DOI 10.1007/s10517-014-2728-1
*Bulletin of Experimental Biology and Medicine, Vol. 158, No. 2, December, 2014 PHARMACOLOGY AND TOXICOLOGY* 

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

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 158, No. 8, pp. 190-194, August, 2014
Original article submitted March 20, 2014

We studied antiulcer activity of the extracts of ecdysteroid-containing plants of the *Caryophyllaceae* family: *Lychnis chalcedonica* L., *Silene viridiflora* 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. viridiflora* 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 viridiflora* 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. viridiflora*, and *S. frivaldszkyana* have been introduced in Siberian Botanical Garden of the National Research Tomsk State 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. viridiflora*, 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)-dehydro-makisterone A. The highest content of the major ecdysteroids, 20-hydroxyecdysone, is characteristic for the aerial portion of *S. frivaldszkyana* (1.5%), *S. viridiflora* (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-*

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*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. 708n 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 and acetylsalicylic ulcerogenesis. Stress injury was caused by the partial immobilization of mice gripping with forceps of the neck fold for 24 h, which led to the development of all types ulcerative lesions of gastric mucosa in animals [4]. For modeling aspirin-induced gastric injury, acetylsalicylic acid was administered through a gastric probe in the dose of 128 mg/kg in 0.5 ml saline twice with 4-h interval [4]. In this case, the test substances were introduced according to the following scheme: extract→in 1 h ulcerogen→in 4 h extract→after 1 h ulcerogen. The rats were sacrificed 18 h after the last administration of acetylsalicylic acid.

Prior to induction of ulcerogenesis, the test compounds were introduced as a course once daily via intragastric tube in doses of 50, 100, and 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 $CO_2$ 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:

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

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-

**TABLE 1.** Effects of the Extracts of *S. frivaldszkyana*, *S. viridiflora* and *L. chalcedonica* on the Process of Neurogenic Ulceration in the Gastric Mucosa of Outbred Female CD1Mice

| Group | Animals with ulcers, % | Number of ulcers per 1 mice ($X \pm m$) | | | Mean number of ulcers per 1 mice ($X \pm m$) | PI | AA, score |
|---|---|---|---|---|---|---|---|
| | | pinpoint | strip | large | | | |
| 6-day course | | | | | | | |
| Control ($n$=10) | 90 | 11.60±2.07 | 3.30±1.29 | 020±0.20 | 15.10±3.04 | 13.59 | – |
| *S. frivaldszkyana* | | | | | | | |
| 50 mg/kg ($n$=9) | 100 | 13.89±3.83 | 1.11±0.81 | 0.78±0.55 | 15.78±4.90 | 15.78 | 0.9 |
| 100 mg/kg ($n$=10) | 90 | 7.90±1.66 | 0.70±0.42 | 0.40±0.31 | 9.00±2.15 | 8.10 | 1.7 |
| 200 mg/kg ($n$=10) | 90 | 7.80±1.61 | 0.50±0.31 | 0 | 8.30±1.66 | 7.47 | 1.8 |
| *S. viridiflora* | | | | | | | |
| 50 mg/kg ($n$=9) | 77.8 | 6.89±2.45 | 0.44±0.44 | 0 | 7.33±2.75* | 5.70 | 2.4 |
| 100 mg/kg ($n$=10) | 90 | 7.00±1.81 | 0.30±0.21 | 0.30±0.21 | 7.60±2.08* | 6.84 | 2.0 |
| 200 mg/kg ($n$=10) | 100 | 7.10±2.11 | 2.10±0.48 | 0.40±0.22 | 9.60±2.90 | 9.60 | 1.4 |
| 4-day course | | | | | | | |
| Control ($n$=10) | 100 | 29.70±3.97 | 2.60±0.88 | 1.50±0.48 | 33.80±4.30 | 33.80 | – |
| *L. chalcedonica* | | | | | | | |
| 50 mg/kg ($n$=9) | 90 | 9.80±2.62** | 0.50±0.27 | 1.00±0.70 | 11.30±3.20** | 10.17 | 3.3 |
| 100 mg/kg ($n$=10) | 90 | 17.70±3.21* | 2.50±0.81 | 1.20±0.33 | 21.40±3.85* | 19.26 | 1.8 |
| 200 mg/kg ($n$=10) | 100 | 20.20±4.45 | 0.60±0.34 | 2.50±0.24 | 23.30±5.06* | 23.30 | 1.5 |

**Note.** Here and in Table 2: *$p$<0.05, **$p$<0.01 in comparison with the corresponding control.

nificant decrease in the mean number of ulcers by 2.1 times (50 mg/kg) and 2.0 times (100 mg/kg) relative to similar values in the control group. It should be noted that in the groups receiving *S. viridiflora*, the effect increased after the dose was reduced from 200 mg/kg to 50 mg/kg (from 1.4 to 2.4, respectively).

In the model of neurogenic injury, the extract of *L. chalcedonica* administered for 4 days exhibited maximum activity (AA score 3.3) in a dose of 50 mg/kg (Table 1). Significant reduction in the number of pinpoint ulcers (by 3 times), number of animals with strip ulcers (30% *vs.* 70% in control) and large lesions (30% *vs.* 80%), mean number of the ulcers (3 times) in comparison with the values in non-treated animals are worthy of note. The extract of *L. chalcedonica* in doses of 100 and 200 mg/kg contributed in the decrease in the number of pinpoint lesions by 1.7 times ($p$<0.01) and 1.5 times, respectively; strip ulcers (200 mg/kg), by 4.3 times in comparison with the control. Moreover, the dose of 200 mg/kg reduced the number of animals with strip lesions (30% *vs.* 70% in the control, $p$<0.05) and large ulcers (40% *vs.* 80%, $p$<0.05). Antiulcer effect in these two groups manifested in significant reduction in the mean number of the ulcers by 1.6 times (100 mg/kg) and 1.5 times (200 mg/kg) in comparison with similar values in non-treated animals.

Thus, expressed gastroprotective effect of the extracts of dried *S. viridiflora* and *L. chalcedonica* was demonstrated in the mouse model of neurogenic gastric mucosal injury. Identified effects may be implemented due to adaptogenic effect of ecdysteroids, which are the main active ingredients of extracts. Presently studied mechanisms of ecdysteroids interacting with membrane receptors as signaling molecules activating second messengers under stress influences, suggests an increased adaptive capacity of the mouse organism in conditions of immobilization stress [1,2,9].

In the rat model of acetylsalicylic gastric mucosal injury, gastroprotective activity of the extract o *S. viridiflora* (25 mg/kg) consisted in a significant decrease in the number of pinpoint ulcers by 1.7 times, the number of animals with strip lesions (55.6% *vs.* 90% in the control), and the number of strip lesions (by 6.6 times in comparison with the control; Table 2). Antiulcer efficacy of the extract (50 mg/kg) manifested in a decrease in the number of large ulcers by 2.1 times ($p$<0.05) in comparison with the corresponding parameter in the control. The use of famotidine in two series of experiments revealed pronounced gastroprotective effect of the test substances in a comparative aspect. Thus, the $H_2$-histamine receptor blocker was more effective (AA score 2.3) that the extract of

**TABLE 2.** Effects of the extracts of *S. viridiflora* and *L. chalcedonica* on the Process of Acetylsalicylic Ulceration in the Gastric Mucosa of Outbred Female CD Rats

| Group | Animals with ulcers, % | Number of ulcers per 1 mice ($X \pm m$) | | | Mean number of ulcers per 1 mice ($X \pm m$) | PI | AA, score |
|---|---|---|---|---|---|---|---|
| | | pinpoint | strip | large | | | |
| 8-day regimen | | | | | | | |
| Control ($n$=10) | 100 | 24.60±3.35 | 5.90±1.62 | 2.70±0.54 | 33.20±4.03 | 33.20 | – |
| Famotidine, 5 mg/kg ($n$=9) | 100 | 12.89±2.71** | 1.22±0.78* | 0.56±0.24** | 14.67±2.62** | 14.67 | 2.3 |
| *S. viridiflora* | 100 | 13.89±3.83 | 1.11±0.81 | 0.78±0.55 | 15.78±4.90 | 15.78 | 0.9 |
| 25 mg/kg ($n$=9) | 100 | 14.89±3.40* | 0.89±0.35* | 4.00±1.35 | 19.78±1.96**+ | 19.78 | 1.7 |
| 50 mg/kg ($n$=10) | 100 | 24.30±2.06 | 4.10±1.46 | 1.30±0.47* | 29.70±2.00++oo | 29.70 | 1.1 |
| Control ($n$=9) | 100 | 17.56±2.17 | 9.78±1.19 | 1.89±0.45 | 29.23±2.24 | 29.22 | – |
| Famotidine, 5 mg/kg ($n$=10) | 100 | 12.10±3.32* | 3.40±1.43** | 0.20±0.20* | 15.70±4.11* | 15.70 | 1.9 |
| *L. chalcedonica* | 90 | 7.00±1.81 | 0.30±0.21 | 0.30±0.21 | 7.60±2.08* | 6.84 | 2.0 |
| 10 mg/kg ($n$=8) | 100 | 15.38±4.05 | 8.38±1.82 | 0.88±0.48 | 24.63±4.14 | 24.63 | 1.2 |
| 25 mg/kg ($n$=9) | 100 | 10.56±2.17** | 5.11±1.03** | 0.22±0.15* | 15.89±2.35** | 15.89 | 1.8 |
| 50 mg/kg ($n$=10) | 90 | 8.70±1.40** | 4.50±1.43** | 0.30±0.21* | 13.50±2.54** | 12.15 | 2.4 |

**Note.** $^{+}p<0.05$, $^{++}p<0.01$ in comparison with famotidine, $^{oo}p<0.01$ in comparison with *S. viridiflora* in a dose of 25 mg/kg.

*S. viridiflora* in doses of 25 and 50 mg/kg (AA score 1.7 and 1.1, respectively).

After introduction of *L. chalcedonica* extract in a dose of 50 mg/kg, pronounced antiulcer effect superior to famotidine activity in the main integral criterion was observed (AA scores 2.4 and 1.9, respectively). The analyzed parameters in the compared groups did not differ significantly. Among the data on the efficacy of the extract of *L. chalcedonica* in doses of 10 and 25 mg/kg, we should note significant decrease in the number of animals with large ulcers (37.5 and 22.2%, respectively, *vs.* 77.8% in the control), the mean and number of different types of lesions (25 mg/kg) in comparison with the corresponding values in untreated animals (Table 2).

Experimental ulcerogenesis reproducing the factors of initiation and chronization of the ulcerative process in the gastroduodenal zone can provide obvious hints to make adequate conclusions about gastroprotective potential of the test substances [8]. A significant reduction in the damaging effect of acetylsalicylic acid as an ulcerogen with the high (166) inhibitory activity against COX-1 and COX-2 suggests that the extracts have a capacity to increase protective function of the mucosal barrier and resistance of the mucous membrane to the factors that disturb the production of prostaglandins. Ecdysteroids are known to stimulate protein-synthetic and reparative processes and prevent the disturbances of the general homeostasis [1,3,9].

Thus, the results of experiments carried out in the model of neurogenic and aspirin ulcerogenesis indicate unidirectional and pronounced gastroprotective action of the extracts of *S. viridiflora* and *L. chalcedonica*. From the obtained data it can be concluded that phytocomplexes can be regarded as a basis for developing new medications to treat gastroenterological diseases.

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