

# The clinical and immunological rationale for the use of prolonged action dental ointment in periodontology

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## ABSTRACT

The purpose of this study was to provide a clinical and immunological rationale for the use of prolonged action dental ointment in periodontology. Study methods: clinical and anamnestic methods; the indices included oral hygiene (OHI-S), periodontal diseases (PI), papillary, marginal, and alveolar (PMA); laboratory indices were quantitative and qualitative content of spontaneously released mixed saliva, its total protein, secretory immunoglobulin A (sIgA), and lysozyme content. The authors have established the high clinical efficacy of using an experimentally developed ointment based on anesthesin, metronidazole and, for the first time, an extract of larvae of a greater wax moth (*Galleria mellonella*) as active substances in combination with additional high-molecular compounds of prolonged action in the complex therapy of inflammatory periodontal diseases. The evaluation of local immunity has shown the followings: an increase in the production of mixed saliva in comparison with the initial data by 2.6 times, the recovery of saliva production, an increase of 1 ml above the control level, an increase in the protein level, lysozyme concentration (1.8 times), and sIgA concentration (by 39%) compared to the initial data. The results of the study showed that the inclusion of prolonged action of dental ointment in the local inflammatory periodontal disease therapy has been an effective means of correcting defects of clinical and laboratory rates of local immunity, which has made it possible to recommend the introduction of a new promising dosage form in practical periodontology.

**Keywords:** *Galleria mellonella*, immunity, immunoglobulin A, lysozyme, periodontitis

## Introduction

According to the World Health Organization, the prevalence of inflammatory periodontal diseases (IPDs) worldwide equals 55-98%.<sup>[1-3]</sup> Today, IPDs have the status of one of the socially significant infectious diseases, which have been characterized by a local inflammatory and destructive process in periodontal tissues, and have had a negative effect on the entire body due to the action of inflammatory and immunopathological development mechanisms.<sup>[4-6]</sup> Dentistry has changed tremendously over the past decade to the benefit of both the clinician and the patient.<sup>[7]</sup> The modern dental industry faces the

need to discover new ways to improve the treatment of IPDs. In the new economic conditions, the search and testing of dosage forms with a high profile of biological activity, safety, and availability of raw materials for manufacturers have been of great theoretical and practical interest. Recently, the development of an analytical method for the determination of active pharmaceutical ingredients and preservative compounds in pharmaceutical products is needed to maintain the quality in order to be in the range of product standards.<sup>[8]</sup> The use of biological preparations based on natural (plant, animal, and mineral) origin can reduce the dependence of clinical medicine on the use of antimicrobial agents of synthetic production.<sup>[9, 10]</sup> Medicinal plants have been utilized for thousands of years ago for curing different diseases of human beings and animals and are still in use by a great percentage of world population for being well.<sup>[11]</sup> One of the promising biological products has been a beekeeping product, the extract of greater wax moth (*Galleria mellonella*) larvae, which has several advantages, such as the availability of raw materials in Bashkortostan, its unique healing properties that affect immunity, the possibility of long-term use, and the absence of significant side effects.<sup>[12]</sup> Taking into account the complex and

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multi-stage etiological, pathophysiological, and immunological mechanisms of the development of IPDs, the authors have experimentally developed a combined composition and technological scheme for the preparation of dental ointment based on the active substances with analgesic, antimicrobial, antiviral, antioxidant, and immunomodulatory properties and a significant prolonged action in combination with additional modern auxiliary substances, such as high-molecular compounds.<sup>[13-15]</sup>

The study of the use of new dental ointment in the complex treatment of IPDs has been important due to the lack of information about its use in periodontology. The purpose of the study was to provide a clinical and immunological rationale for the use of prolonged action dental ointment in periodontology.

## Materials and methods

The study was conducted on patients with IPDs aged from 20 to 75 years. All patients had signed informed consent. The study included 47 men (39.2%) and 73 women (60.8%). The average age of the examined patients was  $44.3 \pm 5.2$  years. The dental diagnosis (K05.1), i. e. chronic catarrhal gingivitis (CCG), and chronic generalized periodontitis (CGP) (K05.3) was determined according to ICD-C on the basis of ICD-10. On the basis of the characteristic clinical manifestations of the disease and according to the data of the main and additional methods of examination, CGP of varying severity was detected in 74% and CCG in 26% of patients.

All patients were divided into three groups: the control group (n=25) – practically healthy individuals who had no dental pathology at the time of the examination; group I (n=120) – patients who had IPDs before the treatment, and group II (n=120) – patients who followed a traditional treatment scheme with the addition of the developed dental ointment with metronidazole, anesthesin and *G. mellonella* extract.<sup>[10]</sup>

The treatment effectiveness evaluation was carried out at the end of the course of treatment according to patients' clinical and laboratory rates. The condition of periodontal tissues was assessed by clinical and anamnestic data and indices: oral hygiene (OHI-S), periodontal diseases (PI), papillary, marginal, alveolar (PMA) indices, as well as by the depth of periodontal pockets by clinical probing at six points of each tooth. Intraoral radiography and orthopantomography of the jaws were performed. The state of local immunity was investigated in terms of quantitative and qualitative content of spontaneously released mixed saliva, and its

total protein, secretory immunoglobulin A (sIgA), and lysozyme content.<sup>[16]</sup>

Statistical processing of the results was performed using generally accepted standard methods. The arithmetic means, the standard error of the arithmetic mean (Se), and the standard deviation were determined. The assessment of the significance of differences in indicators was performed using Student's t-test. The differences were considered statistically significant at  $p < 0.05$ .<sup>[17]</sup>

## Results

According to the data obtained in the control group in practically healthy individuals, they had no complaints of bleeding gums. During the inspection of the oral cavity and radiographic examination of the bone tissue of the alveolar processes, no pathological changes were discovered in the periodontal tissues. In the group I, the medical history of the patients who had IPDs before the treatment revealed that they experienced bleeding and pain while brushing their teeth and eating, the unusual appearance and color of the gums, periodic itching in the gums, mobility, denudation, and dislocation of teeth, as well as bad breath. Clinical and instrumental examination showed that in most cases the mucous membrane of the gums was hyperemic, edematous, with a cyanotic shade. In most cases, it has loosely adhered to the teeth. Gingival bleeding was observed during clinical probing. Abundant soft and hard over- and sub-gingival deposits were noticed on the teeth, soft and pigmented patina. With CGP, the depth of periodontal pockets was from 3 mm and higher, the mobility of teeth varied from I to III degree, the recession in the area of individual teeth varied from 1/4 to 2/3 of the tooth root length and depended on the severity of inflammation. With an increase in the severity of IPDs and the spread of the inflammatory process in the deeper periodontal layers, the patients experienced a decrease in the level of individual oral hygiene, an increase in the number of teeth that needed to be removed or filled due to the complicated and uncomplicated caries, dystopia and overload of preserved teeth, as well as secondary deformation of the tooth rows. Some patients showed the need for surgical care, in particular, implantation of missing teeth, orthodontic and orthopedic care (splinting of mobile teeth and targeted prosthetic treatment). In all patients, the diagnosis was confirmed radiographically. High rates of dental indices (OHI-S, PMA, and PI) were observed (Table 1).

**Table 1: Clinical efficacy of the use of dental ointment in patients with IPDs ( $M \pm m$ )**

Observation groups	Clinical rates			
	Pocket depth (mm)	Green-Vermillion index, units	PMA index, units	Periodontal index, PI units
Group I (n=120)	2.2-7.5	2.32±0.31	46.24±2.11	3.83±0.21
Group II (n=120)	1.5-3.5*	0.34±0.01*	5.32±0.35*	1.14±0.04*

\*differences with the original data are significant at  $p < 0.05$ .

The results of the study of local immunity rates are shown in Table

2.

**Table 2: Local immunity rates in patients with IPDs using prolonged action dental ointment.**

Patient groups	Rates (M±m)			
	Amount of saliva, ml	Protein, g/l	Lysozyme, g/l	sIgA, g/l
Control (n=25)	3.00±0.05	1.50±0.82	14.30±0.70	592.4±24.7
Group I (n=120)	1.54±0.10	1.90±0.10	7.60±0.50	335.0±18.2
Group II (n=120)	4.04±0.41	3.86±0.42	13.52±0.78*	466.4±14.3*

\*difference from the control group is statistically significant at  $p < 0.05$ .

The table shows that the amount of saliva produced in patients of group I was initially two times less than that in the control group. The protein content exceeded the control level by 27%. Along with this, the levels of lysozyme (by 1.9 times) and sIgA (by 43%) were reduced in mixed saliva in comparison with the control group of patients. The data obtained indicated the presence of an inflammatory process, a decrease in antimicrobial protection, and the intensity of the immune response.

In group II, patients with IPDs who received treatment with the use of dental ointment showed positive dynamics of the clinical state of periodontal tissues. In most cases, patients noted the absence of pain when eating, the absence of itching, burning, bad breath, bleeding gums, and tooth mobility. The appearance of the gums was restored to a pale pink color. No signs of inflammation were observed. The gum tightly adhered to the neck and root of the tooth.

Upon completion of the course of treatment, all IPD patients noted that complex therapy with the use of a developed dental ointment contributed to the rapidly advancing positive effect of the treatment including improvement of the general condition, an increase in salivation, the relief of signs of inflammation, and the disappearance of pain by the 2nd or 3rd appointment (1-3 days). Complete epithelization occurred at 5.4-4.2 days, which allowed patients to fully implement individual oral hygiene and gave them a feeling of comfort in the oral cavity. The effectiveness of the treatment of IPD patients was assessed by the following criteria: deterioration, no improvement, improvement, significant improvement (high assessment of effectiveness), and complete disappearance of symptoms (very high assessment of effectiveness). Due to the prolongation effect applied to therapeutic action and a wide range of therapeutic activities of the dental ointment, high clinical efficacy of treatment was observed in 72.3% of cases, of which 41.7% of the patients showed no signs of inflammation, 30.6% had local manifestations of inflammation, and only 6.4% had signs of a generalized spread of inflammation. While preserving the periodontal pocket, its depth decreased by 2.6-3.1 times, the mobility of the teeth decreased due to the restoration of the tooth-gingival attachment, which helped to preserve the teeth and maintain their function for a long period. The effectiveness of treatment was also manifested in the reduction of periodontal indices in patients with IPDs at the end of the course of treatment with the use of dental ointment. The OHI-S indices reduced by 86.6%, PMA by 8.7 times, and PI by 69.7% in comparison with the initial data. The use of dental ointment in case of IPDs contributed to

the selective and more prolonged effect of medicaments on microorganisms and a pathological focus of inflammation, which reduced the number of sessions per course of treatment. With gingivitis and mild CGP, the course of treatment lasted for 3 sessions, with moderate CGP for 5-6 sessions, and with severe CGP for 7-8 sessions.

The evaluation of the state of local immunity in group II in patients with IPDs showed an increase in the production of mixed saliva in comparison with the initial data by a factor of 2.6, an increase of 1 ml above the control level ( $p > 0.05$ ), an increase in the level of protein in the mixed saliva ( $p < 0.05$ ), lysozyme (1.8 times), and sIgA (39%) in comparison with the initial data. Thus, the application of the developed dental ointment led to an improvement in the state of clinical data, dental indices, and rates of local protection factors on the quantitative content of mixed saliva and qualitative state (sIgA and lysozyme).

## Discussion

The results obtained in the group before the treatment showed a pronounced inflammatory process, disorders of the general reactivity of the body, and a decrease in the activity of local factors of natural protection in patients with IPDs, which dictated the need to find effective means of influencing the mechanisms of inflammation and preventing the recurrence of the disease, prolongation of the therapeutic effect, and immunocorrection with multifactorial and polyresistant periodontal pathology. The therapeutic effect could be enhanced by improving the anti-inflammatory therapy in the inflammation focus due to the introduction of pharmacological preparations of a wide range of therapeutic activities. Based on the study of the physicochemical properties, the composition and the technological scheme were experimentally developed for preparing a soft dosage form in the form of an ointment that affected the main pathogenesis of the IPDs, based on the active ingredients with anesthesin, metronidazole, and *G. mellonella* extract. The use of anesthesin has been justified by the analgesic effect on the hyperemic, edematous, painful, and irritated gum tissue inflammation. It reduced the feeling of discomfort in the mouth and hyperesthesia of the exposed necks of hard dental tissues to temperature and other chemical and mechanical stimuli. The etiology and pathogenesis of periodontal disease involved the use of antibacterial medications to reduce the progression of the development of the pathological process and re-colonization of periodontal tissues. Due to this, metronidazole was used, which

has a wide spectrum of action against the simplest, obligate anaerobes including spore-forming and non-spore-forming microbes. Antibacterial drugs helped in fighting various pathogenic microflora, but the normalization of microcirculation in the gum-affected areas of the gums under the action of antibacterial agents occurred indirectly since they did not have the properties of reparation of components of the periodontal complex lost during the pathological process. Therefore, for the first time in periodontology, the *G. mellonella* extract was used, which had immunomodulatory and regenerating effects in the ointment to improve microcirculation and regeneration. The therapeutic properties of the *G. mellonella* extract were conditioned by a wide range of biologically active components. It contained a large set of macro and microelements, bioflavonoids that performed antiviral protection, 19 essential and non-essential amino acids that had a positive effect on tissue metabolism, possessing anabolic, adaptogenic, antioxidant, and immunomodulatory effect that improved the rheological properties of blood and microcirculation.

Additional modern auxiliary substances were used in the technology of ointment production, such as high-molecular compounds employed in the global practice to improve biopharmaceutical properties and increase bioavailability, reduce toxicity and irritant action, and maximize the accumulation of therapeutic concentrations of drugs in pathological foci of infection, in particular, dimethicone, emulsifier T-2, styrene copolymer with maleic anhydride, lutrol, vaseline oil, collidon, and orange seed oil.

The pale pink dental ointment obtained in this study was uniform and stable, had a soft and satisfactory consistency, was easily applied to the gum area with a uniform distribution and the formation of a thin even solid smear, had thixotropy and good adhesion to the mucous surfaces of the gums, good tolerance, pharmaceutical accessibility, characteristic orange aroma, the density of 1.12 g/cm<sup>3</sup>, and pH of 5.9.

It was experimentally established by thin-layer chromatography that the presence of 7% kollidon CL-M in the composition affected the release of metronidazole in the composition of the dental ointment by 80% by the 60th minute, and increased the pharmaceutical accessibility due to the present auxiliary substances. The content of essential orange oil in the amount of 0.5% and kollidon masked the bitter taste of metronidazole. The prolonged effect of the ointment to 5.5 hours was experimentally established, which made it possible to reduce the number of appointments per course of treatment from 3 to 8.

## Conclusion

The results of the study showed that the inclusion of prolonged action dental ointment in local IPD therapy was an effective means of correcting defects of clinical and laboratory rates of local immunity, which allowed expanding the range of newly created pharmacological forms for the local treatment of IPDs and recommending the introduction of a new promising medicinal form in practical periodontology. The pathogenetic

rationale for the use of the experimentally developed combined composition of a prolonged action dental ointment in the complex therapy of IPDs was the immunity responses indirectly contributing to the maintenance, increase of the immunosecreting function of the oral mucosa, and a pronounced anti-inflammatory and immunocorrective effect. The use of dental ointment revealed high clinical efficacy of treatment in 72.3% of cases, and a decrease in the parameters of periodontal indices including OHI-S by 86.6%, PMA by 8.7 times, and PI by 69.7% in comparison with the initial data. The rates of local protection factors were comparable with the comparison group and were even higher and close to the level in the control group, characterized by an increase in the production of mixed saliva by 2.6 times in comparison with the data before treatment, an increase of 1 ml above the control level ( $p > 0.05$ ), lysozyme (1.8 times), sIgA (39%) in comparison with the initial data. The prolonged effect of the ointment to 5.5 hours was experimentally established, which made it possible to reduce the number of appointments per course of treatment. With gingivitis and mild CGP, the course of treatment lasted for 3 sessions, with moderate CGP for 5-6 sessions, and with severe CGP for 7-8 sessions.

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