DOI 10.1007/s10517-015-2841-9
Bulletin of Experimental Biology and Medicine, Vol. 158, No. 5, March, 2015 PHARMACOLOGY AND TOXICOLOGY

Nootropic Effect of Meadowsweet (*Filipendula vulgaris*) Extracts

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 158, No. 11, pp. 609-613, November, 2014
Original article submitted January 17, 2014

The effects of the extracts of the aboveground parts of *Filipendula vulgaris* Moench on the behavior and memory of mice after hypoxic injury and their physical performance in the open-field test were studied using the models of hypoxia in a sealed volume, conditioned passive avoidance response (CPAR), and forced swimming with a load. The extracts improved animal resistance to hypoxia, normalized orientation and exploration activities, promoted CPAR retention after hypoxic injury, and increased physical performance. Aqueous extract of meadowsweet had the most pronounced effect that corresponded to the effect of the reference drug piracetam. These effects were probably caused by modulation of hippocampal activity.

Key Words: *Filipendula vulgaris Moench; conditioned passive avoidance response; open-field test; hypoxia, forced swimming*

Meadowsweet, *Filipendula vulgaris* Moench (*F. hexapetala* Gilib) of the *Rosaceae* family occurs throughout the steppe and forest steppe zones of European Russia and Siberia. Aboveground and underground parts of the plant are used in traditional medicine as an astringent, anti-inflammatory, wound healing, antibacterial, antifungal, diuretic, and hemostatic agent. The roots and rhizome of the plant are used as medicinal plant material (PS 42-49-72) included in the herbal medicinal product for symptomatic treatment in patients with papillomatosis of the bladder, antacid gastritis, and cancer [4]. Aqueous extract of rhizomes and roots exhibits hepatoprotective and immunotropic activity [7], extract of aboveground parts produces an anxiolytic effect [3,5], and flower tincture has cardioprotective [7] and analgesic effects [11]. Aboveground parts of meadowsweet contain phenol glycosides (monotropein), flavonoids (quercetin, kaempferol, luteolin, hyperoside, avicularin, isoquercitrin, quercitrin, spiraeoside,and rutin); catechins, leukoanthocyanidins, chalcones, phenolic acids (salicylic, caffeic, chlorogenic, *p*-coumaric, sinapic, syringic and gallic acids), coumarins, tannins, triterpene and fatty acids, polysaccharides, carotenoids, ascorbic acid, nitrogen-containing compounds, amino acids, major and trace elements [1,9,11-14]. The similarity of the chemical composition between *Filipendula vulgaris* and *Filipendula ulmaria (L) Maxim* possessing nootropic properties [8] as well as preliminary experimental evidence suggest that *Filipendula vulgaris* could also be a source nootropic substances.

The purpose of the work was to study the effect of aqueous and ethanol (40, 70, and 95% ethanol) extracts of the aboveground parts of *Filipendula vulgaris* on the behavior and memory of animals after hypoxic injury as well as on their physical performance.

MATERIALS AND METHODS

The work was carried out on outbred male mice CD-1 (n=170) weighing 20-22 g. First-category mice were obtained from the Laboratory of Experimental Biomodels, E. D. Goldberg Research Institute of Pharmacology (certificate available).

Experiments were carried out in winter from 09.00 to 15.00 h. The animals were kept under standard vivarium conditions on a normal diet in accordance with the rules of the European Convention for the Protec-

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tion of Vertebrate Animals used for experimental and scientific purposes (Strasbourg, 1986).

To obtain the extracts, the aboveground parts of *Filipendula vulgaris* were extracted with water and with 40, 70, and 95% ethanol at 80-85°C for 1.5 h at a raw materials:extractant ratio 1:10. The extracts were evaporated to dryness in vacuum at 50°C. The aqueous extract contains bioactive substances extracted from minced raw material with water; the main constituents were phenolic compounds (simple phenols, flavonoids, hydroxycoumarins, phenol carbonic acids, and hydrolyzable tannins) and triterpene compounds (acids and glycosides) as well as amino acids; the extract also contained 2.56% flavonoids standardized by quercetin glycosides; dry residue constituted at least 15%. The extracts were dissolved in water and administered to the animals intragastrically by gavage daily for 5 days (1 h prior to experimental manipulations) in a dose of 50 mg/kg (according to the preliminary experiments, this dose was considered to be the most pharmacologically active). Piracetam (Nootropyl; Pharma Sector) in a dose of 400 mg/kg was used as the reference drug. Control animals received an equivalent amount of water.

Nootropic activity of the extracts was studied after hypoxic exposure, which was modeled under conditions of hypoxia in a sealed chamber [6]. The animal was placed into sealed 0.5-liter chamber, the hypoxia latency (time to agonal seizure) was measured, the animal was removed to prevent death and 30 min later, orientation and exploratory behavior in the open field test was evaluated [2,10]. Then, passive avoidance response (CPAR) was conditioned; the efficiency of learning was evaluated by the latency of entry into the dark compartment of the chamber, by the percentage of the animals that did not enter the dark compartment, and by the time spent there [2,6]. CPAR was tested in 48 h and on days 14 and 21 after hypoxic injury. The effect on physical performance was evaluated in the forced swimming test with a load (10% body weight) [6] at a water temperature 28°C. The test was done performed twice a day at intervals of 1 h for 5 days. A decrease in physical performance caused by water cooling to 16°C was evaluated on day 6. Under these experimental conditions, the quality of higher nervous activity had maximum effect on performance and adaptation to extreme physical stresses, so this technique was additionally used to confirm the neuroprotective properties.

The obtained data were analyzed using Statistica 6.0 software. The data were normally distributed or close to that, which allowed to use the parametric Student's t test to evaluate the differences between the two groups. Fisher's angular transformation was used to compare the percentages. The differences were significant at $p \leq 0.05$. The results are presented as mean and standard deviation ($\bar{X} \pm m$).

RESULTS

After course treatment with the extracts, locomotor activity in the open-field test in animals not exposed to hypoxia tended to increase or significantly increased (Table 1). The maximum increase in activity by the main indicators was observed after administration of the 40% ethanol extract. Total locomotor activity increased by 2 times, horizontal activity by 1.8 times, vertical rearing postures by 2.5 times, and hole-board activity by 2.3 times. Treatment with 70% ethanol extract primarily stimulated horizontal activity (by 1.7 times); treatment with 95% ethanol extract 1.6-fold increased hole-board activity. After administration of the aqueous extract, the exploratory and orientation activities only tended to increase in animals not exposed to hypoxia and maximally approached to the level of intact controls.

Hypoxia impaired exploratory behavior, increased the latency of unconditioned darkness preference reflex during CPAR formation and deteriorated retention of CPAR (decreased latency of the first entry into the dark compartment and total time in the illuminated

TABLE 1. Effects of *Filipendula vulgaris* Extracts on Indicators of Orientation and Exploratory Behavior in the Open-Field Test in Male Mice not Exposed to Hypoxia (n=10; $\bar{X} \pm m$)

Group		Total locomotor activity	Horizontal activity	Vertical activity	Hole-board test	Grooming	Defecation
Intact control		44.4±8.7	22.1±5.4	2.1±0.7	18.7±3.2	0.4±0.2	1.1±0.4
Extract	aqueous	56.8±6.6	37.4±4.3	2.7±0.7	20.5±2.9	0.5±0.2	1.7±0.5
	40% ethanol	90.5±5.5*	40.2±2.0*	5.3±1.2*	43.3±3.3*	0.8±0.3	0.9±0.5
	70% ethanol	66.1±6.5	38.6±4.5*	3.9±1.5	22.0±2.4	0.6±0.2	1.0±0.3
	95% ethanol	72.5±79.5	35.3±4.9	5.9±1.7	29.9±3.6*	0.7±0.2	0.7±0.4

Note. Here and in Table 2: *$p \leq 0.05$ in comparison with intact controls.

compartment) (Fig. 1) indicating cognitive dysfunction in the animals.

Administration of *Filipendula vulgaris* extracts in a dose of 50 mg/kg increased the resistance of animals to hypoxia (Fig. 1). Administration of aqueous, 70 and 5% ethanol extracts increased the time in sealed camera by 1.2-1.3 times. Aqueous plant extract showed the greatest effect surpassing that of piracetam. Meadowsweet extracts exerted a protective effect on the functional state of CNS in animals exposed to hypoxia. Thus, the use of aqueous, 40, and 95% ethanol extracts increased the levels of physical activity in the open-field test 30 min after hypoxic exposure up to level of intact controls. By this parameter, they exceeded 70% ethanol extract, which was however comparable with piracetam in activity. Treatment with 40, 70, and 95% ethanol meadowsweet extracts practically normalized orientation behavior during CPAR formation after hypoxic exposure (tendency). In case of 40% ethanol extract, this parameter significantly differed from that in intact controls. Significant 1.8-fold improvement of orientation darkness preference behavior was only recorded after administration of aqueous extract. The test meadowsweet extracts improved CPAR retention and restored response performance up to 50-100% when tested in 48 h and 14 and 21 days after hypoxic exposure. The maximum difference between the groups was noted, when checking response after 48 h. Aqueous, 70 and 95% ethanol extracts had a protective effect on CNS after hypoxia in a sealed chamber.

The study of the impact of *Filipendula vulgaris* extracts on physical performance revealed progressive increase in the efficiency in the controls characteristic for adaptation to physical stress for 5 days (Table 2) and a sharp decline when the water temperature decreased to 16°C on experimental day 6. After treatment with all extracts, the duration of swimming increased in comparison with that in intact controls starting from day 1. The longest and statistically significant increase in performance (1.5-1.9-fold) occurred on days 1-4 after administration of the aqueous extract and on days 2 to 4 after administration of 70% ethanol extract (1.6-2.0-fold); 95% ethanol extract significantly

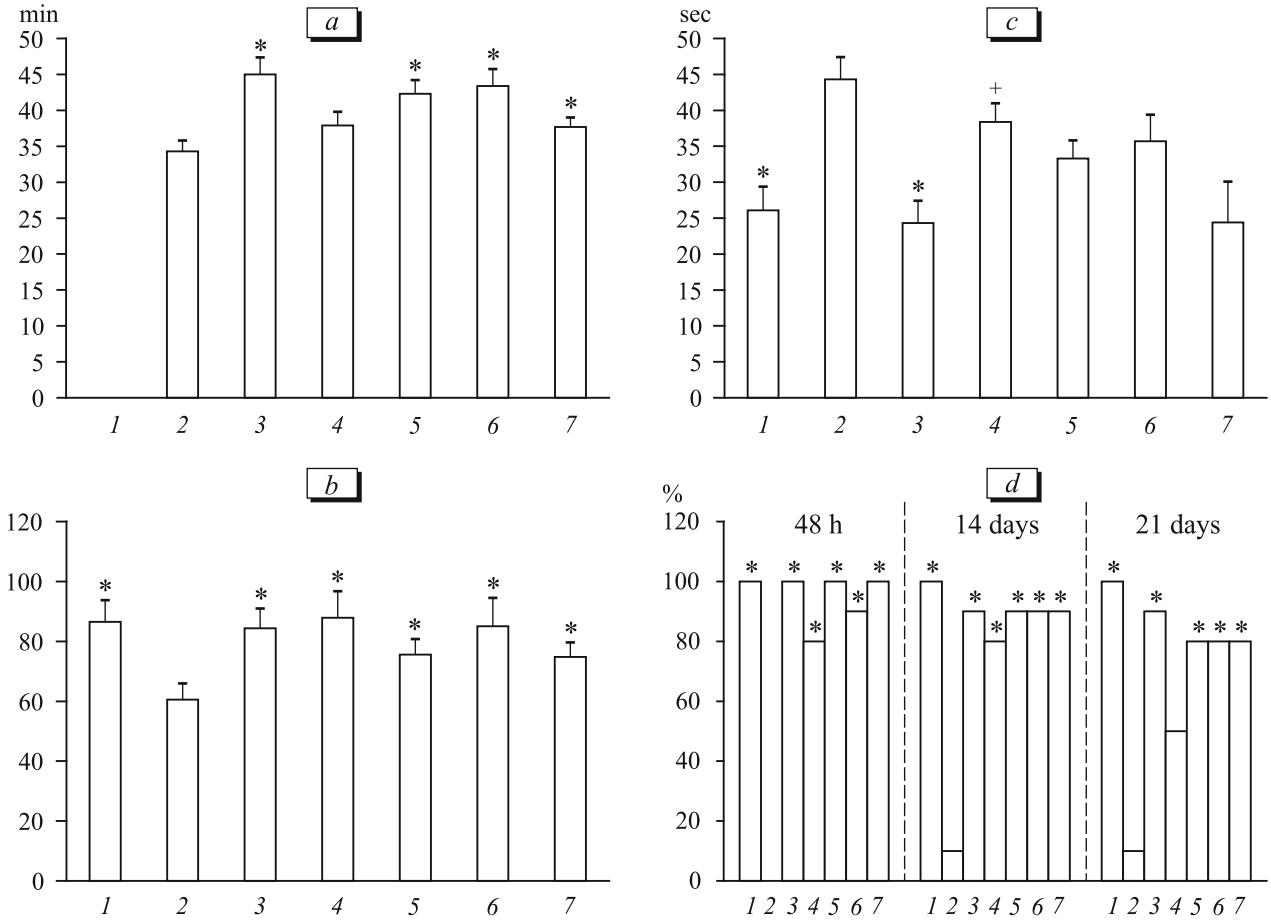

Fig. 1. Effects of *Filipendula vulgaris* extracts on the time spent in sealed chamber (*a*), orientation and exploratory behavior in the open-field test (*b*), formation (*c*) and retention (*d*) of CPAR in mice after hypoxic exposure. *1*) Intact controls; *2*) hypoxia; *3*) aqueous extract; *4*) 40% ethanol extract; *5*) 70% ethanol extract; *6*) 95% ethanol extract; *7*) piracetam. $p \leq 0.05$ in comparison with *hypoxia, +intact controls.

TABLE 2. Effects of *Filipendula vulgaris* Extracts on Physical Performance and Adaptation to Physical Stress in Male Mice not Exposed to Hypoxia (n=10; $\bar{X}\pm m$)

Group		Duration of swimming, sec					
		day 1	day 2	day 3	day 4	day 5	day 6
Intact control		58.0±11.1	50.7±11.2	59.2±7.4	107.8±27.4	128.2±55.5	86.8±14.6
Extract	aqueous	105.0±20.6*	78.9±9.7*	111.9±26.8*	187.0±36.9*	130.1±36.2	140.7±18.4*
	40% ethanol	72.0±11.8	71.3±12.9	85.2±17.5	106.8±21.1	129.1±39.3	125.6±32.8
	70% ethanol	84.8±21.5	81.9±10.0*	117.7±24.3*	190.2±30.2*	139.0±25.4	151.0±34.8*
	95% ethanol	60.6±5.7	72.1±9.3	118.3±17.6*	148.4±31.9	192.4±60.8	161.1±41.1*

elevated this parameter (2.0 times) only on day 3. In animals treated with 40% ethanol extract, this parameter only tended to increase on experimental days 1-3. The decrease in water temperature on experimental day 6 significantly increased this parameter (by 1.6-1.8 times) after treatment with aqueous, 70 and 95% ethanol extracts. The animals reveiving aqueous and 70% ethanol meadowsweet extracts showed the most pronounced and prolonged enhancement of physical performance and adaptation under conditions of forced swimming throughout the experiment.

Thus, the extracts of the aboveground parts of *Filipendula vulgaris* have nootropic activity increasing animal resistance to hypoxia, normalizing orientation and exploratory activities, and enhancing the resistance to extinction of CPAR after hypoxic injury, and also contribute to increased physical performance under experimental conditions. Aqueous extract has the most pronounced activity equal to the effect of piracetam, which is possible due to the characteristics of the chemical structure of its constituent phenol components (simple phenols, flavonoids, and others) and triterpene components (acids and glycosides), amino acids, and their quantitative content. Phenolic compounds carrying hydroxyls with mobile hydrogen atoms neutralize hydroperoxides of polyene fatty acids by metabolizing them into non-toxic hydroxy acids. The lipophilic phenolic compounds incorporate into cell membranes and inhibit the formation of primary and secondary LPO products thereby aborting neuronal apoptosis. Flavonoids normalize tissue homeostasis and therefore reactivity of CNS cells and relax blood vessels. In this case, flavones (apigenin and luteolin) and flavonols (kaempferol and quercetin) prevailing in the extract meadowsweet are the most active as well as combination of rutin and quercetin due to high activity of glycosylated flavonoids. Flavonoids exhibit neuroprotective properties preventing the injury of the hippocampal neurons, selectively interacting with the mitogen-activated protein kinase (MAPK) signaling cascade. Triterpene compounds reduce apoptosis, glycosides possess neurotrophic action activating neurogenesis with increased levels of insulin-like growth factor II and increased gene expression of several antioxidant enzymes; they suppress the NMDA-receptor activation by endogenous exitotoxins and calcium-dependent mechanisms of apoptosis, increase the sensitivity of hippocampal muscarinic cholinergic receptors and activate the central cholinergic system, which in turn can be mediated by antioxidant effects. It was found that the amino acids are also capable to show a stimulating effect on learning and memory via NO formation. Given that metabolic and morphological disorders in hippocampal neurons is an essential component in the pathogenesis of cognitive and memory pathology, it can be assumed that nootropic effects of meadowsweet extracts are associated with membrane-stabilizing and antioxidant properties. The aqueous extract of the aboveground parts of *Filipendula vulgaris* is promising for further research since it could provide a higher therapeutic effect.

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