

## THE EFFECT DEALCOHOLIZED EXTRACT OF ACORUS CALAMUS LEAVES (*Acorus calamus* L.) ON THE BEHAVIOR OF ANIMALS UNDER RESERPINE-INDUCED DEPRESSION

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### Abstract

Depression is a mental disorder characterized by a pathologically low mood with a negative, pessimistic self-assessment, one's position in the surrounding reality, one's past and future. For the treatment of such patients, antidepressants with different mechanisms of action are widely used. However, many antidepressants have several side effects, including hepatotoxic reactions, gastropathy, gastrointestinal bleeding, etc. The use of phytopreparations is promising in this direction.

Our study aimed to investigate the effect of dealcoholized extract of *acorus calamus* leaves (*Acorus calamus* L.) (DEAL) on locomotor activity and tentative research activities of experimental animals with reserpine-induced depression.

**Materials and methods.** The study of the effect of DEAL on locomotor activity and orientation-research activities of animals was performed on white rats. Reserpine-induced depression caused by reserpine at a dose of 4 mg/kg intragastrically as a suspension stabilized with tween-80. DEAL was administered intragastrically at a dose of 5 ml/kg once daily for 2 days, the last time - 60 min before the introduction of reserpine. Four hours after the administration of reserpine in experimental animals, the rectal temperature. Locomotor activity and exploratory activity were studied by the open field method

**Results and discussion.** When using DEAL at a dose of 5 ml / kg significantly (compared to untreated control) increased the number of crossed squares by 1.5 times. Significantly ( $p \leq 0.05$ ) increased the number of inspected holes and vertical uprights by 1.87 and 1.8 times, respectively. The total indicators of exploratory activity increased by 1.67 times compared with untreated animals.

The obtained data indicate the presence of a significant effect of the studied DEAL on locomotor and exploratory activity, as well as the emotional state of the experimental animals. Antidepressant effects of the extract may be due to the presence of hyperoside and some other flavonoids that have antidepressant and other neurotropic properties

**Conclusions.** Dealcoholized extract of *acorus calamus* leaves (*Acorus calamus* L.) under conditions of reserpine-induced depression significantly improves the locomotor and exploratory activities of experimental animals. Prophylactic administration of DEAL reduces the manifestations of hypothermia caused by the introduction of reserpine. The obtained data indicate the feasibility of further studies of DEAL as a promising phytomedicine with antidepressant activity.

**Keywords:** *extract of acorus calamus leaves, reserpine-induced depression, antidepressant activity.*

## Introduction

According to the WHO study "Global Burden of Disease Study 2017", mental and neurological disorders occupy one of the leading places in prevalence and morbidity among the working population of the planet [1]. Depression is a mental disorder characterized by a pathologically low mood with a negative, pessimistic self-assessment, one's position in the surrounding reality, one's past and future [1].

According to the WHO, about 4.4% of the population suffer from depression, and among women, it occurs more often than among men (5.1% vs. 3.6%) [1, 2]. In this case, depression may be an independent disease or be secondary to other pathologies. Among patients with somatic diseases, depressive disorders occur in 22% - 33%, which is almost consistent with the prevalence of hypertension [1, 3].

For the treatment of such patients, antidepressants with different mechanisms of action are widely used [1, 3, 4, 5]. The most commonly used are selective serotonin reuptake inhibitors (fluoxetine, paroxetine, sertraline), melatonin agonist and serotonin antagonist (agomelatine), reversible monoamine oxidase inhibitors (pirlindole), as well as drugs with a dual mechanism of action (venlafaxine, duloxetine, etc.) [3, 4, 5].

However, many antidepressants have several side effects, including hepatotoxic reactions, gastropathy, gastrointestinal bleeding, etc [3, 6]. Therefore, the use of drugs that have gastro- and hepatoprotective effects and have a neuroprotective effect in such patients is appropriate. Also, more than 30% of patients with depression show resistance to the existing antidepressants [6]. This indicates the need to find new psychotropic drugs with a diverse arsenal of pharmacological properties.

The use of phytopreparations is promising in this direction. Thus, in the studies of Bolandghamat S, Moghimi A. antidepressant properties of herbal medicines based on pine have been established [7]. In the studies of Al-Harbi HR, Harthi S [8], the antidepressant effect of barley-based herbal remedies was established. In the treatment of

depressive disorders widely used are drugs based on St. John's wort [3, 4].

Our study aimed to investigate the effect of dealcoholized extract of *Acorus calamus* leaves (*Acorus calamus* L.) on locomotor activity and tentative research activities of experimental animals with reserpine-induced depression. It is known that reserpine combines the properties of sympatholytics and neuroleptics. The drug inhibits the active transport of monoamines, in particular dopamine, in the vesicles, activates MAO, which destroys the cytosolic fraction of the mediator and prevents the formation of norepinephrine [3]. This model is a classic for assessing the effects of drugs on the CNS [9].

The choice of sweet flag leaves for further research is due to the fact that for extracts of sweet flag rhizomes the presence of antidepressant action was found in the experiment [10, 11].

Taking into account the data by P. R. Venskutonis, A. Dagilyte [12] on a significant similarity of the qualitative and quantitative composition of biologically active substances of aerial and underground parts of sweet flag, the study of pharmacological properties of sweet flag leaves is promising.

In our studies, the presence of hepatoprotective, anti-inflammatory, and neurotropic activity for sweet flag leaf extracts was found, which was confirmed in our previous studies [13, 14, 15].

At the Department of Botany of the National University of Pharmacy (Kharkiv, Ukraine) under the supervision of prof. Gontova T.N dealcoholized extract of *Acorus calamus* leaves (DEAL) was obtained.

Dealcoholized extract of *Acorus calamus* leaves (DEAL) was obtained by evaporation of 70% ethanol extract. Evaporation was performed on a rotary evaporator under the following conditions: temperature 50 °C, rotation speed - 100 rpm, and vacuum force - 900 mBar. These conditions allow the removal of ethyl alcohol minimizing the impact on the composition of biologically active components.

The dealcoholized fraction of *Acorus calamus* leaf extract (DEAL) is a liquid dark brown substance, with a strong specific spicy odor. DEAL, as well as alcohol-water extract of sweet flag leaves, contains flavonoids (quantitative content in terms of

hyperoside - 0.75 mg / 100 ml; identified - rutin); hydroxycinnamic acids (quantitative content in terms of rosmarinic acid - 0.39 mg/ 100 mL; identified - ferulic and rosmarinic acids.). The results indicate that the content of flavonoids in terms of hyperoside in 70% alcohol-aqueous extract and the resulting dealcoholized extract are approximately at the same level. Also, the presence of robinin, trifolin, isoorientin, gallic acid has been found in the extracts. It should be noted that azarone was not identified in the dealcoholized fraction of the extract obtained from 70% alcohol-water extract of sweet flag leaves [15].

## Methods

The research was conducted based on the Educational and Scientific Training Center for Medical and Biological Research of the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy (NUPh). During the experiment, the animals were in the vivarium of the NUPh training center at an air temperature of 20-22 °C, natural light regime "day and night", in standard ventilated cages, on a standard diet. All animal manipulations were performed following the requirements of GLP and the European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes [16].

The study of the effect of DEAL on locomotor activity and orientation-research activities of animals was performed on 36 white nonlinear male rats weighing 175-200 g, which were randomly divided into 6 groups of 6 animals each. The intact control group (IC) was administered intragastrically purified water in a volume of 1 mL/kg body weight. The control pathology group (CP) received reserpine (Sigma, USA) at a dose of 4 mg/kg intragastrically as a suspension stabilized with tween-80. DEAL was administered intragastrically at a dose of 5 mL/kg (in our previous studies this dose was established as conditionally therapeutic) once daily for 2 days, the last time - 60 min before the introduction of reserpine [9].

Four hours after the administration of reserpine in experimental animals, the rectal temperature was measured using an electronic thermometer with a flexible tip VEGA MT 519 (Switzerland).

Locomotor activity and exploratory activity were studied by the open field method. Several parameters of rat behavior were determined in a standard-sized open field for 6 min: locomotor activity (number of squares crossed), exploratory activity (number of examined openings and vertical uprights), and vegetative support of emotional reactions (grooming, boluses, and urination). The obtained results were subjected to statistical processing using a package of software (Statistica 6.0, MS Office 2010).

## Results and discussion

The results of the experiment are given in the table and Fig.1.

As can be seen from the results of the experiment under the conditions of the introduction of reserpine, the body temperature of the experimental animals decreased by 3.5 °C. The use of DEAL in the prophylactic mode increased the body temperature by 1.9 °C.

The results of the open field test showed that reserpine causes a significant decrease in the locomotor and exploratory activity of rats (Table 1).

Thus, the number of crossed squares in the group of untreated control decreased 3.7 times, the number of examined holes - 4.7 times, the number of vertical racks - 6 times compared to the intact control ( $p \leq 0.05$ ). In total, the indicators of exploratory activity in the untreated control group decreased by 5.43 times compared with intact animals (Table. 1). It should be noted that after being placed in the "open field" animals of the control group usually crossed only a few peripheral segments and never entered the central ones.

When using DEAL at a dose of 5 mL/ kg significantly (compared to untreated control) increased the number of crossed squares by 1.5 times. Significantly ( $p \leq 0.05$ ) increased the number of inspected holes and vertical uprights by 1.87 and 1.8 times, respectively (Table 1).

The total indicators of exploratory activity increased by 1.67 times compared with untreated animals. This indicates a significant stimulatory effect of DEAL on locomotor activity and exploratory activity of experimental animals under reserpine-induced depression.

Reserpine also affected the indicators of vegetative support of emotional reactions. In the group of animals of control pathology, the number of boluses increased 2.6 times, the number of urination decreased 4 times and grooming 1.8 times. Against the background of the use of DEAL autonomic support of emotional reactions has improved (table1), but these changes were not reliable.

The obtained data indicate the presence of a significant effect of the studied DEAL on locomotor and exploratory activity, as well as the emotional state of the experimental animals.

Given the fact that DEAL does not contain  $\alpha$ - and  $\beta$  azarone, with which many researchers associate the antidepressant properties of sweet flag extracts [17, 18], the antidepressant effects of the extract may be due to the presence of hyperoside and some other flavonoids that have antidepressant and other neurotropic properties [19].

It is known that flavonoids, in most cases flavones, can interact with different zones of GABA- $\alpha$  receptors and, therefore, affect their functioning [19].

In our opinion, the neurotropic properties of DELL under the reserpine-induced depression in rats can be attributed to the presence of apigenin in the DEAL. Apigenin exhibits neuroprotective properties by activating astrocytes and reducing the production of interleukins (IL)- IL-31 and IL-33 [252].

For caffeic acid studies [20] have found the presence of anxiolytic action without changes in locomotor activity in open field tests, for rosmarinic acid, the presence of anxiolytic and (with increasing dose) stimulating effect on the CNS [21] has been found.

Thus, the studies conducted indicate the feasibility of further studies of the extract of *Acorus calamus* leaves as a promising antidepressant.

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Table 1.

Influence of DEAL on the behavior of rats in the open field test under reserpine-induced depression ( $M \pm m$ ,  $n = 6$ )

| Indicator  | Intact control  | Reserpine, 4 mg/kg<br>(untreated control) | Reserpine, 4 mg/kg + DEAL,<br>5 mL/ kg |
|--|-----------------|---|--|
| Locomotor activity (number of squares crossed)           | 24.0 $\pm$ 1.39 | 6.33 $\pm$ 0.4 *                          | 9.5 $\pm$ 0.5 **                       |
| Exploratory activity:<br>– the number of inspected holes | 7.8 $\pm$ 0.6   | 1.66 $\pm$ 0.32 *                         | 2.5 $\pm$ 0.22#                        |
| Number of vertical racks                                 | 11.02 $\pm$ 0.7 | 1.8 $\pm$ 0.37 *                          | 3.3 $\pm$ 0.4 **                       |
| amount   | 18.82 $\pm$ 1.3 | 3.46 $\pm$ 0.69 *                         | 5.8 $\pm$ 0.62 **                      |
| Vegetative support of emotional reactions:               |                 |   |  |
| – boluses  | 0.5 $\pm$ 0.2   | 1.33 $\pm$ 0.33 *                         | 0.66 $\pm$ 0.2                         |
| – urination  | 1.33 $\pm$ 0.3  | 0.33 $\pm$ 0.2 *                          | 0.5 $\pm$ 0.2                          |
| – grooming   | 2.5 $\pm$ 0.25  | 1.33 $\pm$ 0.21 *                         | 1.83 $\pm$ 0.3                         |

Figure 1. The effect of DEAL on the body temperature of rats on the background of reserpine

