Research Results in Pharmacology

Research Results in Pharmacology 9(4): 1–12
UDC: 615:616.311.2:611.018
DOI 10.18413/rrpharmacology.9.10040

Research Article

The effect of zinc complex of N-isopropenylimidazole on the morphological characteristics of gum tissues in experimental endodontic-periodontal lesions in rats

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Academic editor: Mikhail Korokin ♦ **Received** 19 April 2023 ♦ **Accepted** 19 August 2023 ♦ **Published** 04 October 2023

Citation: Galenko-Yaroshevsky PA, Slavinskiy AA, Todorov SS, Popkov VL, Shelemekh OV, Lebedeva SA, Zadorozhniy AV, Zelenskaya AV, Alukhanyan LO, Nektarevskaya IB, Bunyatyan ND, Verevkin AA (2023) The effect of zinc complex of N-isopropenylimidazole on the morphological characteristics of gum tissues in experimental endodontic-periodontal lesions in rats. Research Results in Pharmacology 9(4): 1–12. https://doi.org/10.18413/rrpharmacology.9.10040

Abstract

Introduction: Combined inflammatory and destructive processes affecting the dental pulp and tissues of the periodontal complex are among the most problem diseases of the dental system. Current therapy with use of available pharmacological agents does not always allow achieving the expected positive result. In addition, often the lack of information about morphological processes in the tissues of the dental system, in particular the gums, with endodontic-periodontal lesion (EPL) limits the ability of dentists to carry out targeted pharmacotherapy with both traditional and, in particular, new medications. **The aim of the study** was to evaluate the morphological characteristics of gum tissues in a therapeutic context of N-isopropenylimidazole zinc complex derivative in experimental endodontic-periodontal lesion in rats.

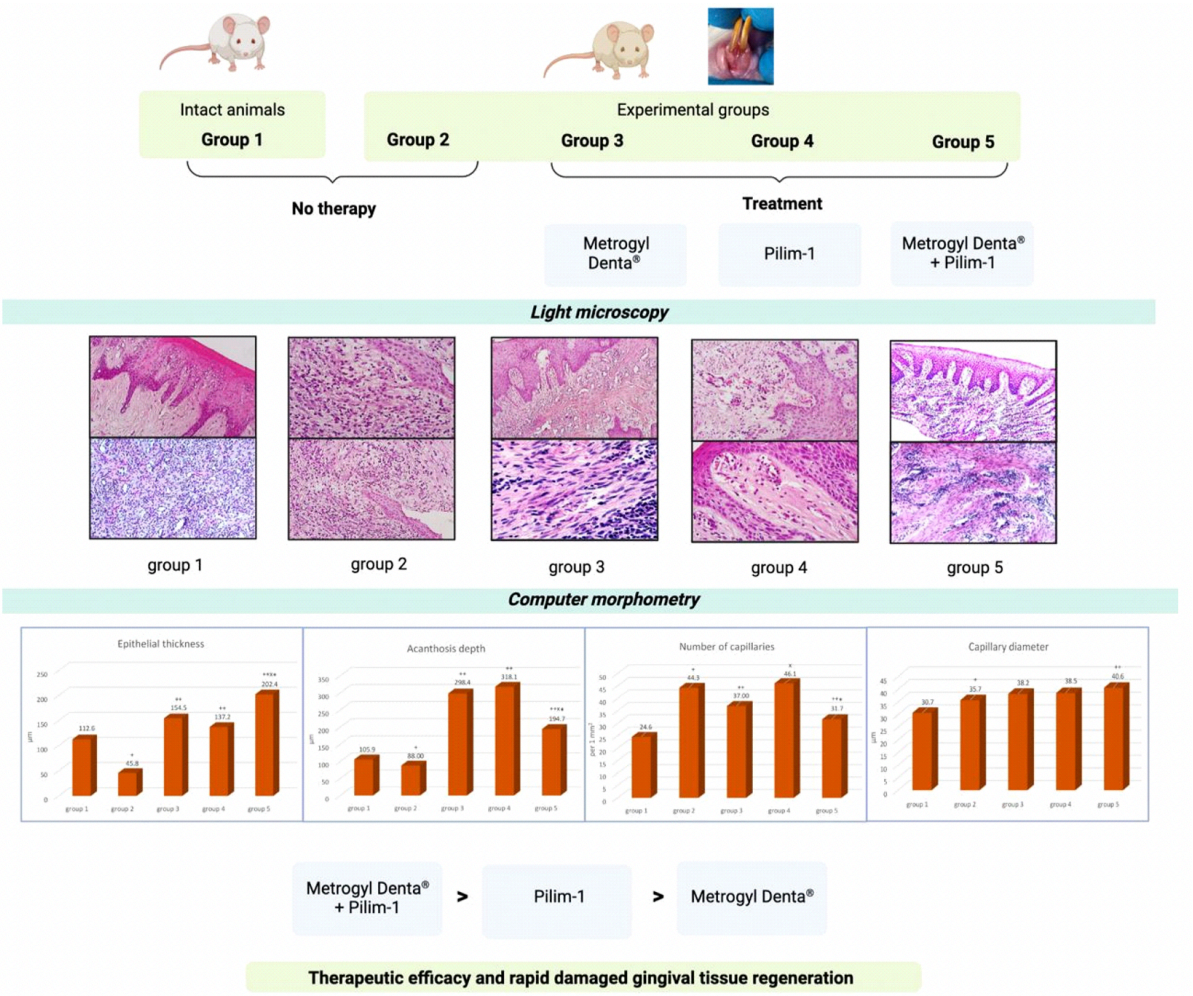
Materials and Methods: A simulation of EPL in rats was performed in two ways: simultaneous induction of acute periodontitis and parodontitis by pulp extraction and natural infection of the pulp cavity, as well as by ligation of the necks of lower incisors. The research protocols included 5 groups of animals: 1st – intact group (control-1); 2nd – animals with simulated EPL (control-2); 3rd – animals with simulated EPL and treated with Metrogyl Denta® gel (M-D); 4th – animals with simulated EPL and treated with N–isopropenylimidazole zinc complex derivative gel under the laboratory code Pilim-1; and 5th – animals with simulated EPL and treated with the combination of M-D + Pilim-1. The gum of the lower incisors was taken for morphological studies. Slides were stained with hematoxylin and eosin. Computer morphometry was performed using the ImageJ software.

Results and Discussion: The substances M-D, Pilim-1 and, especially, the combination of M-D+Pilim-1 (against the background of chlorhexidine bigluconate used as oral rinse) for 14 days in rats with simulated EPL cause a significant improvement of the morphological structure of the gum with minimal residual dystrophy and sclerosis. The combination M-D + Pilim-1 led to a 1.3-time increase in epithelial thickness, and a 1.5-time decrease in acanthosis depth in comparison with M-D, while the number of capillaries and

their diameter had no significant differences. Compared with Pilim-1, the epithelial thickness increased 1.5 times, and the acanthosis depth and the number of capillaries decreased 1.6 and 1.4 times, respectively, whereas the diameter of the capillaries did not change significantly. The pronounced protective effect of the combination M-D + Pilim-1 on the morphological structure of the gingival mucosa of rats with simulated EPL may be associated with antimicrobial, anti-inflammatory, regenerating, angioprotective and antioxidant properties of both M-D and Pilim-1 separately, and, possibly to a greater extent, of the combination M-D + Pilim-1.

Conclusion: The substances M-D, Pilim-1 and, especially, the combination M-D + Pilim-1 (against the background of chlorhexidine bigluconate used as oral rinse) for 14 days in rats with simulated EPL have a protective effect on the epithelial structure and the connective tissue of the proper mucous plate, manifested in active normalization of pathological changes and significant restoration of their organotypic structure.

Gaphical abstract



Keywords

computer morphometry, N-isopropenylimidazole zinc complex derivative, Metrogyl Denta®, pathomorphological changes in gum tissues, experimental endodontic-periodontal lesions in rats

Introduction

Combined inflammatory and destructive processes affecting the dental pulp and tissues of the periodontal complex, called endodontic-periodontal lesions (EPL), are among the most problem diseases of the dental system. This is due to the common origin and anatomical, structural and functional relationship of these structures, which create certain prerequisites for the latent development and rapid spread of this pathology in periodontium with severe lesions of such structures as

pulp, periodont, bone tissue of alveolar processes, the mucous membrane of the gum, etc. (Grudyanov et al. 2013; Miklyaev and Leonova 2019; Morozov and Iordanishvili 2019; Nemcovsky et al. 2019; Denisova and Dedova 2021).

Regardless of the primary lesion of the endodontic-periodontal tissue complex (EPTC), current therapy does not always allow achieving the expected positive result. This is due to several reasons: low awareness of dentists about this pathology, the lack of a clear algorithm for the management of such patients, and often insufficient effectiveness of the pharmacological agents for etiotropic and pathogenetic therapy. In addition, the lack of a fundamental basis in the morphological studies and objective data on the processes occurring in these tissues limits the ability of practicing dentists to effectively influence the lesions in the EPTC and effectively prevent the development of EPL (Morozov and Iordanishvili 2019; Moiseev et al. 2021; Çirakoğlu and Karayürek 2021; Saroch 2021).

In the conditions of rapidly developing dentistry, the use of medicines as an effective pharmacotherapy for patients with EPL is an objective necessity (Orekhova et al. 2021; Weber et al. 2021; Ardila and Bedoya-García 2022). However, current medicines do not always have the expected effectiveness. Some drugs are inactive to pathogenic microflora, others have a limited therapeutic index and duration of pharmacodynamic action and therapeutic effect, still others exhibit various side effects. In this regard, the search for new medicines that will meet all the requirements of dentists is an urgent task. From this point of view, in our opinion, organometallic compounds of zinc are very promising.

Zinc is the only metal represented in more than 300 enzyme systems, an indispensable participant in many physiological processes (Lebedeva et al. 2023). This trace element is an essential component of the antioxidant system, in particular, as part of superoxide dismutase, it prevents the lipid peroxidation process and protects cell membranes from damage, including in inflammatory processes (Prasad 2020). It has been shown that zinc is the primary inducer of the synthesis of cell protective proteins metallothioneins and can participate in the differentiation of monocytes into pro-inflammatory, immunoregulatory and wound-healing macrophages involved in the immunosuppression and subsequent tissue remodeling (Dierichs et al. 2018; Prasad 2020).

Zinc has moderate antibacterial and anti-inflammatory properties due to stimulating the synthesis of antibodies, which allows using zinc-containing drugs for both prevention and treatment of inflammatory processes, as well as using them instead of a number of antimicrobial drugs, including antibiotics (Malinina and Mazalova 2020; Salesa et al. 2021; Elgezawi et al. 2022; Guerra et al. 2022).

Our attention was drawn to the substance N-isopropenylimidazole zinc complex derivative under the laboratory code Pilim-1 with a pronounced antihypoxant effect (Shakhmardanova et al. 2016; Shakhmardanova and Galenko-Yaroshevsky 2017). Previously, the wound-healing effect of Pilim-1 was shown in a linear uninfected wound model, which is probably due to the antihypoxic effect, improvement of microcirculation, normalization of free radical oxidation and cell proliferation (Lebedeva et al. 2021, 2022).

Based on the above, it seemed rational to explore the effect of Pilim-1 on the morphological state of gum tissues in the experimental ELP in rats.

The aim of the study: to evaluate the morphological characteristics of gum tissues in a therapeutic context of N-isopropenylimidazole zinc complex derivative in experimental endodontic-periodontal lesion in rats.

Materials and Methods

Experimental animals

The studies were performed in 35 male Wistar rats weighing 210-320 g, which were kept in the standard vivarium conditions, got a standard food ration according to the rules of good laboratory practice (GOST 33216-2014). The research was conducted in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ed. Strasbourg, 2006), the Federal Law N 498-FZ dated December 27, 2018 (as amended on July 14, 2022) "On Responsible Treatment of Animals and on Amendments to Certain Legislative Acts of the Russian Federation". Good laboratory practice in preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), the provisions of the World Medical Association Declaration of Helsinki (Report of the AVMA Panel on Euthanasia JAVMA, 2001), Guidelines for Maintenance and Care of Laboratory Animals (interstate standard GOST 33216-2014 dated 07/01/2016), and Guidelines for Preclinical Trials of Medicines (Mironov 2012). The experiments were approved by the Ethics Committee of Rostov State Medical University of the Ministry of Health of the Russian Federation (Minutes No. 16/19 of 17/09/2019).

Pharmaceutical substances

Investigated substances were the following: chemical substance N-isopropenylimidazole zinc diacetate under the laboratory code Pilim-1 (Irkutsk Institute of Chemistry of the Siberian Branch of the Russian Academy of Sciences), chlorhexidine bigluconate (JSC Ivanovo Pharmaceutical Factory, Russia) and Metrogyl Denta® (Unique Pharmaceutical Laboratories (a division of J. B. Chemicals & Pharmaceuticals Ltd.), India).

The rats were anesthetized with Telazol (Zoetis Manufacturing & Research Spain, S.L., Spain) and Meditin (Apicenna LLC, Russia) with atropine sulfate premedication (Dalkhimpharm OJSC, Russia).

Experimental design

The rats were randomized into 5 groups of 7 animals in each: group 1 - intact (control-1); group 2 – animals with simulated EPL (control-2); group 3 – animals with simulated EPL, which root canals and periodontal pockets were irrigated with a solution of chlorhexidine bigluconate (CB) and administered with Metrogyl Denta® gel (M-D); group 4 – animals with simulated EPL, which root canals and periodontal pockets were previously irrigated with the solution of CB, and then Pilim-1 gel based on sodium carboxymethyl cellulose (Na-CMC) was injected into these structures (canals and pockets); group 5 – animals with simulated EPL; similarly to the 3rd and 4th groups, the solution of CB and a combination of M-D with Pilim-1 were used (Fig. 1).

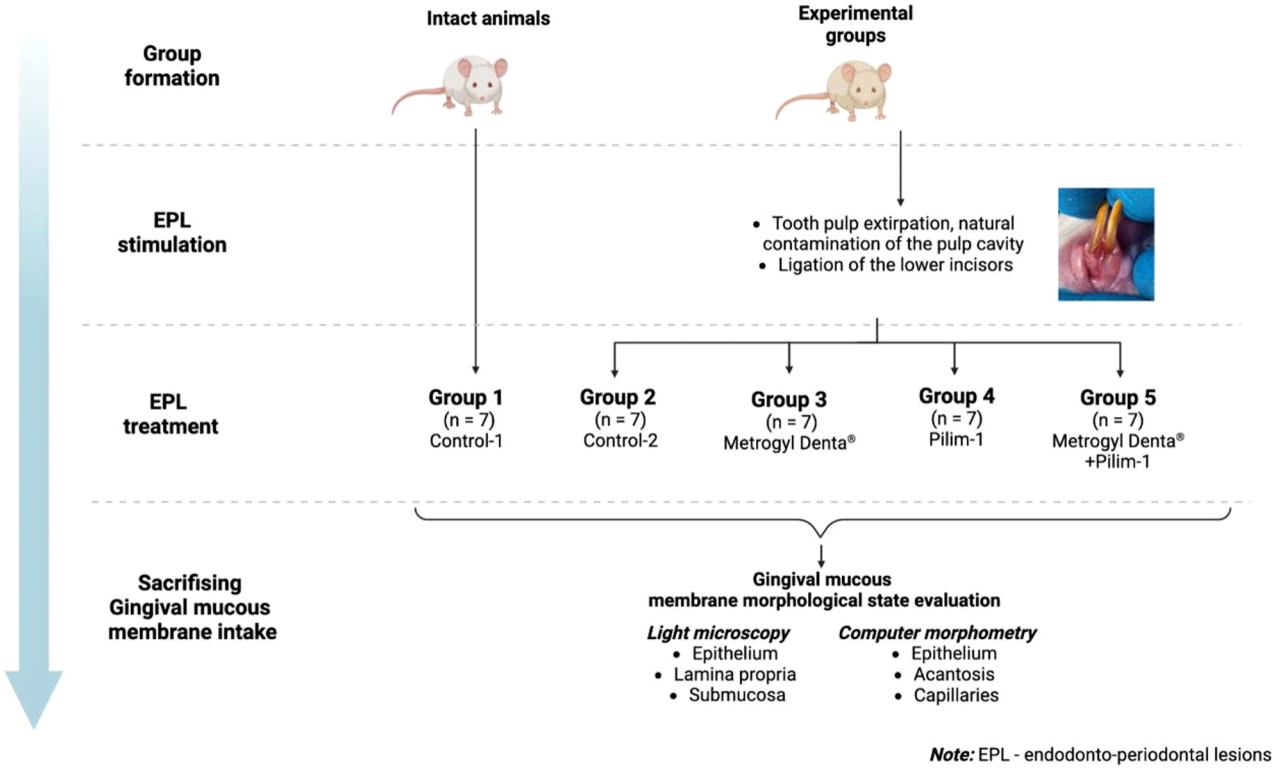


Figure 1. Schematic diagram of the study design.

Research methods

The rats were anesthetized with Telazol (0.3 mg intramuscularly – i/m), Meditin (0.8 mg i/m) and 0.1% solution of atropine sulfate (0.01 mL subcutaneously) per 100 g of animal body weight prior to the studies.

To simulate EPL, a "combined" method of the EPTC lesion was applied, which included a method for induction of acute periodontitis in rats, described by Turova et al. (2010) and Kade et al. (2016), and the ligature method described by Polson and Zander (1983), Keles et al. (2005) and Popkov et al. (2022). To simulate periodontitis, a perforation with a diameter of 1 mm was made on the lingual surface of each lower incisor using a turbine set and a cavity round carbide bur (Frank Dental, Germany). Then pulpotomy of the incisors was performed. The incisor canals were dilated using K-files (Dentsply-Mailifer, Switzerland) and Hedstrom file No. 35-40 (H-Files, Dentsply-Mailifer, Switzerland) to the maximum possible size, and their finalization was completed with ProTaper Manual (ProTaper, Dentsply-Mailifer, Switzerland). The canal orifice was dilated using drills (Gates Drills, Mani, Japan). Plaque accumulation on the vestibular surfaces of the chewing teeth (3/3) was scraped off using Miller broach (KMIZ, Russia) and introduced into the prepared cavities, which were left open for the entry of the content of the oral cavity into the root canal and its infecting with pathogens (Fig. 2, a and b).

Taking into account anatomical characters of the rat's lower incisors and the absence of full-fledged roots in these teeth (Gushchin and Kvanchiani 2020), the simulation of EPL allows achieving the expected result in the near future.

To simulate experimental periodontitis, the necks of the lower incisors were ligated using suture material EUROLON 4/0 (MZKRS suture materials LLC, Russia), followed by the immersion of the ligatures in the gingival groove (Fig. 2, c and d).

Inoculation of the root canals, the apical region of the studied lower incisors and periodontium occurred naturally for 30 days. The perforated cavities of the lower incisors and the ligature applied in the sulcular part of the marginal gum of the animals created optimal conditions for food retention, the formation of microbial plaque in the neck region and the penetration of pathogenic microorganisms into the root canals, which together formed inflammation in the periodontium and apical area of the lower incisors. EPL in rats simulated this way allowed not only studying the pathogenesis of EPL, but also provided an opportunity to analyze the dynamics of changes in the course of the treatment. It should be noted that the EPL described by us is close to a purely combined EPL (according to the classification proposed in 1972 by Simon et al. (Grudyanov et al. 2013)), since the focus of destruction of apical tissues progresses due to their contamination by pathogenic microorganisms from the pulp region through the root canal, connecting with an infected periodontal pocket, which meets three criteria defined by Harrington (1979): the tooth must be devital with the presence of a periodontal pocket communicating with a lateral canal or apical orifice (Grudyanov et al. 2013).

The rats selected for the experiments were randomized into 5 groups of seven animals in each: group 1 - intact (control-1); group 2 – animals with simulated EPL (control-2); group 3 – animals with simulated EPL, which root canals and periodontal pockets were irrigated

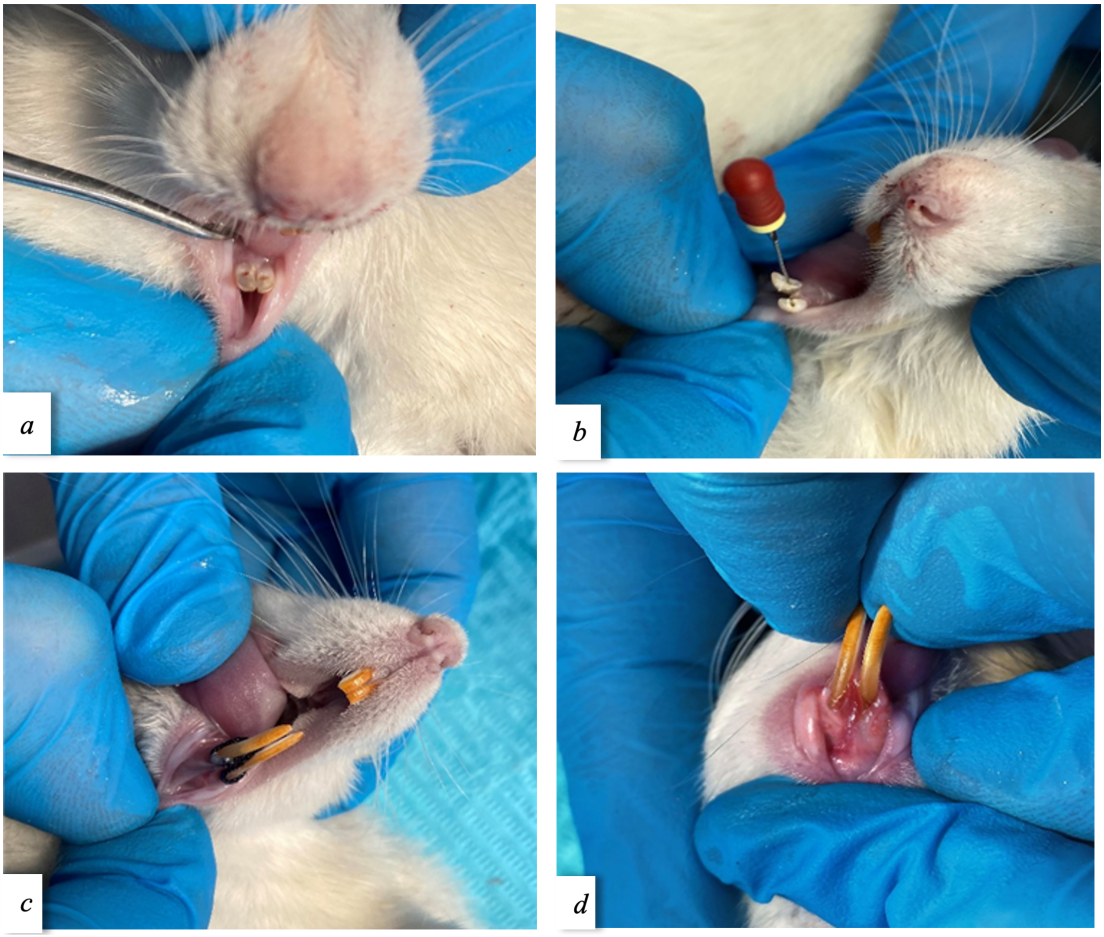


Figure 2. Stages of the ”combined” method (using the methods for induction of periodontitis (**a** and **b**) and parodontitis (**c** and **d**)) of EPL simulation.

with 0.05% solution of chlorhexidine bigluconate (CB), and administered with Metrogyl Denta® gel (M-D); group 4 – animals with simulated EPL, which root canals and periodontal pockets were previously irrigated with 0.05% solution of CB, and then Pilim-1 gel based on sodium carboxymethyl cellulose (Na-CMC) was injected into these structures (canals and pockets); group 5 – animals with simulated EPL, which to similarly to the 3rd and 4th groups, 0.05% solution of CB and a combination of M-D with Pilim-1 were administered.

The therapy with M-D, Pilim-1 and their combination M-D + Pilim-1 was performed in the 3rd-5th groups of rats for 14 days. For pathomorphological studies, biopsy was taken from the gingival margin of the cervical region of the lower incisors on the 45th day of observation, or 15 days after initiation of the treatment.

Rat gum samples were fixed in 10% neutral buffered formalin for 24 hours. Then, standard histological processing of the obtained samples was carried out using isopropyl alcohol in increasing concentration and xylene in a Logos *microwave* tissue processor (Milestone, Italy). After that, the gum samples were embedded in paraffin and histological sections with a thickness of 3 to 5 microns were made using a rotary microtome Leica RM 2255 (Leica, Germany), which were stained with hematoxylin and eosin. The obtained slides were studied using a Leica DM1000 microscope (Leica, Germany) with a Leica ICC50E digital camera with Leica LAS Core software (Leica, Germany) at magnification of a microscope ×100, ×200, ×400 (Yanushevich and Dmitrieva 2018; Grudyanov 2022; Galenko-Yaroshevsky et al. 2023).

For quantitave evaluation of the tissue structures, computer morphometry was performed by image analysis using a Zeiss Axio Lab.A1 microscope (Carl Zeiss MicroImaging GmbH, Germany) with a personal computer and open source software ImageJ (US National Institutes of Health, USA). The epithelial thickness, acanthosis depth, as well as the diameter of blood capillaries were measured using the “Straight Selection” software at the magnification of ×40. The number of blood capillaries per 1 μm² of the sample was calculated using a fine tuned “Analyze particles” tool. The slice area required for calculation was determined manually using the “Free hand selection” or ”Polygon selection” tools (Slavinsky and Verevkin 2022).

Statistical data processing

Statistical analysis of the morphometric study was carried out using ”MedCalc Statistical Software” (Belgium). All samples were tested for the type of distribution using the Shapiro-Wilk W-test. Due to the absence of a normal distribution in most ordered samples, the results were described as a median (Me), lower and upper quartiles (Q1 and Q3, respectively). Pearson’s chi-squared test was used to determine the significance of the differences. The null hypothesis was rejected at the level of statistical significance $p<0.05$.

Results and Discussion

Group 1 (group of intact rats, control-1)

In the animals of this group, the histological structure of the mucous membrane of the gingival margin, represented by a stratified squamous epithelium and proper mucous plate, was unaltered (Fig. 3, A). Occasionally several rows of acaryotic cells were detected in the horny layer on slices, and the phenomena of parakeratosis were noted. Elongated cells of the granular layer, containing keratogyalin in the cytoplasm, were adjacent to the surface horny layer. The spiny layer of the epithelium, as a rule, was represented by polygonal cells, tightly adjacent to each other. Then there were cylindrical cells located on the basement membrane.

The proper mucous plate (connective tissue membrane) of the gum was represented by cellular elements, fibrous structures and extracellular matrix. The papillary layer of the proper mucous plate undulated into the epithelial layer. In the areas of loose connective tissue, cellular elements, mainly fibroblasts, as well as a small number of macrophages, plasma and mast cells were identified among the fibers. There were no dystrophic changes. Signs of inflammatory infiltration were not found in either the papillary or the reticular layer of the proper mucous plate. The acanthosis was moderately pronounced.

Elements of the peripheral microvascular network were determined on the slices in the form of capillaries, precapillaries and small arteries moderately filled with blood corpuscles. Sometimes these vascular structures were visualized as capillary loops and glomeruli. In some areas of the gum, collapsed microcirculatory vessels were detected among the capillaries, their walls were thinned and there were no blood cells in their lumen (Fig. 3, B).

The described morphological pattern of the gingival margin in the samples of the 1st (intact) group of rats conformed to the norm with the absence of any pathological changes in both the surface and deep layers.

Group 2 (animals with simulated EPL without treatment, control-2)

Simulated EPL was characterized by severe changes in the structure of the gingival mucosa of rats. There were pronounced dystrophic changes in the cells of the stratified squamous epithelium, as well as extensive areas of coagulative and colliquative necrosis. Signs of purulent inflammation were presented as pronounced neutrophil infiltration with karyorhexis and karyolysis, with the formation of small apoptotic bodies and areas of connective tissue histolysis (Fig. 3, C).

There was a thickened horny layer, which was represented by parakeratotic cells. There were signs of acanthosis of the epithelial layer into the underlying connective tissue of the proper mucous plate. A characteristic morphological feature of the gum samples in this group of animals was pronounced leukopedesis, manifested by the presence of neutrophilic leukocytes that migrated into the epithelial layers from the underlying proper mucous plate (Fig. 3, D). In the basal layer, cells with hydropic degeneration manifested by vacuole dystrophy in the cytoplasm and chromatolysis of the nuclei were detected. It is important to note that the epithelial layer was significantly thinned.

Massive polymorphocellular infiltrates, including segmented neutrophils, lymphocytes, plasma cells and macrophages, were observed in the proper mucous plate of the gingival mucosa. Areas of sclerosis of connective tissue, newly formed thin-walled capillaries with thickened walls and areas of vascular endothelium proliferation were found in separate parts between cell infiltrates. In some cases, edema, capillary dilation, their fullness of blood, diapedesis, stasis were noted. The collagen fibers of the intercellular substance were often thickened and fragmented.

Group 3 (rats with simulated EPL and administered with Metrogyl Denta® gel (M-D))

In the samples of the rat mucosa after treatment with M-D gel, the structure of the gum tissue complex improved and had features of tissues being restored after damage. The epithelial layer took a linear orientation, typical layering and cellular composition.

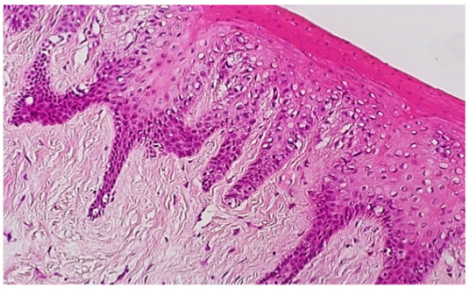
The severity of inflammation was significantly lower, and the area affected by inflammatory cell infiltrates was significantly reduced. There was a sharp decrease in the number of neutrophilic leukocytes and an increase in the fibroblasts, endothelial cells and macrophages. The granular layer of the epithelium undergoes pronounced acanthosis, manifested in the thickening and elongation of the outgrowths of the epithelial lining and their invasion into the underlying connective tissue of the proper mucous plate. Differentiation of the cellular composition was determined in the acanthotic outgrowths. The edema in the papillary and reticular layers of the proper mucous plate significantly decreased (Fig. 3, E). In most slices, moderate mononuclear cell infiltration with angiomatosis and stroma edema was detected under acanthotic outgrowths of epithelial lining along with dystrophic changes.

Moderate perivascular infiltration with stasis of blood vessels persisted in some areas. In some cases, there were perivasculitis in the form of sheaths surrounding the vessels. However, the severity of sclerotic changes in the vascular walls decreased, the lumen of most vessels was filled with blood cells. Sometimes newly formed capillaries surrounded by loose areolar connective tissue with a small number of lymphocytes and plasma cells were visualized.

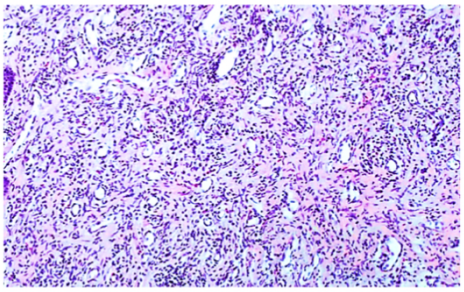
Against the background of attenuation of acute inflammation, proliferative activity of fibroblasts were observed, cords of maturing multicellular fibrous tissue were formed. However, in most areas of the studied slices, the regenerate was represented by coarse-fibred scar tissue with hyalinosis (Fig. 3, F). All these changes were combined with epithelial atrophy, disorders of cell differentiation and keratinization.

Group 4 (rats with simulated EPL and administered with Pilim-1 gel)

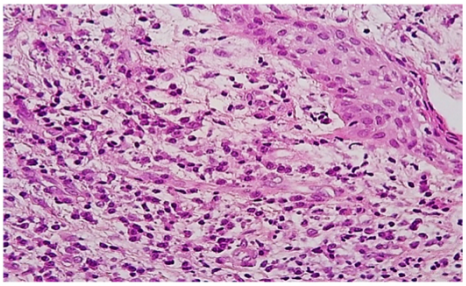
In the samples of the animals of this group, the morphological pattern of the gingival margin tended to normal; however, the exudation was sometimes preserved in some areas, more often in the reticular layer, and there were residual alterative changes in the intercellular matrix of the papillary layer (Fig. 3, G). The number and size of inflammatory cell infiltrates significantly decreased. They covered a small area of the studied gum slices. Neutrophilic leukocytes, lymphocytes and fibroblasts were found in small numbers, mainly they were detected near capillary vessels.



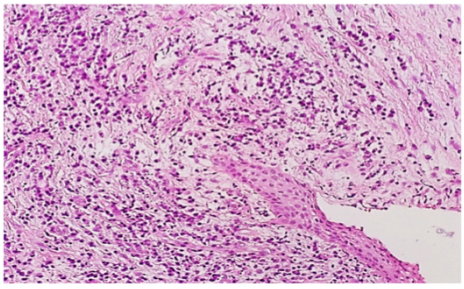
A. Structure of the gum mucosa of the intact rat Stained with hematoxylin and eosin. Magnification ×200.



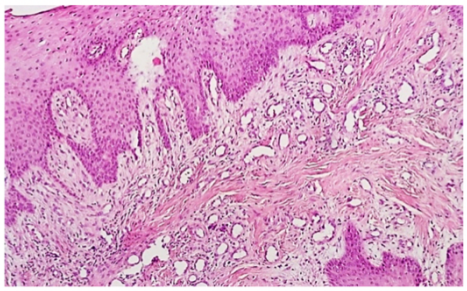
B. The reticular layer of the gum: capillaries, small arteries. Stained with hematoxylin and eosin.



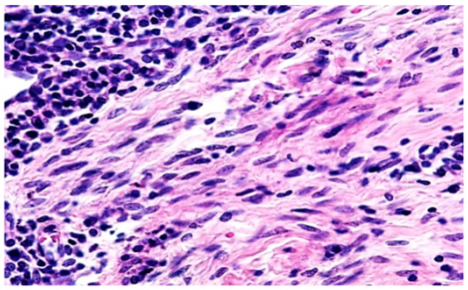
C. Severe purulent inflammation: neutrophil infiltration with the stroma edema, areas of necrosis of the epithelial lining. Stained with hematoxylin and eosin. Magnification ×400.



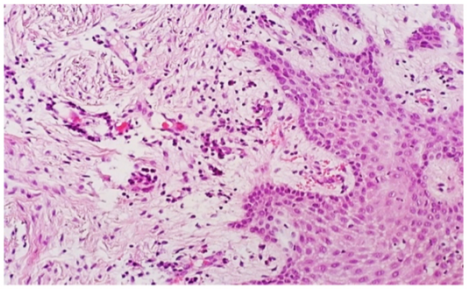
D. Leukodiapedesis in the epithelial lining. Extensive neutrophil infiltration with pronounced edema and areas of necrosis. Stained with hematoxylin and eosin. Magnification ×200.



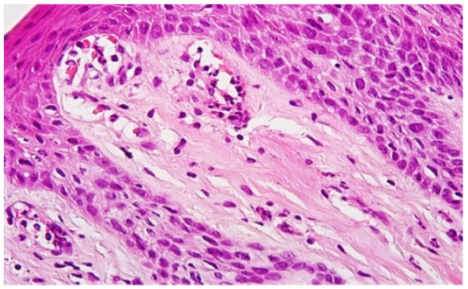
E. Acanthotic outgrowths of the epithelium with signs of cell differentiation, moderate inflammatory cell infiltration. Stained with hematoxylin and eosin. Magnification ×100.



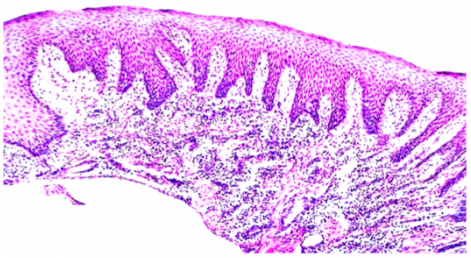
F. Generation of cords of maturing multicellular fibrous connective tissue. Stained with hematoxylin and eosin. Magnification ×400. Magnification ×100.



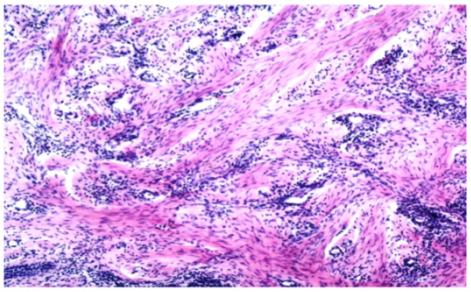
G. Mild inflammatory infiltration, small clusters of lymphocytes and macrophages around the capillaries. Stained with hematoxylin and eosin. Magnification×200.



H. The areas of connective tissue containing capillaries, and a few neutrophils and macrophages between the acanthotic cords. Stained with hematoxylin and eosin. Magnification ×400.



I. The restored structure of the gingival mucosa of rats. Stained with hematoxylin and eosin. Magnification×200.



J. Exudation is eliminated, infiltrates are replaced by loose connective tissue. Stained with hematoxylin and eosin. Magnification×200.

Figure 3. Micrographs of rat gum biopsy specimens.

Against the background of a decrease in the number of cellular inflammatory infiltrates, the neoangiogenesis was observed (Fig. 3, H), which contributed to the improvement of the trophicity of the regenerated cover tissue of the gum and improved its morphological structure. In addition, newly formed collagen fibers were detected, which were united into cords, thickened and located between the cells in reduced infiltrates.

In the greater part of the reticular layer of proper mucous plate, the regenerated tissues in the form of a well-vascularized regenerate took the form of the areas of loose connective tissue with insignificant foci of mononuclear infiltration. This indicates that the inflammatory processes in the gum were not completely eliminated, but their activity was significantly decreased and passed into the stage of chronification, which over time can lead to persistent changes in the gum tissues with the formation of scar tissue.

Group 5 (rats with simulated EPL and administered with the combination of M-D + Pilim-1)

The most significant positive results of the treatment were revealed in the samples of the animals of this group. The regenerative process took more than half of the area of previously affected tissues. Restoration of the structure of the gingival mucosa was observed not only in the proper mucous plate, but also in its epithelial lining. Although the epithelial acanthosis persisted, however, it was significantly less prominent compared to those of animals of groups 3 and 4.

Keratinization was normalized and restoration of keratin formation was noted in the horny layer. Para- and hyperkeratosis were not observed. Exudation and sclerosis in the papillary layer were scarcely noticeable (Fig. 3, I). A well-developed vascular network was determined, around which connective tissue cells and fibrous structures, mainly consisting of collagen fibers, were located. Papillae evenly spaced on the epithelium of the gum. In the reticular layer, thin collagen fibers united into cords, and stroma of the mature connective tissue were detected. Inflammatory cell infiltration was almost absent, and no sclerosis was observed.

The visualized cells were represented by fibroblasts and lymphocytes; an increase in the number of macrophages was noted, which contributed to active neoangiogenesis and improved vascularization of regenerating tissues. There were a small number of mast and plasma cells, as well as single neutrophils. The improvement of trophicity in the gum tissues prevented the atrophic and fibrous sclerotic disorders, and also minimized the formation of scar tissue (Fig. 3, J).

The study of the gum samples of the 5th group of rats allows us to state the positive dynamics in the restoration of the structure of the gingival mucosa after the administration of the therapeutic complex M-D + Pilim-1. This manifested in the elimination of inflammatory cell infiltrates, a decrease in the acanthosis severity, activation of neoangiogenesis and restoration of the proper mucous plate with its fibrous structures.

The results of computer morphometry of rat gum samples revealed a decrease in the thickness of the epithelial lining of the gum 2.5 times ($p<0.05$) and the acanthosis depth 1.2 times ($p<0.05$) in the 2nd group of rats (control-2) with simulated EPL, compared with the 1st group (control-1). At the same time, the number of capillaries increased 1.8 times ($p<0.05$), and their diameter increased 1.2 times ($p<0.05$) (table, Figs 4-7).

In the 3rd group of animals with simulated EPL and treated with M-D, the thickness of the epithelium in the gingival mucosa and the acanthosis depth increased 3.4 times ($p<0.05$) compared with the 2nd group (control-2). The number of capillaries reduced 1.2 times ($p<0.05$), but their diameter has almost not changed (Table, Figs 4-7).

In the 4th group of rats with simulated EPL and treated with Pilim-1, the epithelial lining of the gingival mucosa was 3.0 times thicker ($p<0.05$), and the acanthosis depth increased 3.6 times ($p<0.05$) compared with the 2nd group (control-2). The number of capillaries and their diameter did not change significantly (Table, Figs 4-7).

In the 5th group of animals with simulated EPL and treated with the combination M-D+Pilim-1, the thickness of the epithelial lining of the gum increased 4.4 times ($p<0.05$), while the acanthosis depth remained increased 2.2 times ($p<0.05$) compared with the 2 nd group (control-2). The number of capillaries decreased 1.4 times ($p<0.05$), and their diameter increased 1.1 times ($p<0.05$) (Table, Figs 4-7).

Table. Computer morphometry of the rat's gingival mucosa after administration of M-D, Pilim-1 and the combination of M-D + Pilim-1

Substances and their combinations	Me, Q1 и Q3			
	Epithelial thickness, µm	Acanthosis depth, µm	Number of capillaries per 1 mm²	Capillary diameter, µm
Control-1 [1]	112.6 (91.4; 128.3)	105.9 (82.0; 130.1)	24.6 (19.8; 28.3)	30.7 (25.3; 34.0)
Control-2 [2]	45.8+ (40.7; 64.4)	88.0+ (71.0; 104.1)	44.3+ (37.2; 51.1)	35.7+ (29.1; 43,4)
M-D [3]	154.5++ (133.7; 180.4)	298.4++ (280.3; 332.5)	37.0++ (32.5; 41.1)	38.2 (35.8; 42.2)
Pilim-1 [4]	137.2++ (124.4; 161.0)	318.1++ (250.2; 368.8)	46.1x (44.9; 50.7)	38.5 (32.4; 41.6)
M-D+Pilim-1 [5]	202.4++x* (168.9; 241.0)	194.7++x* (176.5; 211.2)	31.7++* (28.8; 36.7)	40.6++ (35.4; 42.7)

Note: 1. In round parentheses – Q1 and Q3, in square parentheses – group number; 2. + – the difference is significant ($p<0.05$) compared with group 1; + + – the difference is significant ($p<0.05$) compared with group 2; x – the difference is significant ($p<0.05$) compared with group 3; * – the difference is significant ($p<0.05$) compared with group 4.

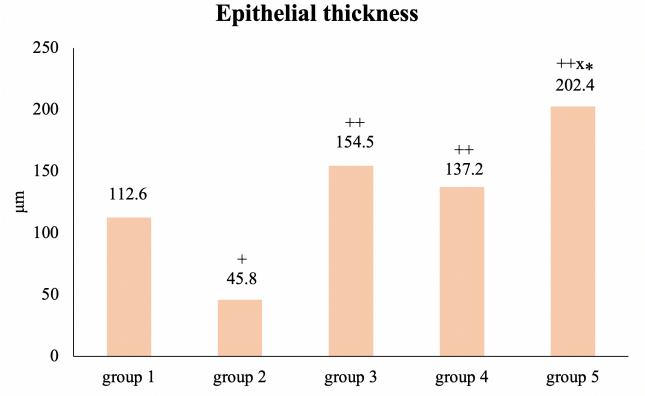


Figure 4. Thickness of the rat's gingival epithelium (microns). ***Note:*** $^{+}$– significant difference ($p<0.05$) from group 1; $^{++}$ – significant difference ($p<0.05$) from group 2; ^{x} – significant difference ($p<0.05$) from group 3; * – significant difference ($p<0.05$) from group 4.

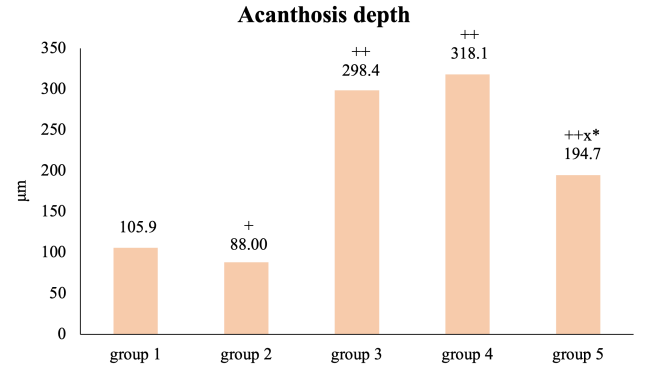


Figure 5. Acanthosis depth in the rat's gingival epithelium (microns). ***Note*:** $^{+}$– significant difference ($p<0.05$) from group 1; $^{++}$ – significant difference ($p<0.05$) from group 2; ^{x} – significant difference ($p<0.05$) from group 3; * – significant difference ($p<0.05$) from group 4.

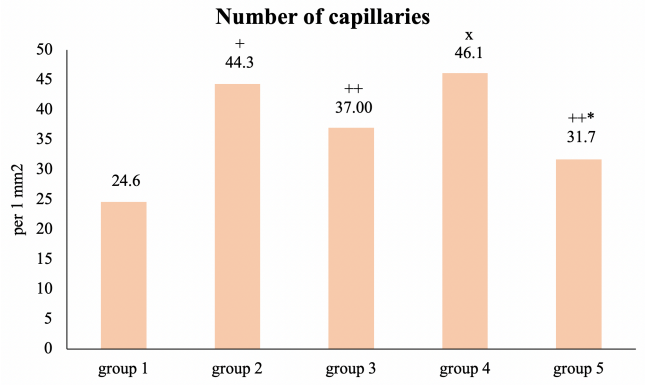


Figure 6. Number of capillaries in the rat's gingival epithelium (per 1 mm^2). ***Note:*** $^{+}$– significant difference ($p<0.05$) from group 1; $^{++}$ – significant difference ($p<0.05$) from group 2; ^{x} – significant difference ($p<0.05$) from group 3: * – significant difference ($p<0.05$) from group 4.

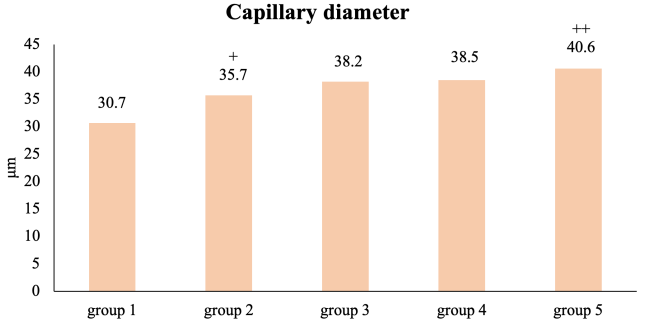


Figure 7. Capillary diameter in the rat's gingival epithelium (microns). ***Note:*** $^{+}$– significant difference ($p<0.05$) from group 1; $^{++}$ – significant difference ($p<0.05$) from group 2.

In the 4th group of rats which had been administered with Pilim-1, epithelial thickness, acanthosis depth and capillary diameter did not change significantly, and the number of capillaries increased 1.2 times ($p<0.05$) compared with the 3rd group treated with M-D (Table, Figs 4-7).

In the 5th group of animals treated with the combination M-D +Pilim-1, the epithelial thickness increased 1.3 times ($p<0.05$), the acanthosis depth decreased 1.5 times ($p<0.05$), the number of capillaries and their diameter had no significant differences compared with the 3rd group treated with M-D, and compared with the 4th group treated with Pilim-1, the epithelial thickness increased 1.5 times ($p<0.05$), the acanthosis depth decreased 1.6 times ($p<0.05$), while the number of capillaries decreased 1.4 times ($p<0.05$). As for the diameter of the capillaries, it had not undergone significant changes (Table, Figs 4-7).

The data of digital computer analysis of the samples indicate that simulation of the endodontic-periodontal lesions caused a pronounced damage of the structures of the gingival mucosa, which was manifested not only by a decrease in the thickness of the surface epithelium, but also by the suppression of acanthosis, or its ability to invade into the underlying connective tissue, the blood vessels of which, as known, supply the epithelium. The sings of a severe acute inflammatory process was a sharp increase in the number of blood capillaries in the gingival mucosa with a moderate increase in their diameter. Regeneration of the mucosal tissues in the course of the treatment was reflected in a somewhat excessive increase in the epithelial thickness and acanthosis depth compared to the norm, which could be explained by the compensatory reaction of the tissues to deep damage. At the same time, the acanthosis depth and the number of capillaries in experimental group 5 (rats with simulated EPL treated with the combination M-D + Pilim-1) showed the nearest approximation to normal indicators.

Thus, the performed pathomorphological studies showed that the administration of M-D in simulated EPL had a moderate positive effect on morphological changes in the affected tissues of the gingival mucosa of rats. Pilim-1 and, more significantly, the combination M-D+Pilim-1 have high therapeutic efficacy in experimental EPL, characterized by relatively rapid restoration of tissue structures of damaged gum.

Conclusion

The administration of M-D, Pilim-1 and, to a greater extent, the combination M-D + Pilim-1 (against the background of chlorhexidine bigluconate used as oral rinse) for 14 days in rats with simulated EPL has a pronounced positive effect on pathomorphological changes in gum tissues characterized by active regeneration processes with elimination of pathological changes and significant restoration its typical structure.

Conflict of interests

The authors declare no conflict of interests.

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