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Exposition to tannery wastewater did not alter behavioral and biochemical parameters in Wistar rats

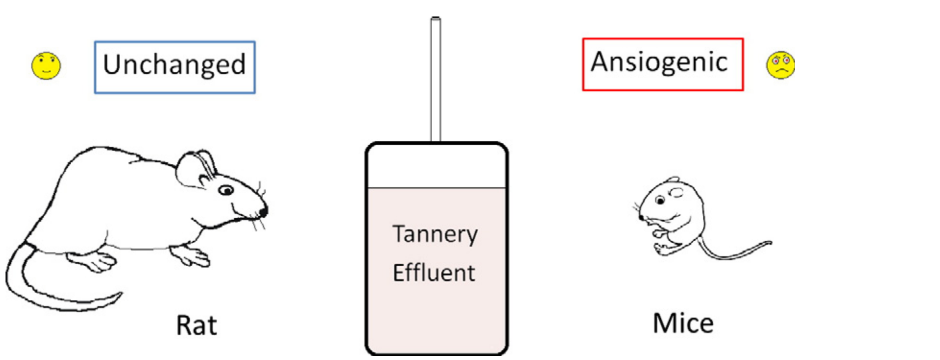
Felipe dos Santos Moysés [a], Karine Bertoldi [a], Christiano Spindler [a], Eduardo Farias Sanches [b], Viviane Rostirola Elsner [a], Marco Antonio Siqueira Rodrigues [c], Ionara Rodrigues Siqueira [a,d,*]

[a] Programa de Pós-Graduação em Ciências Biológicas: Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Programa de Pós-Graduação em Ciências Biológicas: Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Universidade FEEVALE, Novo Hamburgo, RS, Brazil
[d] Unidade de Experimentação Animal, Grupo de Pesquisa e Pós-Graduação do Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

HIGHLIGHTS

- Exposure to tannery wastewater did not induce behavioral changes in Wistar rats.
- Brain acetylcholinesterase activity was unchanged by exposure to tannery wastewater.
- Exposure to tannery wastewater did not alter cerebral oxidative status.
- Photoelectrooxidation treatment did not induce behavioral or neurochemical changes.
- Wistar rats seem not to be useful for ecotoxicological studies with tannery effluents.

GRAPHICAL ABSTRACT



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ABSTRACT

There are scarce data on the neurotoxicity in mammalian induced by tannery wastewaters. Previously, the anxiogenic effect of tannery wastewater was demonstrated in mice, while wastewater submitted to photoelectrooxidation (PEO) process treatment did not affect the anxiety state. Considering that species may response differently to xenobiotics, the aim of the present work was to study the effects of exposure to tannery wastewaters (non-PEO or PEO-treated) on behavioral and neurochemical markers in another species of laboratory animals, specifically Wistar rats. Male Wistar rats were given free access to water bottles containing non-PEO or PEO-treated tannery wastewaters (0.1, 1 and 5% in drinking water). During the exposure, behavioral tests of anxiety (elevated plus-maze, neophobia, open field and light–dark box), depression (forced swimming) and memory (inhibitory avoidance, novel object and discriminative avoidance) were performed. On the 30th day, brain structures were dissected out to evaluate cellular oxidative state (hippocampus, cerebellum and striatum) and acetylcholinesterase activity (hippocampus and striatum). Exposure to tannery effluent with or without photoelectrochemical treatment did not alter any behavioral and neurochemical parameters evaluated. Our data indicate that Wistar rats may not be an adequate species for ecotoxicological studies involving tannery effluents and that POE treatment did not generate other toxic compounds.

1. Introduction

The leather industry is one of the major polluters of many developing countries [30,48]. Buljan [14] reported that the total

* Corresponding author at: Departamento de Farmacologia, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500, sala 202, 90050-170 Porto Alegre, RS, Brazil. Tel./fax: +55 51 33083121.
E-mail address: ionara@ufrgs.br (I.R. Siqueira).

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quantity of chemicals used in hide processing worldwide was nearly 4 million tonnes, producing over 300 million tonnes of wastewater; thus the consumption of water from untreated natural sources might expose human beings and domestic and wild animals to tannery wastewater. In Rio Grande do Sul State, Southern Brazil, there are about 185 companies, which are responsible for about 14 million m^3 of contaminated water per year [33]. Considering the large amount and variety of chemical agents used in hide processing, the wastewaters generated by tanneries are very complex. Major components include organic and inorganic compounds such as tannins (polyphenolic compounds), surfactants, sulfonated oils, dyes, biocides, acrylic resins, organic acids, as well as ammonium, chromium, chloride, and sulfide salts [48,35,55,58].

Some bioassays using *Vibrio fischeri*, *Daphnia magna*, sea urchins and marine microalgae have evaluated toxic effects of tannery wastewater [59,42], while toxicity towards mammals has received only modest attention. There are few studies describing the neurobehavioral alterations are induced by tannery effluents. Recently, it has been demonstrated that the exposure to tannery wastewater induces behavioral changes on the elevated plus maze in mice, indicating an anxiety-like behavior [53]. Considering that different species may show variation in their responses to chemicals, other species of laboratory animals must be tested in order to better understand the toxicity of tannery wastewater, investigating also different behavioral models and biochemical assays. In this context, it has been well-established that xenobiotics metabolism may vary among species, reflecting in dissimilar responses, as species more or less responsive to xenobiotics [3,40,42].

Tannery wastewaters cannot be released into the environment without pretreatment. However, it has been reported that the standard technology for effluent treatment, primary and biological wastewater treatment processes, is unable to significantly reduce all of the polluting parameters [15]. Therefore, additional strategies for the recovery and recycling of chemicals and water have been investigated. The Advanced Oxidation Processes can promote the degradation of several pollutant complexes within a few minutes [29]. The photoelectrooxidation (PEO) process consists of a combination of electrolysis and heterogeneous photocatalyses [45]. Interestingly, it has been demonstrated that the photoelectrooxidation (PEO) process treatment of tannery effluent is effective, since PEO-treated tannery effluents does not affect the anxiety state in mice [53].

Little information exists about cellular and molecular mechanisms underlying the neurotoxic effects of tannery effluents. Several studies have showed that the oxidative stress may be a central biochemical mechanism of toxicity from several xenobiotics [24,50]. Reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical may attack macromolecules such as lipids, proteins and DNA, contributing to a wide range of diseases including cardiovascular and neurodegenerative diseases [20,40,8]. Besides, it has been reported that various xenobiotics can modulate different neurotransmitter systems [1,5], which may alter several neurobehavioral parameters, such as anxiety, depression and memory impairment [27,9,54,19,36]. Organophosphate compounds inhibit the enzyme acetylcholinesterase, leading to toxicity in mammals, throughout the accumulation of acetylcholine and consequent activation of cholinergic receptors [6].

Considering the scarce data on the neurotoxicity of tannery effluents and previous findings with mice, the aim of the present work was to study the effects of exposure to tannery effluents (non-PEO or PEO-treated) on behavioral and biochemical parameters in another species of laboratory animals, specifically Wistar rats. We used the discriminative avoidance, forced swimming, light–dark box, open field, elevated plus-maze and neophobia tests as animal models of depression, anxiety and learning and memory, and also evaluated cellular oxidative state parameters and acetylcholinesterase activity.

2. Materials and methods

2.1. Leather effluent

The samples of an industrial effluent were collected after their treatment by a conventional method (physicochemical, followed by specified units of biological treatment) in the effluent treatment station of SENAI — Technological Center of Leather in Estância Velha/Brazil. The effluent characteristics are presented in Table 1.

All oxidation processes were conducted in the reactor schematically. The reactor had an entrance in the inferior part and an exit in the superior part. In this system, the solution to be treated was pumped into the reactor and returned to the tank for cooling.

The photoelectrochemical degradation experiments were performed in an electrochemical cell. A 400-W high-pressure mercury-vapor lamp was used as the light source. Before each experiment, the UV light was turned on for 15 min to allow the UV energy to become stable. Two pairs of electrodes have been used. The cathode and anode were DSA ($^{70}TiO_2/^{30}RuO_2$). The electrode area inside the cell was 118 cm^2. During the experiments, the reactor was operated in a batch recirculation mode. For each experiment, the effluent was recirculated at a flow rate of 4 L h^{-1} and 20 L of effluents was treated by PEO. The photoelectrochemical oxidation experiments were carried out using a DC power supply with applied current density of 36 mA cm^{-2}. The time spent for this experiment was 2 h.

2.2. Animals and treatment

Adult male Wistar rats aged 3 months, housed with food and water ad libitum under a light–dark cycle of 12 h, were used. The animals were provided by Universidade Federal do Rio Grande do Sul (Centro de Reprodução e Experimentação de Animais de Laboratório — CREAL/UFRGS). "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 1996) was followed in all experiments. All experiments were approved by the Local Institutional Research Committee (Comissão de Ética no Uso de Animais—CEUA/UFRGS, 13002). The room temperature was 22 ± 1 °C and the rats were divided into seven groups: drinking water (control group); 0.1% non-PEO wastewaters; 1% non-PEO wastewaters; 5% non-PEO wastewaters; 0.1% PEO-treated wastewaters; 1% PEO-treated wastewaters and 5% PEO-treated wastewaters. The non-PEO and PEO-treated tannery wastewaters were administered by giving free access to water bottles [42,53]. The exposure by free access to bottles was chosen, since gavage can have potential confounding factors, such as aspiration, pulmonary injury, and/or elicitation of a stress response, we expect that the chronic administration (30 days) by gavage would induce several and more dangerous responses in rats. Indeed, it was demonstrated that mice exposed to tannery effluent show an anxiety-like behavior using this exposure protocol [53]. Water consumption did not differ among the groups (data not shown). Each experimental group consisted of at least 15 animals. Behavioral observations took place in soundproof rooms at the same period of the day. The behavioral tests were divided into two schemes (Fig. 1).

2.3. Behavioral tests

2.3.1. Plus-maze discriminative avoidance task

Plus-maze discriminative avoidance task was performed as previously described [31]. The apparatus employed is a modified elevated plus-maze, made of acrylic, containing two enclosed arms with side walls and no top (50 × 50 × 10 cm), opposite to two open arms (50 × 10 cm). A 100-Watt lamp and a bell were placed exactly over the middle of one of the enclosed arms (aversive enclosed arm). Temperature was the same in both arms of the apparatus and the experimental room (22–23 °C). At the training session (day 1), each rat was placed in the center of the apparatus