Acta Biologica Hungarica 61(3), pp. 250–261 (2010)
DOI: 10.1556/ABiol.61.2010.3.2

EFFECTS OF *XYLOPIA AETHIOPICA* (ANNONACEAE) FRUIT METHANOL EXTRACT ON γ-RADIATION-INDUCED OXIDATIVE STRESS IN BRAIN OF ADULT MALE WISTAR RATS

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(Received: September 4, 2009; accepted: November 3, 2009)

Xylopia aethiopica (XA) (Annonaceae) possesses great nutritional and medicinal values. This study was designed to investigate the effects of XA fruit methanol extract on oxidative stress in brain of rats exposed to whole body γ-radiation (5 Gy). Vitamin C (VC) served as standard antioxidant. Forty-four rats were divided into 4 groups of 11 rats each. One group served as control, two different groups were treated with XA and VC (250 mg/kg), 6 weeks before and 8 weeks after irradiation, and fourth group was only irradiated. Rats were sacrificed 1 and 8 weeks after irradiation. The antioxidant status, viz. Lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and glutathione (GSH) were estimated. Results indicate a significant increase ($p < 0.05$) in levels of brain LPO after irradiation. LPO increased by 90% and 151%, after 1 and 8 weeks of irradiation, respectively. Irradiation caused significant ($p < 0.05$) decreases in levels of GSH and GST by 61% and 43% after 1 week and, 75% and 73%, respectively, after 8 weeks of exposure. CAT and SOD levels were decreased by 62% and 68%, respectively, after 8 weeks of irradiation. Treatment with XA and VC ameliorated the radiation-induced decreases in antioxidant status of the animals. These suggest that XA could have beneficial effect by inhibiting oxidative damage in brain of exposed rats.

Keywords: Antioxidant enzymes – brain – oxidative stress – radioprotection – vitamin C

INTRODUCTION

Living organisms are constantly exposed to a shower of ionizing radiation from natural sources such as cosmic rays and radio nuclides present in the earth's crust (telluric), or through artificial sources like medical and industrial radiation, nuclear exposure, industrial accidents, etc. Ionizing radiations are thus integral part of our life. According to the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) report in 1986, acute doses above 2.0 Gy, 2-0.2 Gy, below 0.2 Gy are regarded as high, intermediate and low-doses, respectively [32]. Either used for medical purpose or accidental discharge, ionizing radiation affects normal cellular activities leading to the production of reactive oxygen species (ROS). The major types of ROS or ROS-producing species are superoxide anion ($O_2^{-\bullet}$), hydrogen per-

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oxide (H_2O_2), and hydroxyl ($O^{\bullet}$) radicals [9]. ROS present a paradox in their biological function: on the one hand, they prevent diseases by assisting the immune system, mediating cell signaling and playing an essential role in apoptosis. On the other hand, they can damage many biologically active molecules, leading to tissue damages and cell death, other side-effects include, nausea, vomiting, diarrhea, etc. [14]. Therefore, the design of strategies capable of protecting normal tissues from the lethal actions of radiation is of great interest in radiation biology. Currently, no efficient radioprotectant is clinically available. Amifostine (WR 2721), approved by the U.S. Food and Drug Administration in 1999, is still used in radiation oncology clinics but with low potency due to the stoichiometric nature of its action, very low bioavailability and toxicity [11, 17]. The ability of a compound to act as an antioxidant by quenching ROS is linked to its potential as a radioprotectant. This is not surprising given that the production of ROS is one indirect effect of radiation that can irrevocably damage DNA and lead to cell death in dividing cells. A number of radioprotective compounds have been identified from medicinal plants. Some of these plants include; *Phyllantus amaurus, Aaegle marmelos, Vernonia amygdalina, Amarathus paniculatus,* etc. [1, 16, 18, 20]. It is pertinent to look inwards to other herbs with antioxidant property as radioprotectants. In the present study, the possible protective effect of methanolic extract from *Xylopia aethiopica* was investigated against γ-radiation induced oxidative stress in brain of adult male Wistar rats.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid and thiobarbituric acid were purchased from BDH Chemical Ltd., Poole, UK. Other chemicals were procured from Sigma Chemical Co., Saint Louis, MO USA and were of analytical grade and purest quality available.

Preparation of plant extracts

Samples of fresh fruit of *Xylopia aethiopica* were obtained in Ibadan, Nigeria. Their botanical identification and authentication were confirmed at the Department of Botany, University of Ibadan, Nigeria, where voucher specimen were kept at the herbarium (Voucher number UI-036187). The fruits were sliced into pieces and air-dried at room temperature and then powdered. The powdered samples (1.2 kg) were de-fatted with *n*-hexane (2.5 litres) and extracted with 75% methanol (2.5 litres) overnight in a soxhlet extractor. The methanol extract was concentrated and evaporated to dryness at 50 °C with a rotary evaporator under reduced pressure. The yield of the preparation was 5.8%. Prior to the experiments, the methanol extract was dissolved in corn oil at a concentration of 4 g/100 ml overnight. Aliquots of different concentrations were given orally to animals with a gavage needle.

Animals

Inbred 8–9 weeks old male Wistar albino rats weighing 235–250 g were purchased from the Animal House of the Physiology Department, University of Ibadan, Nigeria. The animals were kept in well-ventilated cages at room temperature (28–30 °C). They were maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. All animal experiments were conducted without anaethesia in the present study and, the protocol conforms to the guidelines of National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.

Irradiation

The animals were treated with a single dose of gamma radiation of 500 rads (5 Gy). The source of radiation was a ^{60}Co gamma chamber (Model-220, Atomic Energy of Canada Ltd.) used in the Radiotherapy unit of the University College Hospital, Ibadan, Nigeria. The animals were kept in specially designed well-ventilated cages, their movements were restricted and no anaesthesia was administered. The animals were exposed to whole body radiation at a rate of 1.4 Gy/min in a field size of about 25×25 cm^2 and at a distance of 70 cm from the source.

Experimental design

Forty-four rats were used for the experiment, and the animals were randomly divided into four groups of eleven animals each. The treatment of the groups is given in Table 1.

Animals in the test groups were treated with XA and VC for 6 weeks prior to and 8 weeks after irradiation. XA and VC were administered orally at a dose of 250 mg/kg body weight daily. VC was dissolved in water and XA in corn oil (vehicle). Furthermore, all animals, except the N group were exposed to whole body radiation

Table 1
Treatment of groups

Groups	Treatment
N	Normal (Un-irradiated animals)
XA	Irradiated animals treated with XA extract (250 mg/kg body wt. p.o.)
RA	Irradiated control treated with vehicle p.o.
VC	Irradiated animals treated with vitamin C (250 mg/kg body wt. p.o.)

N = Normal, XA = *Xylopia aethiopica*, RA = Irradiated alone, VC = Vitamin C, p.o. = per oral.

(5 Gy). The body weights of all animals were determined a day prior to irradiation and every third day thereafter. One week after irradiation, five animals in each group (n = 5) were sacrificed by cervical dislocation, and the remaining surviving animals sacrificed after 8 weeks of irradiation. Whole brain was immediately dissected out and washed in ice-cold 1.15% KCl to remove the blood stain. The brain tissues were weighed and 10% tissue homogenate was prepared with 5 mM phosphate buffer, pH 7.4. After centrifugation at 10,000 *g* for 15 minutes to obtain post-mitochondrial supernatant fraction (PMF), the clear supernatant was used to measure the protein contents, levels of lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and reduced glutathione (GSH).

Determination of protein contents and antioxidant profile

The total protein level in the brain was determined according to the method of Lowry et al. [21] using bovine serum albumin as standard.

Superoxide dismutase activity (SOD) was measured by the nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich [25]. To the supernatant (0.5 ml), 0.025 M sodium pyrophosphate buffer (pH 8.3), phenazine methosulphate, NBT and NADH were added, and incubated at 30 °C for 90 seconds. The reaction was stopped by the addition of glacial acetic acid and mixed with *n*-butanol. The intensity of the chromogen in the *n*-butanol was measured at 560 nm using Beckman DU 70^R spectrophotometer.

Glutathione-s-transferase (GST) activity was determined by the method of Habig et al. [15], the method is based on the rate of conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB). Thirty µl of GSH was introduced into the blank and test tubes. It was followed by 150 µl of enzyme substrate (CDNB) to blank and test. Then 30 µl of sample was added to the test alone, and the tubes were made up to total volume of 3.0 ml with 0.1 M phosphate buffer, pH 6.5. The reaction was made to run for 60 seconds before taken the absorbance against blank at 340 nm with Beckman DU 70^R spectrophotometer.

Catalase (CAT) activity was assayed by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi [2], briefly, reaction mixture contained phosphate buffer (0.01 M, pH 7.0), sample and 2.0 M H_2O_2. The reaction was stopped by the addition of dichromate-acetic acid reagents (5% potassium dichromate and glacial acetic acid were mixed in a ratio of 1 : 3).

Glutathione (GSH) level was assayed by measuring the rate of formation of chromphoric product in a reaction between DTNB (5,5[1]-dinitrobis-(2-nitrobenzoic acid) and free sulphydryl groups (such as reduced glutathione) according to the method of Moron et al. [26], briefly, 1.0 ml of the supernatant was treated with 0.5 ml Ellman's reagent (19.8 mg of 5,5[1]-dinitrobis-2-nitrobenzoic acid in 100 ml of 0.1% sodium nitrite) and 3.0 ml of 0.2 M phosphate buffer (pH 8.0). The absorbance of the colour formed was read at 412 nm with Beckman DU 70^R spectrophotometer.

The extent of lipid peroxidation (LPO) was estimated by the method of Buege and Aust [7], the method involves the reaction between malondialdehyde (MDA) (end product of LPO) and thiobarbituric acid (TBA). A 1.0 mg/ml final concentration of sample was incubated for 6 hours at 37 °C with or without 1 mM FeSO4; 1 mM ascorbate and 0.2M H_2O_2 (final concentration). A 0.5 ml of 0.75% TBA in 0.1M HCl was added to 0.5 ml of the incubation mixture already quenched with 0.5 ml of 10% TCA. The mixture was heated at 90–95 °C for 25 minutes in a boiling water bath and then cooled. The mixture was then centrifuged at 3,000 rpm for 10 minutes, and the absorbance of supernatant read at 532 nm with Beckman DU 70^R spectrophotometer.

Table 2
Effect of dried fruits extract of *Xylopia aethiopica* on the body weight of wistar albino rats before and after exposure to gamma radiation (5 Gy)

Grouping	Body weight (g)		
	Before irradiation (n=11)	1 week after irradiation (n=5)	8 weeks after irradiation (n=3–6)
N	209.1±6.13	215.7±3.31	288.5±8.00
XA	228.2±8.06	216.4±6.69	166.9±7.50*
RA	223.0±6.09	186.4±3.07**	117.4±5.66*
VC	211.3±7.45	201.2±7.083	154.4±3.08*

Values are mean ± S.D. of n = 3–11 according to the groups. *Significantly different from normal ($p<0.05$). **Significantly different from normal, XA and VC ($p<0.05$). N = Normal (un-irradiated animals), XA = *Xylopia aethiopica* (250 mg/kg) treated before irradiation, RA = Irradiated alone, VC = Vitamin C (250 mg/kg) treated before irradiation.

Table 3
Effect of dried fruits extract of *Xylopia aethiopica* on the weight of brain, relative weight and protein contents in rats exposed to gamma radiation (5 Gy)

Grouping	Protein content (mg/g tissue)		Weight of brain (g)		Relative weight of brain (% of body weight)	
	1 wk	8 wk	1 wk	8 wk	1 wk	8 wk
N	0.60±0.06	0.63±0.07	2.51±0.82	2.91±0.83	1.16±0.24	1.01±0.41
XA	0.62±0.05	0.55±0.06	2.71±0.75	2.26±0.69	1.25±0.48	1.35±0.43
RA	0.59±0.07	0.24±0.03*	1.99±0.79	1.43±0.38**	1.07±0.37	1.22± 0.38
VC	0.58±0.05	0.31±0.03	2.11±0.72	2.18±0.67	1.05±0.38	1.41±0.51

Values are mean ± S.D. of 5–6 animals per group. *Significantly different from normal and XA ($p<0.05$). **Significantly different from normal, XA and VC ($p<0.05$). N = Normal (un-irradiated animals), XA = *Xylopia aethiopica* (250 mg/kg) treated before irradiation, RA = Irradiated alone, VC = Vitamin C (250 mg/kg) treated before irradiation.

Statistical analysis

All values were expressed as the mean ± S.D. of five animals sacrificed after 1 week of irradiation and 3–6 animals after 8 weeks of irradiation. Data were analyzed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis of biochemical data using spss (10.0) statistical software. Values were considered statistically significant at $p<0.05$.

RESULTS

The body weight of irradiated animals significantly ($p<0.05$) decreased after 1 and 8 weeks of exposure. However, treatments with XA and VC ameliorated the radiation induced weight loss after 1 week of exposure. In contrast, the body weights of XA- and VC-treated animals significantly ($p<0.05$) decreased when compared to the control after 8 weeks of irradiation (Table 2). There were no significant ($p>0.05$) changes in the brain protein contents, total and relative weight of irradiated animals after 1 week of irradiation, whereas, significant decreases ($p<0.05$) were observed in protein contents and total weight of brain in the irradiated animals after 8 weeks of exposure. However, treatments with XA and VC significantly ($p<0.05$) ameliorated the adverse effects of radiation on the brain protein contents and total weight after 8 weeks of irradiation (Table 3). In Figure 1, γ-radiation caused a significant increase ($p<0.05$) in the levels of brain lipid peroxidation (LPO) after 1 and 8 weeks of irradiation. Precisely, brain LPO levels were increased by 90% and 151%, after 1 and 8 weeks of irradiation, respectively. The radiation-induced increases in brain LPO were significantly ($p<0.05$) attenuated in XA- and VC-treated animals after 1 week of

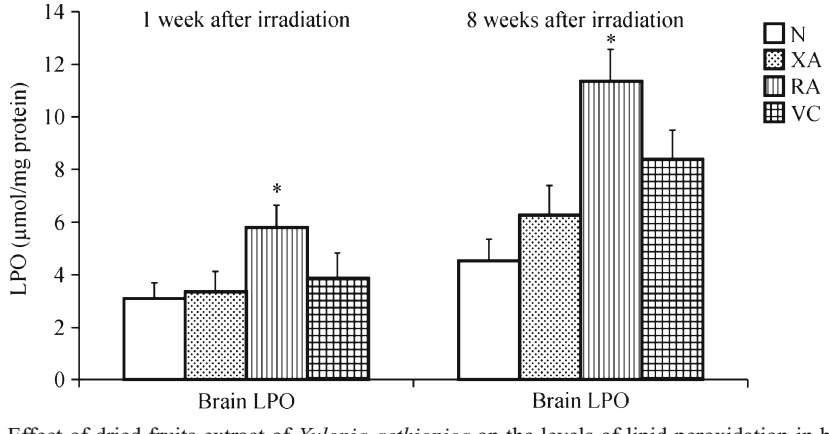

Fig. 1. Effect of dried fruits extract of *Xylopia aethiopica* on the levels of lipid peroxidation in brain of rats exposed to gamma radiation (5 Gy). *Significantly different from normal, XA and VC ($p<0.05$). N = Normal (un-irradiated animals), XA = *Xylopia aethiopica* (250 mg/kg) treated before irradiation, RA = Irradiated alone, VC = Vitamin C (250 mg/kg) treated before irradiation, LPO = Lipid peroxidation

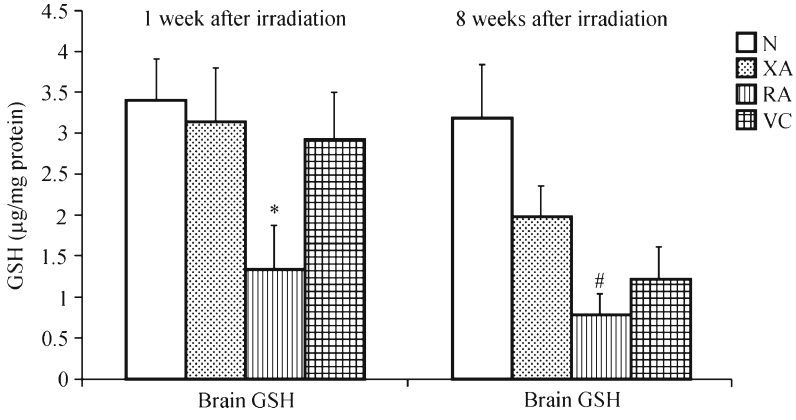

Fig. 2. Effect of dried fruits extract of *Xylopia aethiopica* on the levels of reduced glutathione in brain of rats exposed to gamma radiation (5 Gy). *Significantly different from normal, XA and VC ($p<0.05$). #Significantly different from normal and XA ($p<0.05$). N = Normal (un-irradiated animals), XA = *Xylopia aethiopica* (250 mg/kg) treated before irradiation, RA = Irradiated alone, VC = Vitamin C (250 mg/kg) treated before irradiation, GSH = Reduced glutathione

irradiation. Furthermore, in XA-treated animals, brain LPO levels were restored to values that are statistically similar to normal after 8 weeks of exposure. Furthermore, γ-radiation caused significant ($p<0.05$) decreases in levels of brain reduced glutathione (GSH) and glutathione-s-transferase (GST) after 1 and 8 weeks of exposure (Figs 2, 3). Brain GSH and GST levels were decreased by 61% and 43% after 1 week, and by 75% and 73% after 8 weeks of exposure, respectively. Treatment with XA completely restored the brain GSH and GST levels of irradiated animals after 1 and 8

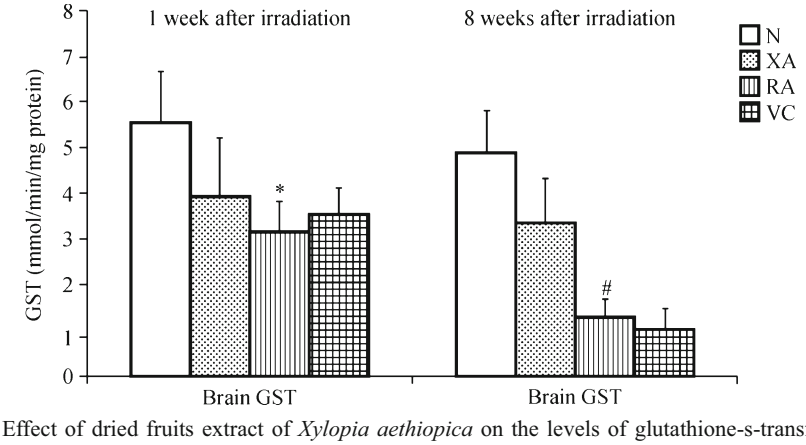

Fig. 3. Effect of dried fruits extract of *Xylopia aethiopica* on the levels of glutathione-s-transferase in brain of rats exposed to gamma radiation (5 Gy). *Significantly different from normal ($p<0.05$). #Significantly different from normal and XA ($p<0.05$). N = Normal (un-irradiated animals), XA = *Xylopia aethiopica* (250 mg/kg) treated before irradiation, RA = Irradiated alone, VC = Vitamin C (250 mg/kg) treated before irradiation, GST = Glutathione-s-transferase

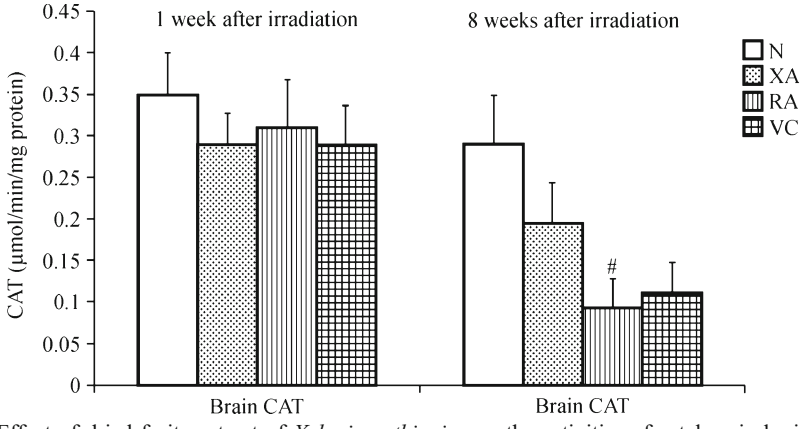

Fig. 4. Effect of dried fruits extract of *Xylopia aethiopica* on the activities of catalase in brain of rats exposed to gamma radiation (5 Gy). #Significantly different from normal and XA ($p<0.05$). N = Normal (un-irradiated animals), XA = *Xylopia aethiopica* (250 mg/kg) treated before irradiation, RA = Irradiated alone, VC = Vitamin C (250 mg/kg) treated before irradiation, CAT = Catalase

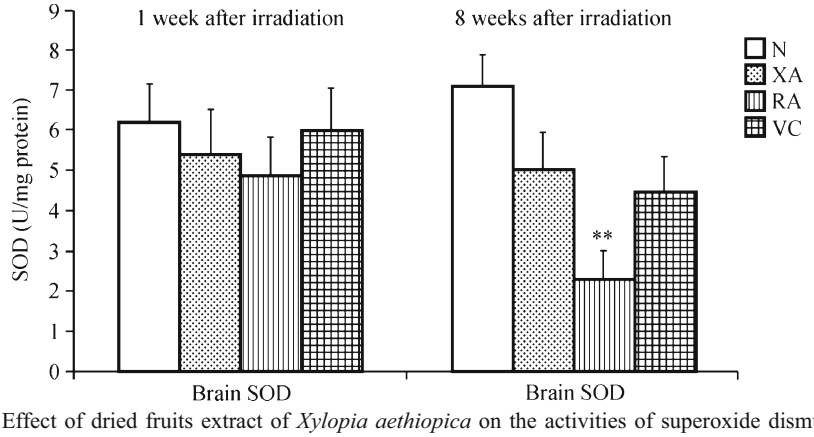

Fig. 5. Effect of dried fruits extract of *Xylopia aethiopica* on the activities of superoxide dismutase in brain of rats exposed to gamma radiation (5 Gy). **Significantly different from normal, XA and VC ($p<0.05$). N = Normal (un-irradiated animals), XA = *Xylopia aethiopica* (250 mg/kg) treated before irradiation, RA = Irradiated alone, VC = Vitamin C (250 mg/kg) treated before irradiation, SOD = superoxide dismutase

weeks of exposure, while treatment with VC could only restored the depleted brain GSH levels after 1 week of irradiation (Figs 2, 3). In Figures 4 and 5, γ-radiation decreased the brain CAT and SOD levels of irradiated animals by 62% and 68%, respectively, after 8 weeks of exposure. However, the radiation-induced decrease in brain CAT was significantly ($p<0.05$) attenuated in XA-treated animals, while both XA- and VC-treated animals had their SOD levels elevated by 121% and 97%, respectively, when compared to irradiated animals.

DISCUSSION

Free radicals arising from either normal metabolism or induced by environmental sources interact continuously in biological systems. Oxidants/antioxidants must be kept in balance to minimize molecular, cellular and tissue damage [30]. Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals are formed in the course of normal cellular metabolism and their productions accelerated in irradiated animals. The deleterious biological consequences of ionizing radiation, especially with respect to causing mutation and carcinogenesis, are well documented. Due to the high concentration of water in metabolizing cells, exposure of biological systems to radiation primarily leads to its radiolysis furnishing ROS [6]. The biological damage induced by radiation is mostly indirect and mediated by ROS such as $^{\bullet}OH$, $O_2^{-\bullet}$, and H_2O_2. These species are known to cause degradation of important macromolecules including DNA and membranes [31]. Thus, the high level of unsaturated lipids is most susceptible to oxidative damage, resulting in disruption of cellular integrity, inactivation of cellular components, and so forth, that lead to cytotoxicity [13] and cause several diseases and aging [29]. Likewise, DNA molecules are also prone to radiation-induced lesions due to the presence of various reactive sites (base and sugar) in them [28]. For a variety of tissues, the pathophysiologic importance of ROS-mediated oxidative injury caused by the exposure to radiation has been widely evaluated [19].

In the current study, the capacity of XA in preventing γ-radiation-induced lipid peroxidation (LPO), attenuating the inhibition of GSH, SOD, CAT and GST was assessed in adult male Wistar albino rats. Several studies have demonstrated a high level of TBARS and hydroperoxides (LPO markers) in brain of various stress exposed animal models [33]. This observation was confirmed in this study in which the LPO levels of irradiated animals were significantly elevated after 1 and 8 weeks of exposure. This may be because; the brain contains relatively high concentration of easily peroxidizable fatty acids [8]. In addition, it is known that certain regions of the brain are highly enriched in iron, a metal that, in its free form, is catalytically involved in production of damaging oxygen species via the metal catalyzed Haber-weiss reaction or Fenton reaction [10]. Thus, suggesting increased production of ROS in the brain of irradiated animals. Administration of extract from XA and VC effectively decreased LPO levels in the brain of irradiated animals. This clearly showed that XA effectively reduced the radiation-induced oxidative stress. This observation is similar to the findings of Manikandan et al. [23], which observed that the methanol extract from *Acorus calamus* decreased brain LPO levels of animals exposed to noisy environment. It has been suggested that ROS are responsible for radiation-induced toxicity, therefore destruction of superoxide radical or H_2O_2 by SOD or CAT would ameliorate such toxicity, as it means that the enzymes would be able to scavenge the ROS generated [22]. Vulnerability of brain to oxidative stress induced by ROS seems to be due to its high oxygen demand, since the brain utilizes about one fifth of total oxygen intake, and on the other hand, it is not particularly enriched, when compared to other organs, in any of the antioxidant enzymes. Thus, the relatively low levels of these

enzymes may be responsible in part for the vulnerability of this tissue [4]. It not surprising, therefore that the activities of the antioxidant enzymes (SOD, CAT and GST) were significantly decreased in the irradiated animals. The altered balance of the antioxidant enzymes caused by the decreased CAT, SOD and GST activities may be responsible for the inadequacy of the antioxidant defenses in combating ROS mediated damage during irradiation. The decreased activities of CAT and SOD may be a response to increased production of H_2O_2 and $O_2^{-\bullet}$ due to radiation exposure. These enzymes have been suggested as playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to oxidants attack [5]. Treatment with extract from XA and VC increased the activity of these enzymes and may help to control ROS generated by radiation. The increase in SOD activity may protect CAT and GST against inactivation by superoxide anions, which has been shown to inactivate CAT and GST [3]. Under *in vivo* conditions, GSH acts as an antioxidant and could be taken as the most accurate single indicator of health of the cell, as GSH depletion determines the vulnerability to oxidants attack. In this study, a significant decrease in GSH levels in brain of irradiated animals was observed. The decrease in GSH levels represents increased utilization due to oxidative stress [24]. The depletion of GSH content may be the reason for the lowered GST activity [34]. The increased GSH content in the brain of rats treated with XA and VC may be a factor responsible for inhibition of LPO. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies ROS generated from exposure to radiation [34]. The significant increase in GSH contents and GSH dependent enzyme (GST) in irradiated animals treated with XA indicates an adaptive mechanism in response to oxidative stress. Significantly lower levels of lipid peroxides in brain of XA-treated rats and, increased activities of enzymic and non-enzymic antioxidants in brain suggest that XA may reduce oxidative stress by quenching free radicals. Diterpenoids (such as 7α-hydroxytrachyloban-19β-oic acid) and alkaloids (such as oxophoebine, liriodenine, oxoglaucine, O-methylmoschatoline and lysicamine) have been isolated from *Xylopia aethiopica* and were reported to have free radical scavenging and antioxidant capacities [12, 27], which may be responsible for the increased antioxidant status in irradiated animals treated with XA.

CONCLUSION

Extract from *Xylopia aethiopica* effectively prevents the γ-radiation-induced oxidative stress in brain of exposed rats. This anti-stressor effect might be due to an increase in brain antioxidative capacity which in turn could be achieved by protection of decreasing GSH, SOD, CAT and restoring the GST detoxifying capacity of the brain. This is the first report to provide a direct evidence for the anti-stressor effect of *Xylopia aethiopica* in the brain of irradiated animals. Since the induction of antioxidant enzymes is considered to be a reliable marker for evaluating the radioprotective

efficacy of medicinal plants, these findings are suggestions of possible use of *Xylopia aethiopica* as a radioprotectant to improve general health conditions in human subjects during and after radiotherapy. Extensive safety and toxicological studies are required before recommending the use of *Xylopia aethiopica* in clinical settings.

ACKNOWLEDGEMENT

This study was partly supported by Senate Research Grant number SRG/COM/2006/36A of the University of Ibadan, Ibadan, Nigeria awarded to OAA.

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