ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN
2019 International Nuclear Atlantic Conference - INAC 2019
Santos, SP, Brazil, October 21-25, 2019

Agência Brasileira do ISBN
ISBN 978-85-99141-08-3
9 788599 141083

RESVERATROL RADIOMODIFIER EFFECT ON *DAPHNIA SIMILIS*

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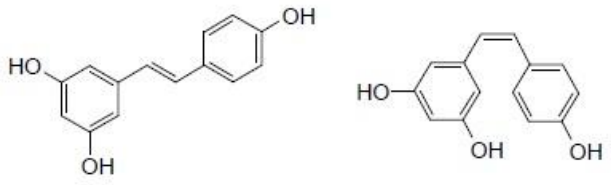
ABSTRACT

Resveratrol has beneficial properties as a potential antioxidant, protecting cells from free radicals, responsible for the natural aging process, anti-inflammatory action, acts against cardiovascular diseases in addition to showing a radiomodifying effect. Resveratrol is synthesized by a wide variety of plants in response to ultraviolet radiation or the action of certain pathogens. Due to the increasing use of this compound, due to its biological properties, studies are being carried out in the field of Ecotoxicology in order to provide information on the toxicity and radiomodifying effect of resveratrol for the development and adoption of criteria in order to improve environmental quality. It is known that resveratrol has radioprotective or radiosensitizing action, depending on its concentration. The objective of this study was to verify in which concentration resveratrol presents radioprotective effect. To achieve this goal, previous studies were carried out about the toxicity of resveratrol and gamma radiation effect on *Daphnia similis* to determine the lethal dose (LD50) of radiation and the EC50 (effective concentration) of resveratrol. Based on these data, the study of resveratrol radiomodifier effect on *Daphnia similis* demonstrated a higher resistance of this organism to gamma radiation at 55 Gy dose when previously exposed to resveratrol at concentrations of 6 and 7 μM, suggesting a radioprotective effect.

1. INTRODUCTION

Resveratrol (3,4',5 – trihydroxystilbene) is a polyphenol belonging to phytoalexins group of compounds and synthesized by a large variety of plants in response to UV radiation exposure or also by mechanic stress produced by pathogens action or chemical and physical agents [1]. Among these plants: peanut, eucalyptus, Kon-jo-kon (*Polygonum cuspidatum*) and grapes (*Vitis vinifera and Vitis labrusca*) the vines are considered a species with high capacity to produce resveratrol, and their synthesis occurs in the fruit film [2]. In wines, especially in red one, the resveratrol concentration is relatively high. Industrialized grape juice is considered a good source of resveratrol for abstemious, although its concentration is inferior compared with that wines [3].



Trans-**R** *Cis*-**R**

Source: Anisimova *et al* 2008 [4].

Figure 1: Resveratrol isomers: trans (trans-3,5,4',5- trihydroxystilbene) and cis (cis-3,5,4',5- trihydroxystilbene).

Free radicals are considered toxic products and are found in physiological natural processes of cell aging or by incidence of ionizing radiation in the body. The protective effects exerted by resveratrol during the process of damage to the cells are due to its properties as: anti-inflammatory response, antitumor activity, prevention or inhibition of degenerative diseases, decrease of cardiovascular diseases incidence, etc. It is known that resveratrol has radioprotective or radiosensitizing action, depending on its concentration.

The increasing use of resveratrol due to its multiple biological activities has led to the constant concern about determine the toxicity in several species of biological interest. In the Ecotoxicology field studies are being carried out in order to provide information of toxicity and radiomodifying effect of resveratrol on the aquatic biota for the development and adoption of criteria to improvement of environmental quality.

Many species of organism can be used as indicators of environmental problems, since contaminants present in aquatic ecosystems can have impacts on all biota. According to Usepa (2002) [5], the test organisms should be easy to collect and maintenance in the laboratory and should be available through all the year. In addition, it is of extreme importance the choice of autochthonous organisms to ensure greater representative ecological results. Cladoceras are widely used as test organisms because their extreme sensitivity to toxicants in the environment and their easy handle in the laboratory. The most Cladoceras used are *Daphnia similis*, *Daphnia magna*, *Ceriodaphnia dubia* and *Ceriodaphnia silvestri*.

Acute ecotoxicity tests are performed to obtain the toxicity of certain substance or environmental sample, important to provide fundamental and rapid information in order to develop and adopt criteria for the environmental quality improvement [6]. They are characterized by the short period of the test organism exposition to a sample. From these tests the gamma radiation Lethal Concentration (LC50), and the resveratrol Effective Concentration (EC50) were established, according to ABNT NBR - 12713 [7] and determined previously [8].

Another point that cause great concern is the disposal of radioactive material in the aquatic environment, allowing the biota exposure to ionizing radiation which can trigger potential and even irreversible effects [9].

In this sense, aquatic organisms are exposed to ionizing radiation in the vicinity of nuclear facilities, and to chemical proceeds of daily use in highly industrialized regions [10].

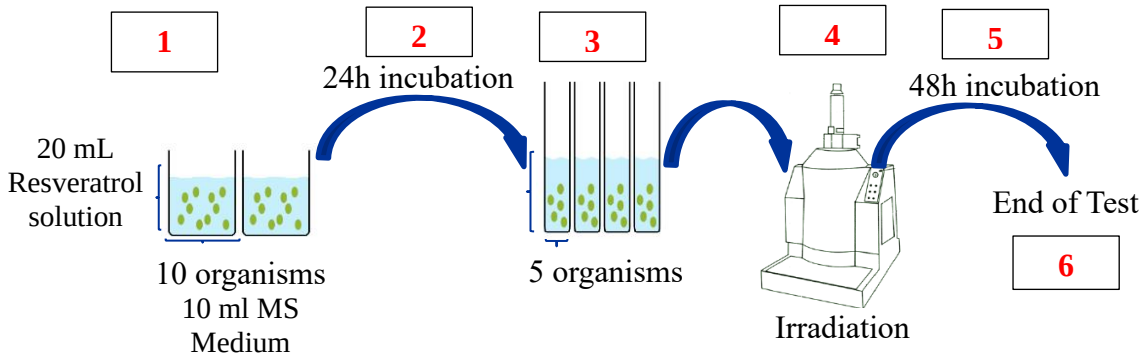
The aim of this work was the evaluation of resveratrol radiomodifier effect on *Daphnia similis* using adapted acute ecotoxicity test based on ABNT NBR – 12713 [7].

2. METHODOLOGY

The EC50 of resveratrol and Lethal Dose (LD50) of gamma radiation on *Daphnia similis* were obtained in the previous work, which showed that for resveratrol EC50 without the presence of gamma radiation was 6.04 μM, and the LD50 for gamma radiation alone was 585.43 Gy, both tests under the same conditions as in this study [8]. These data were used to establish the gamma radiation dose and the concentrations of resveratrol to evaluate the radiomodifier effect of resveratrol on *Daphnia similis*.

2.1. Resveratrol Radiomodifier Test

The resveratrol radiomodifier effect on *D. similis* assay was adapted following ABNT NBR – 12713[7]. The culture and dilution medium (MS medium) used was reconstituted deionized water with a hardness of 40 to 48 mg L^{-1} $CaCO_3$ and pH between 6.5 and 7 .



Source: The author.

Figure 2: Scheme of the radiomodifier effect test in *D. similis*.

This assay was carried out by 6 procedures, showed in the Fig.2: Exposure of resveratrol (1), First Incubation - 24h (2), Preparation for irradiation (3), Irradiation (4), Second Incubation - 48h (5) and End of Test (6):

1. Exposure of resveratrol on *D. similis*: For each dilution of resveratrol: 3, 4, 5, 6 and 7 µM were prepared two beakers containing 20 mL of each concentration and were placed 10 neonates aged from 6-24h in each beaker, totalizing 20 organisms per concentration. In the control the neonates were placed in MS medium, 2 beakers with 10 organisms each.
2. First incubation: All these beakers were kept in incubator at 22 ± 2 °C, during 24h, with a photoperiod of 12h light.
3. Preparation for irradiation: After incubation, 5 neonates were transferred to each prepared 4 falcon tubes containing 10 mL of MS medium for control of assay. For sample were prepared 4 falcon tubes containing 10 mL of each concentration of resveratrol where it was transferred 5 neonates on each tube. All tubes were capped.
4. Irradiation: The organisms were transported to a gamma cell source of 60 Co (Gamma-Cell 200) and irradiated at 55 Gy dose, corresponding to about 10% of gamma radiation LD50 on *D. similis*. The control tubes stayed in local with the same temperature for the same time of irradiation.
5. Second incubation: After irradiation the organisms were maintained in incubator at 22 ± 2 °C, during 28h, with a photoperiod of 12h light.
6. End of the test: The evaluated final effect was the viability of the organisms in each tube and with the obtained results was calculated the EC50 of *D. similis* after resveratrol exposition and gamma irradiation.

3. RESULTS AND DISCUSSION

In the Table 1 and Fig. 4 are shown the immobility results of the resveratrol radiomodifying effect after the exposition of organisms to resveratrol concentrations of 0, 3, 4, 5, 6 and 7 µM, and submitted to gamma irradiation at a dose of 55 Gy. The effect observed at the end of the test was the viability of the *Daphnia similis*.

Table 1. Radiomodifier effect of resveratrol on *Daphnia similis*: viable organisms results in the Acute Ecotoxicity assay after irradiation.

Concentrations of Resveratrol (µM)	Viable organisms (Replicate) 1	2	3
0	16	17	17
3	16	17	17
4	17	18	19
5	18	18	19
6	20	20	20
7	20	20	20

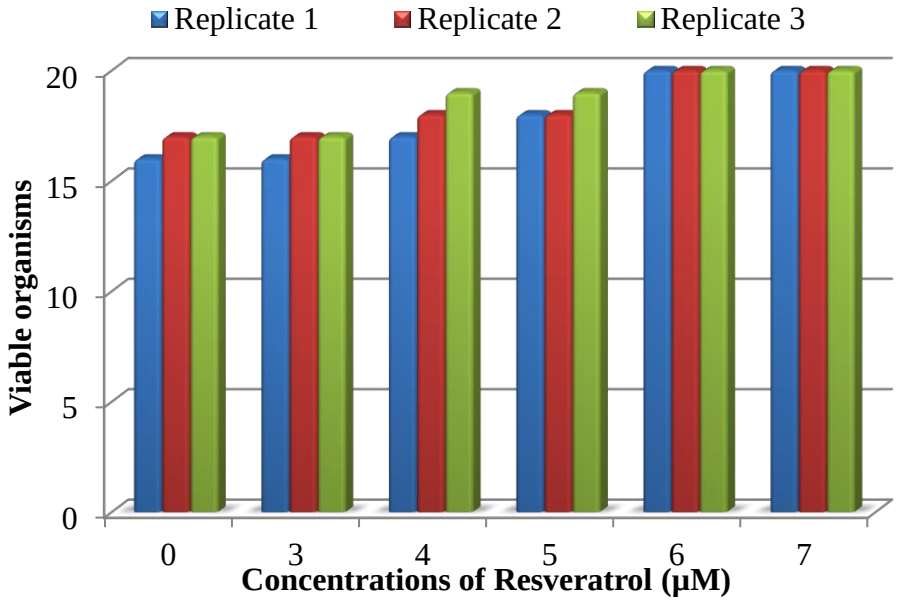


Figure 4. Radiomodifier effect of resveratrol on *D. similis*, irradiated with gamma radiation in the presence of resveratrol.

In the Fig.4 we can observe that the viability of the *D. similis* increasing with the resveratrol concentration increases. No differences where observed between the control group and resveratrol 3 µM. We found 100% of viability at resveratrol concentrations of 6 and 7 µM, which indicate some inconclusive difference compared to the control, where we had a viability of 83.3%.

Using the Trimmed Spearman-Karber statistical software [11], we were able to calculate the EC50 of resveratrol and the result was 4.69 μM, which means that at this concentration resveratrol can protect 50% of the organism in the assay.

This study corroborates with previous studies in the literature, demonstrating a radioprotective effect of resveratrol, by Moreno, 2009 [12], when identified this same effect at concentrations of 12.5 to 25 μM of the phytochemical in mouse connective tissue cells, NCTC, Clone 929, exposed to a dose of 3 Gy of gamma radiation.

4. CONCLUSIONS

The study of the resveratrol radiomodifier effect on *Daphnia similis* evidenced a higher resistance of the organisms when irradiated at 55 Gy dose, in the presence of 6 and 7 μM resveratrol concentrations, corroborating, with previous studies, a possible radioprotective effect. More related studies have be done to confirm this previous data.

ACKNOWLEDGMENTS

To CNPq for PIBIC scholarship that enabled this work to be developed, and Elizabeth Sebastiana Ribeiro Somessari of CTR-IPEN for the help in the part related to the irradiation of organisms.

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