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Research Article

Study of anti-inflammatory, antioxidant, antimicrobial and mineralizing effects of an N-isopropenylimidazole zinc metal complex derivative in experimental endodontic-periodontal lesions in rats

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Abstract

Introduction: The combination treatment of patients with dentition problems including endodontic-periodontal lesions (EPL) is known to widely involve anti-inflammatory drugs, including non-steroidal anti-inflammatory agents, and antimicrobial drugs, in particular antibiotics and antiseptics, from which the former often lead to the development of gastro- and neuropathy, lesions of kidneys and liver, as well as the cardiovascular system, and the latter, in addition to serious side effects, lead to the development of steady resistance of pathogenic microorganisms and dysbacteriosis. This necessitates search for and development of new molecules with a wide range of therapeutic effects aimed to block the infectious and inflammatory process and to restore the functional ability of teeth in EPLs as much as possible (depending on a lesion severity). **The aim of the study** was to examine the anti-inflammatory, antioxidant, antimicrobial and mineralizing effects of an N-isopropenylimidazole zinc metal complex derivative in experimental endodontic-periodontal lesions in rats.

Materials and Methods: EPL simulation in experiments on the anesthetized rats was conducted in the following way: first, the pulp cavity of lower incisors was opened and left exposed to infection; then periodontal inflammation was induced using a ligature. Thirty days later, EPL developed in the animals. An N-isopropenylimidazole zinc metal complex derivative (laboratory code Pilim-1, prepared in form of gel),

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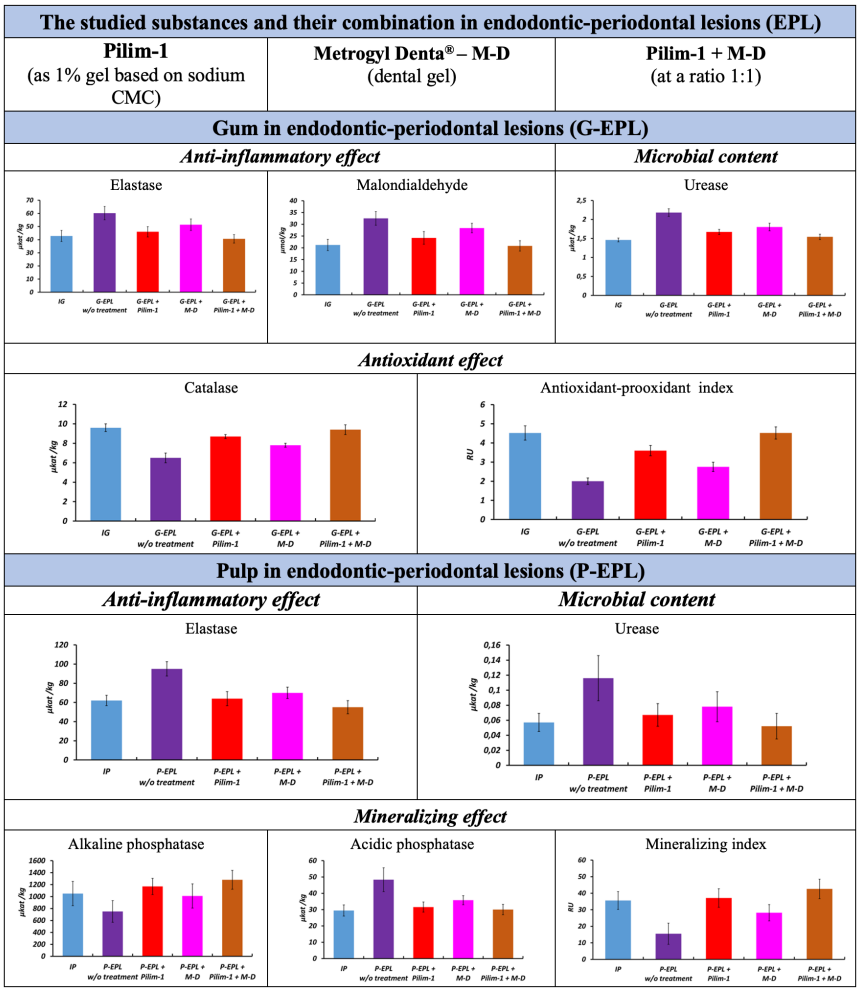
reference drug Metrogyl Denta® (M-D, dental gel), as well as the combination Pilim-1 + M-D were applied daily for 14 days on the lower incisors and appropriate gums, and also placed in the gingival pockets and pulp cavities, with pulp partially removed beforehands. After euthanasia of the animals on the 15th day of the experiments, gingival homogenates were assayed for the activity of elastase, urease, catalase, the level of malondialdehyde, and the antioxidant-prooxidant index was calculated, while pulp homogenates were assayed for the activity of elastase, urease, alkaline and acidic phosphatases, and the mineralizing index was calculated.

Results and Discussion: In the gum in EPL, Pilim-1 and, especially, the combination Pilim-1+M-D more significantly than M-D have a pronounced anti-inflammatory, antioxidant and antimicrobial (reduce a microbial content level) effects. In the pulp in EPL, Pilim-1 and, to a greater extent, the combination Pilim-1 + M-D in a more pronounced way than M-D have anti-inflammatory, antimicrobial (reduce a microbial content level) and mineralizing effects. In the therapeutic action mechanisms of Pilim-1 and, especially, the combination Pilim-1 + M-D in EPL conditions, their ability to have an antihypoxic effect and an inhibitory effect on cyclooxygenase and 5-lipoxygenase is likely to play a very important role, in addition to their identified effect on the examined metabolic processes. Besides, a certain role may be played by the presence of zinc and imidazole in the structure of Pilim-1, as they have a wide range of biological activity.

Conclusion: The presented results of studying Pilim-1 and its combination with M-D indicate their high therapeutic efficiency, especially when using the latter in the experimental EPL in rats, which makes it possible to consider Pilim-1 and Pilim-1 + M-D as potential therapeutic agents to treat EPL.

Graphical abstract



Keywords

antioxidant effect, N-isopropenylimidazole zinc metal complex derivative, microbial content, mineralizing effect, anti-inflammatory effect, endodontic-periodontal lesion

Introduction

The combination treatment of patients with dentition problems including endodontic-periodontal lesions (EPL) is known to widely involve anti-inflammatory drugs, including non-steroidal anti-inflammatory agents, and antimicrobial drugs, in particular antibiotics and antiseptics, from which the former often lead to the development of gastro- and neuropathy, lesions of kidneys and liver, as well as the cardiovascular system (Karateev et al. 2018; Khoroshun and Lazareva 2022; Kamchatnov et al. 2023; Minhas et al. 2023), and the latter, in addition to serious side effects, lead to the development of steady resistance of pathogenic microorganisms and dysbacteriosis (Bakhit et al. 2018; Kuzmina et al. 2019; Orekhova et al. 2020). It is generally believed that in the development and course of EPL, an important role is played by free-radical oxidation, disturbance of which leads to the imbalance of antioxidant and pro-oxidant systems, which causes a significant increase in the level of free radicals and the formation of "oxidative stress", resulting in damage to cells and an increase in vascular and tissue permeability (Grebenchikov et al. 2016; Men'shchikova and Zenkov 2016; Atabay et al. 2017; Chen et al. 2019). All this necessitates search for and development of new molecules with a wide range of therapeutic effects aimed to block the infectious and inflammatory process and to restore the functional ability of teeth in EPL as much as possible (depending on a lesion severity). A very significant problem of EPL should be solved in a comprehensive and targeted manner by using drugs against which microorganisms develop no resistance and which can exert high anti-inflammatory, antimicrobial, detoxification, antioxidant, and regenerative effects (Tsepov et al. 2018; Ushakov and Tsaryov 2019).

In view of the above-mentioned data, our attention settled on a N-isopropenylimidazole zinc metal complex derivative with laboratory code Pilim-1, which, according to our earlier studies, has polyfunctional properties – anti-inflammatory, analgetic, antimicrobial, antioxidant, antihypoxic and wound-healing (Shakhmardanova and Galenko-Yaroshevsky 2015; Lebedeva et al. 2021, 2022, 2022a, 2023, 2023a; Galenko-Yaroshevsky et al. 2023, 2024) and can be of interest as a potentially promising agent in an EPL combination therapy.

The aim of the study was to examine the anti-inflammatory, antioxidant, antimicrobial, and mineralizing effects of an N-isopropenylimidazole zinc metal complex derivative in experimental endodontic-periodontal lesions in rats.

Materials and Methods

Test compound and reference drug

The substance of N-isopropenylimidazole zinc diacetate with laboratory code Pilim-1 (AE Favorsky Irkutsk Institute of Chemistry, Siberian Branch of the Russian Academy of Sciences, Russia) as 1% gel based on sodium carboxymethylcellulose (sodium CMC; RossPolimer LLC, Russia); dental gel Metrogyl Denta® – M-D (Unique Pharmaceutical Laboratories, India), which contains metronidazole, chlorhexidine digluconate, propylene glycol, carbomer-940, edetate disodium, sodium saccharin, levomenthol, sodium hydroxide and water.

Animals

The studies were performed on 400 white Wistar rats weighing 210-280 g, kept in the vivarium standard conditions on a standard diet in accordance with the rules of good laboratory practice (GOST 33216-2014). The studies were conducted in compliance with the European Convention on the Protection of Vertebrates Used for Experiments or Other Scientific Purposes (as amended in Strasbourg, 2006); Law of the Russian Federation "On the Protection of Animals from Cruel Treatment" dated June 24, 1998; Good Laboratory Practice for Conducting Preclinical Trials in the Russian Federation (GOST 3 51000.3-96 and GOST P 53434-2009), Helsinki Declaration designed by the World Medical Association on Humane Treatment of Laboratory Animals (Report of the AVMA Panel on Euthanasia JAVMA, 2001); Guidelines on Maintenance and Care of Laboratory Animals (interstate standard GOST 33216-2014 dated July 1, 2016); Guidelines for Conducting Preclinical Trials of Drugs (Mironov 2012) and Guidelines for Working with Laboratory (Experimental) Animals in Preclinical (Non-clinical) Trials (Recommendations by the board of the Eurasian Economic Commission No. 33 of November 14, 2023, Moscow). The experiments were approved by the Ethical Committee of Rostov State Medical University of the Ministry of Health of Russia (Minutes No. 16/19 of September 17, 2019).

Experiment design

To study Pilim-1, reference drug M-D and the combination Pilim-1 + M-D, according to the aim, five groups of animals were formed:

1. Gingival homogenates were assayed for:
   - elastase activity – 5 groups of 10 rats: Group 1 – intact gums (IG), Group 2 – experimental endodontic-periodontal lesion (E-EPL) without treatment (with applying only gel based on sodium-CMC), Group 3 – E-EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 – E-EPL + Pilim-1 + M-D (at a ratio of 1:1; 0.3 mL on teeth and gums);
   - level of malondialdehyde (MDA) – 5 groups of 10 rats: Group 1 – IG, Group 2 – E-EPL without treatment, Group 3 – E-EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 – E-EPL + Pilim-1 + M-D (at a ration of 1:1; 0.3 mL on teeth and gums);
   - urease activity – 5 groups of 10 rats: Group 1 – IG, Group 2 – E-EPL without treatment, Group 3 – E-EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 – E-EPL + Pilim-1 + M-D (at a ratio of 1:1; 0.3 mL to teeth and gums);
   - catalase activity – 5 groups of 10 rats: Group 1 – IG, Group 2 – E-EPL without treatment, Group 3 – EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 – E-EPL + Pilim-1 + M-D (at a ratio of 1:1; 0.3 mL to teeth and gums).
2. Pulp homogenates were assayed for:
   - elastase activity – 5 groups of 10 rats: Group 1 – intact pulp (IP), Group 2 – E-EPL without treatment, Group 3 –E-EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 –E-EPL + Pilim-1 + M-D (at a ratio of 1:1; 0.3 mL on teeth and gums);

- urease activity – 5 groups of 10 rats: Group 1 – IP, Group 2 – E-EPL without treatment, Group 3 – E-EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 –E-EPL + Pilim-1 + M-D (at a ratio of 1:1; 0.3 mL on teeth and gums);
- activity of alkaline phosphatase (APP) – 5 groups of 10 rats: Group 1 – IP, Group 2 – E-EPL without treatment, Group 3 – E-EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 – E-EPL + Pilim-1 + M-D (at a ratio of 1:1; 0.3 mL on teeth and gums);
- activity of acidic phosphatase (AP) – 5 groups of 10 rats: Group 1 – IP, Group 2 – E-EPL without treatment, Group 3 – E-EPL + Pilim-1 gel (0.3 mL on teeth and gums), Group 4 – E-EPL + M-D (0.3 mL on teeth and gums), Group 5 – E-EPL + Pilim-1 + M-D (at a ratio of 1:1; 0.3 mL on teeth and gums) (Fig. 1).

Experimental research models

The animals were randomized for each studied indicator into 5 groups of 10 individuals each. E-EPL was simulated under Telazol-Meditin anesthesia (Telazol (Zoetis, Spain) – 0.3 mg (intramuscularly), Meditin (API-SAN LLC, Russia) – 0.8 mg (intramuscularly) and 0.1 % Atropine sulfate solution (Federal state unitary enterprise "Moscow Endocrine Plant", Russia) – 0.01 mL (subcutaneously), per 100 g of animal weight). The pulp cavity of lower incisors was opened and left exposed to infection, and then periodontal inflammation was induced using a ligature method described by Polson and Zander (1983), Keles et al. (2005), Popkov et al. (2022), which involves applying ligatures made of EvroLen 4/0 suture material (EuroType, Russia) on the lower incisor necks, with further ligature placement in the gingival sulcus. Inoculation of the root canal system, the apical region of the studied teeth and periodontal tissues in rats occurred naturally within 30 days.

For preparing gel, sodium-CMC was pre-mixed with glycerin at a ratio of 1:2. Then, water heated to 40°C was gradually poured into this mixture, while constantly stirring with a Brayer BR1303 mixer (Brayer, China) until a homogeneous gel was obtained. The prepared solution was left for 2 hours for complete dissolution of sodium-CMC.

Pilim-1, M-D and the combination Pilim-1 + M-D were applied daily at a dose of 0.3 mL for 14 days on the lower incisors and the corresponding gums and also placed into the gingival pockets and pulp cavities after partial removal of pulp. Before applying the studied substances, the lower incisors and gums had been treated with a 0.05% chlorhexidine bigluconate solution (Tula Pharmaceutical Factory, Russia) for 3-5 minutes. After the studied substances and their combination had been applied, the rats had limited access to the feed and water for 2 hours.

The animals were eliminated from the experiment on the 45th day after its onset, i.e. on the 15th day after the treatment, with the help of chloroform (in small doses, drops) under an airtight glass cover. Then, pulp and gums were sampled from the area of lower incisors and homogenates were prepared, which were placed in 0.05 M of Tris-HCl buffer at pH 7.6. The obtained homogenates were kept in the refrigerator for 30 min at t+2-3°C and then centrifuged at 3500 rpm and +4°C for 15 minutes. Supernatant was placed in clean vials for biochemical studies (Levitsky et al. 2018).

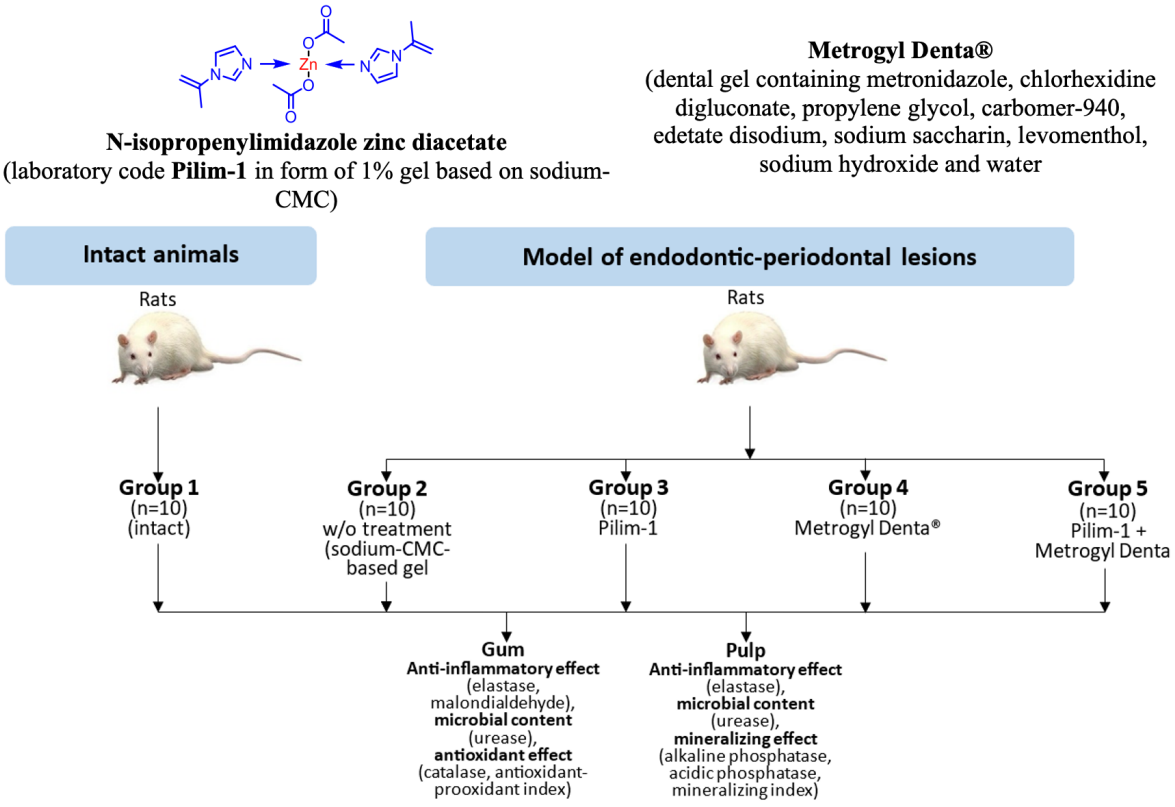


Figure 1. Flowchart of research design.

The elastase activity in the gums and pulp was assayed by the degree of hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester (BOC) (Sigma, USA) according to the method described by Levitsky et al. (2018) and Visser and Blout (1972). The enzyme activity was calculated using formula (1):

$$A = \frac{[(E_5 - E_0) - E] \times n \times 1000}{1.0 \times C \times 300 \times m}, \text{ µkat /kg} \quad (1)$$

where E_0 – extinction at the 0th minute; E_5 – extinction at the 5th minute; E – control for BOC; 1.0 – volume of saliva, mL; 1000 – to convert grams into kilograms; n – sample dilution; 300 – incubation time, sec; C – conversion factor for expressing the extinction value as the concentration of p-nitrophenol; m – mass of tissue in 1 mL of buffer, g.

The activity of elastase was expressed in microkatals per 1 kg of tissue.

The MDA level in gingival homogenates was determined according to the method described by Stal'naya and Garishvilly (1977) and Levitsky et al. (2018), using a reaction with thiobarbituric acid forming stained trimethine complex, with peak absorption at 532 nm. The amount of MDA was calculated using formula (2):

$$A = \frac{E \times 10^6 \times 1000}{1.56 \times 10^5 \times 0.5 \times m}, \text{ µmol /kg} \quad (2)$$

where E – extinction of the experimental sample; 0.5 – volume of the added sample, mL; 1.56 x 10^5 – molar coefficient; 10^6 – to convert moles into micromoles; 1000 – to convert grams into kilograms; m – mass of tissue in 1 mL of buffer, g.

The results were expressed in µmol/kg of tissue.

The urease levels in homogenates of gums and pulp were determined using the method described by Gavrikova and Segen' (1996) and Levitsky et al. (2018). A urea solution was used as a substrate. Sample extinction was measured using a photoelectric colorimeter (APEL AP-101, Japan) at 440 nm. The enzyme activity was calculated using formula (3):

$$U = \frac{(Ets - Ec) \times 1000}{C \times t \times 0.2 \times m}, \text{ µkat /kg} \quad (3)$$

where Ets – extinction of a test sample; E_c – extinction of a control sample; C – conversion factor for expressing extinction as µmol NH_4; t – incubation time, sec.; 1000 –- calculation per 1 kg of tissue; m – mass of tissue, g.

The activity of urease was expressed in microkatals per 1 kg of tissue.

The catalase activity in gingival homogenates was assayed using the method described by Levitsky et al. (2010). The color intensity was measured on a spectrophotometer with a wavelength of 410 nm, the color intensity being opposite to the activity of catalase expressed in µkat/kg of tissue. The enzyme activity was calculated using formula (4):

$$A = \frac{(Eblank - (Ets - Est)) \times 1000}{22.2 \times 0.1 \times 600 \times m}, \text{ µkat /kg} \quad (4)$$

where E_{blank} – extinction of a blank sample; E_{TS} – extinction of a test sample; E_{st} – extinction of stain control; 1000 – to convert grams into kilograms; 0.1 – volume of the added sample, mL; 600 – incubation time, sec; 22.2 (M^{-1} cm^{-1}) – molar extinction coefficient of H_2O_2; m – mass of tissue in 1 mL of buffer, g.

The antioxidant-prooxidant index (API) was calculated according to Levitsky et al. (2010) using formula (5):

$$API = \frac{[(Acat \times 10)}{Cmda}, \quad (5)$$

where A_{cat} – catalase activity (mkat/kg); C_{MDA} – MDA concentration (µmol/kg).

The activity of alkaline and acidic phosphatases in pulp was determined according to Bessey's method modified in Levitsky et al. (2018). The color intensity of each sample was determined on a spectrophotometer with a wavelength of λ 400 nm. The activity of enzymes was calculated using formula (6):

$$API = \frac{\Delta E \times n \times 1000}{C \times T \times V \times m}, \text{ µkat /kg} \quad (6)$$

where ΔE – difference in extinctions between the test sample and stain control; n – sample dilution; 1000 – to convert grams into kilograms; t – incubation time, sec; V (0.1) – volume of biomaterial, mL; C – conversion factor for expressing extinction as the concentration of p-nitrophenol, µmol, determined by the calibration curve; m – mass of tissue in 1 mL of buffer, g.

The activity of alkaline phosphatase (APP) and acidic phosphatase (AP) was expressed in microkatals per 1 kg of tissue (µkat/kg).

The mineralizing index (MI) was calculated according to Levitsky et al. (2018), using formula (7):

$$MI = \frac{APP}{AP}, \quad (7)$$

where APP – activity of alkaline phosphatase; AP – activity of acidic phosphatase.

Statistical analysis

The results (the calculation of arithmetic means – M and standard errors of the mean – m) were statistically processed using Statistica Version 6.0 program (StatSoft Inc., USA), as well as special IBM PC software programs developed at the Department of Pharmacology of Kuban State Medical University. The hypotheses about the means were checked using Student's t-test. The differences were considered reliable at $p < 0.05$.

Results

Influence of Pilim-1 on inflammatory markers – activity of elastase and the MDA level – in the gum in EPL (G-EPL)

Elastase activity

The activity of elastase in G-EPL without treatment was found to have increased 1.4 times when compared to that in IG.

The use of Pilim-1 and reference drug M-D, as well as the combination Pilim-1 + M-D caused a 1.3-, 1.2- and 1.5-time decrease, respectively, in the elastase activity when compared to that in G-EPL without treatment. It is worth mentioning that under the influence of Pilim-1 and even more significantly under the influence of the combination Pilim-1 + M-D, the activity of this enzyme was comparable to that in IG (Table 1, Fig. 2).

Table 1. Influence of Pilim-1, M-D and the combination Pilim-1 + M-D on the activity of elastase and urease, and MDA level in G-EPL in rats (M ± m, n = 10)

Indicators and units of measurement	Groups of animals				
	IG [1]	G-EPL w/o treatment [2]	G-EPL + Pilim-1 [3]	G-EPL + M-D [4]	G-EPL+ Pilim-1+M-D [5]
Elastase, μkat/kg	42.8 ± 1.8 (38.6 ÷ 47.0)	60.2 ± 2.3 (55.1 ÷ 65.3) $p_{1-2} < 0.001$	46.0 ± 1.9 (41.6 ÷ 50.4) $p_{1-3} > 0.05$ $p_{2-3} < 0.001$	51.4± 1.7 (47.5 ÷ 55.3) $p_{1-4} < 0.02$ $p_{2-4} < 0.01$ $p_{3-4} < 0.05$	40.6 ± 1.4 (37.4 ÷ 43.8) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} < 0.05$ $p_{4-5} < 0.001$
MDA, μmol/kg	21.2 ± 1.1 (18.8 ÷ 23.6)	32.5 ± 1.3 (29.6 ÷ 35.4) $p_{1-2} < 0.001$	24.2 ± 0.9 (22.2 ÷ 26.,2) $p_{1-3} < 0.05$ $p_{2-3} < 0.001$	28.4 ± 1.2 (25.7 ÷ 31.1) $p_{1-4} < 0.001$ $p_{2-4} < 0.05$ $p_{3-4} < 0.02$	20.8 ± 1.0 (18.6 ÷ 23.0) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} < 0.05$ $p_{4-5} < 0.001$
Urease, μkat/kg	1.46 ± 0.02 (1.41 ÷ 1.51)	2.18±0.04 (2.08 ÷ 2.28) $p_{1-2} < 0.001$	1.67 ± 0.05 (1.57 ÷ 1.77) $p_{1-3} < 0.001$ $p_{2-3} < 0.001$	1.80 ±0.03 (1.73 ÷1.87) $p_{1-4} < 0.001$ $p_{2-4} < 0.001$ $p_{3-4} < 0.05$	1.54 ± 0.03 (1.47 ÷ 1.61) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} < 0.05$ $p_{4-5} < 0.001$

Note: 1. IG – intact gum, G-EPL – gum in endodontic-periodontal lesion, M-D – Metrogyl Denta®. 2. In parentheses – confidence interval at $p < 0.05$, in square brackets – animal group number.

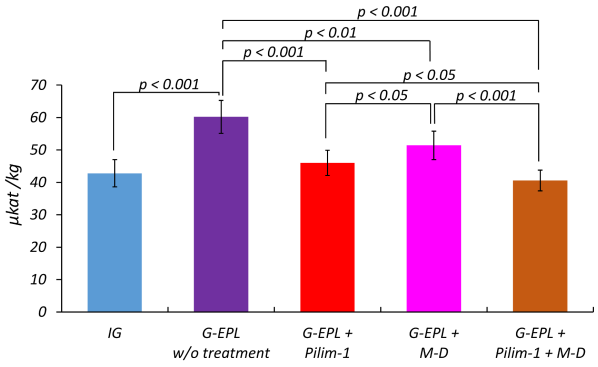


Figure 2. Comparative assessment of the influence of Pilim-1, M-D and the combination Pilim-1 + M-D on the activity of elastase in G-EPL in rats. ***Note:*** IG – intact gum, G-EPL – gum in endontodic-periodontal lesion, M-D – Metrogyl Denta®.

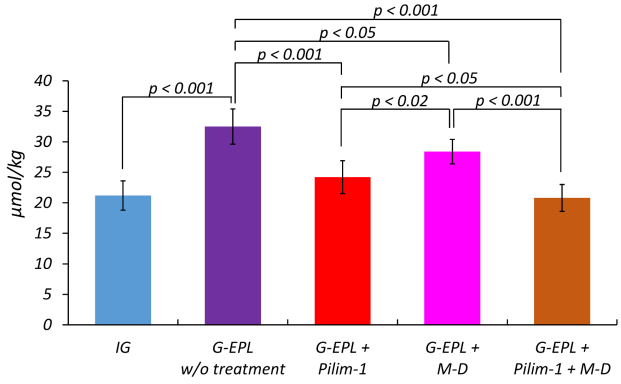


Figure 3. Comparative assessment of the influence of Pilim-1, M-D and combination Pilim-1 + M-D on the MDA level in G-EPL in rats. ***Note:*** IG – intact gum, G-EPL – gum in endontodic-periodontal lesion, MDA – malondialdehyde, M-D – Metrogyl Denta®.

MDA level

Similar changes (as described above for elastase) were observed when determining the MDA level in G-EPL. In G-EPL without treatment, the level of MDA, when compared to that in animals with the IG, increased 1.5 times, and, when applying Pilim-1, M-D and the combination Pilim-1 + M-D, it decreased 1.3, 1.1 and 1.6 times, respectively. It is noteworthy that the MDA level reaches the baseline (as in IG) only when using the combination Pilim-1 + M-D (Table 1, Fig. 3).

Iinfluence of Pilim-1 on the microbial content marker – the activity of urease – in G-EPL

The activity of urease in G-EPL without treatment was found to have increased 1.5 times when compared to that in IG.

The use of Pilim-1, M-D and the combination Pilim-1 + M-D caused a decrease in the urease activity by 1.3, 1.2 and 1.4 times, respectively. It is noteworthy that the activity of urease was almost comparable with that in IG only when Pilim-1 + M-D was applied (Table 1, Fig. 4).

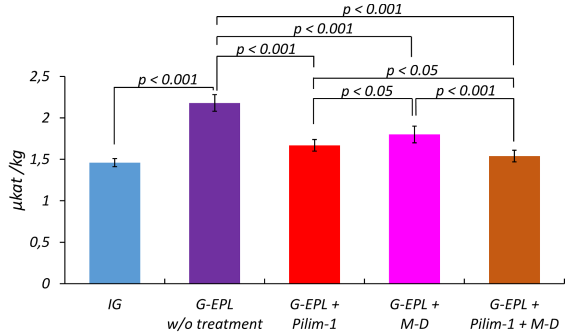


Figure 4. Comparative assessment of the influence of Pilim-1, M-D and the combination Pilim-1 + M-D on the urease activity in G-EPL in rats. ***Note***: IG – intact gum, G-EPL – gum in endontodic-periodontal lesion, M-D- Metrogyl Denta®.

Table 2. Influence of Pilim-1, M-D and the combination Pilim-1 + M-D on the catalase activity in G-EPL and API in rats (M ± m, n = 10)

Indicators and units of measurement	Groups of animals				
	IG [1]	G-EPL w/o treatment [2]	G-EPL + Pilim-1 [3]	G-EPL + M-D [4]	G-EPL+ Pilim-1+M-D [5]
Catalase, μkat/kg	9.6 ± 0.1 (9.4 ÷ 9.8)	6.5 ± 0.2 (6.0 ÷ 7.0) $p_{1-2} < 0.001$	8.7 ± 0.1 (8.5 ÷ 8.9) $p_{1-3} < 0.001$ $p_{2-3} < 0.001$	7.8 ± 0.1 (7.6 ÷ 8.0) $p_{1-4} < 0.001$ $p_{2-4} < 0.001$ $p_{3-4} < 0.001$	9.4 ± 0.2 (8.9 ÷ 9.9) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} < 0.01$ $p_{4-5} < 0.001$
API, RU	4.52 ± 0.16 (4.15 ÷ 4.89)	2.0 ± 0.08 (1.83 ÷ 2.17) $p_{1-2} < 0.001$	3.60 ± 0.11 (3.36 ÷ 3.84) $p_{1-3} < 0.001$ $p_{2-3} < 0.001$	2.75 ± 0.12 (2.48 ÷ 3.02) $p_{1-4} < 0.001$ $p_{2-4} < 0.05$ $p_{3-4} < 0.001$	4.52 ± 0.14 (4.20 ÷ 4.84) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} < 0.001$ $p_{4-5} < 0.001$

Note: 1. IG – intact gum, G-EPL – gum in endodontic-periodontal lesion, M-D – Metrogyl Denta®, API – antioxidant-prooxidant index. 2. In parentheses – confidence interval at $p < 0.05$, in square brackets – animal group number.

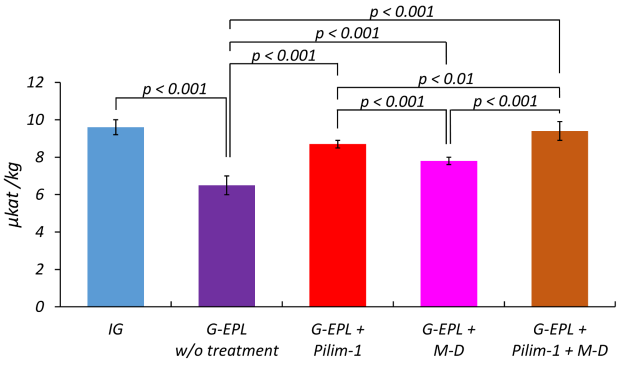


Figure 5. Comparative assessment of the influence of Pilim-1, M-D and combination Pilim-1 + M-D on the catalase activity in G-EPL in rats. ***Note***: IG – intact gum, G-EPL – gum in endontodic-periodontal lesion, M-D- Metrogyl Denta®.

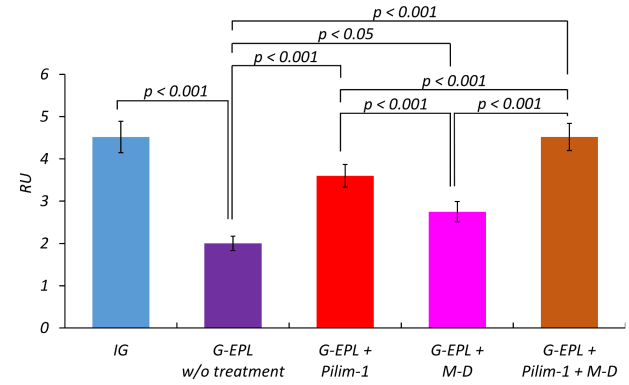


Figure 6. Comparative assessment of the influence of Pilim-1, M-D and combination Pilim-1 + M-D on the catalase activity in G-EPL in rats. ***Note***: IG – intact gum, G-EPL – gum in endontodic-periodontal lesion, API – antioxidant-prooxidant index, M-D- Metrogyl Denta®.

Influence of Pilim-1 on the activity of antioxidant enzyme catalase and API in G-EPL in rats

Catalase activity

The activity of catalase in G-EPL without treatment was found to have decreased 1.5 times when compared to that in IG.

The use of Pilim-1, M-D and the combination Pilim-1 + M-D induced an increase in the catalase activity 1.3, 1.2 and 1.4 times, respectively in comparison with such in G-EPL without treatment. It is noteworthy that the activity of catalase was almost comparable to such only when using the combination Pilim-1 + M-D (Table 2, Fig. 5).

API

The calculation of API showed that in EPL without treatment, this index decreased 2.3 times when compared to that in IG.

With the use of Pilim-1, M-D and the combination Pilim-1 + M-D, API when compared to that in G-EPL without treatment increased 1.8, 1.4 and 2.3 times, respectively. And, like in the case of catalase, when applying the combination Pilim-1 + M-D, API was almost comparable to that in IG (Table 2, Fig. 6).

Influence of Pilim-1 on the activity of elastase and urease in the pulp in EPL (P-EPL)

Elastase activity

The activity of elastase in P-EPL without treatment was found to have increased 1.5 times when compared to that in IP.

The use of Pilim-1 and reference drug M-D, as well as the combination Pilim-1 + M-D, caused a 1.5-, 1.4- and 1.7-time decrease in the elastase activity, respectively, when compared to that in P-EPL without treatment. These data indicate that Pilim-1, Pilim-1 + M-D, as well as reference drug M-D, reduce the activity of elastase to the level of that in IP. Nevertheless, Pilim-1 and especially the combination Pilim-1 + M-D are more preferable in terms of anti-inflammatory action than M-D, although only a trend was observed in this regard (no statistically significant differences were found). When comparing the indicators of elastase activity under the influence of Pilim-1 and M-D, no differences were found, but when comparing the activity of this enzyme under the influence of the combination Pilim-1 + M-D with reference drug M-D and Pilim-1, the first indicator statistically significantly exceeded the second and the third – 1.3 and 1.2 times, respectively (Table 3, Fig. 7).

Table 3. Influence of Pilim-1, M-D and the combination Pilim-1 + M-D on the activity of elastase and urease in P-EPL in rats (M ± m, n = 10)

Indicators and units of measurement	Groups of animals				
	IP [1]	P-EPL w/o treatment [2]	P-EPL + Pilim-1 [3]	P-EPL + M-D [4]	P-EPL+ Pilim-1+M-D [5]
Elastase, μkat/kg	62.0 ± 2.4 (56.6 ÷67.4)	95.0 ± 3.4 (87.5 ÷ 102.5) $p_{1-2} < 0.001$	64.0 ± 2.6 (58.1 ÷ 69.9) $p_{1-3} > 0.05$ $p_{2-3} < 0.001$	70.0 ± 3.2 (62.7 ÷ 77.3) $p_{1-4} > 0.05$ $p_{2-4} < 0.001$ $p_{3-4} > 0.05$	55.0 ± 3.0 (48.1 ÷ 61.9) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} < 0.05$ $p_{4-5} < 0.01$
Urease, μkat/kg	0.057±0.007 (0.042 ÷ 0.072)	0.116±0.012 (0.086 ÷ 0.146) $p_{1-2} < 0.001$	0.067±0.009 (0.047 ÷ 0.087) $p_{1-3} > 0.05$ $p_{2-3} < 0.001$	0.078±0.007 (0.063 ÷ 0.093) $p_{1-4} < 0.05$ $p_{2-4} < 0.002$ $p_{3-4} > 0.05$	0.052±0.008 (0.035 ÷ 0.069) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} > 0.05$ $p_{4-5} < 0.02$

Note: 1. IP – intact pulp, P-EPL – pulp in endodontic-periodontal lesion, M-D – Metrogyl Denta®, 2. In parentheses – confidence interval at $p < 0.05$, in square brackets – animal group number.

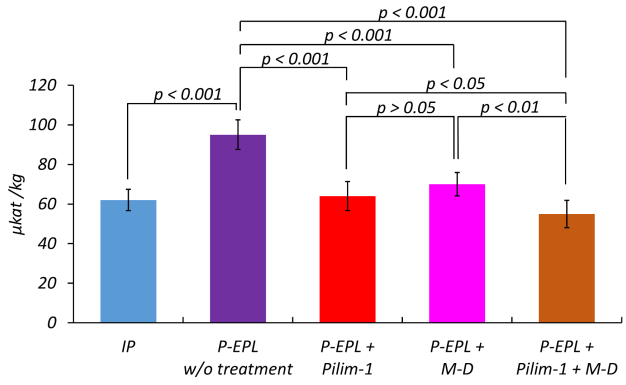


Figure 7. Comparative assessment of the influence of Pilim-1, M-D and combination Pilim-1 + M-D on the elastase activity in P-EPL in rats. ***Note:*** IP – intact pulp, P-EPL – pulp in endodontic-periodontal lesion, M-D – Metrogyl Denta®.

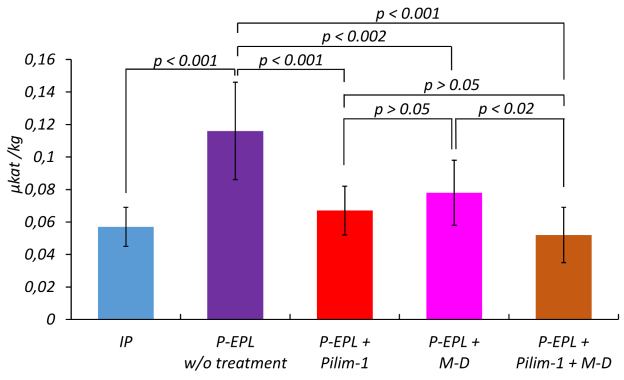


Figure 8. Comparative assessment of the influence of Pilim-1, M-D and the combination Pilim-1 + M-D on the urease activity in P-EPL in rats. ***Note***: IP – intact pulp, P-EPL – pulp in endodontic-periodontal lesion, M-D – Metrogyl Denta®.

Urease activity

The activity of urease in P-EPL without treatment was found to have increased 2 times when compared to that in IP, which indicates a significant microbial content in the pulp.

The use of Pilim-1, M-D and the combination Pilim-1 + M-D led to a 1.7-, 1.5- and 2.2-time decrease in urease activity, respectively. It is noteworthy that under the influence of Pilim-1 and, to a greater extent, the combination Pilim-1 + M-D, the activity of urease was almost comparable to that in IP, whereas the activity of this enzyme when using reference drug M-D was statistically significantly – 1.4 times – higher than that in IP. It is important to mention that no statistically significant differences between the urease activity when using the combination Pilim-1 + M-D and Pilim-1 were observed, although there was a tendency to its more pronounced decrease under the influence of the former (Table 3, Fig. 8).

Influence of Pilim-1 on markers of osteoblast and osteoclast – the activity of APP and AP – and their correlation – MI in P-EPL

APP activity

The studies have shown that the APP activity in P-EPL without treatment reduced 1.4 times when compared to that in IP.

The use of Pilim-1, M-D and the combination Pilim-1 + M-D caused a 1.6-, 1.3- and 1.7-time increase in the APP activity, respectively, when compared to that in P-EPL without treatment. The results obtained indicate that Pilim-1, Pilim-1 + M-D, as well as reference drug M-D increase the APP activity to the level of that in IP. When comparing the indicators of the APP activity under the influence of Pilim-1 and M-D, no differences were found, but when comparing the activity of this enzyme under the influence of the combination Pilim-1 + M-D with reference drug M-D and Pilim-1, the first indicator statistically significantly – 1.3 times – exceeded the second indicator and was almost comparable with the third, with a tendency to a more pronounced increase in the APP activity (Table 4, Fig. 9).

Table 4. Influence of Pilim-1, M-D and combination Pilim-1 + M-D on the activity of APP and AP, MI in P-EPL in rats (m ± m, n = 10)

Indicators and units of measurement	Groups of animals				
	IP [1]	P-EPL w/o treatment [2]	P-EPL + Pilim-1 [3]	P-EPL + M-D [4]	P-EPL+ Pilim-1+M-D [5]
APP, μkat/kg	1050 ± 90 (847 ÷ 1253)	750 ± 80 (569 ÷ 931) $p_{1-2} < 0.05$	1170 ± 90 (967 ÷ 1373) $p_{1-3} > 0.05$ $p_{2-3} < 0.01$	1010 ± 60 (875 ÷ 1145) $p_{1-4} > 0.05$ $p_{2-4} < 0.02$ $p_{3-4} > 0.05$	1280 ± 70 (1123 ÷ 1437) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} > 0.05$ $p_{4-5} < 0.01$
AP, μkat/kg	29.46 ± 1.52 (26.06 ÷ 32.86)	48.32 ± 3.24 (41.02 ÷ 55.62) $p_{1-2} < 0.001$	31.56 ± 1.24 (28.76 ÷ 34.36) $p_{1-3} > 0.05$ $p_{2-3} < 0.001$	35.76 ± 1.38 (32.66 ÷ 38.86) $p_{1-4} < 0.01$ $p_{2-4} < 0.002$ $p_{3-4} < 0.05$	30.05 ± 1.42 (26.85 ÷ 33.25) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} > 0.05$ $p_{4-5} < 0.01$
MI, RU	35.6 ± 2.4 (30.2 ÷ 41.0)	15.5 ± 2.8 (9.1 ÷ 21.9) $p_{1-2} < 0.001$	37.1 ± 2.2 (32.2 ÷ 42.0) $p_{1-3} > 0.05$ $p_{2-3} < 0.001$	28.2 ± 2.5 (22.6 ÷ 33.8) $p_{1-4} < 0.05$ $p_{2-4} < 0.001$ $p_{3-4} < 0.02$	42.6 ± 2.6 (36.7 ÷ 48.5) $p_{1-5} > 0.05$ $p_{2-5} < 0.001$ $p_{3-5} < 0.05$ $p_{4-5} > 0.001$

Note: 1. IP – intact pulp, P-EPL – pulp in endodontic-periodontal lesion, M-D – Metrogyl Denta®, APP – alkaline phosphatase, AP – acidic phosphatase, MI – mineralizing index. 2. In parentheses – confidence interval at p <0.05, in square brackets – animal group number.

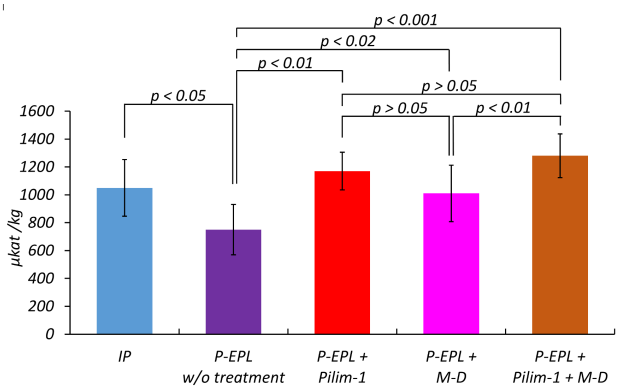


Figure 9. Comparative assessment of the influence of Pilim-1, M-D and combination Pilim-1 + M-D on APP activity in P-EPL in rats. ***Note:*** IP – intact pulp, P-EPL – pulp in endodontic-periodontal lesion, APP – alkaline phosphatase, M-D – Metrogyl Denta®.

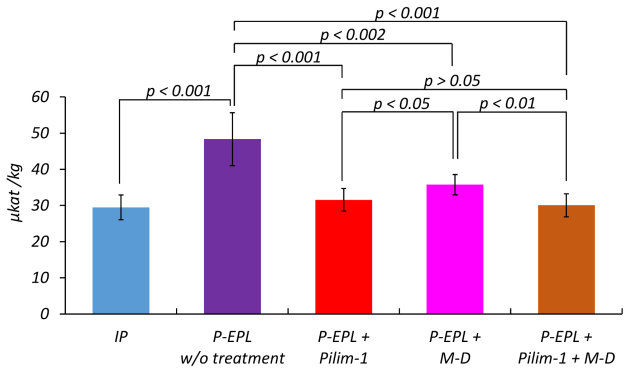


Figure 10. Comparative assessment of the influence of Pilim-1, M-D and combination Pilim-1 + M-D on AP activity in P-EPL in rats. ***Note***: IP – intact pulp, P-EPL – pulp in endodontic-periodontal lesion, AP – acidic phosphatase, M-D – Metrogyl Denta®.

AP activity

AP activity in P-EPL without treatment was found to have increased 1.6 times when compared to that in IP.

The use of Pilim-1, M-D, as well as the combination Pilim-1 + M-D caused a 1.4-, 1.5- and 1.6-time decrease in the AP activity, respectively, when compared to that in P-EPL without treatment. It is noteworthy that the AP activity was almost comparable to that in IP only when using Pilim-1 and the combination Pilim-1 + M-D. In addition, it is worth mentioning that the AP activity when using Pilim-1 was comparable to that when using the combination Pilim-1 + M-D. It is also important to emphasize that Pilim-1 and, especially, the combination Pilim-1 + M-D statistically significantly – 1.1 and 1.2 times, respectively – exceeded the reference drug M-D in inhibiting AP. As for the influence of Pilim-1 and the combination Pilim-1 + M-D on the AP activity, no statistically significant differences were found between them (Table 4, Fig. 10).

Mineralizing index (MI)

When calculating MI, this indicator in EPL without treatment was found to have decreased 2.3 times when compared to that in IP.

The use of Pilim-1, M-D, as well as the combination Pilim-1 + M-D induced a 2.4-, 1.8- and 2.7-time increase in MI, respectively, when compared to that in P-EPL without treatment. It is important to note that MI was almost comparable to that in IP when using Pilim-1 and the combination Pilim-1 + M-D. At the same time, when using Pilim-1 and the combination Pilim-1 + M-D, MI indicators were not significantly different. Besides, Pilim-1 and, especially, the combination Pilim-1 + M-D statistically significantly – 1.3 and 1.5 times, respectively – exceeded reference drug M-D in terms of MI (Table 4, Fig. 11).

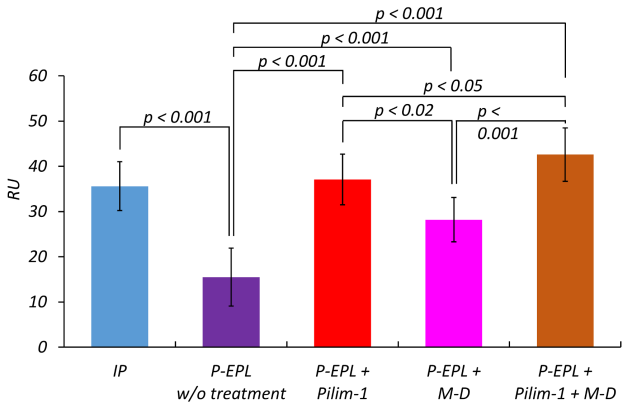


Figure 11. Comparative assessment of the influence of Pilim-1, M-D and combination Pilim-1 + M-D on MI in P-EPL in rats. ***Note:*** IP – intact pulp, P-EPL – pulp in endodontic-periodontal lesion, MI – mineralizing index, M-D – Metrogyl Denta®.

Discussion

Pronounced anti-inflammatory, antimicrobial (a decreased microbial content), antioxidant and mineralizing effects of Pilim-1 in D-EPL and P-EPL can be due to the inhibitory effect of this substance on cyclooxygenase and 5-lipoxygenase (Galenko-Yaroshevsky et al. 2024), as well as to Pilim-1 having antihypoxic, membrane-stabilizing, bacteriostatic and reparative properties resulting from the combined action of its structural components – N-isopropenylimidazole and zinc (Shakhmardanova and Galenko-Yaroshevsky 2015, 2016).

The synthetic derivatives of imidazole, including a number of drugs – metronidazole, naphthyzin, phentolamine, ethimizol, ketoconazole, mebendazole, mercazolil, mercaptopurine, etc. – are known to exert high anti-inflammatory, analgesic, antimicrobial and other types of activity (Fadhil and Qhanim 2020; Gas et al. 2020; Rajam et al. 2020; Valls et al. 2020; Vidhya and Malar 2020; Gurevich et al. 2021; Pham et al. 2021; Patel et al. 2022; Kaldybaeva et al. 2023).

Zinc, as a trace element, participates in the formation of antioxidant status and stabilization of cellular structures (Panasenko et al. 2018), has an anti-inflammatory and antibacterial effect, which involves the suppression of pro-inflammatory cytokines – TNF-D, IL-1β, IL-6, IL-8, IL-12 – by reducing the activation of NF-kB (nuclear factor-kappa B), which is involved in the regulation of genes expression affecting the functioning of the immune system and inflammatory reactions. Moreover, an important role in the mechanism of the anti-inflammatory action of zinc is played by its ability to increase the expression of zinc finger protein A20, known as tumor necrosis factor, alpha-induced protein 3 or A20, which is the central regulator of the NF-kB (Liu et al. 2017; Voelkl et al. 2018; Ho et al. 2020; Nakano et al. 2020; Tobita et al. 2020; Lebedeva et al. 2023).

It is worth mentioning that the suppression of the inflammatory process may be due to zinc inhibiting the activity of serine kinase IKKβ (inhibitory kappa B kinase beta) in the cytosol (Liu et al. 2013; Gammoh and Rink 2017; Notov et al. 2022).

The Pilim-1-caused reduction of impaired bone mineralization in EPL can be due to the fact that zinc contained in Pilim-1, along with copper, manganese, and calcium, through accumulating in the bone system (about 22%), participates in the bone-forming activity of osteoblasts (Persicov and Brodsky 2002; Zakharova et al. 2012; Trisvetova 2021).

The capacity of combination Pilim-1 + M-D exert a more pronounced anti-inflammatory, antimicrobial (a decrease in microbial content), antioxidant and mineralizing effects in the gums and pulp, compared to that of Pilim-1 and M-D, can be due with the synergetic effect of the components. But further in-depth studies are needed to confirm this assumption.

From the results of the studies, it can be concluded that Pilim-1 and, to a greater extent, the combination Pilim-1 + M-D have pronounced anti-inflammatory, antioxidant, antimicrobial (reduce microbial content) and mineralizing effects in D-EPL and P-EPL in rats, surpassing reference drug M-D in this respect.

Conclusion

The presented results of the study of Pilim-1 and its combination with M-D indicate their high therapeutic efficiency, especially when using the latter, in experimental EPL in rats, which makes it possible to view Pilim-1 and Pilim-1 + M-D as a potential therapeutic agent for treating EPL.

Conflict of interest

The authors declare the absence of a conflict of interests.

Data availability

All of the data that support the findings of this study are available in the main text.

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