

## EXPERIMENTAL STUDY OF NEUROTROPIC PROPERTIES OF DEALCOHOLIZED EXTRACT OF ACORUS LEAF

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### Abstract

Every year there is an increase in mental and neurological disorders resulting from the constant increase in the impact of stressors and socio-economic tensions. In the complex therapy of these disorders are widely used herbal medicines.

This study aimed to investigate the neuroprotective properties of dealcoholized extract of Acorus leaf (*Acorus calamus* L.) (DEAL) when administered to intact rats.

**Materials and methods.** An experimental study of psychotropic and neurotropic properties of sweet flag leaf extract was performed on intact white randomized rats weighing 180-210g. DEAL was administered intragastrically in two doses (1 and 5 mL/kg) once 60 minutes before the study. Control animals were administered intragastrically purified water in a similar volume (1 mL per 100 g of body weight). The reference product - ginkgo biloba leaf extract (GBE) at a dose of 100 mg/kg, was dissolved in water and administered in the same mode. The effect of DEAL on locomotor activity, orientation research activity, and the emotional sphere was studied using a standard open field test. The test of swimming with a load was performed at a water temperature of + 21-22° C by using a load of 10% of the body weight of the rats attached to the tail.

At the end of the experiments, the animals were killed under conditions of euthanasia for further study of acetylcholine, dopamine, serotonin, and acetylcholinesterase content in the brain.

In the following series of experiments studied the stress-protective effect of DEAL in white rats under predatory stress (fear of the victim to the predator), exposing them to the smell of the predator (100 mL cat urine on wood fillers for cat toilet in Petri dishes, which were covered with nylon cloth) for 10 minutes daily for 4 days. On the 4th day of the experiment, the level of 17-oxycorticosteroids (17-OHCS) in the daily urine of animals was determined.

**Results and discussion.** We have found a dose-dependent stimulatory effect of DEAL on locomotor, exploratory activity and a moderate actoprotective (anti-fatigue) effect. The neurotropic activity of DEAL is due to the effect on the content of biogenic amines, acetylcholine (ACh), and the activity of acetylcholinesterase (AChE) in brain tissue. In terms of neurotropic activity, DEAL exceeded the effect of GBE.

Under conditions of predatory stress, DEAL has a stress-protective effect in both doses, which is confirmed by both the corresponding neurophysiological reactions and a decrease in the level of 17-OHCS.

**Conclusion.** Thus it has been established that DEAL has a dose-dependent stimulatory effect on locomotor, exploratory activity, stress-protective activity as well as the emotional state of intact animals; has a moderate actoprotective effect.

**Keywords:** dealcoholized extract of Acorus leaf (*Acorus calamus* L.), neurotropic properties.

## Introduction

Every year there is an increase in mental and neurological disorders resulting from the constant increase in the impact of stressors and socio-economic tensions [1]. The high frequency of psychoneurological disorders is due to the increase in emotional and information load in terms of accelerating scientific and technological progress and the pace of life in general [2]. As a manifestation of dysregulation and "failure" of adaptive-compensatory reactions in the CNS in general practice, the most common is asthenic syndrome [3].

Many patients with diseases of the gastrointestinal tract usually have astheno-neurotic and astheno-depressive disorders [4]. Seeking medical attention due to asthenic symptoms can reach 50-64% [5, 6].

According to the etiopathogenetic classification, there are primary, or reactive, and secondary (somatogenic) asthenia. Reactive asthenia occurs in initially healthy individuals as a result of the strain of adaptive capabilities of the body under stress (biological or emotional), as well as in the period of convalescence of diseases, characterized by transient nature of the asthenic syndrome, a clear connection with the provoking factor and its nonspecificity. Secondary asthenia develops against the background of somatic, infectious, endocrine, mental diseases and in its pathogenesis a significant role is played by CNS damage, depression, exposure to comorbid factors, the action of drugs used to treat the underlying disease, etc. [6, 7].

In the complex therapy of these disorders are widely used herbal medicines. The use of drugs developed on the basis of raw materials of plant origin is promising, as they have a number of advantages over synthetic: a wide range of pharmacological activity, a gradual increase in pharmacological effect, low toxicity, and no adverse reactions with prolonged use [8, 9].

In our previous studies, the presence of gastro- and hepatoprotective properties of dealcoholized extract of sweet flag leaf and its favorable toxicological profile [9, **Errorre. L'origine riferimento non è stata trovata.**] have been demonstrated.

This study aimed to investigate the neuroprotective properties of dealcoholized extract

of sweet flag leaf (*Acorus calamus* L.) when administered to intact rats.

## Methods

An experimental study of psychotropic and neurotropic properties of sweet flag leaf extract was performed on intact white randomized male rats weighing 180-210 g. [11, 12, 13].

DEAL was administered intragastrically in two doses (1 and 5 mL/kg) once 60 minutes before the study. Control animals were administered intragastrically purified water in a similar volume (1 mL per 100 g of body weight). The reference product - Ginkgo biloba leaf extract (GBE) at a dose of 100 mg/kg, was dissolved in water and administered in the same mode [11].

The choice of GBE as a reference product is due to the widespread use of Ginkgo biloba leaf extract in the complex therapy of neurological and somatic diseases.

The effect of DEAL on locomotor activity, orientation research activity, and the emotional sphere was studied using a standard open field test [**Errorre. L'origine riferimento non è stata trovata.**]. After being in a darkened cage for 5-6 min, the rat was placed in the center of the platform and the countdown was started. During the 3 min stay in the "field", the locomotor activity of the animal was evaluated by the number of crossed squares, approximate research activity by the number of vertical racks and openings inspected, as well as emotional state and its vegetative support by the number of fecal boluses, urination, and grooming acts [11, 11, **Errorre. L'origine riferimento non è stata trovata.**].

The test of swimming with a load was performed at a water temperature of + 21-22 ° C by using a load of 10% of the body weight of the rat attached to the rats tail [11,14, 15]. The load was fixed at the base of the tail of animals. Swimming time in seconds was recorded. The 10% of body weight load used in the experiment was chosen due to the fact that the load of less than 5% is considered a model of aerobic work, and swimming with a load of more than 10% of body weight - an example of anaerobic work. The criterion of complete exhaustion was three unsuccessful attempts to float to the surface or the

refusal of such attempts followed by sinking to the bottom [15].

At the end of the experiments, the animals were killed under euthanasia for further study of dopamine, serotonin, acetylcholine content and acetylcholinesterase activity [16].

The content of biogenic amines in the brain of experimental animals was determined by column chromatography followed by quantitative analysis of fractions by spectrofluorometric method. The calculation of the content of biogenic amines in the samples was performed according to the calibration curves constructed according to the fluorescence data of standard samples by Sigma

The content of acetylcholine (ACh) in the homogenate obtained from the large hemispheres of the brain was determined spectrophotometrically [177]. Acetylcholinesterase (AChE) activity was determined by the method of Hestrin and co-authors [18].

The following series of experiments studied the stress-protective effect of DEAL in white rats under predatory stress (fear of the victim to the predator), exposing them to the smell of the predator (100 mL of cat urine on wood fillers for cat toilet in Petri dishes, which were covered with nylon cloth) for 10 minutes daily for 4 days[19]. Unlike other experimental models of stress (stress-restress, immobilization stress, etc.), stress with the presence of the smell of a predator does not bring the animal physical suffering and is purely psychological stress. This model of predatory stress is valid for symptoms such as anxiety, fear, traumatic memory, and panic attacks.

When conducting the experiment in experimental animals directly at the time of predatory stress recorded the number of behavioral acts in response to the stimulus. This took into account the reactions of fear, when the animal froze in contact with the smell of a cat, grooming; research reactions, i.e. sniffing of packed in a Petri dish filler with cat urine. Stimulus avoidance reactions manifested in the digging of Petri dishes with filler deep into the litter; fearless reactions when rats jumped on a cup with filler, as well as manifestations of aggression when rats tried to tear the nylon fabric covering the Petri dish with the appropriate filler were also taken into account [19].

DEAL and the comparator were administered intragastrically in two doses (1 and 5 mL/kg) 60 minutes before testing throughout the study. The intact control group was placed for an appropriate period of time with a Petri dish with wood filler for the cat toilet, covered with nylon cloth.

On the 4th day of the experiment, the level of cortisol derivative - 17-oxycorticosteroids (17-OHCS) in the daily urine of animals was determined by conventional methods [200]. Research results were statistically processed using a standard software package Microsoft Office 2007 and "STATISTICA for Windows 6.0". The probability of intergroup differences according to experimental data was established using Student's t-test. The level of statistical significance of differences in research results is  $p < 0.05$  [15].

All procedures with animals were performed in accordance with international rules and regulations for the treatment of laboratory animals [21].

## Results and discussion

Due to the fact that in previous studies we have noted the stimulating effect of DEAL on CNS, and considering the conflicting data from the literature on the effects of sweet flag on the CNS, [22, 23] we have conducted a number of studies to establish the neurotropic properties of sweet flag leaf extract.

The effects of DEAL (doses of 1 mL/kg and 5 mL/kg) and GBE on the parameters of the open field test and the physical endurance of rats in the test "swimming with load" have been studied. The choice of a higher dose of DEAL (5 mL/kg) is due to the fact that under the conditions of neurophysiological tests it is possible to track certain signs of an overdose of neurotropic action medicinal products.

The research results are shown in tables 1-4.

As a result of the conducted researches, it has been established that at the use of DEAL a dose of 1 mL/kg (hereinafter- DEAL1) significantly (compared to IC) increased the number of crossed squares 1.3 times and 1.45 times when using DEAL at a dose of 5 mL/kg (hereinafter - DEAL5). Significantly increased the number of inspected holes and upright racks by 1.5 and 1.3 times, respectively, when using DEAL1 and 1.6 times and 1.45 times, respectively, when using DEAL5 (Table. 1).

When using GBE, the number of crossed squares increased 1.13 times; the number of inspected holes increased 1.16 times and the number of vertical uprights increased 1.2 times ( $p \geq 0.05$ ).

Thus, the sum of indicators of exploratory activity revealed a significant stimulatory effect of DEAL in both doses studied on locomotor activity and exploratory activity of experimental animals and the tendentious stimulating effect of Ginkgo biloba leaf extract. Vegetative support of emotional reactions in all studied groups was almost unchanged (table. 1)

The next stage of our research was to study the physical endurance of rats in the test "swimming with a load" under the influence of DEAL<sub>1</sub>, DEAL<sub>5</sub> and GBE (Table. 2).

It was found that the use of DEAL in both doses increases the physical endurance of rats in the test "swimming with a load". When using DEAL 1 duration of swimming to complete exhaustion increased by 18.9% relative to IC, by 21.6% when using DEAL 5 and 13.3% when using the comparison drug (Table. 2).

These data indicate the presence of moderate actoprotective activity in the studied extracts normothermically.

That is, we have found a dose-dependent stimulatory effect of DEAL on locomotor, exploratory activity, and a moderate actoprotective effect. In terms of neurotropic activity, DEAL exceeded the effect of Ginkgo biloba leaf extract.

In our opinion, the dose-dependent neurotropic activity of DELL is obviously due to the effect on neurotransmission. The latter is confirmed by the results of changes in the content of biogenic amines, acetylcholine (ACh) and acetylcholinesterase (AChE) activity in brain tissue under the action of DEAL<sub>1</sub> and DEAL<sub>5</sub>. (Table 3).

We have observed a tendency for both DEAL and GBE to increase acetylcholine, dopamine, and decrease acetylcholinesterase activity. The content of serotonin under the influence of the studied pharmaceuticals has not changed.

Thus it has been established that DEAL has a dose-dependent stimulatory effect on locomotor, exploratory activity, as well as the emotional state of intact animals; has a moderate actoprotective effect. In our opinion, this is due to the composition

of the biologically active substances of the extract. DEAL has a high content of identified oxycinnamic acids, primarily ferulic, caffeic, and p-coumaric acids, for which antioxidant, membrane stabilizing, anti-inflammatory, cerebroprotective activities have been established [24, 25].

In the literature for caffeic and ferulic acids it is described that their use restores the volumetric velocity of cerebral blood flow in the simulation of total cerebral ischemia, and they do not significantly affect the performance of systemic hemodynamics [24, 25].

Neurotropic properties of Ginkgo biloba are well known. Preparations of Ginkgo biloba are powerful activators of the cognitive sphere, they improve blood supply to the brain and have both nootropic and vasotropic effects [26].

Probably the neurotropic effects of DEAL are realized both through cholinergic structures and through the dopamine system of the brain.

The next stage of our research was to study the effect of DEAL and the reference drug on the behavior of animals under predatory stress. The results of the experiment are shown in Table 4.

The study was conducted on the 4th day of the experiment. It has been found that exploratory reactions dominated in intact animals (75%). Stimulus avoidance reactions and fearlessness reactions were observed in 12.5% of experimental animals. There were no reactions of aggression.

Stimulus avoidance reactions (37.2% of experimental animals) and fear reactions (25% of animals) predominated in the control group under the action of a stress agent. Research reactions and fearlessness reactions were observed in 12.5% of animals. No aggression reactions were observed.

When using DEAL<sub>1</sub> exploratory reactions were observed in 50% of animals, stimulus avoidance reactions in 25% of experimental animals, fear reactions and fearlessness reactions were observed in 12.5% of animals. Against the background of DEAL<sub>5</sub> administration exploratory reactions were observed in 62.5% of animals, stimulus avoidance reactions, fear reactions and fearlessness reactions were observed in 12.5% of animals respectively. When using GBE in experimental animals, exploratory reactions and stimulus avoidance reactions predominated (37.5% of experimental

animals). No aggression reactions were observed in all groups of experimental animals.

One of the indicators of stress is the excretion of 17-OHCS, which reflects the intensity of the stress-implimenting link of the adaptation system. The content of 17-OHCS in the urine of animals on day 4 of the study against the background of DEAL is shown in the figure 1.

It has been found that under conditions of acute predatory stress the excretion of 17-OHCS in the daily urine of experimental animals increases 2.4 times. Against the background of DEAL 1 mL/kg the level of 17-OHCS decreased by 1.3 times compared with control animals; with the introduction of DEAL at a dose of 5 mL/kg - 1.5 times, with the administration of GBE - 1.2 times (Fig.).

Thus, it is possible to draw a preliminary conclusion about the dose-dependent stress-protective effect of DEAL and the need for further in-depth research in this direction.

### CONCLUSIONS:

1. DEAL and GBE have a moderate stimulant effect on locomotor, tentative research activity, as well as the emotional state of intact animals in open field tests and improve the physical endurance of intact rats in the test "swimming with a load".
2. Dose-dependent neurotropic activity of DEAL is apparently due to the effect on neurotransmission, which is confirmed by changes in the level of dopamine, acetylcholine, and acetylcholinesterase activity in the brain of experimental animals.
3. Under conditions of acute predatory stress in rats, DEAL has a dose-dependent stress-protective effect.

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**Table 1.** Indicators of the behavior of rats in the open field test using DEAL ( $M \pm m$ ,  $n = 8$ )

Indicator (for 3 minutes)	IC	DEAL at a dose of 1 mL/kg	DEAL at a dose of 5 mL/kg	GBE, 100 mg/kg
Locomotor activity (crossed squares)	15.20 ± 3.23	19.60 ± 3.89 * /#	22.16 ± 4.33 * /#	17.3 ± 2.7
Exploratory activity:				
– holes	7.8 ± 0.6	11.7 ± 0.72 *	12.6 ± 0.62#	9.05 ± 0.8
– racks	7.02 ± 0.7	9.13 ± 0.6 * /#	10.18 ± 0.5 * /#	8.62 ± 0.72
– amount	14.1 ± 1.3	20.83 ± 1.32	22.78 ± 1.12	17.6 ± 1.52
Vegetative support of emotional reactions:				
– boluses	0.5 ± 0.2	0.7 ± 0.30	0.86 ± 0.25	0.9 ± 0.19
– urination	0.6 ± 0.3	1.38 ± 0.18	1.38 ± 0.18	1.5 ± 0.19
– grooming	1.68 ± 0.18	2.25 ± 0.16	2.38 ± 0.18	2.37 ± 0.18
– amount	2.78 ± 0.22	4.33 ± 0.25	4.62 ± 0.23	4.77 ± 0.18

Notes:

1. \*  $p < 0.05$  - valuable relative to the intact control group (IC);
2. #  $p < 0.05$  - valuable relative to the group of the comparison drug;
3. n - is the number of animals in the group.

**Table 2.** The effect of DEAL on the physical endurance of rats in the test "swimming with a load" ( $M \pm m$ ,  $n = 8$ )

Group of animals	Duration of swimming to complete exhaustion, s
IC	345.6 ± 22.13
DEAL1, 1 mL/kg	411.13 ± 28.16 *
DEAL 5, 5 mL/kg	420.25 ± 42.15 *
GBE, 100 mg/kg	392.26 ± 33.48

Notes:

1. \*  $p < 0.05$  - valuable relative to the intact control group (IC);
2. n - is the number of animals in the group.

**Table 3.** The effect of DEAL on the content of biogenic amines, acetylcholine (ACh), and acetylcholinesterase (AChE) in the brain of intact rats ( $M \pm m$ ,  $n=8$ )

Acetylcholine (ACh), $\mu\text{g/g}$ tissue	
Intact animals (IC)	2.71+0.04
DEAL 1	3.03+0.06
DEAL 5	3.27+0.14
GBE	3.23+0.06
Acetylcholinesterase (AChE), $\mu\text{M/mg}$ tissue	
Intact animals (IC)	56.40+4.1
DEAL 1	54.84+5.11
DEAL 5	50.15+4.93
GBE	51.05+5.06
Dopamine, $\mu\text{m/g}$ tissue	
Intact animals (IC)	0.51+0.01
DEAL 1	0.58+0.01
DEAL 5	0.66+0.02
GBE	0.60+0.0
Serotonin, $\mu\text{m/g}$ tissue	
Intact animals (IC)	25.55+1.18
DEAL 1	25.76+0.92
DEAL 5	26.00+0.87
GBE	25.97+1.05

**Table 4.** The effect of DEAL on anxiety in rats under predatory stress ( $M \pm m$ ,  $n = 8$ )

Indicator	Intact animals	Control	DEAL at a dose of 1 mL/kg	DEAL at a dose of 5 mL/kg	GBE, 100 mg/kg
Reactions of fear	0	25	12.5	12.5	12.5
Exploratory reactions	75	12.5	50	62.5	37.5
Stimulus avoidance reactions	12.5	37.5	25	12.5	37.5
Reactions of fearlessness	12.5	12.5	12.5	12.5	12.5
Reactions of aggression	0	0	0	0	0



**Figure 1.** The content of 17-OHCS in the urine of animals on the 4th day of the study under acute predatory stress on the background of DEAL administration

