Potentiation of the apoptotic signaling pathway in both the striatum and hippocampus and neurobehavioral impairment in rats exposed chronically to a low–dose of cadmium

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RESEARCH ARTICLE

Potentiation of the apoptotic signaling pathway in both the striatum and hippocampus and neurobehavioral impairment in rats exposed chronically to a low–dose of cadmium

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Abstract

Cadmium (Cd) is a highly toxic heavy metal. It accumulates in biological tissues, especially in fish which constitutes a first rank food for humans, particularly in the coastal areas. This study investigates the effect of long-term exposure to low Cd concentration (17 μg/kg/day) in rat striatum and hippocampus. In this study, the neurobehavioral ability changes were assessed by applying cognitive standard testing at the end of the rats' exposure period. In addition, the examination of mitochondrial swelling was performed at the same time of evaluation of its redox status in the brain regions studied through stress parameters (GSH, MDA, GST, and CAT). This study examined also whether this long-term exposure can modify the apoptotic signaling pathway via assessment of apoptotic markers (caspase-8 and 9, Bax, Bcl-2, and Cyt-c) in cell lysates. The results of this study showed changes in neurobehavioral abilities of animals and a stronger mitochondrial swelling associated with a significant decrease in antioxidant systems (GSH, GST, and CAT) and conversely an increase in the lipoperoxidation end product (MDA) in both the striatal and hippocampal mitochondria. In addition, the results revealed a significant increase in pro-apoptotic intracellular components such as caspase-9, Cyt-c, and Bax, and showed also an evident decrease in Bcl-2 levels. In conclusion, our results reported that chronic exposure to Cd produces behavioral and cognitive perturbations, enhances oxidative stress associated with mitochondrial edema and Cyt-c leakage, and, ultimately, potentiates apoptosis signaling pathway in both brain regions in rats.

Keywords Cd · Striatum · Hippocampus · Neurobehaviour · Oxidative stress · Apoptosis

Introduction

Over the past two decades, the industrial revolution and technological development have led to high discharges of toxic products, which considerably complicate environmental problems and their impacts on public health (Andra et al. 2017; Andjelkovic et al. 2019). Among these pollutants, Cd is a non-biodegradable industrial and environmental pollutant widely distributed which could be concentrated in the food chain (Pi et al. 2017). However, Cd, in minute quantities, has delirious effects in the body causing acute and chronic toxicities in humans (Wang et al. 2015). Recently, the studies of Abdeen and his collaborators (2017, 2019), on the effect of Cd in rat liver and kidney, showed significant increases in MDA and reduction in GSH and CAT contents in parallel to mitochondrial damage, accumulation of unfolded proteins, DNA oxidation, and upregulation of Bax and Bcl-2 expression. The U.S. Agency for the Toxic Substances and Disease Registry

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ranked Cd in the seventh position of hazardous substances and nonessential transition metal and classified as a human carcinogen by the National Toxicology Program (ATSDR 2004; Andjelkovic et al. 2019). Effectively, consultation of the available documentation shows that several scientific articles have mentioned that Cd accumulates in various tissues and cell and produces toxic effects, such as immune and brain dysfunction (Mason et al. 2014; Agnihotri et al. 2015; Xu et al. 2017; Chen et al. 2019). The toxicity of Cd can have several consequences on the human body including brain damages (Agnihotri et al. 2015; Alnahdi and Sharaf 2019). Therefore, any neurotoxic effect could alter and modify body functions in humans and animals (Järup 2003). Effectively, many findings indicate the neurotoxic effects of Cd which are associated with biochemical systems of the cell and functional disorders in the brain, especially when the animals are exposed chronically to Cd (Wang and Du 2013 ; Engwa et al. 2019). Moreover, Cd-dependent neurotoxicity has been also related to neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (PD) (Chin-Chan et al. 2015). Indeed, literature consultation reported that toxic mechanisms of Cd act in cells via an increase in reactive oxygen species (ROS) production that could disturb the equilibrium of intracellular redox status (Jomova and Valko 2011). Numerous references provided evidence which indicate that Cd-induced neuronal toxicity is due to the induction of ROS, which lead to an imbalance of redox status for the benefit of oxidative stress (Chen et al. 2008; 2011). It is also stated in scientific reports that Cd is able to induce apoptosis pathway and memory deficits and therefore leading to neurodegenerative diseases (El-kott et al. 2020). Previously, the results of important research shown that the exposition of neuronal cells to Cd generated ROS in the mitochondria and also activated BID (BH3-interacting domain), which may direct the Fas-receptor/Fas-ligand pathway toward mitochondrial apoptosis (Yuan et al. 2018). It was also noted, some time before, that autophagy could be induced by Cd in neurons (Wang et al. 2015), such results reinforce the apoptotic effect of Cd in the brain leading to neurodegenerative diseases. So, in the light of this state of the art, scientific data reveals that the toxic effect of Cd is evident but it is not always exhaustive. There is even a lack of data and knowledge about the mechanisms of its toxicity, mainly in brain regions. The current study focus on the evaluation of harmful effects of Cd, in long-term exposure in a rat model, using real low concentration (17 μg/kg/day), which was detected in freshwater fish living in Beni-Haroun dam, Algeria, in a previous study carried out by our laboratory (Habila 2018). Thus, this in vivo experimental work examines the impact of Cd on neurobehavioral and cognitive abilities in the exposed animals and also evaluates the effects of this heavy metal on mitochondrial redox status and apoptosis signaling pathway in the striatum and hippocampus.

Materials and methods

Chemicals

Almost all chemicals, Cd chloride included, were purchased from Sigma Aldrich, Germany. Assay Kits for enzymes were purchased from Biomerieux, France. For more realism in our study, a residual content of 17 μg/kg/day of Cd, found in the freshwater fish living in the Beni-Haroun dam, Algeria, was used. This study showed an average concentration of 0.34 μg/g of Cd. To convert the concentration of metals in fish to daily dose in experimental animal studies, a conversion factor was used, which is 0.05 in rats (EFSA (European Food Safety Authority) 2011). Caspases substrates were purchased from Beckman, USA, and ELISA kits from Uscn Life Science Inc. China.

Animal treatments

Female Wistar albino rats, weighing 200–250 g, were obtained from Pasteur Institute of Algeria (PIA). Upon arrival, the rats were housed, 10 per cage. Animals were maintained under a daily 12-h light/dark cycle at a constant temperature (22 ± 2 °C), relative humidity of 55 ± 10%, and free access to food and water. Rats were adapted for 2 weeks before the indicated treatments. All experimental assays were carried out in conformity with international guidelines for the care and use of laboratory animals. In this study, the animals were divided into 2 groups of 10 rats each housed in cages of large dimensions (50 × 35 × 20 cm) with double bottles excluding any crowding effect. Processing is carried out by the administration of the prepared solutions by gastric gavages using a probe attached to a syringe daily for 60 days:

- Control group (control): receiving distilled water by gavage for 60 days.
- Cadmium group (Cd group): daily receiving aqueous solution of Cd chloride (17 μg/kg).

Each rat receives an amount of feeding solution equal to 1 ml per 250 g of body weight. The body weight of the animals was monitored every 10 days throughout the study period.

All animal experiments in the current study are approved by PIA under the ethical code: N° Batna-Univ2.2020.231.

Behavioral and cognitive tests

Behavioral models in animals are crucial for neurobiological research; these models make it possible to study in depth the probable lesions and pathologies which affect the brain (Kalueff et al. 2008). After 50 days of exposure to a low dose of Cd, behavioral and cognitive tests were performed. Several targets were set to be achieved in this present neurobehavioral study,

namely the evaluation of the animals' anxiety by applying open field and elevated plus-maze tests; evaluation of working memory and learning ability of the rats using novel object recognition test; and ultimately assessment of the muscle strength of animals by applying Konziela's inverted screen test. All the tests were carried out between 14:00 and 18:00 p.m. Tests were filmed and all the variables were examined by the same experimenter, by making use of the video tracking program Etho-Vision® from Nolduls Information Technologies.

Anxiety-like measurement

Open field test Open field is considered among the oldest and most common tests; it is widely used to assess emotional behavior in rats (Crawley et al. 1997). This test was first proposed by Hall in 1934 (Gould 2009); it consists of measuring the locomotor activity in rats and is also used to assess their level of anxiety (Crépeaux et al. 2012; Lecorps et al. 2016). In principle, the OF is a closed space in cylindrical, square, or rectangular form with an open roof (Ennaceur and Chazot 2016). In this test, the used device is a square glass enclosure; the floor of the device is divided into 25 squares. The OP test was performed according to the method described by Tatem et al. (2014). Briefly, the rats were acclimated in the examination room at least 10 min before the start of the test; then, we placed each time a rat gently in the center of the device; this test lasts 5 min; the OF device is cleaned between each test with ethanol. The behavior of the rats in this test was filmed and the videos obtained were recorded on a computer for later analysis. In this study, the frequency of ambulation and rearing has been considered to assess anxiety in the rats.

Elevated plus maze The EPM test was performed according to the method described by Baldo and Petersén (2015). EPM is performed using a device with four arms 30 cm long and 6 cm wide, arranged as a plus sign, two open, and two closed. The closed arms are surrounded by 30-cm-high walls and the system is raised 50 cm from the base (ground). Each mouse was placed in the center of the maze and its movements are recorded for 5 min. The conflict between innate curiosity to explore the novel environment and fear of open arms allow the animal's state of anxiety to be determined. In the EPM, this anxiety is expressed by the tendency of rats to spend more time in the enclosed arms.

Evaluation of working memory and learning ability

Novel object recognition test The principle of this test is based on exploiting the ability of rats to recognize a new object in the environment. The NOR test was performed by applying the method described by Antunes and Biala (2011). The test is divided into 3 phases: phase of living in the experimental room within which the test takes place, the second is a familiarization phase, and the third corresponds to the test phase. This test took place in an open field described previously in the OF test; the objects used are cylindrical parts. In the first day of testing, the rats are first used to OF for 5 min as described above; the next day, the rats are subjected to an acquisition phase (familiarization) during which each rat was placed in the OF in front of 2 identical pieces marked A; the test lasts for 10 min. A final retention phase was carried out 3 h after acquisition in an identical manner to that of the familiarization phase except that the rat is confronted with 2 different objects: the familiar object A and the other new not B. The whole procedure was filmed in order to calculate the recognition index (RI) or discrimination index (DI) which is the percentage of the exploration time of the new object compared with the total exploration time of the two objects [RI = 100 × (tB/tA + tB)]. Thus, the RI evaluates the working memory of the rats and their tendency for novelty and learning ability.

Assessment of the muscle strength of treated animals

Konziela's inverted screen test The Konziela inverted screen test is used to assess the muscle strength of animals. It was performed by applying the method of Deacon (2013). The test procedures consist of placing the rat in the center of a porous metallic screen with wooden borders; then, the screen is rotated to an inverted position. In this study, the test was carried out after the acclimatization of the rats in the handling room to eliminate the effect of environmental stress. The time allowing the rat to settle before its fall was scored. If the rat resists for more than 60 s, it takes the highest score (4) and the test is stopped by removing it from the screen. The results were expressed with scores depending on the time allowing the fixation of the rat on the screen before falling. If this time is between 1 and 10 s, the rat takes the score 1; if it is between 10 and 26 s, it takes the score 2; between 26 and 60, she takes the score 3.

Separation of the mitochondrial matrix from striatum and hippocampus

At the end of the rats' exposure period, animals were sacrificed by decapitation after prolonged deep ether anesthesia; the brain was recovered and immediately washed with cold phosphate-buffered saline (PBS) and dissected immediately to separate thoroughly striatum and hippocampus which are considered as the source of the mitochondrial matrix, mitochondria suspension, and cell lysates to evaluate the effect of Cd on redox status in mitochondria, mitochondria swelling, and apoptosis markers.

The whole mitochondrial fractions were extracted as described by the method of Sahu et al. (2014). Briefly, the two

brain regions were suspended in the TSE buffer (10 mM tris, 250 Mm sucrose, and 1 mM EDTA, pH 7.2) at 4 °C and homogenized in the same buffer to obtain a tissue homogenate of 10%. The latter was centrifuged first at 600*g* for 10 min to remove cellular debris and the recovered supernatant which was centrifuged at 10,000*g* for 10 min. The pellet obtained was resuspended in an isolation buffer without EDTA. Then, further centrifugation at 10,000*g* for 10 min was carried out. The final pellet obtained was resuspended in the storage buffer (10 mM Tris, 250 mM sucrose, pH 7.2). The entire isolation procedure was carried out at 4 °C. A fresh amount of this suspension was used in the mitochondria swelling essay and the remainder was frozen to be used in the assessment of mitochondrial oxidative stress.

To recover the mitochondrial matrix, we proceeded to freezing and thawing associated with repeated homogenization, approximately 8 times to burst the mitochondrial membranes; then, we carried out centrifugation at 10.000*g* for 10 min. The supernatant obtained was used as a mitochondrial matrix, the source of MDA, GSH, CAT, GST, and SOD.

Evaluation of oxidative stress in the striatum and hippocampus mitochondria

The assessment of the redox status in striatal and hippocampal mitochondria was carried out on the mitochondrial matrix fractions obtained in Section 2.4 above. Protein content was determined by the Bradford method (Bradford 1976), using bovine serum albumin as standard. The catalase (CAT) activity was evaluated according to the method described by Claiborne (1985). The evaluation of the glutathione S-transferase (GST) activity is carried out according to the method described by Habig et al. (1974). The dosage of reduced glutathione (GSH) in brain tissue is carried out according to the method of Ellman (1959). Assessment of MDA (malondialdehyde) levels was assessed using the Ohkawa method (1979).

Evaluation of mitochondria swelling

Mitochondrial membrane permeability was evaluated in the studied brain areas, in order to examine the effect of Cd on mitochondrial permeability transition pore (MPTP). To assess this swelling, the method of Li et al. (2014) was applied. Briefly, just after mitochondria isolation from fresh tissues of the striatum and hippocampus at 4 °C, equal volumes are distributed in quartz cells and the absorbance is monitored spectrophotometrically at $\lambda = 540$ nm. The lowering of absorbance indicates the enhancement increase of mitochondrial swelling, reflecting the loss of MPTP.

Preparation of striatum and hippocampus lysates

Small pieces of striatum or hippocampus tissues removed before were used to extract cell lysates following the method of Ahmed et al. (2013). Briefly, striatum or hippocampus tissues (0.5 g) were minced and then homogenized in 1 mL lysis buffer (20 mM HEPES (pH 7.5), 150 mM NaCl, 1% NP-40, 0.1% SDS, 1 mM EDTA, and 1.0 mM DTT) with protease inhibitors (2 μg each of aprotinin, leupeptin, pepstatin A, and 0.5 mM phenylmethylsulfonyl fluoride), incubated on ice for 30 min, and then centrifuged at 10,000×*g* at 4 °C for 20 min. The striatum or hippocampus lysates were separated to evaluate the different apoptosis markers.

Assessment of apoptosis markers in striatum and hippocampus lysates

Evaluation of caspase-8 and caspase-9 The assessment of caspase-8 and caspase-9 activities in the striatum and hippocampus tissue lysates was carried out by luminescence assay using caspase-Glo8 and caspase-Glo9 substrates in accordance with manufacturer instructions (Beckman, USA). Both caspase substrates are in the form of a peptide that binds a chromogenic reagent (p-nitroanilide) which can be released after the hydrolytic action of its specific caspase. When substrates are incubated with striatum or hippocampus lysates, they give thus a luminogenic product which is measured in plate reading luminometer by Ultra-Glo ™Recombinant Luciferase. One unit of caspase-8 is the amount of enzyme required to cleave 1 pmol of the substrate (Ac-LETD-pNA) per minute at 30 °C. One unit of caspase-9 is the amount of enzyme required to cleave 1 pmol of the substrate (Ac-LEHD-pNA) per minute at 30 °C. Levels were expressed as units per milligram of proteins.

Bcl-2 protein (B cell lymphoma 2) and Bax (Bcl2-associated X protein) These apoptosis indicators were quantified in the striatum or in hippocampus lysates using ELISA kits (Uscn Life Science Inc.). The procedure was performed according to the instructions of the manufacturer. Levels were expressed as nanograms per milligram tissue proteins.

Statistical analysis

All results were expressed as means ± SD ($n = 10$). The data were analyzed by the Student *t* test to determine the significant difference between the groups. The differences were considered to be not significant at $p > 0.05$, significant at $*p < 0.05$; $**p < 0.01$; and $***p < 0.001$. All statistical analyses were carried out using the Excel SPC software package.

Results

Assessment of behavioral and cognitive changes in rats exposed chronically to Cd

Anxiety-like measurement

Assessment of rats' anxiety by the OF test Figure 1 illustrates the rates of locomotor activity which expresses the anxiety of the rats treated with Cd. The statistical analysis of the OF test results showed a significant decrease ($p < 0.001$, $p < 0.01$) respectively in horizontal activity (ambulation: the number of squares crossed by the animal) and in vertical activity (rearing: the position where the animal rests on his two hind limbs only) in rats treated with Cd compared with the control.

Evaluation of anxiety effects of the animals by the EPM test The results of this test are illustrated in Table 1. They showed a significant increase ($p < 0.05$) in the time spent in enclosed arms in the Cd group compared with control rats. At the same time, there was no significant variation in the time spent by animals in open arms, while the same test recorded a significant variation ($p < 0.01$) in the number of entries in open and closed arms in the Cd group when compared with control rats.

Evaluation of working memory and learning ability

Novel object recognition test The results of the NOR test showed a significant change ($p < 0.01$) between the Cd group and the control group (Fig. 2). In fact, there was an increase in the recognition index expressed in terms of the percentage of the exploration time of the new object compared with untreated rats.

Table 1 Assessment of anxiety effects in the rats exposed chronically to a low dose of Cd using the EPM test

	Time spent in closed arms (s)	Time spent in open arms (s)	Number of entries in open and closed arms
Control	280 ± 11.35	12.10 ± 5.08	4.70 ± 1.56
Cd group	290.70 ± 12.70*	09.70 ± 8.30	7.20 ± 1.31**

Values are means ± SD, $n = 10$; p value was detected compared with the control group

*$p \leq 0.05$

**$p \leq 0.01$

Assessment of the muscle strength of treated animals

Konziela's inverted screen test The variation of the score measuring the muscular strength allowing the fixation of the animals on the inverted perforated screen between the Cd group and control rats in the KIS test is illustrated in Fig. 3. The results showed a significant increase ($p < 0.01$) in time allowing rat fixation in the inverted screen in rats treated with Cd compared with control rats.

Evaluation of antioxidant enzymes activities in the striatum and hippocampus mitochondria, GST, and CAT

The results of CAT and GST activities assessment are illustrated respectively in Figs. 4 and 5. In the present study, a significant decrease ($p < 0.01$) in both striatum and hippocampus mitochondrial CAT activities was observed in the Cd group compared with control. The same results were obtained with the assessment of GST activities in the mitochondrial matrix in both brain regions, where the activity of this enzyme decreased significantly ($p < 0.01$) in the Cd group compared with control.

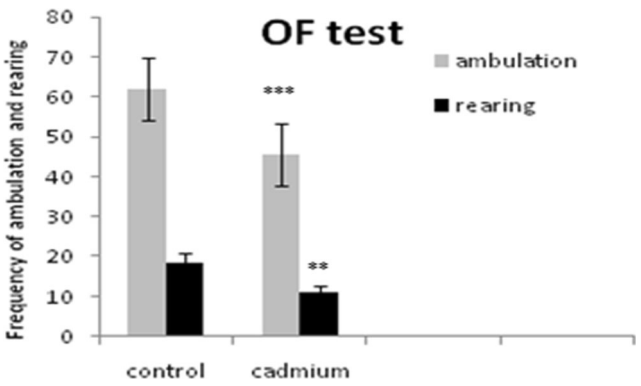

Fig. 1 Evaluation of the level of anxiety in the rats exposed chronically to a low dose of Cd using the open field (OP) test (frequency of ambulation and rearing). Values are means ± SD ($n = 10$); p value was detected compared with the control group. **$p \leq 0.01$; ***$p \leq 0.001$

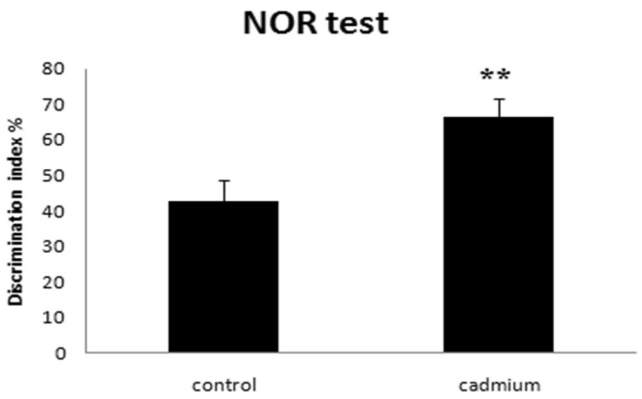

Fig. 2 Evaluation of working memory and learning ability in the rats exposed chronically to a low dose of Cd (cadmium group) using the novel object recognition test (NOR test). Values are means ± SD ($n = 10$); p value was detected compared with the control group. **$p \leq 0.01$

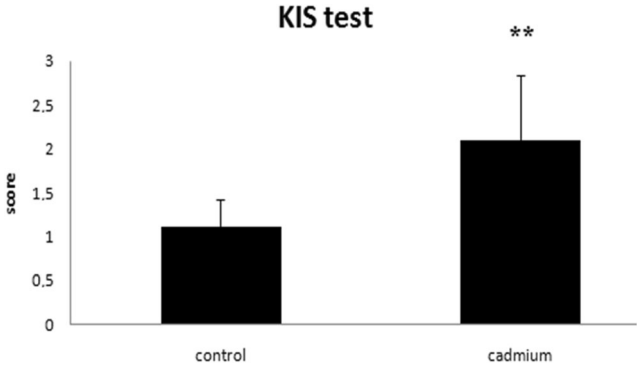

Fig. 3 Evaluation of the muscle strength of the rats exposed chronically to a low dose of Cd (cadmium group) using Konziela's inverted screen test (KIS test). Values are means ± SD (n = 10); p value was detected compared with the control group. **$p \leq 0.01$

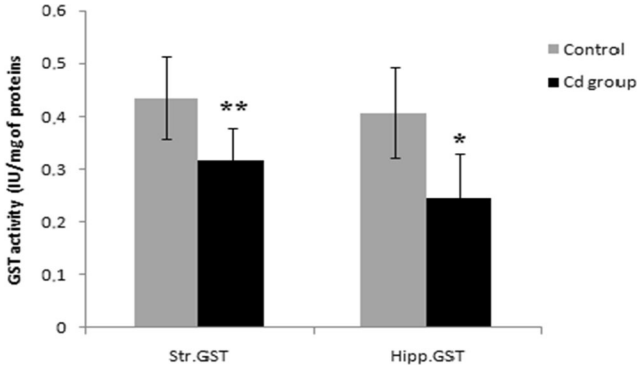

Fig. 5 GST activities in striatum and hippocampus mitochondria in rats exposed chronically to a low dose of Cd. Values are means ± SD (n = 10); p value was detected compared with the control group. *$p \leq 0.05$; **$p \leq 0.01$; Str.GST, striatum glutathione S-transferase; Hipp.GST, hippocampus glutathione S-transferase

Evaluation of mitochondria swelling

In this method, a lowering in absorbance at 540 nm indicates the increase of mitochondrial volume. The results obtained by the mitochondrial swelling assay are presented in Fig. 6. In the current study, a significant increase ($p < 0.01$) in mitochondrial swelling was observed in all rats treated chronically with a low dose of Cd, in comparison with a control group.

Assessment of GSH and lipid peroxidation

The results issuing from the evaluation of mitochondrial GSH and MDA in both studied brain regions in rats after chronic exposure to Cd are illustrated respectively Figs. 7 and 8. Based on statistical analysis, GSH level decreased significantly, at the same time, the rate of MDA increased significantly in striatum and hippocampus mitochondria in the Cd group compared with control.

Assessment of apoptosis markers (Cyt-c, caspase-8 and caspase-9, and Bax and Bcl-2)

Results of apoptosis markers evaluation in striatal and hippocampal cell lysates in rats are illustrated in Table 2. We observed that enzymatic activities of Cyt-c and caspase-9 were significantly increased, with varying degrees of signification ($p < 0.05$, $p < 0.01$), while the activity of caspase-8 was not increased in a meaningful way, in both striatum and hippocampus tissues in the Cd group compared with the control group. On the other hand, outcomes resulting from this study showed a significant increase ($p < 0.01$) and ($p < 0.01$) in the Bax level respectively in both brain area tissues, whereas a decrease in Bcl-2 was detected in a significant manner ($p < 0.05$) in the striatum but not significant in hippocampus tissue when compared with control animals. The ratio Bcl-2/Bax lost about 80% of its value in both studied brain regions.

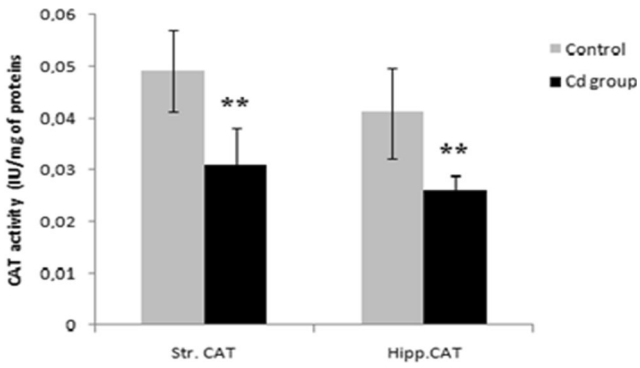

Fig. 4 CAT activities in striatum and hippocampus mitochondria in rats exposed chronically to a low dose of cadmium (Cd). Values are means ± SD (n = 10); p value was detected compared with the control group. **$p \leq 0.01$; Str.CAT, striatum catalase; Hipp.CAT, hippocampus catalase

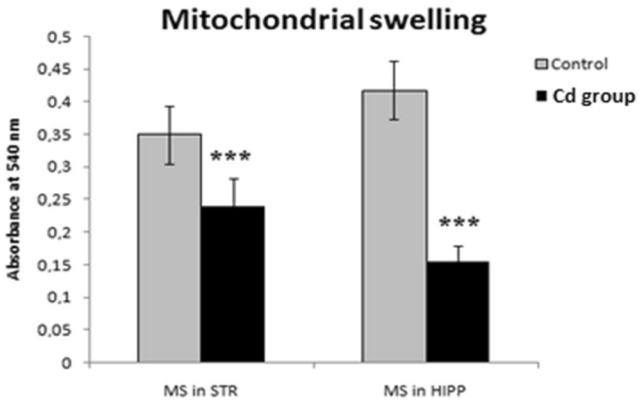

Fig. 6 Evaluation of mitochondrial swelling (MS) in the striatum (STR) and hippocampus (HIPP), in rats, exposed chronically to a low dose of Cd (Cd group). Values are means ± SD (n = 10); p value was detected compared with the control group. ***$p \leq 0.001$

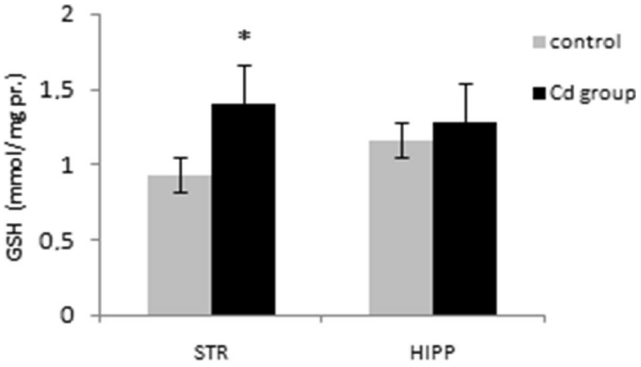

Fig. 7 GSH levels in striatum and hippocampus mitochondria in rats exposed chronically to a low dose of Cd (Cd group). Values are means ± SD ($n = 10$); p value was detected compared with the control group. *$p \leq 0.05$; ns, no significant; Str.GSH, striatum glutathione; Hipp.GSH, hippocampus glutathione

Discussion

The accumulation of different types of pollutants in the environment presents threats to public health and national and international security. These environmental facts never cease to be a source of worry for biologists all over the world. Toxic heavy metals are considered to be themselves highly harmful elements for humans and animals; they accumulate in food matrices, thus exposing humans to a multitude of physiological disorders resulting from the consumption of contaminated food and water (Cobbina et al. 2015). Cd is one of these toxic heavy metals, which takes its place in the blacklist of the most harmful pollutants to human health. Indeed, according to the agency for toxic substances and diseases, the Cd is classified among the top 10 elements the most dangerous heavy metals to health and the environment (Del Pino et al. 2014). This element is considered to be mutagenic, teratogenic, and carcinogenic for humans and animals (Whiteside et al. 2010; Wylly and Pedraza-Chaverrí 2014). In the present study, much attention was devoted to an evaluation of neurotoxicity effects in rats exposed chronically to the environmental dose of Cd (17 μg/kg) which was detected in freshwater fish, such a dose would be far lower than those reported in the literature. Indeed, the main objective of this research work was, firstly, to better determine the effect of chronic exposure to a low dose of Cd on animal behavioral abilities and their cognitive disorders and, secondly, to explore this toxic effect in the striatum and hippocampus by combining biochemical and toxicological approaches to assess mitochondrial integrity and its redox status, besides exploration of the apoptotic signaling pathway in both brain areas.

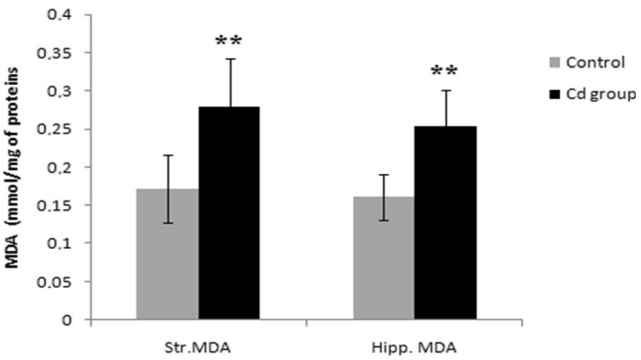

Fig. 8 Evaluation of lipid peroxidation end product (MDA), in striatum and hippocampus mitochondria in rats exposed chronically to a low dose of Cd. Values are means ± SD ($n = 10$); p value was detected compared with the control group. **$p \leq 0.01$; Str.MDA, striatum malondialdehyde; Hipp.MDA, hippocampus malondialdehyde

The results of the OF test showed a significant decrease in rearing and ambulation frequency in rats exposed to Cd. The regression of locomotor activity can be taken as an indication of rats' anxiety. This anxiety is still confirmed by the use of the EPM test which demonstrated a significant increase in the time spent in enclosed arms by the rats, knowing that this test is one of the most used anxiety models (Lister 1987; Kaoud et al. 2010). In the two tests, Cd exerts its anxiogenic effect in rats even with microdoses (17 μg/kg). This anxiogenic-like effect of Cd reported in this study is consistent with other study results obtained in previous works focusing on the link between Cd exposure and neurobehavioral changes in rats, at different higher doses: 1–3 mg/kg, (Haider et al. 2014), 2.5 mg/kg (Abdalla et al. 2014), and 0.25–1 mg/kg (Lamtai et al. 2018). Based on the neurobehavioral study of rats in the NOR test, the current study highlighted some form of cognitive impairment in the Cd group, by undergoing a significant loss of learning ability and working memory, revealed in this test by an increase in RI expressed in terms of percentage of the exploration time of the new object, compared with control. Similar data reported that impaired learning has been demonstrated by a decrease in cholinergic activity in the brain (Kaoud et al. 2010), and exposure to Cd resulted in a decrease in each of the exploratory activity and the recognition of new objects via induction of cell death of cholinergic neurons in the brain (Kim et al. 2016). The results of this study are corroborated by the results of other authors (Haider et al. 2014; Lamtai et al. 2018), despite the lower dose of Cd used in our work. On the other hand, the rats exposed to Cd hanged more in the KIS test than control rats, which indicated significantly an increase in their muscle length compared with control. This extended period in hanging time could traduce the ability of this heavy metal to alter neurobehavioral responses probably by direct influence on acetylcholine esterase activity, thus modifying the duration of postsynaptic neurotransmission (Richetti et al. 2011; Del Pino et al. 2014).

The results of the present study showed a decrease in all antioxidative systems (CAT, GST, and GSH) and an increase in lipid peroxidation end product, MDA. In fact, several research studies indicate that the toxicity of this metal is due in part to its ability to generate oxidative stress (Tobwala et al. 2014), causing damage of the biomolecules as well as the

Table 2 Evaluation of apoptosis indicators in both the striatum and hippocampus in rats exposed chronically to a low dose of cadmium (Cd) (17 μg/kg)

Parameters Brain areas	Striatum		Hippocampus	
	Control	Cd group	Control	Cd group
Caspase-8 (U/mg pr.)	01.1 ± 0.02	1.45 ± 0.04	1.3 ± 0.14	1.9 ± 0.82
Caspase-9 (U/mg pr.)	01.2 ± 0.2	06.9 ± 0.62*	2.2 ± 0.62	9.5 ± 0.41**
Cyt-c (U/mg pr.)	0.069 ± 0.007	0.124 ± 0.02**	0.083 ± 0.008	0.134 ± 0.015**
Bcl-2 (ng/mg pr.)	26.0 ± 1.4	18.3 ± 2.4*	24.1 ± 2.6	20.5 ± 2.1
Bax (ng/mg pr.)	05.5 ± 0.9	21.2 ± 1.8**	08.2 ± 1.1	36.5 ± 3.9***
Bcl-2/Bax ratio	4.72 (100%)	0.86 (18%)	2.93 (100%)	0.56 (19%)

Values are means ± SD (n = 10); p value was detected compared with the control group

*$p \leq 0.05$

**$p \leq 0.01$

***$p \leq 0.001$; (%), residual percentage of the Bcl-2/Bax ratio; *Cyt-c*, cytochrome-c; *Bax*, Bcl2-associated protein X; *Bcl2*, *B cell lymphoma 2*

whole organism (Wylly and Pedraza-Chaverrí 2014; Tobwala et al. 2014). Despite the fact that the molecular mechanisms of Cd brain toxicity are not known yet, it is evident that this metal is implicated in enhancing lipid peroxidation by increasing the production of free radicals that damage enzymatic and non-enzymatic antioxidant barriers (Rios and Méndez-Armenta 2019). This is also supported by the fact that the brain, in a particular way, is quite vulnerable to oxidative stress, due to its richness in polyunsaturated fatty acids, its high consumption of oxygen, and its rather weak antioxidant power (Calabrese et al. 2000). The results obtained in this work showed a significant loss in GSH in both striatum and hippocampus mitochondria. The mitochondrion is an organelle not only very important for the proper functioning of cells but also, constitutes an important site involved in the appearance of cellular lesions following a continuous production of ROS. The imbalance of redox status in mitochondria for the benefit of pro-oxidants is widely associated with depletion of GSH that is considered the first line of defense in neurons against free radicals and pro-oxidant elements (Pathak and Khandelwal 2006; Lahouel et al. 2016), and depletion of GSH contributes to neuronal degeneration (Lakroun et al. 2015). GST is one of the enzymes involved in the metabolism of GSH to combat oxidative stress; it catalyzes also the conjugation of GSH with different xenobiotics (Silvane et al. 2011; Beghoul et al. 2017). In this study, because of their high reactivity and short life, the ROS have been analyzed indirectly in vivo by measuring the changes in mitochondrial antioxidases, such as CAT and GST, which were depleted in both brain areas studied in the Cd group compared with control, giving the accumulation of free radicals which exert direct damage upon brain mitochondria (Shi et al. 2004; Assefa et al. 2005; Lakroun et al. 2015). On the basis of these considerations, intense lipid peroxidation end product and excessive free radicals interact with phospholipids and proteins of mitochondrial membranes impairing their integrity which could induce the mitochondrial pore transition permeability (MPTP) that allows a transition of the osmotic influx of water into the mitochondrial stroma, leading to mitochondrial swelling and mitochondrial membrane potential dissipation (Bonora et al. 2016).

In the present study, results showed an increase in levels of apoptosis markers in cell lysates of striatum and hippocampus in the Cd group compared with control. Indeed, this heavy metal altered MPTP, leading to an increase in mitochondrial edema, until the release of cyt-c into the cytosol, wherein the activity of this enzyme increased significantly in the Cd group compared with control. It is obvious with the current knowledge that mechanisms controlling apoptosis are very complex; one of these mechanisms requires certain proteolytic enzymes called caspases and cytochrome-c (Lakroun et al. 2017; Gasmi et al. 2019). Biologists recognize that there are two apoptotic caspase cascades which are divided into intrinsic and extrinsic pathways (McStay et al. 2008). The literature data suggests that the intrinsic pathway is triggered by the induction of MPTP, occurring Cyt-c release in the cytosol that can be considered a marker of mitochondria perturbations (Clayton et al. 2005). Thereafter, this enzyme activates apoptotic protease-activating factor (APAF-1) and constitutes apoptosome by recruiting and activating caspase-9, which in turn activates caspase-3 and caspase-7 (McStay et al. 2008). Other literature data revealed a decrease in Bcl-2/Bax ratio and mitochondrial membrane potential, release of cytochrome c, and cleavage of caspase-9, in primary cerebral cortical neurons exposed to 20 μM Cd (Yuan et al. 2018). Effectively, our results converge well with some of these findings by demonstrating a significant increase in caspase-9 after cyt-c leakage into the cytosol, but this increase was not significant in the case of caspase-8, in both brain-studied area tissues in the Cd group. On top of that, the same results showed an increase in Bax cytosolic fraction

in the two brain regions and a decrease in Bcl-2 fraction in striatal tissue only, but with a lowering of Bcl-2/Bax ratio.

A review of previous research reports related to Cd neurotoxicity underlined that this metal induced mRNA expression of Bax and reduced mRNA level of Bcl-2 in rat cortex and hippocampus at the millidoses of Cd (1–4 mg/kg) (Mahdavi et al. 2018). Also, the works carried out by Valerio Branca and his collaborators (2019) on an impact assessment of Cd in neuroblastoma cell line exposed to Cd ($CdCl_2$ 10 μM) showed an increase in the subcellular distribution of the cyt-c, as well as the overexpression of the pro-apoptotic protein Bax. Furthermore, the literature review highlighted the involvement of programmed cell death in the emergence of neurodegenerative diseases (Ekshyyan and Aw 2004; El-kott et al. 2020).

In all, our findings in the behavioral study showed a loss of learning ability and working memory in the rats exposed to Cd, such impairment could be originated in the potentiation of neuronal apoptosis pathway occurring in the striatal and hippocampal areas. This explanation is supported by El-Kott et al. (2020) who demonstrated that exposure to $CdCl_2$ induced neuronal apoptosis in the hippocampus and memory deficits in rats. Our results are also corroborated by the study of Yuan et al. (2018) which showed that exposure to Cd leads to neurological disorders in human and animal models such as memory deficits and neuronal apoptosis induction mediated by oxidative stress and mitochondria swelling. In a previous study, an elevated dose of Cd (50 mg/kg) administrated orally weekly in rats exhibited depression and anxiety and impaired memory function with a decrease in serotonin, dopamine, and noradrenalin levels in animals (Batool et al. 2019), while other authors revealed that chronic administration of Cd produces anxiety-like, depression-like, and memory deficits in rats (Lamtai et al. 2018).

Conclusion

This present study devoted the assessment of toxic risks of Cd for both the striatum and hippocampus rats and their behavioral repercussion, associated with chronic exposure to a real low dose of Cd estimated in freshwater fish. The results of the neurobehavioral study showed that Cd has the potential to produce anxiety-like, memory and learning impairments, and, added to this, muscle strength disorders in rats. At the cellular level, the current study revealed that chronic exposure to Cd induced imbalance in redox status and edema in mitochondria, besides, an increase in cytosolic cyt-c, caspases-9, and Bax levels at the same time of a decrease in Bcl-2 rate, in both striatal and hippocampal tissues. Although further empirical research would be required to highlight the nature and the extent of Cd toxicity mechanisms, mainly with regard to the impact of this metal on the genomics issues and brain transmission.

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Compliance with ethical standards All animal experiments in the current study are approved by PIA under the ethical code: N° Batna-Univ2.2020.231.

Conflict of interest The authors declare that they have no conflict of interest.

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