

The Ability of *Alphitobius diaperinus* Homogenates Immobilized on Plant Sorbent to Block the Development of Mouse Parkinsonism

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Insects have long attracted the attention of researchers as a source of biologically active substances present in the tissues of different species of insects at various stages of their life cycle [1, 2]. The analysis of the chemical composition of some species of invertebrates showed that the bee (*Apis mellifera*), grasshoppers (family Acrididae), house-cricket (*Acheta domestica*), American cockroach (*Periplaneta americana*), larval and adult mealworms (*Tenebrio molitor*), as well as larvae of the wax moth (*Galleria mellonella*), silkworm (*Bombyx mori*), blowfly (family Calliphoridae), black soldier fly (*Hermetia illucens*), etc., contain not only proteins and fats, but also chitin, minerals, amino acids, polyunsaturated fatty acids and vitamins, essential oils, and other biologically active substances, including new antimicrobial peptides [3–9]. In the scientific literature, there is scarce information about the possible medical use of extracts from the darkling beetle *Ulomoides dermestoides* from the family Tenebrionidae, which exhibit anti-inflammatory and

immunomodulating effect [10] and cytotoxicity with respect to A549 (lung adenocarcinoma) tumor cells [11]. A species close to *Ulomoides dermestoides* is the beetle *Alphitobius diaperinus*, which is also used in folk medicine to treat various diseases, including one of the most socially important—Parkinson's disease. It was assumed that the *Alphitobius diaperinus* biomass homogenate may exhibit inhibitory activity with respect to MPTP (methylphenyl tetrahydropyridine)—a toxin that causes experimental parkinsonism in C57BL/6JSto mice [12, 13].

The aim of this work was to determine whether the *Alphitobius diaperinus* homogenate immobilized on a plant sorbent can block the development of mouse parkinsonism induced by the administration of the toxin.

The homogenate from the biomass of adult darkling beetles *Alphitobius diaperinus* was obtained using a Waring 800S laboratory blender (Waring, United

Results ($M \pm SE$) of the experiment of blocking the development of parkinsonism in mice with the beetle *Alphitobius diaperinus* homogenate

Parameter	Group		
	C1 (control)	C2 (toxin)	E1 (Toxin + antidote)
Initial body weight, g	24.0 ± 0.8	26.1 ± 0.1	25.5 ± 0.7
Body weight 2 weeks after the toxin injection, g	26.0 ± 0.9	25.6 ± 1.1	27.1 ± 0.9
Changes in body weight, g	+2.0**	−0.6	+1.6**
Average time of staying on rotating rod, min	>10' (15/15)*	<10' (2/12)*	>10' (13/15)*
% of animals that stayed on rotating rod for more than 10 min	100**	17	87**
Number of TH-positive cells in SNpc brain slices	18083 ± 1488**	9704 ± 947	19080 ± 1216**

* Number of mice stayed on the rotating rod longer than 10 min/total number of mice in the sample (in parentheses); ** $p < 0.05$ compared to group C2.

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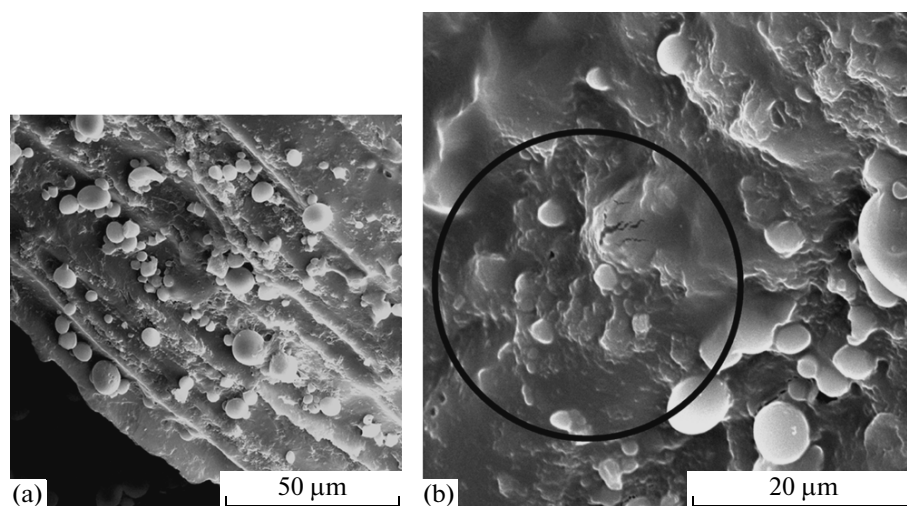


Fig. 1. Surface structure of plant substrate: (a) milk fat globules adsorbed on the bran surface; (b) biofilm of lactic acid bacteria on the bran surface (highlighted).

States) and then immobilized on specially prepared sorbent. Sterile food wheat bran moistened with 1% milk to a 50% weight moisture, the surface of which was coated with a biofilm formed by lactic acid bacteria *Enterococcus* and *Leuconostoc* in the course of solid fermentation of bran, was used as a sorbent. The biofilm formation was monitored using an MW 2300 scanning electron microscope (CamScan, Czech Republic). For this purpose, a drop of isotonic (0.5 M) sucrose solution was loaded on a cover slip ($18 \times 18 \text{ mm}^2$), after which a crude fermentation mass was carefully poured on it. The glass was then air dried at room temperature overnight. The lower part of the glass attached using a double-sided conductive tape to the surface of the microscope stage. The upper part of the cover slip was sprayed with gold under vacuum according to the standard procedure for preparing samples for scanning electron microscopy.

The obtained immobilized preparation of beetle homogenate was stored in a refrigerator and given daily for 1 week to experimental mice. For this purpose, it was added to the feed mixture by fractional mixing and thorough stirring (in a proportion of 4 g of the preparation per 1 kg of feed mixture). The feed mixture consisted of boiled oats and peas supplemented with raw millet and vegetable oil.

Three groups of mice (young mature C57BL/6JSto males, $n = 15$) were formed by the method of weight analogues: C1 (control intact group (animals were not treated with the toxin and did not receive the preparation with food)), C2 (control group of mice that were injected with the toxin but did not receive the preparation with food), and E1 (experimental group (mice that were injected with the toxin and received the preparation with food)).

During the first week of the experiment, mice of group E1 received the preparation with food. Animals

of the control groups C1 and C2 received the same diet without the preparation. Then, the animals of groups E1 and C2 were subcutaneously injected with the MPTP toxin at a dose of 40 mg/kg. Then, over the next two weeks, the mice received the same food as before

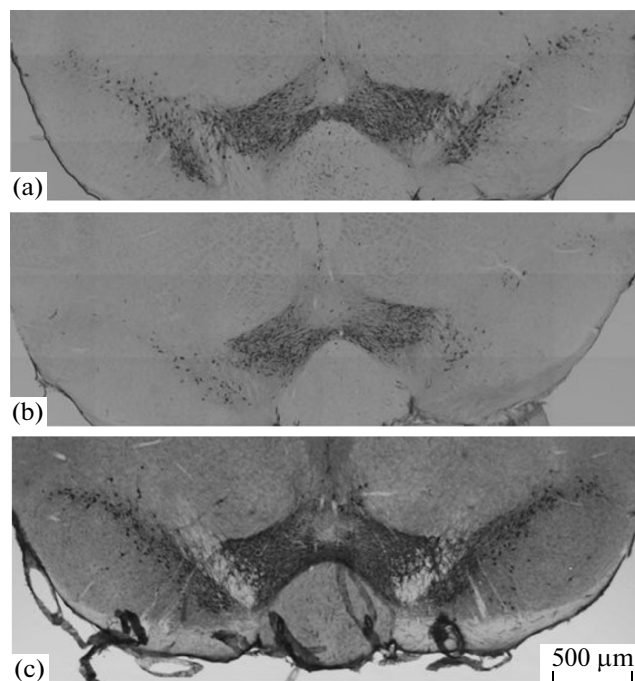


Fig. 2. Cross section of the brain: (a) mice of the control group C1. Cells containing the TH fragment that forms dopamine are darkened. The center of the micrograph shows the ventral midbrain tegmental area; the diverging “wings” show the compact part of the substantia nigra. The same region of the brain of mice from group C2 (b) and group E1 (c) 2 weeks after the subcutaneous injection of the MPTP neurotoxin.

the injection (the experimental animals continued to receive the preparation, and the control animals did not receive it). Group C1 mice throughout the experiment received the standard vivarium diet without the preparation and were not injected with the toxin. Two weeks after the toxin injection, the changes in the body weight of all mice were registered, the coordination of movements was estimated, and the physical endurance was assessed using the Rota-Rod ("rotating rod") apparatus (TSE Systems, Switzerland/Germany). The rotational speed of the rod was 6 rpm, and the exposure duration was 10 min. Statistical analysis was performed using the nonparametric statistics methods (Kruskal–Wallis one-way analysis of variance, Mann–Whitney *U* test, and sign test).

Brain lesion in mice during the development of parkinsonism, associated with the destruction of dopamine-containing neurons, was assessed by counting the dopaminergic neurons immunohistochemistry stained for tyrosine hydroxylase (TH), the key enzyme of the biosynthesis of catecholamines. The animals were euthanized by intraperitoneal injection of a lethal dose of urethane (more than 1 g/kg). The circulatory system was transcardially perfused with a solution of phosphate-buffered saline (PBS, pH 7.4) and then with 4% formaldehyde in PBS. After postfixation in formaldehyde solution at 4°C for 12 h at 4°C, the brain was impregnated with a 30% sucrose solution in PBS at 4°C for 24 h. Subsequently, the brain was cut with a freezing microtome. Frontal sections 40 µm thick, containing the substantia nigra, were collected in PBS. For immunohistochemical staining, every fourth section was placed in a solution of mouse monoclonal anti-TH antibodies (T2928, Sigma, United States) diluted at a ratio of 1 : 200 with PBS supplemented with 2% normal horse serum and 0.3% Triton X-100 (Sigma). Sections were incubated in this solution at 4–8°C for 12 h with continuous stirring. After washing in PBS, the sections were immersed for 1 h in a solution of biotinylated horse antibodies against mouse immunoglobulin (Vector Laboratories, United States), which were diluted at a ratio of 1 : 100 with PBS supplemented with 0.3% Triton X-100 at room temperature. Then, the sections were washed three times with PBS and placed in the ABC complex solution (Vector Laboratories) in PBS at a dilution of 1 : 200 for 1 h. The standard peroxidase reaction was performed using 0.03% diaminobenzidine (Sigma) in PBS supplemented with 0.01% hydrogen peroxide. The stained sections were placed on slides and, coated with 50% glycerol, and covered with cover slips. Quantitative analysis of TH-positive cells on immunohistochemically stained sections was performed using an Olympus IX81 microscope (OLYMPUS Corp., Japan) equipped with a Märzhäuser motorized stage (Märzhäuser Wetzlar, Germany), which was controlled with a computer, and an Olympus DP72 digital camera. Cell were counted with a computer monitor

using the Cell* software (Olympus Soft Imaging Solution GmbH).

The electron microscopy studies of the structure of the used sorbent showed its uniqueness, which is associated with the formation of a complex surface topography of the sorbent during solid-phase cultivation of lactic acid bacteria on wheat bran moistened with milk. Milk fat was present in milk in the form of fat globules 0.2–10 µm diameter, surrounded by a lecithin-protein coat. The fat globule coat exhibits surface activity, which determines the ability of the globules to be adsorbed on bran particles (Fig. 1a). Probiotic bacteria are present in the biofilm (Fig. 1b). Beetle fragments are adsorbed on such sorbent. The experiment showed that the biologically active components of the insect retained their physiological effectiveness.

The results of experiments with mice are summarized in the table. Motor coordination and physical endurance in the rotating rod test were retained in 13 of the 15 mice of the E1 group (antidote + toxin), as well as in the control animals (group C1), which were not injected with the toxin. Conversely, in the group of mice that received no "antidote" with food in the form of a specially prepared *Alphitobius diaperinus* biomass homogenate but were injected with the toxin (group C2), only 2 of the 12 mice were able to stay on the rotating rod for more than 10 min. In group E1 mice, as well as C1 group animals, a weight gain was detected, in contrast to group C2 animals (toxin), in which intoxication caused weight loss.

Histological studies revealed a decrease in the number of TH-containing neurons in the compact zone of the substantia nigra (SNpc) of group C2 mice as compared to the mice of groups C1 and E1. Values for groups C1 and E1 did not differ significantly in this parameter. The results indicate the absence of lesion of dopamine-containing neurons in the brain of E1 mice, which received both the toxin and the antidote, characteristic for the development of parkinsonism (Fig. 2).

Thus, the homogenate of the beetle *Alphitobius diaperinus* immobilized on a plant sorbent demonstrated a pronounced ability to neutralize the effect of the MPTP toxin on mice, simulating parkinsonism. Possibly, this effect was due to inhibition of the enzyme monoamine oxidase B [13].

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