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Detection of Micronuclei and Other Nuclear Abnormalities in *Oreochromis niloticus* Exposed to Potassium Dichromate

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Abstract: The objective of this paper was to analyze the micronuclei (MNs) and other nuclear abnormalities (NAs) frequencies in peripheral blood of Nile tilapia (*Oreochromis niloticus*) treated with potassium dichromate via contaminated water. Blood samples were collected from caudal vessels after potassium dichromate 12 $mg.L^{-1}$ expositions for 24 and 48 hours. The typical micronuclei were not found in control group. In the exposed groups the frequencies were 1.0 ± 1.15 at 24h and 2.43 ± 0.98 at 48h. The nuclear morphological alterations frequencies in control, 24h and 48h were 4.29 ± 4.50, 5.86 ± 3.02 and 11.0 ± 3.74, respectively. The parametric ANOVA showed a very significant difference ($p < 0.01$) in MNs frequencies between control and 48h groups; also there was significant difference ($p < 0.05$) between the two exposition times. In the NAs, there was significant difference only between control and exposed for 48h groups ($p < 0.05$). Results confirm the potentially adverse effects of potassium dichromate. The demonstrated sensitivity to this ion shows that *Oreochromis niloticus* can be used to monitor for the acute effects of pollutants on the basis of hexavalent chromium in freshwater ecosystem.

Key words: Chromium · Mutagenesis · *Oreochromis niloticus* · Micronuclei

INTRODUCTION

Chromium is a transition metal with three oxidation states: +2, +3 and +6, forming various colorful compounds. Trivalent chromium is essential in human nutrition, acting in glucose metabolism, however the hexavalent chromium is the less stable and more biologically reactive form, highly toxic, genotoxic and carcinogenic [1] and may come to have a lethal effect if absorbed through the skin, ingested or inhaled [2]. Chromium VI, as a metallic ion or in the chromate and dichromate salts, acts by inducing mutations through oxidative damages, similar to reactive oxygen species and to ultraviolet light [3].

Among the various mutagen tests used for genotoxicity assays and biomonitoring of contaminated environments, *e.g.* the comet assay [4, 5], nuclear abnormalities [5, 6] and chromosomal aberrations [4, 7], the micronucleus test is relatively simple, reliable and sensitive and has been used to evaluate the effects of mutagen compounds in many different environments [5, 8-12].

Micronuclei (MNs) are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome acentric fragments or from intact whole chromosomes not included in main nucleus after mitosis [8, 13]. Among current cytogenetic techniques, NAs and MNs are considered as indicators of cytotoxicity and genetic toxicology, respectively [14, 15].

Erythrocytes in lower vertebrates such fishes and amphibians are nucleated and undergo cell division in the circulation. These cells are therefore suitable for micronuclei detection which can be readily counted in blood smears [8, 16].

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The main aim of the current study was to assess the genotoxic acute effect in the tilapias *Oreochromis niloticus* exposed to potassium dichromate. The evaluation was made through analysis of the MNs and NAs frequencies in peripheral erythrocytes.

MATERIALS AND METHODS

Animals: *Oreochromis niloticus* (Linnaeus, 1757) commonly known as tilápia was chosen as a model. This species of the Cichlidae from Africa has been introduced for pisciculture in several countries and already occurs naturally in the Amazon Basin occupying different niches. The species was chosen because it is easy to adapt and maintain in laboratory conditions. Specimens of Oreochromis niloticus, with an average length of 16.79 ± 1.78 cm and an average weight of 83.76 ± 19.55 g, were obtained from the Pesque & Pague Company, in Ananindeua, Pará State, Brazil.

Experimental Design: The fishes were transported to the laboratory, where they remained for one-month acclimatization in individual aquariums containing 30 L of dechlorinated water, with constant aeration, a photoperiod of approximately 12 h and 1/3 of total water volume was changed every 48 h. Specimens were firstly used as a negative control and, afterwards, exposed to potassium dichromate12 $mg.L^{-1}$, via contaminated water. The concentration was based in Normann *et al*. [17] that evaluated the potassium dichromate impact in armored catfish *Hypostomus plecotomus* through MN test.

Peripheral Fish Blood Was Collected from the Caudal Vein at Three Times: Before exposure (for negative control) and after each exposure time (24 and 48 hours). Blood samples were smeared and made two slides per animal per treatment. After that, fixation in absolute ethanol was carried for 10 minutes. The slides were stained in 5% Giemsa dye in phosphate buffer (pH 6.8) for 10 minutes, washed with distilled water and air-dried at room temperature.

Two thousand erythrocytes per fish per treatment were examined at 1000 x magnification. MNs were defined as round or oval intracytoplasmatic bodies neither linked nor connected in any way to the main nucleus, with a diameter of 1/30-1/10 of that of the major nucleus and on the same optical plane [8, 18]. Three NAs were considered, viz., buds, lobes and invaginations [18, 19]. The frequencies of MNs and NAs were calculated from the same microscopic slides.

Statistical Analysis: The Kolmogorov-Smirnov test of MNs and NAs data for goodness of fit (p-value > 0.05) revealed no significant departure from normality. After assessing the normality of data distribution, parametric tests were applied for detecting differences at the 0.05 significance level. Differences between mean values were compared by means of one way ANOVA and the Tukey test. All the data were expressed as means ± standard deviation (SD). All the analyses were undertaken with the BioEstat 5.0 statistical package [20].

RESULTS AND DISCUSSION

Aquatic ecosystems are an ultimate destination of agricultural and industrial pollutants. On the other hand, different heavy metals have been used for thousands of years, although their toxic effects were disclosed less than a hundred years ago [21].

As well as typical MNs (Figure 1A), the occurrence register of NAs (Figures 1B and 1C) also has been considered as a real useful indicator in evaluation of genotoxic and cytotoxic effects of contaminants in aquatic organisms [5, 7, 9, 22, 23, 24].

Our results for MNs and NAs after treatments with potassium dichromate 12 $mg.L^{-1}$ are presented in Table 1. NAs described in this study were reported previously by many authors [9, 18, 19, 21, 24]. Although many of these NAs could be easily identified within a particular type, others were dubious as to its characterization and, therefore, all NAs were computer together.

The spontaneous (or basal) frequency of MNs in fish is normally very low [7]. In our study, the typical MNs were not observed in the control group and showed low frequencies in treated groups, but was still possible to observe an extremely significant increase in the 48h exposed group ($p < 0.01$). There were significant changes in MNs frequencies at the two exposition times ($p < 0.05$). NAs frequencies were greater than those of MNs and only showed significant difference between the control and 48h treated groups ($p < 0.05$).

A significant increase in MNs frequency and binucleated cells was detected in erythrocytes and gill cells of Nile tilapia *Orechromis niloticus* exposed to chromium effluents [22]. Matsumoto *et al*. [5] evaluate the genotoxicity and mutagenicity of water contaminated with tannery effluents by the MNs test and comet assay using *O. niloticus*. The greatest damages occurred with water from a chromium-containing tannery effluent discharge site, supporting the hypothesis that chromium residues can be genotoxic and mutagenic.

Fig. 1: Peripheral erythrocytes of tilápias showing micronucleus (A), lobed nucleus (B) and notched nucleus (C)

Table 1: Mean ± SD of micronuclei (MNs) and nuclear morphological alterations (NAs) per 2,000 cells in *Oreochromis niloticus* exposed to potassium dichromate at 12 mg.L^{-1}

	Mean ± SD		
Treatments	MNs	NAs	MNs+NAs
Control	0.0±0.0^{a}	4.29±4.50^{b}	4.29±4.50^{b}
24h	1.0±1.15^{a}	5.86±3.02^{b,c}	6.86±3.08^{b,c}
48h	2.43±0.98^{b}	11.0±3.74^{c,d}	13.43±3.64^{d}

Same letters indicate no statistical difference among the groups (ANOVA, $p < 0.05$)

Normann *et al*. [17] reported that the exposition to potassium dichromate 12 mg.L^{-1} increase significantly the frequency of clastogenic and/or aneugenic events, with micronuclei formation in *Hypostomus plecotomus*. In our study, with the same salt concentration, there was also a significant increase, although low percentage in MNs frequency.

The MN test in fish erythrocytes responded positively to a large number of experimental carcinogens, such aflatoxins, benzidine, ethylmethanesulfonate, chlorinated hydrocarbons [8], phenol and phenolic compounds [10] cyclophosphamide [18, 25] and also to the most common carcinogenic environmental pollutants, such as polycyclic aromatic hydrocarbons [8, 26], pesticides [25] and heavy metals [8, 9, 14, 17, 24].

Nas in fish erythrocytes, as reported in several previous works, have been interpreted such nuclear lesions micronuclei analogous. However, further investigations are necessary in order to know the mechanism of formation of these nuclear abnormalities, as well as to investigate their genotoxic origin [18].

Among current cytogenetic techniques, MNs and certain NAs are considered to be sensitive indicators of genotoxicity and cytotoxicity, some reports indicating that chromium induces both MNs and NAs [5, 22]. In the present case, *O. niloticus* only seems to be sensitive to chromium genotoxicity and cytotoxicity after 48h of exposition.

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