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Protective Effect of Aged Garlic Extract Against the Oxidative Stress Induced by Acute Ionizing Irradiation on Hepatic Antioxidant Enzymes in Rats

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To cite this article:

Abstract: Ionizing radiations damage cells, tissues and organs among which the liver through a cascade of molecular events that are triggered by reactive oxygen species (ROS), lipid peroxidation and nitrogen species (NS). Aged Garlic Extract (AGE) has been demonstrated to possess free radical scavenging capacity and antioxidant activity. Therefore, the present study has been focused in analyzing the properties of AGE against the lipid peroxidation and oxidative damages of rat liver tissues induced by acute radiation. 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 Lipoïc Acid. Then, biochemical analyses, lipid peroxidation, total Protein and antioxidants assessment were made from blood samples and liver tissue homogenates. Exposure of rats to gamma radiation caused a significant increase in the level of Malondialdehyde, Nitrite, transaminase enzymes, alkaline phosphatase, and Bilirubin (Total Serum Bilirubin, Direct Bilirubin and Unconjugated Bilirubin) level while a significant decrease was recorded in serum total proteins, glutathione content, superoxide dismutase, catalase activities and total protein level. AGE treated rats revealed a significant improvement in all previous parameters. From these results, it can be concluded that AGE may have significant anti-radiation properties in rat's liver after radiation exposure.

Keywords: Irradiation, Oxidative Stress, AGE, Liver, Antioxidant Enzymes, Rats

1. Introduction

Whole body exposure of male rats to gamma radiation increased lipid peroxidation in the liver resulting in bio-membrane damage of sub-cellar structures and release of their enzymes. In several studies, a significant increase in lipid peroxidation (LPO) levels was observed in gamma radiated group, whereas a significant decrease in GSH content was recorded in liver of gamma radiated group [1-3]. This is evidenced by increase of Thiobarbituric Acid Reactive Substances (TBARS) in mitochondria, lysosomes and microsomes [4]. Changes in the content of reduced glutathione (GSH), glutathione peroxidase (GSHpx), glucose-6- phosphate dehydrogenase (G-6-PD), superoxide dismutase (SOD) and catalase (CAT) in blood, liver and spleen were also evaluated in different rat groups [2].

The first report of the use of chemicals to protect mammals against radiation-induced damage appeared in 1949, when Patt et al. [5] reported that cysteine protected mice and rats against radiation-induced sickness and mortality. Since then, several chemical compounds and their analogues have been screened for their radioprotective effects. However, the practical applicability of the majority of these synthetic compounds has been limited because of toxicity at radioprotective doses [6]. Thus it was considered important to explore alternatives to the synthetic compounds that would be radioprotective at nontoxic doses. Plants have been used to treat various ailments in humans since time immemorial, and herbal preparations have usually been considered safer and less toxic than synthetic compounds. Moreover, phytoconstituents and herbal medicine are important to manage pathological conditions of diseases caused by free radicals [7]. Therefore, it is natural that the choices of alternative radioprotectors include plants and plant products.

Recently, AGE has received particular attention because its radio-protective and anti-oxidative efficacy has been reported [8-10].

ALA and Vitamin E had been reported to have highly protective effect on lipid peroxidation and administration of ALA + Vitamin E had significant protective effect on blood, liver and muscles against oxidative damage [11].

In view of these considerations, the main objective of this study was to assess the role of Aged Garlic Extract against radiation induced oxidative stress associated with some biochemical disorders in male Wistar albino rats livers after total body exposure to acute ionizing irradiation using Vitamin E and Lipoïc 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 [12-14]. The content of water-soluble compounds is relatively high whereas that of oil-soluble compounds is relatively low [14]. 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 (IIII 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 Lipoïc 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 [15].

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

2.5.1. Blood Samples

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 dry tubes and allowed to clot (stand for 30 min) and centrifuged at 3 000 rpm for 15 min. The supernatant (serum) obtained was gathered in Eppendorf tubes and stored at -20°C for biochemical analysis of alkaline phosphatase, transaminase enzymes (Aspartate transaminase and Alanine transaminase), Bilirubin (Total Serum Bilirubin and Direct Bilirubin) and Total proteins.

2.5.2. Tissue Samples

A vertical midline thoracic and abdominal incision was done to explore the rat's viscera. Because of administration of distilled water, AGE, Lipoïc acid + vitamin E for consecutive days and whole body irradiation at 4.5 Gy, liver of each rat was excised, cleaned from their surrounding fat and connective tissue, washed with normal saline, blotted with filter paper, examined macroscopically (form modification, size, consistency and color) and weighed.

2.6. Biochemical Assay

Liver biomarkers assessment: the levels of aspartate transaminase (AST) and alanine transaminase (ALT) enzymes were estimated in the sera of the blood samples using commercial kits (Inmesco GmbH – Wiedtalstrasse 11&18-D-53577 Neustadt/Wied-Germany) according to Kaplan [16] and alkaline phosphatase level estimated in accordance with Prahlad and Conaway method [17]. Also, serum total protein was determined using Biuret reaction [18] and Bilirubin was assayed according to calculation (Unconjugated Bilirubin) and the method of Balistreri and Shaw (Total Serum Bilirubin and Direct Bilirubin) [19] as well.

Lipid peroxidation, total Protein and antioxidants assessment in tissue homogenates: Homogenate 20% was prepared by adding 2 mL of 50 mM, Tris-HCl buffer to 0.40 g of liver. Homogenate obtained was centrifuged at 3500 rpm for 25 minutes at 4°C after grinding in a mortar on ice tray. The supernatants were collected for the measurement of catalase (CAT), superoxide dismutase (SOD), Nitrite (NO^{2-}), total proteins, the levels of reduced glutathione (GSH), and malondialdehyde (MDA). GSH was determined in accordance with the method of Ellman [20] and SOD activity according to the method of Misra and Fridovish [21]. CAT activity was estimated by measuring the decomposition of hydrogen peroxide, according to the method of Sinha [22] and Nitrite assay according to Slack [23]. The marker of lipid peroxidation (MDA) was determined according to the method of Wilbur et al. [24].

2.7. 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. Liver Biomarkers of Rats

3.1.1. Effect of γ-Radiation and AGE Administration on Protein Profile

From Figure 1, it is clear that irradiation caused a significant increase (P < 0.001) in serum total protein of about 39.64% 12.20±0.2 Vs 8.74±0.42 mg/dL) in the group "Irradiation + 25 mg / kg AGE" and a significant drop (P < 0.001) in the range of 56.03% (3.84±0.26 Vs 8.74±0.42 mg/dL) and 2.93% (6.18±0.26 Vs 8.74±0.42 mg/dL) in groups "Irradiation + Distilled Water" and "Irradiation + Vitamin E and Lipoïc Acid" compared to the negative control group ("Sham Irradiation + Distilled Water"). Among the irradiated groups, a significant increase (P < 0.001) in serum protein was observed in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" respectively, in order of 217.60% (12.20±0.2 Vs 3.84±0.26 mg/dL) and 154.82% (9.79±0.06 Vs 3.84±0.26 mg/dL). In addition, compared to the positive control group ("Irradiation + Vitamin E and Lipoïc Acid"), a significant increase (P < 0.001) in serum proteins was observed in the the group "Irradiation + 25 mg / kg AGE" in order of 97.38% (12.20±0.2 Vs 6.18±0.26 mg/dL) and of 58.36% (9.79±0.06 Vs 6.18±0.26 mg/dL) in those of the group "Irradiation + 50 mg / kg AGE".
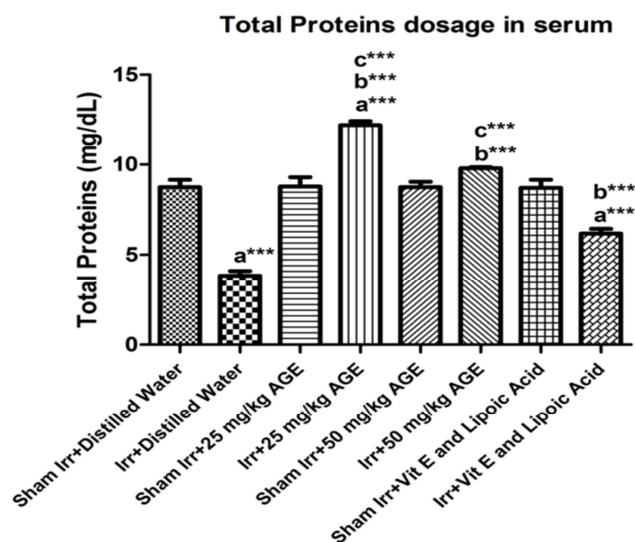

Figure 1. *Effects of γ-radiation and AGE on serum total protein.*

Each bar represents the Mean±ESM, (n = 5: number of animals in each group). 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).

3.1.2. Alanine Transaminase (ALT)

The levels of alanine transaminase increased significantly after irradiation, (p < 0.001) in order of 52.8% (114.6±3.97 Vs 75±4.44 U/L) in the "Irradiation + Distilled Water" group and decreased significantly (p < 0.001 and p < 0.01) in the range of 57.33% (32.0±2.17 Vs 75±4.44 U/L) and 37.87% (46.6±3.06 Vs 75±4.44 U/L) in the groups "irradiation + 25 mg / kg AGE "and" irradiation + 50 mg / kg AGE". Compared to the group "Irradiation + Distilled Water", the decrease of this rate was significant (p < 0.001) in the range of 72.08% (32.0±2.17 Vs 114.6±3.97 U/L) and 59.34% (46.6±3.06 Vs 114.6±3.97 U/L) in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE". This reduction remained significant in order of 43.06% (32.0±2.17 Vs 56.2±3.25 U/L) in the group "Irradiation + 25 mg / kg AGE" compared to the positive control group "Irradiation + Vitamin E and Lipoïc Acid" (Figure 2).
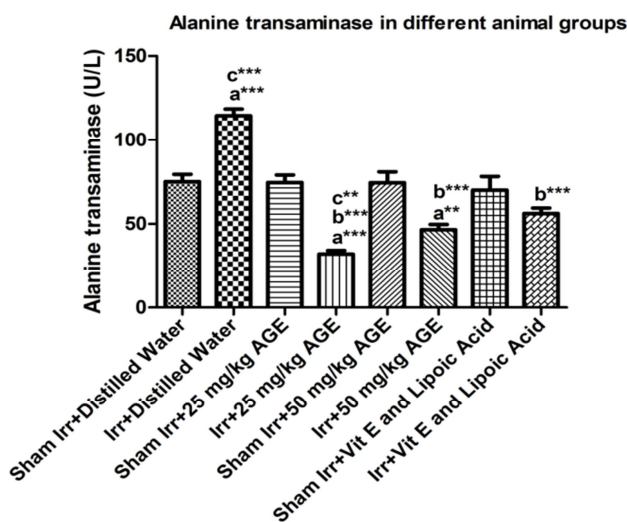

Figure 2. *Effects of γ-radiation and AGE on Alanine transaminase rate.*

Each bar represents the Mean±ESM, (n = 5: number of animals in each group). 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).

3.1.3. Aspartate Transaminase (AST)

The irradiation resulted in a significant increase (p < 0.01) of aspartate transaminase in the group "Irradiation + Distilled Water" in order of 38.08% (190.0±8.15 Vs 137.6±7.19 U/L)

and a significant reduction in the group "Irradiation + 25 mg / kg AGE" (p < 0.001) in the range of 44.33% (76.6±6.72 Vs 137.6±7.19 U/L). Animals receiving AGE at doses of 25 and 50 mg / kg, showed a significant decrease (P < 0.001), in aspartate transaminase levels compared to the group "Irradiation + Distilled Water "(Figure 3). This decrease is respectively in order of 59.68%, (76.6±6.72 Vs 190.0±8.15 U/L) and 43.79% (106.8±8.32 Vs 190.0±8.15 U/L). Similarly, compared to the group "Irradiation + Vitamin E and Lipoïc Acid" a significant decrease (p < 0.01) was observed in the group "Irradiation + 25 mg / kg AGE" in order of 34.97% (76.6±6.72 Vs 117.8±7.96 U/L) and a non-significant decline (P > 0.05) in the group "Irradiation + 50 mg / kg AGE" in order of 9.34% (106.8±8.32 Vs 117.8±7.96 U/L).
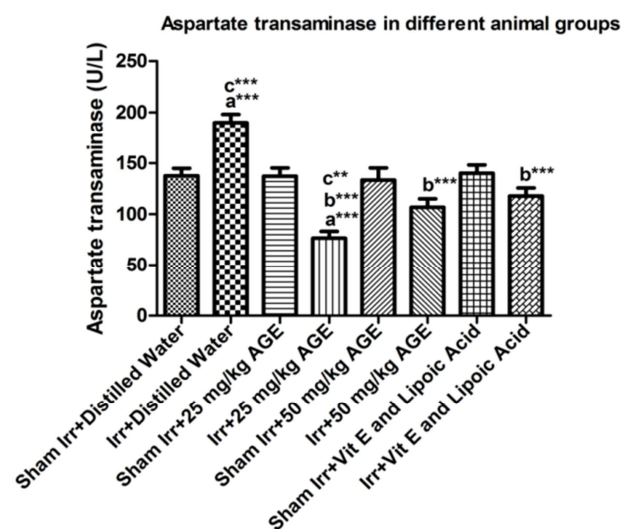

Figure 3. *Effects of γ-radiation and AGE on Aspartate transaminase rate.*

Each bar represents the Mean±ESM, (n = 5: number of animals in each group). 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).

3.1.4. Alkaline Phosphatase (ALP)

Figure 4 reveals that alkaline phosphatase increased significantly (p < 0.001) in the group "Irradiation + Distilled Water" in order of 36.4% (68.2±1.32 Vs 50.00±1.10 U/L) and significantly decreased (p < 0.001) in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" in the range of 42.8% (28.6±1.08 Vs 50.00±1.10 U/L) and 24% (38.±1.67 Vs 50.00±1.10 U/L). Compared to the group "Irradiation + Distilled Water", Figure 4 shows a significant decrease (p < 0.001) in the rate of alkaline phosphatase in

order of 58.06% (28.6±1.08 Vs 68.2±1.32 U/L) and 44.28% (38.±1.67 Vs 68.2±1.32 U/L) in the groups receiving AGE at doses of 25 and 50 mg / kg. Compared to the positive control group "Irradiation + Vitamin E and Lipoïc Acid", the decline was significant (p < 0.001) in the groups "Irradiation + 25 mg / kg AGE" in order of 46.64% (28.6±1.08 Vs 53.6±3.17 U/L) and "Irradiation + 50 mg / kg AGE» in order of 29.10% (38.±1.67 Vs 53.6±3.17 U/L).
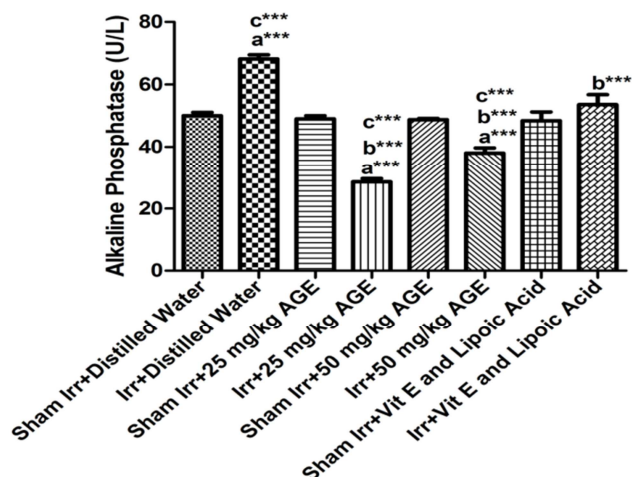

Figure 4. *Effects of γ-radiation and AGE on alkaline phosphatase rate.*

Each bar represents the Mean±ESM, (n = 5: number of animals in each group). 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).

3.1.5. Bilirubin
i. Bilirubin Total

After irradiation, Figure 5 shows a significant increase (P < 0.001) of total Bilirubin level in animals of group "Irradiation + Distilled Water" in order of 52.84% (2.79±0.25 Vs 1.83±0.18 mg/dL), similarly a significant decrease (P < 0.001 and P < 0.05) in the range of 76.59% (0.43±0.08 Vs 1.83±0.18 mg/dL) and 42.89% (1.04±0.15 Vs 1.83±0.18 mg/dL) in groups treated with AGE at a dose of 25 and 50 mg / kg. Moreover, this rate decreased significantly (P < 0.001) in the range of 84.68% (0.43±0.08 Vs 2.79±0.25 mg/dL) and 62.63% (1.04±0.15 Vs 2.79±0.25 mg/dL) in groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" compared to the group "Irradiation + Distilled Water". The total Bilirubin levels also decreased significantly (P < 0.01) in the groups "Irradiation + 25 mg / kg AGE" and not significantly (P > 0.05) in the group "Irradiation + 50 mg / kg AGE" respectively in order of 68.53% (0.43±0.08 Vs 1.36±0.11 mg/dL) and 23.24% (1.04±0.15 Vs 1.36±0.11

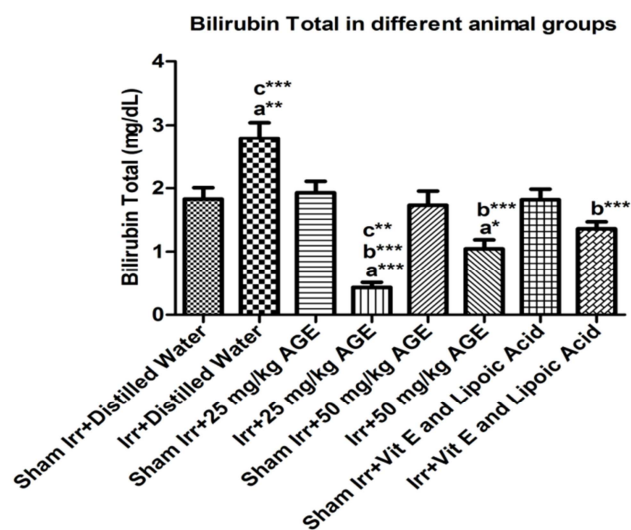mg/dL) compared to the positive control "Irradiation + Vitamin E and Lipoïc Acid."

Figure 5. *Effects of γ-radiation and AGE on total bilirubin rate.*

Each bar represents the Mean±ESM, (n = 5: number of animals in each group). 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).

ii. Direct Bilirubin

Figure 6 shows the effect of irradiation and AGE intake on direct Bilirubin. Compared to the negative control group, irradiation resulted in a significant increase of direct Bilirubin level(P < 0.01) in the range of 73.17% (0.85±0.04 Vs 0.49±0.08 mg/dL) in the group "Irradiation + Distilled Water "and a significant decrease (P < 0.01) in the range of 70.73% (0.14±0.01 Vs 0.49±0.08 mg/dL) and a non-significant drop (P > 0.05) in the range of 36.18% (0.31±0.07 Vs 0.49±0.08 mg/dL) in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE". A significant decrease (P < 0.001) in the ranges of 83.10% (0.14±0.01 Vs 0.85±0.04 mg/dL) and 63.15% (0.31±0.07 Vs 0.85±0.04 mg/dL) occurred by comparing groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" with the group "Irradiation + Distilled Water". The decrease in direct Bilirubin was significant (P < 0.001) in the group "Irradiation + 25 mg / kg AGE" in order of 64.88% (0.14±0.01 Vs 0.41±0.04 mg/dL) and not significant (P > 0.05) in the group "Irradiation + 50 mg / kg AGE" in order of 23.41% (0.31±0.07 Vs 0.41±0.04 mg/dL) compared to the positive control group "Irradiation + Vitamin E and Lipoïc Acid."
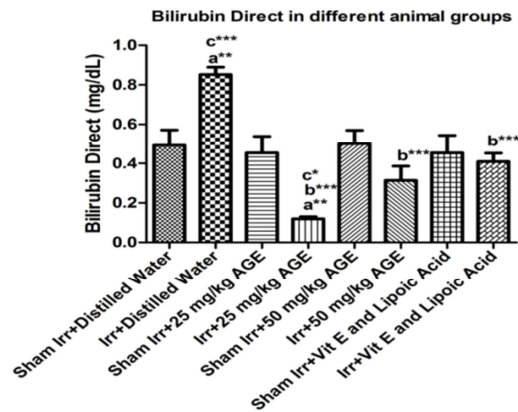
***Figure 6.** Effects of γ-radiation and AGE on direct bilirubin rate.*

Each bar represents the Mean±ESM, (n = 5: number of animals in each group). 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).

iii. Unconjugated Bilirubin

Figure 7 shows the effect of irradiation and AGE intake on the direct Bilirubin. Indeed, compared to the negative control "Sham Irradiation + Distilled Water" a non-significant increase (P > 0.05) was observed in the group "Irradiation + Distilled Water" in order of 45.36% (1.94±0.24 Vs 1.34±0.24 mg/dL); and a significant decrease (P < 0.01) in the range of 76.95% (0.31±0.08 Vs 1.34±0.24 mg/dL) in the irradiated group receiving AGE at a dose of 25mg / kg. The comparison of groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" with "Irradiation + Distilled Water" group shows a significant decrease (P < 0.001) in the range of 84.14%

(0.31±0.08 Vs 1.94±0.24 mg/dL) and 62.41% (0.73±0.14 Vs 1.94±0.24) in these groups. This decline remains not significant (P > 0.05) when comparing the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" with the group "Irradiation + Vitamin E and Lipoïc Acid" respectively in order of 67.58% (0.31±0.08 Vs 0.95±0.11 mg/dL) and 23.16% (0.73±0.14 Vs 0.95±0.11 mg/dL).
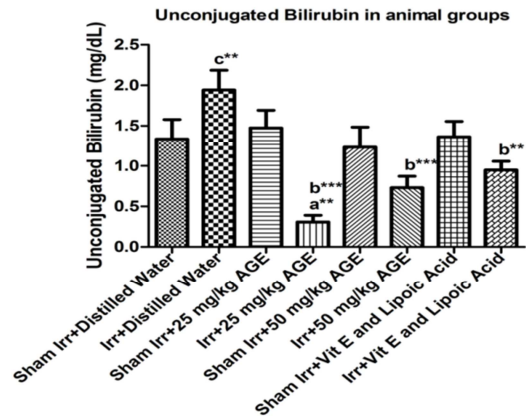

***Figure 7.** Effects of γ-radiation and AGE on unconjugated bilirubin rate.*

Each bar represents the Mean±ESM, (n = 5: number of animals in each group). 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).

3.2. Antioxidants and Lipid Peroxidation

The different effects of γ-radiation and AGE are represented in this table below.

***Table 1.** Effects of γ-radiation and AGE on oxydative stress marker.*

-	Glutathione (μmol/mg of tissue)	Superoxide dismutase (μmol/mg of protein)	Catalase (μmoles H_2O_2/minute/mg of protein)	Nitrite (μmol/mL)	Malondialdehyde (μmol/mg of tissue)
Sham Irradiation+ Distilled Water	88.67±2.37	66.50±0.65	7.51±0.50	0.038±0.001	0.24±0.01
Irradiation+ Distilled Water	59.26±3.02 a***,	39.82±0.39 a***, c***	4.38±0.45 a**,	0.054±0.003 a*,	0.33±0.03 a*,
Sham Irradiation+ 25 mg/kg AGE	88.17±3.92	66.74±0.49	7.65±0.54	0.037±0.005	0.24±0.01
Irradiation+ 25 mg/kg AGE	114.83±2.49 a***, b***, c***	91.62±0.33 a***, b***, c***	9.88±0.75 a*, b***, c***	0.020±0.002 a**, b***, c***	0.14±0.01 a*, b***, c***
Sham Irradiation+ 50 mg/kg AGE	88.04±3.86	66.48±0.51	7.62±0.46	0.037±0.003	0.24±0.02
Irradiation+ 50 mg/kg AGE	101.87±6.10 b***, c***	74.62±0.41 a***, b***, c***	8.71±0.60 b***, c*	0.029±0.004 b***, c*	0.18±0.0 b***, c*
Sham Irradiation+ Vitamin E and Lipoïc Acid	88.61±2.66	66.10±0.57	7.47±0.44	0.038±0.004	0.24±0.02
Irradiation+ Vitamin E and Lipoïc Acid	73.49±3.60 a*,	49.60±0.37 a***, b***	6.23±0.52	0.045±0.004	0.27±0.04

Data are expressed as mean±SEM (n = 5: number of animals in each group). 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).

3.2.1. Reduced Glutathione (GSH)

γ-radiation and AGE intake have led to a significant decrease (P < 0.001) in glutathione reduced levels in the group "Irradiation + Distilled Water" in order of 33.16% (59.26±3.02 Vs 88.67±2.37 µmol/mg of tissue) and a significant increase (P < 0.001) in the group "Irradiation+25 mg/kg AGE" in the range of 29.50% (114.83±2.49 Vs 88.67±2.37 µmol/mg of tissue) compared to the negative control group "Sham Irradiation + Distilled Water" (Table 1). Among the irradiated groups, a significant increase (P < 0.001) was observed in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" respectively, in order of 93.75% (114.83±2.49 Vs 59.26±3.02 µmol/mg of tissue) and 71.89% (101.87±6.10 Vs 59.26±3.02 µmol/mg of tissue) when taking a look to the group "Irradiation + Distilled Water". In addition, compared to the positive control group ("Irradiation + Vitamin E and Lipoïc Acid"), a significant increase (P < 0.001) in glutathione reduced was observed in the group "Irradiation + 25 mg / kg AGE" in order of 56.26% (114.83±2.49 Vs 73.49±3.60 µmol/mg of tissue) and of 38.63% (101.87±6.10 Vs 73.49±3.60 µmol/mg of tissue) in those of the group "Irradiation + 50 mg / kg AGE ".

3.2.2. Superoxide Dismutase (SOD)

From Table 1, it is clear that irradiation caused a significant increase (P < 0.001) in SOD of about 37.77% (91.62±0.33 Vs 66.50±0.65 µmol/mg of protein) in the group "Irradiation + 25 mg / kg AGE" and a significant decrease (P < 0.001) in the range of 40.12% (39.82±0.39 Vs 66.50±0.65 µmol/mg of protein) and 25.41% (49.60±0.37 Vs 66.50±0.65 µmol/mg of protein) in groups "Irradiation + Distilled Water" and "Irradiation + Vitamin E and Lipoïc Acid" compared to the negative control group "Sham Irradiation + Distilled Water". A significant increase (P < 0.001) was observed in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" respectively, in order of 130.09% (91.62±0.33 Vs 39.82±0.39 µmol/mg of protein) and 87.39% (74.62±0.41 Vs 39.82±0.39 µmol/mg of protein) when comparing those groups with the group "Irradiation + Distilled Water". In addition, compared to the positive control group ("Irradiation + Vitamin E and Lipoïc Acid"), a significant increase (P < 0.001) in SOD was observed in the group "Irradiation + 25 mg / kg AGE" in order of 84.72% (91.62±0.33 Vs 49.60±0.37 µmol/mg of protein) and of 50.44% (74.62±0.41 Vs 49.60±0.37 µmol/mg of protein) in

those of the group "Irradiation + 50 mg / kg AGE ".

3.2.3. Catalase (CAT)

Irradiation and AGE intake have led to a significant decrease (P < 0.01) in CAT level in the group "Irradiation + Distilled Water" in order of 41.66% (4.38±0.45 Vs 7.51±0.50 µmoles H_2O_2/minute/mg of protein) and a significant increase (P < 0.05) in the group "Irradiation+25 mg/kg AGE" in order of 31.53% (9.88±0.75 Vs 7.51±0.50 µmoles H_2O_2/minute/mg of protein) compared to the negative control group "Sham Irradiation + Distilled Water" (Table 1). When taking a look at the group "Irradiation + Distilled Water", a significant increase (P < 0.001) was observed in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" respectively, in order of 125.46% (9.88±0.75 Vs 4.38±0.45 µmoles H_2O_2/minute/mg of protein) and 98.73% (8.71±0.60 Vs 4.38±0.45 µmoles H_2O_2/minute/mg of protein). In addition, compared to the positive control group ("Irradiation + Vitamin E and Lipoïc Acid"), a significant increase (P < 0.001 and P < 0.05) in Catalase was observed in the group "Irradiation + 25 mg / kg AGE" in order of 58.50% (9.88±0.75 Vs 6.23±0.52 µmoles H_2O_2/minute/mg of protein) and of 39.71% (8.71±0.60 Vs 6.23±0.52 µmoles H_2O_2/minute/mg of protein) in the group "Irradiation + 50 mg / kg AGE " (Table 1).

3.2.4. Nitrite (N0^{2-})

In comparison with the to the negative control group "Sham Irradiation + Distilled Water" (Table 1), irradiation and AGE administration induced a significant increase in nitrite level (P < 0.05) in order of 40.97% (0.054±0.003 Vs 0.038±0.001 µmol/mL) in the group "Irradiation + Distilled Water" while a significant decrease (P < 0.01) of about 48.33% (0.020±0.002 Vs 0.038±0.001 µmol/mL) occurred in the group "Irradiation + 25 mg / kg AGE" (Table 1). Furthermore, a significant decline in N0^{2-} level (P < 0.001) was observed in the animals irradiated and receiving AGE at doses of 25 mg / kg and 50 mg / kg compared to those of "Irradiation + Distilled Water" group. This decrease was respectively in order of 63.35% (0.020±0.002 Vs 0.054±0.003 µmol/mL) and 45.48% (0.029±0.004 Vs 0.054±0.003 µmol/mL). Similarly, compared to the irradiated positive control group receiving Vitamin E and Lipoïc Acid, a significant decrease (P < 0.001 and P < 0.01) in Nitrite level was also observed in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation 50 mg / kg AGE" respectively in the range of 56.60% (0.020±0.002 Vs 0.045±0.004 µmol/mL) and 35.45% (0.029±0.004 Vs 0.045±0.004 µmol/mL).

3.2.5. Malondialdehyde (MDA)

The effects of radiation and AGE intake on MDA levels are shown in Table 1. Comparison of the groups with the negative control "Sham Irradiation + Distilled Water" revealed a significant increase of MDA level (P < 0.05) in order of 35.92% (0.33±0.03 Vs 0.24±0.01 µmol/mg of tissue) in the group "Irradiation + Distilled Water" while a significant decrease (P < 0.05) of about 42.47% (0.14±0.01

Vs 0.24±0.01 µmol/mg of tissue) occurred in the group "Irradiation + 25 mg / kg AGE". Furthermore, a significant decline in MDA level (P < 0.001) was observed in the animals irradiated and receiving AGE at doses of 25 mg / kg and 50 mg / kg compared to those of "Irradiation + Distilled Water" group. This decrease was respectively in order of 57.68% (0.14±0.01 Vs 0.33±0.03 µmol/mg of tissue) and 44.49% (0.18±0.02 Vs 0.33±0.03 µmol/mg of tissue). Similarly, compared to the irradiated positive control group receiving Vitamin E and Lipoïc Acid, a significant decrease (P < 0.001) in MDA level was also observed in the groups "Irradiation + 25 mg / kg AGE" in the range of 48.93% (0.14±0.01 Vs 0.27±0.04 µmol/mg of tissue).

4. Discussion

All types of ionizing radiation generate ions which can lead to the formation of free radicals and reactive oxygen species (ROS). Excess production of free radicals or decrease in antioxidants level leads to oxidative stress. It is a harmful process that induces damage to cell structures, lipids, proteins, RNA and DNA which leads to number of diseases [25]. The use of plants, natural products are thoughts to be beneficial in protecting against radiation-induced damage, they are less toxic compared to synthetic compounds used at their optimum protective dose levels [26-27] thence the interests has always existed in development of potential drug of plant origin, been a good sources of potent but non-toxic radioprotectors [28]. The investigations made; revealed AGE ameliorates the toxic side effects of different substances through its antioxidant and radical scavenging activities [29]. The aim of this study was to investigate the possible protective effect of AGE against the disturbances induced by radiations in rat's liver.

Serum proteins are synthesized and secreted by several cell types depending on the nature of the individual serum protein [30]. An important function of serum protein is the maintenance of the normal distribution of body water by controlling the osmotic balance between the circulating blood and the membrane of tissues, and the transport of lipids, hormones and inorganic materials [31]. The results obtained in this work showed that, there is significant decrease in serum total proteins post irradiation. The decrease in serum protein in irradiated rats might be the result of damage of vital biological processes or due to changes in the permeability of liver, kidney and other tissues resulting in leakage of protein via the kidney [32-35]. The decrease in blood total protein might be due to the slow rate in synthesis of all protein fractions after irradiation [36-37]. This decrease coincides with the decrease in serum total protein reported by other workers in irradiated rats, which may be due to radiation damage to the liver [38]. The decrease in protein in irradiated rats might be the result of either damage of biological membranes or to changes in the permeability of the liver [39-41]. Several investigations indicated that exposure to radiation increases free radical activity which is considered to be the primary cause of damaging effects.

Radiation induced lipid peroxidation, reduce protein synthesis and cause disturbances in the enzyme activity of the liver [42].

In the present study, there was a decrease in contents of total proteins in serum of rats irradiated with gamma radiation, indicating liver injury [1, 43]. These results are in accordance with other studies using high-energy radiation from cobalt source [44]. Therefore, it is suggested that oxidative stress as a result of gamma-irradiation is linked to the organ damage following exposure to ionizing radiation. Kempner explained that this decrease in proteins level due to gamma radiation can damage or inactivate proteins by two different mechanisms. First, it can rupture the covalent bonds in target protein molecules as a direct result of a photon depositing energy into the molecule. Second, it can act indirectly, link with a water molecule, producing free radicals and other non-radical reactive oxygen species that are in turn responsible for most (99.9%) of the protein damage [31, 45].

Oral administration of AGE one hour after irradiation on day 1 after acclimatization has caused a significant increase in serum total proteins in AGE groups. The increase was more important in "Irradiation+25 mg/kg AGE" group than in "Irradiation+50 mg/kg AGE" group or "Irradiation + Vitamin E and Lipoïc Acid". AGE and SAC were shown to scavenge ROS [10] and to inhibit lipid peroxide formation in several studies [14, 46]. These antioxidant effects can be due to allixin, SAC, SMAC and diallyl polysulfides, whose radical-scavenging action increased with the number of sulfur atoms [47]. Or, due to to N-fructosyl arginine and N-fructosyl glutamate which showed antioxidant effects by spin resonance spectroscopy [12].

Gamma radiation induced an increase in ALP activity in liver tissue on the first and seventh post-exposure day [48]. This may be attributed to the possible release of this enzyme from different tissues associated with the obstruction of the blood stream to the liver [49]. The function and integrity of liver cells are well evaluated with transaminases activities [50], thus change in tissue permeability due to irradiation could enhance the release of transaminases enzymes from their subcellular sites of production to extracellular process and consequently to blood circulation [51]. Radiation exposure caused damage to the cell membrane that increased the ALP activity. This change in ALP activity might be due to radiation induced changes in the amino acid residue and catalytic activity of ALP [52] and due to destruction of this enzyme by radiation [53]. Furthermore, liver responds to hepatobiliary injury by synthetizing more enzymes which inter the circulation, raising the enzyme level in serum [54].

In the present study, the rats irradiated showed elevation of serum levels of ALT, AST, ALP and Bilirubin (Total Serum Bilirubin, Direct Bilirubin and Unconjugated Bilirubin) as compared with saline control group. In agree with our results, El-Kafif *et al.* explained that this increase may be ascribed to the irradiation-induced damage to hepatic parenchymal cells as well as extra hepatic tissues with a subsequent release of the enzymes into the blood stream [39]. It may also be attributed to the structural damage in spleen, lymphnodes and

mature lymphocytes [55]. Moreover, the destruction of erythrocytes due to ionizing radiation and the release of their enzymes cannot be excluded as a causative factor for the rise in these enzymes [56]. The increased activity of serum ALP by gamma radiation agrees with Tabachnick *et al.* who attributed it to the enzyme release from the tissues to the blood stream or to liver disturbances [57], particularly due to defects in cell membrane permeability [58]. The variation in transaminases activities may be due to certain damage in some tissue like heart, liver, kidney and skeletal muscles. Fahim *et al.* mentioned that whole body gamma radiation of rats showed significant changes in the activities of transaminases which are dependent on the time lapses after irradiation and the type of tissue containing the enzyme [59]. These results may be attributed to the state of hypoxia of parenchyma for contracting fibrous tissue and the increased permeability of hepatic cell membrane due to irradiation exposure with release of ALT enzyme to circulation. The elevation in the serum activity of ALT indicates lesions in the liver cells [60]. It is also a sign of liver parenchymal cell destruction induced by whole body gamma radiation [61].

The clinical and diagnostic values associated with changes in blood enzymes concentrations such as AST, ALT, ALP and bilirubin have long been recognized [62]. Increased levels of these diagnostic markers of hepatic function in irradiated rats are implicative of the degree of hepatocellular dysfunction caused by the radiation [61]. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases was the clear indication of cellular leakage and loss of functional integrity of the cell membrane [63]. Omran *et al.* revealed that significant elevation in AST, ALT, ALP and bilirubin were recorded post exposed to gamma-irradiation which reflects detectable changes in liver functions [64]. Such elevation was in agreement with Hassan *et al.* [65]. They reported that this elevation is directly due by interaction of cellular membranes with gamma-rays or through an action of free radicals produced by this radiation.

Oral administration of AGE one hour after irradiation on day 1 after acclimatization significantly reduced radiation toxic effect on serum levels of AST, ALT, ALP and Bilirubin (Total Serum Bilirubin, Direct Bilirubin and Unconjugated Bilirubin) compared to untreated rats. The reduction was significant in groups receiving AGE at a dosage of 25 mg/kg compare to those receiving it at a dosage of 50 mg/kg or to those receiving Vitamin E and Lipoïc Acid after irradiation. This reduction of the liver enzymes following AGE intake in the serum levels of AST and ALT was also noticed by other authors [66-68] then Nada and Hawas observed it as well with the administration of other herbal plants [69]. According to Pradeep *et al.*, free radical induced oxidative damage in the liver but the antioxidants of AGE decrease the lowering of liver enzymes by stabilizing the membrane permeability and reducing the leakage of enzymes into the blood [70]. Hepatoprotective effect also belongs to Garlic properties [71-72] and AGE protects the liver as well by benzopyrene or benzo(a) pyrene and aflatoxin B_1 by the means of SAC and SAMC [73-75].

Ionizing radiations produced peroxidation of lipids leading to structural and functional damage to cellular membranous molecules directly by transferring energy or indirectly by generation of oxygen derived free radical (OH), superoxide (O_2^-) and nitric oxide (NO) which are the predominant cellular free radicals [76-77]. Oxidative stress leads to over production of NO, which readily reacts with superoxide to form peroxynitrite ($ONOO^-$) and peroxynitrous acid which they can initiate lipid peroxidation [78]. Under normal conditions, the inherent defense system, including the enzymes superoxide dismutase, which dismutates superoxide; catalase and glutathione peroxidase, which destroy toxic peroxides, and small molecules including glutathione, protects against oxidative damage. Excessive liver damage and oxidative stress caused by γ-radiation might be responsible for the depletion of GSH [79-81]. Irradiation has been reported to cause renal GSH depletion and lipid peroxides accumulation in different organs [82-84]. It was found that the level of elevation in lipid peroxidation after irradiation is in proportion to radiation dose and elapsed time [85]. Moreover, the formation of lipid peroxidation ultimately would alter the composition of the glomerular basement membrane [86]. Evidence of radiation induced organs injury via a mechanism of oxidative stress caused by increased MDA and nitrite, reduced GSH levels and decreased activity of CAT, SOD were demonstrated by various studies [87-91]. Such oxidative stress was mediated through the generation of ROS that induced disturbance of membrane permeability and severe cell damage [92-93].

In the present study, radiation induced higher MDA level and nitrite, while decreasing SOD, CAT activities and GSH level in the homogenate of rat liver tissue. Increase MDA level enhanced the lipid peroxidation and increased ROS production with subsequent disturbance of membrane function and integrity [94]. These results are in accordance with those of Halliwell, and Gutterige, [95] who observed a significant decrease in SOD and catalase activity after exposure to irradiation due to the excess production of hydroxyl radicals and other reactive oxygen species. SOD is an important endogenous antioxidant enzyme which acts as the first line defense system against ROS and converts the superoxide radicals to H_2O_2. Glutathione peroxidase present in the cytoplasm of the cells removes H_2O_2 by coupling its reduction to H_2O with oxidation of GSH. Glutathione reductase regenerates GSH from oxidized glutathione in the presence of NADPH. GSH is a tripeptide and a powerful antioxidant present within the cytosol of cells and is the major intracellular non protein thiol compound. SH groups present in GSH react with H_2O_2 and the OH• radical and prevent tissue damage and GSH is also capable of scavenging ROS directly or enzymatically via glutathione peroxidase [96]. The decrement of GSH level would be attributed to the decreased activity of glucose-6- phosphate dehydrogenase (G-6-PD) that generates reduced NADPH which generates GSH from oxidized glutathione (GSSG) under the effect glutathione reductase [97]. Moreover, Dahm *et al.* attributed the decrease in liver GSH content to the

inhibition of GSH efflux across hepatocytes membranes [98]. The presence of adequate amount of GSH, SOD and catalase minimize lipids peroxidation [92].

In several studies, natural products with antioxidants components were used to protect against oxidative stress induced by radiations [99-101]. Capasso, Ana *et al.*, revealed AGE exhibited potent antioxidant and free radical scavenging activities [27-28].

Administration of AGE, one hour after irradiation on day 1 after acclimatization induced significant increase in CAT, SOD and GSH activities accompanied with significant decrease in MDA level and nitrite in radiation-treated rat's liver. These effects have been more pronounced with the lower dose of AGE (25 mg/kg) than with the higher (50 mg/kg) or the administration of Vitamin E and Lipoïc Acid. Suggesting in accordance with Khalid S Al-Numair that garlic extracts may have a beneficial effect on antioxidant status by improving antioxidant metabolic indices in rat's plasma [102]. This positive effect can be explained by the presence of S-allyl cysteine, S-allyl mercaptocyteine, allicin, and selenium; compounds responsible for the antioxidant activity of AGE [27]. In vivo, S-allyl cysteine significantly increased antioxidant activities and reduced as well lipid peroxidation widespread [103]. Thus, AGE ameliorates lipid peroxidation [104] and acts as a protective mechanism against oxidative damages of rat liver tissues induced by acute radiation [105-106].

In accordance with this study, significant increase in CAT, SOD and GSH activities were reported in animals treated with AGE as well as significant decrease in MDA and nitrite levels [68, 103, 107]. Garlic has been reported to modulate lipid peroxidation levels and enhance the status of antioxidant [106, 108-109]. It also elevates the levels of SOD, GSH-Px and Catalase [110-111]. The presence of non-enzymatic antioxidants such as selenium, copper metals, vitamin C and organosulphur compounds are also responsible for the beneficial effects of garlic; according to Prasad *et al.* [112]. AGE increases cellular glutathione and other ROS scavenging enzymes in several cells including liver and mammary tissue due to organosulfur components and phenolic compounds [110, 113]. Thus, AGE radioprotective effects [114] relies on its capacity to scavenge free radicals [110] and enhance scavenging systems in the cell, including glutathione, SOD, catalase and glutathione peroxidase [110, 115].

5. Conclusion

From the present study, it can be concluded that γ-radiation induced damages in rat's liver through oxidative stress and lipid peroxidation but these changes are being improved by the intake of AGE, suggesting its radioprotective efficacy. Furthermore, the study revealed that the radioprotective effects of AGE was 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 Lipoïc Acid concerning radio-protective properties.

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