UDC 615.015.4:615.322

INFLUENCE OF DRY HERBAL EXTRACTS ON PENTYLENETETRAZOLE-INDUCED SEIZURES IN MICE: SCREENING RESULTS AND RELATIONSHIP "CHEMICAL COMPOSITION – PHARMACOLOGICAL EFFECT"

© V. Tsyvunin, S. Shtrygol', Y. Prokopenko, V. Georgiyants, N. Blyznyuk

Aim. *Screening of 48 dry extracts of plants belonging to 18 species of 8 families not studied before on anticonvulsant activity, to analyze the chemical composition for the extracts with proved anti- and proconvulsant activity, and to estimate the connection of the effect of herbal products and their phytochemical composition.*

Methods. *On the model of pentylenetetrazole-induced seizures in mice the influence of 48 dry herbal extracts with varied chemical composition has been investigated. Extracts were standardized by the content of alkaloids, flavonoids and polyphenols according to European Pharmacopoeia methods (HPTLC, HPLC, UPLC, GC-MS).*

Results. *By the integral indicator of efficacy – reduction of the mortality rate – it has been found that 11 of herbal preparations have significant anticonvulsant properties, 10 extracts act as proconvulsants, and the remaining 27 extracts have practically no effect on experimental convulsions. It has been established that there is a relationship between the pharmacological effect of preparations and phytochemical composition of the plant being extracted as well as the host species (for parasitic species – Viscum album) and the degree of polarity of the extracting agents. For the extracts with expressed anti- and proconvulsant features the chemical composition has been investigated.*

Conclusion. *The detailed analysis of relationship between phytochemical composition and influence of the extracts on the experimental seizures has found that anticonvulsant peculiarities of extracts most probably depend on rutin high content, but for the herbal medicines with proved proconvulsant properties the role of individual biologically active compounds has not been defined*

Keywords: *medicinal plants, extracts, seizures, screening, "chemical composition – pharmacological effect" relationship*

Мета. *Провести фармакологічний скринінг 48 сухих екстрактів, отриманих із 18 видів рослин 8 родин, протисудомні властивості яких раніше не вивчались, здійснити аналіз хімічного складу екстрактів із виразними анти- та проконвульсивними властивостями, а також оцінити взаємозв'язок між фармакологічним ефектом рослинних препаратів та їх фітохімічним складом.*

Методи. *На моделі пентилентетразолових судом у мишей було встановлено ефект 48 сухих рослинних екстрактів із різним хімічним складом. Екстракти стандартизували за вмістом алкалоїдів, флавоноїдів та поліфенольних сполук відповідно до вимог Європейської Фармакопеї (ВЕТШХ, ВЕРХ, УЕРХ, ГХ з масдетектором).*

Результати. *За інтегральним показником ефективності – зниженню летальності – встановлено, що 11 рослинних екстрактів мають виразні протисудомні властивості, 10 фітопрепаратів діють як проконвульсанти, а інші 27 екстрактів практично не вливають на експериментальні судоми. Визначений зв'язок між фармакологічним ефектом та фітохімічним складом рослин, з яких одержували екстракти, а також видовою приналежністю рослини-хазяїна (для паратизуючого виду – омели білої) та ступенем полярності екстрагенту. Для екстрактів із вираженими анти- та проконвульсивними властивостями визначено їх хімічний склад.*

Висновки. *Детальний аналіз взаємозв'язку між фітохімічним складом та впливом екстрактів на перебіг судом показав, що протисудомна дія екстрактів найвірогідніше обумовлена високим вмістом рутину, проте для рослин із виразними проконвульсивними властивостями роль окремих груп біологічно активних сполук залишається не визначеною*

Ключові слова: *лікарські рослини, екстракти, судоми, скринінг, взаємозв'язок «хімічний склад – фармакологічний ефект»*

1. Introduction

The aimed searching for anticonvulsive features of the plants in the world flora, medical products originating from them, as well as individual components and fractions has been conducted for more than fifty years and still underway. By far the considerable evidence has been collected about anticonvulsive activity of more than 400 species.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

The most overwhelming review [1] includes 342 species of plants, 7 known classes of biologically active components, 20 fractions and 133 individual substances. But this review practically does not include the data about the flora of moderate climatic zones with a variety of equatorial, sub-

equatorial, tropical, and subtropical plants included. Besides, more than 50 % of the species described are endemics, which makes it difficult to search for herbal substances and cultivate these plants, and even may restrict the storage only to the framework of one geographical region.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

It has already been stated that pharmacological features of herbal medicines are connected not with individual active components but with a balanced complex of functionally active compounding of plants [2]. Investigation of extracts received using the extracting agents with different polarity helps to determine the influence of the complex or fraction of biologically active substances of the same plant on the functioning of experimental convulsions and also to standardize the composition of the future medical product.

Our method of searching for potential herbal anticonvulsants presupposes the screening of different (aqueous, aqueous-ethanol, ethanol) extracts from the above-ground parts of cosmopolitan plants with different chemical composition on the basic model of pentylenetetrazole-induced seizures in mice [3].

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

Lack of herbal raw material and problematic availability of plants present a serious argument against further industrial development of potential medicines.

5. Formulation of goals (tasks) of Article

The aims of the research are to make screening of 48 dry extracts of plants belonging to 18 species of 8 families not studied before on anticonvulsant activity, to analyze the chemical composition for the extracts with proved anti- and proconvulsant activity, and to estimate the connection of the effect of herbal products and their phytochemical composition.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

48 dry extracts from above-ground parts of plants belonging to 18 species were studied:

1. *Berberis thunbergii* DC., *Berberidaceae* (aqueous extract);
2. *Corylus avellana* (L.) H. Karst, *Betulaceae* (aqueous, aqueous-ethanol, ethanol extracts);
3. *Weigela hybrida* Jaeg., *Caprifoliaceae* (aqueous extract);
4. *Fumaria schleicheri* Soy-Willem., *Fumariaceae* (aqueous, aqueous-ethanol, ethanol extracts);
5. *Ocimum viridae* Willd., *Lamiaceae* (aqueous, aqueous-ethanol, ethanol extracts);
6. *Origanum vulgare* L., *Lamiaceae* (aqueous, aqueous-ethanol extracts);
7. *Hyssopus officinalis* L., *Lamiaceae* (aqueous extract);
8. *Thymus serpyllum* L., *Lamiaceae* (aqueous extract);
9. *Stachys annua* L., *Lamiaceae* (aqueous extract);
10. *Ligustrum vulgare* L., *Oleaceae* (aqueous extract);
11. *Jasminum officinale* L., *Oleaceae* (aqueous extract);
12. *Syringa vulgaris* L., *Oleaceae* (aqueous, aqueous-ethanol, ethanol extracts);
13. *Forsythia europaea* Geg. et Bald., *Oleaceae* (aqueous extract);
14. *Lycium barbarum* L., *Solanaceae* (aqueous, aqueous-ethanol extracts);
15. *Petunia hybrida* Vilm., *Solanaceae* (aqueous, aqueous-ethanol, ethanol extracts);
16. *Nicotiana tabacum* L., *Solanaceae* (aqueous, aqueous-ethanol, ethanol extracts);
17. *Capsicum annuum* L., *Solanaceae* (aqueous, aqueous-ethanol, ethanol extracts);
18. *Viscum album* L., *Santalacea*e, growing on:

18.1. Maple – *Acer platanoides* L., *Sapindaceae* (aqueous, aqueous-ethanol, ethanol extracts);

18.2. Hawthorn – *Crataegus sanguinea* Pall., *Rosaceae* (aqueous, aqueous-ethanol, ethanol extracts);

18.3. Rowan – *Sorbus aucuparia* L., *Rosaceae* (aqueous, aqueous-ethanol, ethanol extracts);

18.4. Linden – *Tilia cordata* Mill., *Malvaceae* (aqueous, aqueous-ethanol, ethanol extracts);

18.5. Willow – *Salix alba* L., *Salicaceae* (aqueous, aqueous-ethanol, ethanol extracts).

The studied extracts were received by the method of bismaceration according to the demands of the European Pharmacopoeia. Herbal material was washed with water and air-dried. Dry herbal material was dispersed up to the condition when the particles were such size they could pass through the sieve with meshes 0.35–0.5 mm large. Then the herbal material was placed into the extractor and extracting agent was added (distilled water, the mixture of 96 % ethanol-water 1:1, 96 % ethanol) with the material–extracting agent ratio 1:10. The extraction was held at a temperature of 80–90 °C during 2 hours. The process was repeated 3–4 times until the full extraction of the complex of biologically active substances from the material. The received extracts were combined, filtered and concentrated in the vacuum evaporator at a temperature of 50–60 °C and the pressure of 80–87 kPa into the state of stiff consistency. The received semi-product was dried in vacuum drier into the state of final humidity of 5 %.

Extracts were standardized by the content of alkaloids, flavonoids and polyphenols according to European Pharmacopoeia methods.

HPTLC Method

The analysis was carried out using Camag HPTLC system equipped with a semi-authomatic TLC sampler Linomat 5, Visualizer, integrated software WinCATS 1.4.9., and Videoscan. TLC plates Si 60 F254 (Merck, Germany) of 0.2 mm layer thickness were used. TLC separation was carried out using mobile phase of water, ethyl acetate, anhydrous formic acid and anhydrous acetic acid (17,5:67,5:7,5:7,5 V/V/V/V).

After the mobile phase draw up the plate until it is approximately 0,5 cm from the end, the plate was dried, proceed with 2-aminoethyl diphenylborinate and macrogol, and after that, examined in UV-light at 365 nm.

Flavonoids and phenolic acids reference solutions were used.

GC-MS Method

The Agilent Technologies gas chromatograph 6890 series with mass spectroscopy detector 5973 series was used; fitted with a column (30 m×0.25 mm i.d.).

The following oven temperature program was used: 50 °C for 1 min then 4 °C / min up to 320 °C: this final temperature was held for 9 min.

Carrier gas was helium with a constant flow rate of 1.2 mL/min. Starting of the samples introduction into the column began when the temperature of the detector reached 250 °C. The other compounds were approximately identified by using the Nist 05 and Wiley 138 library databases of the GC-MS system. The composition was computed from the GC peak areas according to the method without using any correction factors.

HPLC Method

Determination of compounds in herbal samples was carried out with Acquity H-class HPLC system (Waters, USA).

Gradient elution was performed with purified water (solvent A) and acetonitrile (solvent B) with the flow rate set to 0.4 ml/min. Linear gradient profile was applied with following proportions of solvent A. Xevo TQD triple quadrupole mass spectrometer detector (Waters Millford, USA) was used to obtain MS/MS data.

UPLC Method

Determination of compounds was carried out with UPLC system (Shimadzu Inc., Japan), comprised of a binary pump, diode array detector, auto-sampler, controller, degasser. A reverse-phase column (Shimpack, Shimadzu Inc. Japan) was used for analysis at a temperature of 40 ˚C. Peak areas were determined at 254 nm and 310 nm for phenolic acids.

All standards of phenolic acids were purchased from Sigma-Aldrich. All standards were dissolved in methanol. The LC grade water was obtained from milliQ water purification system (Millipore, USA).

Animals and Treatment

In total 327 random bred albino male mice with the body weight of 18–24 g were used. The animals were kept in the vivarium of the Central Scientific-Research Laboratory of National University of Pharmacy in accordance with the norms and principles of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg 1986). The animals were randomized into 50 groups: 1 – control (n=20) in which the animals received intragastrically distilled water (0.1 ml per 10 g); 2 – animals receiving the classical anticonvulsant sodium valproate (n=8) at a dose of 300 mg/kg in the same regimen; 3–50 – groups in which animals received dry extracts (n=5–7). The extracts were administered into the stomach as water solutions or thin suspensions at the empiric dose of 100 mg/kg (volume 0.1 ml per 10 g of body weight) in a preventive mode during 2 days. The last dose was given 30 minutes before injecting the convulsive agent. A dose of 100 mg/kg in our previous studies turned out to be appropriate for the screening of several *Lamiaceae* plants on anticonvulsant activity [4].

Convulsive agent – $GABA_A$-receptors blocker pentylenetetrazole – was administered subcutaneously in the form of water solution at a dose of 80 mg/kg [5].

Anticonvulsant activity was estimated according to the following markers: latency period of clonic and tonic seizures, number of clonic-tonic attacks for 1 mouse, severity of seizures, duration of convulsive period and lethality. If convulsions have not occurred during 1 hour the latency period was recorded as 60 minutes. The severity of seizures was defined in points: 1 – trembling, 2 – circus movement, 3 – clonic seizures, 4 – clonic-tonic seizures with a lateral position, 5 – tonic extension, 6 – tonic extension that leads to the animal's death [6]

Chemicals and Reagents

Pentylenetetrazole was purchased from Sigma-Aldrich (USA).

Sodium valproate was used in the form of syrup 57.64 mg/1 ml (trade name Depakine, Sanofi-Aventis, France).

Statistical Analysis

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

The studied herbal extracts varied significantly in their influence on the experimental convulsions. Among them the species producing anticonvulsant effect of varying intensity (table 1); convulsants (table 2) and species without considerable influence on seizures (table 3) have been outlined.

According to the integral indicator of efficacy – decrease in lethality rate which in the control group reached 75 %, the following extracts possess clear anticonvulsant features (table 1) approximating to the reference drug sodium valproate: *Corylus avellana* aqueous extract (17 %, $p<0,01$), *Syringa vulgaris* aqueous-ethanol and ethanol extracts (33 % each, $p<0,05$), *Fumaria schleicheri* aqueous extract (29 %, $p<0,05$), *Nicotiana tabacum* aqueous and aqueous-ethanol extracts (33 % each, $p<0,05$), *Ocimum viridae* aqueous-ethanol (33 %, $p<0,05$) and ethanol (17 %, $p<0,01$) extracts, and also aqueous and aqueous-ethanol extracts of *Viscum album* growing on maple (17 % each, $p<0,01$) and ethanol extract of *Viscum album* growing on willow (33 %, $p<0,05$).

Besides decreasing lethality, anticonvulsant features were manifested in the clear reducing of the latency period of seizures (*Corylus avellana* aqueous extract, *Syringa vulgaris* ethanol extract, *Fumaria schleicheri* aqueous extract); decreasing of the number of clonic-tonic attacks for 1 mouse (*Fumaria schleicheri* aqueous extract, *Ocimum viridae* ethanol extract, aqueous extract of *Viscum album* growing on maple), severity of convulsions (*Corylus avellana* and *Fumaria schleicheri* aqueous extracts, *Ocimum viridae* ethanol extract, aqueous and aqueous-ethanol extracts of *Viscum album* growing on maple) and duration of convulsive period (*Fumaria schleicheri* and *Viscum album* (maple) aqueous extracts).

Proconvulsant features were manifested in a statistically significant ($p<0.05$) increase in lethality rate up

to 100 % in the groups receiving *Thymus serpyllum* aqueous extract, *Berberis thunbergii* aqueous extract, *Weigela hybrida* aqueous extract, *Ligustrum vulgare* aqueous extract, *Petunia hybrida* aqueous extract, *Fumaria schleicheri* ethanol extract, *Capsicum annuum* aqueous, aqueous-ethanol and ethanol extracts and aqueous-ethanol extract of *Viscum album* growing on hawthorn (Table 2).

Statistically significant decrease in the number of clonic-tonic attacks for 1 mouse (*Weigela hybrida* aqueous extract, *Fumaria schleicheri* ethanol extract, *Capsicum annuum* aqueous-ethanol and ethanol extracts) and the duration of convulsive period (*Thymus serpyllum* and *Weigela hybrida* aqueous extracts, *Fumaria schleicheri* ethanol extract) in the groups of animals receiving herbal medicines-proconvulsants (Table 2) specify the potentiation of pentylenetetrazole convulsant effect resulting in the quick death of animals registered after 1–2 attacks.

Other extracts did not have considerable influence on convulsion syndrome (Table 3).

The comparison of the individual indicators of experimental groups receiving herbal preparations with control (Table 3) does not allow detecting the clear pro- or anticonvulsant effect. Special emphasis should be given only to the aqueous extract of *Viscum album* growing on maple and aqueous-ethanol extract of *Viscum album* growing on linden. Administration of these extracts resulted in the clear increase in the latency period of seizures, decrease in the number of clonic-tonic attacks for 1 mouse, decrease in the duration of the convulsive period and, in the group receiving aqueous-ethanol extract of *Viscum album* growing on linden, there was also a decrease in the severity of convulsions. But under the influence of these extracts considerable decrease in the lethality rate did not take place.

In the group, which was administered the reference drug sodium valproate, such changes were registered as the clear decrease in lethality, increase in the latency period of seizures, decrease in the number of clonic-tonic attacks for 1 mouse, decrease in the severity of seizures and in the duration of the convulsive period (Tables 1–3).

Chemical composition of extracts with proved anti- and proconvulsant properties are presented in tables 4 and 5, respectively.

Table 1

The efficacy criteria of dry herbal extracts which showed anticonvulsant properties on the screening model of pentylenetetrazole-induced seizures in mice

Animal group, drug or extract, plant, type of extract	**Latency period, min**	**Number of clonic-tonic attacks for 1 mouse**	**Severity of seizures, points**	**Duration of convulsive period, min**	**Letha-lity, %**
Control	4.4±0.4	3.0±0.2	5.4±0.2	10.4±1.5	75
Sodium valproate	32.7±10.4***	1.4±0.6**	3.0±1.1**	3.9±1.9*	38*
Corylus avellana (aqueous)	9.9±0.4***	2.2±0.5	3.5±0.2***	7.0±3.6	17**
Syringa vulgaris (aqueous-ethanol)	4.7±1,2	2.2±0,4	4.3±0,6	7.8±2,6	33*
Syringa vulgaris (ethanol)	7.4±2.2*	2.3±0.5	4.5±0.6	7.8±2.8	33*
Fumaria schleicheri (aqueous)	11.6±1.5***	1.7±0.2**	3.9±0.6**	4.2±1.0*	29*
Nicotiana tabacum (aqueous)	5.6±1.2	2.8±0.8	4.5±0.5	11.7±5.8	33*
Nicotiana tabacum (aqueous-ethanol)	5.9±0.8	3.3±0.8	4.7±0.5	10.4±2.6	33*
Ocimum viridae (aqueous-ethanol)	5.7±0.7	2.8±0.3	4.7±0.4	13.0±2.0	33*
Ocimum viridae (ethanol)	4.1±1.0	1.7±0.2**	4.0±0.5*	6.2±2.4	17**
Viscum album (maple) (aqueous)	7.1±2.8	1.8±0.5*	4.0±0.5*	3.6±2.2*	17**
Viscum album (maple) (aqueous-ethanol)	6.2±1.1	2.3±0.4	4.2±0.4*	7.2±3.0	17**
Viscum album (willow) (ethanol)	5.5±1.4	2.0±0.5	4.7±0.5	6.1±3.6	33*

*Note: Results are means ± SEM. * – Significant at p<0.05 compared with control group. ** – Significant at p<0.01 compared with control group. *** – Significant at p<0.001 compared with control group*

Table 2

The effect of dry herbal extracts which showed proconvulsant properties on the screening model of pentylenetetrazole-induced seizures in mice

Animal group, drug or extract, plant, type of extract	**Latency period, min**	**Number of clonic-tonic attacks for 1 mouse**	**Severity of seizures, points**	**Duration of convulsive period, min**	**Lethality, %**
Control	4.4±0.4	3.0±0.2	5.4±0.2	10.4±1.5	75
Sodium valproate	32.7±10.4***	1.4±0.6**	3.0±1.1**	3.9±1.9*	38*
Thymus serpyllum (aqueous)	5.1±2.0	2.9±0.5	6.0±0.0	4.3±1.0*	100**
Berberis thunbergii (aqueous)	3.2±0.6	2.4±0.4	6.0±0.0	6.5±1.7	100**
Weigela hybrida (aqueous)	3.0±0.3	1.7±0.3*	6.0±0.0	4.2±1.8*	100*
Ligustrum vulgare (aqueous)	3.5±1.1	2.8±0.7	6.0±0.0	6.4±1.9	100*
Petunia hybrida (aqueous)	4.9±0.8	2.6±0.5	6.0±0.0	7.3±1.7	100**
Fumaria schleicheri (ethanol)	3.4±0.4	1.9±0.4*	6.0±0.0	2.8±1.3***	100**
Capsicum annuum (aqueous)	3.3±0.4	3.0±0.4	6.0±0.0	7.3±1.2	100*
Capsicum annuum (aqueous-ethanol)	6.1±1.7	1.8±0.3*	6.0±0.0	6.0±1.9	100*
Capsicum annuum (ethanol)	4.9±1.0	2.0±0.4*	6.0±0.0	5.0±1.6	100*
Viscum album (hawthorn) (aqueous-ethanol)	2.9±0.4	2.0±0.5	5.7±0.3	7.6±3.8	100*

*Results are means ± SEM. * – Significant at p<0.05 compared with control group. ** – Significant at p<0.01 compared with control group. *** – Significant at p<0.001 compared with control group*

Table 3

The results of screening of the herbal dry extracts which did not show any pro- and anticonvulsant properties on model of pentylenetetrazole-induced seizures in mice

Animal group, medicine, plant, type of extract	**Latency peri-od, min**	**Number of clonic-tonic attacks for 1 mouse**	**Severity of seizures, points**	**Duration of convulsive period, min**	**Lethality, %**
1	2	3	4	5	6
Control	4.4±0.4	3.0±0.2	5.4±0.2	10.4±1.5	75
Sodium valproate	32.7±10.4***	1.4±0.6**	3.0±1.1**	3.9±1.9*	38*
Origanum vulgare (aqueous)	3.0±0.5	2.7±0.4	4.9±0.6	6.5±1.6	57
Origanum vulgare (aqueous-ethanol)	3.2±1.0	3.3±0.6	4.9±0.5	13.2±4.3	43
Stachys annua (aqueous)	3.1±0.9	3.6±0.5	4.1±0.5*	11.6±2.0	43
Hyssopus officinalis (aqueous)	2.5±0.6*	2.0±0.4*	4.5±0.7	5.7±2.4	50
Corylus avellana (aqueous-ethanol)	5.1±0.7	3.2±0.6	5.0±0.5	13.5±3.5	50
Corylus avellana (ethanol)	6.8±2.1	3.0±0.9	4.2±0.6*	12.3±4.2	50
Forsythia europaea (aqueous)	6.4±3.1	2.1±0.4	5.6±0.4	4.5±1.7*	86
Jasminum officinale (aqueous)	4.6±0.7	1.5±0.2**	5.7±0.3	5.6±3.6	83
Lycium barbarum (aqueous)	5.8±0.9	2.1±0.3	5.0±0.5	12.6±3.6	57
Lycium barbarum (aqueous-ethanol)	4.4±0.8	2.6±0.4	5.3±0.5	9.3±2.2	71
Petunia hybrida (aqueous-ethanol)	4.7±1.6	2.0±0.4*	5.3±0.5	5.0±1.7	71

Continuation of Table 3

1	2	3	4	5	6
Petunia hybrida (ethanol)	4.5±0.9	2.2±0.6	5.5±0.5	3.7±1.6*	83
Syringa vulgaris (aqueous)	5.0±0.9	1.5±0.3**	5.0±0.6	5.4±3.3	67
Fumaria schleicheri (aqueous-ethanol)	5.2±1.3	1.3±0.2***	4.7±0.6	3.6±2.3*	57
Nicotiana tabacum (ethanol)	4.8±0.9	3.2±0.9	4.6±0.6	10.4±3.1	40
Ocimum viridae (aqueous)	7.8±1.7**	1.7±0.3**	5.0±0.6	7.7±4.2	67
Viscum album (maple) (ethanol)	15.0±9.1*	0.8±0.2***	5.0±1.0	0.3±0.1***	83
Viscum album (hawthorn) (aqueous)	2.7±0.8	2.2±0.6	4.3±0.6	4.7±1.9	50
Viscum album (hawthorn) (ethanol)	5.2±1.8	2.2±0.2	5.4±0.6	5.7±1.8	80
Viscum album (linden) (aqueous)	3.9±0.6	1.7±0.4*	5.5±0.5	1.8±0.9**	83
Viscum album (linden) (aqueous-ethanol)	23.4±11.6**	1.0±0.5***	3.7±1.2*	2.1±1.9**	50
Viscum album (linden) (ethanol)	5.0±1.2	2.8±0.5	5.5±0.3	7.1±2.1	83
Viscum album (willow) (aqueous)	6.8±2.2	2.2±0.5	5.5±0.5	3.8±1.7*	83
Viscum album (willow) (aqueous-ethanol)	5.7±1.3	1.8±0.3*	5.5±0.5	5.1±1.7	83
Viscum album (rowan) (aqueous)	4.7±0.7	2.3±0.4	5.3±0.4	7.2±2.0	67
Viscum album (rowan) (aqueous-ethanol)	4.1±0.6	2.3±0.3	5.2±0.4	9.2±2.7	50
Viscum album (rowan) (ethanol)	4.6±1.1	3.7±0.8	5.5±0.5	4.6±1.0	83

*Note: Results are means ± SEM. * – Significant at p<0.05 compared with control group. ** – Significant at p<0.01 compared with control group. *** – Significant at p<0.001 compared with control group*

Table 4

Chemical composition of the herbal extracts with anticonvulsant features

Plant, herbal material	**Extracting agent**	**Substances**	**Content (mg/kg)**
1	2	3	4
Corylus avellana L. leaves	water	**Flavonoids**	
		rutin	82.3
		quercitrin	11.8
		isoquercitrin	12.4
		quercetin	33.1
		Phenylpropanoids	
		caffeic acid	8.8
		chlorogenic acid	3.6
		neochlorogenic acid	2.5
		ferulic acid	1.2
Syringa vulgaris L. leaves	water:ethanol	**Phenylethanoids**	
		oleuropein	47.4
		acteoside	124.1
		Flavonoids	
		rutin	260.5
		kaempferol	143.3
	ethanol	**Flavonoids**	
		rutin	170.8
		kaempferol	39.5

Continuation of Table 4

1	2	3	4
Fumaria schleicheri Soy.-Willem. herb	water	**Isoquinoline alkaloids**	
		protopine	47.2
		hydrastine	34.5
		sanguinarine	29.5
		Flavonoids	
		rutin	130.2
		quercitrin	138.5
		Dicarbonic acids	
		fumaric acid	9.3
		Phenylpropanoids	
		chlorogenic acid	29.2
		neochlorogenic acid	15.1
Nicotiana tabacum L. leaves	water	**Tropane alkaloids**	
		scopolamine	51.1
		hyoscyamine	37.4
		Steroid alkaloids	
		solasodine	19.7
		Flavonoids	
		rutin	154.5
	water:ethanol	**Tropane alkaloids**	
		scopolamine	28.2
		hyoscyamine	10.7
		Flavonoids	
		rutin	160.8
Ocimum viridae Willd. herb	water:ethanol	**Flavonoids**	
		rutin	173.3
		Pentacyclic triterpenoids	
		ursolic acid	148.8
		Phenylpropanoids	
		rosmarinic acid	422.1
		caffeic acid	10.3
	ethanol	**Flavonoids**	
		rutin	103.5
		Phenylpropanoids	
		rosmarinic acid	187.9
Viscum album L. (maple) herb	water	**Flavonoids**	
		rutin	110.9
		Pentacyclic triterpenoids	
		ursolic acid	553.2
		Phenylpropanoids	
		chlorogenic acid	41.6
		neochlorogenic acid	48.8
	water:ethanol	**Flavonoids**	
		rutin	103.8
		Pentacyclic triterpenoids	
		ursolic acid	403.7
Viscum album L. (willow) herb	ethanol	**Flavonoids**	
		rutin	97.6
		Pentacyclic triterpenoids	
		ursolic acid	598.6

Table 5

Chemical composition of the herbal extracts with proconvulsant features

Plant, herbal material	Extracting agent	Substances	Content (mg/kg)
1	2	3	4
Thymus serpyllum L. herb	water	**Monoterpenoid phenols**	
		carvacrol	374.1
		Phenylpropanoids	
		rosmarinic acid	102.7
		caffeic acid	84.1
Berberis thunbergii DC. leaves	water	**Flavonoids**	
		kaempferol	106.3
		flavan-3-ol	110.8
Weigela hybrida Jaeg. herb	water	**Flavonoids**	
		quercetin	85.5
		kaempferol	136.9
		cyanidin	107.1
		Phenylpropanoids	
		caffeic acid	7.4
Ligustrum vulgare L. herb	water	**Phenylpropanoids**	
		hydroxycinnamate	132.3
		coumaric acid	39.8
		Flavonoids	
		quercetin	94.4
		luteolin	118.2
		Phenylethanoids	
		oleuropein	9.6
Petunia hybrida Vilm. herb	water	**Tropane alkaloids**	
		hyoscyamine	26.7
		Pyridine alkaloids	
		gentianine	19.1
		Flavonoids	
		pelargonidin	55.3
		cyanidin	62.9
		Phenylpropanoids	
		caffeic acid	11.2
Fumaria schleicheri Soy.-Willem. herb	ethanol	**Isoquinoline alkaloids**	
		protopine	38.8
		Flavonoids	
		rutin	76.4
		quercitrin	65.6
Capsicum annuum L. herb	water	**Protoalkaloids**	
		capsaicin	34.2
		Steroid alkaloids	
		solasodine	6.8
		Phenylpropanoids	
		hexanoic acid	147.8
		caffeic acid	2.2
	water:ethanol	**Protoalkaloids**	
		capsaicin	29.7
		homocapsaicin	28.6
		Steroid alkaloids	
		solasodine	8.1
		Flavonoids	
		rutin	3.8
		Phenylpropanoids	
		hexanoic acid	9.9
		caffeic acid	4.1
	ethanol	**Protoalkaloids**	
		capsaicin	30.1
		homocapsaicin	27.3
		Phenylpropanoids	
		hexanoic acid	84.7
		caffeic acid	3.2

Continuation of Table 5

Viscum album L. (hawthorn) herb	water:ethanol	**Pentacyclic triterpenoids**	
		ursolic acid	602.5
		Phenylpropanoids	
		chlorogenic acid	136.9
		neochlorogenic acid	20.1

Chemical analysis of the extracts showed a variety of components, heterogeneity of the qualitative and quantitative composition of the herbal preparations. It is noted, however, that without exception, all of 11 herbal anticonvulsants contain flavonoid rutin, and among proconvulsants rutin was detected only in 2 extracts from 10. There are also significant ($p<0.05$) differences in the quantitative content of rutin in the extracts with anticonvulsant features – 140.75±15.35 mg/kg (82.3–260.5 mg/kg) and proconvulsant properties – 40.10±36.30 mg/kg (3.8 and 76.4 mg/kg).

The difficulty of predicting the pharmacological properties of herbal medicines proceeds from the complexity of their composition. This is especially important to consider when developing new neurotropic medicines, in particular anticonvulsants. Molecular basis of epilepsy pathogenesis are not fully disclosed, however, a multiplicity of mechanisms of seizures development is generally accepted [7].

A divergent herbal drug action, due to the simultaneous influence of several active components of the extract on the functionally antagonistic neurochemical processes, can be expressed in the strengthening, weakening or perversion of the pharmacological effect up to the change of its direction. Voltage gated sodium channels, neuronal calcium channels, components of excitatory and inhibitory neurotransmitter systems (receptors, enzymes), cytokines and growth factors are all potential targets of biologically active substances [1]. The type of the resulting effect depends not only on the chemical structure of biologically active substances causing specific conformational changes of the receptors, but also on its concentration (expressed dose-dependent effect is characteristic of many psychotropic drugs), as well as pharmacological features of other components of the extract, including minor ones.

The plants with various chemical composition containing much alkaloids, flavonoids and polyphenols can show both pro- and anticonvulsant properties.

So, what components determine the character and severity of the pharmacological effect of the studied herbal medicines?

It is known that multimodal activity is inherent in flavonoids (including rutin), fully extractable with water and a mixture of water and ethanol [8]. Rutin modulatory effect on $GABA_A$-receptors and voltage gated ion channels has been proven [1].

In addition to the rutin, that is supposed to determine the anticonvulsant properties of the investigated herbal medicines to a great extent, the anticonvulsant effect of aqueous and aqueous-ethanol extracts of *Nicotiana tabacum* can be mediated by a tropane alkaloid scopolamine. Epoxy bridge, presence in the scopolamine structure distinguishes it from other tropane alkaloids, particularly hyoscyamine. Thus, suppressing properties are inherent only in scopolamine, while other tropane derivatives even at low doses have a stimulating effect on the central nervous system [9, 10]. Both extracts of *Nicotiana tabacum* that possess anticonvulsant properties contain tropane alkaloids with the opposite influence on the CNS – hyoscyamine and scopolamine. However, in both preparations the alkaloid with stimulating properties – hyoscyamine – is a minor component with the content 1.37 and 2.64 times less than that of scopolamine, in aqueous and aqueous-ethanol extracts, respectively.

Phenylpropanoids chlorogenic, neochlorogenic, and rosmarinic acid also, apparently, contribute to the anticonvulsant effect of the extracts, however, they are identified not in all of the investigated herbal medicines, which may point to their auxiliary role in the realization of the anticonvulsant effect.

In-depth study of the chemical composition of *Fumaria schleicheri* extracts was performed. On the example of *Fumaria schleicheri* the influence of polarity of extracting agent on the pharmacological effect can be demonstrated in the brightest way. The aqueous extract possesses clear anticonvulsant features, ethanol extract shows proconvulsant activity, and aqueous-ethanol extract does not affect on seizure scourse. When comparing the chemical composition of water and ethanol extracts of *Fumaria schleicheri* herb, it is noted that in both extracts isoquinoline alkaloid protopine, as well as flavonoids rutin and quercitrin have been identified. However, by the content of these three biologically active compounds the ethanol extract is significantly inferior to the water extract. Additionally, in the water extract isoquinoline alkaloids sanguinarine and hydrastine, fumaric, neochlorogenic and chlorogenic acids have been found. Thus, the differences in qualitative and quantitative composition of the extracts of the *Fumaria schleicheri* herb and, as a consequence, the opposite character of the effect on seizures, are distinctly associated with the nature of the extracting agent.

The role of isoquinoline alkaloids, in particular protopine, in the anticonvulsant effect of the preparations obtained from plants of *Fumaria* genus requires clarification. Anti-seizure properties of protopine are probably associated with the inhibition of glutamate excitotoxicity by suppression of Ca^{2+} influx [11].

Similar dependence of the effect on extracting agent polarity is also a characteristic of the herbal extracts of *Syringa vulgaris*, *Corylus avellana*, *Ocimum viridae*, and *Viscum album*. Pharmacological activity of the extracts from *Viscum album* as a parasitic species depends on the chemical composition of the host plant which explains the difference in the effects of extracts obtained from *Viscum album* growing on maple, linden, rowan, hawthorn, and willow. The significant qualitative

differences in the chemical composition of *Viscum album* extracts consisted only in the presence of rutin in aqueous and aqueous-ethanol extracts of the herbal material grown on maple.

As for the extracts with pronounced proconvulsant features, there were no significant regularities in their qualitative and quantitative composition which could allow an univocal conclusion about their active substances. Most herbal medicines contain components typical for the respective herbal material (e. g. protoalkaloids capsaicin and homocapsaicin, pyridine alkaloid gentianine). It is noteworthy, however, that a lot of proconvulsant extracts (6 of 10) contain caffeic acid, while among the extracts with anticonvulsant properties caffeic acid was only identified in aqueous extract of *Corylus avellana* and aqueous-ethanol extract of *Ocimum viridae* (2 of 11).

A number of species of *Solanaceae* family (*Lycium*, *Petunia*, *Capsicum*, *Nicotiana*) was included to our study proceeding from the data about the protective effect of several extracts of these species in picrotoxin-induced convulsions [12] despite the known ability of tropane alkaloids and nicotine to excite the central nervous system [9]. The results show proconvulsant features of *Petunia hybrida* and *Capsicum annuum* extracts and anticonvulsant activity of *Nicotiana* extracts which demands in-depth phytochemical and pharmacological research. Both extracts of *Lycium barbarum* did not affect on convulsions in mice.

Developing new herbal anticonvulsants should be considered a perspective aim of neuropharmacology, as the majority of known synthetic antiepileptic medicines have a wide range of dangerous side effects including teratogenicity [13, 14]. Special emphasis should be given to *Corylus avellana*, *Fumaria schleicheri* and *Viscum album* growing on maple.

7. Findings from the research and prospects of further development of this area

Screening study of 48 dry extracts of plants with various phytochemical composition (18 species from 8 families) on experimental pentylenetetrazole-induced seizures in mice allowed outlining the herbal extracts with both clear pro- and anticonvulsant features, as well as the extracts without clear effect on convulsive syndrome.

Possessing pro- and anticonvulsant properties by herbal extracts depends on their chemical composition due to the extracting agent polarity. Pharmacological features of plants-parasites (*Viscum album*) considerably differ depending on the species of the host plant.

The detailed analysis of the relationship between phytochemical composition and influence of the extracts on the experimental seizures has shown that anticonvulsant peculiarities of extracts most probably depend on rutin high content. Still it was not possible to identify an individual biologically active compound or a group of biologically active compounds which are responsible for the proconvulsant features of the herbal extracts.

Among the preparations with anticonvulsant features, a special emphasis should be given to aqueous extracts of *Corylus avellana*, *Fumaria schleicheri*, and *Viscum album* parasitizing on maple which showed clear anti-seizure effect.

References

1. Kumar, S. Plants and plant products with potential anticonvulsant activity – a review [Text] / S. Kumar, R. Madaan, G. Bansal, A. Jamwal, A. Sharma // Pharmacognosy Communications. – 2012. – Vol. 2, Issue 1. – P. 3–99. doi: 10.5530/pc.2012.suppl1.2

2. Nsour, W. M. Review on phytotherapy in epilepsy [Text] / W. M. Nsour, C. B.-S. Lau, I. C. K. Wong // Seizure. – 2000. – Vol. 9, Issue 2. – P. 96–107. doi: 10.1053/seiz.1999.0378

3. Pitkänen, A. Models of seizures and epilepsy [Text] / A. Pitkänen, P. A. Schwartzkroin, S. L. Moshé. – Amsterdam: Elsevier, 2005. – 712 p.

4. Tsyvunin, V. V. The screening investigation of anticonvulsant activity of dry extracts from 8 species of plants of the families Solanaceae, Papaveraceae, Lamiaceae and Polemoniaceae [Text] / V. V. Tsyvunin, S. Y. Shtrygol', Y. S. Prokopenko, V. A. Georgiyants // Clinical Pharmacy. – 2012. – Vol. 16, Issue 4. – P. 47–50.

5. Akdogan, I. Experimental Epilepsy Models and Morphologic Alterations of Experimental Epilepsy Models in Brain and Hippocampus [Text] / I. Akdogan, N. Goksin // Underlying Mechanisms of Epilepsy. – 2011. – Vol. 4. – P. 269–283. doi: 10.5772/19928

6. Tsyvunin, V. V. Experimental defining of anticonvulsant action of perspective phytogenic anticonvulsants [Text] / V. V. Tsyvunin, S. Y. Shtrygol', Y. S. Prokopenko, E. L. Toryanik // Ukrainian Biopharmaceutical Journal. – 2014. – Vol. 3, Issue 32. – P. 45–49.

7. Rho, J. M. Epilepsy: mechanisms, models, and translational perspectives [Text] / J. M. Rho, R. Sankar, C. E. Stafstrom. – Boca Raton: CRC Press, 2010. – 684 p. doi: 10.1201/ebk1420085594

8. Medina, J. H. Overview – Flavonoids: A New Family of Benzodiazepine Receptor Ligands [Text] / J. H. Medina, H. Viola, C. Wolfman // Neurochemistry Research. – 1997. – Vol. 22, Issue 4. – P. 419–425.

9. Rang, H. P. Pharmacology [Text] / H. P. Rang, M. Maureen Dale. – Edinburgh: Churchill Livingstone, 2003. – 797 p.

10. Sweta, V. R. Pharmacological profile of tropane alkaloids [Text] / V. R. Sweta, T. Lakshmi // Journal of Chemical and Pharmaceutical Research. – 2015. – Vol. 7, Issue 5. – P. 117–119.

11. Gupta, P. C. A Review on Ethnobotany, Phytochemistry and Pharmacology of Fumaria indica (Fumitory) [Text] / P. C. Gupta, N. Sharma, C. V. Rao // Asian Pacific Journal of Tropical Biomedicine. – 2012. – Vol. 2, Issue 8. – P. 665–669. doi: 10.1016/s2221-1691(12)60117-8

12. Reza, H. M. Effect of methanolic extract of Hyoscymus niger L. on the seizure induced by picritoxin in mice [Text] / H. M. Reza, H. Mohammad, E. Golnaz // Pakistan Journal of Pharmaceutical Sciences. – 2009. – Vol. 22, Issue 3. – P. 308–312.

13. Crawford, P. CPD – Education and self-assessment: Epilepsy and pregnancy [Text] / P. Crawford // Seizure. – 2001. – Vol. 10, Issue 3. – P. 212–219. doi: 10.1053/seiz.2001.0562

14. Holmes, L. B. The teratogenicity of anticonvulsant drugs [Text] / L. B. Holmes, E. A. Harvey, B. A. Coull, K. B. Huntington, S. Khoshbin, A. M. Hayes, L. M. Ryan // The New England Journal of Medicine. – 2001. – Vol. 344, Issue 15. – P. 1132–1138. doi: 10.1056/nejm200104123441504

References

1. Kumar, S., Madaan, R., Bansal, G., Jamwal, A., Sharma, A. (2012). Plants and Plant Products with Potential

Anticonvulsant Activity – A Review. Pharmacognosy Communications, 2 (1), 3–99. doi: 10.5530/pc.2012.suppl1.2

2. Nsour, W. M., Lau, C. B.-S., Wong, I. C. K. (2000). Review on phytotherapy in epilepsy. Seizure, 9 (2), 96–107. doi: 10.1053/seiz.1999.0378

3. Pitkänen, A., Schwartzkroin, P. A., Moshé, S. L. (2005). Models of seizures and epilepsy. Amsterdam: Elsevier, 712.

4. Tsyvunin, V. V., Shtrygol', S. Y., Prokopenko, Y. S., Georgiyants, V. A. (2012). The screening investigation of anticonvulsant activity of dry extracts from 8 species of plants of the families Solanaceae, Papaveraceae, Lamiaceae and Polemoniaceae. Clinical Pharmacy, 16 (4), 47–50.

5. Akdogan, I., Goksin, N. (2011). Experimental Epilepsy Models and Morphologic Alterations of Experimental Epilepsy Models in Brain and Hippocampus. Underlying Mechanisms of Epilepsy, 4, 269–283. doi: 10.5772/19928

6. Tsyvunin, V. V., Shtrygol', S. Y., Prokopenko, Y. S., Toryanik, E. L. (2014). Experimental defining of anticonvulsant action of perspective phytogenic anticonvulsants. Ukrainian Biopharmaceutical Journal, 3 (32), 45–49.

7. Rho, J. M., Sankar, R., Stafstrom, C. E. (2010). Epilepsy: mechanisms, models, and translational perspectives. Boca Raton: CRC Press, 684. doi: 10.1201/ebk1420085594

8. Medina, J. H., Viola, H., Wolfman, C. (1997). Overview – Flavonoids: A New Family of Benzodiazepine Receptor Ligands. Neurochemistry Research, 22 (4), 419–425.

9. Rang, H. P., Maureen Dale, M. (2003). Pharmacology. Edinburgh: Churchill Livingstone, 797.

10. Sweta, V. R., Lakshmi, T. (2015). Pharmacological profile of tropane alkaloids. Journal of Chemical and Pharmaceutical Research, 7 (5), 117–119.

11. Gupta, P. C., Sharma, N., Rao, C. V. (2012). A review on ethnobotany, phytochemistry and pharmacology of Fumaria indica (Fumitory). Asian Pacific Journal of Tropical Biomedicine, 2 (8), 665–669. doi: 10.1016/s2221-1691(12)60117-8

12. Reza, H. M., Mohammad, H., Golnaz, E. (2009). Effect of methanolic extract of Hyoscymus niger L. on the seizure induced by picritoxin in mice. Pakistan Journal of Pharmaceutical Sciences, 22 (3), 308–312.

13. Crawford, P. (2001). CPD – Education and self-assessment: Epilepsy and pregnancy. Seizure, 10 (3), 212–219. doi: 10.1053/seiz.2001.0562

14. Holmes, L. B., Harvey, E. A., Coull, B. A., Huntington, K. B., Khoshbin, S., Hayes, A. M., Ryan, L. M. (2001). The Teratogenicity of Anticonvulsant Drugs. New England Journal of Medicine, 344 (15), 1132–1138. doi: 10.1056/nejm200104123441504

Дата надходження рукопису 14.04.2016

Blyznyuk Nataliya, Quality, standardization and certification department, Institute of Pharmacy Professionals Qualification Improvement of National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: natasha.bliznyuk@mail.ru

Prokopenko Yuliya, PhD, associate professor, Department of Quality, Standardization and Certification of Medicines, Institute of Pharmacy Professionals Qualification Improvement of National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: yuliya.prok@gmail.com

Georgiyants Victoriya, Professor, Doctor of pharmaceutical sciences, head of Pharmaceutical chemistry Department , National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: vgeor@ukr.net

Tsyvunin Vadim, PhD Student, Pharmacology and Drugs Toxicology Department, National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: tsyvunin-vad@ukr.net

Shtrygol' Sergey, Professor, Doctor of medical sciences, head of Pharmacology and Drugs Toxicology Department, National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: shtrygol@mail.ru