AGRICULTURAL BIOLOGY,
2021, V. 56, Iss. 4, pp. 763-771
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

## Tissue biostimulants

*UDC 619:615.36:575.224.46*

doi: 10.15389/agrobiology.2021.4.763eng
doi: 10.15389/agrobiology.2021.4.763rus

# ANTICLASTOGENIC ACTIVITY OF AMINOSELETON UNDER THE EFFECT OF CYCLOPHOSPHAMIDE ON THE BONE MARROW OF MICE

**S.V. SHABUNIN, G.A. VOSTROILOVA ✉, P.A. PARSHIN, D.I. SHABANOV, N.A. KHOKHLOVA**

*All-Russian Research Veterinary Institute of Pathology, Pharmacology and Therapy*, 114-b, ul. Lomonosova, Voronezh, 394087 Russia, e-mail gvostroilova@mail.ru (✉ corresponding author), nina_xoxlova@mail.ru, vnivipat@mail.ru, doktor.57@mail.ru, am7d@mail.ru;
ORCID:
Shabunin S.V. orcid.org/0000-0002-2689-6998
Vostroilova G.A. orcid.org/0000-0002-2960-038X
Parshin P.A. orcid.org/0000-0002-8790-0540
Shabanov D.I. orcid.org/0000-0002-1574-1317
Khokhlova N.A. orcid.org/0000-0001-6861-2554
The authors declare no conflict of interests
*Received April 29, 2021*

Abstract

The tissue drug aminoseleton, designed at the All-Russian Veterinary Research Institute of Pathology, Pharmacology and Therapy, was obtained from the spleen of cattle by cryogenic fractionation. Adaptogenic, membrane stabilizing, stress-protective, antioxidant and immunomodulatory properties of aminoseleton have been already shown. In this work, the anticlastogenic effect of the tissue drug aminoseleton on the bone marrow cells of mice exposed to the experimental mutagen was revealed for the first time. In addition, the preservation of cytogenetic stability and mitotic activity in bone marrow cells of healthy animals was shown when using the study drug. The objective of this work was to assess the effect of aminoseleton on the cytogenetic stability of bone marrow cells in healthy mice and mice exposed to the experimental mutagen, as well as to identify the antimutagenic properties of the drug in relation to the genotoxic effect of cyclophosphamide (CP) using a micronucleus test. The experiments were carried out on outbred white mice (*Mus albus officinarum*), which were divided into six groups subjected to the following treatments: i) intramuscular administration of sterile isotonic sodium chloride solution in a volume of 0.2 ml (negative control, $n = 12$); ii) intraperitoneal injection of 0.2 ml of CP (Baxter Oncology GmbH, Germany) at a dose of 20.0 mg/kg of body weight (positive control, $n = 12$); iii) intramuscular single injection of 0.2 ml of aminoseleton at a therapeutic dose of 0.5 ml/kg ($n = 12$); iv) intramuscular single injection of 0.2 ml of aminoseleton at a tenfold therapeutic dose of 5.0 ml/kg ($n = 12$); v) intramuscular single injection of 0.2 ml of aminoseleton at a dose of 0.5 ml/kg with intraperitoneal injection of 0.2 ml of CP at a dose of 20.0 mg/kg in 72 h ($n = 6$); vi) intramuscular fivefold injection of 0.2 ml of aminoseleton at a dose of 0.5 ml/kg with 24 h intervals and intraperitoneal administration of CP is similar to animals of other groups 72 h after the fifth injection ($n = 6$). To determine the amount of chromosomal aberrations in the bone marrow, 2.5 h before euthanasia, mice were injected intraperitoneally with 0.025 % colchicine (PanEco, Russia). Bone marrow cells were washed out of the femurs using Hanks' buffer solution (pH 7.4), the cell suspension was incubated in 0.075 molar hypotonic KCl solution, then the cells were fixed with acetoalcohol cooled to 4 °C and stained by Romanowsky-Giemsa procedure. The mitotic index (MI) was assessed by the number of dividing cells per 1000 bone marrow cells. The number of cells with chromosomal aberrations was counted in 100 metaphase plates per animal. Single and paired fragments, exchanges and achromatic gaps, as well as cells with multiple pathologies were counted. To study the frequency of micronuclei (micronucleus test) of polychromatophilic erythrocytes (PCE), the obtained bone marrow cells were added to 1 % albumin solution in Hanks' buffer solution (pH 7.4) and applied to glass slides, then the samples were dried, fixed with methanol and stained by Romanowsky-Giemsa protocol. The frequency of micronuclei per 1000 PCE was determined; a total of 2000 PCE per animal was studied. The proportion of PCE per 500 normochromic erythrocytes (NE) and PCE was also calculated. The frequency of chromosomal aberrations and micronuclei when administering the drug at the studied doses did not statistically significantly differ from that in animals of their negative control group that was 1.0±0.40 and 0.2±0.06 %, respectively. The administration of aminoseleton also had no effect on the mitotic index of bone marrow cells in experimental animals.

The course administration of aminoseleton reduced the clastogenic effect of cyclophosphamide, assessed by the number of micronuclei in polychromatophilic erythrocytes of the bone marrow, from 2.3±0.21 % in mice from the positive control group to 1.0±0.40 % in animals after a course of aminoseleton injections. Thus, the clastogenic activity of cyclophosphamide decreased by 51.3 % that was probably due to the correction of the prooxidant-antioxidant system of the animal body with the studied drug. A decrease in the number of micronuclei induced by cyclophosphamide in polychromatophilic erythrocytes of the bone marrow indicates the presence of an anticlastogenic potential in aminoseleton.

Keywords: aminoseleton, cyclophosphamide, mutagenicity, anticlastogenic properties, micronuclei, chromosomal aberrations, bone marrow, white mice, polychromatophilic erythrocytes

Reducing the negative impact of stress factors on the animal body through the use of adaptogen preparations is one of the areas of research in veterinary medicine [1]. Among the drugs of this class, a group of drugs can be distinguished that contain extracts of animal organs and tissues as part of the extract. Since the spleen serves as a source of a significant amount of cytokines of various types, it became the basis for several Russian and foreign organic preparations [2]. For many of them, immunomodulatory and adaptogenic properties have been shown [3, 4]. However, the species and age parameters of the animals from which the organs were obtained, as well as the technological features of the processing of raw materials during the production of the drug, can to some extent change the final chemical composition of the drug and, therefore, affect its biological activity and therapeutic efficacy [4].

At the All-Russian Veterinary Research Institute of Pathology, Pharmacology and Therapy, a new tissue drug aminoseleton was developed, obtained by cryogenic fractionation of the spleen of cattle. Previously, the adaptogenic, stress-protective, antioxidant, and immunomodulatory properties of aminoseleton were shown [5, 6].

Assessment of the genotoxic properties of new drugs is one of the mandatory stages of preclinical studies in their development [7]. Chemical mutagens are widespread in the environment and can cause hereditary and congenital diseases, carcinogenesis, aging, and mitochondrial diseases [8, 9]. The mutagenic and carcinogenic effects of various genotoxic substances also include the formation of free radicals that overload the endogenous antioxidant defense systems that inhibit oxidative stress, one of the causes of DNA damage. In general, all antioxidant agents can be considered as potential inhibitors of mutagenesis and carcinogenesis [10].

Potentially, anti-mutagenic substances include preparations of plant or animal origin, the use of which is associated with their lower toxicity, the affinity of biologically active substances that make up the drugs and are present in the body of animals, and their availability from an economic point of view. However, the effect of natural remedies on the hereditary apparatus of cells has not been sufficiently studied. The accumulation of data on the assessment of the clastogenic and antimutagenic properties of drugs used in veterinary practice is of scientific interest and of great practical importance. According to the authors' data, aminoseleton induced a decrease in the amount of malondialdehyde and nitric oxide metabolites, as well as an increase in the total antioxidant activity of the blood serum of animals. The drug modulated the enzymatic and non-enzymatic links of the antioxidant defense, for example, increased the activity of glutathione peroxidase and catalase, the concentration of vitamins A and E [11]. In this regard, it is advisable to evaluate not only the safety of aminoseleton but also its potential antimutagenic properties in a model of cyclophosphamide-induced (CP-induced) mutagenic action in mouse bone marrow cells [7]. CP is an alkylating drug used in oncology. This substance is activated by cytochrome P-450 in the liver, forming nitrogen mustard, which through a chain of reactions has an alkylating effect on DNA and causes the formation of cross-links between DNA strands at the guanine nitrogenous base,

which can cause mutations and cell death [12].

In the present work, the anticlastogenic effect of a tissue drug aminoseleton on the bone marrow cells of mice exposed to an experimental mutagen was revealed for the first time. In addition, the preservation of cytogenetic stability and mitotic activity in bone marrow cells of healthy animals was shown when using the study drug.

The aim of this work is to assess the effect of aminoseleton on the cytogenetic stability of bone marrow cells in healthy and exposed mice, as well as to identify the antimutagenic properties of the drug in relation to the genotoxic effect of cyclophosphamide using a micronucleus test.

*Materials and methods.* The experiments were carried out on outbred white mice (*Mus albus officinarum*) ($n$ = 60 divided into six groups) according to the guidelines for preclinical trials of drugs [7]. The experimental animals were kept under standard vivarium conditions (air temperature 18-23 °C, relative humidity 45-60%). Access to water and feed was free. Experimental manipulations were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (Strasbourg, 1986, reaffirmed in 2006). Aminoseleton was obtained by cryofractionation in the form of a liquid suspension.

Group I (negative control, $n$ = 12) was single administered intramuscularly with 0.2 ml sterile isotonic sodium chloride solution, Group II (positive control, $n$ = 12) was single injected intraperitoneally with 0.2 ml CF (Baxter Oncology GmbH, Germany) at a dose of 20.0 mg/kg of body weight [15]. Group III ($n$ = 12) was single injected intramuscularly with 0.2 ml aminoseleton at 0.5 ml/kg (a therapeutic dose). Group IV ($n$ = 12) was single injected intramuscularly with 0.2 ml aminoseleton at 5.0 ml/kg (a 10-fold therapeutic dose). Group V ($n$ = 6) was single injected intramuscularly with of 0.2 ml aminoseleton (0.5 ml/kg) followed with intraperitoneal injection of 0.2 ml CP (20.0 mg/kg) in 72 hours. Group VI ($n$ = 6) was injected intramuscularly with 0.2 ml aminoseleton (0.5 ml/kg), five times with a 24-hour intervals, followed by intraperitoneal injection of 0.2 ml CP (20.0 mg/kg) in 72 hours after the fifth injection of aminoseleton.

To determine the amount of chromosomal aberrations in the bone marrow, 2.5 hours before euthanasia, mice were injected intraperitoneally with 0.025% colchicine (PanEco, Russia). Then, bone marrow cells were washed out of the femurs using Hanks buffer solution (pH 7.4) with a 5 ml syringe, the cell suspension was incubated in 0.075 molar hypotonic KCl solution (25 min at 37 °C), then the cells were fixed using cooled to 4 °C acetoalcohol (methanol: acetic acid − 3: 1) and stained according to Romanovsky-Giemsa. The mitotic index (MI) was assessed by the number of dividing cells per 1000 bone marrow cells in the studied samples. The number of cells with chromosomal aberrations in 100 metaphase plates was counted for each animal [7, 14]. The samples were examined at a magnification of ×800 and ×1000 using a Bioskop-1 microscope (LOMO-Microanalysis LLC, Russia). Single and paired fragments, interchanges and achromatic gaps, as well as cells with multiple pathologies were taken into account [7]. Six animals from each group were examined, except for Groups V and VI.

To determine the frequency of micronuclei (micronucleus test) of polychromatophilic erythrocytes (PCEs) [15], the obtained bone marrow cells were added to a 1% albumin solution in Hanks buffer solution (pH 7.4) [16] and applied to glass slides, then the preparations were dried and fixed with methanol and stained according to Romanovsky-Giemsa [15]. Bone marrow preparations were examined at ×800 and ×1000 magnifications. The frequency of micronuclei was set at 1000 PCE; a total of 2000 PCE per animal was studied. The proportion of

PCE per 500 normochromic erythrocytes (NE) and PCE was also taken into account [7]. The study was carried out on 6 animals from each group.

Statistical processing of the obtained results was carried out in the STADIA 8.0 program (NGO Informatika and Computers, Russia). Mean values (*M*) and standard errors of means (± SEM) were calculated. The nonparametric van der Waerden test was used. Differences were considered statistically significant at $p \leq 0.05$.

*Results.* The metaphase plates of the bone marrow cells of the mice of the studied groups were analyzed (Table 1).

**1. Frequency of chromosomal aberrations in bone marrow cells in outbred white mice (*Mus albus officinarum*) exposed to various doses of aminoseleton** ($n$ = 12, $M$±SEM)

| Group | Number of metaphases | Number of abnormalities per 100 examined cells, % | | | | | Total number of cells with abnormalities, % |
|---|---|---|---|---|---|---|---|
| | | gaps | single fragments | paired fragments | interchanges | MP | |
| I | 600 | 0.5±0.37 | 0.2±0.18 | 0 | 0.2±0.18 | 0.2±0.18 | 1.0±0.40 |
| II | 600 | 0.5±0.24 | 12.7±0.92* | 2.8±0.34* | 2.0±0.49* | 0.3±0.23 | 11.2±0.52* |
| III | 600 | 0.7±0.23 | 0.2±0.18 | 0 | 0.8±0.44 | 0 | 1.7±0.46 |
| IV | 600 | 1.0±0.40 | 0 | 0 | 0.5±0.24 | 0.5±0.24 | 2.2±0.59 |

N o t e. MP — multiple abnormalities (more than five per cell). For a description of the groups, see the "Materials and methods" section.
* The difference with the negative control (Group I) is statistically significant at $p \leq 0.05$.

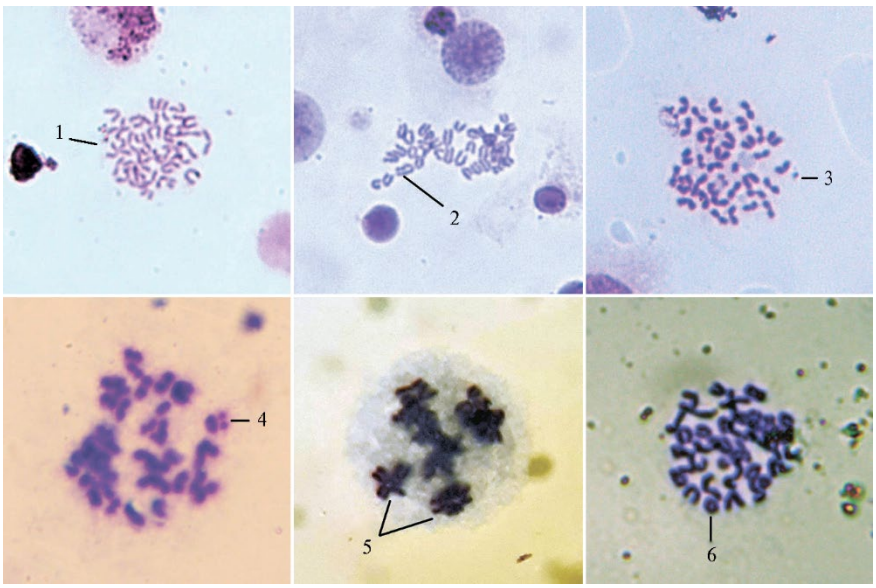


**Fig. 1. Micrographs of metaphase bone marrow plates of outbred white mice (*Mus albus officinarum*) with the introduction of intramuscularly sterile isotonic sodium chloride solution in a volume of 0.2 ml (negative control):** 1 — chromosomes without abnormalities, 2 — achromatic gap, 3 — single fragment, 4 — paired fragment, 5 — chromosomal and chromatid interchanges, 6 — ring chromosome (1-3 — magnification ×800, 4-6 — ×1000; a Bioskop-1 microscope, LOMO-Microanalysis LLC, Russia).

A single intramuscular injection of aminoseleton both at a therapeutic (0.5 ml/kg) and 10-fold (5 ml/kg) dose did not induce a statistically significant increase in the proportion of cells with chromosomal aberrations (Fig. 1) relative to the negative control group (Group I) after 24 hours (see Table 1). The clastogenic effect of CP (20 mg/kg), on the contrary, led to a 10-fold increase in the frequency of cells with pathologies. The introduction of aminoseleton also had no effect on MI in bone marrow preparations: in Groups I-IV, it was 4.0 ± 0.73, respectively; 3.1 ± 0.41; 3.5 ± 0.79 and 3.6 ± 0.75%.

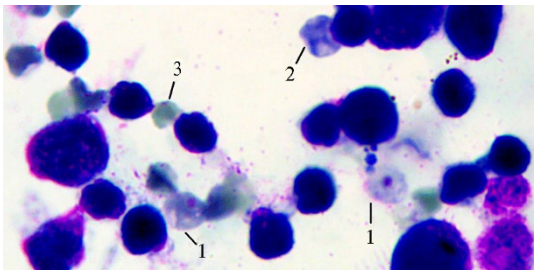


**Fig. 2. Micrograph of a bone marrow preparation of an outbred white mouse (*Mus albus officinarum*) with the introduction of intramuscularly sterile isotonic sodium chloride solution in a volume of 0.2 ml (negative control):** 1 — polychromatophilic erythrocyte with a micronucleus, 2 — polychromatophilic erythrocyte, 3 — normochromic erythrocyte (magnification ×1000, a Bioskop-1 microscope, LOMO-Microanalysis LLC, Russia).

The administration of aminoseleton at d 0.5 and 5 ml/kg of body weight in mice did not lead to a change in the frequency of PCE micronuclei (Fig. 2) as compared to the negative control (0.23±0.06%) (Table 2). CP injection induced a statistically significant increase in the frequency of micronuclei in PCE in mice from Groups II, V, and VI to 2.2±0.21, 2.1±0.26, and 1.1±0.09%, respectively. We found the anticlastogenic effect of aminoseleton, which was used, before CP administration, five times at 0.5 ml/kg (Group VI). Thus, the frequency of micronuclei in PCE in mice from Group VI was 51.3% ($p \leq 0.003$) lower than in animals from Group II, which received only CP. A single administration of aminoseleton (Group V) did not lead to a statistically significant decrease in the frequency of micronuclei. The research team also estimated the proportion of PCE in the bone marrow of mice, which can be an indicator of the toxic effect of xenobiotics [7]. No statistically significant difference occurred in this indicator between animals of all study groups, including the difference with the positive control.

**2. Frequency of polychromatophilic erythrocytes** (PCE) **with micronuclei in the bone marrow of healthy outbred white mice (*Mus albus officinarum*) and animals that received an injection of cyclophosphamide, with the preliminary administration of the drug aminoseleton in various doses** (*M*±SEM)

| Group | PCE with micronuclei/1000 PCE, % | PCE/( NE + PCE), % |
|---|---|---|
| I (*n* = 12) | 0.2±0.06 | 49.9±0.93 |
| II (*n* = 12) | 2.3±0.21[a] | 49.0±1.57 |
| III (*n* = 12) | 0.2±0.07 | 48.9±0.65 |
| IV (*n* = 12) | 0.3±0.11 | 49.5±1.43 |
| V (*n* = 6) | 2.1±0.26[a] | 48.2±1.79 |
| VI (*n* = 6) | 1.1±0.09[a, b] | 50.9±1.86 |

N o t e. NE – normochromic erythrocytes. For a description of the groups, see the "Materials and methods" section. [a], [b] Differences with negative (Group I) and positive (Group II) controls, respectively, are statistically significant at $p \leq 0.05$.

A number of drugs containing spleen extract are used in clinical practice as immunomodulators or drugs to reduce the side effects of anticancer therapy [4, 17]. The obtained data are consistent with the information on the absence of mutagenic properties in other drugs based on the spleen of animals. For example, the Polyerga™ drug recommended for clinical use (HorFerVit Pharma GmbH, Germany) showed no genotoxic effect in the Ames test [18]. In contrast to our data on the absence of the effect of aminoseleton on the proportion of PCEs in the bone marrow of mice, the results of Lu et al. [17] testify to the protective effect of the spleen extract of newborn calves in relation to mice with cyclophosphamide-induced inhibition of hematopoietic activity in the bone marrow. This can be explained by the significantly higher dose of CP (100 mg/kg body weight) used by the authors in the model of cyclophosphamide-induced hematopoiesis suppression [17].

The anticlastogenic effect of aminoseleton discovered by the authors and the absence of mutagenic properties in it is to some extent confirmed by the studies of Dychko et al. [19], who demonstrated a decrease in the number of micronuclei

in erythrocytes when spleen extract was administered to mice exposed to X-rays. The authors believe that the radioprotective effects of the spleen extract were due to its membrane-stabilizing effect on mouse cells, which was also shown by us for aminoseleton [20].

Currently, various mechanisms of antimutagenic action have been identified [21]. CP-induced genotoxicity and cytotoxicity towards bone marrow cells can be partially leveled out due to antioxidant activity [22]. Probably, the anticlastogenic effect of aminoseleton is due to the complex of biologically active substances included in the drug (amino acids, phospholipids, vitamins, oligopeptides, trace elements, nucleic acids) [20], which have an antioxidant effect [21]. Thus, ascorbic acid reduces the frequency of PCE with micronuclei induced by CP in bone marrow cells [23]. In addition, it has been shown that the introduction of aminoseleton increases the activity of glutathione peroxidase and leads to an increase in the content of reduced glutathione, the amount of which decreases with the introduction of CP and plays an important role in the antioxidant defense of the body [11, 24]. However, the antimutagenic effect of the same substances can occur through different mechanisms [25].

Since in the present studies, aminoseleton manifested itself as an immunomodulator of the cellular and humoral links of the immune system [6], its antimutagenic effect could be due to the induction of the synthesis of endogenous cytokines with a gene-protective effect, such as interferon [8]. Thus, a number of studies have shown the anticlastogenic and anticarcinogenic effects of interferons and their inducers, apparently due to the activation of post-replicative DNA repair [26, 27]. In a study by Jia et al. [28], the administration of an extract of spleen of newborn calves to mice with CP-induced immunosuppression led to an increase in the content of INF-$\alpha$ and INF-$\gamma$ in the blood serum, which may testify in favor of the proposed hypothesis.

The sensitivity of cells to the action of genotoxicants depends on the intake of B vitamins with antioxidant properties, micronutrients, and other trace elements (such as magnesium or zinc) involved in reparative processes, maintenance of cell homeostasis, and antioxidant protection [8]. Their replenishment can have a systemic effect on the antioxidant defenses of the body. The prolonged anticlastogenic effect of aminoseleton even 72 h after the final administration is due to the prolonged activation of the antioxidant system upon administration of the drug [11].

Thus, the tissue drug aminoseleton obtained by cryofractionation from the bovine spleen in therapeutic (0.5 ml/kg) and high (5.0 ml/kg) doses had no destabilizing effect on the cytogenetic characteristics of cells, which was assessed by the number of PCEs with micronuclei and chromosome aberrations in bone marrow cells of mongrel mice. The drug did not affect the activity of cell division, assessed by the mitotic index. At the same time, aminoseleton showed an anticlastogenic effect when combined with the genotoxicant cyclophosphamide, reducing the frequency of occurrence of PCEs with micronuclei in the bone marrow cells of mice by 51.3% relative to the positive control group.

## REFERENCES


1. Uchasov D.S., Yarovan N.I., Sein O.B. *Vestnik Orlovskogo gosudarstvennogo agrarnogo universiteta*, 2013, 1(40): 129-131 (in Russ.).
2. Plenina L.V., Soroka N.F., Chudakov O.P., Tret'yak S.I., Bykadorova L.G., Mit'kovskaya N.P., Golynskii A.B., Maksimovich A.V., Evseenko V.M., Ivanovskii G.L., Alikevich I.N., Drazhina L.S., Grak N.N., Skuratovskaya L.I. *Retsept*, 2008, 1(57): 104-106 (in Russ.).
3. Fedulova L.V., Vasilevskaya E.R. *Myasnye tekhnologii*, 2016, 12(168): 37-39 (in Russ.).
4. Chernenkova M.L., Styazhkina S.N., Valinurov A.A. *Zhurnal nauchnykh statei zdorov'e i obrazovanie v XXI veke*, 2017, 19(8): 161-163 (in Russ.).

5. Vostroilova G.A., Khokhlova N.A., Parshin P.A., Cheskidova L.V., Bryukhova I.V., Sashnina L.Yu., Kantorovich Yu.A., Kartashov S.S. *Veterinarnyi farmakologicheskii vestnik,* 2018, 2(3): 37-41 (doi: 10.17238/issn2541-8203.2018.2.37) (in Russ.).
6. Shabunin S.V., Vostroilova G.A., Parshin P.A., Khokhlova N.A., Sashnina L.Yu., Mikhailov E.V., Tyurina E.V. *Veterinarnaya patologiya*, 2018, 3(65): 39-46 (doi: 10.25690/VETPAT.2018.65.20143) (in Russ.).
7. *Rukovodstvo po provedeniyu doklinicheskikh issledovanii lekarstvennykh sredstv. Chast' pervaya* /Pod redaktsiei A.N. Mironova [Guidelines for conducting preclinical studies of drugs. Part one. A.N. Mironov (ed.)]. Moscow, 2012 (in Russ.).
8. Durnev A.D. *Fiziologiya cheloveka*, 2018, 44(3): 116-137 (doi: 10.7868/S013116461803013X) (in Russ.).
9. *Genetic toxicology: principles and methods.* J.M. Parry, E.M. Parry (eds.). Springer, New York, 2012 (doi: 10.1007/978-1-61779-421-6).
10. Delarmelina J.M., Dutra J.C.V., Batitucci Mdo C. Antimutagenic activity of ipriflavone against the DNA-damage induced by cyclophosphamide in mice. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*, 2014, 65: 140-146 (doi: 10.1016/j.fct.2013.12.028).
11. Shabunin S.V., Shakhov A.G., Vostroilova G.A., Parshin P.A., Ermolova T.G., Khokhlova N.A., Bliznetsova G.N. *Dostizheniya nauki i tekhniki APK*, 2019, 33(7): 71-74 (doi: 10.24411/0235-2451-2019-10716) (in Russ.).
12. Emadi A., Jones R.J., Brodsky R.A. Cyclophosphamide and cancer: golden anniversary. *Nature reviews. Clinical Oncology*, 2009, 6(11): 638-647 (doi: 10.1038/nrclinonc.2009.146).
13. Nau H., Spielmann H., Lo Turco Morter C.M., Winckler K., Riedel L., Obe G. Mutagenic, teratogenic and pharmacokinetic properties of cyclophosphamide and some of its deuterated derivatives. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 1982, 95(2-3): 105-118 (doi: 10.1016/0027-5107(82)90250-0).
14. Preston R.J., Dean B.J., Galloway S., Holden H., McFee A.F., Shelby M. Mammalian in vivo cytogenetic assays Analysis of chromosome aberrations in bone marrow cells. *Mutation Research/Genetic Toxicology*, 1987, 189(2): 157-165 (doi: 10.1016/0165-1218(87)90021-8).
15. Hayashi M. The micronucleus test—most widely used in vivo genotoxicity test. *Genes and Environment*, 2016, 38: 18 (doi: 10.1186/s41021-016-0044-x).
16. Agarwal D.K., Chauhan L.K. An improved chemical substitute for fetal calf serum for the micronucleus test. *Biotechnic & Histochemistry*, 1993, 68(4): 187-188 (doi: 10.3109/10520299309104695).
17. Lu W., Jia D., An S., Mu M., Qiao X., Liu Y., Li X., Wang D. Calf Spleen Extractive Injection protects mice against cyclophosphamide-induced hematopoietic injury through G-CSF-mediated JAK2/STAT3 signaling. *Scientific Reports*, 2017, 7(1): 8402 (doi: 10.1038/s41598-017-08970-3).
18. Hartleb M., Leuschner J. Toxicological profile of a low molecular weight spleen peptide formulation used in supportive cancer therapy. *Arzneimittel-Forschung*, 1997, 47(9): 1047-1051.
19. Dychko K.A., Ryzhova G.L., Kravtsova S.S., Kir'yanova N.L., Kuvshinov N.N., Gridneva V.I. *Sposob polucheniya sredstva s adaptogennym i protivoluchevym deistviem. Patent RU 2142284 C1 (RF) MPK$^6$ A 61 K 35/28. Tomskii gosudarstvennyi universitet (RF). № 95110155/14. Zayavl. 14.06.1995. Opubl. 10.12.1999* [Method of obtaining a substance with adaptogenic and antiradiation effects. Patent RU 2142284 C1 (RF) MPK$^6$ A 61 K 35/28. Tomsk State University (RF). № 95110155/14. Appl. 14.06.1995. Publ. 10.12.1999] (in Russ.).
20. Khokhlova N.A., Lobodina T.E., Grigor'eva N.A., Topol'nitskaya A.V., Fedorova N.M., Panina T.A. *Veterinarnyi farmakologicheskii vestnik*, 2018, 1(2): 25-30 (doi: 10.17238/issn2541-8203.2018.1.25) (in Russ.).
21. Goncharova R.I., Kuzhir T.D. *Ekologicheskaya genetika*, 2005, 3(3): 19-32 (in Russ.).
22. Hosseinimehr S.J., Karami M. Chemoprotective effects of captopril against cyclophosphamide-induced genotoxicity in mouse bone marrow cells. *Archives of Toxicology*, 2005, 79(8): 482-486 (doi: 10.1007/s00204-005-0655-7).
23. Vijayalaxmi K.K., Venu R. In vivo anticlastogenic effects of L ascorbic acid in mice. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 1999, 438(1): 47-51 (doi: 10.1016/s1383-5718(98)00161-2).
24. Manda K., Bhatia A.L. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. *Cell Biology and Toxicology,* 2003, 19(6): 367-372 (doi: 10.1023/b:cbto.0000013342.17370.16).
25. De Flora S., Ramel C. Mechanisms of inhibitors of mutagenesis and carcinogenesis. Classification and overview. *Mutation Research*, 1988, 202(2): 285-306 (doi: 10.1016/0027-5107(88)90193-5).
26. Bolzán A.D., Lacunza E., Bianchi M.S. Effect of recombinant interferon-alpha on streptonigrin-induced chromosome aberrations and sister-chromatid exchanges in hamster cells. *Mutation Research*, 2003, 522(1-2): 127-134 (doi: 10.1016/s0027-5107(02)00304-4).
27. Novitskii V.V., KHlusova M.YU., Ternovaya S.V., Saratikov A.S. *Antimutagennoe sredstvo. Patent RU 2189232 C2 (RF) MPK$^7$ A 61 K 31/415, A 61 P 43/00. NII farmakologii Tomskogo nauchnogo tsentra RAMN (RF). Sibirskii meditsinskii universitet (RF). № 2000118409/14. Zayavl.*

*10.07.2000. Opubl. 20.09.2002* [Antimutagenic agent. Patent RU 2189232 C2 (RF) MPK[7] A 61 K 31/415, A 61 P 43/00. Research Institute of Pharmacology of the Tomsk Scientific Center of the Russian Academy of Medical Sciences (RF). Siberian Medical University (RF). № 2000118409/14. Appl. 10.07.2000. Publ. 20.09.2002] (in Russ.).

28. Jia D., Lu W., Wang C., Sun S., Cai G., Li Y., Wang G., Liu Y., Zhang M., Wang D. Investigation on immunomodulatory activity of calf spleen extractive injection in cyclophosphamide-induced immunosuppressed mice and underlying mechanisms. *Scandinavian Journal of Immunology*, 2016, 84(1): 20-27 (doi: 10.1111/sji.12442).