Effect of Complex Phytoadaptogen on MPTP-Induced Parkinson's Syndrome in Mice

E. V. Bocharov, V. G. Kucheryanu, G. N. Kryzhanovskii, O. A. Bocharova*, V. S. Kudrin, and S. A. Belorustseva**

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Oral administration of 10% solution of Phytomix-40 (multicomponent plant phytoadaptogen) to C57Bl/6 mice with MPTP-induced Parkinson's syndrome alleviated symptoms (oligokinesia and muscle rigidity), compensated for the deficiency of dopamine and its metabolites (DOPAC and homovanillic acid), and reduced the level of lipid peroxides in the striatum. *In vitro* Phytomix-40 in a concentration of 3.3×10^{-2} g/liter exhibited a pronounced antioxidant effect (5-fold decreased MDA level in mouse brain homogenate in Fe^{2+}-ascorbate-dependent LPO).

Key Words: *Parkinson's syndrome; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; adaptogens; Phytomix-40*

Parkinson's disease is characterized by selective and progressive damage and death of dopaminergic neurons in the substantia nigra leading to a drop of dopamine level in the striatum. Important causes of death of nigral dopaminergic neurons are formation of free radicals and oxidative stress, impairment of the mitochondrial respiratory function and energy deficit, activation of NMDA-glutamate receptors, *etc.* [1,2,13]. Saponins and Rb_1 and Rg_1 ginsenosides, the main active components of ginseng, are characterized by antioxidant, neuroprotective, and neurotrophic properties [3-5,11]. Ginsenosides block NMDA receptors [7], reduce activity of caspase-3 [4], intracellular Ca^{2+} content [6,7], and glutamate neurotoxicity [8], act as scavengers of free peroxyl ($LOO^{\bullet}$) and hydroxyl ($^{\bullet}OH$) radicals [10], and exhibit geroprotective and nootropic effects [3].

The use of Shengmai san preparation containing ginseng completely blocked the formation of LPO products and prevented tinhibition of glutathion peroxidase during brain ischemia in rats [15].

In culture with high content of dopaminergic neurons isolated from fetal mouse midbrain, Rb_1 and Rg_1 ginsenosides improved neuronal survival and increased the length and number of axons damaged by MPP^+ (1-methyl-4-phenylpyridinium) [12].

Oral administration G-115 ginseng extract to mice with MPTP-induced (1-methyl-4-phenylpyridinium-1,2,3,6-tetrahydropyridine) Parkinson's syndrome (PS) or rats with MPP^+-induced PS prevented death of tyrosine hydroxylase-containing nigral neurons [14].

We studied the therapeutic effects of Phytomix-40, a plant preparation containing adaptogens, in experimental parkinsonism.

MATERIALS AND METHODS

We studied the effect of oral treatment with Phytomix-40 on the development of parkinsonism symptoms, levels of LPO products, serotonin, dopamine and its metabolites DOPAC and homovanil-

Laboratory of General Pathophysiology of Nervous System, Institute of Pathology and Pathophysiology, Russian Academy of Medical Sciences; *N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences; **Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** vkucheryanu@mail.ru. V. G. Kucheryanu

lic acid (HVA) in the striatum of mice with MPTP-induced PS.

Parkinson's syndrome was induced in C57Bl/6 mice by multiple intraperitoneal injections of MPTP neurotoxin (20 mg/kg twice a day with 12-h intervals for 10 days). The development of the syndrome was seen from the severity of oligokinesia and muscle rigidity. Oligokinesia was evaluated by changed horizontal motor activity and number of rearings. Motor activity of animals was evaluated in the open field test in an automated mode using Opto-Varimex-3 system (Columbus Instruments). Muscle rigidity was evaluated by the "hunchback" symptom and expressed in points. Phytomix-40 was given orally as 2 and 10% solution for 7 days before and 10 days after the start of MPTP treatment. The intensity of LPO in brain homogenates and striatum was evaluated by the level of MDA (measured spectrofluorometrically). The content of dopamine and its metabolites (DOPAC and HVA) was measured by HPLC on an LC-304T chromatographer (BAS, West Lafayette) with electrochemical detection. For evaluation of antioxidant activity of Phytomix-40 *in vitro*, LPO in mouse brain homogenate was induced by ascorbate (2×10^{-4} M) and Fe^{2+} (10^{-5} M). Phytomix-40 concentrations in samples were 3.3×10^{-2}, 3.3×10^{-3}, and 3.3×10^{-4} g/liter.

RESULTS

Phytomix-40 in a concentration of 3.3×10^{-2} g/liter *in vitro* exhibited high antioxidant activity. MDA content increased almost 5-fold (483%) in comparison with the initial level (100%) after iron ascorbate induction and decreased 40 min after addition of Phytomix-40 virtually to the control level (115%). Mexidol (antioxidant) under similar conditions less effectively reduced the content of LPO products (2-fold; Table 1).

Experiments on the model of MPTP-induced PS showed that Phytomix-40 (10% solution) significantly restored ($p<0.05$) motor activity of mice on days 5 and 10 of MPTP treatment. Phytomix-40 (2% solution) improved motor activity in mice with PS only on day 5 ($p<0.05$); no changes in motor activity were observed by day 10 (Fig. 1).

TABLE 1. Effect of Phytomix-40 on the Level of MDA under Conditions of Fe^{2+} Ascorbate-Dependent LPO *In Vitro*

Group	Duration of incubation	
	before incubation	after 40 min
Control	100±3	483±11*
Phytomix-40	100±3	115±5

Note. *$p<0.01$ compared to the corresponding parameter before incubation.

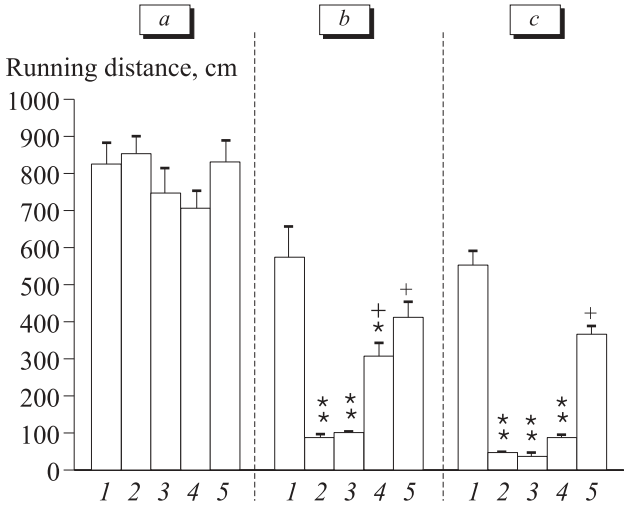

Fig. 1. Effect of Phytomix-40 on motor activity of mice with MPTP-induced Parkinson's syndrome (PS) before (*a*) and 5 (*b*) and 10 (*c*) days after the start of MPTP treatment. *1*) NaCl; *2*) MPTP+NaCl; *3*) MPTP+ethanol; *4*) MPTP+2% Phytomix-40; *5*) MPTP+10% Phytomix-40. *$p<0.05$,**$p<0.01$ compared to NaCl, $^{+}p<0.05$ compared to MPTP+ethanol. Here and in Figs. 2, 3: 8-10 animals per group.

High concentration of Phytomix-40 reduced muscle rigidity in mice receiving MPTP from 2.75 to 1.0 score ($p<0.01$) by day 10 of the neurotoxin injections. Low concentration of the preparation suppressed rigidity in animals with experimental PS during the first 5 days of MPTP injections ($p<0.05$). Hence, Phytomix-40 in both concentrations similarly inhibited the development of oligokinesia and rigidity in animals with MPTP-induced PS (Fig. 2).

Oral treatment with Phytomix-40 modified the content of neurotransmitters and their metabolites in the striatum of mice with experimental PS (Fig. 3). Intraperitoneal injections of MPTP appreciably reduced the levels of dopamine, HVA, and DOPAC in mouse striatum. Chronic treatment with 10% Phytomix-40 solution restored dopamine level by 46%. The concentrations of DOPAC (indicator of dopamine intracellular metabolism) and HVA (indicator of postsynaptic transformation of dopamine) increased 4.1 and 2.3 times, respectively, during treatment with Phytomix-40. The content of serotonin in the striatum of mice with MPTP-induced PS decreased. The preparation in low concentration (2%) did not modify the content of neurotransmitters and their metabolites (Fig. 3).

Phytomix-40 in low (2%) and high (10%) concentrations suppressed the level of MDA in the striatum of mice with MPTP-induced PS by 80%. This parameter was equal to 3.05±0.05 nmol/mg protein in mice treated with NaCl and to 3.90±0.03

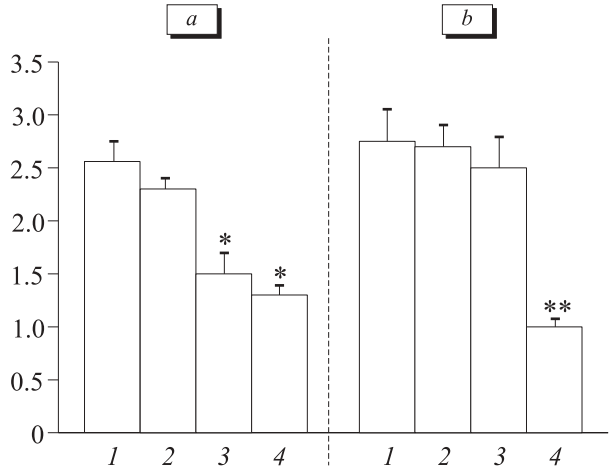

Fig. 2. Effect of oral treatment by Phytomix-40 on rigidity score in mice with MPTP-induced PS after 5 (*a*) and 10 (*b*) days of MPTP treatment. *1*) MPTP+NaCl; *2*) MPTP+ethanol; *3*) MPTP+2% Phytomix-40; *4*) MPTP+10% Phytomix-40. **p*<0.05, ***p*<0.01 compared to MPTP+ethanol.

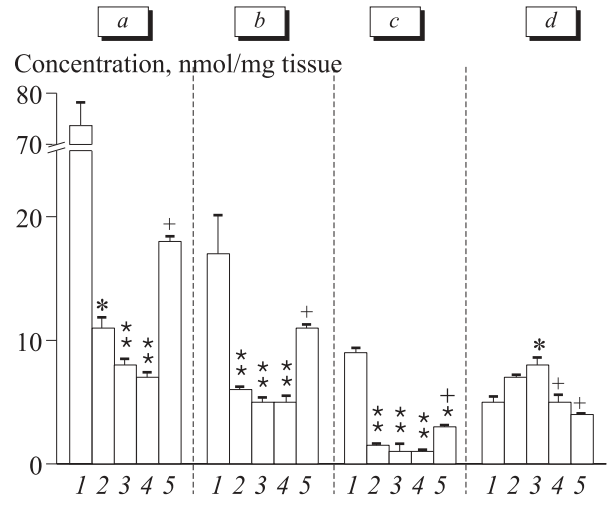

Fig. 3. Effect of Phytomix-40 on the levels of dopamine (*a*), DOPAC (*b*), homovanillic acid (*c*), and serotonin (*d*) in the striatum of mice with MPTP-induced PS after 11 days of MPTP treatment. *1*) NaCl; *2*) MPTP+NaCl; *3*) MPTP+ethanol; *4*) MPTP+2% Phytomix-40; *5*) MPTP+10% Phytomix-40. **p*<0.05, ***p*<0.01 compared to NaCl, ^{+}p<0.05 compared to MPTP+ethanol.

nmol/mg protein in animals with MPTP-induced PS, injected with NaCl. In three other groups with MPTP-induced PS the levels of MDA were 4.00±0.04% in animals injected with ethanol, 3.30±0.04 in those injected with 2% Phytomix-40, and 3.20±0.03 nmol/mg protein in those injected with 10% Phytomix-40. The latter two values differed significantly (p<0.05) from those in animals with MPTP-induced PS injected with ethanol.

Hence, complex plant adaptogen preparation Phytomix-40 is an active antioxidant. This is probably due to the presence of natural antioxidants a-tocopherol and ascorbic acid in its composition. Long oral administration of 10% Phytomix-40 solution to mice with experimental PS reduced the severity of oligokinesia and rigidity and partially restored the deficiency of dopamine and its metabolites, which attests to antiparkinsonic activity of the phytopreparation.

Antiparkinsonic activity of Phytomix-40 can be due to its neuroprotective and antioxidant effects (it arrest degeneration and death of dopamine-producing neurons in the substantia nigra).

Phytomix-40 consists of 40 plant extracts. Complex pharmacological effects of the preparation seem to be due to structural and functional relationships between many its components (panaxosides, aralosides, eleuterosides, salidroside, schizandrin, ursulic acid, oleanolic acid, glycyrrhizic acid, betulin, *etc.*) and steroids, which are polyphysiological due to their structure. They easily penetrate through the plasma membrane, bind to receptors, modify lipid-protein interaction, and modify the genome [3]. The possibility of Rb_2 gensinoside binding to AP_2 transcription factor was shown, which can cause stimulation of SOD_2 activity [9].

Animal experiments showed that Rg_1 gensinoside (ginseng component) protected substantia nigra neurons from MPTP-induced damage and death through inhibition of iNO-synthase and activation of antiapoptotic factor Bcl-2 located on the outer mitochondrial membrane, nuclear membrane, and endoplasmic reticulum and involved in cascade reactions protecting the cells from death [4]. The majority of gensinosides modify the configuration of plasma membranes and microviscosity of the lipid bilayer, interact with proteins, including the membrane ATPase molecules, thus modifying their activity [3]. For example, Rb_1 gensinoside stimulates activities of Na^+-K^+- and Ca^{2+}-Mg^{2+}-ATPases in neuronal membranes, which reduced intracellular calcium content [6].

Our experiments demonstrated antiparkinsonic and antioxidant effects of Phytomix-40 and confirm the necessity of its further clinical studies in combined therapy of patients with Parkinson's disease.

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