Neurotoxicology and Teratology 61 (2017) 123–127

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Brief communication

Memory and depressive effect on male and female Swiss mice exposed to tannery effluent

Abraão Tiago Batista Guimarães [a,b], Raíssa de Oliveira Ferreira [b], Aline Sueli de Lima Rodrigues [b,c], Guilherme Malafaia [a,b,c,d,*]

[a] Programa de Pós-Graduação em Conservação de Recursos Naturais do Cerrado, Laboratório de Pesquisas Biológicas, Instituto Federal Goiano – Campus Urutaí, GO, Brazil, Post-Graduation Program in Conservation of Cerrado Natural Resources, Biological Research Laboratory, Goiano Federal Institute – Urutaí Campus, GO, Brazil
[b] Laboratório de Pesquisas Biológicas, Instituto Federal Goiano – Campus Urutaí, GO, Brazil. Biological Research Laboratory, Goiano Federal Institute – Urutaí Campos, GO, Brazil
[c] Departamento de Ciências Biológicas, Programa de Pós-Graduação em Conservação de Recursos Naturais do Cerrado, Instituto Federal Goiano – Campus Urutaí, GO, Brazil. Biological Sciences Department, Post-Graduation Program in Conservation of Cerrado Natural Resources, Goiano Federal Institute – Urutaí Campus, GO, Brazil
[d] Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Goiás – Campus Samambaia, Goiânia, GO, Brazil. Post-Graduation Program in Animal Biodiversity, Federal University of Goiás – Samambaia Campus, Goiânia, GO, Brazil

ARTICLE INFO

Article history:
Received 9 December 2016
Received in revised form 5 March 2017
Accepted 8 March 2017
Available online 10 March 2017

Keywords:
Agro-industrial waste
Experimental models
Tannery industries
Xenobiotics

ABSTRACT

Although tannery industries generate substantial profits to the countries they are located in, they work with one of the most environmentally harmful human activities. Tannery effluents (TE) are highly toxic; thus, their improper release into water bodies may cause severe problems to individuals depending on this water. Therefore, the aim of the current study is to assess the effects of oral exposure to TE on the anxiety-, memory deficit- and depression-predictive behaviors in male and female Swiss adult mice. The following experimental groups were set in order to do so, control, positive control (reference drugs) and effluent. The animals in the effluent group were treated with 5% TE diluted in potable water for 15 consecutive days. The neurobehavioral tests started on the 12th experimental day. The results found through the elevated plus-maze test (for anxiety prediction) showed no anxiogenic or anxiolytic effects on animals exposed to TE. On the other hand, animals treated with TE showed short- and long-term memory deficit in the object recognition test, as well as depression-predictive behavior in the forced swimming test. These results may concern the high concentration of heavy metals and neurotoxic organic compounds in the TE. Therefore, the oral exposure to TE, even for a short period-of-time, has effects on the central nervous system (CNS) that lead to neurobehavioral changes. Thus, the current study broadens the knowledge on this research field by demonstrating the neurotoxicity of xenobiotics to male and female Swiss mice.

1. Introduction

The tannery sector in Brazil and in other countries such as India, Pakistan and China is an important socio-economic development source. Tannery industries purchase raw material (skin) from slaughterhouses, provide jobs and export fur and leather to furniture, footwear, clothing and automotive industries, besides being one of the main input suppliers in different sectors (Sabumon, 2016). Italy, Hong Kong, China, Brazil, USA, South Korea, Germany, India and Argentina are among the greatest leather exporters in the world (Sabumon, 2016).

Although tannery has great socio-economic importance, it is also of great concern, because of its most environmentally polluting activities (Manivasagam, 1987). The pollution potential of tannery industries, mainly of the small ones, is directly linked to the large amount of solid or liquid untreated or inefficiently treated residues they discharge into waterways. The main components in this effluent are sulphide, chromium, volatile organic compounds, solid wastes, suspended solids - such as animal hair - and trimmings, which have negative impacts on ecosystems and on the health of living beings (Shakir et al., 2012).

Recently, the research team involved with the present research found that the animal's sex (mainly mice), as well as exposure time and route (oral or dermal) are factors to be taken into consideration at the time to assess the effects of the herein studied xenobiotics on mammals (Souza et al., 2016a). However, these team's studies did not use positive pharmacological controls, fact that has limited the researcher's understanding about the observed behavioral changes. The complexity of tannery effluents (TE) comes from the combination between the different inorganic and organic substances found in them and the metabolic and biotransformation mechanisms developed by mammals. Such combination may have underlain the herein observed results.

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

http://dx.doi.org/10.1016/j.ntt.2017.03.003
0892-0362/

Thus, the aim of the current study is to assess the effects of the oral exposure to TE on the memory, anxiety and depression behavior of male and female Swiss mice. Given the variety of inorganic and organic compounds found in TE, mice treated with these xenobiotics are expected to have neurotoxic effects that could result in neurobehavioral disorders; thus, the present article emerges as an incremental step in a series of studies on TE.

2. Materials and methods

2.1. Animals and experimental design

The sample comprised 180 (−90 males and 90 females) - adult (3 months old) and nulliparous Swiss mice who were provided by the Biological Research Laboratory of the Goiano Federal Institute – Urutaí Campus, Brazil. The subjects were kept under standard conditions: 12-h light/dark cycle, 22 ± 3 °C and 55–60% humidity. All the adopted procedures were approved by the Ethics Committee on Animal Use of the Goiano Federal Institute, GO, Brazil (protocol n. 17/2014).

The animals were divided in experimental groups exposed (or not) to TE. The number of mice per group changed depending on the applied behavioral test. The effluent group was composed of animals exposed to 5% wet-blue TE diluted in water, which was made available *ad libitum* to the mice through water dispensers. The positive control group – which was composed of animals treated with intraperitoneal injections of clonazepam (0.5 mg/Kg) and fluoxetine (30 mg/kg), before the behavioral tests - were used to validate the sensitivity of the elevated plus maze and forced swimming tests (Costa et al., 2013). Each experimental group subjected to depression and anxiety-predictive tests was composed of 10 mice ($n = 10$ males and $n = 10$ females). The groups not treated with clonazepam or fluoxetine, received i.p. injections of the drug vehicle (phosphate buffered saline (PBS)). On the other hand, the control and effluent groups subjected to the memory deficit-predictive test were composed of 15 animals ($n = 15$ males and $n = 15$ females). Body mass and water or feed consumption did not differ among the herein analyzed groups (data not shown).

2.2. Tannery effluent

The TE used in the current study was provided by a tannery industry located in Goiás State, Brazil. The analysis applied to the organic compounds found in the TE was performed in a desorption electrospray ionization device coupled to a high-resolution mass spectrometer (HRMS), as described by Guimarães et al. (2016). The physicochemical and chemical analyses applied to the raw TE, to the potable water and to the water added with 5% TE were carried out according to recommendations by the American Public Health Association (APHA, 1997). All the analyzed contaminants found in the tannery effluent are identified and shown in Table 1.

The rate of TE diluted in water bodies depends on hydrological factors such as the large variation in tannery effluent production by the industry, or on local climatic factors, which are often estimated. The effluent dilution rate (5%) in the current study is probably higher than the one typically faced by animals or humans. Accordingly, the aim was to simulate illegal effluent discharge scenarios and dilutions found throughout long drought periods or during months of increased bovine skin processing production.

2.3. Neurobehavioral tests

2.3.1. Elevated plus maze (EPM) test

The EPM test was performed on the 12th experimental day. The EPM device consisted of two opposing open arms (30 × 5 × 25 cm) and two opposing closed arms (30 × 5 × 25 cm) extending from a common central platform (5 × 5 cm). The adopted apparatus was made of wood and elevated 45 cm from ground level. The edges (0.25 cm) of the open arms were tested to prevent the mice from falling. The behavior rehearsal room was soundproof and the light intensity was kept at 100 lx. All experimental groups were kept in the rehearsal room for 30 min for acclimatization purposes. Subsequently, each animal was individually placed in the center of the EPM device with its face turned to one of the open arms. The animal was allowed to freely explore the apparatus for 5 min. All mice were tested once. The EPM device was cleaned with 10% ethanol before each test. The anxiety index was calculated as follows: Anxiety index = 1 - [([time the animal stayed in the open arms, in seconds/test duration in seconds (300 s)] + [input frequency in the open arms/total number of entries])/2]. The total number of entries was defined as the sum of the number of times the animal entered the open and closed arms. An input was accounted each time the animals' four paws overtook the initial limit of the arm. The locomotor activity of the animals in this test was accounted as the total number of entries (line crossing) in the arms. In addition, the frequency of open arm entries and the exploration time spent in the open arms were assessed.

2.3.2. Object recognition test

The object recognition test was performed on the 13th and 14th experimental days using a (30 × 20 × 13 cm^3) box. The test was divided in three sessions, the training session was followed by two test sessions (1 h after training and another 24 h after training). The animals were exposed to two identical objects (in size, form, and color) defined as familiar objects, F1 and F2 (squared Lego toys), for 5 min during the training session. A familiar object was replaced by a new object (N) during the test sessions, so that the animals could explore a familiar object and a new one for 3 min. A triangular Lego toy was used 1 h after the training test; and a circular Lego toy, 24 h after it.

At the beginning of each trial, the animals were placed in front of the objects with their faces turned to the wall. The time each animal spent exploring each object was recorded. A crossed-over design was used in all test sessions, so that the positions of the new and familiar objects was alternated in order to exclude the potential preference of the animals for a certain spatial location of the objects in the box. Exploration behaviors consisted of having the animals smelling and touching the objects with their nose or forepaws, and standing 2 cm or closer to the objects. The recognition index of each animal was calculated according to Rabelo et al. (2016), and expressed by the ratio: TOX/(TF + TN), wherein TOX = time spent exploring the familiar (F) or new (N) object; TF = time spent exploring the familiar object; TN = time spent exploring the new object. The boxes used in the tests were cleaned with 10% alcohol after each session.

2.3.3. Forced swimming test

The forced swimming test was performed on the 15th experimental day. It consisted of individually placing the mice in a cylindrical tank (height 39.0 cm, diameter 20.0 cm) containing water at 25 °C (20.0 cm depth) for 6 min. Subsequently, the animals were removed from the water and left to dry under light heating. Next, they were taken back to their crates. All test sessions were video recorded using a camera located 30 cm above the tank. The videos were used to assess the time the animals were motionless. Such time is often used as depression predictor in the forced swimming test (Petit-Demouliere et al., 2005). Immobility was herein defined as the absence of movement in the whole body - the mouse stops fighting and keeps motionless, floating in water, or only does the necessary movements to keep its head above the water.

The time spent swimming was used to measure the locomotor activity in the forced swimming test, as described by Costa et al. (2013). The understanding of swimming concerns the large and horizontal movements of the forepaws leading the body displacement around the cylinder. The swimming parameter was recorded for the first 2 min of the test, as described by Costa et al. (2013). Three trained observers (blind to the treatments) reviewed the videos, each video was analyzed twice, thus totaling inter-observer consistency >85%.