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Oxidative stress in mollusks *Biomphalaria glabrata* exposed to gamma radiation

Estresse oxidativo em moluscos *Biomphalaria glabrata* expostos a radiação gama

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Ionizing radiation can cause biological changes in different organisms such as mollusks from *Biomphalaria glabrata* species, in which alterations could be observed in the reproductive system of the specimens, prejudicing fertility and fecundity. As the changes may occur due to the lipid peroxidation caused by the action of free radicals on the gonads, the objective of this work was to evaluate the oxidative damage caused by the exposure of *B. glabrata* mollusks to different doses of ^{60}Co gamma radiation. In addition, efforts were carried out to standardize a sensitive and low-cost technique for detecting negative effects caused by high doses of ionizing radiation. For this, each mollusk group (n = 10) was submitted to 0 (control), 10, 15, 20 and 25 Gy (gammacell ^{60}Co, dose rate 3.53 kGy/h). The TBARS method was applied for the quantification of lipid peroxidation of the gonads of the mollusks after 24 and 48 h. ANOVA, followed by the mean comparison (Tukey) at the 5% of significance level ($p<0.05$), indicated high concentrations of TBARS in the gonads after 24 h. Otherwise, after 48 h, differences for TBARS concentrations were not significant, determining that the action of free radicals from ionizing radiation on cell membranes mainly occurred within 24 h after irradiation. Therefore, the TBARS assay could be applied for detecting oxidative stress caused by short exposure of *B. glabrata* to ionizing radiation.
Keywords: *Biomphalaria glabrata*, Radiation, TBARS

A radiação ionizante pode causar alterações biológicas em diferentes organismos. Em moluscos *Biomphalaria glabrata*, estas alterações ocorrem principalmente no sistema reprodutor devido aos efeitos negativos observados na fecundidade e fertilidade dos espécimes. Como as alterações podem ser resultantes da ação dos radicais livres sobre as gônadas causando lipoperoxidação, o objetivo deste trabalho foi avaliar o dano oxidativo causado pela exposição dos moluscos *B. glabrata* a diferentes doses de radiação gama proveniente do ^{60}Co. Além disso, procurou-se padronizar uma técnica sensível e de baixo custo para detecção dos efeitos ambientas causados por altas doses de radiação ionizante. Cinquenta moluscos foram submetidos a 0 (controle), 10, 15, 20 e 25 Gy (gammacell-Co^{60}, taxa de dose 3,53 kGy/h), com as análises realizadas 24 e 48 h após a irradiação. Foi realizada a quantificação da lipoperoxidação das gônadas dos moluscos por meio do método TBARS. A análise estatística foi realizada por meio da ANOVA, seguido pela comparação entre médias (Tukey) em nível de significância de 5% ($p<0,05$). Os resultados demonstraram alta concentração de TBARS nas gônadas 24 h após a irradiação, contrastando com os resultados da análise após 48 h de irradiação, cujas diferenças com relação ao grupo controle não foram significativas. Assim, a ação dos radicais livres provenientes da radiação ionizante sobre as membranas celulares ocorreu nas primeiras 24 h após a irradiação. Com esse trabalho, demonstrou-se a eficácia do ensaio do TBARS em moluscos *B. glabrata* para a detecção de efeitos ambientais causados por radiação ionizante.
Palavras-chave: *Biomphalaria glabrata*, Radiação, TBARS

1. INTRODUCTION

Radiation is a type of energy, emitted by a source that propagates in the form of particles or electromagnetic waves. Ionizing radiations are those that have enough energy to pull electrons

from the atom, producing pairs of electrons. These can interact with biological systems either directly or indirectly [1].

The direct effect of radiation may occur when it interacts with macromolecules such as DNA, proteins, and lipids, and can cause structural changes. On the other hand, the indirect effect occurs when there is an interaction of the radiation with a medium, producing free radicals and these, in turn, reach the target molecules. Such free radicals originated from the action of radiation can generate reactive oxygen species (ROS), among which singlet oxygen ($^1O_2$), superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) [2]. These radicals are electronically unstable and can exert two functions on the biomolecules: electron acceptors, acting as oxidizing agents, and electron donors, acting as reducing agents [3].

However, when ROS are produced in excess, they can cause damage to the major cellular structures [4]. The high level of ROS can lead organisms to oxidative stress by triggering a cascade of biochemical events that result in lipid peroxidation [5].

The lipoperoxidation process occurs when free radicals act under the unsaturated lipids of cell membranes, leading to the destruction of their structure, failure of metabolic exchange mechanisms and, in an extreme condition, to cell death [6]. Changes in membranes cause permeability disorders, altering the ionic flow, resulting in loss of selectivity for entry and exit of toxic nutrients or metabolites [7]. Due to the increasingly use of ionizing radiation, whether for peaceful purposes such as medical diagnostics, therapy, energy production or war purposes, such as nuclear tests in the desert or oceanic regions, there is an interest in developing techniques capable of quantifying biological changes that may reach the organism.

The quantification of the products generated by lipid peroxidation may be analyzed by the formation of the thiobarbituric acid reactive substances (TBARS). This method consists of the analysis of the final products of lipoperoxidation (lipid peroxides, malondialdehydes, and other low molecular weight aldehydes) after reacting with 2-thiobarbituric acid (TBA). Such complexes are colored and their concentration can be determined spectrophotometrically. Thus, the analysis of TBARS formation can be taken as an index for lipid impairment after oxidative damage resulting from a stressful situation for the organism [8].

Among the experimental models, the *Biomphalaria glabrata* is highly important, since this organism has been used as a biomonitor both for physical and for chemical agents, besides showing sensitivity in studies at the cellular level and also as a bioindicator for pollutants [9,10]. According to reports found in the literature, this mollusk combines in its biology, primordial characteristics to a good environmental bioindicator, such as greater sensitivity to pollutants, not presenting a physiological adaptation to them under controlled conditions, has continuous and rapid reproduction throughout the year, allowing the performance of experiments at room temperature. In addition to these characteristics, *B. glabrata* also has a wide geographic distribution, low dispersion, easy capture and maintenance to laboratory conditions, short life cycle, low physical space and low-cost maintenance [11,12,13].

Therefore, the objective of this work was to evaluate the oxidative damage caused by the exposure of *B. glabrata* mollusks to gamma radiation, using sensitive and low-cost techniques.

2. MATERIALS AND METHODS

2.1. Experimental model

We used pigmented *Biomphalaria glabrata* mollusks from São Lourenço da Mata - PE and kept in the molluscary of the Department of Biophysics and Radiobiology of the Universidade Federal de Pernambuco. Fifty animals were selected according to the following criteria: young adult, sexually mature, shell diameter between 10 to 14 mm and a minimum age of 2 months. For each experimental group, 10 animals were used.

2.2. Irradiation

The mollusks were irradiated, based on the work carried out by Carvalho and colleagues [14], at doses of 10, 15, 20 and 25 Gy in the ^{60}Co Gammacell® source (dose rate of 3.532 kGy/h) from the Department of Nuclear Energy of the Universidade Federal de Pernambuco. The control group was kept under the same conditions as the others, besides not being exposed to ionizing radiation. All groups were irradiated in triplicate. Subsequently, the quantification of lipid peroxidation was performed 24 and 48 hours after the beginning of the experimental protocol with the objective of determining the presence of ROS in a short interval (acute) after irradiation.

2.3. Assay TBARS

The determination of TBARS was performed according to the methodology proposed by Ohkawa [15] with modifications. The animals were sacrificed in ice bed for the collection of the gonads. The gonads were then weighed and placed in 0.2% saline solution (1:10) and homogenized with the aid of the homogenizer and centrifuged for ten minutes at 0 °C with a rotation of 1000 x g. Subsequently, 200 µL of the supernatant from each sample was withdrawn and placed in a test tube. The 80 µL of 8.1% SDS, 600 µL of 20% acetic acid and 600 µL of 0.8% TBA were added to each tube. The tubes were heated for 30 minutes in the water bath at 100 °C. After the time, 600 µL of n-butanol was added to each tube and centrifuged at 2500 x g for 10 minutes. Then, with the help of a pipette, 30 µL of each tube was withdrawn and placed in a 96-well plate for spectrophotometer reading (Spectro UV-VIS RS-LaboMed, Inc.) using the wavelength of 595 nm. The quantification of proteins was done by the method of Bradford [16].

2.4. Statistical analysis

Statistical comparisons between groups and their TBARS concentrations, by radiation dose, were performed using ANOVA and the Tukey test with a significance level of 5%. Statistical analysis was performed using the software GraphPad Prism 5.0 (San Diego, CA, EUA).

3. RESULTS AND DISCUSSION

The results demonstrated an increase in lipid peroxidation of irradiated animals and analyzed 24 h after exposure (Figure 1).

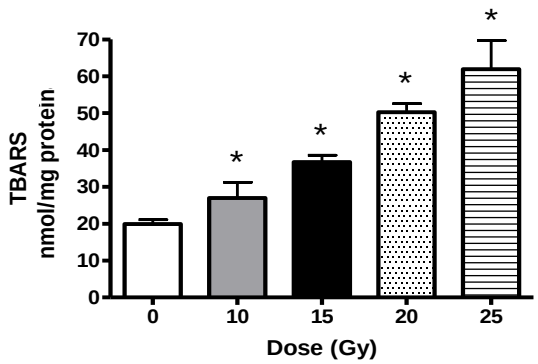

*Figure 1: Quantification of TBARS in mollusks analyzed 24 h after irradiation. The symbol * represents a significant difference ($p<0.05$) compared to the control group.*

It is possible to observe that the levels of thiobarbituric acid reacted with MDA, increased according to dose, demonstrating that the samples from irradiated animals showed an increase in lipid peroxidation reactions in organisms from the action of ROS such as (O^{2-}), (H_2O_2) and (OH^-) [17,18]. According to the statistical tests used, doses of 15, 20 and 25 Gy showed significant differences in relation to the control group. These results differ from those obtained after 48 h of

irradiation (Figure 2), as TBARS levels in the 24 h animals were high reaching the concentration of 60 nmol TBARS/mg protein, however, after 48 h of exposure to gamma radiation, the TBARS/mg protein concentration was lower than 40 nmol.

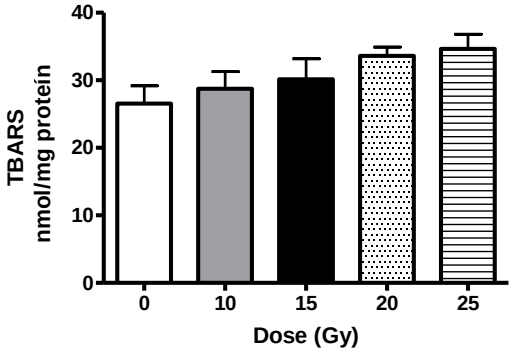

Figure 2: Quantification of TBARS in mollusks analyzed 48 h after irradiation.

An important study conducted by Bergayou and colleagues involving bivalve mollusks (*Scrobicularia plana* and *Cerastoderma edule*) exposed to pollutants from the Oued Souss River, located in Morocco, has shown high TBARS values for both species [19]. It was observed that the contaminants induced oxidative stress and lipoperoxidation. Furthermore, tests performed with freshwater molluscs (*Bellamya bengalensis*) have shown that the increase in global temperature also induces an increase in oxidative stress, since the higher temperature, metabolic rate is increased with increased O_2 consumption and oxygen-radicals generation in the tissues [20]. These results were similar to the high levels of TBARS observed in experiments with *Biomphalaria glabrata*, where it was verified that irradiation deregulated the mechanisms of antioxidant compensation present in the organism [21].

High TBARS levels were also found in mollusks (*Megapitaria squalida*) exposed to heavy metals from Bahia de La Paz in Mexico [22]. Significant differences were found in TBARS levels and in the activity of antioxidant enzymes present in tissues. The highest level of TBARS was found in a group of mollusks living in areas with high cadmium content [22,23].

The results obtained 48 h after irradiation were not significant when compared to the control group, that is, no changes were observed in the concentrations of TBARS/mg protein between the groups. This fact may be related to the expected interval for quantification of lipid peroxidation, since this time was sufficient for antioxidant enzymes to be able to bind to the free radicals generated by the exposure to ionizing radiation, reversing the oxidative damage caused in the organism [24,25].

4. CONCLUSION

According to the results obtained, it can be concluded that gamma radiation induced a high production of radical, which consequently increased TBARS levels 24 h after irradiation. Moreover, it is suggested that after 48 h, the damage caused by ionizing radiation is repaired by the own antioxidant system of the organism. Therefore, the TBARS test proved to be feasible for the detection of free radicals (O^{2-}), (H_2O_2) and (OH^-) from the interaction of ionizing radiation with biological systems. Although this methodology can not be considered as specific, it shows advantages in rapidness and coast-effectiveness,. In addition, *Biomphalaria glabrata* can be used as an environmental biomonitor and can be frequently collected for laboratory analysis.

5. ACKNOWLEDGMENT

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