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JSM Anxiety and Depression

Research Article

Parental Exposure to Tannery Effluent Cause Anxiety-and Depression-Like Behaviors in Mice Offspring

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Submitted: 29 April 2016

Accepted: 11 May 2016

Published: 13 May 2016

Keywords

- C57Bl/6J mice
- Heavy metals
- Toxicity
- Xenobiotic
- Epigenetic effect

Abstract

Few studies have assessed the effects of tannery effluents intake in mammalian animal models, despite the discharge of tannery effluents into water bodies without treatment are environmental and public health problems in many countries. This study therefore aimed to evaluate the effects of the mice parental generation exposure to tannery effluent on the anxiety and depression behaviors in its offspring, based in previous findings that demonstrate central nervous system damage in adult mice exposed to xenobiotic. For this, adult mice C57BL/6J were exposed for 60 days to the experimental treatments: tannery effluent diluted in water at concentrations of 7.5%, 15% or only drinking water (control group). Male and female mice were put for mating and the pregnant females continued exposure to effluent in the treatment groups, until lactation period. After weaning the offspring were submitted to behavioral tests, elevated plus-maze test (predictive of anxiety) and forced swim test (predictive of depression). Our date demonstrated the tannery effluent effects the offspring, increasing their anxiety index, decreasing the time in open-arms ratio and the frequency of open-arms entries, indicating an increase in anxiety-like behavior. Regarding forced swim test, we observed increased time in immobility in the experimental groups, indicating depression-like behavior. Thus, our findings support the hypothesis that parental exposure to tannery effluents, containing neurotoxic substances, cause anxiety-and depression-like behavior in the offspring.

ABBREVIATIONS

EPM: Elevated Plus-Maze; FST: Forced Swim Test

INTRODUCTION

Industrial processes and human activities typically generate specific wastes, which are composed of different substances and can be harmful to the environment and human health [1,2]. Among all the industrial wastes, tannery effluents are ranked as the highest pollutant [3] and produce a considerable pollution load by discharging untreated effluents directly into the environment [4].

This problem is intensified due the incorrect discharge of produced effluents into water bodies, causing a serious risk of environmental contamination [4-6]. Tannery effluent, even after treatment, contains considerable organic and inorganic substances, such as acids, phenols, sulfates and sulfides, and toxic elements, such as chromium, used during the tanning process [7,8]. The heavy metals and organic compounds released are one of the key factors that exert negative effects in humans and environment causing toxicity to plants and other forms of biotics that are continually exposed to potentially toxic heavy metals. Nowadays, the intense growth of this type of industry in certain localities has shown how the waste can cause irreversible damage to the water bodies and their vicinity [9].

Tanneries can generate a considerable pollution load by discharging untreated effluents directly into the environment due to the poor enforcement of law [10]. For example, there are nearly 300 tanneries in Hazaribagh, Dhaka, which are discharging daily about 18,000 L of liquid waste, 115 tons of solid wastes during off-peak time [11]. According to Rusal *et al.,* [12], 60,000 tons of raw hides and skins are processed in these tanneries every year, which release daily approximately 95,000 L of untreated effluents into the environment, resulting into a dead river, named Buriganga.

Toxicological studies demonstrated different effects of tannery effluents, like teratogenicity in sea urchin species, microalgae growth reduction and a variety of toxic effects in micro-crustaceans [13]. However, few studies investigated the tannery effluents effects in mammals. Siqueira *et al.,* [14] demonstrated that mice exposed to different concentrations of tannery effluent exhibited a state of anxiety. Our group has studied the exposure of C57Bl/6J mice to tannery effluent and recently we showed that mice exposure, for 120 days, to 0.1%, 1% and 5% of the tannery effluent diluted in water, presented anxiety-and depression-like behaviors [15] and memory deficit [16]. In female C57Bl/6J mice our data demonstrated that exposure to 7.5% and 15% tannery effluent (60 days) increased the anxiety-like behaviors. Regarding forced swim test, we did not observe changes in the evaluated behaviors [17].

Besides the neurobehavioral effects in mice exposed to tannery effluents from drinking water, a possible environmental contamination is a transgenerational way, which is transmitted to the offspring by different routes [18-22]. Therefore, considering that heavy metals such as chromium, cadmium, nickel and lead (presents in high concentrations in tannery effluents) cause reproductive disruptions in mice [23-27], it is possible that the exposure of parental to the tannery effluent may cause behavioral disruptions on the offspring. Studies about the effects of tannery effluents on the offspring behavior do not exist in the literature. Our hypothesis is that chronic exposure to tannery effluents of the parents (male and female mice) induces anxiety- and depression-like behaviors in the offspring of C57Bl/6J mice.

MATERIALS AND METHODS

Animals and experimental groups

This study used 36 adult mice (18 males and 18 females) C57Bl/6J (3 months old), nulliparous which were housed in the Laboratory of the Biological Research of the *Instituto Federal Goiano - Campus Urutaí* (Urutaí, GO, Brazil). The mice were randomized by body mass and housed in polypropylene boxes (30.3 x 19.3 x 12.6 cm, maximum of three animals per cage). All animals were housed in light/dark cycle of 12/12 h in shelf ventilated under controlled temperature and humidity (22-25ºC and humidity of 55-60%). All procedures were approved by the Ethics Committee on Animal Use (CEUA) of the *Instituto Federal Goiano – Campus Urutaí* (GO, Brazil) (protocol No. 17/2014). Meticulous efforts were made to ensure minimal suffering of animals and reduce external sources of stress, pain and discomfort. We used only a minimum number of animals to produce reliable scientific data.

Initially the animals were distributed into three experimental groups (n = 6 per group; 6 males and 6 females): the control group, in which the animals received only drinking water and two effluent groups which the animals were exposed to tannery effluent diluted in water at 7.5% and 15%. The mice (males and female) were exposed for 60 days before mating. After this period, the females were put in males' boxes, forming six mating couples in each experimental group for a period of 15 days.

The pregnant females, kept in separated boxes, continue to receive treatment (water, 7.5% or 15% of tannery effluent) until the weaning, which occurred 28 days after the puppies birth. Thus, exposure to tannery effluent covered the pre-pregnancy, pregnancy and lactation in the maternal mice. After weaning, the offspring of each experimental group (n = 36 in control group; n = 24 in 7.5% group ; n = 30 in 15% group) was tested to predictive of anxiety and depression behaviors in rodents, as detailed in Figure 1, which illustrate the temporal distribution and the experimental design.

Elevated plus-maze (EPM)

The elevated plus-maze (EPM) test has been widely used to measure anxiety in ro-dents [28-30]. The apparatus used for the EPM test is in the configuration of a + and comprises two open arms (25 x 5 x 0.5 cm) across from each other and perpendicular to two closed arms (25 x 5 x 16 cm) with a center platform (5 x 5 cm). The open arms have a very small wall (0.5 cm) to decrease the number of falls, whereas the closed arms have a high wall (16 cm) to enclose the arm [29]. The behavior testing room was soundproofed, and the illumination levels were maintained at 100 lx. The mice were placed individually in the center zone of the maze, facing an open arm, and were allowed 5 min of free exploration. All mice were tested just once. Before each test, the arena was cleaned with 70% ethanol. EPM test was performed between 1:00 and 5:00 p.m. The anxiety index was calculated according to Cohen *et al.* [31], Contreras et al. [32] and Estrela *et al.* [33] as follows: Anxiety Index = 1 − [([Open arm time/Test duration] + [Open arms entries/Total number of entries])/2]. Furthermore, we evaluated the locomotor activity of the animals in the EPM (total number of entries = open arms + closed arms), frequency of open-arms entries and time spent in open arms ratio, according to Rodgers & Dalvi [28] and Walf & Frye [29].

Were also evaluated ethological components (time), to include: stretched-attend postures (exploratory postures in which the body is stretched forward then retracted to the original position without any forward locomotion), head-dipping (exploratory movements of head/shoulders over the side of the maze) and self-cleaning (sequences of movements oriented to clean and maintain the fur and skin of the head and body). These ethological elements have been linked through factor analysis to risk assessment, directed exploration, and displacement activity, respectively, according to Rodgers *et al.* [34]. Freezing was also evaluated, operationally defined as the total absence of movement of the body and vibrissa, except the ones required for respiration, for at least 6 s, according to Reimer *et al.* [35].

Behavioral analysis for the EPM test was performed using the PlusMZ software. Three trained observers reviewed the videos; each video was analyzed twice, yielding an inter-observer concordance greater than 85%, for all parameters and tests evaluated.

Forced swim test (FST)

The FST (according to Costa *et al.* [36]) consisted of individually placing the mouse into cylindrical tank (height 18.5

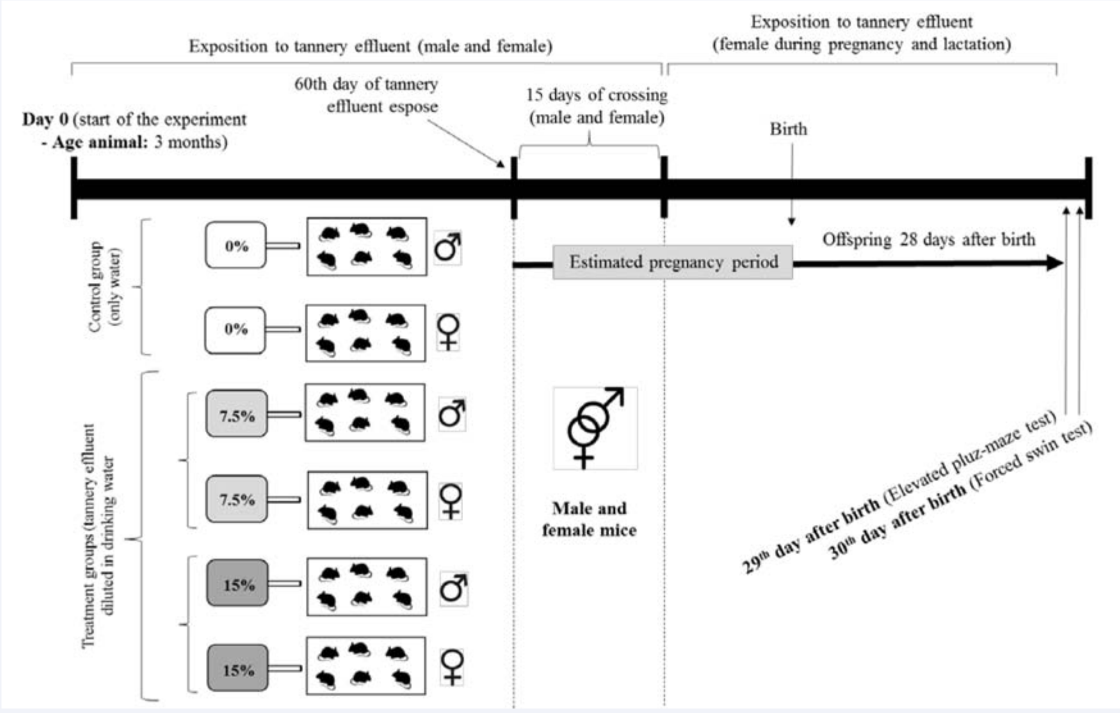

Figure 1 Temporal distribution of the experiment and realized analysis.

cm, diameter 12.5 cm) containing clean water at 25°C (13.5 cm deep). After the test (6 min), the mice were taken out of the water and dry with towel and hairdryer before being returned to their home cages. The FST took place between 1:00 and 5:00 p.m. All test sessions were videotaped using a video camera located 30 cm at the side of the tank, to allow further evaluation of the time spent in swimming, climbing and immobility. Immobility was defined as a lack of motion of the whole body, when mice ceased struggling and remained floating motionless in the water, making only those movements necessary to keep the head above the water. Swimming was recorded when large and horizontal movements of the forepaws were performed, leading to displacement of the body around the cylinder. Climbing was considered when displayed vigorous vertical movements of the forepaws, directed against the wall of the tank, leading to body displacement around the cylinder. These animals' behavioral parameters were recorded during the first 2 minutes of the test. The decision to examine the first 2 min of the test was based in Costa *et al.* [36]). The authors suggest that the first 2 min of the forced swim test in mice is an appropriate period to evaluate the effects of antidepressants on immobility.

During neurobehavioral tests the offspring was kept with their mothers to prevent stress from maternal separation, which has been used for over two decades to study the early stress results in rodent models including changes in hypothalamic-pituitary-adrenal axis [37,38]. Furthermore, the physical development and offspring reflexes were not evaluated in order to prevent that the manipulation of the offspring might provide potential interferences in neurobehavioral test performed in this study.

Tannery effluent and determination of concentrations

The tannery effluent used is classified as wet-blue, generated from the tanning stage, and obtained by a tannery industry in the state of Goiás (Brazil) (Table 1). The definition of tannery effluent concentrations used in this study is due the effluent generation data by the grantor company, taking as a basis the operating system and amount of skins processed daily in the company. Moreover, it was considered to calculate the concentrations a hydrological available data about two rivers that served illegally as receiving bodies of these effluents. The concentration of 7.5% tannery effluent was determined considering the watercourse in the dry season and the concentration of 15% was determined considering the reception of such waste in the rainy season. The grantor company name and the receptors river were purposely omitted for ethical reasons.

Statistics

All data were analyzed using the ASSISTAT software (version 7.7 beta). Data normality was tested using Anderson-Darling and Kolmogorov-Smirnov tests. The data were compared using one-way ANOVA followed by Tukey's test. Statistical differences were considered significant when the P value was below 0, 05.

RESULTS AND DISCUSSION

We found behavioral changes in the offspring from the parents mice exposed to tannery effluents in EPM and forced swim tests. In the EPM test, the offspring from the 15% group presented increased anxiety index ($F(2, 87) = 7.195$, $p = 0.008$), decreased time spent in open-arms ratio ($F(2, 87) = 8.336$, $p = 0.005$) (Figure 2A) and decreased frequency of open-arms entries ($F(2, 87) = 4.467$, $p = 0.035$) (Figure 2B), compared to

Table 1: Physicochemical and chemical characterization of the water, tannery effluent (7.5%, 15% and 100%) used in the present study.

Parameters[1]	Tannery effluent (100%)	Drinking water	Tannery effluent (7.5%)	Tannery effluent (15%)	WHO Guidelines for drinking-water Quality[2]	
					Normally found in fresh water/surface water/ground water	Health-based guideline by the WHO
pH at 25ºC (UpH)	4.05	7.19	5.17	4.87	No guideline	6.5 – 8.5
Total dissolved solids (mg.L-1)	37,380.00	80.00	2,877.5	5,675.0	No guideline	No guideline
Zn (mg.L-1)	0.30	0.03	0.05	0.07	No guideline	3.00 mg.L-1
Na (mg.L-1)	9,690.00	5.01	731.4	1,457.76	<20 mg.L-1	200 mg.L-1
Ca (mg.L-1)	601.20	4.00	48.8	93.58	No guideline	No guideline
Mg (mg.L-1)	364.80	2.43	29.6	56.79	No guideline	No guideline
Pb (mg.L-1)	0.32	‘<0.01	‘<0.01	0.05	No guideline	0.01 mg.L-1
As (mg.L-1)	<0.01	<0.01	<0.01	<0.01	No guideline	0.01 mg.L-1
Cr (mg.L-1)	859.00	<0.05	64.4	128.85	<0.002 mg.L-1	0.05 mg.L-1
Cd (mg.L-1)	0.95	<0.001	0.1	0.14	<0.002 mg.L-1	0.003 mg.L-1
Ni (mg.L-1)	5.50	<0.01	0.4	0.83	<0.02 mg.L-1	0.02 mg.L-1

[1]The analysis of the raw tannery effluent and water followed the methodology recommended by the American Public Health Association [74].
[2]The whose guidelines for drinking-water quality, set up in Geneva, 1993, are the international reference point for standard setting and for drinking-water safety

offspring from control group. Moreover, offspring from the 15% group presented decreased locomotor activity, in comparison with control group, measured indirectly by the total crossings (F (2, 87) = 5.251, p = 0.023) (Figure 2B).

The EPM revels anxiety-like behaviors in pups from tannery effluents groups. The time spent in open-arms and frequency of open-arms entries in EPM is based on the mice exploratory behavior and their natural aversion to open area [28-30]. Therefore, the elevated anxiety index, the reduced frequency of open arms ratio and open arms entries indicate anxiety-like behaviors induced by chemicals presents in the tannery effluent.

Regarding evaluation of complementary ethological elements, we observed an increase in time ($F_{(2,87)}$ = 4.669, p = 0.031) (Figure 3A) and frequency of the freezing behavior ($F_{(2,87)}$ = 5.903, p = 0.016) (Figure 3B); increase in the time of the stretched-attend postures ($F_{(2,87)}$ = 21.522, p < 0.001)and decrease in time of the episodes of head-dipping ($F_{(2,87)}$ = 8.281, p = 0.005) in the offspring of7.5% and 15% tannery effluent groups (Figure 3A). The time of the self-cleaning behavior was higher in the offspring from mice of the 15% group ($F_{(2,87)}$ = 3.313, p = 0.042), in comparison with control group (Figure 3A). The frequency of the stretched-attend postures was higher in the offspring from mice of the 7.5% group ($F_{(2,87)}$ = 9.392, p = 0.003) and the frequency of the self-cleaning was higher in the offspring from mice of the 15% group ($F_{(2,87)}$ = 6.865, p = 0.010), in comparison with control group (Figure 3B). The frequency of the head-dipping was minor in the offspring from mice of the 15% group ($F_{(2,87)}$ = 9.540, p = 0.003) (Figure 3B).

Our results corroborate to the primary behaviors observed in the EPM test. The offspring from parental generation exposed to tannery effluents exhibited less time in freezing behavior, head-dipping and stretched-attend postures, which is consistent with an anxiogenic effect of the treatments. In relation to the head-dipping behavior, it is inversely associated with anxiety, as discussed by Anseloni & Brandao [39], which is consistent in this study at an anxiogenic behavior in animals exposed to tannery effluent diluted in water. Regarding the behavior stretched-attend postures, it can be defined as a risk assessment behavior [40]. Therefore, an increase in time of this behavior found in this study represents increasing animal anxiety during the exposition to the open area on the EPM.

Furthermore, when a mouse explore a new place potentially dangerous or a threatening situation, it gets immobile, alert, with tense muscles, read for quick and vigorous action, defined as “freezing”. Alternating with the immobility, the animal can perform careful exploitation of the environment, making a risk assessment. Thus, an increase in the time of this risk assessment behavior, as seen in our study, is consistent with an anxiogenic behavior, as discussed in different studies [41,42].

Regarding the FST, we observed that offspring from female mice exposed to tannery effluent (7.5% and 15% groups) showed a higher immobility time (in 120 s) (F(2,87) = 2.613, p = 0.022), compared to offspring control. The immobility in the FST was originally considered as a model of depression [43,44] and thought to represent the psychomotor retardation shown by many depressed patients. For the time spent in swimming ($F_{(2,87)}$ = 0.613, p = 0.551) and climbing ($F_{(2,87)}$ = 0.345, p = 0.711), there were no differences between the experimental groups (data not shown).

There are multiple mechanisms through which parents can influence their offspring and recently studies showed environmentally induced epigenetic disease and disease risk [45]. The transgerational epigenetic effects on the brain can be inherited from paternal or maternal, by different pathways [21-22]. Though, the study did not aimed to evaluate how is the heritability of the effluent effects or the maternal and paternal roles in the observed effects [46]. However, there is more

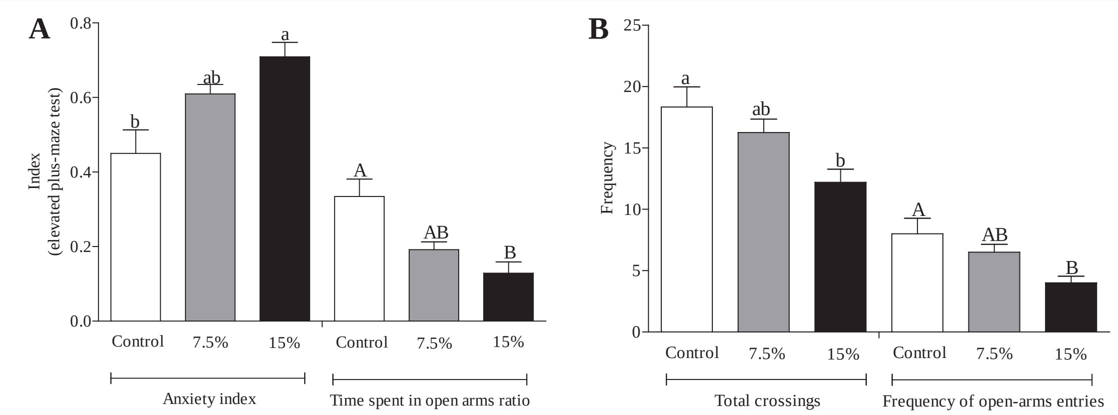

Figure 2 (A) Anxiety index and time spent in open arms ratio by elevated plus-maze test and (B) frequency of open-arms entries and total crossings by elevated plus-maze test of the offspring from mice exposed or no to tannery effluent in different concentrations diluted in water (control, 7.5% and 15%). In "A", distinct lowercase and uppercase indicate significant differencesamong the experimental groups for anxiety index and time spent in open arms ratio by elevated plus-maze test, respectively (one-way ANOVA followed by Tukey's test at 5% probability). In "B", distinct lowercase and uppercase indicate significant differencesamong the experimental groups for total crossings and frequency of open-arms entries by elevated plus-maze test, respectively (one-way ANOVA followed by Tukey's test at 5% probability). Control group = 36 pups; 7.5% group = 24 pups and 15% group = 30 pups.

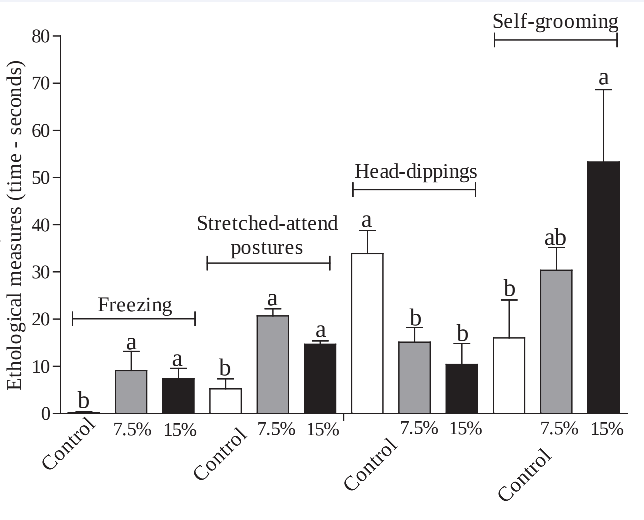

Figure 3 Ethological complementary elements in the elevated plus-maze test of offspring from mice exposed or no to tannery effluent in different concentrations diluted in water (control, 7.5% and 15%). Distinct lowercase indicated significant differences among the experimental groups for each ethological elements evaluated (time and frequency), one-way ANOVA followed by Tukey's test at 5% probability. Control group = 36 pups; 7.5% group = 24 pups and 15% group = 30 pups.

evidence on the epigenetic effects of literature in the offspring, caused by pollutants, inherited from mothers [19,47].

Regarding the pre and neonatal exposure, there is a growing body of evidence that different pollutants can alter the epigenetic programming and/or increase the risk of F1, F2 and even F3 present some diseases or neurobehavioral changes [20], evaluated the impact in the offspring of mice from exposure to heavy metals [47-50], smoke [51-53], air pollutants [54,55] and endocrine disrupters discharging on the environment [56,57].

Few studies involving the exposure of mammals to tannery effluents and their neurobehavioral effects were developed [14,16,58]. Moreover, these studies have assessed the effects of tannery effluents in adult mice, without evaluate the offspring effects (which is not the case in this study), and possess characteristics that make it difficult to compare results. Siqueira *et al.,* [14] demonstrated that male Swiss mice (adults) exposed to effluent tannery diluted in water at a concentration of 1% for 21 days showed predictive anxiety behavior, suggesting affect to the animals' nervous system central.

Moysés *et al.,* [58] investigated possible neuro and hepatotoxic effects and predictive behaviors of anxiety, depression and memory deficits induced by chronic exposure to tannery effluents in adult male Wistar rats. These authors did not observe any change in the evaluated parameters and suggest that the experimental model used in the study may not be an appropriate type for toxicological studies of tannery effluents. However, we have recently demonstrated that male and female Swiss mice

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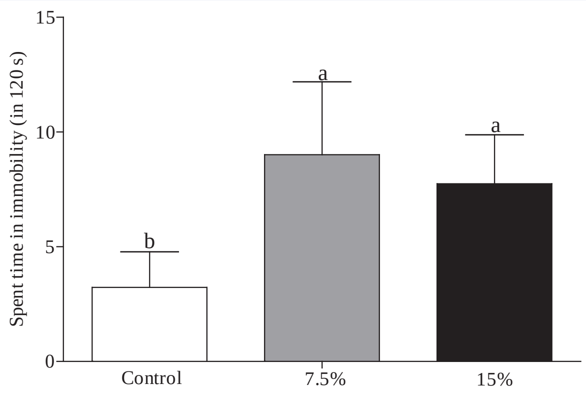

Figure 4 Spent time in immobility (in 120 s) in the forced swim test of offspring from mice exposed or no to tannery effluent in different concentrations diluted in water (control, 7.5% and 15%). Distinct letters indicate significant differences among the experimental groups by one-way ANOVA followed by Tukey's test at 5% probability. Bars indicate mean + standard deviation. Control group = 36 pups; 7.5% group = 24 pups and 15% group = 30 pups.

exposed for 15 days at 1% of effluent tannery diluted in water and offered by gavages to the animals (unlike the studies mentioned above, which they offered to the tannery effluent animals diluted in water troughs of the animals), caused memory deficits [16].

Other particularity of these papers refers to the type of effluent tannery used in the experiments ranging [16,17,58], the animal's strain used as experimental model (our work is the unique to use inbred mouse strain) and the period of exposure to xenobiotics.

Conforming discussed by Shakir et al., [59], the tannery effluents are xenobiotics, which the chemical composition is complex and varies greatly between tannery industries. In a study that aimed to analyze the effects of in vitro exposure of tannery wastewater on the activity of different enzymes in mice, rats and *Drosophila melanogaster*, Moysés [58] identified more than 20 organic chemicals in the effluent, which allows inferences about the difficulty to understand which components of these effluents are responsible for the observed effects.

Therefore, it is interesting that the effects of these effluents intake on the health of organisms can differ, depending on the species and evaluated lines, shape and length of exposure, age and sex of the animals, and on the exposure of parental generation to xenobiotics in question. Our findings suggest effects of tannery effluents beyond the oral ingestion of the residue by animals in adulthood. We noted behavioral disruptions in the offspring, caused possibly by impairment in the central nervous system.

Although analysis of the organic composition of the effluents was not performed, we suggest that constituents (inorganic and/or organic) of the effluent may have disturbed the production/action of neurotransmitters in the central nervous system organs or a dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis which could be related to anxiety and depression. Anyway, although detailed analysis of the organic composition of the effluents have not been conducted in this study, hampering the understanding of mechanisms related to the anxiogenic and depressive-like effects observed in the animals, it is suggested that constituents (organic and/or inorganic) of the effluent may have acted in the animals' body antagonistic or contrary to benzodiazepines, drugs commonly used to treat anxiety disorders [60].

As discussed by Kryger& Roth [61], this group of substances is characterized by the neurotransmitter action in the gamma-amino butyric acid system (GABA) that is the major inhibitory neurotransmitter of the central nervous system (CNS). GABA and its agonists, such as benzodiazepines, barbiturates, imidazopiridinos derivatives, besides the alcohol, act in a transmembrane structure of the GABA-receptor called GABAA complex [62]. The GABAA complex, in turn, is mainly made up of five protein subunits (2 alphas, 2 betas and one gamma) with extramembrana receptors for various substances [61]. The main mechanism of action of these substances is characterized by binding to receptors in the GABA complex, and the action, directly or indirectly of these substances in those receptors, opening the chloride channel with subsequent anion influx into the neuron and resulting in hyper polarization of the cell, causing an anxiety-like effect [60]. Although rare, work involving contaminants in rodents demonstrate behaviors similar to those seen in this study [14], supporting the hypothesis that the effluent from tannery used may have acted antagonistically to these drugs. Moreover, it can be assumed that the effluent from tannery or its constituents may have interfered negatively on serotonergic transmission and/or HPA axis. Different studies have shown the implication of serotonin in the control of anxiety, suggesting that an anxiogenic effect may be induced by increase the level of serotonergic activation, in rodents [63,64].In addition, disturbances in the HPA axis has caused changes compatible with a depressive-like behavior [65-67].

Among the chemical elements present in high concentrations in the tannery effluent used in this study (Table 1), which the neurotoxic effects are well known, refers to cadmium. Although rare, some studies have demonstrated effect of the cadmium in mice central nervous system, when exposed female in pregnancy/lactating or their offspring, what could be related to anxiety and depression [68,69].

Another heavy metals found in high concentrations in the effluent used is nickel (Table 1). Despite the lack of studies about parental exposure of nickel on predictive behaviors of anxiety and depression in the offspring of rodents, there is evidence that the intake of the element by mothers constitute threat to progeny. Saini *et al.,* [49] showed that Swiss mice exposed to Ni^{2+} and hexahydrate nickel chloride ($NiCl_2.6H_2O$) showed a significant effect on the offspring, like decreased of brood size, body mass, higher mortality and increased frequency of morphological abnormalities in eyes, limbs and tail.

The lead is another important element present in the tannery effluent and its effects are sufficiently studied. Harmful effects caused by lead have been observed in the rat neurons structural plasticity, causing debilitation of spatial memory and learning processes [70]. Recently Yu *et al.,* [71] demonstrated changes in key synaptic proteins (such as PSD-95, nNOS and SYP) in the hippocampus of lead-exposed offspring, suggesting that changes in these proteins in the hippocampus can influence the properties of synaptic transmission and cause nerve damage which may induce anxiety disorders, depression and deficits in learning ability and memory. Thus, whereas the hippocampus is an important brain region related to anxiety disorders and depression [72,73], the finding of Yu *et al.,* [71], together with the other aforementioned studies support the hypothesis that predictive anxiety and depression behavior observed in the offspring derived from 7.5% and 15% tannery effluent groups can be related to hippocampal damage caused by one or more constituents of the tannery effluent.

Interestingly, our data provide the first report about tannery effluent effect on the offspring behavior and reinforce the recent evidences that parental exposure of diverse environmental chemicals dysregulates the offspring epigenome, with potential disorders and diseases [20]. The mechanism from tannery effluent which induce anxiety-and depression-like behavior, the major substances and the influence on the maternal and paternal roles are urgent questions to contribute for risk assessment on human and animal health.

CONCLUSION

The offspring of parental C57Bl/6J mice exposure to tannery effluents (7.5 and 15%) presented predictive behaviors of anxiety and depression, soon after the weaning, confirming our hypothesis. We demonstrate in the first time the impairment effects of tannery effluents in the mice offspring, which allows inferring that the tannery effluent intake by parents constitutes a threat to the progeny.

ACKNOWLEDGEMENTS

The authors are grateful for BrazilianFunding Agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Proc. No. 467801/2014-2), Secretaria de Educação Profissional e Tecnológica and Instituto Federal Goiano – Campus Urutaí.

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Cite this article

Batista Guimarães AT, de Oliveira Ferreira R, da Silva WAM, Castro ALS, Malafaia G (2016) Parental Exposure to Tannery Effluent Cause Anxiety-and Depression-Like Behaviors in Mice Offspring. JSM Anxiety Depress 1(1): 1005.