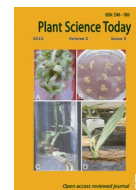




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Research Article

***Cochorous olitorious* and *Adasonia digitata* leaves extracts protects against gamma radiation induced anaemia**

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Abstract

This paper proposes that exposure to radiation could generate free radicals, which could lead to disorders such as hemolysis-induced anaemia. We evaluated the radioprotective potentials of *Cochorous olitorious* and *Adasonia digitata* methanol leaves extract in gamma radiation induced anaemia. Fifty four adult male Wistar rats were divided into nine groups of 6 animals. Except for the control group, the other the animals were treated with a single dose of whole body gamma radiation of 6Gy and received either 500 or 1000 mg/kg body weight (bwt) of *A. digitata* and *C. olitorious* either singly or combination and vitamin C was used as reference. *A. digitata* and *C. olitorious* were screened for phytochemical content and had saponin (16.59±1.85 and 22.12±0.24), tannins (311.98±0.01 and 287.07±0.16), polyphenols (170.90±0.68 and 330.07±0.32), alkaloids (81.56±0.56 and 68.65±2.05) and flavonoids (25.38±2.88 and 157.38±0.38) respectively. There was significant loss in body weight, depletion in red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (HBC) in untreated rats exposed to gamma radiation. Administration of the plant extract to rats exposed to radiation was able to attenuate and ameliorate loss in body weight and changes in blood cells (HBC, PCV, PLT and RBC) especially in radiated rats on combination therapy of both extracts at 1000 mg/kg bwt group. Hepatoprotective and safety evaluation was done by measuring the serum ALT, AST and ALP, these parameters were significantly (p<0.05) increased in untreated rats exposed to gamma radiation compared to normal control rats and these decreased in rats on plant extract.

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Introduction

Ionizing radiation and other radioactive sources of high electron leakages, produce free radicals, which could lead to deleterious conditions such as hemorrhage induced anaemia, cancer, ischemia reperfusion diseases, atherosclerosis, diabetes and several other pathological disorders (Haliwell and Gutteridge, 2000). Ionizing radiation initiates the formation of free radicals, with the potential to attacking molecular oxygen thus producing a variety of reactive oxygen species (ROS) (Sadani and Nadkarni, 1997; Anderson *et al.*, 2001). These reactive species react with the biomolecules such as nucleic acids (DNA and RNA), mostly membrane proteins and lipids, resulting to chain of reaction leading to lipid peroxidation, protein oxidation and Nucleic acid-base breakage (Parihar *et al.*, 2007). ROS in membranes could cause alterations to the integrity, fluidity, permeability and biological functions of membranes; while they could generate in low density lipoprotein (LDL) proatherogenic

and proinflammatory response as well as generates toxic products (Greenberg *et al.*, 2008). One of the pathological conditions resulting from human exposure to radiations is anaemia, a blood disorder which affects all age groups especially the elderly, pregnant, lactating women and infants been more susceptible. There are different types of anemia and all types lead to depletion in red blood cells (WHO, 1986; Ogbe *et al.*, 2010). Iron deficiency is the most common cause of nutritional anaemia, a condition which affects over 600 million especially in low-income countries (Oladiji *et al.*, 2007).

Corchorus olitorius (Linn) is a leafy vegetable that belongs to the family Tiliaceae, and is known as "Ewedu" and Krin-krin in South western and South Eastern, Nigeria. The leaves (either fresh or dried) are cooked into a thick viscous soup or added to stew or soup and are rich sources of vitamins and minerals (Branda *et al.*, 2004).

Nutritionally, *C. olitorius* on an average contain 85-87g H₂O, 0.7g oil, 5g carbohydrate, 1.5g fiber, 250-266mg Ca, 4.8mg Fe, 1.5mg vitamin A, 0.1mg thiamine, 0.3mg riboflavin, 1.5mg nicotinamide, and 53-100mg ascorbic acid per 100g (Branda *et al.*, 2004). Leaves decoction is used for treating iron deficiency, folic acid deficiency, as well as treatment of anemia and as blood purifier, while the leaf twigs are used against heart troubles (Krivanek *et al.*, 2007).

Adansonia digitata L. is a tree found widely throughout Africa and known locally in African countries as the tree of life. *A. digitata* is commonly used traditional plant which are consumed in food or used in the direct treatment of several diseases such as cancer, anaemia, diabetes, ischemia reperfusion diseases, and inflammatory bowel syndrome in South-western Nigeria (Lewanda *et al.*, 2007). The baobab tree is an important food, water and shelter source in many African countries (Byrd-Bredbenner *et al.*, 2007). Chemical composition include tannins, phlorotannins, terpenoids, glycosides, saponin and terpenoids as well as antioxidants including flavonoids and polyphenols. Drying baobab leaves in the shade protects against deterioration of provitamin, other authors mention the carotenoid content of baobab leaves. This result is almost 1000 times lower than composition and nutritional value of baobab foods the one reported by Vertuani *et al.* (2002), content of flavonoids and other antioxidant (Krivanek, 2007).

These considerations led us to evaluate the effect of *C. olitorous* and *A. digitata* on rats exposed to radiotherapy and to compare the protective effect of individual plant extract, combined effect of administration of both extracts and to compare that with standard antioxidant drug Vitamin C.

Materials and Methods

Plants materials

The leaves of *C. olitorous* and *A. digitata* were purchased at Bodija market, Ibadan, Oyo State. The plants were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN). Leaves were dried, ground into a coarse powder and 500g each of the plants macerated in 2000 ml of methanol (solvent) for 72h. Extract was filtered and concentrated using rotatory evaporator, the percentage yield of *C. olitorous* was 4.6% and *A. digitata* was 14.8 %.

Animals, radiation exposure and treatment

Ninety adult male albino rats weighing 190-200g were purchased from the Animal house of the Department of Physiology, University of Ibadan. These rats were initially

acclimatized for a week in well-ventilated cages in Animal house of Department of Biochemistry. Rats had free access to water and rat feed bought from Ladokun Feeds, Mokola, Ibadan.

Animals in the irradiated groups were treated with samples, 1 week before radiation exposure and 1 week after radiation exposure. The animals were treated with a single dose of whole body gamma radiation of 6Gy. Last dose of sample administration was 24hours before irradiation whereas the feed was withdrawn 12hours before irradiation. The radiation exposure system was done at Radiotherapy Department, College of Medicine, UCH, Ibadan. The animals were kept in an improvised cage to restrict their movement and to ensure uniform and effective exposure.

Experimental design

Fifty four adult male Wistar rats weighing between 190-200g, were purchased in the Animal house of Department of Biochemistry, University of Ibadan, and was randomly selected and distributed into nine groups of 6 rats each. The designs of the 9 group members are given in Table 1.

Table 1. Animal grouping and treatment

Control	Non-irradiated, non-treated
IR	Irradiated, non-treated (R)
IR + 500mg/kg	Irradiated animals treated with 500mg/kg body weight (RE _{1 500})
IR + 1000mg/kg	Irradiated animals treated with 1000mg/kg body weight (RE _{1 1000})
IR + 500 mg/kg	Irradiated animals treated with 500mg/kg body weight (RE _{2 500})
IR + 1000mg/kg	Irradiated animals treated with 1000ml/kg body weight (RE _{2 1000})
IR + 500 mg/kg	Irradiated animals treated with 500mg/kg body weight (R E _{1+E₂ 500})
IR + 1000mg/kg	Irradiated animals treated with 1000mg/kg body weight (R E _{1+E₂ 1000})
IR + Vitamin C	Irradiated animals treated with vitamin C (100 mg/kg body weight)

Phytochemical screening

The phytochemicals such as flavonoids, tannins, saponins, alkaloids, polyphenols and anthraquinones were identified by chemical method and as modified by Harborne (1996) and Sofowora (1993).

Table 4. Effects of Methanolic extract of *A. digitata* and *C. olitorous* body weight (g) of rats after exposure to radiation (6Gy)

Treatment Group	Initial b.wt	Final b.wt	Changes b.wt.
NORMAL	199.42 ± 0.59	209 ± 0.00	9.58± 0.59
IR	201.22 ± 0.78	143 ± 0.33	58.22± 0.45
IR (E ₁ 500)	200.33 ± 0.33	166 ± 0.37	34.33±0.04
IR (E ₁ 1000)	201.67 ± 0.33	186 ± 0.58	15.67±0.25
IR (E ₂ 500)	210.00 ± 0.58	169 ± 0.58	41.00±0.00
IR (E ₂ 1000)	197.67 ± 0.33	189 ± 0.58	8.67±0.25
IR (E ₁ +E ₂ 500)	190.00 ± 0.58	194 ± 0.58	4.00±0.00
IR (E ₁ +E ₂ 1000)	197.67± 0.33	208 ± 0.33	11.67±0.00
IR (VIT C)	200.33± 0.33	169 ± 0.33	31.33± 0.00

Values expressed as means ± SEM; n=9, IR: Irradiated; E₂: *C. olitorous*; E₁: *A. digitata*. Significant difference of *p*<0.05.

Table 5. The hematological analysis of rats exposed to radiation (6Gy) on methanolic extracts of *A. digitata* and *C. oltoruous*.

Treatment Group	PCV (%)	HB (%)	RBC (%)	WBC (10 ⁹ /L)	LYPMS	PLT (10 ⁹ /μL)
NORMAL	36.29±0.36 ^a	12.11±0.21 ^a	7.84±0.06 ^a	8.55±0.04 ^a	47.23±0.39 ^a	134227.00±330.06 ^a
IR	26.97±0.89 ^b	6.40±0.12 ^b	3.76±0.01 ^b	13.51±0.33 ^b	81.52±0.29 ^b	67139.67±204.81 ^b
R(E ₁ 500)	26.70±0.16 ^b	8.82±0.10 ^c	5.37±0.04 ^c	10.16±0.03 ^c	65.48±0.29 ^c	81876.33±1406.43 ^c
R(E ₁ 1000)	32.81±0.42 ^c	10.89±0.26 ^a	8.44±0.26 ^a	11.96±0.04 ^d	69.33±0.33 ^d	97921.33±39.48 ^d
R(E ₂ 500)	27.14±0.32 ^b	9.54±0.03 ^c	5.31±0.05 ^c	10.07±0.07 ^c	65.48±0.29 ^c	81525.00±635.02 ^c
R (E ₂ 1000)	31.99±0.20 ^c	11.07±0.12 ^a	9.07±0.04 ^a	11.05±0.08 ^d	69.20±0.31 ^d	98485.33±257.36 ^d
R(E ₁ +E ₂ 500)	33.51±0.38 ^c	13.00±0.11 ^a	10.99±0.01 ^a	11.99±0.02 ^d	71.74±0.13 ^d	108666.33±605.87 ^e
R(E ₁ +E ₂ 1000)	39.21±0.34 ^a	15.33±0.17 ^a	13.73±0.09 ^d	14.85±0.09	74.99±0.07	129044.67±29.356
RVIT.C	27.40±0.81 ^b	8.20±0.11 ^a	5.34±0.02 ^c	10.38±0.31	64.82±0.24	81709.00±595.84

Values are Expressed as Means ± SEM; n=9, R: Irradiated, E₂: *C. oltoruous*; E₁: *A. digitata*. Significant difference of $p < 0.05$.

Table 6. Effect of methanolic extracts of *A. digitata* and *C. oltoruous* on serum AST, ALT and ALP levels (U/L) of rats exposed to radiation (6Gy).

Treatment Group	AST	ALT	ALP
NORMAL	95.39 ± 0.89 ^a	58.23 ± 0.51 ^a	24.68 ± 0.16 ^a
IR	143.33 ± 0.89 ^b	98.00 ± 0.57 ^b	76.49 ± 0.77 ^b
R(E ₁ 500)	126.32 ± 0.36 ^c	72.71 ± 0.16 ^c	62.53 ± 0.24 ^c
R(E ₁ 1000)	101.58 ± 0.27 ^d	53.15 ± 0.45 ^a	46.15 ± 0.57 ^d
R(E ₂ 500)	121.37 ± 0.71 ^c	69.89 ± 0.23 ^c	60.73 ± 0.37 ^c
R (E ₂ 1000)	98.34 ± 1.03 ^a	57.73 ± 0.37 ^d	43.82 ± 0.30 ^d
R(E ₁ +E ₂ 500)	87.26 ± 0.50 ^a	54.45 ± 0.29 ^a	39.07 ± 0.14 ^e
R(E ₁ +E ₂ 1000)	75.89 ± 0.11 ^e	48.67 ± 0.67 ^e	24.30 ± 0.39 ^a
RVIT.C	120.33 ± 0.33 ^c	66.67 ± 0.88 ^c	60.00 ± 0.58 ^c

Values are expressed as means ± sem; n=9, R: Irradiated, E₂: *C. oltoruous*; E₁: *A. digitata*. Significant difference of $p < 0.05$.

Determination of hematological and biochemical parameters

Whole blood was collected from the eyes by ocular puncture and were put in ethylenediamine tetracetate (EDTA) bottles. The packed cell volume or the haematocrit and White blood cell count (WBC) was determined by the method of Baker and Silverton (1985), Hemoglobin (Hb) concentration was determined using the cyanomethemoglobin method (Jain, 1986) while platelets were determined by following the method of Mitruka and Rawnsley, (1997).

Determination of serum enzyme assay

Alanine transaminase (ALT) and aspartate transaminase (AST) activities and alkaline phosphatase (ALP) serum level was estimated were determined using the Randox kits by Cypress diagnostics (Belgium).

Statistical analysis of data

Results was expressed with confidence interval as mean ± standard deviation and were analyzed using the Analysis of Variance 'ANOVA, F-ratio and student's *t* test where applicable. Values of $P \leq 0.05$ were regarded as significant in comparison with appropriate controls.

Results and Discussions

Table 2 shows the results of qualitative phytochemical screening of methanolic leaf extract and the presence of Saponin, alkaloids, flavonoids, polyphenols, tannins and terpenoids were detected. Anthraquinone, cardiac glycosides and phlobatannins were not found in *C. oltoruous* and *A. digitata*. Quantitative phytochemical screening showed that tannins and polyphenols were the most abundant phytochemicals present in both leaf extracts as shown on Table 3. Alkaloids have shown efficient therapeutic properties as an anticancer, analgesic, antispasmodic and antibacterial properties (Yakubu *et al.*, 2009; Branda *et al.*, 2004). Both natural and synthetic

alkaloids have been used as basic medicinal agent. Polyphenol have been shown to be responsible for the induction of several phase-2 (Farombi and Shur, 2006).

Table 2. Qualitative phytochemical screening

Metabolites	<i>Adasonia digitata</i>	<i>Corchorus oltorius</i>
Alkaloids	++	++
Anthraquinone	-	-
Cardiac glycosides	-	+
Flavonoids	++	++
Polyphenols	++	+++
Phlobatannins	-	-
Saponin	++	++
Taninn	+++	++
Terpenoids	+	++
Steroids	+	+

+: Faintly Present ++: Moderately Present +++: Excessively Present -: Absent

Table 3. Quantitative phytochemical screening

Metabolites	<i>Adasonia digitata</i>	<i>Corchorus oltorius</i>
Alkaloids	81.56 ± 0.56	68.65 ± 2.05
Saponin	16.59 ± 1.85	22.17 ± 0.24
Tanins	311.98 ± 0.01	287.07 ± 0.16
Flavonoids	25.38 ± 2.88	157.38 ± 0.38
Polyphenols	170.90 ± 0.68	330.07 ± 0.32

Changes in body weight (bwt) changes in observed in rats in this study is shown on Table 4. Initially the bwt of the rats was 190-200g, but the untreated radiated rats had loss in weight of over 50g. Generally all animals exposed to gamma radiation had loss in bwt but animals on treatment with either vitamin C and plant extract all gained weight with animals on *C. oltoruous* and *A. digitata* combination therapy at 1000 mg/kg bwt having similar weight gain as the control rats that was not exposed to gamma radiation.

Hematological parameters for rats on *C. oltoruous* and *A. digitata* methanol extract exposed to gamma radiation

is shown on Table 5. Untreated radiated rats had significantly decreased PCV, haemoglobin concentration and red blood cells, while platelets, lymphocytes and white blood cells compared to control un-radiated rats. Treatment with plant extract significantly attenuated the deleterious effects of irradiation, especially in animals on 1000 mg/kg.

For the biochemical parameters in the Table 5, statistical analysis showed that the methanolic leaf extract of *C. olitorius* and *A. digitata* recorded a significant ($P=0.05$) increase for total proteins level for all treatment groups; C (60.20 ± 0.46), D (68.97 ± 0.23), and E (60.40 ± 0.30), F (70.14 ± 0.10), G (59.85 ± 0.20), H (79.63 ± 0.80), I (67.89 ± 3.90) when compared with negative control B (54.10 ± 0.58). But as observed in the Bilirubin and the MDA level which reduces upon extract administration when compared with the negative control groups that was exposed to radiation but not treated with the plant extracts.

Saponin has been established by Yakubu *et al* (2007) to prevent haemoglobin and platelets aggregation and protein oligomerization because saponin containing herbs have been successfully used in the management of liver inflammation, Polyphenol in the plant extracts has shown free radical scavenging free radicals and flavonoid have shown ability to prevent oxidative stress induced diseases (Kryston *et al.*, 2011).

Free radicals has the potentials to attack iron containing proteins like haemoglobin resulting to the formation of a disulfide bonds, which disrupt protein structure giving rise to abnormal haemoglobin known as Heinz body. This in combination with other membrane and cytosolic proteins could trigger phagocytic degradation, resulting into iron loss, reduction in the RBC level and packed cell volume, all are pointers to anaemia. There was significant reduction in the PCV, RBC, HBC and PLT level in untreated rats exposed to radiation, when compared to the normal control group, this is suggestive of the effect of free radicals generated from radiation attacked on haemoglobin for destruction resulting into altered haemoglobin culminating anaemia. However, the administration of the methanolic extracts of *C. olitorius* and *A. digitata* on the anaemic irradiated rats was able to ameliorate the decrease in PCV, RBC, PLT and HBC as shown on Table 5. The Table 5 shows that upon extracts administration, the PCV, HB, RBC and PLT level that was initially reduced significantly ($P<0.05$) in the negative control group when compared with positive control group was significantly ($P<0.05$) increased. There was significant ($P>0.05$) reduction in white blood cell count (WBC) on the radiated groups co-treated with plant extract and this could be due to the presence of bioactive agents like polyphenols, flavonoids and tannins as shown on Tables 1 and 2 which have been implicated in free radical scavenging (Oyedepi *et al.*, 2013).

Radiation increased the activities of AST, ALT, and ALP significantly and these were attenuated by administration of *C. olitorius* and *A. digitata* either singly or the combination treatment as shown on Table 6. The high level of ALT, AST and ALP in the serum is an indication of the degree of damages to the liver caused by the radiation. This is in agreement with the work of Albano, (2002) and Cederbaum, (2009) that rise in transaminase is an indication of severe hepatotoxicity thus allowing their escape from the tissues. The reduction in the levels of ALT,

AST and ALP activity in the groups co-administered with *C. olitorius* and *A. digitata* at 500 and 1000 mg/kg may be due to the phytochemicals present in the plant extracts such as the flavonoids and polyphenols (Arteel, 2003).

For AST levels, the extract recorded a significant ($P=0.05$) decrease for all treatment groups; C (126.32 ± 0.36), D (101.58 ± 0.27), E (121.37 ± 0.71), F (98.34 ± 1.03), G (87.26 ± 0.50) H (75.89 ± 0.11) I (120.33 ± 0.33) when compared with negative control B (143.33 ± 0.89). Similar trend were also observed in ALT and ALP levels. The Group 9 (Vitamin C treated group) was able to compete with the plant extract for treatment of irradiation induced anaemia at 500 mg/kg body weight, but above this concentration (1000 mg/kg) the combination therapy of *C. olitorius* and *A. digitata* had better effect than ascorbic acid. In conclusion *C. olitorius* and *A. digitata* extracts at 1000 mg/kg bwt showed better therapeutic efficacy when compared to 500 mg/kg bwt and combination of both plants (500 mg/kg and 1000 mg/kg bwt) was efficacious more than ascorbate alone.

Competing interests

The authors declare that they have no competing interests.

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