



The Study of the Radiation Protection of Aged Garlic Extract to the Radiation Effects in Male Rat's Sperm

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To cite this article:

Kouam Foubi Brice Bertrand, Chuisseu Djamen Dieudonné Pascal, Dzeufiet Djomeni Paul Désiré, Samba Nganou Odette, Moifo Boniface, Zeh Odile Fernande, Guegang Goujou Emilienne, Mbede Maggy, TIEDEU Alain Bertin, Gonsu Fotsin Joseph. The Study of the Radiation Protection of Aged Garlic Extract to the Radiation Effects in Male Rat's Sperm. *International Journal of Biochemistry, Biophysics & Molecular Biology*. Vol. 1, No. 2, 2016, pp. 36-41. doi: 10.11648/j.ijbbmb.20160102.11

Received: October 22, 2016; **Accepted:** November 23, 2016; **Published:** January 5, 2017

Abstract: Irradiation results in a depression of the sperm count. The risk of damage to genes and chromosomal of spermatozoa exists and increases with increasing dose, potentially causing sterility or developmental defects in children. Reputed to be a powerful natural antioxidant that may cause inhibition of radical processes, the protection of the macromolecules essential for cell survival, and limiting oxygen effect, Aged garlic extract (AGE) has been demonstrated to possess several physiological activities in experimental animals thus, the present study aimed to assess if AGE was able to modulate the effects of gamma radiation-induced injury to sperm count. 80 healthy male rats were randomly allotted according to the duration of the experiment into 2 lots (Lot 1: preventive aspect and Lot 2: Curative aspect) of 40 rats each. The animals of each Lot were randomly divided into four equally and double male rat groups, five rats each, among which, 20 irradiated and 20 Sham Irradiated. Sperm motility was appreciated the 8th day post irradiation by counting under a microscope the spermatozoa contained in a determined volume of homogenate of the tail of the epididymis and the sperm density determined using the cell MALASSEZ. Exposure of rats to gamma irradiation caused a significant disturbance in sperm count but in rats exposed to radiation then pre-treated and treated with AGE, the results showed an improvement. Our results prove that AGE has protective effects against radiation-induced changes in sperm count post irradiation. Thus, it could be concluded that AGE might reduce the biological hazards induced by gamma irradiation in rat's sperm.

Keywords: Irradiation, Sperm, AGE, Epididymis, Rats

1. Introduction

Acute Radiation Syndrome (ARS) generate Reactive Oxygen Species, lipid peroxidation and nitrogen species responsible for toxicity through its oxidative stress injury and suppression of the antioxidant defense system [1-2]. It is a harmful process that induces damage to cell structures [3-6] and induction of chromosomal abnormalities, mutations and

cancer [7]. Irradiation of the male reproductive organs may interfere with spermatogenesis (the generation of spermatozoa), resulting in a significant but reversible depression of the sperm count after a brief exposure to about 0.1 Gy. The threshold dose for temporary sterility lasting several weeks is about 0.15 Gy; the spermatogenesis restarts if a sufficient number of stem cells spermatogonia remain viable. Under conditions of prolonged exposure the dose rate threshold is for permanent sterility is about 3.5–6 Gy [8-10].

Many natural and synthetic compounds have been investigated for their efficacy to protect against irradiation damage [11]. AGE is reputed to be a powerful natural antioxidant that may cause inhibition of radical processes, the protection of the macromolecules essential for cell survival, and limiting oxygen effect [12].

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 sperm using Vitamin E and Lipoic Acid as positive control group because the positive effect of Alpha Lipoic Acid includes protection against radiation damage [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 the ICH guidelines from animal lab Mountain University, Bagangte 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, 80 male

rats were randomly allotted according to the duration of the experiment into 2 lots (Lot 1: preventive aspect and Lot 2: Curative aspect) of 40 rats each (Lot 1: Administration of Distilled water, AGE, Vitamin E and Lipoic acid via gavages for 5 consecutive days before acute irradiation and for 7 consecutive days after irradiation and Lot 2: Administration of Distilled water, AGE, Vitamin E and Lipoic Acid via gavages for 7 consecutive days after irradiation). The animals of each Lot 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 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 [16].

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. Determination of Mobility and Density of Sperm in Cauda Epididymis

The tail of the epididymis of each rat was charged immediately after the sacrifice, and then shredded in a foot glass containing 10 mL of 0.9% NaCl previously incubated in a water bath at 36°C [17-20].

Sperm motility was appreciated by direct examination of the previous solution. Thus, 20µl of this solution was placed between slide and cover slip at 400 magnification. The mobile and immotile sperm were quickly counted on 4 random fields and the percentage of mobile forms was determined from the formula:

$$\% \text{ mobile sperm} = \frac{\text{Number of motile sperm}}{\text{Total Number of sperm}} \times 100 \quad (1)$$

The sperm density was determined using the cell

MALASSEZ. Thus, 20 μ L of macerated epididymis was collected with a micropipette and deposited on the cell MALASSEZ then covered with a cover slip. The sperm count was done at 10x40 magnification with a fluorescence microscope Cyscope® (X 400). The number of sperm per mL was estimated in 4 grids of 20 small squares of the cell MALASSEZ by the following formula:

$$N = \frac{n}{a.v} . df \quad (2)$$

N=number of cells per unit volume (number of spermatozoa), n=number of cells counted, df= dilution factor: 20, a=number of counting units of 20 small squares of the cell MALASSEZ enumerated, v=volume of metering unit: 0,01mm³ or 0.01.10⁻³ mL

2.6. Statistical Analyses

Results were expressed as mean \pm Standard Error of the Mean (SEM). Comparison of means was done by Dunnett test as post hoc test. P values less than 0.05 were considered statistically significant. Statistical evaluation was conducted using one way analysis of variance (ANOVA) software Graph Pad Prism 5.03. With the 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. Preventive Aspect of AGE

3.1.1. Motility

It is clear from Figure 1 that irradiation caused a significant decrease ($P < 0.01$ and $P < 0.001$) in the percentage of motile sperm in animals groups "Irradiation + 25 mg / kg AGE" and "Irradiation + Vitamin E and Lipoic Acid" respectively in order of 24.01%, (65.2 ± 0.75 Vs $85.8 \pm 1.87\%$) and 25.17% (64.2 ± 0.61 Vs $85.8 \pm 1.87\%$) compared to the negative control "Sham Irradiation + Distilled Water". Furthermore, the statistical analysis revealed a significant increase ($P < 0.05$ and $P < 0.01$) in the percentage of motile sperm in order of 21.78% (79.4 ± 1.31 Vs $65.2 \pm 0.75\%$) in animals of group "Irradiation 25 mg / kg AGE" and in order of 26.07% (82.2 ± 1.46 Vs $65.2 \pm 0.75\%$) in animals of group "Irradiation + 50 mg / kg AGE" compared to the group "Irradiation + Distilled Water". This increase was also significant ($P < 0.05$ and $P < 0.01$) in the range of 23.68% (79.4 ± 1.31 Vs $64.2 \pm 0.61\%$) and 28.04% (82.2 ± 1.46 Vs $64.2 \pm 0.61\%$) in groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" compared to the positive control "Irradiation + Vitamin E and Lipoic Acid."

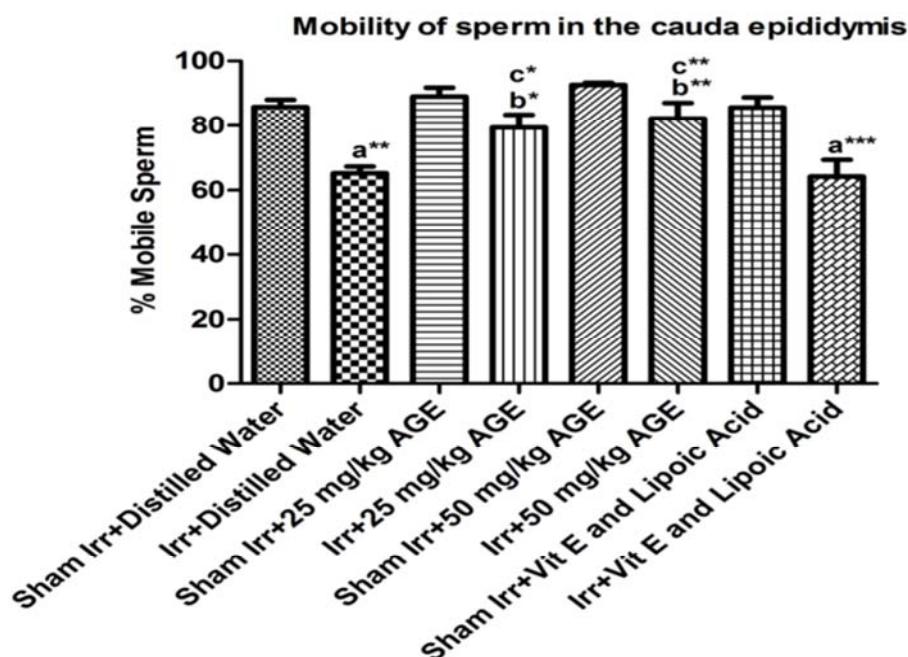


Figure 1. Effects of γ -radiation and AGE on mobility of sperm.

Each bar represents the Mean \pm 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 Lipoic Acid Group » (c).

3.1.2. Density

Figure 2 shows a significant decrease ($P < 0.001$) in the number of sperm per tail of epididymis following irradiation in animal of groups "Irradiation + Distilled Water" and

"Irradiation + Vitamin E and Lipoic Acid", respectively in order of 28.66% and 29.30% compared with those of the negative control group "Sham Irradiation + Distilled Water". This decrease in sperm count was also significant ($P < 0.05$ and $P < 0.001$) in order of 25% and 33.04% when comparing the group "Irradiation + Distilled Water" to the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE". Furthermore, the comparison with the positive control group "Irradiation + Vitamin E and Lipoic Acid" showed a significant decline ($P < 0.05$ and $P < 0.001$) of the sperm count as well as in rats treated with the extract at 25 mg / kg in order of 26.13% than in those treated with a dose of 50 mg / kg in order of 34.23%.

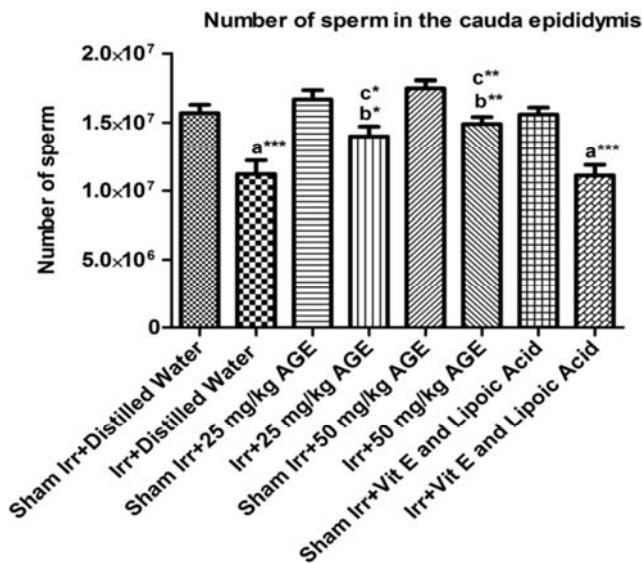


Figure 2. Effects of γ -radiation and AGE on number of sperm in the cauda epididymis.

Each bar represents the Mean \pm 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 Lipoic Acid Group » (c).

3.2. Curative Aspect of AGE

3.2.1. Motility

γ -radiation caused a significant decrease ($P < 0.001$) in the percentage of motile sperm in groups "Irradiation + 25 mg / kg AGE" and "Irradiation + Vitamin E and Lipoic Acid" respectively in the range of 27.11%, (58.6 ± 0.75 Vs $80.4 \pm 1.87\%$) and 28.36% (57.6 ± 0.61 Vs $80.4 \pm 1.87\%$) compared to the negative control "Sham Irradiation + Distilled Water" (Figure 3). Furthermore, the statistical analysis revealed a significant increase ($P < 0.05$ and $P < 0.01$) in the percentage of motile sperm in order of 20.82%

(70.8 ± 1.31 Vs $58.6 \pm 0.75\%$) in the group "Irradiation+25 mg/kg AGE" and in order of 29.01% (75.6 ± 1.46 Vs $58.6 \pm 0.75\%$) in the group "Irradiation+50 mg/kg AGE" compared to the "Irradiation+Distilled Water". This increase was also significant ($P < 0.05$ and $P < 0.01$) in the range of 22.92% (70.8 ± 1.31 Vs $57.6 \pm 0.61\%$) and 31.25% (75.6 ± 1.46 Vs $57.6 \pm 0.61\%$) in groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" compared to the positive control "Irradiation + Vitamin E and Lipoic Acid."

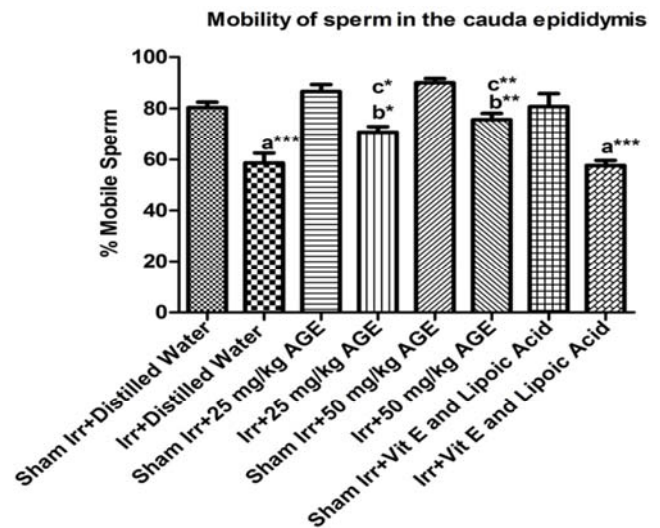


Figure 3. Effects of γ -radiation and AGE on mobility of sperm.

Each bar represents the Mean \pm 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 Lipoic Acid Group » (c).

3.2.2. Density

A significant decrease ($P < 0.01$) in the number of spermatozoa per tail of epididymis was observed following irradiation (Figure 4) in groups "Irradiation + Distilled Water" and "Irradiation + Vitamin E and Lipoic Acid", respectively in order of 32.91% and 32.28% compared with those of the negative control group "Sham Irradiation + Distilled Water". This decrease in sperm count was also significant ($P < 0.05$ and $P < 0.001$) in the range of 37.74% and 44.34% when comparing the group "Irradiation + Distilled Water" with groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE". Furthermore, the comparison with the positive control group "Irradiation + Vitamin E and Lipoic Acid" showed a significant decline ($P < 0.05$ and $P < 0.01$) of the sperm count as well as in rats treated with AGE at 25 mg / kg in order of 36.45% than in

those treated with a dose of 50 mg / kg in the range of 42.99%.

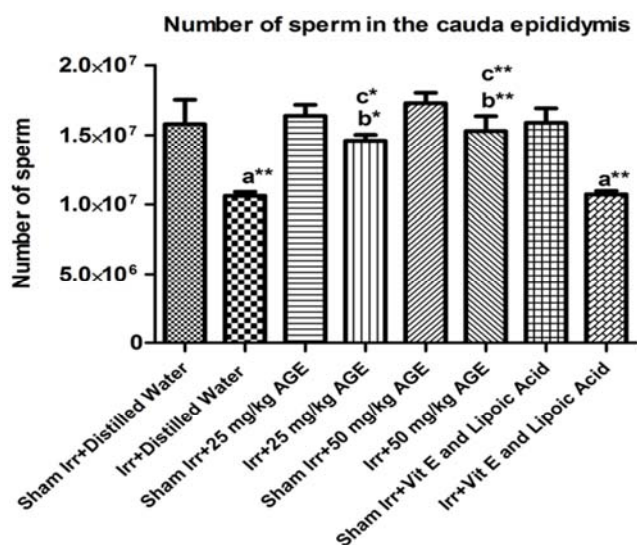


Figure 4. Effects of γ -radiation and AGE on number of sperm in the cauda epididymis.

Each bar represents the Mean \pm 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 Lipoic Acid Group » (c).

4. Discussion

Ionizing radiations are known to induce oxidative stress through the generation of reactive oxygen species resulting in an imbalance in the pro-oxidant, antioxidant status in the cells [21]. Multiple processes may lead to cellular damage under irradiation but the generation of oxygen free radicals following by lipid peroxidation may be one of the key components in this cascade of events [22]. Radiation generates reactive oxygen species that interact with cellular molecules, including DNA, lipids, and proteins [23].

Aged Garlic Extract is reputed to be a powerful natural antioxidant that may cause inhibition of radical processes, the protection of the macromolecules essential for cell survival, and limiting oxygen effect [24]. AGE has also received particular attention because of studies that have reported that it is a highly efficient antioxidant and has free radical scavenging capacity [22, 25]. The study focused on the in vivo evaluation of the use of aged garlic extract of *Allium sativum* L. as radioprotective against injuries induced by ionizing radiation. So, in order to verify the potentially beneficial role of AGE against hematopoietic syndrome post-

acute radiation of male rats, preventive and curative effects of Aged Garlic Extract were studied.

Radiation is one of the cytotoxicants that can kill testicular germ cells and so produce sterility. Selective destruction of the differentiating spermatogonia at low doses of irradiation [2–6 gray (Gy)] is a general phenomenon observed in rodents and humans, resulting in a temporary absence of spermatogenic cells [26–27]. However the stem spermatogonia are relatively radioresistant, they immediately repopulate the seminiferous epithelium as indicated in mice [26]. In this study, a marked significant decrease in sperm count in cauda epididymis and percentage of motile sperm was observed in irradiated rats especially in “Irradiation+Distilled Water” and “Irradiation+Vitamin E and Lipoic Acid” groups. But, the oral administration of AGE, one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days increased the number and the mobility of sperm in the cauda epididymis. The improvement effect of AGE on testicular function may be attributed to the powerful active components of AGE. This improvement has been more pronounced with the lower dose of AGE (25 mg/kg) than with the higher dose (50 mg/kg). These results are similar to those of Khaki *et al.* which have also observed a significant increase in motility and sperm count after 20 days treatment of albino rats with the crude extract of *Allium cepa* [28].

5. Conclusion

The present study revealed that γ -radiation induced different changes in rats sperm count but AGE intake prior and/or after whole body gamma radiation (4.5 Gy) result in an improvement. This radioprotective effect 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 Lipoic Acid.

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