Antiulcer Activity of Extracts of Ecdysteroid-Containing Plants of Genera *Lychnis* and *Silene* of the Caryophyllaceae Family

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We studied antiulcer activity of the extracts of ecdysteroid-containing plants of the Caryophyllaceae family: *Lychnis chalcedonica* L., *Silene viridi flora* L.Sp.Pl., and *Silene frivaldszkyana* Hampe. Experiments on the model of neurogenic and aspirin-induced ulcerogenesis showed unidirectional and pronounced gastroprotective effects of *S. viridi flora* and *L. chalcedonica* extracts comparable to the efficacy of famotidine. In these models, a course of intragastric treatment with the extracts reduced ulcerative lesions of all types.

Key Words: *Lychnis and Silene* extracts; antiulcer activity; ecdysteroids

Plants containing ecdysteroids are of great interest as a source of raw materials for the production of new medicines. A lot of drugs and biologically active additives were formulated on the basis of ecdysteroids in various fields of medicine, such as cardiology, endocrinology, transplantology, and immunology [1,10]. More than 460 ecdysteroids, polyhydroxylated sterols of different structures, are known, most of them are found in more than 100 representatives of the families of angiosperms. However, not all plant species characterized by a high content of ecdysteroids (up to 1-3%) are suitable for practical use in the technologies of obtaining phytoecdysteroids [1,3,9]. In this regard, the search for new sources of ecdysteroids or substances on their basis, which would be characterized by high activity, minimum doses, non-toxicity, rapidly excretion, and low cost, seems relevant to develop new drugs for therapy of socially significant diseases, in particular, gastrointestinal pathologies [10].

The following Caryophyllaceae plants were used in the study: *Lychnis chalcedonica* L., *Silene viridi flora* L.Sp.Pl., and *Silene frivaldszkyana* Hampe. Plants of this family synthesize secondary metabolites of different groups, such as flavonoids, triterpene glycosides, alkaloids, polyphenols, and ecdysteroids. Until recently, the plants of genera *Lychnis* and *Silene* were the least studied members of the Caryophyllaceae family in terms of chemistry and pharmacology, and information on their use mainly referred to traditional medicine [3]. *L. chalcedonica*, *S. viridi flora*, and *S. frivaldszkyana* have been introduced in Siberian Botanical Garden of the National Research Tomsk University as promising ecdysteroid-containing species [1,12]. A number of ecdysteroids was isolated from each plant species (2,22-diacetate 20,26-dihydroxyecdysone, 3,22-diacetate 20,26-dihydroxy-ecdysone from *S. viridi flora*, and 26-hydroxy integristeron-A from *S. frivaldszkyana* were isolated for the first time); their structures were determined by means of HPLC, nuclear magnetic resonance, and mass spectrometry [3,11,13]. Studied species of plants synthesize ecdysteroids, both common to all species *Lychnis* and *Silene*, and rare ones, namely, 26-hydroxy polipodin B, sileneoside A, sileneoside D, stachisterone D, viticosterone E, and 4(28)-dehydromakisterone A. The highest content of the major ecdysteroids, 20-hydroxyecdysone, is characteristic for the aerial portion of *S. frivaldszkyana* (1.5%), *S. viridi flora* (1.1%), and *L. chalcedonica* (0.4%). Flavonoid content in these plants was 1.3, 1.2 and 2.7%, respectively.

Pharmacological studies of *Lychnis* and *Silene* plants revealed antifungal, radioprotective (L. chalce-
donica), hemorheological (L. chalcedonica, S. tatarica, S. dioica) and antitumor (L. chalcedonica, S. viridiflora) activities previously unknown for the examined species and compounds isolated from them [2,3,6].

Here we studied antiulcer effect of dry extracts obtained by original technologies from *Lychnis chalcedonica*, *Silene viridiflora*, and *Silene frivaldszkyana* using generally accepted models of experimental ulcerogenesis.

**MATERIALS AND METHODS**

The work carried out on adult outbred female CD1 mice (*n*=108) and outbred female CD rats (*n*=84). First-category conventional animals were obtained from the Laboratory of Experimental Biomodels, E. D. Goldberg Research Institute of Pharmacology (certificate from October 7, 2012, veterinary certificate c.270 No. 0007293 from November 28, 2013). Maintenance of animals and design of experiments were approved by the ethics committee of E. D. Goldberg Research Institute of Pharmacology. Experiments were carried out in accordance with the rules established by European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and with Order of the Ministry of Health Care and Social Development of the Russian Federation No. 708н of August 23, 2010.

Initial extracts were prepared by exhaustive extraction of the dry-crushed raw material with 70% ethanol at 55°C. The ethanol extract was concentrated using an IKA RV 10 digital Rotary Evaporator at 40°C. Standardization of all three extracts was carried out by content of ecdysteroids chromatospectrophotometrically followed by spectrophotometry of ecdysteroid-containing eluates at 235-250 nm on a Shimadzu UV-1800 spectrophotometer [3-5].

Antiulcer effect of *S. viridiflora*, *S. frivaldszkyana*, and *L. chalcedonica* was studied in the models of neurogenic injury to the gastric mucosa, AA of the extracts of dry *S. frivaldszkyana* and *S. viridiflora* administered for 6 days depended on the dose and the tested object (Table 1). The extract of *S. frivaldszkyana* in doses of 100 and 200 mg/kg caused a trend of reduction in pinpoint ulcers (by 1.5 times) and strip ulcers (by 4.7 and 6.6 times, respectively). The suppression of large lesions formation in comparison with untreated animals was noted for the dose of 200 mg/kg. Despite the decrease in the mean number of ulcers by 1.7 times (100 mg/kg) and 1.8 times (200 mg/kg) in a volume of 0.2 ml of solvent per mouse; 25 and 50 mg/kg (*S. viridiflora*), 10, 25, and 50 mg/kg (*L. chalcedonica*) in a volume of 0.4 ml of solvent per rat. In control groups, animals received an equivalent volume of solvent (distilled water) in a similar mode. Famotidine, a second-generation histamine receptor blocker (5 mg/kg, rats), was used as the reference drug in a similar mode of administration. The proposed dose of the reference drug corresponded to the mean therapeutic dose for humans in accordance with the table of dose conversion [7]. In all experiments, the last dose of the test drugs was given one hour before ulcerogen.

The animals were sacrificed by cervical dislocation (mice) or CO₂ inhalation (rats). At necropsy, the stomachs were removed, cut along the lesser curvature, and rinsed with cold saline. The number and area of lesions were evaluated macroscopically in bright light using a magnifying lens; the lesions were subdivided into pinpoint, strip, and large. The mean number of ulcers per animal in the group and percentage of animals with ulcers were calculated. Pauls index (PI) was defined as the integral measure of the number of lesions by the formula:

\[
\text{PI} = \frac{\text{mean number of ulcers} \times \% \text{ of animals with ulcers}}{100}
\]

Antiulcer activity (AA) of the preparations was determined as the ratio of PI in the control group to PI on the experimental group. The test substance was considered active at AA≥2 [4].

The results were processed using the nonparametric Mann–Whitney test (*U*) and Fisher’s transformation (*φ*) [5].

**RESULTS**

In mouse model of neurogenic injury to the gastric mucosa, AA of the extracts of dry *S. frivaldszkyana* and *S. viridiflora* administered for 6 days depended on the dose and the tested object (Table 1). The extract of *S. frivaldszkyana* in doses of 100 and 200 mg/kg caused a trend of reduction in pinpoint ulcers (by 1.5 times) and strip ulcers (by 4.7 and 6.6 times, respectively). The suppression of large lesions formation in comparison with untreated animals was noted for the dose of 200 mg/kg. Despite the decrease in the mean number of ulcers by 1.7 times (100 mg/kg) and 1.8 times (200 mg/kg) in the gastric mucosa of the animals treated with the extract of *S. frivaldszkyana*, AA was <2 in both groups (Table 1).

High AA was revealed in 6-day course administration of *S. viridiflora* in doses of 50 and 100 mg/kg (Table 1). The decrease in the number of pinpoint and strip-like ulcers and suppressed formation of large ulcers (50 mg/kg) was expressed in statistically sig-