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EFFECTS OF HIGH DOSE RATE GAMMA RADIATION ON SURVIVAL AND REPRODUCTION OF *Biomphalaria glabrata*

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ABSTRACT

Ionizing radiations are known as mutagenic agents, causing lethality and infertility. This characteristic has motivated its application on animal biological control. In this context, the freshwater snail *Biomphalaria glabrata* can be considered an excellent experimental model to study effects of ionizing radiations on lethality and reproduction. This work was designed to evaluate effects of ^{60}Co gamma radiation at high dose rate (10.04 kGy/h) on *B. glabrata*. For this purpose, adult snails were selected and exposed to doses ranging from 20 to 100 Gy, with 10 Gy intervals; one group was kept as control. There was not effect of dose rate in the lethality of gamma radiation; the value of 64,3 Gy of LD_{50} obtained in our study was similar to that obtained by other authors with low dose rates. Nevertheless, our data suggest that there was a dose rate effect in the reproduction. On all dose levels, radiation improved the production of embryos for all exposed individuals. However, viability indexes were below 6% and, even 65 days after irradiation, fertility was not recovered. These results are not in agreement with other studies using low dose rates. Lethality was obtained in all groups irradiated, and the highest doses presented percentiles of dead animals above 50%. The results demonstrated that doses of 20 and 30 Gy were ideal for population control of *B. glabrata*. Further studies are needed; nevertheless, this research evidenced great potential of high dose rate gamma radiation on *B. glabrata* reproductive control.

1. INTRODUCTION

Ionizing radiations can cause mutations, infertility and even mortality in the exposed organisms [1, 2], and for this reason it has been applied for animal biological control [3].

The choice of biological models with short physiological cycle, easy maintenance in laboratory and fast reaction to stimulation is relevant for the follow-up of experiments. Taking these characteristics into account, the freshwater snail *Biomphalaria glabrata* (SAY, 1818) is considered a useful laboratory model, due its physiology and environmental behavior [4]. This species has great importance in tropical public health, mainly in the Northeast of Brazil, as intermediate host of the life cycle of *Schistosoma mansoni*, the trematode responsible for causing schistosomiasis [5, 6].

Many agents such as plant extracts and chemical compounds have been used for control *B. glabrata* where it occurs, but most of them can not be directed to the species of interest and represent danger of environmental contamination [7, 8, 9, 10]. Thus, besides its role as experimental model for radiobiology studies, the *B. glabrata* population control through ionizing radiation could represent an alternative for the control of schistosomiasis.

Studies on mutagenic effects of ionizing radiations in fish [11], amphibians [12] and molluscs [13, 14, 15, 16, 17] revealed the radiation potential for injurious effects on the biological functions and reproduction. It is established that the radiation effects depend on many factors, such as the time of exposure and the applied dose; also, it is known that, in most cases, an augment in the dose rate at which cells are irradiated results in an increase in toxicity [2,18].

Despite the reasonable number of publications using molluscs as experimental model, there are no data on high dose rate sources to control *B. glabrata*; except in [5], that used a high dose rate ^{60}Co source to eliminate *B. glabrata* snails; however, the high dose rate and the short time employed in this study prevented the achievement of data on the physiological and reproductive effects.

The aim of the present study was to contribute for the stablishment of gamma radiation at high dose rate on the population control of aquatic molluscs, not only of *B. glabrata*, but of other species as well. So, besides the action of high dose rate gamma radiation on survival, this work also focused on the effects on reproduction of the *B. glabrata*, by observing the alterations on embryos viability.

2. MATERIALS AND METHODS

2.1 Animals

A pigmented wild-type strain of *B. glabrata* obtained from São Lourenço da Mata (State of Pernambuco, Brazil), reared for several generations in the Laboratory of Radiobiology at the Biophysics and Radiobiology Department (DBR) at the Federal University of Pernambuco (UFPE) was used. Adult animals, *S. mansoni* negative, were maintained in plastic aquaria with filtered and dechlorinated water, changed once a week, pH around 7.0, at room temperature around 25 °C, and fed with fresh lettuce (*Lactuca sativa*).

2.2 Animal Selection

A population of 300 snails was pre-selected and kept under reproductive isolation during five days to confirm their sexual maturity. For the experiments, 200 adult individuals were separated as sexually mature, with shell length between 10 and 16 mm of diameter, distributed in ten groups of 20 individuals each one; one non-irradiated group served as control, and the others were irradiated in a Gammacell ^{60}Co-source (model 220 Excel-MDS Nordion, activity of 15.575 Ci and dose rate about 10.04 kGy/h) at the Nuclear Energy Department of UFPE.

2.3 Irradiation

Experimental groups received doses of 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy. These doses were chosen based in pilot-experiments previously performed in our laboratory, and in the results obtained by [17] for the $LD_{50/30}$ of *B. glabrata* irradiated under a low dose rate from a ^{60}Co source.

During irradiation the snails were kept in Falcon tubes (50 mL), in groups of five snails per tube, separated by a thin cotton cushion, in order to avoid sexual contact. The tubes that would receive the same dose were kept together in the irradiator.

2.4 Reproduction analysis

After irradiation, animals were reared in individual aquaria, under the same environmental conditions as before irradiation. Plastic sheets of nearly 2 X 2 cm were placed on the water surface to ease oviposition. Egg masses were daily collected, transferred to cell culture plates, filled with filtered water, and maintained in climate chambers at 25 °C until the end of the analysis, done under stereoscopycal microscopy.

The viable embryos percentage in each egg mass, according to [19] was observed since the beginning of the embryonic development after oviposition until the hatching day considered as the tenth day after oviposition.

The apparently normal embryos that did not hatch until the tenth day, as well those one with nonspecific malformations and specific malformations such as head, eyes and shell abnormal structure; as well as exogastrula, were considered under the general denomination of malformed embryos.

2.5 Irradiated adult animals survival

The animals maintained in individual aquaria after irradiation were observed during 30 days for determining the survival percentage. Thus, as referenced by [20], the irradiated animals were maintained under observation for 60 days, aiming to determinate the time for fertility recovering.

2.6 Statistical analyses

The statistical diferences among all exposed groups were evaluated by the Kruskal-Wallis test, considering the non-significance threshold as $p>0.05$; analyzed by BioEstat software (version 5.0).

To calculate de lethal dose capable of exterminate the molluscs in 30 days ($LD_{50/30}$) we used the probit test (version 3.1), available at United States Environmental Protection Agency (EPA) website [21].

3. RESULTS

Table 1 shows the percentages of adult animals dead during the period of 30 days after irradiation. An increase in the number of dead animals for the highest doses was observed.

A value of 64,3 Gy for the $LD_{50/30}$ was determined using the probit test. Lethal effects started six days after the exposure, and more than 90% of deaths occurred eight days after the irradiation.

Regarding the radiation effects on *B. glabrata* reproduction, the analysis of the number of embryos was considered a more reliable pattern for evaluation, taking in consideration that it is not rare to find empty eggs or even two or more embryos in one egg.

The analysis of the ionizing radiation effects on the fertility of exposed individuals was based in the following parameters: death, malformation and hatching embryos percentage.

In spite of the increase in the fecundity in the irradiated animals, their descendants did not reach the final embryonic developmental stages, and died before hatching, as indicates the low percentage of hatched embryos in the irradiated groups (Table 1).

Table 1. Effects of gamma radiation at high dose rate on *B. glabrata*.

Dose (Gy)	Lethality (adults dead - %)	Fecundity (n° of embryos)	Fertility (embryos viability)	
			Viable embryos (hatched - %)	Unviable embryos (Dead and malformed - %)
0	0	871	98.8	1.2
20	5	1434	5.9	94.1
30	5	1474	1.4	98.6
40	35	869	0.8	99.2
50	20	1742	0.5	99.5
60	25	2022	0.6	99.4
70	75	1373	0	100.0
80	80	1761	0.1	99.9
90	85	1517	0	100.0
100	55	1828	0	100.0

4. DISCUSSION

The data obtained in this study suggest that the dose rate did not have effect on the lethality of ionizing radiation. The value of 64,3 Gy for the $LD_{50/30}$ determined in this study was similar to that observed for *B. glabrata* by [17] with ^{60}Co gamma radiation (59 Gy) and by [13] with x ray (66 Gy). Nevertheless, low dose rates of 0.97 Gy/min and 10 to16 Gy/min, respectively, were used in these studies.

The irradiated groups produced more egg masses than the control one; such observations differ from [16] results, which compared the action of low dose rate gamma radiation on *B. straminea* fecundity in colony and sexually isolated. They found that ionizing radiation reduced the fecundity of snails.

We obtained egg masses with 13 to 16 eggs per egg mass. These values were in agreement with those described in [4, 22]. Some spawnings presented empty eggs with abnormal forms and size; the number of empty eggs was higher in the animals irradiated with the highest radiation dose. Some irradiated animals did not recover its normal fertility level, laying only empty eggs with abnormal forms and size.

Although the irradiation had caused a substantial improvement in the fecundity, its descendants were not able to reach the last stages of embryonic development, dying before hatching. Table 1 shows that less than 6% of embryos finished the embryonic development. This suggests a low level of cellular repair of the lesions induced by ionizing radiation. The doses above 40 Gy showed embryos hatching below 1%.

Comparing effects of different dose rates of gamma radiation on fishes, [18] found interesting results that support the results described here. This group investigated effects on fecundity and fertility, and confirmed that the effects of the high dose rate were more pronounced than lower dose rate.

A factor should be taken into account in the survival embryos analysis: the absence of unification in hatching times. Around the 8^{th} day 90% of the control embryos hatched, while the remainder embryos hatched in the 9^{th} or 10^{th} days; for the irradiated ones the analysis finished in the 10^{th} day and all the embryos hatched after this period were inserted in the malformed category, indicating a possible alteration in the hatching structure, caused by ionizing radiation, as preconized by [13], [23] and [24].

Despite the absence of effects of dose rate on the lethality of ionizing radiation, a remarkable effect was observed in the fertility of irradiated snails. Unlike [20], exposed animals did not recovered fertility, even with the lowest doses. Fertility and fecundity did not returned to background levels, even 65 days after irradiation.

The increase in the number of viable embryos was not uniform for all analyzed groups. The snails irradiated with the lowest doses (20 and 30 Gy) started the recovery of the spawning earlier, 40 days after the irradiation, while the animals irradiated with higher doses showed a fertility recovery only 50 days after the irradiation.

Aiming to evaluate the minimal dose of radiation to deplete the reproduction, the Kruskal-Wallis test was employed to analyze the differences between the responses with the different doses in this experiment. There were no statistically significant differences on the effects in the fecundity of groups exposed to 20 and 30 Gy. This information has particular importance when establishing protocols for population control, where is necessary that the results be obtained in a short time, with minimal radiation source manipulation.

5. CONCLUSIONS

Observing the effects of ^{60}Co gamma radiation on survival and reproduction of molluscs *B. glabrata*, our findings indicate that high dose rate gamma radiation affects lethality of snails in a similar way of low dose rate. On the other hand, fertility was remarkably influenced by the high dose rate, and did not recover, even 65 days after irradiation. Further studies, concerning on subcellular mechanism of effects should be carried out.

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