Effect of Aged Garlic Extract on Prodromal Phase After Acute Radiation Syndrome

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Abstract: Acute radiation syndrome results in 4 four phases among which the prodromal one characterized by malaise, diarrhea, vomiting, and anorexia. It last a few hours, but from irradiation to the 7th day after, some other signs (unspecified, mild, moderate or severe) are observed. Thus, the aim of this study was to assess if Aged garlic extract (AGE) could offer radioprotection by preventing signs of prodromal phase after acute radiation syndrome. Knowing, AGE is a natural product containing different compounds with antioxidant activity and possessing several physiological activities in experimental animals. 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. The signs observed and evaluated (behavioral changes, salivation, state stool, water and food intake, weight loss, relative weight of organs, fatigue/weakness, temperature and bleeding) revealed a significant change in irradiated groups although AGE-pretreated rats prior and after irradiation revealed significant amelioration in radiation-induced disturbances. Therefore AGE can be considered having radioprotective effects against radiation-induced changes in prodromal phase signs.

Keywords: Irradiation, Radiation Syndrome, Prodromal Phase, AGE, Rats

1. Introduction

Humans are exposed to ionizing radiation during diagnostic, therapeutic and industrial purposes. Apart from this, humans also get exposed to ionizing radiations during air and space travel, background radiation nuclear accidents, and use of electronic devices. Nuclear terror attacks are not distant possibility; therefore it is essential to protect humans from ionizing radiations by pharmacological intervention [1]. When a person is subjected to a brief and intense exposure to a radioactive source, acute radiation syndrome can be observed [2-3]. Furthermore, exposure to high amounts of ionizing radiation results in several syndromes [4] and each syndrome can be divided into 4 phases: prodromal, latent, manifest illness, and recovery or death. The prodromal phase of the acute radiation syndrome usually occurs in the first 48 hours but may develop up to 6 days after exposure [5]. It is also characterized by malaise, diarrhea, vomiting, and
anorexia. It last a few hours, but from irradiation to the 7th day, some other signs (unspecified, mild, moderate or severe) are observed [6-8].

Recently; 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 that could protect humans against the deleterious effects of ionizing radiation in clinical and other conditions as well as during nuclear terror attack [1]. AGE has been demonstrated to possess several physiological activities in experimental animals [9-10] and has received particular attention because it is a highly efficient antioxidant and has free radical scavenging capacity [10-11].

In view of these considerations, the main objective of this study; was to test if Aged Garlic Extract could offer radioprotection for male Wistar albino rats after total body exposure to acute ionizing radiation by preventing the appearance of signs of prodromal phase.

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 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 [12-13]. The content of water-soluble compounds is relatively high whereas that of oil-soluble compounds is relatively low [13]. 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 for 12 days (5 consecutive days prior to acute irradiation and one hour after irradiation on day 6 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 [14].

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. Sings of Prodromal Phase Determination

Observations were made in about 1, 4, 8, 24 to 36 hours and on days 6 and 7 to detect signs of prodromal phase (nausea, vomiting, anorexia, diarrhea, asthenia, swelling, itching, and redness of the skin) and their duration [8, 15-17].

<table>
<thead>
<tr>
<th>Signs</th>
<th>Sings duration: From/on:</th>
<th>Means of appreciation after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral changes</td>
<td>from day 1 to 7</td>
<td>-Social interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Aggressiveness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Reaction to noise</td>
</tr>
</tbody>
</table>
The signs to observe were appreciated with some means among which: behavioral changes, evaluation of food and water consumption, grip strength test and temperature.

2.5.1. Behavioral Changes Observations

Acute radiation syndrome exposed to psychological symptoms [16] and post-traumatic stress disorder which include anxiety disorders and depression [17]. Thus, observations were made for behavioral changes: communication, locomotion, aggressiveness, state of the tail, reaction to noise and reaction to pinch. When the rats are gathered together, it is an indicator of communication (i.e. normal social interaction); they are said to be in activity (i.e. locomotion) when they are roaming in the cage; they are said to be aggressive when in any attempt to touch them they react by biting; normal reaction to noise is when the rats are unsettled on hearing a noise; the cries of rats when pinched on their tail is an indicator of normal reaction to pinch; the tail is normal when it is flexible (i.e. not rigit); a rigid tail is a sign of anger [19].

2.5.2. Evaluation of Food and Water Consumption [20]

The evaluation of food consumption was made by weighing daily (using a scale: KERN EMB 600-2 Max 600 g d = 0.01g) quantities of food distributed, remaining and obtained (after sieving chip) to calculate individual food consumption. So, Food Portion Consumed (Fpc) was calculated as follow:

\[ F_{pc} = \frac{\text{Amount of food distributed} - \text{Amount of uneaten food}}{\text{day}} \]

Individual Food Consumption (Ifc) average was determined by the following formula:

\[ I_{fc} = \frac{\text{Food portion consumed (Fpc)}}{\text{Total number of rats per group}} \]  

The evaluation of water consumption was made by measuring daily quantities of water distributed and remaining to calculate individual water consumption. So, Water Portion Consumed (WPC) in mL was calculated as follow:

\[ W_{PC} = \text{Amount of water distributed} \div \text{day} - \text{Amount of remaining water} \div \text{day} \]

Individual Water Consumption (IWC) average was determined by the following formula:

\[ I_{WC} = \frac{\text{Water portion consumed (WPC)}}{\text{Total number of rats per group}} \text{ (mL)} \]  

2.5.3. Evolution of Body Weight Post irradiation and/or AGE Administration

Animals were weighed from first day of experiment to prior sacrifice using a balance (KERN EMB 600-2 Max 600 g d = 0.01g). The percent change in weight was calculated using the following formula:

\[ \text{WG} = \frac{P_n - P_0}{P_0} \times 100 \]  

WG = weight gain (%); \( P_n \) = measurement of body weight prior to sacrifice; \( P_0 \) = measurement of body weight on the first day of experiment;

2.5.4. Grip Strength Test

Animals from different groups were subjected to strength testing on day 3 after irradiation to assess fatigue/weakness. The test was done. This test, known as "Muscular strength test", was to suspend the animal by its back legs to a fixed horizontal bar maintained by a suitable device [21]. The time taken to the bar by each animal (in seconds) was observed using a stopwatch. The animals took the test three times and the best time was chosen [22]. For this, each subject was suspended from a metal bar placed horizontally.
2.5.5. Temperature Determination

Central temperature was determined by pointing the laser radiation emitted by the TW2 Pocket Infrared Thermometer professional 6:1 optics to the anus of the rat. Rat’s temperature is influenced by several external factors such as room temperature in which they live, diet, health status, humidity and stress. This makes it difficult to have neutral environments to establish a correct average temperature [23]. Therefore, in order to eliminate such influences, daily; the raw results of each group were assigned a factor as it leads to a constant average temperature of witnesses that day. Thus, the variation observed in irradiated animals should be related to the influence of irradiation. Therefore, the calculations allowed eliminating variations due to causes other than radiation.

2.6. Statistical Analyses

Results were expressed as mean ± 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 α 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

3.1. Determination of Mortality Rates and Behavioral Changes After γ-Radiation/AGE Administration

3.1.1. Behavioral Changes

Animals of the different groups were gathering together on day 12 before sacrifice except those of groups VII (sham irradiation + Vitamin E and Lipoïc Acid) and VIII (Irradiation + Vitamin E and Lipoïc Acid) which were also settled on hearing a noise. Compare to the others, animals of this two groups were not interacting, they were aggressive and more sensitive to noise. They were also prompt to cries when pinched on their tail (constantly rigid) (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups observed day before sacrifices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social interaction (rats are gathered together)</td>
<td>I  II  III  IV  V  VI  VII  VIII</td>
</tr>
<tr>
<td>Activity (Locomotion =Roaming in cages)</td>
<td>+    +    +    +    +    -    -</td>
</tr>
<tr>
<td>Aggressiveness (biting when to any attempt to touch them)</td>
<td>+    -    +    +    +    +    +</td>
</tr>
<tr>
<td>Reaction to noise (unsettled on hearing a noise)</td>
<td>+    +    +    +    +    +    +</td>
</tr>
<tr>
<td>Reaction to pinch (cries of rats when pinched on their tail)</td>
<td>+    +    +    +    +    +    +</td>
</tr>
<tr>
<td>State of the tail</td>
<td>f    f    f    f    f    r    r</td>
</tr>
</tbody>
</table>

(+)= normal; (++)= increased; (+++) = profoundly increased; (-)= reduced; (- -)= profoundly reduced; (f)= flexible, (r)= rigid (sign of anger)

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 Lipoïc Acid”, Group VIII: “Irradiation+Vitamin E and Lipoïc Acid”

3.1.2. Mortality Rate

The mortality rate during the 12 days was 0% because no rat has been found dead. Therefore, no autopsy was performed.

3.2. Observation of Nausea, Vomiting and Diarrhea (NVD)

3.2.1. Nausea and Vomiting

The observation snouts and the chip have revealed no salivation or no rejection.

3.2.2. Diarrhea

State stool of the rats was observed between 4th and 8th hour after global irradiation and the results shown in the table below.

<table>
<thead>
<tr>
<th>State of stools</th>
<th>Groups observed 4 to 8 hours after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I   II  III  IV  V  VI  VII  VIII</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>NO  NO  NO  NO  NO  NO  NO  NO</td>
</tr>
<tr>
<td>Soft and mucous stools</td>
<td>NO  O   NO  NO  NO  NO  NO  O</td>
</tr>
<tr>
<td>Ordinary stools (granular)</td>
<td>O    O    O    O    O    O    O    O</td>
</tr>
</tbody>
</table>

Ordinary stool: consistent, a bit hard and granular NO: Non observed O: Observed

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 Lipoïc Acid”, Group VIII: “Irradiation+Vitamin E and Lipoïc Acid”

The consistent stools somewhat hard and granular were observed in all groups. The diarrheia was observed in any group; but soft and mucous stools were noted in the groups “Irradiation + Distilled Water” and “Irradiation + Vitamin E and Lipoïc Acid” (Table 3).
### 3.3. Anorexia

#### 3.3.1. Food and Water Consumption

The consideration of amounts distributed the day of the irradiation (day 6) and those picked up the next day (day 7) as well as those distributed on day 7 and those collected on day 8 indicates that Total Body Irradiation (TBI) has caused a decline in food and water consumption in groups “Irradiation+Distilled Water”, “Irradiation+25 mg/kg AGE”, “Irradiation+50 mg/kg AGE” and “Irradiation+Vitamin E and Lipoïc Acid” (Tables 4 and 5). AGE administration resulted in a lesser reduction of water and food taken in groups “Irradiation+25 mg/kg AGE” and “Irradiation+50 mg/kg AGE” compared to groups “Irradiation+Distilled Water” and “Irradiation+Vitamin E and Lipoïc Acid”.

### 3.3.2. Effects of γ-Radiation/Extracts and Evolution of Body Weight

The effects of TBI and AGE intake on weight changes are shown in Figure 1. These effects vary depending on the order of weight increase of 105.05% and 66.24% respectively for groups “Irradiation+25 mg/kg AGE” and “Irradiation+50 mg/kg AGE”. Age administration resulted in a lesser reduction of water and food taken in groups “Irradiation+25 mg/kg AGE” and “Irradiation+50 mg/kg AGE” compared to groups “Irradiation+Distilled Water” and “Irradiation+Vitamin E and Lipoïc Acid”.

#### 3.3.2.1. Food and Water Consumption

The effects of TBI and AGE intake on weight changes are shown in Table 4. Food and Water Consumption (mL) per Rat before/after γ-radiation and AGE administration are presented in Table 5. The weight change between 7-6 days, 8-7, 9-8, 10-9 and 11-10 indicate an order of weight increase of 105.05% and 66.24% respectively for groups “Irradiation+25 mg/kg AGE” and “Irradiation+50 mg/kg AGE”. Age administration resulted in a lesser reduction of water and food taken in groups “Irradiation+25 mg/kg AGE” and “Irradiation+50 mg/kg AGE” compared to groups “Irradiation+Distilled Water” and “Irradiation+Vitamin E and Lipoïc Acid”.

### Table 4. Food Consumption per Rat before/after γ-radiation and AGE administration.

<table>
<thead>
<tr>
<th>Food Consumption (g)</th>
<th>Day 1-2</th>
<th>Day 2-3</th>
<th>Day 3-4</th>
<th>Day 4-5</th>
<th>Day 5-6</th>
<th>Day 6-7</th>
<th>Day 7-8</th>
<th>Day 8-9</th>
<th>Day 9-10</th>
<th>Day 10-11</th>
<th>Day 11-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Irr+Distilled Water</td>
<td>17.6</td>
<td>18.8</td>
<td>19.6</td>
<td>19.4</td>
<td>19.2</td>
<td>19.4</td>
<td>20.0</td>
<td>19.6</td>
<td>19.4</td>
<td>20.4</td>
<td>19.9</td>
</tr>
<tr>
<td>Irr+Distilled Water</td>
<td>17.4</td>
<td>17.9</td>
<td>18.2</td>
<td>18.4</td>
<td>17.6</td>
<td>16.2</td>
<td>15.4</td>
<td>15.4</td>
<td>15.0</td>
<td>14.8</td>
<td>14.4</td>
</tr>
<tr>
<td>Sham Irr+25 mg/kg AGE</td>
<td>18.8</td>
<td>19.0</td>
<td>18.6</td>
<td>18.8</td>
<td>19.2</td>
<td>19.5</td>
<td>19.2</td>
<td>20.2</td>
<td>19.0</td>
<td>19.3</td>
<td>19.6</td>
</tr>
<tr>
<td>Irr+25 mg/kg AGE</td>
<td>19.2</td>
<td>18.8</td>
<td>18.6</td>
<td>19.2</td>
<td>19.0</td>
<td>17.6</td>
<td>17.4</td>
<td>18.0</td>
<td>17.8</td>
<td>17.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Sham Irr+50 mg/kg AGE</td>
<td>18.4</td>
<td>18.9</td>
<td>19.8</td>
<td>19.6</td>
<td>20.3</td>
<td>20.0</td>
<td>19.8</td>
<td>20.4</td>
<td>19.9</td>
<td>19.4</td>
<td>18.9</td>
</tr>
<tr>
<td>Irr+50 mg/kg AGE</td>
<td>17.9</td>
<td>18.4</td>
<td>19.6</td>
<td>18.8</td>
<td>18.1</td>
<td>17.0</td>
<td>16.8</td>
<td>17.6</td>
<td>17.2</td>
<td>17.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Sham Irr+Vitamin E and Lipoïc Acid</td>
<td>17.4</td>
<td>18.4</td>
<td>19.2</td>
<td>18.9</td>
<td>18.4</td>
<td>19.0</td>
<td>18.8</td>
<td>19.0</td>
<td>19.2</td>
<td>20.0</td>
<td>19.6</td>
</tr>
<tr>
<td>Irr+Vitamin E and Lipoïc Acid</td>
<td>17.6</td>
<td>18.6</td>
<td>19.3</td>
<td>18.6</td>
<td>18.0</td>
<td>16.1</td>
<td>15.6</td>
<td>15.8</td>
<td>15.6</td>
<td>15.2</td>
<td>14.8</td>
</tr>
</tbody>
</table>

### Table 5. Water Consumption per Rat before/after γ-radiation and AGE administration.

<table>
<thead>
<tr>
<th>Water Consumption (mL)</th>
<th>Day 1-2</th>
<th>Day 2-3</th>
<th>Day 3-4</th>
<th>Day 4-5</th>
<th>Day 5-6</th>
<th>Day 6-7</th>
<th>Day 7-8</th>
<th>Day 8-9</th>
<th>Day 9-10</th>
<th>Day 10-11</th>
<th>Day 11-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Irr+Distilled Water</td>
<td>10.08</td>
<td>10.78</td>
<td>10.86</td>
<td>10.96</td>
<td>11.02</td>
<td>10.74</td>
<td>11.10</td>
<td>11.16</td>
<td>11.58</td>
<td>11.70</td>
<td>11.66</td>
</tr>
<tr>
<td>Irr+Distilled Water</td>
<td>09.96</td>
<td>10.26</td>
<td>10.44</td>
<td>10.42</td>
<td>10.08</td>
<td>09.42</td>
<td>09.36</td>
<td>10.28</td>
<td>10.00</td>
<td>09.80</td>
<td>09.44</td>
</tr>
<tr>
<td>Sham Irr+25 mg/kg AGE</td>
<td>10.78</td>
<td>10.84</td>
<td>10.66</td>
<td>10.54</td>
<td>10.60</td>
<td>10.52</td>
<td>10.58</td>
<td>10.98</td>
<td>11.52</td>
<td>11.04</td>
<td>10.98</td>
</tr>
<tr>
<td>Irr+25 mg/kg AGE</td>
<td>11.02</td>
<td>10.96</td>
<td>10.64</td>
<td>10.76</td>
<td>10.90</td>
<td>10.86</td>
<td>10.70</td>
<td>10.78</td>
<td>10.64</td>
<td>10.52</td>
<td>10.44</td>
</tr>
<tr>
<td>Sham Irr+50 mg/kg AGE</td>
<td>10.54</td>
<td>10.84</td>
<td>10.76</td>
<td>10.90</td>
<td>11.00</td>
<td>10.94</td>
<td>11.12</td>
<td>10.98</td>
<td>11.34</td>
<td>11.34</td>
<td>11.04</td>
</tr>
<tr>
<td>Irr+50 mg/kg AGE</td>
<td>10.26</td>
<td>10.54</td>
<td>10.86</td>
<td>10.72</td>
<td>10.36</td>
<td>10.82</td>
<td>10.54</td>
<td>10.72</td>
<td>10.32</td>
<td>10.20</td>
<td>09.96</td>
</tr>
<tr>
<td>Sham Irr+Vitamin E and Lipoïc Acid</td>
<td>09.96</td>
<td>10.54</td>
<td>10.62</td>
<td>10.76</td>
<td>10.54</td>
<td>11.04</td>
<td>11.00</td>
<td>10.78</td>
<td>11.36</td>
<td>11.40</td>
<td>11.32</td>
</tr>
<tr>
<td>Irr+Vitamin E and Lipoïc Acid</td>
<td>10.08</td>
<td>10.66</td>
<td>10.68</td>
<td>10.48</td>
<td>10.32</td>
<td>9.78</td>
<td>09.62</td>
<td>10.44</td>
<td>10.20</td>
<td>09.90</td>
<td>09.70</td>
</tr>
</tbody>
</table>

3.3.2. Effects of γ-Radiation/Extracts and Evolution of Body Weight

The effects of TBI and AGE intake on weight changes are shown in Figure 1. These effects vary depending on the weight change between days.

**Weight change between days 2-1, 3-2, 4-3 and 5-4:** analysis of weight evolution curve shows no significant differences (P> 0.05) over a period ranging from 1 to Day 5.

**Weight change between days 6-5:** The weight change between days 5 and 6 revealed a non-significant (P> 0.05) in all 8 groups compared to that of 5 and 4 days.

**Weight change between 7-6 days, 8-7, 9-8, 10-9 and 11-10:** The weight change between these days reveals that irradiation and AGE administration have caused a non-significant increase in weight between non-irradiated groups (P> 0.05) and a non-significant decline (P> 0.05) in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" and significant (P<0.001) between the groups "Irradiation + Distilled Water" and "Irradiation + Vitamin E and Lipoïc Acid" "compared to the group "Sham Irradiation + Distilled Water". The weight change between these days shows a non-significant (P> 0.05) in the group "Irradiation + 25 mg / kg AGE" in the order of 91.38% as compared to negative control and, a significant drop in weight (P<0.001) among other irradiated groups, compared to the negative control "Sham Irradiation + Distilled Water". This decrease is respectively about 271% for the group "Irradiation + Distilled Water", 157.62% for the group "Irradiation + 50 mg / kg AGE" and 240.13% for the group "Irradiation + Vitamin E and Lipoïc Acid". The weight change between days 12 and also 11 indicate an order of weight increase of 105.05% and 66.24% respectively for groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE "compared to the group" Irradiation + Distilled Water ". This increase is also of the order of 106.15% and 58.88% in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" relative to the positive control "Distilled + Irradiation Water".
Figure 1. Effects of γ-radiation and AGE on animals weight evolution.

3.3.3. Weight of the Organs
Rats TBI and AGE administration have resulted in no significant effect (P > 0.05) on the relative weight of the different organs compared to the weight of the animals of the negative control "Sham Irradiation + Distilled Water". Furthermore, macroscopic observation of the organs (appearance, presence or absence of nodules, necrosis) revealed no physical change (Table 6).

Table 6. Effects of γ-radiation and AGE on relative organs weight (expressed as a percentage of body weight).

<table>
<thead>
<tr>
<th>Relative Weight of the organs</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.66 ± 0.03</td>
<td>0.75 ± 0.02</td>
<td>0.67 ± 0.04</td>
<td>0.68 ± 0.03</td>
<td>0.67 ± 0.02</td>
<td>0.64 ± 0.03</td>
<td>0.73 ± 0.06</td>
<td>0.72 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>0.63 ± 0.02</td>
<td>0.71 ± 0.03</td>
<td>0.68 ± 0.03</td>
<td>0.63 ± 0.04</td>
<td>0.63 ± 0.02</td>
<td>0.69 ± 0.03</td>
<td>0.75 ± 0.03</td>
<td>0.75 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.296 ± 0.008</td>
<td>0.295 ± 0.011</td>
<td>0.288 ± 0.016</td>
<td>0.285 ± 0.015</td>
<td>0.261 ± 0.016</td>
<td>0.298 ± 0.016</td>
<td>0.289 ± 0.008</td>
<td>0.283 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>0.053 ± 0.006</td>
<td>0.064 ± 0.007</td>
<td>0.055 ± 0.007</td>
<td>0.056 ± 0.004</td>
<td>0.059 ± 0.003</td>
<td>0.069 ± 0.007</td>
<td>0.051 ± 0.004</td>
<td>0.073 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2.24 ± 0.06</td>
<td>2.38 ± 0.08</td>
<td>2.16 ± 0.09</td>
<td>2.39 ± 0.04</td>
<td>2.21 ± 0.12</td>
<td>2.58 ± 0.12</td>
<td>2.37 ± 0.12</td>
<td>2.49 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Left kidney</td>
<td>0.25 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.27 ± 0.02</td>
<td>0.28 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Right kidney</td>
<td>0.26 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.3 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>0.079 ± 0.002</td>
<td>0.082 ± 0.003</td>
<td>0.090 ± 0.04</td>
<td>0.086 ± 0.004</td>
<td>0.085 ± 0.006</td>
<td>0.089 ± 0.002</td>
<td>0.083 ± 0.003</td>
<td>0.084 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>0.022 ± 0.003</td>
<td>0.028 ± 0.003</td>
<td>0.025 ± 0.002</td>
<td>0.025 ± 0.003</td>
<td>0.024 ± 0.002</td>
<td>0.024 ± 0.003</td>
<td>0.025 ± 0.002</td>
<td>0.028 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>Vertebrum</td>
<td>3.38 ± 0.24</td>
<td>3.78 ± 0.18</td>
<td>3.47 ± 0.24</td>
<td>3.71 ± 0.18</td>
<td>3.33 ± 0.20</td>
<td>3.67 ± 0.21</td>
<td>3.48 ± 0.23</td>
<td>3.99 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Femur</td>
<td>0.21 ± 0.02</td>
<td>0.24 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.26 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0.25 ± 0.02</td>
<td>0.24 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.27 ± 0.01</td>
<td>0.31 ± 0.02</td>
<td>0.29 ± 0.02</td>
<td>0.29 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.28 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Right Testis</td>
<td>0.49 ± 0.01</td>
<td>0.51 ± 0.03</td>
<td>0.48 ± 0.02</td>
<td>0.50 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.49 ± 0.03</td>
<td>0.48 ± 0.03</td>
<td>0.51 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Right Epididymis</td>
<td>0.125 ± 0.006</td>
<td>0.127 ± 0.005</td>
<td>0.109 ± 0.010</td>
<td>0.130 ± 0.009</td>
<td>0.125 ± 0.005</td>
<td>0.133 ± 0.007</td>
<td>0.122 ± 0.002</td>
<td>0.129 ± 0.006</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 5). Significant differences are:
- a*P < 0.05; a**P < 0.01; a***P < 0.001: when comparing groups to control (Sham Irradiation + Distilled Water) (a) or
- b*P < 0.05; b**P < 0.01; b***P < 0.001: when comparing groups to «irradiation+Distilled Water Group» (b) or
- c*P < 0.05; c**P < 0.01; c***P < 0.001: when comparing groups to «irradiation+Vitamin E and Lipoïc Acid Group» (c).


3.4. Fatigue/Weakness
Statistical analysis of the data obtained on the 3rd day after irradiation during the "gripping" test indicates a non-significant difference (P > 0.05) between the groups of animals treated with AGE at doses 25 and 50 mg / kg compared to the group "Sham Irradiation + Distilled Water". A significant decrease (P < 0.05) has been registered the 3rd day after irradiation when comparing the negative control "Sham Irradiation + Distilled Water" and groups "irradiation + distilled water" in order of 13.67% (24.0 ± 0.75 Vs 27.8 ± 1.87s) and "irradiation + Vitamin E and Lipoïc Acid" in order of 13.67% (24.0 ± 0.61 Vs 27.8±1.87s) (Figure 2).
Each bar represents the Mean ± ESM, n = 5. Significant differences are:
* a*P ≤ 0.05; a**P ≤ 0.01; a***P ≤ 0.001; when comparing groups to control (Sham Irradiation + Distilled Water) (a)

### 3.5. Temperature and Fever

There is a significant increase (P ≤ 0.05, P ≤ 0.01 and P < 0.01) in temperature from day 5. Compared to day 1, this increase is in order of 1.81% on day 5, 2.14% on day 6 and 1.96% on day 7 (Figure 3).

On day 6: the temperature increase in irradiated groups was not significant (P > 0.05) compared to the negative control "Sham Irradiation + Distilled Water". This increase was in order of 1.21% (38.46 ± 0.50 Vs 38.0 ±0.24°C) for the group "Irradiation + Distilled Water", 0.68% (38.26 ± 0.54 Vs 38.0 ±0.24°C) for the "Irradiation+25 mg/kg AGE", 1.0% (38.38 ± 0.45 Vs 38.0 ±0.24°C) for the group "Irradiation+50 mg/kg AGE" and 1.89% (38.72 ± 0.16 Vs 38.0 ±0.24°C) for the group "Irradiation+Vitamin E and Lipoïc Acid".

On day 7: the temperature increase is significant (P <0.05) in the "Irradiation + Distilled Water" group and remains not significant (P> 0.05) compared to the negative control "Sham Irradiation + Distilled Water" in groups "Irradiation + 25 mg / kg AGE", "Irradiation + 50 mg / kg AGE" and "Irradiation + Vitamin E and Lipoïc Acid". This increase was in order of 4.06% (38.96±0.22 Vs 37.44±0.30°C) for the group "Irradiation + Distilled Water", 1.92% (38.16±0.45 Vs 37.44±0.30°C) for the group "Irradiation + 25 mg / kg AGE", 2.24% (38.28±0.54 Vs 37.44±0.30°C) for the group "Irradiation + 50 mg / kg AGE" and 2.62% (38.42 ± 0.50 Vs 37.44±0.30°C) for the group "Irradiation + Vitamin E and Lipoïc Acid."
3.6. Infection, Bleeding, Anemia, Difficulty in Breathing and Hematological Studies

The inspection of rat’s orifices (nose, mouth, anus) of different groups and chip the 7th and 8th day revealed no bleeding (Table 7).

Table 7. Nose, mouth, anus and chip observation for bleeding on day 7 and 8 after irradiation.

<table>
<thead>
<tr>
<th>Observed</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Mouth</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Anus</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Paddy</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

NO: Non observed O: Observed


4. Discussion

4.1. Mortality Rates and Behavioral Changes After γ-Radiation and AGE Administration

4.1.1. Mortality Rate

The exposure of animals to radiation results in gamma radiation-induced sickness and mortality [24]. It is generally accepted that death of mammals after lethally doses around the LD90/30d is a result of damage to the hematopoietic system [25]. For mice irradiated with X or γ-radiation, the deaths are confined to the period between 6 to 20 days and are completed by day 30 [26]. The dose of 4.5 Gy and the oral administration of distilled water, AGE or vitamin E + Lipoïc Acid, one hour after irradiation/sham irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days did not kill any animals during this study in agreement with earlier reports because death within 10 days postirradiation is due to gastrointestinal damage and the one from 11 to 30 days to hematopoietic damage [27-29]. The Dose Reduction Factor (DRF) was not calculated because the animals were sacrificed 8 days after irradiation in order to highlight the hematopoietic syndrome. However, the dose of 4.5 Gy used for the study represents a significant dose, causing acute radiation syndrome within days, but not lethal to a month. Indeed, the LD90/30d (the lethal dose to 99.9% of subjects, 30 days after irradiation) in rats is of the order of 9 Gy and LD50/30d is close to 7.5 Gy [30-31]. The percentage survival depends on the exposure dose, the route of introduction in the organism; of course, the form in which the radioprotective is administered [32]. The percentage of survival observed post irradiation (post irradiation mortality rate: 0%) may indicate that AGE administration prior and after irradiation protects against oxidative damage and lowers the risk of injury to vital molecules and to varying degrees [11, 33-35]. In addition, it would let glimpse that AGE are orally active (only way to truly be usable easily) whether they are administered 5 consecutive days before irradiation or immediately after irradiation and for 7 consecutive days. This is very important because just some radioprotective are administered by this route [32].

4.1.2. Behavioral Changes

Acute radiation syndrome exposed to psychological symptoms [16] and post-traumatic stress disorder which include anxiety disorders and depression [11]. Generally, irradiation and oral administration of distilled water and AGE one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days did not reveal any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma in rats. However, behavioral changes were observed in groups receiving Vitamin E and Lipoïc Acid 5 consecutive days before acute irradiation or 7 consecutive days after, such as absence of interaction and high reaction in noise, suggesting that this extract may have a depressant effect on the central nervous system. The administration of this extract increased the reaction to pinch. This increased sensitiveness may be due to increase in prostaglandin levels, since prostaglandin have been reported to function in regulating the perception of pain [36]. Prostaglandins are not stored, and their release is dependent on biosynthesis [37]. Evidently, various medications that prevent the perception of pain inhibit the conversion of arachidonic acid by inhibiting the release of prostaglandin synthetase or by interfering in some other way with the synthesis of prostaglandins [37]. Nevertheless, further studies are warranted to investigate the active principles responsible for the behavioral changes in Vitamin E and Lipoïc Acid.

4.2. Nausea, Vomiting and Diarrhea (NVD) Syndrome

Less than one hour after irradiation at doses comprised between 3 and 5.3 Gy severe vomitting are observed in patients. These become moderate between the 4th and the 24th hour after irradiation to the 2nd day [38]. The observation of snouts and chip rats after irradiation has revealed no salivation or no rejection in groups receiving orally distilled water, AGE or Lipoïc Acid and Vitamin E probably because the capacity salivating is very limited in rats [39] and vomiting is impossible because of the anatomy of the anastomosis of the esophagus to the stomach [40].

At doses between 3 and 5.3 Gy, diarrhea is observed in 10% of individuals between the 4th and 8th hour after irradiation [38]. Furthermore, exposure to 4 Gy or more of radiation results in gastrointestinal syndrome, in which the person becomes severely dehydrated in the first 2 days, then has a respite of 4 to 5 days in which the patient "feels well" but then dehydration returns with blood-filled diarrhea as the bacteria from the digestive tract starts invading all of the body, creating infections [6-7, 41-42]. State stool of the rat’s observation between 4th and 8th hour after irradiation revealed the absence of diarrhea in the irradiation treated rats with AGE and the presence of Soft and mucous stools in groups...
“Irradiation+Distilled Water” and “Irradiation+Vitamin E and Lipoïc Acid”. Several studies proved that plants and their active ingredients are having anti-emetic and anti-inflammatory activities and they could give good radiation protection against nausea, vomiting and inflammation during radiation disaster [42].

4.3. Effects of γ-Radiation and AGE Administration on Anorexia and Weight Lose

4.3.1. Food and Water Consumption

The consideration of amounts distributed the day of irradiation (day 1) and those collected the next day (day 2) as well as those distributed on day 2 and those collected on day 3 post-irradiation; shows that radiation has caused a decrease in food and water consumption in irradiated groups. Indeed, irradiation at doses between 3 and 5.3Gy lead to a mild or unspecified anorexia 4 hours after irradiation until Day 2 [38]. However, compared to groups "Irradiation + Distilled Water" and "Irradiation + Vitamin E and Lipoïc Acid", oral administration of AGE, one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days resulted in a slight decline in water and food intake. The increase of food and water consumption was more important in "Irradiation+25 mg/kg AGE” group than in "Irradiation+50 mg/kg AGE” group. Therefore AGE decrease the amplitude of the change in weight after irradiation. Confirming statements of Abd-El Allatif, A. and K. Ebraheem, that; using of garlic increased body gain, feed intake and feed efficiency [43].

4.3.2. γ-Radiation/AGE Administration and Evolution of Body Weight

One of the general criteria reflecting the effect of irradiation is the weight loss experienced by irradiated animals [31]. All irradiated animals, protected or not, have lost weight. But the weight loss is less sensitive in those receiving orally AGE one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days. Weight loss is greater in the groups "Irradiation + Distilled Water" and "Irradiation + Vitamin E and Lipoïc Acid" than in the one receiving AGE orally. It is also noteworthy that, this effect has been more pronounced with the lower dose of AGE (25 mg/kg) than with the higher dose (50 mg/kg). Therefore AGE decrease the amplitude of the change in weight after the irradiation. Monitoring the weight change and food and water consumption is a sensitive indicator of the general health of the animal [44].

4.3.3. Observation and Weight of the Organs

Although, allergic reactions and contact dermatitis to raw garlic have been reported [45-55] and no matter the fact raw garlic is known to cause gastric irritation if taken in high doses by sensitive individuals [56], the safety of Aged Garlic Extract has been confirmed by toxicological studies including the following acute toxicity, sub-acute toxicity, chronic toxicity and mutagenicity tests [57]. Thus macroscopic observation of the organs (appearance, presence or absence of nodules, necrosis) of the rats revealed no physical change. Moreover, in this study, irradiation and the oral administration of distilled water, AGE or vitamin E + Lipoïc, one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days did not result in any significant effect on the relative weight of the different organs compare to the weight of the animals of the negative control group “Sham Irradiation+Distilled Water”. Nakagawa et al. [58] found that no growth retardation, stomach injuries, or morphological abnormalities were observed in models given Aged Garlic Extract for three to 21 days. The authors concluded that when garlic is aged, the toxicity is greatly reduced since none of the side effects observed in the raw garlic group were observed in the Aged Garlic Extract group [59].

4.4. Fatigue/Weakness Observations

Fatigue/weakness is present moderately between the 8th time and 3rd day after irradiation. It is reduced or unspecified between the 3rd and 7th day after irradiation [38]. This could explain fatigue/weakness recorded in the negative control groups "Sham Irradiation + Distilled Water" and "irradiation + Vitamin E and Lipoïc Acid" at the 3rd day after irradiation even a non-significant difference was observed between animals non-irradiated and those receiving orally AGE one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days. There is therefore a positive correlation between irradiation, AGE and muscle strength. In support of the above, Hamlaoui-Gasmi et al. showed that AGE exhibited profound antianemic and antifatigue [59].

4.5. Temperature and Fever

Rat temperature is like humans, an important indicator of health [60]. One to two hours after irradiation, the temperature increases as the 6th and 7th day after irradiation [38]. The mean rectal temperature of the rat is between 35.9 and 37.5°C [61] or 38-39°C. But according to Harkness and Wagner, that are average values; which do not represent an interval of values for a particular sample [62]. This is a value at rest that can be influenced by several external factors [23]. A significant and prolonged exercise or exposure to radiation may of course increase this value.

The irradiation resulted in a nonsignificant increase in temperature in the irradiated groups at 7th day especially in irradiated groups receiving orally distilled water or vitamin E + Lipoïc Acid. This increase is lower in groups receiving AGE one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days. The increase was slight important in “Irradiation+25 mg/kg AGE” group than in “Irradiation+50 mg/kg AGE” group. The temperature regulation is under the control of the hypothalamus. It receives and analyzes information from the receivers of the body temperature and sends control signals if required to
bring to normal in case of overshoot [60]. The increase in post irradiation temperature could be explained either by an action of gamma radiation on the hypothalamus or the presence of an underlying infection resulting from additional post radiation injury. Additional injuries, such as mechanical trauma or burns (the combined injury syndrome), are expected to occur in 60% to 70% of patients after exposure [16, 63]. Patients with burns [64-66] and trauma [67] may develop lymphopenia as a result of these injuries alone. These injuries significantly complicate the management of patients with the hematopoietic syndrome and significantly lower the LD₅₀/₆₀. Prognosis is grave in patients with the combined injury syndrome and radiation exposure [68]. In case of prolonged Whole Body Hyperthermia (WBH), rat body temperature rises, blood pressure drops and heart rate accelerates (tachycardia). It is always accompanied by dehydration, the rat is weak, it does not drink and it does not eat. If nothing is done it can be accompanied by neurological sequelae [60].

The temperature drop in irradiated groups receiving orally AGE at doses of 25 and 50 mg / kg may indicate the absence of underlying infection or potentiation of the thermal self-regulation mechanism of the rat knowing that the sweating mechanism which is used for cooling in humans does not exist in the rat. Grooming enables depositing saliva on his body partially offset this handicap. Its tail also offers him a large heat exchange surface and plays a role in temperature regulation [60]. Thus, in case of hypothermia, rat circulated little blood in his cock and he does not lose heat while in case of hyperthermia, he circulated a lot and it loses its heat [22].

4.6. Infection, Bleeding, Anemia, Difficulty in Breathing and Hematological Studies

Moderate bleeding is usually observed from the 7th day in patients following radiation [38]. However, the rat has very poor eyesight but a highly developed sense of smell. It has particular lacrimal gland, named Harder gland, which secretes tears rich in fat and porphyrins. Normally these tears lubricate the eye, but in case of severe stress or acute disease, they flow through the internal angle of the eye and nose, revealing red scabs that are not blood (chromodacryorrhea) [62, 69-70]. 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 particular lacrimal gland, named Harder gland, which poor eyesight but a highly developed sense of smell. It has

5. Conclusion

Acute radiation syndrome end up with four phases among which the prodromal one characterized by significant disturbances in: behavioral changes, salivation, state stool, water and food intake, weight loss, relative weight of organs, fatigue/weakness, temperature and bleeding. The present study revealed that the pre-treatment of AGE provided a beneficial role in radiation-induced prodromal phase signs, suggesting, AGE may be considered as a useful dietary supplementary compound to patients irradiated. This provides a cheap protective strategy in the management of radiation-induced prodromal phase signs.

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[18] Data adapted by 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.


Hibiscus subdariffa

Association

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