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Evaluacion de los efectos del herbicida glifosato en el pez Prochilodus lineatus a traves del test de aberracion cromosomica

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Abstract:
El uso generalizado del glifosato ha sido objeto de numerosas controversias, ya que la exposición prolongada a bajas concentraciones de este agroquímico podría dar lugar a efectos no deseados o nocivos. Ello favorecería la aparición de patologías en el mediano y largo plazo, lo que aumenta progresivamente el riesgo de exposición en seres humanos y animales, a través del consumo de agua y alimentos contaminados. Teniendo en cuenta esta premisa, el objetivo del trabajo fue evaluar el posible efecto mutagénico del herbicida a través de la prueba de aberraciones cromosómicas en peces de la especie Prochilodus lineatus. Los peces fueron expuestos a 0,1 ug/l de glifosato durante 70 días. Después de este período una muestra de la porción anterior del riñón fue extraída para el análisis de aberraciones cromosómicas (AC). En los individuos expuestos al pesticida se registró mayor número de AC. El análisis estadístico de los datos mostró diferencias significativas entre los grupos control y tratado. Los resultados sugieren la existencia de una respuesta genética causada por la exposición y el contacto de los especímenes con el herbicida.

Palabras clave: pez, exposición al glifosato, aberración cromosómica, mutagénesis.

The widespread use of glyphosate has been subject of numerous controversies since the prolonged exposure to low concentrations of this substance could lead to toxic effects. Then, the appearance of pathologies in medium and long term progressively will increase because of the exposure of people and animals through the consumption of contaminated water or food. Considering this, the aim of this study was to evaluate the potential mutagenicity of glyphosate herbicide in fish (Prochilodus lineatus) using the test of chromosome aberrations. The fish were exposed to 0,1 ug/l of glyphosate for 70 days. After this period, samples of the anterior portion of the kidney were extracted for chromosomal aberrations (CAs) analysis. A greater number of CAs (gaps, breaks, stickiness, endomitosis, fragmentations, and pulverizations) were observed in individuals exposed to the pesticide. The statistical analysis of the data showed significant differences between the control and treated groups. In this way, the results suggested the existence of a genetic response as a consequence of pesticide exposure in fish.

Key words: fish, glyphosate exposure, chromosome aberrations, mutagenesis.

Full Text:
Evaluation of herbicide glyphosate effects in the fish Prochilodus lineatus using chromosome aberration test.

INTRODUCTION

Argentina produces and exports raw materials and agricultural inputs. Its' farming system, like most of the countries in the world, has undergone remarkable process of intensification in order to increase the hectare's productivity to satisfy nutritional needs.

This change turned crops more sensitive to disease causing organisms, so pesticides are used to control them. Waste and chemical substances resulting from this practice are dumped to watersheds and contaminating it, causing a serious impact on the environment (15,19).

Fishes are used as environmental toxicity bioindicators, because of their sensitiveness and usefulness in the evaluation of ecological risk by chemical contaminants (4,17,26). Currently, glyphosate is one of the pesti cides which use has been widespread. Glyphosate (N-phosphonomethyl glycine) is a systemic herbicide, that affects all the organs of a plant, is non-selective and of broad spectrum. It is applied to the foliage at the stage of post-emergence. According to the categorization of the USEPA (United States Environmental Protection Agency) it's a class II (moderately toxic) with acid character substance (2,25,28).

Many authors evaluated mutagenic and genotoxic effects of glyphosate in its Roundup[R] commercial formulation through micronuclei and comet trials in aquatics organisms (6,8,13,14,16). Nevertheless, there are sparse data about chromosomal aberrations induced by being in contact with this herbicide.

The aim of this study was to evaluate the mutagenicity of herbicide glyphosate (Roundup Full II[R]) by testing chromosomal aberrations in fish (Prochilodus lineatus).

MATERIAL AND METHODS

The methodological procedures used in this study were endorsed by the Committee of Ethics and Biosecurity of the Faculty of Veterinary Science, Northeast National University (UNNE), protocol 0033.

Animals. Eight young and healthy specimens of Prochilodus lineatus (Order Characiformes, Family Prochilodontidae), from the aquaculture center of the Institute of Ichthyology from the Northeast (INICNE) of the Veterinary Sciences Faculty, UNNE. They have a period of a week to get accustomed, being fed with an appropriate ration every 48 h, before the bioassay had started. Fish were kept in 300 liters tanks, with continuously aerated water, temperature around 20[degrees]C, ph 7.0 and a photoperiod of 12 h light.

Experimental design. After the adaptation period fishes were divided into two groups (treated and control), each one containing 4 animals. The animals were kept in aquariums of 7 liters, in a density of 1 g of fish by every liter of water. Control aquariums were loaded only with artesian water, while the treated tanks were filled with a solution of glyphosate in a concentration of 0,1 ug/l of water. The treated group received every 7 days a dose of herbicide with the renewal of the water and the water was also renewed in the control group. Food was provided every 48 h. Fish were kept in the aquariums in a chronic toxicity test for a period of 70 days.

Preparations of chromosomes. After the period of experimentation the fishes-were sacrificed with an overdose of the anesthetic drug Tricaíne Methanesulfonato MS-222 (Finquel[R]) dissolved in water. The somatic chromosomes were prepared according to the conventional technique for the of mitotic's chromosomas (12), both in control and treated groups. A total of 50 metaphases per individual were analyzed.

Statistical analysis. The differences in the frequencies of chromosomal aberrations were determined by comparison of the treated group with the control one, through the U Mann-Whitney non-parametric test. The statistical program SPSS was used for the analysis.

RESULTS

Diploid number in P. lineatus is 54 chromosomes (metasubmetacentrics), fundamental number 108. There are not chromosomal differences between sexes.

We analyzed a total of 339 metaphases not being possible to find the 50 established in two specimens, belonging one to the control group and the other from the treated one. A total of 168 metaphases in control group were counted, of which 124 were normal and 44 had chromosomal aberrations (ACs). Whereas in the treated group, 171 metaphases were analyzed, identifying 30 normal and 141 ACs. These data showed an increase of the CAs in the treated group. The abnormalities found were breaks, stickiness, endomitosis, gaps, fragments and pulverization (Figure 1). Differences in aberration's frequencies between the treated and the controls groups were statiscally significant ($p<0.05$, Figure 2).

DISCUSSION

In previous studies, it was determined that the species Prochilodus lineatus presents 54 chromosomes (meta-sub-metacentrics), equal to 108 fundamental number, not observing sex chromosomal heteromorfism (5). The conservative nature of the karyotype structure is characteristically observed in all species of the genus Prochilodus (P. vimboides, P. lineatus, P. affinis, P. marggravii, P. cearensis, P. argenteus and P. nigricans), exhibiting a karyotype consisting of 54 bibraquials chromosomes (20,21).

The presence of 0-2 microchromosomes Bs was also observed, which varied in number not only between but also in the same karyotypic snap. The occurrence of supernumerary chromosomes or Bs in P. lineatus from different sampling points of the Paraná River (Brazil) reached a number of 0-7 in certain populations (3,5,7,18,20,21).

There are scarce research considering pesticides in teleosts, including the chromosome aberration test. Being this one a valuable genetic biomarker of environmental pollution. Chromosomal aberrations found in specimens of P. lineatus exposed to glyphosate (Roundup Full II) were gaps, breaks, stickiness, endomitosis y pulverization. Similar CAs were observed in laboratory fish bioassays where other pesticides were used.

An experience carried out on specimens of Channa punctatus (23) subjected to a dose of 0.01 ppm of Dichlorvos (o,o-Dimethyl-2, 2-dichlorovinyl phosphate) in an acute assay, exhibited several alterations at chromosomal level, identified as gaps, breaks, fragments and stickiness. In Etroplus suratensis (10) exposed for 96 h to the action of methyl parathion in concentrations of 0.05, 0.1, and 0.2 ppm; and phosphamidon at a dose of 0.5, 1.0 and 2.0 ppm, had shown significant increase of different types of aberrations (gaps and fragmentations).

Intramuscular injections with azadirachtin (biological metabolite of the plant Azadirachta indica, which features insecticides) in Oreochromis mossambicus caused significant injuries at cytogenetic level, being the chromosomal breaks a frequent structural anomaly (9).

Experiences with individuals of Channa punctatus 11 subjected to the action of pentachlorophenol and 2,4-dichlorophenoxyacetic acid during 48, 72, and 96 hours revealed the occurrence of structural chromosomal aberration (breaks, stickiness and pulverization chromosomal). Specimens of Oreochromis niloticus (Nile tilapia) exposed to the action of the herbicide Butataf (N-Butoxymethyl-2-chloro-2,6-diethylacetanilide) (22) during a month at concentrations of 0.02, 0.002, 0.004 and 0.008 ppm, showed a notable increase of injuries at chromosomal level, such as fragmentation, breaks, stickiness and deletion.

Active substances of pesticides (27) induce lesions in the DNA (aberrations) and the type of problem found is subject to the nature of the pesticide concentration and the period of exposure. Such adverse effects could lead through different mechanisms, damage genetic material (1,24).

In conclusion, the results obtained in the present study showed that chronic exposure to the herbicide glyphosate (Roundup Full II), even at sublethal doses, can induce chromosomal damage in Prochilodus lineatus. These specimens are an excellent natural model for the detection of mutagenic agrochemicals effects.

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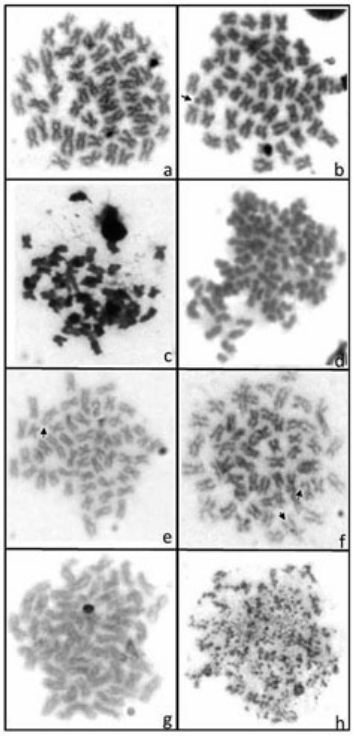

Figure 1. Photomicrograph slide of kidney cells showing (a) normal metaphase without chromosome B; (b) normals metaphases with chromosome B; (c) chromosome stickiness; (d) endomitosis; (e) gaps; (f) breaks; (g) chromosome fragments, and (h) pulverization of *P. lineatus*.

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Caption: Figure 1. Photomicrograph slide of kidney cells showing (a) normal metaphase without chromosome B; (b) normals metaphases with chromosome B; (c) chromosome stickiness; (d) endomitosis; (e) gaps; (f) breaks; (g) chromosome fragments, and (h) pulverization of P. lineatus.

Caption: Figure 2. Box plot showing number of chromosomal aberrations on each group, control and treated, in P. lineatus at each period of exposure (70 days). with significant differences between groups (p<0.05)

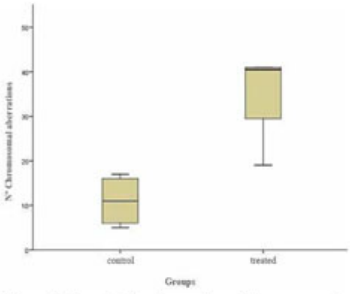

Figure 2. Box plot showing number of chromosomal aberrations on each group, control and treated, in *P. lineatus* at each period of exposure (70 days). with significant differences between groups (p<0.05).

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