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Alkaloids from the Delphinium Flexuosum Bieb. and Their Pharmacological Activity

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ABSTRACT

Background: The aerial and underground parts of D. flexuosum Beeb. have been studied regarding the content of diterpene alkaloids, and a preliminary estimation of their pharmacological potency has been conducted. Diterpenoid alkaloids from larkspur species are claimed to have diverse pharmacological activity, including analgesic, anticonvulsant, muscle relaxant, etc. In this regard, searching for additional natural resources rich in alkaloids within larkspur species growing in Georgia seems reasonable.

Objectives: This study aims to comprehensively investigate the diterpene alkaloid content and pharmacological potency of D. flexuosum M. Bieb's aerial and underground parts, which have yet to be sufficiently explored in previous research.

Methods: Alkaloid fractions from aerial and underground parts of D. flexuosum were isolated using the polybuffer separation method with citrate-phosphate buffers (pH 8.0 → 2.0). The antinociceptive activity was evaluated using the hot plate test, a standard method for assessing analgesics with central mechanisms. In contrast, the anticonvulsant activity was examined using the pentylenetetrazol (PTZ) induced seizures test.

Results: The composition of 8 alkaloid fractions (4 each for aerial and underground parts) has been studied, revealing six dominant alkaloids. Diterpene bases methyllycaconitin, anthranoyllycoctonine, delcosine, and lycoctonine predominate in both aerial and underground organs of D. flexuosum, while the underground organs additionally contain alkaloids songorine, norsongoramine, and delectin. Pharmacological tests revealed that three fractions from the ground and two from the underground parts exhibited a short-time analgesic activity. In contrast, none of the studied fractions appeared to have notable anticonvulsant activity.

Conclusions: The observed activity of the investigated alkaloids is based on central rather than peripheral mechanisms of analgesia by breaking impulse conductivity at different stages of passing through nociceptive pathways.

Keywords: Analgesic activity; Delphinium flexuosum; Diterpene alkaloids.

BACKGROUND

Due to diterpene alkaloids, Delphinium (Larkspur) species are considered very toxic plants. The latter are neurotoxic agents, causing bradycardia, muscle system spasms, hypotension, and death by arrest of respiration. At the same time, many studies claim that the crude extracts from the whole plant containing diterpene alkaloids exhibit diverse pharmacological effects: antibacterial, analgesic, anti-inflammatory, antidepressant, and muscle relaxant.[1] In recent years, more than 150 alkaloids, mainly diterpene ones, were isolated from plants of Delphinium, including C-18, C-19 alkaloids, and C-20 alkaloids. Chemical investigations were mainly focused on D. grandiflorum, D. anthriscifolium var., D. elatum, D. brunonianum, D. tiantaishanense, and D. pseudoaemulans.[1-7] Among 18 species of Delphinium represented in the flora of Georgia,[7] D. flexuosum M. Bieb. is poorly investigated; therefore, the present study aimed to study both the aerial and underground parts of D. flexuosum on the content of diterpene alkaloids and estimate their pharmacological potency.

METHODS

Materials and methods

D. flexuosum specimens in the flowering phase were harvested from Tsikhijvari, Georgia. A voucher specimen labeled TBPH-21405 is preserved at the Herbarium of the TSMU I. Kutateladze Institute of Pharmacochemistry.

Obtaining of alkaloids

The aerial and underground parts of D. flexuosum were air-dried and powder-grinded, treated with a 5% sodium carbonate solution to remove non-alkaloid impurities, and extracted with chloroform. The chloroform extracts were concentrated to one-fifth of their original volume, and alkaloids were extracted using a 5% aqueous solution of sulfuric acid. After cooling, the acidic extract was rinsed with diethyl ether, alkalinized with sodium carbonate to a pH of 9, and the alkaloids were then extracted using chloroform. After dehydration with anhydrous sodium sulfate and vacuum concentration, crude alkaloids were fractionated according to basicity using a modified polybuffer separation method.[8] Fractionation with citrate-phosphate buffers (pH

8.0 → 2,0) yielding eight fractions (4 each for aerial and underground parts of D. flexuosum).

Qualitative analysis of alkaloids in comparison with reference samples was conducted using TLC Silica gel 60 F_{254} plates (Merck, Germany) in the following systems: I – chloroform: methanol (6:1), II – chloroform: benzene: 95% ethanol: 25% ammonia (40:40:10:0.2). Detection of alkaloids was done using Dragendorff's reagent.[9,10]

United fractions with a pH of 2-5 and a pH of 6-8 were separated on a silica gel column (100/400), eluted with chloroform, and six mixtures of chloroform-methanol (100:1; 90:1; 50:1; 25:1; 5:1, 1:1). Alkaloids were identified using the 7890B GC System and the 5977B Single Quadrupole GC/MSD System (Agilent Technologies, USA). Alkaloids' melting point was determined by the IA 9100 melting point apparatus (Wenk Lab Tec, Germany).

Experimental animals

Inbred male albino mice (n=72, body weight: 26±2 g) were utilized and randomly divided into six groups. Animals were housed under standard conditions: temperature of 20±2°C, humidity at 40%, with a 12/12 hr. light/dark cycle, and provided with a standard rodent diet and water ad libitum. All animal maintenance and experimental procedures adhered to internationally accepted ethical guidelines.[10,11] Statistical analysis of experimental data was conducted using Student's t-test. All animal experiments were approved by the Tbilisi State Medical University Ethical Committee on Animal Experiments (approval #AP-61-2023).

PTZ-induced seizures assay

D.flexuosum fractions were administered intraperitoneally at doses of 10 mg/kg. Thirty minutes after administration, each animal was injected intraperitoneally with 80 mg/kg pentylenetetrazol (PTZ, Sigma, USA). After PTZ injection, mice were placed in a plexiglass acrylic cage, and seizures were recorded until convulsions stopped and the animals were fully recovered. Occurrence, severity, latency, and duration of seizures were analyzed, as well as the number of animals displaying seizures and death due to seizures.[12,13]

The severity of seizures was estimated by the following scale: 0 - no changes in behavior; 1 - isolated myoclonic jerks; 2 - atypical minimal seizures (unilateral, incomplete); 3 - total clonic seizure; 4 - a pattern of tonic-clonic seizures with a suppression of tonic phase; 5 - generalized tonic-clonic seizure and status epilepticus; Mortality percentage was recorded over 60 minutes, with animals surviving beyond one hour deemed protected.

"Hot plate" assay

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

RESULTS AND DISCUSSION

The study's findings reveal that the diterpene bases methyllycaconitin, anthranoyllicoctonin, delkosin, and lycoctonin predominate in both aerial and underground organs of D.flexuosum. In contrast, the underground organs additionally contain alkaloids songorine, norsongoramine, and delectin (Tab.1 and Fig.1).

TABLE 1. Dominant alkaloids from the aerial and underground parts of D.flexuosum Bieb.

Plant part	Fraction	pH	Dominant alkaloids	Cont (%)	m.p.	Cont (%)
Aerial	DFA-1	2.0 - 4.0	Methyllycaconitine	32	amorph.	682(M+), 667, 651(100), 649
	DFA-2	4.0 - 6.6	Delcosine Anthranoyllycoctonine	13 11	203-204 153-155	453(M+), 438(100), 436, 422, 420 586(M+), 571, 569, 555(100), 538, 523
	DFA-3	6.6 - 8.0	Lycoctonine	18	136-140	467(M+), 452, 450, 436(100), 418
Undergraund	DFU-1	2.0 - 4.0	Methyllycaconitine Delectine	30 4	amorph. 107-109	682(M+), 667, 651(100), 649 572 (M+), 557, 555, 541(100), 539, 120
	DFU-2	4.0 - 8.0	Anthranoyllycoctonine Delcosine Songorine Norsongoramine Lycoctonine	8 9 5 4 14	153-155 203-204 201-203 286-288 136-140	586(M+), 571, 569, 555(100), 538, 523 453(M+),438(100), 436,422,420 357(M+100), 340, 328, 315, 298, 246,180 327(M+), 310, 299, 281(100) 467(M+), 452, 450, 436(100), 418

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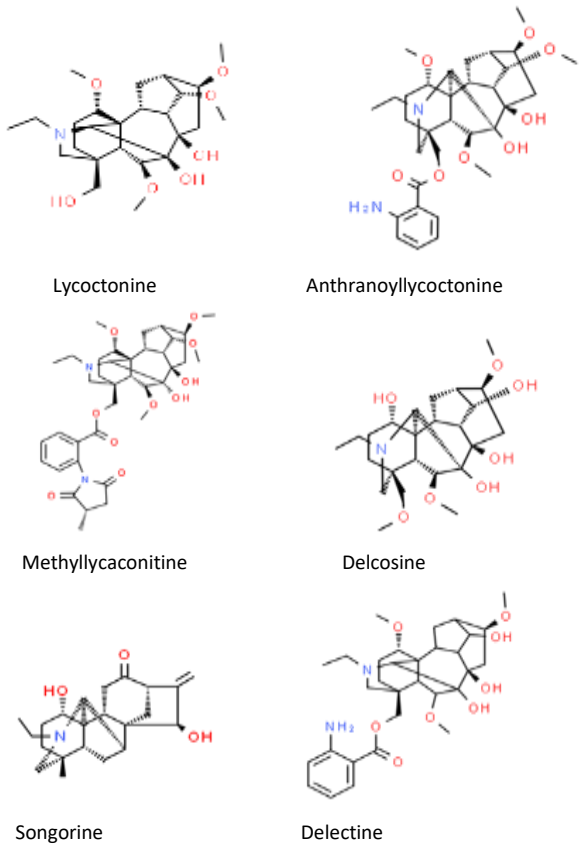
FIGURE 1. Dominant alkaloids from the aerial and underground parts of D.flexuosum Bieb.



Fractions DFU-1 and DFU-2 from the underground parts and DFA-1, DFA-2, and DFA-3) from the above-ground parts of D.flexuosum were evaluated in mice on the "Hot plate" model. The study compounds were administered intraperitoneally in doses of 10 mg/kg. The experiment results revealed a short-term analgesic activity of both DFU-1 and DFU-2 (Tab.2 and Fig.2).

TABLE 2. Antinociceptive effects of D.flexuosum fractions in "Hot plate" model in mice

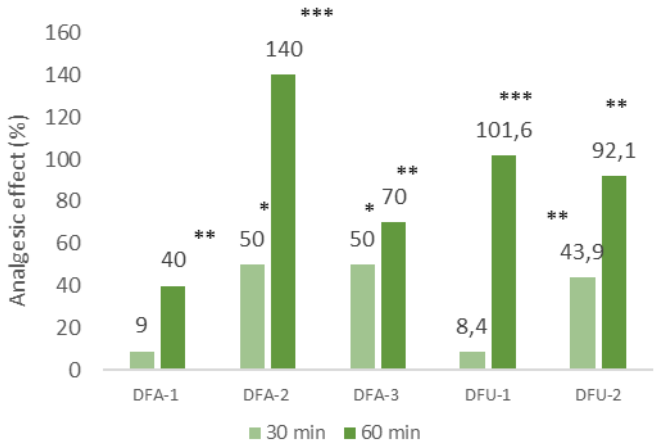
	Reaction latency (in seconds)					
Recroding time	**Control**	**DFA-1**	**DFA-2**	**DFA-3**	**DFU-1**	**DFU-2**
Basic	10.5±1.1	10.8±1.4	10.9±0.9	10.7±1.1	11.1±1.2	10.4±0.9
30 min	10.7±0.8	11.7±0.6	16.3±1.1*	16.1±1.8*	11.± 1.6	15.2±1.4**
60 min	9.9 ± 0.4	15.1±1.2**	26.1±2.1***	18.2±1.7**	21.3±1.4***	20.3±1.9***
90 min	10.2 ± 0.2	12.4 ± 0.8	19.1 ± 1.8	14.3 ± 0.8	16.1 ± 1.9	14.1 ± 1.2
120 min	10.1 ± 0.7	11.6 ± 1.3	12.1 ± 1.3	10.9 ± 1.4	10.0 ± 0.7	9.8 ± 0.6

Data represented as mean±S.E.M (n=6). * - p<0.05; ** - p<0.01 *** - p<0.001 vs control.

In a mouse model of pentylenetetrazol-induced convulsions (PTZ, 80 mg/kg intraperitoneally), it was determined that fractions neither from underground (DFU-1, DFU-2) nor aerial (DFA-1, DFA-2, and DFA-3) parts do not exhibit notable anticonvulsant properties. However, it should be mentioned that DFU-1 decreased the mortality of animals by 25%.

It is also important to acknowledge that doses exceeding 10 mg/kg for each fraction revealed toxic effects. Determining its composition and dominant LD50 component is necessary to establish suitability for additional DFA-2 testing.

FIGURE 2. Analgesic activity of D.flexuosum fractions in mice. Data represented as mean±S.E.M (n=6). * - p<0.05; ** - p<0.01 *** - p<0.001 vs control



CONCLUSIONS

The aerial and underground parts of D. flexuosum Beeb. were analyzed for their diterpene alkaloid content, and a preliminary assessment of their pharmacological efficacy was conducted. Our findings suggest that the observed activity of the investigated alkaloids is likely mediated through central rather than peripheral mechanisms of analgesia, involving the modulation of impulse conductivity at various stages along nociceptive pathways. These results are consistent with existing literature documenting the analgesic properties of diterpene alkaloids.

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