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ON TECHNOLOGIES, SAFETY AND PHARMACOLOGICAL PROPERTIES OF NEW ALNIDENT MEDICINAL PREPARATION

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ABSTRACT

The data on the substantiation of composition, technology and research activity of phytopreparation in the form of dental gel based on aloe and nimesulide was given. Its advantages over other compounds and forms were proved. Pharmacological properties of a new drug-dental gel Alnident developed in the Tashkent Pharmaceutical Institute have been studied. The results showed that the drug has the expressed wound-healing and strong anti-inflammatory properties. According to the classification of substances on toxicity "Alnident" preparation appeared relatively harmless. Preparation "Alnident" when applied to the skin of guinea pigs had no irritative action. In the studied doses of "Alnident" preparation - dental gel has pronounced wound-healing effect and a strong anti-inflammatory effect. Effect of "Alnident" preparation was close to "Dentashan" preparation, produced by Isshaan Healthcare Pvt. Ltd, India, while "Kamistad®" preparation produced by Stada Arzneimittel AG, Germany had a weaker effect. According to studies results of technological and biopharmaceutical factors it can be concluded that the gel has developed satisfactory dental technological and biopharmaceutical properties which allow to recommend it for use in dentistry.

KEYWORDS: periodontal disease, dental gel, wound-healing effect, anti-inflammatory effect.

INTRODUCTION

One of the urgent problems of modern dentistry is periodontal disease - periodontitis. It was found that persistent generalized inflammation of the marginal periodontium is the most constant clinical and morphological manifestations of periodontitis, in this regard, special significance acquire prevention and relief of inflammatory changes in periodontal tissues. In spite of the existing tools and methods for treatment of diseases of periodontal tissues, there are no optimal methods of influence on the mechanism of development and pathogenesis of this disease. The role of a trigger in the development of inflammatory and degenerative-inflammatory processes in the periodontium play microorganisms and their toxins, which, combined with other local factors induce microcirculatory disorders, immunological reactivity, trophism and contribute to periodontal destruction.[1,9] This is especially significant in the elderly in the period of adaptation to removable dentures. Not always medicines used in medical practice in order to reduce adaptation periods lead to positive results in the elderly.[2,7]

The many factors of the disease, chronic process, difficulties in achieving positive results of conservative and surgical treatment, the emergence of drug-resistant forms of microorganisms require the creation of new drugs for the treatment of periodontal disease.

All the above indicates that the perspective direction today is to develop drugs with prolonged action, which provide local and uniform release of active substance from the dosage form, making it a high therapeutic concentration at the site of application without significantly affecting the level of drug in the systemic circulation.

In this regard, the topical was the development of soft dosage form as a combination of gel for the prevention and complex treatment of inflammatory and degenerative - inflammatory periodontal diseases. It was created by the employees of the Tashkent Pharmaceutical Institute dental gel with the conditional name "Alnident" ("Al" - aloe, "ni" - nimesulide "dent" - dental) for the treatment of inflammatory dental diseases.

Due to the constant increase in the incidence in recent years the problem of periodontal disease is of particular relevance. Periodontitis takes the 4th place among non–infectious diseases, and the age of patients does not play

a role: to 30 years the disease is recorded in 50% of the population.

In the development of periodontitis are involved exogenous and endogenous factors as well, such as the composition of saliva, microflora, formation of tartar, etc. Immune and hormonal factors are the primary to launch the mechanism of development severe forms of inflammatory - destructive periodontal disease. Periodontitis is characterized by the fact that in patients are affected all structures of parodont with general periodontal bone resorption of the alveolar process, resulting in death of the retaining teeth and loss of teeth apparatus. By the treatment of periodontal disease, it is necessary to accelerate the processes of neoangio - and osteogenesis and restore all tissues affected by the disease.

Actual direction today is considered the development of long-acting preparations which provide local and uniform release of active substance from the dosage form, making its high therapeutic concentration in the place of application without significant affecting the level of medicinal substance in the systemic circulation.

These requirements, in addition to films correspond gels, sustained action of those is achieved by immobilizing the active ingredients in a variety of polymeric carriers.[1,3,4]

The purpose of this study was

1. Development of composition and technology, as well as the study of safety on parameters acute toxicity and locally – irritating action of soft dosage form in the form of combined gel "Alnident" for the prevention and complex treatment of inflammatory and degenerative - inflammatory periodontal diseases.
2. Pharmacological properties of the new "Alnident" product - gel developed by employees of the Tashkent Pharmaceutical Institute in comparison with the drugs Kamistad® "- gel for topical use, produced by Stada Arzneimittel AG, Germany and "Dentashan"- Gel for gums, produced by Isshaan Healthcare Pvt. Ltd, India.

MATERIAL AND METHODS

Research is to create dental gel, as a biologically active substance of vegetable origin. We selected the extract of aloe arborescent, (lat. Aloe arborescens, also known as agave) and synthetic substance nimesulide.

Characteristics of raw matrrials:

- Aloe – Aloe arboresscens Mill, (dry extract). FC Uz-0570-2013.
- Nimesulid– Nimesuidum, (powder). VFC 42-2550-95.
- Na CMC – Carboxymethlcellulose, VFC 42-1091-81.
- Clycerin – Giycerin, FC 42-2202-84.
- Propylene glycol – Propyleneglycol, VFC 42-1594-86.
- Peppermint water – Aquae Menthae piperitae, GF, X 14.2.5.

Aloe arborescens is widely used in medicine, possesses a variety of pharmacological properties. Aloe is applied in dentistry for inflammation of the oral mucosa, damaging of mucosa after irradiation, enhances cell regeneration and accelerates healing.

Nimesulide is characterized with pronounced anti-inflammatory, analgesic and antipyretic effects, has antiphlogistic, antioxidant and immunotropic activity and also has high safety and relatively low risk of side effects when applied topically.[6]

RESULTS

To select an optimal basis were conducted structural-mechanical, biopharmaceutical, microbiological research, allowed to choose gel base Na-CMC - 1.6 at a concentration of 1%, and as the hydrophilic nonaqueous solvents of gel were chosen PEO-400 and propylene glycol in an osmotically active concentration of 20%.[10,14]

Based on the experimental research we have ascertained the optimal concentration of active substances. 10% concentration of aloe extract was selected based on conducted microbiological screening relative to test strains, and anaerobic pathogens of dental diseases.

Pharmaco-technological research ascertained the degree of dispersion of nimesulide and the expediency of its introduction into the gel. Taking into account that one of the major pathogenetic mechanisms of periodontal tissues diseases is an inflammation[11], the concentration of nimesulide in model samples is selected using screening of its anti-inflammatory activity. It was found that 0.5% concentration of nimesulide provides optimal microbicidal activity of the entire dental product.[12,13]

The technology of gel preparation

The studied drug: "Alnident" - Gel - the original drug, the pharmacological effect is associated with its constituent active ingredients: aloe liquid extract and nimesulide. Aloe extract in 1.0ml vials is used in dentistry for treatment of inflammation of the oral mucosa, mucosal damage after irradiation, intensifies cell regeneration and accelerates healing. For nimesulide are characteristic pronounced anti-inflammatory, analgesic and antipyretic effects, it has antiphlogistic, antioxidant and immunotropic activity and has a high safety and a relatively low risk of side effects when applied topically.

Into a vacuum reactor of homogenizer mixer with MG UGM of 2 liters volume equipped with a stirrer are added through the gage tank 200 ml of purified water at 20 ^{0}C, to it under stirring is loaded 20 g of Na CMC and

stirring is continued for 20-30 minutes, then left for 40 minutes for the swelling process. After the mass swollen under stirring to the gel mass is added 30 ml of glycerol and stirring is continued for 20-30 minutes. Separately, in 50 ml of water and 6 ml glycerol are dissolved 5 g of nimesulide, after dissolving is added into the reactor through the gage tank and stirring is continued for 20 minutes. Also separately in 50 ml of water 10 g of dry aloe extract is dissolved, after dissolving it is added into the reactor through the gage tank and stirring is continued for up to a homogeneous mass. The resulting mass is transferred to the stage of packing. Prepacking and packing of finished product. Finished product is prepacked in aluminium tubes by 30 g.

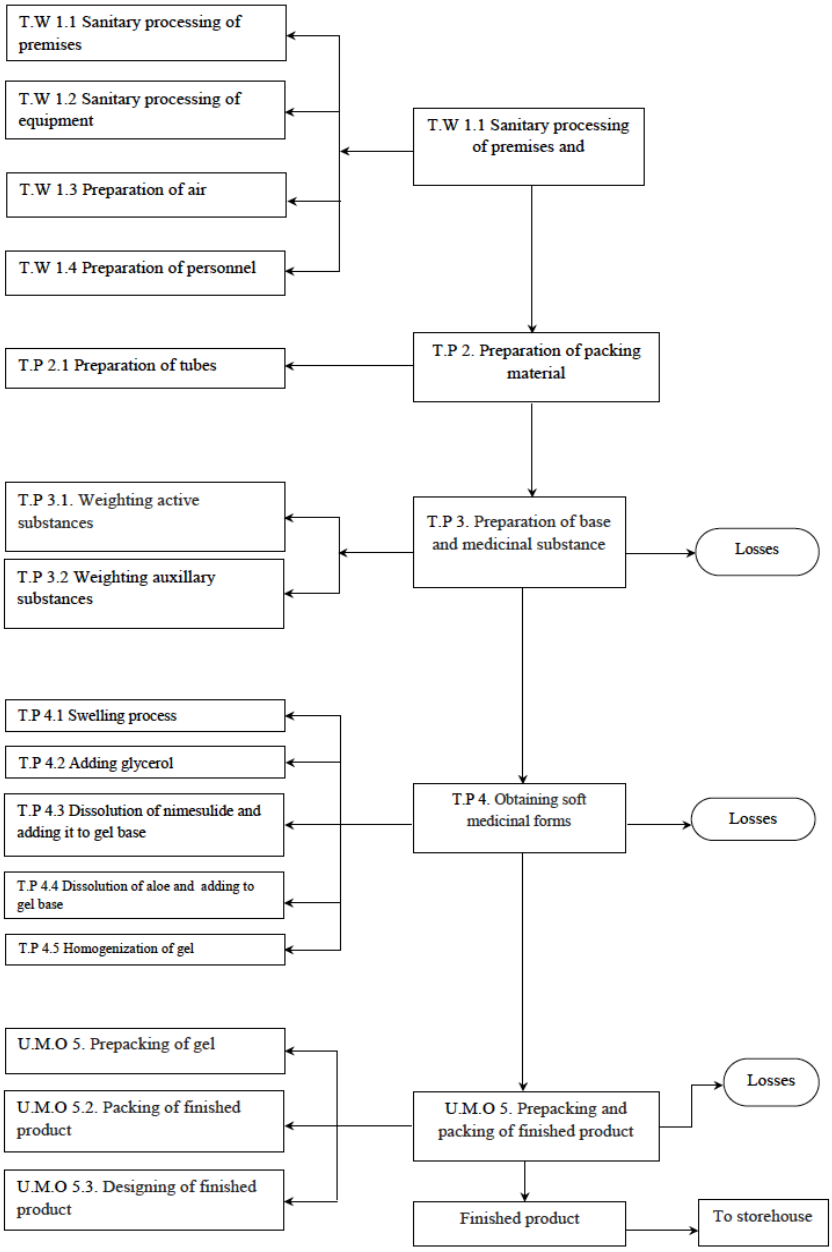

Fig. 1: Technological scheme of production Alnident gel

1. Acute toxicity was studied using conventional method described in the literature, a single intragastric administration and a single dermal administration of drugs with determination of LD50 and toxicity class.[3,5]
2. The studied drug: "Alnident" - Gel - the original drug, the pharmacological effect is associated with its constituent active ingredients: aloe liquid extract and nimesulide. Aloe extract in 1.0 mL vials is used in dentistry for treatment of inflammation of the oral mucosa, mucosal damage after irradiation, intensifies cell regeneration and accelerates healing. For nimesulide are characteristic pronounced anti-inflammatory, analgesic and antipyretic effects, it has antiphlogistic, antioxidant and immunotropic activity and has a high safety and a relatively low risk of side effects when applied topically.

The type and number of animals

For the experiment were used white outbred male mice of 10 animals, weighing 19-21g and 8 male albino rats, weighing 180 - 200g, kept in quarantine for 14 days.

Carrying out the experiment

The experiment to study the acute toxicity of compared preparations was carried out in two series. In the first series of experiment, acute toxicity of preparations was studied in male albino mice, which one-time was injected intraperitoneally the drug in amounts: 0.4 ml; 0.6 ml and 0.8 ml, dose: 25000 mg / kg; 37,500 mg / kg and 50,000 mg / kg, respectively.

OBSERVATION

On the first day of the experiment the animals were monitored every hour in the laboratory, and the appearance indicators were recorded (wool condition, mucous membranes, etc.); functional status (survival experience, general condition, possible convulsions and death) and behavior. Then daily, for 2 weeks in a vivarium, animals of all groups were observed for general condition and activity patterns of behavior, reaction to tactile, pain, auditory and visual stimuli, the frequency and depth of the respiratory movements, heart rate, condition of the hair and skin, tail position, the number and consistency of faeces, urinary frequency, weight change, and other indicators. All experimental animals were kept in the same conditions and on the general diet with free access to food and water.[5,10]

In the second series of experiment, acute toxicity of "Alnident" preparation was studied on 8white rats. The rats were housed in standard plastic cages on sawdust bedding. One day before the experimental studies on the skin of the back the hair was shorn, on a area measuring 2 x 2 cm. Shorn area of rats' skin was lubricated with the studied preparation "Alnident" at a dose of 5 g / kg. The animals were observed every hour for the first day of the experiment. Then, for 2 weeks, the animals in both groups were observed for general condition and activity, taking into account the behavioral responses. All experimental animals were kept in the same conditions and on the general diet with free access to food and water.[5,11] Acute toxicity was evaluated by the change in body weight and neuro-somatic indicators: general state of the animal, patterns of behavior, the intensity and the nature of motor activity, the presence and nature of seizures, coordination of movements, reaction to tactile, pain, auditory and visual stimuli, the frequency and depth of respiratory movements, condition of hair and skin, as well as macroscopic changes in the skin.

Locally – irritating action of "Alnident" preparation was studied by application of the drug on the unbroken skin of guinea pigs. For the experiment were used 6 guinea pigs of white colour weighing 300 - 350 g.[2,7] The animals for the period before the test was performed control of body temperature, general condition, evaluation of hair cover of back skin. One day prior to experimental research on the side surface of the body of guinea pigs, hair was shorn with scissors on the area size of 4 x 4 cm on both sides of the spinal column. On the shorn skin area of the right side surface of the body of guinea pigs were applied by 10 g/kg of "Alnident" preparation over which were applied tissues. On the left shorn side surface of guinea pigs was applied the gel base without the active substances. The exposure time was 4 hours. After the acute (single) exposure of drugs was evaluated the state of the skin when observed after 1 hour, 24 hours and 72 hours in points according to the system of classification of skin reactions in accordance with SSt R ISO 10993.[10-99]

Reference preparations

1. "Kamistad®" – combined preparation, gel for topical application, produced by Stada Arzneimittel AG, Germany. Composition: lidocaine hydrochloride, chamomile flower extract. Lidocaine has a local anesthetic effect, which contributes to the rapid and lasting pain reduction in inflammation and damage to the mucous membrane of the mouth and gums. Chamomile extract has anti-inflammatory, antiseptic and regenerating properties.

2. "Dentashan" - gel for gums, produced by Isshaan Healthcare Pvt. Ltd, India. Ingredients: metronidazole benzoate, chlorhexidine gluconate. Dentashan gel contains metronidazole chlorhexidine. Metronidazole according to therapeutic classification is antiprotozoal and antibacterial agent which is active against obligate anaerobic microorganisms such as bacteria and protozoa. Chlorhexidine is an antiseptic and antibacterial agent effective against gram-positive and gram-negative aerobic and anaerobic bacteria. It does not disturb the functional activity of lactobacilli.

Wound healing effect and anti-inflammatory activity of the preparation "Alnident" and reference drugs was studied in a rat model of periodontal disease by introducing into the gum around the neck of the upper and lower incisors of ethyl alcohol in a concentration of 70%.[3,8] Ethyl alcohol leads to pathological changes in the periodontium. For the experiment were used 30 albino rats, weighing 180 - 205 g of both sexes. Throughout the experiment, the rats were given pulpy food excluding self-cleaning of teeth. Signs of inflammation in the gingiva of rats in experimental groups treated with ethyl alcohol were observed on the 5th day of the experiment. On the 10 - 13th day was observed cyanosis of the gums, in the area of the incisors the appearing of periodontal recesses was observed. Clearly periodontal lesion in rats was observed after 9-13 days. After 1 day the treatment with studied and reference preparations was begun.

For the experiment, the animals were divided into 5 groups of 6 animals in each group, reference drugs were administered as follows:

1. Intact group - no manipulations were carried out with rats.
2. Control group - in rats gum with syringe was administered 0.1 ml of 70% ethanol, without treatment;
3. Experimental group - 0.1 ml of 70% ethanol + gums were treated with cotton turundas with "Alnident" preparation;
4. Experimental group - 0.1 ml of 70% ethanol + gums were treated with cotton turundas with "Kamistad®" preparation produced by Stada Arzneimittel AG, Germany;
5. Experimental group - 0.1 ml of 70% ethanol + gums were treated with cotton turundas with "Dentashan" preparation produced by Isshaan Healthcare Pvt. Ltd, India.

Treatment with drugs was carried out daily until complete recovery.

In rats with a damaged passive gums was observed passive meal, moreover, the inflammatory process is accompanied by changes in body temperature. In this regard, the clinical status of the animals was evaluated by the total of (measured body weight and body temperature) also recorded the period of wound healing (days).

The data obtained are statistically processed using STATISTICA for Windows 95 software.

DISCUSSION

These experiments showed that after a single intragastric administration of "Alnident" drug in doses of 25,000 mg/kg; 37,500 mg/kg and 50,000 mg/kg in animal behavior and the functional status no visible changes were observed (table).

Table 1: Determination of acute toxicity (LD50) of «Alnident»

No. Groups	«Alnident»			
	dose		Application method	Number of dead mice
	mg/kg	ml		
1	25000	0,4	Per os	0/6
2	37500	0,6	Per os	0/6
3	50000	0,8	Per os	0/6

According to the classification of substances on toxicity "Alnident" preparation was relatively harmless[4];

The conducted experiments of the second series of experiments showed that after a single epicutaneous application to the skin of white rats "Alnident" preparation at a dose of 5g/kg, death of rats was not observed. Visual symptoms of the pathological changes in the form of disorder of intensity and nature of motor activity, motor coordination, skeletal muscle tone were not observed. Behavioral response did not deviate from the norm. The response to tactile, pain, sound and light stimuli - unchanged. Condition of the hair and skin, the color of the mucous - without pathologic changes.

Observations of the experimental skin area condition of guinea pigs in comparison with the control after 1, 24 and 72 hours showed that when applying "Alnident" preparation visible changes in the skin were not observed.

Results obtained by studies have shown that inflammation of the gums in rats was observed after only 1 day after the administration of ethyl alcohol, which is manifested in the form of swelling, redness, swelling and bleeding of gums, a slight increase in body temperature. Rats in the control group showed weakness, stiffness, passive meal. When measured at 10th day of the experiment body weight showed that the rats do not gain weight, even a weight reduction was observed at 2.1% in comparison with the original, while the rats in the intact group on the contrary for 10days was observed an increase in body weight by 21.3% compared with the initial weight.

Measurement of body temperature on the 5th day showed that in rats in the control group was observed an increase of body temperature by 0,9 º C. Complete healing of gums in rats occurred after 42 ± 14 days.

In the group of rats in which the inflamed gingiva was treated with "Alnident" preparation, a day after treatment decreased gingival swelling and hyperemia of the gums. Animals willingly ate soft food. Measurement of body weight of rats on 10th day showed that in animals was observed an increase in weight by 16.5% compared with the initial weight, indicating their good nutrition. The body temperature of the rats on the 5th day of the experiment did not differ from the original. Complete healing of the gingiva of rats in the treatment with "Alnident" preparation was observed after 28,2 ± 0,7 days, which is 32.9% faster compared to the untreated control group (Table 2).

Table 2: The influence of the preparations "Alnident", "Kamistad®" and "Dentashan" on progress dynamics of periodontal inflammation in the of rats

No of animal	Initial body weight, g	Body weight on 10th day, g	Initial body t, ºC	Body t on 5th day, ºC	Complete recovery, days
Intact group					
M ± m	192,0 ±8,0	233,2 ± 6,8	37,3 ± 0,3	37 ± 0,2	-
Control group					
M ± m **P**	191 ± 8,5	187 ± 15 P < 0,05	37,4 ± 0,2 P > 0,5	38,5 ± 0,2 P < 0,05	42 ± 14 P < 0,05
«Alnident»					
M ± m **P**	194 ± 8,2	226 ± 7,0 P < 0,05	37,3 ± 0,2 P > 0,5	38 ± 0,14 P < 0,05	28,2 ± 0,7 P < 0,05
«Kamistad®», Stada Arzneimittel AG, Germany					
M ± m **P**	191 ± 6,4	209 ± 15 P < 0,05	37,4 ± 0,2 P > 0,5	38,2 ± 0,12 P < 0,05	38,1 ± 2,5 P < 0,05
«Dentashan`», Isshaan Healthcare Pvt. Ltd, India					
M ± m **P**	191 ± 7,6	228 ± 16 P < 0,05	37,4 ± 0,18 P < 0,05	38,0 ± 0,14 P < 0,05	27,5 ± 3,1 P < 0,05

Similar results were obtained in the treatment of inflamed gums of rats with preparations "Kamistad®" produced by Stada Arzneimittel AG, Germany and "Dentashan" produced by Isshaan Healthcare Pvt. Ltd, India.

However, as shown by the results of studies on the therapeutic action of the drug "Alnident" was close to the "Dentashan" preparation, produced by Isshaan Healthcare Pvt. Ltd, India, while drug "Kamistad®" produced by Stada Arzneimittel AG, Germany had a weaker effect.

All the data obtained in the study of the pharmacological properties of "Alnident" preparation in comparison with the preparations Kamistad® " produced by Stada Arzneimittel AG, Germany and" Dentashan" produced by Isshaan Healthcare Pvt. Ltd, India are presented in the table.

Thus, the data obtained in the study of wound healing effect on the model of inflammation in periodontium of white rats showed that the preparation "Alnident" has a pronounced wound-healing, anti-inflammatory action and its effect is not inferior to foreign analogues preparations "Dentashan" produced by Isshaan Healthcare Pvt. Ltd, India, and slightly larger than the preparation "Kamistad®" produced by Stada Arzneimittel AG, Germany.

CONCLUSION

Pharmacological studies of "Alnident" drug - dental gel showed:

1. According to the classification of substances on toxicity "Alnident" preparation appeared relatively harmless[4];
2. Preparation "Alnident" when applied to the skin of guinea pigs had no irritative action.
3. In the studied doses of "Alnident" preparation - dental gel has pronounced wound-healing effect and a strong anti-inflammatory effect.
4. Effect of "Alnident" preparation was close to "Dentashan" preparation, produced by Isshaan Healthcare Pvt. Ltd, India, while "Kamistad®" preparation produced by Stada Arzneimittel AG, Germany had a weaker effect.

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