

Preclinical evaluation of a gel composition based on a flavonoid complex for the treatment of periodontal diseases in orthodontic patients

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The aim of the work was a preclinical assessment of acute toxicity, skin resorptive, irritant effects, cumulative and catalase activity, as well as sensitizing properties of the local gel composition "Benzidafaziverdine" (GCB) used for the treatment of periodontal diseases in orthodontic patients.

Materials and methods. 119 animals were involved in the experiment, assigned to seven main and two control groups. GCB was administered intragastrically in doses of 300–600 mg/kg and intradermally of 200 µg into the outer surface of the ear. The native solution of GCB was applied to the skin and mucous membranes, administered orally by the method of "subchronic toxicity" and to the surface of the chorioallantoic membrane (CAM) of chicken embryos. The intensity of lipid peroxidation (LPO) was assessed by the level of diene conjugates (DCs) and malondialdehyde (MDA), and the antioxidant system by catalase activity. The specific leukocyte agglomeration reaction (SLAR), the specific leukocyte lysis reaction, and neutrophil damage indicators were used.

Results. The median lethal dose LD₅₀ for rats and mice of both sexes exceeded 5000 mg/kg. The irritant effect of GCB on the mucous membranes was manifested by hyperemia on the second day. Symptoms of irritation disappeared after 3–4 days without medical intervention. An analysis of the CAM blood vessels after exposure to GCB in two observations at the 120th second showed the beginning of hemorrhages. In one observation, GCB caused minor hemorrhages at the 300th second of the experiment.

It was found that the coefficient of GCB irritant action was 5 (the mean score of Me (Q1; Q3) was 5 (4; 5)). The coefficient of cumulation (K_{сум}) exceeded 8.2. An insignificant increase in the median or mean values of catalase enzyme activity, DCs, and the amount of LPO end product such as MDA was observed compared to the control group animals. The SLAR test indicated the development of a delayed-type allergic reaction under the influence of GCB in a 1:10 dilution. One-hundred-fold dilution did not cause significant changes in the indicator in the main group compared to the control one.

Conclusions. GCB belongs to the 4th class of toxicity – practically non-toxic substances, does not have sex- and species sensitivity, has weak cumulative activity, minimal effect on the system of LPO. GCB can be recommended for the use in clinical periodontology for medical support of orthodontic patients.

Ключові слова:

експеримент, лабораторні тварини, пародонтальна гелева композиція, флавоноїди, токсикологічні дослідження, сенсibiлізація, перекисне окиснення ліпідів, ортодонтічні пацієнти.

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Доклінічне оцінювання гелевої композиції на основі флавоноїдного комплексу для лікування захворювань пародонта в ортодонтічних пацієнтів

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Мета роботи – доклінічне оцінювання гострої токсичності, шкірно-резорбтивної, іритативної та подразнювальної дії, кумулятивної та каталазної активності, а також сенсibiлізувальних властивостей гелевої композиції місцевого застосування «Бензидафлазівердин» (ГКБ) для лікування захворювань пародонта в ортодонтічних пацієнтів.

Матеріали та методи. В експеримент залучено 119 тварин, яких поділили на 7 основних і 2 контрольні групи. ГКБ вводили внутрішньошлунково у дозах 300–600 мг/кг та внутрішньошкірно 200 мкг у зовнішню поверхню вуха. Робили аплікації нативного розчину ГКБ на шкіру та слизові оболонки, перорально вводили за методом «субхронічної токсичності» та наносили на поверхню хоріоалантоїсної оболонки (ХАО) курячих ембріонів.

Інтенсивність перекисного окиснення ліпідів (ПОЛ) оцінювали за рівнем дієнових кон'югатів і малонового діальдегіду, антиоксидантну систему – за активністю каталази. Використовували реакцію специфічної агломерації лейкоцитів (РСАЛ), реакцію специфічного лізису лейкоцитів і показники пошкодження нейтрофілів.

Результати. Середня летальна доза LD₅₀ для щурів і мишей обох статей перевищувала 5000 мг/кг. Іритативну дію ГКБ на слизові оболонки спостерігали на другу добу як гіперемію. Симптоми подразнення зникали через 3–4 доби без медикаментозного втручання. Аналіз судин ХАО після впливу ГКБ у двох спостереженнях на 120 секунд показав початок крововиливу. В одному спостереженні ГКБ викликала дрібні крововиливи на 300 секунд експерименту.

Встановлено, що коефіцієнт подразнювальної дії ГКБ становив 5 (середній бал Me (Q1; Q3) дорівнював 5 (4; 5)). Коефіцієнт кумуляції перевищував (K_{сум} >8,2). Спостерігали недостовірне збільшення медіанних або середніх значень активності ферменту каталази, дієнових кон'югатів і кількості кінцевого продукту ПОЛ малонового діальдегіду порівняно з тваринами контрольної групи. Тест РСАЛ показав виникнення алергічної реакції сповільненого типу під впливом ГКБ у розведенні 1:10. Розведення у 100 разів не викликало достовірних змін показника в основній групі порівняно з контролем.

Висновки. ГКБ належить до 4 класу токсичності – майже нетоксичні речовини, не характеризується статевою та видовою чутливістю, має слабку кумулятивну активність, мінімальний вплив на систему ПОЛ. ГКБ можна рекомендувати для використання у клінічній пародонтології для медикаментозного супроводу ортодонтічних пацієнтів.

Increasing the treatment effectiveness of maxillofacial anomalies will continue to be an urgent problem of modern dentistry. However, over the years, numerous studies have established a high percentage (68 %) of the risk of developing periodontal tissue diseases against the background of maxillofacial anomalies among patients of various ages. In approximately 80 % of cases, such patients undergo orthodontic treatment with the use of fixed appliances, during which the severity of the pathological process course in the periodontal complex tissues only worsens [1,2,3].

Under the influence of a local stressor effect of a bracket system on the periodontium, there is a focal loosening of the epithelium, leukocyte infiltration, edema and dilation of blood vessels. According to some researchers, in the realization of damaging mechanisms to periodontal soft tissues under local stress, hypoxia plays a leading role, which is associated with disturbances in external respiration and gas exchange, transport and utilization of oxygen [4]. In turn, the inflammatory process, stress reaction, and hypoxia cause a loss of balance between lipid peroxidation (LPO) and antioxidant systems (AOS), which is one of the important links in the pathogenesis of many diseases, particularly periodontal tissues.

Oxidative stress occurs when intracellular concentrations of reactive oxygen forms exceed physiological values [5]. It is during orthodontic treatment that two different situations coexist potentiating the occurrence of inflammatory processes and oxidative stress: on the one hand, it is bracket systems, and on the other hand, the biomechanics of tooth movement [6]. Thus, violations of oxygen transport and utilization, respiratory functions of mitochondria, prooxidant-antioxidant homeostasis in soft and hard periodontal tissues, as well as the occurrence of oxidative stress when using bracket systems are the basis for using local pharmacological agents with anti-inflammatory, analgesic, anti-hypoxic and antioxidant effects.

The data of numerous studies indicate that flavonoids have a pronounced antibacterial, anti-inflammatory and antioxidant effects – their phenolic structure enables molecules to interact with free radicals, reducing the intensity of lipids, resulting in inhibition of the main negative factor formation – malondialdehyde (MDA) [7,8]. Thus, the drug “Proteflazid®” (“ECOFARM,” Ukraine) based on flavonoid glycosides (liquid extract 1:1), obtained from a mixture of *Deschampsia caespitosa herba* and *Calamagrostis epigeios herba*, has pronounced antioxidant and immunotropic properties, protects mucous membranes, normalizing indicators of local immunity (lactoferrin, secretory immunoglobulin A, lysozyme and complement component 3).

Also, among the drugs used in dentistry, a non-steroidal low-toxic drug benzydamine hydrochloride with antimicrobial, analgesic and anti-exudative properties and an active anti-inflammatory activity, is also effective. On the basis of the above-mentioned medicinal products as active components in the composition of the gel base (sodium alginate, nipagin and water for injections), an extemporaneous gel composition “Benzidaflaziverdine” (GCB) was developed for a local use for periodontal dressings in the treatment of gingivitis and periodontitis in orthodontic patients before and during active orthodontic treatment to eliminate signs of inflammation and reduce oxidative stress from the loading effect of bracket systems [9].

Aim

The aim of the work was a preclinical assessment of acute toxicity, skin resorptive, irritant effects, cumulative and catalase activity, as well as sensitizing properties of the local GCB gel composition used for the treatment of periodontal diseases in orthodontic patients.

Materials and methods

To carry out toxicological studies of GCB for compliance with the requirements of DSanPiN 2.2.9.027-99 [10], four types of laboratory animals of both sexes were used equally: 54 non-linear sexually mature rats (weighing 180–200 g), 34 white sexually mature mice (weighing 17–23 g), 30 light-colored guinea pigs (weighing 300–350 g) and one rabbit (male, weighing 560 g), which were kept in vivarium conditions at Danylo Halytsky Lviv National Medical University (LNMU) on a standard diet [11].

During animal research, the principles of bioethics, legal norms and requirements in accordance with the principles of humanity set forth in the Directive of the European Community [12], in accordance with the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), as well as the General Ethical Principles of Animal Experiments adopted at the First National Congress on Bioethics (September 20, 2004, Kyiv, Ukraine) were followed. The study was approved by Protocol No. 8 of the Committee on the Ethics of Scientific Research, Experimental Developments and Scientific Works of Danylo Halytskyi LNMU dated October 18, 2021.

Each group of experimental animals was formed by compiling ranked rows and numbering and labeling according to initial body weight. The results obtained during the research were compared with standard reference indicators of 20 intact animals that made up the control groups [13]. Anesthesia was performed by intramuscular injection of 2 % “Xylazine” (Alfasan, the Netherlands) at a dose of 5 mg/kg of body weight and “Zoletil 50” (Virbac, France) at a dose of 0.5 mg/kg of body weight. The studied material was collected during the experiments by cardiac puncture under anesthesia.

Acute toxicity was determined in rats ($n = 24$) and mice ($n = 24$) by intragastric administration of GCB in doses of 3000–6000 mg/kg followed by determination of the degree and nature of acute oral toxicity. For 14 days, after the drug administration, the animals were followed up daily to register the presence of toxicity clinical signs or death. The following indicators were evaluated: lethality (death dates of animals in each group, daily); manifestations of toxicity (daily), including appearance; body weight dynamics. The criterion of GCB acute toxicity was the median lethal dose (LD_{50}). After the end of the experiment, euthanasia, dissection and macroscopy of internal organs were performed in randomly selected 5 animals [14].

The assessment of the skin resorptive and irritative effect of GCB consisted in studying changes in the structural and functional state of the skin and mucous membrane with an assessment of the risk of acute manifestations on their surface under laboratory conditions. The study was conducted by immersing mouse tails ($n = 10$) into a native solution of GCB 10 times for 4 hours (exposures of 4 hours

per day 5 days a week) and applying this agent to the mouse skin ($n = 10$). A conclusion was made based on toxicological indicators: lethal effect, time and degree of intoxication sign manifestations, changes in body weight. The irritant effect was examined by applying GCB to the conjunctival sac of rabbit eyes [15].

The chorioallantoic membrane (CAM) of 9-day-old chicken embryos was used to determine the irritating effect of GCB [16]. Fresh chicken eggs (up to seven days after oviposition) weighing from 50 g to 60 g. Incubation in an inverter incubator was carried out at a temperature of 38.3 ± 0.2 °C. In order to provide a base level when evaluating the experimental results, sterile 0.9 % sodium chloride (NaCl) solution was tested as a negative control. As a positive control, a 1 % solution of sodium dodecyl sulfate was used as a generally recognized irritant based on the results of *in vivo* experiments. Three eggs were taken to study each substance. To avoid a traumatic reaction, eggs were incubated for 30 minutes, the inner membrane was pre-moistened with a 0.9 % NaCl solution, and then it was removed with tweezers. GCB was assessed in the native state by applying 0.3 ml directly to the CAM surface using a disposable glass pipette.

Reactions to CAM were monitored for 300 seconds using a micro-camera endoscope. The following reactions and changes in CAM were monitored and recorded in points with mandatory photo-fixation after 30, 120 and 300 seconds from the time of substance application: 1 – vascular lysis (breakdown of blood vessels): 30 seconds – 5 points; 120 seconds – 3 points; 300 seconds – 1 point; 2 – hemorrhages (bleeding from blood vessels): 30 seconds – 7 points; 120 seconds – 5 points; 300 seconds – 3 points; 3 – coagulation (intra- and extravascular protein denaturation): 30 seconds – 9 points; 120 seconds – 7 points; 300 seconds – 5 points.

The irritation index was calculated as the median value from the total scores of all test repetitions. The ratio of the numerical values of the irritating effect (irritation index) and the risk category of developing an irritating effect was as follows: 0.0–0.9 – not causing an irritating effect; 1.0–4.9 – weak irritating action; 5.0–8.9 – moderate irritating effect; 9–21 – pronounced irritating effect.

The presence of a toxic-cumulative effect upon GCB oral administration into rat bodies ($n = 10$) – the severity degree of the toxic “accumulation” effect in a living organism (cumulation coefficient – K_{cum}) – was determined by the method of “subchronic toxicity” [17, 18]. GCB was administered in its native form, starting with a dose of 600 mg/kg with a dose increase of 1.5 times every 4 days. Animals were removed from the experiment after 28 days.

The state of lipid peroxidation (LPO) was determined in rats ($n = 10$) of the main group by the content of active thiobarbituric acid (TBK-AP) products in the blood via reaction with thiobarbituric acid [19]. The intensity of LPO processes was assessed with diene conjugates (DCs) – by the intensity of heptane fraction and secondary products – MDA light absorption [20]. The antioxidant system state was assessed by the main enzyme of antioxidant protection – catalase activity [21]. The control group consisted of intact rats ($n = 10$).

The sensitizing properties of GCB were evaluated during complex sensitization of the main group guinea pigs ($n = 10$), which was carried out by intradermal injection of

200 µg (in 0.02 ml) of the agent into the outer surface of the ear [22]. Animals of the control group ($n = 10$) were injected with 0.02 ml of solvent (physiological solution). The gel was then applied to the left half of the animal bodies for 10 days. The degree of sensitization was defined after conducting intradermal tests at dilutions 1:10, 1:100. The body reaction was evaluated by visual examination of the skin surface at the place of sample injection after 20–30 minutes, 4–5 hours and 24 hours after administration and according to the results of clinical and immunological tests.

After completion of the experiment, changes in peripheral blood parameters were studied: content and qualitative composition. The obtained data were expressed in percentages and in absolute units per 1 liter of blood ($10^9/l$) [23]. We calculated the lymphocyte-monocyte ratio index (LMRI), the neutrophil-monocyte ratio index (NMRI), and the neutrophil-eosinophil ratio index (NERI). After sensitization of the animals, a method for detecting the reaction of blood cells to the allergen “*in vitro*” was used for its quantitative assessment – the specific leukocyte agglomeration reaction (SLAR), the specific leukocyte lysis reaction (SLLR) and indicators of neutrophil damage (IND) [23].

Statistical processing of the results was carried out using the Microsoft Excel package. Compliance of the obtained data with the normal distribution law was checked using the Shapiro–Wilk test. Provided the normality of the distribution was met, the significance of the obtained differences in the compared values was assessed using the Student’s test or the Mann–Whitney test in cases where there was a non-parametric data distribution. Non-normally distributed data were presented in the form of Me (Q1; Q3), where Me was the median and (Q1; Q3) were the upper and lower quartiles. Changes with a level of more than 95 % ($p < 0.05$) were considered significant [24].

Results

The study on the GCB acute toxic effect nature within 14 days made it possible to reveal that after a single intragastric administration of the agent, no signs of intoxication were observed in the experimental animals. The animals were active, responded to light and sound stimuli, urination and defecation processes were normal, there was no respiratory disturbance, reflex excitability was preserved. The body weight dynamics of the animals that received the studied drug was within the physiological norm. There were no fatal cases during the entire follow-up period, after which, at an autopsy of 5 randomly selected animals, no abnormalities were noted in the internal organs. LD_{50} for rats and mice of both sexes exceeded 5000 mg/kg. According to the toxicological classification, the developed GCB was assigned to the 4th class of toxicity – practically non-toxic substances. It was also found that GCB did not have sex- and species-related sensitivity.

In the study on the skin resorptive effect, no symptoms of intoxication were noted after the application of the studied GCB to the guinea pig skin. When examining the treated skin area of the white mouse tails, no signs of irritation were seen. It was detected that functional changes such as the appearance of erythema, edema, and cracks did not occur after the GCB application to the intact skin of experimental animals.

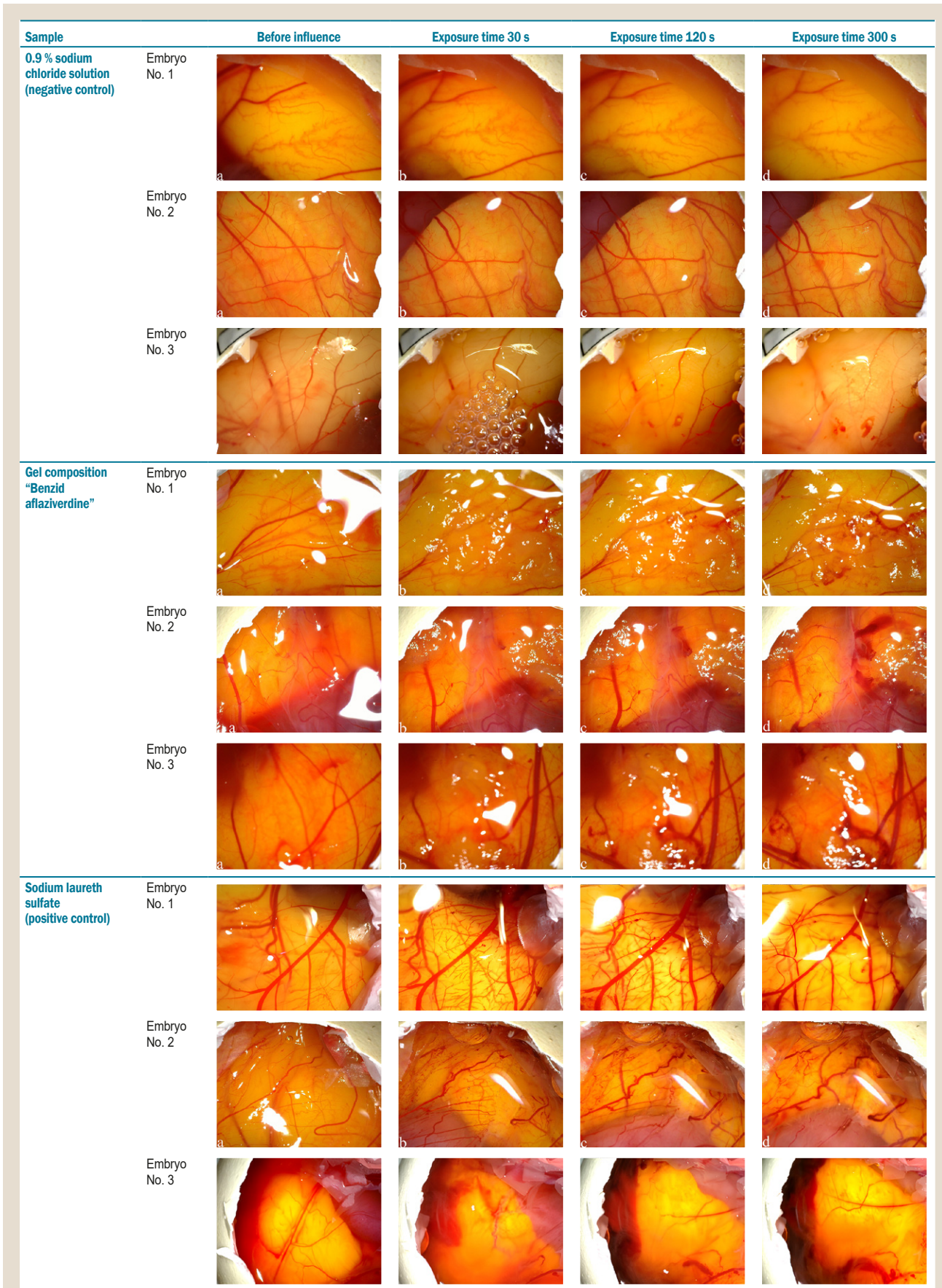


Fig. 1. Photofixation of irritating effect of gel composition "Benzidafaziverdine" on the chicken embryo chorioallantoic membrane (macrophoto).

Table 1. Manifestations of irritant action on the chicken embryo chorioallantoic membrane

Effect/experiment	Lysis (points)			Hemorrhages (points)			Coagulation (points)			Total score
	30 s	120 s	300 s	30 s	120 s	300 s	30 s	120 s	300 s	
Physiological solution (0.9 % sodium chloride solution). Negative control										
Observation 1	–	–	–	–	–	–	–	–	–	0
Observation 2	–	–	1	–	–	–	–	–	–	1
Observation 3	–	–	–	–	–	–	–	–	–	0
Irritation index Me (Q1; Q3)										0 (0.0; 0.5)
Gel composition "Benzidafaziverdine" in its native state										
Observation 1	–	–	–	–	–	3	–	–	–	3
Observation 2	–	–	–	–	5	–	–	–	–	5
Observation 3	–	–	–	–	5	–	–	–	–	5
Irritation index Me (Q1; Q3)										5 (4; 5)
Sodium laureth sulfate (1 % solution of sodium dodecyl sulfate). Positive control										
Observation 1	5	–	–	7	–	–	–	–	–	12
Observation 2	5	–	–	7	–	–	–	–	–	12
Observation 3	5	–	–	7	–	–	–	–	–	12
Irritation index Me (Q1; Q3)										12 (12; 12)

Days of administration	1–4	5–8	9–12	13–16	17–20	21–24	25–28
An administered daily dose LD ₅₀	0.1	0.15	0.22	0.34	0.5	0.75	1.12
Total dose for 4 days LD ₅₀	0.4	0.6	0.88	1.36	2.0	3.0	4.5
Total dose for the periods		1.0					
			1.88				
				3.24			
					5.24		
						8.24	
							12.74

Fig. 2. Scheme of conducting the "subchronic toxicity" test.

The irritant effect of GCB on the rabbit conjunctiva was manifested on the second day by hyperemia of the mucous membrane (intensity 0–1 points), edema (0 points), secretions (0 points). Symptoms of irritation disappeared after 3–4 days without medical intervention.

The study on the GCB irritating effect using an alternative model – the chicken embryo CAM allowed us to obtain the following results (Fig. 1, Table 1). Physical testing solution (0.9 % sodium chloride solution) as a negative control demonstrated vessel lysis at the 300th second in one experimental observation. Analysis of the CAM blood vessels after exposure to GCB showed the beginning of hemorrhage in two observations at the 120th second. In one observation, small hemorrhages were caused by the tested agent at the 300th second of the experiment.

Testing of sodium laureth sulfate (1 % solution of sodium dodecyl sulfate) as a positive control showed hemorrhage and lysis of vessels at the 30th second in all experimental observations (Fig. 1, Table 1).

The obtained results of recording negative changes in the CAM for a given time period are presented in Table 1.

The obtained irritation index served as a criterion for classifying the irritative activity of the studied GCB. The compound was considered to have a moderate irritant effect on mucous membranes based on the detected coefficient of irritation.

Cumulation coefficient – K_{cum} was determined by the method of "subchronic toxicity" [20] in a subacute experiment with repeated administration of increasing doses

(starting with a dose of 0.1 LD₅₀ to 0.5 LD₅₀) by 1.5 times every 4 days according to the relevant scheme (Fig. 2).

A subchronic experiment to determine the GCB cumulative properties made it possible to show satisfactory general condition and behavior of rats throughout the experiment; the animals consumed food and water, responded adequately to external stimuli, and an increase in body weight was observed. No deaths of experimental animals were registered. The K_{cum} exceeded 8.2, which indicated a weak GCB cumulative activity.

After the subchronic administration of GCB, the results of blood analysis in experimental animals based on LPO indicators – catalase activity, concentrations of MDA and DCs were as follows. The catalase index (nmol H₂O₂/ml × min) in the experimental group animals was on average 3.4 (2.3; 5.0) in comparison with the index of 2.27 (2.1; 3.2) in the control group animals (a p value significance of 0.18) (Me (Q1; Q3); M ± m). The level of MDA in the experimental group animals was on average 89.38 ± 46.10 mmol/l in comparison with the level of 62.24 ± 11.60 mmol/l in the control group animals (a p value significance of 0.088). The index of DCs in the experimental group animals was on average 1.61 ± 0.68 units of A/ml in comparison with the indicator of 1.28 ± 0.25 units of A/ml in the control group animals (a p value significance of 0.162).

The study results showed a slight and insignificant increase in the median or mean values of the enzyme activity of catalase, DCs and the MDA amount, an end product of LPO, in the experimental animals compared to those in the control group animals. This testified to the minimal effect of GCB components on the LPO system.

When conducting intradermal tests in sensitized guinea pigs, GCB in doses of 1:10 and 1:100 (solvent – physiological solution) did not cause changes on the skin surface. As can be seen from the data presented in Table 2, the values of leukocyte absolute number and the percentage content and absolute number of basophils, eosinophils, neutrophils, monocytes and lymphocytes in the experimental group animals did not change significantly compared to those in the control group.

The sensitizing effect of GCB was manifested by an increase in the SLLR value. In animals exposed to the agent at a dilution of 1:10, a persistent tendency to increase leu-

colysis was noted. Exceeding its critical value of 10 % was observed in 70 % of experimental animals. In the control, it almost did not exceed the critical 10 %. The mean group values of this indicator in the experimental animals exceeded the control indicators by two times, which characterized the development of allergic cytotoxic-type reaction in their body. At the same time, at a 1:100 dilution, the lysis values in the experimental group indicated the absence of allergic reactions (Table 3).

The phenomenon of leukocyte agglomeration is the first phase of allergic reaction. The SLAR test results indicated the development of a delayed-type hypersensitivity under the influence of GCB at a 1:10 dilution. One-hundred-fold dilution did not cause significant changes in the indicator of the main group compared to the control.

For IND in the group for which the 1:10 dilution was analyzed, fluctuations in the value from 0.05 to 0.07 were noted (exceeding the critical value of 0.05 was noted in 50 % of animals from the main group), while the median IND in this group was within normal limits. In a dilution of 1:100 and in the control group animals, this indicator did not exceed 0.05 in all experimental guinea pigs. An increase in IND observed in some experimental animals under the influence of GCB in a dilution of 1:10 was due to the effect on the maturation of neutrophilic granulocytes which have amoeboid activity, indicating the potential of GCB for specific sensitization of the organism.

Discussion

Thus, the conducted experimental studies indicate the absence of toxic effects and the relative safety of the developed GCB due to the two active components. This is benzydamine hydrochloride for the local use in the oral cavity, which belongs to non-steroidal anti-inflammatory drugs, which are inferior to steroids in terms of their mechanism of action but have a very low toxicity. At the same time, benzydamine hydrochloride is characterized by high virucidal effect as well as antimicrobial activity against periodontopathogens [25].

The drug "Proteflazid[®]" is characterized by significant antioxidant activity associated with the presence of free and glycosidated flavonoids, an important property of which is the involvement in redox reactions as donors or acceptors of electrons and protons [26]. "Proteflazid[®]" has a wide spectrum of biological effects on numerous pathogenetic links of hypoxic tissue damage, including preventing blockage of microcirculation vessels by leukocyte plugs, inhibiting the synthesis of leukotrienes from arachidonic acid, reducing capillary permeability, restoring the sensitivity of platelets and increasing their duration of action, reducing the adhesive property of blood elements, protects endotheliocytes from damage, reduces perivascular edema [27]. In addition, this drug has a positive effect on the gastrointestinal tract state, normalizes peristalsis, vegetative-vascular disorders, increases the body non-specific resistance due to the induction of endogenous α - and γ -interferons, as well as a detoxifying effect [26,27].

The low sensitizing potential of GCB was revealed using such tests as SLAR, SLLR and IND, which made it possible to detect a delayed type of allergic reaction. SLLR is based on the change of sensitized cells under the action of a specific

Table 2. The results of hematological parameters of guinea pigs with percutaneous exposure to gel composition "Benzidaflaziverdine" (Me (Q1; Q3); M \pm m)

Parameters, units of measurement	Control animals (n = 10)	Sensitized animals (n = 10)	The significance value, p
Leukocytes, g/l	12.96 \pm 2.60	12.40 \pm 1.99	0.59
Basophils, %	0 (0; 0)	0 (0; 1)	0.65
Basophils, g/l	0.00 (0.00; 0.00)	0 (0.00; 0.12)	0.69
Eosinophils, %	3 (2; 3)	3 (2; 4)	0.18
Eosinophils, g/l	0.33 \pm 0.04	0.43 \pm 0.15	0.07
Neutrophils, %	17.0 (15.0; 23.0)	17.5 (16.0; 21.0)	0.82
Neutrophils, g/l	1.93 (1.80; 3.24)	2.35 (2.30; 2.47)	0.31
Monocytes, %	3 (2; 3)	3 (2; 3)	0.56
Monocytes, g/l	0.35 \pm 0.10	0.38 \pm 0.14	0.54
Lymphocytes, %	75.9 \pm 4.9	74.5 \pm 5.8	0.57
Lymphocytes, g/l	9.80 \pm 1.60	9.70 \pm 1.97	0.96
LMRI	26.4 (23.3; 39.0)	25.4 (24.5; 32.3)	0.57
NMRI	7.8 (5.7; 8.6)	6.2 (5.7; 7.1)	0.54
NERI	6.36 (5.70; 8.00)	5.33 (4.60; 8.50)	0.18

Table 3. Assessment of the sensitizing effect of gel composition "Benzidaflaziverdine" (GCB) in the experimental animals and *in vitro* allergy tests

Parameters		Control	GCB	
			1:10 dilution	1:100 dilution
SLLR	N	1/10	7/10	0/10
	%	6.18 \pm 2.70	11.09 \pm 1.90* (p = 0.0002)	6.98 \pm 2.0
SLAR	N	0/10	4/10	0/10
	%	1.20 \pm 0.07	1.4 \pm 0.1	0.89 \pm 0.2
IND	N	0/10	5/10	0/10
	%	0.025 \pm 0.008	0.045 \pm 0.008* (p = 0.00005)	0.020 \pm 0.001

N: numerator – the number of animals with positive (supernormal) results, denominator – all in the experiment; *: significant differences compared to the control, p > 0.05 (Tukey's HSD test) and its value was omitted in the table.

allergen and is associated with the complement involvement in the implementation of the immune complex formation on the cell surface resulting in their damage and lysis. The SLAR is based on the effect of strengthening the blood cell adhesion when a specific allergen is added to it, which is one of the first phases of the specific allergic reaction of blood cells. The IND reflects the increase in their mobility under the influence of an allergen and serves to assess the early phase of the allergic reaction of blood cells [23].

As there is convincing evidence that inflammation of periodontal tissue is one of the main sources of reactive oxygen species (ROS) in the oral cavity [5] coupled with the activation of aggressive periodontopathogenic microorganisms [28], it is likely to indicate that aseptic inflammation in orthodontic patients may also be related to damage caused by oxidative stress.

Finally, it is a proven fact that fixed orthodontic appliances are a source of corrosion, and due to the simultaneous influence of deformation, friction and mechanical action, the degradation of orthodontic brackets and arches occurs causing higher concentrations of metal ions in the oral cavity. Corroded appliances induce the release of metal ions, which can cause increased levels of ROS through metal-catalyzed free radical reactions. Chromium, iron, nickel, cobalt, titanium and molybdenum are a group of transition metals that can undergo redox reactions with the ROS formation [29].

Therefore, it is decisive to include GCB in the drug

“Proteflazid[®]” as an inducer of the endogenous α - and γ -interferon synthesis up to a physiologically active level, which increases the non-specific body resistance to viral and bacterial infections, has antioxidant properties, does not have an immunotoxic effect, does not cause refractoriness (hyporeactivity) of the immune system. The drug inhibits the course of free radical processes, prevents the accumulation of LPO products, increases the antioxidant status of cells, and thus reduces stressor intoxication [5]. In this study, it has been confirmed by the main enzyme of antioxidant protection activity – catalase and the studied level of primary LPO products – DCs, secondary products – MDA, which are responsible for damage to cell membranes and vessel walls, as one of the leading factors in the development of inflammatory process and its chronicity [20].

Therefore, the developed and preclinically evaluated GCB is a novel approach to the local treatment of periodontal tissue diseases in orthodontic patients with the aim of avoiding oxidative stress by means of the synergistic antioxidant action of the flavonoid complex and the non-steroidal drug antimicrobial properties.

Conclusions

Through the experimentally detected toxicity parameters, the effect of periodontal gel composition “Benzidafaziverdine”, based on the flavonoid complex and benzydamine hydrochloride, on the body of warm-blooded animals was analyzed. It has been proven that this gel composition:

1) is classified as the 4th class of toxicity – practically non-toxic substances. LD₅₀ for white rats and white mice exceeds 5000 mg/kg; does not have sex and species sensitivity;

2) has weak cumulative activity, the cumulation coefficient exceeds 8.2;

3) does not have skin-resorptive and local-irritating effects when applied to the skin;

4) has a minimal effect on the lipid peroxidation system, which suggests the ability of GCB to reduce oxidative stress in periodontal tissues of orthodontic patients.

5) may cause an allergy and insignificant changes in peripheral blood when experimental animals are sensitized;

6) has a moderate irritating effect on mucous membranes.

Prospects for further research are to continue the study on the developed gel composition “Benzidafaziverdine” when used in clinical settings as a means of local direction for the treatment of inflammatory and dystrophic-inflammatory diseases of periodontal tissues in orthodontic patients.

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