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RADIOPROTECTIVE EFFECT OF THE EXTRACT OF *ZIZIPHUS JOAZEIRO* AND *ANACARDIUM OCCIDENTALE* ON EMBRYOS OF *BIOMPHALARIA GLABRATA* SUBMITTED TO IONIZING RADIATION

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ABSTRACT

Electromagnetic radiations are energies that can be classified as non-ionizing and ionizing. This type of energy is propagated by a material medium and the vacuum. The important characteristic of ionizing radiation is the localized release of large amounts of energy. The biological effects of radiation result principally from damage to DNA, which is the critical target. Given these harmful effects caused by radiation highlights the importance of acquiring knowledge about the radioprotective substance, because they act to protect the living tissue, decreasing the damage he caused by the effects of radiation. In this study we investigated the radioprotective effect of extract hydroalcoholic of *Ziziphus joazeiro* and *Anacardium occidentale* on embryos of *Biomphalaria glabrata*. The embryos of *Biomphalaria glabrata*-pigmented were divided into 18 groups of 100 specimens. The experimental groups were exposed to the extracts at a concentration of 200 ppm and then irradiated. For irradiation, we used a source of ^{60}Co (Gammacell of Radionics Labs. Dose rate = 4.359 Gy/h). The viability of the embryos was examined using a stereoscopic microscope and statistical analysis was performed using the test Student-Newman-Keuls and χ^2. Our results showed that the extracts of hydroalcoholic *Ziziphus joazeiro* showed radioprotective effect and that the aqueous extract of the bark of *Anacardium occidentale* exhibited a reduction in its embryotoxic effect.

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1. INTRODUCTION

Radiation is energy that is characterized by propagation through a medium or a vacuum. Because of its energy charge and interaction with matter can be classified as ionizing radiation, when they have enough energy to remove electrons from an atom electrosphere, causing the ionization of the same, and non-ionizing, when the energy interacts with the electrons of the atoms of arousing same. May be cited as examples of ionizing radiation, alpha particles, beta, protons, and non-particulate, gamma, neutron radiation and X-ray ranges. In relation to non-ionizing radiation may be cited as an example, ultraviolet, infrared, radio waves, lasers, microwaves and visible light [1,2].

Ionizing radiation when interacting with the biological system can have deleterious effects on living organisms at the molecular level, caused mainly during replication or during repair of DNA damage. Ionizing radiation is capable of breaking the DNA strand causing errors in cell division, may cause mutations and even death of the organism [3].

In the 1950 it was hypothesized that the damage caused by radiation and oxygen poisoning are related to the formation of reactive oxygen species [4]. The biological consequences of exposure to ionizing radiation are mediated by a series of physical phenomena, chemical, biochemical and cellular initiated after the absorption of radiation by the biological system [5].

Studies performed in bacteria [6], cell culture [7], fish [8], shell of molluscs [9], embryos of *Biomphalaria glabrata* [10,11,12] erythrocyte ghosts [13] and lymphocyte culture [14] were used as models for assessment of harmful effects caused by radiation on biological systems. Molluscs *Biomphalaria* and their embryos have proved to be excellent experimental models for evaluation of the biological effects of ionizing radiation, because they have characteristics such as short life cycle, easy to maintain in the laboratory, low maintenance cost, good reproducibility and rapid response and accurate [10,11,15].

Due to the increased use of ionizing radiation in various aspects of human life especially in areas pertaining to radiotherapy of cancer, food preservation, agriculture, industry and power generation, there is a need to develop an effective and non-toxic radioprotector. However, these studies today have little applicability due to high toxicity.

The radioprotective are substances that have the property to protect the living tissue, reducing the damage to it caused by radiation. Its action is due to chemical interaction between these substances and reactive oxygen species produced by radiation, preventing the harmful effects [16]. An ideal radioprotector should be cheap, does not have toxic implications in a wide dose range, orally administered, rapidly absorbed, possesses a reasonably good dose reduction factor and can act through multiple mechanisms. The plant and natural products have all these qualities. They are usually non-toxic, relatively cheap, can be orally administered and could act through multiple mechanisms due to the presence of many chemicals. Therefore, screening of plants and natural products is a useful paradigm for radioprotection. The advantage of plants and natural products is that they are used in several traditional systems of medicines. They are usually considered non-toxic and widely accepted by humans. Their use as

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radioprotectors needs scientific evaluation and validation. Once this is done their use, as radioprotectors could be more successful than synthetic chemicals [17].

A preliminary study was undertaken to elucidate the radioprotective effect of aqueous extracts of *Ziziphus joazeiro* and *Anacardium occidentale* using embryos of *Biomphalaria glabrata*.

2. MATERIALS AND METHODS

2.1. Collect the samples

The *Ziziphus joazeiro* and *Anacardium occidentale* plants were collected in the Agronomic Institute in the Experimental Station of Caruaru, state of Pernambuco, Brazil. After botanical identification, voucher specimens were deposited in the Herbarium at the Department of Botany, Federal University of Pernambuco.

Plants were selected portion of previously demarcated within the fragment, in places with higher humidity and better state of conservation, considered preferred for the development of plants [18]. The samples were collected from individuals with diameter at ground level DNS $\geq$ 10 cm. The criteria for the collection of plant material were undamaged leaves located one meter from the ground and barks have shown no marks extraction. Subsequently the material was properly stored for obtaining the hidroalcoholic extracts.

2.2. Obtaining the hydroalcocholic extracts

The samples were dried in a ventilated, shaded area at the end of this procedure, crushed. Then the material was sifted in a sieve mesh of 2,0 x 2,0 mm. Subsequently the samples were put in contact with 70% ethanol for 72 hours for the completion of maceration. At the end of the previous procedure, the hydroalcoholic extracts were subjected to filtration and evaporation the dark on the rotary evaporator (Fisaton 803). Then the samples were weighed for use in bioassays.

2.3. Concentrations of the aqueous extracts

The aqueous extracts of *Ziziphus joazeiro* and *Anacardium occidentale* subjected to gamma radiation of ^{60}Co were used at concentration of 200 ppm.

2.4. Irradiation of embryos in the presence of extracts

Extracts of stem bark and leaves of *Ziziphus joazeiro* and *Anacardium occidentale* were prepared and placed in petri dishes. Then the embryos were placed on the boards irradiated at a time using a Gammacell ^{60}Co source (Radionics Labs and the dose rate 4,359 Gy/h) situated at the Nuclear Energy Department, Federal University of Pernambuco, Brazil. The total absorbed dose used was 2,5 and 5,0 Gy . The irradiated embryos were immersed in the solution of the extracts during the entire period of observation.

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2.5. Bioassay with embryos of *Biomphalaria glabrata*

Embryos were used 4500 species of mollusks *Biomphalaria glabrata* - pigmented. In order to facilitate the collection of egg masses of snails were placed small strips of colorless polyethylene (5x5 cm) above the water surface of aquaria breeding. After 24 hours, the strips were removed from the polyethylene tank that was preceded individualization of spawning. Then the embryos were analyzed for viability and separated into groups, with about 100 specimens for testing of embryotoxicity and analysis of radioprotective effect of the extract.

2.6. Radioprotective effect of *Anacardium occidentale* and *Ziziphus joazeiro*

To study the radioprotective capacity of the extracts was performed as follows:

a) Control group with water and subjected to irradiation;
b) Control group with extracts of leaf and bark of the cashew *Ziziphus joazeiro* and non-irradiated at a concentration of 200 ppm. The concentration chosen was due to previous tests in our laboratory (unpublished data);
c) Group with extracts at a concentration of 200 ppm of leaf and bark of the cashew *Ziziphus joazeiro* and irradiated with 2,5 and 5,0 Gy.

For analysis of embryos was used a stereomicroscope (Tecnival-SQZ). The evaluation of effects was carried out through the methodology standardized by Okazaki et al., 1996.

2.7. Statistical analysis

The results were expressed as mean ± S.E.M. statistical analysis was performed by means of the Student-Newman-Keuls and $\chi 2$ (chi-square test) for multiple comparisons. $P < 0,05$ were considered to be statistically significant.

3. RESULTS

3.1. Test of the radioprotective effect of extract of *Ziziphus joazeiro*

In the figure 1, you can see the graphics for embryos that were exposed to gamma radiation from ^{60}Co at doses of 2,5 and 5,0 Gy in the presence of aqueous extracts of leaf and bark of *Ziziphus joazeiro*.

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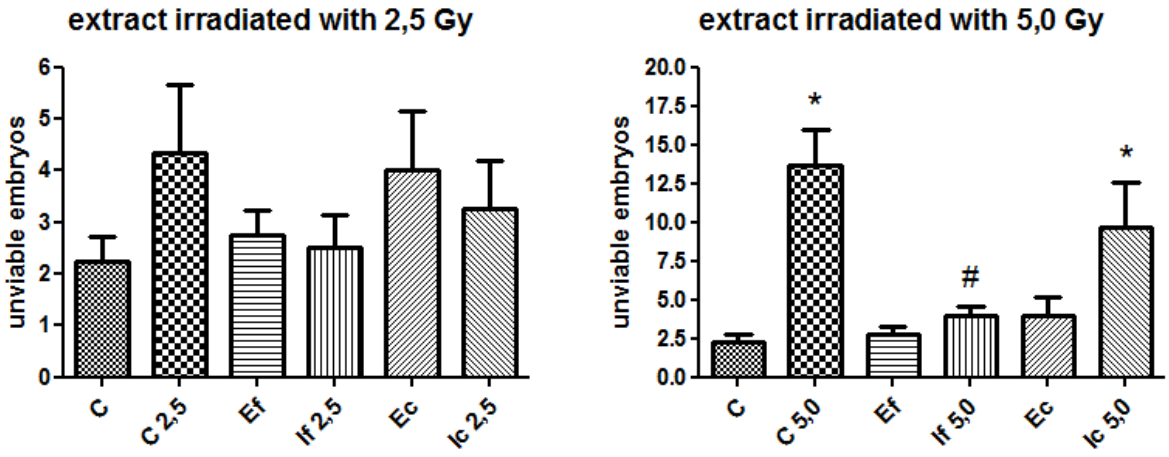

Figure 1. Embryos exposed to ionizing radiation at a dose of 2,5 and 5,0 Gy in the presence of leaf and bark extracts of *Ziziphus joazeiro*. Data were expressed ± S.E.M., p <0,05: * vs. C # vs. C 5,0.

In the left panel, with representatives of the groups that were irradiated with a dose of 2,5 Gy, no significant difference between groups experimental and control groups. All groups showed a low percentage of malformed or dead embryos. Regarding the groups that were irradiated with a dose of 5,0 Gy, it can be seen in the figure (right graph) than the group irradiated in the presence of aqueous extract of leaf (If 5,0) showed a lower number of embryos unviable when compared to the irradiated control group (C 5,0), the leaf extract when irradiated with 5,0 Gy showed a radioprotective effect, minimizing the damage caused by ionizing radiation. However the group irradiated in the presence of aqueous extract of the bark (Ic 5,0) was not significantly different compared to the irradiated control group (C 5,0).

3.2. Test of the radioprotective effect of extract of Anacardium occidentale

In figure 2, is can observe the graphics for embryos that were exposed to gamma radiation from ^{60}Co at doses of 2,5 and 5,0 Gy in the presence of aqueous extracts of leaf and bark of *Anacardium occidentale*.

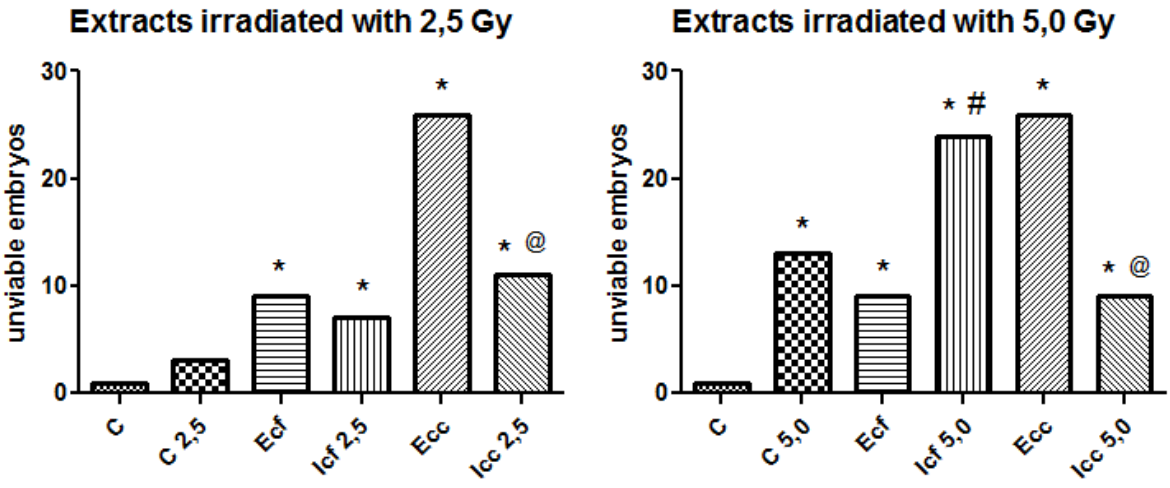

Figure 2. Embryos exposed to ionizing radiation at a dose of 2,5 and 5,0 Gy in the presence of leaf and bark extracts of *Anacardium occidentale*. The data were analyzed using the $\chi 2$ test, $p < 0.05$: * vs. C # vs. Ec.

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To procedures where the embryos were subjected to radiation at a dose of 2,5 Gy (left panel), we observed that the groups in the presence of leaf and bark (Ef and Ec) had an embryotoxic effect in the control group (C), and the toxicity of the bark more pronounced when compared with the sheet. However, when the aqueous extract of the bark was irradiated with 2,5 Gy (Ic 2,5), there was a marked reduction in the embryotoxic effect.

For the embryos that were exposed to radiation at a dose of 5,0 Gy in the presence of extracts, similar to the results of the accompanying graph, it appears that the groups in the presence of the extract at a concentration of 200 ppm of the leaf and bark (Ef and Ec) were toxic to embryos and that the extract of the bark irradiated with 5,0 Gy (Ic 5,0) showed a significant reduction of the embryotoxic effect.

4. DISCUSSION

The radioprotective effect of leaf extract (figure 1) can increase is related to the possible antioxidant activity of secondary metabolites present in leaves of *Ziziphus joazeiro* and that are possibly present in small concentrations in the bark. However further tests are needed, as well as the use of a more sophisticated methodology for elucidating the molecular mechanisms involved in the activity of radioprotective substances in the extract.

Konopacka et al. [19] evaluated the radioprotective effect of beta-carotene, a major supplier of vitamin A in bone marrow and bladder of mice irradiated with single dose of 2,0 Gy, whereas the effectiveness the substance had already been certified in radiation protection of splenocytes, reticulocytes and spermatids [20]. When administered in different doses (1 to 20 mg/ml) the antioxidant vitamins C, E and β-carotene in cultured lymphocytes, before and after irradiation, these substances can promote radioprotective effect on chromosomes, and its effectiveness depends on the dose, time and frequency of administration [21]. Konings and Drijver [22] reported the role of vitamin E in inhibiting the formation of free radicals. In 1975, Hoffer and Roy [23] showed that a diet deficient in vitamin E became more fragile red blood cells to the action of ionizing radiation, due to the high radiosensitivity by these cells.

Studies conducted by Mutlu-Turkoglu et al. [24] regarding the administration of selenium, vitamin E and combination of both, prior to abdominal irradiation in rats showed that administration of antioxidants before irradiation, particularly selenium and vitamin E, have protective effects against intestinal injury induced by ionizing radiation.

In 2000, Tuji [25] evaluated the effect of sodium selenite in the process of tissue repair in rats irradiated with 6,0 Gy, where it was observed that the repair process in irradiated animals and pre-treated with sodium selenite was given so similar groups of non-irradiated animals.

Shimoi et al. [26] found that plants containing flavonoids have shown antioxidant activity both *in vitro* and *in vivo*, where the radioprotective effect can be attributed to a debugging activity of free radicals. Radioprotective effect was attributed to similar extracts of *Centella asiatica* [27] and *Perna viridis* [28].

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Tests conducted with aqueous extract of *Mentha piperita* indicated radioprotection of hematopoietic tissue in mice subjected to ionizing radiation [29].

Studies also have shown that the extract of *Panax ginseng* has radioprotective effect, and that it is due to its antioxidant capacity exerted by certain chelating metal ions and the elimination of free radicals responsible for damage to the structure of DNA [30,31].

The results of experiments performed with *Anacardium occidentale* (figure 2) showed a significant reduction of the embryotoxic effect, suggesting that reducing the number of non-viable embryos may correlate with the increased presence of phenolic compounds and/or tannins in the bark of *Anacardium occidentale* extract and after irradiation these compounds potentiated its antioxidant activity.

Kubo et al. [32] analyzed the pharmacological activity of phenolic lipids from the extract of *Anacardium occidentale*, due to its antioxidant properties.

Haslam [33] evidenced that natural phenolic compounds inhibit lipid peroxidation and the activity of lipooxygenase *in vitro*. These compounds are present in significant concentrations, especially in the bark extract of *Anacardium occidentale* [34,35].

Chaves et al. [36] showed that the ethanolic extract of *Anacardium occidentale* bark has a high antioxidant potential in the DPPH assay, stable free radical used to assess antioxidant activity of extracts and pure substances [37,38] mainly phenolic compounds.

However, extracts of leaf and bark alcoholic *Anacardium occidentale* exhibited molluscicidal properties, possibly related to the presence of acid anacardic [39].

5. CONCLUSION

In conclusion, our research conducted so far suggests that extract of *Ziziphus joazeiro* is effective and non-toxic radioprotector. However, further research is necessary before implementation in human protection against ionizing radiation.

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