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Effect of Cadmium Chloride Toxicity on Olfactory Mucosa

Efecto de la Toxicidad del Cloruro de Cadmio en la Mucosa Olfativa

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HAZNEDAR, B.; ERTUGRUL, G. & DEVECI, E. Effect of cadmium chloride toxicity on olfactory mucosa. *Int. J. Morphol., 40(6)*:1574-1578, 2022.

SUMMARY: Cadmium is a highly toxic metal and affects the respiratory mucosa. The aim of the study is to show the inflammation and degenerative effect of cadmium on the olfactory mucosa. In this study, eight-week-old Wistar rats with an average weight of 170-190 g were divided into two groups (control and experiment) with 20 animals in each group and used in the experiments.The rats in the experimental group were given 2 mg/kg/day powdered cadmium chloride dissolved in water intraperitoneally every day for two weeks. At the end of the experiment, the nasal cavity was completely removed with anesthesia. Concha nasalis superior was separated, fixed with zinc-Formalin solution and decalcified with 5 % EDTA (Ethylene-diaminetetraacetic acid).After routine histopathological procedure, APAF-1 antibody was used for expression of Hematoxylin-Eosin (HE) and immunohistochemistry. Histopathological examination revealed interruptions in the basement membrane structure due to cadmium and degenerative changes in stem cells, degeneration in sensory cells and pycnosis in nuclei, dilatation in blood vessels and increased inflammation in connective tissue. APAF-1 expression was found to increase in epithelial cells and olfactory glands (Bowman gland) cells. It has been thought that cadmium toxicity increases cell degeneration and inflammation in the olfactory mucosa and may significantly affect cell death and olfactory metabolism by inducing the pro-apoptotic process.

KEY WORDS: Cadmium chloride; Olfactory mucosae; APAF-1 (Apoptotic Protease Activating Factor-1); Rat; Histopathology.

INTRODUCTION

Cadmium is a metal used in the industrial field, and it is known that cadmium causes serious damage to the lungs, brain, testicles, kidneys and bones by cellular degeneration (Bernard, 2008; Deveci *et al.,* 2010). Cadmium metal passes to the fetus during pregnancy and crosses the blood-brain barrier (BBB), causing neuronal degeneration and cell apoptosis (Pulido *et al*., 2019). Epidemiological studies on toxic effects on olfactory mucosa have reported that exposure to metal compounds containing cadmium, chromium and nickel and formaldehyde may impair the human sense of smell (Werner & Nies, 2018). Programmed cell death is the process that begins with the degenerative pathway mediated by the mitochondria apoptotic cascade. Proteins of the Bcl-2 (B-cell leukemia/lymphoma 2) family primarily control and ensure the continuation of the proapoitotic process (Gokalp-Ozkorkmaz *et al*., 2018).

Critical regulators of apoptosis are both BAX and BCL-2', for example translocation of BAX. to the internal mitochondrial matrix releases cytochrome C that binds to APAF-1 (apoptotic protease activating factor 1), inducing apoptosome formation (Chan & Yu, 2004; Ladokhin, 2020; Christgen *et al*., 2022). The aim of this study is to examine the histopathological changes and APAF-1 expression in the olfactory mucosa after cadmium application.

MATERIAL AND METHOD

All experimental protocols were approved by the Dicle University Animal Care and Use Committee. 170-190 g weighing eight-week-old Wistar rats were divided in two groups (control and experimental), with 20 animals in each group and used in the trials. Control group rats were given 1 ml of 0.9 % NaCl intraperitoneally every day for two weeks, while experimental group rats were given 2 mg/kg/day powdered cadmium chloride dissolved in water intraperitoneally every day for two weeks (Gök & Deveci,

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2022). An intraperitoneal injection of 5 mg/kg xylazine HCl (Rompun) and 40 mg/kg ketamine HCl (Ketalar) were used to anesthesize the rats and they were allowed to breathe spontaneously.

The skins were removed as well as all the soft tissues surrounding the olfactory region. Then, the bony - framework of the nasal cavity including concha nasalis superior were nibbled out by bone-nibbler. The olfactory region were fixed with zinc-Formalin solution and decalcified with 5 % EDTA (Ethylene-diaminetetraacetic acid) (Gem *et al*., 2021).Tissues were passed through ascending alcohol series for about 12 hours. Tissues were washed with xylene 2x25 minutes and incubated within paraffin wax. 4-6 µm sections were cut with microtome . Sections were stained with routine Hematoxylin and Eosin (Dag & Ermis, 2021; Özgökçe et al., 2022; Tasin *et al*., 2021).

Histopathological method. The samples taken will be placed in 10 % formaldehyde and dehydrated in ethanol series rising 70-100 %. Then, for paraffin sections, they were placed in paraffin baths at 58°C. Then, sections taken using a rotary microtome were stained with Hematoxylin-Eosin (H-E) and evaluated to prepare 4-6 mm sections from paraffin blocks (Durgun & Asir, 2022).

Immunohistochemical Method. Formaldehyde-fixed tissues were embedded in paraffin. Deparaffinization of microtome cut sections was performed in absolute alcohol. Antigen retrieval was performed in citrate buffer solution (pH: 6.0) twice, first for 7 min, then for 5 min, then at 90 ºC at 700 W in a microwave oven. Boiled for 3 min. Afterwards, it will be cooled at room temperature for 20 min and then washed in distilled water for 6 min. Endogenous peroxidase activity was blocked in 0.1 % hydrogen peroxide (catalogue #TA-015-HP, Thermo Fisher Scientific, US) for 20 min. Ultra V block (TA-125-UB, Thermo Fisher Scientific, US) was applied for 10 min prior to the application of primary antibodies, which were left on overnight APAF-1 antibody (1:100 dilution). The slides were then exposed to streptavidin-peroxidase for 20 min. Chromogen diaminobenzidine (DAB; Invitrogen) was used. Control slides were prepared as described above with the exclusion of primary antibodies. Finally, the slides were mounted with protective entelen after counterstaining with hematoxylin, washing for 10 minutes in tap water, and soaking in distilled water for 15 minutes (Ermis, 2021; Ermis & Deveci, 2021; Ozgokce et al., 2022; Yüselmis & Ermis, 2022).

Statistical analysis. was performed by the IBM SPSS 25.0 software (IBM, Armonk, New York, US). Data distribution was analyzed with Shapiro-Wilk test. The data were recorded as median (minimum – maximum) with mean rank value. Binary group comparisons were evaluated with Mann-Whitney U and. P <0.05 was accepted as the significance level.

RESULTS

Statistical analysis of histological parameters (vascular dilatation/congestion, inflammation, degenerated epithelial cells, APAF1 expression) were shown in Table I. Compared to control group, vascular dilatation/congestion, inflammation, degenerated epithelial cells and APAF-1 expression were significantly increased in cadmium group. Box plot of Table I was shown in Figure 1, and results in Figures 2 to 5.

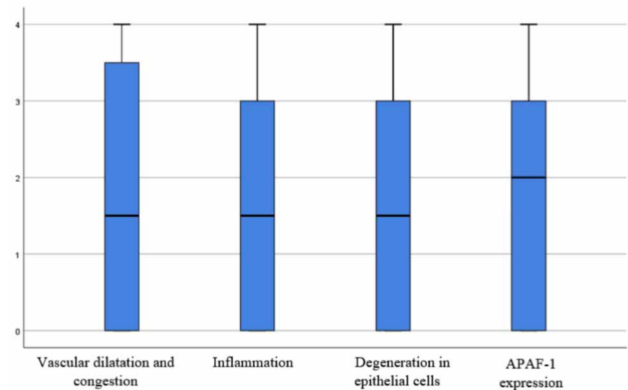

Fig. 1. Box plot of vascular dilatation/congestion, inflammation, degenerated epithelial cells, APAF1 expression.

Table I. Histological parameters of control and cadmium groups.

Parameters	Groups	n	Median (Min-Max)	Mean Rank	p value
Vascular dilatation and congestion	Control	10	0.00 (0.00-1.00)	5.50	<0.001
	Cadmium	10	3.50 (2.00-4.00)	15.50	
Inflammation	Control	10	0.00 (0.00-1.00)	5.50	<0.001
	Cadmium	10	3.00 (2.00-4.00)	15.50	
Degeneration in epithelial cells	Control	10	0.00 (0.00-1.00)	5.50	<0.001
	Cadmium	10	3.00 (2.00-4.00)	15.50	
APAF-1 expression	Control	10	0.00 (0.00-2.00)	5.60	<0.001
	Cadmium	10	3.00 (2.00-4.00)	15.40	

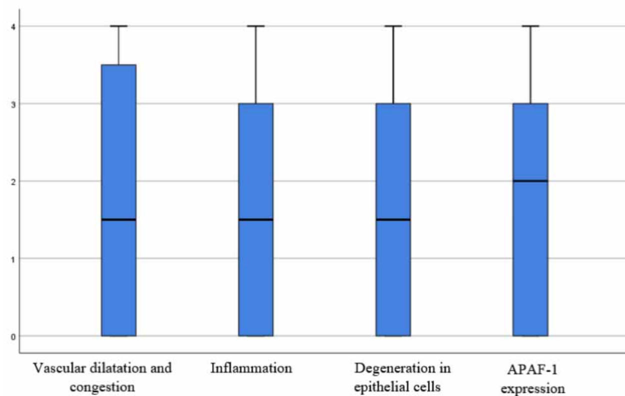

Fig. 2. Control group. In the transversal section passing through the concha nasalis superior of the control group, olfactory epithelial cells, sensory cells extending towards the apical region with chromatin-rich nucleus located on the basal membrane, and regular support cells in between were observed.

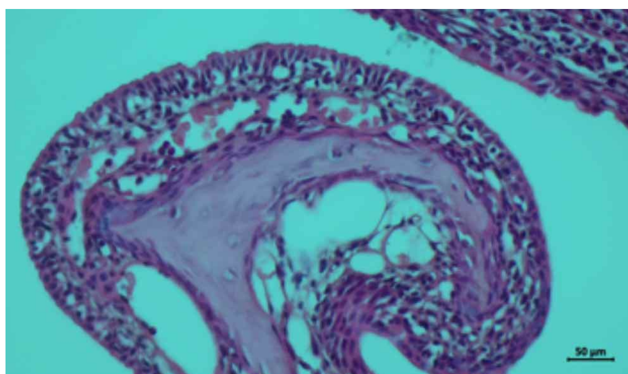

Fig. 3. Cadmium group. In the histopathological section of the cadmium applied group, interruptions in the basement membrane structure and degenerative changes in the stem cells and pycnosis in the nuclei, deformity of the sensory cells, and occasional spaces were formed between the support cells. it was observed that the integrity of the structure was lost, dilatation and free dispersed erythrocyte infiltration increased.

DISCUSSION

Intranasal administration of 400 mg of CdCl2 to mice has been shown to cause partial damage to the olfactory epithelium, reversible loss of olfactory discrimination, and specific cadmium deposition in the olfactory bulb (Bondier *et al*., 2008). In the olfactory epithelium, enzymes that metabolize toxic substances and thus can both detoxify and toxic are Sustentacular cells, especially cytochrome P450 (CYP). It expresses CYP proteins responsible for the oxidation of xenobiotics (Özevren *et al.*, 2018). If these cells are damaged or destroyed, the metabolic activity of CYP enzymes has also been observed to be inhibited. In our study, in the histopathological examination of the group exposed

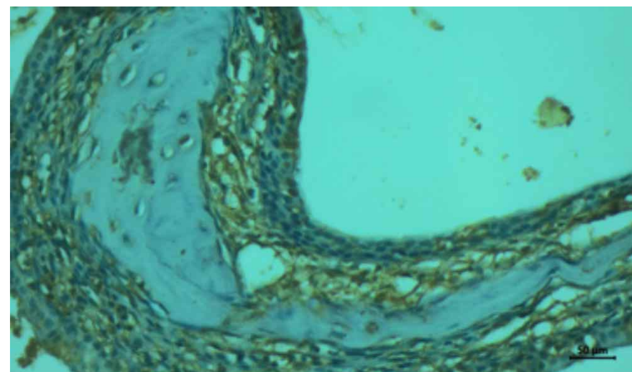

Fig. 4. Control group. Caspas-9 activity was negative in most of the olfactory epithelial cells and caspas-9 expression was negative in some supporting cells. Caspas-9 activity was observed to be mild in cells in the connective tissue area in the lamina propria.

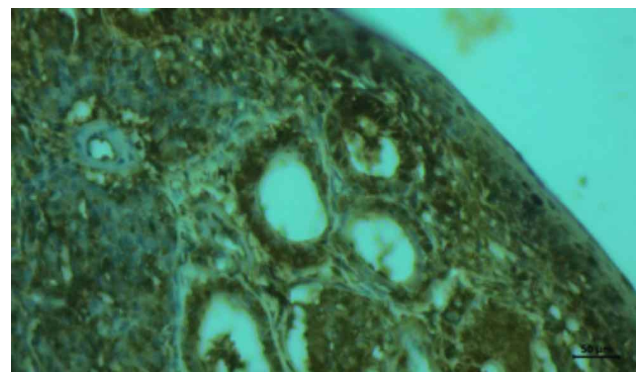

Fig. 5. Cadmium group. Caspas-9 reaction was positive in most of the olfactory epithelial cells in the cross-section of the cadmium applied group. Inflammatory cells in the lamina propria and caspas-9 in some endothelial cells in the cells of the olfactory glands (Bowman glands).

to cadmium application, degenerative changes were observed in the cells located in the basement membrane of the olfactory epithelium, in some of the sensory cells and support cells.Dilatation and congestion in the blood vessels in the lamina propria, and inflammatory cell infiltration around the vessel were observed (Fig. 3).

Shimada *et al*. (2005) stated that the olfactory mucosa of rats exposed to mercury vapor affected olfactory glands (Bowman's gland) cells. In our study, no changes were observed in secretion and cell degeneration in olfactory glands in the application of cadmium.

In apoptosis in cells, the death-causing signals combine in the mitochondria, causing the release of cytochrome c (Baloglu *et al.*, 2020). Cytosolic cytochrome c binds to APAF-1 and causes recruitment of procaspase-9 and formation of apoptosome (Wright *et al*., 2004). In a study, it was shown that the production of reactive oxygen and

nitrogen species increases after IR injury, which impairs ATP production in mitochondria. Thus, they stated that the mitochondrial membrane was damaged by the disruption of the ionic balance in the cell and in the organelles, triggering apoptotic cell death (Bektas *et al*., 2016). It has been reported that inhibition of apoptosis may lead to the development of autoimmune and inflammatory diseases, and that genetic and epigenetic factors stimulate inflammatory responses, including chronic rhinosinusitis (Lam *et al*., 2015). It was observed that APAF-1 expression, which is the precursor of proapoptotic signal, increased with the increase in inflammation due to cadmium toxicity, both in epithelial cells and in olfactory glands (Fig. 5). This indicated that the process started in cell apoptosis.

It has been thought that cadmium toxicity increases cell degeneration and inflammation in the olfactory mucosa and may significantly affect cell death and olfactory metabolism by inducing the pro-apoptotic process.

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RESUMEN: El cadmio es un metal altamente tóxico que afecta la mucosa respiratoria. El objetivo fue mostrar el efecto inflamatorio y degenerativo del cadmio sobre la mucosa olfativa. En este estudio, ratas Wistar de ocho semanas de edad con un peso promedio de 170-190 g se dividieron en dos grupos (control y experimental) con 20 animales en cada grupo . Las ratas del grupo experimental recibieron 2 mg/kg/día de cloruro de cadmio en polvo disuelto en agua por vía intraperitoneal todos los días durante dos semanas. En los animales se exirpó la cavidad nasal bajo anestesia. Se separó la concha nasal superior, se fijó con solución de zinc-Formalina y se descalcificó con EDTA (ácido etilendiaminotetraacético) al 5 %. Después del procedimiento histopatológico de rutina, Hematoxilina-Eosina (HE) e inmunohistoquímica, se utilizó el anticuerpo APAF-1 . El examen histopatológico reveló interrupciones en la estructura de la membrana basal debido al cadmio y cambios degenerativos en las células madre, degeneración en las células sensoriales y picnosis en los núcleos, dilatación de los vasos sanguíneos y aumento de la inflamación en el tejido conjuntivo. Se encontró que la expresión de APAF-1 aumenta en las células epiteliales y en las células de las glándulas olfatorias (glándulas de Bowman). Se ha pensado que la toxicidad del cadmio aumenta la degeneración celular y la inflamación en la mucosa olfativa y puede afectar significativamente la muerte celular y el metabolismo olfativo al inducir el proceso proapoptótico.

PALABRAS CLAVE: Cloruro de cadmio; Mucosas olfativas; APAF-1 (Factor Activador de Proteasa Apoptótica-1); Rata; Histopatología.

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