Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

CHARACTERIZATION OF PHYSICO-CHEMICAL PARAMETERS AND TOXICOLOGICAL PROPERTIES OF NEOCYTIN

Umidat Shamilevna Dzhabrailova[1], Vagab Malikovich Vagabov[2*], Zumrud Nasirovna Akhaeva[2], Zamira Zairbekovna Kasimova[2], Sergei Pavlovich Kolesnikov[3], Natalia Grigorievna Bondarenko[4]

1. ***Department of Pediatrics, Faculty of Pediatric, University of Astrakhan State Medical, Astrakhan, Russia.***
2. ***Department of Therapy, Faculty of Medicine, University of Dagestan State Medical, Makhachkala, Republic of Dagestan, Russia.***
3. ***Department of Therapy, Faculty of Medicine, University of Stavropol State Medical, Stavropol, Russia.***
4. ***Department of History and Philosophy of Law, Institute of Service, Tourism, and Design, University of North Caucasus Federal, Pyatigorsk, Stavropol Territory, Stavropol.***

ARTICLE INFO

Received:
17 Jul 2022
Received in revised form:
01 Oct 2022
Accepted:
10 Oct 2022
Available online:
28 Oct 2022

Keywords: Neocytin, Toxicological properties, Physico-chemical parameters, Drug

ABSTRACT

In the mid-50s of the last century, reports began to appear about the ineffectiveness of sulfonamides and antibiotics in bacterial infections. And soon the widespread spread of numerous strains of antibiotic-resistant microorganisms was noted. The fight against drug-resistant bacteria is carried out in several directions: obtaining new chemotherapeutic drugs, chemical modification of known antibiotics, or the use of inhibitors that suppress the activity of bacterial enzymes that inactivate antibiotics. Neocytin is a complex chemotherapeutic drug, which is a solution of tetracycline, levomycitin, novocaine, and ascorbic acid. In this scientific work, some physico-chemical properties of Neocytin are investigated, as well as the main toxicological indicators: acute toxicity, chronic toxicity, the irritating effect of the drug, and the effect of the drug on the mucous membranes. In addition, the effect of the drug on the change in body weight of the studied animals was studied. The drug is a low-toxic agent for warm-blooded animals, both in acute and chronic experiments. Neocytin has mild acute toxicity, without causing severe toxicosis at a dose of 0.3 ml/kg of body weight, and belongs to the 4th hazard class (minor hazardous substances). Long-term use of the drug in doses exceeding therapeutic by 3-5 times does not adversely affect the general condition of animals and other indicators of their clinical status, does not have a harmful local effect, does not adversely affect the main types of metabolism, does not violate the functions and structure of vital systems, organs, and tissues.

To Cite This Article: Dzhabrailova US, Vagabov VM, Akhaeva ZN, Kasimova ZZ, Kolesnikov SP, Bondarenko NG. Characterization of Physico-Chemical Parameters and Toxicological Properties of Neocytin. Pharmacophore. 2022;13(5):44-50. https://doi.org/10.51847/igW0BabCma

Introduction

Antibiotics are compounds with a strong antimicrobial effect and the least toxicity compared to other antibacterial drugs, which provides them with high therapeutic efficacy [1]. They are used both in pure form and in combination with other means, taking into account their compatibility, potentiation, and synergy [2].

The successful use of antibiotics, sulfonamides, and nitrofurans, which dramatically reduced the death of people and animals from many infectious diseases, contributed to the creation of an opinion about the unlimited possibilities of these drugs in the fight against pathogenic and conditionally pathogenic microorganisms [3]. However, already in the 50s, separate reports began to appear about the ineffectiveness of sulfonamides and antibiotics in bacterial infections. And soon the widespread spread of numerous strains of antibiotic-resistant microorganisms was noted [4]. This led to a sharp decrease in the therapeutic effectiveness of previously highly active (penicillins, tetracyclines, streptomycin, etc.) drugs.

Corresponding Author: Vagab Malikovich Vagabov; Department of Therapy, Faculty of Medicine, University of Dagestan State Medical, Makhachkala, Republic of Dagestan, Russia. E-mail: ruslankalmykov777@yandex.ru.

Enterococci are increasingly becoming the cause of infections. Their significance is not only in their potential pathogenicity but also in the rapid formation of antibiotic resistance. They are naturally resistant to some commonly used antibiotics, in particular to cephalosporins, due to their selective pressure with frequent administration [5]. Over the past two decades, they have acquired resistance to chloramphenicol, erythromycin, and tetracycline [6].

Unfortunately, there are no effective medicines in the future that could take the place of drugs active against multiple resistant strains of pathogens.

In Russia, strains of pathogenic and conditionally pathogenic microorganisms resistant to many antibiotics are also widespread in all regions. Thus, the percentage of E. coli and salmonella strains resistant to tetracyclines and "old" aminoglycosides can reach 67.3-89.6% and 56.2-73.1%, respectively [7].

There is a lot of data on the release of enzymes by various drug-resistant microorganisms that inactivate tetracycline and aminoglycoside antibiotics, penicillins, levomycitin, etc., which significantly reduces the effectiveness of treatment [8, 9].

The fight against drug-resistant bacteria is conducted in several directions. This is the preparation of new chemotherapeutic drugs that differ from the existing mechanisms of antibacterial action. Another way is the chemical modification of known antibiotics (group radicals). The third way is to use inhibitors that suppress the activity of bacterial enzymes that inactivate antibiotics [10].

Within the framework of this research work, a new generation antibiotic, Neocytin, was developed, and the studies carried out allowed us to determine its basic physico-chemical and toxicological properties.

Production of a Neocytin

Neocytin is a complex chemotherapeutic drug, which is a solution of tetracycline, chloramphenicol, novocaine, and ascorbic acid in propylene glycol. It is soluble in most organic solvents: polyethylene glycol, xylene, acetone, hexane, methanol, etc. The organoleptic and physico-chemical properties of the resulting preparation are indicated in **Table 1**.

Table 1. Physico-chemical properties of the drug Neocytin

The name of the indicator	Characteristics and norms
Appearance, color	Light brown liquid
Mechanical inclusions	Not allowed
Authenticity:	
Chloramphenicol	Cherry color
Tetracycline hydrochloride	Brown color
Novocaine	Greenish color
Ascorbic acid	Sediment formation
Sterility	Sterile
Toxicity, test dose per mouse subcutaneously, ml	0,05

Neocytin is a liquid of light brown color, transparent, odorless, bitter taste. According to the value of LD50 when injected into the stomach, it belongs to the class of moderately dangerous substances.

All work on the manufacture of the drug must be carried out in compliance with the safety standards and regulations used when working with toxic substances, as well as the general rules for the use of personal protective equipment in accordance with standard standards approved in accordance with the established procedure.

Materials and Methods

The main object of research was the drug NEO-antibiotic, manufactured in the conditions of the educational laboratory of the Astrakhan State Medical University. To determine the appearance, color, and presence of impurities, all vials with the drug are viewed visually on a white background. If there are defects in individual vials, they are discarded.

In order to determine the sterility of the Neocytin, the test sample of the drug is diluted in a liquid thioglycol medium in various concentrations. Preparation for the test consists of the preparation of a one-billion suspension of the test microbe Staphylococcus aureus 209P.

Preclinical and clinical study of the drug was carried out in accordance with the requirements of the relevant "Guidelines for the experimental study of new pharmacological substances" and "Guidelines for conducting clinical trials of new drugs" for the selection of experimental animals, control, maintenance, and feeding, accounting for the physiological and pathological condition of animals in the experiment and taking into account its results [11, 12].

The general toxic properties of the Neocytin were evaluated by determining acute, and chronic toxicity, as well as possible side effects and long-term consequences in accordance with the "Guidelines for determining the toxic properties of drugs used in veterinary medicine and animal husbandry" and "Scientific and methodological aspects of the study of the toxic properties of pharmacological drugs for animals" [13].

The parameters of acute toxicity were studied in white mice using the Kerber method [14]. Neocytin was administered to mice intraperitoneally. After administration, the general condition of the animals, the peculiarities of their behavior, motor activity, reaction to stimuli, feed and water consumption, quantity, and indicators of feces and urine were recorded.
The chronic toxicity of the Neocytin was studied on white rats with an internal, intraperitoneal method of administration. Intraperitoneal Neocytin was administered at a dose of 1/5 and 1/10 of LD50, the drug was administered in volumes not exceeding the maximum prescribed by the guidelines.
Observation of animals and registration of the intoxication pattern was carried out daily. As diagnostic indicators, the consumption of feed and water, the condition of the hair and mucous membranes, behavior, respiration, pulse, and body temperature were noted.
The effect of the Neocytin on the function of the kidneys and digestive tract in chronic experiments was assessed by changes in the physico-chemical properties of urine, while taking into account its color, odor, the concentration of hydrogen ions, specific gravity, presence and amount of protein, carbohydrates, bile pigments, among the indicators of feces, their color, smell and consistency, the presence of blood and blood pigments with the help of benzidine reagent, bile pigments with iron chloride, fat and fatty acids with Sudan-3, starch coloring with iodides [15]. The skin-resorptive effect of Neocytin was evaluated on rabbits by the method of skin applications [11].
Determination of the sterility of a Neocytin. From the samples selected for control, crops are carried out on a thioglycol medium in a volume of 10 ml. From each sample, the samples are seeded into ten test tubes. Of these, five tubes are for the growth of stable microflora and five for sensitive [15, 16].
Antibiotic-resistant cultures of micrographs are detected at a concentration of 1000 micrograms/ml of the total activity of antibacterial drugs included in the Neocytin. For this purpose, the initial samples in the volume of 1 ml (100,000 micrograms/ml) are diluted in 9 ml of triglycol medium, and then 1 ml is added to each of the five tubes with 9 ml of the same medium [17].
Antibiotic-sensitive microorganisms are detected at a concentration of 0.01 micrograms/ml. For this purpose, the initial samples in the volume of 1 ml are sequentially diluted in two flasks and two test tubes. The flasks contain 99 ml and one tube of 9 ml of sterile water, the second tube contains 9 ml of thioglycol medium. 1 ml of the initial sample of the drug is added to the first flask, mixed and 1 ml is transferred to the second flask. Then take 1 ml from the second flask and transfer it to a test tube with water, from which 1 ml is transferred to a test tube with a thioglycol medium. From this tube, 1 ml is added to 5 tubes with 9 ml of thioglycol medium. A control test microbe Staphylococcus aureus 209P is introduced into a test tube with a thioglycol medium without antibiotics at the rate of 250 microbial cells per 1 ml of medium. One billion daily agar culture is successively diluted in two vials containing 100 ml of sterile isotonic sodium chloride solution. 0.1 ml of the culture of the control test microbe is introduced into the first vial, 0.5 ml is mixed and transferred to the second vial, from which 0.5 ml is introduced into test tubes with a thioglycol medium [18].
Crops from each sample: three tubes with a high concentration of the drug, three tubes with a low concentration, and a tube with a control culture of staphylococcus are kept at 37 ± 0.5 ° C, and four tubes - at 26 ± 0.5 ° C for 7 days. The crops should remain sterile, except for the test tube with the control [19].
Test for harmlessness (toxicity). The method consists of the fact that with subcutaneous administration of the maximum tolerated dose of 0.05 ml of the drug dissolved in isotonic sodium chloride solution, healthy white mice weighing 18-20 g should remain alive. To do this, 1 ml of the drug is dissolved in 9 ml of sterile isotonic sodium chloride solution for injection, and 0.5 ml of the resulting solution is injected subcutaneously into five mice in the back area. The observation period is 48 hours. Neocytin is considered harmless if all the mice remain alive. Each mouse is used in the experiment once.

Results and Discussion

Acute Toxicity of the Drug
The acute toxicity of the Neocytin was determined on mongrel white mice weighing 20.0-22.0 g, selected according to the principle of paired analogs.
5 groups of white mice were formed, with 6 heads in each group. The animals of the experimental and control groups were monitored daily for 14 days. Special attention was paid to the development of signs of toxicosis, their severity, duration, time of recovery, or death of animals were assessed.
In the first series of experiments (5 groups), the drug was administered intraperitoneally, in a volume for white mice from 1.0 to 6.0 ml/kg.
It was established that the clinical manifestation of the toxic effect of the Neocytin was manifested by single tetanic seizures of a weak degree, depression, and decreased motor activity. A moderate degree of intoxication was characterized by muscular tetanus, which after 40-60 minutes was replaced by general depression. A pronounced clinic of intoxication was observed in the form of multiple spastic contractions of the mouse, incessant tetanus for 2-3 hours, stomach spasms, abdominal wall tension, and further depression.
With an increase in the dose of Neocytin, there was a steady tendency to more intense functioning of the excretory system and liver. However, the average value of these indicators in the mice of the experimental group exceeded the upper limits of the norm for this type of animal. With an increase in the dose of neocytin, a significant increase in the bactericidal activity of blood serum was also observed.

The clinical picture of acute poisoning in white mice was characterized by a picture of initial brief excitement, then clonic convulsions followed after half an hour or an hour, and then a sharp depression ending in the death of animals, usually during the first day.

Chronic Toxicity of the Drug

When studying cumulative properties, the total doses of Neocytin – 114,000 mg/kg for white mice and 108,000 mg/kg for white rats - did not cause the death of animals. This did not allow us to calculate the cumulation coefficient according to the lethal effect indicator, but given that the doses received are 4LD50, the possible cumulation coefficient exceeds 4 and the Neocytin refers to substances with weakly expressed cumulation.

When studying the chronic toxicity of a Neocytin, the deaths of mice and rats were not recorded throughout the experiment.

The results of determining the body weight of white mice during the experiment are presented in **Figure 1**.

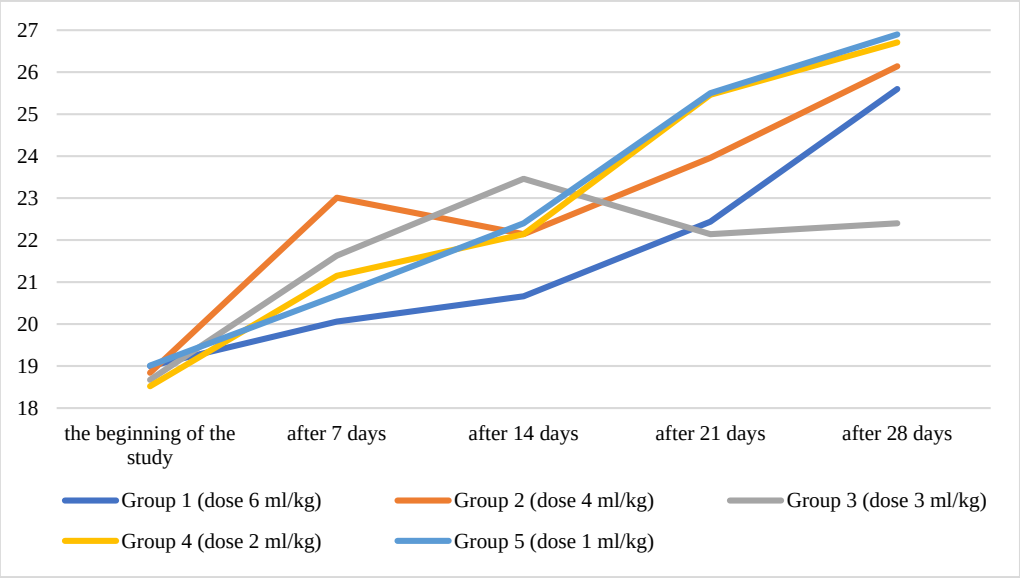

Figure 1. Average body weight of mice during the experiment (g)

In group 1 mice that received the highest dose of the drug (6 ml/kg), the smallest increase in body weight was noted throughout the experiment, which indicates the presence of toxic properties. However, 11 days after discontinuation of the drug, the weight gain indicators approach the control ones, which indicates the rapid reversibility of changes that occurred in the body of white mice under the influence of the drug. The decrease in body weight gain observed on the 14th day of group 2 animals can be explained as a minor manifestation of the toxic effect of the drug. At the same time, the largest increase in body weight was recorded in animals of groups 2 and 4 on days 21 and 28.

The effect of the Neocytin on the functional activity of the digestive system of animals was judged by the physico-chemical properties of feces, which were collected at the beginning of the study and every subsequent 7 days until the end of the experiment. At the same time, an organoleptic assessment was carried out, paying attention to the shape, consistency, smell, and color, as well as the presence of foreign impurities.

The physico-chemical properties of feces were studied by determining pH using litmus papers, as well as - the presence of blood – a benzidine sample, bile pigments - a Terquay sample, bilirubin - a Fouche sample, fat and starch – microscopic Sudan III and Lugol solution.

The conducted studies have established that the acts of defecation in animals of the experimental and control groups were carried out in a natural pose for this type of animal, painlessly, without tension. In the studied stool samples, impurities of blood, mucus, pus, and gas bubbles were not found, and helminth eggs and protozoa were absent.

Microscopic examination characterizing the digestive capacity of the gastrointestinal tract revealed single starch grains and neutral fat.

Conducted chemical studies in the feces did not reveal blood pigments, bile pigments were released within the normal range.

The pH response ranged from 7.0-7.4, confirming the normal functioning of the intestinal microflora.

Thus, the Neocytin in therapeutic doses many times higher than therapeutic, with prolonged administration, did not negatively affect the digestive processes.

Irritating Effect of a Neocytin

Determination of the irritating effect of a Neocytin with intradermal administration was carried out on 2 rabbits. Experimental animals were fixed in the dorsal position; the fur was trimmed on the abdomen. The trimmed skin area was divided into 6 fields with an area of approximately 10 cm2. In the center of three fields, each rabbit was injected with a Neocytin. The drug was administered intradermally in a volume of 0.3 cm^3. 20 minutes after administration of the drug, a 1% solution of trypan

blue was administered intravenously at a dose of 1 cm3 / kg of rabbit body weight. After 30, 60, and 180 minutes after the introduction of the dye, the color of the skin zones at the injection sites of the Neocytin was examined.

The irritating effect of the Neocytin in the intradermal method of administration was assessed by the intensity of tissue staining on an 8-point scale.

In the experiment, it was found that 30 minutes after the introduction of the dye, a weak irritating effect of neocytin was noted, after 60 and 180 minutes, the irritating effect of the drug was moderate, after 4 hours - weak, and after 5 hours it was absent. The effect of a Neocytin on the intensity of tissue staining is presented in **Table 2**.

Table 2. The effect of a Neocytin on the intensity of tissue staining

Rabbit	Indicators	Study time (hour)					
		0	**0.5**	**1**	**3**	**4**	**5**
№1	Score in points	0	4	6	6	4	0
	effect	absent	weak	moderate	moderate	weak	absent
№2	Score in points	0	4	6	6	4	0
	effect	absent	weak	moderate	moderate	weak	absent

The Effect of the Neocytin on the Mucous Membranes

When visually assessing the condition of the conjunctiva, cornea, and eyelids of rabbits, it was found that the drug neocytin causes mild irritation of the conjunctiva 2-3 hours after instillation, which took place by the 4th hour **(Table 3)**.

Table 3. The effect of the drug Neocytin on the rabbit's eye

Rabbit	Indicators	Study time (hour)							
		0	**0.5**	**1**	**2**	**3**	**4**	**5**	**6**
№1	Score in points	0	0	0	2	2	0	0	0
	effect	absent	absent	absent	weak	weak	absent	absent	absent
№2	Score in points	0	0	0	2	2	0	0	0
	effect	absent	absent	absent	weak	weak	absent	absent	absent

Thus, the Neocytin does not have a pronounced irritant effect when applied externally.

The clinical state of the rabbits' bodies after applying neocytin to the conjunctiva of the eyes remained within the normal range, i.e. no changes in body temperature, pulse rate, and number of respiratory movements were detected **(Table 4)**.

Table 4. Data on the clinical condition of rabbits

Rabbit	Indicators	Study time (hour)							
		0	**0.5**	**1**	**2**	**3**	**4**	**5**	**6**
№1	Body temperature	38,65	38,65	38,65	38,6	38,7	38,75	38,75	38,7
	Heart rate	126	126	125	125	126	125	127	126
	Respiratory rate	54	53	52	53	52	52	53	53
№2	Body temperature	38,7	38,7	38,7	38,65	38,6	38,8	38,8	38,75
	Heart rate	128	128	126	126	128	128	128	127
	Respiratory rate	55	56	56	55	56	57	57	57

Thus, the data obtained in the experiment indicate that the Neocytin does not have a pronounced irritating effect when applied externally.

Conclusion

Neocytin is a new complex drug, which includes chloramphenicol and tetracycline. The control of qualitative and quantitative indicators of the drug is carried out in accordance with the approved specifications for organoleptic and physico-chemical indicators characterizing their appearance, smell, taste, sterility, microbiological purity, and toxicity.

It was found that the clinical manifestation of the toxic effect of the Neocytin was manifested by single tetanic seizures of a weak degree, depression, and decreased motor activity. A moderate degree of intoxication was characterized by muscular tetanus, which after 40-60 minutes was replaced by general depression. A pronounced clinic of intoxication was observed in the form of multiple spastic contractions of the mouse, incessant tetanus for 2-3 hours, stomach spasms, abdominal wall tension, and further depression.

The effect of the Neocytin on the functional activity of the digestive system of animals was judged by the physico-chemical properties of feces, which were collected at the beginning of the study and every subsequent 7 days until the end of the

experiment. In the studied stool samples, impurities of blood, mucus, pus, and gas bubbles were not found, and helminth eggs and protozoa were absent. Thus, the NEO-antibiotic in therapeutic doses many times higher than therapeutic, with prolonged administration, did not negatively affect the digestive processes.

When studying the irritating effect of the Neocytin, it was found that 30 minutes after the introduction of the dye, a weak irritating effect of neocytin was noted, after 60 and 180 minutes, the irritating effect of the drug was moderate, after 4 hours - weak, and after 5 hours it was absent.

When visually assessing the condition of the conjunctiva, cornea, and eyelids of rabbits, it was found that the drug Neocytin causes mild irritation of the conjunctiva 2-3 hours after instillation, which took place by the 4th hour. Thus, the Neocytin does not have a pronounced irritant effect when applied externally.

The clinical state of the rabbits' organism after applying the Neocytin preprate to the conjunctiva of the eye remained within the normal range, i.e. no changes in body temperature, pulse rate, and a number of respiratory movements were detected.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Also, the experimental work with animals did not contradict the internal regulations of the ethical councils of the Astrakhan State Medical University, Dagestan State Medical University and Stavropol State Medical University.

References

1. Allison MG, Heil EL, Hayes BD. Appropriate Antibiotic Therapy. Emerg Med Clin North Am. 2017;35(1):25-42. doi:10.1016/j.emc.2016.08.003
2. Walger P. Rational use of antibiotics. Internist (Berl). 2016;57(6):551-68. doi:10.1007/s00108-016-0071-5
3. Pithie AD, Ellis CJ. Review article: antibiotics and the gut. Aliment Pharmacol Ther. 1989;3(4):321-32. doi:10.1111/j.1365-2036.1989.tb00219.x
4. Wright GD. Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv Drug Deliv Rev. 2005;57(10):1451-70. doi:10.1016/j.addr.2005.04.002
5. Nnadozie CF, Odume ON. Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes. Environ Pollut. 2019;254(Pt B):113067. doi:10.1016/j.envpol.2019.113067
6. de Vera ME, Simmons RL. Antibiotic-resistant enterococci and the changing face of surgical infections. Arch Surg. 1996;131(3):338-42. doi:10.1001/archsurg.1996.01430150116021
7. Gut AM, Vasiljevic T, Yeager T, Donkor ON. Salmonella infection - prevention and treatment by antibiotics and probiotic yeasts: a review. Microbiology (Reading). 2018;164(11):1327-44. doi:10.1099/mic.0.000709
8. Moser C, Lerche CJ, Thomsen K, Hartvig T, Schierbeck J, Jensen PØ, et al. Antibiotic therapy as personalized medicine - general considerations and complicating factors. APMIS. 2019;127(5):361-71. doi:10.1111/apm.12951
9. Waglechner N, Culp EJ, Wright GD. Ancient Antibiotics, Ancient Resistance. EcoSal Plus. 2021;9(2):eESP-0027. doi:10.1128/ecosalplus.ESP-0027-2020
10. Walsh C. Where will new antibiotics come from? Nat Rev Microbiol. 2003;1(1):65-70. doi:10.1038/nrmicro727
11. Blinov AV, Nagdalian AA, Povetkin SN, Gvozdenko AA, Verevkina MN, Rzhepakovsky IV, et al. Surface-Oxidized Polymer-Stabilized Silver Nanoparticles as a Covering Component of Suture Materials. Micromachines. 2022;13(7):1105. doi:10.3390/mi13071105
12. Osipchuk GV, Povetkin SN, Nagdalian AA, Rodin IA, Rodin MI, Ziruk IV, et al. The Issue of Therapy Postpartum Endometritis in Sows Using Environmentally Friendly Remedies. Pharmacophore. 2019;10(2):82-4.
13. Ziruk IV, Egunova AV, Kopchekchi ME, Frolov VV, Babina KI, Povetkin SN. Morphometric of Pig Livers under Different Doses of Minerals in Feed Allowance. Int Trans J Eng Manag Appl Sci Technol. 2020;11(14):1-0. doi:10.14456/ITJEMAST.2020.277
14. Lopteva, MS, Povetkin SN, Pushkin SV, Nagdalian AA. 5% Suspension of Albendazole Echinacea Magenta (Echinacea Purpurea) Toxicometric Evaluation. Entomol Appl Sci Lett. 2018;5(4):30-4.
15. Maslova AY, Tskaeva AA, Ashurova ZA, Abazova A, Ismailov MM, Ismailova MM, et al. Study of the effect of Baricitinib on the Course of COVID-19. J Pharm Res Int. 2021;33(35):204-13.
16. Raevskaya AI, Belyalova AA, Shevchenko PP, Karpov SM, Mishvelov AE, Simonov AN, et al. Cognitive Impairments in A Range of Somatic Diseases Diagnostics, Modern Approach to Therapy. Pharmacophore. 2020;11(1):136-41.
17. Ranjha MM, Shafique B, Rehman A, Mehmood A, Ali A, Zahra SM, et al. Biocompatible Nanomaterials in Food Science, Technology, and Nutrient Drug Delivery: Recent Developments and Applications. Front Nutr. 2022;8:1-15. doi:10.3389/fnut.2021.778155

18. Siddiqui SA, Bahmid NA, Taha A, Abdel-Moneim AM, Shehata AM, Tan C, et al. Bioactive-loaded nanodelivery systems for the feed and drugs of livestock; purposes, techniques and applications. Adv Colloid Interface Sci. 2022;308:102772. doi:10.1016/j.cis.2022.102772
19. Maslova AY, Mishvelov AE, Jamarzaevna M, Dudusheva GA, Minaev SV, Shchetinin EV, et al. Using a Decision Tree with a Feedback Function to Select Therapeutic Tactics for Viral Infection of the Respiratory Tract in the Medical Expert System. Int Trans J Eng Manag Appl Sci Technol. 2022;13(8):1-0. Available from: http://TUENGR.COM/V13/13A8J.pdf doi:10.14456/ITJEMAST.2022.157