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ANTIHYPOXIC ACTIVITY OF NATIVE HUMIC ACIDS OF TOMSK LOWLAND PEAT

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The antihypoxic activity of humic acids of Tomsk lowland peat was studied experimentally. It was established that native humic acids of peat (HAP) had pronounced antihypoxic activity after a preventive intragastric administration to mice, increasing the lifespan of animals and decreasing the lethality under hypobaric hypoxic and histotoxic hypoxia conditions. The discovered antihypoxic properties of HAP were probably due to their protective antioxidant properties, which prevent free-radical injury to cells and organelles under hypoxia conditions.

Keywords: humic acids, antihypoxic activity, peat.

One of the principal causes of injury to various cellular structures during hypoxia is known to be activation of lipid peroxidation (LPO). Accordingly, the ability to inhibit LPO processes is responsible for the application of antioxidants as antihypoxants under conditions where LPO processes are activated [1 – 6].

Another harmful factor during hypoxic states can be Ca^{2+} ions, the accumulation of which in the cytosol facilitates injury of cellular membranes, in particular, mitochondrial membranes, through the action of phospholipase A_2, which is localized in the inner mitochondrial membrane [7, 8].

According to the literature, humic acids of peat (HAP) exhibit pronounced antioxidant properties [9 – 12], possess pronounced chelating properties [13 – 16], and can form complexes with metal ions, including Ca^{2+} [17 – 20]. Thus, the antioxidant and chelating properties of the investigated HAP indicate that they may potentially have antihypoxic properties. This prompted us to evaluate their antihypoxic activity in various hypoxic state models.

EXPERIMENTAL CHEMICAL PART

Humic acids (HA) were extracted from lowland woody-grassy peat from Klyukvennoe, Tomsk District, by NaOH solution (0.1 N) without heating, precipitated from the extract by HCl solution (10%), rinsed with purified H_2O until the rinsings were neutral to litmus, and dried at room temperature. The investigated HAP were standardized according to the criteria developed by us earlier that were based on IR spectroscopy [Nicolet 5700 IR Fourier spectrometer, Thermo Electron Corp., USA; pressed KBr pellets (1:10 ratio); frequency range from 500 to 4000 cm^{-1}], elemental analysis (ashing on a Carlo Erba Strumentazione Model 1106 C, H,N analyzer, Italy; oxygen content by difference), UV spectroscopy [Uvikon 943spectrophotometer, Italy; wavelength range 190 – 700 nm, 1-cm quartz cuvette, aqueous solutions (0.001%) of HA], and exclusion chromatography [HPLC on a Supelco Progel-TSK GMPXL exclusion column (300 × 7.8 mm), Japan; 13 μm, column efficiency 11,000 theor. plates, mobile phase H_2O (1 mL/min); Agilent 1100 chromatograph, Germany; vacuum degasser, four-channel gradient pump, column thermostat].

The obtained HAP were a dark-brown odorless crystalline powder. The IR spectrum of the HAP contained characteristic absorption bands at 3500 – 3300, 3250 – 3200, 2920, 2860, 1460 – 1440, 700 – 900, 2600 – 2500, 1725 – 1700, 1625 – 1610, 1510 – 1500, 1250 – 1225, and 1050 – 1150 cm^{-1}. The UV absorption spectrum of the HAP appeared as a sloping curve with continuous absorption in the range from 220 to 800 nm that increased sharply toward the short-wavelength side. It had two absorption maxima in the regions 245 ± 2 and 294 ± 2 nm. The extinction coefficient at

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TABLE 1. Effect of Five Intragastric Administrations of HAP Solution (3 mass%, 25 – 100 mg/kg) on Lifespan of Mice Under Hypobaric Hypoxic Conditions ($X \pm \Delta X$)

Animal group	Number of animals	Lifespan, min (Student t-criterion)	Lethality from hypoxia, % (χ^2-criterion)
Control	25	11.88 ± 1.13	92
HAP (25 mg/kg)	25	13.72 ± 1.24	84
HAP (50 mg/kg)	25	16.44 ± 1.19*	76
HAP (100 mg/kg)	25	17.80 ± 1.31*,**	64

* Statistically significant difference ($p < 0.05$) compared with the control; ** statistically significant difference ($p < 0.05$) compared with humic acids (25 mg/kg).

TABLE 2. Effect of Five Intragastric Administrations of HAP Solution (3 mass%, 25 – 100 mg/kg) on Lifespan of Mice Under Histotoxic Hypoxia Conditions ($X \pm \Delta X$)

Animal group	Number of animals	Lifespan	
		in min	in % of control
Control (sodium nitroprusside)	25	9.45 ± 2.78	100
HAP (25 mg/kg) + sodium nitroprusside	25	11.83 ± 1.73*	125.20
HAP (50 mg/kg) + sodium nitroprusside	25	14.10 ± 1.87*	148.80
HAP (100 mg/kg) + sodium nitroprusside	25	16.32 ± 1.48*,**	172.60

* Statistically significant difference ($p < 0.05$) compared with the control; ** statistically significant difference ($p < 0.05$) compared with humic acids (25 mg/kg).

465 nm (E_{465}) was 0.020 ± 0.002; at 650 nm (E_{650}), 0.0041 ± 0.0004. The chromaticity coefficient (E_{465}/E_{650}) calculated from them was 4.88 ± 0.05. The HAP according to elemental analysis contained (mass%) C, 47.0; H, 5.5; N, 3.8; and O, 43.5. The molecular-weight (MW) distribution of the HA showed a characteristic spectrum. The HAP MW was 1000 – 1200 kDa.

EXPERIMENTAL BIOLOGICAL PART

Experiments were carried out using mature mongrel male white mice (18 – 22 g). Laboratory animals were kept under conditions corresponding to rules adopted by the European Convention on the Protection of Vertebrate Animals. Experiments were conducted according to good laboratory practices (GLP); Order of the RF Ministry of Health No. 267 of June 19, 2003, "On approval of good laboratory practices"; and the *Handbook of Experimental (Preclinical) Study of New Drugs* (2005) [21]. Antihypoxic activity of the investigated HAP was estimated using histotoxic hypoxia (tissue) and hypobaric hypoxia (chamber hypoxia) models.

Histotoxic hypoxia was induced by i.p. administration to mice of sodium nitroprusside at a dose of 20 mg/kg in isotonic saline [21]. The lifespan of the animals after injection of sodium nitroprusside was recorded. Animals of test groups received intragastrically beforehand once daily for 5 d the drug at doses of 25 – 100 mg/kg as an aqueous solution (3 mass%). The last administration was made 2 h before injecting sodium nitroprusside. Animals of the control group received intragastrically equal volumes of isotonic saline.

Hypobaric hypoxia was modeled in an Oka-MT pressure chamber by rarefying air to conditions equivalent to an altitude of 10,500 m with a 20-min exposure [21, 22]. The lifespan of animals under hypoxia conditions was recorded. The percent lethality was also calculated. Animals of test groups received intragastrically beforehand for 5 d the drug at doses of 25 – 100 mg/kg as a solution (3 mass%). The last administration was made 2 h before inducing hypoxia. Control animals received intragastrically equal volumes of isotonic saline.

The statistical significance of the differences was estimated using the Wilcoxon—Mann—Whitney criterion for significance level 5 mass% ($p < 0.05$).

RESULTS AND DISCUSSION

Judging from our results, HAP under hypobaric hypoxia conditions at doses of 50 – 100 mg/kg possessed pronounced antihypoxic activity, increasing by 38 and 50% the lifespan of mice (Table 1) and reducing the lethality of hypoxia by 24 and 36%, respectively.

Analogous results were obtained for the histotoxic tissue hypoxia model. HAP at doses of 25 – 100 mg/kg under histotoxic hypoxia conditions exhibited antihypoxic activity, increasing by 72.6% the lifespan of mice (Table 2).

Thus, preventive intragastric administration of HAP to mice had pronounced antihypoxic activity, increasing the lifespan of animals and reducing the lethality under various hypoxic conditions. Possible mechanisms of the HAP antihypoxic properties were their pronounced antioxidant and chelating properties.

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