Chemosphere 183 (2017) 491–502

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The genotoxicity and cytotoxicity of tannery effluent in bullfrog (*Lithobates catesbeianus*)

Mateus Flores Montalvão [a], Joyce Moreira de Souza [b], Abraão Tiago Batista Guimarães [b], Ivandilson Pessoa Pinto de Menezes [c], André Luis da Silva Castro [d], Aline Sueli de Lima Rodrigues [e], Guilherme Malafaia [e, f, *]

[a] *Laboratório de Pesquisas Biológicas, Instituto Federal Goiano – Campus Urutaí, GO, Brazil*
[b] *Programa de Pós-Graduação em Conservação de Recursos Naturais do Cerrado and Laboratório de Pesquisas Biológicas, Instituto Federal Goiano – Campus Urutaí, GO, Brazil*
[c] *Departamento de Ciências Biológicas, Laboratório de Genética e Biologia Molecular and Programa de Pós-Graduação em Conservação de Recursos Naturais do Cerrado, Instituto Federal Goiano – Campus Urutaí, GO, Brazil*
[d] *Departamento de Ciências Biológicas, Laboratório de Zoologia and Programa de Pós-Graduação em Conservação de Recursos Naturais do Cerrado, Instituto Federal Goiano – Campus Urutaí, GO, Brazil*
[e] *Departamento de Ciências Biológicas, Laboratório de Pesquisas Biológicas and Programa de Pós-Graduação em Conservação de Recursos Naturais do Cerrado, Instituto Federal Goiano – Campus Urutaí, GO, Brazil*
[f] *Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Goiás – Campus Samambaia, GO, Brazil*

HIGHLIGHTS

- Exposure to tannery effluent has adverse effects on *Lithobates catesbeianus*.
- Tannery effluent increased MN frequency and other nuclear abnormalities.
- Genotoxic effects induced by exposure to tannery effluent in bullfrog were evaluated.

ARTICLE INFO

Article history:
Received 3 December 2016
Received in revised form
2 May 2017
Accepted 13 May 2017
Available online 16 May 2017

Handling Editor: Frederic Leusch

Keywords:
Xenobiotics
Amphibians
Aquatic contamination
Agro-industrial waste

ABSTRACT

Some of the most polluting activities occur in bovine skin processing. Tannery generates effluents containing high concentrations of heavy metals and organic compounds. The phases composing the leather production process generate a large volume of tannery effluents that are often discarded in aquatic environments without any previous treatment. However, the effect these xenobiotics have on adult representatives belonging to the class Amphibia remains unknown. Thus, the aim of the present study is to assess the geno- and cytotoxic effects of tannery effluent on adult male bullfrogs (*Lithobates castesbeianus*) exposed to it. Accordingly, the animals were divided into the following groups: negative control (tannery effluent-free water), positive control (cyclophosphamide), and effluent (water added with 5% tannery effluent). The animals were euthanized for blood collection, and erythrocyte analyses were conducted after 35 and 90 days of exposure. The micronuclei (MN) frequency and the frequency of other nuclear abnormalities in each of the animals in the experimental groups were assessed in 2000 erythrocytes. According to the present results, the exposure to tannery effluents increased MN frequency as well as other nuclear abnormalities (i.e., lobed nuclei, binucleated cell, kidney-shaped nuclei, notched nuclei, and apoptotic cell) in the erythrocytes of animals in the effluent group and in the positive control group after 35 and 90 exposure days. Thus, the current study corroborated the hypothesis that the tannery effluent has aneugenic and clastogenic potential in adult male bullfrogs (*L. castesbeianus*). The present study is the first to report such effect.

1. Introduction

The increased generation of solid and liquid waste containing substances of negative impact on the environment and organisms

* Corresponding author. Laboratório de Pesquisas Biológicas, Instituto Federal Goiano – Campus Urutaí, Rodovia Geraldo Silva Nascimento, 2,5 km, Zona Rural, Urutaí, CEP: 75790-000, GO, Brazil.
E-mail address: guilhermeifgoiano@gmail.com (G. Malafaia).

http://dx.doi.org/10.1016/j.chemosphere.2017.05.080
0045-6535/

has resulted from the population growth recorded in recent years as well as from industrial processes (Claxton et al., 1998; Segura et al., 2009). The various types of waste generated include those that are produced by profitable industrial activities, such as bovine skin processing. Studies have shown that the industrial activity is one of the most environmental pollution-causing activities (Tare et al., 2003; Prabakaran et al., 2007).

Problems caused by tannery effluents are worsened by their improper discharge, which leads to high environmental contamination risk (Mohan and Routray, 2015). According to Bhat (2011) and Isaac (2013), the tannery effluent contains high concentrations of organic or inorganic agents. These agents are highly toxic to the aquatic biota, even when they are previously treated.

The significant decline in the population of semi-aquatic organisms such as amphibians is another environmental and biological concern, because it has led to the reduced abundance and biodiversity of these species (Stuart et al., 2004). Thus, it is worth to conduct more specific research to find the cause of such decline. Several studies have shown that waterbody contamination by chemicals is strongly related to losses in amphibian species (Egea-Serrano et al., 2012; Mann et al., 2009; Crane et al., 2016).

Different effects such as teratogenicity in *Paracentrotus lividus*/*Sphaerechinus granularis*; reduced growth in the alga species *Pseudokirchneriella subcapitata* (Tigini et al., 2011) and *Selenastrum capricornutum* (Oral et al., 2005); genotoxicity to *Allium cepa*; toxicity to *Daphnia magna*, *Ceriodaphnia dubia*, and *Hyalella azteca* (Júnior et al., 2007) have been noted in organisms assessed in the experimental field and exposed to tannery effluents (Júnior et al., 2007). Other effects caused by such exposure were mutagenic activity in *Salmonella* microsome (Tagliari et al., 2014) and the increased mortality and cytotoxic actions in *Etroplus suratensis* (Taju et al., 2012), in addition to immune response reduction in *Oreochromis mossambicus* (Tagliari et al., 2014) and genotoxicity in *Oreochromis niloticus* (Matsumoto et al., 2006a, b).

These organisms are suitable for lethality determination, because they are good ecotoxicological models. However, more complex organisms such as vertebrates may respond differently from these ecotoxicological models; therefore, further investigations using vertebrates must be carried out. Although some studies have already reported the adverse effects of tannery effluent on some groups of vertebrates such as fish (Nagpure et al., 2015; Aich et al., 2015; Walia et al., 2015; Walia and Handa, 2016) and mammals (Almeida et al., 2016; Rabelo et al., 2016; Silva et al., 2016; Guimarães et al., 2016a, b; Souza et al., 2016), the effects of this xenobiotic on adult amphibians remain completely unknown. Amphibians are good environmental health indicators because of their high sensitivity to toxicological effects linked to chemical contaminants (Blaustein and Wake, 1990; Wyman, 1990; Lips, 1998). According to Wells (1977), amphibians live in the water and on land as well as feed on plants and animals as part of their biology and life cycle; these feeding habits may serve as an important pollutant uptake route. Furthermore, the amphibians' semi-permeable skin can facilitate the absorption of harmful organic substances such as pesticides and hydrocarbons. Thus, it is important to broaden the awareness of the effects of this xenobiotic on the biota, besides its effects on fish and mammals.

The disposal of tannery effluent without prior treatment into watercourses is a real concern in different countries, mainly in developing countries such as Brazil, China, Pakistan, and India (Gödecke et al., 2012). The probable direct or indirect effects of this xenobiotic (even at small concentrations) on the genetic material of organisms open an exciting research field. The micronuclei (MN) frequency and the frequency of other nuclear abnormalities in peripheral blood erythrocytes have been often used to assess the genomes of amphibians and the pollutant cytotoxicity to them (Bosch et al., 2011; Lajmanovich et al., 2014; Josende et al., 2015; Veronez et al., 2016; Pollo et al., 2016). Studies concerning the aforementioned topics are interesting, because the cellular concentration in pollutants that can cause adverse effects at the genetic level can be lower than that in pollutants causing severe toxicity in animals, as well as can lead to high mortality rates. Therefore, these studies can serve as useful tools to assess the early impacts of the exposure to xenobiotics.

The present study therefore aims to assess the geno- and cytotoxic effects of water containing tannery effluent on adult *Lithobates castesbeianus* (Amphibia, Anura, Ranidae), assuming that the xenobiotic has high concentrations of heavy metals and organic compounds, and presents aneugenic and clastogenic potential. Another aim of the current research is to broaden the knowledge on the effect of this tannery effluent on aquatic vertebrates. The present study is the first to report the adverse effects of the exposure to tannery effluent on amphibians.

2. Materials and methods

2.1. Animals and experimental design

Lithobates catesbeianus was used in the current study. The species, also known as American bullfrog, is native to North America. It has been introduced in more than 40 countries because of its economic potential (Ficetola et al., 2007). It is found in several countries and is distributed in natural environments; it lives in water bodies, where it feeds and reproduces at high rates (Lowe et al., 2004). *L. catesbeianus* was chosen as the experimental model, because it has been successfully used in environmental toxicology studies involving several xenobiotics such as heavy metals (Ossana et al., 2013; Veronez et al., 2016), pesticides (Freitas et al., 2016; Rissoli et al., 2016), and contaminants generated from paper milling processes and human sewage (Wirz et al., 2005).

The animals were provided by a commercial frog farm located in Goiás State - Brazil. The subjects were kept in mini bays (30.5 cm × 39.0 cm x 23.0 cm) installed in an experimental room at *Instituto Federal Goiano* (Goiano Federal Institute) (Urutaí, GO, Brazil). The boxes were placed on a workbench fixed at 35° slope to form a dry area in its upper side and a water pool to the frogs in the lower side of the box. According to the *Anfigranja* system proposed by Lima and Agostinho (1992), each experimental unit in the strategic environmental sites had small and fixed-size plastic containers to make the food available to the animals.

The frogs were fed *ad libitum* with commercial feed for carnivorous fish (Guarantee levels: 45% crude protein, 14% ether extract, 5% crude fiber, 14% mineral matter, and 87% dry matter) once a day. The frogs were fed and the stalls were kept clean during management procedures. A complete water exchange was performed in the swimming pool. The minimum and maximum temperatures in the experimental room were 21 °C and 32.1 °C, respectively, and the mean temperature was 25.7 °C. The mean minimum temperature was 22.7 °C, and the mean maximum temperature, 26.5 °C.

The animals were divided in three groups according to the factorial arrangement wherein three treatments and two tannery effluent exposure periods were used after the acclimatization period (seven days). The treatments were as follows: i) negative control group, animals exposed to clean water pools (0% tannery effluent; n=5); ii) positive control group, animals kept under the same conditions imposed on animals in the negative control group, but in this case, the animals were treated with cyclophosphamide (40 mg/kg), which was intraperitoneally administered 48 h before euthanasia (n = 5); and iii) effluent group, water pools containing 5% tannery effluent (n = 5). Five (5) animals from each experimental group were assessed after 35 and 90 days of exposure,