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Toxicity and Cumulative Effect Study of "Benzpaya - S" Dental Gel

Umida M. Tillaeva
Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan

Andrey A. Prisny
Belgorod State National Research University (BSU), Institute of Pharmacy, Chemistry and Biology, Russia

Gulnora U. Tillaeva
Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan

Diyorakhon Z. Abbosova
Belgorod State National Research University (BSU), Institute of Pharmacy, Chemistry and Biology, Russia

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Abstract

Studies were conducted to investigate the toxicological effects—specifically the safety and cumulative action—of a new combined dosage form in the gel format based on benzketozone and papaya extract. Experiments were performed on oral mucosal wounds and cutaneous wounds in a rat model. A comparative analysis of the wound-healing activity of the new gel composition "Benzpaya" was carried out against the reference drugs Asepta gel and Solcoseryl gel, demonstrating the clear advantage of the former. The developed gel has the potential to become an alternative agent for the treatment and prevention of dental diseases with a minimal risk of side effects. The new composition of "Benzpaya" gel at a dose of 150 mg/kg possesses wound-healing activity, reducing wound cleansing and healing time by 4–8 days. A comparative analysis with the reference drugs Asepta gel and Solcoseryl revealed the superiority of the evaluated formulation.

Results of the acute toxicity study of the new composition "Benzpaya-S" gel demonstrated that it belongs to Class VI of relatively harmless substances. The LD50 in mice upon oral administration was greater than or equal to 5000 mg/kg (≥ 5000 mg/kg). Furthermore, an absence of both cumulative action and habituation (tolerance) was noted.

Keywords: Dental diseases, nonsteroidal anti-inflammatory drugs, phenylglyoxylic acid, benzketozone, papaya, gingivitis, stomatitis.

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1. Introduction

Dental diseases, including inflammatory processes in the oral cavity such as stomatitis and gingivitis, remain a pressing health issue among the population of Uzbekistan. According to WHO data, approximately 60% of the adult population in the country suffers from oral cavity diseases (1,2,3). One of the promising directions is the utilization of domestic pharmaceutical developments and biologically active substances based on natural and synthetic

components.

Benzketozone, a phenylglyoxylic acid derivative and a domestically produced nonsteroidal anti-inflammatory drug, was approved for clinical use by the Main Directorate for Quality Control of Medicines and Medical Equipment of the Ministry of Health of the Republic of Uzbekistan in the form of tablets (0.1 g of active substance; VFS 42 Uz-0663-2003) and ointments (0.5% and 3%; FSP 42 Uz-0186-2007). In accordance with the order of the Ministry of Health of the Republic of Uzbekistan, 3% Benzketozone ointment is authorized for the treatment of inflammatory viral diseases (conjunctivitis, colpitis) as part of complex therapy (FSP 42 Uz-0186-2007) (4,5). The efficacy of Benzketozone in clinical trials was evaluated in comparison with a steroid anti-inflammatory drug—0.5% hydrocortisone eye ointment. Experiments on various animal species have shown that benzketozone possesses high anti-inflammatory activity and low toxicity. It is also worth noting the importance of using enzyme preparations in combination with nonsteroidal anti-inflammatory drugs, as a synergistic/enhancing effect has been reported (6).

Objective of the Study

To study the toxicological profile—including acute toxicity and cumulative action (on a stomatitis model)—as well as the wound-healing activity of "Benzpaya-S," a new combined dosage form in the format of a gel based on benzketozone and papaya extract.

2. Methods

Dental gel "Benzpaya-S" (20 g aluminum tubes, containing 0.1 g of benzketozone and 10 g of papaya fruit extract; Lot 10 09 23) experimental laboratory batches were developed and prepared at the Department of Pharmaceutical Production Organization and Quality Management of the Tashkent Pharmaceutical Institute.

Combined gel "Asepta" containing propolis (reference drug; Lot 12.01.21, manufactured by JSC VERTEX, Russia) and "Solcoseryl" gel (medicinal product manufactured by LEGACY PHARMACEUTICALS SWITZERLAND, GmbH, Switzerland) were used. The research was conducted at the Belgorod Institute of Pharmacy, Chemistry and Biology, in the Pharmacology Laboratory, under the scientific consultation of Prof. A. A. Prisny, D.Sc. in Biology.

General effect and acute toxicity of the new combined composition "Benzpaya-S" gel were studied according to the Sanotsky method. To determine the parameters of acute toxicity, the Litchfield and Wilcoxon method was applied (7). The study of acute toxicity of the preparation was conducted on male outbred white mice with a body weight of 20 ± 2.0 g, divided into groups of 5 animals each (total N = 25 mice). The dental gel was applied into the oral cavity at doses of 2000, 2500, 3000, 4000, and 5000 mg/kg. The latter two doses were administered fractionally at 1-hour intervals.

All pharmacological studies were carried out on healthy, sexually mature animals (mice) that underwent quarantine for at least 10–14 days. General condition, behavior, and mortality of the animals were monitored. Observations were carried out hourly during the first day of the experiment under laboratory conditions; parameters assessing functional status included survival rate throughout the test, general condition, presence of convulsions, and lethality. Subsequently, daily monitoring was conducted in the animal facility for 2 weeks, assessing general status and activity, behavioral characteristics, respiratory rate and depth, state of skin and hair coat, tail position, body weight changes, and other indicators.

All experimental animals were housed under standard laboratory vivarium conditions at a temperature of 23–26°C, maintained on a standard diet with ad libitum access to water and food. At the end of the experiment, the median lethal dose (LD50) and toxicity class were determined (8,9).

The cumulative properties of "Benzpaya-S" gel were evaluated using a subchronic toxicity test according to the method of Lima et al., which allows assessment of both accumulation and drug habituation/tolerance. Experiments were conducted on 10 rats of both sexes with a body weight of 200 ± 15.0 g. Treatment was initiated 2 hours after inducing the experimental disease model by applying the test gel to the oral cavity of guinea pigs / rats.

Following the assessment of cumulative properties, animals were euthanized (decapitated) for pathomorphological examination of internal organs, calculating an integrative index—the organ-to-body weight ratio (mass coefficient, MC). Analysis of this parameter allows identification of target organs associated with pathological effects. Organs harvested at necropsy were weighed wet to prevent desiccation; paired organs were weighed together. The mass coefficient was calculated using the formula:

$$MC = (\text{Organ Mass (g)} / \text{Body Mass (g)}) \times 100\%$$

Statistical processing of the obtained data was performed using Microsoft Office Excel and Statistica 6.0 software packages.

In the second series of experiments, the anti-inflammatory activity of "Benzpaya-S" gel was studied on a model of experimental stomatitis, and reparative (wound-healing) activity was evaluated on a model of alterative cutaneous wound inflammation.

Experimental Stomatitis Model

Anti-inflammatory evaluation was conducted on 25 male guinea pigs weighing 300–350 g, divided into 5 animals per experimental group. The experimental stomatitis inflammation model was induced by the intraoral administration of 0.15 mL of 25% formalin into the oral cavity of the guinea pigs.

Treatment was initiated 2 hours post-induction of the experimental disease model by intraoral application of "Benzpaya-S" gel to the guinea pigs at a dose of 150 mg/kg. The dose calculation was performed in accordance with the guidelines for the development of drugs and biologically active substances [7]:

1600 mg/human ÷ 70 kg = (mg/kg) × 39 (human coeff.) ÷ 6 (guinea pig coeff.) mg/kg

The reference products included combined "Asepta" gel containing propolis and "Solcoseryl" gel (a medicinal product containing 4.15 mg of deproteinized hemodialysate from healthy calves' blood, calculated as dry matter, per 20 g tube)—an anti-inflammatory and regenerative agent.

Systematic monitoring of changes in the oral mucosa and skin was carried out under experimental conditions on days 3, 7, 14, and 21 after the oral administration of formalin. The severity of the developing disease was evaluated based on the general condition (overall appearance, coat condition, oral discharge, body weight dynamics) and behavior (locomotor activity, aggressiveness, appetite). Concurrently, rectal temperature was recorded using an electronic thermometer. In addition, peripheral blood parameters—hemoglobin concentration (g/L), erythrocyte count ($\times 10^{12}$/L), and leukocyte count ($\times 10^{9}$/L)—were analyzed using a Diatron Ltd. hematology analyzer (2002, Hungary).

Model of Alterative Inflammation (Cutaneous Wound Model)

The cutaneous wound model was implemented on 25 male rats weighing 200–215 g, with 5 animals per experimental group. In rats anesthetized with sodium ethaminal (50 mg/kg, i.p.), standard full-thickness cutaneous wounds measuring 1.0 x 1.0 cm with a depth of 0.5 mm were created under aseptic conditions on a pre-depilated area of the dorsal skin. The wounds were left open [2,3].

Treatment commenced immediately following the induction of the experimental pathology. The test gels were applied until complete recovery (21 days). Animals in the control group received no treatment, and their wounds remained open. Observation intervals were days 1, 3, 7, 14, and 21 from the start of the experiment. Wound healing kinetics were evaluated based on:

1. Presence of wound exudate and its cellular composition;
2. Condition of adjacent tissues;
3. Reduction in wound surface area (mm^2)

3. Results

Studies on the general activity and acute toxicity of "Benzpaya-S" gel were carried out on healthy laboratory animals—male outbred white mice weighing 18–22 g. Prior to the study, animals were held in quarantine for 10 days. As stated above, the preparation was administered at doses of 2000, 2500, 3000, 4000, and 5000 mg/kg.

Animal observations following application were conducted for 2 days under laboratory conditions and for 14 days under vivarium conditions. During the monitoring period, primary attention was given to general clinical status, skeletal muscle and limb tone relaxation, pupil response, and reactivity to external stimuli.

Experimental findings demonstrated that intraoral administration of the new composition "Benzpaya-S" gel at doses of 2000–5000 mg/kg induced no adverse reactions or pathological alterations in the animals.

Analysis of the animals' general state indicated no statistically significant body weight variations in those treated with the new "Benzpaya-S" gel formulation. Satisfactory appetite was maintained across all test groups. No animal mortality was recorded during the entire observation period (Table 1).

Table 1

Acute Toxicity Parameters Following Oral Administration of the New Composition "Benzpaya-S" Gel to Mice

Drug / Test item, Animal species, Route of administration	Gender	Doses (mg/kg)	Number of animals per group / number of deaths	LD16 ± m (mg/kg)	LD50 ± m (mg/kg)	LD84 ± m (mg/kg)	General effects
Mice, intraoral administration	Males	2000	6/0	-	≥5000		No negative reactions or pathological alterations were observed in the animals following administration of the test substance.
		2500	6/0				
		3000	6/0				
		4000	6/0				
		5000	6/0				

Consequently, the acute toxicity evaluation results of the new "Benzpaya-S" gel composition demonstrated that it belongs to Class VI of relatively harmless substances. The oral LD_{50} in mice was greater than or equal to 5000 mg/kg ($\ge 5000\text{ mg/kg}$).

In the second series of experiments, the anti-inflammatory activity of the studied formulations and their therapeutic effect on experimental stomatitis were evaluated.

Against the background of the experimental stomatitis model induced by a 25% formalin solution, treatment was administered in five groups: animals in the first group received a placebo gel and served as the control group; the second group received "Benzpaya-S" gel at a dose of 150 mg/kg; the third and fourth groups were treated for experimental stomatitis using the reference drugs—combined "Asepta" gel containing propolis and "Solcoseryl" dental gel, respectively.

At the moment of formalin intraoral administration, agitation expressed by increased locomotor activity and tachypnea was observed in all animals. After 3–4 hours, the animals exhibited marked aggressiveness, which persisted in the control group until the end of the experiment.

In experimental animals of the control group, both local inflammatory response and its systemic manifestations progressed one day after pathology induction. The most prominent symptoms during this period included severe oral erythema, with the mucosa being hyperemic and edematous. In subsequent days, local inflammatory reactions developed, characterized by blood on the mucosa, mucous discharge, and swelling. On days 3–7 of the study, hemorrhagic and purulent aphthous lesions were observed. Concurrently, the guinea pigs became pasty. On days 14–21 of the experiment, the general condition of the animals returned to baseline. The overall duration of experimental stomatitis ranged up to 14–21 days.

Treated animals tolerated the damaging effect of formalin on the oral mucosa significantly better. The initial clinical signs of the disease, as in the control group, appeared 24 hours following pathology reproduction.

In the experimental group treated with the test gel and reference drugs, these pathological changes manifested in a mild form. Local inflammatory symptoms were more pronounced on day 3 of the experiment; however, blood with mucus, swelling, and oral discharge were reduced on average by 1.5–2 times compared to control animals. Bleeding was not observed and presented in only 50% of animals. Nevertheless, after 5–7 days of treatment, the overall state of the treated animals improved. The guinea

pigs displayed their normal physical activity.

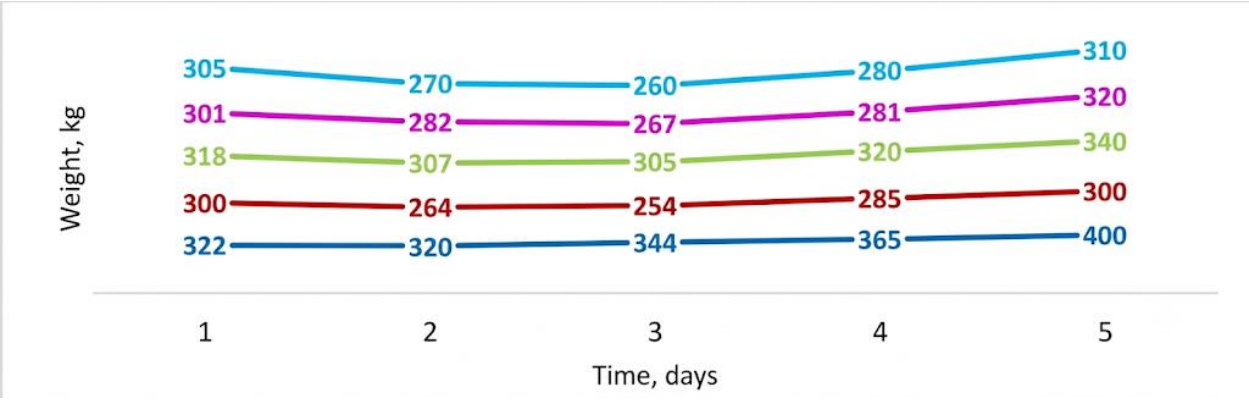
On day 7 of the study, a reduction in body weight was recorded in experimental animals: by 26% in the control group, by 10% in the experimental group receiving the dental gel composition, and by 22.3% and 24.4% in the reference groups treated with Asepta and Solcoseryl, respectively. By day 21, body weight in the control group and reference drug groups returned to baseline values, whereas in the group treated with the "Benzpaya-S" gel composition, body weight recovery was achieved on day 14. Notably, mortality of 2 out of 5 control animals occurred on days 6–7, accompanied by a sharp drop in body weight. No animal mortality was recorded in the experimental groups (Table 2, Fig. 1).

Table 2

Effect of the New Composition "Benzpaya-S" Gel on the Body Weight of Guinea Pigs with Experimental Stomatitis (M ± m; n = 5)

Experimental condition, dose, mg/kg	Animal weight, g / study days				
	Baseline	3	7	14	21
Intact animals (or: Intact control)	322±16,3	330±12,4	344±12,5	365±14,5	400±15,0
Control (untreated animals)	300±16,3	264±12,4	254±12,5	285±14,5	300±15,0
New "Benzpaya-S" gel composition	318±13,5	307±13,0	310±13,8	320±13,9	340±14,8
Asepta gel	301±13,5	282±13,0	267±3,8	281±13,9	320±14,8
Solcoseryl gel	305±13,0	270±11,0	260±3,1	280±13,4	310±13,0

P < 0.05 vs. control group



- **Intact animals**
- **Control**
- **New "Benzpaya-S" gel composition**
- **Asepta gel**
- **Solcoseryl gel**

Fig. 1. Effect of the new "Benzpaya-S" gel composition on the body weight of guinea pigs with experimental stomatitis.

Manifestations of local inflammation severity and systemic intoxication included an increase in rectal temperature by

0.6–0.8 °C on days 3–7. In animal groups treated with the new "Benzpaya-S" gel composition and the reference drugs (Asepta gel and Solcoseryl gel), the febrile response was attenuated and short-lived, with the peak temperature increase not exceeding 0.3 °C (Table 3).

Table 3

Effect of the new "Benzpaya-S" gel composition on the rectal temperature of guinea pigs (M ± m; n = 5)

· **Experimental condition, dose, mg/kg**	Rectal temperature, °C / study days				
	Baseline	3	7	14	21
· **Control (untreated animals)**	38,7±3,2	39,3±3,2*	39,5±3,2*	38,9±3,2*	38,5±3,2*
· **New "Benzpaya-S" gel composition**	39,0±3,2	39,1±3,2	39,3±3,2	38,9±3,2	39,0±3,2
· **Asepta gel**	39,0±3,2	39,2±3,2	39,3±3,2	38,8±3,2	39,0±3,2

P < 0.05 vs. baseline

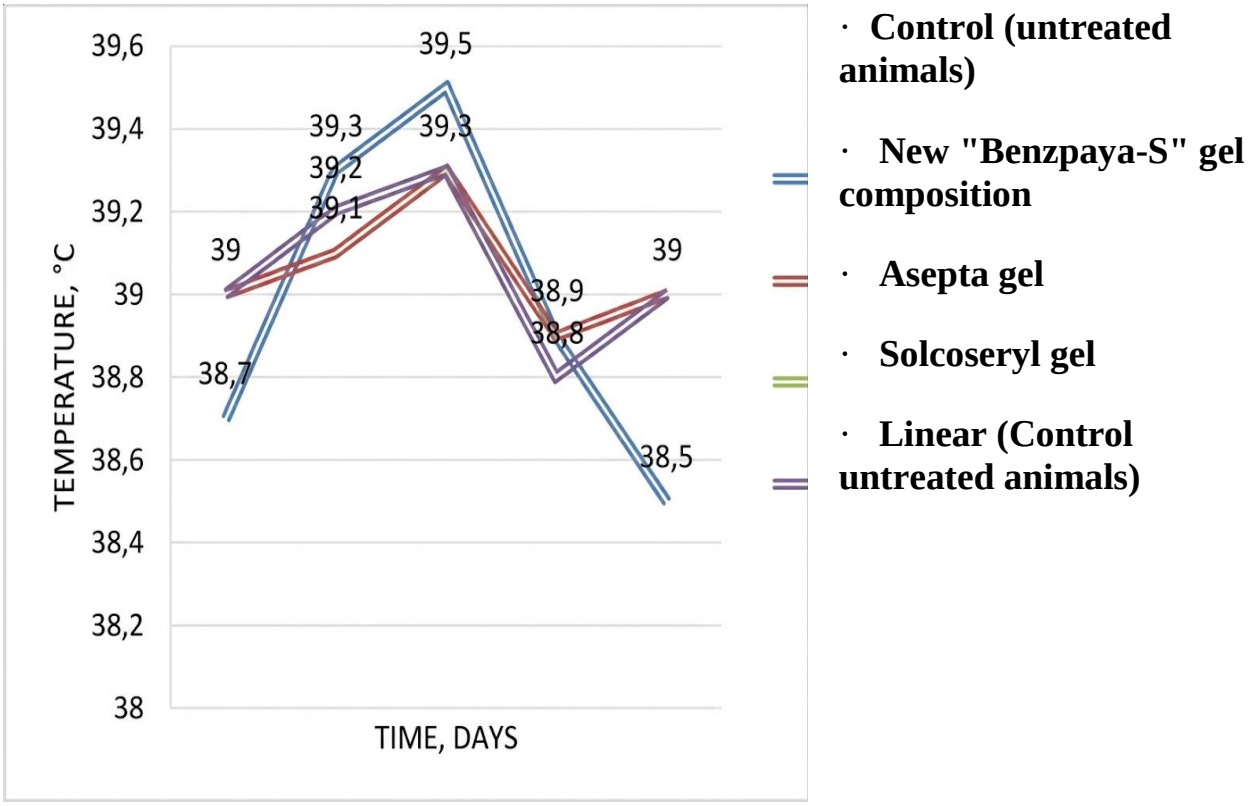


Fig. 2. Effect of the new "Benzpaya-S" gel composition on the rectal temperature of guinea pigs with experimental stomatitis.

Significant changes were observed in peripheral blood parameters. During the first three days after the pathology induction, the leukocyte count increased by 30%, with peak leukocytosis noted on days 7–14 (65–100%).

As the inflammatory process progressed, all animals showed signs of impaired erythropoiesis. In the control group, hemoglobin levels decreased by 21–10% and erythrocyte concentration by 30–31% at 7–14 days from the beginning of the experiment, returning to normal values by day 21.

Changes in the peripheral blood of treated animals exhibited certain features: leukocytosis was generally less pronounced. Hemoglobin levels decreased to a lesser extent than in the control group and reached baseline values by day 7. The decrease in erythrocyte concentration was not statistically significant (Table 4).

Table 4

Effect of the new "Benzpaya-S" gel composition on the morphological composition of peripheral blood in guinea pigs with experimental stomatitis (M ± m; n = 5)

Parameters	Study days				
	Intact	Control	"Benzpaya-S" gel composition	Asepta gel	Solcoseryl gel
After 3 days					
Hemoglobin, g/L	10,5±0,4	8,8±0,3	10,6±0,2	9,3±0,2	8,9±0,5
Erythrocytes, 10^{12}/L	5,9±0,5	4,6±0,2	5,8±0,3	4,3±0,3	4,0±0,4
Leukocytes, 10^{9}/L	9,2±1,0	13,5±1,1	10,0±1,2	9,8±1,1	11,2±1,5
After 7 days					
Hemoglobin, g/L	11,0±0,4	8,2±0,3	11,3±0,2	9,3±0,2	9,0±0,1
Erythrocytes, 10^{12}/L	6,0±0,5	4,0±0,2	7,0±0,3	4,8±0,3	4,8±0,3
Leukocytes, 10^{9}/L	9,0±1,0	19,2±1,1	9,25±1,2	8,2±1,1	8,0±1,1
After 14 days					
Hemoglobin, g/L	11,0±0,4	8,8±0,3	12,0±0,2	10,1±0,2	9,5±0,2
Erythrocytes, 10^{12}/L	6,0±0,5	4,3±0,2	7,4±0,3	5,2±0,3	5,0±0,3
Leukocytes, 10^{9}/L	10,0±1,0	15,25±1,1	11,0±1,2	10,0±1,1	9,0±0,8
After 21 days					
Hemoglobin, g/L	10,5±0,4	9,6±0,3	11,0±0,2	10,3±0,2	10,0±0,1
Erythrocytes, 10^{12}/L	5,8±0,5	5,0±0,2	6,8±0,3	5,7±0,3	5,3±0,2
Leukocytes, 10^{9}/L	10,5±1,0	12,25±1,1	9,0±1,2	10,8±1,1	10,8±1,1

P < 0.05 vs. control

Based on the results of the pharmacological study, it can be concluded that the new "Benzpaya-S" gel formulation exerts anti-inflammatory and reparative effects characteristic of its active components, favorably influencing the course of experimental stomatitis.

4. Conclusions

Based on the results of the pharmacological study, it can be concluded that the new "Benzpaya-S" gel formulation exerts anti-inflammatory and reparative effects characteristic of its active components, favorably influencing the course of experimental stomatitis.

The new "Benzpaya" gel formulation at a dose of 150 mg/kg possesses wound-healing efficacy, reducing the wound cleansing and healing time by 4–8 days. A comparative analysis with reference drugs (Asepta gel and

Solcoseryl gel) demonstrated the superiority of the former.

The results of acute toxicity studies of the new "Benzpaya-S" gel formulation indicated that it belongs to Class VI (relatively harmless substances). The oral LD50 in mice was ≥ 5000 mg/kg.. The investigation of cumulative properties carried out using the subchronic toxicity method according to the Lima method demonstrated not only the absence of cumulation but also the absence of tolerance (habituation).

References

1. Dental diseases (Stomatitis, Gingivitis). https://www.sm-stomatology.ru/
2. Ahmetkan D.A., Turgunbaeva A.A., Burasheva G.Sh., Ustenova G.O. Study of a dental gel with dry extract of camel thorn. Proc. XIII Int. Sci.-Pract. Conf. "Priorities of Pharmacy and Dentistry: from Theory to Practice", dedicated to the 135th anniversary of S.D. Asfendiyarov. NJSC KazNMU, Almaty, 2024, pp. 1–21.
3. Sapovskaya A.V., Sampiev A.M., Nikiforova E.B. Current issues of nomenclature, composition, and technology of dental gels. Modern Problems of Science and Education, 2015, no. 1, p. 128.
4. Zakirov U.B., Maksudov A.A., Azizov U.M. Study of the efficacy of benzketozone in the treatment of eye burns. Pharmaceutical Journal, Tashkent, 2004, no. 1, pp. 90–92.
5. Pharmacopoeial Monograph. Benzketozone Substance. 42 Uz-0850-2010, 42-Uz-0850-2020.
6. Tillaeva U.M., Nazarkulov M.S., Rahmanov Z.A., Tillaeva G.U., Gaibnazarova D.T. Analysis of drugs containing enzymes in the pharmaceutical market of the Republic of Uzbekistan. World Journal of Pharmaceutical and Life Sciences, 2020, 6(1), pp. 01–04.
7. Testing methods for the effects of chemical products on the human body. Acute oral toxicity – Acute Toxic Class Method. (OECD, Test No. 423: 2001, IDT). Minsk, 2013.
8. Methodological recommendations for preclinical studies of dermatotropic drugs. In: Guidelines for Preclinical Trials of Medicines. Part One. Moscow, 2012, pp. 738–746. (Ed. Mironov A.N.).
9. Glyantsev S.P., Annaeva A.G., Savina T.V. Morphological rationale for choosing the composition and structure of a biologically active composition based on sodium alginate for wound treatment. Bull. Exp. Biol. Med., 1999, no. 1, pp. 65–67.
10. Hsu C.Y., Saadh M.J., Mutee A.F., Mumtaz H., Tillaeva G.U., Mirzaei M., Da'i M., Mascarenhas-Melo F., Salem-Bekhit M.M. Assessing the metronidazole adsorption by an iron-enhanced nanocone along with DFT calculations regarding the conjugated system formations for developing the drug delivery platforms. Inorganic Chemistry Communications, 2024, vol. 165, p. 112496. https://doi.org/10.1016/j.inoche.2024.112496
11. Bautista M.C., Cortés-Arriagada D., Shakerzadeh E., Anota E.C. Acetylsalicylic acid interaction with Boron nitride nanostructures–a density functional analysis. J. Mol. Liq., 2022, vol. 355, p. 118980.
12. Asif M., Sajid H., Ayub K., Gilani M.A., Anwar N., Mahmood T. Therapeutic potential of oxo-triarylmethyl (oxTAM) as a targeted drug delivery system for nitrosourea and fluorouracil anticancer drugs: a first principles insight. J. Mol. Graph. Model., 2023, vol. 122, p. 108469.