

Effects of Aged Garlic Extract on Rat's Blood Cells Parameters After Whole-Body Exposure to Ionizing Radiation

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Abstract: Exposure to high amounts of ionizing radiation results in damage to the hematopoietic system characterized by the destruction of hematopoietic cells in bone marrow, thus reducing the production of blood cells. Infections and bleeding that result can be fatal. Aged garlic extract (AGE) has been demonstrated to possess several physiological activities in experimental animals thus, the present study aimed to evaluate the effect of AGE on the hematopoietic syndrome post whole-body exposure to ionizing radiations. Eight groups, five healthy male rats each were used (20 irradiated and 20 Sham Irradiated), among which some were receiving via gavages distilled water, the others AGE at different doses (25 mg/kg and 50 mg/kg) and the rest vitamin E + Alpha Lipoic Acid. Blood samples were collected the 8th day post irradiation to investigate blood picture. Exposure of rats to gamma radiation caused a significant disturbance in the level of white blood cells count (WBCs), Lymphocytes (LY), mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells (MID), Granulocytes (GR), red blood cells count (RBCs), hemoglobin content (Hb), Mean Corpuscular Volume (MCV), hematocrit value (HTC), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCH_C) and platelets count (PLT). In rats exposed to radiation then treated with AGE, the results showed an improvement in all previous parameters. The results of the present investigation showed that AGE intake confers radiation protection to rat's blood cells parameters after whole-body exposure to ionizing radiation.

Keywords: Irradiation, Ionizing Radiation, Oxidative Stress, AGE, Blood, Rats

1. Introduction

Exposure to high amounts of ionizing radiation results in damage to the hematopoietic, gastrointestinal and central nervous systems depending on radiation dose [1]. Hematopoietic system is one of the most sensitive systems to evaluate the hazards effects of radiations in humans and

animals [2-4]. It is seen with significant partial-body or whole-body radiation exposures exceeding 1 G y [5]. Mitotically active hematopoietic progenitors have a limited capacity to divide after a whole-body radiation dose greater than 2 to 3 G y [6]. In the ensuing weeks after exposure, a hematologic crisis occurs, characterized by hypoplasia or aplasia of the bone marrow. These changes result in

pancytopenia predisposition to infection, bleeding, and poor wound healing, all of which contribute to death. The hematopoietic tissues are among the most radiosensitive tissues in the body following irradiation. Damage of the hematopoietic system is a major factor in the mortality following acute radiation exposure. The first to be affected after irradiation are the red cells precursors causing a decrease in the amount and life span of peripheral red cells, next is those of the white blood cells and last the platelets precursors. The decrease in the number of platelets increases the incidence of hemorrhage [7-8].

The hematopoietic system is very well protected by vitamin E and Alpha Lipoic acid (ALA) because Vitamin E promotes the natural formation of red blood cells and its half-life is prolonged by Lipoic acid which thereby enhances its antioxidant activities [9]. Supplementation with α -tocopherol and α -lipoic acid enhanced the erythrocyte antioxidant defense [10]. But, recently; apart from synthetic antioxidants, focus of radiation protection has shifted to test the radioprotective potential of plants and herbs in the hope that one day it will be possible to find a suitable pharmacological agent/s that could protect humans against the deleterious effects of ionizing radiation in clinical and other conditions as well as during nuclear terror attack [11]. AGE, an odorless garlic preparation have been shown to possess several physiological activities in experimental animals [12-13]. Its antioxidant property and free radical scavenging capacity have also been investigated [13-14]. Although these antioxidant and radioprotective activities of AGE have been reported, there is paucity of data on such effects on the hematologic aspect particularly post irradiation.

The present investigation was undertaken to assess the radioprotective property, if any, of AGE on some hematological parameters of rats after whole-body exposure to ionizing radiation. The study was performed using Vitamin E and Lipoic Acid as positive control group.

2. Material and Methods

2.1. Animals

Eighty healthy Albino male rats (*Rattus norvegicus*) of Wistar strain (3 to 4 months old) ranging from 214-230g body weight were obtained according to the ICH guidelines from animal lab Université des Montagnes, Bangangté and Douala University in Cameroon. Their acclimatization to laboratory conditions took place at room temperature, relative humidity and natural light-dark cycle (12 hours light and 12 hours dark). The rats were given ad libitum tap water and food of a commercial balanced diet. Five animals were housed per plastic cage containing paddy husk (procured locally) as bedding and fasted night before sacrifice. The experimental protocol and the maintenance of the experimental animals was done in accordance with the regulations of the Organization for Economic Cooperation and Development (OECD) guide since in Cameroon the ethics committee focuses only on clinical studies.

2.2. Chemical

Aged Garlic Extract (KYOLIC® Aged Garlic Extract™ Liquid) is prepared by soaking sliced raw garlic (*Allium sativum* Linn) with a quality plan program (QPP-003) in 15-20% aqueous ethanol for 20 months at room temperature. The extract is then filtered and concentrated under reduced pressure according to the guidelines of Good Manufacturing practices established by the World Health Organization. The garlic is grown under strictly controlled organic conditions (without herbicides or pesticides of any kind), harvested at full maturity, cleaned, sliced and stored in stainless steel tanks under carefully controlled conditions without the use of a heating process [15-17]. The content of water-soluble compounds is relatively high whereas that of oil-soluble compounds is relatively low [17]. The AGE used in this study is standardized with S-Allyl Cysteine and contained 30% extracted solids (300 mg/ml), and S-allyl cysteine present at 1.47 mg/ml.

2.3. Experimental Design

Two weeks after acclimatization and conditioning, the animals were randomly divided into four equal and double male rat groups in separate plastic cages, five rats each. Two negative control groups receiving 10 mL/kg of distilled water (I and II), two AGE-treated groups at dose of 25 mg/kg AGE (III and IV), two AGE-treated groups at dose of 50 mg/kg AGE (V and VI) and two positive control groups (receiving 50 mg/kg Vitamin + 25 mg/kg of Lipoic Acid) (VII and VIII) were used. Among the double groups, 20 were irradiated (rats of groups II, IV, VI and VIII) and 20 sham irradiated (rats of groups I, III, V and VII). The rats of each group were fed via gavages one hour after irradiation on day 1 and for 7 consecutive days and weighed daily during the experiment. The experimental protocol and the maintenance of the experimental animals was done in accordance with the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 [18].

2.4. Irradiation

The Albino Wistar rats were placed in collective cages made of plastic for whole-body exposure after at least two weeks of acclimatization and conditioning. Rats were exposed using the facilities provided by the Oncology and Radiotherapy department of the Douala General Hospital. Irradiation was delivered by an ALCYON-II model cobalt-60 teletherapy unit (General Electric/GE Healthcare). The rats in an area of 36 x 36 cm were exposed to a single dose of 4.5 Gy applied as single shot dose at a dose rate of 0.55 Gy/min. Five animals were irradiated at once and sham-irradiated animals were treated in the same manner but were not exposed to the source. After irradiation, the rats were brought back to the animal Lab of Douala University for the follow up and the tests.

2.5. Sample Collection and Hematological Studies [19]

The animals were put to fast during the night before their blood test (7th day post irradiation). The day of sacrifice (8th day post irradiation), arterio-venous blood was collected in Ethylene Diamine Tetra-Acetic (EDTA) tubes for determination of hematological parameters: white blood cells count (WBCs), Lymphocytes (LY), mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells (MID), Granulocytes (GR), red blood cells count (RBCs), hemoglobin content (Hb), Mean Corpuscular or Cell Volume (MCV), hematocrit value (HTC), Mean corpuscular or cell hemoglobin (MCH), Mean corpuscular or cell hemoglobin concentration (MCHC), platelets count (PLT).

2.6. Statistical Analyses

Results were expressed as mean \pm Standard Error of the Mean (SEM). Comparison of means was done by Dunnett test as post hoc test. P values less than 0.05 were considered statistically significant. Statistical evaluation was conducted using one way analysis of variance (ANOVA) software Graph Pad Prism 5.03. With the risk of 5%, statistically significant differences are reported in the tables and figures with an asterisk (*), the highly statistically significant

differences are marked with two stars (**) and statistically highly significant differences are indicated by three stars (***).

3. Results

WBCs: white blood cells count, LY: Lymphocytes, MID: mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells, GR: Granulocytes, RBCs: red blood cells count, Hb: hemoglobin content, MCV: Mean Corpuscular or Cell Volume, HTC: hematocrit value, MCH: Mean corpuscular, MCHc: Mean Cell hemoglobin concentration, PLT: platelets count.

Group I: "Sham Irradiation + Distilled Water", Group II: "Irradiation + Distilled Water", Group III: "Sham Irradiation + 25 mg/kg AGE", Group IV: "Irradiation + 25 mg/kg AGE", Group V: "Sham Irradiation + 50 mg/kg AGE", Group VI: "Irradiation + 50 mg/kg AGE", Group VII: "Sham Irradiation + Vitamin E and Lipoic Acid", Group VIII: "Irradiation + Vitamin E and Lipoic Acid"

The irradiation resulted in a non-significant variation ($P > 0,05$) of blood constituents in groups "Sham Irradiation+Distilled Water", "Irradiation+25 mg/kg AGE", "Irradiation+50 mg/kg AGE" and "Irradiation+Vitamin E and Lipoic Acid" as shown below (Table 1). In the group.

Table 1. Effects of γ -radiation and AGE on blood parameters.

Blood Count	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
WBCs ($10^9/\mu\text{l}$)	15.48 \pm 0.95	12.07 \pm 1.11	15.96 \pm 0.61	13.98 \pm 0.79	15.65 \pm 0.55	13.23 \pm 0.77	15.39 \pm 1.59	12.95 \pm 0.70
LY ($10^9/\mu\text{l}$)	12.88 \pm 1.85	8.70 \pm 0.72	12.97 \pm 1.21	11.80 \pm 1.10	12.95 \pm 1.91	10.97 \pm 0.92	12.78 \pm 0.92	8.99 \pm 0.90
MID ($10^9/\mu\text{l}$)	0.54 \pm 0.07	0.38 \pm 0.05	0.55 \pm 0.06	0.45 \pm 0.06	0.54 \pm 0.07	0.43 \pm 0.06	0.52 \pm 0.06	0.38 \pm 0.07
GR ($10^9/\mu\text{l}$)	4.09 \pm 0.65	2.23 \pm 0.42a*	4.22 \pm 0.45	3.43 \pm 0.45	4.20 \pm 0.50	3.23 \pm 0.24	4.04 \pm 0.53	2.34 \pm 0.41
RBCs ($10^9/\mu\text{l}$)	7.18 \pm 0.18	4.63 \pm 0.37 a***	7.38 \pm 0.18	6.5 \pm 0.55b**, c*	7.2 \pm 0.18	6.19 \pm 0.45 b*	7.09 \pm 0.32	4.92 \pm 0.39a***
Hb (g/dl)	14.52 \pm 1.47	11.10 \pm 0.83	14.92 \pm 0.60	13.64 \pm 0.35	14.38 \pm 1.01	13.00 \pm 1.37	14.02 \pm 1.51	11.10 \pm 1.09
MCV (fl)	57.8 \pm 1.47	50.50 \pm 2.17 a*	56.15 \pm 3.17	58.88 \pm 1.03 b*, c*	56.85 \pm 1.96	58.64 \pm 0.98 b*, c*	57.35 \pm 1.06	51.25 \pm 1.76
HTC (%)	41.4 \pm 0.86	23.3 \pm 2.17 a***	41.37 \pm 2.22	38.86 \pm 3.70b***, c**	41.39 \pm 1.80	36.39 \pm 3.03 b**, c*	40.67 \pm 2.23	25.38 \pm 2.59 a***
MC (pg)	20.35 \pm 2.20	24.31 \pm 2.02	20.27 \pm 1.04	21.44 \pm 2.13	19.74 \pm 1.31	21.89 \pm 3.82	20.20 \pm 2.92	22.57 \pm 1.16
MCHc (g/dl)	34.9 \pm 3.42	48.52 \pm 4.80	36.53 \pm 2.80	36.67 \pm 4.23	35.06 \pm 3.13	37.35 \pm 6.64	35.15 \pm 4.86	44.31 \pm 2.86
PLT ($10^9/\mu\text{l}$)	1157.60 \pm 3.36	845.20 \pm 2.31a***, c**	1163.00 \pm 4.06	1096.80 \pm 2.4a***, b***, c***	1159.80 \pm 3.22	1080.00 \pm 4.09a***, b***, c***	1150.00 \pm 4.47	890.40 \pm 3.08a***, b**

"Sham Irradiation+Distilled Water": the decline was in order of 22.02% (12.07 \pm 1.11 Vs 15.48 \pm 0.95 $10^9/\mu\text{l}$) for WBCs: white blood cells count, of 32.43% (8.70 \pm 0.72 Vs 12.88 \pm 1.85 $10^9/\mu\text{l}$) for LY: Lymphocytes, of 29.63% (0.38 \pm 0.05 Vs 0.54 \pm 0.07 $10^9/\mu\text{l}$) for MID: mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells and of 23.55% (11.10 \pm 0.83 Vs 14.52 \pm 1.47 g/dL) for Hb: hemoglobin content. The increase was in order of 19.50% (24.31 \pm 2.02 Vs 20.35 \pm 2.20 pg) for MCH: Mean corpuscular hemoglobin and of 38.68% (48.52 \pm 4.80 Vs 34.99 \pm 3.42 g/dl) for MCHc: Mean corpuscular hemoglobin concentration.

"Irradiation+25 mg/kg AGE": the decline was in order of 9.69% (13.98 \pm 0.79 Vs 15.48 \pm 0.95 $10^9/\mu\text{l}$) for WBCs: white blood cells count, of 8.36% (11.80 \pm 1.10 Vs 12.88 \pm 1.85 $10^9/\mu\text{l}$) for LY: Lymphocytes, of 17.41% (0.45 \pm 0.06 Vs 0.54 \pm 0.07 $10^9/\mu\text{l}$) for MID: mid-size population of monocytes, basophils, eosinophils, blasts, and other

immature cells, of 16.13% (3.43 \pm 0.45 Vs 4.09 \pm 0.65 $10^9/\mu\text{l}$) for GR: Granulocytes, and of 6.06% (13.64 \pm 0.35 Vs 14.52 \pm 1.47 g/dl) for Hb: hemoglobin content. The increase was in order of 5.37% (21.44 \pm 2.13 Vs 20.35 \pm 2.20 pg) for MCH: Mean corpuscular or cell hemoglobin and of 4.81% (36.67 \pm 4.23 Vs 34.99 \pm 3.42 g/dl) for MCHc: Mean corpuscular or cell hemoglobin concentration.

"Irradiation+50 mg/kg AGE": the decline was in order of 14.53% (13.23 \pm 0.77 Vs 15.48 \pm 0.95 $10^9/\mu\text{l}$) for WBCs: white blood cells count, of 14.80% (10.97 \pm 0.92 Vs 12.88 \pm 1.85 $10^9/\mu\text{l}$) for LY: Lymphocytes, of 19.63% (0.43 \pm 0.06 Vs 0.54 \pm 0.07 $10^9/\mu\text{l}$) for MID: mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells, of 21.11% (3.23 \pm 0.24 Vs 4.09 \pm 0.65 $10^9/\mu\text{l}$) for GR: Granulocytes and of 10.47% (13.00 \pm 1.37 Vs 14.52 \pm 1.47 g/dl) for Hb: hemoglobin content. The increase was in order of 7.57% (21.89 \pm 3.82 Vs 20.35 \pm 2.20 pg) for MCH: Mean corpuscular or cell hemoglobin and of 6.75%

(37.35 ± 6.64 Vs 34.99 ± 3.42 g/dl) for MCHc: Mean corpuscular or cell hemoglobin concentration.

"Irradiation+Vitamin E and Lipoic Acid": the decline was in order of 16.34% (12.95 ± 0.70 Vs 15.48 ± 0.95 $10^9/\mu\text{l}$) for WBCs: white blood cells count, of 30.18% (8.99 ± 0.90 Vs 12.88 ± 1.85 $10^9/\mu\text{l}$) for LY: Lymphocytes, of 29.63% (0.38 ± 0.07 Vs 0.54 ± 0.07 $10^9/\mu\text{l}$) for MID: mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells, of 42.82% (2.34 ± 0.41 Vs 4.09 ± 0.65 $10^9/\mu\text{l}$) for GR: Granulocytes and of 23.55% (11.10 ± 1.09 Vs 14.52 ± 1.47 g/dl) for Hb: hemoglobin content. The increase was in order of 11.34% (51.25 ± 1.76 Vs 57.80 ± 1.47 f L) for MCV: Mean Corpuscular or Cell Volume, of 10.94% (22.57 ± 1.16 Vs 20.35 ± 2.20 p g) for MCH: Mean corpuscular or cell hemoglobin and of 26.64% (44.31 ± 2.86 Vs 34.99 ± 3.42 g/dl) for MCHc: Mean corpuscular or cell hemoglobin concentration.

The radiation also caused a significant variation of blood constituents in groups "Sham Irradiation+Distilled Water", "Irradiation+25 mg/kg AGE", "Irradiation+50 mg/kg AGE" and "Irradiation+Vitamin E and Lipoic Acid" according to the following parameters (Table 1).

Granulocytes (GR): irradiation resulted in a significant decrease ($P < 0.05$) in order of 45.45% (2.23 ± 0.42 Vs 4.09 ± 0.65 $10^9/\mu\text{l}$) in the group "Irradiation+Distilled Water".

Red blood cells count (RBCs): a significant reduction ($P < 0.001$ and $P < 0.001$) was observed in the group "Irradiation+Distilled Water" in order of 35.48% (4.63 ± 0.37 Vs 7.18 ± 0.18 $10^9/\mu\text{l}$) and 31.42% (4.92 ± 0.39 Vs 7.18 ± 0.18 $10^9/\mu\text{l}$) in the group "Irradiation+Vitamin E and Lipoic Acid" after irradiation. This rate increased significantly ($P < 0.01$ and $P < 0.05$) in the groups "Irradiation+25 mg/kg AGE" and "Irradiation+50 mg/kg AGE" respectively in order of 41.94% (6.57 ± 0.55 Vs 4.63 ± 0.37 $10^9/\mu\text{l}$) and of 53.05% (6.19 ± 0.45 Vs 4.63 ± 0.37 $10^9/\mu\text{l}$) compared to the group "Irradiation+Distilled Water". The increase was also significant ($P < 0.05$) in the group "Irradiation+25 mg/kg AGE" in order of 33.58% (6.57 ± 0.55 Vs 4.92 ± 0.39 $10^9/\mu\text{l}$) compared to the group "Irradiation+Distilled Water".

Mean Corpuscular or Cell Volume (MCV): a significant reduction ($P < 0.05$) of this rate was observed in the group "Irradiation+Distilled Water" in order of 12.64% (50.50 ± 2.17 Vs 57.80 ± 1.47 f L) compared to the group "Sham Irradiation+Distilled Water". This rate increased significantly ($P < 0.05$ and $P < 0.05$) in the groups "Irradiation+25 mg/kg AGE" and "Irradiation+50 mg/kg AGE" respectively in order of 16.60% (58.88 ± 1.03 Vs 50.50 ± 2.17 f L) and of 16.12% (58.64 ± 0.98 Vs 50.50 ± 2.17 f L) compared to the group "Irradiation + Distilled Water". The increase was also significant ($P < 0.05$ and $P < 0.05$) in the groups ("Irradiation + 25 mg/kg AGE" and "Irradiation+50 mg/kg AGE") of approximately 14.89% (58.88 ± 1.03 Vs 51.25 ± 1.76 f L) and 14.42% (58.64 ± 0.98 Vs 51.25 ± 1.76 f L) compared to the positive control group "Irradiation + Vitamin E and Lipoic Acid".

Hematocrit value (HTC): the decrease in this parameter was significant ($P < 0.001$) in the groups "Irradiation + Distilled Water" and "Irradiation + Vitamin E and Lipoic after irradiation, respectively in order of 43.55% (23.37 ± 2.17 Vs

$41.40 \pm 0.86\%$) and of 38.71% (25.38 ± 2.59 Vs $41.40 \pm 0.86\%$). The Hematocrit value increased significantly ($P < 0.001$ and $P < 0.01$) in order of 66.28% (38.86 ± 3.70 Vs $23.37 \pm 2.17\%$) and of 55.68% (36.39 ± 3.03 Vs $23.37 \pm 2.17\%$) in the groups "Irradiation+25 mg/kg AGE" and "Irradiation+50 mg/kg AGE" compared to the group "Irradiation+Distilled Water". This increase was also significant ($P < 0.01$ and $P < 0.05$) in the range of 53.13% (38.86 ± 3.70 Vs $25.38 \pm 2.59\%$) and of 43.38% (36.39 ± 3.03 Vs $25.38 \pm 2.59\%$) by comparing the groups "Irradiation+25 mg/kg AGE" and "Irradiation+50 mg/kg AGE" to the positive control group "Irradiation+Vitamin E and Lipoic Acid".

Platelets count (PLT): after irradiation, the decrease in this parameter was significant ($P < 0.001$) in the group "Irradiation + Distilled Water" in order of 26.99% (845.20 ± 2.31 Vs 1157.60 ± 3.36 $10^9/\mu\text{l}$), in the group "Irradiation+25 mg/kg AGE" in order of 5.25% (1096.80 ± 1.24 Vs 1157.60 ± 3.36 $10^9/\mu\text{l}$), in the group "Irradiation + 50 mg/kg AGE", in order of 6.70% (1080.00 ± 4.09 Vs 1157.60 ± 3.36 $10^9/\mu\text{l}$) and in the group "Irradiation + Vitamin E and Lipoic Acid" in order of 23.08% (890.40 ± 3.08 Vs 1157.60 ± 3.36 $10^9/\mu\text{l}$). Platelet counts increased significantly ($P < 0.001$) in order of 29.77% (1096.80 ± 1.24 Vs 845.20 ± 2.31 $10^9/\mu\text{l}$) and of 27.78% (1080.00 ± 4.09 Vs 845.20 ± 2.31 $10^9/\mu\text{l}$) in the groups "Irradiation+25 mg/kg AGE" and "Irradiation+50 mg/kg AGE" compared to the group "Irradiation + Distilled Water". This increase remains also increased significantly ($P < 0.001$) in order of 23.18% (1096.80 ± 1.24 Vs 890.40 ± 3.08 $10^9/\mu\text{l}$) and of 21.29% (1080.00 ± 4.09 Vs 890.40 ± 3.08 $10^9/\mu\text{l}$) in the group "Irradiation + Vitamin E and Lipoic Acid" compared to groups "Irradiation+25 mg/kg AGE" and "Irradiation+50 mg/kg AGE".

4. Discussion

Hematopoietic system is one of the most sensitive systems to evaluate the hazardous effects of radiations in humans and animals [2-4]. The hematopoietic tissues are among the most radiosensitive tissues in the body following irradiation, there is inhibition of mitosis and diminution in cellularity. Damage of the hematopoietic system is a major factor in the mortality following acute radiation exposure. The first to be affected after irradiation are the red cells precursors causing a decrease in the amount and life span of peripheral red cells, next are those of the white blood cells and last the platelets precursors. The decrease in the number of platelets increases the incidence of hemorrhage [7-8].

In consistency, the present study indicated that irradiation with a dose of 4.5 Gy induced a decrease in the values of WBCs, lymphocytes, MID, GR, Hb and MCV with a significant reduction in RBCs, HTC and platelets counts. An increase in MCH and MCHC was noticed in rats as well. This is in partial agreement with the findings of Shaheen and Hassan, who reported that gamma radiation caused a significant decrease in red blood cells [20]. AGE exhibited profound antianemic, antifatigue, lipid-lowering activity and transaminases lowering and its administration via gavages prior or after irradiation had successfully ameliorated the

hematological disturbances induced by radiations. The amelioration was more important in "Irradiation+25 mg/kg AGE" group than in "Irradiation+50 mg/kg AGE" group. Furthermore, this improvement has been more pronounced with AGE than with vitamin E + Lipoic Acid.

Radiation is known to cause tissue damage via the generation of free radicals like hydroxyl and superoxide radicals. So, the reduction observed in RBCs can be explained by cells death due to oxidative damages; generated by these free radicals. The disturbances in RBCs could reflect an imbalance between its production and loss [21]. According to some authors, gamma radiation caused alterations in the haematological parameters of irradiated rats [22] and among the leukocytes, lymphocytes have been reported to be the most radiosensitive haematologic cells [23-24]. The significant reduction in the mean of the lymphocytes observed in this study 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 [25], which may be deployed in mitigating the effects of the oxidative damage induced by the gamma radiation. Moreover, the significant reduction noticed in platelets count might be due to radiations inhibiting bone marrow activity or could be due to decreased production or increased consumption of platelets or due to the increased platelets aggregation. These results showed that gamma radiation causes oxidative stress in human platelets and lymphocytes, which might reflect on their life expectancy. However, the subsequent decline in the values of WBCs and lymphocytes, MID and GR could be the consequence of absence of infection and inflammation. While, elevation of HTC value in irradiation-treated rats could be related to loss of the body fluid and hemoconcentration that resulted from the damages of gamma radiation on the function of rat kidney.

Changes in blood values are recognized as an asset in determining the extent of radiation exposure. Furthermore, destruction of bone marrow cells or increase osmotic fragility of RBCs allowed to establish an etiological relationship between anemia, difficulty in breathing, infection, fever and irradiation [26]. Thus, radiation injuries might lead to anemia, difficulty in breathing, infection and fever as a result of either suppression the activity of hematopoietic tissues, impaired erythropoiesis, and accelerated RBCs destruction. Yuan *et al.* showed that, the alteration of RBCs membrane permeability, increased RBCs mechanical fragility, and/or defective Fe metabolism [27].

Although, normal concentrations of hematological parameters usually measured can be modified by plants consumption or toxic substance [28-29], the animals receiving orally AGE prior or after irradiation, revealed significant modulation in most of the hematological indices. This modulation has been more pronounced with the lower dose of AGE (25 mg/kg) than with the higher dose (50 mg/kg). AGE was then found to have beneficial effects against gamma radiation-induced suppression in most of hematological parameters and RBCs indices as it increased

number of RBCs and Hb concentration about to normal levels. AGE has been shown to exert antioxidant properties in plasma and erythrocytes as well [30-31]. It also induced great improvement in the reduction of RBCs, HTC and platelets counts with subsequent decline in the values of WBCs and lymphocytes, MID, GR and MCV levels in irradiation-treated animals. The ameliorative effect of AGE could be due to reduction of lipid peroxidation level in cell membrane with subsequent prevention of free radicals induced damage through its antioxidant activity achieved by its active compounds [32]. These results tend to indicate that AGE had preventive and protective effects against radiation-induced hematological changes. The beneficial effects and the improvement have been more pronounced with the lower dose of AGE (25 mg/kg) than with the higher dose (50 mg/kg). Garlic-induced increase in erythrocytes count might be linked either to an increase in erythropoiesis or to the ability of garlic in decreasing membrane rigidity [33].

5. Conclusion

The present study demonstrated that through its antioxidant and free radical scavenging activities, AGE confers significant radiation protection to blood cells parameters followed hematopoietic syndrome induced by γ -radiation. This radioprotection is achieved by its ability as a scavenger for free radicals generated by ionizing radiation. In addition, the study revealed that the preventive and protective effects of AGE were more pronounced with the lower dose of AGE (25 mg / kg) than with the higher (50 mg / kg) and the power of AGE was greater than the one of the positive control group Vitamin E and Lipoic Acid concerning radioprotective properties.

References

- [1] Hosseini-mehr S J, Zakaryae V and Froughizadeh M. Oral oxymetholone reduces mortality induced by gamma irradiation in mice through stimulation of hematopoietic cells. *Mol Cell Biochem.* 2006; 287(1-2), 193-199.
- [2] Parihar VK, Prabhakar KR, Veerapur VP, Priyadarsini KI, Unnikrishnan MK and Rao CM, Anticlastogenic activity of morin against whole body gamma irradiation in Swiss albino mice. *Eur J Pharmacol.* 2007; 557(1), 58-65.
- [3] Zhou Y and Mi MT, Genistein stimulates hematopoiesis and increases survival in irradiated mice. *J Radiat Res.* 2005; 46(4), 425-433.
- [4] Abdel-Magied, N. and Ahmed, A. G. The Protective Role of Lettuce oil (*Lactuca sativa*) against Radiation induced Biological Hazards in Male Rats. *J. Rad. Res. Appl. Sci.* 2011; Vol. 4, 4, No. 3(B), pp. 923 – 938.
- [5] Walker RI, Cervený RJ, eds. Medical Consequences of Nuclear Warfare. Falls Church, VA: Office of the Surgeon General; 1989. Available at www.afri.usuhs.mil.
- [6] Hall EJ. Acute effects of total-body irradiation. In: Hall EJ. Radiobiology for the Radiologist. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2000: 124-35.

- [7] Casarett, A. P. Effect of radiation on blood cells. In: *Radiation Biology*. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 1968; 3,125.
- [8] Kojima N and Okaw J. Prospective analysis of radiation induced cytopenia. *Biotherapy*. 1999; 13(5): 462.
- [9] Venkatachalan S, Chattopadhyay S. Natural radioprotective agents: An overview. *Curr Org Chem*. 2005; 9: 389-04.
- [10] Louise A Lexis, Robert G Fassett and Jeff S Coombes. α -Tocopherol and α -Lipoic Acid enhance the erythrocyte antioxidant defence in Cyclosporine A-Treated Rats. *Basic and Clinical Pharmacology and Toxicology*. 2006, 98: 68–73.
- [11] Ganesh C Jagetia. Radioprotective potential of plants and Herbs against the effects of Ionizing Radiation. *J. Clin. Biochem. Nutr.* 2007; 40: 74-81.
- [12] Tanaka T. Cancer chemoprevention by natural products. *Oncol. Rep.* 1994; 1: 1139–1155.
- [13] Kong Ah-Ng T, Yu R., Chen C, Mandlekar S and Primiano T. Signal transduction events elicited by natural products: Role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch. Pharm. Res.* 2000; 23:1–16.
- [14] Ahmed R S, Seth V and Banerjee B D. Influence of dietary ginger (*Zingiber officinale* Rosc) on antioxidant defense system in rat: Comparison with ascorbic acid. *Indian J. Exp. Biol.* 2000; 38:604–606.
- [15] Amagase H, Petesch B L, Matsuura H, Kasuga S, and Itakura Y. "Intake of garlic and its bioactive components". *Journal of Nutrition*. 2001; 131(3): 955–962.
- [16] Moriguchi T, Saito H, Nishiyama N. Anti-aging effect of aged garlic extract in the inbred brain atrophy mouse model. *Clin. Exp. Pharmacol. Physiol.* 1997; 24: 235–42.
- [17] Balamash K, Albar O, Wang Q and Ahmed N. Effect of Kyolic® aged garlic extract on glycaemia, lipidaemia and oxidative stress in patients with type 2 diabetes mellitus. *Journal of Diabetes Research and Clinical Metabolism*. 2012; 1:p.18.
- [18] EEC: Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities* 1986; L358:1–29.
- [19] Ashraf Y Nasr. Protective effect of aged garlic extract against the oxidative stress induced by cisplatin on blood cells parameters and hepatic antioxidant enzymes in rats. *Toxicology Reports*. 2014; 1: 682–691.
- [20] Shaheen AA, Hassan SM. Radioprotection of whole-body gamma irradiation-induced alteration in some haematological parameters by cysteine, vitamin E and their combination in rats. *Strahlenther Onkol.* 1991; 167(8): 498-501.
- [21] Hassan I, Chibber S, Naseem I. Ameliorative effect of riboflavin on the cisplatin induced nephrotoxicity and hepatotoxicity under photo-illumination. *Food Chem. Toxicol.* 2010; 48 (8–9): 2052–2058.
- [22] Olatunde Owoeye, Silas Kalu Onwuka and Ebenezer Olatunde Farombi. *Vernonia amygdalina* leaf extract and alpha-tocopherol alleviated gamma radiation-induced haematological and biochemical changes in rats. *Int. J. Biol. Chem. Sci.* 2011; 5(5): 1978-1992.
- [23] Jones TC, Hunt, RD, King, NW. Pathologic effects of ionizing radiations. *Veterinary Pathology* (6th edn). Lippincott Williams & Wilkins: Philadelphia. 1997; 681-694.
- [24] Jagetia GC, Baliga MS, Malagi KJ, Kamath, MS. The evaluation of the radioprotective effect of Triphala (an Ayurvedic rejuvenating drug) in the mice exposed to gamma-radiation. *Phytomedicine*. 2002; 9: 99-108.
- [25] Bounous G, Molson JH. The antioxidant system. *Anticancer Research*. 2003; 23: 1411-1416.
- [26] Radiation Emergency Medical management (REMM) from NATO Handbook on the Medical Aspects of NBC Defensive Operations AMedP-6(B), Chapter 6, General Medical Effects of Nuclear Weapons: Diagnosis, Treatment, and Prognosis, 1 February, 1996, available on www.remm.nlm.gov.
- [27] G. Yuan, S. Dai, Z. Yin, H. Lu, R. Jia, X. Song, Y. Shu, X. Zhao, Toxicological assessment of combined lead and cadmium: acute and sub-chronic toxicity study in rats. *Food Chem. Toxicol.* 2014; 65: 260–268.
- [28] Abatan MO, Arowolo RO. Toxicity of *Eugenia uniflora* to rats. *Nig J Animal Production*. 1989; 16:16–19.
- [29] Ajagbonna O P, Onifade K I, Suleiman U. Haematological and biochemical changes in rats given extract of *Calotropis procera*. *Sokoto J Vet Sciences*. 1999; 1: 36–42.
- [30] Avci A, Atli T, Erguder I B, Varli M, Devrim E, Aras S, Durak I. Effects of garlic consumption on plasma and erythrocyte antioxidant parameters in elderly subjects. *Gerontology*. 2008; 54: 173–176.
- [31] Ana L C, Santana R A, Silva-Islas C A, Chanez-Cardenas M E, Santamaria A, Maldonado P D. The antioxidant mechanisms underlying the aged garlic extract- and s-allyl cysteine-induced protection. *Oxid. Med. Cell. Longev.* 2012; 16: <http://dx.doi.org/10.1155/2012/907162>, Article ID 907162.
- [32] Capasso A. Antioxidant action and therapeutic efficacy of *Allium sativum* L. *Molecules* 18. 2013; 1: 690–700.
- [33] Hamlaoui-Gasmi S, Mokni M, Limam N, Limam F, Amri M, Aouani E, Marzouki L. Effect of garlic's mode of administration on erythrocytes and plasma parameters in Wistar rat. *Afr. J. Biotechnol.* 2012; 11 (33): 8259–8263.