

# Screening Study for Finding the Optimal Combination Gel Composition for the Treatment of Periodontal Disease, Which Contains Extracts of *Aloe Vera* and Oak Bark

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## Abstract

**Introduction:** Today in many countries, there is a clear trend to a growing number of major diseases of the oral cavity, due to the poor state of the environment, unbalanced diet, and concomitant chronic diseases of the gastrointestinal tract. Topical problem of modern medicine and pharmacy is optimization of pharmacotherapy of inflammatory periodontal diseases. The aim of the study was to establish optimal combination of new gel for the treatment of periodontal disease. **Materials and Methods:** The experimental studies were conducted on white male rats. Research of membrane protective activity was carried out on the standard model of spontaneous hemolysis of erythrocytes by Jager. The research of antimicrobial activity was carried out by method of diffusion in agar. Research of Parodont-protective effect was carried out on the model protamine periodontitis. **Results and Discussions:** Extract of Aloe has powerful membrane protective effect in different doses. The study *in vitro* showed the high sensitivity of thick oak bark extract in the form of a gel to the cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans* that allows to recommend it for the treatment of inflammatory diseases of the mouth. **Conclusions:** According to the results of screening studies, optimal composition of gel that contains *Aloe arborescens* and cortex *Quercus* were selected for the treatment of parodontal diseases. Parodont-protective activity of gel is confirmed on the protamine periodontitis model, the ability to decrease the clinical symptoms, namely, hyperemia, edema and necrotic erosive changes of the gums and mucous membranes of the mouth.

**Key words:** Aloe extract, dental gel, oak bark extract

## INTRODUCTION

Today in many countries there is a clear trend to a growing number of major diseases of the oral cavity, due to the poor state of the environment, unbalanced diet, and concomitant chronic diseases of the gastrointestinal tract. The prevalence of periodontal disease is in range of 75-92% in children and rises to 98-100% of the adult population.<sup>[1,2]</sup> According to medical statistics, periodontitis is a leader in the structure of diseases of the oral cavity (90-95% of cases). This disease is characterized by gingival inflammation, progressive destruction of the normal structure of tooth, jaw and violation of periodontal attachment.

One of the most important etiopathogenetic factors in the development of periodontal disease is an infection that causes or exacerbates the inflammatory response. Among the infectious agents most frequently are declared by the bacterium *Streptococcus (Streptococcus*

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*milleri*, and *Streptococcus mutans*), anaerobic flora (*Peptostreptococcus* spp. *actinomyces* spp. *Fusobacterium* spp.) and main causative agents of periodontal infection with *Porphyromonas gingivalis*, *Eikenella corrodens*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*.<sup>[1]</sup>

The problem is that according to many modern studies, there is a positive correlation between inflammatory periodontal disease and many systemic diseases.<sup>[3-6]</sup> In the Suzuki *et al.*<sup>[7]</sup> research it is shown that the infectious periodontitis is associated with hypertrophy of the myocardium due to the activation of metalloproteinase-2. Another group of researchers Li *et al.*<sup>[8]</sup> is proved that patients with acute myocardial infarct have significantly worse periodontal status. Furthermore, there are many studies that declare that inflammatory periodontal diseases aggravate and cause the progression of chronic hepatitis, atherosclerosis and other systemic diseases.<sup>[9,10]</sup>

Topical problem of modern medicine and pharmacy is optimization of pharmacotherapy of inflammatory periodontal diseases.

There were two main aspects in the development of the new dental gel: The presence of strong anti-inflammatory and antimicrobial activity and safety in long-term use. It is also important that components of the new dosage forms have to be compatible with each other in one medicine and were pharmacologically active during long periods of storage (not <2 years).

Promising from our point of view is standardized thick oak bark extract (TOBE) and dry extract of aloe (DEA).<sup>[11]</sup>

The aim of this work is to conduct screening pharmacological studies on the search for the optimal composition of the new dental gel for the treatment of inflammatory diseases of the oral cavity.

## MATERIALS AND METHODS

The experimental studies were conducted on white male rats with weight 180-220 g. Animals were kept in standard conditions of vivarium of the Central Research Laboratory of NUPh, according to the sanitary hygienic norms in compliance with the principles of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), "general ethical principles of experiments on animals" (Ukraine, 2001) and European regulations on working with experiment animals (2005).<sup>[12]</sup>

Research of membrane protective activity was carried out on the standard model of spontaneous hemolysis of erythrocytes by Jager.<sup>[13]</sup> For this spectrophotometric on SF-46 at 540 nm

was determined extinct of out erythrocyte hemoglobin that enters the blood due to hemolysis of red blood cells caused by peroxide oxidation of lipids by air oxygen. Animals received once the aloe extract in doses of 1, 3, and 5 mg/kg and the comparison drug - tocopheryl acetate at a dose of 18 mg/kg (vitamin E, "Zentiva").

For the study of antimicrobial activity was used standard test strain of *Staphylococcus aureus* ATCC 25923, strain of *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, and *Candida albicans* ATCC 885/653. Microbial load was up to 10 microbial cells per 1 ml in the environment and placed according to the standard turbidity of McFarland. The research was carried out by method of diffusion in agar. For the manufacture of inoculum culture of bacteria suspended in 2 ml of liquid environment (meat-peptonemia broth, pH = 7.0 and 7.2). Bacterial suspension of 1 ml was poured on the surface of environment and evenly distributed through the vibrations of the cup. The excess of liquid was removed by pipette; the cups were dried at room temperature for 30-40 min. The tested samples, TOBE, in the dose range of 1-6% in the form of a gel were added 0.2 ml in the wells using sterile disposable syringes. After adding drug in Petri dishes kept at room temperature for 1 h and then placed in a thermostat and incubated for 18-24 h at 35°C. Each sample was tested in six experiments. Analysis of the results was performed after 24 h by measuring the zones of growth inhibition, including the diameter of the holes. The measurements were performed with an accuracy of 1 mm, while focused on the complete lack of visible growth.

Research of Parodont-protective effect of the selected doses of TOBE and DEA in the form of gel was carried out on the model protamine periodontitis on white male rats weighing 180-250 g. Prata Men model of experimental periodontitis<sup>[14]</sup> was reproduced by the application within 7 days on the gums of the rats 0.5 ml of a gel of carboxymethyl cellulose containing a 10% solution of protamine sulfate (manufacturer: "Indar," Ukraine). The gel contains extract *Aloe arborescens* and *Quercus cortex* added on the gums of experimental animals in an amount of 0.5 ml after 40 min after the addition of protamine gel within 7 days of the experiment. The reference drug Metrogyl Denta (manufacturer: "Unique Pharmaceutical Laboratories", India) was used in a similar way. Effectiveness of the compound and the comparison drug Metrogyl Denta was determined by changing clinical manifestations of experimental periodontitis, namely, the lower lip, gums and teeth-the gingival papilla, carried out through observation. Clinical changes expressed in points (P): Hyperemia is absent (0P), weak (1P), and expressive (2P); edema - absent (0P), weak (1P), covers all the gums around the tooth, dentogingival papilla (2P) covers all of the gums around the teeth, and dentogingival papilla and lip (3P); erosive and necrotic changes are absent (0P), weak (1P), many with a touch of (2P), and numerous small or one large erosion with a touch (3P).

Statistical data processing was performed using the software StatPlus 2009, calculating mean, standard error of mean, confidence interval (*P*) using a parametric criterion of Student's *t*-test. The differences were considered significant with a significance level <95% ( $P < 0.05$ ).

## RESULTS

The results of the study of membrane protective activity of different doses of DEA are given in Table 1.

Level of hemolysis of erythrocytes caused by peroxide lipid arismendy membrane air oxygen at animals of the intact control group is  $31.7\% \pm 1.34\%$ .

Addition of tocopheryl acetate significantly reduces the level of hemolysis of erythrocytes by 1.5 times.

Membrane stabilizing the effect of aloe extract 1 mg/kg, statistically has no difference from the efficiency of tocopheryl

acetate (level of hemolysis of erythrocytes was  $23.4\% \pm 1.58\%$  and  $21.2\% \pm 1.27\%$ , respectively).

Maximum membrane protective activity established with the addition of *Aloe vera* extracts 3 mg/kg and 5 mg/kg. DEA in doses of 3 mg/kg and 5 mg/kg statistically significantly higher than the efficiency of known drug with antioxidant activity of tocopheryl acetate in 1.2-1.3 times ( $P < 0.05$ ), and membrane protective effect of DEA at a dose of 1 mg/kg 1.3-1.4 times ( $P < 0.05$ ).

The optimal concentration of TOBE was established on its antimicrobial activity, which verified by the diameter of the zones of growth inhibition of microorganisms in the bacterial environment. Antimicrobial activity of TOBE determined in the range of doses from 1% to 6% [Table 2].

The results of the study indicate that TOBE in the range of doses from 1% to 6% in gel form has a significant antimicrobial activity, which is set for lower growth of microorganisms in the bacterial environment.

Was established dependence of the strength of antibacterial activity to TOBE dosage.

The maximum activity against reference strains of Gram-positive cultures has TOBE of 5% to *Bacillus subtilis* ATCC 6633, *S. aureus* ATCC 25923, *g*, and *Candida albicans*, ATCC 885/653. When increasing the concentration to 6 mg/kg antimicrobial activity is not increased significantly. Furthermore, the dose of TOBE 5 significantly exceeds the antibacterial effect of TOBE in doses of 1-4 mg/kg.

Parodont-protective activity of the new gel contains a TOBE - 5%, DEA - 3%, it is confirmed under condition of protamine periodontitis at rats.<sup>[15]</sup> The gel which contains an extract of oak bark and extract aloe, and Metrogyl Denta have effect on clinical parameters of experimental protamine periodontitis on the 7<sup>th</sup> day of the experiment, as it shown in Figure 1.

**Table 1:** Membrane protective activity of different doses of DEA on model of spontaneous hemolysis of erythrocytes by Jager ( $n=8$ )

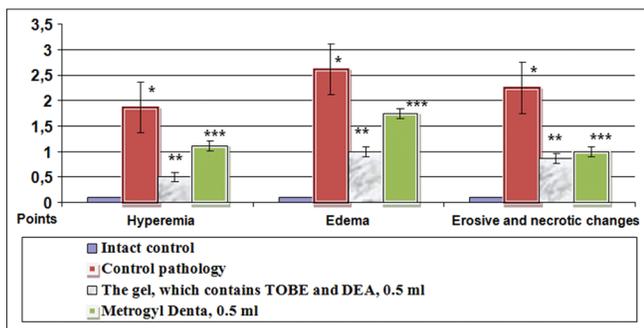
Groups	Level of hemolysis of erythrocytes, %	Membrane protective activity, %	<i>P</i>
Intact control	32.70±0.69	-	-
DEA, 1 mg/kg	24.28±0.73	25.75	$P_{2-1} < 0.01$
DEA, 3 mg/kg	18.13±0.66	44.56	$P_{3-1} < 0.001$ $P_{3-2} < 0.05$ $P_{3-5} < 0.05$
DEA, 5 mg/kg	17.12±0.72	47.64	$P_{4-1} < 0.001$ $P_{4-2} < 0.05$ $P_{4-5} < 0.05$
Tocopheryl acetate, 18 mg/kg	22.01±1.14	31.47	$P_{5-1} < 0.001$

DEA: Dry extract of aloe

**Table 2:** Antimicrobial activity of TOBE in various doses, which is injected in gel form ( $n=6$ )

Groups	The diameters of zones of growth inhibition of microorganisms, mm			
	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Bacillus subtilis</i> ATCC 6633	<i>Candida albicans</i> ATCC 885/653
Control gel	0±0	0±0	0±0	0±0
TOBE, 1%	16.68±0.65*	14.34±0.40*	13.14±0.29*	12.29±1.02*
TOBE, 2%	18.14±0.72*	15.56±0.64*	15.05±0.74*	19.01±1.13**
TOBE, 3%	18.68±0.59*	18.42±0.78**	18.11±0.62**	25.10±0.79***
TOBE, 4%	18.43±0.47*	19.23±0.55**	19.64±0.40**	28.11±0.43***
TOBE, 5%	20.92±0.53****	21.67±0.41****	20.93±0.49****	30.42±0.50****
TOBE, 6%	20.97±0.61****	21.09±0.62****	20.50±0.66****	30.12±0.31****

Significant differences: \*Indicators of control gel ( $P < 0.001$ ), \*\*indicators of TOBE, 1 mg/kg ( $P < 0.05$ ), \*\*\*indicators, TOBE, 2 mg/kg ( $P < 0.05$ ), \*\*\*\*indicators, TOBE, 1 mg/kg ( $P < 0.05$ ); \*\*\*\*\*indicators, TOBE, 1 mg/kg ( $P < 0.05$ ), and TOBE: Thick oak bark extract



**Figure 1:** The gel which contains a thick oak bark extract and D, and Metrogyl Denta have effect on clinical parameters of experimental protamine periodontitis on the 7<sup>th</sup> day of the experiment,  $M \pm m$ ,  $n=8$ . Significant differences: \*With the original value of the intact control ( $P < 0.05$ ), \*\*with values of the control pathology ( $P < 0.05$ ), \*\*\*with values of comparison drug Metrogyl Denta ( $P < 0.05$ )

According to the results of the study, it is proved that on the 7<sup>th</sup> day of the clinical manifestations of experimental protamine periodontitis acquired a significant effect. All animals from control pathology group had a significant redness of the gums, lower lip and oral mucosa - 1.87 points ( $P < 0.05$ ). Hyperemia was accompanied by a significant swelling of the gums and lips - 2.62 points ( $P < 0.05$ ) and numerous erosion we on the gums and mucosal - 2.25 points ( $P < 0.05$ ) [Table 1].

The reference drug Metrogyl gel reduced the symptoms of hyperemia by 1.7 times ( $P < 0.05$ ), and by 1.4 times ( $P < 0.05$ ) decreased symptoms of edema and 2.2 times ( $P < 0.05$ ) reduced the number of erosions that have been registered.

The gel which contains TOBE and DEA more definitely influenced the clinical symptoms of periodontitis: Reducing hyperemia by 3.7 times ( $P < 0.05$ ), edema - 2.6 times ( $P < 0.05$ ), erosive and necrotic changes - by 2.5 times ( $P < 0.05$ ). It should be noted that the new gel which contains TOBE and DEA, exceed the efficiency of the analog and new gel significantly exceeds the influence of Metrogyl Denta by the ability to reduce swelling.

## DISCUSSION

On the model of spontaneous hemolysis of erythrocytes by Jager established a distinct membrane stabilizing the effect of tocopheryl acetate. This is because vitamin E is the universal protector of cell membranes against oxidative damage. Vitamin E prevents contact of oxygen with the unsaturated lipids of membranes, forming hydrophobic complexes and preventing destruction of the membrane. Hydroxyl chromatic core of tocopheryl acetate directly interacts with oxygen free radicals of unsaturated fatty acids and their peroxides. Tocopheryl acetate stabilizes the mitochondrial membrane, contributes to spragens oxidative phosphorylation and other processes of energy metabolism of the cell. Tocopheryl

acetate at preclinical and clinical researches<sup>[3]</sup> consider the reference drug with antioxidant activity.

DEA have powerful membrane protective effect in different doses because it consists of biologically active substances, which have antioxidant, immunomodulator, and membrane protective activity. It is known that standardized DEA contains antraglycosides in 2.8-3.3%: Barbaloin - 10-glucopyranoside, aloe emodin anthrone, aloe emodin ( $\approx 1.7\%$ ), nataloin, and rabarbaras; resinous substances - to 20%, among which are identified aloesin and alainn a and b; carotenoids, organic acids, and ascorbic acid; malic acid and trace elements: K, Mg, Cu, Se, Zn, Li, and Ba.

According to the literature,<sup>[12]</sup> it is known that natural antraglycosides have a pronounced antioxidant and anti-inflammatory activity, mechanism of implementation which, along with anti lecturenobel activity is the ability to stabilize cell membranes.

The study *in vitro* showed the high sensitivity of TOBE in the form of a gel to the cultures of *S. aureus*, *Pseudomonas aeruginosa*, *B. subtilis*, and *C. albicans* that allow to recommend it for the treatment of inflammatory diseases of the mouth, which is accompanied by the accession of bacterial flora. The nature of the antibacterial effect of TOBE depends on the concentration. The basis of the mechanism of antibacterial activity of TOBE is the ability of biological active substances of the extract, namely, derivatives of gallic acid and tannins to induce denaturation of the proteins with which they directly interact. At low concentrations, TOBE has bacteriostatic activity, in high doses - bactericidal effect.

On the model of protamine periodontitis in rats has a significant Parodont-protective activity, the effectiveness of the new gel containing TOBE (5%) and DEA (3%), significantly exceeds the efficiency of known drug Metrogyl gel. The above said, due to the complex composition of the new gel, where membrane stabilizing, antioxidant activity of DEA is complemented by anti-inflammatory and angioprotective activity of TOBE.

## CONCLUSIONS

According to the results of screening studies, optimal composition of gel that contains *Aloe arborescens* and cortex *Quercus* was selected for the treatment of periodontal diseases.

It is established that the studied DEA in a dose of 3 mg/kg has a pronounced membrane stabilizing activity that significantly exceeds membrane protective activity of DEA of 1 mg/kg, the reference drug tocopheryl acetate at a dose of 18 mg/kg and does not have a statistically significant difference on the pharmacological activity in comparison with aloe extract 5 mg/kg.

TOBE shows huge antibacterial activity in comparison with the reference test strains of *S. aureus* ATCC 25923, *E. coli* ATSS 25922, *B. subtilis* ATCC 6633, and *C. albicans* ATCC 885/653 at a dose of 5 mg/kg. Further increase in dose of extract of oak bark to 6 mg/kg has no statistically significant differences.

Parodont-protective activity of gel is confirmed on the protamine periodontitis model, the ability to decrease the clinical symptoms, namely, hyperemia, edema and necrotic erosive changes of the gums and mucous membranes of the mouth.

The new gel which contains an TOBE (5%) and DEA (3%) can be considered a promising target for further pharmacological studies with the aim of creating a new effective Parodont-protective drug.

## REFERENCES

1. Miller AL, Long IM, Hrstyan GE. Integral characteristics of infectious and inflammatory diseases of the mouth. *Clin Exp Patol* 2015;1:215-20.
2. Weston P, Yaziz YA, Moles DR, Needleman I. WITHDRAWN: Occlusal interventions for periodontitis in adults. *Cochrane Database Syst Rev* 2016;11:CD004968.
3. Han K, Ko Y, Park YG, Park JB. Associations between the number of natural teeth in postmenopausal women and hormone replacement therapy. *Maturitas* 2016;94:125-30.
4. Guideline for periodontal therapy. *Pediatr Dent* 2016;38:397-401.
5. Holmstrup P, Reinholdt J, Poulsen AH. Periodontitis is one of the most commonly occurring inflammatory diseases. *Ugeskr Laeger* 2010;172:3029-32.
6. Sakalauskiene J, Giedrimiene D, Gleiznys D, Gleiznys A, Gleizniene R, Vitkauskiene A. Peripheral blood leukocytes interleukin-1 beta (IL-1 $\beta$ ) cytokine hyper-reactivity in chronic periodontitis. *Med Sci Monit* 2016;22:354-9.
7. Suzuki JI, Sato H, Kaneko M, Yoshida A, Aoyama N, Akimoto S, *et al.* Periodontitis and myocardial hypertrophy. *Hypertens Res* 2016;10:142-7.
8. Li P, He L, Sha YQ, Luan QX. Periodontal status of patients with post-acute myocardial infarction. *Beijing Da Xue Xue Bao* 2013;45:22-6.
9. Perrell-Jones C, Ireland RS. What factors influence patient compliance with supportive periodontal therapy in a general practice setting? *Br Dent J* 2016;221:701-4.
10. Marin MJ, Figuero E, Herrera D, González I, O'Connor A, Diz P, *et al.* Quantitative analysis of periodontal pathogens using real-time polymerase chain reaction (PCR). *Methods Mol Biol* 2017;1537:191-202.
11. Sundarkar P, Govindwar R, Nyamati SB, Alladwar N, Thombre V, Soni A, *et al.* Use of aloe in dentistry. *J Indian Acad Oral Med Radiol* 2011;23:389-91.
12. De Simone FI, Serratos J. Biotechnology, animal health and animal welfare within the framework of European Union legislation. *Rev Sci Tech* 2005;24:89-99.
13. Stefanova AV. Preclinical Studies of Drugs: Methodical Recommendations. Kyiv: Avicenna; 2001. p. 528.
14. Borisenko AV, Weaver OB, Linovizky OV. Experimental substantiation of application nanogold drugs for the treatment of periodontal diseases. *Dent Prak* 2014;1:58-62.
15. Tsubanova NA, Khokhlenkova NV, Zhurenko DS, Matthew IE. Research of efficiency new gel containing an extract of oak bark and extract of aloe vera under condition protamine periodontics. *Ukr Biopharm J* 2016;2:27-31.

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