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Research Article

Comparative study of anti-inflammatory and analgesic activity of diterpene alkaloid songorine obtained from *Aconitum barbatum* and its cell culture

Yulia V. Nesterova[1], Anna P. Chernova[2], Tatiana N. Povetyeva[1], Nikolay I. Suslov[1], Gleb N. Zyuz`kov[1], Olga G. Afanasyeva[1], Pavel V. Kulpin[1], Vadim V. Zhdanov[1]

1 E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk National Research Medical Center; 3 Lenin St., Tomsk 634028 Russia; 2 National Research Tomsk Polytechnic University; 30 Lenin St., Tomsk 634050 Russia.

Corresponding author: Yulia V. Nesterova (nes-yuliua@yandex.ru)

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Abstract

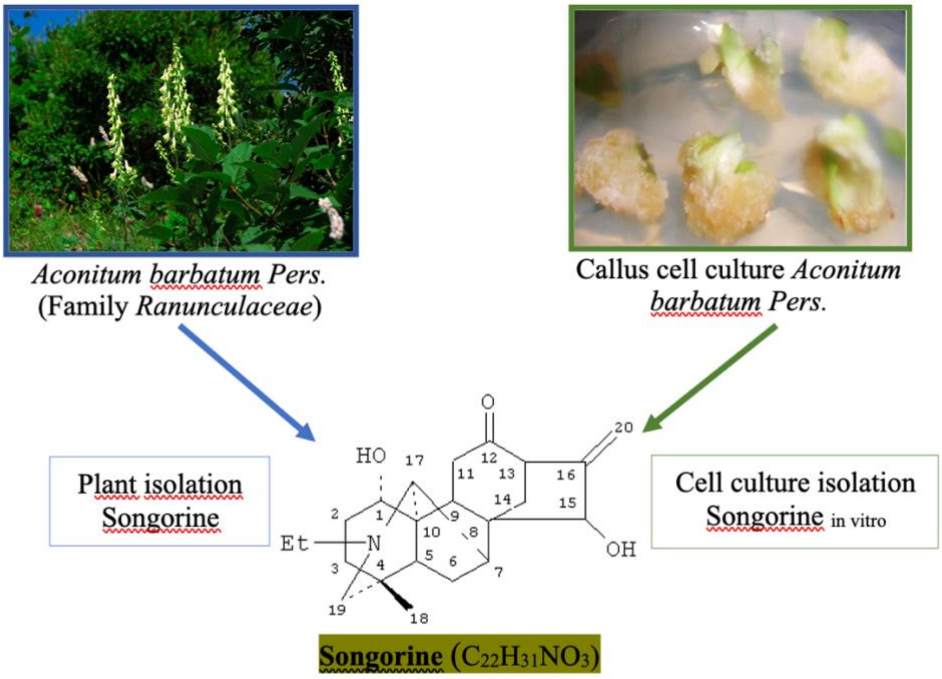
Introduction: The study was aimed at a comparative analysis of the anti-inflammatory and analgesic activities of diterpene alkaloid songorine obtained from *Aconitum barbatum* and its cell culture.

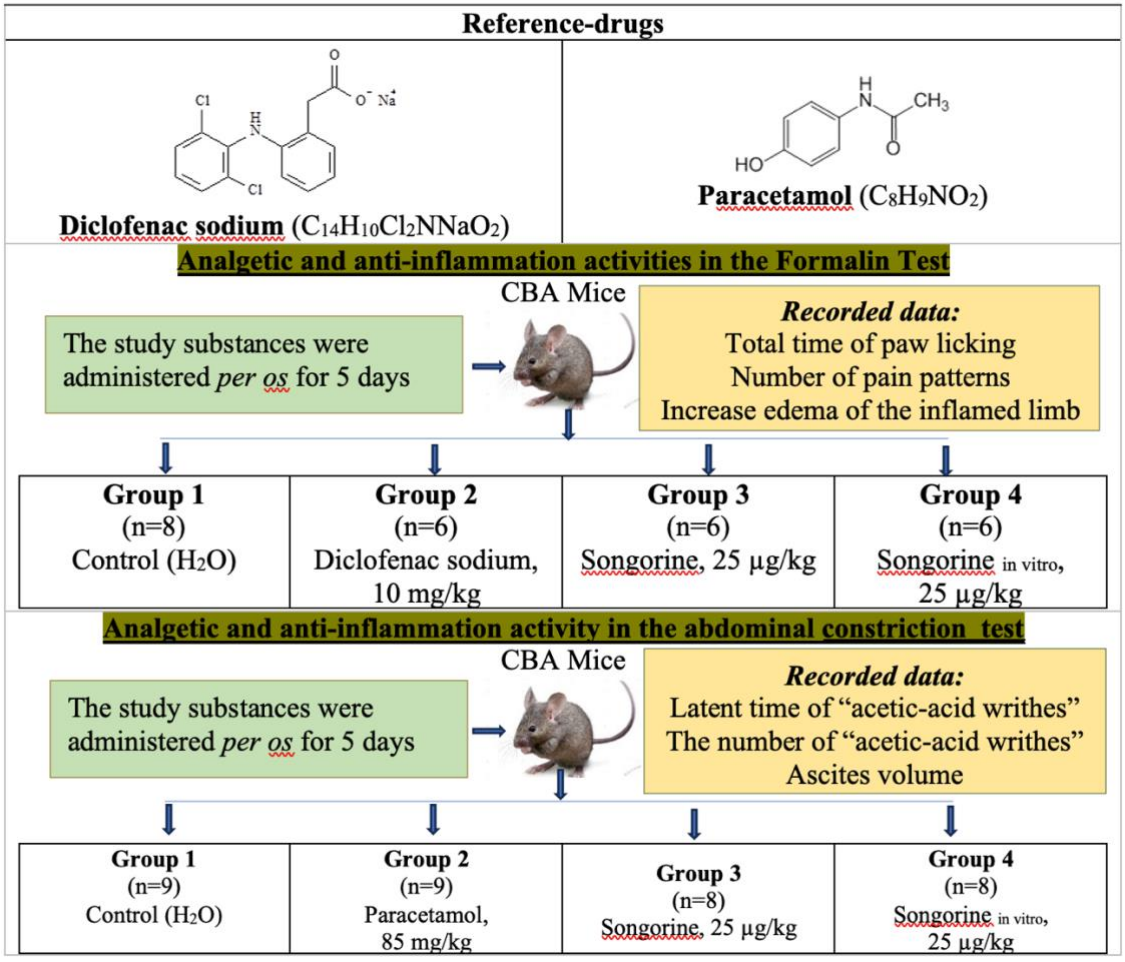
Materials and Methods: Experiments were performed on 60 male mice of the CBA line. The object of the study was the diterpene alkaloid of songorine (25 µg/kg) isolated from *Aconitum barbatum*, as well as obtained from the callus culture of cells. Comparative study of analgesic and anti-inflammatory activities of the alkaloid obtained from different sources was carried out under conditions of the formalin test and abdominal constriction test. Diclofenac sodium (10 mg/kg) and paracetamol (85 mg/kg) were used as comparison drugs. Control animals received distilled water. All substances were administered to mice *per os* for 5 days, the last time – 1 hour before the onset of the damaging effect.

Results and Discussion: Under the formalin test conditions, the analgesic activity of songorine from both sources was manifested in the second phase of pain response. The anti-inflammatory activity in this model of both “standard” and ”culture” songorine was comparable to that of diclofenac sodium. Probably, pharmacological activity of alkaloid in the formalin test is provided by suppression of proinflammatory mediators and TRPA1-receptors. In the abdominal constriction test, all investigated substances showed a pronounced analgesic effect maximum under the action of songorine in vitro. In addition, songorine from both sources, in contrast to the reference drug, provided a reliable phlogolytic effect. Probably, the pharmacological activity of songorine in this model is provided by its suppressive action against endogenous mediators of pain and inflammation and of TRPV1-receptors.

Conclusion: Comparative analysis of the analgesic and anti-inflammatory effects of songorine (25 µg/kg) from *Aconitum barbatum* after a 5-dose regimen demonstrated comparability with “culture” songorine (25 µg/kg).

Graphical Abstract





Keywords

diterpene alkaloid, songorine, *Aconitum barbatum*, anti-inflammatory and analgesic activity

Introduction

The diterpene alkaloid songorine is a secondary metabolite, which is mainly produced by plants of the genus *Aconitum* and, like many representatives of this class of compounds (Pereira et al. 2023), has a wide spectrum of pharmacological action. Experiments on animals revealed a pronounced antinociceptive activity of songorine, comparable to the action of both non-narcotic analgesics (Nesterova et al. 2014) and the opioid drug of mixed mechanism of action – tramadol (Nesterova et al. 2024). High phlogolytic properties of the alkaloid, not inferior to the action of non-steroidal anti-inflammatory drugs, have been demonstrated on various models of acute and chronic inflammation (Nesterova et al. 2011; Nesterova et al. 2014a). Significant regenerative (Zyuz'kov et al. 2012), anxiolytic (Nesterova et al. 2015; Nesterova et al. 2024), antidepressant (Nesterova et al. 2011), nootropic (Nesterova et al. 2018) and anticonvulsant activities of songorine has been established. Convincing data have been obtained on the prospects of using this diterpene alkaloid as an active ingredient of a fundamentally new cerebroprotective drug with neuroregenerative activity (Zyuz'kov et al. 2015). The potential for high efficiency of its clinical application for the treatment of neurodegenerative and other neurological diseases is determined by the uniqueness of the mechanism of action of this alkaloid, which consists in stimulation of the functions of regenerator-competent cells of nervous tissue (Zyuz'kov et al. 2015; Zyuz'kov et al. 2016). Given the significant therapeutic potential and low toxicity profile of songorine (III class of hazard according to GOST 12.1.007-76), as well as the absence of ulcerogenic effect (Nesterova et al. 2014a), the creation of effective and low-toxicity drugs based on it seems very promising.

The use of plant raw materials as a source of songorine in an amount sufficient for industrial production of drugs has a number of disadvantages and limitations. The solution to this problem seems possible within the framework of using the technology of cultivation of plant cells (aconites) to isolate the desired substance from them (Yenikeev et al. 2011; Popova et al. 2021). In order to find an original approach to obtain the required amount of songorine for its use in the pharmaceutical industry, the Department of Analytical Chemistry of Tomsk Polytechnic University developed a technology for obtaining songorine *in vitro* from the callus culture of cells of wolfsbane (*Aconitum barbatum Pers.*). However, it is known that substances obtained from plant cell culture can significantly differ in their properties from those isolated directly from plants (Kochkin et al. 2019, 2023). This phenomenon to varying degrees can be related both to a change of the primary structure of the compound synthesized under *in vitro* conditions and to its isomerization due to the specificity of growth and functioning of plant cells in the culture medium (Popova et al. 2021). Therefore, it was of interest to conduct a comparative study of the pharmacological properties of songorine obtained from the above-ground part of *Aconitum barbatum* and songorine isolated from aconite cell culture (songorine $_{\text{in vitro}}$). The anti-inflammatory and antinociceptive activities of songorine isolated by the standard method and "culture" songorine was evaluated as the most pronounced in the studied alkaloid and relatively easy to detect.

Materials and Methods

Tested substances

The object of the study was songorine, a diterpene alkaloid of the atizin series, isolated from the above-ground parts of *Aconitum barbatum* according to the standard methodology (Schmidt-Traube et al. 2022). Above-ground parts of plants collected during flowering in Irkutsk and Tomsk regions were ground to a particle size of less than 5 mm, treated with sodium carbonate solution and subjected to continuous extraction with chloroform for 5 days. The chloroform extract was evaporated to a small volume and thoroughly extracted with 5% sulfuric acid. The acidic extract was alkalized with sodium carbonate to pH 9–10 and extracted sequentially first with ether, then with chloroform. The ether extract was evaporated to dryness, dissolved in a small amount of ether and chromatographed on deactivated aluminum oxide in the hexane-acetone system (90→50%). The ether-soluble fraction was subjected to fractional extraction with buffer solutions of increasing pH values. The ether solution remaining after extraction with the most alkaline buffer was evaporated to dryness and chromatographed on aluminum oxide in the hexane-methanol system until elution of songorine. The yield of songorine from the above-ground parts of *Aconitum barbatum* was 0.2±0.1 % of the dry weight of the raw material. Songorine was dissolved in distilled water and administered to animals *per os* at the most effective dose of 25 μg/kg., which was previously established during the screening study.

In addition, songorine was isolated from the callus culture of *Aconitum barbatum* cells. The

yield of songorine from the callus culture of *Aconitum barbatum* cells amounting to approximately 0.4±0.1 % of the dry weight of the raw material. To obtain callus culture of *Aconitum barbatum*, seeds of the plant were used as an explant, as they are the most convenient objects that allow growing the plant cell culture year-round, regardless of weather conditions and time of year. *Aconitum barbatum* seeds were specially prepared and placed in containers on agarized hormone-free nutrient medium Murashige and Skoog (MS) (Table 1) (Filonova et al. 2020).

Table 1. MS culture medium composition

Components	Concentration of components in MS medium, mg/L
Macronutrients	
H4NO3	1650
KNO3	1900
CaCl2 ×2H2O	440
MgSO4 × 7H2O	370
KH2PO4	170
NH4NO3	1650
Micronutrients	
KJ	0.83
H3BO3	6.2
MnSO4 × 4H2O	22.3
ZnSO4 × 7H2O	8.6
Na2MoO4 × 2H2O	0.25
CuSO4 × 5H2O	0.025
CoCl2 × 6H2O	0.025
Chelated irons	
FeSO4 × 7H2O	27.8
Na2 EDTA × 2H2O	37.3
Vitamins and organic compounds	
Nicotinic acid	0.5
Pyridoxine-HCl	0.5
Thiamine -HCl	1
Sucrose	60000
Agar	8000
Distilled H2O	Up to 1 liter
pH	5.8

The containers were sealed with foil and paraffin and placed in a culture room (T= 26ºC, E=1000 lx, humidity – 70%). Under these conditions, the plants were grown for 30 days. The first signs of germination were observed in seeds 9–10 days after the beginning of the experiment (Fig. 1).

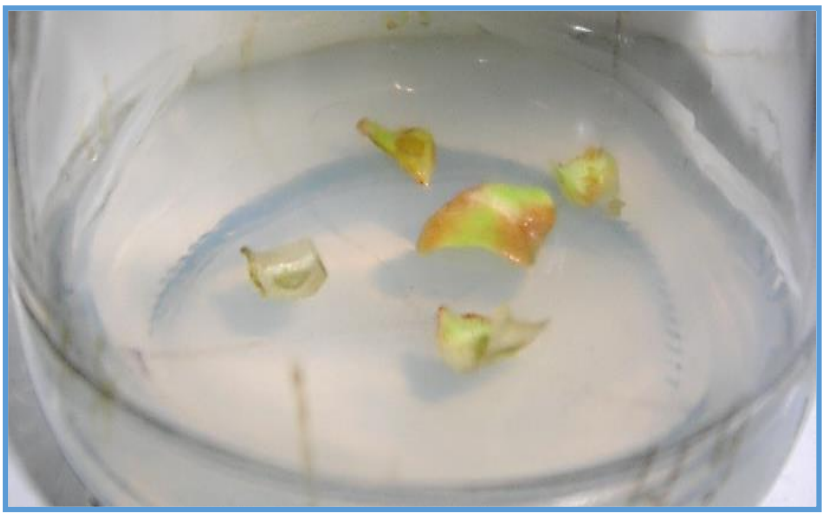


Figure 1. Photograph of a sterile *Aconitum barbatum* plant.

Thirty days after sowing seeds, sterile aconite plants were used to obtain explants (for callus induction). On the 28th day, explants were placed in test tubes on MS nutrient medium with a special hormonal composition promoting intensive growth of the culture and synthesis of secondary metabolites (Lyapkov et al. 1999). Callus tissue formation was observed on the 3rd–5th day of cultivation (Fig. 2).

Figure 2. Callus formation from a leaf explant of *Aconitum barbatum*.

The sum of alkaloids from callus cell culture was isolated by distillation under vacuum. The sum of alkaloids was separated by non-classical affinity chromatography using liquid column chromatography (LCC). Identification of separately isolated alkaloids was carried out by TLC, HPLC and NMR-spectroscopy (Grinkevich 1991; Taigushanov 2015).

Reference preparations

Diclofenac sodium and paracetamol were used as reference drugs. Diclofenac sodium (Chemopharm LLC, Obninsk, Russia) is a drug from the NSAID group, a derivative of phenylacetic acid. In our experiments, diclofenac was dissolved in distilled water and administered to mice *per os* at a dose of 10 mg/kg.

Paracetamol (Asfarma LLC, Russia) is a non-narcotic analgesic and antipyretic agent of central acting from the group of anilides, widely used in clinical practice (Seth 2019; Narzikulov et al. 2021), which is considered the most common analgesic in the world, used in all three stages of intensive pain management. In our studies, we dissolved paracetamol in distilled water and administered to mice *per os* at a dose of 85 mg/kg.

The animals received the study drugs prophylactically for 5 days, the last time 1 hour before the test. The control group of mice was administered solvent (distilled water) according to a similar scheme.

Experimental animals

The experiments were performed on 60 male mice of the CBA line weighing 25–28 g. Animals of the 1st category were obtained from the Department of Experimental Biological Models of E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (Russia). The animals were kept in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg 1986). The study was conducted in accordance with the rules of laboratory practice in the Russian Federation (GOST R 33044-2014), Principles of Good Laboratory Practice (GLP), GOST 7.32-2001 (ISO 5966-82), GOST 32296-2013, Guidelines for Conducting Preclinical Studies of Drugs (Moscow 2013) and approved by the Ethical Committee of E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (the IACUC Protocol No. 191112021 dated 21.12.2021). In the formalin test, 26 mice of the CBA strain were used, and in the acetic acid writhing test, 34 mice of the CBA strain were used.

Experimental protocol

To study analgesic and anti-inflammatory activities of songorine, the formalin test was used. In this experiment, animals were allocated to groups as follows: Group 1 – Control, water (8 CBA mice); Group 2 – Diclofenac sodium, 10 mg/kg (6 CBA mice); Group 3 – Songorine, 25 μg/kg (6 CBA mice); Group 4 – Songorine «cultural», 25 μg/kg (6 CBA mice). Formalin solution (2%,

0.05 mL) was subplantarly injected into the right hind paw of mice, after which the animals were placed in a transparent box, and a timer was simultaneously turned on. The intensity of the pain response in the first (first 5 minutes) and second (40 minutes after formalin injection) phases of the test was evaluated by the number of pain patterns (lifting, shaking, licking) and duration of licking (in seconds) of the injected paw within 15 minutes. The total number of pain patterns was summarized for each animal. One hour after phlogogen injection, the animals were killed; both hind paws were separated and weighed. The intensity of the inflammatory reaction was estimated by the difference between the weight of the healthy and diseased limb according to the formula:

$$I = \frac{M_1 - M_2}{M_1} \times 100\%,$$

where I – edema increment; M_1 – weight of inflamed paw; M_2 – weight of healthy paw.

Antinociceptive and phlogolytic activities were also evaluated against the background of intraperitoneal injection of 1% acetic acid solution (GOST 61-75). During the next 15 minutes after algogen injection, the number of acts of specific pain reaction – writhes –was counted for each animal. The effectiveness of the studied substances as analgesics was judged by the difference in the average number of writhes in the control and experimental groups, as well as by the increase in the latent time of the pain response. Three hours after acetic acid administration, the animals were killed, the abdominal cavity was opened, and exudate was collected. The anti-inflammatory effect was evaluated by decreasing the exudate volume. In this experiment, animals were allocated to groups as follows: Group 1 – Control, water (9 CBA mice); Group 2 – Paracetamol, 85 mg/kg (9 CBA mice); Group 3 – Songorine, 25 µg/kg (8 CBA mice); and Group 4 – Songorine «cultural», 25 µg/kg (8 CBA mice).

Statistics

The results were processed in Statistica 6.0 program (StatSoft, Inc., USA) by the method of variation statistics using Student;s t test and nonparametric Mann-Whitney U test. Data are presented as mean (M) and error of mean (m). The difference between the compared values was considered reliable if the probability of their identity was less than 5% ($p<0.05$).

Results and Discussion

Chromatographic separation of a model mixture of alkaloids and the total alkaloid sum isolated from *Aconitum barbatum* tissue culture using LCC on an azoepoxyadsorbent Sephadex LH-20-NG-EpKG is presented in Figures 3 and 4. As a result of chromatography, the presence of 6 peak fractions was observed, indicating complete separation of the model alkaloid mixture. A similar result was noted when separating the total alkaloid sum obtained from the culture.

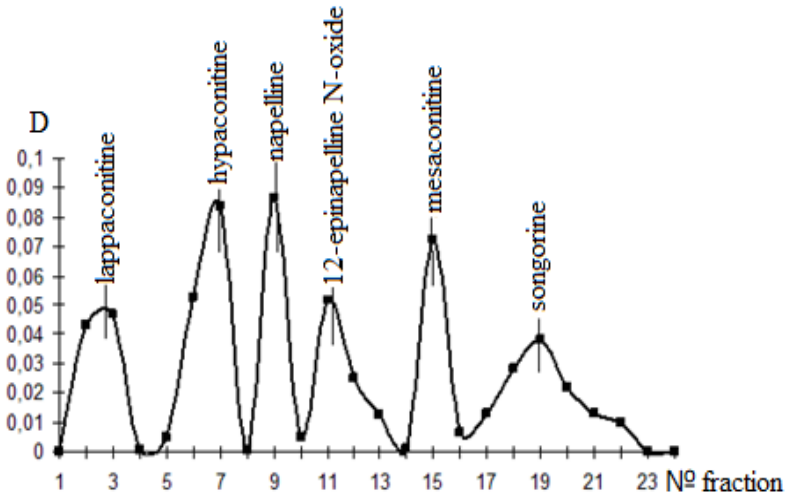


Figure 3. Chromatographic separation profile of an alkaloid model mixture on azoepoxyadsorbent Sephadex LH-20-NG-EpKG.

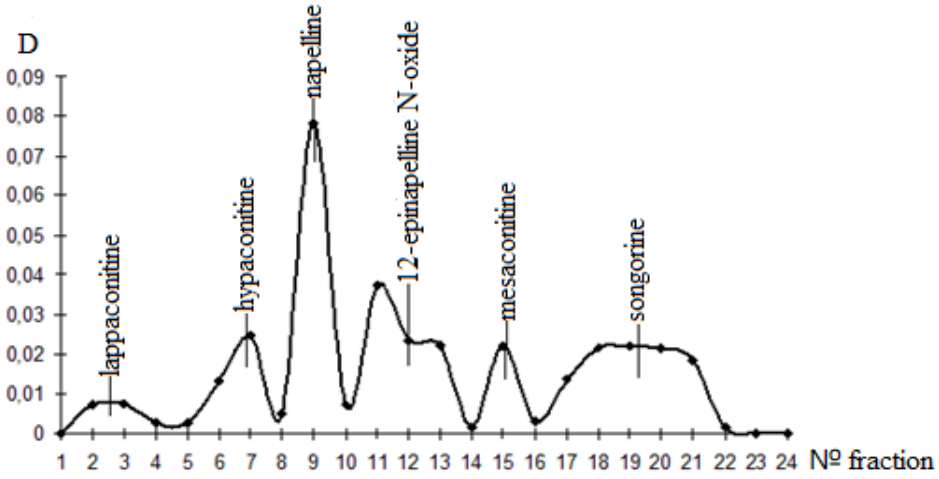


Figure 4. Chromatographic profile of the alkaloid extract from *Aconitum barbatum* tissue culture separated on azoepoxyadsorbent Sephadex LH-20-NG-EpKG.

The UV spectra of the isolated peak fractions were consistent with the UV spectra of the alkaloid standards.

The results of the identification of isolated alkaloids from the tissue culture of *Aconitum barbatum* by thin-layer chromatography are presented in Table 2.

Table 2. Identification of alkaloids by TLC

Alkaloid standards		Fractions from LCC	
standard	**R_f**	**Fraction number**	**R_f**
Lappaconitine	0.65	3	0.65
Hypaconitine	0.75	7	0.75
Napelline	0.10	9	0.10
12-epinapelline N-oxide	0.66	11	0.66
Mesaconitine	0.91	15	0.91
Songorine	0.49	19	0.49

Based on the obtained data, the HPLC chromatograms of the isolated alkaloids correspond to the HPLC chromatograms of the standards (Figs 5 and 6).

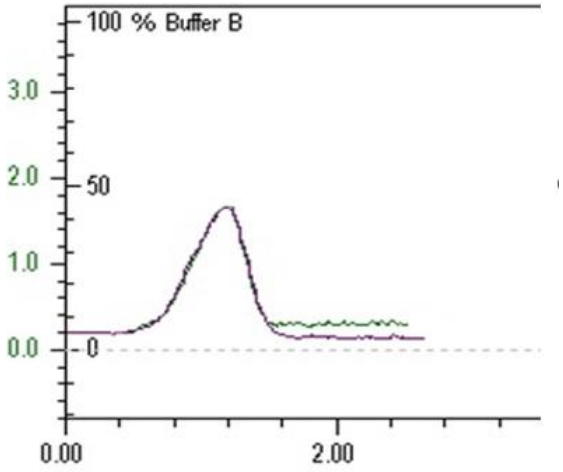


Figure 5. HPLC chromatogram of songorine standard.

A comparative study of songorine samples obtained from *Aconitum barbatum* cell culture and songorine isolated from the above-ground parts of *Aconitum barbatum* was performed using NMR spectroscopy (1H, 13C) on a Bruker AVANCE III HD NMR spectrometer (400 MHz). NMR spectroscopy revealed that *in vitro* songorine obtained from *Aconitum barbatum* cell culture is a structurally similar, related compound to songorine isolated from *Aconitum barbatum*, or its stereoisomer (Figs 7 and 8).

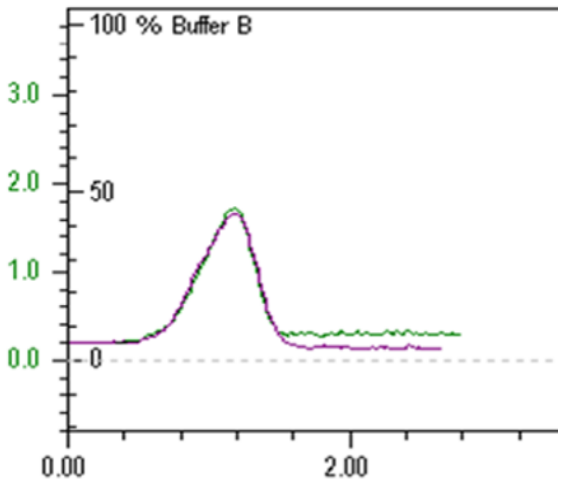


Figure 6. HPLC chromatogram of songorine isolated from cell culture on azoepoxyadsorbent Sephadex LH-20-NG-EpKG.

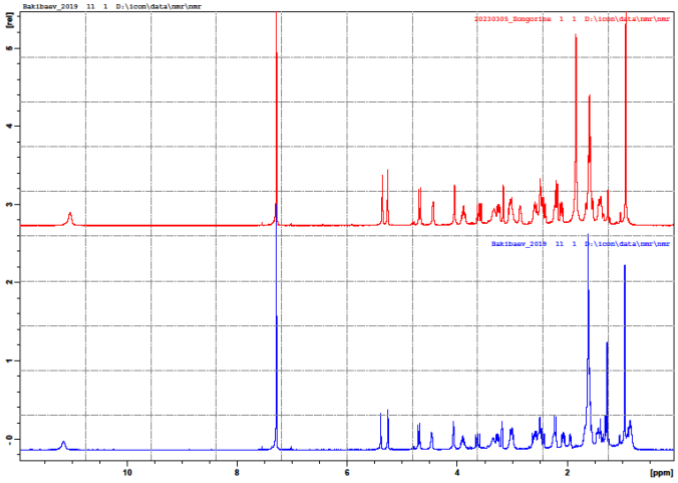


Figure 7. Comparative ^{1}H-NMR spectroscopy, where the blue curve represents the ^{1}H-NMR spectrum of songorine isolated from culture; the red curve represents the ^{1}H-NMR spectrum of songorine isolated from *Aconitum barbatum*.

The formalin test involves recording the nociceptive response of rodents to moderate, continuous pain induced by formalin-induced tissue damage. The test is thought to provide a more effective model of clinical pain than tests with phasic mechanical or temperature stimuli (Bibik et al. 2021).

Two phases of pain are known to develop in response to formalin injection (Lei and Yan 2022). The first phase lasts for 3–5 minutes from the beginning of injection, which is associated with chemical action on nociceptors and activation of C-fibers. After that, there is little or no pain response for 10–15 minutes. The second phase begins 15–20 minutes after injection and lasts for 20–40 minutes, which is associated with the inflammatory response and neuronal activation in the dorsal horns of the spinal cord (Lei and Yan 2022). Substance P and bradykinin are involved in the first phase, while histamine, serotonin, prostaglandins, and bradykinin are involved in the second phase (Sałat and Filipek 2015).

In addition, one of the mechanisms of the nocigenic action of formalin is the activation of TRPA1 channels, which normally respond to cold and stimulate the development of

inflammation (Sałat and Filipek 2015). Opioid analgesics are believed to block both phases of the pain response; NSAIDs mainly inhibit the second phase, and local anesthetics – only the first phase.

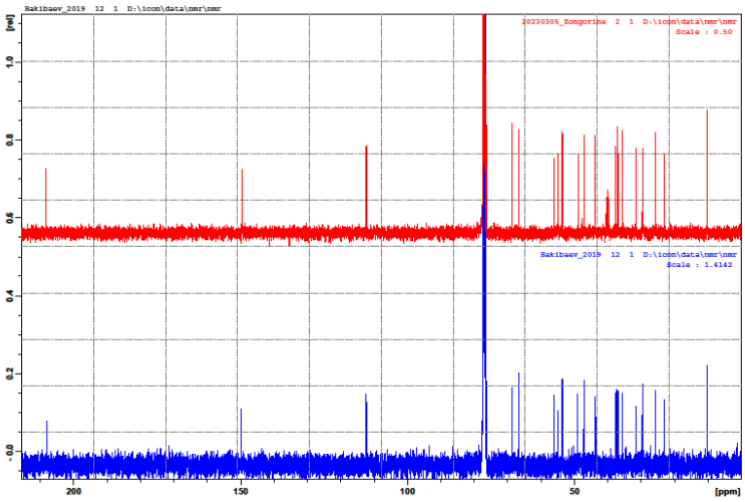


Figure 8. Comparative ^{13}C-NMR spectroscopy, where the blue curve represents the ^{13}C-NMR spectrum of songorine isolated from culture; the red curve represents the ^{13}C-NMR spectrum of songorine isolated from *Aconitum barbatum*.

The experimental study showed that the course administration of diclofenac sodium and, to a greater extent, songorine from the above-ground part of *Aconitum barbatum* to mice reduced the duration of licking the injected limb during the first phase of the pain response, but this effect was not statistically significant (Fig. 3). Another recorded parameter – the total number of pain patterns significantly decreased (1.7 times) only under the influence of the reference drug (Fig. 9).

First phase of inflammation (acute pain)

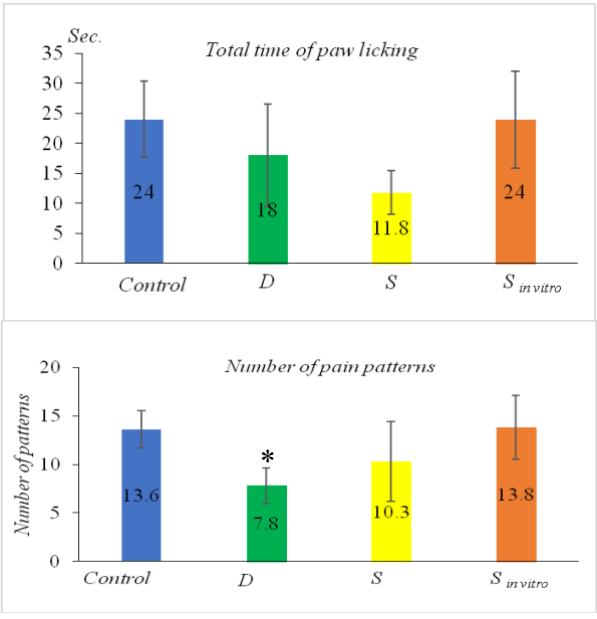


Figure 9. Comparative study of analgesic activity of songorine isolated from *Aconitum barbatum* and songorine obtained from *Aconitum barbatum* cell culture in the formalin test. ***Note:*** D – diclofenac sodium (10 mg/kg), S – songorine from *Aconitum barbatum* (25 μg/kg), S $_{\text{in vitro}}$ – songorine from *Aconitum barbatum* cell culture, * – $p<0.05$ compared to control.

In the second phase of the pain response, analgesic activity was detected in all studied groups. Under the action of diclofenac sodium and songorine isolated from *Aconitum barbatum*, the total licking time significantly reduced by 4.5 and 18 times, respectively, compared to that in the control (Fig. 10). Course administration of songorine isolated from cell culture also reduced the

duration of licking the injured paw, but this effect was not statistically significant (Fig. 10). All tested substances provided a significant reduction in the total number of pain patterns (by 3.3–13.3 times compared to the negative control) and, thus, had an antinociceptive effect comparable to the action of the classical NSAID diclofenac sodium. Maximum efficacy was observed under the action of songorine obtained from the above-ground part of *Aconite barbatum*. The pronounced analgesic activity of songorine from both sources, observed in the second phase of the formalin test (inflammatory nociception), once again proves that the antinociceptive effect of this alkaloid, in addition to central mechanisms (Nesterova et al. 2024), is mediated by the effect on peripheral mechanisms of analgesia and is possibly related to the effect on TRPA1 channels (Logashina et al. 2019).

Second phase of inflammation (tonic pain)

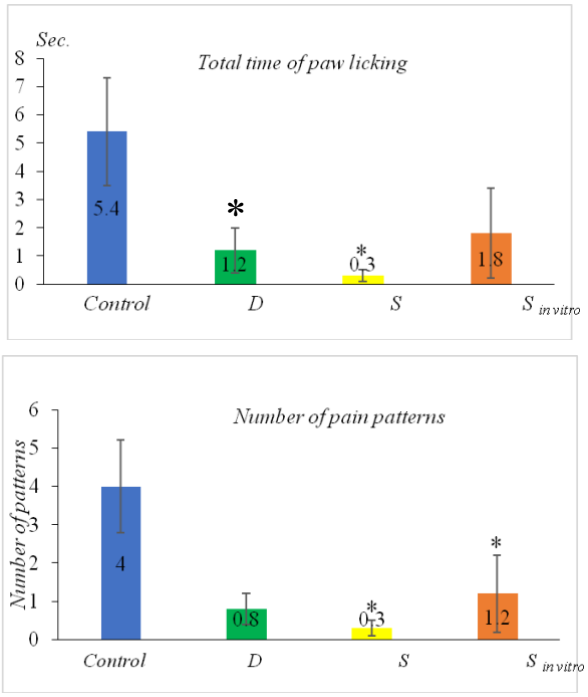


Figure 10. Comparative study of analgesic activity of songorine isolated from *Aconitum barbatum* and songorine obtained from *Aconitum barbatum* cell culture in the formalin test. ***Note:*** D – diclofenac sodium (10 mg/kg), S – songorine from *Aconitum barbatum* (25 μg/kg), S $_{in\ vitro}$ – songorine from *Aconitum barbatum* cell culture, * – $p<0.05$ compared to control.

Effect of songorine on acute exudative inflammation induced by subplantar injection of formalin

Formalin-induced edema is a model of arthritis in rodents because it resembles arthritis in humans (Vashist et al. 2012). Subplantar injection of formalin induces the development of proliferative inflammation of the mouse paw (Gowayed et al. 2021), which is formed as a result of cellular damage that provokes the release of endogenous mediators (histamine, serotonin, prostaglandins, bradykinin) (Sachan and Singh 2013) as well as IL-6 and TNF-α (Abdelhady et al. 2021). In addition, as described above, formalin stimulates temperature-sensitive TRPA1 channels, also responsible for the development of the inflammatory response (Krylova et al. 2020).

As a result of testing, it has been shown that the efficacy of songorine obtained from different study subjects is approximately the same. Course administration of songorine in all experimental groups provided a 1.4-fold decrease in edema compared to the control (Fig. 11). Similarly, the reference drug – diclofenac sodium – reduced edema (Fig. 11). Hence, in the formalin-induced edema model, administration of songorine isolated from *Aconitum barbatum* and *Aconitum barbatum* cell culture prevented inflammation to the same extent and was comparable to the efficacy of diclofenac sodium. Probably, the mechanism of anti-inflammatory activity of songorine includes the effect on TRPA1-receptors.

Anti-inflammatory activity

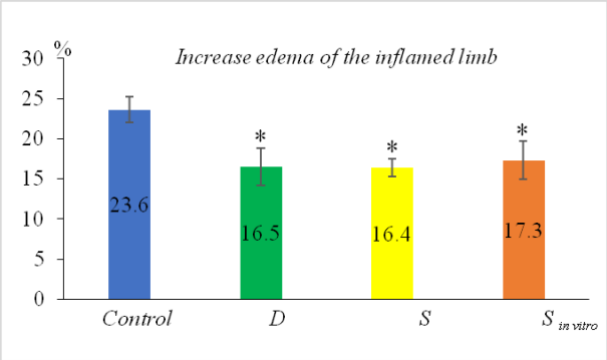


Figure 11. Comparative study of the anti-inflammatory activity of songorine isolated from *Aconitum barbatum* and songorine obtained from *Aconitum barbatum* cell culture in the formalin test. ***Note:*** D – diclofenac sodium (10 mg/kg), S – songorine from *Aconitum barbatum* (25 μg/kg), S $_{in\ vitro}$ – songorine from *Aconitum barbatum* cell culture, * – $p<0.05$ compared to control.

Study of analgesic activity of songorine on the model of acetic-acid writhes in mice

It is known that intraperitoneal injection of acetic acid into animals promotes general activation of the nociceptive system: local release of bradykinin, histamine, serotonin, prostaglandins, and leukotrienes, which leads to the development of spontaneous contractions of the abdominal press muscles – writhes – alternating with their relaxation, which are accompanied by extension of the hind limbs, the arching of the back, and resemble pain during peritonitis (Lei and Yan 2022). This test is a well-established model of visceral nociception and is designed to study the peripheral analgesic activity of new substances (Gowayed et al. 2021; Lei and Yan 2022). In addition, it is known that downward changes in extracellular pH (pH <6.0) can activate the non-selective cation channel TRPV1 (Abbas 2020; Xu et al. 2023). TRPV1 function has been found to be closely related to the formation and maintenance of inflammation in almost all pathological processes. TRPV1 channels are widely expressed in nociceptive neurons of the peripheral nervous system and are responsible for the neurogenic component of inflammation (Duitama et al. 2020). Therefore, modeling chemical pain irritation with acetic acid is one of the most common tests reflecting the effect of the investigated substances on TRPV1 receptors (Gladkikh et al. 2021; Galenko-Yaroshevsky et al. 2024).

As a result of the study, it was found that the efficacy of the comparison drug – paracetamol in this case – was lower than that of songorine. The test showed that the course administration of "standard" songorine and "culture" songorine increased the latent time of pain response by 2.4 and 2.8 times relative to that in the control and significantly reduced the number of writhes – by 3.2 and 5.3 times, respectively (Fig. 12). The maximum effect was manifested under the action of songorine isolated from cell culture of *Aconite barbatum*, which significantly exceeded the activity of the reference drug (Fig. 12).

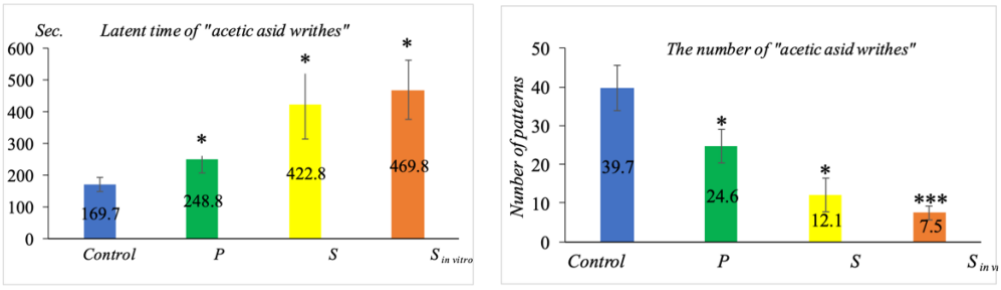


Figure 12. Comparative study of analgesic activity of songorine isolated from *Aconitum barbatum* and songorine obtained from *Aconitum barbatum* cell culture in the acetic acid writhing test. ***Note*:** P – paracetamol (85 mg/kg), S – songorine from *Aconitum barbatum* (25 μg/kg), S $_{in\ vitro}$ – songorine from *Aconitum barbatum* cell culture, * – $p<0.05$ compared to control, ** – $p<0.05$ compared to paracetamol.

Analgesic activity

This experimental study demonstrated high analgesic activity during prophylactic administration of songorine isolated both from the above-ground part of *Aconitum barbatum* and its cell culture.

The efficacy of songorine obtained from *Aconitum barbatum* culture was comparable to that of songorine obtained from *Aconitum barbatum* plant and superior to the antinociceptive response of the reference drug, paracetamol. Since the onset of pain upon exposure to acetic acid is primarily induced by endogenous kinins formed under low pH condition, as well as other algogenic compounds such as histamine, serotonin, acetylcholine, and prostaglandins; hence, the analgesic effect of songorine is related to its inhibitory effect on these substances (Nesterova et al. 2014a). In addition, since vanilloid TRPV1 receptors are activated under low pH conditions (Abbas 2020), it is possible that songorine has an inhibitory effect on these receptors.

Study of anti-inflammatory activity of songorine on the model of peritonitis in mice

It has been proved that the model of experimental peritonitis caused by intraperitoneal injection of 1% acetic acid solution (Zhao et al. 2012) can serve as an indicator of the effect of the studied substances on the permeability of vascular-tissue barriers. Acetic acid increases the level of proinflammatory mediators in the peritoneal fluid, which, in turn, leads to characteristic vascular changes: dilation of capillaries and venules and disturbance of their permeability.

The experimental study showed that the course administration of songorine from both sources to mice provided a reliable decrease in the acute inflammatory reaction – the volume of exudate in the experimental groups decreased 1.9–2.8 times compared to that in the control (Fig. 13). The maximum anti-inflammatory activity was exerted by songorine isolated from the cell culture *Aconitum barbatum*. In the group that received the comparison drug, the anti-exudative effect was shown only as a tendency.

Since the diterpene alkaloid songorine obtained by the classical method and songorine isolated from *Aconitum barbatum* cell culture showed comparable analgesic and antiexudative properties in the test with acetic acid, it is likely that the studied substances equally prevent vascular changes occurring against the background of the inflammatory process by inhibiting the activity of endogenous phlogogens: kinins, histamine, and serotonin (Nesterova et al. 2014a) and due to inhibition of vanilloid TRPV1 receptors.

Anti-inflammatory activity

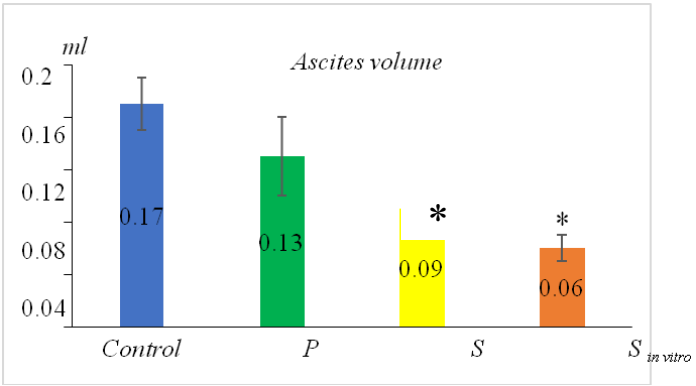


Figure 13. Comparative study of the anti-inflammatory activity of songorine isolated from *Aconitum barbatum* and songorine obtained from *Aconitum barbatum* cell culture in acetic acid writhing test. ***Note*:** P – paracetamol (85 mg/kg), S – songorine from *Aconitum barbatum* (25 μg/kg), $S_{in\ vitro}$ – songorine from *Aconitum barbatum* cell culture, * – $p<0.05$ compared to control.

Our results revealed some differences in the pharmacological activity of "culture" songorine compared to that of the "standard" one. This can be explained by the fact that substances obtained from plant cell cultures can significantly differ in their properties from those isolated directly from plants (Kochkin et al. 2019). This phenomenon can be attributed to either a degree of alteration in the primary structure of the compound synthesized *in vitro*, or its isomerization, caused by the specific growth and functioning of plant cells in the culture medium (Popova et al. 2021).

The identified pharmacological properties of "culture" songorine, compared to songorine of natural origin, suggest probable differences in its structure, which was confirmed by NMR-spectroscopy (Figs 7 and 8).

Conclusion

A comparative study of anti-inflammatory and analgesic activities of the diterpene alkaloid songorine isolated from the above-ground part of *Aconitum barbatum* and obtained from the

callus culture of *Aconitum barbatum* cells showed that the alkaloid from both sources had comparable activity. In the formalin test conditions, songorine (25 μg/kg), from both sources, administered orally five times, exhibited antinociceptive activity primarily in the second phase of inflammation, and its phlogolytic activity was comparable to that of a classic NSAID, sodium diclofenac. Under conditions of chemical pain irritation induced by acetic acid, the analgesic effect of songorine $_{\text{in vitro}}$ significantly exceeded the activity of the reference drug paracetamol. In addition, in contrast to the reference drug "standard" and "culture" songorine showed a pronounced anti-inflammatory activity in the model of peritonitis.

Thus, the results of comparative study of pharmacological properties of songorine isolated from cell culture of *Aconitum barbatum* showed comparability of effects with songorine obtained from the above-ground part of the plant. This indicates the expediency of such studies to ensure resource conservation of raw materials and accelerate the introduction of drugs based on pharmacologically active substances of plant origin in clinical practice. The obtained data indicate the prospect of practical use of biotechnologies for obtaining individual compounds from plant cell culture, since their implementation can provide large-scale production of substances with a given/known spectrum of activities for the pharmaceutical industry.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

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The authors have no funding to report.

Ethics statement

Studies of Drugs (Moscow 2013) and approved by the Ethical Committee of E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (the IACUC Protocol No. 191112021 dated 21.12.2021).

Data availability

All of the data that support the findings of this study are available in the main text.

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Author Contributions

- **Yulia V. Nesterova**, Doctor Habil. of Medical Sciences, Senior Researcher of Laboratory of Phytopharmacology and Special Nutrition, E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk, Russia; e-mail: nes-yuliya@yandex.ru; **ORCID ID:** https://orcid.org/0000-0002-1382-926X. The author analyzed and interpreted the results obtained, carried out the writing of the article, and performed the experimental work.
- **Anna P. Chernova**, Candidate of Chemical Sciences, Associate Professor of the Department of Physical and Analytical Chemistry, Tomsk Polytechnic University, Tomsk, Russia; e-mail: apa2004@mail.ru; **ORCID ID:** https://orcid.org/0000-0001-7002-492X. The author carried out work on cultivation of aconite cell culture and isolation of alkaloids from it.
- **Tatiana N. Povetyeva**, Doctor Habil. of Biological Sciences, Professor, Senior Researcher of Laboratory of Phytopharmacology and Special Nutrition, E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk, Russia; e-mail: ptn@bk.ru; **ORCID ID:** https://orcid.org/0000-0001-8644-489X. The author carried out experimental work and participated in planning the experiments.
- **Nikolay I. Suslov**, Doctor Habil. of Medical Sciences, Professor, Head of Laboratory of Phytopharmacology and Special Nutrition, E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk, Russia; e-mail: nis-51@mail.ru; **ORCID ID:** https://orcid.org/0000-0002-7993-5639. The author made substantial contributions to the conceptualization of the article and later participated in drafting the article.
- **Gleb N. Zyuz'kov**, Doctor Habil. of Medical Sciences, Professor, Member of the Russian Academy of Sciences, Head of Laboratory of Pathophysiology and Experimental Therapy, E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk, Russia; e-mail: zgn@pharmso.ru; **ORCID ID:** https://orcid.org/0000-0003-0384-333X. The author provided general supervision of writing the article and editing the final manuscript.
- **Olga G. Afanasyeva**, Candidate of Biology Sciences, Researcher of Laboratory of Phytopharmacology and Special Nutrition, E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk, Russia; e-mail: olgaafanasjeva@mail.ru; **ORCID ID:** https://orcid.org/0000-0002-7374-5586. The author carried out experimental work and participated in planning the experiments.
- **Pavel V. Kulpin**, Junior Researcher of Laboratory of Phytopharmacology and Special Nutrition, E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk, Russia; e-mail: pavellevap@inbox.ru; **ORCID ID:** https://orcid.org/0000-0002-0261-5645. The author carried out experimental work and participated in planning the experiments.
- **Vadim V. Zhdanov**, Doctor Habil. of Medical Sciences, Corresponding member of the Russian Academy of Sciences, Professor, Director of E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, Tomsk, Russia; e-mail: zhdanov_vv@pharmso.ru; **ORCID ID:** https://orcid.org/0000-0002-9516-0204. The author provided general supervision of writing the article and editing the final manuscript.