidant systems and lipid peroxidation in testis after 24 hours, days 15 and 30 post irradiation are presented in Figs. 1a- c and Figs. 2a-c. A significant (p<0.05) decrease in the activities of antioxidant enzymes SOD and GSH-Px as well as GST, a phase-II xenobiotic detoxifying enzyme was observed in testes within 24 hours post irradiation and continued till days 15 and 30 post irradiation by 65, 50 and 70% for SOD; 60, 75, and 50% for GSH-Px and 50, 65 and 70% for GST as shown in Figs. 1a, b and c. There was a concomitant decrease in reduce GSH by 40, 65 and 55% as shown in Fig. 2c, pretreatment with TOE at doses of 400 and 800mg/kg of b. wt reversed these values by 55-85% when compared with control values as shown in Figs 1a, b, c and 2a, b, c.

However, the levels of hydrogen peroxide (H_2O_2) generation, and lipid peroxidation, increased significantly (p< 0.05) at 24 hours, days 15 and 30 post irradiation compared with control values, the levels of H_2O_2 generated in testes increased by 40, 70 and 72% as shown in Fig. 2b and MDA levels increased by 63, 70 and 60% in testes respectively as shown in Fig. 2a these also was reversed by pretreatment with TOE at doses of 400 and 800mg/kg of b.wt when compared with control values from 60-80% as shown in Fig. 2a and b.



Fig. 1a. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced decrease in superoxide dismutase (SOD) enzyme levels. R (Radiation only -2 Gy), R + 50mg VE (Radiation +Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05



Fig. 1b. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced decrease in glutathione peroxidase (GSH-P_x) enzyme levels. R (Radiation only -2 Gy), R + 50mg VE (Radiation + Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05



Fig. 1c. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced decrease in glutathione-S-transferase (GST) enzyme levels. R (Radiation only -2 Gy), R + 50mg VE (Radiation +Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05



Fig. 2a. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced alteration in lipid peroxidation (LPO) levels. R (Radiation only- 2 Gy), R + 50mg VE (Radiation +Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05



Fig. 2b. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced alteration in the levels of hydrogen peroxide generated. R (Radiation only- 2 Gy), R + 50mg VE (Radiation +Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05.</p>



Fig. 2c. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced alteration in reduced glutathione levels. R (Radiation only - 2 Gy), R + 50mg VE (Radiation +Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05.

3.2 Sperm Analysis

Fig. 3 shows the data on, sperm motility, sperm count, morphological abnormalities assay and live/dead ratio. There was a decrease in sperm motility, and count as shown in Figs 3a, b. Compared with controls, live/dead ratio decreased by 34, 36, and 38%, respectively, at 24 hours, days 15 and 30 post irradiation as shown in Fig. 3d and the decreases in sperm motility were 40, 41 and 43%, respectively as shown in Fig. 3a, while decrease in sperm count was 40, 41 and 39 at 24 hours, days 15 and 30 respectively as shown in Fig. 3b. The percentage of morphologically abnormal sperm increased by 47, 45 and 44 respectively when compared with control as shown in Fig. 3c. The altered sperm parameters were prevented by TOE pretreatment at doses of 400 and 800 mg/kg of b.wt at various points.



Fig. 3a. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced alteration in sperm motility. R (Radiation only- 2 Gy), R + 50mg VE (Radiation +Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05



Fig. 3b. Effects of *Telfairia occidentalis* (TOE) on gamma radiation induced alteration in sperm count. R (Radiation only -2 Gy), R + 50mg VE (Radiation +Vitamin E), R + 400mg (Radiation + 400mg dose of TOE), R + 800mg (Radiation + 800mg dose of TOE), R + 1600mg (Radiation + 1600mg dose of TOE), *p<0.05

British Journal of Medicine & Medical Research, 4(12): 2431-2445, 2014









3.3 Histopathology

Histological sections of radiation only rats showed drastic pathological lesions in the tubular architecture when compared with the control or TOE (400 and 800 mg/kg of b.wt). Distorted architecture of seminiferous tubules was seen in the form of foci of germinal erosion, sloughing with clumps of germinal cells in the lumen of the tubules, reduced height of germinal epithelium, severe germinal epithelial erosion in some tubules and moderate subcapsular congestion. The TOE and Vit E pretreatment groups remarkably offers significant radioprotection as evident in the form of intact germinal epithelium of the seminiferous tubules, mild interstitial oedema, moderate sub-capsular congestion at day 1 and 15 post radiation as shown in Figs. 4a and 4b and a virtually normal testicular architecture was visualized by the end of experiment day 30 as shown in Figs. 4c. Over all result revealed a possible protective effects of TOE at doses of 400 and 800 mg/kg of b.wt when compared with 50 mg/kg of b.wt TOE pretreatment group, there was no protection indicating that the radiation induced testicular toxicity might be potentiated by a high dose of TOE.



Fig. 4A. Amelioration of radiation-induced histological changes in testes of Wistar rats after 24 hours post-treatment by *T. occidentalis* extract. G 1– (Control):, G 2– (Radiation only): reduced height of germinal epithelium of seminiferous tubule (bar), defoliated germinal cells in the lumen of the tubules (asterick), G 3 - (vitamin E): no visible lesion, G 4 – (R+400mg): no visible lesion, G 5 – (R+800mg): mild germinal epithelial erosion (thin arrow), G 6 – (R+1600mg): reduced height of seminiferous tubular germinal epithelium (bar). Magnification: × 400; Stain; Haematoxylin and Eosin



Fig. 4B. Amelioration of radiation-induced histological changes in testes of Wistar rats at day 15 post-treatment by *T. occidentalis extract*. G 1 – (Control): shows normal architecture of testis , G 2 – (Radiation only): foci of germinal erosion (thin arrow), sloughing with clumps of germinal cells in the lumen of the tubules (asterick) and reduced height of germinal epithelium (bar) G 3 - (vitamin E): mild seminiferous tubular germinal epithelia erosion (thin arrow), G 4 – (R+400mg): no visible lesion, G 5 – (R+800mg): mild subcapsular congestion (dark thick arrow), G 6 – (R+1600mg): severe seminiferous tubular germinal epithelial erosion (thin arrow) with few clumps of germinal cells in lumen (asterick). Magnification: × 400; Stain; Haematoxylin and Eosin



Fig. 4C. Amelioration of radiation-induced histological changes in testes of Wistar rats at Day 30 post-treatment by *T. occidentalis* extract. G 1 – (Control): shows normal architecture of testis , G 2 – (Radiation only): severe germinal epithelial erosion (thin arrow) in some tubules and moderate subcapsular congestion (dark thick arrow), G 3 - (vitamin E): no visible lesion , G 4 – (R+400mg): no visible lesion, G 5 – (R+800mg): mild interstitial oedema (white thick arrow), G 6 – (R+1600mg): mild seminferous tubular germinal epithelium erosion (thin arrow). Magnification: × 400; Stain; Haematoxylin and Eosin

4. DISCUSSION

Exposure to ionizing radiation severely increases oxidative load on the testicular system. Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, iron oxygen complexes, hydrogen peroxide and lipid peroxides are generated due to the ionization reactions mediated by gamma radiations [6]. When ROS generation is immense, as it is during gamma irradiation exposure, the cytotoxic effect is not merely local but may result in intracellular and extracellular transmission. This increases the interaction of the radicals with phospholipid structures, induce peroxidation and increase free radical induced DNA damage [10].

The significant increase in testicular LPO and concomitant accumulation of H_2O_2 in irradiated group as compared to control was a consequence of increased oxidative stress and cellular damage. However, decreases in levels of LPO and H_2O_2 generation was observed in animals pretreated with TOE, prior to gamma irradiation exposure suggesting that TOE at doses of 400 and 800 mg/kg of b.wt strengthened the antioxidant defense system and prepared the animals to withstand the damaging effects of irradiation. Antioxidant enzymes i.e., SOD, GSH-Px and GST activity in testes showed significant decrease in gamma irradiated group probably due to the excessive generation of free radicals and increased oxidative stress. The superoxide anions are generally dismutated by SOD to form H_2O_2 , which is broken down by CAT and GSH-Px, however GSH-Px is more prominent in testicular milieu [7,25]. It is suggested that decrease in testicular antioxidant enzymes activity in gamma irradiated animals may be due to the denaturation of enzymes by gamma irradiation exposure. Moreover, decrease in GSH-Px activity in animals exposed with gamma irradiation was probably due to the inactivation of enzyme, as a flux of superoxide anions have been shown to reduce GSH-Px activity [9,26]. Our results were in concurrence with the

earlier studies, in which gamma irradiation exposure to animals showed marked increase in LPO and inhibition of SOD, GSH-Px and GST activity [10,27].

GST is directly responsible for the elimination of electrophilic oxidants at the expense of GSH [17]. The decrease in GST activity observed in testes of irradiated rats may be the result of the decrease in the availability of substrate (GSH) and also alterations in its protein structure under oxidative conditions [28]. Interestingly, pretreatment with VE 50 mg/kg of b.wt and TOE 400 and 800 mg/kg of b.wt showed radioprotection by increasing antioxidant capacity of the testicular micro environment. However higher dose of TOE (1600mg/kg of b.wt) tends to exacerbate the radiation induced testicular toxicity.

Exposure to gamma radiation markedly decreased epididymal sperm count, sperm motility and live/dead ratio, with a visible increase in sperm abnormalities in rats. Therefore, the observed decrease in testicular and epididymal sperm numbers may indicate that radiation affects the early stages of spermiogenesis. Abnormalities in sperm morphology observed in this study may be the consequence of increased lipid peroxidation of polyunsaturated fatty acids in the sperm plasma membrane. Impaired sperm motility may result in infertility caused by failure of the sperm to reach the site of fertilization as well as their ability to penetrate zonal pellucida [28]. It is commendable to note that pretreatment of TOE with its rich antioxidant properties prior to irradiation was able to improve sperm quality of rats. Histopathological studies showed that exposure to gamma radiation produced significant testicular damage in rats. The histological studies also suggest that pretreatment with TOE prior to gamma irradiation reduced the extent of testicular radiation damage and accelerated the recovery process at the cellular level. These effects may be attributed to the inhibition of oxidative stress and radioprotective function against gamma irradiation exposure by TOE.

TOE pretreatment protected or reduced the severity of irradiation induced testicular toxicity. Results from this experiment showed that the pretreatment with TOE (400 and 800 mg/kg of b.wt.) significantly increased activities of SOD, GSH-Px, GST, GSH content and reduced testicular damage.

5. CONCLUSION

Telfairia occidentalis has potent antioxidative potential and radioprotective effect against Gamma irradiation induced oxidative assault.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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