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The Effect of Selenase on Inflammatory and Cytoprotective Markers in Experimental Chronic Generalized Periodontitis

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Epidemiological studies in recent decades have revealed a significant increase in the number of patients with periodontal diseases leading to tooth loss. Modern realities require improvement of drug treatment of periodontitis. The antioxidant Selenase, selenium derivative, is an interesting treatment strategy for periodontitis. The study was carried out with the aim to evaluate the healing effectiveness of Selenase in rats with chronic generalized periodontitis (CGP) by its effect on markers of inflammation and cytoprotection. Experimental CGP was modulated in Wistar rats by a calcium-deficient diet with the inclusion of a prooxidant. Selenase (50 mcg/kg) and Mexidol (ethylmethylhydroxypyridine succinate, 250 mg/kg) were administered intragastrically for 30 days. Levels of IL-1â, HIF-1á, HSP_{70}, and TNF-á were determined in the blood after treatment using the enzyme immunoassay method. Experimental CGP was characterized by the development of hyperemia, swelling, and bleeding of the gums; mobility of teeth; and gingival pockets up to 8 mm against the background of increased inflammatory markers (IL-1â, TNF-á), and molecular markers of cytoprotection (HIF-1á, HSP_{70}) in the blood, indicating a homeostatic response of the periodontium in response to inflammation and subsequent hypoxia. Administration of Selenase to rats with CGP produced pronounced healing effects: the reduction in the depth of periodontal pockets by 42.55 %, cessation of bleeding, and disappearance of swelling against the background of a decrease of inflammatory markers: IL-1â – by 44.6 %, and TNF-á – by 65.9 % ($p < 0.05$). HIF-1á increased by 36.8 %, and HSP_{70} – by 71.1 % compared to those of the control group, which was not given the treatment ($p < 0.05$). The results obtained suggest a significant influence of Selenase on HSP_{70}-dependent mechanisms of endogenous cytoprotection. The results of the study found that the use of Selenase in experimental CGP is more effective than Mexidol.

Keywords: Antioxidants; Chronic generalized periodontitis; Inflammation; Mexidol; Selenase.

Epidemiological studies conducted from 1990 to 2020 revealed a significant rise in patients with periodontal diseases leading to tooth loss. Thus, among the adult population of 17 countries, periodontitis was found in 62 %, and its severe form – in 23.6 %[1]. As international clinical trials show, the current therapeutic strategy for the treatment of periodontitis does not provide significant success[2]. Currently, medications obtained from various plant parts (bark, leaves, fruits, flowers, roots and rhizomes) are widely used in the treatment of periodontitis. The therapeutic effectiveness

of essential oils that have anti-inflammatory, antibacterial, and wound-healing properties in inflammatory periodontal diseases, has been most studied[3,4]. Precious metal nanomaterials are also used in the treatment of periodontitis[5,6].

Oxidative stress is of great interest as a promising target in the treatment of periodontitis. Bacteria in inflamed gum tissue and virulence factors entering the bloodstream cause an exaggerated inflammatory response of the body. IL-1â and TNF-á stimulating signaling pathways such as NF-êB (*nuclear factor ê-light-chain enhancer of activated B cells*), and mitogen-activated protein kinases, enhance the production of reactive oxygen species (ROS). Increased accumulation of ROS during periodontitis contributes to damage to DNA, proteins, and lipidss[7,8].

Herb extracts from medicinal plants with antioxidant activity such as bioflavonoids, carotenoids, organic acids, and polyphenols, are administered in periodontitis. However, practice shows that phytoantioxidants are not very effective due to low bioavailability and the peculiarity of the mechanism of antioxidant action[9]. Antioxidants such as melatonin, alpha-tocopherol, thiotriazoline, recombinant human superoxide dismutase (SOD), and mexidol have been shown to be effective in the treatment of periodontitis[10-14].

Selenium and selenium-containing compounds have interested researchers and dentists worldwide owing to their promising applications in periodontitis. Selenium-based drugs have been found to have antioxidant, anti-inflammatory, cytoprotective properties, and are low-toxic form of selenium[15,16].

Sodium selenite stimulates the conversion of methionine to cysteine, and enhances glutathione synthesis. Due to its effect on cysteine-dependent domains, sodium selenite activates the expression of transcription factors such as p53 and NF-kB. Selenase exhibits cardio- and neuroprotective properties in myocardial infarction and cerebral ischemia. Selenase also has cardioprotective and neuroprotective action in myocardial infarction and cerebral ischemia[32,42]. All of the above predetermined the topicality and prospects of this study. Our scientific activities were aimed at studying the healing efficacy of Selenase (*Sodium selenite*), selenium-containing medication, under the conditions of modeling chronic generalized periodontitis CGP in rats by its effect on markers of inflammation and cytoprotection.

MATERIALS AND METHODS

The study was conducted on 40 adult female Wistar rats (weight 180-230 g). All experiments were conducted in accordance with national and international guidelines for the humane treatment of laboratory animals. [17,18]. The Bioethics Commission of ZDMPU confirmed compliance with ethical standards (Protocol No. 3, dated March 22, 2021). Maintenance of animals during the acclimatization period, and during the experiment: rats were kept in accordance with the rules for the design, equipment and maintenance of vivaria.

Experimental model

Experimental CGP was simulated by a calcium-deficient peroxide diet for 8 weeks. Drinking water was replaced with a 2 % aqueous solution of calcium complexon disodium salt of ethylenediaminetetraacetic acid (Trilon B). In parallel, rats were administered *Delagil* (*Chloroquine phosphate*, produced by ICN Hungary S.A.) at a daily dose of 30 mg/kg of body weight using a metal probe. Animals in the experiment received soft foods[19] to reduce chewing function. Animals received the investigational drugs after CGP formation. The animals were divided into four groups, each with ten animals (n = 10):

Group 1 – healthy animals (*intact*), received normal saline solution (0.9 % NaCl) intragastrically for 30 days;

Group 2 (control) – after introduction of chloroquine phosphate and Trilon B for 8 weeks, received 0.9 % NaCl intragastrically for 30 days;

Group 3 – after introduction of chloroquine phosphate and Trilon B for 8 weeks, received Selenase 50 ìg/kg (*Mivolis, Germany*) intragastrically using an atraumatic metal probe[19] for 30 days;

Group 4 – after introduction of chloroquine phosphate and Trilon B for 8 weeks, received the reference medication Mexidol (*Mexicor, PJSC "Technolog", Ukraine*) 250 mg/kg intragastrically daily[14] for 30 days.

After 86 days of the study, blood was

taken from the abdominal aorta after laparotomy in anesthetized rats. (*Sodium thiopental, 40 mg/kg intraperitoneally*) using a special syringe. The obtained blood was subjected to centrifugation to obtain serum. To do this, tubes with blood were placed in the rotor cells of Eppendorf 5804R centrifuge (Germany) and centrifugation was carried out at +4°C, at 1500 rpm, 20 min.

Levels of hypoxia inducible factor-1á (HIF-1á) in the serum were assessed using the solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) method. The ELISA Kit HIF-1 alpha ELISA kit ab275103 (Abcam Limited, UK) was used according to the instructions. Levels of heat shock protein 70 (HSP_{70}) were calculated using the HSP_{70} High-Sensitivity StressXpress ELISA Kit #MBS806878 (MyBioSource, Canada) according to the instructions supplied with the kits. IL-1â levels were calculated using the Rat Interleukin 1â test, IL-1â ELISA Kit #CSB-E08055r (CUSABIO TECHNOLOGY, USA) according to the instructions supplied with the kits.

The TNF-á content was determined with the Rat TNF alpha ELISA Kit #ab 108913 (Abcam, USA) in accordance with the instructions supplied with the kits. These analyses were conducted on a complete plate enzyme immunoassay analyzer (SIRIO-S, Seac, Italy).

Statistical analysis

Normality of distribution was analyzed using the Kolmogorov-Smirnov (D) and Lilliefors tests, and the Shapiro-Wilk (W) test[14]. In addition, the magnitude of asymmetry and excess of the data distribution were assessed as a criterion of agreement. If it was impossible to reject the null hypothesis about statistically significant differences in the distribution of variables from normal, nonparametric methods of data analysis were used. In other cases, parametric methods were used. If there were variants sharply deviated from the mass of observations, based on the properties of the standard normal distribution, these were excluded from further analysis if their absolute value was greater or less than the critical value, calculated as the sum of the sample mean and the triple sample of mathematical expectation value. Based on the properties of the standard normal distribution, variants that sharply deviate from the mass of observations, which in absolute value were greater or less than the critical value, calculated as the sum of the sample mean and the triple value of the sample mathematical expectation, were excluded from further analysis. Analysis of variance (ANOVA) was used to calculate independent variables in more than two samples. Data were presented as the mean and standard error of representativeness of the sample mean. Data processing and statistical analysis of the results were carried out using the statistical package of the licensed program "STATISTICA® for Windows 6.0" (StatSoft Inc., No. AXXR712D833214FAN5), as well as "SPSS 16.0", "Microsoft Excel 2003". Statistical procedures and algorithms were performed in the form of specially written macros in the corresponding programs. Differences were judged as statistically significant if p-value was less than 0.05 for all analyses.

RESULTS

Data presented in tables 1 and 2 show that an 8-week administration of Chloroquine

Table 1. Concentration of IL-1β and TNF-α in the blood of rats with experimental CGP after administration of pharmacological medications

Markers studied	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n = 10)	Group 4 (n = 10)
Periodontal pocket depth, mm	0	8.0 ± 0.431	4.6 ± 0.691*	6.0 ± 0.931*
IL-1β, ng/mL	0.13 ± 0.014	0.56 ± 0.109[1]	0.31 ± 0.028*[1]	0.397 ± 0.06[1]
TNF-α, ng/mL	0.112 ± 0.053	0.907 ± 0.107[1]	0.33 ± 0.02*[1#]	0.577 ± 0.03*[1]

Notes:
*–in comparison with the control group (CGP) ($p < 0.05$);
[1] –in comparison with the intact group ($p < 0.05$);
[#]–in comparison with the Mexidol group ($p < 0.05$)

Table 2. Concentration of HIF-1α and HSP_{70} in the blood of rats with experimental CGP after administration of pharmacological medications

Markers studied	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n = 10)	Group 4 (n = 10)
HIF-1α,pg/ml	1874.1 ± 121.1	2761.2 ± 117.1[1]	3778.3 ± 109.2*[1#]	3117.5 ± 112.3*[1]
HSP70, ng/ml	17.4 ± 0.82	24.2 ± 1.32[1]	41.4 ± 4.22*[1#]	27.0 ± 4,1[1]

Notes:
* – compared to the control group (CGP) ($p<0.05$);
[1] – compared to the intact group ($p<0.05$);
[#] – compared to the mexidol group ($p<0.05$)

phosphate and Trilon B to rats led to typical symptoms of periodontitis: hyperemia, swelling, and bleeding of the gums; mobility of teeth; and the formation of periodontal pockets up to 8 mm. A pronounced healing effect characterized by a pronounced decrease in pocket depth to 4.6 mm; reduction of hyperemia, swelling and bleeding in rats with CGP taken Selenase. Rats with CGP receiving Mexidol had a less pronounced healing effect compared to the group receiving Selenase (Table 1). In animals of this group bleeding persisted when probing the periodontal pocket with a button probe; the depth of the periodontal pocket was about 6 mm; swelling of the gums and mobility of teeth remained, but were less compared to the control group.

Molecular studies of the peripheral blood of rats of the control group revealed pronounced elevation (*several times*) of concentration of proinflammatory cytokines such as IL-1â ($p<0.05$) and TNF-á ($p<0.05$) in plasma in comparison with the intact group.

The use of Selenase in rats with CGP contributed to a decrease in IL-1â by 44.6 % ($p<0.05$) and TNF-á – by 65.9 % ($p < 0.05$) in comparison with the control group. The use of Mexidol to rats with CGP contributed to the decrease in the levels of TNF-á by 36.3% ($p < 0.05$) compared to the control group without affecting the levels of IL-1â. As can be seen from the data in Table 1, Selenase was superior to Mexidol in reducing the levels of TNF-á. ($p < 0.05$).

The data characterizing the markers of endogenous cytoprotection HSP_{70} and HIF-1á presented in Table 2 show an increase in the levels of HSP_{70} in rats with CGP by 1.4 times compared to intact group, and the levels of HIF-1á – by twice compared to the intact group. Administration of Selenase led to the increase in the concentration of HIF-1á by 36.8 % ($p < 0.05$) compared to control group. Concentration of HSP_{70} increased by 71.1 % compared to the control group, and by 138.0 % ($p<0.05$) compared to the intact group. These statistically significant results indicate a remarkable action of selenase on HSP_{70}-dependent mechanisms of endogenous cytoprotection. Administration of Mexidol contributed to the increase in HIF-1á by 12.9 % ($p < 0.05$) in comparison with the control group, without affecting the levels of HSP_{70} in animals with CGP.

DISCUSSION

The results of the study indicate the development of a pronounced inflammatory process in the periodontium of animals with experimental CGP, which is also confirmed by our early research[14]. The results of the enzyme immunoassay obtained in this work demonstrated changes in the levels of proinflammatory cytokines and cytoprotective factors during modeling of periodontitis, which are in line with ideas about the pathogenesis of this disease and do not contradict the data of other scientists[21,22].

Bacterial colonization of the surface of teeth and gum tissue leads to the initiation of inflammatory reactions that trigger molecular mechanisms in periodontal degradation and the development of periodontitis. Leukocytes release proinflammatory mediators that play an essential role in the progression of chronic periodontitis in response to bacterial colonization.

As a result of the described events, the production of both pro-inflammatory cytokines such as IL-1á, IL-1â, IL-6, IL-12, and regulatory cytokines such as TNF-á, IL-4, IL-10 and IL-1RA increases. The increased production of chemokine interferon-inducible protein-10 (IP-10) is also noted. Marked expression of pro-inflammatory cytokines against the background of increased levels of prostaglandin E_2, interferon-ã, and macrophage colony-stimulating factor enhance osteoclast function and activate bone resorption[23].

IL-1â promotes the destruction of periodontal tissue, bone resorption and induces the production of proteinases that destroy bone tissue. TNF-á and IL-1â can trigger molecular reactions leading to the activation of nitrosative and oxidative stress[7]. The bacterial cell and its components, IL-1â and TNF-á enhance molecular activation mechanisms of hypersensitive polymorphonucleocytes involved in the production of ROS[24]. Another source of ROS formation during periodontitis is neutrophil NADPH oxidase, the activity of which increases during inflammation and correlates with an increase in the levels of proinflammatory cytokines[25]. Pathological concentrations of IL-1â and TNF-á enhance the expression of inducible nitric oxide synthases (iNOS). iNOS involved in the mechanisms of inflammation, the production of ROS, oxidative and nitrosative stress[26,27]. ROS, free radicals and stable products of oxidative stress (*malondialdehyde, 4-hydroxy-2-transnonenal, etc*.) enhance the production of proinflammatory mediators, and gingival inflammation; reduce the expression of antioxidant enzymes, HIF-1á, and contribute to alveolar bone loss. Many recent studies confirm a close relationship between the severity of periodontitis and oxidative stress[28-30].

The increase in HSP_{70} and HIF-1á in animals with CGP found in this study clearly corresponds to modern ideas about inflammatory periodontal diseases. IL-1, INF-ã and TNF-á produced in inflamed periodontal tissues function as triggers to induce HSP_{70} production. Lipopolysaccharides also increase hyperthermia-induced HSP_{70} levels in monocyte/macrophage cells. HSP_{70} expression levels correlate with IL-1â levels in periodontitis. Increasing the production of HSP_{70} provides an anti-inflammatory effect, protection of cells from oxidative stress, and damage to bone matrix proteins in the initial stages of periodontitis[7,31].

HSP_{70} has antioxidant and anti-inflammatory effects, helps in early folding and refolding of proteins, protects the nucleus and lipid membrane from destruction, and prevents cell apoptosis[32]. HIF-1á, a major regulator of metabolism in periodontal tissue and alveolar bone, has critical functions in angiogenesis, erythropoiesis, energy metabolism, and cell fate determination during inflammation. However, the role of this factor in the regulation of cytoprotection/cytodestruction in chronic inflammation is not entirely clear and controversial[33,34]. The results of this research and our previous study[7] show a decrease in HIF-1á gene expression, and an increase in its expression in the protein level in experimental CGP. Apparently, changes in HIF-1á expression depend on both the duration and severity of the inflammatory response, and IL-1â and TNF-á modulate its expression. In our case, a point at which the production of HIF-1á began to decrease, and the mechanisms of endogenous cytoprotection were disrupted, was reached. Many studies manifest both the stimulating effect of IL-1â and TNF-á on the synthesis of HIF-1á[35], and their inhibiting action on HIF-1á expression[36,37]. The results obtained confirm the activation of endogenous cytoprotection mechanisms in response to inflammation in experimental CGP found in our earlier study[14].

The above justifies the use of antioxidants in complex drug therapy of periodontitis. Studies have shown the role of selenium in the body's antioxidant defense, and the significance of its deficiency in the development of periodontitis[38-40]. The protective properties of selenase that we have identified in CGP can be explained from the point of view of both the antioxidant properties of selenium, and its ability to influence HSP_{70}-dependent mechanisms of endogenous cytoprotection. Thus, selenium can increase the expression of glutathione peroxidase 4 (GPR-4), inhibit the oxidation of membrane phospholipids, maintain the concentration of vitamin E, and regulate the thiol-disulfide balance[40-42].

Selenium influences inflammation indirectly by regulating the expression of cyclooxygenase and lipoxygenase through the mitogen-activated protein kinase (MAPK)

pathway[43]. Selenium can promote NF-êB entry into the nucleus and bind to areas of antioxidant/ electrophilic regions of sensing elements (ARE/ EpRE) to enhance the expression of antioxidant genes including GPx4, which reduces formation of pro-inflammatory metabolites of arachidonic acid[44]. Selenase can increase the expression of the endogenous cytoprotection factor HSP_{70} by increasing the levels of reduced thiols, especially glutathione[33,45]. Selenium may influence cytoprotection mechanisms by regulating the levels of HIF-1á through VHL-1[46]. Selenium may also prolong the lifetime of HIF-1á indirectly through HSP_{70}[34].

It was found that Mexidol did not significantly affect the levels of IL-1â in experimental CGP, but significantly lowered the expression of TNF-á, which may be due to the suppression of the expression of the succinate receptor SUCNR1/GPR91[47]. The mechanisms of the antioxidant effect of Mexidol do not provide it with action on the expression of HSP_{70} in animals with periodontitis. We identified a certain effect of Mexidol on the synthesis of HIF-1á in rats with CGP. Apparently, Mexidol modulates the level of HIF-1á through the succinate signaling system[48]. Mexidol due to its antioxidant mechanisms impedes the oxidative modification of macromolecules, providing membrane-protective, antioxidant, and anti-inflammatory effects. Mexidol, due to the presence of a succinic acid residue in its structure, and inhibition of the expression of the succinate receptors SUCNR1/GPR91, reduces the levels of pro-inflammatory cytokines, such as TNF-1á[47].

CONCLUSION

Based on the results of the study, it can be concluded that:

1. Experimental CGP simulation in rats by 8-week administration of the prooxidant Delagil, and adding EDTA to water led to the development of typical symptoms of periodontitis: gum hyperemia, swelling, and bleeding; mobility of teeth; the formation of periodontal pockets up to 8 mm against the background of raised inflammatory markers IL-1â and TNF-á, and molecular markers HIF-1á and HSP_{70}, indicating the homeostatic response of the periodontium in response to inflammation and subsequent hypoxia.

2. The course of treatment with a selenium derivative Selenase (50 mcg/kg) in a therapeutic regimen to rats with CGP produced pronounced healing effects: the reduction in the depth of periodontal pockets to 4.6 mm; cessation of bleeding; and disappearance of swelling against the backdrop of declining levels of inflammatory markers: IL-1â – by 44.6 % ($p < 0.05$), and TNF-á – by 65.9 % ($p < 0.05$) and compared to the group of untreated animals.

3. The introduction of Selenase led to a rise in HIF-1á by 36.8 % ($p < 0.05$); an increase in HSP_{70} by 71.1 % in comparison with untreated animals; and by 138 % ($p < 0.05$) in comparison with the intact animals. These data indicate a strong effect of Selenase on HSP_{70}-dependent mechanisms of endogenous cytoprotection.

4. Selenase was significantly superior to the reference medication Mexidol in experimental CGP by its action on the studied parameters.

5. The results obtained justify further study of Selenase as a promising treatment for CGP.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability

The manuscript includes data obtained from the study.

Ethics Statement

Experiments and all manipulations with animals were carried out in accordance with the regulations on the use of animals in biomedical experiments (Strasbourg, 1986, as amended in 1998) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. The experimental research protocols and their results were approved by the decision of the ZSMPhU Bioethics Commission.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Authors' Contribution

Valerii Salnykov [1]: collection and/or assembly of data, writing the article, Igor Belenichev[2]: research concept and design, data analysis and interpretation, writing the article, critical revision of the article, Iryna Samura[3] ; data analysis and interpretation, writing the article, final approval of the article

REFERENCES

1. Trindade D, Carvalho R, Machado V, Chambrone L, Mendes JJ, Botelho J. Prevalence of periodontitis in dentate people between 2011 and 2020: A systematic review and meta-analysis of epidemiological studies. *J Clin Periodontol*. 2023; 50(5): 604—26. 10.1111/jcpe.13769.
2. Radu C-M, Radu CC, Arbãnaºi E-M, Hogea T, Murvai VR, Chi' I-A, Zaha D.C. Exploring the efficacy of novel therapeutic strategies for periodontitis: a literature review. *Life*. 2024; 14(4): 468. 10.3390/life14040468.
3. Rajendhran J, Gunasekaran P. Human microbiomics. *Indian J Microbiol*. 2010; 50(1): 109-12. 10.1007/s12088-010-0034-9.
4. Sabaoui Z, Lakhdar L. Essential oils in periodontics. What Is the Interest? *Integr. J. Med. Sci.* 2021; 8: 1-3. 10.15342/ijms.2021.499.
5. Nasiri K, Masoumi SM, Amini S, Goudarzi M, Tafreshi SM, Bagheri A, Yasamineh S, Alwan M, Arellano M.T.C, Gholizadeh O.. Recent advances in metal nanoparticles to treat periodontitis. *J Nanobiotechnology*. 2023; 21(1): 283. 10.1186/s12951-023-02042-7.
6. Bapat RA, Chaubal TV, Dharmadhikari S, Abdulla AM, Bapat P, Alexander A, Dubey SK, Kesharwani P. Recent advances of gold nanoparticles as biomaterial in dentistry. *Int J Pharm*. 2020; 586:119596. PMID: 32622805.
7. Shang J, Liu H, Zheng Y, Zhang Z. Role of oxidative stress in the relationship between periodontitis and systemic diseases. *Front Physiol*. 2023; 14: 1210449. 10.3389/fphys.2023.1210449.
8. Belenichev I, Popazova O, Bukhtiyarova N, Savchenko D, Oksenych V, Kamyshnyi O. Modulating nitric oxide: implications for cytotoxicity and cytoprotection. *Antioxidants*. 2024;13:504. 10.3390/antiox13050504.
9. Vo TTT, Chu PM, Tuan VP, Te JS, Lee IT. The promising role of antioxidant phytochemicals in the prevention and treatment of periodontal disease via the inhibition of oxidative stress pathways: updated insights. *Antioxidants*. 2020;9(12):1211. 10.3390/antiox9121211.
10. Castro MML, Duarte NN, Nascimento PC, Magno MB, Fagundes NCF, Flores-Mir C, Monteiro MC, Rösing CK, Maia LC, Lima RR. Antioxidants as adjuvants in periodontitis treatment: a systematic review and meta-analysis. *Oxid Med Cell Longev*. 2019;22:9187978. 10.1155/2019/9187978.
11. Lokes K, Kiptilyi A, Skikevych M, Steblovskyi D, Lychman V, Bilokon S, Avetikov D. Microbiological substantiation of the effectiveness of quercitin and its combination with ethylmethylhydroxypyridine succinate in the complex treatment of odontogenic phlegmon and maxillofacial abscesses. *Front Oral Health*. 2024;5:1338258. 10.3389/froh.2024.1338258.
12. Purpura S, Fernandes GVO, Oliveira FP, de Castro F.C. Effects of melatonin in the non-surgical treatment of periodontitis: a systematic review. *Appl Sci*. 2022;12:11698. 10.3390/app122211698.
13. Zhang H, Sun L, Zhang L, Li J, Liu Y, Chen Z, Wang S, Gao C, Sun X. The role of periodontitis in the link between alpha-tocopherol intake and cognitive performance: A mediation analysis in older adults. *Front Aging Neurosci*. 2023;9(15). 10.3389/fnagi.2023.1129095.
14. Parkhomenko D, Belenichev IF, Kuchkovskyi OM, Ryzhenko V. Characteristics of HIF-1Á and HSP70 MRNA expression, level, and interleukins in experimental chronic generalized periodontitis. *Microrna*. 2024;9. PMID: 38616740.
15. Thomas B, Ramesh A, Suresh S, Prasad BR. A comparative evaluation of antioxidant enzymes and selenium in the serum of periodontitis patients with diabetes mellitus type 2. *Contemp Clin Dent*. 2013;4(2):176-80. 10.4103/0976-237X.114867.
16. Au A, Mojadadi A, Shao JY, Ahmad G, Witting PK. Physiological benefits of novel selenium delivery via nanoparticles. Int J Mol Sci. 2023;24(7):6068. 10.3390/ijms24076068.
17. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities*. 1986;L358:1-29.
18. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the

protection of animals used for scientific purposes. *Official Journal of the European Union*. 2010:276;:33-79. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063.

19. Notsek MS, Gorchakova NO, Belenichev IF, Puzyrenko AM, Chekman Y S. The impact of selenium drugs on the performance of enzyme link of thiol disulfide system in the brain tissue of animals with acute cerebrovascular insufficiency. 2015;4(125):202-5.
20. McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods. 24th Ed., 2021;*Hardback*, ISBN:9780323673204.
21. Leira Y, Iglesias-Rey R, Gómez-Lado N, Aguiar P, Sobrino T, D'Aiuto F, Castillo J, Blanco J, Campos F. Periodontitis and vascular inflammatory biomarkers: an experimental in vivo study in rats. *Odontology*. 2020;108(2):202-212. 10.1007/s10266-019-00461-3.
22. Lee J, Lee JB, Song HY, Son MJ, Li L, Rhyu IC, Lee YM, Koo K.T, An JS, Kim JS, Kim E. Diagnostic models for screening of periodontitis with inflammatory mediators and microbial profiles in saliva. *Diagnostics* (*Basel*). 2020;10(10):820. 10.3390/diagnostics10100820).
23. Ramadan DE, Hariyani N, Indrawati R, Ridwan R.D, Diyatri I. Cytokines and chemokines in periodontitis. *Eur J Dent*. 2020;14(3):483-95. 10.1055/s-0040-1712718.
24. Dahiya P, Kamal R, Gupta R, Bhardwaj R, Chaudhary K, Kaur S. Reactive oxygen species in periodontitis. *J Indian Soc Periodontol*. 2013;17(4):411-6. 10.4103/0972-124X.118306.
25. Sui L, Wang J, Xiao Z, Yang Y, Yang Z, Ai K. ROS-scavenging nanomaterials to treat periodontitis. *Front Chem*. 2020;4(8):595530. 10.3389/fchem.2020.595530.
26. Burke SJ, Updegraff BL, Bellich RM, Goff MR, Lu D, Minkin SC Jr, Karlstad MD, Collier JJ. Regulation of iNOS gene transcription by IL-1â and IFN-ã requires a coactivator exchange mechanism. *Mol Endocrinol*. 2013;27(10):1724-42. PMID: 24014650.
27. Toczewska J, Konopka T, Zalewska A, Maciejczyk M. Nitrosative stress biomarkers in the non-stimulated and stimulated saliva, as well as gingival crevicular fluid of patients with periodontitis: review and clinical study. *Antioxidants* (*Basel*). 2020;9(3):259. 10.3390/antiox9030259).
28. Toraman A, Arabaci T, Aytekin Z, Albayrak M, Bayir Y. Effects of vitamin C local application on ligature-induced periodontitis in diabetic rats. *J Appl Oral Sci*. 2020;28:e20200444. 10.1590/1678-7757-2020-0444.
29. Toker H, Balci Yuce H, Lektemur Alpan A, Gevrek F, Elmastas M. Morphometric and histopathological evaluation of the effect of grape seed proanthocyanidin on alveolar bone loss in experimental diabetes and periodontitis. *J Periodontal Res*. 2018;53(3):478-486. 10.1111/jre.12536.
30. Shang J, Liu H, Zheng Y, Zhang Z. Role of oxidative stress in the relationship between periodontitis and systemic diseases. *Front Physiol*. 2023;14:1210449. 10.3389/fphys.2023.1210449.
31. Furuse N, Takai H, Ogata Y. Effects of initial periodontal therapy on heat shock protein 70 levels in gingival crevicular fluid from periodontitis patients. *J Clin Med*. 2020;9(10):3072. 10.3390/jcm9103072.
32. Belenichev IF, Aliyeva OG, Popazova OO, Bukhtiyarova NV. Involvement of heat shock proteins HSP70 in the mechanisms of endogenous neuroprotection: the prospect of using HSP70 modulators. *Front Cell Neurosci*. 2023;17:1131683. 10.3389/fncel.2023.1131683.
33. Taylor CT, Scholz C.C. The effect of HIF on metabolism and immunity. *Nat Rev Nephrol*. 2022; 18: 573–87. 10.1038/s41581-022-00587-8.
34. Shan C, Xia Y, Wu Z, Zhao J. HIF-1á and periodontitis: novel insights linking host-environment interplay to periodontal phenotypes. *Prog Biophys Mol Biol*. 2023; 184: 50-78. 10.1016/j.pbiomolbio.2023.
35. Ng KT, Li JP, Ng KM, Tipoe GL, Leung WK, Fung M.L. Expression of hypoxia-inducible factor-1á in human periodontal tissue. *J Periodontol*. 2011; 82(1): 136-41. 10.1902/jop.2010.100100.
36. Basic VT, Jacobsen A, Sirsjö A, Abdel-Halim SM. TNF stimulation induces VHL overexpression and impairs angiogenic potential in skeletal muscle myocytes. *Int J Mol Med*. 2014; 34(1): 228-36. 10.3892/ijmm.2014.1776.
37. Li X, Lou X, Xu S, Du J, Wu J. Hypoxia inducible factor-1 (HIF-1á) reduced inflammation in spinal cord injury via miR-380-3p/ NLRP3 by Circ 0001723. *Biol Res*. 2020; 53(1): 35. 10.1186/s40659-020-00302-6.
38. Huang H, Yao J, Yang N, Yang L, Tao L, Yu J, Gao Y, Liu Z. Association between levels of blood trace minerals and periodontitis among United States adults. *Front Nutr*. 2022; 9: 999836. 10.3389/fnut.2022.999836.
39. Thomas B, Ramesh A, Suresh S, Prasad BR. A comparative evaluation of antioxidant enzymes and selenium in the serum of periodontitis patients with diabetes mellitus type 2. *Contemp Clin Dent*. 2013; 4(2): 176-80. 10.4103/0976-

237X.114867.

40. Hondal RJ, Marino SM, Gladyshev VN. Selenocysteine in thiol/disulfide-like exchange reactions. *Antioxid Redox Signal*. 20131; 18(13): 1675-89. 10.1089/ars.2012.5013.
41. Ingold I, Berndt C, Schmitt S , Doll S, Poschmann G, Buday K, Roveri A, Peng X, Porto Freitas F, Seibt T, Mehr L, Aichler M, Walch A, Lamp D, Jastroch M, Miyamoto S, Wurst W, Ursini F, Arnér ESJ, Fradejas-Villar N, Schweizer U, Zischka H, Friedmann Angeli JP.. Conrad M S. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell*. 2018; 172(3): 409-22. PMID: 29290465.
42. Belenichev I, Gorchakova N, Bukhtiyarova N, Samura I, Savchenko N, Nefedov A, Bak P. Modulation of HSP70-dependent mechanisms of endogenous neuroprotection with selenium derivatives under conditions of ischemic-type acute cerebrovascular accident modeling. *Pedagogy and Psychology of Sport*. 2020; 6(4): 99-108. 10.12775/PPS.2020.06.04.009.
43. Barchielli G, Capperucci A, Tanini D. The role of selenium in pathologies: an updated review. *Antioxidants* (*Basel*). 2022; 11(2): 251. 10.3390/antiox11020251.
44. Vaghari-Tabari M, Jafari-Gharabaghlou D, Sadeghsoltani F, Hassanpour P, Qujeq D, Rashtchizadeh N, Ghorbanihaghjo A. Zinc and selenium in inflammatory bowel disease: trace elements with key roles? *Biol Trace Elem Res*. 2021; 199(9): 3190-3204. 10.1007/s12011-020-02444-w.
45. Rivera RE, Christensen VL, Edens FW, Wineland MJ. Influence of selenium on heat shock protein 70 expression in heat stressed turkey embryos (Meleagris gallopavo). *Comp Biochem Physiol A Mol Integr Physiol.* 2005; 142(4): 427-32. 10.1016/j.cbpa.2005.09.006.
46. Romanelli-Credrez L, Doitsidou M, Alkema MJ, Salinas G. HIF-1 has a central role in *Caenorhabditis elegans* organismal response to selenium. *Front Genet.* 2020; 11: 63. 10.3389/fgene.2020.00063.
47. Õu J, Zheng Y, Zhao Y, Zhang Y, Li H, Zhang A, Wang X, Wang W, Hou Y, Wang J. Succinate/IL-1â signaling axis promotes the inflammatory progression of endothelial and exacerbates atherosclerosis. *Front Immunol*. 2022; 13: 817572. 10.3389/fimmu.2022.817572.
48. Selak M.A, Armour S.M, MacKenzie E.D, Boulahbel H, Watson D.G, Mansfield K.D, Pan Y, Simon M.C, Thompson C.B, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell.* 2005; 7(1): 77-85. 10.1016/j.ccr.2004.11.022.