



Original Paper

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***Vernonia amygdalina* leaf extract and alpha-tocopherol alleviated gamma radiation-induced haematological and biochemical changes in rats**

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ABSTRACT

The methanolic extract of *Vernonia amygdalina* (MEVA) has previously been shown to possess antioxidant property and provide radioprotection in the brain and liver of rats, but this effect has not been tested in blood cells. The objective of this study was to test if MEVA could offer radioprotection for blood cells of rats using alpha-tocopherol (TOCO) as a standard antioxidant. Forty-two male albino Wistar rats, aged 12-14 weeks were randomly divided into seven groups of six rats each. The control group received distilled water orally, while other groups received either MEVA, MEVA with radiation, or radiation alone. Rats were treated for 14 days, irradiated on the 15th day, euthanized on the 16th day, and their blood investigated using standard methods. Data were analyzed using ANOVA with post-hoc test. Results showed that radiation caused a reduction in the haemoglobin, red blood cells and packed cell volume, which pretreatment with MEVA did not improve, whereas TOCO caused a significant increase in the values. The radiation-induced reduction of lymphocytes and increases in the liver enzymes was mitigated by pretreatment with both MEVA and TOCO. This study demonstrated that MEVA and TOCO provided radio-protection for rat's lymphocytes and the liver enzymes.

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INTRODUCTION

The beneficial effects of radiation therapy in the management of cancers have been documented (Jagetia et al., 2005). Radiation therapy (RT) is the use of high-energy rays to damage cancer cells, thereby stopping the malignant cells from further growing and dividing. Although RT regimes are designed to maximize tumoricidal effects while causing minimal damage to normal

organs, in reality, it may cause damage to adjacent non-cancerous cells (Belka et al., 2001).

The use of total body irradiation (TBI) may be indicated in some medical conditions, for example, as in preparation of patients for renal transplant (Aunapuu et al., 2003). TBI may cause acute radiation syndrome (ARS), defined as an acute illness caused by a dose greater than 0.5-1 Gy of penetrating radiation

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to most or all of the body in a short time, usually a matter of minutes (Waselenko et al., 2004; MedicineNet.com, 2011). Syndromes associated with ARS may include the haematologic, the gastrointestinal, and the cardiovascular (CVS) / central nervous system (CNS) syndromes. The haematologic and liver aspects of these associated syndromes are the subject of this investigation. The progenitor cells of the haemopoietic system resident in the bone marrow, and the epithelium of the gastrointestinal tract are highly radiosensitive, whereas the red cells are relatively radio resistant (Hla et al., 2003). Among the leukocytes, lymphocytes have been reported to be the most radiosensitive haematologic cells (Jones et al., 1997; Jagetia et al., 2002).

Radiation side effects have been attributed to generation of free radicals (FR), which when in excess interact with biological systems and may attack various biomolecules including DNA, proteins, and membrane lipids, eventually leading to significant cellular damage. Antioxidants have been reported to mitigate the effects of free radical damage (Farombi et al., 2008; Prabhakar et al., 2006). Apart from synthetic antioxidants, studies have shown that commonly used medicinal plants, are also good sources of antioxidants that offer radioprotection in untried models. Examples include *Aegele marmelos*, *Hibiscus sabdariffa*, and *Vernonia amygdalina* (Jagetia et al., 2004; Adaramoye et al., 2008; Owoye et al., 2010). *Vernonia amygdalina*, commonly called "bitter leaf" is a popular African vegetable whose health benefits have been reported (Igile et al., 1994). Although the antioxidant and radioprotective activities of the methanolic extract of *Vernonia amygdalina* leaf extract have been reported, there is paucity of data on such effects on the haematologic aspect. It is well reported that alpha-tocopherol (vitamin E) is a radical scavenger and should therefore have a modulatory effect on irradiated rat's blood cells (Shaheen and Hassan, 1991; Hla et al., 2003).

This study was designed to investigate the modulatory effect of the methanolic extract of *Vernonia amygdalina* leaf extract on the haematological and associated liver enzymes changes in gamma irradiated rats, using alpha-tocopherol as a reference antioxidant compound. The study should provide an answer to the question: "Can *Vernonia amygdalina* leaf extract modulate the gamma radiation-induced alterations in the blood cells and liver enzymes of rats?" The significance of this study is its potential relevance to patients who might need treatment for cancer, because the degree of positive response of patients to any form of chemotherapy, surgery or radiotherapy depends on the status of their blood parameters *ab initio*. For example, patients who were anaemic or leukopaenic at the onset of medical treatment may not respond optimally to therapy.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (12 -14 weeks; 180-200 g, body weight) were maintained in the animal house of Department of Anatomy, University of Ibadan, Ibadan, in transparent plastic cages with wood shavings in a fly-proof, freely ventilated and naturally illuminated environment. Animals were fed dry pellets (Ladokun Feeds Nig. Ltd, Mokola, Ibadan), and given drinking water *ad libitum*. The experimental protocols were approved and carried out according to the guidelines set by the University of Ibadan Ethical Committee, which conformed to the acceptable guidelines on the ethical use of animals in research (Clarke et al., 1996).

Experimental design

The rats were randomly divided into seven groups of 6 rats per group. They were allowed 5 days to acclimatize to laboratory environment before treatment commenced. The details of grouping and treatment are presented in Table 1. All the treatments were given orally by gavage. Gamma-radiation was

given as a single dose of 2.0 Gy on Day 15 and rats in all groups were sacrificed on the 16th day of the experiments.

Leaf extract preparation

The leaves of *Vernonia amygdalina* Del ('bitter leaf') were harvested at a farm in Ibadan, Nigeria, in May, 2006. Botanical identification and authentication was done at the Forest Research Institute of Nigeria, Ibadan, Nigeria, where the voucher sample number FHI 107408, was deposited for reference. The leaves were rendered pest-free and 4.55 kg were extracted with pure MeOH (3 x 17 L) at room temperature with weekly changes of solvent, over a period of three weeks. The solvent was evaporated with a rotary vacuum evaporator (Eyela N.21, Tokyo) to afford a methanolic extract of *V. amygdalina* (MEVA) weighing 700 g, a yield of 15.4%.

Preparation and administration of MEVA

Based on the report of Abosi and Raseroka (2003), the stock solution of MEVA, of two different concentrations was prepared for this experiment. 1 ml of the prepared stock solution containing 250 mg/kg/day or 500 mg/kg/day was administered orally by gavage.

Drugs

Each capsule of 100 mg vitamin E acetate (G. A. Pharmaceuticals, Athens, Greece) was aspirated with a size 21G needle, attached to an oral gavage, and administered orally at a dose of 500 mg/kg daily for 14 days. This dose was based on Viana et al. (2003).

Irradiation procedures

Rats in Groups IV-VII (Table 1) were anaesthetized with Ketamine hydrochloride injection (Rotex medica, Trittau, Germany, batch 40092) at 10 mg/kg body weight and Diazepam injection (Roche, Switzerland) at 3 mg/kg body weight for muscle relaxation. Radiation as a single fraction of 2.0 Gy of

gamma-rays at a dose rate of 117.338 cGy/min, for 2.0 minutes was delivered by an AECL Theratron 780C Teletherapy machine with energy of 1.25 MeV. The source to surface distance was 72 cm, at a depth of 4 cm, and a field size of 18 cm by 18 cm, with an equivalent square area of 324 cm² and the percentage depth dose was 85.32%. There was no shielding of any part of the rat's body, and the rats were returned to their cages and transferred to the animal room for recovery from anaesthesia after irradiation.

Sample collection

On day 16th of the experiment, all the animals were euthanized using Ketamine at 10 mg/kg to each rat *i.p.* Through a cardiac puncture, blood was withdrawn from the left ventricle with some transferred into ethylenediamine tetra-acetic acid (EDTA) coated plastic tube and the remaining into plain tubes.

Preparation of serum

The blood in the plain tubes was centrifuged at 3000 revolutions per minute for ten minutes, and the supernatant was decanted into labeled Ependorf tube with the aid of a Pasteur pipette and then stored at -20 °C until subsequent analysis which in all cases was usually conducted within 24 hours or less of sample collection.

Determination of haematological values

The haematocrit or packed cell volume (PCV) and haemoglobin (Hb) values were determined by the microhaematocrit and cyanomethaemoglobin methods respectively. The red blood cell count (RBC) and white blood cell count (WBC) were assessed using the improved Neubauer haemocytometer (SpencerR). Erythrocytic indices namely mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) were calculated from the data obtained using standard formulae (Green and Ezeilo, 1978; Penington et al., 1978). The leucocyte

differential counts were determined according to the methods described by Jain (1986).

Assessment of hepatic integrity in the studied animals by enzymic and non-enzymic biochemical parameters

Determination of aspartate aminotransferase (AST)

The activity of AST in the serum was determined based on the method of Reitman and Frankel (1957). Briefly, 0.1 mL of diluted sample was mixed with solution of phosphate buffer, L-aspartate and α -oxoglutarate. The mixture was incubated for exactly 30 minutes at 37 °C. Then, 0.5 mL of solution of 2, 4-dinitrophenylhydrazine was added to the reaction mixture and allowed to stand for exactly 20 minutes at 25 °C. Then 0.5 mL of NaOH was added and the absorbance read against reagent blank after 5 minutes at 546 nm using a spectrophotometer.

Determination of alanine aminotransferase (ALT)

The activity of AST in the serum was determined based on the method of Reitman and Frankel (1957). Briefly, 0.5 mL of buffer was put into the test tubes of reagent blank and test sample. Then, 0.1 mL of distilled water was added to reagent blank test tube followed by the addition of 0.1 mL sample (serum) to test sample. These were mixed and incubated for 30 minutes at 37 °C, and 0.5 mL of 2, 4-dinitrophenylhydrazine was added to both reagent blank and test sample tubes. This was mixed, allowed to stand for exactly 20 minutes at 20-25 °C, after which 5.0 mL of sodium hydroxide (0.4 mol/L) was added to both reagent blank and test sample tubes and then mixed. The absorbance of sample was read against reagent blank after 5 minutes at 530-550 nm using spectrophotometer.

Determination of alkaline phosphatase (ALP)

The plasma activity of alkaline phosphatase (ALP) was determined using the colorimetric method of Williamson (1972). Briefly, ALP activity was measured by

monitoring the concentration of *p*-nitrophenol formed when ALP reacts with *p*-nitrophenol phosphate [PNPP] at 405 nm using spectrophotometer.

Estimation of serum albumin

The serum albumin level was determined photometrically by a modified method of Spencer and Price (1977). The method is based on the principle of the affinity of bromocresol green (BCG) for albumin at pH 4.2. The level of BCG-albumin complex formed was then determined spectrophotometrically.

Statistical analysis

Results were expressed as mean \pm S.D. of five animals. Data were analysed using one-way ANOVA, followed by Tukey post-test performed for multiple comparisons using GraphPad Prism version 4.0 (2003) for Windows GraphPad Software, San Diego, California, USA, (www.graphpad.com). Differences were considered statistically significant at $p < 0.05$.

RESULTS

General

All control, M250, and M500 animals survived the whole duration of the experiment. One rat each from the R, R + M500, and R + TOCO treatment groups died during anaesthesia or irradiation.

Haematological parameters

Red blood cell parameters

Red blood cell count (RCC)

Table 2 shows the non-significant decrease of the mean of R treatment when compared with the control ($p > 0.05$). The R+M250 and R+M500 treatments did not improve the effect of radiation whereas R+TOCO pretreatment caused a significant increase in the mean of RCC ($p < 0.001$) as compared with R.

Packed cell volume (PCV)

Table 2 shows the non-significant decrease caused by radiation on the PCV

which R+M250 and R+M250 treatments did not improve. However, R+TOCO pretreatment caused a significant increase in the mean ($p < 0.05$) when compared with R.

Haemoglobin concentration (Hb)

The significant reduction of haemoglobin concentration by radiation is shown in Table 2. The improvement of pretreatment was significant in the R+TOCO treatment group ($p < 0.001$), whereas it was non-significant in the R+M250 and R+M250 treatments.

Mean corpuscular volume (MCV)

Table 3 shows that the slight reduction of the MCV by radiation was not significant. While the slight increases in the R+M250 and R+M250 treatments were insignificant, that of R+TOCO treatment was significant when compared with R ($p < 0.05$).

Mean corpuscular haemoglobin (MCH)

In Table 3, the slight reduction of the MCH by radiation was not significant. Although the slight increases in the R+M250 and R+M250 treatments were insignificant, that of R+TOCO treatment was significant when compared with R ($p < 0.05$).

Mean corpuscular haemoglobin concentration (MCHC)

As shown in Table 3, the slight reduction of the MCHC by radiation was not significant. Although the slight increases in the R+M250 and R+TOCO treatments were insignificant, that of R+M500 treatment was significant when compared with R ($p < 0.05$).

White cell parameters

The absolute and differential counts of the white blood cells of rats are stated below:

Total white cell count (WBC)

Table 4 shows a general overview of the total white cell count. Radiation caused a non-significant reduction ($p > 0.05$) in the mean of WBC relative to the control group. Similarly, the alterations in the pretreatment groups were not significant.

Differential white blood cell counts

Neutrophils count

Table 4 shows that radiation caused a non-significant reduction ($p > 0.05$) in the mean of WBC relative to the control group. The alterations in the pretreated groups were not significant.

Lymphocyte count

In Table 4, radiation caused a significant reduction ($p < 0.001$) of the mean of the lymphocyte count when compared with the control. However, in the pretreated groups, R+M250, R+M250 and R+TOCO, there were significant increases in the means ($p < 0.001$) relative to R treatment.

Biochemical parameters

Rat serum albumin (RSA)

Radiation caused significant elevation of serum albumin ($p < 0.001$) which was significantly reduced by pretreatment as shown in the values obtained in the pretreated groups (Figure 1).

Aspartate aminotransferase (AST)

Table 5 and Figure 2, show the significant elevation (142%) of AST ($p < 0.001$) by gamma radiation when compared with the control. This was lowered significantly ($p < 0.001$), by pretreatment as the Table shows a reduction of AST levels in groups R+M250 (61.6%), R+M250 (46.5%), and R+TOCO (43.2) when compared with R.

Alanine aminotransferase (ALT)

A non-significant reduction in ALT levels was observed for the M250 and M500 treatment groups. Radiation caused a non-significant 16% rise in ALT level as compared with the control, which was reduced significantly by pretreatment with MEVA and TOCO ($p < 0.001$) as shown in Table 5 and Figure 3.

Alkaline phosphatase activity (ALP)

Table 5 and Figure 4, show the significant elevation (128%) of the ALP ($p < 0.001$), which was reduced in the pretreated groups of R+M250 (58%), R+M250 (60%), and R+TOCO (64%), and this was significant ($p < 0.001$).

Table 1: Experimental design.

Code	Groups	Treatment
I	Control	Normal rats
II	M250	250mg MEVA <i>p.o.</i> x 14 days
III	M500	500mg MEVA <i>p.o.</i> x 14 days
IV	R	Irradiation alone on day 15
V	R+M250	250mg MEVA <i>p.o.</i> + irradiation
VI	R+M500	500mg MEVA <i>p.o.</i> + irradiation
VII	R+TOCO	500mg TOCO <i>p.o.</i> + irradiation

Note: MEVA - methanolic extract of *V. amygdalina*; R - 2.0 Gy gamma radiation treatment as a single dose on day 15 of experiment; Control - 1 ml distilled water (d.w)/oral daily for 14 days; M 250 - 250 mg/kg/day/oral of MEVA in 1 ml d.w daily for 14 days; M 500 - 500 mg/kg/day/oral of MEVA in 1 ml d.w daily for 14 days; TOCO - 500 mg/kg/day/oral of α -Tocopherol for 14 days; *p.o.*- per oram.

Table 2: Effect of MEVA, α -tocopherol and γ -irradiation on the red cell count (RCC), packed cell volume (PCV), and haemoglobin concentration (Hb) of Wistar rat.

Group	Control	M 250	M 500	R	R + M250	R + M500	R + TOCO
RCC (x 10 ¹² /l)	7.36 ± 0.09	6.31 ± 0.89	7.28 ± 0.56	6.89 ± 0.53	6.25 ± 0.84	5.91 ± 0.71	8.57 ± 0.12 ^{b***}
PCV (%)	43.2 ± 3.31	38.6 ± 5.12	41.4 ± 4.54	42.4 ± 3.26	38.0 ± 4.47	38.0 ± 2.1	54.4 ± 3.2 ^{a*}
Hb (g/dl ³)	14.96 ± 0.43	13.46 ± 1.58	14.04 ± 1.03	12.92 ± 1.47 [*]	13.02 ± 1.55	12.92 ± 1.48	17.72 ± 1.37 ^{b***}

Values are given as means ± S.D., (n = 5). MEVA - methanolic extract of *Vernonia amygdalina*; R - Gamma radiation; M 250 - MEVA at 250 mg/kg; M 500 - MEVA at 500 mg/kg; TOCO - α -Tocopherol; * p < 0.05 versus control; ^{a*} p < 0.05 versus R; ^{b***} p < 0.001 versus R.

Table 3: Effect of MEVA, α -tocopherol and γ -irradiation on the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) of Wistar rat.

Group	Control	M250	M500	R	R+ M250	R+ M500	R+ TOCO
MCV (fl)	59.6 \pm 0.46	59.6 \pm 1.34	58.2 \pm 0.75	59.4 \pm 1.36	61.8 \pm 3.42	61.2 \pm 2.71	63.4 \pm 2.57*
MCH (pg)	19.8 \pm 0.4	21.4 \pm 4.36	19.4 \pm 0.49	19.6 \pm 1.36	21.4 \pm 2.06	21.6 \pm 1.85	21.2 \pm 0.75*
MCHC (g/dl)	32.8 \pm 0.4	34.2 \pm 2.48	33.0 \pm 0	32.6 \pm 0.8	34.2 \pm 2.04	34.8 \pm 1.6*	32.8 \pm 0.4

Values are given as means \pm S.D., (n = 5). MEVA - methanolic extract of *Vernonia amygdalina*; R - radiation; M 250 - MEVA at 250 mg/kg; M 500 - MEVA at 500 mg/kg; TOCO - α -Tocopherol;
* p < 0.05 versus R.

Table 4: Effect of MEVA, α -tocopherol and γ -irradiation on the total white blood cell count, neutrophils, and lymphocytes of Wistar rat.

Group	Control	M 250	M 500	R	R + M250	R + M500	R + TOCO
WBC total (x 10 ³)	6.34 1.32	5.70 \pm 0.52	6.24 \pm 0.25	5.25 \pm 0.56	4.46 \pm 0.76	4.73 \pm 0.44	5.65 \pm 0.47
Neutrophils (x 10 ³)	1.92 \pm 1.00	2.11 \pm 1.10	2.22 \pm 1.23	1.87 \pm 0.33	1.79 \pm 0.37	1.60 \pm 0.41	2.25 \pm 1.13
Lymphocytes (x 10 ³)	4.30 \pm 1.28	3.36 \pm 0.04	4.01 \pm 0.11	2.21 \pm 0.15 ^{a***}	2.64 \pm 0.13 ^{b***}	3.09 \pm 0.11 ^{b***}	3.30 \pm 0.16 ^{b***}

Values are given as means \pm S.D., (n = 5). MEVA - methanolic extract of *Vernonia amygdalina*; R - radiation; M 250 - MEVA at 250 mg/kg; M 500 - MEVA at 500 mg/kg; TOCO - α -Tocopherol; ^{a***} p < 0.001 versus control; ^{b***} p < 0.001 versus R.

Table 5: Effect of MEVA, α -tocopherol and γ -irradiation on the relative changes in liver enzymes of Wistar rats.

Group	Control	R	%	R +	%	R +	%	R +	%
Enzyme			increase	M250	decrease	M500	decrease	TOCO	decrease
AST	15.3	37	42	14.2	61.6	19.8	46.5	21	43.2
ALT	15.0	17.4	16	4	77.0	6.02	65.4	13.2	24
ALP	6.4	14.6	128	6.1	58	5.8	6.0	5.2	64.4

Values are means of 5 animals per treatment. MEVA = methanolic extract of *V. amygdalina*; R - 2.0 Gy gamma radiation treatment as a single dose on day 15 of experiment; Control - 1 ml distilled water (d.w)/oral daily for 14 days; M 250 - 250 mg/kg/day/oral of MEVA in 1 ml d.w daily for 14 days; M 500 - 500 mg/kg/day/oral of MEVA in 1ml d.w daily for 14 days; TOCO - 500 mg/kg/day/oral of α -Tocopherol for 14 days; AST - aspartate aminotransferase ; ALT - alanine aminotransferase; ALP - alkaline phosphatase. Values of columns 5, 7 and 9 were compared with R.

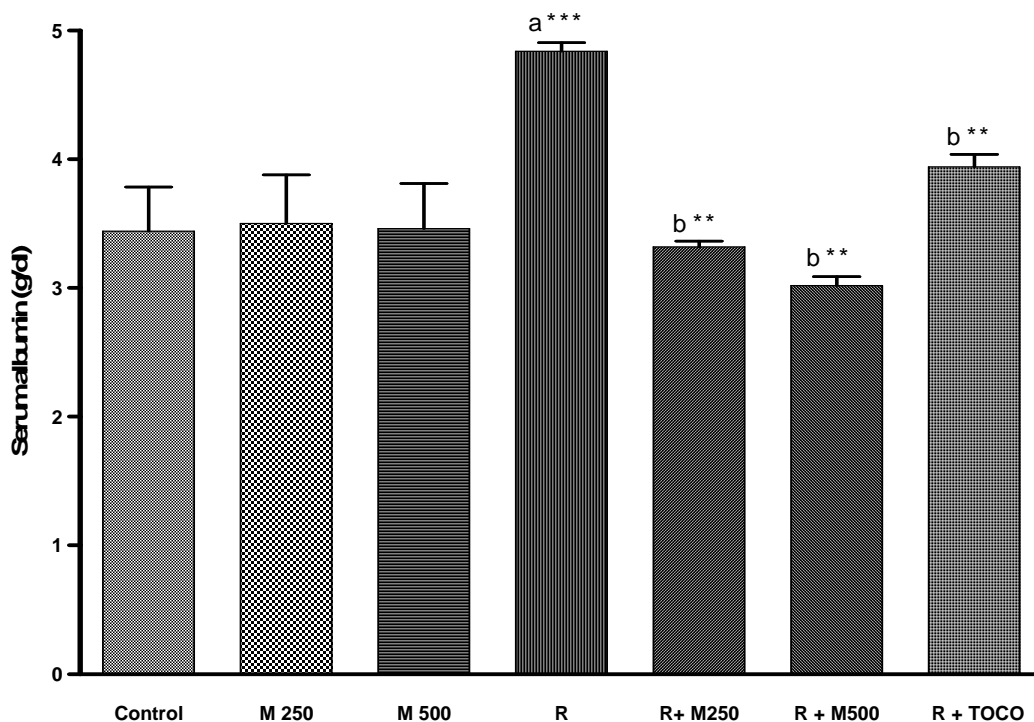


Figure 1: Effect of MEVA, α -tocopherol and γ -radiation on serum albumin of Wistar rats. Values are given as means \pm S.D. (n = 5). MEVA - methanolic extract of *Vernonia amygdalina*; R - radiation; M 250 - MEVA at 250 mg/kg; M 500 - MEVA at 500 mg/kg; TOCO - α -Tocopherol; ****p < 0.001 versus control; **p < 0.01 versus R.

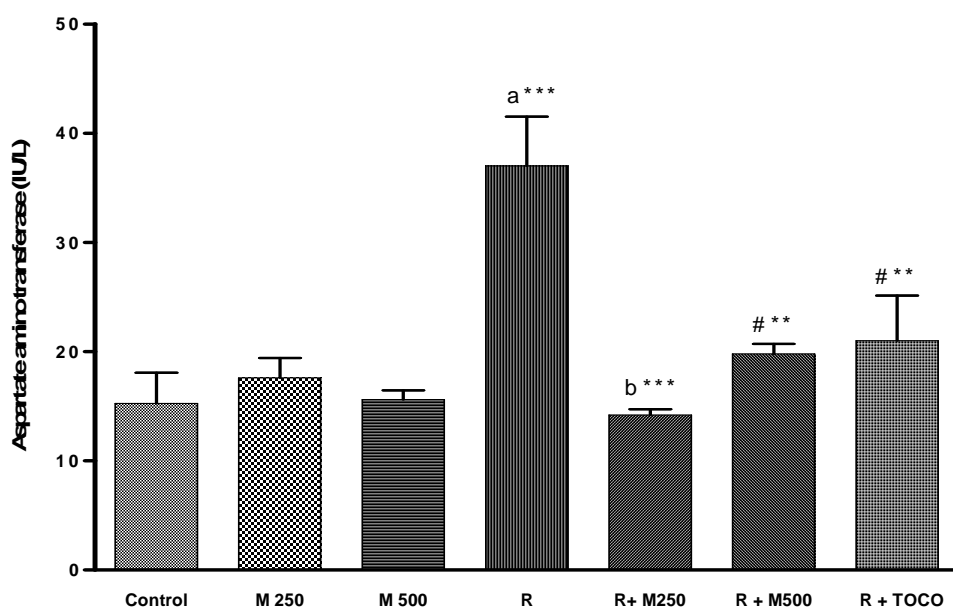


Figure 2: Effect of MEVA, α -tocopherol and γ -radiation on aspartate amino transferase (AST) in the serum of Wistar rats.

Values are given as means \pm S.D., (n = 5). MEVA - methanolic extract of *Vernonia amygdalina*; R - radiation; M 250 - MEVA at 250 mg/kg; M 500 - MEVA at 500 mg/kg; TOCO - α -Tocopherol; ***p < 0.001 versus control; b***p < 0.001 versus R; #**p < 0.01 versus R.

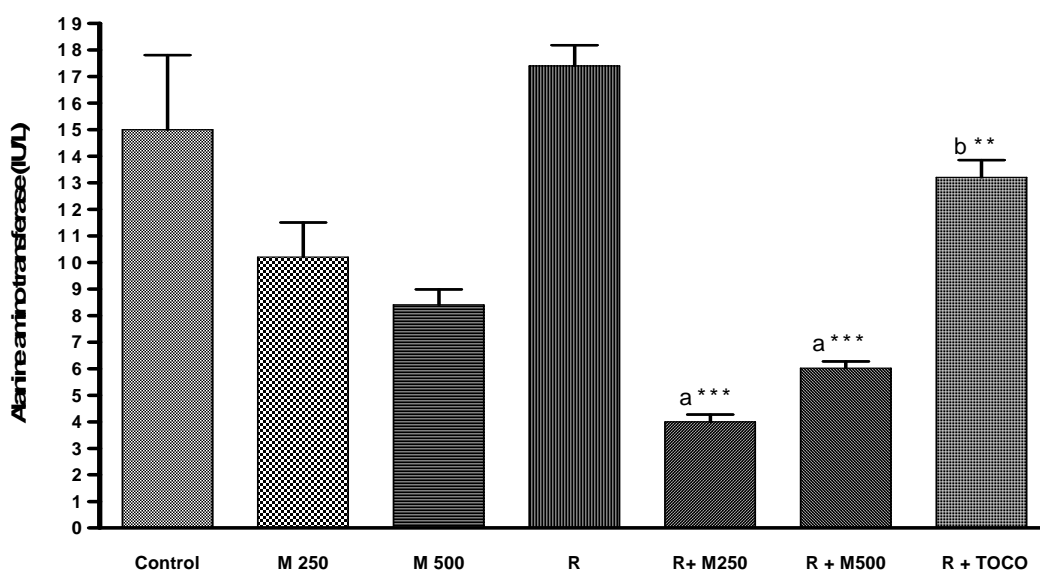


Figure 3: Effect of MEVA, α -tocopherol and γ -radiation on alanine amino transferase (ALT) in the serum of Wistar rats.

Values are given as means \pm S.D., (n = 5). MEVA - methanolic extract of *Vernonia amygdalina*; R - radiation; M 250 - MEVA at 250 mg/kg; M 500 - MEVA at 500 mg/kg; TOCO - α -Tocopherol; ***p < 0.001 versus R; b**p < 0.01 versus R.

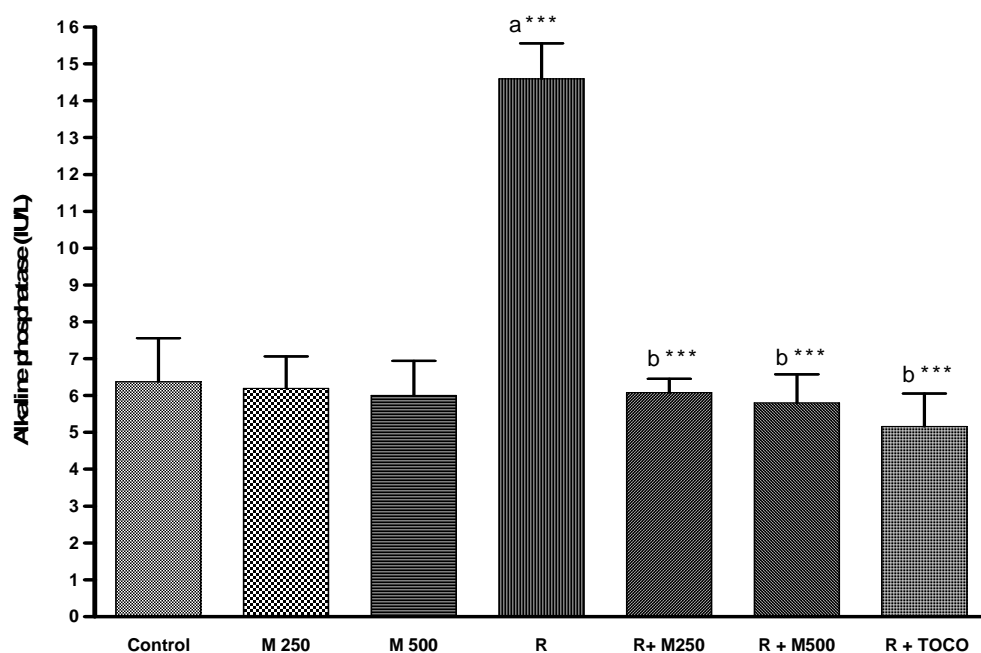


Figure 4: Effect of MEVA, α -tocopherol and γ -radiation on alkaline phosphatase (ALP) in the serum of Wistar rats.

Values are given as means \pm S.D., (n = 5). MEVA - methanolic extract of *Vernonia amygdalina*; R - radiation; M 250 - MEVA at 250 mg/kg; M 500 - MEVA at 500 mg/kg; TOCO - α -Tocopherol, ***p < 0.001 versus control; ****p < 0.001 versus R.

DISCUSSION

In this work, we addressed the question: "Can *Vernonia amygdalina* leaf extract modulate gamma-radiation induced alterations in the blood cells and liver enzymes of rats?" The study demonstrated that gamma radiation caused alterations in the haematological parameters, liver enzymes, and serum albumin of irradiated rats. These alterations were, however, alleviated by the MEVA and TOCO.

Changes in blood values are recognized as an asset in determining the extent of radiation exposure. Reports of immature and dividing blood cells being the most radiosensitive, the mature or non-dividing cells being radioresistant, and the pattern of white blood cells decrease in response to irradiation occurring in the sequence: lymphocytes, thrombocytes, neutrophils have been documented (Andersen, 1970).

Lymphocytes being the most radiosensitive of the leucocytes are sensitive to as low as 0.25 Gy of ionizing energy, whereas erythrocytes (red blood cells) are relatively radioresistant than other cellular components of blood, and can resist to some 30 Gy, though developing erythrocytes are sensitive to 0.5 Gy (Andersen, 1970, Jones et al., 1997; Hla et al., 2003). Newer findings have shown that as low as 2.25 Gy of gamma radiation can induce haematological changes in the peripheral blood (Rana et al., 1992).

In this study, 2.0 Gy gamma radiation reduced the total red blood cell count, the haematocrit (PCV), and the haemoglobin (Hb). This is in partial agreement with the findings of Shaheen and Hassan, (1991), who reported that gamma-irradiation, caused a significant decrease in red blood cells (RBCs) count with insignificant change in hemoglobin level, 24 and 48 hrs post-irradiation in male

rats, and Rana et al. (1992) who reported similar findings in chicks respectively. Radiation is known to cause tissue damage via the generation of free radicals like hydroxyl and superoxide radicals, which then cause oxidative damage which may lead to cell death, hence reducing RBCs population.

The significant elevation of the means of RBCs, PCV, and Hb, in the R+TOCO rats might be due to the reported effect of TOCO on the blood whereby, high doses of orally administered TOCO (vitamin E) elicited a significantly higher red blood cell glutathione (GSH) than in the placebo controls (Costagliola et al., 1985). GSH plays an important part in protecting red cell sulphhydryl groups, especially those of Hb, against oxidation (Penington et al., 1978), a fact which may explain the greater stability of the RBCs and Hb against irradiation damage. Of note were the findings that TOCO inhibited free radical formation in cell membranes, thus stabilizing the cells, and thus demonstrating that the administration of vitamin E preceding gamma-radiation exposure gave a significant radioprotection against radiation injury to RBCs in male rats (Schafer et al., 2002; Shaheen and Hassan, 1991). Even in humans, elevated levels of vitamin E have been demonstrated to increase human RBCs resistance to radiation-induced haemolysis (Brown, 1983).

The mean volume of the red blood cells evaluated by the MCV, which is a function of the red cell count and the PCV, demonstrated that the RBCs were normocytic. The significant increase in the mean of the TOCO group as compared with R group suggests an enhancement of the haemopoietic system during the pretreatment. The average weight of Hb contained in each red blood cell was evaluated in the animals by assessing the MCH. This parameter, a function of the Hb content and the total red cell count, was significant in the TOCO group. This can be explained by the high values of the Hb in the TOCO group. The MCHC, which represents the average concentration of Hb of RBCs was

unaltered, implying the rats were generally normochromic (Schalm, 1970). As already reported, a true increase in MCHC value does not normally occur because increased MCHC is almost always as a result of *in vivo* and *in vitro* haemolysis (Duncan et al., 1994). However, none of the rats used in the study had clinical features suggestive of haemolysis as no evidence of epistaxis, haematemesis, haematuria, haematochezia, or bleeding in an open injury was observed, though internal bleeding was not monitored.

Total leukocyte count in rats was reported to be influenced both by the site of blood withdrawal and the type of anaesthesia (Schalm, 1970). Blood obtained from clipping the tail and cardiac puncture after stunning the rats was compared by Quimby et al. (1948), who reported the finding of 3.5 times as many leukocytes in tail blood than in heart blood. To ensure uniformity, all the animals had the same form of Ketamine injection anaesthesia, and blood was obtained from cardiac puncture. The total leukocyte count exhibited slight insignificant alterations. There was relative leukopaenia in the groups that had pretreatment (R+M250, R+M500, and R+TOCO). Since these rats did not suffer any observable medical condition that might precipitate a leukopaenia e.g. lymphoma, aplastic anaemia or liver disease (Falase and Akinkugbe, 2007), it is safe to assume that the radiation energy depressed the white blood cells and this effect was not mitigated by the pretreatment possibly due to the short interval of 24 hours sacrifice after irradiation.

The significant reduction in the mean of the lymphocytes of the R group might have been due to the radiosensitivity of lymphocytes to gamma rays. Lymphocytes could offset oxidative damage by their capacity to regenerate intracellular stores of reduced glutathione (GSH) (Bounus and Molson, 1999), which may be deployed in mitigating the effects of the oxidative damage induced by the gamma radiation. The significant reduction of the means of the lymphocytes of the MEVA and TOCO groups

might be due to the inability of these substances, including the GSH expected to be generated by lymphocytes themselves to protect lymphocytes from radiation injury. The fact that the neutrophils did not show any significant group differences suggests the possibility of radioresistance of neutrophils to the irradiation dose used in this study as reported by Schalm (1970). The leukopaenia, especially the lymphopaenia and neutropaenia observed in these groups may have been due either to ineffective granulopoiesis or reduced granulopoiesis or reduced survival of leukocytes in the blood in response to radiation toxicity (Coles, 1986; Falase and Akinkugbe, 2007).

As the major detoxification system of the body, deranged liver function tests suggests a lesion of the organ (Giannini et al., 2005; Kierzenbaum, 2007). The administration of a toxicant (2.0 Gy gamma rays) as TBI activated the elevation in the levels of liver enzymes AST, ALT, and ALP, and also that of the rat serum albumin (RSA). The elevated levels of the enzymes were indicative of hepatic injury after radiation exposure, which probably enhanced the leakage of the enzymes into the blood. The observation of more than double in the increase of the values of the enzymes AST and ALP as shown in Table 5, may however, not be entirely due to liver damage since there are other sources of these enzymes, namely: heart and skeletal muscle, kidney, brain and RBCs in the case of AST; and bone and intestine in the case of ALP (Giannini et al., 2005). Since ALT has been found to be relatively liver-specific and also to increase in the plasma during hepatocellular necrosis or degeneration (Nagiev and Karpovich, 1994), the slight increase in its level of the R group might suggest that the hepatocytes were mildly affected. That pre-treatment with MEVA and TOCO markedly reduced the elevated levels of AST, ALT, and ALP suggest that both MEVA and TOCO might act as an indirect or direct antioxidant by combining with toxic free radicals produced

by radiation or inactivate them, thus preventing hepatocyte injury. This antioxidant activity would have led to the subsequent inhibition of the leakage of these enzymes into the circulation (Bender and Mayes 2003; Barone, 2004; Iwalokun et al., 2006; Owoeye et al., 2010).

Since albumin is a protein made specifically by the liver (Akinosun et al., 2006), its elevation by gamma radiation might be due to gamma rays energizing the hepatocyte mRNA specific for albumin synthesis to be translated into more albumin molecules, hence the albumin synthesis. This finding contradicts the findings of Sharma et al. (2001) and Yammani et al. (2002) who reported hypo-albuminaemia due to albuminuria in the rat following single dose TBI although Yammani and his colleagues had irradiated rats with 9.5 Gy of X-rays as a single dose. This contradiction in the result of the present study may be due to the fact that a lower radiation dose of 2.0 Gy was used, apart from the fact that gamma rays and not X-rays were used as in the study of Yammani et al. The result is, however in agreement with the findings of Adaramoye et al. (2008), who used gamma rays at a dose of 5.0 Gy and observed an elevated the RSA of the irradiated rats. Of importance was the finding that pretreatment with MEVA and TOCO significantly reduced the elevated level of RSA to a level comparable to the control value. This may have been due to the stabilizing role of the antioxidants present in MEVA (Adaramoye et al., 2008; Owoeye et al., 2010), and TOCO (Schafer, 2002). TOCO is preferentially retained in the body by the action of the alpha-tocopherol transfer protein (α -TTP) in the liver, which preferentially incorporates it into lipoproteins that are circulated in the blood and ultimately delivers it to different tissues in the body. TOCO as a radical scavenger in chemical and biological systems, protects cellular structures against damage by reactive oxygen species especially hydroxyl and superoxide radicals. This is in addition to its contribution to cell membrane stability which

has been demonstrated in this study (Bender and Mayes, 2003; Mayes and Botham, 2003; Cerecetto and Lopez, 2007).

Taken together, the results of this study have demonstrated that the methanolic extract of *Vernonia amygdalina* and alpha-tocopherol were able to modulate the effects of gamma radiation-induced injury to blood cells, liver enzymes, as well as rat serum albumin. These results may be applicable in further research aimed at enhancing patient protection during radiotherapy.

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REFERENCES

- Abosi AO, Raseroka BH. 2003. *In vivo* antimalarial activity of *Vernonia amygdalina*. *Br. J. Biomed. Sci.*, **60**(2): 89-91.
- Adaramoye O, Ogungbenro B, Anyaegbu O, Fafunso M. 2008. Protective effects of extracts of *Vernonia amygdalina*, *Hibiscus sabdariffa* and Vitamin C against radiation- induced liver damage in rats. *J. Radiat. Res.*, **49**: 123-131.
- Akinosun OM, Arinola OG, Salimonu LS. 2006. Immunoglobulin classes and liver function tests in Nigerian petrol attendants. *Indian J. Occup. Environ. Med.*, **10**: 58-61
- Andersen AC. 1970. Effects of ionizing radiation on blood. In *Veterinary Haematology* (2nd edn), Schalm OW (Ed). Lea and Febiger: Philadelphia; 471-477.
- Aunapuu M, Pekhter I, Roosaar P, Gershkevich E, Mar'iamiagi MM, Arend A, Kol'ts I, Ots M. 2003. Morphological changes in the kidney nephrectomy and low-dose radiation. *Tsitologia*, **45**(6): 555-63.
- Barone S. 2004. Vitamins. In *Modern Pharmacology with Clinical Applications* (6th Edn), Craig CR, Stitzel RE (Eds). Lippincott Williams & Wilkins: Philadelphia; 777-779.
- Belka C, Budach W, Kortmann RD, Bamberg M. 2001. Radiation induced CNS toxicity-molecular and cellular mechanisms. *Br. J. Cancer*, **85**(9): 1233-1239.
- Bender DA, Mayes PA. 2003. Vitamins and minerals. In *Harper's Illustrated Biochemistry* (26th Edn), Murray RK, Granner DK, Mayes PA, Rodwell VW (Eds). Lange Medical Books/McGraw-Hill: New York; 481- 497.
- Bounous G, Molson JH. 2003. The antioxidant system. *Anticancer Research*, **23**: 1411-1416.
- Brown MA. 1983. Resistance of human erythrocytes containing elevated levels of vitamin E to radiation-induced hemolysis. *Radiat. Res.*, **95**(2): 303-16.
- Cerecetto H, Lopez GY. 2007. Antioxidants derived from vitamin E: An overview. *Mini- Reviews in Medicinal Chemistry*, **7**: 315-338.
- Clarke JD. 1996. The guide for the care and use of laboratory animals. National Academy of Science Revision Committee.
- Coles EH. 1986. *Veterinary Clinical Pathology* (4th Edn). W.B. Saunders Co: Philadelphia; 1- 486.
- Costagliola C, Libondi T, Menzione M, Rinaldi E, Auricchio G. 1985. Vitamin E

- and red blood cell glutathione. *Metabolism*, **34**(8): 712-714.
- Duncan JR, Prasse KW, Mahaffey EA. 1994. *Veterinary Laboratory Medicine: Clinical Pathology* (3rd edn). Iowa State University Press: Ames.
- Falase AO, Akinkugbe OO. 2007. *A Compendium of Medicine* (New Edition). Spectrum Books Limited: Ibadan. 185-188, 370-379, 582.
- Farombi EO, Ugwuezumba MC, Ezenwadu TT, Oyeyemi MO, Martins E. 2008. Tetracycline-induced reproductive toxicity in male rats: Effects of vitamin C and N-acetylcysteine. *Exp. & Toxicol. Pathol.*, **60**: 77-85.
- Giannini EG, Testa R, Savarino V. 2005. Liver enzyme alteration: a guide for clinicians. *CMAJ.*, **172**(3): 367-379.
- Green JH, Ezeilo GC. 1978. The blood. In *An Introduction to Human Physiology* (4th edn). Oxford University Press: Ibadan; 5-22.
- Hla K, Starykovich LS, Chaika IaP, Vyhovs'ka TV, Pauk HM, Datsiuk LO, Trykulenko OV, Velykyi MM, Donchenko HV, Kuz'menko IV. 2003. Activity of antioxidants in enterocytes of the small intestine and blood cells after ionizing irradiation and administration of vitamin E in rats. *Ukr Biokhim Zh.*, **75**(6): 87-94.
- Igile GO, Oleszek W, Jurzysta M, Burda S, Fafunso M, Fasanmade AA. 1994. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. *J. Agric. Food Chem.*, **42**: 2445-2448.
- Iwalokun BA, Efedede BU, Alabi-Sofunde JA, Oduala T, Magbagbeola OA, Akinwande AI. 2006. Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. *J. Med. Food*, **9**(4): 534-30.
- Jagetia GC, Baliga MS, Malagi KJ, Kamath, MS. 2002. The evaluation of the radioprotective effect of Triphala (an Ayurvedic rejuvenating drug) in the mice exposed to gamma-radiation. *Phytomedicine*, **9**: 99-108.
- Jagetia GC, Venkatesh P, Balinga MS. 2004. Fruit extract of *Aegle marmelos* protects mice against radiation-induced lethality. *Integr. Cancer, Ther.*, **3**: 323-332.
- Jain WC. 1986. *Schalm's Veterinary Haematology* (4th Edn). Lea and Febiger: Philadelphia; 69-71.
- Jones TC, Hunt, RD, King, NW. 1997. Pathologic effects of ionizing radiations. *Veterinary Pathology* (6th edn). Lippincott Williams & Wilkins: Philadelphia; 681-694.
- Kierszenbaum, AL. 2007. *Histology and Cell Biology, an Introduction to Pathology* (2nd Edn). Mosby Elsevier: Philadelphia; 221-226.
- Mayes PA, Botham KM. 2003. Biologic Oxidation. In *Harper's Illustrated Biochemistry* (26th Edn), Murray RK, Granner DK, Mayes PA, Rodwell VW (Eds). Lange Medical Books/McGraw-Hill: New York; 86-91.
- MedicineNet.com. Retrieved on 24/03/11 from <http://www.medterms.com/script/main/art.asp?articlekey=18311>
- Nagiev ER, Karpovich GA. 1994. Activity of alanine- and aspartate- aminotransferases of organs of albino rats subjected to whole body gamma-radiation and physical exercise. *Radiats. Biol. Radioecol.*, **34**: 639-644.
- Owoeye O, Adesida A, Onwuka SK, Farombi EO. 2010. Gamma radiation effects on the brain of rats: antioxidant and radioprotective properties of *Vernonia amygdalina* leaf extract. *Int. J. Biol. Chem. Sci.*, **4**(6): 2324-2336.
- Pennington DG, Rush B, Castaldi PA. 1978. The red cell anaemia. *G.C. de Gruchy Clinical Haematology in Medical Practice* (4th edn). The English Language Book Society and Blackwell Scientific Publications: Oxford; 31-58.
- Prabhakar KR, Veeresh VP, Vipani K, Sudheer M, Priyadarsini KI, Satish RBSS, Unnikrishnan, MK. 2006. Bioactivity

- guided fractionation of *Coronopus didymus*: A free radical scavenging perspective. *Phytomedicine*, **13**: 591-595.
- Quimby, FH, Saxon, PA, Goff, LC. 1948. Total white cell counts of peripheral and heart blood of the rat. *Science*, **107**: 447.
- Rana K, Malhotra N, Malhotra RK. 1992. Effect of radiation on some haematological parameters and its modification by vitamin E in chicks. *Indian J. Exp. Biol.*, **30**(1): 60-61.
- Reitman S, Frankel S. 1957. A colorimeter method for the determination of serum glutamate-oxaloacetate and pyruvate transaminases. *Am. J. of Clin. Path.*, **28**: 56-63.
- Schafer FQ, Wang HP, Kelley EE, Cueno KL, Martin SM, Buettner GR. 2002. Comparing beta-carotene, vitamin E and nitric oxide as membrane antioxidants. *Biol. Chem.*, **383**(3-4): 671-81.
- Schalm OW. 1970. Normal values in blood of laboratory, fur-bearing, and miscellaneous zoo and wild animals. In *Veterinary Haematology* (2nd edn). Lea and Febiger: Philadelphia; 309-303.
- Shaheen AA, Hassan SM. 1991. Radioprotection of whole-body gamma-irradiation-induced alteration in some haematological parameters by cysteine, vitamin E and their combination in rats. *Strahlenther Onkol.*, **167**(8): 498-501.
- Sharma M, Sharma R, Ge XL, Fish BL, McCarthy ET, Savin VJ, Cohen EP, Moulder JE. 2001. Early detection of radiation-induced glomerular injury by albumin permeability assay. *Radiation Research*, **155**(3): 474-480.
- Spencer K, Price CP. 1977. Influence of reagent quality and reaction conditions on the determination of serum albumin by the Bromocresol Green Dye-binding Method. *Ann. Clin. Biochem.*, **14**: 105.
- Viana M, Castro M, Barbas C, Herrera E, Bonet B. 2003. Effect of different doses of vitamin E on the incidence of malformations in pregnant diabetic rats. *Ann. Metab.*, **47**(1): 6-10.
- Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, Tsu H, Confer DL, Coleman NC, Seed T, Lowry P, Armitage JO, Dainiak N. 2004. Medical Management of the Acute Radiation Syndrome: Recommendations of the Strategic National Stockpile Radiation Working Group. *Annals of Internal Medicine*, **140**(12): 1037-1051.
- Williamson TA. 1972. Comparison between the phosphatase and phenyl phosphate methods of alkaline phosphatase assay. *Med. Lab. Technol.*, **29**: 182-187.
- Yammani RR, Sharma M, Seetharam S, Moulder JE, Dahms NM, Seetharam B. 2002. Loss of albumin and megalin binding to renal cubilin in rats results in albuminuria after total body irradiation. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.*, **283**: R339-R346.