Effects of Complex Phytoadaptogens in the Treatment of Experimental Periodontitis Associated with Chronic Stress

Dzampaeva Zh.V.[1]*, Khadartsev A.A.[2]

1. Department of chronopathology and health recreation, Institute of Biomedical Investigations – the Affiliate of Vladikavkaz Scientific Centre of Russian Academy of Sciences / Russia.
2. Medical Institute, Tula State University / Russia.

Abstract

The aim of this study was to increase the effectiveness of complex treatment of periodontitis associated with chronic stress by including complex phytoadaptogens (CFA) as adjuvant therapy. The experiment was performed on 40 adult Wistar male rats, weighing 230±20 g, divided into four groups (n = 10): 1 – Control (healthy periodontium - control); 2 - LP+CD (ligature placement +high carbohydrate diet); 3 - LP+CD+S (ligature placement + high carbohydrate diet+ chronic immobilization stress); 4 - LP+CD+S+CFA (ligature placement + high carbohydrate diet+ chronic immobilization stress + complex phytoadaptogens). To assess the severity of inflammation process in periodontium histological structure of periodontium around the ligature was monitored and also developed index SP (severity of periodontitis) ($p<0.05$). Stress influence on adrenal glands was evaluated as they are the main stress responsive organs. Microcirculation disorders were monitored by Doppler ultrasound.

The LP+CD group presented clinically lower intensity of the inflammatory process and fewer inflammatory cells in gingiva around fixed ligature and no changes in adrenal glands. Compared to the LP+CD group, LP+CD+S group showed a higher intensity of the inflammatory response, a greater bone loss (SP index 3 points), and a higher violation of periodontal structure and adrenal glands in D7 ($P < 0.05$). The LP+CD+S+CFA group presented intact periodontium and adrenal glands. The results prove that 28-day application of complex phytoadaptogens (CFA) reduce inflammation in periodontium associated with chronic stress, alveolar bone loss, normalizes microcirculation disorders and histological structure of gum and adrenal glands.

Experimental article (J Int Dent Med Res 2021; 14(3): 988-995)

Keywords: Acanthopanax senticosus, chronic stress, experimental periodontitis, Gglycyrrhiza glabra, Rhodiola rosea.

Received date: 28 April 2021 **Accept date:** 30 May 2021

Introduction

Periodontitis is a polietiologiclocal chronic inflammatory disease. Progressive destruction of the periodontal structures [1-5] is mainly associated with periodontal pathogens – *Porphiramonas gingivalis, Treponema denticola, Tannerella forsythia* (local factor).[6,7] Periodontal tissue destructs in two ways.[8] Primary - toxins, lipopolysaccharide, enzymes of microorganisms.[9] Secondary - activation of the immune system, which produces neutrophils, macrophages, lymphocytes, high concentration of cytokines and other inflammatory mediators, activation of osteoclasts.[10-13] Additionally, many risk factors such as reduced reactivity, smoking, food culture (common factors) aggravate periodontium destruction. In connection with the growth of civilization, more and more recent studies demonstrate high correlation between chronic periodontal diseases and stress.[14,15] In several studies stress was associated with poor periodontal treatment outcomes and thus stress should be considered in periodontitis treatment protocols.[16,17]

Currently, many herbal preparations are used in dentistry for complex treatment of periodontitis.[18] We propose a new cocktail composed of complex phytoadaptogens, which includes *Glycyrrhiza glabra, Rhodiola rosea, Acanthopanax senticosus* (RF Patent No.

Corresponding author:
Dzampaeva Zh.V.
Department of chronopathology and health recreation, Institute of Biomedical Investigations – the Affiliate of Vladikavkaz Scientific Centre of Russian Academy of Sciences / Russia
E-mail: dzhanaeva_1991@mail.ru

2019137264). These are immunomodulators, antioxidants, they possess anti-stress activity - modulate the synthesis of cortisol and adrenocorticotropic hormone under stress, increase neurohormone levels (endorphins, dopamine), exhibit neuroprotective activity, thereby affecting the etiological factors and pathogenic mechanisms of periodontitis.[19-21] The use of CFA leads to nonspecifically increased resistance.[21] CFA can be used for long-term prophylaxis and treatment, since they rarely cause side effects and are easily integrated in the biochemical processes of the body.[22]

The aim of this study was to increase the effectiveness of complex treatment of periodontitis by including complex phytoadaptogens (CFA) to modify destructive aspects of inflammatory process in periodontium associated with chronic stress on new experimental model of periodontitis in rats.[23]

Materials and methods

Animals

The experiment was performed on 40 adult *Wistar* male rats, weighing 230±20 g. The animals were kept in cages (5 animals in each) in natural light (43°01'00"N 44°41'00"E, Vladikavkaz) with free access to food and water. Food was given once a day (from 9.00 to 10.00 o'clock a.m.). The rats were kept in a room with controlled temperature (21±1ºC) and humidity (50-55%) and in natural light.

Ethical approval

The study was approved by the Biomedical Ethics Committee of the Institute of Biomedical Investigations – the Affiliate of Vladikavkaz Scientific Centre of Russian Academy of Sciences (protocol 6, January 24, 2019). The study was carried out in accordance with the ethical standards established by the 1964 Declaration of Helsinki.

Ligature-induced periodontitis and sample treatment

The rats were divided into 4 groups (n=10). First group – animals with healthy periodontium (control). In the 2 group periodontitis was modeled by applying a metal ligature (LP) at the gingival margin of the lower incisions, using "Zoletil" as an analgesic at a dosage of 0.1 ml/100g. The ligature was fixed to the crest of the alveolar process with a silk thread, disturbing the integrity of the ameloblastic epithelium (hydrostatic cushion) on the vestibular side, where it has the smallest thickness in order to create an entrance for microflora. The animals were fed with wheat porridge with milk (30%), starch (20%) and sugar (15%) (high-carbohydrate diet according to A. I. Evdokimov).[23] In addition to applying the ligature and high carbohydrate diet in the same way as in 2 group, in the 3 and 4 groups from the 2nd to the 7th day of the modeling, each laboratory animal was daily placed in a cage with an area of 0.0008 m^2 for 6 hours to reproduce chronic immobilization stress (S), as one of the risk factors of periodontitis. In 4 group periodontitis was treated with 28-day use of complex phytoadaptogens (CFA) – 14 days before the periodontitis modeling process and 7 days during and after it (therapeutic and prophylactic use). The sample size was determined based on previous studies.[23] In 2-4 groups after experimental modeling of periodontitis on D7 soft plaque was removed, periodontal pockets and mouth of each rat were irrigated with 0,05% chlorhexidine gluconate to times daily for 7 days.

Preparation of ethanol extract of complex phytoadaptogens

CFA is composed from official 70% tincture of *Glycyrrhiza glabra* and 40% tincture of *Rhodiola rosea, Acantopanax senticosus* in the ratio 2:1:1.[23] The dose was calculated taking into account the average daily volume of liquid consumed and the coefficient (x10) for small laboratory animals (0.1 ml/100g) per day. CFA was administered with drinking water in a therapeutic and preventive mode - 14 days before the experimental simulation and 14 days throughout the simulation (4 group).

Since the CFA contains alcohol extracts of FA, additional control group of rats was formed. 1,6 % ethanol solution was administered to these animals (the concentration of ethyl alcohol in the applied dose of CFA) in the therapeutic and preventive mode. No significant changes were detected, so we combined the animals into common group.

Clinical examination

The clinical picture was monitored daily (D1, D7, D14). To assess the severity of the inflammatory process in rat periodontium and intersystem correlation analysis, we developed an index reflecting the severity of peritonitis (SP). Evaluation scale: 0 – intact periodontal (gums are pale pink, do not bleed); 1-gums are pale pink, when probing bleeds, periodontal pockets are not detected; 2 – gums are hyperemic, edematous, loose, bleed when probed, periodontal pockets up to 1.5 mm deep, mobility of the first degree; 3–gums are hyperemic, edematous, profusely bleed when probed, periodontal pockets depth up to 3 mm, mobility of the II degree.

Histopathological study

Gum and adrenal gland tissue were taken for histological examination on D1, D7, D14 in anesthetized rats by intraperitoneal injection of 0.1 ml/100g Zoletil. Pieces of lower incisors (including the free gingiva, the interdental papilla and the attached gingival, lower incisors, alveolar bone process part) were fixed in 10% buffered formalin for histopathological studies. The fixed tissues were washed in running tap water, dehydrated in acetone, cleared in benzene, and immersed in paraffin wax (melting temperature 60-62°C). Paraffin sections were cut at the thickness of 4-5 µ and stained with Lily Mayer's hematoxylin and 2% water soluble eosin. The study was carried out using a polarizing microscope with a digital camera ZEISS Axio Lab.A1 (Germany). In periodontium the following histological parameters were evaluated: 1) intensity of the inflammatory process; 2) structural pattern of the connective tissue; 3) alveolar bone process structure.

Adrenal gland tissue was evaluated the following histological parameters: 1) structural pattern of cortical substance; 2) structural pattern of medulla.

Doppler ultrasound

Doppler ultrasound was used to access microcirculation (MC) disorders (device "Angiodin-PC", Russia, 16MHz probe). For this task each rat was fixed on a wooden board in the supine position, upper and lower jaws were anchored in an open position. In the attached gingiva of the transition fold near the lower incisors an area where large blood vessels do not pass was selected to examine the liquid exchange in the tissues - systolic (S), diastolic (D), medium (M) blood flow velocity, PI - pulsatility index (Gosling index), RI – resistivity index (Pourcelot index), SD - systolic-diastolic index (Stewart index). Examination was performed on D1, D7, D14.

Statistical methods

Data were presented as mean ± standard deviation. One-way analysis of variance followed by Tukey's and unpaired t tests were used to determine statistical significance between groups. Data were compared on D1, D7, D14. The value of $p < 0.05$ was accepted as statistically significant.

Results

Rats oral examination

The dynamics of the SP index indicators are shown in figure 3. The control healthy gingiva with a clear gingival margin along the cervix, when probing does not bleed, the teeth are motionless on D1, SP index –0 (Figure 2A).

In the 2 group (LP+CD) on D7 the cervical gingiva was swollen due to the accumulation of dental plaque with the ligature, hyperemia, easily bleeds when probing, there is an abundance of soft plaque, presence of periodontal pockets up to 1,5 mm deep, I degree of mobility of the lower incisors, confirming severe periodontal inflammation, SP index - 2 points. In the 2 group on D14 after irrigation with 0,05% chlorhexidine gluconate of periodontal pockets and mouth for 7 days the inflammatory process in the periodontium has become less pronounced, but there is still easily bleeds when probing, periodontal pockets up to 1,5 mm deep, SP index - 1 point.

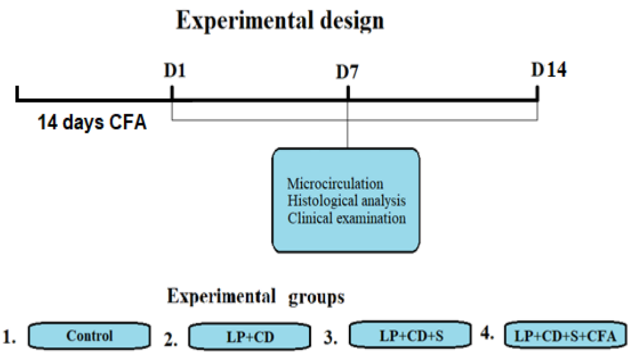

Figure 1. Scheme depicting the experimental design. Procedures performed during the experimental period in the different groups. Groups: 1 – Control (healthy periodontium - control); 2 - LP+CD (ligature placement +high carbohydrate diet); 3 - LP+CD+S (ligature placement + high carbohydrate diet+ chronic immobilization stress); 4 - LP+CD+S +CFA (ligature placement + high carbohydrate diet+ chronic immobilization stress + complex phytoadaptogens).

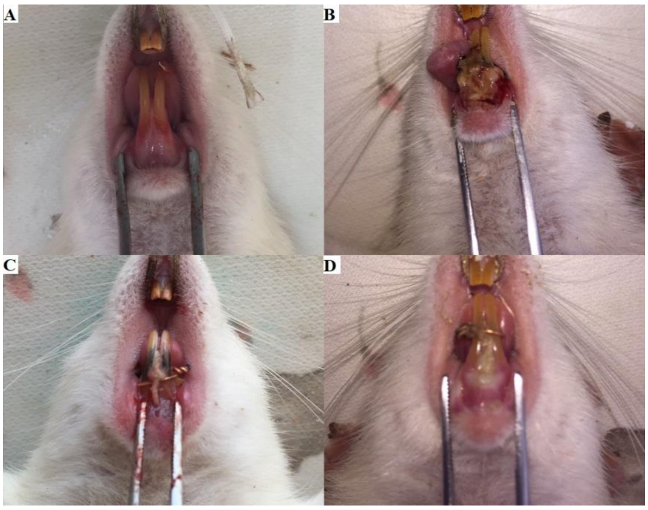

Figure 2. Oral images of experimental groups.

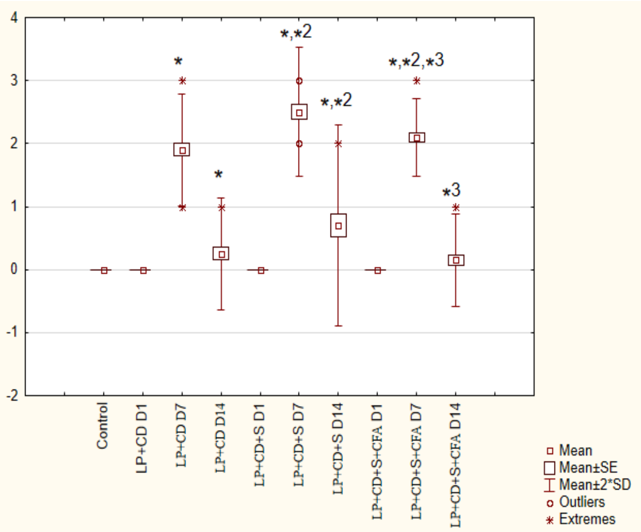

Figure 3. Dynamics of SP index (severity of periodontitis) in all experimental groups (n=10). *Statistically significant difference compared to control; $*^{n}$ to the group in the upper index in relevant day, n – number of experimental group; P<0,05.

In the 3 group (LP+CD+S) on D7 the cervical gingiva was hyperemic, edematous, easily bleeds when probing, there is an abundance of soft plaque, purulent discharge and the presence of periodontal pockets up to 3 mm deep, II degree of mobility of the lower incisor, SP index - 3 points (Figure 2B). In the 3 group (LP+CD+S) on D14 after irrigation with 0,05% chlorhexidine gluconate the inflammatory process in the periodontal tissues subside, but persist periodontal pockets 3 mm deep, II degree of mobility of the lower incisor, SP index - 2 points (Figure 2C).

In the 4 group (LP+CD+S+CFA) on D14 after 28-day treatment of periodontitis with complex phytoadaptogens animals had intact periodontium, SP index - 0 points (Figure 2D).

Dynamics of microcirculatory parameters

In the 2 group (LP+CD) on D7 there was a significant increase in systolic blood flow velocity (S)by 34%, pulsatility index (PI) and Stewart index (SD) were lower by 29% and15% to control, which indicates a decrease in vascular wall density, peripheral resistance to blood flow in microvessels, which is characteristic of for acute periodontal inflammation (Table 1).

Experimental groups		Microcirculation parameters					
		S, sm/sec	D, sm/sec	M, sm/sec	PI, relative units	RI, relative units	SD, relative units
1. Control	D1	1.9±0.64	1.3±0.47	1.85±0.67	0.76±0,23	0.51±0.1	2.14±0.47
2. LP+CD	D7	$2.55\pm0.6^{*}$	1.5±0.76	1.95±0.51	$0.54\pm0.17^{*}$	0.45±0.1	$1.8\pm0.36^{*}$
	D14	1.85±0.67	1.35±0.58	1.45±0.6	0.78±0.24	0.54±0.11	2.12±0.55
3. LP+CD+S	D7	$2.95\pm0.82^{*}$	$2.3\pm0.66^{*,*2}$	$2.75\pm0.78^{*,*2}$	$0.6\pm0.13^{*}$	0.47±0.1	$1.68\pm0.27^{*}$
	D14	2.05±0.51	$1.9\pm0.45^{*}$	1.95±0.39	0.82±0.2	$0.61\pm0.1^{*}$	2±0.28
4. LP+CD+ S+CFA	D7	$1.9\pm0.3^{*3}$	$1.85\pm0.37^{*,*3}$	$1.95\pm0.22^{*3}$	0.66±0.16	$0.4\pm0.09^{*}$	1.8±0.23
	D14	1.75±0.72	$1.45\pm0.6^{*3}$	1.65±0.67	0.7±0.14	$0.54\pm0.07^{*3}$	1.97±0.22

Table 1. Dynamics of microcirculation parameters in all experimental groups.
Parameters: S – systolic (Vas, mm/sec); M - mean (Vam, mm/sec), D – diastolic (Vad, mm/sec) blood flow velocity; pulsatility index (Gosling index), RI – resistivity index (Pourcelot index), SD – Stewart index in the region of attached gingiva of transitional fold; *Statistically significant difference compared to control; $*^{n}$ to the group in revalent day, n – number of experimental group; P<0,05. Values are presented as mean**±**SD (n=10).

In the third group(LP+CD+S) on D7 the systolic (S), diastolic (D), mean (M) blood flow velocity significantly increase of control by 55%, 77% and 49%, pulsatility index (PI) and Stewart index (SD) were lower by 21% and22% to control. On D14microcirculation parameters were approaching the control after irrigation with 0,05% chlorhexidine gluconate for 7 days, but diastolic (D) blood flow velocity and resistivity index (RI) significantly increase of normal indicators by 31% and 12%. Statically significant differences were also observed between the indicators of microcirculation on D7 in the 2 and 3 group.

In the 4 group (LP+CD+S+CFA) on D7after 21-day treatment of periodontitis with complex phytoadaptogens the diastolic (D) blood flow velocity significantly increase of normal indicators by 42%, resistivity index (RI) were significantly lower by 21% to control. On D14 after 28-day treatment of periodontitis with complex phytoadaptogens microcirculation parameters were approaching the control, but diastolic (D) blood flow velocity and resistivity index (RI) significantly increase of normal indicators by 31% and 12%. Statically significant differences were also observed between the indicators of microcirculation on D7 in the 2 and 3 group. More pronounced changes in diastolic (D) and medium (M) flow velocity are associated with stress experienced by animals during experimental modeling of periodontitis in 3group. In the 2 group on D14 microcirculation parameters returned to the control range and compared to 3 group not receiving treatment with CFA diastolic (D) blood flow velocity and resistivity index (RI) were significantly lower by 24% and 11%, what proves the greater effectiveness of soft plaque removal, periodontal pockets, mouth irrigation with 0,05% chlorhexidine gluconate to times daily for 7 days.

Histological analyses of gum tissue

In control group on D1 gum was represented by its own plate of the mucous membrane, covered with stratified squamous keratinizing epithelium (Figure 4A(1,2)). The lamina propria of the mucous membrane consisted of relatively thin collagen fibers arranged in an orderly manner with low-activity fibrocytes lying between them (Figure 4A(3)). Relatively few vessels in the form of arterioles, venules and capillaries are also determined. No signs of bone resorption (Figure 4A(4)).

On D7 in the 2 group mild gingivitis was observed: vasodilatation and perivascular oedema in the lamina propria, increase in the number of histiocytes. On D14 in the 2 group persists perivascular oedema in the lamina propria.

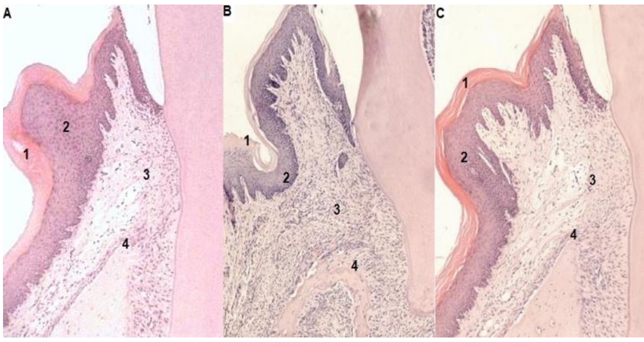

Figure 4. Histological picture of inflammatory process in periodontium (hematoxylin-eosin staining (H & e × 100) experimental groups: 1 – epithelial layer, 2 - stratified squamous keratinized epithelium, 3 - lamina propria of the mucous membrane, 4 - alveolar bone.

On D7 in the 3 group animals showed more pronounced activity of the inflammatory reaction in comparison with the animals of 2 group, extensive areas of necrosis of the collagen stroma of the own mucosal plate, an increase in the number of histiocytes, lymphocytes and plasma cells (Figure 4B(3)). Marked thinning of the epithelial layer with signs of acanthosis (Figure 4B(1)), spinous layer (Figure 4B(2)), deeply penetrating in lamina propria of the mucous membrane with signs of inflammatory process (Figure 4B(3)), inflammatory resorption of the alveolar bone (Figure 22B(4)).

Figure 4C presents periodontal tissue after 28-day application of CFA in 4 group. Its structure is close in its histological structure to the group of intact animals, but in the epithelial layer there are signs of acanthosis (Figure 4C(1)), a spiny layer penetrate into the own plate of the mucous membrane (Figure 4C 3), without areas of alveolar bone loss (Figure 4C(4)).

Histological analyses adrenal gland tissue

The adrenal glands play a major role in adaptation; therefore, their histological structure directly affects this function. In the 1 and 2 groups on D1, D7, D14 adrenal glands had a normal structure: zona glomerulosa cells were arranged in clusters, zona fasciculata cells were arranged in straight columns and zona reticulosa cells were arranged as anastomosing cords (Figure 5A). Lamellar separations of the capsule with areas of ghost cells were observed in zona glomerulosa and zona fasciculata (Figure 5B). In the 3groupthere was a change in the width of the zones, thickening of the connective tissue capsule, a sharp expantion of sinusoidal capillaries, some sections of the bundle zone were destroyed (Figure 5C). Differentiation into light and dark endocrinocytes is not expressed. The connective tissue place between the cortex and the medulla is thickened. The blood vessels are dilated. Spindle cell hyperplasia and vacuolated cell-areas were detected in zona reticulosa in the group 3 (Figure 5D). In the 4group on D1, D7, D14 adrenal glands had a normal structure against the background of taking complex phytoadaptogens.

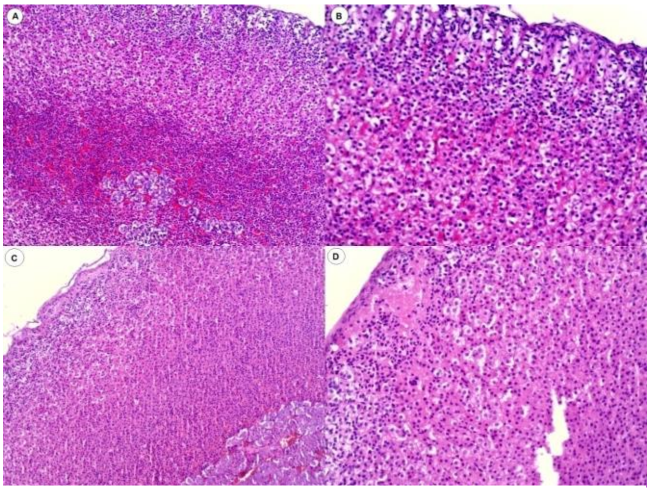

Figure 5. Histological picture of adrenal gland tissue (hematoxylin-eosin staining (H&e × 100).

Discussion

The LP+CD group presented clinically lower intensity of the inflammatory process and fewer inflammatory cells in gingiva around fixed ligature and no changes in adrenal glands. Compared to the LP+CD group, LP+CD+S group showed a higher intensity of the inflammatory response, a greater bone loss (SP index 3 points), and a higher violation of the structure of connective tissue and adrenal glands in D7 ($P < 0.05$). The LP+CD+S+CFA group presented intact periodontium, adrenal glands in D14 after 28-day use of complex phytoadaptogens (CFA) – 14 days before the periodontitis modeling process and 7 days during and after it (therapeutic and prophylactic use).

These results prove that application of CFA, along with the absence of adverse reactions, greatly reduces the inflammatory process and its damages to periodontal structures and prevent the negative effects of stress.

More pronounced inflammatory changes in 3 group, were periodontitis was induced by ligature placement and chronic stress is explained by the fact that stress influence the immune system through two major pathways - the neural (autonomic nervous system) and endocrine systems (hypothalamic–pituitary–adrenal axis).[24-27] During a stress response hypothalamus throws out corticotrophin releasing factor and arginine vasopressin, which stimulates the release of adrenocorticotrophic hormone from the pituitary into the circulation. Adrenocorticotrophic hormone influences on the adrenal cortex and stimulates the glucocorticoid hormones production and release, which, in turn, suppress the immune response by reducing the circulating macrophages and T-lymphocytes number. Glucocorticoid hormones also depress the function of macrophages and polymorphonuclear neutrophils, in terms of chemotaxis, secretion and degranulation. Thus, stress has a significant effect on the immune system (cellular aspects).

Glucocorticoids affect periodontal structures in different ways. On the one hand, during inflammation, glucocorticoids help maintain the integrity of the cell membrane even in the presence of toxins, which reduces edema of periodontal soft tissues (enhances lipomodulin synthesis, decreases histamine secretion). On the other hand, glucocorticoid exposure leads to rapid bone loss due to the combined effect of decreasing osteosynthesis and increasing bone resorption, since glucocorticoids promote osteoclastogenesis by inhibiting osteoprotegerin (OPG).[15,28]

CFAs modulate the synthesis of cortisol and adrenocorticotropic hormone during stress, increase levels of neurohormones (“happiness hormones” – endorphins, dopamine), exhibit neuroprotective activity, prolong the stage of resistance of Selye’s triad.[20,21] Secondary metabolites of adaptogens initiate adaptation of cells to stress, which is called the phenomenon of hormesis or preconditioning;[29] under the influence of Fapri, transcription factors NF-KB and FOXO, neurons adapt to stress that plays a role in the adaptation of the NEIM system to the photoperiod;[30,31] glycyrrhizin *Glycyrrhiza glabra* significantly inhibits RANKL-induced osteoclastogenesis by suppressing the expression of nuclear factor activated T cells 1 (NFATc1); glycyrrhizin significantly reduces the secretion of tumor necrosis factor-α (TNF-α), IL-1β and IL-6, reduces the production of ROS in osteoclasts, inducing phosphorylation of AMPK (AMP-activated protein kinase) and nuclear transfer of NRF2 (nuclear factor-erythroid 2 related factor 2), which leads to an increase in the activity of antioxidant enzymes.[32] The use of CFA makes it possible to obtain a synergistic effect in various pathological conditions.[21,33]

Complaining of microcirculation parameters of animals of 2 and 3 groups on D7 showed that the blood flow velocity characteristics significantly increase in 3 group ($P<0.05$), which is associated with tissue blood flow compensatory response to inflammatory process in the periodontium. Gosling index (PI) (P<0.05) was significantly reduced, which is associated with a reduction in the elasticity of vascular wall due to gain vascular constrictor response in connection with difficult outflow. Pourcelot index (RI) was tendency to reduce indicating a reduction in the density of the vascular wall, peripheral resistance to blood flow in the microvasculature periodontal bed. Hyperperfusion of periodontal tissues develops, which is a mechanism of tissue blood flow compensation in response to inflammatory process.[34,35]

The poor periodontal treatment outcomes of 0,05% chlorhexidine gluconate may also be due to the fact that stress can influence on wound healing may (pathophysiological effects of cell mediators' changes). Proinflammatory cytokines (interleukin-1, tumor necrosis factor-alpha) are critical in the early healing events, and thus any stress-induced alterations may impair wound healing, especially in the early stages. Recent studies in rats also demonstrate decreased expression of basic fibroblast growth factor and slower recovery of expression of basic fibroblast growth factor in the stress groups.[36] Finally, reduced blood flow, as a result of increased catecholamine levels in response to stress, can, in turn, affect oxygen-dependent healing mechanisms, such as angiogenesis, collagen synthesis and epithelialization.[37,38] Thus, chronic stress is a reason of poor periodontal treatment outcomes.

The data support the concept that stress and distress are important risk factors for the development and progression of periodontal diseases and should be taken into account in patients' complex treatment. Complex phytoadaptogens application in patients with chronic periodontitis is more than appropriate due to their immunomodulatory, anti-inflammatory, antioxidant and stress-limiting effects.

Acknowledgements

No external funding, apart from the support of the authors' institution, was available for the study.

Declaration of Interest

The authors report no conflicts of interest pertaining to any of the products or companies discussed in this article.

References

1. Eke, P.I., Dye, B.A., Wei, L., Slade, G.D., Thornton-Evans, G.O., Borgnakke, W.S., Taylor, G.W., Page, R.C., Beck, J.D., Genco, R.J. Update on prevalence of periodontitis in adults in the United States: NHANES 2009 to 2012. J Periodontol 2015;86(5):611–2.
2. Zhang, Q., Li, Z., Wang, C., Shen, T., Yang, Y., Chotivichien, S., Wang, L. Prevalence and predictors for periodontitis among adults in China, 2010. Glob Health Action 2014;7:24503.
3. Graves, D. T., Li, J., & Cochran, D. L. Inflammation and uncoupling as mechanisms of periodontal bone loss. Journal of Dental Research 2011;90(2):143–3.
4. Garlet, G. P. Destructive and protective roles of cytokines in periodontitis: A reappraisal from host defense and tissue destruction viewpoints. Journal of Dental Research 2010;89(12):1349–3.
5. Sanz, M., Beighton, D., Curtis, M. A., Cury, J. A., Dige, I., Dommisch, H., et al. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. Journal of Clinical Periodontology 2017;44(18):5–1.
6. Frederick, J.R., Sarkar, J., McDowell, J.V., Marconi, R.T. Molecular signaling mechanisms of the periopathogen, Treponemadenticola. J Dent Res2011;90(10):1155–3.
7. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, Hernandez M, Gamonal, J. Host response mechanisms in periodontal diseases. J Appl Oral Sci2015;23(3):329–5.
8. Kornman, K. S. Contemporary approaches for identifying individual risk for periodontitis. Periodontol 2018;78:12–9.
9. Lamont, R.J., Koo, H., Hajishengallis, G. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol 2018;16(12):745–9.
10. Preshaw, P. M., & Taylor, J. J. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? Journal of Clinical Periodontology 2011;38(11):60–4.
11. Graves, D. Cytokines that promote periodontal tissue destruction. The Journal of Periodontology 2008;79(8):1585–1.
12. Graves, D. T., & Cochran, D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. The Journal of Periodontology 2003;74(3):391–1.
13. Yucel-Lindberg, T., &Båge, T. Inflammatory mediators in the pathogenesis of periodontitis. Expert Reviews in Molecular Medicine 2013;15:7.
14. Haririan, H., Andrukhov, O., Böttcher, M., Pablik, E., Wimmer, G., Moritz, A., Rausch-Fan, X.J. Salivary neuropeptides, stress, and periodontitis. Periodontol 2018;89(1):9-8.
15. Pitzurra, L, Loos, BG. Stress and periodontitis. Ned Tijdschr Tandheelkd 2020;127(6):358-4.
16. Axtelius, B., Soderfeldt B., Nilsson A., Edwardsson, S., Attstrom, R. Therapy-resistant periodontitis. Psychosocial characteristics. J Clin Periodontol 1998;25:482–1.
17. Wimmer, G, Kohldorfer, G, Mischak, I., Lorenzoni, M., Kallus, K.W. Coping with stress: its influence on periodontal therapy. J Periodontol 2005;76:90–8.
18. Safiaghdam, H., Oveissi, V., Bahramsoltani, R., Farzaei, M.H., Rahimi, R. Medicinal plants for gingivitis: a review of clinical trials. Iran J Basic Med Sci 2018;21(10):978-1.
19. Erik, I., Denia, A.T., Daniel, S.S., Nabila, E.C., Valencia, A.H., Dewi, F.S., et al. Inhibition of Alveolar Bone Destruction by Roselle Extract (Hibiscus Sabdariffa L.). Journal of International Dental and Medical Research 2020; 13(3):830-5.

20. Panossian, A., Seo, E.J., Efferth, T. Effects of anti-inflammatory and adaptogenic herbal extracts on gene expression of eicosanoids signaling pathways in isolated brain cells. Phytomedicine 2019;60:15288.
21. Panossian, A., Seo, E.J., Efferth, T. Novel molecular mechanisms for the adaptogenic effects of herbal extracts on isolated brain cells using systems biology. Phytomedicine. 2018;50:257-4.
22. Jówko, E., Sadowski, J., Długołęcka, B., Gierczuk, D., Opaszowski, B., Cieśliński, I. Effects of Rhodiola rosea supplementation on mental performance, physical capacity, and oxidative stress biomarkers in healthy men. J Sport Health Sci 2018;7(4):473-0.
23. Dzampaeva, Zh. V., Datieva, F. S., Dzaraeva, Z. R., Dzhioev, I. G., Seleskeridi, V. V., Vakushina, E. A. Assessment of the efficiency of the application of complex phytoadaptogens in the treatment of inflammatory diseases of periodontal. Medical News of North Caucasus 2020;15(2):262-5.
24. Boyapati, L., Wang, H.L. The role of stress in periodontal disease and wound healing. Periodontol 2000 2007;44:195– 0.
25. Breivik, T., Thrane, P.S., Murison, R., Gjermo, P. Emotional stress effects on immunity, gingivitis and periodontitis. Eur J Oral Sci 1996;104:327–4.
26. Genco, R.J., Ho, A.W., Grossi, S.G., Dunford, R.G., Tedesco, L.A. Relationship of stress, distress, and inadequate coping behaviors to periodontal disease. J Periodontol 1999;70: 711–3.
27. LeResche, L., Dworkin, S.F. The role of stress in inflammatory disease, including periodontal disease: review of concepts and current findings. Periodontol 2000 2002;30: 91– 3.
28. Tobeiha, M., Moghadasian, M.H., Amin, N., Jafarnejad, S. RANKL/RANK/OPG Pathway: A Mechanism Involved in Exercise-Induced Bone Remodeling. Biomed Res Int2020:6910312.
29. Panossian, A.G., Efferth, T., Shikov, A.N., Pozharitskaya, O.N., Kuchta, K., Mukherjee, P.K., et al. Evolution of the adaptogenic concept rom traditional use to medical systems: Pharmacology of stress- and aging-related diseases. Med Res Rev 2020;1:630-3.
30. Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, X., et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004;303:2011-5.
31. Mattson, M.P., Meffert, M.K. Roles for NF-kappaB in nerve cell survival, plasticity, and disease. Cell Death Differ 2006;5:852-0.
32. Li, Z., Chen, C., Zhu, X., Li, Y., Yu, R., Xu, W. Glycyrrhizin Suppresses RANKL-Induced Osteoclastogenesis and Oxidative Stress Through Inhibiting NF-κB and MAPK and Activating AMPK/Nrf2. Calcif Tissue Int 2018;3:324-7.
33. Bocharova, O.A., Karpova, R.V., Bocharov, E.V., Solov'yov, Y.N. Effects of Phytoadaptogen on Hepatoma Development in Cancer-Prone CBA Mice. Bull Exp Biol Med 2015;5:655-7.
34. Krechina, E.K., Smirnova, T.N. Modern approaches to periodontal microcirculatory parameters assessment. J Dent 2017;1:28-2.
35. Zyulkina, L.A., Sabaeva, M.N., Ivanov, P.V., Shastin, E.N. Periodontal microcirculation: causes of disorders and correction mechanisms. J Modern problems of science and education 2017;2:61.
36. Zhao, Y-J., Li, Q., Cheng, B-X., Zhang, M., Chen, Y-J. Psychological stress delays periodontitis healing in rats: the involvement of basic fibroblast growth factor. Mediators Inflamm 2012;2012:9–3.
37. Whitney, J. Physiologic effects of tissue oxygenation on wound healing. Heart Lung 1989;18:466–4.
38. Faisal, G.G, Radeef, A.S. Depression, Anxiety and Stress among Diabetic and Non-Diabetic patients with Periodontitis. Journal of International Dental and Medical Research 2017;10(2):248-2.