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Study of the radiomodifier effect of *Pityrocarpa moniliformis* extract

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ABSTRACT

Ionizing radiation has been applied in several areas of knowledge, among them the study of the radiomodifier activity of natural substances. These substances can modify the cellular response to the damage induced by the radiation. Therefore, this work aimed to evaluate the radiomodifier action of *Pityrocarpa moniliformis* extract on *Biomphalaria glabrata* embryos exposed to ^{60}Co gamma radiation. Initially, toxicity tests were performed on the extract against the *B. glabrata* embryos for the choice of concentration that did not cause death and embryonic malformation. Then, the antioxidant activity of the *P. moniliformis* extract with flavonoids and phenolic compounds was evaluated by means of the ABTS method. To evaluate the radiomodifier activity of the extract, embryos were selected in the blastula stage and irradiated with 7.5 Gy in a ^{60}Co source (gammacell-Co^{60}). Then, the embryos were exposed for 24 h to the extract of P. moniliformis at a concentration of 250 µg/mL. The results showed that the extract of *P. moniliformis* presents flavonoids and enzymatic inhibition by ABTS, which demonstrates the presence of antioxidant compounds. However, the tests of the radiomodifier activity did not present radioprotective effect for embryos exposed to ionizing radiation.

Keywords: Biomphalaria glabrata, *Pityrocarpa moniliformis*, Radiation.

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1. INTRODUCTION

Ionizing radiation is the electromagnetic wave or high energy particle that, when interacting with the absorber medium, has the property of transferring, in whole or in part, energy to the atoms and molecules of the medium, resulting in the phenomenon known as ionization [1]. In living organisms, the incidence of ionizing radiation can compromise the functioning of a cell, because, when interacting with the biological system, there is the frequent formation of free radicals that, being unstable and highly reactive, are considered the main responsible for the cellular damage caused by radiation [2].

Since ionizing radiation has the ability to alter the DNA molecule, one of its possible consequences would be the induction of mutations in the germ cells that would produce the offspring of the affected individuals. However, there is great difficulty in finding evidence that a given organic change is the exclusive consequence of exposure to ionizing radiation [3]. Another important issue is that the effects of ionizing radiation depend, among other factors, on the total dose and dose rate at which the organism was exposed [4]. In addition, there is a latent period before the detection of any response where it can be prolonged for decades, when exposed to low doses of radiation occurs, or for a very short period of time (minutes or hours), if it occurs in high doses and/or high rates, thus making the radiation effects difficult to observe [5].

Exposure to radiation and its effects have been described as Acute Radiation Syndrome [6]. With the advancement of radiobiology, not only a better understanding of the radio-induced cellular response, but also the development of modern prognostic, diagnostic and therapeutic measures in radioprotection were obtained. Thus, there are many derivatives of plant extracts or genetically modified plant sources which are used for the deleterious effects of ionizing radiation and are administered prior to irradiation. The interest in new sources of products for treatment after irradiation has been the subject of studies [7]. The radiomodifiers, which are agents that modify biological responses, include natural and chemical compounds [8]. However, the chemical compounds have a high toxicity, which contributed to the realization of research related to new alternatives of products with low toxicity, thus generating the products or compounds isolated from natural sources.

Ionizing radiation has been widely used in different areas of knowledge, medicine (radiodiagnosis and radiotherapy), agriculture, and industry. With increasing use of radiation, the interest and the need to find substances that can modify the cellular response to the damage induced by radiation. The *Biomphalaria glabrata* is a mollusk that is considered sensitive to toxic agents present in the environment and has been used as a biological model for the toxicity and embryotoxicity tests of chemical substances [9]. The plant kingdom has contributed significantly to the production of biologically active substances and its study has helped discover new compounds useful for various purposes, such as antibiotics or medicines [10].

Therefore, investigating substances of plant origin that may perform functions of pharmacological interest, such as radiomodifiers, are of great importance because they may present less toxicity and greater efficacy when compared with synthetic substances used for the same purpose [11].

Thus, the objective of this work was to evaluate the radiomodifier action of the aqueous extract of *Pityrocarpa moniliformis* on embryos of *B. glabrata* exposed to ionizing radiation.

2. MATERIALS AND METHODS

2.1. Phytochemical analysis

The antioxidant activity of the P. moniliformis extract quantified by ABTS methods, following the methodology of Re at al. [12], the dosage of phenolic compounds by the Folin-Ciocalteau method described by Li et al. [13], and dosage of flavonoids, following the methodology described by Woisky and Salatino [14].

2.2. Bioassays with *B. glabrata* embryos

2.2.1. Evaluation of the toxicity of the aqueous extract

Embryos of *Biomphalaria glabrata* in the blastula stage were separated into groups of approximately 100 embryos and exposed for 24 h to the aqueous extract of *P. moniliformis* (125, 250, 500 e 1000 µg/mL). Two control groups were used: one containing filtered water and the other group with 0.5% DMSO (dimethylsulfoxide) in filtered water. After exposure, the embryos were stored in containers with filtered water until their hatching and were later counted as viable and unviable (dead and malformed). The experiment was performed in triplicate.

2.2.2. Dose determination

The embryos (divided into groups of 100) of *B. glabrata* were packed in microtubes (Axygen Scientific, Inc., Union City, CA 94587 USA) containing 1 mL of filtered water. Subsequently, the animals were exposed to doses of 5, 7.5, 10, 20, 25, 30 and 35 Gy of gamma radiation of ^{60}Co (model II 200 Excel - MDS Nordion with dose rate of 3.532 kGy/h) at 25 °C (± 2) for determination of the dose to be used in the radiomodifier test.

2.2.3. Radiomodifier assay

To perform the radiomodifier test, the embryos were exposed to the extract of *P. moniliformis* at the concentration of 250 µg/mL after being irradiated at the dose of 7.5 Gy to evaluate its radiomodifier activity. The animals were divided into 5 groups: control with filtered and dechlorinated water (C), DMSO, exposed only to extract (E), irradiated (I) and submitted to extract and radiation (E + I).

2.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 software. The ANOVA and Student Newman-Keuls tests were used. The data were expressed as a mean ± standard error of the mean, where differences were significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

The phytochemical analysis confirmed the presence of antioxidant compounds (Table 1). The quantification of flavonoids was performed, where the presence of the extract of *Pityrocarpa moniliformis* (15.4783 µEQ/mg).

Similar results on flavonoid content were observed by Silva [15] in analyses performed with P. moniliformis extract. Through the ABTS method, the aqueous extract of *P. moniliformis* demonstrated the ability to sequester free radicals. This finding is compatible with those observed by Silva [15], who evaluated the antioxidant activity of *P. moniliformis* through the DPPH method. However, phenolic compounds were not detected in the extract.

Table 1: Results of the dosage tests of flavonoids, ABTS, and phenolic compounds.

	Flavonoids	**ABTS**		**Phenolic Compounds**
	(µEQ/mg)	**(µEQ/mg)**	**(E.I.)**	**(µEQ/mg)**
P. moniliformis	15.4783%	171.11%	15.84%	ND

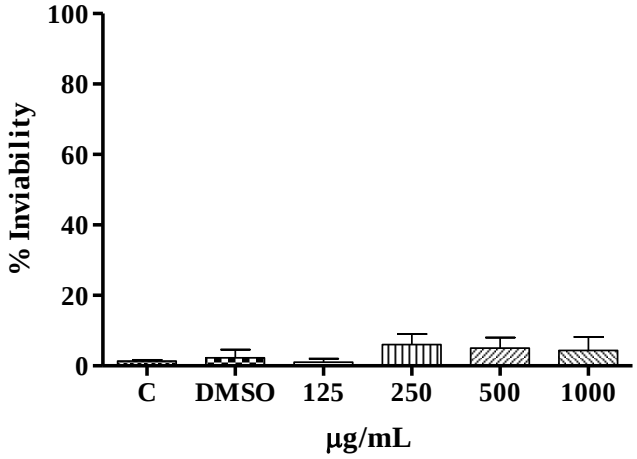
* Results expressed in µEQ/mg and Enzymatic Inhibition (E.I.). ND = Not detected.

In Figure 1 it is possible to observe the result regarding the exposure of the embryos to the extract of *P. moniliformis* in different concentrations, where no significant difference was observed in the percentage of inviability between the groups submitted to the extracts when compared to the control groups.

A result similar to that observed by Rocha-Filho et al. [16] after exposing embryos of *B. glabrata* to the *Moringa oleifera* extract to evaluate the embryocidal capacity of the substance, however, no

significant differences were observed between the groups of mollusks exposed to the extract when correlated to the animals belonging to the control group.

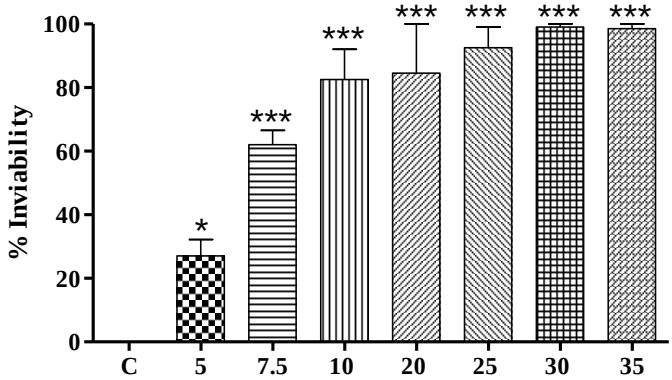
Figure 1: *The graph shows the result of the exposure of the B. glabrata embryos to the aqueous extract of P. moniliformis in different concentrations.*



In Figure 2 it is possible to observe the percentage of embryonic infeasibility of 27, 62, 82.5, 84.5, 92.5, 98.5 and 99% for the exposed embryos at doses of 5, 7.5, 10, 20, 25, 30 and 35 Gy, respectively. The 7.5 Gy dose was selected because it was the closest to LD_{50} (Lethal Dose to 50%) for the embryos studied.

Analysis of the results (Figure 2) showed a high percentage of inviability in the groups exposed to ionizing radiation, where even the lowest dose tested (5 Gy) induced a significant percentage (27%) of nonviable embryos.

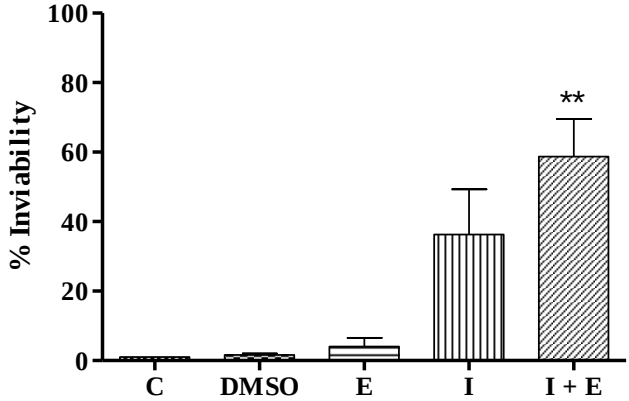
Figure 2: *In the figure, the effect of the ^{60}Co gamma radiation on embryos of B. glabrata can be observed at different doses.*



Okazaki et al. [17], after a study to evaluate the radiosensitivity of *B. glabrata* embryos, observed that the radiation caused death and development of several malformations, such as head malformations and shell malformations, with non-specific malformations being the most frequent.

Figure 3 shows the results of the radiomodifier test, where a 12% increase in the percentage of nonviable embryos belonging to the I + E group was observed when related to the group exposed to radiation alone, indicating that the *P. moniliformis* extract damage to embryos of *B. glabrata* after exposure to ionizing radiation.

Figure 3: *The graph shows the result of the radiomodifier test. Where, E = extract of P. moniliformis (250 μg/mL); I = irradiated (7.5 Gy); I + E = irradiated and later exposed to P. moniliformis extract.*



The results found in this work were similar to those demonstrated by Siqueira et al. [18], where it was observed that after exposure to ionizing radiation embryos of *B glabrata*, in the presence of the aqueous extract of the *Anacardium occidentale* leaf, an increase in the frequency of unviable

embryos occurred and that this effect possibly was caused by the potentiation of the activity of secondary metabolites of embryotoxic action present in the medium.

4. CONCLUSIONS

The phytochemical analysis showed the presence of flavonoids in the analyzed species, besides showing antioxidant activity through the ABTS test. However, the presence of phenols in their composition was not identified.

The *Pityrocarpa moniliformis* extract did not show a significant difference in the percentage of inviability for embryos of *Biomphalaria glabrata* at the concentrations studied making it impossible to determine the LC_{50} (Lethal Concentration for 50%) for mollusks.

B. glabrata embryos are sensitive to ionizing radiation, presenting a dose-dependent relationship with their rate of inviability.

The extract of *P. moniliformis* potentiated the damage caused to *B. glabrata* embryos after exposure to ionizing radiation.

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