

Antidepressant Activity of Diterpene Alkaloids of *Aconitum baicalense* Turcz

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Course treatment with diterpene alkaloids of *Aconitum baicalense* in mice reduced the time of immobilization in the tail suspension test and produced an antiexudative effect in mouse model of serotonin-induced edema. In the open field test, application of alkaloids did not change the total motor activity, orientation and exploratory behavior, and emotional reactions of animals. Experimental data suggest that diterpene alkaloids of *Aconitum baicalensis* exhibit antidepressant properties, possibly due to modulation of sensitivity to serotonin.

Key Words: diterpene alkaloids; antidepressant activity; serotonin edema

In modern medicine, many natural bioactive substances, e.g. alkaloids serve as the source of highly effective drugs providing correction of disturbed physiological functions of the organism [4]. Alkaloids exhibit a wide spectrum of pharmacological properties, among them tranquilizing and stimulating effects on the central nervous system, hypertensive and hypotensive action, vasoconstrictor and vasodilator effects on the cardiovascular system, different effects on transmitter functions and activities of the muscular system.

Diterpene alkaloid methyllycaconitine is an active matter of the drug mellicline known as a muscle relaxant. Furthermore, it was recently discovered that methyllycaconitine exhibits high affinity and selectivity for *n*-choline receptors of the nervous system in mammals, which makes it possible to use this alkaloid when diagnosing Alzheimer's disease [6]. Another representative of the diterpene alkaloids, lappaconitine, is used since 1987 for the treatment of antiarrhythmic cardiovascular disease as an active matter of officinal drug allapinine. The psychotropic and analgesic effects of lappaconitine were also reported. On the basis of

alkaloid songorine, a local anesthetic 1-benzoyl-napelline hydrochloride was obtained, which is superior by activity and duration of the action to novocaine amide and not inferior to cocaine. Neither songorine, nor any of its derivatives were used in medicine production until now. The study of another representative of alkaloids, mesaconitine, showed that its analgesic properties are due to the influence of the noradrenergic system [7]. Antidepressant properties of diterpene alkaloids are also known. Diverse and pronounced effect of the alkaloids on the transmitter systems in humans and animals suggests the possibility of their impact on serotonin metabolism, especially as we had previously shown antiinflammatory activity of diterpene alkaloids extracted in bulk from *Delphinium elatum* L. under conditions of acute serotonin edema [5].

It is currently accepted that the main psychoactive effect of antidepressants is associated with blockade of reuptake of neurotransmitters, serotonin and norepinephrine, in the synapse. Since serotonin receptors are located not only in the central nervous system, but also on platelets and in the vascular wall, a clear-cut relationship between despair behavior in the tail suspension test (TST) and the degree of exudation induced by peripheral administration of serotonin was demonstrated [3]. Therefore, the aim of this study was to study the antidepressant effect of *Aconitum baicalense*

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alkaloids under conditions of despair behavior and analysis of the relationships of this activity with the severity of serotonin edema in mice.

MATERIALS AND METHODS

Experiments were carried out on albino outbred mature female mice weighing 20-30 g (conventional mouse strain obtained from the nursery of the Institute of Pharmacology, certificate is available). All animals were kept under standard vivarium conditions with 12-hour light regime on full-balanced diet (GOST R 50258-92) with free access to food and water. Experiments were carried out in compliance with international recommendations of the European Convention for the Protection of Vertebrate Animals Used in Experimental Studies (1998) as well as Rules and laboratory practice in the conduct of preclinical studies in Russia (GOST R 51000.3-96 and GOST R 51000.4-2008). The animals were sacrificed in accordance with the Regulations of the work with experimental animals approved by the Ministry of Health of Russia. We studied separately extracted diterpene alkaloids: mesaconitine, hypaconitine, napelline, songorine, 12-epinapelline N-oxide. The extraction of above-ground parts (herbs) of *Aconitum baicalense* was performed at the laboratory of Irkutsk Institute of Organic Chemistry, Siberian Division of the Russian Academy of Sciences, by the extraction method for raw materials. Well-known antiphlogistic orthophen (10 mg/kg) was used as the reference drug. Alkaloids of *Aconitum baicalense* in a dose of 0.025 mg/kg and orthophen were administered *per os* as a 5-day preventative course, and for the last time 1 hour before the exposure.

In all animals, the duration of immobilization was initially studied in TST, which is used to identify agents possessing antidepressant activity [9].

The behavior of animals was observed in a chamber (51×25×15) divided into 3 compartments by 16 cm. The mouse was hung by the tail on the adhesive plaster at a distance of 1.5 cm from the tip. The distance from the floor to the animal nose was 10 cm. The duration of immobilization (motionless hanging) was visually recorded for 6 min. In addition, the latency of immobilization, *i.e.* the time before motionless hanging, was determined. The criterion of antidepressant activity was statistically significant reduction in the time of immobilization in experimental animals compared to controls in TST.

After completion of the test, acute exudative reaction was modeled. Serotonin creatinine sulphate (Reanal) was injected under hind paw aponeurosis (volume 0.05 ml in 0.9% NaCl, 0.5 mg/ml). One hour after the subaponeurotic injection of serotonin and equivolume injection of 0.9% NaCl into contralateral paw, exarticulation at the ankle joints was performed. The severity of inflammatory edema was assessed gravimetrically and expressed as a percentage of the weight increment of experimental paw compared to control paw [2].

Additionally, the effect of diterpene alkaloids on mouse behavior in the open field test was studied [1]. Individual behavior was considered as a complex of discrete behavioral acts and postures: horizontal activity, vertical activity, grooming, defecations, hole board exploratory behavior. We calculated the proportion of each pattern among other behavioral elements. Total motor activity, orientation and exploratory activity (number of rearings and explored holes), and emotional reaction by Brady were evaluated as integral criteria.

The significance of differences was evaluated using parametric (Student's *t* test) and nonparametric (Mann-Whitney *U*) tests.

TABLE 1. Effect of Course Treatment with Diterpene Alkaloids from *Aconitum baicalense* on Antidepressant Activity in Inbred Female Mice under Conditions of TST ($\bar{X} \pm m$)

Drug	Dose, mg/kg	Number of animals	Latency of immobilization, sec	Total time of immobilization, sec
Control (no drug)	—	10	62.3±17.2	118.9±13.8
Orthophen	10	6	40.3±10.5	132.3±17.5
Napelline	0.025	7	109.3±17.9 ⁺	74.0±11.9 ⁺⁺
Songorine	0.025	6	102.7±24.5 ⁺	64.8±17.1 ⁺⁺
12-Epinapelline N-oxide	0.025	6	76.3±19.6	87.8±12.9 ⁺
Hypaconitine	0.025	6	114.7±32.3 ⁺	69.3±14.4 ⁺⁺
Mesaconitine	0.025	6	95.4±7.8	68.8±16.9 ⁺⁺

Note. Here and in Tables 2, 3: $p < 0.05$ compared to ⁺control, ⁺⁺orthophen.

TABLE 2. Effect of Course Treatment with Diterpene Alkaloids from *Aconitum baicalense* on Exudative Reaction Caused by Peripheral Administration of Serotonin in Inbred Female Mice ($X \pm m$)

Drug	Dose, mg/kg	Number of animals	Weight increment of the limb, %	Inhibition of edema, %
Control (no drug)	—	10	34.7±2.4	—
Orthophen	10	6	16.4±2.0*	52.7
Napelline	0.025	7	30.2±3.5	12.8
Songorine	0.025	6	24.4±3.3*	29.7
12-Epinapelline N-oxide	0.025	6	29.2±4.7	15.9
Hypaconitine	0.025	6	20.9±2.6*	39.6
Mesaconitine	0.025	6	26.1±2.3*	24.8

RESULTS

Experimental studies showed that the studied diterpene alkaloids affected the latency of immobilization and duration of despair behavior in TST (Table 1). In all groups, the time before immobilization stably tended to increase by 1.2-1.8 times, while the total time of immobilization significantly decreased by 37.8-45.5% in comparison with the control. Songorine produced the most pronounced antidepressant effect. Administration of 12-epinapelline N-oxide also decreased the total immobility time by 26% compared to controls, but this effect was statistically insignificant (Table 1). In contrast, orthophen (reference drug) did not reduce and even somewhat increased the total time of immobilization (Table 1).

Peripheral serotonin administration induced significant weight increment of the affected limb in the control group (Table 2). Administration of the test substances helped to reduce the severity of serotonin edema in animals of the experimental groups. Most effective were the alkaloids hypaconitine and songorine reducing limb weight increment by 1.4-1.7 times compared to the control. The course of

napelline and 12-epinapelline N-oxide injections to experimental mice provided only a slight alleviation of edema (Table 2). Orthophen caused the most pronounced antiinflammatory effect: the weight of inflamed limb decreased 2-fold compared to the control (Table 2).

The duration of immobilization in TST can vary not only under the influence of antidepressant effect of the test substances, but also in case they exhibit stimulating or psychosedative activity. To determine the possible psychosedative and psychostimulating effects, the alkaloids were tested in the open field test. It was found that none of the test substances significantly affected general locomotor activity and individual behavioral elements, such as movement, hole board exploratory behavior, and grooming. Orientation and exploratory behavior (Table 3) and emotional response of mice also remained at the control levels. Only the number of rearings significantly decreased in mice receiving 12-epinapelline N-oxide and mesaconitine (Table 3). The latter also significantly reduced the number of defecation boluses (Table 3), which together with reduction in vertical activity can be regarded as a sign of sedation.

TABLE 3. Effect of Course Treatment with Diterpene Alkaloids of *Aconitum baicalense* on Orientation and Exploratory Behavior of Mice and the Number of Individual Behavioral Acts: Rearing and Defecation under Conditions of Open Field Test

Drug	Orientation and exploratory behavior	Number of rearings	Number of defecations
Control	11.6±1.4	5.8±0.8	0.5±0.2
Songorine	9.6±3.0	4.0±1.1	0.4±0.2
Napelline	11.7±3.7	4.0±0.7	0.6±0.3
Hypaconitine	11.8±3.0	3.0±1.3	0.7±0.2
12-Epinapelline N-oxide	9.0±1.7	2.2±0.8*	0.4±0.2
Mesaconitine	9.8±0.4	1.6±1.2*	0.0±0.0*

Thus, our studies revealed antidepressant properties of individual diterpene alkaloids of *Aconitum baicalense* (as increased activity): napelline < hypaconitine < mesaconitine < songorine. At the same time, alkaloids also exhibited antiinflammatory properties and reduced exudative reaction caused by peripheral administration of the neurotransmitter serotonin. Similar properties were revealed in sertraline, a representative of the selective serotonin reuptake inhibitors [3], which simultaneously reduced serotonin edema and shortened the duration of despair behavior in TST. It is known that changes in sensitivity to serotonin in the prolonged administration of selective serotonin reuptake inhibitor are the major mechanism of their antidepressant activity [8]. It is possible that changes in sensitivity to serotonin contribute to the development of antidepressant effect during the course of treatment with diterpene alkaloids from *Aconitum baicalense*.

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