

ORIGINAL ARTICLE

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**LOCAL USE OF APISAN GEL, A NEW ORAL CARE PRODUCT IN THE  
TREATMENT OF EXPERIMENTAL PERIODONTITIS AGAINST THE  
BACKGROUND OF HYPERACID GASTRITIS AND INTOXICATION WITH  
TOBACCO SMOKE**

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**ABSTRACT**

**Objectives:** The aim of the work is to substantiate the use of the newly created oral care product in the treatment of the periodontal disease reconstructed against the background of hyperacidic gastritis under the conditions of tobacco smoke intoxication.

**Materials and methods:** The study was conducted in 2 stages. At the first stage, all experimental animals were divided into 4 groups: 1 - intact, 2 - with simulated periodontitis, 3 - with reproduced periodontitis against the background of reproduced hyperacidic gastritis, 4 - with reproduced periodontitis against the background of hyperacid gastritis under the conditions of tobacco smoking. Biochemical studies of the gum homogenate with periodontitis in rats were conducted to determine the impact of stomach pathology and tobacco smoke as endogenous and exogenous risk factors. At the stage 2, the effectiveness of local therapy with the use of the newly created oral care product and comparator was studied in rats with reproduced periodontitis against the background of hyperacidic gastritis under the conditions of smoking.

**Results:** In the experimental periodontitis against the background of hyperacidic gastritis under the conditions of tobacco smoking, there were significant changes in the periodontal tissues characteristic of the inflammatory process: the activity of POL increased and activity of the antioxidant system decreased, inflammatory markers increased when non-specific protection was reduced. Local therapy in the rats using the new "Apisan" gel resulted in correction of certain metabolic disorders, faster

elimination of the harmful effects of the damaging factors and restoration of the condition of periodontal tissues than with the use of the comparator - the Asepta gel.

**Conclusions:** The therapeutic effectiveness of the new Apisan gel is due to the normalizing effect on the processes of POL (peroxide oxidation of lipid), inflammation and activation of the oral cavity protective systems during inflammatory periodontal disease occurring against the background of concomitant stomach pathologies - hyperacidic gastritis.

**Key words:** Apigel, periodontitis, hyperacidic gastritis, tobacco smoking, inflammation.

## INTRODUCTION

Changes in the tissues of the oral cavity with manifestations of inflammation are often associated with diseases of the gastrointestinal tract (GIT), which is due to the morphofunctional unity of the digestive apparatus. There are ulcerative lesions, erosion, aphtoe of the oral cavity mucosa (OCM) in the GIT pathology due to trophic disorders, The presence of harmful habits, especially smoking, in patients with concomitant diseases of GIT further enhances the severity of the inflammatory symptoms in the oral cavity. Dental deposit of smokers is a major provocative factor in the development of gum diseases and caries<sup>[1,2]</sup>.

In inflammatory diseases of the oral cavity, gastritis among the concomitant GIT pathology makes more than 70% of cases, the form and duration of which affects the functional state of OCM. Changes in the oral cavity during chronic gastritis depend on the secretion and acid forming function of the stomach. Increased acidity in the stomach is accompanied by hypersalivation, pallor, edema and gingival inflammation, bleeding, decreased capillary stability and reactivity of OCM<sup>[3]</sup>. By weakening the immune defence of the body, the concomitant GIT diseases create conditions for the affecting influence on OCM, and periodontium by the microflora present in the oral cavity and the endogenous periodontal pathogenic factors. In addition, current data suggest a negative impact of Helicobacter pylori infection<sup>[4]</sup>, tobacco smoking<sup>[5]</sup> on the incidence of the OCM and periodontium diseases.

The mechanisms underlying the influence of changes in GIT on the pathobiochemical reactions of the inflammatory process in the periodontal tissues and OCM, the possibility of treating such a combined pathology, taking into account the harmful habit of tobacco smoking require a specific study. Therefore, we consider relevant and promising for dentistry to search for and study the effectiveness of new

means of local therapy of OCM and periodontal diseases, as a concomitant pathology of hyperacidic gastritis under the conditions of tobacco smoking.

The aim of the work is to substantiate the local application of the newly created oral care product in the treatment of the periodontal disease reconstructed against the background of hyperacidic gastritis under the condition of intoxication with tobacco smoke.

## MATERIALS AND METHODS

The study was conducted on 72 Wistar male rats, 1-1.5 months of age, weighing 180-220 g, which were in the vivarium of Odessa National Medical University on a standard feed for laboratory rats. According to the tasks of the experiment, the study was conducted in 2 stages. At the first stage the rats were divided into 4 groups (10 animals in each). The first group consisted of intact rats (control). The second group of rats had modeling of periodontitis. The third group consisted of rats, in which, after reproduction of hyperacidic gastritis, periodontitis was modelled. The fourth group included rats, which, against the background of reproduced hyperacidic gastritis, were modelled periodontitis under the condition of dosing of tobacco smoke. After conducting the first series of the experiment to determine the effect of concomitant stomach and tobacco smoke on metabolic disorders in the tissues of the oral cavity of rats at the reproduction of periodontitis, the effectiveness of local treatment with the use of the newly created drug was studied on the basis of apioproducts and other biologically active substances with anti-inflammatory, antimicrobial, antioxidant effects<sup>[6]</sup> and the comparator preparation - aseptic gel. Experimental animals were also divided into 4 groups: 1 - intact (control); 2 - rats with reproduced model of periodontitis against the background of hyperacidic gastritis under the conditions of exposure to tobacco smoke; 3 - the main one, which included newly treated rats with periodontal disease reproduced against the background of hyperacidic gastritis under the conditions of intoxication with tobacco smoke; 4 - a comparison group, which included rats with a periodontitis model in the 2,3 group received a local treatment with Asepta gel.

Affection of the gastro-duodenal zone in rats was induced by the addition of ammonium acetate 2 g / l to drinking water for 10 days, then 0.4 mg of Helicobacter pylori suspension  $5 \times 10^8$  KU / ml given per os twice daily for 7 days in 3 days<sup>[7,8]</sup>. Hyperacidic gastritis was reproduced by the introduction of a single 5% solution of the acetic acid in the account of 4 ml / kg of mass through the probe 5 days before

the trial. For control intragastric Ph-metry was made under intraperitoneal anesthesia using sodium thiopental at a dose of 20 mg / kg of the rat's mass by insertion of the glass electrode (EL-40) into the cavity of the stomach in supramedial laparotomy using a pH meter (pH-340). The level of basal acidity in the simulation of hyperacidic gastritis was 1.80-2.00.

The rats of the 3rd and 4th groups after the reproduction of hyperacidic gastritis and of group 2 on the first day of the experiment in the first series of experiments under the thiopental anesthesia (20 mg / kg) were modelled periodontitis by applying a ligature on the central incisor. The essence of the model consists in the formation of a retention point for the dental plaque, which initiates the development of inflammation and destruction of the periodontal tissues<sup>[9]</sup>. The rats of the 4th group in the first series of the experiment and of the 2nd, 3rd and 4th groups of the second series were created the conditions for tobacco smoking by exposure to the effect of the tobacco smoke.

To reproduce the conditions for tobacco smoking, a plastic hermetic chamber of 28 liters with three different sections was used, to which tobacco smoke from 15 cigarettes (Primma red with a content of 1.0 mg of nicotine and 10 mg of resin in 1 cigarette) was introduced inside by pressure of the engine, through the holes for 30 minutes, daily, for 15 days. At the same time, there were 7 animals in the chamber. During cigarette smoking, the behavioral reactions of the rats were observed: initially, the supply of tobacco smoke to the chamber made the rats anxious, looking for a place for normal breathing, in 10 minutes they calmed down and fell asleep. After finishing the inhalation of tobacco smoke and supply of fresh air, the rats activated, began to breathe frequently, 15 minutes later they became normal.

The animals were removed from the experiment in several stages. Euthanasia of rats of the 1st, 2nd, 3rd, 4th groups of the first series was performed immediately after the last procedure of inhalation of tobacco smoke (on the 15th day) under thiopental anesthesia (20 mg / kg) by total bloodletting from the heart. All animals treated after reproduced periodontitis against the background of hyperacidic gastritis under tobacco-smoking conditions was slaughtered partly on the 8th and 14th days after treatment beginning.

A tissue sampling of the gum for biochemical studies was taken. In the supernatant fluid of the gum homogenates the final level of the POL-malondialdehyde (MDA) by the thiobarbituric method was determined<sup>[10]</sup>, the

antioxidant protection status (AOP) was assessed by activity of catalase<sup>[11]</sup> as well as inflammation level by elastase activity<sup>[12]</sup>, non-specific activity protection index lysozyme<sup>[13]</sup>. The antioxidant prooxidant index of API was calculated by the ratio of catalase activity to MDA concentration.

During the study, there were used general principles of animal experiments approved at the National Congress on Bioethics (Kyiv, Ukraine, 2001) and in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, France, 1985). The statistical processing of the data was carried out using the program "Statistica 6.0" using the Student t-criterion. The changes were considered to be significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The experimental animals had the intact mucous membrane of the gum before the reproduction of pathological conditions, with no apparent pathological changes; gum bleeding was not found on probing. There were manifestations of clinical symptoms of the periodontal tissue inflammation, namely, hyperemia, edema, bleeding of the gum in the area of incisors in all rats after ligature-induced periodontitis on the third day. In 5 days, the inflammation was seen in the area of molars, that is, there was a generalization of the inflammatory process in the periodontal tissues. The animals that were reproduced hyperacidic gastritis, became week, ate little, there were recorded inflammatory symptoms of OCM in their oral cavity: hyperemia and edema. After modeling periodontal disease in these animals there was an explicit picture of inflammation in the gums in the form of edema and hyperemia of the marginal region, gingivitis on the 2nd day. There were metabolic changes in the periodontal tissues of the rats, as evidenced by the biochemical parameters of the gum biopsy in the rats of the 2<sup>nd</sup> and 3<sup>rd</sup> groups compared to intact animals. The analysis of changes in the biochemical markers of inflammation in the gum tissues of animals determined the most significant deviations in the rats with periodontitis against the background of hyperacidic gastritis under the conditions of intoxication with tobacco smoke (group 4).

Taking into account that one of the main factors that trigger the inflammatory processes is the activation of free radical lipid oxidation, the prooxidant system has been studied for the level of MDA and the activity of antioxidant protection in the activity of catalase. Modeling of periodontitis led to an increase in the content of MDA

in the gum tissues, which pointed to intensification of peroxide oxidation of lipid (POL) with decreased activity of the antioxidant protection (catalase activity decreased) in the periodontal tissues. Even more significant similar changes occurred in the gum biopsy of the animals, in which periodontitis was reproduced against the background of hyperacidic gastritis. The most expressed metabolic disorders in the tissues of the oral cavity were found in the 4th groups of rats, in which the simulation of periodontitis was made against the background of hyperacidic gastritis under the conditions of smoking, when the combination of the influences of two affecting factors, especially in the system of POL - AOP, was observed. The process of POL activation as the main factor of the cell membrane damage due to the action of an excessive stimulus was realized in all tissues, including the mucous membranes of the digestive system. In the tissues of rats, there is a system for counteracting damage and activation of POL, such counteracting factor is AOP, in particular, the enzyme catalase. The activity of catalase was reduced as a result of its consumption during active participation in the processes of decontamination of the POL products. In the 4th group, the animals showed the lowest activity of catalase in the gum ( $4.96 \pm 0.39$  mcat / kg) and the highest level of MDA ( $19.70 \pm 1.30$  mcmol / kg), which 2.3 times exceeded this index in the intact animals ( $p < 0.05$ ) and 1.3 times - in rats of the 2nd group with periodontitis (Table 1).

**Table 1. Changes in biochemical parameters in the gum tissues in the rats in reproduction of periodontitis against the background of hyperacid gastritis**

Group of animals	Contents of MDA, mcmol/kg	Elastase, mccat/kg	Catalase, mcat/kg	Lysozyme, u/kg	API
Intact (control), N=10	8.42±0.34	34.0±2.00	7.18±0.33	276±24	8.52±0.32
Periodontitis, N=10 P	14.70±0.62 <0.05	40.0±3.00 >0.05	6.74±0.41 >0.05	188±14 <0.05	4.58±0.52 <0.05
Hyperacidic gastritis + periodontitis, N= 10	17.80±1.20	43.0±3.00	5.86±0.48	176±22	3.29±0.84

P	<0.05	<0.05	<0.05	<0.05	<0.05
P <sub>1</sub>	<0.05	>0.05	>0.05	>0.05	>0.05
Hyperacidic gastritis + periodontitis+tobacco smoke	19.70±1.30	46.0±4.00	4.96±0.39	154±22	2.52±0.60
N= 10	<0.05	<0.05	<0.05	<0.05	<0.05
P	<0.05	>0.05	<0.05	>0.05	<0.05
P <sub>1</sub>	>0.05	>0.05	>0.05	>0.05	>0.05
P <sub>2</sub>					

Notes: P - probability relative to the control group;

P<sub>1</sub> - probability of the periodontitis group;

P<sub>2</sub> - probability of differences between the 3rd and 4th groups

Elastic activity, which characterizes the development of inflammation in the gum during reproduction of periodontitis was most increased under the conditions of combined stomach pathology and the action of tobacco smoke, which indicated a high activity of neutrophils that in a large number infiltrated the periodontal tissues in the development of inflammation.

In the tissues of rats with periodontitis against the background of hyperacidic gastritis, which was influenced by tobacco smoke, elastase activity was 1.35 times higher than in intact animals ( $p <0.05$ ), without toxication with tobacco smoke, the studied index increased by 25% less ( $p <0.05$ ). Concomitant hyperacid gastritis significantly influenced the degree of metabolic disorders of the oral cavity tissues in animals with induced inflammation of the periodontal tissues, enhancing the manifestations of oxidative stress, suppressing the functional state of the system of antioxidant protection, causing damage to biological membranes, structural and functional changes in the gum mucosa with elements of inflammation. Elastase activity in the gum tissues was increased in rats with periodontitis against the background of hyperacid gastritis 1.26 times compared to intact ones, exceeding values in the rats without combined pathology. At the same time, there was a decrease in the local resistance of the tissues of the oral cavity in groups 3 and 4, which was evidenced by 36.3% and 44.3% less than lysozyme activity in the gum homogenates compared with intact animals. It is known that lysozyme has a local

anti-inflammatory and immunomodulating effect: it inhibits the chemotaxis of neutrophils and the production of toxic oxygen radicals<sup>[14]</sup>. Reduction of the activity of lysozyme may be the cause of maintaining a local inflammatory process.

The application of a new mucosal apigel Apisan in local therapy of the periodontal disease reconstructed against the background of hyperacidic gastritis under the conditions of intoxication with tobacco smoke contributed to the reduction of the influence of the affecting factors on the animal oral cavity and the restoration of the tissue condition. During the examination of the oral cavity, much less damage to the mucosa was detected, namely, swelling and redness of the gums decreased. After the local use of the Apisan gel, the condition of the periodontal tissues improved already in 5 days after the beginning of treatment, and when using the application of the comparator - the Asepta gel - only in 10 days. The results of the biochemical studies showed that the newly created drug significantly reduced the markers of inflammation in the gum tissue. Their parameters in the substrate that was studied in the animals made applications with a new gel to the areas with induced periodontitis, were recorded to be in reduced values compared with the data of the comparison group. On the 8th day after treatment with a new gel, most animals (86%) were found to have normalization of the antioxidant-prooxidant system, markers of inflammation in the gum tissues.

**Table 2. Correction of metabolic disorders in the periodontal tissues of rats in the local treatment of reconstructed periodontitis against the background of hyperacidic gastritis ( $M \pm t$ )**

Group of animals	Contents of MDA, mcmol/kg	Elastase, mccat/kg	Catalase, mcat/kg	Activity Lysozyme, u/kg	API
Intact (control) n=10	8.42±0.34	34.0±2.0	7.18±0.33	276±24	8.52±0.32
Hyperacidic gastritis + periodontitis+tobacco smoke N= 10	19.7±1.30 <0.05	46.0±4.0 <0.05	4.96±0.39 <0.05	154±34 <0.05	2.52±0.80 <0.05

P					
The main group, N=10	9.87±0.48	37.0±2.0	6.88±0.58	218±28	7.07±0.53
P	<0.05	>0.05	>0.05	>0.05	>0.05
Comparison group, N=10	13.31±0.74	40.0±3.0	6.23±0.42	197±22	4.68±0.58
P	<0.05	>0.05	<0.05	<0.05	<0.05
P <sub>1</sub>	<0.05	>0.05	<0.05	>0.05	<0.05
P <sub>2</sub>	<0.05	>0.05	>0.05	>0.05	<0.05

Notes: P - probability relative to the control group;

P<sub>1</sub> - probability relative to the data before treatment;

P<sub>2</sub> - probability of differences between the main group and the comparison group.

During the application of the Asepta gel the positive effect is determined only in 38% of rats on the 8th day after the beginning of application, and metabolic disturbances, which were eliminated mainly at the end of the observation were recorded in the remaining animals (62%). In the course of a comprehensive study it has been found that the Apisan gel<sup>[6]</sup> gave a more pronounced therapeutic effect than the Asepta gel that was characterized by the improvement of biochemical indices of the rat periodontal tissues (Table 2).

Thus, summing up the results of experimental studies, it is possible to state that rats during the reproduction of periodontitis against the background of hyperacidic gastritis under the conditions of intoxication with tobacco smoke had increased activity of the prooxidant system and decreased activity of the antioxidant system, elastase activity increased in decreasing nonspecific protection in the periodontal tissues, indicating the development of the inflammatory process. The application of the newly created Apisan apigel as applications in the periodontal disease in rats against the background of hyperacidic gastritis after intoxication with tobacco smoke significantly reduced the processes of inflammation in the periodontal tissues, affecting the normalization of the POL processes, inflammation and activation of the protective systems of the oral cavity.

## CONCLUSIONS

1. In experimental periodontitis against the background of hyperacidic gastritis under the conditions of intoxication with tobacco smoke, changes in the periodontal tissue are characteristic of the inflammatory process: the activity of lipid peroxidation

increases and the activity of the antioxidant system decreases, the markers of inflammation increase in decreasing nonspecific protection.

2. Local therapy of the periodontal disease reconstructed against the background of hyperacidic gastritis with the effect of tobacco smoke in rats using the newly created apigel Apisan led to the correction of certain metabolic disorders in the gum homogenate, faster elimination of the harmful effects of the damaging factors and restoration of the state of the periodontal tissues than in comparative application of the Asepta gel.
3. The therapeutic efficacy of the Apisan gel is due to a normalizing effect on the processes of POL, inflammation and activation of the oral protective systems.
4. The results of the studies give grounds for recommending the local application of the new APISAN apigel to prevent inflammatory processes in the tissues of the oral cavity against the background of concomitant hyperacidic gastritis and to create optimal conditions for the elimination of structural and functional disorders caused by endogenous and exogenous risk factors as tobacco smoke [15].

## **PROSPECTS FOR FURTHER RESEARCH**

The results obtained in the experiment indicate the expediency of studying the effect of the developed treatment on indices of nonspecific resistance and immune reactivity in the oral cavity during periodontitis against the background of concomitant gastrointestinal tract pathology and the creation of recommendations for its application in the complex therapy of dental diseases.

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