EFFECT OF EXPOSURE TO GLYPHOSATE BASED HERBICIDE - ROUNDUP ORIGINAL® - AND NUTRITIONAL THERAPY WITH FOLIC ACID AND SELENIUM ON CARDIAC HISTOGENESIS OF BULLFROG (*LITHOBATES CATESBEIANUS*, SHAW - 1802)

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Resumo

Os anfíbios são suscetíveis à toxicidade, devido à absorção cutânea dos produtos químicos presentes no ambiente. O uso de herbicidas à base de glifosato é comum e pode ser transportado pelas águas superficiais para os locais de reprodução. O glifosato possui propriedades oxidantes e inibe a ação de enzimas-chave na regulação do ciclo celular. O ácido fólico e o selênio possuem propriedades antioxidantes, que podem reduzir o estresse oxidativo gerado por pesticidas. Ovos de rã-touro (n = 47) foram mantidos em aquários com aeração constante, temperatura controlada (25 ± 2 ° C) e com ciclo claro e escuro de 12/12 horas. Os ovos foram divididos em: Grupo Controle (GC, n = 12), Grupo Exposto ao Roundup Original® (GR, n = 11); grupo tratado com ácido fólico exposto ao Roundup Original® (GAFR, n = 12) e Grupo tratado com selênio exposto ao Roundup Original® (GSR n = 12). As larvas foram mantidas em condições experimentais durante os três primeiros dias de desenvolvimento e depois foram realocadas para aquários com água desclorinada. Após 12 dias, os girinos tiveram sua massa e comprimento aferidos, anestesiados por hipotermia e fixados para o preparo histológico. Os animais expostos ao herbicida exibiram diminuição da massa corporal, comprimento e redução no diâmetro dos cardiomiócitos, enquanto os animais tratados com ácido fólico e selênio não apresentaram alterações nas medidas corporais nem nas trabéculas do tecido cardíaco. Os resultados mostram que o glifosato tem efeitos prejudiciais ao desenvolvimento da rã-touro e a suplementação periconcepcional com antioxidantes pode mitigar danos.

Palavras-chave: Anfíbio; Estresse oxidativo, Toxicologia do desenvolvimento, Histopatologia

Abstract

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Amphibians are susceptible to toxicity, due to the easy cutaneous absorption of chemicals present in the environment. Using of glyphosate-based herbicides is very common and becomes susceptible to be carried by surface waters to breeding sites. Glyphosate has oxidizing properties and inhibits the action of key enzymes for the regulation of the cell cycle. Folic acid and selenium have antioxidant properties, which may help to reduce oxidative stress generated by contact with pesticides. Bullfrog eggs (n = 47) were kept in aquariums with constant aeration and controlled temperature (25 ± 2 ° C) with a light and dark cycle of 12/12 hours. Subjects were divided in Control Group (CG, n = 12), Group Exposed to Roundup Original® (GR, n = 11); group Treats with Folic Acid Exposed to Roundup Original® (FARG, n = 12) and Group Treated with Selenium Exposed to Roundup Original® (SRG n = 12). Larvae were maintained under experimental conditions during the first three days of development and then relocated to aquariums with dechlorinated water. After 12 days, tadpoles had their mass and length measured, then were anesthetized by hypothermia and fixed to histological preparation. Animals exposed to herbicide exhibited decreased body mass, length, and reduction in cardiomyocyte diameter, while animals treated with folic acid and selenium showed no changes in body measurements neither in trabeculae of heart tissue. Results show that glyphosate has detrimental effects on the development of bullfrog tadpoles and periconceptional supplementation with antioxidants can mitigate damages.

Keywords: Amphibian; Oxidative Stress, Developmental Toxicology, Histopathology.

EFFECT OF ROUNDUP ORIGINAL® ON BULLFROG CARDIAC HISTOGENESIS

1. INTRODUCTION

Amphibians play a variety of ecological functions, being important energy and matter carriers between aquatic and terrestrial ecosystems as they travel from one to another, and act as key species in diverse communities (MURPHY et al., 2000). Due to its anatomy and physiology adapted to skin breathing made possible by the highly vascularized thin integument and development dependent on aquatic environments, this group became highly sensitive to both natural and anthropogenic environmental disturbances (DUELLMAN and TRUEB, 1994). Due to many environmental requirements to develop, amphibians have suffered a drastic reduction in species abundance and richness in recent years. Habitat fragmentation, solar radiation, diseases, and water bodies contamination by pesticides are factors that have a great impact on these animal populations (ARNTZEN, 2017).

Currently, herbicide use is a recurring practice around the world. When used without adequate control, they are leached and deposited in water bodies that may be important nurseries for amphibian species (THOMPSON et al., 2004). Glyphosate is a widely used herbicide, with broad-spectrum and high-efficiency (DUKE and POWLES, 2008). It is absorbed by leaves of plants and acts on several enzymatic complexes blocking the synthesis of some amino acids (AMRHEIN et al., 1980). Acute exposure to glyphosate is considered from low toxicity but interferes with some enzymatic pathways, reducing the activity of enzymes that regulates the synthesis of sex steroids and blocking enzymes that act in cell cycle regulation which may alter embryonic development (DEFARGE, 2016; TOWNSEND et al., 2017). However, in 2015 the International Agency for Research on Cancer (IARC)

classified glyphosate as a probably carcinogenic substance (TARAZONA et al., 2017) such as Brazilian National Agency of Sanitary Surveillance (ANVISA) also has altered glyphosate classification to possibly carcinogenic based on recent researches (ANVISA, 2017).

In the context of the threat of amphibian populations, antioxidants have the potential to improve the survival rate and mass gain in tadpoles by being able to reduce oxidative stress (SZUROCZKI et al., 2016). Among them, folic acid stands out because it plays an important role in DNA synthesis, repair, and methylation. This vitamin has antioxidant properties that can protect the body from free radicals and consequently avoid oxidative stress and lipid peroxidation (JOSHI et al., 2001). Also, it is important to emphasize its protective activity against the malformation of the neural tube (WALD et al. 1991) and acts in the prevention of heart disease (WELCH et al., 1998). Like folic acid, selenium is another important nutrient in the diet (HAMILTON et al., 2004) and plays important role in maintaining the metabolism of the immune and endocrine systems (ARTHUR et al., 2003). Selenium is also part of the composition of important enzymes of redox metabolism, acting as a protector against oxidative stress (BURK and HILL, 1993).

This study aimed to analyze acute exposure effect of glyphosate-based herbicide - Roundup Original® - on bullfrog histogenesis, evaluating possible histopathological changes in cardiac tissue generated by exposure during early development, and to evaluate whether nutritional therapy with folic acid or selenium is capable of attenuating or inhibiting its possible teratogenic effects.

2. MATERIAL AND METHODS

2.1 *Nutritional therapy*

To investigate if folic acid and selenium have protective effects on the development of amphibian embryos heart, the supplement should be available to developing myocytes, when differentiation and the heartbeat onset depends exclusively on yelk accumulated in the oocyte. Therefore, to ensure the presence of antioxidants in yelk composition, we feed the female frogs in reproductive age with feed enriched with these nutrients, based on the method proposed by Dugas and coworkers (2013), where adult frogs (*Oophaga pumilio*) were fed with flies supplemented with carotenoids. The result of this study showed that supplementation improved the reproductive success of these animals due to the improvement in the quality of the eggs generated during reproduction.

Some commercial food used for bullfrog growth are already enriched with folic acid (0.750 $mg.kg^{-1}$) and selenium (0.225 $mg.kg^{-1}$) (PAP, 2017), however, due to restricted supply of this feed type, many ranches use commercial feed formulated for carnivorous fish, such as Supra Carnivorous 40® (Supra Alimentos S/A), which already has in its composition 1 $mg.kg^{-1}$ of folic acid and 0.3 $mg.kg^{-1}$ of selenium being this, the ration used by the ranch that worked in collaboration with the research group.

As the objective of this study was to ensure that folic acid and selenium were incorporated into the yelk, we chose to work with high doses in the diet of adult frogs, thus ensuring that during the formation of yelk at the moment preceding the fertile period, supplement in high concentration in maternal organism be incorporated into developing oocytes. Vitellogenesis is the process by which the vitellogenin that was synthesized in the liver and carried to the ovary by the female's bloodstream, is incorporated into the oocyte by micropinocytosis. As pinocytosis is not a selective process, substances that are in high concentrations in maternal fluids end up being internalized to the forming oocytes (GILBERT, 2010).

The folic acid dose was determined according to therapy used to evaluate the benefits of B vitamins in humans with cancer, where the daily dose of 0.8 mg of folic acid raised serum folate levels in the blood of 3.9 to 29.9 $ng \cdot mL^{-1}$ (EBBING et al., 2009). As commercial ration already contains 1 $mg.kg^{-1}$ of folic acid, 159 mg of folic acid was added per kilogram of animal ration used for the treatment of females. Group treated with selenium, received a ration enriched with 2 $mg \cdot Kg^{-1}$ of sodium selenite. The Control group was fed with the commercial formulation.

2.2 *Animal Handling*

The protocol for use of animals in experiment number 01/2012 was endorsed by the Ethics Committee on Use of Animals of UFSJ - CEUA / UFSJ at a meeting held on March 30, 2012. Experiments were performed with bullfrog eggs (*Lithobates catesbeianus*) which were supplied by *RANAMIG Ranch* – Betim - Brazil. One reproductive female was fed with commercial ration, another was fed with folic acid-enriched ration and a third matrix was fed with selenium-enriched ration. Eggs from these animals were transported to laboratory accommodated inside sealed plastic bags filled with water and aeration to maintain oxygenation inside a thermal box.

Eggs were kept in aquariums with a volume of 5 liters in agreement with the maximal density of 40 tadpoles per liter, used in other works with tadpoles in early stages of development (COOK, 2011).

2.3 *Exposure to Roundup original®*

Each aquarium received a 5 mg dose of Roundup Original® obeying final concentrations of 1 $mg.L^{-1}$. This is a sub-lethal dose (COSTA et al., 2008) and simultaneously is a concentration found in water bodies of agricultural areas in Brazil (0.49-3.75 $mg.L^{-1}$) (LIMA et al., 2015).

2.4 *Experimental exposure*

Eggs were divided into four experimental groups: Control Group (CG, n = 12), Group exposed to Roundup Original® (RG, n = 11), Folic Acid Treated Group and exposed to Roundup Original® (FARG, n = 12) Selenium Treated Group and exposed to Roundup Original® (SRG, n = 12). CG eggs were kept in aquariums with dechlorinated water during the entire experimental period. RG, FARG, and SRG eggs were kept in aquariums with dechlorinated water with a dilution of Roundup Original® (1 $mg \cdot L^{-1}$). Aquariums of all experimental groups received constant aeration and had controlled temperature (25 ± 2 °C) with a light and dark cycle of 12/12 hours. RG, FARG, and SRG groups were maintained under the experimental conditions mentioned above during the first three days of development and were then relocated to aquariums with dechlorinated water. Hatched tadpoles were fed with commercial ration given once daily, *ad libitum* for 12 days for growth, after which they were anesthetized by hypothermia and euthanized.

2.5 *Morphometry*

Living animals had their measurements of mass (in milligrams) and length (in millimeters) measured using a precision analytical balance and pachymeter. Results obtained were expressed as means ± standard deviation of the mean (S.D.).

2.6 *Histological preparation*

Tadpoles were fixed whole due to their small size, which makes difficult dissection and identification of internal organs, after immersion in the Clark solution (95% Ethanol and glacial acetic acid 3: 1) for 2 hours at 20 ° C. Fixing solution was removed, and the material was stocked in 70% alcohol. Then tadpoles were progressively dehydrated and underwent diaphanization and infusion with

liquid paraffin heated at 65 ° C for 12 hours, and then placed in appropriate inclusion molds. Paraffin blocks containing the samples were cut in a microtome (Leica® RM2235), where thin sections of 7 µm thickness were made. Slices were stained with hematoxylin and eosin. Images were analyzed under an optical microscope with aid of the software Motic Plus 2.0 and were measured the mean diameter of trabeculae of uncompacted cardiac tissue.

2.7 *Statistical analysis*

Morphological characteristics were quantified and presented as means ± standard deviation (S.D.) and subsequent comparison of the values obtained in different experimental protocols One - way analysis of variance (ANOVA) with Dunnet 's posthoc was performed to compare the values obtained from different experimental groups compared to each other. All statistical tests were performed using GraphPad Instat software version 3.00 (GraphPad Software, San Diego, CA, USA). Only the differences at the 5% level of significance (P <0.05) were considered.

3. RESULTS

Mean values of total mass (TM) of bullfrog tadpoles are represented in the graph below (Figure 1. A). A significant reduction in mass was observed only in RG (8.1 mg ± 0,43) when compared to CG (15 mg ± 0.29), FARG (16.3 mg ± 0.36) or SRG (14.9 mm ± 0.51), however, there was no significant increase neither reduction in mass of tadpoles from FARG and SRG when compared to CG, demonstrating that there were deleterious effects in body mass acquisition of the tadpoles that were exposed to the commercial formulation of glyphosate but not in those that received nutritional therapy. The mean values of the body length (BL), from the rostrum to the tip of the tail of the bullfrog tadpoles are also represented in the graph below (Figure 1. B). Significant reduction in length was observed only in the group exposed to glyphosate (6.4 mm ± 0.75) when compared to CG (10 mm ± 0.58). There was no significant difference between FARG (10.3 mm ± 1.06) and CG or between SRG (8.8 mm ± 0.35) and CG.

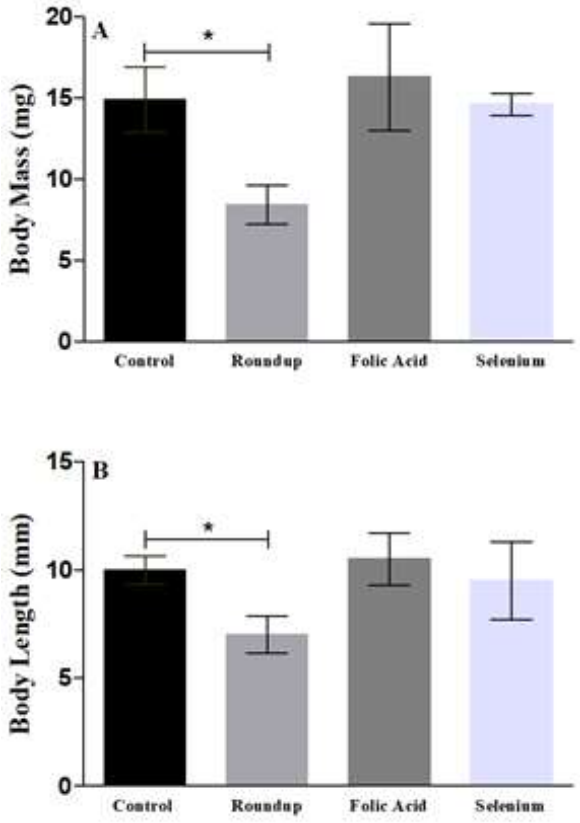

Figure 1. Mean of the Body Mass (MM) (n = 47) of the Control Group (CG, n = 12), Group exposed to Roundup Original® (RG, n = 11) and Group Treated with Folic Acid and exposed to Roundup Original® (FARG, n = 1 2) 6 146 and Selenium Treated Group and exposed to Roundup Original® (SRG, n = 12) (A). Mean length (ML) CG, (n = 12), RG (n = 12), FARG (n = 12) and SRG (n = 12) (B). * indicates a significant difference between treatments with 95% confidence. Values of the mean of the length ± S.D.M

The morphometric analysis of the cardiac tissue (Figure 2) demonstrated a

significant reduction in the mean diameter of the trabeculae in the RG (2.65 µm ± 0.28), whereas the FARG (8.2 µm ± 1,22) and SRG (6,4 µm ± 0,85) groups showed a significant increase in the mean diameter of trabeculae concerning the control group (3.9 µm ± 0.16) (Figure 3).

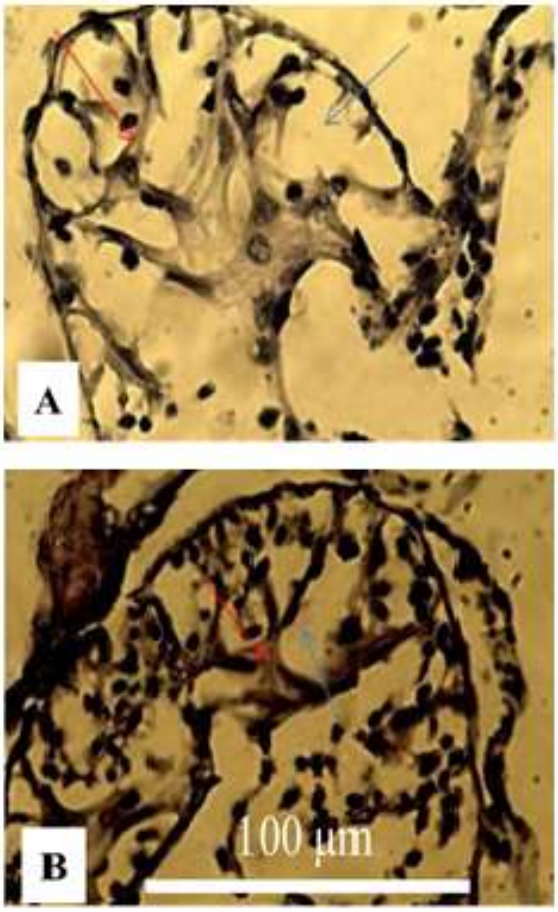

Figure 2. Photomicrographs of the spongy ventricular tissue of the experimental groups. CG: A and GR B. 400x magnification. Trabeculae indicated with red arrows and inter-trabecular spaces with blue arrows.

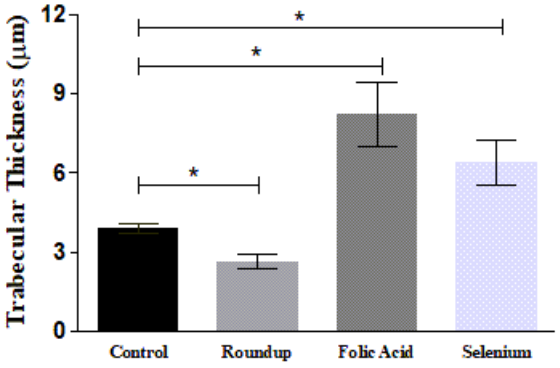

Figure 3. Mean trabeculae Thickness (TT) (n = 47) from the Control Group (CG, n = 12), Group exposed to Roundup Original® (RG, n = 11) and Group Treated with Folic Acid and exposed to Roundup Original® (FARG, n = 12) and Selenium Treated Group and exposed to Roundup Original® (SRG, n = 12). * indicates a significant difference between treatments with 95% confidence. Values of the mean of the length ± S.D.M.

The analyzes also showed a significant increase in the mean diameter of the empty spaces between the trabeculae (figure 4) in the RG (23 µm ± 0.57) when compared with CG (18 µm ± 2.02), while the FARG and SRG groups showed a significant reduction in the mean diameter of these spaces to the control group (14 µm ± 0.1 and 15 µm ± 0.13 respectively), suggesting that antioxidants protected animals against the teratogenic herbicidal effect.

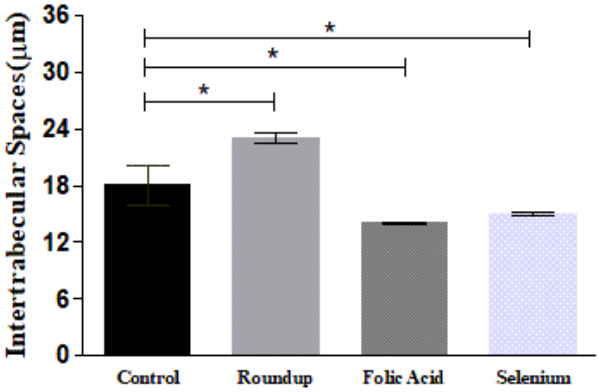

Figure 4. Mean thickness of intertrabecular spaces (IS) (n = 47) from the Control Group

(CG, n = 12), Group exposed to Roundup Original® (RG, n = 11) and Group Treated with Folic Acid and exposed to Roundup Original® (FARG, n = 12) and Selenium Treated Group and exposed to Roundup Original® (SRG, n = 12). * indicates a significant difference between treatments with 95% confidence. Values of the mean of the length ± S.D.M.

4. DISCUSSION

Aquatic environments are most susceptible to the contamination that is related to dumping of domestic, industrial, and agricultural wastes, which are carried primarily by surface waters to other aquatic environments. Today, the economic model and the human population lifestyle have strongly contributed to the contamination of water bodies with toxic waste. In rural areas, pesticides used in agriculture have been responsible for much of aquatic environments pollution where they deposit in the sediment, continue in suspension, or be absorbed by non-target organisms (VOULVOULIS et al., 2016). Roundup Original® is a glyphosate-based herbicide used to control terrestrial and aquatic weeds. The Corporation that produces it, indicates the direct application in aquatic environments in the concentration of 3.7 $mg{\cdot}L^{-1}$ to control of aquatic macrophytes (Roundup WG, 2014), which further aggravates the aquatic contamination problem.

Commercial formulations are presented as a 48% dilution and pulverization indication is on average 5 $L{\cdot}ha^{-1}$ (Roundup WG, 2014). Glyphosate's molecular formula is $C_3H_8NO_5P$. It is a polar solid with high solubility in water and low solubility in organic solvents. It has a density of 0,5 g. $(cm^3)^{-1}$ and does not undergo photodegradation (DUKE AND POWLES, 2008).

Initially, its application was restricted to the moments before sowing the crop or after, to avoid contact with crops. Creation and patenting of glyphosate-resistant plant strains by the same company which produces it has allowed them to be sprayed at any time, which makes its use indiscriminate (GREEN, 2016). In contact with soil, it forms interactions with metals and humic acids adhering to soil particles, remaining there for a period ranging from thirty to ninety days (KUMARI et al., 2016). Acute exposure to glyphosate is considered to be of low toxicity by manufacturers, however, several studies show that this herbicide may have pathological effects on animals. Besides, the offspring of Wistar rats exposed to glyphosate during gestation presented endocrine disruptions, behavioral changes, and defects in the histological structure of the gonads (ROMANO, et al., 2012).

The heart is the first organ to become functional in the embryo. Initially, it is a contorted S-shaped tubular muscular organ capable of contracting. It is formed by an atrium and a ventricle that throughout the development have separate chambers and valves formed. The heart, as well as vessels and blood cells, originate in the mesodermal tissues of the lateral plaque. In amphibians, the cardiogenic regions are in the anterior part of the mesodermal blanket (MOHUN, 2000).

In 1960 Gosner synthesized the stages of development of tadpoles into 46 stages, beginning in fertilization and ends with a complete metamorphosis. When the animal reaches stage 19 heart is a longitudinal tube made up of arterial cone, ventricle, and sino-auricular complex and in the next stage (20) it begins to beat. In stage 22 heart is ventrolateral repositioned and posterior to the pharynx. In stage 23, valves of conus arteriosum and conus septum differ from the endothelial line of conus arteriosum at the bifurcation of the aortic trunk. From stage 25 heart does not transform until complete metamorphosis occurs (MCDIARMID AND ALTIG, 1999).

Folic acid (FA) is a soluble vitamin B complex and has antioxidant properties. FA

also acts in the formation and maintenance of new cells due to its action in the prevention of inadequate DNA synthesis. A deficiency is associated with embryo bad formation and the development of neoplastic and pre-neoplastic cells. FA is important for the stabilization of a cofactor of the endothelial NO synthase enzyme (eNOS), responsible for the synthesis of nitric oxide (NO). Besides, NO can react with reactive oxygen species (ROS), reducing oxidative stress, and promoting protective effect in cells (FERREIRA et al., 2010; MOENS et al., 2008).

Selenium (Se) is an element with similar properties to sulfur and is part of the composition of selenoproteins such as glutathione peroxidase (GPx), important for the protection of tissues against the harmful effects of ROS. Selenium deficiency is also associated with the incidence of cancer, vascular changes, and reproductive deficit (DOS REIS, 2017).

Cyclin-dependent kinases (CDK's) are monomeric proteins that are inactive in the cell until they bind to a cyclin. Activation of this enzymatic complex is responsible for the phase's progression in the cell cycle. During interphase, the association of CDKs with cyclins occurs through phosphorylation, and then cell enters mitosis. Then the complex dissociates and the cell enters interphase again. In higher eukaryotes, cyclin B regulates the mitotic phase beginning, which in association with phosphorylated CDC2, which is renamed CDK1. After association occurs the formation of active mitotic promoter factor (MPF) which allows cells to enter into mitosis (TERZOUDI, et al., 2000).

Glyphosate may interfere with some enzymatic pathways, such as reducing the activity of aromatase, the enzyme responsible for estrogen synthesis (DEFARGE, 2016) in addition to reducing the activity of the CDK1/cyclin B complex, besides disrupting the general synthesis of proteins, which may alter cell cycle regulation and embryonic development (MARC et al, 2002). CDK1 / cyclin B complex inactivation by glyphosate may explain the damage caused by this herbicide observed in these experiments. Mass and length reduction observed in these animals may be a direct consequence of the interruption of the cell cycle before the mitotic phase (TERZOUDI, et al., 2000) or a consequence of the oxidative stress caused by it (COSTA et al., 2008).

Chronic exposure to glyphosate may cause malformation in some animal species when doses are high and for long periods. The occurrence of weight loss, digestive dysfunctions, and death of pregnant females were recorded, besides causing problems in puberty progression, morphological alterations in testicles, endocrine and behavioral dysregulation and 50% reduction of oxidative phosphorylation in mitochondria isolated from Wistar rats (PEIXOTO, 2005), which may corroborate our results.

Animals that received supplementation with folic acid and selenium, even after exposure to the herbicide, did not present changes in mass and length when compared to the control group. Folic acid periconceptional supplementation reduces the chances of congenital malformation (OBEID et al., 2019). Rat fetuses treated with retinoic acid antagonists, an important agent in cell differentiation, presented cardiac defects, thymic hypoplasia, and various central nervous system abnormalities. When animals were submitted to nutritional therapy with folic acid and methionine, there was a significant reduction in the incidence of congenital heart disease (CIPOLLONE, et al., 2009).

Low concentrations of selenium are required in animal and human nutrition to maintain normal development and can be stored (DOS REIS, 2017). However, high concentrations of this nutrient can cause severe poisoning (MONTEIRO, et al., 2009). Concentrations between 1 and 2 $mg.kg^{-1}$ of sodium selenite demonstrated benefits for the growth and increase of GPx activity in *Salmo salar* (LORENTZEN, 1994).

Animals in this study that received supplementation with both antioxidants did not present external morphological alterations and, in parallel, showed an increase in the diameter of cardiac tissue trabeculae, indicating that supplementation was able to reduce deleterious effects of this herbicide to cardiac development of these animals.

In the study carried out by Costa and collaborators (2008), the occurrence of oxidative stress in bullfrog tadpoles by increasing the superoxide dismutase and catalase activity, inducing the generation of reactive oxygen species (ROS) and increased lipid peroxidation (LPO) causing damage to the cell membrane and tachycardia occurred due to stress-induced adrenergic stimulation after exposure to Roundup. Small concentrations of Roundup Original® (1 $mg \cdot L^{-1}$) used in the experimental condition of this study were enough to cause impacts on development, which, in a field situation of environmental contamination that may jeopardize the performance and success of tadpoles during metamorphosis, causing population decline (CAUBLE and WAGNER, 2005).

Changes observed in trabeculae diameter and spaces between them can be used as a parameter to infer reduction or increase in mass and volume of cardiac tissue (HEYMSFIELD et al., 1978). Tadpoles exposed to herbicide had an increase in the spaces between trabeculae and a decrease in trabecular diameter, while animals treated with folic acid and selenium showed a reduction in spaces between trabeculae and an increase in trabecular diameter to CG. The morphological changes observed in cardiac tissue can be explained by the reduction of CDK1/cyclin B complex activity (TERZOUDI, et al., 2000) as well as the response of stress-induced cardiac adjustment mechanisms (COSTA et al., 2008), in the case of the group exposed to Roundup Original®.

Costa and coworkers (2008) demonstrated that bullfrog tadpoles exposed to sublethal concentrations of Roundup Original® present a reduction of ventricular mass leading to a reduction in cardiac output, suggesting that it is an effect of reduction of myocyte diameter, but did not find data in the literature that explained this fact. Increased trabecular lumen observed in this experiment and consequently, reduction in myocyte diameter may be explained as a strategy to more easily recruit Ca^{2+} from the extracellular medium to compensate for cardiac output deficit (BARNI et al., 1994). Nevertheless, molecular tests are required to characterize the cell signaling pathways involved with the failure of tissue development described above.

In addition to its pathological effects, glyphosate causes imbalances in the floristic composition of habitats of several animals, altering its fitness (DUKE and POWLES, 2008). Several factors can influence the ecology of these animals at all stages of development (MEINDL et al., 2020; PENG et al., 2020; PARSLEY et al., 2020) Larvae undergo pressure from biotic components such as intra and interspecific predation and competition, as well as diseases and parasites. To these animals to overcome adversities, it is necessary for great phenotypic plasticity that allows the manifestation of behaviors of escape and searches for resources. For this, larvae depend on the reproductive success of their parents. They should choose a suitable location for spawning and the female needs to accumulate enough yelk to provide energy for the initial transformations. Anurans depend on the oscillation of the ambient temperature, the availability, and level of agitation of the water, pH, availability of nutrients and oxygen, and environmental contamination at all life stages. Disturbance in environmental balance may reduce the survival rates of these animals (MCDIARMID and ALTIG, 1999; BENKE and HURYN, 2010). Supplementation with antioxidants may be used in the future as a strategy to increase survival rates in both zootechnical production and conservation programs.

5. CONCLUSION

The acute exposure of bullfrog tadpoles to herbicide Roundup Original® based on glyphosate causes delayed development of larvae causing body mass and length reduction in addition to causing atrophy of the cardiac muscle fibers, a limiting factor for the survival of these animals. In addition to this fact, periconceptional supplementation with folic acid or selenium can alleviate the deleterious effects of this xenobiotic may due to its antioxidant properties, and may have potential to be used as a strategy to mitigate the deleterious effects of glyphosate by increasing survival rates of amphibians. Further studies are needed to elucidate the biochemical mechanisms involved with the histopathological changes caused by this herbicide.

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