
# Saponins of Dipsacus Azureus Plant and Their Hypoglycemic Activity

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ABSTRACT

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].

**Keywords:** Dipsacaceae, herbaceous plant

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 hypoglycemic activity of the aqueous layer and the total 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 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 hypoglycemic activity of the studied substances [16]. The experiment was 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 before the intraperitoneal administration of glucose solution 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.

The glycemic level was 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. LD50 for mice was 9500.0 (8333.3 - 10830.0) mg/kg, for rats - 9850.0 (8716.8 -11136.1) mg/kg.

The results of studying the influence of the studied substances on the level of glycemia are presented in Table. 1 and 2. In control animals, the level of glucose in the blood after intraperitoneal administration of glucose solution after 3 hours increased to 12.4 mmol/l.

**Table 1**

The effect of an aqueous extract of *Dipsacus azureus* on the level of glycemia in rats after a single injection (M±m, n=6)

| Experiment conditions | Dose, mg/kg | Blood glucose level, mmol/l | | Effect, % |
|---|---|---|---|---|
| | | initial | after 3 hours | |
| Control | 0.2 ml dis. water | 3.4±0.6 | 12.4±1.3 | - |
| Water extract D. azureus | 50.0 | 3.3±0.4 | 11.3±2.1* | 8.8 |
| | 100.0 | 3.2±0.9 | 10.1±1.8* | 18.5 |
| | 150.0 | 4.4±0.5 | 9.7±1.3* | 21.7 |
| | 200.0 | 3.4±0.7 | 9.2±1.9* | 25.8 |
| Metformin | 30.0 | 3.6±0.8 | 8.0±1.1* | 35.4 |

*Note. * - Reliably in relation to the initial level (p<0.05)*

Both extracts of *Dipsacus azureus* in healthy animals showed insignificant hypoglycemic activity, inferior to the activity of metformin (Metforvin) (Mediwin Pharmaceuticals, India). The most pronounced hypoglycemic effect of the studied substances was noted at the 3rd hour after a single injection at a dose of 150.0 - 200.0 mg/kg, for the aqueous extract - 21.7 and the total of saponins - 26.8%.

**Table 2**

Influence of alcohol extract (total saponins) on the level of glycemia in healthy rats after a single injection (M±m, n=6)

| Experiment conditions | Dose, mg/kg | Blood glucose level, mmol/l | | Effect, % |
|---|---|---|---|---|
| | | initial | after 3 hours | |
| Control | 0.2 ml dis. water | 3.4±1.2 | 13.4±2.3 | - |
| Total saponins | 50.0 | 3.5±1.8 | 12.7±1.7 | 5.2 |
| | 100.0 | 4.2±1.5 | 12.4±2.5 | 7.4 |
| | 150.0 | 4.5±2.1 | 9.8±1.9 | 26.8 |
| | 200.0 | 3.8±1.6 | 10.5±2.7 | 21.6 |
| Metformin | 30.0 | 3.6±1.8 | 8.0±2.3 | 40.2 |

*Note. * - Reliably in relation to the initial level (p<0.05)*

**Conclusions.** Thus, the conducted studies have shown that the studied compounds have a hypoglycemic effect, significantly inferior to the action of the drug metformin. According to the parameters of acute toxicity, the studied substances belong to the class of low-toxic substances.

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