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The effect of a new 3-hydroxypyridine derivative LHT-2-20 on free radical oxidation in experimental periodontitis

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ABSTRACT

Aim. To study the effect of a new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) on free radical oxidation in experimental periodontitis.

Materials and methods. The experimental study was performed on 195 white mongrel mice weighing 19–23 g and 137 white mongrel rats weighing 180–220 g. The effect of a new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) on the intensity of free radical oxidation and the local state of periodontal tissues during a course of intragastric administration was studied on the experimental model of periodontitis. Statistical processing of the results was carried out using the SPSS Statistics 20.0 software package with the analysis of variance (ANOVA) and the parametric Tukey's test.

Results. The LHT-2-20 compound reduced elevated levels of primary and secondary lipoperoxidation products (conjugated dienes, malondialdehyde in plasma and in erythrocytes during spontaneous and iron-induced oxidation) already at the early stages of the experiment, bringing the studied parameters closer to the reference values by the end of the course of treatment. The use of the compound LHT-2-20 contributed to an increase in the activity of the main antioxidant enzymes (catalase and superoxide dismutase), normalizing them to baseline values by the end of the experiment. With the correction of free radical processes, the use of LHT-2-20 limited the local inflammatory response in periodontal tissues, which was confirmed by a decrease in gingival edema and hyperemia, bleeding, depth of periodontal pockets, and tooth mobility.

Conclusion. The results of this study confirm the anti-inflammatory potential of the compound and the multiplicity of its effects due to the impact on the mechanisms of oxidative stress. The expediency of further study of the drug is justified by the prospect of creating a new drug and its subsequent wide clinical application as part of the complex therapy of periodontal inflammation.

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Влияние нового производного 3-гидроксипиридина ЛХТ-2-20 на процессы свободнорадикального окисления при экспериментальном пародонтите

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РЕЗЮМЕ

Цель исследования: изучение влияния нового комплексного соединения ЛХТ-2-20 (2-этил-6-метил-3-гидроксипиридиния-2-(3-бензоилфенил)-пропаноата) на процессы свободнорадикального окисления при экспериментальном пародонтите.

Материалы и методы. Экспериментальное исследование выполнено на 195 белых беспородных мышах массой 19–23 г, 137 белых беспородных крысах массой 180–220 г. На модели экспериментального пародонтита изучено влияние нового комплексного соединения ЛХТ-2-20 (2-этил-6-метил-3-гидроксипиридиния-2-(3-бензоилфенил)-пропаноата) на интенсивность свободнорадикального окисления и местное состояние тканей пародонта при курсовом внутрижелудочном его применении. Статистическая обработка результатов проводилась с использованием программного комплекса SPSS Statistics 20.0 с применением дисперсионного анализа (ANOVA) и параметрического критерия Тьюки.

Результаты. Соединение ЛХТ-2-20 снижало повышенные уровни первичных и вторичных продуктов липопероксидации (диеновых конъюгатов, малонового диальдегида в плазме и в эритроцитах при спонтанном и железоиндуцированном окислении) уже в ранние сроки эксперимента, приближая исследуемые показатели к референсным значениям к концу курсового лечения. Применение соединения ЛХТ-2-20 способствовало росту активности основных энзимов антиоксидатной системы (каталазы и супероксидисмутазы), нормализуя данные показатели к концу эксперимента до исходных значений. На фоне коррекции свободнорадикальных процессов применение ЛХТ-2-20 способствовало ограничению местной воспалительной реакции тканей пародонта, что подтверждалось уменьшением отека и гиперемии десневого края,

кровоточивости, глубины пародонтальных карманов и степени подвижности зубов.

Заключение. Результаты данного исследования подтверждают противовоспалительный потенциал соединения и множественность его эффектов за счет воздействия на механизмы оксидативного стресса. Целесообразность последующего изучения средства обоснована перспективой создания нового лекарственного препарата и его последующего широкого клинического применения в составе комплексной терапии воспалительных процессов тканей пародонта.

Ключевые слова: производные 3-гидроксипиридина, пародонтит, оксидативный стресс, кровоточивость, патологическая подвижность

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источники финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Currently, periodontal diseases represent an important medical and social problem which is due to the widespread prevalence of this pathology. Globally, chronic periodontitis ranks 11th among all dental diseases [1]. A similar trend can be observed in the Russian Federation where the assessment of dental morbidity among the adult population revealed high prevalence of inflammatory periodontal diseases (89%), with the peak of morbidity (94.3%) occurring at the age of 40–45 years [2]. Therefore, chronic periodontitis is characterized by a marked trend toward an increase in morbidity rates [1].

Activation of free radical oxidation processes and inhibition of the antioxidant system (AOS) are of great importance in the pathogenesis of inflammatory and destructive periodontal diseases [3]. As a result, an imbalance develops between the prooxidant and antioxidant systems, leading to increased production of reactive oxygen species (ROS). These pathological changes predetermine the emergence of oxidative stress, characterized by increased production of ROS and destruction of cells [4]. Emerging disorders of oxidative homeostasis and capillary blood flow and increased vascular permeability contribute to the formation of local inflammatory changes in the periodontium, characterized by gingival hyperemia, hemorrhagic manifestations, and a pathological increase in the depth of periodontal pockets followed by pathological tooth mobility [5, 6].

The pronounced prevalence of chronic periodontitis, its continuous recurrent course, and an increase in the number of disease forms with complications demand the development of new methods for the treatment of this pathology [7]. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) used in various forms are components in the treatment of chronic periodontitis [8]. However, despite their pronounced anti-inflammatory effect, this class of drugs has limited use due to frequent complications resulting from their ulcerogenic activity [9]. In addition, NSAIDs have low antioxidant, membrane protective, and antihypoxic activity, which is manifested by their inability to correct the imbalance between two interdependent systems in order to increase antioxidant potential [8]. The above disadvantages make it necessary to include drugs with complex antioxidant and anti-inflammatory effects in periodontal therapy, which will contribute to the potentiation of therapeutic effects and leveling of adverse drug reactions.

The aim of the research was to study the effect of a new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) on free radical oxidation in experimental periodontitis.

MATERIALS AND METHODS

The experimental study was performed on 195 white mongrel mice weighing 19–23 g and 137 white mongrel rats weighing 180–220 g divided into 5 groups. All animals were obtained from the SMK STEZAR bionursery (Vladimir, Russia) and were kept under laboratory conditions at standard temperature and air humidity. The study was carried out in accordance with the requirements of the European Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes and the rules of Good Laboratory Practice (Order No. 267 of the Ministry of Health of the Russian Federation of 19.06.2003). The experimental study was approved by the Ethics Committee (Protocol No. 102 of 31.01.2021).

A new complex compound 2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate (laboratory name LHT-2-20) was synthesized at the Department of Chemistry and Technology of Synthetic Drugs and Analytical Control of the All-Union Research Center for Safety of Biologically Active Compounds (Patent of the Russian Federation for invention No. 2793537) [10]. The NSAIDs ketoprofen (2-(3-benzoylphenyl)-propanoic acid, Velpharm, Russia), which has anti-inflammatory effects, and mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate, Pharmacoft, Russia), which has antioxidant effects, were selected as reference-listed drug.

The model of experimental periodontitis was reproduced in accordance with the patented method developed by K.D. Shkolnaya et al. [11]. Prednisolone at a dose of 12 mg / kg was administered to the rats on days 1, 3, and 5. On day 5 of the experiment, under general anesthesia with 0.03 ml of zoletil 100 (Virbac Sante Animale, France) intramuscularly, the interdental papilla between the first and second maxillary molars was sutured with suture fixation and filled with Filtek Bulk Fill (3M, USA) from the vestibular side.

Group 1 included intact rats ($n = 15$) with a healthy periodontium. In group 2 (control; $n = 30$), the model of periodontitis without treatment was reproduced. Animals in groups 3 and 4 ($n = 30$ in each group) were administered ketoprofen and mexidol at doses corresponding to 2% and 5% of the LD_{50} value, respectively. Rats in group 5 ($n = 32$) received LHT-2-20 at a dose of 11.54 mg / kg corresponding to 2% of the LD_{50}. After preliminary dissolution in 1.5 ml of starch, the studied compounds were administered intragastrically (i.g.) in a volume of 1.5 ml once a day for 10 days. The animals were euthanized under isoflurane anesthesia by decapitation on day 25.

Acute toxicity of LHT-2-20 was studied in white mongrel mice of both sexes divided into groups of 5 animals each. After preliminary dissolution in starch, the compound was administered i.g. in a volume of 0.3 ml at increasing concentrations. LD_{50} was calculated using the Litchfield and Wilcoxon's method.

Free radical processes in plasma were assessed by biochemiluminescence on the Fluorate–02-ABLF-T biochemical analyzer (Promecolab, Saint Petersburg), by the level of primary and secondary lipid peroxidation products – conjugated dienes (CD), determined in blood plasma by the modified Placer method (1976), as well as by the level of malondialdehyde (MDA) in plasma and erythrocytes in spontaneous (MDA) and iron-induced oxidation (Fe-MDA), determined by the method proposed by S.G. Konyukhova (1989). Antioxidant potential was studied by the activity of catalase (CAT) in plasma and erythrocytes determined in accordance with the method developed by M.A. Korolyuk (1988) and by the activity of superoxide dismutase (SOD) in plasma determined by the method proposed by E.E. Dubinina (1983).

Local periodontal changes were identified based on the state of the oral mucosa; the severity of bleeding gums (0–3 points); grades of tooth mobility (grade 0–2); and the depth of periodontal pockets (mm).

Statistical processing of the results was carried out using the SPSS Statistics 20.0 software package. The data were presented as the mean and the standard deviation ($M \pm \sigma$), absolute and relative values (n (%)), and a 95% confidence interval (CI). The normality of data distribution was checked using the Shapiro – Wilk test. Intergroup comparisons were conducted using the two-tailed Fisher's exact test and the analysis of variance (ANOVA) with the Tukey's post hoc test. The correlation analysis was carried out using the Spearman's rank correlation coefficient. The differences were considered statistically significant at $p < 0.001$.

RESULTS

Determination of acute toxicity of LHT-2-20

LD_{50} of LHT-2-20 in mice with i.g. administration of the compound was 1,130 mg / kg. The result shows that the compound is 2.97 times less toxic

than ketoprofen, but 1.88 times more toxic than mexidol (Table 1).

Table 1

Acute toxicity of LHT-2-20 in mice

Substance (compound)	LD_{50}, mg / kg	95% CI
Ketoprofen	380	358–402
Mexidol	2,120	2,010–2,230
LHT-2-20	1,130 [A B]	1,040–1,220

[A] – the differences are significant compared to ketoprofen; [B] – the differences are significant compared to mexidol ($p = 0.001$, one-way analysis of variance (ANOVA) with the Tukey's test).

Anti-inflammatory and antioxidant effects of LHT-2-20 in experimental periodontitis

LHT-2-20 administered i.g. suppressed free radical oxidation, which was confirmed by the following changes. The plasma CD level was 50% lower ($p_1 < 0.001$) compared to the control group, thereby approaching intact values. In addition, compared to the ketoprofen and mexidol groups, it was 73.3% ($p_2 < 0.001$) and 26.7% lower ($p_3 = 0.344$), respectively. The MDA values in plasma and erythrocytes during spontaneous oxidation in the LHT-2-20 group were 6.2 ± 0.6 and 9.6 ± 0.6 μmol / l, which is 32.6% ($p_1 = 0.46$) and 28.1% ($p_1 = 0.002$) lower than the control values, respectively.

Fe-MDA levels in plasma and erythrocytes were 37.6% ($p_1 < 0.001$) and 29.5% lower ($p_1 = 0.124$) than the control values, respectively. Compared to i.g. administration of ketoprofen and mexidol, plasma MDA in the group receiving the new compound was 42.9% ($p_2 < 0.001$) and 8.8% ($p_3 = 0.417$) lower, plasma Fe-MDA was 55.6% ($p_2 < 0.001$) and 22.7% ($p_3 < 0.001$) lower, MDA in erythrocytes was 34% ($p_2 < 0.001$) and 17.5% ($p_3 = 0.328$) lower, and Fe-MDA in erythrocytes was 40.4% ($p_2 < 0.001$) and 16.8% ($p_3 < 0.001$) lower, respectively. Moreover, on day 25 of the experiment, more effective restoration of the antioxidant potential was revealed in the LHT-2-20 group: CAT activity in plasma was 0.89 ± 0.06, which is 23.9% ($p_2 < 0.001$) and 10.3% ($p_3 = 0.035$) higher than the same parameter in the ketoprofen and mexidol groups, respectively.

CAT in erythrocytes was 16.8% ($p_2 < 0.001$) and 7.9% higher ($p_3 = 0.026$), and SOD activity in plasma was 39.7% ($p_2 < 0.001$) and 22.1% higher ($p_3 < 0.001$), compared to groups 3 and 4, respectively. When conducting a biochemiluminescence study of plasma with the use of the new compound, the I_{max} and S values were 27.2% ($p_1 < 0.001$) and 37.2% lower ($p_1 < 0.001$), compared to the control values, respectively. The I_{max} value was 31.9% ($p_2 < 0.001$) and 6.0% lower ($p_3 = 0.596$), and S was 35.5% ($p_2 < 0.001$) and 10.2% lower ($p_3 = 0.001$), compared to the ketoprofen and mexidol groups, respectively (Table 2).

Table 2

Effect of LHT-2-20 on free radical processes in experimental periodontitis, $M \pm \sigma$

Parameter	Group 1 ($n = 15$)	Experimental groups			
		Group 2 ($n = 30$)	Group 3 ($n = 30$)	Group 4 ($n = 30$)	Group 5 ($n = 32$)
CD in plasma, U / ml	0.15 ± 0.02	0.34 ± 0.05*	0.29 ± 0.05	0.22 ± 0.04*^	0.18 ± 0.04*^A
Plasma MDA, mmol / l	4.89 ± 0.21	9.12 ± 0.71	8.25 ± 0.54*	6.58 ± 0.52*^	6.15 ± 0.53*^A
Fe-MDA in plasma, mmol / l	9.56 ±0.44	19.80 ± 1.52	17.68 ± 0.69*^	14.53 ± 0.63*^	12.36 ± 0.51*^AB
MDA in erythrocytes, mmol / l	8.05 ± 0.49	13.36 ± 0.71*	12.34 ± 0.88*	10.18 ± 0.59*^	9.60 ± 0.58*^A
Fe-MDA in erythrocytes, mmol / l	17.49 ± 1.42	30.26 ± 1.01	28.41 ± 0.86*^	24.28 ± 0.87*^	21.33 ± 0.82*^AB
CAT in plasma, kcat / s·l	1.17 ± 0.13	0.51 ± 0.08*	0.62 ± 0.07*	0.77 ± 0.08*^	0.89 ± 0.06*^A
CAT in erythrocytes, mkcat / s·l	2.27 ± 0.13	1.29 ± 0.11*	1.59 ± 0.13*^	1.79 ± 0.10*^	1.97 ± 0.14*^A
SOD in plasma, AU	1.31 ± 0.12	0.52 ± 0.08*	0.65 ± 0.08*	0.88 ± 0.07*^A	1.17 ± 0.06^AB
I_{max} in plasma, mV / sec	1.85 ± 0.14	3.53 ± 0.23*	3.16 ± 0.17*^	2.68 ± 0.17*^	2.57 ± 0.14*^A
S in plasma, mV / sec	26.77 ± 1.06	48.80 ± 1.76*	40.18 ± 1.73*^	33.39 ± 1.29*^	30.66 ± 1.63*^A

* – significance compared to reference values ($p < 0.001$); ^ – significance compared to control values ($p_1 < 0.001$); [A] – significance compared to the values of the ketoprofen group ($p_2 < 0.001$); [B] – significance compared to the values of the mexidol group ($p_3 < 0.001$) (one-way analysis of variance (ANOVA), Tukey's test).

When assessing the state of the oral cavity in the laboratory animals of the control group, pronounced gingival hyperemia was determined. Heavy bleeding, manifested immediately after probing (3 points), as well as an increase in tooth mobility up to grade 2 was detected in 30 rats of the control group (100%). When determining the depth of periodontal pockets, we noted their increase by 1.3 ± 0.2 mm ($p < 0.001$). These changes indicate the existing inflammatory and degenerative changes in periodontal tissues leading to the formation of pathological tooth mobility and tooth loss.

By the end of the study, the examination of the oral cavity in the ketoprofen and groups revealed a decrease in the severity of local signs of inflammation, which was manifested by a decrease in gingival edema and hyperemia. The determined depth of periodontal sulcus probing was less than the control values in the context of ketoprofen administration (by 40% ($p_1 < 0.001$)) and intragastric mexidol administration (by 32.1% ($p_1 < 0.001$)). When assessing bleeding on a 3-point scale, the following dynamics was determined in the ketoprofen group: 1 rat (3.3%) scored 1 point, 21 rats (70%) – 2 points, and 8 rats (26.7%) – 3 points. In the mexidol group, 1 rat (3.3%) scored 1 point, 18 rats (60%) – 2 points, and 11 rats (36.7%) – 3 points. We also assessed the grade of pathological tooth mobility during ketoprofen treatment and revealed that 6 rats (20%) had grade 1, and 24 rats (80%) had grade 2 mobility. During mexidol therapy, 14 rats (46.7%) and 16 rats (53.3%) had grades 1 and 2, respectively. The results obtained indicate a decrease in the percentage of animals with bleeding gums of 2–3 points and grade 1–2 pathological tooth mobility. However, despite the positive dynamics, these changes indicate insufficient effectiveness of the studied compounds in experimental periodontitis.

In the LHT-2-20 group, a pronounced decrease in the intensity of inflammatory changes was determined as evidenced by the absence of gingival hyperemia. Using a button probe, we determined a decrease in the severity of hemorrhagic manifestations. We detected 1 and 2 points in 24 rats (75%) and 8 rats (25%), respectively; animals with intense bleeding gums (3 points) were not detected. When assessing the severity of damage to the tooth-supporting apparatus by the degree of pathological toot mobility, grade 0 was determined in 12 rats (37.6%), grade 1 – in 20 rats (62.4%), and grade 2 was not diagnosed in the studied groups of rats. Assessing the depth of the periodontal pocket by probing revealed that it was 67.2% less ($p_1 < 0.001$) compared to the control group (0.4 ± 0.01 mm), which is 27.8% ($p_2 < 0.001$) and 35.7% ($p_3 < 0.001$) less compared to the ketoprofen and mexidol groups, respectively (Table 3).

Table 3

The effect of LHT-2-20 on the parameters of the local state of periodontium in experimental periodontitis

Parameter	Group 1 (n = 15)	Group 2 (n = 30)	Group 3 (n = 30)	Group 4 (n = 30)	Group 5 (n = 32)
Depth of periodontal pockets, mm, $M \pm \sigma$	0.3 ± .1	1.3 ± 0.2*	0.8 ± 0.02*^	0.9 ± 0.02*^^A	0.4 ± 0.01*^AB
Bleeding gums, points, n (%):					
1	15 (100%)	0 (0%)*	1 (3.3%)*	1 (3.3%)*	24 (75%)^ AB
2	0 (0%)	0 (0%)	21 (70%)*^	18 (60%)*^	8 (25%)*^ AB
3	0 (0%)	30 (100%) *	8 (26.7%)*^	11 (36.7%)*^	0 (0%) ^ AB
Tooth mobility, grade, n (%):					
0	15 (100%)	0 (0%)*	0 (0%)*	0 (0%)	12 (37.6%) *^ AB
1	0 (0%)	0 (0%)	6 (20%)*^	14 (46.7%)^ A	20 (62.4%)*^ AB
2	0 (0%)	30 (100%)*	24 (80%)*^	16 (53.3%)^ A	0 (0%) ^ AB

* – significance compared to the reference values ($p < 0.001$); ^ – significance compared to the control values ($p_1 < 0.001$); A – significance compared to the values in the ketoprofen group ($p_2 < 0.001$); B – significance compared to the values in the mexidol group ($p_3 < 0.001$) (one-way analysis of variance (ANOVA), Tukey's test, Fisher's exact test).

The correlation analysis revealed a positive correlation between the parameters of free radical oxidation and the data on the local status of periodontal tissues. A strong correlation was revealed between CD, MDA, and Imax biochemiluminescence values in plasma and parameters of gum bleeding, depth of periodontal pockets, and grade of tooth mobility. A very strong correlation was detected between the values of Fe-MDA and biochemiluminescence S in plasma

and the grade of tooth mobility. The assessment of the relationship between the levels of the main antioxidant enzymes and the parameters of the periodontal tissue revealed a negative correlation. Thus, a strong correlation was revealed between the levels of CAT and SOD in plasma and data on local periodontal tissue status, except for the SOD value and the grade of tooth mobility, whose correlation was very strong, thereby indicating the interrelation between the studied parameters (Table 4).

Table 4

Correlation between some parameters of free radical oxidation, the antioxidant system, and local periodontal tissue status in experimental periodontitis in rats

Parameter	Spearman's rank correlation coefficient						
	Plasma MDA, mmol / l	Fe-MDA in plasma, mmol / l	CD in plasma, U / ml	CAT in plasma, kcat / s·l	SOD in plasma, AU	I max in plasma, mV / sec	S in plasma, mV / sec
Bleeding gum, points	0.71	0.83	0.67	–0.74	–0.73	0.75	0.88
Depth of periodontal pockets, mm	0.76	0.87	0.73	–0.82	–0.88	0.77	0.89
Tooth mobility, grade	0.85	0.94	0.73	–0.84	–0.95	0.84	0.93

DISCUSSION

As a result of the study, it was found that free radical oxidation processes play an important role in the pathogenesis of experimental periodontitis, contributing to the formation of local inflammatory changes in periodontal tissues.

During the experiment, we observed enhanced free radical oxidation determined by biochemiluminescence data and the level of lipid peroxidation products in plasma and red blood cells, associated with a strong correlation with the parameters of the local status of periodontal tissues. Oxidative stress and the inflammatory response at the local and systemic levels are interconnected and involved in periodontal tissue damage during the development of periodontitis.

The course of ketoprofen therapy did not allow to inhibit free radical oxidation at the systemic level, as evidenced by the preservation of a high level of lipid peroxidation parameters compared to reference values. At the same time, the treatment course limited the inflammatory process in periodontal tissues, which was confirmed by the trend toward decreased values of the corresponding parameters by the end of the experiment, and improved the local state of periodontal tissues.

During mexidol therapy, more effective inhibition of free radical damage mechanisms was determined compared to the control group. However, no significant differences were observed compared to the ketoprofen group.

The use of a new complex compound 2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate led to the inhibition of free radical processes and activation of the antioxidant system, contributing to the normalization of the studied parameters to the values in the intact animals. Along with correction of free radical oxidation processes, we observed elimination of the local inflammatory response and a decrease in the destructive processes in periodontal tissues.

CONCLUSION

In experimental periodontitis, an increase in the activity of free radical oxidation processes was determined with a simultaneous decrease in the antioxidant enzyme potential in erythrocytes and blood plasma. The data obtained indicate the development of oxidative stress along with an increasing local and systemic inflammatory response which promotes destructive processes in the connective tissue matrix of the periodontium and contributes to the progression of the disease.

In the rats with experimental periodontitis, the new complex compound LHT-2-20 (2-ethyl-6-methyl-3-hydroxypyridine-2-(3-benzoyl phenyl)-propanoate) administered intragastrically at a dose of 11.54 mg / kg per day for 10 days led to a significant decrease in the levels of lipid peroxidation parameters and a decrease in the severity of local inflammatory changes in periodontal tissues by the end of the experiment. The results obtained confirm the presence of anti-inflammatory and antioxidant activity of the compound and also justify the relevance of its further study to create a drug for

the complex therapy of inflammatory periodontal diseases.

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Authors' contribution

Poryadin G.V. – conception and design, analysis and interpretation of the data, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Zakhvatov A.N., Skachilova S.Ya., Yasnetsov V.V. – conception and design, analysis and interpretation of the data, drafting and editing of the manuscript, final approval of the manuscript for publication. Khaydar D.A. – drafting and editing of the manuscript, final approval of the manuscript for publication. Tarasova T.V., Zakharkin I.A., Parshina A.Yu., Simakina E.A. – analysis and interpretation of the data, drafting and editing of the manuscript, final approval of the manuscript for publication.

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