Protective Effect of Kolaviron, a Biflavonoid from *Garcinia kola* Seeds, in Brain of Wistar Albino Rats Exposed to Gamma-Radiation

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This study was designed to evaluate the protective effect of kolaviron (KV), a biflavonoid from *Garcinia kola* seeds, against γ -radiation (5 Gy)-induced oxidative stress in brain of Wistar rats. Vitamin C (VC) served as standard antioxidant. Forty-four rats were divided into 4 groups of 11 animals each. One group was un-irradiated (normal), two groups were treated with KV and VC (250 mg/kg) for 6 weeks prior to and 8 weeks after irradiation, and fourth group was only irradiated. Rats were sacrificed 1 and 8 weeks after irradiation. Cellular alterations were monitored using changes in the levels of malondialdehyde (MDA)—an index of lipid peroxidation, superoxide dismutase (SOD), glutathione-S-transferase (GST), reduced glutathione (GSH), catalase (CAT), alanine and aspartate aminotransferases (ALT and AST), urea and creatinine. MDA levels increased significantly ($p < 0.05$) by 90% and 151% after 1 and 8 weeks of irradiation. Furthermore, levels of GSH and antioxidant enzymes were significantly ($p < 0.05$) decreased in γ -irradiated animals. GSH and GST decreased by 61% and 43% after 1 week, and by 75% and 74%, after 8 weeks of exposure, respectively. γ -Irradiation decreased SOD and CAT levels by 53% and 68%, respectively, and caused significant ($p < 0.05$) increases in serum ALT, AST and urea after 8 weeks of exposure. Treatment with KV and VC significantly decreased the levels of MDA, ALT, AST and urea. The antioxidant indices were significantly ameliorated in KV-treated animals. These data suggest that kolaviron may protect against γ -radiation-induced oxidative stress in brain of exposed rats.

Key words antioxidant enzyme; brain; radioprotection; kolaviron; vitamin C

Ionizing radiation is an important environmental risk factor for various cancers and also a major therapeutic agent for cancer treatment. Exposure of mammalian cells to radiation induces several types of damage to DNA, including double and single-strand breaks, base and sugar damage, as well as DNA–DNA and DNA–protein cross-links.¹⁾ During radiotherapy, ionizing radiation interacts with biological systems to induce excessive oxygen free radicals or reactive oxygen species (ROS), which attack various cellular components including DNA, proteins and membrane lipids, thereby leading to significant cellular damage. ROS also negatively affects intracellular concentration of antioxidants.²⁾ The major types of ROS or ROS-producing species generated by radiation are superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl ($OH^{\cdot}$) radicals.³⁾ ROS present a paradox in their biological functions: on 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,^{4,5)} other side effects of irradiation include; nausea, vomiting, diarrhea, etc.⁶⁾ Therefore, the design of strategies capable of protecting normal tissues from the lethal actions of radiation is of great interest in radiation biology.

Plants have been the companion of man and formed the basis of useful drugs for the treatment of various ailments. The use of plants may be beneficial in protecting against the radiation-induced damage, since they are less toxic than synthetic compounds at their optimum doses.⁷⁾ Therefore, screening of plants present a major avenue for the discovery of new radioprotective drugs. A number of radioprotective compounds that have been identified from medicinal plants are known to elicit strong antioxidant potentials, these include; *Phyllanthus amarus*, *Tinospora cordifolia*, *Hibiscus*

sabdariffa, *Aegle marmelos*, *Vernonia amygdalina*, *Amaranthus paniculatus*, etc.^{8–12)} In view of the above, it is pertinent to look inwards to other medicinal plants that can protect against radiation.

Garcinia kola HECKEL (family Guttiferae) is a herb grown in Nigeria and has a characteristic astringent, bitter and resinous taste. Extracts of the plant are used in ethnomedicine to treat laryngitis, cough and liver diseases.¹³⁾ Chemical investigations of the seeds revealed the presence of a GB (*Garcinia* biflavanone)-type biflavonoids, xanthenes, triterpenes, benzophenones, cycloartenol and its 24-methylene derivative.¹⁴⁾ Kolaviron (KV), the predominant constituent of the kola is a biflavonoid complex containing the C-3/8"-link biflavanones; GB-1, GB-2, GB-1a and kolaflavanone. KV has been reported to prevent hepatotoxicity mediated by carbon tetrachloride, paracetamol, thioacetamide, D-galatosamine, Aflatoxin B1 and 2-acetylaminofluorene.^{15,16)} Similarly, Adaramoye *et al.*¹⁷⁾ reported the anti-atherogenic effect of KV in rats fed on high cholesterol diet. Also, KV has been reported to elicit strong antioxidant activity in both *in vivo* and *in vitro* experimental models.^{18,19)} Accordingly, this study was designed to evaluate the role of KV, when given orally, in protecting brain of rats from oxidative stress caused by 5 Gy of γ -radiation.

MATERIALS AND METHODS

Materials Trichloroacetic acid and thiobarbituric acid were purchased from BDH Chemical Ltd., Poole, U.K. Other chemicals were procured from Sigma Chemical Co., Saint Louis, MO, U.S.A. and were of analytical grade and purest quality available.

Preparation of Kolaviron *Garcinia kola* seeds were obtained commercially in Ibadan, Nigeria and certified at the

herbarium in the Department of Botany, University of Ibadan, Nigeria, where a voucher specimen already exists (UI-00530). Three kilogram of peeled seeds was sliced, pulverized with an electric blender and air-dried in the laboratory (25–28 °C). Extraction of KV was achieved by the method of Iwu *et al.*²⁰⁾ Briefly, powdered seeds were extracted with light petroleum ether (bp 40–60 °C) in a soxhlet extractor. The defatted, dried marc was repacked and then extracted with methanol. The extract was concentrated and diluted to twice its volume with distilled water and extracted with ethyl acetate (6×250 ml). The concentrated ethyl acetate fraction gave a yellow solid known as kolaviron (KV) (Fig. 1) with a percentage yield of 6%. Prior to the experiments, KV was dissolved in corn oil at a concentration of 4 g/100ml 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 anaesthesia 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 γ -radiation of 500 rads (5 Gy). The source of radiation was a ⁶⁰Co 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 cm² 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.

Groups	Treatment
N	Normal (Un-irradiated animals)
KV	Irradiated animals treated with KV (250 mg/kg body weight <i>p.o.</i>)
RA	Irradiated control treated with vehicle <i>p.o.</i>
VC	Irradiated animals treated with vitamin C (250 mg/kg body weight <i>p.o.</i>)

Animals in the test groups were treated with KV and VC for 6 weeks prior to and 8 weeks after irradiation. KV and VC were administered orally at a dose of 250 mg/kg body weight daily. The dose of KV was established from previous experiment in our laboratories.¹⁷⁾ VC was dissolved in water and KV in corn oil (vehicle). Furthermore, all animals, except the N group were exposed to whole body radiation (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 surviv-

Fig. 1. Structure of Kolaviron (KV)

ing animals sacrificed after 8 weeks of irradiation.

Preparation of Post-mitochondrial Fraction (PMF)

Whole Brain was immediately dissected out and washed in ice-cold 1.15% KCl to remove blood stains. The brain tissues were weighed and 10% tissue homogenate was prepared with 5 mM phosphate buffer, pH 7.4. After centrifugation at 10000 *g* for 15 min 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).

Preparation of Serum Rats were dissected and blood collected from inferior *vena cava* of the heart into clean centrifuge tubes and allowed to stand for 1 h. Serum was prepared by centrifugation at 3000 *g* for 15 min in a mesenchymal stromal cells (MSC) bench centrifuge. The clear supernatant was used for the estimation of serum enzymes (ALT and AST), urea and creatinine.

Protein Determination Protein contents of serum and PMF were determined according to Lowry *et al.*²¹⁾ using bovine serum albumin as a standard. Briefly, the method involved the reduction of phospho-18 molybdc tungstic complex by phenolic groups present in amino acids to blue complex at alkaline pH. The absorbance of the complex was read at 720 nm.

Alanine and Aspartate Aminotransferases (ALT and AST) Determination

Serum ALT and AST activities were determined using a combination of the methods of Mohun and Cook²²⁾ and Reitman and Frankel.²³⁾ Briefly, 1.0 ml of the enzyme substrate was pre-incubated at 37 °C for 10 min in both test and blank tubes. To the test, 0.2 ml of serum was added and incubated for another 60 min. For AST, 0.05 ml of aniline citrate reagent was added and then followed by 0.2 ml of serum to the blank. (For ALT, the incubation time was 30 min and aniline citrate reagent was not added). The pyruvate formed was reacted with 1.0 ml of 2,4-dinitrophenyl hydrazine to form a coloured hydrazone. The reaction was stopped with 10.0 ml of 0.4 N NaOH and the absorbance read at 505 nm using spectrophotometer.

Creatinine and Urea Determination Serum creatinine and urea levels were estimated by the methods of Jaffe²⁴⁾ and, Talke and Schubert,²⁵⁾ respectively.

Glutathione-S-transferase (GST) Determination The PMF GST level was determined spectrophotometrically at

37°C according to the procedure of Habig *et al.*²⁶⁾ The method is based on the rate of conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB). Thirty microliters of reduced GSH was introduced into the blank and test tubes. It was followed by 150 µl of enzyme substrate (1-chloro-2,4-dinitrobenzene) to blank and test. Then 30 µl of enzyme source (PMF) 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 allowed to run for 60 s before the absorbance was read against the blank at 340 nm.

Reduced Glutathione (GSH) Determination PMF GSH level was assayed by measuring the rate of formation of chromophoric product in a reaction between 5,5'-dinitrobis-2-nitrobenzoic acid (DTNB) and free sulphhydryl groups (such as GSH) at 412 nm as described by Moron *et al.*²⁷⁾ To the homogenate, 10% trichloroacetic acid was added and centrifuged. 1.0 ml of the supernatant was treated with 0.5 ml Ellman's reagent (19.8 mg of 5,5'-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.

Superoxide Dismutase and Catalase Determination Superoxide dismutase activity (SOD) was measured by the nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich.²⁸⁾ 0.5 ml of tissue homogenate was mixed with ethanol and chloroform mixture and then centrifuged. To the supernatant, 0.025 M sodium pyrophosphate buffer (pH 8.3), phenazine methosulphate, nitroblue tetrazolium and NADH were added and incubated at 30°C for 90 s. The reaction was stopped by the addition of glacial acetic acid and mixed with *n*-butanol. The intensity of the chromogen in the butanol was measured at 560 nm. Catalase (CAT) activity was assayed by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi.²⁹⁾ The reaction mixture contained phosphate buffer (0.01 M, pH 7.0), tissue homogenate and 2 M H₂O₂. 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).

Lipid Peroxidation (LPO) Determination LPO in the PMF was assayed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method, as described by Walls *et al.*³⁰⁾ 1.0 mg/ml final concentration of sample (PMF) was incubated for 6 h at 37°C with or without 1 mM FeSO₄; 1 mM ascorbate and 0.2 M H₂O₂ (final concentration). 0.5 ml of 0.75% TBA in 0.1 M HCl was added to 0.5 ml of the incubation mixture already quenched with 0.5 ml of 10%

trichloroacetic acid (TCA). The mixture was heated at 90–95°C for 25 min in a boiling water bath and then cooled. The mixture was then centrifuged at 3000 rpm for 10 min, and the absorbance of supernatant read at 532 nm.

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

Table 1 shows that the body weight of irradiated animals significantly ($p < 0.05$) decreased after 1 and 8 weeks of exposure by 20% and 46%, respectively, relative to normal. Treatment with KV and VC attenuated the γ -radiation induced weight loss after 1 week of exposure. In 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 exposure. However, significant decreases ($p < 0.05$) were observed in the brain protein contents, total and relative weight of the irradiated animals after 8 weeks of exposure. These observed decreases were significantly ($p < 0.05$) ameliorated in animals treated with KV and VC. In Table 3, γ -irradiation caused significant increases ($p < 0.05$) in the activities of serum ALT and AST after 8 weeks of exposure. Precisely, serum ALT and AST were increased by 69% and 82%, respectively, after 8 weeks of irradiation. However, the γ -radiation-induced increases in ALT and AST

Table 1. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Body Weight of Wistar Albino Rats before and after Exposure to γ -Radiation (5 Gy)

Grouping	Body weight (g)		
	Before irradiation (<i>n</i> = 11)	1 week after irradiation (<i>n</i> = 5)	8 weeks after irradiation (<i>n</i> = 3–6)
N	209.1 ± 6.13	225.7 ± 3.31	288.5 ± 8.00
KV	220.1 ± 8.64	226.3 ± 7.01	185.1 ± 5.33*
RA	223.0 ± 6.09	181.4 ± 3.07*#	157.1 ± 5.66*#
VC	211.3 ± 7.45	207.2 ± 7.083	178.9 ± 3.08*

Values are mean ± S.D. of *n* = 3–11 according to the groups. Where *n* = 6, 5, 3 and 4 for N, KV, RA and VC groups, respectively, after 8 weeks of irradiation. * Significantly different from normal ($p < 0.05$). # Significantly different from KV and VC ($p < 0.05$). N = normal (un-irradiated animals), KV = kolaviron (250 mg/kg), RA = irradiated alone, VC = vitamin C (250 mg/kg).

Table 2. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Weight of Brain, Relative Weight and Protein Contents in Rats Exposed to γ -Radiation (5 Gy)

Grouping	Protein content (mg/g tissue)		Weight of brain (g)		Relative weight of brain (% of body weight)	
	1 week	8 weeks	1 week	8 weeks	1 week	8 weeks
N	0.60 ± 0.06	0.63 ± 0.07	2.51 ± 0.82	2.91 ± 0.83	1.11 ± 0.24	1.35 ± 0.40
KV	0.54 ± 0.04	0.58 ± 0.08	2.67 ± 0.59	2.51 ± 0.60	1.18 ± 0.27	1.36 ± 0.41
RA	0.59 ± 0.07	0.24 ± 0.03*.*	1.99 ± 0.79	1.43 ± 0.38*.*	1.09 ± 0.37	0.91 ± 0.22*.*
VC	0.58 ± 0.05	0.31 ± 0.03*	2.11 ± 0.72	2.18 ± 0.67	1.09 ± 0.41	1.22 ± 0.47

Values are mean ± S.D. of 3–6 animals per group. Where *n* = 5 for all groups after 1 week of irradiation, and *n* = 6, 5, 3 and 4 for N, KV, RA and VC groups, respectively, after 8 weeks of irradiation. * Significantly different from normal ($p < 0.05$). ** Significantly different from KV ($p < 0.05$). # Significantly different from KV and VC ($p < 0.05$). N = normal (un-irradiated animals), KV = kolaviron (250 mg/kg), RA = irradiated alone, VC = vitamin C (250 mg/kg).

Table 3. The Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Levels of Serum Aminotransferases, Creatinine and Urea in Rats Exposed to γ -Radiation (5 Gy)

Grouping	ALT (U/l)		AST (U/l)		Creatinine (mmol/l)	
	1 week	8 weeks	1 week	8 weeks	1 week	8 weeks
N	46.21 $\pm$ 8.17	52.43 $\pm$ 10.35	219.53 $\pm$ 21.48	215.15 $\pm$ 18.52	436.60 $\pm$ 36.18	473.54 $\pm$ 20.30
KV	47.80 $\pm$ 7.58	61.05 $\pm$ 9.94	209.33 $\pm$ 17.02	227.08 $\pm$ 16.35	424.36 $\pm$ 25.11	461.20 $\pm$ 30.05
RA	49.35 $\pm$ 9.27	88.49 $\pm$ 11.27* [#]	232.80 $\pm$ 20.71	391.56 $\pm$ 23.58* [#]	490.61 $\pm$ 32.40	508.07 $\pm$ 21.01
VC	48.20 $\pm$ 8.43	55.36 $\pm$ 9.80	206.42 $\pm$ 19.59	223.33 $\pm$ 19.60	437.11 $\pm$ 18.90	490.63 $\pm$ 31.80

Values are mean $\pm$ S.D. of 3–6 animals per group. Where $n=5$ for all groups after 1 week of irradiation, and $n=6, 5, 3$ and 4 for N, KV, RA and VC groups, respectively, after 8 weeks of irradiation. * Significantly different from normal ($p<0.05$). [#] Significantly different from KV and VC ($p<0.05$). N=normal (un-irradiated animals), KV=kolaviron (250 mg/kg), RA=irradiated alone, VC=vitamin C (250 mg/kg).

Table 4. Effect of Kolaviron (KV), a Biflavonoid Complex from *Garcinia kola* Seeds, on the Antioxidant Enzymes in Wistar Albino Rats before and after Exposure to γ -Radiation (5 Gy)

Antioxidant indices	KV-treated rats			Normal rats ($n=5$)
	Before irradiation ($n=5$)	1 week after irradiation ($n=5$)	8 weeks after irradiation ($n=3-6$)	
GSH	4.86 $\pm$ 0.73*	3.43 $\pm$ 0.52	2.45 $\pm$ 0.47*	3.46 $\pm$ 0.50
GST	6.91 $\pm$ 0.81*	5.11 $\pm$ 0.66	4.01 $\pm$ 0.71*	5.54 $\pm$ 1.03
CAT	0.32 $\pm$ 0.04	0.33 $\pm$ 0.04	0.23 $\pm$ 0.05*	0.35 $\pm$ 0.05
SOD	6.31 $\pm$ 1.12	5.78 $\pm$ 1.02	5.34 $\pm$ 1.22	6.89 $\pm$ 0.78
LPO	2.05 $\pm$ 0.47*	3.74 $\pm$ 0.61	6.49 $\pm$ 0.89*	3.05 $\pm$ 0.65

Values are mean $\pm$ S.D. of $n=3-11$ according to the groups. * Significantly different from normal ($p<0.05$). Units: GSH (μ g/mg protein), GST (mmol/min/mg protein), CAT (μ mol/min/mg protein), SOD (U/mg protein), LPO (μ mol/mg protein).

Fig. 2. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Levels of Lipid Peroxidation in Brain of Rats Exposed to Gamma Radiation (5 Gy)

* Significantly different from normal, KV and VC ($p<0.05$). ** Significantly different from RA ($p<0.05$).

activities were significantly ($p<0.05$) attenuated in KV- and VC-treated animals after 8 weeks of exposure. Furthermore, there were no significant differences ($p>0.05$) in the levels of serum creatinine of irradiated animals when compared to others after 1 and 8 weeks of irradiation. In Table 4, pretreatment with KV boosted the antioxidant status of the rats as evidenced by significantly elevation of GSH and GST levels when compared to the normal.

In Figs. 2 and 3, there were significant increases ($p<0.05$)

Fig. 3. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Levels of Serum Urea in Rats Exposed to Gamma Radiation (5 Gy)

* Significantly different from normal and KV ($p<0.05$).

Fig. 4. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Levels of Reduced Glutathione in Brain of Rats Exposed to Gamma Radiation (5 Gy)

[#] Significantly different from KV and VC ($p<0.05$). * Significantly different from normal ($p<0.05$). ^a Significantly different from KV ($p<0.05$).

Fig. 5. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Levels of Glutathione-S-transferase in Brain of Rats Exposed to Gamma Radiation (5 Gy)

* Significantly different from normal and KV ($p < 0.05$). ** Significant difference from RA ($p < 0.05$).

Fig. 7. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Activities of Superoxide Dismutase in Brain of Rats Exposed to Gamma Radiation (5 Gy)

* Significantly different from normal, KV and VC ($p < 0.05$).

Fig. 6. Effect of Kolaviron, a Biflavonoid Complex from *Garcinia kola* Seeds, on the Activities of Catalase in Brain of Rats Exposed to Gamma Radiation (5 Gy)

* Significantly different from normal and KV ($p < 0.05$).

in the levels of brain lipid peroxidation (LPO) and serum urea of irradiated animals when compared to normal. Specifically, brain LPO increased by 90% (5.80 ± 0.82 versus 3.05 ± 0.65) and 151% (11.33 ± 1.21 versus 4.51 ± 0.81) after 1 and 8 weeks of irradiation, respectively, while the level of serum urea in irradiated animals was increased by 57% (5.54 ± 0.67 versus 3.52 ± 0.78) after 8 weeks of exposure. Treatment with KV and VC significantly ($p < 0.05$) ameliorated the radiation-induced increases in the levels of brain LPO. Also in KV-treated animals, the radiation-induced increase in serum urea was ameliorated. Importantly, in KV-treated animals, the levels of LPO and urea were restored to values that are statistically similar to normal.

In Figs. 4 and 5, γ -radiation caused significant ($p < 0.05$)

decreases in the levels of brain reduced glutathione (GSH) and glutathione-S-transferase (GST) of irradiated animals. Brain GSH and GST levels were decreased by 61% (1.33 ± 0.54 versus 3.41 ± 0.50) and 43% (3.17 ± 0.65 versus 5.54 ± 1.13) after 1 week, and by 75% (0.79 ± 0.25 versus 3.18 ± 0.61) and 74% (1.29 ± 0.39 versus 4.89 ± 0.92) after 8 weeks of exposure, respectively. Treatment with KV completely restored the brain GSH and GST levels of irradiated animals after 1 and 8 weeks of exposure, while treatment with VC could only restore the depleted brain GSH levels after 1 week of irradiation (Figs. 4, 5).

In Figs. 6 and 7, γ -radiation decreased the brain CAT and SOD levels of irradiated animals by 68% (0.093 ± 0.04 versus 0.290 ± 0.05) and 53% (3.26 ± 0.92 versus 6.89 ± 0.89), respectively, after 8 weeks of exposure. The radiation-induced decrease in brain CAT was significantly ($p < 0.05$) attenuated in KV-treated animals, while both KV- and VC-treated animals had their SOD levels elevated by 64% (5.34 ± 1.02 versus 3.26 ± 0.73) and 83% (5.95 ± 0.87 versus 3.26 ± 0.73), respectively, when compared to irradiated and untreated animals after 8 weeks of irradiation.

DISCUSSION

During radiotherapy normal brain can undergo undesirable tissue injury especially in the treatment of cerebral tumors. Moreover, central nervous system detrimental effects have been found in radiation exposed children after Hiroshima and Nagasaki atomic bomb blasts as well as after the Chernobyl accident.^{31,32} For these reasons, the present study aimed at exploring the possible radioprotective effect of kolaviron (a biflavonoid from *Garcinia kola* seeds) on γ -radiation-induced oxidative stress in brain of rats. The goal of radiation treatment is to deliver precisely measured doses of ionizing radiation to a defined tumor volume with minimum acceptable injury to surrounding healthy tissue while eliminating the tumor cells.³³ Ionizing radiation is known to induce ox-

oxidative stress through generation of ROS including superoxide, hydroxyl radicals, singlet oxygen and hydrogen peroxide. These free radicals react with critical cellular components, such as DNA, RNA, proteins and membranes, leading to cell dysfunction and death. Excess ROS may lead to an imbalance in pro-oxidant and antioxidant status in the cells.³⁴⁾

Today, there is considerable interest in free radical mediated damage to biological systems due to xenobiotics or radiation exposure. A large number of xenobiotics have been identified to have potential to generate free radicals in biological systems.³⁵⁾ Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction and injury to the brain and other organs. Lipid peroxidation has been suggested as one of the molecular mechanisms involved in radiation-induced toxicity.¹²⁾ In the present study; increased levels of malondialdehyde (MDA, an index of lipid peroxidation) was observed in brain tissues of γ -irradiated rats. This may be due to the free radical attack on cell membrane phospholipids and circulating lipids and, thus, MDA acts as a sensitive biomarker for oxidative stress that occurs as part of the pathogenesis of various diseases.^{36,37)} The present study found that KV from *Garcinia kola* seeds and VC significantly reduced the MDA levels. The decrease in the activities of SOD, CAT and GST, and the decreased level of GSH in the brain tissues may be due to their utilization by the enhanced production of ROS.³⁸⁾ In agreement with this finding, Bhatia and Jain³⁹⁾ observed a significant depletion in the antioxidant system, and an increased lipid peroxides in animals exposed to whole body γ -irradiation. The decreased levels of GSH in brain of irradiated animals may have resulted from the activity of GST and glutathione peroxidase (GPx) in reducing lipid hydroperoxide to stable non-radical lipid alcohols or GSH is directly utilized as an antioxidant in terminating free radical reaction initiated by irradiation. Under normal conditions, the inherent defense system, including glutathione and the antioxidant enzymes ability to protect against oxidative damage. These endogenous enzymes, namely SOD, CAT, GST and GPx, are responsible for the deactivation of ROS and are the primary antioxidant system in cells. SOD catalyzes dismutation of the superoxide ion ($O_2^{\cdot-}$) and converts it to H_2O_2 .⁴⁰⁾ In this study, SOD activity was decreased by γ -irradiation, but it was significantly restored when rats were given KV and VC after 8 weeks of exposure. In the antioxidant cascade, CAT decomposes H_2O_2 to H_2O and O_2 .⁴¹⁾ Administration of KV and VC significantly restored the radiation-induced suppression of CAT. GSTs are family of soluble proteins, which conjugate xenobiotics with glutathione.⁴²⁾ Metabolites after glutathionylation are more hydrophilic and thus biologically inactive. Therefore, they are readily excreted in bile or urine as conjugates. This action is thus believed to be a major mechanism for the detoxification of reactive species. It was observed that the administration of KV and VC significantly inhibited GSH depletion in brain of irradiated animals and up-regulated GST activity, pointing to the protective role of KV against γ -radiation-induced adverse reactions in the exposed animals.

It is clear that γ -irradiation caused significant increases in the levels of serum ALT, AST and urea, while the total pro-

tein contents of brain dropped significantly after 8 weeks of exposure. These parameters measure the excretory and synthetic functions of the liver and, indicate an injury, impaired functions and damage of liver of irradiated animals. It is not surprising that the liver functions were impaired in the irradiated animals knowing fully well that ROS are generated during radiation exposure. The ability of KV to ameliorate radiation-induced increases in serum ALT, AST and urea levels, and also restore the protein contents of irradiated animals point to its possible protective role in these animals. It is therefore reasonable to state that the oxidative stress elicited by irradiation might have been nullified due to the antioxidant activity of KV. In addition, the ability of VC to attenuate the antioxidants status of the irradiated animals further confirmed its antioxidant and protective effects.

In conclusion, the current study finds kolaviron (bioflavonoid from *Garcinia kola* seeds) protective against γ -radiation induced oxidative stress in brain of exposed rats. Therefore, the consumption of *Garcinia kola* seeds may be encouraged in patients undergoing radiotherapy to improve general health conditions.

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