Protective Effects of Extracts of *Vernonia amygdalina, Hibiscus sabdariffa* and Vitamin C against Radiation-induced Liver Damage in Rats

Oluwatosin ADARAMOYE\textsuperscript{†*}, Bayo OGUNGBENRO, Oluchi ANYAEGBU and Michael FAFUNSO

Radioprotection/*Vernonia amygdalina*/Hibiscus sabdariffa//Radiation/Antioxidants.

The radioprotective efficacy of methanolic extracts of leaves of *Vernonia amygdalina* (VA) and *Hibiscus sabdariffa* (HS), and vitamin C (VIT C) against gamma radiation (4 Gy) induced liver damage was studied in male Wistar albino rats. VIT C was administered at a dose of 250 mg/kg body weight, while VA and HS were administered at doses; 200, 400 and 800-mg/kg body weight, orally for 4 weeks prior to radiation and 5 weeks after irradiation. The rats were sacrificed at 24 hours and 5 weeks after irradiation. Treatment with VIT C and VA (800 mg/kg) significantly ($p < 0.05$) decreased the gamma radiation-induced increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities at 24 hours after irradiation, whereas, HS (400 mg/kg) significantly ($p < 0.05$) decreased the serum ALT activity only. Similarly, treatment with VIT C and VA (800 mg/kg) significantly ($p < 0.05$) decreased the serum conjugated bilirubin levels by 56% and 29%, respectively at 24 hours. Furthermore, VIT C, VA and HS significantly ($p < 0.05$) decreased the levels of serum lipid peroxidation (LPO) and increased the hepatic superoxide dismutase (SOD) activities at 24 hours. Treatment for 5 weeks after irradiation with VITC, VA and HS significantly ($p < 0.05$) decreased the levels of un conjugated bilirubin, while VIT C and VA alone decreased the levels of conjugated bilirubin. Furthermore, treatment with VA (400 and 800 mg/kg) decreased the serum ALT activities by 25% and 34%, respectively, at 5 weeks after irradiation. Similarly, alkaline phosphatase and LPO levels were significantly ($p < 0.05$) attenuated following treatment with VITC and VA (400 and 800 mg/kg) at 5 weeks after irradiation. In addition, treatment with VIT C, VA (800 mg/kg) and HS (400 and 800 mg/kg) significantly ($p < 0.05$) elevated the levels of reduced glutathione (GSH) by 61%, 56%, 41% and 44%, respectively, at 5 weeks. Similar elevation of antioxidant enzymes; SOD, glutathione-s-transferase and catalase were obtained in animals treated with VIT C and extracts at 5 weeks. Taken together, the results suggest that the extracts of VA and HS, and VIT C could increase the antioxidant defense systems and may probably protect animals from radiation-induced liver damage.

INTRODUCTION

Radiotherapy is an important modality for cancer cure and it is estimated that about one half of cancer patients derive benefits from it.\textsuperscript{1)} Radiotherapy is used in the treatments of loco-regional growths that cannot be excised by surgery such as those of advanced lung, head and neck cancers.\textsuperscript{1)} Although radiotherapy is a chief modality in the treatment of cancer, it faces major drawbacks mainly due to the severe side effects generated as a result of damage to tissues.\textsuperscript{2)} Radiation produces various pathological changes in living systems, such changes include; nausea, vomiting, diarrhea, etc.\textsuperscript{3)} In such situations, an agent that can render a therapeutic differential between a cancer cell cytotoxicity and normal tissue toxicity may be of great help. This therapeutic differential may be achieved with chemical radiation sensitizers or protectors.\textsuperscript{1)} The design of strategies capable of protecting normal host tissues from the lethal actions of radiation without compromising their anti-cancer activity is of great interest in radiation biology.\textsuperscript{4)} The use of several chemicals such as WR 2721 (Amifostine),\textsuperscript{5)} natural antioxidants such as
GSH (Glutathione, reduced form), biological response modifiers such as cytokines, immune stimulators and some products were found to provide good radioprotection in experimental animals. However, the inherent toxicity of these agents at the radioprotective doses warranted further search of a safer and effective radioprotectors.\(^7\) Therefore, the development of effective, inexpensive and non-toxic protective agents is still an active area of research in radiation biology.\(^8\)

Recent studies have shown that commonly used medicinal plants and herbs are good sources of radioprotection in experimental models and in patients receiving radiotherapy.\(^9\) The herbs include; \textit{Phyllanthus amarus},\(^12\) \textit{Tinospora cordifolia},\(^7\) \textit{Aaegle marmelos},\(^1\) \textit{Amarathus paniculatus},\(^13\) etc. In the light of the aforementioned, it is pertinent to look inwards to other medicinal plants with potent antioxidant activity and within the reach of all for protection against radiation in normal tissues.

\textit{Hibiscus sabdariffa} (HS) commonly called ‘Roselle’ or ‘red roselle’ in English, ‘Kardesh’ in Arabic and ‘Sobo’ in Nigeria,\(^10\) is a herb, whose flower extracts are used in folk medicine for treatment of high blood pressure, liver disease and fever.\(^15\) The herb is also known to elicit potent antioxidant and anticancer activities in several \textit{in vivo} and \textit{in vitro} studies.\(^18\)\(^19\)

\textit{Vernonia amygdalina} (VA), commonly called, bitter leaf, is a popular African vegetable,\(^20\) purported to have several health benefits.\(^21\) VA is also used in the treatment of parasite-related diseases.\(^22\) Extracts from VA has been reported to elicit cytotoxic effects in human carcinoma nasopharynx cells\(^23\) and subsequently, Hissaka \textit{et al.}\(^24\) showed that vernodalone and vernolid (saponins from VA) elicited antitumoral activities in leukemia cells. Recently, Izevbie\(^25\) reported that some isolated peptides from aqueous extract of VA are potent inhibitor of mitogen-activated proteins kinases (MAPKs), which are crucial for breast tumour growth. However, no study has been reported so far on the radioprotective activity of these herbs. Also, on the basis of the medicinal properties of VA and HS, the present study has been undertaken to investigate the use of \textit{Vernonia amygdalina} and \textit{Hibiscus sabdariffa} as radioprotective agents in experimental animal models.

**MATERIALS AND METHODS**

**Preparation of plant extracts**

Samples of fresh leaves of \textit{Vernonia amygdalina} and, the calyces of \textit{Hibiscus sabdariffa} were obtained from a local market, Sasa 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 nos. UI-02567 and UI-02568). The leaves and calyces were air-dried at room temperature and then powdered. The powdered samples (1 kg each) were de-fatting with n-hexane (2.5 Litres) and then extracted with 75% methanol (2.5 Litres) overnight in a soxhlet extractor. The methanolic extracts of the two herbs were concentrated and evaporated to dryness at 50°C with a rotary evaporator under reduced pressure. The yields of the preparations were 6.8% and 8.5% for VA and HS, respectively. The extracts were dissolved in water at a concentration of 4 g/100ml, and aliquots of different concentrations were given orally to animals with a gavage needle.

**Animals**

Inbred 5–6 weeks old male Wistar albino rats weighing 195–210 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) and under controlled light cycles (12 h light/12 h dark). They were maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water \textit{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 radiation of 400 rads (4 Gy). The source of radiation was a \textit{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 cm\(^2\) and at a distance of 70 cm from the source.

**Experimental design**

Ninety rats were used for the experiment, and the animals were randomly divided into nine groups of ten animals each.

Animals in the test groups were treated with VA, HS and vitamin C (250 mg/kg body wt. p.o)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>UI</td>
<td>Un-irradiated control</td>
</tr>
<tr>
<td>RA</td>
<td>Irradiated control treated with vehicle p.o</td>
</tr>
<tr>
<td>VA1</td>
<td>Irradiated animals treated with VA extract 200 mg/kg body wt. p.o</td>
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<tr>
<td>VA2</td>
<td>Irradiated animals treated with VA extract 400 mg/kg body wt. p.o</td>
</tr>
<tr>
<td>VA3</td>
<td>Irradiated animals treated with VA extract 800 mg/kg body wt. p.o</td>
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<tr>
<td>HS1</td>
<td>Irradiated animals treated with HS extract 200 mg/kg body wt. p.o</td>
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<tr>
<td>HS2</td>
<td>Irradiated animals treated with HS extract 400 mg/kg body wt. p.o</td>
</tr>
<tr>
<td>HS3</td>
<td>Irradiated animals treated with HS extract 800 mg/kg body wt. p.o</td>
</tr>
<tr>
<td>VIT C</td>
<td>Irradiated animals treated with vitamin C 250 mg/kg body wt. p.o</td>
</tr>
</tbody>
</table>

\(^7\) abdomen
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\[ http://jrr.jstage.jst.go.jp \]
VIT C for four weeks prior to irradiation. Furthermore, all animals, except the UI group were exposed to whole body radiation (4 Gy), and treated with the extracts (VA and HS) and VIT C for five weeks after irradiation. The body weights of all animals were determined a day prior to irradiation and every third day thereafter. Twenty-four hours after irradiation, half of the animals in each group (n = 5) were sacrificed by cervical dislocation, and blood collected from the inferior vena cava of the heart into plain centrifuge tube. Blood were allowed to stand for 1 hour and then centrifuged at 3,000 g for 15 minutes in a bench centrifuge to obtain serum, which was used for the estimation of serum enzymes. Five weeks after irradiation, the surviving animals in each group were sacrificed as mentioned above. Liver from the animals were quickly removed and washed in ice-cold 1.15% KCl solution, dried and weighed. The liver were homogenized in 4 volumes of 5mM phosphate buffer, pH 7.4 and centrifuged at 10,000 g for 15 minutes to obtain post-mitochondrial supernatant fraction (PMF), which was used for the assay of antioxidant profile of the animals.

**Determination of protein contents and the antioxidant profile**

The total protein levels in the blood and liver of animals were determined according to the method of Lowry et al.\(^{30}\) using bovine serum albumin as standard.

Superoxide dismutase activity (SOD) was measured by the nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich.\(^ {27}\) Glutathione-s-transferase (GST) activity was determined by the method of Habig et al.,\(^ {28}\) the method is based on the rate of conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB). Catalase (CAT) activity was assayed by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi.\(^ {29}\) Glutathione (GSH) level was assayed by measuring the rate of formation of chromphoric product in a reaction between DTNB (5, 5'-dinitro bis (2-nitrobenzoic acid) and free sulphhydryl groups (such as reduced glutathione) at 412 nm according to the method of Moron et al.\(^ {30}\) The extent of lipid peroxidation in the blood and liver was estimated by the method of Buege and Aust.\(^ {31}\) The method involves the reaction between malondialdehyde (MDA) (product of lipid peroxidation) and thiobarbituric acid (TBA) to form a pink precipitate, which was read at 535 nm with spectronic-20 spectrophotometer.

**Assessment of Liver Integrity**

The integrity of liver in the studied animals was assessed using both enzymic and non-enzymic biochemical indices. Serum alanine and aspartate aminotransferases (ALT and AST) activities were assayed by the method of Reitman and Frankel,\(^ {32}\) the method involves the reaction between pyruvate (end product of transamination reaction catalyzed by ALT and AST) and 2,4-dinitrophenyl hydrazine to produce intensely coloured hydrazone, which was read at 546nm with spectronic-20 spectrophotometer. The estimation of alkaline phosphatase (ALP) activities was based on the method of Williamson.\(^ {33}\) ALP activity was measured by monitoring the concentration of p-nitrophenol formed when ALP reacts with p-nitrophenyl phosphate (PNPP) at 405 nm.

The bilirubin levels (Total and Direct) were assayed by the method of Rutkowski and Debaare,\(^ {34}\) the method involves the reaction between bilirubin and diazotized sulfanilic acid in alkaline medium to form a blue coloured complex, which was read at 546nm. The indirect bilirubin (unconjugated bilirubin) is obtained by subtracting the value of direct bilirubin (conjugated bilirubin) from total bilirubin.

**Statistical analysis**

All values were expressed as the mean ± S.D. of five animals sacrificed after 24 hours 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**

All rats in the un-irradiated group (UI), 80% in groups treated with vitamin C, VA (400 and 800 mg/kg) and HS (800 mg/kg) survived at 5 weeks after irradiation. In con-

**Table 1.** Five-week percentage survival of wistar albino rats pre- and post-treated with extracts of VA and HS after exposure to 4 Gy of gamma ray radiation.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Weeks after irradiation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
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<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
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<td>60</td>
<td>60</td>
<td>40</td>
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<td>100</td>
<td>80</td>
<td>80</td>
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<tr>
<td>VA2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>80</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>VA3</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>HS1</td>
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<td>80</td>
<td>60</td>
<td>60</td>
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<tr>
<td>HS2</td>
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<td>80</td>
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<tr>
<td>HS3</td>
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<td>100</td>
<td>100</td>
<td>80</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>VIT C</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>80</td>
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</tr>
</tbody>
</table>

At first week, \(n = 5\) (100%) for all groups except HS1

UI = Un-irradiated rats, RA = Radiation alone, VA1 = Vernonia amygdalina 200 mg/kg, VA2 = Vernonia amygdalina 400 mg/kg, VA3 = Vernonia amygdalina 800 mg/kg, HS1 = Hisbiscus sabdariffa 200 mg/kg, HS 2 = Hisbiscus sabdariffa 400 mg/kg, HS3 = Hisbiscus sabdariffa 800 mg/kg, VIT C = Vitamin C

trast, only 40% of the irradiated and untreated rats survived at 5 weeks after irradiation (Table 1). A significant decrease ($p < 0.05$) in protein contents (serum and liver) was observed in irradiated animals when compared to extracts-treated animals at 5 weeks after irradiation. Similarly, gamma radiation caused a significant decrease ($p < 0.05$) in glutathione (GSH) content of the animals at 5 weeks after irradiation. However, the gamma-radiation induced decrease in GSH content was significantly attenuated ($p < 0.05$) in animals treated with VA (800 mg/kg), HS (400 and 800 mg/kg) and

| Table 2. | The effect of varying doses of VA and HS on the levels of serum protein, and liver glutathione and protein of rats exposed to gamma radiation (4 Gy). |
| --- | --- | --- |
| Grouping | Protein (Serum) (mg/100 ml) | Protein (Liver) (mg/g tissue) | GSH (Liver) (µg/ml) |
| | 24 h | 5 wk | 24 h | 5 wk | 24 h | 5 wk |
| UI | 2.35 ± 0.98 | 2.41 ± 0.08* | 10.53 ± 2.38 | 10.5 ± 2.23* | 25.0 ± 3.82 | 30.14 ± 4.00* |
| RA | 2.68 ± 0.35 | 0.89 ± 0.05 | 9.80 ± 1.48 | 1.80 ± 0.56 | 23.0 ± 4.32 | 14.60 ± 2.49 |
| VA1 | 2.49 ± 0.70 | 1.90 ± 0.07* | 9.26 ± 1.68 | 4.53 ± 0.56* | 22.7 ± 4.73 | 18.31 ± 3.57 |
| VA2 | 2.60 ± 0.70 | 1.52 ± 0.09* | 10.35 ± 2.39 | 4.81 ± 1.51* | 27.0 ± 4.08 | 16.54 ± 2.70 |
| VA3 | 2.92 ± 0.43 | 1.70 ± 0.04* | 10.27 ± 2.04 | 5.38 ± 1.12* | 25.3 ± 3.30 | 22.89 ± 3.64* |
| HS1 | 2.39 ± 0.56 | 1.88 ± 0.06* | 9.49 ± 1.10 | 4.37 ± 1.35* | 23.6 ± 4.44 | 15.80 ± 5.01 |
| HS2 | 2.70 ± 0.51 | 1.75 ± 0.09* | 9.73 ± 1.16 | 5.40 ± 1.27* | 24.0 ± 2.16 | 20.55 ± 6.36* |
| HS3 | 2.36 ± 0.97 | 2.00 ± 0.05* | 9.49 ± 1.54 | 5.83 ± 1.20* | 26.0 ± 2.20 | 21.06 ± 4.12* |
| VITC | 2.73 ± 0.75 | 2.10 ± 0.08** | 10.43 ± 3.08 | 9.86 ± 1.71** | 26.1 ± 5.30 | 23.51 ± 3.71* |

Values are mean ± S.D. of five animals per treatment
* Significantly different from irradiated group (RA) ($p < 0.05$)
** Significantly different from irradiated group (RA), but statistically similar to un-irradiated group (UI) ($p < 0.05$).
UI = Un-irradiated rats, RA = Radiation alone, VA1 = Vernonia amygdalina 200 mg/kg, VA2 = Vernonia amygdalina 400 mg/kg, VA3 = Vernonia amygdalina 800 mg/kg, HS1 = Hibiscus sabdariffa 200 mg/kg, HS 2 = Hibiscus sabdariffa 400 mg/kg, HS3 = Hibiscus sabdariffa 800 mg/kg, VIT C = Vitamin C

| Table 3. | The effect of varying doses of VA and HS on the activities of liver glutathione-s-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) in rats exposed to gamma radiation (4 Gy). |
| --- | --- | --- | --- |
| Grouping | SOD (IU/mg protein) | GST (nmol/min/mg protein) | CAT (µmol/ mg protein) |
| | 24 h | 5 wk | 24 h | 5 wk | 24 h | 5 wk |
| UI | 2.15 ± 0.89 | 2.35 ± 0.81* | 1.58 ± 0.36 | 1.93 ± 0.09* | 0.74 ± 0.20 | 0.76 ± 0.09* |
| RA | 1.82 ± 0.75 | 1.03 ± 0.45 | 1.48 ± 0.40 | 0.86 ± 0.04 | 0.63 ± 0.12 | 0.27 ± 0.01 |
| VA1 | 1.85 ± 0.67 | 1.44 ± 0.61* | 1.45 ± 0.53 | 1.28 ± 0.47* | 0.66 ± 0.13 | 0.48 ± 0.05* |
| VA2 | 1.89 ± 0.65 | 1.56 ± 0.67* | 1.39 ± 0.63 | 1.49 ± 0.68* | 0.70 ± 0.19 | 0.52 ± 0.07* |
| VA3 | 1.87 ± 0.50 | 1.55 ± 0.35* | 1.55 ± 0.67 | 1.39 ± 0.33* | 0.68 ± 0.16 | 0.50 ± 0.05* |
| HS1 | 1.81 ± 0.66 | 1.50 ± 0.62* | 1.43 ± 0.45 | 1.25 ± 0.51* | 0.61 ± 0.15 | 0.46 ± 0.03* |
| HS2 | 1.91 ± 0.64 | 1.56 ± 0.80* | 1.49 ± 0.55 | 1.35 ± 0.61* | 0.65 ± 0.20 | 0.40 ± 0.05* |
| HS3 | 1.92 ± 0.60 | 1.88 ± 0.73* | 1.46 ± 0.73 | 1.34 ± 0.78* | 0.68 ± 0.14 | 0.50 ± 0.04* |
| VITC | 1.96 ± 0.70 | 1.82 ± 0.53* | 1.42 ± 0.68 | 1.47 ± 0.83* | 0.64 ± 0.15 | 0.68 ± 0.06** |

Values are mean ± S.D. of five animals per treatment
* Significantly different from irradiated group (RA) ($p < 0.05$)
** Significantly different from irradiated group (RA), but statistically similar to un-irradiated group (UI) ($p < 0.05$).
UI = Un-irradiated rats, RA = Radiation alone, VA1 = Vernonia amygdalina 200 mg/kg, VA2 = Vernonia amygdalina 400 mg/kg, VA3 = Vernonia amygdalina 800 mg/kg, HS1 = Hibiscus sabdariffa 200 mg/kg, HS 2 = Hibiscus sabdariffa 400 mg/kg, HS3 = Hibiscus sabdariffa 800 mg/kg, VIT C = Vitamin C

vitamin C at 5 weeks (Table 2). There were no significant differences ($p > 0.05$) in the activities of superoxide dismutase (SOD), glutathione-s-transferase (GST) and catalase (CAT) of irradiated animals when compared to un-irradiated group at 24 hours after irradiation. However, 5 weeks after irradiation, the mean activities of SOD, GST and CAT decreased significantly ($p < 0.05$) in the irradiated animals when compared to un-irradiated group. Specifically, gamma-radiation decreased SOD, GST and CAT levels by 57%, 55% and 71%, respectively. Furthermore, treatment with VA, HS

**Fig. 1.** Effect of different doses of *Vernonia amygdalina* and *Hisbiscus sabdariffa* on the activities of serum alanine and aspartate aminotransferases of rats exposed to radiation (4 Gy).

UI = Un-irradiated rats, RA = Radiation alone, VA1 = Vernonia amygdalina 200 mg/kg, VA2 = Vernonia amygdalina 400 mg/kg, VA3 = Vernonia amygdalina 800 mg/kg, HS1 = Hisbiscus sabdariffa 200 mg/kg, HS2 = Hisbiscus sabdariffa 400 mg/kg, HS3 = Hisbiscus sabdariffa 800 mg/kg, VIT C = Vitamin C. ALT = Alanine aminotransferase, AST = Aspartate aminotransferase

* significantly different from irradiated group (RA) ($p < 0.05$)

** significantly different from irradiated group (RA) ($p < 0.05$), but statistically similar to un-irradiated group (UI) ($p > 0.05$)

**Fig. 2.** Effect of different doses of *Vernonia amygdalina* and *Hisbiscus sabdariffa* on the levels of serum unconjugated and conjugated bilirubin in rats exposed to radiation (4 Gy).

UI = Un-irradiated rats, RA = Radiation alone, VA1 = Vernonia amygdalina 200 mg/kg, VA2 = Vernonia amygdalina 400 mg/kg, VA3 = Vernonia amygdalina 800 mg/kg, HS1 = Hisbiscus sabdariffa 200 mg/kg, HS2 = Hisbiscus sabdariffa 400 mg/kg, HS3 = Hisbiscus sabdariffa 800 mg/kg, VIT C = Vitamin C. Unbil = Unconjugated Bilirubin, Conbil = Conjugated Bilirubin

* significantly different from irradiated group (RA) ($p < 0.05$)

** significantly different from irradiated group (RA) ($p < 0.05$), but statistically similar to un-irradiated group (UI) ($p > 0.05$)
and vitamin C at the tested doses significantly ($p < 0.05$) attenuated the radiation-induced decrease in the activities of these antioxidant enzymes at 5 weeks after irradiation (Table 3). In Fig. 1, gamma radiation caused an elevation of serum alanine aminotransferase (ALT) by 158% in the irradiated group at 24 hours. However, treatment with VA (800mg/kg), HS (400mg/kg) and Vitamin C significantly decreased ($p < 0.05$) the serum ALT levels in these animals at 24 hours. Furthermore, the activities of serum ALT decreased significantly ($p < 0.05$) in rats treated with VA (400mg/kg and 800mg/kg) and Vitamin C by 25%, 34% and 27%, respectively, at 5 weeks after irradiation. Likewise, serum AST activity was significantly increased ($p < 0.05$) in irradiated animals at 24 hours, while treatment with VA (800mg/kg)

![Graph](image1)

**Fig. 3.** Effect of different doses of *Vernonia amygdalina* and *Hisbiscus sabdariffa* on the levels of serum and liver lipid peroxidation (LPO) product in rats exposed to radiation (4 Gy). UI = Un-irradiated rats, RA = Radiation alone, VA1 = *Vernonia amygdalina* 200 mg/kg, VA2 = *Vernonia amygdalina* 400 mg/kg, VA3 = *Vernonia amygdalina* 800 mg/kg, HS1 = *Hisbiscus sabdariffa* 200 mg/kg, HS2 = *Hisbiscus sabdariffa* 400 mg/kg, HS3 = *Hisbiscus sabdariffa* 800 mg/kg, VIT C = Vitamin C, LPO = Lipid peroxidation

* Significantly different from irradiated group (RA) ($p < 0.05$)

** Significantly different from irradiated group (RA) ($p < 0.05$), but statistically similar to un-irradiated group (UI) ($p > 0.05$)

![Graph](image2)

**Fig. 4.** Effect of different doses of *Vernonia amygdalina* and *Hisbiscus sabdariffa* on the activities of serum alkaline phosphatase (ALP) of rats exposed to radiation (4 Gy). UI = Un-irradiated rats, RA = Radiation alone, VA1 = *Vernonia amygdalina* 200 mg/kg, VA2 = *Vernonia amygdalina* 400 mg/kg, VA3 = *Vernonia amygdalina* 800 mg/kg, HS1 = *Hisbiscus sabdariffa* 200 mg/kg, HS2 = *Hisbiscus sabdariffa* 400 mg/kg, HS3 = *Hisbiscus sabdariffa* 800 mg/kg, VIT C = Vitamin C, ALP = Alkaline phosphatase

* Significantly different from irradiated group (RA) ($p < 0.05$)
and Vitamin C significantly decreased ($p < 0.05$) the serum AST activities at 24 hours. In addition, there were no significant differences ($p > 0.05$) in serum AST levels of extracts treated groups when compared to irradiated group at 5 weeks. Furthermore, gamma radiation caused a significant increase ($p < 0.05$) in the levels of serum unconjugated and conjugated bilirubin in irradiated animals (Fig. 2). Precisely, serum unconjugated and conjugated bilirubin levels were elevated by 88% and 118%, respectively at 24 hours. Furthermore, treatment with VA at a dose of 800mg/kg significantly ($p < 0.05$) decreased the level of unconjugated bilirubin at 24 hours, while the same parameter was significantly ($p < 0.05$) decreased in animals treated with VA and HS (200,400 and 800mg/kg) and vitamin C at 5 weeks after irradiation. Specifically, the level of unconjugated bilirubin in vitamin C treated animals was statistically similar ($p > 0.05$) to un-irradiated group at 5 weeks. The lipid peroxidation (LPO) levels in the liver of irradiated and extracts-treated animals were significantly higher ($p < 0.05$) than the un-irradiated animals at 24 hours (Fig. 3). However, hepatic LPO level was significantly decreased ($p < 0.05$) in animals treated with vitamin C when compared to irradiated group at 24 hours. Five weeks after irradiation, levels of hepatic LPO were significantly lowered ($p < 0.05$) in animals treated with VA (800mg/kg) and vitamin C. Similarly, the radiation-induced increase in the levels of serum LPO was significantly attenuated ($p < 0.05$) in VA, HS and vitamin C treated animals at 24 hours. Furthermore, 5 weeks after irradiation, animals treated with VA (800mg/kg), HS (400 and 800mg/kg) and vitamin C had significantly lowered ($p < 0.05$) serum LPO than irradiated group. In addition, gamma radiation caused a significant increase ($p < 0.05$) in serum ALP activities of the animals at 24 hours and 5 weeks after exposure (Fig. 4). At 24 hours, there were no significant differences ($p > 0.05$) in the serum ALP levels of extracts-treated animals and the irradiated group. However, at 5 weeks after irradiation, the serum ALP levels were significantly decreased ($p < 0.05$) in VA (400 and 800 mg/kg) and vitamin C treated animals.

**DISCUSSION**

The results of the present study indicate that treatment with vitamin C and, extracts of *Vernonia amygdalina* and *Hibiscus sabdariffa* may protect the hepatic tissue from the damaging effects of ionizing radiation using biochemical indices. The degree of cell damage caused by radiation depends on several factors, these include; the radiation dose, stage of the cell within the cell cycle, levels of cellular antioxidant defense system, time of administration and the availability of oxygen in tissues during irradiation. The biological damages induced by ionizing radiation are mostly indirect, and mediated by reactive oxygen species (ROS) such as hydroxyl radical (OH), superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O), etc, generated by the radiolysis of water. These reactive species are known to cause degradation of important macromolecules including DNA and membranes. ROS significantly induces lipid peroxidation of biological membranes, thereby producing damaging effects on cells. ROS also affects the hemopoeitic system and considerably decreases its cellular components. In the present study, gamma radiation significantly decreased the antioxidant defense mechanisms; it reduced the liver GSH content, and also decreased the activities of SOD, GST and CAT. The observed effect of radiation on the levels of antioxidant enzymes in this study is in consonance with the findings of Kumar and Kuttan, and Krishna and Kumar. One of the effects of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. Lipid peroxidation (LPO) can be initiated by radiolytic products, such as hydroxyl and hydroperoxy radicals formed by the effect of radiation on water in the cells. In the present study, the plant extracts and vitamin C significantly reduced the levels of lipid peroxidation in the blood of these animals at 5 weeks after irradiation. The potency of vitamin C and VA (800 mg/kg) in particular as anti-liperoxidative agents against radiation-induced LPO was obvious in the liver of the treated animals. Inhibition of LPO in bio-membranes can be achieved by antioxidants, for example, α-tocopherol and β-carotene have been reported to react directly with peroxyl radical involved in lipid peroxidation in *vitro* thereby terminating the adverse effect of this radical. The ability of *Hibiscus sabdariffa* (HS) to attenuate the LPO levels in irradiated animals is not very strange. HS contained gossypetin, glucoside, bibiscin, anthocyanin and protocatechuic acid, which have been reported to elicit several biological activities. Protocatechuic acid and anthocyanin from HS are known to elicit strong antioxidant effects by inhibiting the activity of xanthine oxidase, decreasing the formation of malondialdehyde and effectively scavenging 1,1-diphenyl-1,2-picrylhydrazide (DPPH) radicals in different models. Similarly, the reduction in LPO levels of irradiated animals pretreated with extracts of VA could be linked to the presence of flavonoids, such as; luteolin, luteolin-7-O, β-glucuronoside and luteolin-7-O, β-glucoside, present in the leaves of this plant. Vitamin C, as a reference antioxidant in this study protected the hepatic tissues of the irradiated animals. Extensive animal, clinical and epidemiological studies have confirmed the role of vitamin C in the prevention of diseases. Vitamin C is an important dietary, water-soluble antioxidant and, has been reported to decrease the adverse effect of ROS and nitrogen species generated *in vivo* in animals by scavenging or neutralizing an array of these radicals. In addition, vitamin C can regenerate other antioxidants such as α-tocopherol, urate and β-carotene from their cation radical species. Thus, vitamin C acts as a co-antioxidant and may improve the total antioxidant status of animals exposed to oxidative stress. Due to the aforementioned reasons, vitamin C was used as a reference antioxidant in this study. The ability of vitamin C to attenuate the enzymic
and non-enzymatic antioxidant status of the irradiated animals further confirmed its antioxidant potency. Furthermore, we assessed the integrity of liver from irradiated animals using relevant biochemical indices. It is an establish fact that increased level of ROS generated by radiation will expose the liver (being central to metabolism) to attack. In this study, gamma radiation caused significant increase in the levels of serum aminotransferases (ALT and AST) and alkaline phosphate by enhancing their leakages to the blood. The observed increase in the levels of aminotransferases, in particular ALT, is in conformation with the findings of Kuzin et al.\(^{50}\) and, Nagiev and Karpovich.\(^{51}\) Serum ALT is a relatively sensitive indicator of hepatic damage in certain animal species (rats inclusive) and also cellular injury accompanied with membrane damage and bile formation.\(^{51}\) The fact that vitamin C and, extracts from VA and HS could ameliorate gamma radiation-induced increase in serum aminotransferases point to their hepatoprotective ability,\(^{50,51}\) perhaps mediated by their ability to scavenge ROS generated by the ionizing radiation. Similar explanation holds for the attenuation of radiation-induced increases in the levels of conjugated and unconjugated bilirubin.

The present study showed that treatment of animals with 200, 400 and 800 mg/kg body weight of extracts from VA and HS, as well as vitamin C could attenuate the biochemical indices of toxicity elevated in animals exposed to gamma radiation (4 Gy). This attenuation may be partly due to the scavenging or suppressing of ROS formation, detoxification of radiation-induced species or enhancing the recovery of antioxidant enzymes. This study further supports the use of vitamin C in cancer radiotherapy and, also provides preliminary information on the potential use of VA and HS against radiation-induced damage as well as other ROS mediated disorders such as cancer, rheumatoid arthritis, diabetes, etc. However, further studies are warranted to determine the exact component of VA and HS responsible for the observed effects and the mechanization involved.

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