

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

Recommended by Doctor of Medicine, professor A.I. Bereznyakova

UDC 615.322:615.451.16:615.213

ANTIEPILEPTIC POTENTIAL OF *FUMARIA SCHLEICHERI* AND *OCIMUM BASILICUM* DRY EXTRACTS

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Key words: medicinal plants; extracts; antiepileptic drugs

The results of investigation of the antiepileptic activity of promising herbal anticonvulsants – Fumaria schleicheri (FSDE) and Ocimum basilicum (OBDE) dry extracts – are presented. The herbal remedies were administered intragastrically in the conditionally effective dose of 100 mg/kg in the therapeutic and preventive mode during 3 days with the last time of 30 minutes before the experiments. As reference drugs sodium valproate and carbamazepine were chosen. Under the conditions of maximal electroshock (MES) test the ability of the chosen dry extracts to prevent the development of primarily generalized convulsions was studied. For the in-depth study of antiepileptic properties of FSDE and OBDE the model of pentylenetetrazole (corasole)-induced kindling has been chosen. In general the researched dry extracts in the condition of the MES test have shown the anticonvulsant effect on the level of sodium valproate, but they were slightly inferior in efficacy compared with carbamazepine. On the model of pentylenetetrazole-induced kindling it has been shown that FSDE unlike OBDE and sodium valproate has the ability to prevent convulsions under the conditions of experimental chronic epileptogenesis. Thus, it has been found that OBDE is able to prevent the development of primary generalized seizures, while FSDE prevents acute paroxysms stimulated by electrical impulse and chronic epileptogenesis.

Under conditions of progressing development and spreading of nervous and psychic diseases the problem of improving the quality of treating epilepsy is topical. According to the data of the WHO almost 0.68 per cent of the world population suffers from this disease and this figure has been increasing steadily [11]. It is well known that treating chronic diseases, including epilepsy, is a rather long-term if not lifelong process [3]. That is why in this case the use of herbal medicines is relevant as they are highly safe even in the situation of a long-term application [12].

In the previous studies on different models of chemo-induced convulsions, including those caused by pentylenetetrazole (the main screening model [1]), picrotoxin, thiosemicarbazide, strychnine and camphor, the high level of anticonvulsant properties was shown by the dry extract of fumitory (*Fumaria schleicheri* Soy.-Willem., *Fumariaceae*) and the dry extract of basil (*Ocimum basilicum* L., *Lamiaceae*) [7, 8]. Taking into account the absence of herbal medicines with the proven anticonvulsant activity at the domestic and international pharmaceutical markets the profound study of the antiepileptic properties of these dry extracts is reasonable.

The aim of this work is to study the antiepileptic potential of the promising herbal anticonvulsant drugs – dry extracts of *Fumaria schleicheri* (FSDE) and *Ocimum basilicum* (OBDE) obtained from the aerial part

of the plants according to the requirements of the State Pharmacopoeia of Ukraine – taking into consideration their ability to prevent primarily generalized convulsions on the model of seizures induced by the maximal electroshock and the ability to inhibit epileptogenesis under conditions of pentylenetetrazole-induced kindling.

Materials and Methods

The experimental part was performed on 86 white random-bred mice. The animals were kept in the standard conditions of the vivarium of the Central Research Laboratory at the National University of Pharmacy according to the hygiene norms and principles of the European Convention on laboratory animals protection (Strasbourg, 1986). In the research period the animals were kept in the vivarium at the temperature of 19-24°C, with humidity of not more than 50%, in the “day-night” natural light mode in plastic cages with the standard nutrition and free access to water and food. All research processes were performed according to the “General ethical principles of the experiments with animals” [6] and according to the methodological recommendations for pre-clinical study of specific activity of potential anticonvulsant medicines [1].

In the conditions of maximal electroshock (MES) test the ability of the chosen dry extracts to prevent the development of primarily generalized convulsions was studied [1]. The experiments were conducted on 55 random-

bred male albino mice with the body weight of 21-27 g that were randomly divided into 5 groups: 1 – control; 2-3 – the groups where animals received aqueous solutions of the given dry extracts; 4-5 – comparison groups where animals received reference drugs such as sodium valproate and carbamazepine. The reference drugs were chosen taking into account the recommendations [1, 4].

The dry extracts studied were obtained by Yu.S. Prokopenko, the assistant of the Department of Quality, Standardization and Certification of Medicines, the Institute of Pharmacy Professionals Qualification Improvement at the National University of Pharmacy. The aerial parts of *Fumaria schleicheri* Soy.-Willem. and *Ocimum basilicum* L. were collected in the phase of blooming in different regions of Ukraine (Kharkiv, Luhansk, Donetsk, Ternopil, Rivne, Zhytomyr, Kyiv regions and the Republic of Crimea). The raw material was washed with water and air-dried. The dried material was kept at the room temperature. A relatively dry herbal material was powdered and extracted with distilled water (1:10) for 2 hours at the temperature of 80°C. The process was repeated 3-4 times up to the full extraction of the biologically active substances from the raw material. The received extracts were mixed, filtered and concentrated in vacuum device at the temperature of 50-60 °C and the pressure of 80-87 kPa up to thick consistency. The received semi-product was dried in vacuum-drying device up to the remaining humidity of 5% [5].

FSDE was standardized according to the content of alkaloids (the noscapine group) and flavonoids (flavonoles and flavons) using the method of absorption UV-spectrophotometry [5], and OBDE – according to the content of flavonoids.

The animals of experimental group received intragastrically the aqueous solutions of the given dry extracts in the conditionally effective dose 100 mg/kg [7] in the treating and preventive mode during 3 days with the last time 30 minutes before conducting the experiment. The comparison groups received intragastrically classic antiepileptic medicines – sodium valproate (the syrup “Depakine”, Sanofi-Aventis, France) in the dose of 300 mg/kg and carbamazepine (“Finlepsin”, TEVA, Israel/Poland) in the dose of 40 mg/kg in the same mode. The second reference drug was injected in the form of a thin aqueous suspension solubilized by Tween-80. The mice in the control group received intragastrically distilled water (0.1 ml per 10 g of the body weight).

Then the animals through the corneal electrodes were affected by electric stimuli with duration of 0.2 sec, frequency of 50 Hz and current of 50 mA. Under these conditions 100% of animals in the control group had maximum tonic extension of hind limbs. The anticonvulsant action was estimated according to the following indicators: duration of convulsions, the time of the survived mice recovery, the time of the animals' death and the percentage of lethality in the experimental groups [4].

For the profound study of antiepileptic properties of the extracts the model of pentylentetrazole (corasole)-induced kindling was chosen [1, 4].

Kindling is a phenomenon based on the occurrence of focal convulsion afterdischarge, behavioral automatisms and generalized convulsion attacks in response to multiple epileptic stimulations of subthreshold intensity that at first do not cause convulsions. The kindling model is characterized with the unique methodological advantages for experimental determination of the effect of potential anticonvulsant drugs on pathophysiology of seizures [1, 4]. Under conditions of the kindling model one can clearly check the start of convulsions, the stages of their development and preserving. Behavioral patterns in this model are characterized by high repeatability, easy visual control of their intensity, severity and duration. The kindling model reflects pathophysiological and clinical specificities of epilepsy in the most adequate way and the phenomenon of “swaying” is viewed as an universal mechanism, which takes part in epilepsy not only experimentally, but it is also practically characteristic for the human brain [9].

For the experiment 31 random-bred albino male mice with the body weight of 22-29 g were selected. The animals were divided into 4 groups (n=7-9): 1– control; 2-3 – groups of animals that received aqueous solutions of the given dry extracts; 4 – group of comparison that received sodium valproate as a reference drug.

The animals of experimental groups received intragastrically the aqueous solutions of appropriate dry extracts in the conditionally therapeutic dose of 100 mg/kg [7] in the therapeutic and preventive mode during 27 days once a day 30 minutes before injecting the convulsant. The group of comparison received intragastrically a classic anticonvulsant drug sodium valproate (the syrup “Depakine”, Sanofi-Aventis, France) in the dose of 300 mg/kg in the same mode. The mice from the control group received intragastrically distilled water (0.1 ml per 10 g of the body weight). The aqueous solution of pentylentetrazole (corasole) was injected intraperitoneally in the subthreshold dose of 30 mg/kg.

After injecting of the convulsant every mouse was placed into a separate plastic cylinder box with the diameter of 20 cm and the height of 35 cm. The state of every mouse was being observed for 30 min. The anticonvulsant activity was estimated daily according to the following indicators: the percentage of mice with convulsions in each group, the number of days with paroxysms in the group, as well as according to the most informative indicator – the day of occurrence of the first convulsions in the group [4].

The results was expressed as mean \pm standard error of mean (SEM). Statistical differences between groups were analyzed using Student's t-test (in case of normal distribution), Mann-Whitney U test and Fisher angular transformation (for the alternative form of analysis). The level of statistical significance was considered as $p < 0.05$.

Results and Discussion

The maximal electroshock in 100% of animals from the control group was followed by immediate development of several convulsions in the form of clonic-tonic paroxysms with prevailing of the tonic component. The

Table

The effect of *Fumaria schleicheri* and *Ocimum basilicum* dry extracts, sodium valproate and carbamazepine on the seizures induced by the maximal electroshock in mice ($M \pm m$)

| Animal Group | Dose, mg/kg | n | Duration of convulsions, sec | Time of recovery, sec | Time of death, sec | Lethality, % |
|------------------|-------------|----|------------------------------|-----------------------|--------------------|--------------|
| Control | – | 15 | 19.40±3.66 | 18.71±7.89 | 20.00±1.85 | 53.3 |
| FSDE | 100 | 10 | 9.20±2.28*/** | 6.14±1.68** | 16.33±4.70 | 30***** |
| OBDE | 100 | 10 | 9.88±3.47** | 1.83±0.98*# | 21.75±2.93 | 40***** |
| Sodium valproate | 300 | 10 | 8.60±2.37*/** | 4.29±0.47** | 18.67±3.18 | 30***** |
| Carbamazepine | 40 | 10 | 1.70±1.07****# | 1.70±1.07* | – | 0*** |

Note: 1. Statistically significant differences: * – compared to control ($p < 0.05$); ** – compared to the group receiving carbamazepine ($p < 0.05$); *** – compared to control ($p < 0.01$); **** – compared to control ($p < 0.001$); ***** – compared to the group receiving carbamazepine ($p < 0.01$); # – compared to the group receiving sodium valproate ($p < 0.05$).

2. Abbreviations: FSDE – *Fumaria schleicheri* dry extract; OBDE – *Ocimum basilicum* dry extract.

death of 53.3% of mice was caused by the tonic extension of hind limbs (Table). It corresponds to the existing data, which show the variation of the lethality level in the wide range from 0 to 100% [10, 13] depending on many factors.

In the MES test the herbal drugs studied showed marked anticonvulsant properties. FSDE decreased duration of convulsions by 2.1 times compared to control ($p < 0.05$), and also decreased the time of recovery of the survived animals by 3 times ($p > 0.05$). Such indicators as the time of death and the lethality level in the group were not seriously influenced by FSDE.

OBDE decreased duration of the tonic extension of hind limbs almost by 2 times compared to the control group, but because of the high dispersion of data this difference did not reach the level of statistical significance. But OBDE significantly decreased the time of the mice recovery by more than 10 times compared to the indicator in the control group. The positive effect of OBDE on the time of death and lethality in the experimental group was not recorded.

Sodium valproate showed the effect at the level of the studied dry extracts – it statistically significantly de-

creased the duration of convulsions compared with the control group by 2.3 times and showed the tendency to decrease the recovery time by 4.3 times without the influence on the death time and lethality level in the group.

Carbamazepine showed the most expressed anticonvulsant activity, which significantly exceeded the similar effect of the studied herbal drugs and sodium valproate. Under its action not only duration of convulsions, but also the recovery time decreased by 11.4 and 11.0 times, respectively. Besides, carbamazepine completely prevented the death of animals ($p < 0.01$).

On the model of pentylenetetrazole-induced kindling according to the most informative indicator – the day of the first convulsions occurrence – FSDE continued the latent period of the first convulsions occurrence with statistical significance (Fig. 1): in the control group spontaneous seizures were observed on the 23rd day of the study, while FSDE continued the latent period of attack occurrence up to the 27th day ($p < 0.01$).

According to the data of Fig. 1 it can also be observed that FSDE decreased the percentage of mice with convulsions in the group from the 23rd to the 27th day,

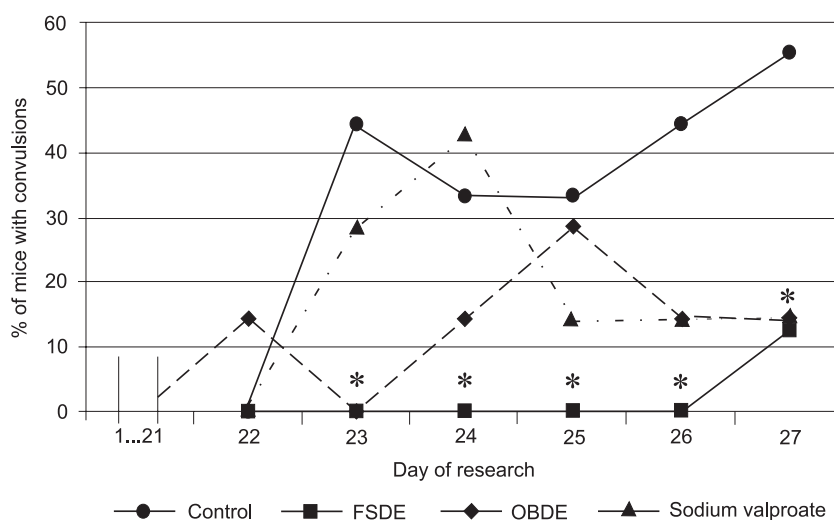


Fig. 1. Dynamics of the anticonvulsant activity of *Fumaria schleicheri* and *Ocimum basilicum* dry extracts and sodium valproate under conditions of pentylenetetrazole-induced kindling.
Note: * – statistically significant difference compared to control ($p < 0.05$).

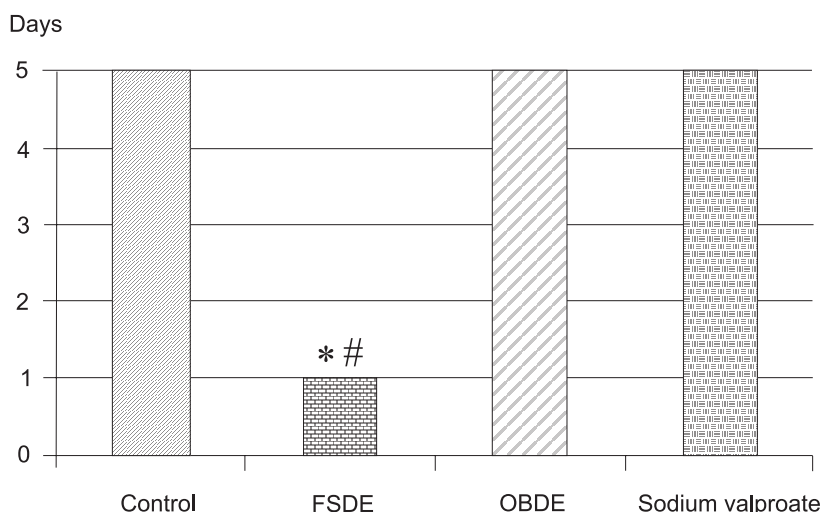


Fig. 2. The number of days with convulsions in the experimental groups on the model of pentylenetetrazole-induced kindling.
Note. Statistically significant difference: * – compared to control ($p < 0.01$); # – compared to sodium valproate ($p < 0.01$).

including the last one ($p < 0.05$), while OBDE at the same dose decreased the corresponding indicator only on the 23rd and the 27th days. The reference drug sodium valproate on this model showed relatively weak antiepileptic properties: significant decrease of the percentage of mice with convulsions was observed only on the 27th day of the study. Besides, in the groups where animals received OBDE and sodium valproate no increase of the latent period of spontaneous seizures occurrence was observed.

As can be seen from the data of Fig. 2, only FSDE significantly decreased the total number of days with convulsions compared to control ($p < 0.01$). Neither OBDE, nor sodium valproate affected this indicator under the same conditions.

As it is known, the anticonvulsant properties are characteristic to combinations of different chemical compounds [2, 12]. However, most representatives with the above-mentioned pharmacological activity belong to such classes of biologically active substances as flavonoids, alkaloids, as well as terpenes and volatile components of essential oils [2]. The role of individual components

and fractions of FSDE and OBDE in providing the anticonvulsant effect requires further verification and is a subject of the next research stage.

CONCLUSIONS

1. The antiepileptic properties of *Fumaria schlei-cheri* dry extract (FSDE) and *Ocimum basilicum* dry extract (OBDE) have been studied.

2. Under conditions of the maximal electroshock test it has been found that FSDE and OBDE in the dose of 100 mg/kg showed considerable anticonvulsant properties that were not inferior to the effect of sodium valproate in the dose of 300 mg/kg, but did not reach the level of carbamazepine in the dose of 40 mg/kg.

3. On the model of pentylenetetrazole-induced kindling it has been shown that FSDE unlike OBDE and sodium valproate has the ability to prevent convulsions under conditions of the experimental chronic epileptogenesis.

4. The obtained data allow recommending FSDE for further research with the aim of developing the first Ukrainian herbal antiepileptic drug.

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ПРОТИЕПИЛЕПТИЧНИЙ ПОТЕНЦІАЛ СУХИХ ЕКСТРАКТІВ РУТКИ ШЛЕЙХЕРА ТА БАЗИЛІКУ КАМФОРНОГО

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Ключові слова: лікарські рослини; екстракти; протиепілептичні засоби

В роботі представлені результати дослідження протиепілептичної активності перспективних рослинних антиконвульсантів – сухих екстрактів рутки Шлейхера (СЕРШ) та базилику камфорного (СЕБК). Рослинні препарати вводили внутрішньошлунково в умовно ефективній дозі 100 мг/кг у лікувально-профілактичному режимі протягом трьох днів, востаннє за 30 хв до експерименту. В якості препаратів порівняння було обрано натрію вальпроат та карбамазепін. За умов тесту максимального електрошоку (МЕШ) вивчали здатність обраних сухих екстрактів запобігати розвитку первинно-генералізованих судом. Для поглибленого дослідження протиепілептичних властивостей СЕРШ та СЕБК було обрано модель пентилентетразолового (коразолового) кіндлінгу. Загалом досліджувані сухі екстракти за умов тесту МЕШ виявили виразний протисудомний ефект на рівні натрію вальпроату, проте поступалися за ефективністю карбамазепіну. На моделі пентилентетразолового кіндлінгу було встановлено, що СЕРШ, на відміну від СЕБК та натрію вальпроату, має здатність запобігати розвитку судом за умов експериментального хронічного епілептогенезу. Отже, встановлено, що СЕБК виявляє антиконвульсивну активність на моделі первинно-генералізованих судом, у той час як СЕРШ запобігає розвитку як гострих електростимульованих пароксизмів, так і хронічного епілептогенезу.

ПРОТИВОЭПИЛЕПТИЧЕСКИЙ ПОТЕНЦИАЛ СУХИХ ЭКСТРАКТОВ ДЫМЯНКИ ШЛЕЙХЕРА И БАЗИЛИКА КАМФОРНОГО

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Ключевые слова: лекарственные растения; экстракты; противозепилептические средства

В работе представлены результаты исследования противозепилептической активности перспективных растительных антиконвульсантов – сухих экстрактов дымянки Шлейхера (СЭДШ) и базилика камфорного (СЭБК). Растительные препараты вводили внутрижелудочно в условно эффективной дозе 100 мг/кг в лечебно-профилактическом режиме в течение трех дней, в последний раз за 30 мин до эксперимента. В качестве препаратов сравнения выбраны натрия вальпроат и карбамазепин. В условиях теста максимального электрошока (МЭШ) изучали способность исследуемых сухих экстрактов предотвращать развитие первично-генерализованных судорог. Для углубленного изучения противозепилептических свойств СЭДШ и СЭБК выбрана модель пентилентетразолового (коразолового) киндлинга. В целом исследуемые сухие экстракты в условиях теста МЭШ выявили значительный противосудорожный эффект на уровне натрия вальпроата, однако уступали по эффективности карбамазепину. На модели пентилентетразолового киндлинга было установлено, что СЭДШ, в отличие от СЭБК и натрия вальпроата, имеет свойство предотвращать развитие судорог в условиях экспериментального хронического эпилептогенеза. Итак, установлено, что СЭБК оказывает антиконвульсивную активность на модели первично-генерализованных судорог, в то время как СЭДШ предотвращает развитие как острых электростимулируемых пароксизмов, так и хронического эпилептогенеза.