Research Article

Neuroprotective Effects of Melatonin Against Neurotoxicity Induced by Intrahippocampal Injection of Aluminum in Male Wistar Rats: Possible Involvement of Oxidative Stress Pathway

Oussama Zghari*, Mouloud Lamtai, Sofia Azirar, Mohamed Yassine El-Brouzi, Hajar Benmhammed, Aboubaker El-Hessni, Ali Ouichou, Abdelhalem Mesfioui

Laboratory of Biology and Health, Neurosciences, Neuroimmunology and Behaviour Unit, Faculty of Science, Ibn Tofail University, Kenitra, Morocco.

Abstract | Aluminum (Al) is a well-established neurotoxicant, affecting various regions of the brain and causing many neuropathological and neurobehavioral abnormalities as well as oxidative stress (OS). Conversely, melatonin (MEL) has been considered as an antidepressant, anxiolytic substance and protects neurons from OS. The present study was designed to evaluate the neuroprotection effect of MEL against Al neurotoxicity and OS in male Wistar rats. Rats received an intraperitoneal and/or a single intrahippocampal injection of NaCl, MEL or $AlCl_3$. Before two weeks of intrahippocampal surgery period, MEL (4 mg/kg) was intraperitoneally injected (Group II and Group IV). Thereafter, control group (group I) received 2 µl NaCl (0.9%) and groups III and IV received 2 µl $AlCl_3$ (2 mg/kg) intracerebrally into the right ventral hippocampus. Five days after treatment period, all the rats were subjected to the neurobehavioral tests. The animals were then decapitated and the hippocampus was removed. Biochemical parameters of OS and the histology of *Cornu Ammonis* 3 (CA3) area of the hippocampus were evaluated. The results clearly showed that Al induced anxiety and depressive-like behaviour and cognitive impairment. In the hippocampus, Al also increased the levels of lipid peroxidation (LPO) and nitric oxide (NO) and reduced the activity of superoxide dismutase (SOD) as well mediates brain damage in CA3 hippocampal area. These alterations were reversed by MEL. It can be concluded that, by exerting its properties against the oxidative action of this metal in the hippocampus, MEL may play a potential role in protecting against the behavioral and histopathological alterations induced by Al.

Keywords | Aluminum, Melatonin, Neurobehavioral alterations, Oxidative stress, Biochemical indices, Histopathology

Received | February 18, 2023; **Accepted** | March 21, 2023; **Published** | March 25, 2023
***Correspondence** | Oussama Zghari, Laboratory of Biology and Health, Neurosciences, Neuroimmunology and Behaviour Unit, Faculty of Science, Ibn Tofail University, Kenitra, Morocco; **Email:** zghari.oussama.91@gmail.com, oussama.zghari@uit.ac.ma
Citation | Zghari O, Lamtai M, Azirar S, El-Brouzi MY, Benmhammed H, El-Hessni A, Ouichou A, Mesfioui A (2023). Neuroprotective effects of melatonin against neurotoxicity induced by intrahippocampal injection of aluminum in male Wistar rats: Possible involvement of oxidative stress pathway. Adv. Anim. Vet. Sci. 11(5): 711-719.
DOI | https://dx.doi.org/10.17582/journal.aavs/2023/11.5.711.719
ISSN (Online) | 2307-8316

INTRODUCTION

Aluminum (Al) neurotoxicity is a major factor influencing affective and cognitive disorders (Zghari et al., 2018). A growing body of literature suggests that heavy metals, including cadmium, copper and nickel, produce affective and cognitive disorders in rat brain (Lamtai et al., 2018, 2019, 2020a, b, 2021; El-Brouzi et al., 2020). Neurodegenerative diseases such as Alzheimer's disease (AD) have also been linked to Al exposure (Esparza et al., 2018).

After chronic exposure, there are reports that Al crosses the blood-brain barrier (BBB) and accumulates in the hippocampus, the striatum and the cerebral cortex. Additionally, investigations have reported that Al promotes oxidative stress (OS) in these areas and causes substantial neuronal and synaptic damage, especially in the hippocampus (Maya et al., 2016).

Much scientific investigation has focused on developing new prevention approaches against the neurotoxic effects of heavy metals. Of particular interest for this research, melatonin (MEL) has been described as an effective antidepressant and anxiolytic substance (Ouakki et al., 2013). This neurohormone is mainly produced in the pineal gland (Bubenik and Konturek, 2011). In several brain regions, it binds to MEL-specific receptors on neuronal membranes. MEL is well known for its low toxicity and BBB permeability. Additionally, it is a powerful antioxidant and may protect against Al-induced neurotoxicity (Esparza et al., 2018).

The aim of this study was to evaluate whether pretreatment with MEL could modulate the affective and cognitive disorders and brain damage produced by a single intrahippocampal injection of Al into male Wistar rats and the implication of OS.

MATERIALS AND METHODS

Animals and experimental conditions

A total of 28 male Wistar rats from the Faculty of Life Sciences, Ibn Tofaïl University, initially weighing 250 ± 50g were chosen as the animal model in this study. They were placed in plastic cages, with stainless steel cover, in four groups of seven animals each, with two subgroups of four rats in one cage and three rats in the other. The experiments were conducted under standard temperature (21±1°C) and photoperiod LD12/12 (12h light/dark). Animals were given free access to food and water *ab libitum*. All animal procedures were approved by the University Animal Experiments Ethics Committee and were performed in accordance with the Animal Scientific Procedure.

Rats were injected intraperitoneally and/or intrahippocampally with physiological saline, Aluminum chloride ($AlCl_3$) (SEGMA-ALDRICH) or MEL (SIGMA France). Before two weeks of intrahippocampal surgery period, MEL (4 mg/kg) was injected intraperitoneally (Group II and Group IV). After this period, control group was microinjected with 2 µl of NaCl (0.9%) and groups III and IV were intracerebrally injected with 2 µl of $AlCl_3$ (2 mg/kg) into the right ventral hippocampus as follows:

- Control: Rats treated with normal saline (0.9%)
- MEL group: Rats given *MEL* at the dose of 4 mg/kg
- Al group: Rats injected with $AlCl_3$ at the dose of 2 mg/kg
- Al + MEL group: Rats received 2 mg/kg of $AlCl_3$ + 4 mg/kg of MEL.

Stereotaxic surgery and injection procedures

The protocol that has been adapted for stereotactic surgery is the one of (Ferry and Vogt, 2014). Using the bregma as reference, our right ventral hippocampal target is located according to the following stereotactic coordinates: Anteroposterior (AP): -2.4 mm; mediolateral (ML): + 1.6 mm; dorsoventral (DV): - 3.4 mm (Paxinos and Watson, 2007).

The rats were injected with 2 µl of $AlCl_3$ or saline using a Hamilton syringe with a 0.3 mm diameter cannula. This was done at a rhythm of 1 µl/min for 2 minutes, then left in place for 2 minutes to permit passive diffusion through. Finally, to limit the reflux along the injection route, the cannula was then slowly withdrawn.

To facilitate physiologic recovery, rats were caged individually for 5 days immediately after intrahippocampal surgery. A series of neurobehavioral tests were then performed on the rats.

Neurobehavioral tests

Anxiety-like measurement

Open field test (OFT): The OFT is used to assess the rats-anxiety-like behavior following the administration of Al (Gentsch et al., 1987; Carola et al., 2002). When the ten-minute test begins, the rats were gently placed in the center of the apparatus (100-L × 100-W × 40-H cm) and its behavior was videotaped for subsequent analysis. The quantified parameters were the time spent in the center area (TCA), the number of returns to the center (NRC) and the number of total squares (NTS). TCA and NRC perimeters are seen as indicator of anxiety. While the NTS parameter is a reliable index of locomotors activity. The device was cleaned between each rat with 7% alcohol solution to remove odor clues.

Elevated plus maze (EPM)

The EPM is a model of anxiety-like behavior in rats brought about by novelty and repulsion due to the elevation and the illumination of the maze (2 open arms (50 × 10 cm), 2 closed arms (50 × 10 × 40 cm), and a central region (10 × 10 cm)) (Naranjo-Rodriguez et al., 2000). The test was carried out on the 2nd day following the OFT. At the start of the five-minute test, rat ware placed on the central area of the labyrinth in front of an open arm and its behavior like number of entries in open arms (EOA), the time spent in these arms (TOA) and the number of full entries into the arms (TAE) was recorded for later analysis. The TOA and EOA parameters were reported as the criteria of open space-induced anxiety-like behavior. Whereas the

total number of the entries into all arms provides general locomotors activity. 7% alcohol was used to clean the apparatus prior to the introduction of each rat to eliminate any lingering olfactory cues.

Depression-like measurement

Forced swimming test (FST)

The FST, also known as behavior despair test, is an excellent maze used to evaluate the depressive-like behavior in rodents (Porsolt et al., 1978). The Five-minute swimming sessions were conducted by placing the rat in a transparent cylindrical glass (50 cm high and 30 cm in diameter) filled with purified water (35 cm, 23±2°C) to forcing it to swim while the camera is recording it for later analysis to determine the immobility times (TIM). An increase in TIM parameter is related to depressive-like behavior (Porsolt et al., 1978; Benabid et al., 2008).

Cognitive measurement

Y-maze test

Spatial working memory was tested by the Y-maze spontaneous alternation test (Sierksma et al., 2014). When the eight-minute test begins, each rat was placed on the central area of the apparatus and allowed to explore freely through the three arms (A, B and C; 61 × 35 × 12 cm3) and the sequences of arms entries was recorded (i.e., ACBCABACABCABAC, etc.) and calculated by the following formula: Spontaneous alternation% = [(Number of alternations)/(Total arms entries-2)] × 100. Spontaneous alternation behavior, which reflects the spatial working memory, has been defined as the entry into the three different arms when making consecutive choices. Between each examination and to eliminate the remaining olfactory signs, the maze was cleaned using 7% alcohol solution.

Morris water maze test

The morris water maze (Morris, 2008) are used to evaluate cognitive functions related with learning and memory in rodents. It consisted of a big round water tank (110 cm in diameter and 20 cm high) that was filled with opaque water and maintained a temperature at 22°C. The apparatus was divided into four quadrants of equal area with a circular platform (13 cm high, 9 cm in diameter), submerged 0.5 cm beneath the water surface and placed at the center of the northeast zone. the test is carried out in two phases: Acquisition and probe trial (Wong and Brown, 1984). At the first phases (4 trials/day for 4 days), each rat was placed individually into the water maze facing the wall of the pool and allowed to locate the hidden platform within a maximum swimming time of 60 seconds. If the rat couldn't locate the platform, we guided it and let it stay there for 10 s. On the fifth day, rats were tested for spatial memory in a 60 s probe trial (Kahloula et al., 2014).

At the end of the tests, the rats were decapitated and their brains were removed and prepared for biochemical examination and histological analysis.

Biochemical examination

The biochemical examination is based on the measurement of oxidative stress markers in the hippocampus, of which lipid peroxidation (LPO), nitric oxide (NO) and superoxide dismutase (SOD) activity have been selected. This requires preparing hippocampal supernatants by carefully isolating hippocampal tissue on ice, homogenizing in phosphate buffer at pH 7.4 and centrifuging for 10 minutes at 1500rpm.

The LPO assay was analyzed according to the method of (Draper and Hadley, 1990) by measuring thiobarbituric acid reacting substances (TBARS) in the cells (expressed as µmol/g hippocampal tissue). Absorbance at 535 nm was used to measure the TBARS (Freitas et al., 2004). The activity of NO (expressed as µmol/g of hippocampal tissue) was evaluated according to the method of (Chao et al., 1992), while the activity of SOD (expressed as U/g of hippocampal tissue) was assayed by the Beauchamp and Fridovich method (Beauchamp and Fridovich, 1971).

Histological analysis

To evaluate the histology of CA3 area of the hippocampus, brains were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4 (Freire et al., 2012). Subsequently, hippocampal sections (30 mm slices) were cut with a vibratome (VT 1000 S, Leica Microsystems). The sections were then mounted on gelatinized slides, stained with cresyl violet, dehydrated and cover slipped. The CA3 area was selected for light microscopy at × 20 magnification (Optika microscope, Italy). Photomicrographs were then taken and analyzed using ImageJ software (Zhu et al., 2015; Bittencourt et al., 2022). Figure 1 is an outline of the experimental protocol for all steps of the study

Statistical analysis

To determine the differences between the experimental groups regarding behavioral, biochemical and histological data, which are presented as mean±standard error of the mean (S.E.M.), statistical analysis was performed using SPSS version 22 software (IBM Corp., Armonk, NY, United States), by two-way ANOVA with Tukey's test for post hoc comparisons and ANOVA repeated measures for the MWM test. When $p<0.05$, differences were considered significant.

RESULTS AND DISCUSSION

Effects of Al and MEL on the levels of anxiety-like measured in the OFT

The study's findings revealed that Al administration

induced an anxiogenic action in the OFT, characterized by a substantial reduction in the TCA and NRC parameters in comparison with control animals ($p<0.001$ and $p<0.01$, respectively), and that MEL treatment reduced this action ($p < 0.01$), with no changes in the NTS parameter which representing locomotors activity ($p > 0.05$) (Table 1).

Table 1: Effect of treatment with Aluminum (2 mg/kg) and melatonin (4 mg/kg) on total amount time spent in the center (TCA), number of return into center area (NRC); and number of total squares (NTS) determined in the open field test in male Wistar rats.

Groups	TCA (S)	NRC	NTS
Control	22.28±03.59	15.43±02.30	114.29±13.89
MEL	22.00±03.27$^{\#\#\#}$	16.71±03.99$^{\#\#\#}$	118.14±13.73
Al	09.29±02.81***	07.71±02.69**	106.86±12.69
Al+MEL	17.29±03.90$^{\#\#}$	15.00±03.74$^{\#\#}$	117.00±20.36

Results are represented as mean ± SEM. $^{*}p < 0.05$ vs. control group; and $^{\#}p < 0.05$ vs. Al treated group

Effects of Al and MEL on anxiety levels measured in EPM

In addition to the TCA and NRC in OFT, the Al administration modified another parameters indicative of anxiety-like behavior, the TOA and EOA indicators in EPM, which were significantly reduced in comparison with control groups ($p < 0.001$ and $p < 0.05$, respectively). However, following the administration of MEL, TOA and EOA were significantly increased as compared to Al groups ($p < 0.001$). Besides, the TEA was unaffected in all groups ($p > 0.05$) (Table 2).

Table 2: Effect of treatment with Aluminum (2 mg/kg) and melatonin (4 mg/kg) on the total amount of time spent in exposed arms (TOA); number of entries in exposed arms (EOA); and total number of arms entries (TEA) determined in the elevated plus maze test in male Wistar rats.

Groups	TOA (S)	EOA	TEA
Control	61.29 ± 07.87	03.86 ± 01.35	06.57 ± 01.27
MEL	59.86 ± 10.07 $^{\#\#\#}$	02.57 ± 00.98	05.29 ± 02.06
Al	31.29 ± 07.78 ***	01.43 ± 01.81 *	05.57 ± 03.46
Al + MEL	55.43 ± 10.49 $^{\#\#\#}$	03.29 ± 02.14	07.71 ± 01.80

Results are represented as mean ± SEM. $^{*}p < 0.05$ vs. control group; and $^{\#}p < 0.05$ vs. Al treated group.

Effects of Al and MEL on depressive-like performances measured by FST

As depicted in Figure 2, Al administration significantly increased the TIM when compared to control animals ($p < 0.001$). Furthermore, treatment with MEL reduce this depress genic effect induced by decreasing significantly the TIM as compared to Al-treated groups ($p < 0.001$).

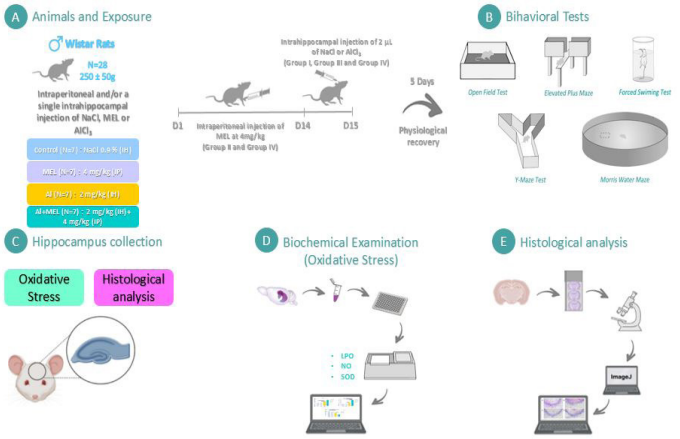

Figure 1: Experimental design of all methodological steps of the study. (A) Sample description of experimental groups and exposure to NaCl (0.9%) or $AlCl_3$; (B) after the intrahippocampal injection, rats were submitted to the behavioral tests assessment through Open Field test (OFT), Elevated Plus Maze (EPM), Forced Swimming Test (FST), Y-maze and Morris Water Maze (MWM) test; (C) The animals were euthanized and the hippocampus was taken; (D) Thereafter, oxidative stress markers (lipid peroxidation (LPO), nitric oxide (NO) levels, and superoxide dismutase (SOD) activities) and (E) histology of CA3 area of the hippocampus were evaluated.

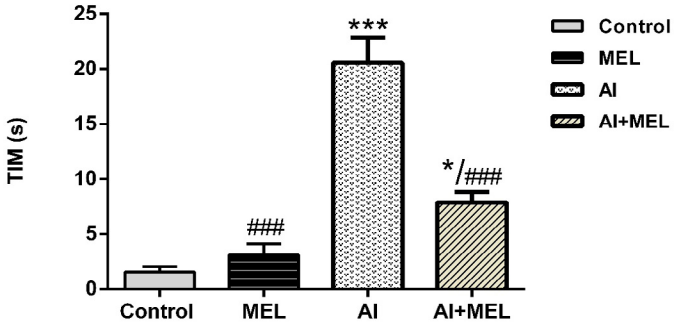

Figure 2: Effect of treatment with Aluminum (2 mg/kg), melatonin (4 mg/kg), and their combination on immobility time (TIM) expressed in seconds in forced swimming test in male Wistar rats. Results are represented as mean ± SEM. $^{*}p < 0.05$ vs. control group; and $^{\#}p < 0.05$ vs. Al-treated group.

Al and MEL effects on memory

Y-maze test

Statistical analysis demonstrated that the Al intoxicated rats group had a lower % of alternation when compared to control animals ($p<0.001$). Furthermore, the MEL administration was able to reverse the decrease in the % of alternation percentage ($p<0.001$), as illustrated in Figure 3.

Morris water maze

Figure 4A revealed that male rats treated with Al, showed longer escape latencies were in comparison with control group ($p < 0.01$). Conversely, exposure to MEL

ameliorated the effects of Al ($p > 0.05$). Furthermore, in the probe test, the % of time spent in the correct quadrant was significantly reduced following Al administration as compared to control ($p < 0.001$). However, the increase by Al was abolished by MEL ($p < 0.001$) (Figure 4B).

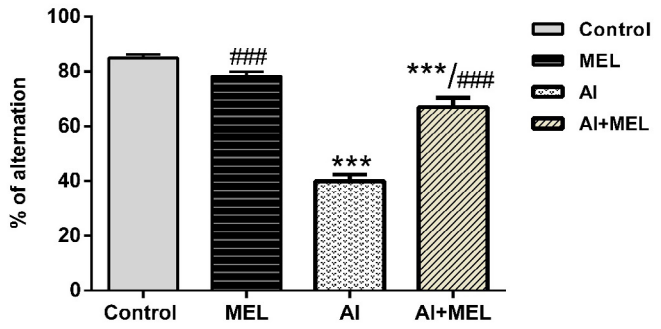

Figure 3: Effects of Al (2 mg/kg) and melatonin (4 mg/kg) on percentage of alternation measured in Y-maze test in male Wistar rats. Results are represented as mean ± SEM. *p < 0.05 vs. control group; and #p < 0.05 vs. Al-treated group.

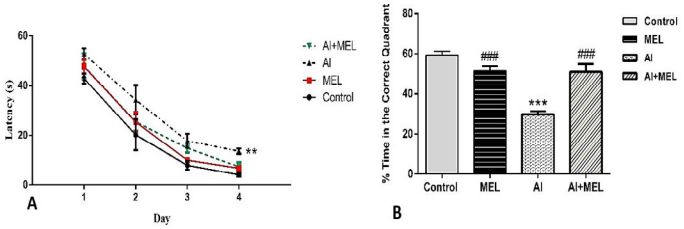

Figure 4: Effects of Al (2 mg/kg) and melatonin (4 mg/kg) on memory performance measured in MWM test in male Wistar rats. (A) Latency to reach the hidden platform on each of the 4 days of learning phase. (B) Percentage of time spent in the correct quadrant. Results are represented as mean ± SEM. *p < 0.05 vs. control group; and #p < 0.05 vs. Al-treated group.

Table 3: Effect of melatonin treatment on Al induced oxidative stress in the hippocampus of male Wistar rats: thiobarbituric acid reactive substances (TBARS) content, nitric oxide (NO) and superoxide dismutase (SOD) activities.

Groups	TBARS (nmol /g of tissue	NO (µmol/g of tissue)	SOD (Units/g of tissue)
Control	12.82±00.27	2617.08±228.63	01.55±00.05
MEL	13.62±03.11###	2213.09±30.18###	01.44±00.04###
Al	26.96±00.58***	9027.51±494.93***	00.54±00.04***
Al+ MEL	17.80±02.45##	5388.73±217.48**/##	01.30±00.05**/###

Values are expressed as mean ± S.E.M. *p < 0.05 vs. control group, and #p < 0.05 vs. Al treated group.

Al and MEL effects on oxidative stress

The OS markers were determined in the hippocampus of all animals, as represented in Table 3. Statistical analysis showed that Al rats have higher levels of TBARS and NO ($p < 0.001$) and lower activity of SOD ($p < 0.001$) than control groups. Moreover, MEL administration reduce Al provoked-OS an cell damage in the hippocampus by reducing the LPO and NO levels ($p < 0.01$) and enhancing the SOD activity ($p < 0.001$) in comparison with the Al-treated animals.

Histopathological results

Statistical analysis in Figure 5 showed that, in CA3 hippocampal area, the number of intact pyramidal neurons is significantly reduced following Al administration in comparison with control animals ($p < 0.01$). Interestingly, MEL totally prevented the number of intact pyramidal neurons decrease caused by Al ($p < 0.05$). Additionally, in control and MEL groups, a normal histological appearance was observed in the histopathological analyses of the CA3 pyramidal neurons illustrated in Figure 6. In contrast, compared to control animals, the Al group showed neuronal loss and cellular disorganization. These cellular lesions caused by this metal were attenuated following MEL administration.

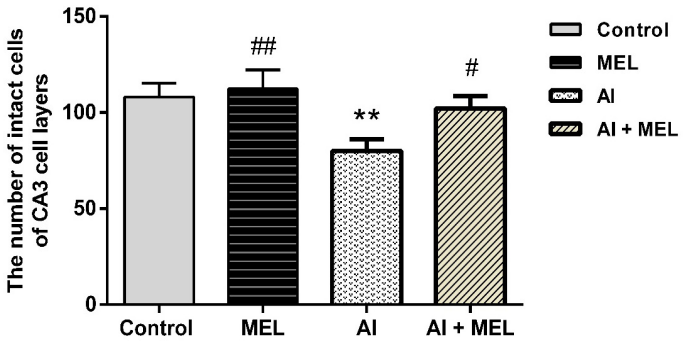

Figure 5: Effect of Al (2 mg/kg), MEL (4 mg/kg) and Al+MEL on the number of neurons in 0.18 mm^2 area of CA3 region of hippocampus in male rats. Results are represented as mean ± SEM. *p < 0.05 vs. control group; and #p < 0.05 vs. Al-treated group.

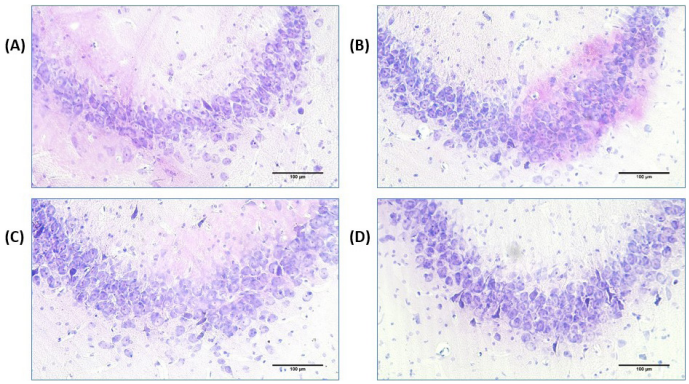

Figure 6: Light micrographs of hippocampus with cresyl violet staining in the control (A), MEL (B), Al (C) and Al+MEL (D) groups in CA3 cell layer of hippocampus in male rats. Bar represents 100µm.

The interest of this study was to determine the neuroprotective effect of MEL on anxiety, depression, cognitive dysfunction, OS level and hippocampal CA3 morphological assessment by intrahippocampal Al injection in male Wistar rats.

To our knowledge, in the literature, poorly experiments on intrahippocampal Al injection have been realized and, for the first time, a complete study was carried out in this experiment, covering neurobehavioral, biochemical and histological aspects to investigate neurotoxic effects of Al and protective properties of MEL against Al-induced neurotoxicity.

Based on neurobehavioral tests, our data clearly demonstrate that, into the right ventral hippocampus, a single intrahippocampal injection of Al causes anxiety-like, depressive-like and cognitive disorders. These results are in line with an earlier publication by our team, which found that chronic administration of Al at very low doses (0.125, 0.25, 0.5 and 1 mg/kg) for 8 weeks in female and male Wistar rats induced anxiety, depression and promoted working memory impairment (Zghari et al., 2018). Recent study have also shown similar results (Auti and Kulkarni, 2019). As well, in Wistar rats treated with Al lactate intragastrically for 12 weeks, Al causes cognitive dysfunction and negatively affects spatial learning capacities (Sharma et al., 2013). Another study by Taïr et al. (2016) reported that chronic intraperitoneal Al exposure for 90 days causes anxiety in the OFT. The same results were observed in male Prague Dawley rats who received for 5 weeks an intraperitoneal injection of $AlCl_3$ at 70 mg/kg (Ali et al., 2016).

In addition, based on biochemical assay for markers of OS in hippocampal tissues, our data clearly demonstrate that Al-induced brain OS, this is due on the fact that Al exposure significantly elevated TBARS and NO levels whereas reducing SOD activity. Our results are in total agreement with recent published data (Auti and Kulkarni, 2019; Shaik et al., 2019). Similarly, the reports of Al-Amin et al. (2019) are in line with our results in which Al treatment enhanced the level of LPO and NO in the hippocampus as well as a decrease in antioxidant status. Although, some authors have reported elevated SOD activity (Benyettou et al., 2017) or unchanged TBARS levels in the hippocampus (Sánchez-Iglesias et al., 2009). We note that the apparently conflicting findings in the literature may be explained by the use of various chemical forms of Al exposure and/or routes of administration.

Because the mechanism of Al neurotoxicity is poorly understood, the results obtained in this study open a path of reflection to explain the origin of anxiety-like, depression-like and memory impairment and that these neurobehavioral changes may be related to OS. LPO is considered as a key biomarker of oxidative damage, an increase in this parameter reflects the onset of OS. Commonly, the increased LPO level is, at least in part, due to a reduction in antioxidant enzyme activity such as SOD, catalase and glutathione which play an important part in fighting the damage caused by free radicals in the brain (Abdel-Moneim et al., 2013). Furthermore, Al provides a favorable environment for ROS generation by increasing the intracellular concentration of free form of iron and accelerates iron-mediated lipid peroxidation induced neuronal damage (Berihu et al., 2015). As well, Al may induce microglia and astrocytes to produce NO (Zaky et al., 2013). The reduction in antioxidant enzymes activities and the high levels of free radicals such as NO induce oxidative damage in the mitochondria (Lin and Beal, 2006), leading to cell damage and death (Xu et al., 2010) and consequently altered behavior.

On the other hand, MEL pretreatment has both anxiolytic and antidepressant effects and a positive effect on memory and learning in rat. In addition, MEL was found to reduced Al induced-OS and cell damage in the hippocampus by reducing LPO and NO levels and enhancing SOD activity. These results are in line with the current literature, which shows that MEL could counteracts anxiety-like, depressive-like, cognitive disorders and OS in Wistar rats (Garcia-Santos et al., 2012; Corrales et al., 2013; Zhang et al., 2013; Allagui et al., 2014). As well, Al-Olayan et al. (2015) reports that treatment with MEL (10 mg/kg) for 7 days, decreased LPO and NO levels and increased antioxidant enzymes activities in brains of Al treated rats. By reducing OS and inhibiting mitochondrial cell death pathways, MEL might protect against Al-induced neurotoxicity.

The literature suggests that MEL is an antioxidant and a potent direct scavengers free radical, prevents overproduction of free radicals, OS and neuronal damage by enhancing the activity of various antioxidant enzymes (Reiter et al., 2001). In addition, MEL or its metabolites can detoxify ROS such as ONOO (Koh, 2008; Reiter et al., 2008), and prevent the neurons from LPO damage by up-regulates the gene expression of antioxidant enzymes like SOD and CAT (Reiter et al., 2013). As well as, MEL reducing the NO levels by enhancing the enzymatic activity of NO synthase (NOS) which leads to a down-regulation of pro-oxidant enzyme gene expression, such as NOS (Tan et al., 2007).

Histological analysis showed that $AlCl_3$ induced progressively more severe changes in the hippocampus compared to the control group, characterized by disorganized pyramidal cell and reduced CA3 neuron density. Interestingly, MEL administration reduced brain

damage and improved affective and cognitive outcome as observed in behavioral studies.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the data from the present study support the observation that Al causes anxiety, depression, memory impairment and brain damage in the hippocampus characterized by pyramidal cell disorganization and reduced neuronal density in the CA3 area which are associated to OS. However, MEL ameliorates/prevents the Al-provoked behavioral alterations in Al-exposed rats. This finding has been linked to the regulation of the levels of OS markers in the hippocampus. Further research is needed to determine the exact mechanisms by which MEL responds.

Our work has yielded interesting results and could be continued in various research directions, leading to a better understanding the exact mechanisms of action of the interaction between MEL and Al. This work opens up many perspectives.

- Expanding the number of brain areas studied, as aluminum accumulates in different regions besides the hippocampus in the striatum and cortex, as these structures are involved in brain pathologies such as anxiety, depression and memory disorders.
- Determine the concentrations of MEL and Al in the brain tissue, since both molecules cross the BBB, to determine the concentration that accumulates after chronic intraperitoneal administration and to compare it with the intracerebral administration, especially for Al, to determine the concentration that can alter behaviour, inducing oxidative stress, neurodegeneration in the brains of rats and ultrastructural changes in the neurons of the hippocampus.
- Perform immunohistochemical studies of the hippocampus and other brain structures, and measuring various neurotransmitters involved in affective and cognitive disorders, including serotonin, dopamine and acetylcholine.

ACKNOWLEDGEMENTS

The Biology and Health Laboratory of the Ibn Tofail University has experienced the most difficult time after the loss of one of its eminent professors, Mr. Ali Ouichou, may his soul rest in peace. He was a special person and a great academic professor who contributed to the growth of the Moroccan neuroscience community. We send our sincere and warmest thoughts and prayers to his family and friends and promise to always work to make the laboratory worthy of his legacy. Forever in our memory.

NOVELTY STATEMENT

The novelty of this study is to evaluate whether pretreatment with MEL could modulate the affective and cognitive disorders, OS and brain damage induced by a single intrahippocampal injection of Al.

AUTHOR'S CONTRIBUTION

All authors have co-authored the manuscript equally.

Abbreviations

Al: Aluminum; AlCl3: Aluminum chloride; BBB: Blood-brain barrier; CA: Cornu Ammonis; EOA: Entries in open arms; EPM: Elevated Plus Maze; FST: Forced Swimming Test; LPO: Lipid peroxidation; MEL: Melatonin; MWM: Morris Water Maze; NO: Nitric oxide; NRC: Number of returns to the center; NTS: Number of total squares; OFT: Open Field Test; OS: Oxidative stress; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TAE: Number of full entries into the arms; TCA: Time spent in the center area; TIM: Immobility times; TOA: Time spent in these arm.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdel Moneim AE, Othman MS, Mohmoud SM, El-Deib KM (2013). Pomegranate peel attenuates aluminum-induced hepatorenal toxicity. Toxicol. Mech. Methods, 23(8): 624–633. https://doi.org/10.3109/15376516.2013.823634

Al-Amin MDM, Chowdury MDIA, Saifullah ARM, Alam MN, Jain P, Hossain M, Alam MDA, Kazi M, Ahmad A, Raish M, Alqahtani A, Reza HM (2019). Levocarnitine Improves AlCl3-Induced Spatial Working Memory Impairment in Swiss albino Mice. Front. Neurosci., 13(March): 1–11. https://doi.org/10.3389/fnins.2019.00278

Ali AA, Ahmed HI, El-samea HAA, El-demerdash E (2016). The potential effect of caffeine and nicotine co-administration against aluminum-induced Alzheimer's disease in rats. J. Alzheimers Dis. Parkinsonism. 6(3). https://doi.org/10.4172/2161-0460.1000236

Allagui MS, Feriani A, Saoudi M, Badraoui R, Bouoni Z, Nciri R, Murat JC, Elfeki A (2014). Effects of melatonin on aluminium-induced neurobehavioral and neurochemical changes in aging rats. Food Chem. Toxicol., 70(May): 84–93. https://doi.org/10.1016/j.fct.2014.03.043

Al-olayan EM, El-khadragy MF, Moneim AEA (2015). The protective properties of melatonin against aluminium-induced neuronal injury. Int. J. Exp. Pathol., 96(3): 196–202. https://doi.org/10.1111/iep.12122

Auti ST, Kulkarni YA (2019). Neuroprotective effect of cardamom oil against aluminum induced neurotoxicity in rats. Front. Neurol., 10(APR). https://doi.org/10.3389/fneur.2019.00399

Beauchamp C, Fridovich I (1971). Superoxide dismutase:

Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44(1): 276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Benabid N, Mesfioui A, Ouichou A (2008). Effects of photoperiod regimen on emotional behaviour in two tests for anxiolytic activity in Wistar rat. Brain Res. Bull., 75(1): 53–59. https://doi.org/10.1016/j.brainresbull.2007.07.016

Benyettou I, Kharoubi O, Hallal N, Benyettou HA, Tair K, Belmokhtar M, Aoues A, Ozaslan M (2017). Aluminium-induced behavioral changes and oxidative stress in developing rat brain and the possible ameliorating role of omega-6/omega-3 ratio. J. Biol. Sci., 17(3): 106–117. https://doi.org/10.3923/jbs.2017.106.117

Berihu BA, Afwerk M, Debeb YG, Gebreslassie A (2015). Review on histological and functional effect of aluminium chloride on cerebral cortex of the brain. Int. J. Pharm. Sci. Res., 6(8): 1105–1116.

Bittencourt LO, Damasceno-silva RD, Alana W, Arag B, Eir L, Carolina A, Oliveira A, Fernandes RM, Freire MAM, Carvalho S, Dionizio A, Buzalaf R, Cassoli JS, Cirovic A, Cirovic A, Ferraz S, Lima RR (2022). Global proteomic profile of aluminum-induced hippocampal impairments in rats: Are low doses of aluminum really safe? Int. J. Mol. Sci., 23(20): 12523. https://doi.org/10.3390/ijms232012523

Bubenik GA, Konturek SJ (2011). Melatonin and aging: Prospects for human treatment. J. Physiol. Pharmacol., 62(1): 13–19.

Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav. Brain Res., 134(1–2): 49–57. https://doi.org/10.1016/S0166-4328(01)00452-1

Chao CC, Hu S, Molitor TW, Shaskan EG, Peterson PK (1992). Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. J. Immunol., 149(8): 2736–2741. https://doi.org/10.4049/jimmunol.149.8.2736

Corrales A, Martínez P, García S, Vidal V, García E, Flórez J, Sanchez-Barceló EJ, Martínez-Cué C, Rueda N (2013). Long-term oral administration of melatonin improves spatial learning and memory and protects against cholinergic degeneration in middle-aged Ts65Dn mice, a model of Down syndrome. J. Pineal Res., 54(3): 346–358. https://doi.org/10.1111/jpi.12037

Draper HH, Hadley M (1990). Malondialdehyde determination as index of lipid peroxidation. Meth. Enzymol., 186: 421–431. https://doi.org/10.1016/0076-6879(90)86135-I

El-Brouzi MY, Lamtai M, Zghari O, Ouakki S, Azizoun I, El Hessni A, Mesfioui A, Ouichou A (2020). Intrahippocampal effects of nickel injection on the affective and cognitive response in Wistar rat: Potential role of oxidative stress. Biol. Trace Elem. Res., 199(9): 3382-3392. https://doi.org/10.1007/s12011-020-02457-5

Esparza JL, Gómez M, Domingo JL (2018). Role of melatonin in aluminum-related neurodegenerative disorders: A review. Biol. Trace Elem. Res., 188(1): 60-67. https://doi.org/10.1007/s12011-018-1372-4

Ferry B, Gervasoni D, Vogt C. (2014). Stereotaxic neurosurgery in laboratory rodent: handbook on best practices. No. 15550. Springer Paris. https://doi.org/10.1007/978-2-8178-0472-9

Freire MAM, Faber J, Picanço-Diniz CW, Franca JG, Pereira A (2012). Morphometric variability of nicotinamide adenine dinucleotide phosphate diaphorase neurons in the primary sensory areas of the rat. Neurosci. 205: 140–153. https://doi.org/10.1016/j.neuroscience.2011.12.029

Freitas RM, Sousa FCF, Vasconcelos SMM, Viana GSB, Fonteles MMF (2004). Pilocarpine-induced status epilepticus in rats: lipid peroxidation level, nitrite formation, GABAergic and glutamatergic receptor alterations in the hippocampus, striatum and frontal cortex. Pharmacol. Biochem. Behav., 78(2): 327–332. https://doi.org/10.1016/j.pbb.2004.04.004

Garcia-Santos G, Martin V, Rodriguez-Blanco J, Herrera F, Casado-Zapico S, Sanchez-Sanchez AM, Antolin I, Rodriguez C (2012). Fas/Fas ligand regulation mediates cell death in human Ewing's sarcoma cells treated with melatonin. Br. J. Cancer, 106(7): 1288–1296. https://doi.org/10.1038/bjc.2012.66

Gentsch C, Lichtsteiner M, Feer H (1987). Open field and elevated plus-maze: A behavioural comparison between spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats and the effects of chlordiazepoxide. Behav. Brain Res., 25(2): 101–107. https://doi.org/10.1016/0166-4328(87)90003-9

Kahloula K, Eddine D, Adli H, Slimani M, Terras H, Achour S (2014). Effet de l'exposition chronique au nickel sur les fonctions neurocomportementales chez les rats Wistar pendant la période de développement. Toxicol. Anal. Clin. 26(4): 186–192. https://doi.org/10.1016/j.toxac.2014.09.056

Koh PO (2008). Melatonin regulates nitric oxide synthase expression in ischemic brain injury. J. Vet. Med. Sci., 70(7): 747–750. https://doi.org/10.1292/jvms.70.747

Lamtai M, Azirar S, Zghari O, Ouakki S, El Hessni A, Mesfioui A, Ouichou A (2021). Melatonin ameliorates cadmium-induced affective and cognitive impairments and hippocampal oxidative stress in rat. Biol. Trace Elem. Res., 199(4): 1445–1455. https://doi.org/10.1007/s12011-020-02247-z

Lamtai M, Chaibat J, Ouakki S, Berkiks I, Rifi E, Essamri A, Ouichou A (2018). Effect of chronic administration of cadmium on anxiety-like, depression-like and memory deficits in male and female rats: Possible involvement of oxidative stress mechanism. J. Behav. Brain Sci., 8(5): 240–268. https://doi.org/10.4236/jbbs.2018.85016

Lamtai M, Ouakki S, Zghari O, Mesfioui A, Hessni A El, Ouichou A (2019). Affective Behavior Dysregulation Was Induced by Chronic Administration of Copper in Wistar Rats. Neurosci. Med., 10(2): 134–149. https://doi.org/10.4236/nm.2019.102009

Lamtai M, Zghari O, Azirar S, Ouakki S, Mesfioui A, Hessni A, Berkiks I, Marmouzi I, Ali O (2020a). Melatonin modulates copper-induced anxiety-like, depression-like and memory impairments by acting on hippocampal oxidative stress in rat. Drug. Chem. Toxicol., 45(4): 1707-1715. https://doi.org/10.1080/01480545.2020.1858853

Lamtai M, Zghari O, Ouakki S, Marmouzi I, Mesfioui A, Hessni A, Ali O (2020b). Chronic copper exposure leads to hippocampus oxidative stress and impaired learning and memory in male and female rats. Toxicol. Res., 13; 36(4): 359-366. https://doi.org/10.1007/s43188-020-00043-4

Lin MT, Beal MF (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature, 443: 787–795. https://doi.org/10.1038/nature05292

Maya S, Prakash T, Madhu K Das, Goli D (2016). Multifaceted effects of aluminium in neurodegenerative diseases: A review. Biomed. Pharmacother., 83: 746–754. https://doi.

org/10.1016/j.biopha.2016.07.035

Morris R (2008). Developments of a water1maze procedure for studying spatial learning in the rat. Search 11: 7336–7336. https://doi.org/10.1016/0165-0270(84)90007-4

Naranjo-Rodriguez EB, Osornio AO, Hernandez-Avitia E, Mendoza-Fernandez V, Escobar A (2000). Anxiolytic-like actions of melatonin, 5-metoxytryptophol, 5-hydroxytryptophol and benzodiazepines on a conflict procedure. Prog. Neuropsychopharmacol. Biol. Psychiatry, 24(1): 117–129. https://doi.org/10.1016/S0278-5846(99)00075-5

Ouakki S, El-Mrabet FZ, Lagbouri I, El-Hessni A, Mesfioui A, Pévet P, Challet E, Ouichou A (2013). Melatonin and diazepam affect anxiety-like and depression-like behavior in Wistar rats: Possible interaction with central GABA neurotransmission. J. Behav. Brain Sci., 3(November): 522–533. https://doi.org/10.4236/jbbs.2013.37055

Paxinos G, Watson C (2007). The rat brain in stereotaxic coordinates, 6th Edition.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978). Behavioural despair in rats: A new model sensitive to antidepressant treatments. Eur. J. Pharmacol., 47(4): 379–391. https://doi.org/10.1016/0014-2999(78)90118-8

Reiter R, Tan D-X, Rosales-Corral S, C. Manchester L (2013). The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. Mini. Rev. Med. Chem., 13(3): 373–384. https://doi.org/10.2174/1389557511313030006

Reiter RJ, Acuña-Castroviejo D, Tan DX, Burkhardt S (2001). Free radical-mediated molecular damage. Mechanisms for the protective actions of melatonin in the central nervous system. Ann. N. Y. Acad. Sci., 939: 200–215. https://doi.org/10.1111/j.1749-6632.2001.tb03627.x

Reiter RJ, Tan D-X, Jou M-J, Korkmaz A, Manchester LC, Paredes SD (2008). Biogenic amines in the reduction of oxidative stress: melatonin and its metabolites. Neuro Endocrinol. Lett., 29(4): 391–398.

Sánchez-Iglesias S, Méndez-Álvarez E, Iglesias-González J, Muñoz-Patiño A, Sánchez-Sellero I, Labandeira-García JL, Soto-Otero R (2009). Brain oxidative stress and selective behaviour of aluminium in specific areas of rat brain: Potential effects in a 6-OHDA-induced model of Parkinson's disease. J. Neurochem., 109(3): 879–888. https://doi.org/10.1111/j.1471-4159.2009.06019.x

Shaik A, Shenoy S, Anupama V, Rao K, Amuthan A (2019). Antidepressants modulate behavioral, biochemical, and histological alterations induced by chronic aluminum chloride administration in wistar rats. J. Pharmacol. Pharmacother., 10(1): 16–21. https://doi.org/10.4103/jpp.JPP_135_18

Sharma DR, Wani WY, Sunkaria A, Kandimalla RJL, Verma D, Cameotra SS, Gill KD (2013). Quercetin protects against chronic aluminum-induced oxidative stress and ensuing biochemical, cholinergic, and neurobehavioral impairments in rats. Neurotox. Res., 23(4): 336–357. https://doi.org/10.1007/s12640-012-9351-6

Sierksma ASR, Van Den Hove DLA, Pfau F, Philippens M, Bruno O, Fedele E, Ricciarelli R, Steinbusch HWM, Vanmierlo T, Prickaerts J (2014). Improvement of spatial memory function in APPswe/PS1dE9 mice after chronic inhibition of phosphodiesterase type 4D. Neuropharmacology, 77: 120–130. https://doi.org/10.1016/j.neuropharm.2013.09.015

Taïr K, Kharoubi O, Taïr OA, Hellal N, Benyettou I, Aoues A (2016). Aluminium-induced acute neurotoxicity in rats: Treatment with aqueous extract of Arthrophytum (*Hammada scoparia*). J. Acute Dis., 5(6): 470–482. https://doi.org/10.1016/j.joad.2016.08.028

Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ (2007). One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? J. Pineal Res., 42(1): 28–42. https://doi.org/10.1111/j.1600-079X.2006.00407.x

Wong AA, Brown RE (1984). Age-related changes in visual acuity, learning and memory in C57BL/6J and DBA/2J mice. Neurobiol. Aging, 11(10): 47–60. https://doi.org/10.1016/0165-0270(84)90007-4

Xu SC, He M Di, Zhong M, Zhang YW, Wang Y, Yang L, Yang J, Yu ZP, Zhou Z (2010). Melatonin protects against Nickel-induced neurotoxicity *in vitro* by reducing oxidative stress and maintaining mitochondrial function. J. Pineal Res., 49(1): 86–94. https://doi.org/10.1111/j.1600-079X.2010.00770.x

Zaky A, Mohammad B, Moftah M, Kandeel KM, Bassiouny AR (2013). Apurinic/apyrimidinic endonuclease 1 is a key modulator of aluminum-induced neuroinflammation. BMC Neurosci., 14: 1–12. https://doi.org/10.1186/1471-2202-14-26

Zghari O, Rezqaoui A, Ouakki S, Lamtai M, Chaibat J (2018). Effect of chronic aluminum administration on affective and cognitive behavior in male and female rats. J. Behav. Brain Sci., 8(4): 179–196. https://doi.org/10.4236/jbbs.2018.84012

Zhang L, Zhang H-Q, Liang X-Y, Zhang H-F, Zhang T, Liu F-E (2013). Melatonin ameliorates cognitive impairment induced by sleep deprivation in rats: role of oxidative stress, BDNF and CaMKII. Behav. Brain Res., 256: 72–81. https://doi.org/10.1016/j.bbr.2013.07.051

Zhu Y, Liu F, Zou X, Torbey M (2015). Comparison of unbiased estimation of neuronal number in the rat hippocampus with different staining methods. J. Neurosci. Methods, 254: 73–79. https://doi.org/10.1016/j.jneumeth.2015.07.022