Original article

Evolution of Glycaemia in Albino Wistar Male Rats Irradiated in the Presence of Aged Garlic Extract

Introduction. Whole body irradiation results in blood glucose disturbances from the first hours post irradiation up to several days later. Aged garlic extract (AGE) has been demonstrated to possess several physiological activities in experimental animals thus; the present study was aimed at evaluating the possible protective effect of AGE on evolution of Glycaemia in Albino Wistar male rat’s post-acute whole body irradiation. Materials and Methods: 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. A slight bite to the distal tip (lateral vein) of rat tail enables to get a slight bleeding which was deposited on a reactive dipstick and the blood sugar reading was done using a glucometer. Results. Exposure of rats to gamma irradiation caused a significant elevation of blood glucose at 4:00, 24:00, a non-significant elevation at 48:00, then a non-significant increase at 72:00 and 96:00. In rats receiving AGE orally via gavage for 5 consecutive days prior to acute irradiation and one hour after irradiation on day 6 and for 7 consecutive days, the results showed an improvement in blood glucose evolution. Conclusion. AGE seems to have protective effects against radiation-induced changes in evolution of glycaemia.

Keywords: irradiation, glycaemia, AGE, rats.
INTRODUCTION

The study of blood glucose variation in the patient in which the incident radiation was essentially consisting of gamma rays and about 10% fission neutrons after an accidental global irradiation occurred Venus reactor at Mol, dated 30.12.1965 revealed rapid hyperglycemia, intense and early in the first hours, then hypoglycemia in the current of the second day and finally a second and very discreet hyperglycemia until the 7th day [1-3].

The first measurable phenomenon after irradiation appears to be mild hypoglycemia fickle from 4th hours [4] that can be attributed with some authors [4-6] to decrease of intestinal absorption of glucose. Then follows, hyperglycemia noticed by all authors [5, 7-9], and a secondary hypoglycemia observed while the assimilation of dietary glucose is made very difficult by the condition of the intestinal mucosa. This secondary hypoglycemia appears quite relative and comes down to either a return of blood sugar to normal or to a depletion liver glycogen stores with a tendency to disappear completely [4, 9-10]. Similarly, the muscle tissues have their reservations lowered to a third or a quarter of the normal value. This secondary high blood sugar goes very often unnoticed in most cases and on that date the gastrointestinal syndrome begins to fade and restored intestinal mucosa may resume its digestive role vis-a-vis the exogenous glucose [4].

Recently; focus of radiation protection has shifted to test the radioprotective potential of plants and herbs in the hope that one day it will be possible to find a suitable pharmacological agent/s that could protect humans against the deleterious effects of ionizing radiation in clinical and other conditions [11]. AGE has been demonstrated to possess several physiological activities in experimental animals [12-13]. Furthermore, Alpha Lipoic acid (ALA) has shown good ability to protect hematopoietic system [14] and reduce the level of sugar in blood [15] in different studies.

Therefore, in the present study, the possible protective effect of AGE on evolution of Glyceremia in rat’s post acute ionizing radiation was examined using Vitamin E and Lipoic Acid as positive control group.

MATERIAL AND METHODS

Animals

Eighty healthy Albino male rats (Rattus norvegicus) of Wistar strain (3 to 4 months old) ranging from 214-230g body weight was obtained according to the ICH guidelines from animal lab Université des Montagnes, Banganté 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 on clinical studies.

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 [16-17]. The content of water-soluble compounds is relatively high whereas that of oil-soluble compounds is relatively low [17]. The AGE used in this study is standardized with S-Alllyl Cysteine and contained 30% extracted solids (300 mg/ml), and S-allyl cysteine present at 1.47 mg/ml.

Experimental Design

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

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.

Glucose assay [19]
A slight bite to the distal tip (lateral vein) of albino Wistar rat tail will get a slight bleeding (1-2 µL) which was deposited on a reactive dipstick (Accu-Check Active). The blood sugar reading was done using the glucometer (Accu-Check Active), 5 seconds after depositing the drop of blood on the strip. Blood was collected before irradiation (0:00: served to define reference value of glycaemia to rat) 4, 24, 48, 72 and 96 hours after irradiation. The raw results of blood sugar are subject to daily changes related to the time of collection, to animal feed as well as repeated blood samples. Repeated blood withdrawals are the cause of largest error because in the daily blood collection sets in the same animal, there is always a drop in blood sugar probably caused by anorexia subjects. The time of blood collection and sunshine influence the diurnal fasting of rats. This; results in significant differences in the results depending on the time of collection and weather conditions [20]. These variations affect at the same time all the animals of the same group or of the same series that live in the same conditions, the same room and suffer the same blood collection. Therefore, changes in blood glucose of control groups also exist and identically in irradiated animals. In order to remove these differences, the raw results of each group were assigned a factor as it leads to constant average blood glucose of witnesses group that day, so that the variation observed in irradiated animals should be related to the influence of irradiation. The calculations made therefore eliminated the variations due to causes other than radiation and the role of the constant chosen to fix the control mean of all series is to let the results normal value despite the transformation they have suffered [20].

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 (**__).

RESULTS
The figure below shows the change in blood glucose The figure below shows the change in blood glucose over time (0:00, 4:00, 24:00, 48:00, 72:00 and 96:00) following irradiation and administration of AGE. Calculations have allowed eliminate variations due to causes other than radiation and constant chosen to fix the control mean of all series is the average gross measurements among 40 animals before irradiation is 82.48 mg/dL at 0:00 with ESM 5.5.

Figure 1. effects of γ-irradiation and AGE on blood glucose over time
Data are expressed as mean ± SEM (n = 5). Significant differences are:

- aP < 0.05; a**P < 0.01; a***P < 0.001: when comparing groups to control (Sham Irradiation + Distilled Water) (a)
- bP < 0.05; b**P < 0.01; b***P < 0.001: when comparing groups to “Irradiation+Distilled Water Group” (b)
- cP < 0.05; c**P < 0.01; c***P < 0.001: when comparing groups to “Irradiation+Vitamin E and Lipoïc Acid Group” (c).

The effects of irradiation and AGE administration follow a more or less significant variation depending on the time of observation. Thus, to:

0:00: No significant difference (P > 0.05) in blood glucose levels were recorded by comparing glucose levels of different groups.

4:00: Irradiation resulted in a significant elevation of blood glucose in groups “Irradiation + Distilled Water” (P < 0.01) and “Irradiation + Vitamin E and Lipoïc Acid” (P < 0.05) in the range of 16.94% (103.94 ± 0.93 Vs 88.88 ± 0.78 mg/dL) and 15.99% (103.08 ± 1.29 Vs 88.88 ± 0.78 mg/dL) compared to the negative control “Sham Irradiation + Distilled Water”.

Figure 1 shows a significant decrease in blood glucose (P < 0.05) in the group “Irradiation + 25 mg / kg AGE” in order of 13.76% (89.64 ± 3.42 Vs 103.08 ± 1.29 mg/dL) compared to the positive control “Irradiation + Vitamin E and Lipoïc Acid” in order of 11.72% (92.70 ± 4.09 Vs 103.94 ± 0.93 mg/dL). While there was a non-significant decrease (P < 0.05) in the group “Irradiation + 50 mg / kg AGE” in order of 10.81% (91.37 ± 2.24 Vs 103.08 ± 1.29 mg/dL) compared to “Sham Irradiation + Distilled Water”.

24:00: Increased blood glucose is still significant in the groups “Irradiation + Distilled Water” (P < 0.001) and “Irradiation + Vitamin E and Lipoïc Acid” (P < 0.001 mg / dL) in order of 19.75% (101.43 ± 2.31 Vs 84.70 ± 2.56 mg/dL) and 19.37% (101.10 ± 2.83 Vs 84.70 ± 2.56 mg/dL) compared to the negative control “Sham Irradiation + Distilled Water” and a significant decline (P < 0.001 and P < 0.05) in the groups “Irradiation + 25 mg / kg AGE” and “Irradiation + 50 mg / kg AGE” in order of 14.20% (87.03 ± 2.42 Vs 101.43 ± 2.31 mg/dL) and 9.87% (91.42 ± 2.02 Vs 101.43 ± 2.31 mg/dL) compared to the group “Irradiation + Distilled Water”. The comparison with the positive control “Irradiation + Vitamin E and Lipoïc Acid” shows a significant decrease (P < 0.01 and P < 0.05) of blood glucose in the range of 13.92% (87.03 ± 2.42 Vs 101.10 ± 2.83 mg/dL) in the group “Irradiation + 25 mg / kg AGE” and in order of 9.58% (91.42 ± 2.02 Vs 101.10 ± 2.83 mg/dL) in the group “Irradiation + 50 mg / kg AGE”.

48:00: The blood sugar increase was not significant (P > 0.05) in the groups “Irradiation + Distilled Water” and “Irradiation + Vitamin E and Lipoïc Acid” in order of 15.76% (98.92 ± 3.15 Vs 85.45 ± 1.61 mg/dL) and 15.37% (98.58 ± 2.14 Vs 85.45 ± 1.61 mg/dL) compared to the negative control “Sham Irradiation + Distilled Water” and the decline was not significant (P > 0.05) in the groups “Irradiation + 25 mg / kg AGE” and “Irradiation + 50 mg / kg AGE” in order of 13.24% (85.82 ± 1.97 Vs 98.92 ± 3.15 mg/dL) and 9.97% (89.06 ± 5.20 Vs 98.92 ± 3.15 mg/dL) compared to the group “Irradiation + Distilled Water”. The comparison with the positive control “Irradiation + Vitamin E and Lipoïc Acid” also shows a non-significant decline (P > 0.05) in blood glucose levels in the range of 12.94% (85.82 ± 1.97 Vs 98.58 ± 2.14 mg/dL) in the group “Irradiation + 25 mg / kg AGE” and in order of 9.66% (89.06 ± 5.20 Vs 98.58 ± 2.14 mg/dL) in the group “Irradiation + 50 mg / kg AGE”.

72:00: In view of the negative control “Sham Irradiation + Distilled Water”, a non-significant increase (P > 0.05) in blood glucose was observed in the groups “Irradiation + Distilled Water” and “Irradiation + Vitamin E and Lipoïc Acid” in order of 11.72% (95.84 ± 4.41 Vs 85.79 ± 2.77 mg/dL) and 9.87% (94.26 ± 1.50 Vs 85.79 ± 2.77 mg/dL). Figure 1 shows a non-significant decrease in blood glucose (P > 0.05) in groups “Irradiation + 25 mg / kg AGE” and “Irradiation + 50 mg / kg AGE” in order of 9.62% (86.62 ± 0.93 Vs 95.84 ± 4.41 mg/dL) and 7.96% (88.21 ± 1.96 Vs 95.84 ± 4.41 mg/dL) compared to the group “Irradiation + Distilled Water”. This reduction remains not significant (P > 0.05) in the groups “Irradiation + 25 mg / kg AGE” and “Irradiation + 50 mg / kg AGE” respectively of about 8.10% (86.62 ± 0.93 Vs 94.26 ± 1.50 mg/dL) and 6.41% (88.21 ± 1.96 Vs 94.26 ± 1.50 mg/dL) compared to the positive control group “Irradiation + Vitamin E and Lipoïc Acid.”

96:00: A non-significant increase of blood glucose (P > 0.05) was observed in the group “Irradiation + Distilled Water” in order of 7.45% (91.79 ± 0.83 Vs 85.42 ± 2.17 mg/dL) and in the group “Irradiation + Vitamin E and Lipoïc Acid” in order of 6.97% (91.37 ± 2.24 Vs 85.42 ± 2.17 mg/dL) compared to the group “Sham Irradiation + Distilled Water”.

**DISCUSSION**

While the blood glucose rate drops within 24 hours after irradiation, to keep almost constant in the control groups, it is different in irradiated rats. The irradiation triggers an adrenaline hypersecretion causing hyperglycemia [3, 10, 21-26]. Over a period of 15 days, this early hyperglycemia (observed on Day 3) is preceded by a
slight inconstant hypoglycemia (observed 4 hours after the start of the study until the 2\textsuperscript{nd} day), followed by a delayed hypoglycemia (observed the 5\textsuperscript{th} day and the amplitude and changes will vary depending on the dose to the animal) and then a secondary hyperglycemia (observed on the 11\textsuperscript{th} day) for a dose of 4.75 Gy \[20\]. The study of the variation of blood glucose in a patient in which the incident radiation was essentially consisting of gamma rays and 10\% fission neutrons revealed rapid hyperglycemia, intense, early in the first hours then hypoglycemia in the current of the second day and finally a second very discreet hyperglycemia until the seventh day \[27, 1-2\]. Some authors have meanwhile noted a rise in blood glucose after irradiation with the maximum between 24 and 48 hours after irradiation both in rats that fasted than in rats fed \[22, 28\]. This increase is followed the 3\textsuperscript{rd} day by the occurrence of hypoglycaemia which evolution varies according to the dose the 4\textsuperscript{th} day. Thus, the 4\textsuperscript{th} day, there may be a return of blood glucose to normal for low doses (2.5 to 5 Gy); a slow persistence or aggravation of hypoglycemia in lethal doses (7.5 to 10 Gy) or a net worsening of hypoglycemia for supralethals doses (beyond 10 Gy) \[26\].

Early hyperglycemia was observed from the 4\textsuperscript{th} to the 48\textsuperscript{th} hour, followed by hypoglycemia in the 3\textsuperscript{rd} to the 4\textsuperscript{th} day after irradiation of rats at a dose of 4.5 in groups receiving distilled water and vitamin E + Lipoïc Acid orally. The elevation in glucose level may be attributed to the diminished utilization of glucose by irradiated tissues, increased blood amino acids level which is considered as an important source for glucose formation through the processes of deamination and transamination \[29\]. The observed hyperglycemia may appear due to an increase of the action of catecholamines by stimulation of glycogenolysis; increased during irradiation. The amplitude of this hyperglycemia is explained by the relatively sudden release of an existing reservation of glucose; which is the case of liver and muscle glycogen \[30\]. This high blood sugar can also be explained by the direct action of radiation on the liver without intervention from the pituitary \[8-9\]. Radiations alter in vitro intracellular ionic ratio Na/K for sodium. This increase in the concentration of sodium ion in the intracellular medium of hepatocytes causes a parallel increase in the production of glucose-1-phosphate with an inhibitory action on the phosphorylase phosphatase \[31-32\]. The hyperglycemia condition induced by irradiation could also be attributed to the inhibition of insulin secretion, diminished utilization of glucose by irradiated tissue or to increased blood amino acids level which are considered as an important source for glucose formation through the processes of deamination and transamination \[33\].

The oral administration of AGE, one hour after irradiation on day 1 after acclimatization and on day 6 for the duration of 12 days acted as hypoglycemic and stabilizers especially at a dose of 25 mg / kg where hypoglycemic effect is greater than with dose of 50 mg / kg. AGE significantly reduced the effects of hyperglycemia caused by irradiation and created a relatively stable blood sugar levels in the groups "Irradiation + 25 mg / kg AGE" and "Irradiation + 50 mg / kg AGE" up to the 4\textsuperscript{th} day. Several studies have also shown that garlic contains active hypocholesterolemic and hypoglycemic components, known as diallyl disulfide and dipropyl disulfide \[34-37\]. S-allyl cysteine sulphoxide (SACS), a sulphur containing amino acid of garlic decreased concentration of serum lipids, blood glucose and activities of serum enzymes, like ALP \[38\]. This condition was attributed to improving of the antioxidant system in cell of pancreas to produce insulin. These results agree with those of Kumar and Reddy \[36\] and Thomson \[39\] who found that feeding mice with garlic induced significant decrease of serum glucose levels. Oral administration of AGE is associated with hypoglycaemia, as it promotes insulin sensitivity, thus lowering insulin resistance in irradiated rats by regulating the cell energy metabolism or reducing free radical fatty acids \[34-37\].

**CONCLUSION**

The present study revealed that \(\gamma\)-irradiation induced different changes of blood glucose in rats post irradiation. However, AGE intake prior and after whole body radiation ameliorated such previous changes particularly 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 radioprotective properties. Suggesting, AGE may be considered as a useful dietary supplementary compound to patients irradiated. This provides as well a natural radioprotective; easily accessible to all at a lower cost with a suitable activity/toxicity report which in the long term; could be used in medicine for the preservation of healthy tissues or for the protection of people acting in contaminated environment. Thus; it’s a cheap preventive and protective strategy in the management of radiation-induced blood injuries.

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**TRANSPARENCY DOCUMENT**

The Transparency document associated with this article can be found in the online version.

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REFERENCES