



Screening Study for Anticonvulsive Activity of Lipophilic Fractions from *Empetrum nigrum* L.

Ekaterina A. Bezverkhniaia^{1,2}, Tatyana N. Povet'eva³, Tatyana V. Kadyrova¹, Nikolai I. Suslov³, Yuliya V. Nesterova³, Ol'ga G. Afanas'eva³, Pavel V. Kul'pin³, Yuliya G. Yusova¹, Elena V. Ermilova¹, Alexandr G. Miroshnichenko¹, Konstantin S. Brazovskii^{1,2}, Mikhail V. Belousov^{1,2}

1 Siberian State Medical University, 2 Moskovsky Hwy, Tomsk 634050 Russia

2 Tomsk Polytechnic University, 30 Lenin St., Tomsk 634050 Russia

3 Russian Academy of Sciences, Tomsk National Research Medical Center, E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, 3 Lenin St., Tomsk 634028 Russia

Corresponding author: Ekaterina A. Bezverkhniaia (yekaterinabezv@mail.ru)

Academic editor: Mikhail Korokin ♦ Received 1 September 2020 ♦ Accepted 21 September 2020 ♦ Published 25 September 2020

Citation: Bezverkhniaia EA, Povet'eva TN, Kadyrova TV, Suslov NI, Nesterova YuV, Afanas'eva OG, Kul'pin PV, Yusova YuG, Ermilova EV, Miroshnichenko AG, Brazovskii KS, Belousov MV (2020) Screening Study for Anticonvulsive Activity of Lipophilic Fractions from *Empetrum nigrum* L.. Research Results in Pharmacology 6(3): 67–73. <https://doi.org/10.3897/rrpharmacology.6.55015>

Abstract

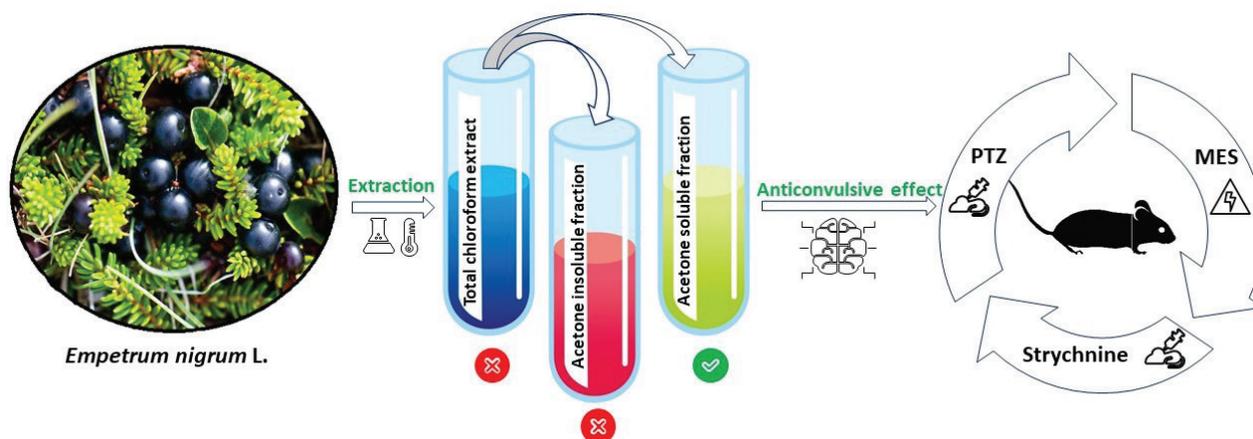
Introduction: The plants of genus *Empetrum*, which are used in the traditional medicine to cure seizures and neurodegenerative diseases, can be considered as potent antiepileptic drugs. This paper focuses on a comparative analysis of an anticonvulsive activity of lipophilic fractions from *Empetrum nigrum* L.

Materials and methods: The experiments were conducted using mature outbred CD-1 male mice. The lipophilic fractions from aerial parts of *Empetrum nigrum* L. were administered through a catheter into the stomach at a dose of 150 mg/kg for 5 days. The anticonvulsive effects were studied using the acute seizure tests: strychnine-, pentylenetetrazole – and maximal electroshock (MES) induced tests. Carbamazepine was used as a positive control drug at a dose of 100 mg/kg.

Results and discussion: The acetone-soluble fraction (ASF) of the chloroform extract from *Empetrum nigrum* L. showed a pronounced anticonvulsive effect on seizures induced by strychnine (1.5 mg/kg) and pentylenetetrazole (150 mg/kg). In comparison to the control group, the time from seizures to death increased by 1.5 for the strychnine-induced seizures, and 1.9 times in case of pentylenetetrazole model. The survival rate of the animals was 22.2% and 20%, correspondingly. The survival rate in the MES test was 77.8%. Overall, ASF demonstrates a remarkable anticonvulsive activity in all the tests, especially in the MES test.

Conclusion: Our study for the first time shows a potent antiepileptic effect of ASF from *Empetrum nigrum* L., containing triterpene compounds and chalcones. The future studies will be focused on investigating the exact mechanisms of anticonvulsive and neuroprotective effects of ASF.

Graphical abstract



Keywords

Anticonvulsive activity, *Empetrum nigrum L.*, maximal electroshock, [pentylene-tetrazole](#), [strychnine](#).

Introduction

Epilepsy is a very common noncontagious chronic neurological disorder affecting more than 50 million patients worldwide, according to the World Health Organization (Duncan et al. 2006). The frequency and prevalence of epilepsy varies by age (Banerjee et al. 2009) and is highest among children under 7 years old and among people over 55 years old (Jalalpure et al. 2009).

According to the modern conception, epilepsy is not a homogeneous disease, but a multifactorial pathological condition. There are several factors which play a significant role in the onset of seizure activity – neurochemical mechanisms and disorders, accompanied by destabilization of ionic permeability (channelopathies), mediators metabolism (glutamate, gamma-aminobutyric acid) and energy processes. All these factors lead to an increase in the permeability of neural cell membranes and, consequently, depolarization of neurons, their hyperexcitability, epileptization and hypersynchronization, and as a result formation of an epileptic source (Dichter 2009). Neuronal death is a dynamic process, which results from the genetic factors, excitotoxicity due to mitochondrial dysfunction, changes in the level of cytokines and oxidative stress due to antioxidant systems inactivity (Shin et al. 2011). Anticonvulsive synthetic drugs (ASD) are used for the pathogenetic treatment of epilepsy and suppress energy production in the epileptogenic source.

The main target for any ASDs, such as [valproic acid](#) and [zonisamide](#), is to block neuronal ion channels (mainly, sodium and calcium); to increase GABA-ergic inhibition ([gabapentin](#)); and to reduce glutamatergic excitation ([lamotrigine](#)). Some ASDs have a multi-target activity. For instance, [zonisamide](#), regulating the permeability of

sodium and calcium channels of neurons, has a unique property: it also reduces overproduction of nitric oxide and free radicals, providing a high neuroprotective effect (Czapinski et al. 2005; Brodie et al. 2011). ASDs used in modern medicine have strong anticonvulsive effects, but their antiepileptogenic properties are significantly weaker. Moreover, ASD sometimes have severe side effects, limiting their use, especially in pediatrics. Long-term ASD therapy leads to pharmacological resistance in 20–30% of all cases (Goldberg and Burdick 2001; Avakian and Avakian 2017). Consequently, finding new anticonvulsive medicines, both effective and safe, is the task of great importance.

Nowadays the tendency is to prescribe herbal medicines that feature a wide therapeutic range along with low toxicity and better patient's tolerance to the side effects. These properties are very desirable for successful treatment of all chronic diseases, in particular, epilepsy (Nsour et al. 2000; Sambukova et al. 2017). The plants of genus *Empetrum* are known to be used in traditional medicine to cure seizures and neurodegenerative diseases (Krasnov 2006; Barnaulov 2018).

Previous studies conducted in Siberian State Medical University (a joint research by the Departments of Pharmacology and Pharmaceutical Analysis) discovered a strong anticonvulsive effect of extracts from *Empetrum nigrum L.*, comparable to the activity of synthetic anticonvulsants, whereas their toxicity was significantly lower (Saratikov et al. 2003). The major outcome of that research was a wasteless technology to extract a lipophilic purified fraction (acetone insoluble fraction of chloroform total extract (AIF)), which demonstrated anticonvulsive effect (Krasnov 2006). Another component was a thick crowberry extract (acetone soluble fraction of chloroform

total extract (ASF)), which showed high antioxidant and antihypoxic activities (Ermilova et al. 2001). At the same time, triterpene compounds and chalcones containing in the ASF can be considered as potent cytoprotective drugs to treat epilepsy and neurodegenerative disorders due to their reported ability to enhance resistance of neurons to damaging factors (Stepkina and Velikorodov 2015; Lipatova et al. 2017; Kun and Zuhua 2019; Nogueira et al. 2019).

The anticonvulsive activity of the chloroform extract (CE) from *Empetrum nigrum* L. and its fractions (AIF, ASF) has never been studied yet within the comparative experiment. This paper focuses on a comparative analysis of an anticonvulsive activity of lipophilic fractions from *Empetrum nigrum* L. to select the most promising candidate for further chemical and pharmacological investigation.

Materials and methods

Plant materials and extraction

Black crowberry aerial parts (*Empetrum nigrum* L.), collected in 2018 in the Altai Region of Russia, were purchased from Horst Company, Barnaul (Russia). The lipophilic fractions (CE, AIF, ASF) from black crowberry were extracted as described earlier (Ermilova et al. 2001). Ground dry aerial parts of *Empetrum nigrum* L. were twice extracted using boiling chloroform in a round-bottom flask with a reflux condenser. The ratio of solvent to the sample was 10 to 1. Extracts were filtered hot through a cloth filter. The combined chloroform extracts were evaporated to dryness in a rotary evaporator at 30 °C and a rotation speed of 60 rpm. The AIF and ASF fractions were obtained from CE through acetone extractions, conducted three times at room temperature with continuous stirring. The ratio of solvent to CE was 10 to 1. The acetone extracts were filtered in vacuum using the Bunsen-Buechner system. The precipitate (AIF) was collected and dried through convection. The filtrates were mixed and dried in a rotary evaporator at 30 °C and a rotation speed of 60 rpm to 1/3 of the initial volume. The acetone extract (ASF) was dried through convection.

Experimental animals

The experiments were conducted using first category conventional mature outbred CD-1male mice, provided by the Department of Biomodelling of Goldberg Research Institute of Pharmacology and Regenerative Medicine. The weight of the animals ranged from 22 to 27 g. Animal welfare and the experimental design were approved by the Bioethical Committee (JACUC protocol No. 164042019). The research protocol was in compliance with the Directive 2010/63/EU of the European Parliament and the Council on the protection of animals as well as Order № 199n of the Ministry of Health of the Russian Federation of August 1, 2016. The animals were adapted to the vivarium in a separate room for 3 days prior to administration. Before the

start of the experiment, each animal within the group was assigned an individual number. The animals were randomly divided into 5 groups (n = 10): control group, carbamazepine, CE, AIF, ASF, using body weight as a criterion.

Drugs

Strychnine was purchased from Sigma-ALDRICH (India), pentylenetetrazole (PTZ) was purchased from Sigma (Germany). Carbamazepine was purchased from Alsi-Pharma (Russia). All the drug solutions were freshly prepared by dissolving in saline, and the solvents used were of analytical grade.

Experimental protocol

The fractions (CE, AIF, ASF), emulsified with tween-80, were injected through a catheter into the stomach at a dose of 150 mg/kg for 5 days. On the day of the experiment, the same dose was given 2 hours before testing. The positive control drug (carbamazepine) was administered in form of aqueous suspension at a dose of 100 mg/kg, following the same scheme. The animals from the control group received only solvent in equivalent amount.

The experimental tests were carried out according to *The Guidelines for Preclinical Trial of Drugs* (Mironov 2012). The protocol to study anticonvulsive effects included seizures induced by strychnine at a dose of 1.5 mg/kg, PTZ at a dose of 150 mg/kg, and MES. Strychnine and PTZ were administered subcutaneously in the cervical region of the back. The animals were observed for 30 minutes after the injection. The anticonvulsant effect of the lipophilic fractions was evaluated using the survival rate and time to death after an injection of chemiconvulsants.

MES stimulation with pulse charge of 4.9 mC was applied through corneal electrodes to induce tonic extension of the hind limbs causing 100 % lethality rate in the control group. A drop of electrolyte solution (0.9% sodium chloride) was applied for better electrode contact (Castel-Branco et al. 2009). The anticonvulsive effect of the extracts was estimated as their ability to prevent the tonic extension. The survival rate after MES was also taken into account (Mironov 2012).

Statistics

The experimental values measured in all the groups were compared using Student's T-test, nonparametric Wilcoxon-Mann-Whitney U test, and Fisher angular transformation in Statistica 6.0 package. The differences were considered significant if the significance level was $p \leq 0.05$ (Sergienko 2006).

Results

It was confirmed that 1.5 mg/kg of strychnine injected subcutaneously leads to a 100% lethality rate in the control

group (Table 1). The preventive course of carbamazepine administered *per os* at the dose of 100 mg/kg significantly increased all the parameters: survival rate, time to death, onset time of the myoclonic, clonic and tonic seizures by 1.4, 1.5, and 2.8 times correspondingly. The ASF from aerial parts of *Empetrum nigrum* L. administered using the same scheme has a stronger anticonvulsive effect than CE and AIF. In this group, time to death increased by 1.5 times and the survival rate – by 22.2% as compared to the control group. Both CE and ASF demonstrated a protective activity close to the activity of the positive control, delaying the onset of seizures.

PTZ injected subcutaneously at a dose of 150 mg/kg also leads to a 100% lethality rate in the control group (Table 2). Carbamazepine reliably increased time to death by 3.4 times and reduced the lethality rate, with 60% of the animals in the positive control group surviving. The ASF is less effective than carbamazepine, but has a stronger anticonvulsive activity in comparison to CE and AIF, increasing time to death by 1.9 times and the survival rate up to 20%.

Maximal electroshock (MES) test induced tonic extensions of the hind limbs (Castel-Branco et al. 2009), causing a 100% lethality rate in the control group (Table 3). The pre-

ventive course of carbamazepine eliminated tonic extensions, but clonic and clonic-tonic seizures still remained.

The ASF demonstrated the highest anticonvulsive activity among all the lipophilic extracts. The survival rate in the ASF group was 77.8%. Despite the fact that MES induced tonic extensions in all the animals, the majority of the group survived. This finding supports the hypothesis about a protective activity of the ASF.

Discussion

The anticonvulsive activity of the three components (chloroform extract (CE) and its fractions: AIF – acetone-insoluble, ASF – acetone-soluble) extracted from the aerial parts of *Empetrum nigrum* L. was compared using different primary seizures models. The ASF features the highest activity among all the lipophilic extracts, demonstrating the best results in MES test.

The models of acute seizures allow studying anticonvulsive and neuroprotective effects of chemical compounds through their impact on cellular and molecular processes that may be involved in both the generation and

Table 1. Anticonvulsive Effect of the Lipophilic Fractions from *Empetrum nigrum* L. in Comparison to Carbamazepine on Seizures Induced by 1.5 mg/kg of Strychnine.

Group, dose, N	Onset time of myoclonic seizures, sec	Onset time of clonic seizures, sec	Onset time of tonic extension of the hind limbs, sec	Time to death, sec	Survival rate, %
1. Control group (n = 11)	324.3 ± 21.2	353.0 ± 19.5	484.5 ± 29.7	535.6 ± 26.2	0
2. Carbamazepine, 100 mg/kg (n = 9)	454.9 ± 39.0**	534.6 ± 57.0*	1348.1 ± 186.0**	1535.2 ± 136.6**	66.7**
3. CE, 150 mg/kg (n = 10)	404.0 ± 33.8*	449.0 ± 39.8*	539.4 ± 55.6	773.9 ± 140.3	10
4. AIF, 150 mg/kg (n = 10)	345.9 ± 28.2	360.9 ± 28.5	474.2 ± 46.0	695.2 ± 79.6	0
5. ASF, 150 mg/kg (n = 9)	391.9 ± 30.6*	407.4 ± 32.9	555.8 ± 66.0	827.2 ± 144.6*	22.2**

Note: * – p < 0.05; ** – p < 0.01 in comparison to the control group; CE – total chloroform extract; AIF – acetone insoluble fraction of CE; ASF – acetone soluble fraction of CE.

Table 2. Anticonvulsive Effect of the Lipophilic Fractions from *Empetrum nigrum* L. in Comparison to Carbamazepine on Seizures Induced by 150 mg/kg of Pentylene-tetrazole.

Group, dose, N	Onset time of myoclonic seizures, sec	Onset time of clonic seizures, sec	Onset time of tonic extension of the hind limbs, sec	Time to death, sec	Survival rate, %
1. Control group (n = 10)	174.2 ± 26.6	210.2 ± 32.6	387.3 ± 42.1	404.9 ± 42.2	0
2. Carbamazepine, 100 mg/kg (n = 10)	158.4 ± 14.6	213.1 ± 23.7	575.4 ± 138.4	1372.1 ± 212.9**	60**
3. CE, 150 mg/kg (n = 10)	198.6 ± 32.0	356.2 ± 45.7*	361.1 ± 45.4	375.8 ± 46.1	0
4. AIF, 150 mg/kg (n = 10)	180.9 ± 24.1	212.0 ± 32.4	466.5 ± 27.8	690.1 ± 153.7*	0
5. ASF, 150 mg/kg (n = 10)	179.4 ± 33.8	289.7 ± 59.1	446.0 ± 30.5	749.5 ± 177.7*	20**

Note: * – p < 0.05; ** – p < 0.01 in comparison to the control group. CE – total chloroform extract; AIF – acetone insoluble fraction of CE; ASF – acetone soluble fraction of CE.

Table 3. Anticonvulsive Effect of the Lipophilic Fractions from *Empetrum nigrum* L. in Comparison to Carbamazepine on Seizures Induced by Maximal Electroshock.

Group, dose, N	Cases with tonic extension, %	Survival rate, %
1. Control (n = 10)	100	20
2. Carbamazepine, 100 mg/kg (n = 9)	0	100**
3. CE, 150 mg/kg (n = 9)	100	30
4. AIF, 150 mg/kg (n = 10)	100	40
5. ASF, 150 mg/kg (n = 10)	100	77.8**

Note: * – p < 0.05; ** – p < 0.01 in comparison to the control group. CE – total chloroform extract; AIF – acetone insoluble fraction of CE; ASF – acetone soluble fraction of CE.

termination of seizures. The most common models are chemically induced seizures, for example, pentylene-tetrazole- and strychnine-induced seizure models. PTZ induces various types of seizures due to binding to the t-butyl bicyclopheosphorothionate site of the GABA_A receptor (Olsen 1981). It is generally agreed that the pentylene-tetrazole-induced seizures can be used to identify the compounds that enhance GABA_A-ergic neurotransmission, or regulate permeability of T-type calcium (Ca²⁺) channels (Mandhane et al. 2007). Strychnine blocks glycine receptors, which are allosteric regulators of the GABA complex (Lehmann et al. 1988).

The anticonvulsive activity of the ASF could be attributed to its interaction with GABA receptors and Ca²⁺ T-type channels because this fraction blocks seizures induced by GABA receptor antagonist (PTZ) and glycine receptor blocker (strychnine), allosteric GABA modulator. These mechanisms play an important role in an anticonvulsive activity of many medicines. It is well known fact that primary mechanisms, such as modulation of GABA-ergic transmission and blocking of sodium and calcium channels, are the most important in preventing seizures and stopping current abnormal neuronal activity.

Previously discovered antioxidant and antihypoxic effects of the ASF (Ermilova et al. 2001) along with its anticonvulsive activity make this fraction a promising candidate for further chemical and pharmacological investigations in order to find specific compounds related to the different effects and explain mechanisms of their biological activity.

Conclusion

The present study, for the first time, reports potent antiepileptic effect of the ASF from *Empetrum nigrum* L. con-

taining triterpene compounds and chalcones. The possible mechanisms for an anticonvulsive effect of ASF could be an allosteric regulation of GABA-system and Ca²⁺ T-type channels. The previously identified antioxidant and antihypoxic properties of ASF determine the uniqueness and complexity of its pharmacological action. Our results support the traditional claims of plants of genus *Empetrum* for the treatment of epilepsy and related disorders. Future studies should be focused on the identification of exact mechanisms of anticonvulsive and neuroprotective effects of triterpene compounds and chalcones from ASF using bioinformatics *in vivo* and *in vitro* models.

Conflict of interests

The authors have no conflict of interests to declare.

Acknowledgements

The reported study was funded by RFBR according to the research project №20-315-90015.

References

- Avakian GN, Avakian GG (2017) Transformation of epileptic system. The status of issue and possible solutions. *Epilepsy and Paroxysmal Conditions [Epilepsia i Paroksizmal'nye Sostoiania]* 9(2): 6–19. <https://doi.org/10.17749/2077-8333.2017.9.2.006-019> [in Russian]
- Banerjee PN, Filippi D, Hauser WA (2009) The descriptive epidemiology of epilepsy. A review. *Epilepsy Research* 85(1): 31–45. <https://doi.org/10.1016/j.eplepsyres.2009.03.003> [PubMed] [PMC]
- Barnaulov OD (2018) Comparative evaluation of cerebroprotective and other pharmacological properties of plants of family Empetraceae. *Traditional Medicine [Traditsionnaya Meditsina]* 1(52): 38–47. [in Russian]
- Brodie MJ, Covanis A, Gil-Nagel A, Lerche H, Perucca E, Sills GJ, White HS (2011) Antiepileptic drug therapy: does mechanism of action matter? *Epilepsy & Behavior* 21(4): 331–341. <https://doi.org/10.1016/j.yebeh.2011.05.025> [PubMed]
- Castel-Branco MM, Alves GL, Figueiredo IV, Falcão A, Caramona MM (2009) The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. *Methods and Findings in Experimental and Clinical Pharmacology* 31(2): 101–106. <https://doi.org/10.1358/mf.2009.31.2.1338414> [PubMed]
- Czapinski P, Blaszczyk B, Czuczwar SJ (2005) Mechanisms of action of antiepileptic drugs. *Current Topics in Medicinal Chemistry* 5(1): 3–14. <https://doi.org/10.2174/1568026053386962> [PubMed]
- Dichter MA (2009) Emerging concepts in the pathogenesis of epilepsy and epileptogenesis. *Archives of Neurology* 66(4): 443–447. <https://doi.org/10.1001/archneurol.2009.1> [PubMed]
- Duncan JS, Sander JW, Sisodiya SM, Walker MC (2006) Adult epilepsy. *The Lancet* 367(9516): 1087–1100. [https://doi.org/10.1016/S0140-6736\(06\)68477-8](https://doi.org/10.1016/S0140-6736(06)68477-8) [PubMed]
- Ermilova EV, Kadyrova TV, Krasnov EA, Hazanov VA, Iljushenko SV, Pisareva SI (2001) The technology of obtaining a thick extract of crowberry, its antioxidant and antihypoxic activity. *Pharmaceutical Chemistry Journal [Khimiko-Pharmatsevticheskii Zhurnal]* 35(11): 26–28. <https://doi.org/10.1023/A:1012759421519> [in Russian]
- Goldberg JF, Burdick KE (2001) Cognitive side effects of anticonvulsants. *The Journal of Clinical Psychiatry* 62(14): 27–33. [PubMed]
- Jalalpure SS, Salahuddin M, Shaikh IM, Manvi FV (2009) Anticonvulsant effects of *Calotropis procera* root in rats. *Pharmaceutical Biology* 47(2): 162–167. <https://doi.org/10.1080/13880200802437008>
- Krasnov EA, Ermilova EV, Kadyrova TV (2000) Phenolic components of *Empetrum nigrum* extract and crystal structure of 2'-methoxy-4'-hydroxy-alpha, beta-dihydrochalcone. *Chemistry of Natural Compounds [Khimiya Prirodnikh Soedinenii]* 5: 389–391. [in Russian]
- Krasnov EA (2006) Flora of Siberia – a source of biologically active substances and medicines. *Bulletin of Siberian Medicine [Bulletin' Sibirskoy Meditsini]*. Appendix 2: 11–18. [in Russian]
- Kun X, Zuhua G (2019) Amyrin exerts potent anxiolytic and antidepressant effects via mechanisms involving monoamine oxidase and gamma-aminobutyric acid in mouse hippocampus. *Tropical Journal of Pharmaceutical Research* 18(8): 1673–1681. <http://dx.doi.org/10.4314/tjpr.v18i8.16>
- Lehmann J, Hutchison AJ, McPherson SE, Mondadori C, Schmutz M, Sinton CM, Tsai C, Murphy DE, Steel DJ, Williams M, Cheney DL, Wood PL (1988) CGS 19755, a selective and competitive N-methyl-D-aspartate-type excitatory amino acid receptor antagonist. *Journal of Pharmacology and Experimental Therapeutics* 246(1): 65–75. [PubMed]
- Lipatova LV, Dubinina EE, Alexeeva DV, Kapustina TV, Egorova DA, Leonov NV (2017) The role of oxidative stress in the pathogenesis of epilepsy. *Siberian Medical Review [Sibirskoe Meditsinskoe Obozrenie]* 1(103): 11–17. <https://doi.org/10.20333/2500136-2017-1-11-16> [in Russian]

- Liu Y, Hu C, Tang Y, Chen J, Dong M, Song T, Zhang X, Zhao J (2009) Clozapine inhibits strychnine-sensitive glycine receptors in rat hippocampal neurons. *Brain Research* 1278: 27–33. <https://doi.org/10.1016/j.brainres.2009.04.043> [PubMed]
- Mandhane SN, Aavula K, Rajamannar T (2007) Timed pentylenetetrazol infusion test: a comparative analysis with s.c. PTZ and MES models of anticonvulsant screening in mice. *Seizure* 16(7): 636–644. <https://doi.org/10.1016/j.seizure.2007.05.005> [PubMed]
- Mironov AN (2012) Guidelines for Pre-clinical Trials of Medicines. Grif i Co, Moscow, 944 pp. [https://doi.org/10.1016/S0140-6736\(06\)68477-8](https://doi.org/10.1016/S0140-6736(06)68477-8) [in Russian]
- Nogueira AO, Oliveira YIS, Adjafre BL, de Moraes MEA, Aragao GF (2019) Pharmacological effects of the isomeric mixture of alpha and beta amyryn from *Protium heptaphyllum*: a literature review. *Fundamental & Clinical Pharmacology* 33(1): 4–12. <https://doi.org/10.1111/fcp.12402> [PubMed]
- Nsour WM, Lau CS, Wong ICK (2000) Review on phytotherapy in epilepsy. *Seizure* 9(2): 96–107. <https://doi.org/10.1053/seiz.1999.0378> [PubMed]
- Olsen RW (1981) The GABA postsynaptic membrane receptor-ionophore complex. *The Biological Effects of Glutamic Acid and Its Derivatives*. Dordrecht, Springer, 261–279. https://doi.org/10.1007/978-94-009-8027-3_17
- Sambukova TV, Ovchinnikov BV, Ganapol'skii VP, Jatmanova AN, Shabanov PD (2017) Prospects for the use of herbal remedies in modern pharmacology. *Reviews on Clinical Pharmacology and Drug Therapy [Obzory po Klinicheskoi Farmacologii i Lekarstvennoi Terapii]* 15(2): 56–63. <https://doi.org/10.17816/RCF15256-63> [in Russian]
- Saratikov AS, Novozheeva TP, Livshiz NS, Burchenkova FI, Kadigachova NU, Melik-Gaikazyan EV, Ahmedzhanov RR, Perevozchikova TV, Bashirova LV (2003) Preclinical toxicological study of extract from aerial parts of *Empetrum nigrum* L. *Plant Sources [Rastitel'nye Resursy]* 39(2): 91–94. [in Russian]
- Sergienko VI (2006) *Mathematical Statistics in Clinical Research*. Moscow: GEOTAR-Media, 303 pp. [in Russian]
- Shin EJ, Jeong JH, Chung YH, Kim WK, Ko KH, Bach JH, Hong JS, Yoneda Y, Kim HC (2011) Role of oxidative stress in epileptic seizures. *Neurochemistry International* 59(2): 122–137. <https://doi.org/10.1016/j.neuint.2011.03.025> [PubMed] [PMC]
- Stepkina NI, Velikorodov AV (2015) The dependence of the biological activity of chalcones on their structure. *Fundamental Research [Fundamental'nye Issledovania]* 3(11): 505–510. <https://doi.org/10.17513/fr.39449> [in Russian]

Author contributions

- **Ekaterina A. Bezverkhniaia**, postgraduate student of Research School of Chemistry & Applied Biomedical Sciences of Tomsk Polytechnic University, laboratory assistant of Department of Pharmaceutical Analysis of Siberian State Medical University, e-mail: yekaterinabezv@mail.ru, **ORCID ID** <https://orcid.org/0000-0001-7699-5719>. The author carried out experimental work, statistical processing and evaluation of anticonvulsive activity of the samples in the experiments.
- **Tatyana N. Povet'eva**, PhD in Biology, Professor, Senior Researcher of Laboratory of Phytopharmacology and Special Nutrition of E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, e-mail: ptn@bk.ru, **ORCID ID** <https://orcid.org/0000-0001-8644-489X>. The author carried out experimental work, as well as conducted analysis and interpreted the results.
- **Tatyana V. Kadyrova**, PhD in Pharmacy, lecturer of Department of Pharmaceutical Analysis of Siberian State Medical University, e-mail: kadyrov2@sibmail.com, **ORCID ID** <https://orcid.org/0000-0002-2119-0259>. The author carried out extraction and fractionation of experimental samples.
- **Nikolai I. Suslov**, PhD in Medicine, Professor, Head of Laboratory of Phytopharmacology and Special Nutrition of E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, 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.
- **Yuliya V. Nesterova**, PhD in Medicine, Researcher of Laboratory of Phytopharmacology and Special Nutrition of E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, e-mail: nes-yuliya@yandex.ru, **ORCID ID** <https://orcid.org/0000-0002-1382-926X>. The author carried out experimental work, participated in planning the experiments.
- **Ol'ga G. Afanas'eva**, PhD in Biology, Researcher of Laboratory of Phytopharmacology and Special Nutrition of E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, e-mail: olgaafanasjeva@mail.ru. The author carried out experimental work, participated in planning the experiments.

- **Pavel V. Kul'pin**, Junior Researcher of Laboratory of Phytopharmacology and Special Nutrition of E.D. Goldberg Research Institute of Pharmacology & Regenerative Medicine, e-mail: pavellevap@inbox.ru. The author carried out experimental work, participated in planning the experiments.
- **Yuliya G. Yusova**, undergraduate student of Siberian State Medical University, e-mail: yuss111@icloud.com. The author participated in drafting the article.
- **Elena V. Ermilova**, PhD in Pharmacy, Professor of Department of Pharmaceutical Analysis of Siberian State Medical University, e-mail: nedel@sibmail.com, ORCID ID <https://orcid.org/0000-0001-6724-0955>. The author carried out planning of extraction and fractionation of experimental samples.
- **Alexandr G. Miroshnichenko**, PhD in Medicine, Professor of Department of Pharmacology of Siberian State Medical University, e-mail: miroshnichenko.ag@ssmu.ru, ORCID ID <https://orcid.org/0000-0003-4035-8341>. The author participated in drafting the article.
- **Konstantin S. Brazovskii**, PhD in Technical Sciences, Researcher of Laboratory of targeted research for medical engineering technologies of Siberian State Medical University, Professor of Research School of Chemistry & Applied Biomedical Sciences of Tomsk Polytechnic University, e-mail: bks_2005@mail.ru, ORCID ID <https://orcid.org/0000-0002-4779-9820>. The author carried out statistical processing, provided technical support, and took part in translation of the article.
- **Mikhail V. Belousov**, PhD in Pharmacy, Head of Department of Pharmaceutical Analysis of Siberian State Medical University, Professor of Research School of Chemistry & Applied Biomedical Sciences of Tomsk Polytechnic University, e-mail: mvb63@mail.ru, ORCID ID <https://orcid.org/0000-0002-2153-7945>. The author participated in drafting the article.