



## RESEARCH ARTICLE

# The radio-protective effects of n-Hexane extracts of *Telfairia occidentalis* Hook. f. and *Cucumeropsis mannii* Naud. seed oils on the liver of irradiated male Wistar rats

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## ABSTRACT

The human population is predisposed to some considerable amounts of radiation especially ionizing radiation which may negatively impact their metabolic processes. Herbal extracts can mitigate these harmful effects. Therefore, this study aims to investigate the protective effect of n-Hexane extracts of *Telfairia occidentalis* and *Cucumeropsis mannii* seeds oils against radiation-mediated oxidative stress in Wistar rats. Sixty male rats were randomly distributed into six groups of six animals each and n-hexane extracts of *T. occidentalis* and *C. mannii* were administered at a dose of 2.4 or 4.8 mg/kg b. wt., orally for 7 days before irradiation and 10 days after irradiation, when they were sacrificed. Lipid peroxidation was measured, hepatic antioxidant status; SOD, CAT, GSH, Gpx and GST were estimated. The activities of liver enzymes: ALT, AST and ALP were measured and histological examination of sections of the liver was carried out. Radiation significantly increased MDA levels, SOD, GPx, AST, ALT and ALP activities but reduced body weights, total proteins, CAT, GSH and GST activities. Administration of the extracts significantly reduces the levels of MDA, SOD, GPx, ALT, AST and ALP activities while they increase the activities of CAT, GSH and GST at a dosage of 4.8 mg/kg. Histological examination showed increased levels of toxicity in radiated and groups administered 2.4 mg/kg extracts. From these findings, extracts of *T. occidentalis* and *C. mannii* at 4.8 mg/kg b. wt are effective herbal remedies in the prevention and amelioration of the consequences of oxidative stress due to exposure to ionizing radiation.

## Introduction

The human population is predisposed to some considerable amounts of radiation especially ionizing radiation (1). Even if considerable research studies on their dangerous consequences have been documented, humans cannot be fully exempted from absorbing rays intentionally or unintentionally in our daily activities (1). Among the ionizing radiations encountered by humans, one of the most harmful is the radiation from gamma rays which have large energy and a short wavelength (2). Gamma rays can break the barriers of living organisms during their transmission, transmit their radiant energy to biological molecules within the cells and induce oxidative stress by the production of reacting oxygen species (ROS) and free radicals (2-4).

Free radicals have been implicated in the incidence, occurrence and distribution of most metabolic abnormalities such as compromised cell membranes integrity, spleen dysfunction, cancer of the lungs and various other malignancies (1-3). The cytotoxic effects induced by ionizing radiation in normal tissues are reduced or ameliorated through the administration of various organic compounds with therapeutic potential as well as the capability of scavenging the free radicals (4, 5). These compounds are known as radioprotective agents and could be of synthetic or of plant origin (6). Those of plant origin are preferred because they pose little or no side effects (7) and Vegetables such as *Telfairia occidentalis* Hook. f. and *Cucumeropsis mannii* Naud. are good examples (6, 7).

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*T. occidentalis* otherwise called 'fluted pumpkin' belonging to the family of Cucurbitaceae, is one of the most common tropical vines cultivated in Sub-Sahara Africa especially the Western region. This vegetable commonly called the 'Ugu' in the most southern parts of Nigeria, is cultivated for its leaves and seeds which are processed as additives to foods (8). *Cucumeropsis mannii* which also belongs to the family of Cucurbitaceae is widely distributed around the tropics and they possess fibrous and shallow roots (9). They are usually called 'melon or egusi' and are processed for their edible seeds which contain various minerals including sodium and potassium as well as vitamins such as retinol and tocopherols (10, 11).

Most studies on plant biodiversities concerning their medicinal and therapeutic potentials have a focus on the plants' leaves, stems and barks and studies on *T. occidentalis* and *C. mannii* are not exceptions. Some studies of the leaves of *T. occidentalis* include its ability to ameliorate damages caused by oxidative stress as a result of a high dosage of alcohol is by controlling the production of biomarkers for oxidative stress (8).

The effect of the aqueous extract of the leaf of *T. occidentalis* on performances and indices of hematology of starter broilers have been studied (12). Also, its effects on hematological parameters such as packed cell volumes, blood cells and hemoglobin on Wistar rats were studied by and these studies postulated that *T. occidentalis* can be used as an effective blood-booster for the management of anemia (13). The methanolic extracts of *T. occidentalis* have been shown to possess antioxidant activities, hypolipidemic, antidiabetic and hepatoprotective potentials when investigated using rats models (14-16). The effect of the phenolic extracts of *C. mannii* seeds has been studied and shown to have inhibitory potentials against arginase and phosphodiesterase enzymes which are key enzymes in the occurrence of erectile dysfunction, thus can be used as an erectogenic herb in the management of erectile dysfunction (17).

Notwithstanding the investigations on the potentials of *T. occidentalis* and *C. mannii* more researches are still needed to fully uncover their medicinal relevances. The investigation of the radio-protective effects of n-Hexane extracts of *T. occidentalis* and *C. mannii* seed oils on the liver of irradiated male Wistar rats is aimed at using the extracts of *T. occidentalis* and *C. mannii* seed oils as a herbal remedy in the prevention and amelioration of the oxidative consequences of exposure to radiation.

## Materials and Methods

### Plant materials and extraction procedure

Fresh seeds of *T. occidentalis* (T.O) and *C. mannii* (C.M) were obtained from the Bodija market, Ibadan, Oyo State, Nigeria. These seeds were identified, authenticated and samples were kept with the Herbarium of Department of Botany, University of Ibadan, Nigeria. The extraction of the seed oils were

by the method described (18). The seeds were aired dried at room temperature in the Laboratory about washing. The dried seeds were kept in clean dried bottles and later blended manually to obtain a powdered form. 30 g of the powdered seeds were extracted with 250 ml n-hexane using a Soxhlet extractor at 70 °C for 6 hr. After extraction, the solvent was removed from the oil using a rotary evaporator to obtain the crude oil extract. The crude oil was clarified by using 0.1M NaOH at 65°C and bleached by using bentonite clay at 105°C for 1 hr. Desolventization of the extraction solvent was by flashing between 80-100° C.

### Experimental design

Sixty adult male Wistar rats (125–150 g) were used for this study. For acclimatization, the rats were housed in a ventilated rat cage for two weeks. They were fed with normal laboratory feeds and water ad libitum. The rats were divided into six groups randomly with 6 rats per group. Rats in group I were negative control and received only pellets feed while group II was positive control (IR) which was irradiated with 6 Gy and fed with pellets feeds. Group III pairs received 2.4 mg/kg b.wt T.O. and C.M. respectively while group IV pairs received 4.8 mg/kg b.wt without radiation. Group V pairs received 2.4 mg/kg b.wt T.O. and C.M. (2.4 mg/kg b.wt T.O + IR and 2.4 mg/kg b.wt C.M. +IR) respectively while group IV pairs received 4.8 mg/kg b.wt (4.8 mg/kg b.wt T.O + IR and 4.8 mg/kg b.wt C.M. +IR) respectively with radiation.

The crude oil extracts were administered orally at a dosage of 2.4 mg/kg b.wt and 4.8 mg/kg b.wt for 7 days, before and 10 days after irradiation. The irradiation was according to the described method of (1). "The animals were exposed to a single dose of 600 rads (6 Gy) of whole-body gamma radiation from a 60 Co source gamma chamber (Model 220, Atomic Energy of Canada Ltd.) used in the Radiotherapy Unit of the University College Hospital (Ibadan, Nigeria)".

### Tissue Preparation for Sample Collection and Histological studies

10 days after irradiation and administration of extracts, blood samples were collected in a plain tube by ocular puncture technique for clotting. Serum was obtained by centrifugation of the clotted blood samples at 4000 g for 15 mins and stored for biochemical analysis (1). The rats in each of the groups were sacrificed and their livers were harvested for liver homogenate preparation. The harvested livers were rinsed in 1.15% KCl buffer, weighed and placed in a Teflon homogenizer of buffer pH 7.4. The liver homogenates were centrifuged (4° C) and the collected supernatant was stored for biochemical analysis (1).

### Biochemical Analysis

The concentration of total proteins was by the Biuret method using bovine serum albumin as a standard (19). Lipid peroxidation (LPO) was quantified by the thiobarbituric acid (TBA) method in which the concentration of MDA; Malondialdehyde formed per mg protein was estimated (20). Activity of Catalase (CAT) was measured by the rate of decomposition of

hydrogen peroxide at 570 nm (21) while superoxide dismutase (SOD) activity was assayed according to described methods respectively (21, 22). Activities of Glutathione enzymes; Glutathione Peroxidase (GPx) and glutathione S-transferase (GST) were determined according to described methods (23, 24). Non-enzymatic glutathione (GSH) concentration was estimated by the described method (25).

Randox commercial Enzyme kits were used to assay for the Liver function biomarkers: Alanine Amino-Transferase (ALT), Aspartate Amino-Transferase (AST) and Alkaline phosphatase (ALP) according to described methods (26). A small portion of the liver was fixed in 10% neutral buffered formalin for histological examination which was carried out at the Department of Veterinary Anatomy, University of Ibadan, Ibadan. This was carried out according to the described methods (1, 16).

### Statistical analysis

Data collected from this study were analyzed using the two-way analysis of variance for the effect of radiation and treatment extracts. This was followed by a post-hoc LSD test using the SPSS statistical analysis software (SPSS-20®). All statistical analysis was performed at a confidence limit of 95 % ( $p = 0.05$ ) and values are presented as mean  $\pm$  standard deviation.

## Results

The results obtained from the investigation of the effects of the extracts of *T. occidentalis* and *C. mannii* in the prevention of the consequences of radiation on the male Wistar rats are given in the tables and figures described below.

Table 1 shows the effects of n-Hexane Extract of *T. occidentalis* and *C. mannii* on body weight (g) after exposure to radiation. Irradiation significantly reduced the body weight of group II by 15.91% compared to other groups. The bodyweights of groups administered the extracts of T.O. and C.M. at 2.4 mg/kg and 4.8 mg/kg were insignificantly increase

radiation showed reduced the total proteins ( $p < 0.05$ ) when compared with group I. The total proteins of the groups administered the extracts of *T. occidentalis* and *C. mannii* at 2.4 mg/kg and 4.8 mg/kg showed increased ( $p < 0.05$ ) mean values when compared with group II (IR) in a concentration-dependent manner.

Table 3 shows the effects of n-hexane extracts of *T. occidentalis* and *C. mannii* on hepatic LPO Levels, SOD and CAT (U/mg proteins) activities after exposure to radiation (6 Gy). MDA levels and SOD activity were increased in group II exposed to radiation but CAT activities were reduced. Administration of the extracts of *T. occidentalis* at 4.8 mg/kg significantly reduced MDA levels and SOD activities while CAT activities were increased. Administration of the extracts of *T. occidentalis* at 2.4 mg/kg insignificant mean values when compared to group exposed to radiation only. Extracts of *C. mannii* significantly decreased values for MDA and SOD at 4.8 mg/kg but was unchanged at 2.8 mg/kg. However, the activities of CAT were significantly increased when 2.4 mg/kg and 4.8mg/kg extracts of *C. mannii* were administered. The effects of n-Hexane Extracts of *T. occidentalis* and *C. mannii* on Hepatic Intracellular Glutathione Enzymes activities after exposure to radiation (6 Gy) were shown in Table 4. Administration of 2.4 mg/kg and 4.8 mg/kg of the extracts of *T. occidentalis* and *C. mannii* significantly decreased GSH and GPx activities while GST activities remained unchanged in groups not exposed to radiation. In groups exposed to radiation, the values of GSH and GPx were unchanged while GST is significantly reduced when extracts were administered at 2.4 mg/kg. Extracts at 4.8 mg/kg significantly increased the concentration of GSH and GST activities while GPx is unchanged.

The effects of n-hexane extracts of *T. occidentalis* and *C. mannii* on serum AST levels (U/L) of rats After exposure to radiation (6 Gy) were shown in Fig. 1. The mean values of AST were higher in groups exposed to radiation only when compared to the control group ( $p < 0.05$ ). AST levels were significantly reduced in groups administered 4.8 mg/kg extracts of *T. occidentalis* and *C. mannii* but remained

**Table 1.** Effects of n-Hexane extract of *T. occidentalis* and *C. mannii* on body weight (g) after exposure to radiation

| Treated Groups          | <i>T. occidentalis</i> |                                 |                     | <i>C. mannii</i>   |                                 |                     |
|-------------------------|------------------------|---------------------------------|---------------------|--------------------|---------------------------------|---------------------|
|                         | Initial b.wt (g)       | Final b.wt (g)                  | %increase/loss      | Initial b.wt (g)   | Final b.wt (g)                  | %increase/loss      |
| Group I (Control)       | 141.96 $\pm$ 13.92     | 156.81 $\pm$ 19.76              | 10.46 <sup>b</sup>  | 141.96 $\pm$ 13.92 | 156.81 $\pm$ 19.76              | 10.46 <sup>b</sup>  |
| Group II (IR)           | 152.34 $\pm$ 18.99     | 128.10 $\pm$ 23.07              | -15.91 <sup>a</sup> | 152.34 $\pm$ 18.99 | 128.10 $\pm$ 23.07 <sup>a</sup> | -15.91 <sup>a</sup> |
| Group III (2.4 ml/kg)   | 135.43 $\pm$ 6.680     | 146.18 $\pm$ 11.97              | 7.94 <sup>b</sup>   | 134.64 $\pm$ 12.11 | 143.44 $\pm$ 10.06 <sup>a</sup> | 6.54 <sup>b</sup>   |
| Group IV (4.8 ml/kg)    | 134.91 $\pm$ 10.90     | 132.29 $\pm$ 10.20              | -1.94 <sup>d</sup>  | 133.01 $\pm$ 9.261 | 127.34 $\pm$ 9.85 <sup>a</sup>  | -4.26 <sup>c</sup>  |
| Group V (IR+2.4 ml/kg)  | 134.60 $\pm$ 10.31     | 126.02 $\pm$ 9.300 <sup>a</sup> | -6.37 <sup>c</sup>  | 140.38 $\pm$ 15.42 | 130.74 $\pm$ 16.26              | -6.87 <sup>c</sup>  |
| Group VI (IR+4.8 ml/kg) | 137.74 $\pm$ 13.89     | 139.62 $\pm$ 10.00 <sup>a</sup> | 1.36 <sup>d</sup>   | 133.46 $\pm$ 0.50a | 136.51 $\pm$ 2.42               | 2.29 <sup>d</sup>   |

Values are expressed as mean  $\pm$  S.D. for n = 6

\*Mean values with different letters represent statistical significance ( $p < 0.05$ )

body weight when compared to groups I and II. The effects of the seed oil extracts of T.O and C.M. on the serum total proteins after exposure to radiation were shown in Table 2. Group II which were exposed to

unchanged at 2.4 mg/kg when compared to the groups exposed to radiation only. Fig. 2 shows the effects of n-hexane extracts of *T. occidentalis* and *C. mannii* on serum ALT levels (U/L) of rats After

**Table 2.** Effects of n-Hexane extracts of *T. occidentalis* and *C. mannii* on Serum Total Proteins (mg/g) after exposure to radiation

| Treated Groups          | Serum Total Proteins (mg/g) |                          |
|-------------------------|-----------------------------|--------------------------|
|                         | <i>T. occidentalis</i>      | <i>C. mannii</i>         |
| Group I (Control)       | 92.81±11.43 <sup>a</sup>    | 92.81±11.43 <sup>a</sup> |
| Group II (IR)           | 63.53±2.73 <sup>c</sup>     | 63.53±2.73 <sup>c</sup>  |
| Group III (2.4 ml/kg)   | 94.31±3.02 <sup>a</sup>     | 95.23±8.93 <sup>a</sup>  |
| Group IV (4.8 ml/kg)    | 91.86±2.08 <sup>a</sup>     | 99.31±6.11 <sup>a</sup>  |
| Group V (IR+2.4 ml/kg)  | 77.65±5.02 <sup>b</sup>     | 76.18±1.08 <sup>b</sup>  |
| Group VI (IR+4.8 ml/kg) | 85.49±3.27 <sup>a</sup>     | 88.17±0.40 <sup>a</sup>  |

Values are expressed as mean ± S.D. for n = 6

\*Mean values with different letters represent statistical significance ( $p < 0.05$ )

**Table 3.** Effects of n-hexane extracts of *T. occidentalis* and *C. manni* on hepatic LPO Levels, SOD and CAT (U/mg proteins) activities after exposure to radiation (6 Gy)

| Treated Groups          | LPO<br>(nU MDA/mg/Protein) |                           | SOD<br>(U/mg proteins)   |                          | CAT<br>(U/mg proteins)    |                          |
|-------------------------|----------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
|                         | <i>T. occidentalis</i>     | <i>C. mannii</i>          | <i>T. occidentalis</i>   | <i>C. mannii</i>         | <i>T. occidentalis</i>    | <i>C. mannii</i>         |
|                         | Group I (Control)          | 10.21±2.28 <sup>b,c</sup> | 10.2±2.28 <sup>b,c</sup> | 5.87±0.68 <sup>b,c</sup> | 5.87±0.68 <sup>b,c</sup>  | 59.55±2.05 <sup>b</sup>  |
| Group II (IR)           | 18.61±1.46 <sup>a</sup>    | 18.61±1.46 <sup>a</sup>   | 7.41±0.29 <sup>a</sup>   | 7.41±0.28 <sup>a</sup>   | 48.39±4.42 <sup>c</sup>   | 48.39±4.42 <sup>c</sup>  |
| Group III (2.4 ml/kg)   | 9.88±1.34 <sup>c</sup>     | 10.13±0.91 <sup>b,c</sup> | 7.38±0.40 <sup>a</sup>   | 4.82±0.22 <sup>c</sup>   | 75.17±9.97 <sup>a</sup>   | 46.32±1.52 <sup>c</sup>  |
| Group IV (4.8 ml/kg)    | 10.82±5.33 <sup>b,c</sup>  | 9.97±1.01 <sup>c</sup>    | 5.57±0.16 <sup>b,c</sup> | 5.46±0.10 <sup>b</sup>   | 85.53±4.17 <sup>a</sup>   | 48.59±2.88 <sup>c</sup>  |
| Group V (IR+2.4 ml/kg)  | 18.13±2.09 <sup>a</sup>    | 19.51±2.34 <sup>a</sup>   | 8.78±1.56 <sup>a</sup>   | 6.48±1.14 <sup>b</sup>   | 50.38±2.84 <sup>b,c</sup> | 61.11±10.34 <sup>b</sup> |
| Group VI (IR+4.8 ml/kg) | 14.52±2.68 <sup>b</sup>    | 14.71±2.11 <sup>b</sup>   | 6.37±0.45 <sup>b</sup>   | 6.88±0.05 <sup>b</sup>   | 55.86±3.44 <sup>b,c</sup> | 64.93±1.27 <sup>b</sup>  |

Values are expressed as mean ± S.D. for n = 6

\*Mean values with different letters represent statistical significance ( $p < 0.05$ )

**Table 4.** Effect of n-Hexane extracts of *T. occidentalis* and *C. mannii* on hepatic intracellular glutathione enzymes activities after exposure to radiation (6 Gy)

| Treatment groups        | GSH (μmol/mg Protein)      |                            | Gpx (μmolGSH/mg protein) |                          | GST (μmol/min/mg protein) |                          |
|-------------------------|----------------------------|----------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
|                         | <i>T. occidentalis</i>     | <i>C. mannii</i>           | <i>T. occidentalis</i>   | <i>C. mannii</i>         | <i>T. occidentalis</i>    | <i>C. mannii</i>         |
| Group I (Control)       | 24.25±4.193 <sup>a,b</sup> | 24.25±4.193 <sup>a,b</sup> | 2.70±0.39 <sup>c</sup>   | 2.70±0.39 <sup>c</sup>   | 1.32±0.27 <sup>a</sup>    | 1.32±0.27 <sup>a</sup>   |
| Group II (IR)           | 11.75±1.291 <sup>c</sup>   | 11.75±1.291 <sup>c</sup>   | 3.67±0.27 <sup>b,c</sup> | 3.67±0.27 <sup>b,c</sup> | 0.84±0.81 <sup>b</sup>    | 0.84±0.81 <sup>b</sup>   |
| Group III (2.4 ml/kg)   | 29.69±3.430 <sup>a</sup>   | 26.50±1.756 <sup>a</sup>   | 4.81±0.76 <sup>a,b</sup> | 3.09±0.35 <sup>b,c</sup> | 1.31±0.16 <sup>a</sup>    | 1.25±0.43 <sup>a</sup>   |
| Group IV (4.8 ml/kg)    | 29.63±1.451 <sup>a</sup>   | 30.33±2.376 <sup>a</sup>   | 4.33±1.04 <sup>a,b</sup> | 2.86±0.22 <sup>c</sup>   | 1.09±0.04 <sup>a,b</sup>  | 0.73±0.029 <sup>b</sup>  |
| Group V (IR+2.4 ml/kg)  | 12.38±0.595 <sup>c</sup>   | 12.33±0.38 <sup>c</sup>    | 5.30±0.54 <sup>a</sup>   | 3.10±0.01 <sup>b,c</sup> | 1.30±0.38 <sup>a</sup>    | 1.31±0.38 <sup>a</sup>   |
| Group VI (IR+4.8 ml/kg) | 18.42±0.577 <sup>b</sup>   | 19.17±0.38 <sup>b</sup>    | 3.59±0.28 <sup>b,c</sup> | 3.78±0.01 <sup>b,c</sup> | 1.33±0.29 <sup>a</sup>    | 1.03±0.11 <sup>a,b</sup> |

Values are expressed as mean ± S.D. for n = 6

\*Mean values with different letters represent statistical significance ( $p < 0.05$ )

exposure to radiation (6 Gy). The mean values of Group II (IR) were higher than group I ( $p < 0.05$ ). Comparison of *T. occidentalis* and *C. mannii* showed that only 4.8 mg/kg extracts of *T. occidentalis* can significantly reduce ALT after radiation. The mean values of serum ALT were higher in groups exposed to radiation only (Fig. 2). These values were significantly reduced when extracts of *T. occidentalis* and *C. mannii* were administered at 4.8 mg/kg but remained unchanged at 2.4 mg/kg. For ALP as shown in Fig. 3, the values were higher in group exposed to radiation ( $p < 0.05$ ). 4.8 mg/kg extracts of both *T. occidentalis* and *C. mannii* significantly reduced ALP ( $p < 0.05$  while extracts administered at 2.4 mg/kg showed insignificant values ( $p > 0.05$ ).

The histological analysis of liver sections on the effect of *T. occidentalis* after exposure to radiation was shown in Fig. 4. Slide B which belongs to groups exposed to radiation only showed the presence of severe portal congestion and vacuolar degeneration

associated with a stressed liver. Slide A which belongs to the control group, showed no visible lesion. Also, slides C and D which belong to groups administered only extracts of *T. occidentalis* showed no visible lesion. Slide F showed slight to moderate vacuolar degeneration while slide E which is group irradiated and administered 2.4 mg/kg of extracts, still maintained severe vacuolar degeneration similar to slide B. The observations of the histological analysis of liver sections on the effects of *C. mannii* after exposure to radiation shown in slides A-F (Fig. 5) were similar to those of the administration of extracts of *T. occidentalis*.

## Discussion

Living organisms are exposed to some amounts of radiation especially ionizing radiations during radiotherapy and transmit their radiant energy to

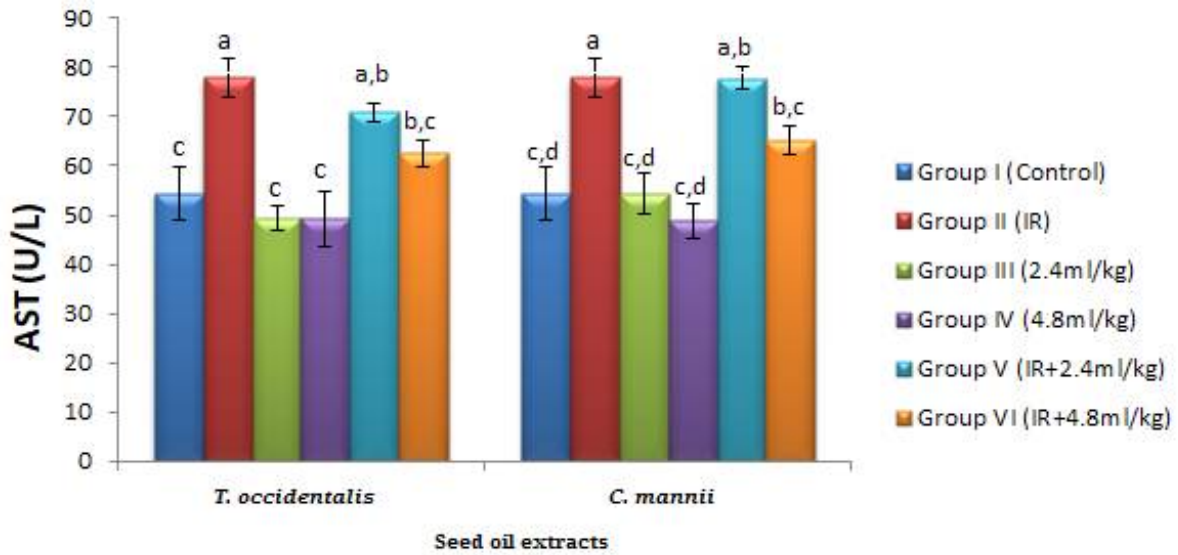


Fig. 1. Effects of n-hexane extracts of *T. occidentalis* and *C. mannii* on serum AST levels (U/L) of rats after exposure to radiation (6 Gy). \*Mean values with different letters represent statistical significance ( $p < 0.05$ )

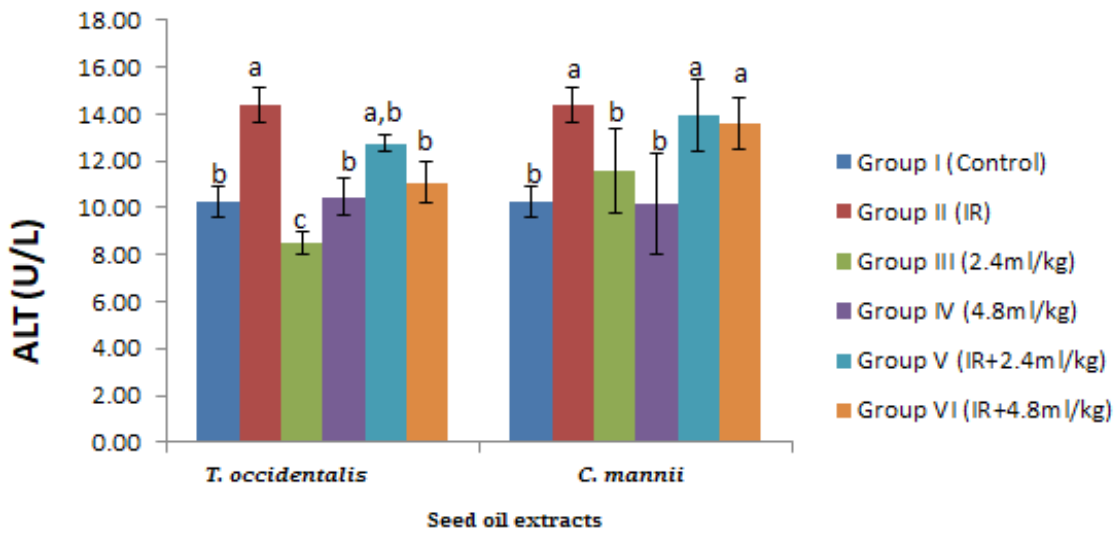


Fig. 2. Effects of n-hexane extracts of *T. occidentalis* and *C. mannii* on serum ALT levels (U/L) of rats after exposure to radiation (6 Gy). \*Mean values with different letters represent statistical significance ( $p < 0.05$ )

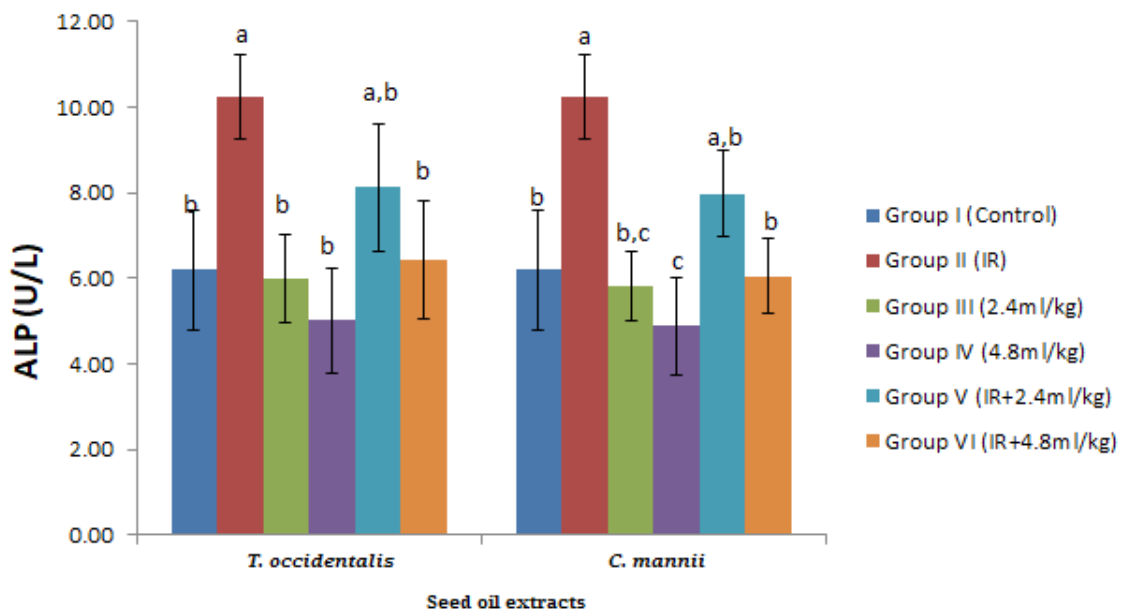
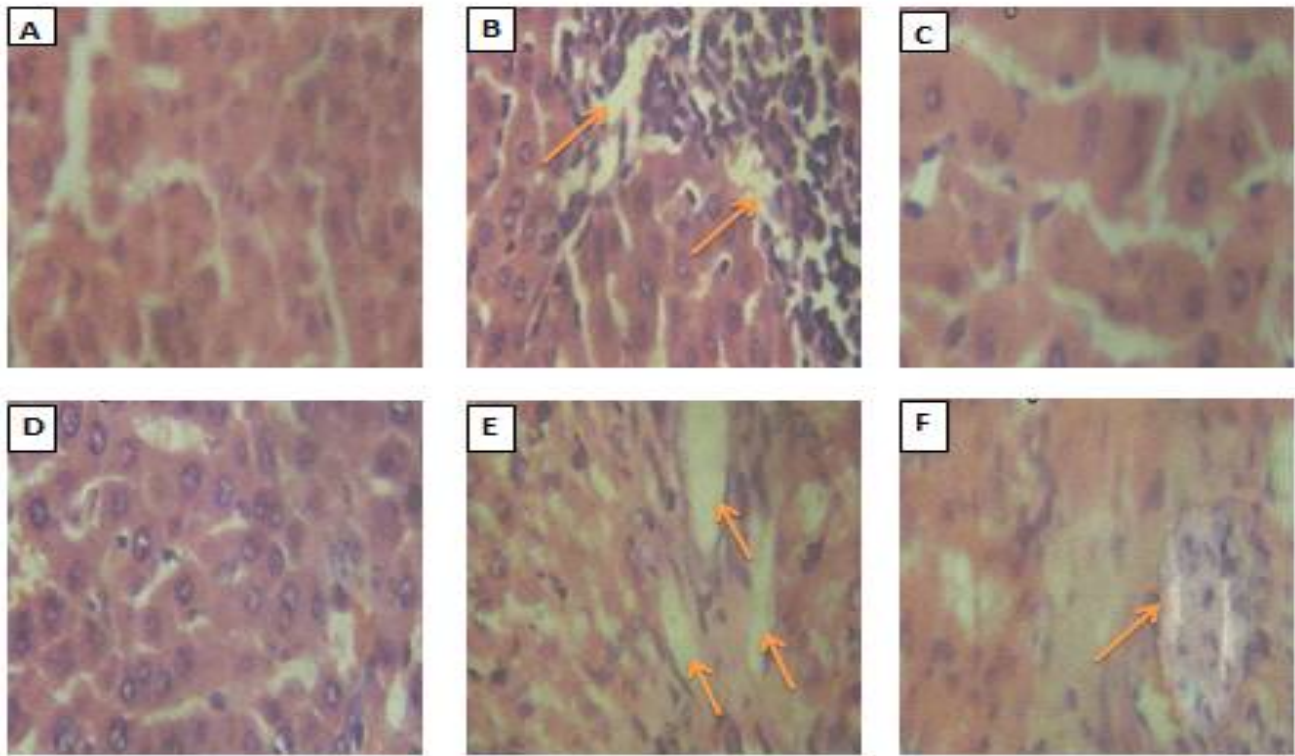
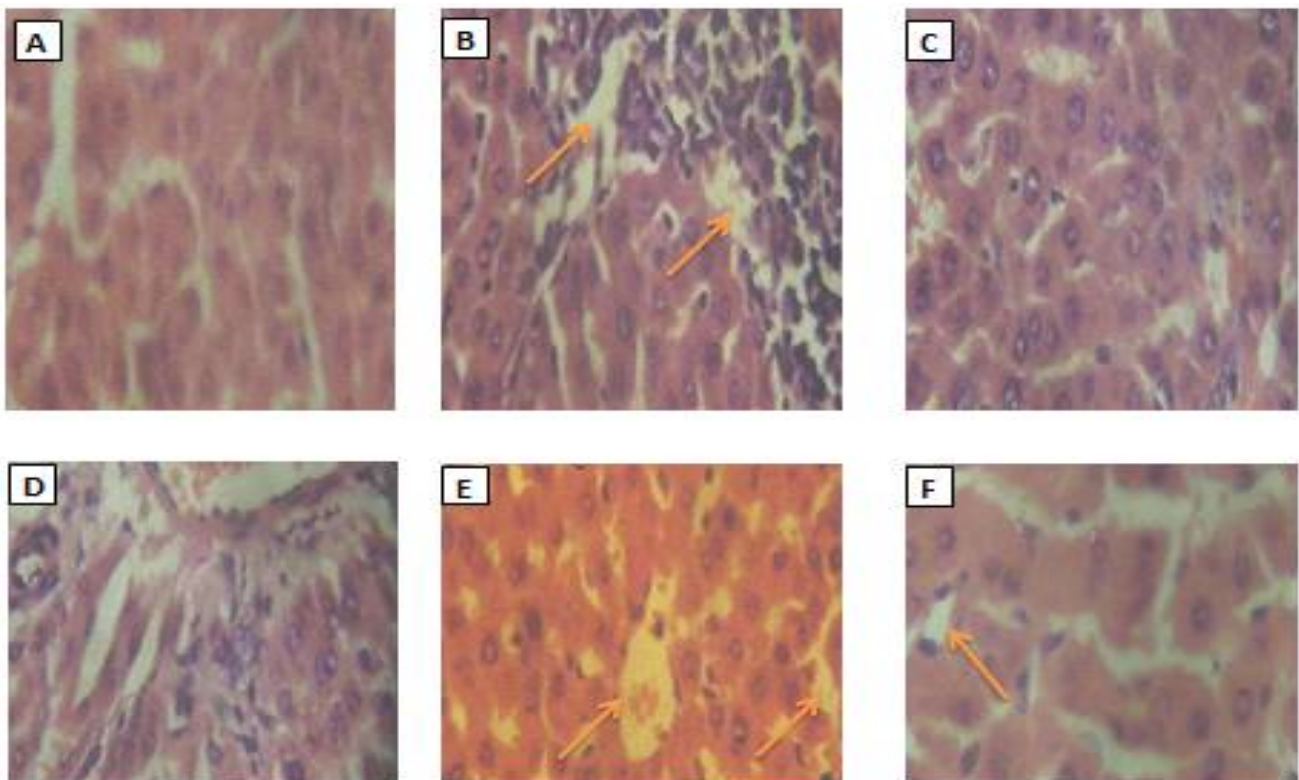


Fig. 3. Effects of n-hexane extracts of *T. occidentalis* and *C. mannii* on serum ALP levels (U/L) of rats after exposure to radiation (6 Gy). \*Mean values with different letters represent statistical significance ( $p < 0.05$ )



**Fig. 4.** Histological analysis of liver sections on the effect of *T. occidentalis* after expose to radiation.  
\*Hepatocytes showing abnormal morphology are represented by the yellow arrow. A: Group I (control), B: Group II (IR only), C: Group III (2.4 mg/kg b. wt.), D: Group IV (4.8 mg/kg b. wt.), E: Group V (IR + 2.4 mg/kg b. wt.) and F: Group VI (IR + 4.8 mg/kg b. wt.)



**Fig. 5.** Histological analysis of liver sections on the effect of *C. mannii* after expose to radiation.  
\*Hepatocytes showing abnormal morphology are represented by the yellow arrow. A: Group I (control), B: Group II (IR only), C: Group III (2.4 mg/kg b. wt.), D: Group IV (4.8 mg/kg b. wt.), E: Group V (IR + 2.4 mg/kg b. wt.) and F: Group VI (IR + 4.8 mg/kg b. wt.)

biological molecules within the cells and induce oxidative stress by the production of reacting oxygen species (ROS)/reacting nitrogen specie (RNS) in the form of free radicals as previously stated (1). These Free radicals production has been implicated in the incidence, occurrence and distribution of most

metabolic abnormalities and ultimately death of cells (27).

The changes in the body weights observed among the groups exposed to radiation show the potential of bodyweight reduction by exposure to gamma radiation (Table 1). Several studies have

shown that oxidative stress can reduce total proteins especially when it interferes with the production of albumin which constitutes more than 60% of the total proteins (1, 28-30). The extracts of *T. occidentalis* and *C. mannii* show their potency in the prevention of weight loss and reduced total proteins due to gamma irradiation (Table 2).

Radiations have been shown to increase lipid peroxidation as a consequence of the overwhelming production of free radicals that subdue the cell antioxidant cascade (31, 32). The extent of this cell membrane compromise is estimated by the measurement of the MDA and GSH levels. Therefore, an increased MDA and reduced GSH values associated with this study are expected when animals are exposed to the 6 Gy gamma radiation (Table 3, 4).

Within tolerable limits of production of free radicals, the cell can be protected from their deleterious effects by the engagement of its endogenous antioxidant defense mechanisms which include the enzymatic and non-enzymatic systems. The enzymatic mechanisms involve the upregulation of the synthesis of antioxidant enzymes such as SOD, CAT, GPx and GST. Superoxide dismutase provides the first-line defense against ROS mediated cellular damages is provided by Superoxide dismutase which converts the superoxide radicals into hydrogen peroxide ( $H_2O_2$ ) and the hydrogen peroxide produced is catabolized by catalase into water ( $H_2O$ ) and oxygen ( $O_2$ ). Thus, ROS generated from oxidative stress is neutralized (33, 34).

Excessive ROS as such associated with radiation will initially increase the activities of SOD and CAT and will be decreased in the long run. This may be a reason for the increased SOD and decreased CAT values observed in our studies when group II was exposed to radiation (Table 3). These findings are supported by several reports (16, 35, 36).

Hydrogen peroxide can also be neutralized by the action of glutathione peroxidase (GPx). GPx uses the reduced glutathione (GSH) to reduce the  $H_2O_2$  into  $H_2O$  and  $O_2$  while GST plays an important role in the conjugation of xenobiotics with GSH in the glutathione redox cycle (37). Our findings showed that radiation significantly reduced GSH and GST while GPx is increased (Table 4) and this is corroborated by the documented findings (28, 38, 39). The increase in GPx may be as a result of the increased hydrogen peroxide which has been shown to upregulate the gene for the synthesis of glutathione peroxidase while low levels of GSH negatively affect the synthesis of GST (40, 41). The extracts of *T. occidentalis* and *C. mannii* significantly reduced MDA, SOD and GPx while GSH levels, CAT and GST activities were increased (Table 3, 4) as supported by documented findings (8, 40-42).

Liver function biomarkers play crucial roles in the assessment of toxicological implications of therapeutics and several extracts to the survival of humans as the liver is the site of detoxification. Hepatocellular damages has been correlated with increased levels of the major biomarkers of the hepatocytes which are AST, ALT and ALP (43). Exposure of rats to gamma irradiation induces

hepatic damage and a significant increase in activities of serum alanine aminotransferases (ALT), aspartate aminotransferases (AST) and alkaline phosphatase (ALP) which is consistent with the previous observations (44, 45). Extracts of *T. occidentalis* and *C. mannii*, reduced AST, ALT and ALP at a dosage-dependent manner with a dosage of 4.8 mg/kg being effective in the reduction of the radiation effect on liver enzymes. These findings are in line with the reports of 35 and 38. Radiation caused severe portal congestion and vacuolar degeneration while the control has no visible lesion. Extracts of *T. occidentalis* and *C. mannii* showed slight or moderate vacuolar degeneration to signify the ameliorating effect of extracts against radiation (Fig. 4, 5). These histological sections give further credence to the increased values of liver enzymes when animals are exposed to irradiation.

The ability of the extracts of *T. occidentalis* and *C. mannii* to reduce lipid peroxidation and improve antioxidant capacities suggests that these extracts, possibly contain some bioactive agents capable of scavenging free radicals and preventing radiation-induced lipid peroxidation. Thus, they could inhibit the disruption of membrane integrity by reactive oxygen species during radiation exposure. The seed of *T. occidentalis* is very rich in oil, especially unsaturated fatty acids which form 61% of the oil. It was reported that fluted pumpkin seeds contain fat, protein and carbohydrate (46). The seed also contains a high level of vitamin A which possesses antioxidant capacity (14, 47). Similarly, seeds of *C. mannii* have been shown to possess a significant quantity of vitamins A and E which is also essential in antioxidant cascade (10).

## Conclusion

From the findings of this study in which there are evidence of reduction in the tissue and serum biochemical markers as well as reduced histological indices of toxicity, which could be due to the suppression of reactive oxygen species generation and induction of the synthesis of the antioxidant enzymes, this study concludes that extracts of *T. occidentalis* and *C. mannii* at 4.8 mg/kg b. wt. are effective herbal remedies in the prevention and amelioration of the consequences of oxidative stress due to exposure to ionizing radiation.

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## Authors' contributions

The study was conceived and supervised by NSO. All authors participated in its design, the procurement and administration of extracts to experimental animals were by UOM. The laboratory analysis was

carried out by UOM and IEJ under the supervision of NSO. All authors were involved in the preparation, reading and approval of the manuscript.

### Conflict of interests

Authors do not have any conflict of interest to declare.

### Ethical approval

Ethical approval and consent to undertake this study was approved by the Animal Care and Use Committee of the Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria.

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