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PHENOLIC GLYCOSIDE – ARBUTIN FROM THE PLANT *ONOBRYCIS GRANDIS* LIPSKY AND ITS BIOLOGICAL ACTIVITY

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ABSTRACT

From the aerial part of the plant Onobrychis grandis Lipsky (Fabaceae) growing in the territory of Uzbekistan, the phenolic glycoside arbutin was isolated. The parameters of the acute toxicity of arbutin were determined, its analgesic activity was studied in the tests "hot plate," "acetic writhing" and "acetylcholine writhing." It was shown that arbutin belongs to low-hazard substances (Class IV according to GOST 12.1.007-76) and practically non-toxic compounds (Class VI according to the classification of A.V. Stefanov).

The compound showed pronounced analgesic activity in thermal and chemical (acetic acid) irritation, but did not show a significant effect in the acetylcholine writhing test.

Introduction

Medicinal plant preparations are widely used in medical practice both for the treatment and prevention of various diseases. Plants of the legume family (Fabaceae), with triterpenic and phenolic glycosides, attract special attention. In this regard, the chemical composition of the plant *Onobrychis grandis* Lipsky, belonging to the Fabaceae family, as well as its acute toxicity and analgesic activity, were studied. Previously we investigated cycloartane triterpenoids from plants of the genus *Astragalus* belonging to the same Fabaceae family [1–3].

The fatty acid composition of the plant *O. grandis* growing in the territory of Turkey was studied earlier [4], however the chemical composition of the plant of this species growing in the territory of the Republic of Uzbekistan has been little studied.

The genus *Onobrychis* includes perennial herbaceous plants of the legume family (Fabaceae). The main center of their diversity extends from Central Asia to Iran. Currently about 150 species are known. These plants have high nutritional value, were previously

widely used as feed for heavy draft horses, and are also an excellent source of nectar for honey production. Purpose of the study: to isolate and characterize individual compounds from the aerial part of *O. grandis*, to study the acute toxicity and analgesic activity of arbutin. In Uzbekistan this plant grows in the mountainous regions of Kashkadarya and Samarkand regions [5, 6].

Materials and Methods

From the aerial part of this plant, an individual phenolic glycoside – arbutin – was isolated (Fig.) [7].

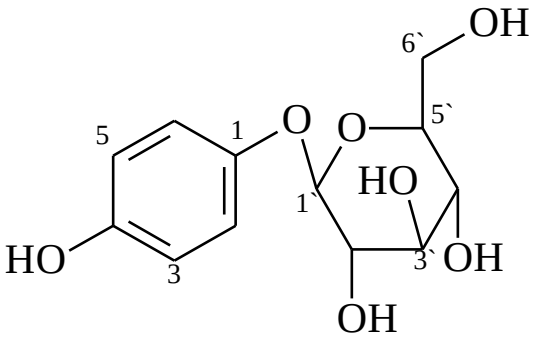

Fig. Arbutin

The structure of arbutin was established based on the analysis of ^{1}H and ^{13}C NMR spectra data (table 1), as well as HSQC and HMBC experiments. Air-dried, ground aerial parts (1 kg) of *O. grandis* were extracted 7 times with 5 L of methanol at 25 °C for 24 hours. The combined alcoholic extract was concentrated by evaporation under reduced pressure and at a temperature of 40–50 °C until a thick resinous mass was obtained.

Table 1.

Chemical shifts of ^{1}H and ^{13}C NMR of arbutin

(CDCl3, 77.16, δ, ppm, J/Hz, 600 MHz)

Atom C	δ_C	δ_H(J/Гц)
1	152.41	
2,6	119.38	6.96, d (8.9)
3,5	116.61	6.69, d (8.9)
4	153.77	
1`	103.61	4.73, d (7.4)
2`	74.96	3.41, m
3`	77.97	3.43, m
4`	71.41	3.37, m
5`	78.00	3.37, m
6`	62.54	3.70, dd (12.1; 5.4); 3.88, dd (12.1; 1.7)

Distilled water (100 ml) was added to the resulting resinous mass with vigorous stirring to obtain a colored homogeneous solution. The resulting aqueous solution was then washed (7 times) sequentially with chloroform (300 ml). After this, the aqueous solution was extracted (5 times) with butanol, the extract was evaporated in a vacuum

rotary evaporator, and the residue was dried. The yield of extractive substances was 146.4 g (14.6% of the air-dry raw material).

The extract was chromatographed on silica gel. Column chromatography was carried out with elution using a chloroform-methanol solvent system with a sequential change in the component ratio (9:1 and 4:1). As a result, 2.1 g of arbutin (0.021%) was isolated, m.p. 197.5 °C (lit. data 199 °C), Rf = 0.4 (solvent system: $CHCl_3$-MeOH-H_2O; 4:1:0.1).

Evaluation of the acute toxicity and analgesic activity of arbutin was carried out on white outbred mice kept under standard vivarium conditions in accordance with the rules adopted by the "International Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [8].

The study of the parameters of acute toxicity was conducted in experiments on white outbred male mice weighing 19–21 g. The test substance was administered orally using an atraumatic metal probe in doses of 1000–3000–8000–10000–15000–20000–30000 mg/kg as a 20% aqueous solution. Each dose of the preparation was tested on 6 mice. After a single administration of the preparation, the condition of the test animals was observed for 14 days. Higher doses (starting from 15000 mg/kg) were administered fractionally. The median lethal dose was determined by the Litchfield and Wilcoxon method [9].

At doses up to 20000 mg/kg, arbutin caused increased motor activity and accelerated heart rate. At a dose of 30000 mg/kg, suppression of the general condition, decreased motor activity, shallow breathing, reduced response to external stimuli, clinic tremor, and death of some animals within 8–10 hours were observed. The median lethal dose (LD_{50}) for oral administration was 27680 (24714–31001) mg/kg. Thus, according to the parameters of acute toxicity for intragastric administration, arbutin belongs to low-hazard substances (Class IV) according to GOST 12.1.007-76, and to practically non-toxic substances (Class VI) according to the classification of A.V. Stefanov [10].

Study of the effect of the test substance on the behavior of mice in the open field The study of the effect of the test substance on the central nervous system was conducted in experiments on white male mice weighing 18–20 g in open field conditions [11]. Observations of the behavior of the test animals were carried out for 2 minutes, 60 minutes after intragastric administration of the test substance at doses of 50.0 – 100.0 – 150.0 – 200.0 mg/kg. Motor activity was assessed by the number of squares crossed by the animals, exploratory activity by the number of hole visits, and the degree of orienting activity by the number of rearings on the hind legs. Each dose of the test substances was tested on 10 mice. The control group of animals under the same experimental conditions and in the corresponding volume received sterile water for injection. Experiments were conducted in comparison with the drug piracetam (Ukraine) at a dose of 400 mg/kg. The control group of animals under the same experimental conditions received sterile isotonic sodium chloride solution. The results of the conducted studies are presented in table 2.

Table 2.

Effect of arbutin on the motor activity of mice in the "Open field" test (n=10)

№	Substance	Dose, mg/kg	Horizontal movements		Vertical struts		Inspection of openings	
			percentage	%	percentage	%	percentage	%
1.	Control (saline solution)	0,2	12,7±1,9	100	5,0±1,7	100	5,2±1,4	100
2.	Piracetam	400.0	15,2±2,2	20,0	8,4±1,4	68,0	11,2±2,4	115,3
3.	Arbutin	50.0	14,0±1,9	10,2	6,5±1,7	30,0	6,2±2,6	19,2
		100.0	15,2±2,3	20,0	12,3±1,9	146,0	10,5±2,4	102,0
		150.0	16,5±2,9	30,0	10,7±1,6	114,0	12,0±2,4	130,0
		200.0	13,7±1,9	7,8	5,4±1,5	8,0	7,8±1,92	50,0

Note: *P=0.05

As can be seen from the presented data, arbutin, depending on the administered dose, increased spontaneous motor activity compared to the control animals. When the optimal dose was exceeded, the stimulating effect of the substance changed to the opposite – the motor activity of the test animals decreased, which is observed with many substances of stimulating action.

The orienting reaction (vertical rearing of the animals) also depended on the administered dose of the substance. At doses of 100.0 – 150.0 mg/kg, the test compound exceeded piracetam in activity.

Comparative study of the analgesic effect of arbutin and ibuklin

The analgesic activity was studied in the hot plate test [12, 13] on 70 white outbred mice weighing 18–20 g. The test substances were administered intragastrically 60 minutes before thermal stimulation. The animals were placed on a metal surface heated to 58 °C and surrounded by a cylinder. The time from placement on the hot surface to the appearance of a behavioral response to pain stimulation (licking of the hind paws, jumping, withdrawal of the hind paw) was recorded. A statistically significant increase in the latency period of the reaction to thermal stimulation after administration of the substance was considered the criterion of the analgesic effect.

Table 3.

Analgesic effect of arbutin and ibuklin in mice under thermal pain stimulation, n=10

№	Substance	Dose, mg/kg	Latent period of pain response		
			Before administration	After administration after 150 min	
			sek.	sek.	%
1	Control, distilled water	0,2 мл	14,2 ± 3,1	16,5±2,9	-
2	Arbutin	50,0	14,0±2,9	23,6±2,2	68,5*
		100,0	14,5±2,6	21,2±2,6	46,2*
		150,0	14,8±2,4	23,7±2,4	60,1*

		200,0	14,2±3,4	22,5±2,9	58,4*
3	Ibuclin	10,0	14,5±3,1	25,2±2.2	73,7*
		20,0	14,0±2,7	27,7±2,6	97,8*

Note: *P=0.05

The experimental results were statistically processed using the R.B. Strelkov coefficient; differences were considered significant at p<0.05 [14]. The results are presented in table 3.

The effect of the studied substances under chemical irritation was examined in the acetic acid writhing test on mice weighing 18–20 g. A 2.5% solution of acetic acid was administered intraperitoneally at a dose of 250 mg/kg. The number of "writhes" was counted over 20 minutes. Arbutin was administered orally at doses of 50.0–100.0–150.0–200.0 mg/kg 60 minutes before the injection of acetic acid. The effect was evaluated by the reduction in the number of "writhes" compared to the control animals, in comparison with ibuklin at doses of 10.0–20.0 mg/kg. The control and experimental groups included 10 mice each [15].

The studies showed that arbutin at all studied doses possesses analgesic activity (table 4).

Table 4.

Analgesic activity of arbutin under chemical irritation (acetic acid writhing test), n=10

№	Substance	Dose, mg/kg	Number of writhings in 20 minutes	Efficiency, %
1	**Control Acetic acid 2.5% 250 mg/kg**	0,2 мл	42,0±3,1	-
2	**Arbutin**	50,0	26,0±2,6	**62,0***
		100,0	21,0±2,2	**50,0***
		150,0	23,3±2,9	**56,0***
		200,0	22,4±2,4	**53,4***
3	**Ibuclin**	10,0	31,0±2,2	**73,9***
		20,0	35,0±2,9	**83,4***

Note: *P=0.05

The effect of the substances on chemical irritation was also studied in the acetylcholine writhing test. The analgesic effect was determined by the number of writhes in each animal within 20 minutes after intraperitoneal administration of acetylcholine at a dose of 3.2 mg/kg. Experiments were conducted on 70 outbred male mice weighing 18–20 g.

Arbutin was administered once into the stomach at doses of 50.0–100.0–150.0–200.0 mg/kg. Under similar conditions, the reference drug ibuklin was administered at

doses of 10.0–20.0 mg/kg [16, 17]. In this test, the studied substance did not show pronounced activity compared to the control (table 5).

Table 5.

Analgesic activity of arbutin and ibuklin under acetylcholine irritation, n=10

№	Substance (n=10)	Dose, mg/kg	Number of writhings in 10 minutes	Efficiency, %
1	Control acetylcholine 3.2 mg/kg	0,2 мл	11,5±1,9	-
2	Arbutin	50,0	10,2±1,4	**11,3**
		100,0	9,8±1,7	**14,7**
		150,0	9,5±2,2	**17,3**
		200,0	11,8±2,4	-
3	Ibuclin (peros)	10,0	9,0±1,9	**21,7**
		20,0	7,5±1,4	**34,7***

Note: *P=0.05

CONCLUSION

Thus, arbutin at doses of 50.0–100.0–150.0–200.0 mg/kg increases the threshold for thermal pain stimulation and exhibits pronounced analgesic activity under chemical irritation, reducing the number of acetic acid writhes, but in the acetylcholine writhing test it does not show pronounced activity.

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