PHARMACOLOGY AND TOXICOLOGY

Corrective Effect of Flavonoid-Containing Preparation Extralife on the Development of Parkinson's Syndrome

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Oral administration of flavonoid-containing preparation Extralife (daily dose 40 mg/kg) to animals with modeled Parkinson's syndrome considerably improved their survival and the main diagnostic and nosological parameters characterizing the state of locomotor functions. The preparation decreased animal mortality, rigidity, disturbances in dynamic muscular work and coordination of movements, and reduced oligokinesia. Experimental data confirm the involvement of mitochondrial enzyme complex I dysfunction in the pathogenesis of the disease and showed the possibility of by-passing this site of the respiratory chain with Extralife.

Key Words: *modeled Parkinson's syndrome; mitochondrial complex I; flavonoid-containing preparation Extralife*

Parkinson's disease is a prevalent neurodegenerative pathology. The main symptoms are locomotor dysfunctions, in particular, disturbances in locomotor activity and coordination and the development of rigidity and tremor. About 1% population above 60 years all over the world suffers from Parkinson's disease. This pathology is related to the deficiency of the dopaminergic system due to degeneration of dopaminergic neurons in the substantia nigra of the midbrain. In 95% cases the disease is sporadic, which attests to possible toxic damage to dopaminergic neurons.

According to current views, the pathogenesis of the disease at the molecular level is determined by two factors: decreased activity of ubiquitin-proteasome system responsible for degradation of proteins accumulating in these brain structures [6,8], which promotes activation of free-radical processes [10] and toxic effects of dopamine analogs [5], on the one hand, and inhibition of mitochondrial enzyme complex I (MEC I) leading to energy deficiency [5,9,10,12], reduced formation of glutathione, and, hence, impairment of the antioxidant defense [11] on the other. Correction of energy disturbances and prooxidant effects is of particular importance in this disease.

Flavonoids, phenol-containing plant pigments, were successfully used in Parkinson's disease as preparations reducing the degree of neurological disturbances and decelerating neurodegenerative processes [4,7,15]. The positive effects flavonoids are related to their antioxidant properties. We previously demonstrated that under conditions of suppressed MEC I activity phenol-containing plant preparations exhibiting pronounced redox properties and cytosolic enzyme DT-diaphorase can form a shunting oxidation pathway, thus restoring electron-transporting function of the cytochrome site of the respiratory chain (vitamin K_3-like effect) [2].

These properties were found in polyphenol complex Extralife obtained from *Pentaphylloides fruticos* and containing 3-5% flavonoids (hesperidin,

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quercitin, kempferol, quercitrin, isoquercitrin, astragalin, catechins, leucoanthocyanines), 9-11% tannins, terpenoids and phenylcarbonic acids.

Here we studied the possibility of using Extralife as an energotropic preparation capable of correcting (due to its redox properties and interaction with DT-diaphorase) disturbances determined by inactivation MEC I in Parkinson's syndrome (PS).

MATERIALS AND METHODS

Symptoms of PS can be modeled by chronic intraperitoneal administration of 1-methil-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin that is selectively bound by dopaminergic neurons and converted in cells into 1-methyl-4-phenyl-pyridine (MPP), an inhibitor of MEC I. This model was recommended by Russian Pharmacological Committee for preclinical testing of drugs intended for the use as neuroprotectors in Parkinson's disease [3]. In animals chronically receiving the toxin in doses of 10-40 mg/kg twice a day, the first symptoms (decrease in locomotor activity and increase in rigidity) developed by the 3rd day and peaked on day 10 after the start of treatment [1,14].

The experiments were performed on male C57Bl/6 mice. PS was induced by chronic intraperitoneal administration of MPTP in a dose of 20 mg/kg in 1 ml/kg physiological saline twice a day (with 12-h intervals) over 10 days. Control animals received 1 ml/kg physiological saline according to the same scheme. Extralife was dissolved in drinking water to a concentration ensuring daily intake of 40 mg/kg preparation. Drinking bowls with the preparation were placed into cages 16 h before the start of MPTP treatment.

Experiments were carried out on 4-month-old (young animals, n=60) and 13-month-old (old animals; n=60) mice. The mice of each age group were divided into 4 subgroups (15 mice each). Group 1 (controls) received intraperitoneal injections of physiological saline and water in drinking bowls; group 2 mice (control for the effect of Extralife) were injected with physiological saline and received Extralife; group 3 mice comprised animals with modeled PS (MPTP treatment) drinking pure water; and group 4 included mice with modeled PS receiving Extralife.

The development of PS was evaluated by oligokinesia, muscular rigidity, dynamic muscular working capacity, and coordination of movements. Oligokinesia was evaluated 24 h before and on days 5 and 9 after the start of MPTP injections by recording horizontal and vertical motor activities in an Opto-Varimex-3 system (Columbus Instruments) using Auto-Track software. For evaluation of muscular rigidity, body length from the ear line to the tail base was measured. Measurements were performed 24 h before and on days 6 and 10 after the start of MPTP treatment; the ratio of each measured body length to the initial value was calculated. Dynamic work and coordination of movements were evaluated on day 10 after the start of MPTP injections by the time of stay on a round polyfoam rod with a diameter of 2 cm rotating at a rate of 20 rpm [15].

The data were processed statistically by ANOVA dispersion analysis and using Statistica software.

RESULTS

The development of PS can led to death of experimental animals and therefore animal survival is an essential parameter of the dynamics of PS development. In the subgroup of young mice receiving MPTP and drinking water, the number of deaths by the 10th day was significantly lower than in the corresponding subgroup of old animals (36 and 48%, respectively). Against the background of Extralife treatment (40 mg/kg body weight with drinking water), animal mortality in both age groups considerably decreased (to 9 and 13%, respectively, p<0.05).

Thus, course treatment with Extralife considerably (3-4-fold) reduced animal mortality in the dynamics of PS.

Coordination of movements in young control animals was much better than in old mice (Table 1). This function was sharply impaired in mice with induced PS in both age groups: the time of stay on a rotating rod on day 10 of the experiment in young and old rats was 19 and 29% of the control level, respectively. In animals with MPTP-induced PS receiving Extralife, the disturbances in movement coordination were less pronounced than in untreated mice: the time of test performance increased to 65 and 54% of the control level, respectively (p<0.05, Table 1). Thus, course treatment with Extralife alleviated disturbances in dynamic muscular work and movement coordination in the dynamics of PS development.

Muscular rigidity is a typical sign of PS, which is well reproduced in the used PS model (Fig. 1).

In untreated animals, muscular rigidity on day 6 after the start of MPTP injections evaluated by the decrease in body length attained appreciable values and was more pronounced in old animals. This process progressed to day 10 and changes in muscular rigidity during this period were similar in both age groups. In mice receiving Extralife, muscular rigidity was considerably less pronounced. In young

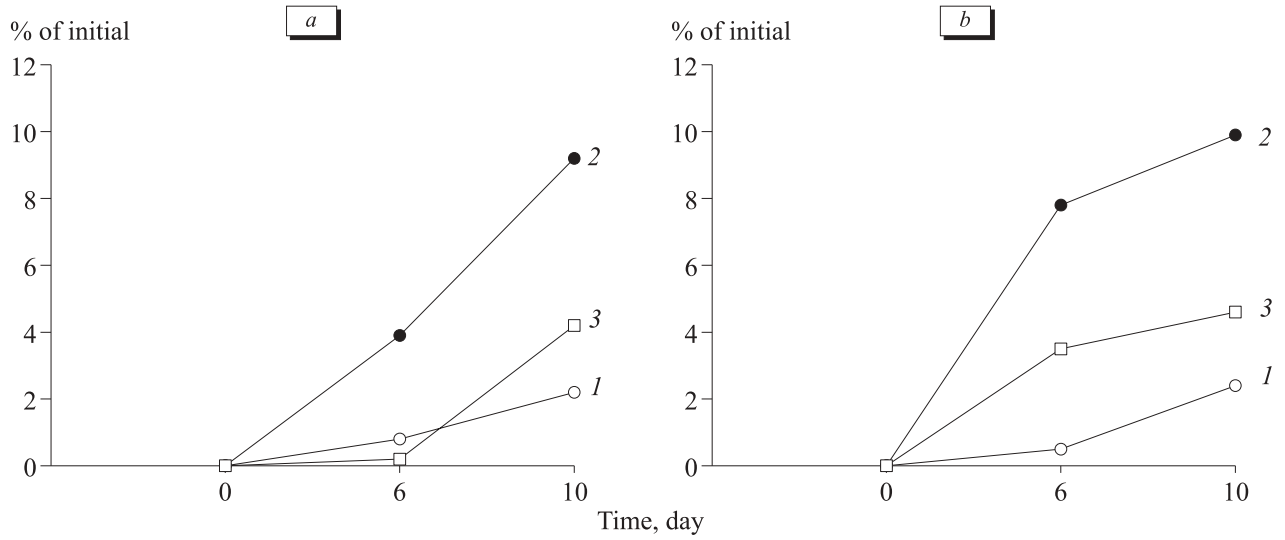

Fig. 1. Effect of Extralife on rigidity (decrease in body length) in young (*a*) and old (*b*) mice in the dynaics of MPTP-induced PS. Here and on Figs. 2, 3: *1*) control (intact) animals; *2*) animals receiving MPTP and physiological saline; *3*) mice receiving MPTP and Extralife.

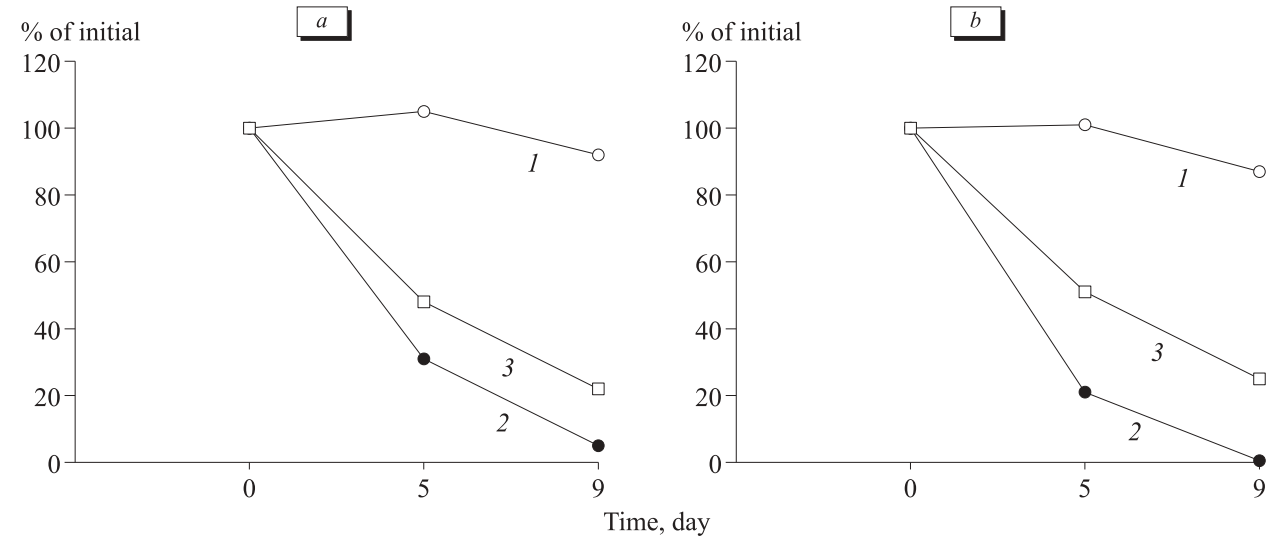

Fig. 2. Effect of Extralife on horizontal activity of young (*a*) and old (*b*) mice with MPTP-induced PS.

mice with PS receiving Extralife, these changes appeared by only day 10 and were less pronounced (by 2 times) compared to animals not treated with the preparation. Extralife did not completely eliminated rigidity in old animals during the first 6 days, but it was less pronounced than in untreated animals. This difference was observed also on day 10. Thus, course treatment with Extralife reduced rigidity accompanying PS development in both old and young animals.

The development of PS during MPTP treatment led to progressing suppression of motor (horizontal) and orientation and exploratory (vertical) activities (Fig. 2, 3). Course administration of Extralife to animals with MPTP-induced PS reduced disturbances in both parameters. The protective effects of Extralife produced better protective effects on exploratory activity than on motor activity. On day 9, the protective effect of the preparation sharply decreased irrespective of animal age, both functions were protected by only 16-18% (Fig. 2, 3).

Thus, treatment with Extralife during the development of PS induced by chronic administration of neurotoxin MPTP reduced the degree of oligokinesia. The protective effect of Extralife was more pronounced during the early stage of the disease and in old animals, was primarily aimed at correction of exploratory activity (and to a lesser degree on motor activity), and decreased at later terms of the disease development.

The positive corrective effect of Extralife on all measured parameters of PS (animal mortality, mus-

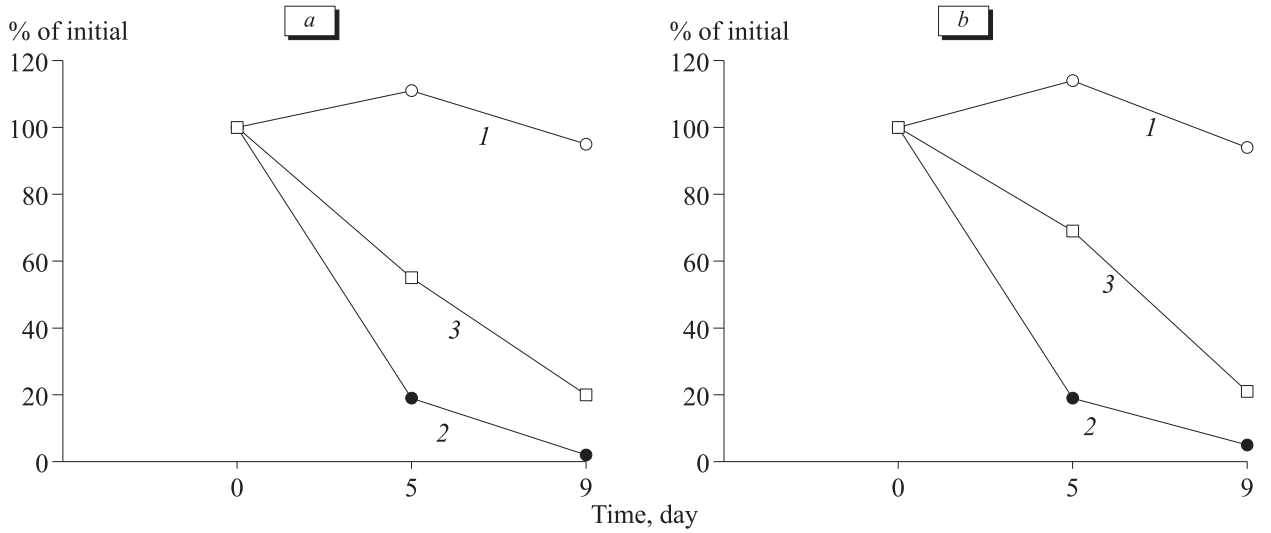

Fig. 3. Effect of Extralife on vertical activity of young (*a*) and old (*b*) mice with MPTP-induced PS.

TABLE 1. Effect of Extralife on Dynamic Muscle Work and Coordination of Movements in Mice with MPTP-Induced PS on Day 10

Subgroup	Time of stay on rod, sec			
	young animals		old animals	
	M±*m*	% of control	*M*±*m*	% of control
Control	60.2±9.4	100	25.3±6.9	100
Extralife	59.5±8.9	99	30.5±5.3	121
MPTP+physiological saline	11.5±3.8*	19	7.3±1.6*	29
MPTP+Extralife	40.5±3.7*+	67	13.6±2.4*+	54

Note. $p<0.05$ compared to: *control group, +mice receiving MPTP and physiological saline.

cular rigidity, oligokinesia, disturbances in dynamic muscular work and coordination of movements) confirms the involvement into pathogenesis of PS of mitochondrial dysfunction related to inactivation of MEC I, which can be corrected by the flavonoid-containing preparation. The latter attests to the possibility of compensatory shunting of this site of the respiratory chain with Extralife. At the same time, only partial protective effect of this preparation in the dynamics of experimental PS attests to complex mechanisms of this process including not only mitochondrial dysfunction, but also other targets.

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