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The influence of lead on gingiva and periodontal tissue

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SUMMARY

Introduction Exposure to lead (Pb) can have harmful effects on the general state of the body, including oral health. The aim of this study was to examine the prevalence of changes in gingiva and periodontal tissue in adult rats intoxicated with lead acetate by histological analysis.

Material and methods The study was conducted on 42 Wistar rats. Intoxication of rats with lead acetate was carried out through drinking water ad libitum. The first group (A1) consisted of 16 rats that received lead-acetate in water at a concentration (1500 ppm) for 14 days, and the second group (A2) consisted of 16 rats that received lead-acetate in water at the same concentration for 30 days.

Results Gingivitis and periodontitis in the first group of rats (A1) were observed in 41.7% of cases, and in the second group (A2) in 84% of cases. A statistically significant difference was found between the investigated groups ($\chi^2 = 6.955$; $p < 0.018$).

Conclusion Since changes in the gingiva and periodontium have been observed in rats intoxicated with lead for a long period of time, it is necessary to propose a preventive program for patients exposed to lead, which includes dental procedures such as oral hygiene training and regular visits to the dentist.

Keywords: lead-acetate, gingivitis; periodontitis; rats

INTRODUCTION

Toxic elements are found in the body at different concentrations due to contamination from the environment (aluminum, mercury, cadmium, lead, bismuth, silver). Among them, lead (Pb) stands out as one of the most important, toxic and prevalent heavy metals in the world. The presence of lead in the environment increases with human activity. Excessive lead concentration can be toxic to human body because it affects the blood flow, nervous, gastrointestinal, cardiovascular and musculoskeletal system [1, 2]. Long-term exposure to lead can cause cheilitis, ulcers and epithelial desquamation of the tongue, palate and other parts of the oral mucosa, damage to the gums, periodontal ligaments and alveolar bone tissue [3]. More recent studies have indicated that lead can also cause oxidative stress in many tissues and organs, including salivary glands. A disorder in the salivary glands also leads to caries, gingivitis and periodontitis, which is later attributed to tooth loss [4, 5].

The results of one of the studies conducted on rats indicated that elevated lead concentration led to decreased salivary flow by 30-40%, which accelerates formation of caries due to insufficient washing of the tooth surface with saliva, and leads to inflammation of gingiva and periodontium [6]. Periodontitis is one of the most common dental diseases. It is a chronic disease of gingiva which, if not treated, leads to the spread of inflammation in the surrounding structures of the tooth, changing bone homeostasis, later destroying supporting and surrounding structures, causing tooth loss [7].

Lee et al. found an association between increased blood lead concentrations and oxidative stress in adults that releases reactive oxygen species (ROS) [8]. ROS can also cause protein and DNA damage, as well as lipid peroxidation, and its increased value can lead to damage to gingival, periodontal ligament and alveolar bone tissue. Although biological link between lead exposure and periodontal disease has not been sufficiently investigated, ROS has been identified as a parameter for lead intoxication, because it is formed in contact with it and causes oxidative stress [9,10]. The level of lead in the teeth is related to the level of lead in the blood. Therefore, teeth are considered a good biological indicator of exposure to environmental lead pollution [11].

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Children tend to absorb more lead in the body than adults because their metabolism is accelerated, and children's gastrointestinal organs are underdeveloped and absorb substances more easily. Some of the studies found that elevated lead concentration in the blood is associated with gingival diseases and plaque deposition in children [12].

Similarly, another study reported that gingivitis was the most common condition among school children living near a shipyard area known to be contaminated with lead. Almost 98% of them had gingivitis. Gingivitis may be a consequence of increased prevalence of *Aggregatibacter actinomycetemcomitans* (17%) in children with high blood lead concentration [7, 13].

According to the study by El-Said et al., which focuses on the risk of gingivitis in workers exposed to lead, the occurrence of gingivitis was found to be associated with lead sulfide, a product of the reaction between lead and hydrogen sulfide during food fermentation, which can cause gingival irritation and gingivitis [14].

Lead intoxication can be determined in the oral cavity by the presence of Burton's lines (purple-blue lead-sulphide deposits at the border between the gums and teeth), which occur when lead in the blood and saliva reacts with sulfur ions released by the action of oral microorganisms [15]. There may be more than one underlying mechanism for the development of Burton's line or Gubler's sign. Various foods and vegetables contain large amounts of sulfates. Oral bacteria modify these sulfates into sulfur compounds e.g. hydrogen sulfide (H2S) [16].

The aim of this study was to examine the prevalence of changes in the gingiva and periodontium in adult rats intoxicated with lead acetate by histological analysis.

MATERIAL AND METHODS

The study was conducted after the approval of the Ethical Committee of the University Clinical Center in Banja Luka (Bosnia and Herzegovina). The sample consisted of 42 Wistar rats. The animals were two months old, with a body weight of 150–200 g. They were kept in group cages made of Plexiglas, with 12 hours of light (07:00 - 19:00), at an air temperature of 22°C (± 2) and a humidity of 60 ± 10%, with free access to food and water during experiment. At the beginning of the experiment, the rats were separated into the corresponding test groups. They were given a 15-day adaptation period. After adaptation, they were divided into two experimental groups (A1 and A2). The first (A1) and second (A2) groups consisted of 16 rats each [17].

Intoxication of adult rats with lead-acetate in a concentration of 1500 ppm was carried out through drinking water ad libitum. Lead poisoning lasted 14 days in the A1 group, and 30 days in the A2 group. All animal procedures, care, experimental treatment, pain-free and stress-free sacrifice were performed in accordance with the Guidelines for the Care of Animals in Experimental Research.

Rats from the first group (A1) were sacrificed after 14 days, and from the second group (A2) after 30 days. For histological analysis, the bones of the upper jaws together with the teeth (after 48 hours of fixation in 10% neutral buffered formalin) were decalcified in nitric acid solution (no longer than 90 minutes). Decalcified samples were then washed under running water and processed in an automated tissue processor Leica TP 1020 (Leica Byosistems) according to a standard protocol: dehydration in increasing concentrations of ethyl alcohol (70%, 96%, 100%), washing in xylene, impregnation with liquid paraffin, after which selected tissue samples were molded into paraffin blocks. For histological analysis, after cooling, paraffin blocks were cut on a sliding microtome (Leica SM 2000R, Leica Byosistems) into sections 4–5 μm thick, and cross-sections of the supporting apparatus were collected on appropriate glass slides and dried at 60°C. In an automatic staining processor (Leica ST4040 Linear stainer, Leica Byosistems), tissue sections were deparaffinized, rehydrated and rinsed in distilled water. After that, they were stained with the standard hematoxylin-eosin (HE) method. Definitive preparations were analyzed with a light microscope (Leica DM 2500, Leica Byosistems) and photographed with a camera connected to the microscope [18].

RESULTS

In the first experimental group (A1), periodontitis and gingivitis were recorded in 41.7% of cases (Table 1). In the second experimental group (A2), periodontitis and gingivitis were recorded in 84% of cases (Table 1, Figure 2). A statistically significant difference was found between the examined groups ($\chi^2 = 6.955$; $p < 0.018$) (Table 1).

Table 1. Periodontitis and gingivitis in the studied groups
Tabela 1. Parodontitis i gingivitis u ispitivanim grupama

			Gingivitis and periodontitis Gingivitis i parodontitis		Total Ukupno
			No / Ne	Yes / Da	
Group Grupa	Pb 14 days Pb 14 dana (A1)	N	7	5	12
		%	58.3%	41.7%	100%
	Pb 30 days Pb 30 dana (A2)	N	4	21	25
		%	16%	84.0%	100%
Everything Sve		N	11	26	37
		%	29.7%	70.3%	100%

DISCUSSION

Lead is one of the most important and widespread environmental pollutants in terms of global contamination and health impact [7, 19]. Children tend to absorb more lead than adults due to their higher metabolic rate, as well as their physical tendency to inhale lead from polluted air. In fact, children's gastrointestinal organs are underdeveloped and absorb lead more easily, leading to many diseases in the body and teeth [20].

Many studies have examined the effects of lead on the oral health of adults. However, the relationship between lead and oral health in children is insufficiently investigated, as is its influence on the gingiva and periodontium [21, 22].

Considering the data that children absorb much more lead and that this issue is insufficiently investigated, especially by

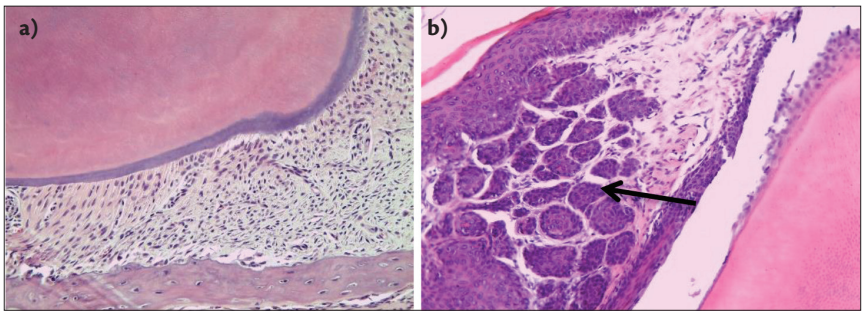

Figure 1. a) Section of a tooth – periodontal ligament, cementum and dentin are observed without morphological changes (H&E, 400×); b) Section of the teeth of rats that received lead for 30 days – in the periapical area chronic inflammation around the teeth (chronic periodontitis) is observed (H&E, 400×).
Slika 1. a) Presek zuba – uočavaju se periodontalni ligament, cement i dentin bez morfoloških promena (H&E, 400×); b) Presek zuba pacova koji su primali olovo 30 dana – u periapikalnom području se uočava hronično zapaljenje u okolini zuba (hronični parodontitis) (H&E, 400×).

histological analysis, our study was conducted on two-month-old rat pups that were intoxicated with lead in drinking water for 14 and 30 days, in order to examine the changes on the gingiva and periodontium of the same. The results of our study indicated a significantly higher incidence of gingivitis and periodontitis in rat pups that drank water with lead for a longer period of time- 30 days (Group A2) in 84% of cases compared to pups that received lead for only 14 days (Group A1) in 41.7% of cases. Our results support the theory that lead is a contributing factor in the development of periodontal disease in humans. Our results are consistent with the results of previous studies that dealt with similar issues in children. One of them was conducted by Youravong et al. among the children of Thailand who live in the industrial zone of the shipyard. The results indicated a significant positive correlation between high blood lead concentration and periodontal diseases, especially deep periodontal pockets [7]. Borany et al. aimed to investigate the relationship between blood lead levels (BLL) and oral health in South Korean children. The examination was based on blood analysis and dental examinations of children with determination of gingival index and plaque index. They found that high blood lead concentration was associated with oral health problems in South Korean children, including plaque deposition and gingival disease, which is consistent with the results of our study [23].

A large number of authors studied the relationship between the influence of lead and inflammation of the tooth's supporting tissue, because in addition to many clinical manifestations, lead-intoxicated patients (including children and young adults) have an increased risk of periodontitis. Periodontitis is a chronic disease of gingiva which, if not treated, leads to the spread of inflammation in the surrounding tooth structures, changing bone homeostasis and destroying the supporting and surrounding structures, causing tooth loss [12, 20, 24].

Periodontitis is primarily caused by an aggravated immune-inflammatory response of the host, which has many triggers, one of them being lead and cadmium, which in higher concentrations stimulate the production of reactive oxygen species (ROS), which can be a potential cause of periodontitis progression [25]. Browar et al. examined the correlation between exposure to cadmium and lead in relation to periodontal diseases using the immunohistochemical method in experimental rats. In their study, male rats received subcutaneous injections of lead and cadmium (0.6 mg/kg/day) for a period of 12 weeks. They came to the result that cadmium and lead have a significant negative effect on the periodontium of rat teeth, which is in agreement with our study, which was also performed using the immunohistochemical method on rats [26].

Determination of lead concentration in teeth is considered a good biological indicator of lead exposure in the environment and at the workplace. This was confirmed by a study by El-Said et al. which indicated that 97.2% of workers in a factory for the production of lead batteries had gingivitis, which was the most prevalent of all periodontal diseases [14]. Lead in blood, saliva, plaque, and food debris can react with oral hydrogen sulfide (produced by fermentation of food deposited in the mouth) to form lead sulfide. This can cause irritation, discoloration and bleeding gums [14, 27].

Exposure to lead mainly comes from informal recycling and manufacturing of batteries, electronic waste, emissions from factories that process ores rich in heavy metals, and excessive intake of foods and spices that contain high concentrations of lead. This was confirmed by one of the studies conducted by Ericson et al. where lead concentration in the blood of children in 34 countries was measured in order to determine the presence of lead in 1,300 million children. They found that 48.5% of children (632 million) had blood lead levels above 5 μg/dL [28].

Some of the studies indicated that the influence of lead affects reduced secretion of saliva in rats by about 30-40%. However, there have been no human studies. The effect of lead on the reduction of saliva can partially explain our study in which rats were intoxicated with lead. Lead reduced the production of saliva in the salivary glands and thus prevented its complete function of protecting and moisturizing gingiva, leading to gingivitis and periodontitis, and this was confirmed by our results [29].

By conducting education on protective measures at workplaces (smelters, battery factories and heavy industries, etc.) by avoiding areas contaminated with lead, as well as the intake of food and water from that area and adopting a healthy lifestyle, the intake of lead in the body and thus dental and mouth diseases will be reduced. Fluoridation of drinking water, salt and milk can help prevent the prevalence of oral diseases caused by lead exposure [30].

CONCLUSION

In the group of rats, that were intoxicated with lead for a longer period of time -30 days, compared to the group, which

was intoxicated with lead for a shorter period of 14 days, a significantly higher prevalence of changes in gingiva and periodontium (gingivitis and periodontitis) was observed. The results of our study indicate a connection between the influence of lead and periodontal diseases, which is why it is necessary to introduce a preventive program and measures for patients and especially for children. This includes educating children from a young age about the importance of maintaining oral hygiene and regular visits to the dentist, which can reduce the presence of dental plaque, soft and hard dental deposits, and enable detection and treatment of initial pathological changes in periodontium.

REFERENCES

1. Tinkov AA, Filippini T, Ajsuvakova OP, Skalnaya MG, Aaseth J, Bjørklund G, et al. Cadmium and atherosclerosis: a review of toxicological mechanisms and a meta-analysis of epidemiologic studies. Environ Res. 2018;162:240–60. [DOI: 10.1016/j.envres.2018.01.008] [PMID: 29358116]
2. Mehrotra N, Singh S. Periodontitis. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2022. [DOI: //www.ncbi.nlm.nih.gov/books/NBK541126/]
3. Kamberi B, Koçani F, Dragusha E. Teeth as Indicators of Environmental Pollution with Lead. J Environment Analytic Toxicol. 2012;2:1–5. [DOI: 10.4172/2161-0525.1000118]
4. Yetkin-Ay Z, Cadir B, Uskun E, Bozkurt FY, Delibas N, Gultepe FM, et al. The periodontal status of indirectly lead-exposed apprentices working in autorepair workshops. Toxicol Ind Health. 2007;23(10):599–606. [DOI: 10.1177/0748233708090906] [PMID: 18717518]
5. Chakraborty S, Tewari S, Sharma RK, Narula SC, Ghalaut PS, Ghalaut V. Impact of iron deficiency anemia on chronic periodontitis and superoxide dismutase activity: a cross-sectional study. J Periodontal Implant Sci. 2014;44(2):57–64. [DOI: 10.5051/jpis.2014.44.2.57] [PMID: 24778899]
6. Khalid M, Abdollahi M. Role of lead in dental diseases. J Environ Sci Health C Toxicol Carcinog. 2020;38(4):329–61. [DOI: 10.1080/26896583.2020.1834313] [PMID: 33382012]
7. Youravong N, Teanpaisan R. The periodontal health of lead-exposed children living in a shipyard industrial area. Toxicol Ind Health. 2015;31(5):459–66. [DOI: 10.1177/0748233712472529] [PMID: 23406954]
8. Watson GE, Davis BA, Raubertas RF, Pearson SK, Bowen WH. Influence of maternal lead ingestion on caries in rat pups. Nat Med. 1997;3(9):1024–5. [DOI: 10.1038/nm0997-1024] [PMID: 9288731]
9. Lee DH, Lim JS, Song K, Boo JDR Jr. Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress-related markers in the US population: results from the third National Health and nutrition examination survey. Environ Health Perspective. 2006;114(3):350–4. [DOI: 10.1289/ehp.8518] [PMID: 16507456]
10. Khalid M, Abdollahi M. Epigenetic modifications associated with pathophysiological effects of lead exposure. J Environ Sci Health Part C: Environ Carcinog Ecotoxicol Rev. 2019;37(4):235–87. [DOI: 10.1080/10590501.2019.1640581] [PMID: 31402779]
11. Tarakji B, Umair A, Prasad D, Alsakran Altamimi M. Diagnosis of oral pigmentations and malignant transformations. Singapore Dent J. 2014;35C(1):39–46. [DOI: 10.1016/j.sdj.2014.03.001] [PMID: 25496584]
12. Shishniashvili TE, Suladze NN, Margvelashvili VV. Primary teeth and hair as indicators of environmental pollution. J Clin Pediatr Dent. 2016;40(2):152–5. [DOI: 10.17796/1053-4628-40.2.152] [PMID: 26950818]
13. American Academy of Pediatrics Committee on Environmental Health. Lead exposure in children: prevention, detection, and management. Pediatrics. 2005;116(4):1036–46. [DOI: 10.1542/peds.2005-1947] [PMID: 16199720]
14. El-Said KF, El-Ghamry AM, Mahdy NH, El-Bestaw NA. Chronic occupational exposure to lead and its impact on oral health. J Egypt Public Health Assoc. 2008;83(5–6):451–66. [PMID: 19493512]
15. Verma S, Santhosh AP, Shukla S, Gupta P, Mandavdhare HS, Dutta U, et al. Gastrointestinal: An oral clue to an unusual cause of abdominal pain: Burton's line. J Gastroenterol Hepatol. 2019;34(4):629. [DOI: 10.1111/jgh.14519] [PMID: 30450668]
16. Cleymaet R, Collys K, Retief DH, Michotte Y, Slop D, Taghon E, et al. Relationship between lead in surface tooth enamel, blood, and saliva from children residing in the vicinity of a non-ferrous metal plant in Belgium. Br J Ind Med. 1991;48(10):702–9. [DOI: 10.1136/oem.48.10.702] [PMID: 1931730]
17. Kuzmanović Radman I, Arbutina A, Josipović R, Marin S, Umićević-Davidović M, Arbutina R, et al. Examination of the presence of periodontitis and gingivitis in rats with induced diabetes mellitus. Dental Gazette of Serbia. 2023;70(3):131–7. [DOI: 10.2298/SGS2303131K]
18. Kuzmanović Radman I, Đeri A, Gajanin R, Arbutina A, Josipović R, Živković S. Expression of a fibronectin in the dental pulp of lead intoxicated rats with experimentally induced diabetes mellitus. Srp Arh Celok Lek. 2021;149(3–4):136–41. [DOI: 10.2298/SARH191022102K]
19. Metryka E, Chibowska K, Gutowska I, Falkowska A, Kupnicka P, Barczak K, et al. Lead (Pb) exposure enhances the expression of factors associated with inflammation. Int J Mol Sci. 2018;19(6):1813. [DOI: 10.3390/ijms19061813] [PMID: 29925772]
20. Gemmel A, Tavares M, Alperin S, Soncini J, Daniel D, Dunn J, et al. Blood lead level and dental caries in school-age children. Environ Health Perspect. 2002;110(10):A625–30. [DOI: 10.1289/ehp.021100625] [PMID: 12361944]
21. Vandal V, Noorani H, Shivaprakash P, Walikar B. Salivary lead concentration in dental caries among normal and children with cerebral palsy. J Indian Soc Pedodontics Preventive Dent. 2018;36(4):381–5. [DOI: 10.4103/JISPPD.JISPPD_200_16] [PMID: 30324929]
22. Cenić-Milošević D, Mileusnić I, Kolak V, Pejanović D, Ristić T, Jakovljević A, et al. Environmental lead pollution and its possible influence on tooth loss and hard dental tissue lesions. Vojnosanit Pregl. 2013;70(8):751–6. [DOI: 10.2298/vsp1308751c] [PMID: 24069824]
23. Borany T, Youn-Hee C, Eun-Kyong K, Yun-Sook J, Mina H, Keun-Bae S, et al. Lead exposure may affect gingival health in children. BMC Oral Health. 2018;18(1):79. [DOI: 10.1186/s12903-018-0547-x] [PMID: 29728081]
24. Dommisch H, Kuzmanova D, Jönsson D, Grant M, Chapple I. Effect of micronutrient malnutrition on periodontal disease and periodontal therapy. Periodontol 2000. 2018;78(1):129–53. [DOI: 10.1111/prd.12233] [PMID: 30198127]
25. Alomary A, Al-Momani IF, Obeidat SM, Massadeh AM. Levels of lead, cadmium, copper, iron, and zinc in deciduous teeth of children living in Irbid, Jordan by ICP-OES: some factors affecting their concentrations. Environ Monit Assess. 2013;185(4):3283–95. [DOI: 10.1007/s10661-012-2790-y] [PMID: 22851195]
26. Browar AW, Koufos EB, Wei Y, Leavitt LL, Prozialeck WC, Edwards JR. Cadmium exposure disrupts periodontal bone in experimental animals: implications for periodontal disease in humans. Toxics. 2018;6(2):32. [DOI: 10.3390/ toxics6020032] [PMID: 29899258]
27. Craan A, Nadon G, P'an AYS. Lead flux through kidney and salivary glands of rats. Am J Phys. 1984;247(5 Pt 2):F773–83. [DOI: 10.1152/ajprenal.1984.247.5.F773] [PMID: 6496745]
28. Ericson B, Hu H, Nash E, Ferraro G, Sinitsky J, Taylor MP. Blood lead levels in low-income and middle-income countries: a systematic review. Lancet Planet Health. 2021;5(3):e145–53. [DOI: 10.1016/S2542-5196(20)30278-3] [PMID: 33713615]
29. Levy SM. An update on fluorides and fluorosis. J Can Dent Assoc. 2003;69(5):286–91. [PMID: 12734021]
30. Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Community Dent Oral Epidemiol. 2004;32(5):319–21. [DOI: 10.1111/j.1600-0528.2004.00175.x] [PMID: 15341615]

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Uticaj olova na gingivu i potporni aparat zuba

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KRATAK SADRŽAJ

Uvod Izloženost olovu (Pb) može imati štetne efekte na opšte stanje organizma, uključujući i oralno zdravlje. Cilj ove studije je bio da se histološkom analizom ispita zastupljenost promena na gingivi i parodoncijumu kod adultnih pacova koji su intoksikovani olovo-acetatom.

Materijal i metode Studija je sprovedena na 42 pacova soja vistar. Intoksikacija pacova olovo-acetatom je vršena putem vode za piće *ad libitum*. Prvu grupu (A1) činilo je 16 pacova koji su 14 dana dobijali olovo-acetat u vodi u koncentraciji od 1500 ppm, a drugu grupu (A2) činilo je 16 pacova koji su 30 dana dobijali olovo-acetat u vodi u istoj koncentraciji.

Rezultati Gingivitis i parodontitis u prvoj grupi pacova (A1) uočeni su u 41,7% slučajeva, a u drugoj u grupi (A2) u 84% slučajeva. Između ispitivanih grupa utvrđena je statistički značajna razlika ($\chi^2 = 6{,}955$; $p < 0{,}018$).

Zaključak S obzirom na to da je kod pacova koji su intoksikovani olovom duži vremenski period uočena promena na gingivi i parodoncijumu, neophodno je predložiti preventivni program za pacijete izložene olovu, koji obuhvata stomatološke procedure kao što su obuke održavanja oralne higijene i redovne posete stomatologu.

Ključne reči: olovo-acetat; gingivitis; periodontitis; pacovi

UVOD

Toksični elementi se u organizmu nalaze u različitim koncentracijama usled kontaminacije iz okoline (aluminijum, živa, kadmijum, olovo, bizmut, srebro). Među njima se posebno izdvaja olovo (Pb) kao jedan od najvažnijih, najtoksičnijih i najzastupljenijih teških metala u svetu. Prisustvo olova u okolini se povećava sa ljudskom aktivnošću. Prekomerna koncentracija olova može biti toksična za čovekov organizam jer utiče na krvotok, nervni, gastrointestinalni, kardiovaskularni i mišićno-skeletni sistem [1, 2]. Dugotrajno izlaganje olovu može izazvati nastanak heilitisa, ulkusa i epitelne deskvamacije jezika, nepca i drugih delova oralne sluznice, oštećenja gngive, parodontalnih ligamenta i alveolarnog koštanog tkiva [3]. Novije studije su ukazale da olovo takođe može izazvati oksidativni stres u mnogim tkivima i organima, uključujući i pljuvačne žlezde. Poremećaj u pljuvačnim žlezdama dovodi i do karijesa, gingivitisa i parodontitisa, kojem se kasnije pripisuje gubitak zuba [4, 5].

Rezultati jedne od studija sprovedene na pacovima ukazali su na to da je povišena koncentracija olova dovela do smanjenog protoka pljuvačke za 30-40%, što ubrzava nastanak karijesa usled nedovoljnog ispiranja površine zuba pljuvačkom, te dovodi do upale gingive i parodoncijuma [6]. Parodontitis je jedno od najčešćih oboljenja zuba. To je hronična bolest gingive koja ako se ne leči, dovodi do širenja upale u okolne strukture zuba, menjajući homeostazu kostiju, kasnije razara potporne i okolne strukture uzrokujući gubitak zuba [7].

Lee i autori su pronašli povezanost između povećane koncentracije olova u krvi i oksidativnog stresa kod odraslih, koji oslobađa reaktivnu vrstu kiseonika (ROS) [8]. ROS takođe može uzrokovati oštećenje proteina i DNK, kao i peroksidaciju lipida, a njegova povećana vrednost može dovesti do oštećenja gingivalnog, parodontalnog ligamenta i alveolarnog koštanog tkiva. Iako biološka veza između izloženosti olovu i parodontoloških oboljenja nije dovoljno ispitana, ipak je ROS označen kao parametar za intoksikaciju olovom, jer nastaje u kontaktu sa njim i uzrokuje oksidativni stres [9, 10].

Nivo olova u zubima je povezan sa nivoom olova u krvi. Zbog toga se zubi smatraju dobrim biološkim indikatorom izloženosti zagađenju okoline olovom [11].

Deca imaju tendenciju da apsorbuju više olova u organizmu nego odrasli jer je kod njih ubrzan metabolizam, a dečji gastrointestinalni organi su nedovoljno razvijeni i lakše apsorbuju supstance. Neke od studija su utvrdile da je povišena koncentracija olova u krvi povezana sa bolestima gingive i taloženjem plaka kod dece [12].

Slično tome, druga studija je objavila da je upala gingive najčešće stanje među školskom decom koja žive u blizini područja brodogradilišta, za koje se zna da je kontaminirano olovom. Skoro 98% njih je imalo zapaljenje gingive. Gingivitis može biti posledica povećane prevalencije bakterije *Aggregatibacter actinomycetemcomitans* (17%) kod dece sa visokom koncentracijom olova u krvi [7, 13].

Prema studiji koju su sproveli El-Said i saradnici, koja se fokusira na rizik od gingivitisa kod radnika izloženih olovu, uvrđeno je da je pojava gingivitisa povezana sa olovnim sulfidom, proizvodom reakcije između olova i sumporovodika tokom fermentacije hrane, što može izazvati iritaciju gingive i gingivitis [14].

Intoksikacija olovom se može utvrditi u usnoj šupljini prisustvom Burtonovih linija (ljubičasto-plavih naslaga olovo-sulfida na granici između desni i zuba), koje nastaju kada olovo u krvi i pljuvački reaguje sa jonima sumpora koji se oslobađaju delovanjem oralnih mikroorganizama [15]. Može postojati više od jednog osnovnog mehanizma za razvoj Burtonove linije ili Gublerovog znaka. Razna hrana i povrće sadrže velike količine sulfata. Oralne bakterije modifikuju ove sulfate u jedinjenja sumpora, npr. sumporovodik (H2S) [16].

Cilj ove studije je bio da se histološkom analizom ispita zastupljenost promena na gingivi i parodoncijumu kod adultnih pacova koji su intoksikovani olovo-acetatom.

MATERIJAL I METODE

Studija je sprovedena posle odobrenja Etičkog odbora Univerzitetskog kliničkog centra u Banjoj Luci (Bosna i Hercegovina). Uzorak se sastojao od 42 pacova soja vistar. Životinje su bile stare dva meseca, telesne težine 150–200 g. Čuvane su u grupnim kavezima od pleksiglasa, na 12 sati svetlosti (07.00 – 19.00 časova), na temperaturi vazduha od 22°C (± 2) i vlažnosti od 60% ± 10%, pri čemu su imali slobodan pristup hrani i vodi tokom eksperimenta. Na početku eksperimenta pacovi su razdvojeni u odgovarajuće testne grupe. Dobili su 15-dnevni period adaptacije. Posle adaptacije podeljeni su u dve eksperimentalne grupe (A1 i A2). Prvu (A1) i drugu (A2) grupu činilo je po 16 pacova [17].

Intoksikacija odraslih pacova olovo-acetatom u koncentraciji od 1500 ppm izvedena je putem vode za piće *ad libitum*. Trovanje olovom je trajalo 14 dana u grupi A1, a 30 dana u grupi A2. Sve procedure na životinjama, negovanje, eksperimentalni tretman, žrtvovanje bez bola i stresa izvedeni su u skladu sa Smernicama za brigu o životinjama u eksperimentalnim istraživanjima.

Pacovi iz prve grupe (A1) žrtvovani su posle 14 dana, a iz druge grupe (A2) posle 30 dana. Za histološku analizu su kosti gornjih vilica zajedno sa zubima (posle 48 sati fiksacije u 10% neutralnom puferovanom formalinu) dekalcifikovane u rastvoru azotne kiseline (ne duže od 90 minuta). Dekalcifikovani uzorci su zatim isprani tekućom vodom i obrađeni u automatizovanom tkivnom procesoru Leica TP 1020 (Leica Byosistems) po standardnom protokolu: dehidracija u rastućim koncentracijama etil-alkohola (70%, 96%, 100%), ispiranje u ksilolu, impregnacija tečnim parafinom, nakon čega su odabrani uzorci tkiva ukalupljeni u parafinske blokove. Za histološku analizu parafinski blokovi su nakon hlađenja sečeni na kliznom mikrotomu (Leica SM 2000R, Leica Byosistems) na preseke debljine 4–5 μm, a poprečni preseci potpornog aparata sakupljani su na odgovarajuća predmetna stakalca i sušeni na 60°C. U procesoru za automatsko bojenje (Leica ST4040 Linear stainer, Leica Byosistems) tkivni preseci su deparafinisani, rehidrirani i ispirani u destilovanoj vodi. Nakon toga su obojeni standardnom metodom hematoksilin-eozina (HE). Definitivni preparati su analizirani svetlosnim mikroskopom (Leica DM 2500, Leica Byosistems) i fotografisani kamerom povezanom sa mikroskopom [18].

REZULTATI

U prvoj eksperimentalnoj grupi (A1) parodontitis i gingivitis su zabeleženi u 41,7% slučajeva (Tabela 1). U drugoj eksperimentalnoj grupi (A2) parodontitis i gingivitis su zabeleženi u 84% slučajeva (Tabela 1, Slika 2). Između ispitivanih grupa utvrđena je statistički značajna razlika ($\chi^2 = 6{,}955$; $p < 0{,}018$) (Tabela 1).

DISKUSIJA

Olovo je jedan od najvažnijih i najrasprostranjenijih zagađivača u životnoj sredini u smislu globalne kontaminacije i uticaja na zdravlje [7, 19].

Deca imaju tendenciju da apsorbuju više olova u organizam nego odrasli zbog veće stope metabolizma, kao i fizičke sklonosti da udišu olovo iz zagađenog vazduha. U stvari, dečji gastrointestinalni organi su nedovoljno razvijeni i lakše apsorbuju olovo, dovodeći do mnogih oboljenja u organizmu i zubima [20].

Mnoge studije su se bavile ispitivanjem uticaja olova na oralno zdravlje odraslih osoba. Međutim, odnos olova i oralnog zdravlja kod dece je nedovoljno ispitan, kao i njegov uticaj na gingivu i parodoncijum [21, 22].

S obzirom na podatke da deca mnogo više apsorbuju olovo i da je ova problematika nedovoljno istražena, posebno histološkom analizom, naša studija je sprovedena na mladuncima pacova starim dva meseca koji su intoksikovani olovom u vodi za piće 14 i 30 dana, u cilju da se ispitaju promene na njihovoj gingivi i parodoncijumu. Rezultati naše studije su ukazali na značajno veću zastupljenost gingivitisa i parodontitsa kod mladunaca pacova koji su pili vodu sa olovom duži vremenski period (30 dana) (Grupa A2) – 84% slučajeva, u odnosu na mladunce koji su olovo dobijali samo 14 dana (Grupa A1) – 41,7% slučaja. Ovi naši rezultati podržavaju mogućnost da je olovo faktor koji doprinosi razvoju parodontalnih bolesti kod ljudi. Naši rezultati su u skladu s rezultatima prethodnih studija koje su se bavile sličnom problematikom kod dece. Jednu od njih je sproveo Youravong sa saradnicima među decom Tajlanda koja žive u industrijskoj zoni brodogradilišta. Rezultati su ukazali na značajnu pozitivnu korelaciju između visoke koncentracije olova u krvi i parodontoloških oboljenja, posebno dubokih parodontalnih džepova [7]. Borany je sa saradnicima imao za cilj da istraži vezu između nivoa olova u krvi (BLL) i oralnog zdravlja kod dece Južne Koreje. Ispitivanje je bilo bazirano na analizi krvi i stomatološkim pregledima dece uz određivanje gingivalnog indeksa i plak indeksa. Utvrdili su da se visoka koncentracija olova u krvi dovodi u vezu s problemima oralnog zdravlja kod dece Južne Koreje, uključujući taloženje plaka i bolesti gingive, što je usaglašeno sa rezultatima naše studije [23].

Veliki broj autora se bavio se proučavanjem veze između uticaja olova i inflamacije potpornog tkiva zuba, jer je pored mnogih kliničkih manifestacija kod pacijenata intoksikovanih olovom (uključujući decu i mlade odrasle osobe) povećan rizik od parodontitisa. Parodontitis je hronična bolest gingive, koja ako se ne leči dovodi do širenja upale u okolne strukture zuba, menjajući homeostazu kostiju, kasnije razarajući potporne i okolne strukture uzrokujući gubitak zuba [12, 20, 24].

Parodontitis je prvenstveno uzrokovan pogoršanim imunološko-upalnim odgovorom domaćina, koji ima mnogo pokretača, a jedan od njih su olovo i kadmijum, koji u većoj koncentraciji podstiču proizvodnju reaktivnih vrsta kiseonika (ROS), što može biti mogući uzrok progresije parodontitisa [25]. Browar je sa saradnicima ispitivao korelaciju između izloženosti kadmijumu i olovu u odnosu na parodontološka oboljenja i to imunohistohemijskom metodom kod eksperimentalnih pacova. U njihovoj studiji su mužjaci pacova dobivali supkutano injekcije olova i kadmijuma (0,6 mg/kg/dan) u periodu od 12 sedmica. Došli su do rezultata da kadmijum i olovo imaju značajan negativni uticaj na parodoncijum zuba pacova, što je usaglašeno sa našom studijom, koja je takođe rađena imunohistohemijskom metodom na pacovima [26].

Određivanje koncentracije olova u zubima smatra se dobrim biološkim pokazateljima izloženosti olovu u životnoj sredini i na radnom mestu. To je potvrdila studija koju su sproveli

El-Said i saradnici. Ona je ukazala da 97,2% radnika u fabrici za proizvodnju olovnih baterija ima gingivitis, koji je bio najzastupljeniji od svih parodontoloških oboljenja [14]. Olovo u krvi, pljuvački, plaku i ostacima hrane može stupiti u reakciju sa oralnim vodonik-sulfidom (koji nastaje fermentacijom hrane koja se taloži u ustima) da bi se formirao olovni sulfid. To može izazvati iritaciju, diskoloraciju i krvarenje desni [14, 27].

Izloženost olovu uglavnom dolazi od neformalnog recikliranja i proizvodnje baterija, elektronskog otpada, emisijom iz fabrika koje obrađuju rude bogate teškim metalima i prekomernim unosom hrane i začina koji sadrže velike koncentracije olova. To je i potvrdila jedna od studija u kojoj je Ericson sa saradnicima određivao vrednosti koncentracije olova u krvi dece u 34 zemlje kako bi se utvrdila zastupljenost olova kod 1300 miliona dece. Došli su do rezultata da je 48,5% dece (632 miliona) imalo nivo olova u krvi iznad 5 μg/dL [28].

Neke od studija su ukazale da uticaj olova utiče na smanjeno lučenje pljuvačke kod pacova čak 30-40%. Međutim, nije bilo studije sprovedene na ljudima. Uticaj olova na smanjenje pljuvačke može delimično objasniti i našu studiju u kojoj su pacovi intoksikovani olovom. Olovo je u pljuvačnim žlezdama smanjilo proizvodnju pljuvačke i tako onemogućilo njenu potpunu funkciju zaštite i vlaženja gingive, dovodeći do gingivitisa i parodontitisa, a to su potvrdili i naši rezultati [29].

Sprovođenjem edukacija o merama zaštite na radnim mestima (topionice, fabrike baterija i teške industrije itd.), izbegavanjem područja kontaminiranih olovom, kao i unosa hrane i vode sa tog područja, te usvajanjem zdravog načina života, smanjiće se unos olova u organizam i samim tim i oboljenja zuba i usta. Fluorizacija vode za piće, soli i mleka može pomoći u sprečavanju prevalencije bolesti usta uzrokovanih izloženošću olovu [30].

ZAKLJUČAK

Kod grupe pacova koja je intoksikovana olovom duži vremenski period – 30 dana, u odnosu na grupu koja je intoksikovana olovom u kraćem vremenskom periodu – 14 dana, uočena je znatno veća zastupljenost promena na gingivi i parodoncijumu (gingivitis i parodontitis) potpornog aparata zuba pacova. Rezultati ove studije ukazuju na povezanost uticaja olova i parodontalnih oboljenja, zbog čega je neophodno uvesti preventivni program i mere za pacijente, a posebno za decu. To obuhvata edukaciju dece od malih nogu o značaju održavanja oralne higijene i redovnih poseta stomatologu, koja može smanjiti zastupljenost zubnog plaka, mekih i tvrdih zubnih naslaga i omogućiti uočavanje početnih patoloških promena parodoncijuma i njihovo lečenje.