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Alkaloids from the Aerial Parts of Larkspur (*Delphinium speciosum* Beeb.) and Their Pharmacological Activity

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ABSTRACT

The aim of the present study was to study alkaloidal spectrum of the aerial parts of larkspur (*Delphinium speciosum* Beeb.) *D. speciosum* Beeb. and estimate their pharmacological potency. Chloroform extraction of the powdered air-dried raw material yielded tertiary sum of alkaloids (TADS). Subsequent pH-guided fractionation of TADS led to the isolation and characterization of diterpene alkaloids methyl lycaconitine, gigactonine, lycoctonine. Preliminary pharmacological study of TADS on rodents revealed its anticonvulsant and moderate analgesic activity in PTZ-induced seizures and “hot plate” tests, correspondingly. The observed pharmacological effects may result from blocking of ligand-gated Na^+-channels that destroy cholinergic transmission and downstream Na^+-current due to selective interaction with nAChRs.

INTRODUCTION

Nowadays, up to 40% of all patients with epilepsy suffer from numerous side effects (e.g. sedation and muscle relaxation), as well as convulsive resistance to modern antiepileptic drugs. On the other site, some medicinal compounds of vegetable origin are claimed to significantly decrease seizure response without aforesaid side effects, hence it is evident that medicinal plants can be considered as a rich source for discovering safer and more effective antiepileptics.

Plants of the genus *Delphinium* belong to the family *Helleboraceae*. 18 species of *Delphinium* are represented in the flora of Georgia. [1]. Delphinium species are a rich source of diterpene alkaloids, possessing a wide range of pharmacological activity: analgesic, non-thyrotoxic, curare-like. In folk and traditional medicine, tinctures and decoctions of larkspur were used in treatment of female and genitourinary diseases, jaundice, liver enlargement, diseases of gastrointestinal tract, pneumonia, pleurisy and whooping cough [2-6]. Due to ability to decrease skeletal muscles tone larkspur alkaloids are used in medical practice as muscle relaxants in treatment of disorders of the motor function associated with diseases of the central nervous system (parkinsonism, multiple sclerosis, etc.). In this regard, it was advisable to search for new sources for the production of alkaloids of larkspur among species growing in Georgia. In particular, we investigated yet poorly studied *D. speciosum* Beeb., widespread in Georgia.

The aim of the study was to study the aerial parts of *D. speciosum* Beeb. on the content of diterpene alkaloids and estimate their pharmacological potency.

MATERIALS AND METHODS

D. speciosum in flowering phase was collected in 2018 in the Tsikhijvari, Georgia. The object of study was alkaloids from the aerial organs of *D. speciosum*.

Air-dried ground aerial parts of larkspur (1.0 kg) were alkalized with a 5% sodium carbonate solution and extracted with chloroform. The chloroform extracts were concentrated to 1/5 of the original volume and the alkaloids were extracted with a 5% aqueous solution of sulfuric acid. The acidic extract was rinsed with ether, then, with cooling, it was alkalinized with sodium carbonate to pH 9, and the alkaloids were extracted with chloroform. After dehydration with anhydrous sodium sulfate and vacuum-concentration, 9.6 g of the tertiary

sum of diterpene alkaloids was obtained. Then crude alkaloids were fractionated according to basicity (pH 10.0-2.0) [7, 8].

Fractions with a pH of 2-6 were separated on a silica gel column (100/400), eluted with chloroform, 6 mixtures of chloroform-methanol (100:1; 90:1; 50:1; 25:1; 5:1, 1:1).

Fractions with a pH of 7-9 were processed on a silica gel column (100/400), eluting with chloroform, a mixture of chloroform-methanol: (100:1; 90:1; 50: 1; 25:1; 5:1, 1:1).

Qualitative analysis of alkaloids was carried out by thin-layer chromatography on TLC Silica gel 60 F_{254} plates (Merck, Germany) in systems: I-chloroform-methanol (6:1), II-chloroform-methanol (4:1), III-chloroform-benzene-95% ethanol-25% ammonia (40:40:10:0.2), in comparison with reference samples of methyllycaconitin, karakoline, delkozin, gigactonine, lycoctonin, lycaconitine [4]. Dragendorff's reagent was used to detect alkaloids [9]. Identification of alkaloids was realized using 7890B GC System and 5977B Single Quadrupole GC/MSD System (Agilent Technologies, USA). Alkaloids melting point was determined by IA 9100 melting point apparatus (WenkLabTec, Germany).

Animals

Inbred male rats, (n=40, b.w. 200±10 g) and mice (n=40, b.w. 24±2 g) were used. Animals were randomly divided in groups of ten in each. All animals were kept in following conditions: 12/12 hr light/dark cycle, temperature 20±2°C, humidity 40%, standard diet for rodents, water access *ad libitum*. Maintenance of animals and all experimental procedures were performed in accordance with internationally accepted ethical guidances [10, 11]. Experimental data statistical processing was done using Student's t-test.

Evaluation of anticonvulsant activity in rats

Total alkaloids of *D. speciosum* (TADS) were administered by oral gavage and intraperitoneally at doses of 10, 25, and 50 mg/kg. Thirty minutes after administration, each rat was injected intraperitoneally 80 mg/kg pentylenetetrazole (PTZ, Sigma, USA). The doses of used drugs were defined in a pilot study of acute toxicity. After PTZ injection, rats were placed in a plexiglass acrylic cage and seizures were recorded until convulsions stopped and rats were fully recovered. Seizure susceptibility was analyzed considering the following

parameters: occurrence, severity, latency and duration of behavioral seizures, number of animals displaying seizures and death due to seizures [12].

The severity of seizures was estimated according to the following scale: 0- no changes in behavior; 1- isolated myoclonic jerks; 2- atypical minimal seizures (unilateral, incomplete); 3- full clonic seizure; 4- pattern of tonic-clonic seizures with a suppression of tonic phase; 5- generalized tonic-clonic seizure and status epilepticus; percentage of mortality was recorded for a period of 60 min. Animals surviving over 1 hour were considered to be protected[13].

Evaluation of analgesic activity in mice

Analgesic activity of alkaloids was evaluated using Eddy's "hot plate" test [14-16]. In brief, animals were placed into a 12 cm wide and 25 cm height transparent polycarbonate cylinder on a hot plate maintained at 55±0.2°C. Experimental mice were injected *Delphinium* extract 5 and 15 mg/kg intraperitoneally 15 min before the experiment. Normal saline 0.2 ml/animal, intraperitoneally, was used as the control. The latency period (in seconds) between placing on the plate and occurrence of response reactions (withdrawal of hind paws, jumping) was recorded. Animals without response within 30 seconds were excluded from the experiment. Latencies were recorded before (base level) and 15, 30, 45, 60 and 120 min after the injection of test and reference compounds.

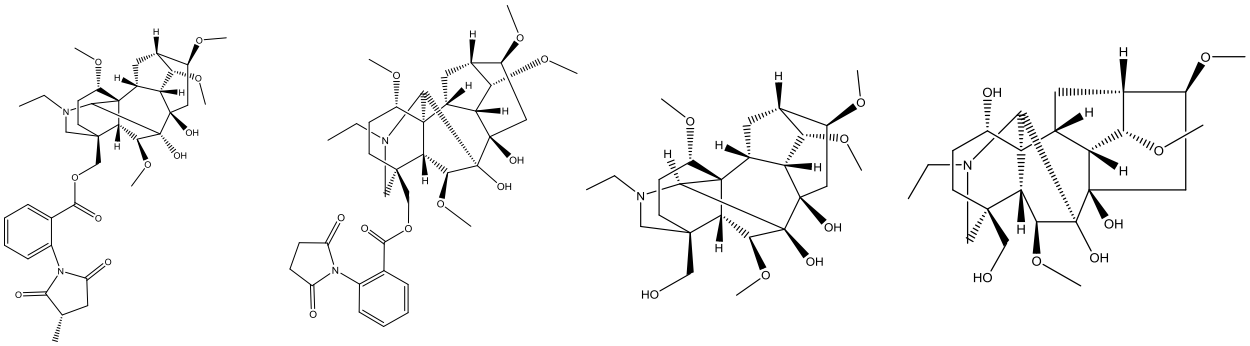
RESULTS AND DISCUSSION

Polybuffer separation of the total alkaloids revealed the presence of four dominant alkaloids (Table 1, Fig.1).

Table No. 1: Dominant alkaloids eluted from the aerial parts of *D. speciosum*

Elution system *	Alkaloid	Content (%)	M.P. (°C)	m/z
CHL + CHL:MET (100:1)	methyllycaconitine	35	amorph.	682(M^+), 667, 651(100), 649
CHL:MET (90:10)	gigactonin	10	168-169°	453(M^+), 438(100), 420, 397
CHL:MET (50:1)	lycoctonin	19	136-140°	467(M^+), 452,450, 436(100), 418
CHL:MET (25: 1)	lycaconitine	8	amorph.	668(M^+), 653, 650, 637(100), 650,434, 248,174

*CHL – chloroform; MET - methanol



Methyllycaconitine Lycaconitine Lycoctonine Gigactonine

Figure No. 1: Alkaloids from the aerial parts of *D. speciosum.*

Anticonvulsant activity

After the analysis of the convulsive behavior of rats treated with **TADS**, it was found that seizure latency and severity was similar in all treated groups: time to seizure onset significantly increase and most of the rats in treated groups corresponding to myoclonus and minimal seizures. It is important to mention that the number of rats that survived to the generalized tonic-clonic seizure, status epilepticus was high (Tabs. 2, 3).

Table No. 2: Anticonvulsant effect of TADS on PTZ-induced seizures in rats*

Dose (mg/kg) p.o.	Time to seizure onset (min)	Death latency (min)	Death rate (%)
control	3.6 ± 0.5	5.5	100
10	15.5 ± 2.4 **	50	10
25	18.0 ± 4.1 **	0	0
50	20.0 ± 3.2 **	0	0

*Data represented as mean±SD(n=10); ****** - $p < 0.01$ vs control.

Table No. 3: Protective effect of TADS on the severity of PTZ-induced seizures in rats*

Seizures severity	PTZ 80mg/kg	**TADS** (mg/kg/p.o.)		
		10	25	50
1	0	0	0	0
2	0	7	8	7
3	0	2	2	3
4	10	1	0	0
5	10	0	0	0

***** n=10 for PTZ and each TADS dose

Analgesic activity

In "hot plate" model the **TADS** in dose-depend manner increases latency time, when compared to control, with maximal effect (approx. 70%) at 30 min after the **TADS** administration (Fig. 2).

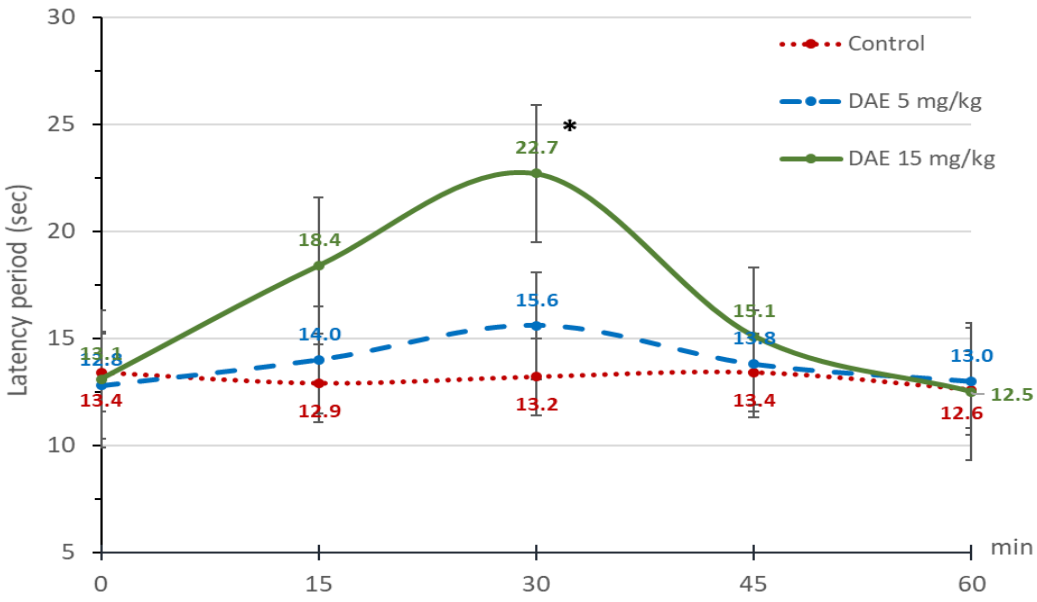


Figure No. 2: Analgesic activity of TADS in mice. * - p<0.05 vs control.

The observed TADS-induced analgesia corresponds with the data on antinociceptive action of some diterpene alkaloids [17] and may result, for example, from blocking of ligand-gated Na^+-channels that destroy cholinergic transmission and downstream Na^+-current due to selective interaction within AChRs. [18]. On the other site, the nAChRs are also involved in the pathogenesis of epilepsy [19]. Moreover, recently it was shown that hippocampal

cholinergic terminals establish effective GABA-ergic synapses as well [20, 21]. The results obtained in PTZ induced convulsion test coincide with data described in [22].

CONCLUSION

According to the results of the study, it was found that the aerial parts of *D. speciosum* growing in Georgia, contain mainly diterpene alkaloids methyllycaconitine; gigactonine, lycoctonine, and lycaconitine. Summarizing the obtained pharmacological data and taking into consideration that methyllycaconitine can antagonize nicotine-induced seizures and antinociception in mice via blocking apha7 nAChR as well [22], it is highly likely that the studied TADS modulated the observed analgesic and anti-seizure effects via cholinergic structures of the CNS due to the diterpene alkaloids methyllycaconitine.

REFERENCES

1. Gagnidze R. Vascular plants of Georgia a nomenclatural checklist. Tbilisi, "Universal" Pubs.; 2005.
2. Xu TF, Liu, S, Meng, LL. *et al.* Bioactive heterocyclic alkaloids with diterpene structure isolated from traditional Chinese medicines. J. Chromatogr. B, 2016;1026:56–66.
3. WangJL, Shen XL, Chen QH *et al*. Structure-analgesic Activity Relationship Studies on the C18-and C19-Diterpenoid Alkaloids. Chem. Pharm. Bull. 2009;57:801–807.
4. Liu F, Yang, CH, Liang, JY, Liu, JH. Advances in the studies on the pharmacological activities and toxicities of C19 diterpenoid alkaloids. Strait Pharm.J.2012;24:1–5.
5. Shan, L, Zhang, J, Gao, F. *et al*. Diterpenoid Alkaloids from *Delphinium anthriscifolium* var. majus. Sci Rep. 2017;**7:**6063.
6. Kintsurashvili L. Alkaloids of some plants of families Helleboraceae and Ranunculaceae growing in Georgia. 3rd International conference on pharmaceutical sciences, Abstract book. Tbilisi, "Universal" Pubs, 2015;116.
7. Pirildar S, UnsalGurer C, Kocyigit M *et al*. Alkaloids from the roots of *Aconitum anthora* and aerial parts of *Delphinium kurdicum*. Chem. Nat. Compd. 2013;48:1115–1116.
8. Kabelianskaia L. Use of mellictin in clinical diseases of the nervous system in spastic paralysis. Farmakologiiai toksikologiia. (Article in Russian). 1959;22(1):38-42.
9. Detection of Alkaloids in TLC. Journal of Chromatography Library. Eds. A. B. Svendsen, R. Verpoorte. 23 (Part A), 11-18. 1983.
10. Directive 2010/63/EU on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276/33, 2010.
11. Guide for the Care and Use of Laboratory Animals, 8th Ed. USA: the National Academies Press: 2011.
12. Li B, Wang L, Sun Z, *et al*. The Anticonvulsant Effects of SR 57227 on Pentylenetetrazole-Induced Seizure in Mice. PLoS ONE. 2014;9(4):e93158.
13. Puig-Lagunes AA, Manzo J, Beltrán-Parrazal L, *et al*. Pentylenetetrazole-induced seizures in developing rats prenatally exposed to valproic acid. PeerJ. 2016; 4:e2709.
14. Wood PL. Animal models in analgesic testing. In MJ Kuhar and GW Pasternak (Eds.), Analgesics: Neurochemical, Behavioral, and Clinical Perspectives.N.Y: Raven Press; 1984.
15. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines, J. Pharmacol. Exp. Ther. 1953;107;385-393.
16. Hunskaar S, Berge, O-G, Hole K. A modified hot-plate test sensitive to mild analgesics. Behavioural Brain Research, 1986;21(2):101–108.
17. Pereira F. Polypharmacology of Aconitum and Delphinium sp. Diterpene Alkaloids: Antiarrhythmic, Analgesic and Anti-Inflammatory Effects. Mini-Reviews in Organic Chemistry. 2017;14 (4).

18. Tursunkhodjaeva F, Dzhakhangirov F. Analgesic and anti-inflammatory activity of diterpenoid alkaloids isolated from the Central Asian species of *Aconitum* and *Delphinium* plants. Materials of the 1st International Electronic Conference on Medicinal Chemistry.2015;doi:/10.3390/ecmc-1-A007.
19. Iha H, Kunisawa N, Shimizu S. *et al*. Nicotine Elicits Convulsive Seizures by Activating Amygdalar Neurons. Front Pharmacol. 2017;8:57.
20. Takács VT, Cserép C, Schlingloff D *et al*. Co-transmission of acetylcholine and GABA regulates hippocampal states. Nat Commun. 2018;9:2848.
21. Johnstone T, Gu Z, Yoshimura RF *et. al.,* Allosteric Modulation of Related Ligand-Gated Ion Channels Synergistically Induces Long-Term Potentiation in the Hippocampus and Enhances Cognition. *The* Journal of pharmacology and experimental therapeutics. 2011;336(3):908-915.
22. Ivy Carroll F, Ma W, Navarro HA *et al*. Synthesis, nicotinic acetylcholine receptor binding, antinociceptive and seizure properties of methyllycaconitine analogs. Bioorg Med Chem.2007;15(2):678-685.

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