African Journal of Biotechnology Vol. 7 (7), pp. 893-896, 3 April, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB07.669
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Micronuclei in red blood cells of armored catfish *Hypostomus plecotomus* exposed to potassium dichromate

Carlos Augusto Borba Meyer Normann[1], José Cláudio Fonseca Moreira[2] and Valesca Veiga Cardoso[1]*

[1]Centro Universitário Metodista - Porto Alegre - RS –Brazil.
[2]Department of Biochemistry-ICBS; UFRGS - Porto Alegre - RS - Brazil.

Accepted 22 February, 2008

The present work aims to evaluate the impact of potassium dichromate in armored catfishes' (*Hypostomus plecotomus*) erythropoiesis, using piscine micronucleus test. Armored catfishes (n = 30) were subjected to 12 mg/L of potassium dichromate, with an equal control group (n = 30). For each 2,000 red blood cells of animals subjected to treatment, it was possible to observe 8.25 ± 0.02% cells with micronuclei and 17 ± 0.005 mg/kg of total chrome in tissues, detected through atomic absorption spectrophotometry. In the control group, the average was 0.75 ± 0.03% for micronuclei and 1.08 ± 0.04 mg/kg of chrome in tissues. The results confirm the clastogenic effect of potassium dichromate. The sensibility to this ion and the economic relevance of *H. plecotomus* show that armored catfish can be used to monitor acute effects of metallic pollutant spills in freshwater.

Key words: Piscine micronucleus test, aquatic pollution, potassium dichromate, *Hypostomus plecotomus.*

INTRODUCTION

Exposure to heavy metals is known to cause alteration in hematological parameters in fishes (Heath, 1995). One parameter used in bioindication is the generation of genetic material fragments, known as micronuclei, due to the activity of clastogenic agents which provoke chromosome breaks. These fragments appear in the cytoplasm when either parts of the chromosomes or chromatids or entire chromosomes are not incorporated in the nuclei of the daughter cells in mitosis, frequently because these fragments do not have centromers; these fragments left behind are incorporated in the secondary nuclei, called micronuclei (Schmid, 1975; Heddle et al., 1983; Ribeiro et al., 2003). Micronuclei have between 1/5 and 1/20 of the original nucleus' size and there is generally more than one per cell (Schmid, 1975).

Micronuclei test in red blood cells and lymphocytes can be used as an indicator of toxic effects in determined target populations (Berces et al., 1993). Since DNA repair system is very sensitive to metallic ions, it is possible to visualize genomic structural damage such as the formation of micronuclei and cellular blebs, the latter typical of apoptosis (Berces et al., 1993; Bagchi et al., 2000).

In fishes, piscine micronucleus tests are usually performed using red blood cells, although liver and gill tissues are also used (Ferraro, 2003; Benincá, 2006). For biomonitor mutagenic elements, the analysis of micronuclei using peripheral blood instead of tissues is very advantageous, since peripheral red blood cells are as sensitive as kidney cells, main hematopoietic organ in fishes, and much easier to get a sample of. In organisms with adequate size, samples of peripheral blood also permit monitoring of the organism's reaction to the pollutant through various samplings (Palhares and Grisolia, 2002).

The present work aims to verify the possible formation of micronuclei in red blood cells of *Hipostomus plecotomus*, subjected to chronic exposure to potassium dichromate in concentrations of 12 mg/liter.

MATERIAL AND METHODS

Specimens of *H. plecotomus* from vivariums (ponds) were supplied by EMATER-NH and kept in a fish tank with 20 liters of water, forced aeration and 14:10 photoperiod, under temperature of 25 ±

*Corresponding author. E-mail: anastrefa@ig.com.br.

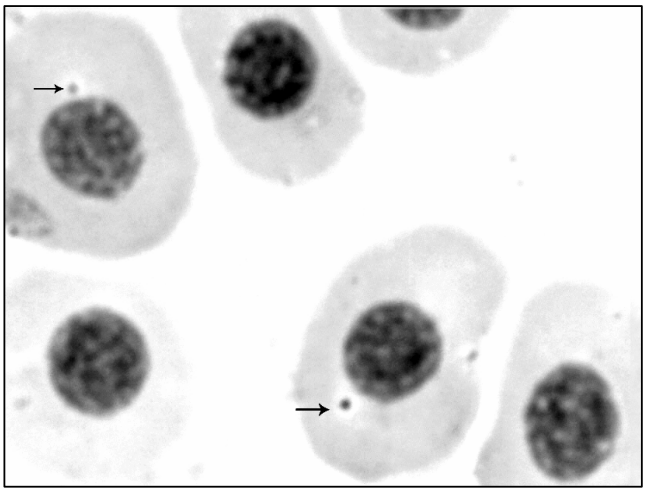

Figure 1. Red blood cells of fishes, subjected to potassium dichromate, 12 mg/L, in smears. Coloration: methylene blue. Arrow-head indicates micronucleus. Magnification: 10x100.

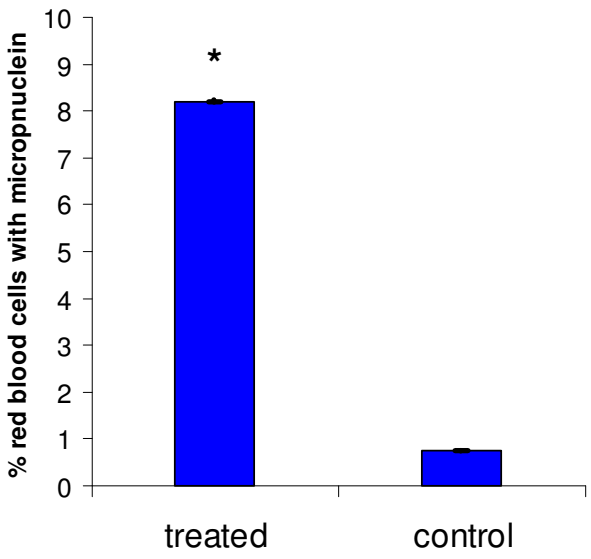

Figure 2. Percentage of micronuclei in red blood cells of fishes (count of 2000 cells/individual). *Indicates significant difference between the treated animals and the controls (t-test; $p < 0.05$).

1.2°C. Thirty fishes of each group were kept for fifteen days, with pellet and flake feed being given *ad libido*. A control group was isolated (n =30) and another group (n =30) kept in a fish tank with 12 mg/L of potassium dichromate, dose previously tested in our laboratory as optimum inductor of micronuclei.

The significant difference between the treated animals and the controls was analyzed by t-test for independent samples and $p < 0.05$.

After anesthesia with ice, the specimens were decapitated and their blood collected for air-dried, Bouin fixed smears. The smears were stained with methylene blue, 0.03%, pH 4.5. 2,000 cells per smear slide were found. Tissue fragments of sampled fishes (n=3) were analyzed by atomic absorption spectrophotometry to quantify absorbed chrome.

Table 1. Quantification of absorbed chrome by atomic absorption spectrophotometry analysis (EAA). Fragments of tissues of treated fishes (n = 3) and controls (n = 3).

Sample	Chrome (mg/kg)
Control	1.08 ± 0.04
Treated	17.0 ± 0.005

RESULTS AND DISCUSSION

It was possible to see (Figure 1), in each animals kept in the fish tank with 12 mg/L of potassium dichromate, 2,000 observed blood cells, 8.25 ± 0.02% cells with micronuclei (Figure 2). These fishes accumulated 17 ± 0.005 mg/kg of total chrome in their tissues, detected by atomic absorption spectrophotometry (Table 1). Control animals presented a count of 0.75 ± 0.03% red blood cells with micronuclei. In these animals, atomic absorption spectrophotometry detected 1.08 ± 0.04 mg/kg of chrome in the tissues (Table 1).

In animals subjected to potassium dichromate 12 mg/L, an increase in the generation of clastogenic accidents was observed, yielding the formation of micronuclei. There was, therefore, a direct relation between bioabsorption of chrome and the formation of micronuclei. This result confirms the data registered by Al-Sabti and Metcalfe (1995), Dillon et al. (1998), Kohlpoth et al. (1999), and Messer et al. (1999).

Hexavalent chromium and potassium dichromate, among the various clastogenics, awaken the interest about its genotoxic activity due to its ample industrial use (Berces et al., 1993; Kohlpoth et al., 1999; Rozgaj et al., 2002; Kasuba et al., 2002). Dillon and his collaborators (1998) detected, in V79 cells, micronuclei and other alterations resulting from genotoxic action of chromium complexes. Kohlpoth et al. (1999) found significant formation of micronuclei in RTG-2 cells exposed to industrial effluents containing between 20 and 100 µM of potassium dichromate. Vaglenov and collaborators (1999) also found, in measurements made in metallurgic workers, high red blood cell concentrations of chrome, besides a significant increase in the number of red blood cells having micronuclei. Messer et al. (1999) also associate nuclear morphological alterations, reflecting genomic alterations, in gingival fibroblasts subjected to chrome.

Chrome VI, as a metallic ion or as chromates and dichromates, acts inducing mutations through oxidative damage, similarly to reactive oxygen species (ROS) and ultraviolet light (Lewin, 2000). Mattagajasingh and Misra (1999) detected crosslinks in DNA-protein complexes in leukemic T MOLT4 lymphocytes induced by potassium dichromate. Flores and Perez (1999) observed, in murine and human cell lines, DNA damage induction by potassium dichromate. According to the authors, the damage

caused by hexavalent chrome would be linked to the production of intermediate oxidative states, such as Cr^{5+}, what is also suggested by Sakurai et al. (1999). Hodges et al. (2001) demonstrated, in pulmonary A549 cells and peripheral blood lymphocytes, a dose-dependent correlation in the increase of breaks in DNA strands by potassium dichromate, with bigger presence of 8-desoxiguanosine in pulmonary cells treated with 10 to 500 µMol of potassium dichromate, for one hour, besides breaks in peripheral blood lymphocytes from healthy volunteers exposed to the compound.

The breaks in genetic material yielding micronuclei in cells that do not divide anymore, such as red blood cells, reflect cytogenetic abnormalities that happen in hematopoietic stem cells of the bone marrow. In human beings, the micronuclei test aims to evaluate the impact of carcinogenic agents in specific populations, such as smokers, alcoholics, etc. (Dietz et al., 2000)

In fishes, the damages are very significant, making it possible to monitor the environmental health state through this parameter, concerning chrome and other metals with clastogenic action. In eels, Sanchez-Galan and collaborators (2001) verified a production of 2.64 micronuclei per 1000 blood cells in animals from rivers with different levels of pollution with metals and injected with Cd solution. Ayllon and Garcia-Vazquez (2000) also saw the generation of micronuclei in sailfin molly (*Poecillia latipinna*), seeking for standardization of the test for this fish. Another fish used for this test, due to its abundance in nature, is the brown trout (*Salmo truta*), which, according to experiments of Sanchez-Galan and collaborators (1999), significantly increases the number of micronuclei in kidney red blood cells, 24 h after intraperitoneal injection of low doses of cadmium chloride.

It was also possible to notice that, even in natural conditions, a percentage of micronuclei formation is observed. Very probably, the metal comes from agrochemicals or other sources. Previous experiments determined the presence of some heavy metals in tissues of fishes commonly eaten by populations on the Rio dos Sinos banks (Normann et al., 2002; Fillion et al., 2006).

Due to the industrial process, the discharge of effluents with high metallic residue levels in the receiving bodies of water becomes more and more worrying. The verification of the impact of this material in natural populations is an alert as to the contamination state of living beings. The fact that many fishes such as the Loricaridae serve as food to poor populations must also be taken into consideration. The social impact of this contamination of natural resources may, in the medium and long run, significantly affect human beings, which should never be forgotten.

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