RESEARCH PAPER

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Sub-chronic administration of lead alters markers of oxidative stress, acetylcholinesterase and Na+K+-ATPase activities in rat brain

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This study investigated the effects of sub-chronic administration of lead (Pb) acetate on thiobarbituric acid reactive substances (TBA-RS), total sulfhydryl content, protein carbonyl content, antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GSH-Px]), acetylcholinesterase (AChE), and Na+K+-ATPase in the cerebral structures of rats. Male Wistar rats aged 60 days were treated with saline (control group) or Pb (treatment group), at various doses, by gavage, once a day for 35 days. The animals were sacrificed twelve hours after the last administration, and the cerebellum, hippocampus and cerebral cortex were removed. The results showed that Pb did not alter the evaluated oxidative stress parameters. Furthermore, Pb (64 and/or 128 mg/kg) altered SOD in the cerebellum, cerebral cortex and hippocampus. Pb (128 mg/kg) altered CAT in the cerebellum and cerebral cortex and GSH-Px in the cerebral cortex. Also, Pb (64 mg/kg and 128 mg/kg) altered GSH-Px in the cerebellum. Moreover, Pb (128 mg/kg) increased AChE in the hippocampus and decreased Na+K+-ATPase in the cerebellum and hippocampus. In conclusion, sub-chronic exposure to Pb (occupational and environmental intoxication) altered antioxidant enzymes, AChE, and Na+K+-ATPase, contributing to cerebral dysfunction.

Key words: lead exposure, oxidative stress, cerebral structures, antioxidant enzymes, Na+K+-ATPase, acetylcholinesterase

INTRODUCTION

Lead (Pb) exposure is a serious health issue due to lead's highly toxic effects on organisms. Several biochemical targets, including the central nervous system (CNS), are especially susceptible to damage by this metal (Bokara et al., 2008; Agrawal et al., 2015). In addition, exposure to Pb unbalances pro-oxidant and antioxidant substances, causing oxidative stress and poisoning (Kim et al., 2017).

Some of the most significant sources of environmental Pb contamination include mining, smelting, and, in some countries, the use of leaded paint and leaded fuels. About three-quarters of global Pb consumption occurs in the manufacturing of Pb-acid motor vehicle batteries (WHO, 2021).

In recent years, the improper management of solid waste has posed a threat to the ecosystem and human health due to the continued release of heavy metals (Sun et al., 2018). Many studies have pointed out the harmful effects of Pb, leading to increased awareness

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and minimized use of this metal. The number of children experiencing Pb intoxication has declined after bans on leaded fuels and paint were implemented. However, even minimal levels have been associated with a decline in cognitive function, and environmental exposure to Pb continues since Pb does not dissipate from the environment (Rocha and Trujillo, 2019).

Pb absorption is affected by the route of exposure, the chemical species formed, dose, frequency, duration, water solubility, and individual physiological variations such as age, sex, lifestyle, and physiological and nutritional state, as well as by the susceptibility of the exposed organism (Arantes et al., 2016). Pb can enter through the respiratory tract, skin contact and digestive tract and accumulate in the body (Adamse et al., 2017).

Once absorbed, Pb is distributed between blood and soft tissues (kidneys, bone marrow, liver, and brain) and mineralized tissues such as bone and teeth (Ahrens et al., 2016). The half-life of Pb in the erythrocyte is 35 days, two years in the brain, and more than ten years in bone (Safety, 2016). Bones are an important storage and mobilization site for Pb, contributing 50% of blood Pb levels (Flora et al., 2012). Studies have shown that children absorb five times more Pb than adult organisms due to the inefficiency of the blood-brain barrier in the developing body. There is also evidence that maternal blood Pb levels may become a source of exposure to the developing fetus through the umbilical cord (Ladele et al., 2019).

In the CNS, this metal tends to concentrate in the grey matter and certain nuclei. The highest concentrations occur in the hippocampus, followed by the cerebellum, cerebral cortex, and bone marrow (Costa et al., 2001). Recent studies highlight the importance of genetic factors, such as the *GRIN2A* and *GRIN2B* genes, in identifying susceptibility to neurotoxicity due to continuous exposure to substances with low Pb content in children as a result of using dental amalgam (Rooney et al., 2018). A recent study also suggests that Alzheimer's disease may be associated with a defect in epigenetic regulation that is possibly caused by Pb exposure (Wang et al., 2020).

Studies have shown that the toxic action of Pb may occur through two mechanisms of action: the first is due to the formation of complex enzymatic functional groups, and the second is due to interference with cell membrane function, altering or preventing the transport of essential substances (Dobrakowski et al., 2017). Pb also competes with essential metals by binding to physiological sites, mimicking their function, thus leading to the bioaccumulation of this heavy metal in the organism (Arantes et al., 2016).

The presence of Pb in biological systems results in the generation of reactive oxygen species (ROS), directly or indirectly causing lipid peroxidation (Kasperczyk et al., 2015). Studies have also demonstrated that impairment of the antioxidant enzymatic system in the brain can result in brain toxicity and neurodegenerative disorders such as Parkinson's and Alzheimer's (Bokara et al., 2008; Halliwell, 2013; Mao et al., 2017; Modabbernia et al., 2016; Huat et al., 2019). Several studies have demonstrated that Pb exposure may cause neurological deficits such as the impairment of learning/memory processes, dysfunctions in neuronal differentiation; neurogenesis; and neuronal regeneration, and impairment of locomotor activity (Basit et al., 2015; Reckziegel et al., 2011).

This study aims to evaluate the effects of sub-chronic Pb exposure on oxidative stress parameters and on the activity of acetylcholinesterase (AChE) and Na+K+-ATPase enzymes in the brain structures of rats. The study stands out by evaluating three different doses of Pb, which were chosen in consideration of the wide range of occupational and environmental Pb exposures in humans (Celik et al., 2005; Lugate and Costa, 2013).

METHODS

Animals and reagents

Sixty-day-old male Wistar rats (220-280g), obtained from Univali University, Itajaí, Brazil, were used in the experiments. The animals were maintained on a 12 h light/12 h dark cycle at a constant temperature (22 ± 1°C), with free access to water and commercial protein chow. The "Principles of Laboratory Animal Care" (NIH publication 85-23, revised 1985) were followed in all the experiments, and the experimental protocol was approved by the Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, under the protocol number 002/2016–PRPPG/CEP. Environmental conditions, lighting, accommodation and nutrition followed the recommendations required by the "Guide for the Care and Use of Laboratory Animals."

All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

Sub-chronic treatment with Pb

Male Wistar rats aged 60 days were treated with saline (control group) or Pb acetate (Sigma Aldrich) at doses of 16 mg/kg, 64 mg/kg or 128 mg/kg by gavage once a day for a period of 35 days (Lugate and

Costa, 2013). The Pb exposure model chosen took into account that the LD50 for oral Pb acetate rats is 4,665 mg/kg (ATSDR, 2007) and doses of 16 mg/kg, 64 mg/kg and 128 mg/kg were based on concentrations found in exposures to humans in the environment according to the literature (Celik et al., 2005; Lugate and Costa, 2013).

The rats were divided into four groups (n=8), as follows: Control group received 1 mL of saline by gavage once a day for 35 days; Treatment group (I) received 16 mg/kg of Pb by gavage once a day for 35 days; Treatment group (II) received 64 mg/kg of Pb by gavage once a day for 35 days; Treatment group (III) received 128 mg/kg of Pb by gavage once a day for 35 days.

The animals were sacrificed twelve hours after the last administration, and the brains were removed.

Tissue preparation

After decapitation, the brain was immediately removed; the cerebral cortex, cerebellum, and hippocampus were dissected and kept chilled until homogenization. The time that elapsed between decapitation and dissection was less than 1 min. The cerebral structures were homogenized in ten volumes (1:10 w/v) of appropriate buffer according to the technique to be performed. Homogenates were prepared using a Potter-Elvehejem homogenizer (Remi Motors, Mumbai, India) by passing five pulses and centrifuging at 800 × g for 10 min at 4°C before discarding nuclei and cell debris. The pellet was discarded, and the supernatant was saved in aliquots and stored at -80°C for assaying the activity of antioxidant enzymes, damage to proteins, lipid peroxidation estimates, cholinesterase and Na+K+-ATPase activities (Delwing et al., 2007).

Thiobarbituric acid reactive substances (TBA-RS) measurement

TBA-RS were determined according to the method described by (Ohkawa, 1979). TBA-RS methodology measures malondialdehyde (MDA), a product of lipoperoxidation, primarily due to hydroxyl free radicals. Briefly, plasma in 1.15% KCl was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBA-RS were determined by the absorbance at 535 nm. A calibration curve was obtained using 1,1,3,3-tetramethoxypropane as the MDA precursor, and each curve point was subjected to the same treatment as that of the supernatants. TBA-RS content was calculated as nanomoles of MDA formed per milligram of protein.

Total sulfhydryl content determination

Total thiol group concentration was determined by the method of Aksenov and Markesbery (2001). Briefly, 50 µL of homogenate was added to 1 mL of phosphate-buffered saline (PBS), pH 7.4, containing 1 mM ethylenediamine tetraacetic acid (EDTA). The reaction was started by the addition of 30 µL of 10.0 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and incubated for 30 min at room temperature in a dark room. Total sulfhydryl content was determined by measuring the absorbance at 412 nm. Analysis of a blank (DTNB absorbance) was also performed.

Results are reported as nmol 3-thio-2-nitrobenzoic acid (TNB)/mg protein.

Protein carbonyl content determination

Carbonyl content was assayed by a method described by Reznick and Packer (1994), based on the reaction of protein carbonyls with dinitrophenylhydrazine, which forms dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm. Briefly, 200 µL of homogenate was added to plastic tubes containing 400 µL of 10.0 mM dinitrophenylhydrazine (prepared in 2.0 M HCl). Samples were kept in the dark for 1 h and vortexed every 15 min. Subsequently, 500 µL of 20% trichloroacetic acid was added to each tube. The mixture was vortexed and centrifuged at 14,000 × *g* for 3 min, and the supernatant obtained was discarded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v), vortexed, and centrifuged at 14,000 × g for 3 min. The supernatant was discarded, and the pellet was resuspended in 600 µL of 6 M guanidine (prepared in a 20.0 mM potassium phosphate solution, pH 2.3) before vortexing and incubating at 60°C for 15 min. Samples were then centrifuged at 14,000 × g for 3 min, and the supernatant was used to measure absorbance at 370 nm (UV) in a quartz cuvette. Results are reported as nmol carbonyl content/mg protein.

Catalase assay (CAT)

CAT activity was assayed by the method of Aebi (1984) using a UV-visible Shimadzu spectrophotometer. The method used was based on the disappearance of hydrogen peroxide (H_2O_2) at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100, 10.0 mM potassium phosphate buffer, pH 7.0, and 0.1-0.3 mg protein/mL. One CAT unit was defined as 1 µmoL of H_2O_2 consumed per minute, and the specific activity was calculated as CAT units/mg protein.

Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured by the method of Wendel (1981) using tert-butyl-hydroperoxide as substrate. NADPH disappearance was monitored at 340 nm using a UV-visible Shimadzu spectrophotometer. The medium contained 2.0 mM GSH, 0.15 U/mL GSH reductase, 0.4 mM azide, 0.5 mM tert-butyl-hydroperoxide and 0.1 mM NADPH. One GSH-Px unit was defined as 1 µmol of NADPH consumed per minute, and the specific activity is presented as GSH-Px units/mg protein.

Superoxide dismutase assay (SOD)

The method used to assay SOD activity is based on the capacity of pyrogallol to autoxidize, a process highly dependent on superoxide ($O_2^{\bullet-}$), which is a substrate for SOD, using the method of Marklund (1985). Briefly, to 15 µL of each sample, 215 µL of a mixture containing 50.0 µM Tris buffer, pH 8.2, 1.0 µM EDTA, and 30.0 µM CAT were added. Subsequently, 20.0 µL of pyrogallol was added, and the absorbance was immediately recorded every 30 s for 3 min at 420 nm using a UV-visible Shimadzu spectrophotometer. The inhibition of autoxidation of pyrogallol occurs in the presence of SOD, whose activity can be indirectly assayed spectrophotometrically. A calibration curve was performed with purified SOD as a reference to calculate the activity of SOD present in the samples. One SOD unit is defined as the amount of SOD necessary to inhibit 50% of pyrogallol autoxidation, and the specific activity is reported as SOD units/mg protein.

Acetylcholinesterase (AChE) activity assay

The brain structures were homogenized in potassium phosphate buffer, pH 7.5. The homogenate was centrifuged at 1,000 x g for 10 min, the pellet was discarded, and the supernatant was used for determining AChE activity and protein concentration. AChE activity was determined according to the colorimetric method of Ellman et al. (1961) with some modifications.

Na+K+-ATPase activity assay

The reaction mixture for the Na+K+-ATPase activity assay contained 5.0 mM $MgCl_2$, 80.0 mM NaCl, 20.0 mM KCl and 40.0 mM Tris–HCl, pH 7.4, in a final volume of 200 µL. The reaction was initiated by the addition of ATP. Controls were treated under the same conditions with the addition of 1.0 mM ouabain. Na+K+-ATPase activity was calculated by the difference between the two assays, as described by Wyse et al. (1998). Inorganic phosphate (Pi) release was measured by the method of Chan et al. (1986). Specific enzyme activity was expressed as nmol Pi released per min per mg of protein.

Protein determination

Protein was measured by the Lowry et al. (1951) or Bradford (1976) methods, using serum bovine albumin as standard.

Statistical analysis

For analyses, data were analyzed by ANOVA followed by the Duncan multiple range test when the F-test was significant. All analyses were performed using the IBM Statistical Package for the Social Sciences (SPSS) for Windows version 20.0 using a PC-compatible computer (IBM Corp. Armonk, NY, USA). The graphs were structured in the GraphPad Prism 6 program. Values of $p<0.05$ were considered to be significant.

RESULTS

Effects of sub-chronic administration of Pb on TBA-RS, total sulfhydryl content, and protein carbonyl content in the brain of rats

We initially assessed the effects of sub-chronic administration of different doses of Pb acetate (16 mg/kg, 64 mg/kg and 128 mg/kg) on TBA-RS, total sulfhydryl content and protein carbonyl content in the brain (cerebellum, hippocampus, and cerebral cortex) of rats. Fig. 1A-C shows that sub-chronic administration of 16 mg/kg, 64 mg/kg and 128 mg/kg Pb did not alter the levels of TBA-RS ($F_{(3,20)}=1.198$; $p>0.05$), ($F_{(3,20)}=1.294$; $p>0.05$) and ($F_{(3,20)}=1.684$; $p>0.05$); total sulfhydryl content ($F_{(3,20)}=1.436$; $p>0.05$), ($F_{(3,20)}=0,660$; $p>0.05$) and ($F_{(3,20)}=0.341$; $p>0.05$); or protein carbonyl content ($F_{(3,20)}=1.041$; $p>0.05$), ($F_{(3,20)}=0.657$; $p>0.05$) and ($F_{(3,20)}=1.115$; $p>0.05$) in the cerebellum, hippocampus and cerebral cortex, respectively, of rats.

Effects of sub-chronic administration of Pb on the activity of antioxidant enzymes in the brain of rats

Subsequently, we analyzed the effects of sub-chronic administration of different doses of Pb acetate

(16 mg/kg, 64 mg/kg, and 128 mg/kg) on the activities of SOD, CAT, and GSH-Px in the brain of rats. As shown in Fig. 2A, sub-chronic administration of Pb acetate (128 mg/kg) significantly increased the activity of SOD in rat cerebellum ($F_{(3,20)}$=16.527; p<0.001) and decreased this enzyme's activity in rat cerebral cortex ($F_{(3,20)}$=3.765; p<0.05); at doses of 64 mg/kg and 128 mg/kg, it decreased SOD activity in the hippocampus ($F_{(3,20)}$=13.492; p<0.001) of rats. Sub-chronic administration of Pb acetate (128 mg/kg) in the cerebellum decreased CAT activity ($F_{(3,20)}$=2.038; p<0.001), and in the cerebral cortex increased CAT activity ($F_{(3,20)}$=7.772; p<0.01), compared to the control group (Fig. 2B). However, this enzyme's activity was not altered in the hippocampus ($F_{(3,20)}$=1.176; p>0.05). Regarding GSH-Px activity (Fig. 3C), sub-chronic administration of Pb

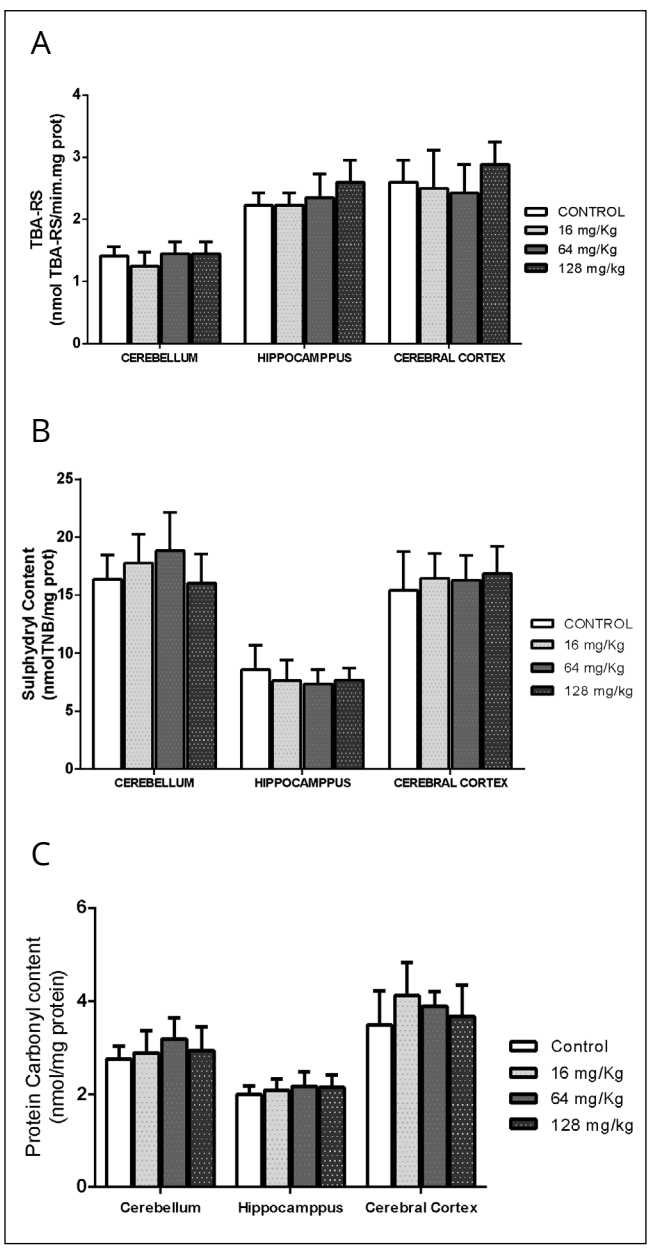

Fig. 1. Effect of increasing doses of Pb acetate (16 mg/kg, 64 mg/kg, and 128 mg/kg) on (A) thiobarbituric acid reactive substances (TBA-RS), (B) total sulfhydryl content, and (C) protein carbonyl content in the cerebellum, hippocampus, and cerebral cortex of 60-day-old rats. Results are expressed as mean ± SD for eight independent experiments (animals) performed in duplicate.

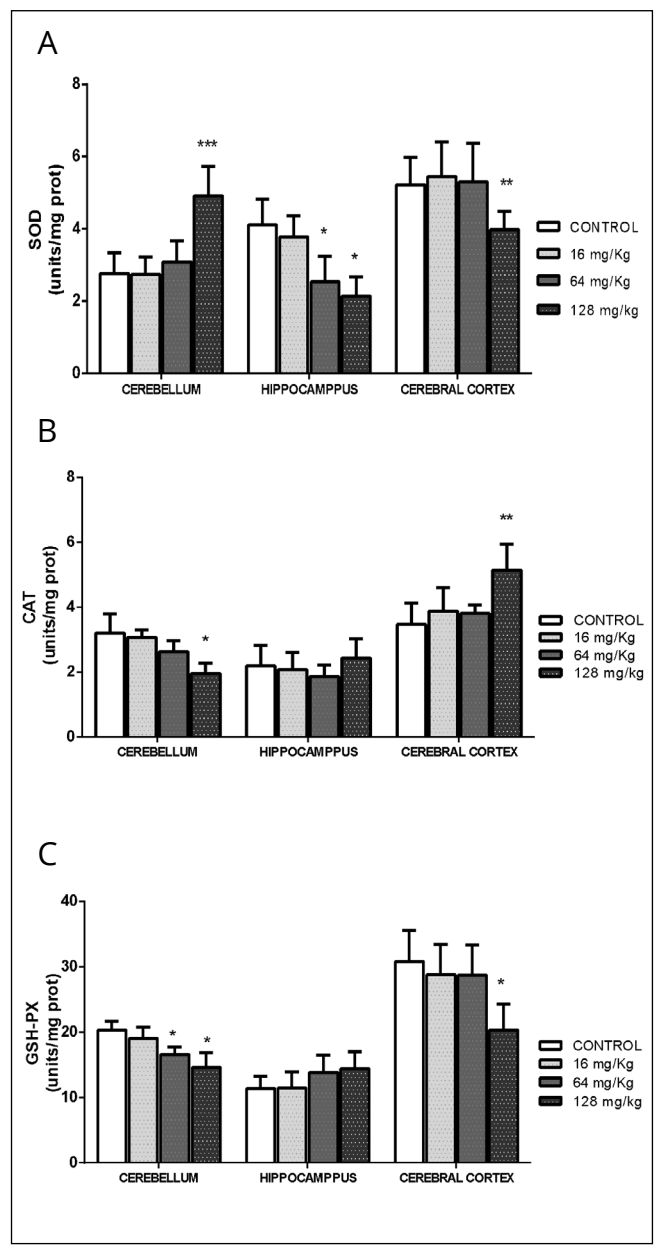

Fig. 2. Effect of increasing doses of Pb acetate (16 mg/kg, 64 mg/kg and 128 mg/kg) on the activities of (A) SOD, (B) CAT and (C) GSH-Px in the cerebellum, hippocampus, and cerebral cortex of 60-day-old rats. Results are expressed as mean ± SD for eight independent experiments (animals) performed in duplicate. ***p<0.001, **p<0.01 and *p<0.05, compared to control group (Duncan's multiple range test).

acetate (64 mg/kg and 128 mg/kg) caused a decrease in both the cerebellum ($F_{(3,20)}$=14.,428; p<0.001) and at (128 mg/kg) in the cerebral cortex ($F_{(3,20)}$=6.443; p<0.01). However, GSH-Px activity in the hippocampus was not altered by the sub-chronic administration of Pb ($F_{(3,20)}$=2.556; p>0.05).

Effects of sub-chronic administration of Pb on the activity of AChE and Na+K+-ATPase in the brain of rats

Finally, this study investigated the effects of sub-chronic administration of Pb acetate (16 mg/kg, 64 mg/kg and 128 mg/kg) on the activity of AChE and sub-chronic administration of Pb acetate (128 mg/kg) on the activity of Na+K+-ATPase in the brain of rats. The activity of AChE (Fig. 3A) was increased by sub-chronic administration of Pb acetate (128 mg/kg) in rats only in the hippocampus ($F_{(3,20)}$=3.78; p<0.05), while in the cerebellum ($F_{(3,20)}$=0.728; p>0.05) and cerebral cortex ($F_{(3,20)}$=1.744; p>0.05), Pb did not change its activity. With regard to Na+K+-ATPase activity (Fig. 3B), sub-chronic administration of Pb acetate (128 mg/kg) decreased activity in the hippocampus (T=2.723; p<0.01) and cerebellum (T=1.891; p<0.05) but did not alter Na+K+-ATPase activity in the cerebral cortex (T=0.9141; p>0.05) of rats.

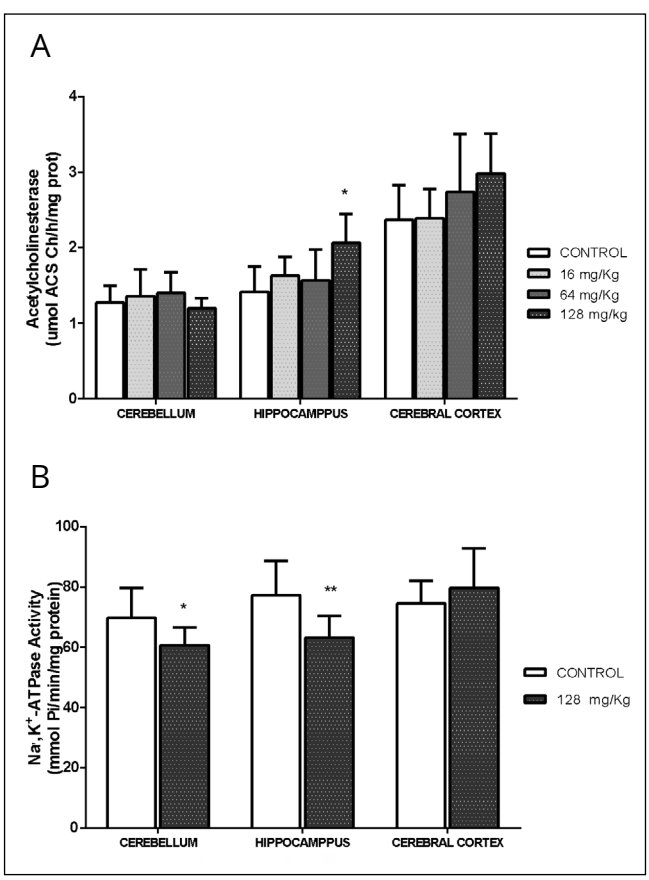

Fig. 3. Effect of increasing doses of Pb acetate (16 mg/kg, 64 mg/kg and 128 mg/kg) on the activity of (A) AChE and (B) Na+K+-ATPase in the cerebellum, hippocampus and cerebral cortex of 60-day-old rats. Results are expressed as mean ± SD for eight independent experiments (animals) performed in duplicate. **p<0.01 and *p<0.05, compared to the control group (Duncan's multiple range test).

DISCUSSION

Pb intoxication has led many countries to take measures to reduce exposure. However, contamination by Pb has remained high due to the utilization of this metal in industry and the difficulty of enforcing regulations, leading to the generation of sources of occupational and environmental exposure, including the inhalation of contaminated air and ingestion of contaminated water and food (Asaduzzaman et al., 2017; Obeng-Gyasi, 2019).

Oxidative stress (OS) has recently been studied as a mechanism of Pb toxicity due to brain damage induced by deregulation of the oxidant/antioxidant balance of nerve cells (Bokara et al., 2008; Dobrakowski et al., 2017; Reckziegel et al., 2011). The brain has a limited capacity to support OS due to the high content of easily oxidizable substrates such as polyunsaturated fatty acids and catecholamines, as well as the relatively low levels of antioxidants (Barkur and Bairy, 2015; Halliwell, 2013). The damage caused by Pb intoxication occurs mainly in the cerebellum, hippocampus, and cerebral cortex, which may result in morphological changes in the brain (Barbary et al., 2011; Sguazzin, 2020).

The brain structures chosen for this study derive from the importance of their functions. The hippocampus is fundamental to processing emotions and information associated with memory (Barkur and Bairy, 2015). The cerebellum plays an important role in motor learning, maintaining balance, controlling muscle tone, and voluntary movements (Bazrgar et al., 2015). The cerebral cortex has overlapping functions related to several processes, such as motor behavior, receiving information, coordinating complex movements, multisensory information processing, and language comprehension (Barkur and Bairy, 2015). The results of this study demonstrate that, among the cerebral structures evaluated, regarding antioxidant enzymes, major changes occurred in the cerebellum, in addition to changes in the cerebral cortex and hippocampus.

The results indicated that Pb causes significant alterations to antioxidant enzymes, thus revealing

an effect on OS. Alterations were different across the analyzed cerebral regions, which suggests that oxidative potential differs in these regions relating to susceptibility to Pb. Prasanthi et al. (2010) reported that the sensitivity to Pb neurotoxicity in the different brain regions is not only related to the accumulation of this metal but possibly to changes in specific biochemical or cellular processes in each region. The changes identified through the analyzed parameters also differed in relation to the dose of Pb used for the intoxication model. For the lowest dose of 16 mg/kg, no statistically significant changes were found; however, for the intermediary dose of 64 mg/kg, changes in SOD and GSH-Px were observed. This demonstrates that OS may occur even when one is exposed to lower doses of Pb, which may occur in occupational exposure in adults or children via water, soil, or air. In children, absorption is greater due to the rapid development of the CNS (Li et al., 2016). Another important effect of low-concentration exposure occurs with internal exposure, as Pb can be deposited in the bone by replacing Ca+2, leading to continuous systemic exposure in the organism (Basit et al., 2015). At the highest dose of 128 mg/kg, all antioxidant enzymes, AChE, and Na+K+-ATPase showed significant changes. This study showed that Pb acetate (16 mg/kg, 32 mg/kg and 128 mg/kg) did not alter TBA-RS, total sulfhydryl content, or protein carbonyl content in the brain structures analyzed, corroborating a study by Dabrowska et al. (1996) performed under similar temporal conditions with similar concentrations of Pb exposure.

Regarding antioxidant enzymes, Pb acetate at a dose of 128 mg/kg increased SOD activity in the cerebellum and decreased it in the cerebral cortex, while doses of 64 mg/kg and 128 mg/kg decreased SOD activity in the hippocampus. SOD is responsible for catalyzing the dismutation of superoxide radicals ($O^{\bullet}$) into H_2O_2 and O_2 (Halliwell, 2013). This decrease may be associated with the consumption of the enzyme by high levels of $O^{\bullet}$ or attributed to the high affinity of Pb for sulfhydryl groups (Moneim, 2012; Prasanthi et al., 2010). The decrease in SOD activity observed in this study may also be associated with Pb mimicking the function of other metals, such as copper and zinc, present as cofactors in the composition of SOD and found in the cytosol, or manganese in the mitochondria, which could have altered the concentration and function of this enzyme (Arantes et al., 2016; Basha et al., 2012; Kamiński and Kurhalyuk, 2007). Our results corroborate those of Abdulmajeed (2015), who reported a decrease in SOD activity in the cerebral structures of Wistar adult rats when exposed to 0.2% Pb in drinking water for 28 days; Bokara (2008) and Soleimani (2016), who reported similar results relating to SOD activity in the hippocampus; and Prasanthi et al. (2010), who found a decrease in SOD in the cerebral cortex.

Sub-chronic administration of Pb acetate (128 mg/kg) decreased CAT activity in the cerebellum. CAT is an important heme enzyme that directly catalyzes the decomposition of H_2O_2 (Basha et al., 2012). Pb is known for reducing iron absorption in the gastrointestinal tract, thus inhibiting heme synthesis and therefore leading to a decrease in CAT activity. Similar to our results, other studies have reported a decrease in CAT activity in the cerebellum (Baranowska-Bosiacka et al., 2011; Bokara et al., 2008; Ghareeb et al., 2010; Prasanthi et al., 2010; Reckziegel et al., 2011). In this study, in the cerebral cortex, Pb acetate (128 mg/kg) increased CAT activity, possibly due to excessive H_2O_2 formation that generated an imbalance in the redox system. The increase in CAT activity probably aided in the detoxification of this reactive species (Antonio et al., 2003; Chibowska et al., 2016; Reckziegel et al., 2011).

Sub-chronic administration of Pb acetate (64 mg/kg and 128 mg/kg) decreased GSH-Px activity in the cerebellum and, at a dose of 128 mg/kg, in the cerebral cortex. This enzyme is responsible for controlling the levels of H_2O_2 and lipid hydroperoxides. In this study, the decrease in GSH-Px activity may be associated with excessive consumption due to the increase in H_2O_2. Another possible explanation for this effect is the competition of Pb with selenium causing a decrease in the activity of GSH-Px (Dobrakowski et al., 2016). Previous studies have also reported similar results for GSH-Px in rats exposed to Pb (Baranowska-Bosiacka et al., 2011; Lalith and Muralidhara, 2014; Jiang et al., 2021).

Regarding AChE activity, results showed that sub-chronic administration of Pb acetate (128 mg/kg) increased AChE activity in rat hippocampus but did not alter it in the cerebellum or cerebral cortex. AChE is a serine protease that interrupts synapse neurotransmission by hydrolyzing acetylcholine into acetic acid and choline (Basha et al., 2012). Similar to our results, previous studies also observed an increase in AChE activity in the hippocampus (Lalith and Muralidhara, 2014; Reddy et al., 2003). This increase in AChE activity may be related to the interaction of heavy metals with acetylcholine receptors, affecting their binding efficiency and resulting in greater enzyme synthesis (Tandon et al., 2003). However, other studies have reported a decrease in AChE activity (Antonio et al., 2003; Basha et al., 2012; Phyu and Tangpong, 2014).

Finally, sub-chronic administration of Pb acetate (128 mg/kg) decreased Na+K+-ATPase activity in the hippocampus and cerebellum but did not alter it in the

cerebral cortex. Na+K+-ATPase stands out as a transmembrane enzyme of the CNS and is essential for the regulation of intracellular pH, cell volume, and calcium concentration, as well as for the exchange of transported solutes (Adefegha et al., 2016). A reduction in Na+K+-ATPase activity may be associated with neural damage caused by an excess of ROS generated as a result of Pb intoxication (Martini et al., 2014). Na+K+-ATPase activity is used as a sensitive indicator of toxicity in brain cells. This decrease in Na+K+-ATPase in the cerebellum and hippocampus is in line with the results of previous studies evaluating the neural system in Pb intoxication, as Moneim (2012) reported a reduction in Na+K+-ATPase activity in the cerebellum in an intraperitoneal route model. Possible mechanisms of inhibition of Na+K+-ATPase include the interaction of Pb with sulfhydryl groups present in its composition (Antonio and Leret, 2000) and/or competition with calcium, inhibiting the release of neurotransmitters. It can also interfere with the regulation of cellular metabolism by binding to receptors, blocking calcium transport through calcium channels and ATPase, competing for protein sites with calcium, and uptake by mitochondria (Kumar et al., 2010; Moneim, 2012).

CONCLUSION

The present study supports the hypothesis that Pb intoxication causes OS and cerebral dysfunction as it alters the activities of the antioxidant enzymes, AChE and Na+K+-ATPase in the brain structures of rats. Furthermore, the results show that depending on the dose and length of exposure to Pb, the effects are distinct in different brain areas. The study evaluated a wide range of exposures to Pb, mimicking different degrees of environmental exposure; thus, it may contribute to remediation studies and, most importantly, to preventive measures protecting the population from future brain damage.

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