Original article DOI: 10.2478/aiht-2021-72-3513

Protective effects of oral melatonin against cadmium-induced neurotoxicity in Wistar rats

İhsan Kısadere[1], Mehmet Faruk Aydın[2], Mustafa Usta[3], and Nurcan Donmez[4]

[1] *University of Balıkesir Faculty of Veterinary Medicine, Department of Physiology, Balıkesir, Turkey*
[2] *University of Balıkesir Faculty of Veterinary Medicine, Department of Histology and Embryology, Balıkesir, Turkey*
[3] *University of Balıkesir Faculty of Veterinary Medicine, Department of Pathology, Balikesir, Turkey*
[4] *University of Selcuk Faculty of Veterinary Medicine, Department of Physiology, Konya, Turkey*

[Received in December 2020; Similarity Check in December 2020; Accepted in June 2021]

The aim of this study was to investigate the effects of oral melatonin on oxidative/antioxidative parameters and histopathological changes in the hippocampal tissue of Cd-exposed Wistar rats, including malondialdehyde (MDA), glutathione (GSH), tumour necrosis factor-alpha (TNF-α), interleukin (IL-6 and IL-10), and gamma-aminobutyric acid (GABA) levels and catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase (AChE) activities. Thirty-two male Wistar rats were divided randomly into four groups as follows: untreated control (n=8), cadmium (Cd) (n=8), melatonin (Mlt) (n=8), and Cd+Mlt (CdMlt) (n=8). Cd (2 mg/kg) was administered orally by gastric gavage three times a week and Mlt (100 mg/kg) five times a week. The control group received standard feed and water only. After four weeks of treatment, the animals were decapitated and tissue samples taken for biochemical and histopathological evaluations. Mlt caused a significant increase in GSH levels and SOD and CAT activities in the CdMlt group compared to the Cd group. Tissue TNF-α and IL-6 levels were significantly higher in the Cd group than other groups ($P<0.05$). This effect was significantly countered by Mlt in the CdMlt group ($P<0.05$). GABA concentrations were significantly higher in the Mlt than other groups ($P<0.05$). Our findings clearly evidence the protective effects of melatonin against Cd-induced neurotoxicity in rats.

KEY WORDS: AChE; antioxidant; CAT; Cd; cytokine; GABA; GSH; hippocampus; IL-6; IL-10; MDA; oxidative stress; SOD; TNF-α

The main sources of occupational exposure to cadmium (Cd) are metal industries (mining, smelting, processing, product formulations, and battery manufacturing), while the sources of non-occupational exposure include contaminated air (smoking included), water, soil, plants, and food. Exposure through skin, however, is rare (1–3).

Although the main target organs of Cd poisoning are the kidney and the liver, in chronic exposure Cd can also accumulate in different parts of the central nervous system (CNS) by damaging and passing the blood-brain barrier (BBB) or through nasal mucosa or olfactory pathways (4, 5). There it causes oxidative stress and through it neurotransmitter dysfunction and hippocampus-dependent learning and memory impairment in humans and animals (6–8). It has also been implicated in the development of Alzheimer's and Parkinson's disease (9).

In recent years, research has been focused on how to counter the harmful oxidative effects of Cd and has included a number of substances with antioxidant and metal-binding properties, especially in Cd-exposed animals (10–12). One of them is melatonin (*n*-acetyl-5-methoxytryptamine), a hormone secreted mainly from the pineal gland with powerful metal-binding (13), antioxidant (14), and free radical scavenging (15) properties due to its small size and lipophilicity. Recent studies have also shown that melatonin plays a neuroprotective role in many central nervous system (CNS) disorders, including Alzheimer's and Parkinson's disease and ischaemic brain injury. It has also shown beneficial effects on memory, posture control, and balance (16) and was reported to protect against Cd-induced neurotoxicity in rats by reducing lipid peroxidation and restoring antioxidant defence parameters and acetylcholinesterase (AChE) activity in the plasma and brain (17).

Considering the important role of the hippocampus in spatial navigation, emotional behaviour, regulation of hypothalamic functions, learning, memory, limbic system (18–20), and its damage in Alzheimer's disease (20, 21), we wanted to complement relatively modest literature (14, 22–24) about beneficial effects of oral melatonin in the hippocampus, especially in regard to cytokines and gamma-aminobutyric acid (GABA) levels. To that effect the aim of our study was to compare common oxidative stress, inflammation, and neurotoxicity parameters [malondialdehyde (MDA) (25), interleukins 6 and 10 (IL-6, IL-10), tumour necrosis factor alpha (TNF-α) (9, 26–28),

Corresponding author: İhsan Kısadere, University of Balıkesir Faculty of Veterinary Medicine, Department of Physiology, Balıkesir, Turkey, E-mail: *ihsan.kisadere@balikesir.edu.tr*, ORCID ID: 0000-0003-0732-0464

GABA (29–36), acetylcholinesterase (AChE) (17, 37, 38)] and antioxidant defence parameters [glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (39)] in the hippocampal tissue of control, Cd-, and melatonin-treated rats. We also wanted to investigate potential neuroprotective effects of melatonin through histopathological changes in the cerebral cortex of rats exposed to Cd.

MATERIALS AND METHODS

Animals and experimental design

Three weeks old male albino Wistar rats weighing ~200±30 g (n=32), purchased from Balıkesir University Experimental Medicine Research and Application Center (BUEMRAC), were first acclimatised to laboratory conditions for two weeks and then divided randomly into four groups: untreated control, cadmium (Cd), melatonin (Mlt), and Cd+Mlt (CdMlt), each consisting of eight animals. The rats were housed in standard plastic rat cages in an air-conditioned room (at 23±2 °C and 55±10 % humidity) with a 12-hour light/dark cycle. They had free access to standard pellet diet and fresh water.

The animals in the Cd and CdMlt groups received 2 mg/kg of Cd (Cat. No: 1002283736; $CdCl_2$) by gavage (1 mL/kg bwt) three times a week for four weeks, as described elsewhere (40). Those in the Mlt and CdMlt groups were receiving 100 mg/kg of melatonin (Cat. No: 1002042514) also by gavage five times a week for four weeks, as described elsewhere (41). The control group received only standard feed and water. After the four-week treatment, rats were sacrificed under anaesthesia with intraperitoneal injection of ketamine/xylazine (0.1 mL/100g/bwt) and then decapitated. Hippocampal/cerebral cortex tissue samples for biochemical and histopathological investigations were taken immediately. All animal procedures were approved and conducted in line with the guidelines of the Balıkesir University Experimental Animal Tests Local Ethics Committee (approval No. 2018/2-2).

Hippocampal tissue MDA, GSH, CAT, and SOD measurements

Hippocampal tissue samples of the animals were placed in liquid nitrogen and stored at -80 °C until analysis. 200 mg of each tissue sample in 800 mL of isotonic sodium chloride solution was homogenised at 9961 *g* in an ultrasonic homogeniser (IKA-T25, Merck, Darmstadt, Germany) and centrifuged at 4025 *g* for 10 min (Sigma18-K, Newtown, Shropshire, UK) to obtain supernatants. MDA and GSH levels and CAT and SOD activities were determined in the supernatants with the enzyme-linked immunosorbent assay (ELISA) using the ELx800 absorbance microplate reader (Biotek Instruments, Winooski, VT, USA) and standard commercial kits (Cat. Nos. 10009055, 703002, 707002, and 706002, respectively; Cayman, Ann Arbor, MI, USA).

Hippocampal tissue cytokine measurements

IL-6, IL-10, and TNF-α were also detected in the obtained supernatants with ELISA (ELx800, Biotek) and commercial kits (Cat. Nos. BMS625, BMS629, and KRC3011, respectively; ThermoFisher Scientific, San Jose, CA, USA) according to the manufacturers' instructions.

Hippocampal tissue GABA and AChE measurements

GABA concentrations were measured using the double-sandwich ELISA kit (Sunred Biological Technology, Shanghai, China) according to the manufacturer's instruction. In brief, tissue samples (supernatants) and kit standards (Cat. No: 201-11-0103) were extracted on an extraction plate, derived using an equalising reagent, and subjected to ELISA in GABA pre-coated microtitre strips. The absorbance of the solution in the wells was read at 450 nm within 15 min with a Multiskan FC microplate reader (ThermoFisher Scientific). GABA concentrations were calculated based on optical density using a standard curve.

AChE activities were also determined using a sandwich ELISA kit (Elabscience, Wuhan, China) according to the producer's instruction. Micro plates provided in this kit were pre-coated with an antibody specific to rat AChE (Cat. No: E-EL-R0355). The absorbance of the solution was immediately read at 450 nm with the above mentioned plate reader.

Cerebral cortex histopathology

Brain tissue samples (cerebral cortex) collected for histopathology were fixed in 10 % buffered formaldehyde solution, embedded in paraffin and cut into 4 μm thick sections, stained with haematoxylin and eosin (H&E), and observed under a light microscope (100x, Nikon, Eclipse Ni, Tokyo, Japan).

In situ *TUNEL assay in the cerebral cortex*

To identify apoptotic cells in the cerebral cortex we used the DeadEnd™ Colorimetric TUNEL System (Cat No: G7130; Promega, Madison, WI, USA) according to the manufacturer's instructions.

Statistical analysis

All statistical analyses were run on IBM SPSS v. 25.0 for Windows (IBM Corp., Armonk, NY, USA) starting with the analysis of variance (ANOVA) followed by Duncan's test. P values ≤ 0.05 were considered significant.

RESULTS

Table 1 shows hippocampal tissue MDA and GSH levels and SOD and CAT activities. MDA levels were the highest in the Cd group compared to the rest (P<0.05). The oxidative stress was significantly reduced by melatonin in the CdMlt compared to the Cd group (P<0.05). As expected, GSH levels and SOD and CAT activities were the lowest in the Cd group, but melatonin treatment improved them significantly in the CdMlt compared to the Cd group (P<0.05).

Table 2 shows cytokine levels in the hippocampal tissue of our rats. TNF-α and IL-6 levels significantly increased in the Cd group (P<0.05), while IL-10 levels dropped compared to the other groups (P<0.05). Again, melatonin significantly countered these effects of Cd (P<0.05).

Hippocampal tissue GABA concentrations were significantly lower in the Cd group than the other groups (P<0.05), but were increased by melatonin in the Mlt and CdMlt groups compared to the Cd group (P<0.05). However, we found no significant differences in AChE activities between the groups (Table 3).

Histopathological findings in the cerebral cortex

The control group showed normal histomorphology (Figure 1a), while the Cd group had severe multifocal histopathological changes in the cerebral cortex. In this location, most of the pyramidal and granular cells were shrunken and had pericellular halos and dark stained nuclei with lost nucleoli. Many vacuoles of variable sizes were noted in some granule cells and the neuropil tissue. These vacuolar changes were most prominent in the pyramidal layer but were also evident in other layers. Hyperaemia was also noted in some locations (Figure 1b). In the CdMlt group, some pyramidal and granular cells showed signs of cellular degeneration. A vacuolar halo around cells with shrunken nuclei was also seen in this group (Figure 1c). Hyperaemia was not observed. Only a few multifocal areas with these cellular changes were observed compared to the many in the Cd group. No histopathological changes were identified in the Mlt group (Figure 1d).

Figure 2 shows apoptotic granular cells, identified only in the Cd group (Figure 2). No apoptotic (TUNEL-stained) cells were observed in other groups.

Table 1 Hippocampal tissue MDA and GSH levels and SOD and CAT activities by the experimental groups of Wistar rats

Pro/antioxidant parameters	Control (n=8) Mean±SD	Mlt (n=8) Mean±SD	CdMlt (n=8) Mean±SD	Cd (n=8) Mean±SD
MDA (ng/mL)	1.717±0.016^{c}	1.738±0.020^{c}	1.785±0.013^{b}	1.877±0.038^{a}
SOD (ng/mL)	0.434±0.022^{a}	0.432±0.016^{a}	0.420±0.027^{a}	0.390±0.014^{b}
GSH (µg/mL)	2.170±0.094^{a}	2.177±0.066^{a}	2.013±0.086^{b}	1.136±0.009^{c}
CAT (U/L)	4.616±0.361^{a}	4.697±0.463^{a}	4.486±0.145^{ab}	4.126±0.551^{b}

$^{a–c}$ Means in the same row with different superscripts differ significantly (P<0.05). Mlt – group receiving melatonin alone; Cd – group receiving cadmium alone; CdMlt – group receiving both melatonin and cadmium. MDA – malondialdehyde; SOD – superoxide dismutase; GSH – glutathione; CAT – catalase; SD – standard deviation

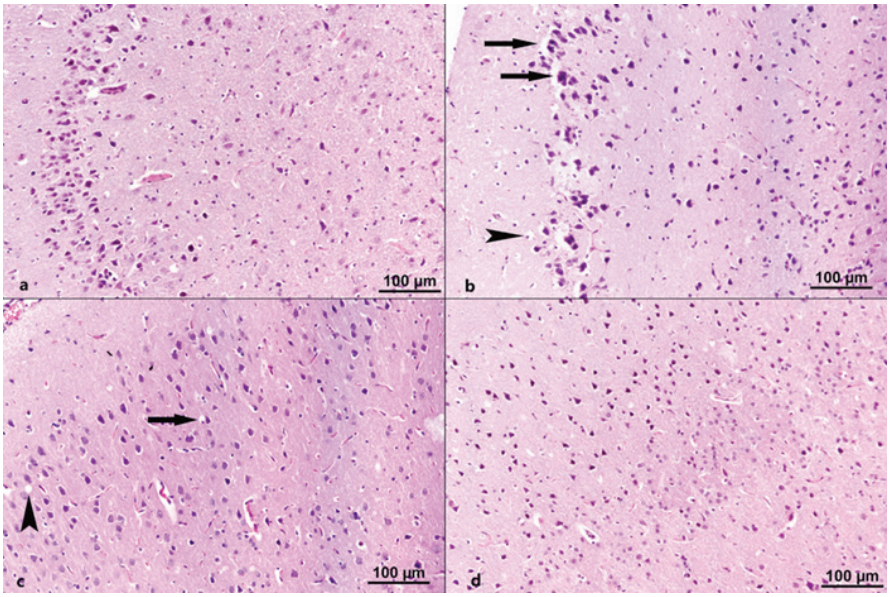

Figure 1 Typical H&E-stained histological images of cerebral cortex sections in Wistar rats by groups (a) normal histological appearance in the control group; (b) pyramidal cells irregular in shape, surrounded by pericellular halos (arrows) and intracellular vacuoles (arrowhead) in the Cd group; (c) more or less normal pyramidal and granular cells and intracellular (arrow) and extracellular vacuoles (arrowhead) in the CdMlt group; (d) histological appearance in the Mlt group

Table 2 Hippocampal tissue cytokine levels by the experimental groups of Wistar rats

Cytokines	Control (n=8) Mean±SD	Mlt (n=8) Mean±SD	CdMlt (n=8) Mean±SD	Cd (n=8) Mean±SD
TNF-α (pg/mL)	0.070±0.001[c]	0.070±0.001[c]	0.081±0.002[b]	0.095±0.001[a]
IL-6 (pg/mL)	0.058±0.003[b]	0.116±0.171[ab]	0.125±0.191[ab]	0.217±0.107[a]
IL-10 (pg/mL)	0.256±0.004[a]	0.259±0.019[a]	0.252±0.005[a]	0.207±0.003[b]

[a–c] Means in the same row with different superscripts differ significantly (P<0.05). Mlt – group receiving melatonin alone; Cd – group receiving cadmium alone; CdMlt – group receiving both melatonin and cadmium. TNF-α – tumour necrosis factor alpha; IL-6 – interleukin 6; IL-10 – interleukin 10; SD – standard deviation

Table 3 Hippocampal tissue GABA concentrations and AChE activities by the experimental groups of Wistar rats

Neurotoxicity parameters	Control (n=8) Mean±SD	Mlt (n=8) Mean±SD	CdMlt (n=8) Mean±SD	Cd (n=8) Mean±SD
GABA (nmol/L)	8.124±0.008[b]	9.610±0.107[a]	7.174±0.082[c]	6.252±0.012[d]
AChE (ng/mL)	0.781±0.001	0.781±0.001	0.780±0.001	0.780±0.001

[a–d] Means in the same row with different superscripts differ significantly (P<0.05). Mlt – group receiving melatonin alone; Cd – group receiving cadmium alone; CdMlt – group receiving both melatonin and cadmium. AChE – acetylcholinesterase; GABA – gamma-aminobutyric acid; SD– standard deviation

DISCUSSION

It is well known that the brain tissue is susceptible to lipid peroxidation due to high use of oxygen and polyunsaturated fatty acids. Our study confirms numerous findings of adverse Cd effects in this respect, showing significant increases in MDA (22, 24, 42, 43) levels and MDA lowering effects in the hippocampal tissue thanks to melatonin administration (42). Several studies have also reported these effects of melatonin in the hippocampal and brain tissue against arsenic (As) and aluminium (Al) poisoning (15, 23, 44).

The same is true for antioxidant response, which is severely affected by Cd (17, 39, 43, 45). Oral administration of melatonin in our study significantly increased and nearly restored GSH, SOD, and CAT, which is in line with earlier findings (17, 42, 44), evidencing its antioxidant action.

Of particular interest is the anti-inflammatory action of melatonin found in our study. Melatonin treatment reversed the elevated levels of TNF-α and IL-6 in the hippocampal tissue of animals receiving Cd, which adds new, even though expected, knowledge, as little has been reported about melatonin effects on IL- 6 and TNF-α levels in the hippocampus of rats exposed to Cd. Other studies found beneficial effects of melatonin in rats with induced breast cancer (46) or against aluminium (23), arsenic (44), and fluoride (47). As for IL-10, a regulatory cytokine which has both immunosuppressive and immunostimulatory properties, our findings confirm earlier reports of inhibitory effects of Cd in rat brain (9, 48) and beneficial effects of melatonin (49), which highlight the immunomodulatory potential of melatonin against chronic Cd poisoning (50).

In line with earlier reports on the GABA-lowering effects of Cd (35, 36), our study found the lowest GABA

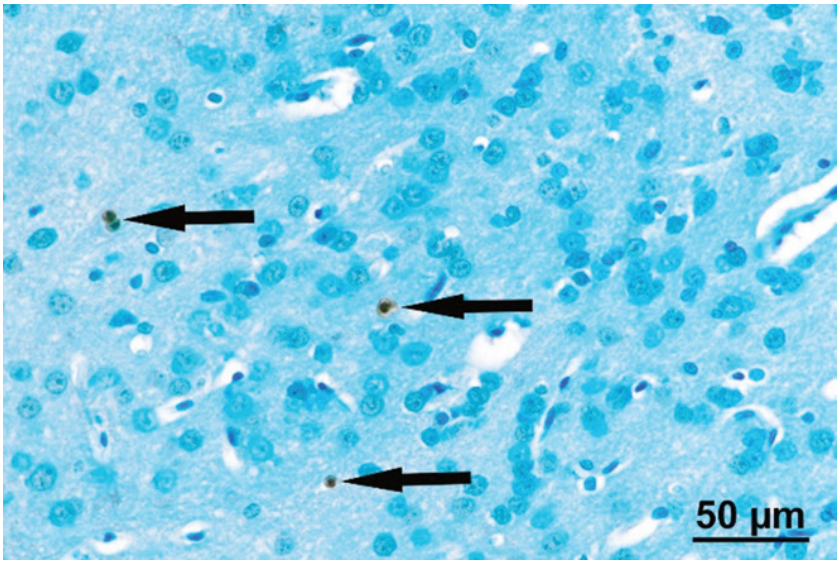

Figure 2 Section showing apoptotic cells (marked with arrows) in the granular layer of a Cd-treated rat (TUNEL method)

hippocampal tissue concentrations in the Cd group. Similar was reported for the hypothalamus of rats treated with Cd (35, 51). As expected, melatonin significantly increased hippocampus GABA concentrations in the CdMlt group in our study, but it is interesting to note that the highest concentration was observed in the Mlt group. It seems that melatonin increases GABA accumulation in different parts of the brain tissue (52).

Another interesting finding was the lack of significant changes in the activity of AChE in the brain tissue in any of the groups in our study. Most importantly, AChE activities were not affected by melatonin treatment. In contrast, other studies reported a rise in the brain tissue of Cd-exposed rats treated with melatonin (17, 42), but this may be due to different dosage regimes.

Our histopathological exam showed that Cd caused severe multifocal changes and apoptotic cells in the cerebral cortex. Apoptosis may have been the result of increased caspase-3/7 activity, as reported by Mahdavi et al. (53). Apoptotic changes induced by Cd in cerebral cortical neurons were also reported by Yuan et al. (54). Melatonin seems to have attenuated these histopathological changes in our study, as we observed but a few multifocal areas and no apoptotic cells in the CdMlt group. This is in line with an earlier report of melatonin (4 mg/kg/day for eight weeks, *i.p.*) treatment reducing the loss in hippocampal neurons through direct blocking of Cd-induced oxidative stress pathways (14). Shagirtha et al. (17) also reported that oral melatonin pretreatment (10 mg/kg/day for four weeks) mitigated Cd-induced histopathological changes such as spongiform necrosis, nuclear vacuolisation, pycnosis, and lymphocytic inflammation in the rat brain tissue.

CONCLUSION

Our findings show that oral melatonin treatment has a great neuroprotective potential, especially against Cd neurotoxicity in the hippocampus and cerebral cortex and provide useful information for its use against metal poisoning.

Conflict of interests

None to declare.

Acknowledgements

This study was funded as part of the Balıkesir University Scientific Research Coordinatorship Project No. 2019-068.

REFERENCES

1. Tobwala S, Wang HJ, Carey J, Banks WA, Ercal N. Effects of lead and cadmium on brain endothelial cell survival, monolayer permeability, and crucial oxidative stress markers in an *in vitro* model of the blood-brain barrier. Toxics 2014;2:258-75. doi: 10.3390/toxics2020258
2. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Cadmium. Atlanta (GA): ATSDR; 1999.
3. Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PEB, Williams DJ, Moore MR. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett 2003;137:65-83. doi: 10.1016/s0378-4274(02)00381-8
4. Lafuente A, Esquifino AI. Cadmium effects on hypothalamic activity and pituitary hormone secretion in the male. Toxicol Lett 1999;110:209-18. doi: 10.1016/s0378-4274(99)00159-9
5. Esquifino AI, Marquez N, Alvarez-Demanuel, Lafuente A. Effects of chronic alternating cadmium exposure on the episodic secretion of prolactin in male rats. J Trace Elem Med Biol 1999;12:205-10. doi: 10.1016/S0946-672X(99)80059-5
6. Goncalves JF, Nicoloso FT, Da Costa P, Farias JG, Carvalho FB, Da Rosa MM, Gutierres JM, Abdalla FH, Pereira JSF, Dias GRM, Barbosa NBV, Dressler VL, Rubin MA, Morsch VM, Schetinger MRC. Behavior and brain enzymatic changes after long-term intoxication with cadmium salt or contaminated potatoes. Food Chem Toxicol 2012;50:3709-18. doi: 10.1016/j.fct.2012.07.016
7. Méndez-Armenta M, Ríos C. Cadmium neurotoxicity. Environ Toxicol Pharmacol 2007;23:350-8. doi: 10.1016/j.etap.2006.11.009
8. Wang F, Fan F, Wang L, Ye W, Zhang Q, Xie S. Maternal cadmium levels during pregnancy and the relationship with preeclampsia and fetal biometric parameters. Biol Trace Elem Res 2018;186:322-9. doi: 10.1007/s12011-018-1312-3
9. Sivaprakasam C, Rajendran V, Mathivanan A, Nachiappan V. Alteration of mitochondrial phospholipid due to the PLA_2 activation in rat brains under cadmium toxicity. Toxicol Res 2016;5:1680-7. doi: 10.1039/c6tx00201c
10. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol 2006;5:1142-5.
11. Fang C. Characterization of Polyphenol Oxidase and Antioxidants from Pawpaw (*Asimina Tribola*) Fruit [master thesis]. Kentucky, USA: University of Kentucky; 2007 [displayed 2 June 2021]. Available at: https://uknowledge.uky.edu/gradschool_theses/477
12. Karabulut-Bulan O, Bolkent S, Yanardag R, Bilgin-Sokmen B. The role of vitamin C, vitamin E, and selenium on cadmium-induced renal toxicity of rats. Drug Chem Toxicol 2008;31:413-26. doi: 10.1080/01480540802383200
13. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. J Biomed Sci 2000;7:444-58. doi: 10.1007/BF02253360
14. Lamtai M, Azirar S, Zghari O, Ouakki S, El Hessni A, Mesfioui A, Ouichou A. Melatonin ameliorates cadmium-induced affective and cognitive impairments and hippocampal oxidative stress in rat. Biol Trace Elem Res 2021;199:1445-55. doi: 10.1007/s12011-020-02247-z
15. Sushma NJ, Priyanka S, Rao KJ. Neuroprotective role of Melatonin against aluminum-induced oxidative stress in the hippocampus of mouse brain. J Appl Pharm Sci 2011;1:126-33.

16. Pandi-Perumal SR, Trakht I, Srinivasan V, Spence DW, Maestroni GJM, Zisapel N, Cardinali DP. Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Prog Neurobiol 2008;85:335-53. doi: 10.1016/j.pneurobio.2008.04.001
17. Shagirtha K, Muthumani M, Prabu SM. Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. Eur Rev Med Pharmacol Sci 2011;15:1039-50. PMID: 22013727
18. Stella F, Cerasti E, Si B, Jezek K, Treves A. Self-organization of multiple spatial and context memories in the hippocampus. Neurosci Biobehav Rev 2012;36:1609-25. doi: 10.1016/j.neubiorev.2011.12.002
19. Gilbert PE, Brushfield AM. The role of the CA3 hippocampal subregion in spatial memory: A process oriented behavioral assessment. Prog Neuropsychopharmacol Biol Psychiatry 2009;33:774-81. doi: 10.1016/j.pnpbp.2009.03.037
20. Zarei M, Beckmann CF, Binnewijzen MAA, Schoonheim MM, Oghabian MA, Sanz-Arigita EJ, Scheltens P, Matthews PM, Barkhof F. Functional segmentation of the hippocampus in the healthy human brain and in Alzheimer's disease. NeuroImage 2013;66:28-35. doi: 10.1016/j.neuroimage.2012.10.071
21. Jia S, Lu Z, Gao Z, An J, Wu X, Li X, Dai X, Zheng Q, Sun Y. Chitosan oligosaccharides alleviate cognitive deficits in an amyloid-β1–42-induced rat model of Alzheimer's disease. Int J Biol Macromol 2016;83:416-25. doi: 10.1016/j.ijbiomac.2015.11.011
22. Kisadere I, Dönmez N, Dönmez HH. The effects of quercetin on antioxidant and cytokine levels in rat hippocampus exposed to acute cadmium toxicity. JCNOS 2019;11:10. doi: 10.37212/jcnos.584684
23. Qusti SY. Selenium and melatonin attenuates inflammation and oxidative stress in the brain of aged rats with aluminum chloride-induced Alzheimer. Int J Appl Pharm Res Allied Sci Res 2017;6:277-89.
24. Kanter M, Unsal C, Aktas C, Erboga M. Neuroprotective effect of quercetin against oxidative damage and neuronal apoptosis caused by cadmium in hippocampus. Toxicol Ind Health 2016;32:541-50. doi: 10.1177/0748233713504810
25. Gaweł S, Wardas M, Niedworok E, Wardas V. [Malondialdehyde (MDA) as a lipid peroxidation marker, in Polish]. Wiad Lek 2004;57:453-5. PMID: 15765761
26. Djokic J, Aleksandrov AP, Ninkov M, Mirkov I, Zolotarevski L, Kataranovski D, Kataranovski M. Cadmium administration affects circulatory mononuclear cells in rats. J Immunotoxicol 2015;12:115-23. doi: 10.3109/1547691X.2014.904955
27. Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. Oxid Med Cell Longev 2015;610813. doi: 10.1155/2015/610813
28. Phuagkhaopong S, Ospondpant D, Kasemsuk T, Sibmooh N, Soodvilai S, Power C, Vivithanaporn P. Cadmium-induced IL-6 and IL-8 expression and release from astrocytes are mediated by MAPK and NF-kB pathways. Neurotoxicology 2017;60:82-91. doi: 10.1016/j.neuro.2017.03.001
29. Bu DF, Erlander MG, Hitz BC, Tillakaratne NJ, Kaufman DL, Wagner-McPherson CB, Evans GA, Tobin AJ. Two human glutamate decarboxylases, 65-kDa GAD and 67-kDa GAD, are each encoded by a single gene. Proc Natl Acad Sci USA 1992;89:2115-9. doi: 10.1073/pnas.89.6.2115
30. Birnir B, Korpi ER. The impact of sub-cellular location and intracellular neuronal proteins on properties of GABA (A) receptors. Curr Pharm Des 2007;13:3169-77. doi: 10.2174/138161207782341330
31. Schmidt MJ, Mirnics K. Neurodevelopment, GABA system dysfunction, and schizophrenia. Neuropsychopharmacology 2015;40:190-206. doi: 10.1038/npp.2014.95
32. Plog BA, Nedergaard M. The glymphatic system in central nervous system health and disease: past, present, and future. Annu Rev Pathol 2018;13:379-94. doi: 10.1146/annurev-pathol-051217-111018
33. Wong CG, Bottiglieri T, Snead OC 3rd. GABA, gamma-hydroxybutyric acid, and neurological disease. Ann Neurol 2003;6:S3-12. doi: 10.1002/ana.10696
34. Bhandage AK, Cunningham JL, Jin Z, Shen Q, Bongiovanni S, Korol SV, Syk M, Kamali-Moghaddam M, Ekselius L, Birnir B. Depression, GABA, and age correlate with plasma levels of inflammatory markers. Int J Mol Sci 2019;20:6172. doi: 10.3390/ijms20246172
35. Caride A, Fernández-Pérez B, Cabaleiro T, Lafuente A. Cadmium chloride exposure modifies amino acid daily pattern in the mediobasal hypothalamus in adult male rat. J Appl Toxicol 2010;30:84-90. doi: 10.1002/jat.1472
36. Alam RTM, Hendawi MY. Protective efficacy of *Spirulina platensis* against cadmium induced neurotoxicity in rats. Glob Vet 2015;14:490-9. doi: 10.5829/idosi.gv.2015.14.04.93239
37. Tsakiris S, Schulpis KH. The effect of galactose metabolic disorders on rat brain acetylcholinesterase activity. Z Naturforsch C J Biosci 2000;55:852-5. doi: 10.1515/znc-2000-9-1032
38. Antonio MT, López N, Leret ML. Pb and Cd poisining during development alters cerebellar and striatal function in rat. Toxicology 2002;176:59-66. doi: 10.1016/s0300-483x(02)00137-3
39. López E, Arce C, Oset-Gasque MJ, Cañadas S, González MP. Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture. Free Radic Biol Med 2006;40:940-51. doi: 10.1016/j.freeradbiomed.2005.10.062
40. Kısadere I, Aydın MF, Undag I. Partial protective effects of melatonin on cadmium-induced changes in hematological characteristics in rats. Biotech Histochem 2021. doi: 10.1080/10520295.2021.1925965
41. Haddadi GH, Fardid R. Oral administration of melatonin modulates the expression of tumor necrosis factor-α (TNF-α) gene in irradiated rat cervical spinal cord. Rep Pract Oncol Radiother 2015;20:123-7. doi: 10.1016/j.rpor.2014.11.003
42. Mukherjee R, Desai F, Singh S, Gajaria T, Singh PK, Baxi DP, Sharma D, Bhatnagar M, Ramachandran AV. Melatonin protects against alterations in hippocampal cholinergic system, trace metals and oxidative stress induced by gestational and lactational exposure to cadmium. EXCLI J 2010;9:119-32. PMCID: PMC5698886
43. Lamtai M, Chaibat J, Ouakki S, Berkiks I, Rifi E, El-Hessni A, Mesfioui A, Hbibi AT, Ahyayauch H, Essamri A, Ouichou A. 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 2018;8:240-68. doi: 10.4236/jbbs.2018.85016
44. Durappanavar PN, Nadoor P, Waghe P, Pavithra BH, Jayaramu GM. Melatonin ameliorates neuropharmacological and neurobiochemical alterations induced by subchronic

exposure to arsenic in Wistar rats. Biol Trace Elem Res 2019;190:124-39. doi: 10.1007/s12011-018-1537-1
45. Pulido G, Treviño S, Brambila E, Vazquez-Roque R, Moreno-Rodriguez A, Rosas UP, Moran-Perales JL, Silva AH, Guevara J, Flores G, Diaz A. The administration of cadmium for 2, 3 and 4 months causes a loss of recognition memory, promotes neuronal hypotrophy and apoptosis in the hippocampus of rats. Neurochem Res 2019;44:485-97. doi: 10.1007/s11064-018-02703-2
46. Gulbahce-Mutlu E, Baltaci SB, Menevse E, Mogulkoc R, Baltaci AK. The effect of zinc and melatonin administration on lipid peroxidation, IL-6 levels, and element metabolism in DMBA-induced breast cancer in rats. Biol Trace Elem Res 2021;199:1044-51. doi: 10.1007/s12011-020-02238-0
47. Jain A, Mehta VK, Chittora R, Mahdi A, Bhatnagar B. Melatonin ameliorates fluoride induced neurotoxicity in young rats: an *in vivo* evidence. Asian J Pharm Clin Res 2015;8:164-7.
48. Akinyemi AJ, Adeniyi PA. Effect of essential oils from ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) rhizomes on some inflammatory biomarkers in cadmium induced neurotoxicity in rats. J Toxicol 2018;2018:4109491. doi: 10.1155/2018/4109491
49. Dehghan F, Shahrokhi N, Khaksari M, Soltani Z, Asadikorom G, Najafi A, Shahrokhi N. Does the administration of melatonin during post-traumatic brain injury affect cytokine levels? Inflammopharmacology 2018;26:1017-23. doi: 10.1007/s10787-017-0417-1
50. Carrillo-Vico A, Lardone PJ, Alvarez-Sánchez N, Rodriguez-Rodriguez A, Guerrero JM. Melatonin: buffering the immune system. Int J Mol Sci 2013;14:8638-83. doi: 10.3390/ijms14048638
51. Lafuente A, Gonz A, Cabaleiro T, Romero A, Esquifino AI. Toxic effects of cadmium on GABA and taurine content in different brain areas of aduh male rats. J Physiol Biochem 2005;61:439-46. doi: 10.1007/BF03168450
52. Rosenstein RE, Cardinali DP. Central gabaergic mechanisms as targets for melatonin activity in brain. Neurochem Int 1990;17:373-9. doi: 10.1016/0197-0186(90)90019-p
53. Mahdavi S, Khodarahmi P, Roodbari NH. Effects of cadmium on Bcl-2/Bax expression ratio in rat cortex brain and hippocampus. Hum Exp Toxicol 2018;37:321-8. doi: 10.1177/0960327117703687
54. Yuan Y, Jiang CY, Xu XE, Sun Y, Hu F, Bian J, Liu X, Gu J, Liu Z. Cadmium-induced apoptosis in primary rat cerebral cortical neurons culture is mediated by a calcium signaling pathway. PLoS One 2013;8(5):e64330. doi: 10.1371/journal.pone.0064330

Djelotvornost peroralne primjene melatonina u ublažavanju neurotoksičnosti izazvane kadmijem u Wistar štakora

Cilj je ovog istraživanja bio utvrditi djelovanje peroralne primjene melatonina na razine malondialdehida (MDA), glutationa (GSH), čimbenika nekroze tumora alfa (TNF-α), interleukina 6 i 10 (IL-6 i IL-10), enzimske aktivnosti katalaze (CAT), superoksid dismutaze (SOD) i acetilkolinesteraze (AChE) te na koncentraciju gama-aminomaslačne kiseline (GABA) u hipokampusnom tkivu Wistar štakora izloženih kadmiju (Cd). Također je cilj bio utvrditi histopatološke promjene u cerebralnom korteksu štakora. U tu je svrhu istraživanje obuhvatilo 32 mužjaka, nasumce raspoređena u četiri skupine: kontrolnu (n=8), skupinu koja je primala samo Cd (n=8), skupinu koja je primala samo melatonin (Mlt) (n=8) i skupinu koja je istodobno primala Cd + Mlt (n=8). Štakori u Cd i CdMlt skupinama primali su Cd u dozi od 2 mg/kg gavažom na usta triput na tjedan odnosno melatonin u dozi od 100 mg/kg na isti način pet puta na tjedan. Kontrolna je skupina primala samo vodu. Nakon četverotjednog tretmana životinje su dekapitirane te su uzeti uzorci tkiva za biokemijsku i histopatološku analizu. Primjena melatonina dovela je u CdMlt skupini do rasta razina GSH i SOD te aktivnosti katalaze u odnosu na skupinu koja je primala samo Cd. Potonja je pak iskazala značajno više tkivne razine TNF-α i IL-6 od ostalih skupina ($P<0,05$). Primjena melatonina dovela je do njihova značajnog sniženja u CdMlt skupini ($P<0,05$). Koncentracije GABA bile su pak značajno više u skupini na melatoninu nego u ostalim skupinama ($P<0,05$). Naši rezultati potvrđuju da melatonin štiti od neurotoksičnosti izazvane kadmijem u štakora.

KLJUČNE RIJEČI: acetilkolinesteraza; antioksidansi; CAT; Cd; citokini; GABA; GSH; hipokampus; IL-6; IL-10; MDA; oksidacijski stres; SOD; TNF-α