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Anti-Inflammatory and Analgesic Activities of the Complex of Flavonoids from *Lychnis chalcedonica* L.

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Anti-inflammatory and analgesic activities of the complex of flavonoids from *Lychnis chalcedonica* L. were studied in the models of acute aseptic inflammation induced by carrageenan, histamine, and serotonin and acetic acid-induced painful chemical stimulation. It is demonstrated that course treatment with flavonoids derived from *Lychnis chalcedonica* L. produced a stable pharmacological effect comparable with that of the reference anti-inflammatory drug diclofenac.

Key Words: *anti-inflammatory and analgesic activities; flavonoids*

Anti-inflammatory drugs have traditionally held a prominent position on the pharmaceutical market ranking third after antibiotics and cardiovascular preparations, but in view of numerous adverse effects [4], consumers' preferences are given to plant-derived antiphlogistics. In this context, the searching for novel plant-derived potent and nontoxic agents for the therapy of inflammatory diseases of different genesis remains a pressing problem.

Flavonoids are promising biologically active compounds for designing medications exhibiting anti-inflammatory and analgesic activities [1,4,14]. This group containing a large number of natural compounds with diverse structures is found in all plants and exhibits a variety of pharmacological activities. Anti-inflammatory, analgesic, wound-healing, antihypoxic, and stress-protecting effects of flavonoids extracted from Siberian plants have been revealed using various experimental models [5,6]. These compounds were also found to have anti-allergic, anti-anaphylactic, spasmolytic, bactericidal, radioprotective, estrogenic, hypotensive, and antitumor properties [4,14]. It is believed that the broad range of pharmacological effects of flavonoids is determined by their antioxidant properties that are essential in the therapy of various diseases [13,14].

Studies of the extracts derived from the aerial parts of *Lychnis chalcedonica* L. revealed their pronounced gastroprotective [2] and antitumor activities and hemorheological and cerebroprotective properties [7,8].

Here we studied anti-inflammatory and analgesic activities of the complex of flavonoids from *Lychnis chalcedonica* L. using on the model of acute aseptic inflammation induced by carrageenan and early inflammation mediators, histamine and serotonin, and on the model of painful chemical stimulation.

MATERIALS AND METHODS

Sixty-five outbred CD1 mice (males and females) and 60 female CBA mice (certified category I animals) were used for the experiments. The animals were obtained from the Laboratory of Experimental Biomodeling, E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (animal health certificate, veterinary certificate 270 No. 0008633). Animal keeping conditions and experimental design were approved by the Ethics Committee of E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (protocol No. 95092015) and com-

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plied with the Directive 2010/63/EU of the European Parliament and the Council of Europe Convention for the Protection of Vertebrate Animals Used for Scientific Purposes and the Order No. 708n of the Ministry of Health of the Russian Federation (August 28, 2010). The animals were euthanized after the experiments by cervical dislocation.

The study agent, complex of flavonoids from *Lychnis chalcedonica* L., was obtained at the Laboratory of Phytochemistry of the Tomsk State University by repeated extraction of air-dried plant material from the aerial part of *Lychnis chalcedonica* L., followed by filtration and drying. Identification of the compounds by HPLC, UV, and mass spectrometry showed that vicenin (C-glycoside apigenin) is the main component of the complex (87.4%), sugar components are presented by glucose and arabinose. Another flavonoid is presented by apigenin C-monoglycoside, neovitexin [12].

Animals of the experimental groups received a solution of flavonoids from *Lychnis chalcedonica* L. in a dose of 16 μg/kg (in 0.4 ml distilled water per mouse), which was found in earlier experiments with a broad range of doses (4-1600 μg/kg) to be the most effective one. Anti-inflammatory drug diclofenac (10 mg/kg) and antihistamine drug cetrine (1.5 mg/kg) were used as the reference drugs. The plant-derived complex and the reference drugs were administered intragastrically once a day for 5 days; the final dose was given 1 h prior to the damaging agent. The control animals received equivalent volume of distilled water by the same scheme. Acute exudative inflammation was simulated by subplantar injection of 0.05 ml of 1% carrageenan (sulfated polysaccharide extracted from carragheen moss), or 0.1% histamine dihydrochloride (Sigma), or 0.01% 5-hydroxytryptamine hydrochloride (Sigma) diluted with 0.9% NaCl into the right hind paw. Three hours after subaponeurotic injection of carrageenan or 60 min after injection of histamine, serotonin, or saline into the contralateral paw, we performed exarticulation at the ankle joints. The anti-inflammatory effect was assessed according to changes in edema weight and expressed in % compared to the control values [10].

Specific pain response was induced by intraperitoneal injection of 0.75% acetic acid solution in a dose of 0.1 ml per 10 g body weight. The latency of pain response (writhings) after acetic acid injection and pain response intensity (number of writhings for 15 min) were recorded [9]. Analgesic activity was assessed by suppression of nociceptive responses (reducing writhing count) and lengthening of pain response latency.

The results were processed by ANOVA using Student's t test and nonparametric Mann—Whitney U test (Statistica 6.0). The differences were significant at $p \leq 0.05$ [3].

RESULTS

Inflammation involves several sequential stages, each being characterized by release of various mediators and modulators of this process. Three phases of mediator release are observed in the model of carrageenan-induced edema: histamine is released during phase I; kinins, during phase II; and prostaglandins (PGs), during phase 3 [11]. Simulation of carrageenan-induced inflammation caused edema in the injured paw. In control mice, the weight of inflamed paw increased by 29.5% in comparison with the contralateral paw (Table 1). Administration of the complex of flavonoids from *Lychnis chalcedonica* L. produced a pronounced phlogolytic effect: increase in the weight of inflamed paw was 1.4-fold lower ($p \leq 0.01$) than in the control; edema inhibition was 29.6% (Table 1). Preventive treatment with diclofenac also prevented the increase in the paw weight by 1.5 times compared to the control values; edema inhibition in this case was 33.7% (Table 1).

Primary inflammatory mediators histamine and serotonin disturbed microcirculation in the pathological zone. In order to assess the effect of the complex of flavonoids derived from *Lychnis chalcedonica* L. on the initial stage of the inflammatory process, we selected the models of histamine- and serotonin-induced edema. Treatment of the animals with flavonoids under study had no anti-inflammatory effect for the hista-

TABLE 1. Effect of the Complex of Flavonoids Extracted from *Lychnis chalcedonica* L. on Anti-Inflammatory Activity in Male Outbred CD1 Mice Exposed to Carrageenan-Induced Inflammation ($\bar{X} \pm m$)

Group	Dose	Paw weight increase, %	Edema suppression, %
Control (n=10)	—	29.5±0.9	—
Diclofenac (n=11)	10 mg/kg	19.6±1.6****	33.7
Complex of flavonoids extracted from *Lychnis chalcedonica* L. (n=10)	16 μg/kg	20.8±1.0***	29.6

Note. Here and in Table 2: *p<0.05, **p<0.02, ***p<0.01, and ****p<0.001 in comparison with the control.

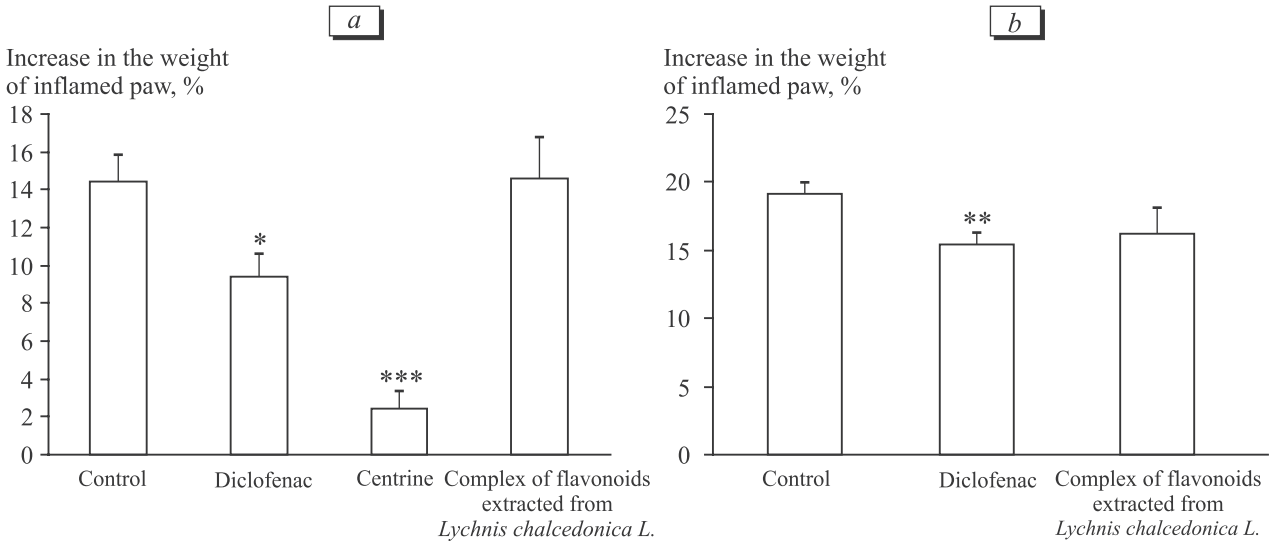

Fig. 1. Effect of the complex of flavonoids extracted from *Lychnis chalcedonica* L. on the development of histamine- (*a*) and serotonin-induced (*b*) acute inflammation in female CBA mice. *p<0.02, **p<0.01, ***p<0.001 in comparison with the control.

mine-induced inflammation model: paw edema did not differ from the control (Fig. 1, *a*). Preventive treatment of mice with the reference drugs under conditions of this model had a stable phlogolytic effect manifesting itself as a significant decrease in edema of the damaged paw with respect to the control values, 1.5-fold for diclofenac and 6-fold for citrine (Fig. 1, *a*). Edema inhibition was 34.9 and 83.2%, respectively. In mice with acute inflammation caused by serotonin, no positive effect of the complex of flavonoids extracted from *Lychnis chalcedonica* L. was revealed (Fig. 1, *b*). Administration of diclofenac in this model produced a significant anti-inflammatory effect (Fig. 1, *b*). Therefore, the complex of flavonoids extracted from *Lychnis chalcedonica* L. in a dose of 16 μg/kg has no phlogolytic properties with respect to early inflammation mediators histamine and serotonin.

The lack of anti-inflammatory effect for the models of histamine- and serotonin-induced edema but pronounced phlogolytic effect for the model of carrageenan-induced inflammation suggest that the complex of flavonoids from *Lychnis chalcedonica* L. has an inhibiting effect on late mediators of carrageenan-induced edema, prostaglandins and kinins.

The development of pain response in the model of acetic acid-induced writhing is known to be mainly associated with activation of kinins formed as a result of reduced pH and PG level [11]. Therefore, analysis of analgesic activity using this model will make it possible to verify the inhibiting effect of the complex of flavonoids under study on these mediators. Painful chemical stimulation contributed to the development of a typical pain response (writhing) in experimental animals (Table 2). Treatment with the complex of flavonoids extracted from *Lychnis chalcedonica* L. reduced the number of writhings by 1.9 times (p<0.02) in comparison with the control, while inhibition of pain response was 46.2% (Table 2). Furthermore, the latency of specific pain response in animals treated with the complex of flavonoids extracted from *Lychnis chalcedonica* L. increased 1.6-fold (p<0.05) in comparison with the control (Table 2).

Administration of the reference drug diclofenac also provided the analgesic effect manifesting in significant reduction of the writhing count by 1.6 times and 1.7-fold increase in the latency of pain response in comparison with the control (Table 2). Diclofenac resulted in 38.7% inhibition of the pain response.

TABLE 2. Effect of the Complex of Flavonoids Extracted from *Lychnis chalcedonica* L. on Analgesic Activity in Female Outbred CD1 Mice under Painful Chemical Stimulation Induced by Intraperitoneal Injection of Acetic Acid Solution ($\bar{X}$±m)

Group	Medication dose	Writhing count	Inhibition of pain response, %	Latency of the onset of writhing, sec
Control (n=12)	—	17.3±1.8	—	218.3±33.7
Diclofenac (n=12)	10 mg/kg	10.6±2.2*	38.7	380.0±62.2*
Complex of flavonoids extracted from *Lychnis chalcedonica* L. (n=10)	16 μg/kg	9.3±2.3**	46.2	351.6±42.3*

Using the resulting experimental data, one can draw a conclusion that the complex of flavonoids derived from *Lychnis chalcedonica* L. in a dose of 16 μg/kg exhibits anti-inflammatory and analgesic activities comparable with those of the reference drug diclofenac probably due to their inhibiting effect on late inflammation mediators (prostaglandins and kinins). Furthermore, the direct effect on the group of enzymes cyclooxygenases (COX) catalyzing the reaction of prostaglandin formation can determine the phlogolytic activity of flavonoids derived from *Lychnis chalcedonica* L. The researchers have recently detected flavonoids with the COX1/COX2 inhibiting effect, with the latter one predominating [1]. This very group of mixed inhibitors of COX of flavonoid nature is most promising to be used as novel anti-inflammatory agents in terms of its gastrointestinal toxicity. The presence of the C_2-C_3 double bond and the pattern of hydroxylation/methoxylation of rings A and B is the key factor in structure of flavonoids partaking in the mechanism of regulation of enzymatic processes with COX-2. Flavones, including apigenin whose derivatives are found in the complex derived from *Lychnis chalcedonica* L., more effectively inhibited COX-2 than flavonols [1]. Previously reported gastroprotective properties of the whole extract [2] support predominant inhibition of inducible COX by flavonoids within the extract, thus making them superior to nonsteroidal anti-inflammatory drugs and opens prospects of designing novel nontoxic antiphlogistics based on the complex of flavonoids extracted from *Lychnis chalcedonica* L.

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