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PROTEINS FROM THE AERIAL PART OF *Delphinium leptocarpum* AND THEIR BIOLOGICAL ACTIVITY

Sh. Kh. Rakhimova,[1*] U. Kh. Kurbanov,[1] L. G. Mezhlumyan,[1] Z. I. Sanoev,[1] U. Zh. Ishimov,[2] Zh. M. Abdurakhmanov,[1] and N. I. Mukarramov[1]

The quantitative protein content of Delphinium leptocarpum *was 17.2%. Proteins, the amino-acid compositions of which featured high contents of leucine, cysteine, and aspartic and glutamic acids, were detected in the aqueous layer after extracting alkaloids from it. The anxiolytic and hypoglycemic activities of the studied protein were evaluated.*

Keywords: *Delphinium leptocarpum*, protein constituents, amino acids, anxiolytic and hypoglycemic activity.

The plant *Delphinium leptocarpum* (Nevski) Nevski is widely distributed in the foothills and on the plains of Uzbekistan [1]. Plants of the genus *Delphinium* contain mainly alkaloids. Leptanine [2], delsoline, delcosine, demethyleneeldelidine, 14-benzoylbrucine, and lycoctonine were isolated from the aerial part of *D. leptocarpum* [3]. The EtOH and $CHCl_3$ extracts of the aerial part of the plant were found to possess insecticidal activity [4].

Diabetes mellitus is currently considered a global medical and social problem in the world health system. According to the International Diabetes Federation (IDF), > 425 million diabetes patients were counted in the world in 2019 [5]. The medical and social importance of diabetes mellitus is explained by the number of serious disease complications, the high level of invalidism, and the mortality. Therefore, studies of the pharmacological and toxicological properties of local medicinal plants [6–10] are important to use them for prevention and treatment of this pathology.

Therefore, studies of proteins from *D. leptocarpum* after removal of alkaloids is a timely task.

A colorimetric method was used for quantitative determination of the protein content in the aerial part of *D. leptocarpum* and of protein after removal of alkaloids [11]. The protein content in the plant was 17.2% of its dry mass. The protein molecular mass was 6 kDa. Figure 1 shows for comparison that the molecular mass of the protein after removal of the alkaloids was also 6 kDa.

A modified Laemmli method of electrophoresis in polyacrylamide gel (PAAG, 12%) in the presence of sodium dodecyl sulfate (SDS PAAG) was used to compare the molecular masses [12, 13]. Protein markers (Invitrogen, USA) were used for the comparison.

PTC (phenylthiocarbamoyl) derivatives of free amino acids were synthesized by the Stiven method [14]. Table 1 presents the results for the amino-acid composition of the protein components from the aqueous layer after purification.

The total amino acids contained the whole set of essential amino acids (Table 1). The amino-acid composition of the proteins was dominated by aspartic and glutamic acids and amino acids with branched hydrocarbon chains, e.g., valine, leucine, and isoleucine. The proteins were balanced in nonessential amino acids. The amino-acid composition included a high content of total essential amino acids.

The acute toxicity (LD_{50}) of the protein from *D. leptocarpum* was determined and gave a value of 197.25 mg/kg in rats after intravenous administration; 10,000 mg/kg, after peroral administration; and 5818 mg/kg, after peroral administration to white mice.

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, 77 Mirzo Ulugbek St., Tashkent, 100170, e-mail: shahnoza2380@mail.ru; 2) A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, 83 Mirzo Ulugbek St., Tashkent. Translated from *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2023, pp. 627–629. Original article submitted October 31, 2022.

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TABLE 1. Composition of Free Amino Acids from *Delphinium leptocarpum* After Removal of Alkaloids

Nonessential amino acid	Concentration, mg/g	Essential amino acid	Concentration, mg/g
Asp	16.32	Thr	6.03
Glu	34.39	Val	9.44
Ser	5.10	Met	1.08
Gly	9.90	Ile	11.86
Cys	13.20	Leu	15.91
Arg	10.85	His	2.31
Ala	9.43	Phe	6.09
Pro	7.68	Lys HCl	6.54
Tyr	6.90	Total	59.26
Total	114.77		

TABLE 2. Effect of Protein from Aerial Part of *D. leptocarpum* on Locomotor and Exploratory Activity by Open Field Method

Sample	Number of locomotor activity	Number of exploratory activity	Number of rearings	Number of feces
Control	9.25 ± 1.2	12.25 ± 1.7	1 ± 0.02	2.75 ± 0.3
Protein, 50 mg/kg	16 ± 1.7* (+73%)	16.5 ± 1.3* (+35%)	0	3.25 ± 0.6
Protein, 100 mg/kg	14.5 ± 2.1* (+57%)	10 ± 1.2 (–19%)	1.25 ± 0.1	4.75 ± 0.8*
Protein, 150 mg/kg	12.25 ± 1.4* (+32%)	10.25 ± 0.8 (–16%)	0.25 ± 0.01	0.2 ± 0.01*

*$P \leq 0.05$ vs. the control group.

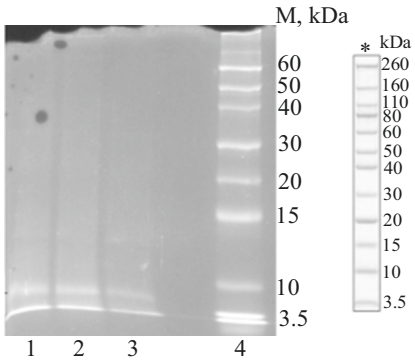

Fig. 1. Electrophoresis of *D. leptocarpum* proteins (1); aqueous layer of EtOH extract (2); after removal of alkaloids (3); and markers (4) in PAAG. *Novex Pre-Stained protein standard (Invitrogen) was used.

The influence of the protein from *D. leptocarpum* on the central nervous system after a single peroral administration was studied by the open field method for exploratory and locomotor activity [15]. Proteins at doses of 50, 100, and 150 mg/kg increased locomotor activity in the open field by 73, 57, and 32%, respectively, as compared to the control group and reduced exploratory activity at doses of 100 and 150 mg/kg by 19 and 16%; at a dose of 50 mg/kg, increased it by 35%. Table 2 presents the experimental results.

The anxiolytic activity of the protein was determined as a function of the number of fecal excretions. The experiment was conducted by studying the fecal excretion activity using the method of Sanberg et al. [16]. Fecal excretion at doses of 50, 100, and 150 mg/kg decreased by 37.5, 62.5, and 41.7% as compared to the control group. Therefore, the protein exhibited anxiolytic activity as compared to the control at all doses.

TABLE 3. Efficacy of Protein on Tolerance and Glucose Load

Sample	Sugar concentration, mM		Change of blood sugar, mM	Hypoglycemic effect, %
	before glucose load	after glucose load		
Control	7.34 ± 0.43	21.8 ± 1.3	14.46 ± 2.1	–
Protein, 50 mg/kg	7.4 ± 0.6	18.5 ± 2.3*	11.1 ± 0.3*	23
Protein, 100 mg/kg	7.5 ± 0.3	17.5 ± 1.7*	10 ± 0.43*	31
Asformin, 50 mg/kg	7.22 ± 0.8	20.54 ± 2.3	13.32 ± 1.3	8
Glempid, 0.2 mg/kg	6.36 ± 0.3	18.24 ± 1.8*	11.88 ± 1.2*	18

*$P \leq 0.05$ vs. the control group.

Various amino acids, including arginine and leucine, and amino acids with branched hydrocarbon chains such as valine, leucine, and isoleucine are known to play important roles in prevention of diabetes. Leucine stimulates insulin production by the pancreas, which normalizes the blood glucose level of diabetes mellitus patients. It was proposed that the amounts of above amino acids in the studied protein could play a key role in determining the level of hypoglycemic properties. The experimental results showed that glycemic curve of control rats changed as follows after glucose administration. First, the glucose level gradually rose. The maximum rise level was observed after 60 min (21.8 mM). Then, it began to decrease gradually. However, it did not reach the initial values (Table 3). The blood glucose level in test rats that received the proteins rose after 30 min and was less than in the control after 60 min by 23 and 31% while the reference drugs asformin (50 mg/kg) and glempid (0.2 mg/kg) caused a statistically significant decrease by 8 and 18%. Then, the glucose load curve began to decrease smoothly to the initial values.

Thus, the results established that the protein from the aerial part of *D. leptocarpum* after removal of alkaloids was a marginally toxic compound with respect to acute toxicity, increased locomotor and exploratory activity, and exhibited pronounced anxiolytic and hypoglycemic activity as compared to the drugs asformin and glempid after a single administration.

EXPERIMENTAL

Isolation and Purification of Protein. The aerial part (1 kg) of *D. leptocarpum* was dried, ground, and extracted with EtOH (80%, 3 × 10 L). The solvent was distilled to produce an aqueous solution (1.8 L) that was purified of impurities. Then, it was made basic (pH 9–10) using aqueous NaOH solution (5%) and extracting with $CHCl_3$ to afford total alkaloids (3.58 g, 0.36%). The aqueous layer retained a large amount of proteinaceous substances. The total protein content identified in the aqueous layer without losses was ~86% [11].

Determination of Amino-acid Composition After Removal of Alkaloids. Proteins or peptides in the aqueous extract were precipitated in centrifuge tubes, for which the studied sample (1 mL) was treated with trichloroacetic acid (1 mL, accurate volume, 20%). The precipitate was separated after 10 min by centrifugation at 8000 rpm for 15 min. The supernatant liquid (0.1 mL) was separated and lyophilized. The hydrolysate was evaporated. The dry solid was dissolved in Et_3N–MeCN–H_2O (1:7:1) and evaporated. The operation was repeated twice to neutralize the acid. Reaction with phenylthioisocyanate produced the phenylthiocarbamoyl (PTC) derivatives of the amino acids by the literature method [14].

PTC-amino-acids were identified on an Agilent Technologies 1200 chromatograph over a Discovery HS C18 column (75 × 4.6 mm) using solution A (0.14 M NaOAc + 0.05% TEA at pH 6.4) and B (MeCN) at flow rate 1.2 mL/min. Absorption at 269 nm was measured. The gradient (%B/min) was 1–6%, 0–2.5 min; 6–30%, 2.51–40 min; 30–60%, 40.1–45 min; 60–60%, 45.1–50 min; 60–0%, 50.1–55 min.

IR spectra of protein were recorded in KBr pellets on a Perkin-Elmer 2000 FTIR spectrophotometer. IR spectrum (KBr, ν_{max}, cm^{-1}): 3155 [-C(O)NH-], 2814 (CH_2), 1690 (C=O), 1405 [-CH_2-C(=O)-] tertiary alcohols (C-OH), 1104 broad peak, secondary alcohols (C-OH), 612 (O=C-N).

Acute Toxicity Study. Tests used outbred male white mice (18–22 g) and white rats (180–250 g) that were quarantined for 14 d under standard vivarium conditions. Acute toxicity of the proteins after removal of alkaloids was studied after peroral and intravenous administration to white rats (180–250 g) and outbred mice (18–22 g).

Hypoglycemic Activity Study. The blood glucose level was studied by peroral administration to rats of protein (50–100 mg/kg) isolated from the aerial part of *D. leptocarpum*. The reference drugs for the hypoglycemic activity study were asformin (50 mg/kg (active ingredient metformin hydrochloride; manufacturer, Turkiye) and glempid 0.2 mg/kg [17] (active ingredient glimepiride; manufacturer, Hungary). Hypoglycemic activity was assessed using the glucose tolerance test [18], i.e., 35% glucose solution at a dose of 3500 mg/kg for 24 h in fasted white rats. Blood from rats of each group was taken from a tail vein with fasting and then 30, 60, and 120 min after glucose administration. The glucose load was induced by intraperitoneal administration of glucose solution (3500 mg/kg) to all groups.

Influence of compounds on locomotor and exploratory activity was studied by the open field method [15] and fecal excretion using the method of Sanberg et al. [16]. Test were conducted on 30 outbred rats (180–250 g) in five groups with six animals in each. All animal experiments were conducted in compliance with requirements of international recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [ETS No. 123, Strasbourg (1986)]. Experimental results were processed by statistical analysis methods.

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