THE EXPERIMENTAL STUDY OF PSYCHOTROPIC AND NEUROTROPIC PROPERTIES OF ACORUS CALAMUS LEAVES

L. V. Derymedvid, L. A. Korang, V. V. Tsyvunin
National University of Pharmacy

One of the most widespread plants in Ukraine is sweet flag (Acorus calamus). Acorus calamus leaves possess the pharmacological properties. In recent years there have been data on the neurotropic properties of Acorus calamus extracts.

**Aim.** To determine the effect of Acorus calamus leaves on the animal’s behavioral responses, anxiety, depression, as well as the muscle tone and the coordination of movements.

**Materials and methods.** The dealcoholized water-alcohol extract of Acorus calamus leaves (conditional name ECL) was obtained by M.S. Yaremenko, a postgraduate student at the Department of Botany of the NUPh under the supervision of prof. T.M. Gontova. The psychotropic and neurotropic properties of the original extract from Acorus calamus leaves in the doses of 1 and 5 ml/kg by the behavioral responses of mice in the open field test, manifestations of depression in the tail suspension test, the course of thiopental-induced anesthesia and physical endurance in the forced swim test were studied.

**Results.** It has been found that the extract from Acorus calamus leaves has a dose-dependent effect on the locomotor, orienting-exploratory activity, as well as the muscle tone and the movement coordination of the experimental animals. In the doses of 1 and 5 ml/kg the extract from Acorus calamus leaves demonstrated a moderate actoprotective activity. The extract from Acorus calamus leaves in the dose of 5 ml/kg showed a moderate analeptic effect. The reference drug Bilobil in a single dose of 100 mg/kg did not show a significant effect on the behavioral responses, manifestations of depression, the course of thiopental-induced anesthesia and physical endurance of mice.

**Conclusions.** The extract from Acorus calamus leaves shows a dose-dependent effect on the animal’s behavioral responses, anxiety, depression, as well as the muscle tone and the coordination of movements. The extract from Acorus calamus leaves demonstrates a moderate actoprotective activity and a moderate analeptic effect. The data obtained indicate the necessity for further in-depth studies of extracts from Acorus calamus leaves to create effective drugs based on them for the correction of the central nervous system disorders.

**Key words:** extract from Acorus calamus leaves; psychotropic properties; neurotropic properties; behavioral tests
Л. В. Деримедведь, Л. А. Коранг, В. В. Цывунин
Национальный фармацевтический университет

Экспериментальное изучение психотропных и нейротропных свойств листьев аира обыкновенного

Одним из самых распространенных растений в Украине является аир обыкновенный (Acorus calamus). Фармакологические свойства имеют и листья аира. В последние годы появились данные об нейротропных свойствах экстрактов Acorus calamus.

Цель исследования. Определение влияния листьев аира обыкновенного на поведенческие реакции животных, тревожность, депрессию, а также мышечный тонус и координацию движений.

Материалы и методы. Декалиголизированный спиртово-водный экстракт листьев аира обыкновенного (Acorus calamus) – условное название ЭКЛ был получен на кафедре ботаники НФА У аспирантом Яременко М. С. под руководством проф. Гонтового Т. М. Исследованы психотропные и нейротропные свойства оригинального экстракта листьев аира обыкновенного (Acorus calamus) в дозах 1 и 5 мл/кг по влиянию на поведенческие реакции мышей в тесте открытого поля, проявления депрессивности в иммобилизационном тесте, течение тиопенталового наркоза и физическую выносливость в тесте «плавание с нагрузкой».

Результаты. Установлено, что экстракт листьев аира обыкновенного проявляет дозозависимое влияние на локомоторную, ориентировочно-исследовательскую активность, а также на мышечный тонус и координацию движений подопытных животных. В дозах 1 и 5 мл/кг экстракт листьев аира обыкновенного оказывает умеренное актопротекторное действие. Экстракт листьев аира обыкновенного в дозе 5 мл/кг оказывал умеренное аналептическое действие. Препарат сравнения «Билобил» при однократном применении в дозе 100 мг/кг не показал существенного влияния на поведенческие реакции мышей, проявления депрессивности, течение тиопенталового наркоза и физическую выносливость.

Выводы. Экстракт листьев аира обыкновенного оказывает дозозависимое влияние на поведенческие реакции, тревожность, депрессивность животных, а также на мышечный тонус и координацию движений. Экстракт листьев аира обыкновенного проявляет умеренную актопротекторную и аналептическую активность. Полученные данные свидетельствуют о необходимости дальнейших углубленных исследований экстрактов листьев аира обыкновенного для создания на их основе эффективных препаратов для коррекции нарушений функции нервной системы.

Ключевые слова: экстракт листьев аира обыкновенного; психотропные свойства; нейротропные свойства; поведенческие тесты

Оne of the most widespread plants in Ukraine is sweet flag (Acorus calamus) [1]. Since ancient times it has been used in folk medicine as a sedative, analgesic, antispasmodic, diuretic and antimicrobial medicine.

Medicines based on the rhizome of sweet flag are widely used in official medical practice. Biologically active substances of these medicines affecting the endings of taste receptors increase appetite, improve digestion, enhance reflex secretion of the gastric juice, enhance the biliary function of the liver, increase the tone of the gall bladder, and increase diuresis [2]. Water extracts from Acorus calamus rhizomes have the tonic, anti-inflammatory, expectorant, choleretic, antispasmodic, anti-ulcer, antibacterial and disinfectant effects.

Acorus calamus leaves possess the pharmacological properties. In recent years, there has been rather interesting information on the neurotropic properties of Acorus calamus extracts. In the studies of Hazra et al. the inhibitory role of Acorus calamus in iron chloride (III)-induced epileptogenesis in rats was demonstrated. [3]. The components of the rhizomes have the sedative effect and cause a behavior modification [4]. In the studies of Muthumaran A., Singh N., the neuroprotective effect of the saponin-rich Acorus calamus L. extract was determined on the model of chronic neuropathic pain caused by squeezing of the gluteal nerve [5].

Clinical studies of the effectiveness of 70 % alcohol extract of Acorus calamus in generalized anxiety disorders in humans showed that the use of the extract not only significantly attenuated anxiety disorders, but also significantly (p<0.001) reduced the effects of stress and its correlated depression. It indicates the presence of the anxiolytic properties of the extract [6].

Analyzing the literature it was found that the neurotropic properties of the most Acorus calamus extracts studied were due to the presence of α- and β-azarones [7]. The latter, in addition to the therapeutic effect, unfortunately, has negative effects, in particular the genotoxic, carcinogenic action (causes hepatocarcinoma, tumors of the small intestine in rats), the mutagenic activity [7, 8].

Due to potential toxicity the use of sweet flag is limited in Europe (MPC 0.1 mg/kg in food and drink). In the USA, the use of the extract from Acorus calamus rhizome and the oil in food is prohibited by the FDA [9].

Unfortunately, the uncontrolled harvesting of the raw material and the decrease in the natural range of Acorus calamus led to a significant decrease in the stock of sweet flag. A number of foreign researchers indicate a considerable similarity of the qualitative and quantitative composition of biologically active substances of aerial and underground parts of sweet flag, which creates prerequisites for in-depth studies of Acorus calamus leaves for their use in medicine and pharmacy [10].

The aim of the study was to determine the effect of Acorus calamus leaves on the animal’s...
behavioral responses, anxiety, depression, as well as the muscle tone and the coordination of movements.

**Materials and methods**

The dealcoholized water-alcohol extract of *Acorus calamus* leaves (conditional name ECL) was obtained by M. S. Yaremko, a postgraduate student at the Department of Botany of the NUPh under the supervision of prof. T. M. Gontova. The characteristic property of ECL is the absence of asarone.

The studies were conducted on white random bred non-linear male mice weighing 22-28 g. Animals were kept under standard conditions of the vivarium of the Central Research Laboratory (NUPh) in standard plastic cages with free access to water and food, at a temperature of 19-24 °C, humidity of not more than 50 %, the natural day-night light mode [11].

The experiments were conducted in accordance with the provisions of the European Convention for the Protection of Laboratory Animals (Strasbourg, 1986), the Law of Ukraine “On the Protection of Animals against Cruelty” No. 3447-IV of 21.02.2006, Order of the Ministry of Education and Science, Youth and Sports of Ukraine “On Approval of the Order of carrying out experiments, experiments on animals by scientific institutions” No. 249 of 01.03.2012.

ECL was administered intragastrically in two doses (1 and 5 ml/kg) once 60 min before the tests. Control animals received intragastrically purified water in the same volume (0.1 ml per 10 g of the body weight). The reference drug was Bilobil (KRKA, Slovenia) – a standardized dry extract of *Gingko biloba* leaves in the dose of 100 mg/kg, it was dissolved in water and administered in a similar manner [12].

The effect of ECL on the locomotor activity, orienting-exploratory activity, as well as on the emotional state was studied using the standard open field test [12, 13]. After being in a dark cell for 5–6 min, a mouse was placed in the center of the platform and the countdown began. Within 3 min of being in the field, the locomotor activity of the animal was estimated by the number of squares crossed, the orienting-exploratory activity – by the number of upright postures and the holes studied, as well as the emotional state and its vegetative support by the number of fecal boli, urinations and acts of grooming [12, 14].

The effect of ECL on the muscle tone and the coordination of movements was studied using the rotating rod test [12]. The criterion for assessing the effects of ECL, as well as the reference drug on the muscle tone and the coordination of movements was the number of mice fallen down over a period of time from a standard rod, which rotated at a constant speed of 10 rpm.

The forced swim test was performed at a water temperature of +21–22 °C using a load of 10 % of the body weight of the mouse attached to the animal tail [14]. The swimming time until the animal was not able to dive out from the water for 10 sec was recorded.

The tail suspension test – the Porsolt’s immobilization test – was used to study the antidepressant properties [15]. Mice were fixed to a tripod by the tip of the tail with an adhesive plaster at a distance of 10 cm from the table surface. The duration of immobilization (fixed hanging) was recorded with a stopwatch for 6 min. The number of immobilization episodes was also recorded.

The barbiturate-induced anesthesia in mice was carried out by the thiopental sodium intraperitoneal administration in the dose of 50 mg/kg [12]. The number of animals in the lateral position, the latent period of the lateral position and the lateral position duration were registered.

To assess the statistical significance of the group differences in the results obtained the Student’s parametric t-criterion was used in the case of normal distribution, and the non-parametric Mann-Whitney U-criterion was applied in the case of its absence, as well as the Fisher angular transformation (when considering data in an alternative form).

**Results and discussion**

The results of the open field test are presented in Table 1.

| Dose (ml/kg) | Number of Squares Crossed | Number of Holes Studied | Number of Upright Postures | Number of Fecal Boli | Number of Urinations |
|-------------|---------------------------|-------------------------|---------------------------|---------------------|----------------------|
| 1           | 145                       | 58                      | 14                        | 2                   | 3                    |
| 5           | 134                       | 56                      | 13                        | 3                   | 4                    |

The results of the study showed that using ECL in the dose of 1 ml/kg (hereinafter – ECL₁) significantly increased (compared to the intact control) the number of crossed squares by 1.48 times and 1.34 times when using ECL in the dose of 5 ml/kg (hereinafter – ECL₅). The number of holes studied and upright postures increased significantly by 1.45 and 1.52 times, respectively, when using ECL₁, and by 1.22 times and 1.34 times, respectively, when using ECL₅ (Tab. 1).

In total, the orienting-exploratory activity increased by 1.5 times when using ECL₁ (p<0.05) and by 1.25 times when using ECL₅. These data suggest only a reliable stimulating effect of ECL₁ on the locomotor activity and the orienting-exploratory activity of the experimental animals, and a trend stimulating effect of ECL₅ on these indicators. The sum total of indicators of all types of activity the use of ECL₁ showed a significant increase by 1.46 times and an increase by 1.27 times when using ECL₅.

The reference drug Bilobil showed no significant changes in the indicators of the locomotor and orienting-exploratory activity; vegetative reactions compared to the control group.

Thus, the data obtained indicate that there is a reliable effect of the ECLs studied in both doses on...
The locomotor and orienting-exploratory activity, as well as on the emotional state of the experimental animals.

The results of the study of the effect of ECL₁, ECL₅, and the reference drug Bilobil on the muscle tone and the coordination of movements in the rotating rod test are shown in Table 2.

The data obtained indicate that ECL₁, ECL₅ and Bilobil do not exert the muscle relaxant effect and do not cause disturbance in the coordination of movements of the experimental animals.

The next stage of our research was the study of the physical endurance of mice in the forced swim test under the effect of ECL₁, ECL₅, and the reference drug Bilobil (Tab. 3).
Both ECLs increased the physical endurance of mice in the forced swim test. Against the background of using ECL$_1$ and ECL$_5$ the duration of swimming to full exhaustion increased by 15.8 % and 17 %, respectively, compared to the intact control group (Tab. 3).

These data indicate the presence of a moderate actoprotective activity in the extracts studied. At the same time, in this route of administration the reference drug Bilobil did not show any actoprotective activity.

The next stage of our experiments was to study the effect of ECLs on depressive behavior of mice in the tail suspension test (Tab. 4).

It was found that against the background of using ECL$_1$ and ECL$_5$ the total immobility time compared to the intact control decreased by 15.8 % and 17 %, respectively. The average duration of one hang when using ECL$_1$ decreased significantly by 1.46 times, and while using ECL$_5$ - by 1.13 times. Compared to the reference drug the average duration of one hang when using ECL$_1$ significantly decreased by 1.46 times, and when using ECL$_5$ - by 1.3 times, indicating the different composition of the drugs. The number of episodes of passive hanging did not change significantly.

The latent period of the first hang-up increased by 1.35 times, and with the introduction of ECL$_5$ - by 1.55 times (p<0.05). Therefore, the data obtained are indicative of the presence of the antidepressant effect of ECL$_1$ and ECL$_5$. The similar changes were observed in comparison with the reference drug.

The effect of extracts from Acorus calamus leaves on the course of thiopental-induced anesthesia in mice was also studied. The results of the experiments indicate that both extracts from Acorus calamus leaves have the stimulating effect on the CNS (Tab. 5).

It was found that against the background of using ECL$_1$ and ECL$_5$ the latent period of the lateral position compared to the intact control decreased by 1.43 and 1.24 times, respectively. The average duration of one hang when using ECL$_1$ decreased significantly by 1.46 times, and while using ECL$_5$ - by 1.13 times. Compared to the reference drug the average duration of one hang when using ECL$_1$ significantly decreased by 1.7 times, and when using ECL$_5$ - by 1.3 times, indicating the different composition of the drugs. The number of episodes of passive hanging did not change significantly.
of ECL, decreased by 1.7 times (p<0.01) compared to the control group and by 3.6 times (p<0.01) under the effect of ECL. Compared to the reference drug ECL, reduced the lateral position time by 1.7 times, and ECL – by 3.8 times (p<0.05).

Thus, ECL exceeded the effect of ECL by 2.1 times, and the effect of the drug Bilobil by 3.8 times in reducing the anesthetic effect.

The data obtained indicate a possible analgesic action of ECL, which may be associated with antagonistic effects with barbiturates. This issue requires further in-depth research.

In our opinion, certain neurotropic properties of extracts from Acorus calamus leaves are due to their composition – the presence of flavonoids (hyperoside, rutin, etc.), phenylpropanoids (ferulic and rosemarinic acids) and other biologically active substances. According to many researchers, all these substances have the antioxidant and cytoprotective properties [16-18].

Thus, the data obtained indicate the necessity for further in-depth studies of extracts from Acorus calamus leaves to create effective drugs based on them for the correction of the central nervous system disorders.

CONCLUSIONS
1. The effects of the extract from Acorus calamus leaves in the doses of 1 and 5 ml/kg on the behavioral reactions of mice in the open field test, manifestations of depression in the tail suspension test, the course of thiopental-induced anesthesia and physical endurance in the forced swim test were studied.

2. The extract from Acorus calamus leaves had a dose-dependent effect on the locomotor, orienting-exploratory activity, as well as the emotional state of the experimental animals.

3. The extracts from Acorus calamus leaves in the doses of 1 and 5 ml/kg demonstrated a moderate actoprotective activity.

4. The extract from Acorus calamus leaves in the dose of 5 ml/kg showed a moderate analgetic effect.

5. The reference drug Bilobil in a single dose of 100 mg/kg did not show a significant effect on the behavioral responses, manifestations of depression, the course of thiopental-induced anesthesia and physical endurance of mice.

Conflict of interests: authors have no conflict of interests to declare.

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