

Radiomodifying Evaluation of Folic Acid in *Danio rerio* Embryos Exposed to Gamma Radiation

ALAVARSE, R. D.¹, ROGERO, J. R.², ROGERO, S. O., VIGILATO, M. A. RODRIGUES³,
VIEIRA, D. P.⁴

¹ *rodrigodelfonso@gmail.com*

² *rogero@ipen.br*

1. Introduction

The projected increase in cancer cases necessitates more radiotherapeutic treatments, often utilizing radiomodifying agents. The zebrafish, with a genome similar to humans, serves as an animal model in toxicity studies and therapies. Zebrafish embryos exposed to gamma radiation exhibit cellular changes comparable to clinical treatments. The role of folic acid, essential for cell division, in radiomodification is not well understood, necessitating further research. [1,2,3].

2. Methodology

Test Organism

The adult fish used for reproduction were provided by CETESB and bred in the ecotoxicology laboratory of CEQMA/IPEN. The aquariums adhere to ABNT - 2007 standards for pH, conductivity, and temperature. Embryo assays follow OECD guidelines for zebrafish. [4,5]

Minimum inhibitory concentration required to inhibit 50% of cells (IC₅₀).

The assays were conducted on mouse NCTC-L929 cells using PBS buffer with AF. Cell viability was measured at 490 nm using a Thermo Multiskan EX spectrophotometer. Analysis was performed using Prism GraphPad software version 10.0.0, utilizing the "Dose-Response Inhibition" equation.

Lethal dose to kill 50% of zebrafish embryos (LD₅₀).

Zebrafish embryo LD₅₀ assays for gamma radiation followed OECD Protocol No. 236 criteria, using batches with specific fertility and survival rates. Embryos were irradiated at 4, 6, and 24 hpf using doses of 5, 10, 15, 20 and 25 Gy and 10, 20,30, 40 and 50 Gy, respectively. Irradiation was done using a cobalt-60 source at the Radiation Technology Center of IPEN. [4]

Acute toxicity of folic acid in *Danio rerio* embryos.

The concentrations of folic acid for the assay of the concentration that kills 50% of zebrafish embryos (LC₅₀) were chosen based on the IC₅₀ obtained in the cytotoxicity assay (Fig.1)

Hours post-fertilization (hpf)	Dilutions (%)	Folic Acid (mg/mL)
2	100	1
	50	0,5
	25	0,25
	12,5	0,125
	6,25	0,0625
	3,125	0,03125

Figure 1: hpf and folic acid concentration per milliliter to which the embryos were exposed, with 100% corresponding to the mother solution.

The CL₅₀ values were determined at 96, 120, and 144 hpf intervals. During these processes, mortality and developmental alterations were noted and recorded. The data were entered into the statistical program ORIGIN 8.0, using the dose-response function to obtain the CL₅₀.

Assay for evaluating the radiomodifying effect of folic acid on zebrafish embryos.

Embryos at 2 hpf were selected for this assay. The defined radiation dose was 10 Gy for the control group, incubated with AF. The AF dose corresponds to 0.5 mg/mL. The incubation time in this solution was 3 hours. After incubation, the embryos were washed twice to remove excess AF and placed in 24-well plates with embryonic medium for irradiation. Subsequently, the embryos were observed at 96, 120, and 144 hours, and the irradiated groups with and without AF presence were compared. The data were collected and analyzed using the statistical program Origin 8.

3. Results and Discussion

IC₅₀

The IC₅₀ obtained for AF+PBS was 2.366 mg/mL (Fig. 2).

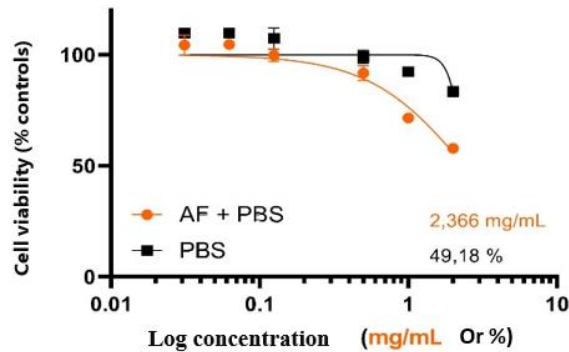


Figure 2: IC₅₀ of the cell viability assay for AF = 2.366 mg/mL, PBS concentration = 49.18%.
Source: the author

LD₅₀

The acute toxicity assays to obtain the LD₅₀ in organisms irradiated at 4 hpf are in Fig 3, at 6 hpf Fig. 4, and at 24 hpf Fig 5.

Hours post-fertilization (hpf)	Time after irradiation (h)	DL ₅₀ (Gy)
4	96	9,77±0,48
	120	8,71±1,28
	144	6,75±0,15

Figure 3: LD₅₀ values obtained for organisms irradiated at 4 hpf.
Source: the author

Hours post-fertilization (hpf)	Time after irradiation (h)	DL ₅₀ (Gy)
6	96	14,68±0,56
	120	12,31±1,78
	144	11,84±0,25

Figure 4: LD₅₀ values obtained for organisms irradiated at 6 hpf.
Source: the author

Hours post-fertilization (hpf)	Time after irradiation (h)	DL ₅₀ (Gy)
24	96	-
	120	16,17±1,09
	144	12,85±1,16

Figure 5: LD₅₀ values obtained for organisms irradiated at 24 hpf.

Source: the author

Damasceno (2018) employed similar doses in this study with zebrafish embryos. The research concluded that chorion removal did not significantly affect the embryos' response to radiation, even with equal doses.[6]

CL₅₀

The CL₅₀ for organisms at 96 hpf could not be calculated due to low lethality. At 120 hpf, the lethality corresponded to 0.67911 mg/mL (Fig. 6-a), and at 144 hpf, a CL₅₀ of 0.54421 mg/mL was obtained (Fig. 6-b).

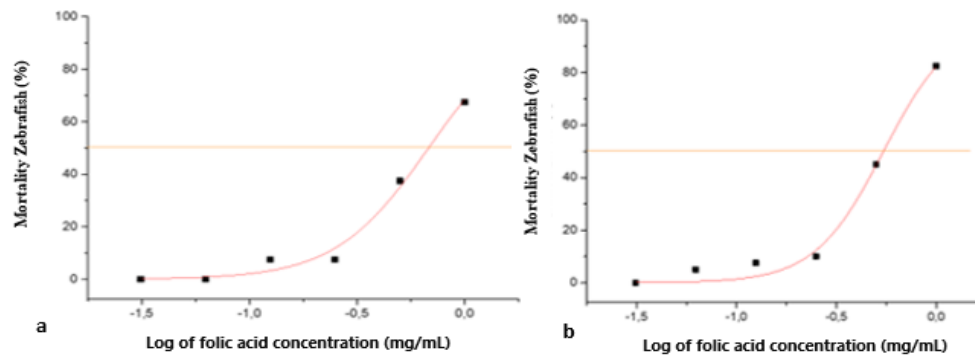


Figure 6: a - CL₅₀ for organisms at 120 hpf = 0.67911 mg/mL, b - CL₅₀ for organisms at 144 hpf = 0.54421 mg/mL.

Source: the author

Nascimento (2022) study on nutrition in zebrafish breeding, it is mentioned that folic acid is one of the main vitamins for development; however, there are no recommended values for its nutrition. [7]

Radiomodifying activity of folic acid in *Danio rerio* embryos

The embryos irradiated after incubation with AF showed higher survival compared to organisms only irradiated (Fig.7).

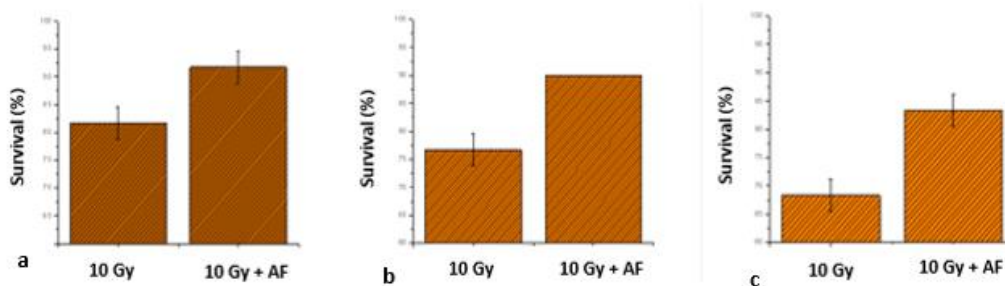


Figure 7: a- embryos at 96 hpf, b- embryos at 120 hpf, c- embryos at 144 hpf. At all observed stages, significant differences in the survival of organisms irradiated with 10 Gy + AF were noted compared to higher mortality in organisms irradiated only with 10 Gy.

4. Conclusions

The IC₅₀ of AF with PBS was 2.366 mg/mL, maintaining a stable pH of 7.2 in the embryonic medium.

The LD₅₀ values in embryos irradiated at 4 hpf varied with development time.

The CL₅₀ of AF in embryos at 96 hpf was not obtained due to low lethality, but decreased with increased exposure time to AF, reaching 0.67911 mg/mL at 120h and 0.54421 mg/mL at 144 hpf.

Organisms irradiated after incubation with AF showed significant survival.

References

[1] CHAL, Juliana Lopes et al. Ionizing radiation radiomodifying vegetables. *Acta Scientiae et Technicae*, v. 8, n. 1, p. 1-33, 2020.

[2] SCHNEIDER, Ana Cláudia Reis et al. Implementation of a new animal experimentation model: zebrafish. *HCPA Journal*. Vol. 29, n. 2 (2009), p. 100-103, 2009.

[3] DE OLIVEIRA SANTOS, Marceli et al. Estimate of cancer incidence in Brazil, 2023-2025. *Brazilian Journal of Cancerology*, v. 69, n. 1, 2023.

[4] OECD. GUIDELINE FOR THE TESTING OF CHEMICALS. Fish Embryo acute toxicity (FET) test, n. 236, July 2013.

[5] BRAZILIAN ASSOCIATION OF TECHNICAL STANDARDS (ABNT). Brazilian regulation standard no. 15,499: aquatic ecotoxicology-short-term chronic toxicity-assay method with fish. 2007.

[6] DAMASCENO, K. Radiomodifying evaluation of resveratrol in *Danio rerio* embryos irradiated with gamma radiation. 2019. Dissertation (Master's in Nuclear Technology), Institute of Energetic and Nuclear Research, IPEN-CNEN/SP, São Paulo. Available at: <www.teses.usp.br> (accessed on: 05/25/19)

[7] NASCIMENTO, Carolina Bulhões do et al. Important aspects of Zebrafish (*Danio rerio*) nutrition in vivariums. 2022.