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INFLUENCE OF *FILIPENDULA ULMARIA* (L.) MAXIM. EXTRACT ON LEWIS LUNG CARCINOMA DEVELOPMENT AND CYTOSTATIC THERAPY EFFECTIVENESS IN MICE

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Meadowsweet extract was prepared by heating the aerial part of *Filipendula ulmaria* (L.) Maxim. in EtOH (70%). Experiments in C57BL/6 mice with Lewis lung carcinoma (LLC) showed that the extract at doses of 50 and 100 mg/kg exhibited dose-dependent antimetastatic action. Combined treatment of animals with LLC using cyclophosphamide and meadowsweet extract at doses of 50 and 100 mg/kg increased the antitumor effect of the cytostatic drug.

Keywords: *Filipendula ulmaria* (L.) Maxim. extract, Lewis lung carcinoma, cytostatic therapy.

Meadowsweet, *Filipendula ulmaria* (L.) Maxim. (Rosaceae), is a perennial herbaceous plant of height up to 150 cm that inhabits forest and forest-steppe zones and mountain-forest belts around the world [1]. It is used in folk medicine as an anti-inflammatory, wound-healing, anticonvulsant, astringent, hemostatic, capillary-strengthening, antimicrobial, and general tonic agent [2]. Herb and root of the plant have been used to treat malignant neoplasms [3]. Meadowsweet flowers are used in official medicine as pharmacopoeial raw material (VFS 42-1777-87) and are approved for treating inflammatory diseases of skin and mucous membranes [2].

The chemical composition of meadowsweet is now well studied. The aerial part has afforded phenolic (phenols, flavonoids, phenolic carboxylic acids, coumarins, tanning agents) and triterpenoid compounds, sterols, polysaccharides, carotenoids, amino acids, essential oil, and inorganic constituents [1 – 3]. Researchers are attracted to meadowsweet as a source of new highly efficacious medicines because of the variety of biologically active compounds (BACs) in it and information from folk medicine about its curative properties. Various plant parts (flowers, aerial part, roots) and various methods for extracting BACs are used in the studies.

Tumor models with spontaneous tumors or those induced by chemical carcinogens or ionizing radiation were used to prove the anticarcinogenic effect of the decoction of meadowsweet flowers [4 – 6]. Experiments *in vitro* found that meadowsweet flower extract inhibited growth of human tumor cells (NCI-H460, lung carcinoma; A375-C5, melanoma; MCF-7, breast adenocarcinoma) although it did not affect apoptosis [7]. Also, the level of protein p21, which is known to inhibit the cell cycle in phase G1 and to stop proliferation, increased in the cells [8, 9].

The goal of the present work was to evaluate the effect of the extract from the aerial part of meadowsweet [*Filipendula ulmaria* (L.) Maxim.] on the development of Lewis lung carcinoma in mice and the effectiveness of cytostatic therapy.

EXPERIMENTAL PART

The aerial part of meadowsweet was collected during flowering in July 2016 in the vicinity of Ol'govka, Tomsk District, Tomsk Region. Air-dried (9.1 ± 0.03% moisture) raw material was milled, sieved (1 – 2 mm), extracted (3×) with refluxing EtOH (70%) (1:18 ratio) at 90°C for 30 min. The resulting extracts were combined, filtered, and evaporated to dryness in vacuo at <50°C. The extract (38.8% yield)

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contained flavonoids (4.21 ± 0.04%) recalculated as quercetin [10].

The experiments used 153 female C57BL/6 mice obtained from the Department of Experimental Biological Models, Goldberg RIPRM (Quality Certificate No. 18805). All procedures (housing, administration of tested compounds, euthanasia) were conducted in compliance with Directive 2010/63/EU *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* [11]. Lewis lung carcinoma (LLC) was transplanted i.m. $(4 – 5)\cdot10^6$ cells per 0.1 mL of normal saline by the usual methods [12].

Meadowsweet extract was administered through a catheter into the stomach at doses of 25, 50, and 100 mg/kg. Tables 1 and 2 present the durations of the courses. Treatment of mice began at various times after tumor transplantation to evaluate the effect of meadowsweet extract alone on LLC, i.e., 24 h after transplantation in series 1 experiments and 7 d, in series 2.

Cytostatic therapy used cyclophosphamide (CP, OOO Biokhimik, Saransk, Russia), which was injected i.p. once at a dose of 125 mg/kg to mice on the 10th (series 3) or 11th day (series 4) after tumor transplantation. Use of meadowsweet extract in the mice started on the 7th day after tumor transplantation.

Treatment efficacy was evaluated on the 17th (series 1), 20th (series 2 and 4), and 21st day (series 3) of the experiment. Tumor mass, percent tumor growth inhibition (TGI), number of lung metastases and their area, incidence of metastasis in percent vs. the control, and incidence of metastasis index (IMI) were determined [12 – 14]. Results were processed using Wilcoxon—Mann—Whitney (U) nonparametric criteria and Fisher's angular transformation (φ) [14, 15].

RESULTS AND DISCUSSION

Regular administration of meadowsweet extract at doses of 25, 50, and 100 mg/kg (series 1) did not affect growth of the main tumor mass (Table 1). The extract at doses of 50 and 100 mg/kg did have an antimetastatic effect with dose-dependent effectiveness. Thus, a dose of 50 mg/kg gave a statistically significant 1.5 times decrease of metastasis area. Increasing the extract dose to 100 mg/kg reduced statistically significantly the number of lung metastases (by 1.3 times) and their area (by 1.7 times) (Table 1).

Series 2 tests produced analogous results. Meadowsweet extract suppressed development of metastases. The tumor mass in mice that received the extract did not differ from that of the control. The number of metastases ($p < 0.05$) and their area decreased with the extract (Table 1).

The effect of meadowsweet extract on cytostatic therapy effectiveness was assessed in the next research stage in series 3 and 4 experiments in mice with LLC. A single administration to the animals of CP (series 3) led to a statistically significant decrease by 1.3 times of tumor mass. The incidence of metastasis and number of metastases decreased by 3.9 times. Their area was significantly less than the control values ($p < 0.01$). The IMI was 78.7% (Table 2).

The cytostatic antitumor effect increased if CP and meadowsweet extract were used together (series 3). Thus, the tumor mass was statistically significantly less in mice that received CP and extract at a dose of 50 mg/kg. The TGI was 47% vs. 25% in animals treated with only CP (Table 2).

TABLE 1. Effect of Meadowsweet (MS) Extract on Development of Lewis Lung Carcinoma in Female C57BL/6 Mice

Test group, drug administration regime (number of animals)	Tumor mass ($X \pm m$), g	TGI, %	Metastasis incidence, %	Number of metastases ($X \pm m$)	Metastasis area ($X \pm m$), mm^2	IMI, %
Series 1						
1. Control (10)	5.1 ± 0.2		100	12.4 ± 0.9	13.32 ± 2.06	
2. MS Extract, 25 mg/kg × 13 (10)	5.2 ± 0.2	–2	100	13.6 ± 1.9	12.51 ± 1.84	– 9.7
3. MS Extract, 50 mg/kg × 13 (10)	4.9 ± 0.2	4	100	11.5 ± 1.0	8.75 ± 2.03 $^{1-3}p < 0.05$	7.3
4. MS Extract, 100 mg/kg × 13 (10)	5.4 ± 0.2	–6	100	9.7 ± 1.1 $^{1-4}p < 0.05$	7.87 ± 1.81 $^{1-4}p < 0.05$	21.8
Series 2						
1. Control, (8)	8.1 ± 0.4		100	10.6 ± 1.3	8.68 ± 1.57	
2. MS Extract, 50 mg/kg × 11 (11)	7.7 ± 0.1	5	100	7.6 ± 1.5 $^{1-2}p < 0.05$	4.77 ± 1.62 $^{1-2}p < 0.05$	28.3
3. MS Extract, 100 mg/kg × 11 (10)	6.8 ± 0.3	16	100	7.5 ± 0.6 $^{1-3}p < 0.05$	5.23 ± 0.85	29.2

Note: Here and in Table 2, the number of compared groups is given before significance level p.

TABLE 2. Effect of Meadowsweet (MS) Extract on Cytostatic Treatment Effectiveness of Lewis Lung Carcinoma in Female C57BL/6 Mice

Test group, drug administration regime (number of animals)	Tumor mass ($X \pm m$), g	TGI, %	Metastasis incidence, %	Number of metastases ($X \pm m$)	Metastasis area ($X \pm m$), mm^2	IMI, %
Series 3						
1. Control (10)	6.20 ± 0.62		100	21.6 ± 1.8	27.02 ± 5.59	
2. CP, 125 mg/kg × 1 (11)	4.64 ± 0.59 $^{1-2}p < 0.01$	25	82 $^{1-2}p < 0.05$	5.6 ± 1.7 $^{1-2}p < 0.01$	1.12 ± 0.69 $^{1-2}p < 0.01$	78.7
3. CP, 125 mg/kg × 1 + MS extract, 50 mg/kg × 11 (10)	3.27 ± 0.57 $^{2-3}p < 0.05$	47	70	3.2 ± 0.7	0.29 ± 0.10	89.6
4. CP, 125 mg/kg × 1 + MS extract, 100 mg/kg × 11 (12)	4.63 ± 0.67	25	58	2.0 ± 0.7	0.14 ± 0.05 $^{2-4}p < 0.05$	94.6
Series 4						
1. Control (8)	8.06 ± 0.38	–	100	10.6 ± 1.3	8.68 ± 1.57	–
2. CP, 125 mg/kg × 1 (11)	6.15 ± 0.35 $^{1-2}p < 0.01$	24	100	5.3 ± 0.9 $^{1-2}p < 0.01$	2.15 ± 0.69 $^{1-2}p < 0.01$	50.0
3. CP, 125 mg/kg × 1 + MS, 50 mg/kg × 11 (11)	5.08 ± 0.25 $^{2-3}p < 0.05$	37	64 $^{2-3}p < 0.01$	1.4 ± 0.4 $^{2-3}p < 0.05$	0.16 ± 0.09 $^{2-3}p < 0.01$	91.5
4. CP, 125 mg/kg × 1 + MS, 100 mg/kg × 11 (11)	5.37 ± 0.26	33	91	3.5 ± 0.6	0.50 ± 0.17 $^{2-4}p < 0.05$	69.9

Both the number (by 1.8 times) and area of metastases (by 3.9 times) tended to decrease. The CP effectiveness for primary tumor did not change if it was used in combined therapy with meadowsweet extract at a dose of 100 mg/kg (Table 2). All parameters indicative of the severity of metastasis had their minimal values in the group receiving CP and meadowsweet extract at a dose of 100 mg/kg. The IMI was 94.6%. Metastases were observed in 58% of mice in this group vs. 82% in mice that received CP. The number of metastases and their area decreased by 2.8 and 8.0 times, respectively.

Series 4 experiments included meadowsweet extract at a dose of 50 mg/kg in a treatment regime that increased the antitumor and antimetastatic activity of CP. All evaluated parameters were statistically significantly less than those of the CP group. Thus, the TGI was 37%; IMI, 91.5% vs. 24% (TGI) and 50.0% (IMI) for the group that received only CP (Table 2). The metastasis area decreased statistically significantly by 4.3 times vs. that of mice that received CP if CP was used in combination with extract at a dose of 100 mg/kg (Table 2).

Thus, studies showed that administration of meadowsweet extract alone at doses of 50 and 100 mg/kg inhibited metastasis development in mice with LLC and increased the antitumor effect of CP if used together with it.

Meadowsweet extract used in the present work was analyzed chemically to detect flavonoids (quercetin, kaempferol, isoquercitrin, quercetin 4′-glucoside, avicularin, rutin), coumarins (esculetin), phenols, organic acids (benzoic, salicylic, *m*-hydroxybenzoic, anisic, vanillic, gentisic, gallic and its ethyl ester, *p*-coumaric, caffeic, chlorogenic, ferulic, etc.), triterpenes (ursolic and oleanolic acids), tanning agents of the hydrolyzed group, steroids, water-soluble polysaccharides, essential oil, carotenoids, amines, amino acids (valine, glutamic acid, histidine), and inorganic constituents [16].

Currently, the effects of flavonoids on carcinogenesis and tumor growth are being broadly investigated. Compounds of this class are known to possess antiproliferative activity, to induce apoptosis [17 – 20], to inhibit cell invasion and metastasis, and to affect angiogenesis [21]. The possibility of treating tumor patients with flavonoids is being studied. Thus, clinical trials are being conducted for quercetin [17, 20, 22], curcumin, epigallocatechin, genistein [23 – 25], and a preparation containing apigenin and epigallocatechin [20]. Data from various phases of clinical trials and the ability to use these compounds to treat tumors of various etiologies are being reported. Flavonoids are an important food component. Epidemiological data suggest that including them in the diet prevents development of prostate, colorectal, and ovary cancer [23, 26, 27].

Various flavonoids present in meadowsweet extract are probably responsible for its antimetastatic effect and its ability to increase the antitumor activity of a cytostatic.

Antitumor medicines are known to have a toxic effect on hemopoiesis and to cause immunosuppression resulting in increased risk of developing infections (fungal, bacterial, viral, parasitic). Serious complications of cytostatic therapy are functional disruption of the gastrointestinal tract, liver, and kidneys. Adjuvant therapy intended to ameliorate adverse effects of cytostatic drugs and improve patient quality of life is widely used during chemotherapeutic procedures of tumor

patients. The literature indicates that meadowsweet extract possesses a broad spectrum of pharmacological activity and can be used not only to increase the effectiveness of antitumor treatment but also as a therapeutic adjuvant. Previous research showed that extracts from the aerial part and roots of meadowsweet reduced damage by cisplatin to rat kidneys and liver [28]. Biochemical and histological studies allowed us to conclude that oxidative stress plays an important role in the pathogenesis of cisplatin-induced toxicity. Meadowsweet extract potentiates the antioxidant system and decreases damage by the cytostatic drug to the liver and kidneys [28]. Aqueous and aqueous EtOH extracts of the aerial part of meadowsweet in mice immunized with thymus-dependent antigen stimulated cellular and humoral responses with immunosuppression induced by CP [29]. Aqueous and aqueous EtOH extracts from the aerial part of meadowsweet were demonstrated to have hepatoprotective and antioxidant activity in a model of toxic hepatitis induced by administering CCl_4 to rats [30]. Extract of the aerial part of meadowsweet possessed analgesic and anti-inflammatory activity and inhibited the enzymes COX-1 and COX-2 [31, 32]. Extract of meadowsweet flowers exhibited antihyperalgesic and antiedematous properties [31]. Furthermore, it is noteworthy that meadowsweet extracts displayed antimicrobial [33] and antifungal activity [16].

The studies and a literature analysis demonstrated that further studies of the possible use of extracts from the aerial part of *F. ulmaria* for complex therapy of oncological patients are promising.

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