ECOTOX - BRASIL

Ecotoxicol. Environ. Contam., v. 12, n. 1, 2017, 133-139
doi: 10.5132/eec.2017.01.15

EEC

Evaluation of resveratrol toxicity in the embryolarval stage of *Danio rerio* fish

Cavalcante, A.K.[1]; Lopes-Ferreira, M.[2] ; Rogero, S.O.[1] & Rogero, J.R.[1]

1- Laboratório de Ecotoxicologia - Centro de Química e Meio Ambiente - Instituto de Pesquisas Energéticas e Nucleares (IPEN) – Comissão Nacional de Energia Nuclear- IPEN/CNEN-SP, São Paulo, Brasil.

2- Laboratório Especial de Toxinologia Aplicada (LETA) - Instituto Butantan, São Paulo, Brasil.

(Received August 03, 2017; Accept November 16, 2017)

Abstract

Human beings concern about healthy life has driven researchers to study new compounds capable of reaching that desire. Resveratrol (3, 4 ‘, 5-trihydroxystilbene) a phenolic compound, is one of these substances which presents a variety of pharmacological actions, as antioxidant potential, anti-inflammatory capacity, protection against heart and cancer diseases. Despite the resveratrol health benefits studies, there is a little evidence of its toxicity in the literature in aquatic organisms, and especially the data on the concentration of resveratrol in the environment, making the present study fundamental for information about resveratrol ecotoxicity in the aquatic system. The aim of this study was to evaluate the toxicity of resveratrol in embryos and larvae of *Danio rerio* (zebrafish). The *in vitro* cytotoxicity and ecotoxicity assays were performed. The IC_{50} obtained in the NCTC-L929 cell line cytotoxicity assay was 38.5 mg L^{-1}. The LC_{50} (96h) obtained in fish embryo toxicity test was 75.3 mg L^{-1} and the mean value of resveratrol LC_{50} (168h) obtained in the short-term chronic ecotoxicity assays performed with zebrafish larvae was 51.4 mg L^{-1}. This work provided data on the toxicity of resveratrol in the embryonic stage of fish of the species *Danio rerio* and the toxic effects are dependent on its concentration.

Key-words: *Danio rerio*; Ecotoxicology; Fish embryo toxicity test; Resveratrol; Zebrafish.

INTRODUCTION

The search for human beings healthy life has driven researchs for new substances capable of meeting that purpose. Among these substances we find the phenolic compound resveratrol (3, 4 ‘, 5-trihydroxystilbene). The phenolic compound resveratrol is a phytoalexin which belongs to the stilbene family (Jeandet *et al.,* 2002). Phytoalexins are antimicrobial compounds produced by several plant species under stress conditions, which may be caused by biotic or abiotic factors, giving plants a protection against different diseases (VanEtten *et al*., 1994).

Resveratrol was identified in 72 plant species, such as blackberry, peanut, eucalyptus and grape. The species *Vitis vinifera* and *Vitis labrusca*, present the greatest resveratrol synthesis capacity, and the grape and its industrialized products are important sources of this compound (Vuong *et al.,* 2014).

In grapes, resveratrol is produced in fruit peels at various concentrations, and in wine, the concentration of the compound depends on several factors, such as geographical origin, wine type, oenological practices, climatic conditions and degree of infection of the plant by fungi (Fernández-Mar *et al*., 2012).

Studies have shown that resveratrol has a wide variety of biological activities, such as antioxidant activity; anti-inflammatory; antimicrobial; anticancer; neuroprotective; cardioprotective; reduction of obesity; inhibition of platelet aggregation and estrogenic activity (Gülçin, 2010; Leonard *et al.,* 2003).

Although it is established that resveratrol exerts beneficial effects on humans and other living beings, its mechanism of action is not well understood. According to Mukherjee

*Corresponding author: Adriana Kuchinski Cavalcante; e-mail: adrianakuchinski@gmail.com

et al. (2010), in low doses resveratrol has anti-apoptotic and cardioprotective function. However, in high doses it presents pro-apoptotic actions, preventing tumor development, but on the other hand, can also cause structural chromosomal aberrations and increase the incidence of myocardial infarction. The functions of the compound tend to indicate that its effects are dose-dependent

It is known that resveratrol is absorbed by the intestine and eliminated rapidly, and the excretion with its metabolites occur with the help of bile and urine (Leonard *et al.,* 2003), however, there is no literature data on the concentration of the compound in the environment. Despite the numerous studies on the health benefits of resveratrol, there is little data in the literature on its toxicity in aquatic organisms, making the present study fundamental for the contribution of new information on the ecotoxicity of resveratrol in the aquatic environment.

Toxicity tests are capable of evaluating environmental impacts, since the knowledge of the contaminants toxicity makes it possible to establish the permissible limits of several chemical substances for the protection of aquatic life (Zagatto & Bertoletti, 2006).

The species *Danio rerio* (zebrafish) is an important animal model used in the areas of developmental biology, genetics and biomedicine (Grunwald & Eisen, 2002) and it is used in ecotoxicological trials through Brazilian and International standards. As zebrafish presents 70% genetic similarity with humans, as well as physiological and anatomical similarities, this model can be used to predict toxicity effects in humans (Hill *et al.,* 2005; Bar-llan *et al.,* 2009). In addition to high reproductive rate and rapid embryonic development (out of the maternal body), the species shows great sensitivity when exposed to chemicals, being able to quickly absorb the compounds that are directly added to the water and accumulate them in various tissues. The organism is easy to obtain, manage and it is low cost (Zagatto & Bertoletti, 2006).

The aim of this study was to evaluate the toxicity of resveratrol in embryos and larvae of *Danio rerio* (zebrafish). For this purpose the *in vitro* cytotoxicity and ecotoxicity assays were performed.

MATERIAL AND METHODS

Chemical

Resveratrol (3, 4', 5- trihydroxy-trans-stilbene) was purchased from Sunrise Chemical Ltd. – China, imported and marketed by Pharma Nostra, Campinas - SP. Resveratrol was extracted from the root of the plant *Polygonum cuspidatum Sieb*, and the companies attest the compound as the standard resveratrol *trans* isomer form with 99.4% purity. All the other chemical compounds used were analytical grade.

Fish Husbandry

Adults zebrafish (*Danio rerio*) are kept in the Special Laboratory of Applied Toxinology (LETA), of the Butantan Institute, in aquariums with reconstituted deionized water, conductivity of 500 µS, pH 7.0 to 7.5, temperature of 28 ± 1 °C, and photoperiod cycle of 14 h light/10 h dark and fed three times daily with commercially available fish food and *Artemia*.

Danio rerio eggs were collected immediately after natural mating and transported to the Ecotoxicology Laboratory of the Chemistry and Environment Center (CQMA) of the Energy and Nuclear Research Institute (IPEN / CNEN-SP). The fertilized eggs were selected under a stereomicroscope. Fertilized eggs were used in the fish embryo toxicity test. To obtain the larvae, the eggs were kept in an incubator at 28 ± 1ºC, with photoperiod cycle of 14 h of light/ 10 h dark and 48 h post fertilization (hpf), hatched larvae were used in short-term chronic ecotoxicity assays.

In vitro cytotoxicity assay

In vitro cytotoxicity assay was performed to determine the half maximal inhibitory concentration (IC_{50} - extract concentration which induces 50% lysis or cell death) of resveratrol following International Standard ISO 10993-5. This assay was performed by the neutral red uptake method. Diluted solutions of resveratrol (6.2%; 12.5%; 25%; 50% and 100%) were placed in contact with NCTC-L929 (CCIAL 020), mouse connective tissue cells, distributed in 96 well microplates. The microplates were supplied by the Nucleus of Cell Cultures of the Adolfo Lutz Institute. In the assay, in addition to cell control, a negative and a positive control were tested. Cell viability was verified by the incorporation of neutral red by the living and intact cells. The optical density reading of the final microplate solution was performed in a spectrophotometer, ELISA reader-SUNRISE, at 540 nm, after cell lysis. The percentage of cell viability was calculated in relation to the control cells and projected on a graphic, as a function of the concentration of resveratrol obtaining a curve that indicated the IC_{50}.

Fish embryo toxicity (FET) test

The assay was based on the OECD 236 (Guideline on Fish Embryo Toxicity Test – FET) (2013), to determine the resveratrol lethal concentration (LC_{50}). Based on the half maximal inhibitory concentration of resveratrol (IC_{50}) obtained in the above test, a preliminary test with zebrafish embryos was performed, the last concentration of resveratrol being 100 mg L^{-1}. From this test, the definitive test was performed.

Sixty eggs per treatment were used and distributed in three 24-well microplates (replicates). Eggs were placed in each well individually with 2 mL of test solution. The treatments used were: control (reconstituted deionized water), solvent control dimethyl sulfoxide (DMSO) and resveratrol test solution at nominal concentrations of 0.5; 1.1; 2.3; 4.8; 10.3; 22; 46.9 and 100 mg L^{-1}. The microplates were maintained in an incubator, in the temperature range of 26 ± 1 °C and photoperiod cycle of 14 h light / 10 h dark, during 96 h.

Test solutions were prepared at the time of the assay by stock solution dilution in reconstituted deionized water. Resveratrol stock solution was prepared by dissolving resveratrol on DMSO and deionized water. The test solutions were renewed 48 h after the start of the assay. Solvent control containneds 15 µL of DMSO, the highest concentration of solvent used in the test.

The organisms were observed by the inverted microscope every 24 h. In the embryonic phase the following parameters were observed: coagulation of fertilized eggs, heartbeats, pigmentation of the eyes and body, formation of somites, tail detachment, hatching and lethality. After hatching, the parameters evaluated in the larvae were: heartbeats, oedemas, spine deformity and lethality. At the end of the test, LC_{50} was determined on the basis of lethality results of organisms.

Short-term chronic ecotoxicity test with fish larvae

The test was performed with adaptations of the norm ABNT NBR 15499 (Short-term chronic toxicity - Fish test method) (2007), to determine the resveratrol lethal concentration (LC_{50}) on zebrafish. The larval less than 24 h after hatching were exposed to different concentrations of resveratrol for a period of 168 h and the effect evaluation was lethality. A preliminary test was performed to determine the concentration range to be used. In this preliminary study, concentrations of resveratrol were: 2.3; 4.8; 10.3; 22; 46.9 and 100 mg L^{-1}, in addition to the control with reconstituted deionized water and solvent control (DMSO). From this test, definitive assay was performed in triplicate (n=3).

The organisms were exposed to the following concentrations of resveratrol: 3.1; 6.2; 12.5; 25; 50 and 100 mg L^{-1}, which were determined by dilution factor 2, as requested by ABNT NBR 15499 (2007). For each resveratrol concentration was used four replicates containing 10 larvae in 250 mL of test solution, prepared by dilution of stock solution (resveratrol on DMSO and deionized water) in reconstituted deionized water. The same proceeding was used for control, utilizing reconstituted deionized water as test solution. In relation to the solvent control, DMSO was used in concentrations of: 2, 4 and 8 mg L^{-1}. The organisms were maintained without feeding, in an incubator at a temperature of 25 ± 2ºC and photoperiod cycle of 14 h light / 10 h dark.

The test solutions were renewed daily, therefore, they were produced daily, as well as the stock solution. The larvae were observed every 24 h during the test, and the lethality was evaluated by the absence of the heartbeats, measured for 2 min using a binocular loupe with a minimum magnification of 80x. At the end of each test, LC_{50} was determined based on the lethality results of the organisms.

Statistical analysis

With the percentage of lethality of the organisms, the estimation of concentrations that had effect on 50% of the exposed organisms was calculated using the non-parametric statistical method Trimmed Spearman-Karber (Hamilton *et al*., 1977). The hatch rates and deformities observed in the organisms during the test were evaluated by Student's *t*-test, to verify if the resveratrol showed statistically significant differences on the organisms in comparison with the control, with significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Information about toxicity data of resveratrol in *Danio rerio* was not available in the literature. Because of that, the discussion was conducted with toxicity information of drugs, metals and insecticides in zebrafish.

In vitro cytotoxicity assay

The results of resveratrol cytotoxicity are presented in the Figure 1. The controls are used to verify the effectiveness of the assay. The sample whose cell viability curve projects above the IC_{50} line is considered non-cytotoxic, as observed in the negative control. However, the sample presenting a cell viability curve below or crossing the IC_{50} line is considered to be cytotoxic, as observed in the positive control. The IC_{50} is estimated at the intersection between the cell viability curve and the line of 50% viability in the graph. Resveratrol had IC_{50} of about 77%, which corresponds to the concentration of 38.5 mg L^{-1}, and the positive control IC_{50} was 68% (34 mg L^{-1}), being 100% corresponding to the concentration of 50 mg L^{-1}, the highest concentration used in the test.

Fish embryo toxicity (FET) test

The fertilized zebrafish eggs were exposed to eight concentrations of resveratrol for 96 h. After 48 h, the control groups (95%) had a normal embryonic development as described by Kimmel et al. (1995). However, in the concentration of 100 mg L^{-1} of resveratrol, observed in 67% of the embryos the lack of pigmentation of the eyes and body

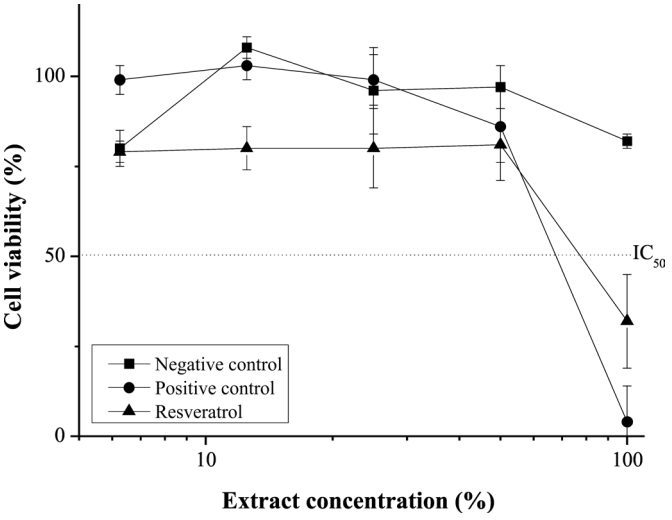

Figure 1: Cell viability curves in the cytotoxicity assay of resveratrol by the Neutral Red Uptake methodology.

(Figure 2) and slow heart rate, representing a statistically significant difference in relation to controls.

Embryos mortality in control was below 10% as required for test validity. Figure 3 shows the lethality curve (%) of the zebrafish in the fish embryo toxicity test of resveratrol (mg L^{-1}). It was observed lethality after 46.9 mg L^{-1} resveratrol concentration and in the lower concentrations the lethality was in the normal range, less than 10%.

The LC_{50} (96h) of resveratrol to zebrafish embryos was 75.3 mg L^{-1}. In study conducted by Damasceno *et al*. (2016), the EC_{50} that is the effective concentration causing immobility in 50% of exposed organisms of resveratrol in *Daphnia similis* was 3.6 mg L^{-1}, what shows that *Daphnia similis* had a more sensitive response than *Danio rerio's* embryos to the resveratrol.

Hatching has been widely used as a parameter in the embryological stage of fish. The percentage of zebrafish hatch observed during the fish embryo toxicity test is presented in the table 1. The test conducted in order to evalute the DMSO toxicity in concentrations used on fish embryo toxicity test was not toxic to zebrafish embryos.

The embryos exposure to resveratrol from 0.5 to 2.3 mg L^{-1} did not show statistically difference in hatching rate in comparison to the control, in the 48 h period. In the concentrations of 100 mg L^{-1} of resveratrol a statistically difference in hatching delay was observed in comparison to the control, in 48 h period.

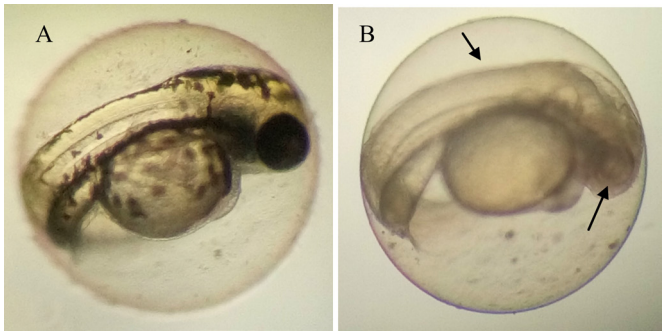

Figure 2: (A) Control with pigmentation of the eyes and body; (B) Embryo without pigmentation of the eyes and body (black arrows) after 48 h of exposition in resveratrol 100 mg L-1.

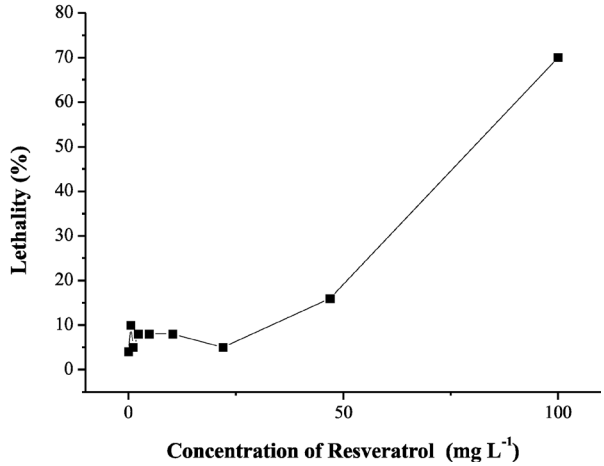

Figure 3: Lethality curve of zebrafish in the embryolarval stage, on relation to resveratrol concentrations after 96 h of exposure. "0*" refers to solvent control.

Table 1- Hatching rate of zebrafish larvae at 48, 72 and 96 h.

Resveratrol (mg L^{-1})	Hatching (%)		
	48 h	72 h	96 h
Solvent Control	37	100	100
0	32	97	100
0.5	63	98	100
1.1	73	100	100
2.3	50	100	100
4.8	35	100	100
10.3	45	98	100
22	28	98	100
46.9	18	84	97
100	7 *	36	76

* Mean significantly different from control (Student´s t-test, $p < 0.05$)

It is known that physical and chemical signals detected by the embryo activate the production of the hatchery-related enzyme (chorionase), which degrades the inner layer of the chorion and allows movements that provide the hatching event (Fuiman, 2002). Different toxic mechanisms may justify delayed hatching or failure, such as induction of abnormal function of the chorionase enzyme and / or the inability of emerging larvae to break eggshells (Hallare *et al.,* 2005; Jezierska *et al.,* 2009).

The organisms hatching process may be dependent on the concentration of resveratrol. The results suggests that resveratrol is able to induce or inhibit the activity of the enzyme chorionase. According to Jezierska *et al.* (2009) some metals may inhibit or accelerate the hatching process of fish. Dave & Xiu (1991) reported the delay in hatching of *Danio rerio* embryos exposed to copper and nickel. The authors mentioned above suggest that copper may inactivate the chorionase enzyme and cause osmotic disorders that can also affect the muscle movements needed to break the egg. The delay of the hatching of *Danio rerio* was also verified in the study of Bai *et al.* (2010); Muller *et al.* (2015) and Oliveira *et al.* (2009). However, premature hatching was observed in the studies of Jesus *et al.* (2013) and Oliveira *et al.* (2013), where there was an increase in the hatch rate of *Danio rerio* exposed to the antiseptic chlorhexidine and the antibiotic amoxicillin.

Deformities and lethality were observed in the organisms exposed to 46.9 and 100 mg L^{-1} during the assay. The observed deformities were: pericardial oedema, oedema of the yolk sac and spine deformity. All deformations were statistically different from the controls.

Short-term chronic ecotoxicity test with fish larvae

Danio rerio larvae were exposed for 168 h to several concentrations of resveratrol. The larvae mortality in control and in solvent control was below 20%, as required for test

validity. In the Figure 4 is shown the mean and standard deviation of the lethality rate (%) according to resveratrol concentrations (mg L^{-1}). It is observed that the rate of lethality increases as the concentration of resveratrol increases.

The table 2 shows the results of short-term chronic ecotoxicity assay of resveratrol on zebrafish larvae.

The mean value of resveratrol LC_{50} (168h) obtained in the short-term chronic ecotoxicity assay performed with zebrafish larvae was 51.4 mg L^{-1}. The result is lower than the LC_{50} (96h) (75.3 mg L^{-1}) obtained in the fish embryo toxicity test. The results show that the larvae stage is considerable more sensitive than the embryonic stage. The difference in the sensitivity of the organisms can be explained by the fact that the embryos when exposed to resveratrol in FET test has the chorion protection, which provided a barrier and reduce the substances exposition.

However, it was verified in FET test a high lethality of organisms (70%) at 100 mg L^{-1} of resveratrol concentration, and in the study performed by Vallverdú-Queralt *et al*. (2015), resveratrol was found in embryos of *Danio rerio* when exposed to the red wine extract, so it can be inferred that the resveratrol crossed the egg chorion and caused effects on the embryos. The recently hatched larvae have no structure to prevent or reduce the exposure to resveratrol in the short-term chronic ecotoxicity test.

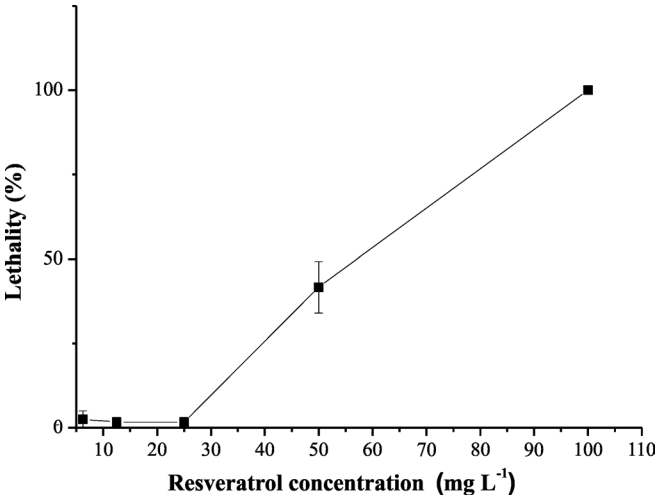

Figure 4: Lethality curve of zebrafish larvae as a function of resveratrol concentrations after 168 h of exposure. Mean and standard deviation (n=3).

Table 2 - Values of the LC_{50}, confidence intervals, mean, standard deviation and variation coefficient for the short-term chronic ecotoxicity assay of resveratrol in zebrafish (n=3).

Replica	LC50 (mg L^{-1})	Confidence limit (mg L^{-1})
1	52.7	(46.7 – 59.4)
2	49.7	(44.3 – 55.7)
3	51.7	(46 – 58.2)
Mean	**51.4**	
Standard deviation	1.5	
Variation coefficient (%)	0.03	

At concentrations of 50 and 100 mg L^{-1} were observed deformities in the organisms and lethality during the assay. The observed deformities were: pericardium oedema, oedema of the yolk sac and a spine deformity showns in figure 5. Such deformities were observed by several authors who studied the toxicity of drugs and some metals: Akande *et al*. (2010); Li *et al*. (2017); Oliveira *et al.* (2009); Oliveira *et al.* (2013); Tesolin *et al*. (2014); Tokunaga *et al.* (2016). All deformations were statistically different from the controls.

Oedema has been one of the malformations most observed in toxicity studies with *Danio rerio* (Tesolin *et al.,* 2014). The presence of pericardial oedema is due to cardiac dysfunction, functioning as an indicator of osmotic or metabolic dysfunction, frequently associated with extravasations of the endothelial vessels (Dejana *et al.,* 2009; Hallare *et al.,* 2005; Ali *et al.,* 2014) or it is due to renal dysfunction, since the regulation system depends on renal involvement (Coelho, 2004).

Spine deformations can be linked with the depletion or deregulation of ions such as calcium and phosphorus, or with reduction of myosin, both necessary for the normal development of the organism (Cheng *et al.,* 2000).

The larvae with deformities showed less activity compared to the control larvae. In the study by Suvarchala & Philip (2016) with insecticide 3,5,6-Trichloro-2-pyridinol (TCP), larvae of *Danio rerio* presented the same deformities observed in this study, as well as altered swimming behavior. These same effects were observed by Lutte (2015) who studied the metabolism of adenosine in a model of fetal alcohol syndrome in *Danio rerio.*

In this study, the deformities observed in the *Danio rerio* exposed to resveratrol were similar in all trials performed, as these deformities were similar to the toxicity tests of drugs, some metals and insecticides.

Crowell *et al.* (2004) conducted a study in rats to verify the toxicity of resveratrol. At the high dose administration (3000 mg/kg/day) adverse effects were observed, such as weight loss and changes in haematological parameters. In another study, with healthy volunteers, a single dose of resveratrol was given at 4 different concentrations: 0.5; 1; 2.5 and 5 g. No adverse effects were observed in volunteers (Boocock *et al*., 2007). According to Gillespie & Lenz (2012), studies suggest that oral doses of up to 1 g per day of resveratrol in humans are well tolerated. Gastrointestinal discomfort and diarrhea were

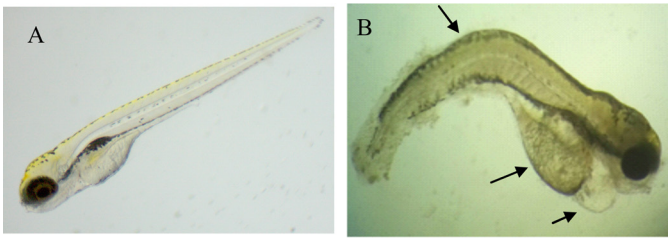

Figure 5: Deformities in zebrafish larvae after exposure to resveratrol during 96 h: (A) Control in the period of 96 h; (B) Larvae at the concentration of 50 mg L-1 with oedema of pericardium, oedema of yolk sac and spine deformity in the period of 96 h (black arrows).

observed with oral administrations of the compound at doses greater than 2.5 g per day

CONCLUSION

Resveratrol is largely present in a variety of foods and beverages, and that there is no data in the literature on its concentration in the aquatic environment, the knowledge of its toxicity is highly necessary. This study provided data about resveratrol toxicity in embryolarval stage on *Danio rerio* fish. The toxic effects of resveratrol in *Danio rerio* are dependent on its concentration, presenting a toxic effect similar to other compounds found in the literature. Resveratrol may have an effect on aquatic biota, which propose on a necessity toassess the disposal of this compound in water bodies.

ACKNOWLEDGEMENTS

The author thanks CAPES for the scholarship which enabled the project realization.

REFERENCES

ABNT (Associação Brasileira de Normas Técnicas). 2007. Ecotoxicologia aquática– Toxicidade crônica de curta duração – Método de ensaio com peixes NBR 15499, Rio de Janeiro RJ.

AKANDE, M.G., ORN, S. & NORRGREN, L. 2010. Evaluation of the toxic effects of clozapine in zebra fish (*Danio rerio*) embryos with the fish embryo toxicity test. IJPBR., 1(4): 90-94.

ALI, S., AALDERS, J. & RICHARDSON, M.K. 2014. Teratological effects of a panel of sixty water-soluble toxicants on zebrafish development. Zebrafish, 11(2): 129-141. http://dx.doi.org/10.1089/zeb.2013.0901

BAI, W., TIAN, W., ZHANG, Z., HE, H., MA, Y., LIU, N. & CHAI, Z. 2010. Effects of copper nanoparticles on the development of zebrafish embryos. J. Nanosci. Nanotechnol., 10(12): 8670-8676. http://dx.doi.org/10.1166/jnn.2010.2686

BAR-LLAN, O., ALBRECHT, R.M., FAKO, V.E. & FURGESON, D.Y. 2009. Toxicity assessment of multisized gold and silver nanoparticles in zebrafish embryos. Small, 5(16):1897-910. http://dx.doi.org/10.1002/smll.200801716

BOOCOCK, D.J., FAUST, G.E.S., PATEL, K.R., SCHINAS, A.M., BROWN, V.A., DUCHARME, M.P., BOOTH, T.D., CROWELL, J.A., PERLOFF, M., GESCHER, A.J., STEWARD, W.P. & BRENNER, D.E. 2007. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agente. Cancer Epidemiol. Biomark. Prev., 16(6): 1246-1252. http://dx.doi.org/10.1158/1055-9965.EPI-07-0022

CHENG, S.H., WING, K.W.A., HUNG, S.C. & SHIU, S.W.R. 2000. Cellular and molecular basis of cadmium-induced deformities in zebrafish embryos. Environ. Toxicol. Chem., 19(12): 3024-3031. http://dx.doi.org/10.1002/etc.5620191223

COELHO, E.B. 2004. Mecanismos de formação de edemas. Medicina Ribeirão Preto, 37(3): 189-198. http://dx.doi.org/10.11606/issn.2176-7262.v37i3/4p189-198

CROWELL, J.A., KORYTKO, P.J., MORRISEY, R.L., BOOTH, T.D. & LEVINE, B.S. 2004. Resveratrol-associated renal toxicity. Toxicol. Sci., 82(2): 614-619. http://dx.doi.org/10.1093/toxsci/kfh263

DAMASCENO, K.C., MAZIERO, J., KUSCHINSKY, A., MAMEDE, F.C.S., MARTINI, G.A., ROGERO, S.O. & ROGERO, J.R. 2016. Avaliação ecotoxicológica do Resveratrol em *Daphnia similis*. In: III Simpósio de Ecotoxicologia, 2016, Rio Claro. Annals…Rio Claro, 2016. p. 104.

DAVE, G. & XIU, R. 1991. Toxicity of mercure, copper, nickel, lead, and cobalt to embryos and larvae of zebrafish, *Brachydanio rerio*. Arch. Environ. Contam. Toxicol., 21(1):126-134. http://dx.doi.org/10.1007/BF01055567

DEJANA, E., TOURNIER-LASSERVE, E. & WEINSTEIN, B.M. 2009. The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications. Dev. Cell., 16(2): 209-221. http://dx.doi.org/10.1016/j.devcel.2009.01.004

FERNÁNDEZ-MAR, M.I., MATEOS, R., GARCÍA-PARRILHA, M.C., PUERTAS, B. & CANTOS-VILLAR, E. 2012. Bioactive compounds in wine: Resveratrol, hydroxytyrosol and melatonin: A review. Food Chem., 130(4): 797-813. http://doi.org/10.1016/j.foodchem.2011.08.023

FUIMAN, L.A. 2002. Special considerations of fish eggs and larval. In: Fuiman, L.A. & Werner, R.G. (ed), Fishery Science: The unique contributions of early life stage. Oxford: Blacwell Science, pp.1-32.

GILLESPIE, N.D. & LENZ, T.L. 2012. Resveratrol: Potential benefits on aging and heart disease. Am. J. Lifestyle Med., 6(5): 390-392. http://dx.doi.org/ 10.1177/1559827612448314

GRUNWALD, D.J. & EISEN, J.S. 2002. Headwater of the zebrafish-emergence of a new model vertebrate. Nat. Rev. Genet., 3(9): 717-724. http://dx.doi.org/10.1038/nrg892

GÜLÇIN, I. 2010. Antioxidant properties of resveratrol: A structure-activity insight. Innov. Food Sci. Emerg. Technol., 11(1): 210-218. http://dx.doi.org/10.1016/j.ifset.2009.07.002

HALLARE, A.V., SCHIRLING, M., LUCKENBACH, T., KÖHLER, H.R. & TRIEBSKORN, R. 2005. Combined effects of temperature and cadmium on developmental parameters and biomarker responses in zebrafish (*Danio rerio*) embryos. J. Therm. Biol., 30(1): 7-17. http://dx.doi.org/10.1016/j.jtherbio.2004.06.002

HAMILTON, M.A., RUSSO, R.C. & THURSTON, R.V. 1977. Trimmed Spearman-Karber Method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol., 11(7): 714-719. http://dx.doi.org/10.1021/es60130a004

HILL, A.J., TERAOKA, H., HEIDEMAN, W. & PETERSON, R.E. 2005. Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol. Sci., 86(1): 6-19. http://dx.doi.org/10.1093/toxsci/kfi110

ISO (International Organization for Standardization). 2009. Biological evaluation of medical devices, Part 5, Tests for in vitro cytotoxicity, Switzerland, 10 993-5.

JEANDET, P., DOUILLET-BREUIL, A.C., BESSIS, R., DEBORD, S., SBAGHI, M. & ADRIAN, M. 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J. Agric. Food Chem., 50(10): 2731-2741. http://dx.doi.org/10.1021/jf011429s

JESUS, F.T., OLIVEIRA, R., SILVA, A., CATARINO, A.L., SOARES, A.M., NOGUEIRA, A.J. & DOMINGUES, I. 2013. Lethal and sub lethal effects of the biocide chlorhexidine on aquatic organisms. Ecotoxicology, 22(9): 1348-1358. http://dx.doi.org/10.1007/s10646-013-1121-6

JEZIERSKA, B., LUGOWSKA, K. & WITESKA, M. 2009. The effects of heavy metals on embryonic development of fish (a review). Fish Physiol. Biochem., 35(4): 625-640. http://dx.doi.org/10.1007/s10695-008-9284-4

KIMMEL, C.B., BALLARD, W.W., KIMMEL S.R., ULLMANN,

B. & SCHILLING, T.F. 1995. Stages of embryonic development of zebrafish. Dev. Dyn., 203(3): 253-310. http://dx.doi.org/10.1002/aja.1002030302

LEONARD, S.S., XIA, C., JIANG, B.H., STINEFELT, B., KLANDORF, H.; HARRIS, G.K. & SHI, X. 2003. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochem. Biophys. Res. Commun., 309(4): 1017-1026. http://dx.doi.org/10.1016/j.bbrc.2003.08.105

LI, K., WU, J.Q., JIANG, L.L., SHEN, L.Z., LI, J.Y., HE, Z.H., WEI, P., LV, Z. & HE, M.F. 2017. Developmental toxicity of 2,4-dichlorophenoxyacetic acid in zebrafish embryos. Chemosphere, 171: 40-48. http://dx.doi.org/10.1016/j.chemosphere.2016.12.032

LUTTE, A.H. 2015. Avaliação do metabolismo de adenosina em um modelo de síndrome alcoólica fetal em zebrafish (*Danio rerio*). MSc. Dissertation. Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, 81p.

MUKHERJEE, S., DUDLEY, J.I. & DAS, D.K. 2010. Dose-dependency of resveratrol in providing health benefits. Dose Response, 8(4): 478-500. http://dx.doi.org/10.2203/dose-response.09-015.Mukherjee

MULLER, E.B., LIN, S. & NISBET, R.M. 2015. Quantitative adverse outcome pathway analysis of hatching in zebrafish with CuO nanoparticles. Environ. Sci. Technol., 49(19): 11817-11824. http://dx.doi.org/10.1021/acs.est.5b01837

OECD (Organization for Economic Co-operation and Development). 2013. Guideline for the testing of chemicals. Fish embryo acute toxicity (FET) test Nº 236, Paris.

OLIVEIRA, R., DOMINGUES, I., GRISOLIA, C.K. & SOARES, A.M. 2009. Effects of triclosan on zebrafish early-life stages and adults. Environ. Sci. Pollut. Res. Int., 16(6): 679-688.http://dx.doi.org/10.1007/s11356-009-0119-3

OLIVEIRA, R., McDONOUGH, S., LADEWIG, J.C.L., SOARES, A.M.V.M., NOGUEIRA, A.J.A. & DOMINGUES, I. 2013. Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (*Danio rerio*). Environ. Toxicol. Pharmacol., 36(1): 903-912. http://dx.doi.org/10.1016/j.etap.2013.07.019

SUVARCHALA, G. & PHILIP, G.H. 2016. Toxicity of 3,5,6-trichloro-2-pyridinol tested at multiple stages of zebrafish (*Danio rerio*) development. Environ. Sci. Pollut. Res. Int., 23(15): 15515-15523. http://dx.doi.org/10.1007/s11356-016-6684-3

TESOLIN, G.A.S., MARSON, M.M., JONSSON, C.L., NOGUEIRA, A.J.A., FRANCO, D.A.S., ALMEIDA, S.D.B., MATALLO, M.B. & MOURA, M.A.M. 2014. Avaliação da toxicidade de herbicidas usados em cana-de-açúcar para o Paulistinha (*Danio rerio*). Mundo Saúde, 38(1): 86-97. http://dx.doi.org/10.15343/0104-7809.20143801086097

TOKUNAGA, S., WOODIN, B.R. & STEGEMAN, J.J. 2016. Plant lignan secoisolariciresinol suppresses pericardial edema caused by dioxin-like compounds in developing zebrafish: Implications for suppression of morphological abnormalities. Food Chem. Toxicol., 96(1): 160-166. http://dx.doi.org/10.1016/j.fct.2016.07.012

VALLVERDÚ-QUERALT, A., BOIX, N., PIQUÉ, E., GÓMEZ-CATALAN, J., MEDINA-REMON, A., SASOT, G., MERCADER-MARTÍ, M., LLOBET, J.M. & LAMUELA-RAVENTOS, R.M. 2015. Identification of phenolic compounds in red wine extract samples and zebrafish embryos by HPLC-ESI-LTQ-Orbitrap-MS. Food Chem., 181(1): 146-151. http://dx.doi.org/10.1016/j.foodchem.2015.02.098

VANETTEN, H.D., MANSFIELD, J.W., BAILEY, J.A. & FARMER, E.E. 1994. Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". Plant Cell., 6(9): 1191-1192. http://dx.doi.org/10.1105/tpc.6.9.1191

VUONG, T.V., FRANCO, C. & ZHANG, W. 2014. Treatment strategies for high resveratrol induction in *Vinis vinifera L.* cell suspension culture. Biotechnol. Rep. (Amst)., 1(2): 15-21. https://doi.org/10.1016/j.btre.2014.04.002

ZAGATTO, P.A. & BERTOLETTI, E. 2006. Ecotoxicologia aquática – Princípios e Aplicações. Rima, São Carlos, SP. 478p.