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Protective effects of aged garlic extract against oxidative stress induced by total body irradiation in albino Wistar rat's kidney

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Abstract: Aged garlic extract (AGE) is widely used as a powerful antioxidant that protects tissues against oxidative stress by inhibition of free radical processes and limiting oxygen effect. The present study was designed to determine the protective effect of Aged garlic extract (AGE) against oxidative kidney damage induced by gamma-irradiation. 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, after sacrifice; the 8th day post irradiation, biochemical analyses, lipid peroxidation, total Protein and antioxidants assessment were made from blood samples and kidney tissue homogenates. The data obtained revealed that exposure of rats to gamma radiation caused a significant decrease in the level of glutathione content, superoxide dismutase, catalase activities and total protein level while a significant increase in the level of Creatinine, Malondialdehyde and Nitrite. Oral administration of AGE to rats from the first

hour after irradiation on day 1 up to day seventh revealed a significant improvement in all previous parameters. It could be concluded that Aged garlic extract (AGE) administration after whole body gamma-irradiation might be capable to attenuate gamma radiation induced kidney injury.

Keywords: gamma radiation, kidney, Aged garlic extract, rats

1. INTRODUCTION

Many authors reported that ionizing radiation greatly affected renal function[1,2]. They explained that irradiation leads to biochemical changes in the irradiated animals, which may suffer from continuous loss in body weight and this could be attributed to disturbances in nitrogen metabolism usually recognized as negative nitrogen balance[1, 2]. Accordingly, it could be expected that this may cause an increase in the urea level, ammonia concentration and amino acid contents in blood and urine due to great protein destruction induced by gamma radiation[3]. Also, increases of Creatinine have been reported after exposure to radiation; it is an evidence of marked impairment of kidney function[4]. A single dose of radiation (6 Gy) caused renal damage manifested biochemically as an increase in blood urea[5]. Also, exposure of female albino rats to whole body gamma irradiation at a dose level of 6 Gy caused the mean activity values of blood urea, and total cholesterol to be significantly elevated[6]. Moreover, a single dose of total body gamma radiation 6.5 Gy to mice induced significant elevation in plasma Creatinine, urea and in urine protein concentration and also detectable decrease in plasma total protein and urine Creatinine levels[7]. Irradiation of rats caused significant drop in serum total protein, elevation in serum uric acid, urea and Creatinine[1]. Also, irradiation significantly elevated serum urea, uric acid and Creatinine while it declined total proteins and albumin. Creatinine was significantly increased in sera of irradiated rats with dose of 2 and 4 Gy[8].

Many natural and synthetic compounds have been investigated for their efficacy to protect against irradiation damage[9]. AGE is reputed to be a powerful natural antioxidant that may cause inhibition of free radical processes, the protection of the macromolecules essential for cell survival, and limiting oxygen effect[10].

The present study aims not to synthesize new agents of protection against ionizing radiation but to investigate the possible protective role of Aged Garlic Extract (AGE) against injuries induced by whole body gamma irradiation (4.5 Gy) in male rat's kidney using Vitamin E and Lipoïc Acid as positive control group because the positive effect of Alpha Lipoïc Acid includes protection against radiation damage[11]. Furthermore, ALA and Vitamin E have been reported to have highly protective effect on lipid peroxidation and their positive effect includes protection against radiation damage[12,13].

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 good clinical practice (GCP: VICH GL9) of the International Conference on Harmonization (ICH) guidelines from animal lab Mountain University, Bagangté and Douala Universities 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[14,15]. The content of water-soluble compounds is relatively high whereas that of oil-soluble compounds is relatively low[15]. 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[16]; EEC Directive 86/609/EEC, of the 24th November 1986.

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 Creatinine 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, kidney 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

2.6.1. **Creatinine assessment**: The level of Creatinine was estimated in the sera of the blood samples using commercial kit (Inmesco GmbH-Wiedtalstrasse 11&18-D-53577 Neustadt/Wied-Germany) according to Bartels and Bohmer method[17]. Serum total protein was determined as well using Biuret reaction[18].

2.6.2. **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[19] and SOD activity according to the method of Misra and Fridovish[20]. CAT activity was estimated by measuring the decomposition of hydrogen peroxide, according to the method of Sinha[21] and Nitrite assay according to Slack[22]. The marker of lipid peroxidation (MDA) was determined according to the method of Wilbur *et al.*[23].

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. Effects of γ-radiation and AGE administration on Creatinine rate: The effects of radiation and AGE intake on Creatinine levels are shown in **Figure 1**. Comparison of the groups with the negative control "Sham Irradiation + Distilled Water" revealed a significant increase of Creatinine level ($P < 0.05$) in order of 33.2% **(**1.65 ± 0.06 Vs. 1.24 ± 0.08 mg/dL) in the group "Irradiation + Distilled Water" while a significant decrease of about 59.5% **(**0.5 ± 0.15 Vs. 1.24 ± 0.08 mg/dL) occurred in the group "Irradiation +25 mg/kg AGE". Furthermore, a significant decline in Creatinine 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 69.6%, **(**0.5 ± 0.15 Vs. 1.43 ± 0.06 mg/dL) and 39.35% **(**1.00 ± 0.08 Vs. 1.43 ± 0.06 mg/dL). 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 Creatinine level was also observed in the groups "Irradiation +25 mg/kg AGE" and "Irradiation 50 mg/kg AGE "respectively in the range of 64.8%, **(**0.5 ± 0.15 Vs. 1.0 ± 0.08 mg/dL) and 29.71% **(**1.00 ± 0.08 Vs. 1.0 ± 0.08 mg/dL).

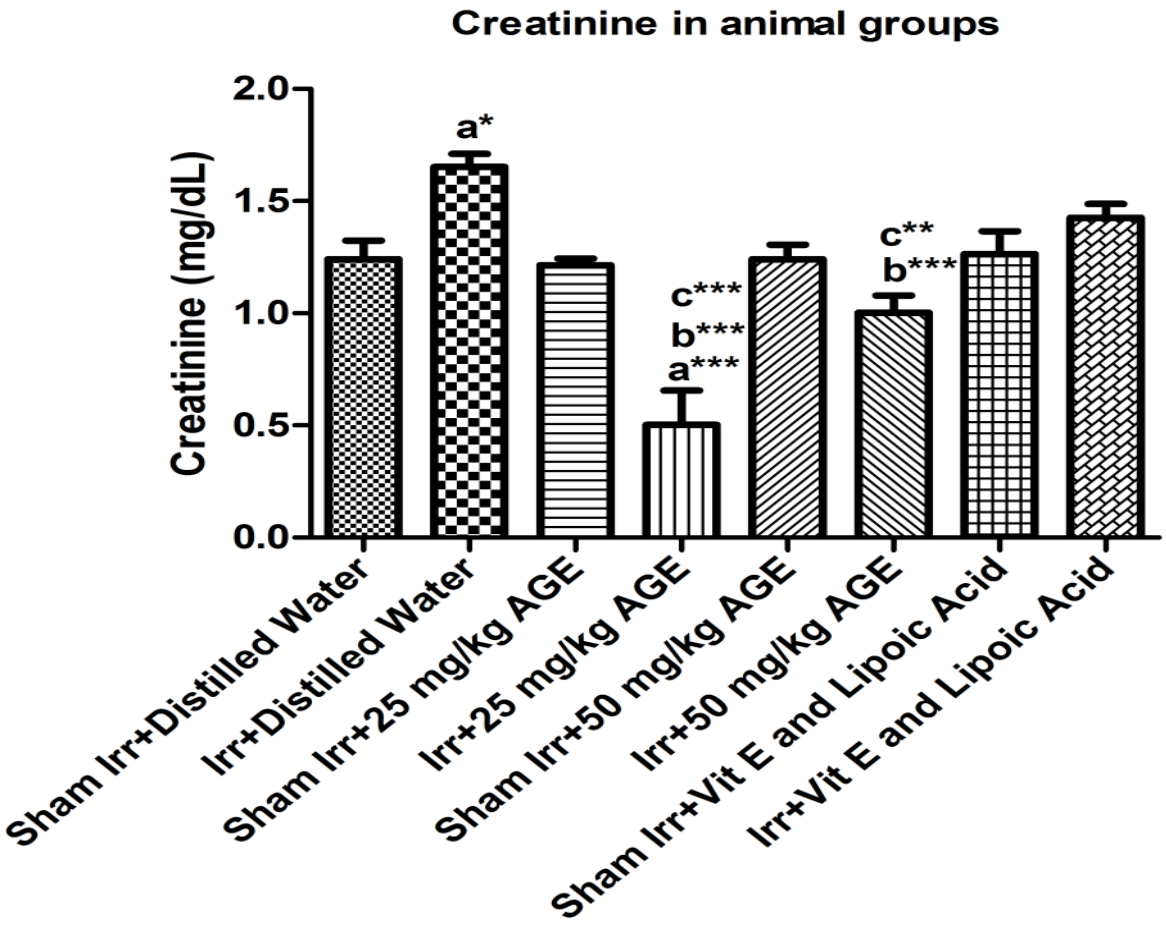

Figure 1: effects of γ-radiation and AGE on Creatinine rate

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)** 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, Lipid Peroxidation and Total tissues Proteins: 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)	**Proteins** (mg/mL)
Sham Irradiation+ Distilled Water	63.29±4.41	44.70±0.52	7.48±0.84	0.122±0.003	0.26±0.02	1.16±0.08
Irradiation+ Distilled Water	32.57±2.50 **a***, c***	32.32±0.51 **a***, c****	4.12±0.40 **a****	0.157±0.013 a*	0.37±0.01 **a****	0.60±0.02 **a***, c***
Sham Irradiation+ 25 mg/kg AGE	63.38±2.80	44.74±0.61	7.82±1.18	0.122±0.006	0.26±0.01	1.18±0.07
Irradiation+ 25 mg/kg AGE	83.22±4.63 **a**, b***, c*****	54.96±0.63 **a***, b***, c*****	11.24±0.74 **a**, b***, c*****	0.061±0.006 a***, b***, c***	0.16±0.02**a**, b***, c*****	1.88±0.10 **a***, b***, c*****
Sham Irradiation+ 50 mg/kg AGE	63.98±4.22	44.68±0.48	7.68±0.49	0.122±0.005	0.26±0.01	1.17±0.10
Irradiation+ 50 mg/kg AGE	71.35±6.28**b***, c****	51.80±0.70 **a***, b***, c*****	9.36±0.52**b***, c***	0.095±0.004 b***, c**	0.21±0.03**b***, c****	1.34±0.06 **b***, c****
Sham Irradiation+ Vitamin E and Lipoïc Acid	63.22±2.70	44.50±0.48	7.46±0.59	0.122±0.007	0.26±0.01	1.16±0.06
Irradiation+ Vitamin E and Lipoïc Acid	51.89±3.04 **b***	35.64±0.43 **a***, b****	6.18±0.32	0.143±0.013	0.31±0.02	0.94±0.06 **b***

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 48.54% (32.57±2.50 Vs. 63.29±4.41 µmol/mg of tissue) and a significant increase (P <0.01) in the group "Irradiation+25 mg/kg AGE" in the range of 31.50 % (83.22±4.63 Vs. 63.29±4.41 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 155.53 % (83.22±4.63 Vs. 32.57±2.50 µmol/mg of tissue) and 119.05 % (71.35±6.28 Vs. 32.57±2.50 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 in glutathione reduced was observed in the group "Irradiation + 25 mg / kg AGE" (P <0.001) in order of 60.39 % (83.22±4.63 Vs. 51.89±3.04 µmol/mg of tissue) and of 37.49 % (71.35±6.28 Vs. 51.89 ±3.04 µmol/mg of tissue**)** in those of the group "Irradiation + 50 mg / kg AGE " (P <0.01).

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 22.96 % (54.96±0.63 Vs. 44.70±0.52 µmol/mg of protein) in the group "Irradiation + 25 mg / kg AGE" and a significant decrease (P <0.001) in the range of 27.69 % (32.32±0.51Vs. 44.70±0.52 µmol/mg of protein) and 20.26 % (35.64 ±0.43 Vs. 44.70±0.52 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 70.05 % (54.96±0.63 Vs. 32.32±0.51 µmol/mg of protein) and 60.27 % (51.80±0.70 Vs. 32.32±0.51 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 54.21 % (54.96±0.63 Vs. 35.64 ±0.43 µmol/mg of protein) and of 45.34 % (51.80±0.70 Vs. 35.64 ±0.43 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 44.89% (4.12±0.40 Vs. 7.48±0.84 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 50.26 % (11.24±0.74 Vs. 7.48±0.84 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 172.64 % (11.24±0.74 Vs. 4.12±0.40 µmoles H_2O_2/minute/mg of protein) and 127.05 % (9.36±0.52 Vs. 4.12±0.40 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 81.82 % (11.24±0.74 Vs. 6.18 ±0.32 µmoles H_2O_2/minute/mg of protein) and of 51.42 % (9.36±0.52 Vs. 6.18 ±0.32 µmoles H_2O_2/minute/mg of protein) in the group "Irradiation + 50 mg / kg AGE " (**Table 1**).

3.2.4. Nitrite **(**NO^{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 28.23% (0.157±0.013 Vs. 0.122±0.003 µmol/mL) in the group "Irradiation + Distilled Water" while a significant decrease (P <0.01) of about 89.68 % (0.061±0.006 Vs. 0.122±0.003 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 61.11 % (0.061±0.006 Vs. 0.157±0.013 µmol/mL) and 39.34 % (0.095±0.004 Vs. 0.157±0.013).

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 57.31 % (0.061±0.006 Vs. 0.143±0.013 µmol/mL) and 33.42 % (0.095±0.004 Vs. 0.143±0.013).

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 40.15 % (0.37±0.01 Vs. 0.26±0.02 µmol/mg of tissue) in the group "Irradiation + Distilled Water" while a significant decrease (P <0.05) of about 40.15 % (0.16±0.02 Vs. 0.26±0.02 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 57.29 % (0.16±0.02 Vs. 0.37±0.01 µmol/mg of tissue) and 43.06 % (0.21±0.03 Vs. 0.37±0.01 µmol/mg of tissue). Similarly, compared to the irradiated positive control group receiving Vitamin E and Lipoïc Acid, a significant decrease in MDA level was also observed in the groups "Irradiation + 25 mg / kg AGE" (P <0.001) and "Irradiation + 50 mg / kg AGE" (P <0.001) in the range of 49.02 % (0.16±0.02 Vs. 0.31 ±0.02 µmol/mg of tissue) and 32.02 % (0.21±0.03 Vs. 0.31 ±0.02 µmol/mg of tissue.

3.2.6. Total tissues Proteins rate:From **Table 1**, it is clear that irradiation caused a significant increase (P <0.001) in serum total protein of about 61.81 % (1.88 ±0.10 Vs. 1.16±0.08 mg/dL) in the group "Irradiation + 25 mg / kg AGE" and a significant drop (P <0.001) in the range of 48.00 % (0.60±0.02 Vs. 1.16±0.08 mg/dL) in the group "Irradiation + Distilled Water" 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 211.20 % (1.88 ±0.10 Vs. 0.60±0.02 mg/dL) and 122.04 % (1.34±0.06 Vs. 0.60±0.02 mg/dL). In addition, compared to the positive control group ("Irradiation + Vitamin E and Lipoïc Acid"), a significant increase in serum proteins was observed in the group "Irradiation + 25 mg / kg AGE" (P <0.001) in order of 99.54 % (1.88 ±0.10 Vs. 0.94 ±0.06 mg/dL) and of 42.37 % (1.34±0.06 Vs. 0.94 ±0.06 mg/dL) in those of the group "Irradiation + 50 mg / kg AGE " (P <0.01).

4. DISCUSSION

Ionizing radiations are known to induce oxidative stress through the generation of reactive oxygen species resulting in an imbalance of the prooxidant and antioxidant status in the cells[24, 25]. The present study and several others reported an increase in blood Creatinine after exposure to irradiation[26-28]. Elevation, attributed by some authors to the interaction of radiations with the Creatinine sites of biosynthesis[29] and by others to the back leakage of the filtered Creatinine, which may occur through the damaged tubular epithelium along the concentration gradient established by salt and water reabsorption[30,31]. AGE intake one hour after irradiation significantly decreased rat's serum concentration of Creatinine. The reduction was more pronounced with AGE than with Vitamin E and Lipoïc Acid and more important in groups receiving AGE at a dosage of 25 mg/kg compare to those receiving it at a dosage of 50 mg/kg. Stipulating, garlic has ameliorative activity on Creatinine. This amelioration is attributed to allicin[32]. The lowering in Creatinine might cause a decrease in urinary protein extraction,

attenuation of lipid upsets, decreased oxygen consumption and the hypertrophy of the kidney [33], but AGE improves kidney function by maintaining cellular hydration and by retaining the balance between lipogenesis and lipolysis in the kidney to counteract the hyperlipidemia associated renal damage. Hence, AGE supplementation could be helpful in preventing the progression of radiation injuries and can thus be consider as nephroprotective[34]. The renoprotective effects of AGE could be associated with the antioxidant properties of SAC[35].

Ionizing radiation leads to structural and functional damage to cellular membranous molecules through oxidative stress which can initiate lipid peroxidation[36,37]. It has also been reported to cause renal GSH depletion and lipid peroxides accumulation in different organs[38-40]. Moreover, the composition of the glomerular basement membrane is being altered by the formation of lipid peroxidation[41]. According to some studies, radiation induced organs injury via increased MDA and nitrite, reduced GSH levels and decreased activity of CAT and SOD[42-46]. These organs injury is via a mechanism of oxidative stress mediated through the generation of ROS that induced severe cells damages[47, 48]. In the present study, higher MDA level and nitrite were obtained, while decreasing SOD, CAT activities and GSH level were noticed in the homogenate of rat kidney tissues after irradiation. These results are in accordance with those of some authors who observed a significant decline in SOD and catalase activity after exposure to irradiation[49]. 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 which will be removed by glutathione peroxidase present in the cytoplasm[50]. The presence of adequate amount of GSH, SOD and catalase minimize lipids peroxidation[47]. AGE intake induced significant increase in CAT, SOD and GSH activities accompanied with significant decrease in MDA level and nitrite in radiation-treated rat's kidney. 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[51-53].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. Capasso, Ana *et al.*, revealed AGE exhibited potent antioxidant and free radical scavenging activities[54-55]. It ameliorates lipid peroxidation and acts as a protective mechanism against oxidative damages of rat tissues[56-57]. This positive effect can be explained by the presence of S-allyl cysteine, S-allylmercaptocyteine, allicin, and selenium compounds in AGE[54]. Garlic has been reported to modulate lipid peroxidation levels and enhance the status of antioxidant[53, 58, 59]. It also elevates the levels of SOD, GSH-Px and Catalase[60, 61]. Hence, AGE radioprotective effects rely on its capacity to scavenge free radicals and enhance scavenging systems in the cell[61-63].

The present study revealed significant reduction in the content of kidney total proteins in rats submitted to γ-radiation. This decline may be attributed to excessive protein loss through injury of kidney[64]. Oral administration of AGE one hour after irradiation on day 1 and for 7 consecutive days improved the radiation-induced reduction in total protein level in kidney tissues in both irradiated groups. This effect has been more pronounced with AGE than with Vitamin E and Lipoïc Acid. The improvement 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. This could be attributed to the physiological role of AGE as antioxidants in minimizing radiation induced injuries because AGE and SAC were shown to scavenge ROS[65] and to inhibit lipid peroxide formation in several studies[66,67]. These antioxidant effects can be due to allixin, SAC, SMAC and diallylpolysulfides, whose radical-scavenging action increased with the number of sulfur atoms [68].

5. CONCLUSION

Based on the results obtained in the current study, it appears that, Aged garlic extract showed a radioprotective impact against ionizing-radiation-induced kidney injury due to its free radical scavenging and antioxidant properties. The attenuation of the severity of biochemical disorders in the kidney 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. Hence, AGE may be used to delay the progression of renal damage post irradiation.

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