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Saponins of *Dipsacus Azureus* Plant and Their Hypocholesterolemic Activity

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Abstract: The aerial part of the plant *Dipsacus azureus* was extracted with 80% ethanol. The extract was concentrated and precipitated with acetone (sum of saponins), and the remaining residue was fractionated into aqueous, *n*-butanol and chloroform parts. The acetone-precipitated precipitate (sum of saponins) and aqueous fractions were examined for hypocholesterolemic properties.

Keywords: saponins, *Dipsacus azureus*, hypocholesterolemic activity, extract, fraction.

Introduction. The genus *Dipsacus* belongs to the family Dipsacaceae, represented by 92 species worldwide, of which 2 species grow in Uzbekistan: *D. laciniatus* (L) and *D. azureus* (Schrenk) [1-2].

The perennial herbaceous plant *Dipsacus azureus* Schrenk (azure hairweed) belongs to the Dipsacaceae family, distributed mainly along the northern slope of the Kyrgyz Ala-Too ridge in the Chui region, in Uzbekistan in the Tashkent, Fergana, Surkhandarya, Samarkand and Andijan regions [2].

According to the literature data, *D. azureus* differs from all known saponin-containing plants by the highest content of saponins, up to 18.9% of the root mass [3]. Previous phytochemical study of *D. azureus* roots revealed the presence of triterpenoids, alkaloid, coumarin, flavonoid, and triterpene glycosides dipsacoside A4 and dipsacoside B [4–9].

Saponins are a diverse group of naturally occurring active compounds widely found in the plant kingdom, and they are active components of over 100 families, including terrestrial and marine endophytic fungi [10]. From a chemical point of view, the term "saponin" refers to a specific group of molecules, including glycosylated steroids, steroidal alkaloids and triterpenoids. Saponins are divided into two main classes: triterpene and steroid [11]. A number of studies have shown that saponins from various sources reduce serum cholesterol levels in various animals, including humans [12].

The *D. azureus* plant has been used as a traditional medicinal plant for rheumatism, skin ulcers, and stomach cancer. In the experiment, it has an analgesic and stimulating effect on the cardiovascular system [13].

Materials and methods. As a promising source of triterpene saponins, for the purpose of detailed study, the aerial part of *D. azureus* was collected during the flowering period in the Tashkent region of the Republic of Uzbekistan in the city of Tashkent. Raw material identified by O.M. Nigmatullaev in the laboratory of medicinal and industrial plants of the Institute of Chemistry of Plant Substances. acad. S.Yu.Yunusov Academy of Sciences of the Republic of Uzbekistan (herbarium number 2027).

The whole plant was air-dried, packed in paper bags and stored in a cool, dark place. The air-dry aerial part of *Dipsacus azureus* (3 kg) was crushed and extracted with 80% aqueous ethanol at room temperature, and 300 g of dry extract was obtained after vacuum evaporation (saponins precipitated with acetone). The dry extract was suspended in water (1 L), then successively extracted with chloroform (1x2 L), *n*-butanol (1x2.0 L), the resulting extracts were concentrated in a vacuum, as a result, fractions of chloroform, *n*-butanol extracts and an aqueous layer were obtained.

In vivo screening for the hypocholesterolemic activity of the aqueous layer and the amount of saponins obtained from this plant was carried out.

Experimental pharmacological part. The acute toxicity of the studied substances from *Dipsacus azureus* was assessed on white outbred mice - females weighing 18-20 g and on rats weighing 180-220 g, kept under standard vivarium conditions in accordance with the rules adopted by the "International Convention for the

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Protection of Vertebrate Animals used for experimental and scientific purposes" (Strasbourg, 1986) [14]. The test substances were administered orally using an atraumatic metal probe at doses ranging from 1000.0 to 13000.0 mg/kg. Each dose was tested on 6 mice and 6 rats. After a single application of the extracts, the condition of the experimental animals was observed for 14 days. The mean lethal dose was determined by the Litchfield and Wilcoxon method [15].

We have studied the hypocholesterolemic activity of the studied substances [16]. The experiments were carried out on white outbred rats weighing 200-220 g. Blood was taken by puncture of the tail vein. The studied substances were administered orally using an atraumatic metal probe at a dose of 3500 mg/kg. Endogenous hyperlipidemia was induced by daily fasting of animals after prophylactic administration of the drug. The experiments were carried out with daily, 5- and 10-day prophylactic administration of the studied extracts.

Cholesterol levels were determined in serum using enzymatic colorimetric tests manufactured by Langdorpsesteenweg, Langdorp-Belgium on a Basic SECOMAM biochemical analyzer, Anova Analytics company, FRANCE, following the manufacturer's instructions at a wavelength of 505 nm and a temperature of 37°C, a 1 cm cuvette.

Results and discussion. With oral administration of an aqueous extract at a dose of 1,000.0-2,000.0 mg/kg, limitation of motor activity, depression of the general condition, weakness, and drowsiness were noted after 2-3 minutes. The reaction to pain and sound stimuli is preserved. The death of experimental animals within 14 days of observation was not observed.

With an increase in the dose (from 5,000.0 to 10,000.0 mg/kg), the picture of intoxication became more pronounced, respiratory failure, head tremor, convulsions and death of some of the experimental animals were noted within 30-150 minutes after oral administration of the extract. The initial lethal dose for mice was 10,000.0 mg/kg. 100% death of mice was observed with the introduction of a dose of water extract 12,500.0 mg/kg. The picture of poisoning in rats did not differ from the picture of intoxication in mice. LD_{50} for rats was 12,100.0 (10,614.0 - 13,794.0) mg/kg, for mice - 11,700.0 (10,173.9 - 3,455.0) mg/kg.

With the introduction of the sum of saponins at a dose of 1000.0 to 6000.0 mg/kg, mice experienced a noticeable depression of the general condition, limitation of motor activity, increased tone and tension of the motor muscles, and short-term increased respiration. When the dose was increased from 7,000.0 to 10,000.0 mg/kg, limitation of motor activity, tremor, individual muscle twitches, convulsions from the second minute after administration, and a lateral position were observed. At a dose of 9300.0 mg/kg, the death of 2 animals was observed, at a dose of 9500.0 mg/kg - 3 animals, at a dose of 9700.0 mg/kg - 4 animals out of six within 4-90 minutes. A dose of 10,300 mg/kg was absolutely lethal. A similar picture of poisoning was observed in experiments on rats. LD_{50} for mice was 9500.0 (8333.3 - 10830.0) mg/kg, for rats - 9850.0 (8716.8 -11136.1) mg/kg.

The study of the effect of *Dipsacus azureus* extracts on the cholesterol content in the blood serum of rats in comparison with Roxera (rosuvastatin) (KRKA, Slovenia) under conditions of endogenous hyperlipidemia caused by daily fasting showed a difference in the effect on cholesterol levels. In the control group of animals during starvation, an increase in serum cholesterol by 54.4% was observed. An aqueous extract of Dipsacus azureus at a dose of 200 mg/kg after 5 days of administration caused an effect similar to Roxer's preparation at a dose of 1.0 and 5.0 mg/kg. The cholesterol level under the influence of these drugs decreased by 42.1 and 47.1%, respectively, compared with the control (table 1).

Table 1

The effect of 5-day administration of an aqueous extract of *Dipsacus azureus* on the cholesterol content in the blood serum of animals with endogenous hyperlipidemia caused by daily fasting (M+m, n=6)

№	Experience conditions	Dose, mg/kg	Cholesterol, mg/dl	Effect, %
1	Intact		48.7±0.384	-
2	Control+GP		75.2±0.297*	+54.4
3	Water extract D. azureus + GP	50.0	66.4±0.317**	- 11.7
		100.0	59.7±0.348**	- 20.6
		150.0	51.3±0.369**	- 31.7

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		200.0	43.5±0.275**	- 42.1
4	Roxera+GP	1.0	41.2±0.306**	- 45.2
		5.0	39.8±0.268**	- 47.1

*Note. (p<0.05) GP-hyperlipidemia; *-significance of differences with intact animals. **-significance of differences with the control group.*

With a 10-day administration of the aqueous extract in hyperlipidemic rats, cholesterol levels decreased in proportion to the administered dose (Table 2).

Table 2

Effect of water extract of *Dipsacus azureus* on cholesterol content in blood serum of rats after 10-day administration (M+m, n=6)

№	Experience conditions	Dose, mg/kg	Cholesterol, mg/dl	Effect, %
1	Intact		27.6±0.269	-
2	Control+GP		38.2±0.324*	+38.4
3	Water extract D. azureus + GP	50.0	32.5±0.285**	- 14.9
		100.0	31.0±0.314**	- 18.8
		150.0	25.7±0.348**	- 32.7
		200.0	23.2±0.279**	- 39.2
4	Roxera+GP	1.0	21.8±0.367**	- 42.9
		5.0	20.1±0.304**	- 47.3

*Note. (p<0.05) GP-hyperlipidemia; *-significance of differences with intact animals. **-significance of differences with the control group.*

The data showed that in the control group of animals during starvation, the content of cholesterol in the blood serum increases by 38.4%. An aqueous extract of *Dipsacus azureus* at a dose of 200 mg/kg after 10 days of administration showed a similar effect with Roxer's preparation at a dose of 1 mg/kg. At the same time, the cholesterol level decreased by 39.2 and 42.9%, respectively, compared with the control.

The sum of saponins at a dose of 200 mg/kg with a 5-day administration causes a similar effect with Roxer's drug at a dose of 5 mg/kg. The level of cholesterol under the influence of these substances at the indicated doses decreased by 52.3 and 59.7%, respectively, compared with the control (table 3).

Table 3

Effect of total saponins on serum cholesterol in rats with endogenous hyperlipidemia after 5-day administration (M+m, n=6)

№	Experience conditions	Dose, mg/kg	Cholesterol, mg/dl	Effect, %
1	Intact		47.3±0.436	-
2	Control+GP		72.6±0.375*	+53.4
3	The sum of saponins + HP	50.0	50.2±0.285**	- 30.8
		100.0	46.7±0.348**	- 35.6
		150.0	37.1±0.413**	- 48.8
		200.0	34.6±0.298**	- 52.3
4	Roxera+GP	1.0	32.4±0.362**	- 55.3
		5.0	29.2±0.394**	- 59.7

*Note. (p<0.05) GP-hyperlipidemia; *-significance of differences with intact animals. **-significance of differences with the control group.*

The lipid-lowering effect of the sum of saponins under conditions of endogenous hyperlipidemia was also studied with a longer administration (10 days).

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Table 4

Influence of total saponins on cholesterol content in blood serum of rats with endogenous hyperlipidemia after 10-day administration (M+m, n=6)

№	Experience conditions	Dose, mg/kg	Cholesterol, mg/dl	Effect, %
1	Intact		45.7±0.345	-
2	Control+GP		63.2±0.426*	+38.2
3	The sum of saponins + HP	50.0	50.3±0.349**	- 20.4
		100.0	48.5±0.263**	- 23.2
		150.0	39.2±0.321**	- 38.0
		200.0	25.7±0.276**	- 59.3
4	Roxera+GP	1.0	27.9±0.382**	- 55.8
		5.0	22.4±0.431**	- 64.5

*Note. (p<0.05) GP-hyperlipidemia; *-significance of differences with intact animals. **-significance of differences with the control group.*

The data showed that the control group of animals had an increase in serum cholesterol by 38.2% (Table 4). On the 9-10th day of administration, high doses of the sum of saponins (150-200 mg/kg) led to a decrease in cholesterol levels lower than in intact animals, a similar effect was exerted by Roxera at a dose of 5 mg/kg. At the same time, the cholesterol level decreased by 59.3 and 64.5% compared with the control, respectively.

Conclusions. Thus, the conducted studies showed that the studied compounds have a hypocholesterolemic effect at doses of 1/60-1/50 LD50 were comparable with the activity of Roxera (rosuvastatin) at a dose of 5 mg/kg. According to the parameters of acute toxicity, the studied substances belong to the class of low-toxic substances.

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