Neurotoxicology and Teratology 55 (2016) 45–49

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Brief communication

Memory deficit in Swiss mice exposed to tannery effluent

Letícia Martins Rabelo [a], Bianca Costa e Silva [a], Sabrina Ferreira de Almeida [a], Wellington Alves Mizael da Silva [a], Bruna de Oliveira Mendes [a], Abraão Tiago Batista Guimarães [a], Anderson Rodrigo da Silva [b], André Luis da Silva Castro [c,e], Aline Sueli de Lima Rodrigues [a,e], Guilherme Malafaia [a,d,e,*]

[a] Laboratório de Pesquisas Biológicas, Instituto Federal Goiano — Campus Urutaí, GO, Brazil
[b] Laboratório de Estatística Experimental, Instituto Federal Goiano — Campus Urutaí, GO, Brazil
[c] Laboratório de Zoologia, Instituto Federal Goiano — Campus Urutaí, GO, Brazil
[d] Programa de Pós-graduação em Biodiversidade Animal, Universidade Federal de Goiás — Campus Samambaia, GO, Brazil
[e] Programa de Pós-graduação em Conservação de Recursos Naturais do Cerrado, Instituto Federal Goiano — Campus Urutaí, GO, Brazil

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 27 March 2016
Accepted 31 March 2016
Available online 6 April 2016

Keywords:
Experimental models
Xenobiotic
Cognition
Tannery effluent

ABSTRACT

Although it is known that tannery effluents constitute highly toxic pollutants whose effects in humans represent public health problems in several countries, studies involving experimental mammalian models are rare. In this context, the objective of the present study was to assess the effect of the exposure to tannery effluent on the memory of male and female Swiss mice. Animals of each sex were distributed into two experimental groups: the control group, in which the animals received only drinking water and the effluent group, in which the mice received 1% of gross tannery effluent diluted in water. The animals were exposed to the effluent by gavage, oral dosing, for 15 days, ensuring the administration of 0.1 mL of liquid (water or effluent)/10 g of body weight/day. On the 14th and 15th experimental days the animals were submitted to the object recognition test. It was observed that the new object recognition indices calculated for the animals exposed to the effluent (males and females) were significantly lower than those obtained with the control group. The exposure to tannery effluent caused memory deficit in Swiss mice in a similar way for both sexes, reinforcing previous findings that these pollutants affect the central nervous system. It contributes to the knowledge in the area by attesting harmful effects to the cognition of such animals.

1. Introduction

The process of fast industrial growth, especially in developing countries, has led to increasing production and release of pollutants. One of the pollutants produced in large scale worldwide is the tannery industry effluent, which represents high environmental and ecologic risk in several countries, such as Brazil, China, Pakistan and India (Prabakaran et al., 2007; Shakir et al., 2012). Such residues are produced in different phases of the bovine hide processing, which require varied mechanical and chemical treatment processes, conferring to the effluents high toxic potential to the environment and health of both organisms and humans, once they are discarded in water bodies with no processing or treatment (Shakir et al., 2012). The tannery effluent presents organic and inorganic components, such as tannins, surfactants, dyes and heavy metals, especially chromium, cadmium and lead (Lofrano et al., 2013).

Studies in humans have shown the relationship between the exposure to tannery effluent and related diseases, such as ear-nose-throat, skin, eye, respiratory, reproductive and neural problems (Cuberos et al., 2009; Rumin et al., 2013; Chandrasekaran et al., 2014). In the experimental field, the effects of tannery effluents have already been reported in the teratogenicity in sea urchin species, in reducing microalgae growth and a variety of toxic effects on micro crustaceans (Oral et al., 2005). Other studies have shown harmful effects in fish, plants and bacteria (Tagliari et al., 2014; Matsumoto et al., 2006; Júnior et al., 2007; Tigini et al., 2011; Taju et al., 2012). However, research on the effect of these pollutants in more complex organisms, such as mammalian, is still scarce.

Just a few authors have assessed the effect of the exposure to tannery effluents in mammals, for example Kumar et al. (2008), Siqueira et al. (2011), Moysés et al. (2014), Silva et al. (2015), Lemos et al. (2015), and Ferreira et al. (2015). In the pioneer study of Kumar et al. (2008), who chronically exposed male Wistar rats to tannery effluent, an increase in the mass of the androgen-dependent organs, such as prostate and seminal vesicle, was observed, besides deformations in the seminiferous tubules with signs of testicular hyperplasia.

Siqueira et al. (2011) showed that male Swiss mice exposed to ingestion of 1% untreated tannery effluent diluted in water for only 15 days presented anxiogenic behavior. On the other hand, Moysés et al. (2014), studying neurotoxicity and hepatotoxicity induced by the chronic exposure of male Wistar rats to tannery effluents, have

* Corresponding author at: Rodovia Geraldo Silva Nascimento, 2.5 km, Zona Rural, Urutaí, GO CEP: 75790-000, Brazil.
E-mail address: guilhermeifgoiano@gmail.com (G. Malafaia).

http://dx.doi.org/10.1016/j.ntt.2016.03.007
0892-0362/

observed no changes in the assessed variables. In turn, Silva et al. (2015), Lemos et al. (2015), and Ferreira et al. (2015) tried to determine lethal doses of tannery effluents diluted in water in different concentrations, using rats of different lineages and sex. Despite being considered pioneer studies related to the assessment of the acute toxicity and determination of lethal tannery effluent doses in rats, these studies have not investigated any neurobehavioral effects in such animals.

Thus, from the findings of Siqueira et al. (2011), who observed anxiogenic behavior in male Swiss mice exposed to tannery effluent for a short time period, it is suggested that the effluents constitute xenobiotics capable of affecting the central nervous system. In this context, our hypothesis was that the tannery effluent ingested by Swiss mice promotes memory deficit. Thus, our objective was to assess the effect of tannery effluent ingestion in the memory of Swiss mice by means of the object recognition test, which is one of the classical and popular predictive tests for memory deficit applied to experimental models. Since males can differently respond to xenobiotics (Mugford and Kedderis, 1998), we also assessed the effect of the tannery effluent separately. Our results represent an advance for the knowledge of the effects of the exposure to tannery effluent, subsidizing more specific studies on the neurotoxic mechanisms of action of this pollutant in mice.

2. Material and methods

2.1. Animals, experimental groups and experimental design

Fifty-six (from 36- to 44-days old) male and female Swiss mice were used. The animals were obtained from colonies of the Central Animal Facility of the *Universidade Federal de Goiás* (Goiânia, Goiás State, Brazil), and were kept in the Central Animal Facility of the Laboratory of Biological Research of the *Instituto Federal Goiano* – Campus Urutaí (Urutaí, Goiás State, Brazil), under sanitary conditions of a conventional animal facility, with controlled temperature (22 to 24 °C) and luminosity (12-hourlight cycle). The backcrossing strategy used to avoid genetic drift and creation of a subline was based on Andrade et al. (2002).

The animals were kept collectively in standard polypropylene boxes for mice (30 × 20 × 13 cm), having antioxidant-treated galvanized wire-mesh lids. A maximum of four animals was kept in each box. The boxes were cleaned three times a week, with change of sawdust and food. Rodent standard diet (Nuvilab CR 1) and water were provided *ad libitum*. The whole procedure adopted in this study was approved by the Ethics Committee on Animal Use of the *Instituto Federal Goiano* (IF Goiano) (Goiás State, Brazil) (protocol # 17/2014).

After the equitable division of body mass, the animals of each sex were distributed into two experimental groups: the control group, in which the animals received distilled water only, and the effluent group, in which the mice received 1% of gross tannery effluent diluted in distilled water. Each experimental group was composed of 14 animals. The effluent concentration was established according to Siqueira et al. (2011). It also corresponds to 1/60 of the median lethal dose (DL_{50}) determined for Swiss mice, according to Ferreira et al. (2015). The animals were exposed to oral dosing for 15 days (exposure period adopted by Siqueira et al., 2011). Diluted effluent and distilled water were offered by gavage, ensuring the administration of 0.1 mL/10 g of body mass/day for each animal, additionally to the drinking water taken by the animals from the drinking bottles, offered *ad libitum*.

2.2. Characterization of the water and the tannery effluent

The tannery effluent used in the experiment was obtained from a tannery industry located in Inhumas, Goiás State, Brazil. The effluent is referred to as of wet-blue type, obtained during the bovine hide tanning stage, in which large quantities of chromium salts and chlorides are usually used. The physical-chemical and chemical characterization of the tannery effluent used confirms the presence of high heavy metal concentrations, such as chromium, nickel and cadmium (Table 1). The drinking water used in the experiment came from the water supply system of the IF Goiano – Campus Urutaí, Goiás State, Brazil, whose physical-chemical and chemical characterization are shown in Table 1.

2.3. Object recognition test

The object recognition test was performed on the 14th and 15th experimental days in a (30 × 20 × 13 cm^3) box, as modified after Bevins and Besheer (2006). The test was divided into three sessions: a training session, followed by two test sessions (1 h after training and another 24 h after training). During the training session, the animals were exposed to two identical objects (in size, form, and color), defined as familiar objects (F1 and F2), for 5 min (square Lego toys). During the test sessions, a familiar object was replaced by a new object (N) (1 h after training we used a triangular Lego toy and 24 h after training we used a circular Lego toy), so that the animals could explore a familiar object and a new one for 3 min. At the beginning of each trial the animals were placed in front of the objects, facing the wall, according to Akkerman et al. (2012). The time spent to explore each object was recorded. A crossed-over design was used in all test sessions, so that the new and the familiar objects were placed alternately, in order to exclude potential preference for a certain spatial location of the objects in the box. Exploration was considered smelling and touching objects with the nose or forepaws and when the animal was at a distance ≤2 cm from the objects (Ennaceur and Delacour, 1988; Rajagopal et al., 2014).The recognition index for each object was calculated for each animal, as described by Piéta-Dias et al. (2007), and expressed by the ratio: TOX/(TF + TN) [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]. Between sessions, the boxes used in the tests were cleaned with alcohol 70%.

2.4. Statistical analysis

Initially, the residual normality was checked by means of the Shapiro-Wilk test and the Bartlett test was used to check residual homoscedasticity. In order to validate the object recognition test, we analyzed the data recorded from the training of the control group (male and female mice) and the recognition index for each object (N and F) of the control groups (males and females mice). For this, we applied the Student's *t*-test with Bonferroni adjustment for multiple pairwise comparisons. The resulting data relating to the new object recognition indices were subjected to analysis of variance (ANOVA) with factors "treatment" (effluent and water) (factor 1), "sex" (male and female mice) (factor 2) and "time" (1 and 24 h) (factor 3). In case of significant ($p < 0.05$) *F* value, Fisher LSD test was applied at 5% probability. The statistical analyses were performed using the software R version 3.0.3 (R Core Team, 2014).

3. Results and discussion

The recognition indices for familiar objects (F1 and F2) in the training session of the control group animals (male and female mice) differed from zero and did not show significant difference ($p > 0.05$), according to the Student's *t*-test (Fig. 1). This result constitutes one of the validation indicators of the test, once it demonstrates that the random exploration of the objects in the training session resulted in an equal exploration of both objects, besides excluding potential preference for a certain spatial location of the objects placed in the test box. On the other hand, the control group (male and female mice) yielded higher recognition indices for the new object in the test session performed 1 h after training, when comparing to the indices of the familiar object, which indicates success in retaining the memory of the familiar object. Similar result was observed in the test performed 24 h after the training session, both for male and female mice. These data