Biological Activity of the Tenebrionidae Beetle Antioxidant Complex in a Murine Neurotoxic Model of Parkinson's Disease

Vladimir M Kovalzon[1,2*], Aleksandr V Ambaryan[1], Aleksandr V Revishchin[3], Galina V Pavlova[3], Ekaterina Y Rybalkina[4], Aleksandr I Bastrakov[1], Nina A Ushakova[1]

[1]Laboratory of Innovation Technology, and [2]Laboratory of Behavior & Behavioral Ecology, Severstov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia

[3]Laboratory of Genetics and Developmental Genetics, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia

[4]Laboratory of Tumor Cell Genetics, Blokhin Russian Cancer Research Center, Ministry of Health of the Russian Federation, Moscow, Russia

ABSTRACT

We have previously shown that the aqueous extract of the *Ulomoides dermestoides* darking beetle (the Tenebrionidae family) biomass contains a powerful complex of antioxidant substances of protein and non-protein nature. Considering the crucial role of ROS (Reactive Oxygen Species) in the development of neurodegeneration, we set out to test the biological activity of this extract in a mouse neurotoxic model of Parkinson's disease. The beetle extracts were administrated continuously with food and their effects on parkinsonism caused by twice injected defoliant paraquat to experimental mice was evaluated. The motor activity of the animals was analyzed in behavioral tests using a rotarod and a vertical pole. The number of tyrosine hydroxylase-immunopositive neurons in the ventral part of the substantia nigra of the midbrains of experimental and control mice was studied by immunohistochemistry. In the model *in vitro* system with SH-SY5Y human neuroblastoma, the effect of the extracts on cell proliferation was examined in the absence and presence of the neurotoxin MPP+ (1-methyl-4-phenylpyridinium). The isolation of biologically active substances from raw biomass using cavitation effects made it possible to obtain extracts with protective properties in the model of an early stage of Parkinson's disease used in this study.

Keywords: Parkinson's disease, Neurotoxic model, Early stage, Biologically active substances from insects

*Correspondence: Vladimir M Kovalzon, Laboratory of Innovation Technology, and Laboratory of Behavior & Behavioral Ecology, Severstov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia, E-mail: somnolog43@gmail.com

INTRODUCTION

Insects and their by-products are widely used in traditional folk medicine across the globe and have long attracted the attention of researchers as a source of biologically active substances (see reviews: Adamski Z, *et al.*, 2019; Deyrup ST, *et al.*, 2021; Dutta P, *et al.*, 2019; Seabrooks L and Hu L, 2017 for references). For example, *Apis mellifera* bees, the larvae and adults of the large flour beetle *Tenebrio molitor*, carrion flies of the Calliphoridae family, *Galleria mellonella* wax moths and *Bombyx mori* silkworm contain new antimicrobial peptides, chitin-melanin complexes, flavonoids, aminoacids and organic acids (Adamski Z, *et al.*, 2019; Deyrup ST, *et al.*, 2021; Dutta P, *et al.*, 2019; Seabrooks L and Hu L, 2017; Ushakova NA, *et al.*, 2021). Darkling beetles of the Tenebrionidae family, which are used in traditional medicine to treat a wide range of diseases, are of special interest (Adamski Z, *et al.*, 2019; Deyrup ST, *et al.*, 2021; Dutta P, *et al.*, 2019; Seabrooks L and Hu L, 2017; Flores DR, *et al.*, 2020; Mendoza-Meza DL, España-Puccini P, 2016; Ushakova NA, *et al.*, 2021). These insects synthesize protective secretions, which are a mixture of repellent and blocking chemoreceptor substances. They are found in cuticular inclusions or abdominal glands and are released when the beetles are stressed. It is believed that the secretions of these beetles are also necessary for the insect to prevent drying out and protect against pathogenic microorganisms (Adamski Z, *et al.*, 2019; Deyrup ST, *et al.*, 2021; Dutta P, *et al.*, 2019; Seabrooks L and Hu L, 2017; Flores DR, *et al.*, 2020; Mendoza-Meza DL, España-Puccini P, 2016; Ushakova NA, *et al.*, 2021). It has been shown that these secrets are a source of pharmacologically active compounds that are promising for the treatment of respiratory diseases (Adamski Z, *et al.*, 2019; Deyrup ST, *et al.*, 2021; Dutta P, *et al.*, 2019; Seabrooks L and Hu L, 2017). There is information about the possibility of medical use of substances recently isolated from extracts of a representative of this family, the *Ulomoides dermestoides* darkling beetle. These substances have anti-inflammatory and immunomodulatory properties, as well as cytotoxicity in relation to the cells of some tumors (Adamski Z, *et al.*, 2019; Deyrup ST, *et al.*, 2021; Dutta P, *et al.*, 2019; Seabrooks L and Hu L, 2017; Flores DR, *et al.*, 2020; Mendoza-Meza DL, España-Puccini P, 2016; Ushakova NA, *et al.*, 2021). *U. dermestoides* is used in traditional Argentinian medicine to treat asthma, diabetes, arthritis, HIV-induced diseases, cancer and Parkinson's disease (PD) (Seabrooks L and Hu L, 2017).

We assumed that some components of biomass extracts of a representative of the same family, the *Alphitobius diaperinus* beetle, have an inhibitory activity against the delayed effects of the proneurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which causes experimental parkinsonism in C57Bl/6 mice (Ushakova NA, *et al.*, 2015; Ushakova NA, *et al.*, 2018; Koval'zon VM, *et al.*, 2017). Our experiments using the model of the early clinical PD stage showed that the primary aqueous extract and, especially, the secondary aqueous-methanol extract of the biomass of this beetle had a powerful protective influence in response to the neurotoxic effect of MPTP, both according to behavioral tests and the results of neuromorphological analysis.

Recent studies have revealed a strong antioxidant activity of aqueous extracts of Tenebrionidae beetles (Flores DR, *et al.*, 2020; Ushakova NA, *et al.*, 2021) suggesting that the extracts may also have a neuroprotective effect (Nuzzo D, 2021). The present study evaluated the activity of aqueous extracts of biomass of the adult beetle *Ulomoides dermestoides* in relation to the delayed effects

of paraquat defoliant causing early experimental parkinsonism in experimental animals (Bastías-Candia S, *et al.*, 2019; Brooks AI, *et al.*, 1999). The motor activity of animals was analyzed in behavioral tests on a rotating rod (rotarod) and a vertical pole (Matsuura K, *et al.*, 1997; Ogawa N, *et al.*, 1985). Changes in the number of tyrosine hydroxylase-immunopositive (TH+) neurons in the ventral part of the substantia nigra of the midbrain (SNM) of mice were studied by immunohistochemistry (Revishchin A, *et al.*, 2017). In a model system with SH-SY5Y-human neuroblastoma, we examined the effect of the extract on cell proliferation without and in the presence of the neurotoxin MPP+ (Xicoy H, *et al.*, 2017).

MATERIALS AND METHODS

The laboratory population of *Ulomoides dermestoides* beetles was cultivated at the Severstov Institute Ecology/Evolution of the Russian Academy of Sciences on a nutrient mixture of wheat bran (70%), milk powder (5%), corn flour (20%) and sunflower meal (5%) in a climatic chamber at 28°C and 60%-70% humidity, conditions similar to those for the cultivation of *Alphitobius diaperinus* (Zagorinsky AA, *et al.*, 2020). Sexually mature adult beetles of both sexes separated from the substrate were immobilized by cooling at -18°C, and the resulting biomass was divided into two parts. One part was crushed in distilled water using cavitation on a rotary pulsation unit followed by separation of the sediment by centrifugation in an Ohaus frontier 5706 for 15 min at 5000 g. The received extract was named "cavitation extract". Beetle substances were extracted from the second part of the biomass by the method of electro-pulse plasma-dynamic extraction (EPP-DE) in distilled water at 23°C. EPP-DE extraction was carried out using a special setup (Ushakova NA, *et al.*, 2021). Extraction parameters: The power of the transmitted electric discharge was 38,000 Volts, the pulse frequency was one pulse per second, the distance between the electrodes was 5 mm, the extraction time was 7 min. After exposure, the extract was separated from the solid fraction by centrifugation at 5000 g for 15 min. The received extract was named "lightning extract". Antibacterial processing of the extracts was carried out by radiation decontamination using a beam of accelerated electrons using a Compact Radiation Sterilization Unit with local biosecurity (CRSU) of the Moscow Radiotechnical Institute, Russian Academy of Sciences, at 15 KGy. The energy of the accelerated electrons was 5 MeV; the power of the electron beam was 1.5 kW.

The "lightning extract" contained 20 mg/ml of dry matter, and the "cavitation extract" contained 12 mg/ml of dry matter. The obtained extracts were immobilized on sterile food wheat bran; the final moisture content of the mass was 8%. The preparations were stored in a refrigerator and fed to experimental mice, for which dry preparations were added to the main food mixture for mice by fractional and thorough mixing (at the rate of 4 g of the "lightning preparation" and 8 g of the "cavitation preparation" per 1 kg of the food mixture). The main food mixture consisted of porridge, including boiled oats and peas, with the addition of vegetable oil.

To study the biological activity of the extracts, we used a neurotoxic model of an early stage of Parkinson's disease in male C57Bl/6jsto mice administered paraquat (Bastías-Candia S, *et al.*, 2019; Brooks AI, *et al.*, 1999; Uversky VN, 2004; Bastías-Candia S, *et al.*, 2015; Bastías-Candia S, *et al.*, 2019; McCormack AL, *et al.*, 2005; McCormack AL, *et al.*, 2002; Marsova M, *et al.*, 2020). The animals were divided into 4 groups. Group 1 (n=12): toxin+"cavitation extract"; the animals were injected i.p. twice (with an interval of 1 week) with 10 mg/kg of paraquat, dissolved in 0.3 ml of saline; an extract obtained by cavitation was added to the food as an antidote as described above; the supplementation began one week before the first injection of the toxin and continued throughout the entire experiment. Group 2 (n=11): Toxin+"lightning extract": The animals were injected with paraquat and received the beetle extract with the food; everything was performed according to the same regimen as Group 1. Group 3 (n=12): Intact control; the animals were not injected with paraquat and did not receive anything additional to the food. Group 4 (n=24): Toxin-control;

the animals were injected with paraquat like Groups 1 and 2, but they did not receive any antidote. Four days after the second injection of the toxin, the motor activity of all the animals was tested using a rotarod and then a vertical pole.

The mice were placed on a rod rotating at a constant speed of 6 rpm for a period of 600 sec, then the rotation speed was increased automatically by 1 rpm every 30 sec up to 20 rpm. The entire duration of the test was 990 sec. We recorded how long each mouse could hold out on the rod without falling at a constant speed and at what maximum speed of rotation. If the mouse was able to stay on the rotarod for 990 seconds and did not fall at 20 rpm within 30 sec, the test was considered 100% complete. Statistical analysis was performed using the nonparametric U-test (Mann-Whitney). The test for locomotor activity on a vertical pole was used in accordance with the previously described method (Matsuura K, *et al.*, 1997; Fernagut PO, *et al.*, 2003; Ogawa N, *et al.*, 1987; Ogawa N, *et al.*, 1985). Individual testing was performed in a familiar environment for the animals. At the beginning of testing, a vertical pole with an unpolished rough surface 50 cm high and 1 cm in diameter was placed in the cuvette. A mouse was placed on it close to the top of the pole so that its head was oriented upward. Once on the pole, the mouse reoriented its body position with its head down and began descent from the pole to the bottom of the cage. Even if the animal, after reorientation, did not go down the pole completely and jumped off the pole, the time it took to reach the bottom of the cage was recorded. In our variant of the method, 3 tests were carried out with each mouse. The minimum time interval between tests was 30 seconds. If the mouse did not reorient its body and start descending within the first 3 minutes from the start of testing in two or all three trials, data were not included in the statistical analysis. All the experiments were recorded using a Panasonic HC-VX1 video camera and then processed by the BORIS (Behavioral Observation Research Interactive Software) 7.9.8 program for the analysis and presentation of behavioral data. In the statistical analysis, only the duration of the descent of the mouse from the pole was taken into account. The groups were compared in terms of maximum and mean time of descent from the pole from three attempts. When analyzing the data, the nonparametric Kruskal-Wallis test was used. Pairwise post hoc comparisons were made using the Steel-Dvas-Critchlow-Flyiner test. In addition, intergroup comparisons were made for maximal and mean pole exposure relative to maximal possible duration in three attempts (180 sec). The relations were compared using the χ^2 test. For pairwise comparisons of the groups, the Marasquilo procedure was used. Statistical analysis was performed using the XLSTAT 2019 2.2 and Analyze-it 5.66 software.

After the completion of the behavioral tests, the animals were perfused under deep general anesthesia through the heart with phosphate buffered saline (PBS) and then 4% formaldehyde in PBS. The brain was removed, and after additional fixation in a perfusion solution for 12 hours at 4°C, it was soaked in a 30% sucrose solution in PBS for 24 hours. A series of coronal 20 μm sections was obtained using a freezing microtome with a spacing between the sections of 160 μm. The sections containing substantia nigra of the midbrain were immunohistochemically stained for TH using monoclonal antibodies against TH (T2928 Sigma), diluted 1:300 in a PBS solution with the addition of 2% normal horse serum, 0.5% Triton X100 detergent (Sigma, USA) and 0.01% sodium azide (Sigma, USA). The free-floating sections were kept in the primary antibody solution with stirring at 4°C for 48 hours. Then, after the reaction in a solution of biotinylated horse antibodies against mouse immunoglobulin (Vector Laboratories USA), these sections were processed in a solution of the ABC complex (Vector Laboratories, USA). A standard procedure for staining for peroxidase was carried out using a 0.03% diaminobenzidine solution (Sigma, USA) in PBS with the addition of 0.01% hydrogen peroxide. Stained sections were placed on glass slides, covered with 50% glycerol and a cover slip.

The quantitative analysis of TH+ cells was performed using an Olympus IX81 microscope equipped with a Märzhäuser motorized (FRG) computer controlled stage and an Olympus DP72 digital camera. Cells were counted on a computer monitor using the Cell* software (Olympus Soft Imaging Solution GmbH, Germany). At a low magnification (lens 10x), an overview image of the section with the SNM and the Ventral Tegmental Area (VTA) was obtained. Then, at a high magnification (lens 40x), the number of TH+ cells was counted using a method of the optical fractionator. The position of the test square measuring 50×50 μm was changed with a step of 200 μm along the X axis and 100 μm along the Y axis within the ventral part of the midbrain. An uninformed operator counted the number of TH+ cells in the test square, using unstained nuclei of TH+ cells as the object of counting. A cell stained for TH was included in the count if its nucleus lay inside the test square or touched two of its adjacent sides *(Figure 1)* and did not touch the other two sides marked in this figure with a thicker outline. Since the calculated fraction was 1/8 of the area of the investigated section, to determine the total number of TH+ cells in the section, the counted number was multiplied by 8. Subsequently, the number of cells in 9 consecutive sections containing SNM was determined. Intergroup comparisons of the number of TH+ cells were performed using the Kruskal-Wallis test. Since the compared samples were small, the Conover-Iman test was used for pairwise *a posteriori* comparisons.

The effect of darkling beetle extracts on SH-SY5Y human neuroblastoma cells were evaluated using the MTT test. This test is based on the ability of mitochondrial dehydrogenases of living cells to convert "yellow" MTT (3-(4,5-dimethylthiazolyl-2)2,5-diphenyl tetrazolium bromide) into "blue" formazan, insoluble in aqueous solutions. For analysis, cells are placed in 96-well plateaus at 5000 cells per well in 100 μl of standard culture medium. After 24 hours of incubation, 10 μl (1/10 of the medium volume) of the tested extract at various concentrations was added to the cells. The cells were incubated for another 48 or 72 hours (the incubation time depended on the rate of cell proliferation). After that, 20 μl of MTT preparation (diluted in saline solution 5 μg/ml) was added to each well for 3 hours. Then, the solution was removed from the wells, and 60 μl of dimethyl sulfoxide (DMSO) was added to each well and they were shaken thoroughly until the formazan crystals completely dissolved. The quantitative determination of formazan was carried out on a brand multichannel photometer with a 530 nm filter. Cell viability was assessed by the ratio of optical density in the control wells without the tested extract and in the wells with the extract (Mosmann T, 1983).

RESULTS

The rotarod test, assessing the biological effect of beetle extracts in the diets of mice under conditions of a neurotoxic model of an early stage of Parkinson's disease, revealed *(Figure 2 and Table 1)* that the continuous administration of "cavitation extract" with food caused a slight, insignificant increase in the mean rotation speed that the mouse could sustain before falling down as compared with the toxin control group (from 12.5 ± 1.2 to 15 ± 1.6 rpm; M ± S.E.M. ; p>0.1). The mean time of holding the mouse on the rotarod before falling increased significantly (from 701 to 825 sec, p<0.05, n=12/24, U-test).

The "lightning extract" preparation caused a sharp increase in the ability of
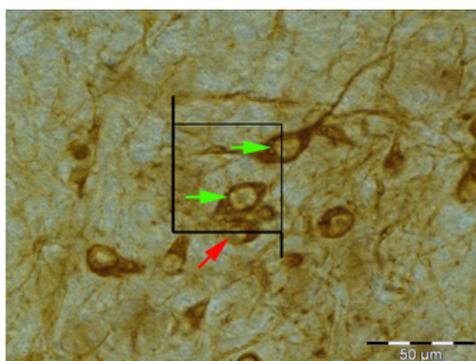
Figure 1: Representative micrograph of a TH-stained mouse brain slice with an overlaid test square. The green arrows indicate the nuclei of the cells included in the count; the red arrow indicates the nucleus of the excluded cell
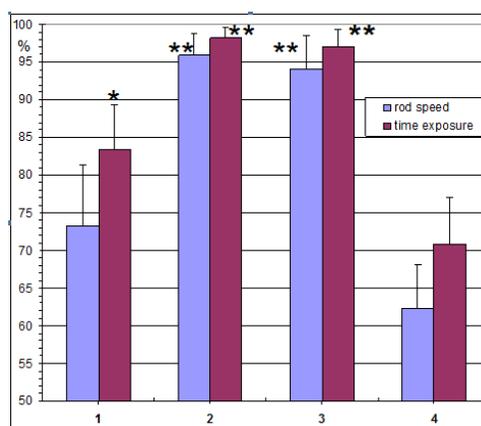

Figure 2: Results of the behavioral test on rotarod. Groups of mice: 1-toxin+"cavitation extract", n=12; 2-toxin+"lightning extract", n=11; 3-intact control; n=12; 4-toxin control, n=24. M ± S.E.M differences from group 4, *p<0.05, **p<0.001, U-test. The y-scale shows the percentage of the test completion (see "Methods")

Table 1: The rotarod testing

	"Cavitation extract"		"Lightning extract"		Intact control		Paraquat control	
	Rod speed (rpm)[a]	Time of exposure (sec)[b]	Rod speed (rpm)	Time of exposure (sec)	Rod speed (rpm)	Time of exposure (sec)	Rod speed (rpm)	Time of exposure (sec)
Mean	14.7	825.3*	19.2**	972.9**	18.8**	960.9**	12.5	700.8
SD	5.6	208.4	1.9	42.8	3	77.3	5.8	300.6

[a] revolutions per minute-an indicator of the sustained rotarod rotation speed
[b] time duration of holding the mouse on the rotarod at a rotation speed of 6 rpm
*p<0.05, **p<0.001, difference from the paraquat-control group

the animal to hold on the rotarod rotating both with constant and increasing speed. Improvement occurred with both parameters: rotarod rotation speed and mouse retention time *(Figure 2 and Table 1)*. The parameters in this group did not differ from those of the intact control, with a significant difference from the toxin control group (for both parameters, p<0.001).

The results of intergroup comparisons of the maximum, minimum and mean durations of exposure on the vertical pole are presented in *Table 2*.

The comparison of the maximum, minimum and mean times (out of three attempts) spent by mice on the pole using the Kruskal-Wallis test showed that there were significant differences between the groups in maximal and mean times only (maximum time: H3=10.19, P=0.017; mean time: H3=11.10, P=0.011); therefore, in the statistical analysis, the minimal time was excluded from the account.

Pairwise *a posteriori* comparisons using the Steel-Dvas-Critchloo-Flyiner test showed that the maximal time spent on the pole was significantly higher in the "cavitation extract" group than in the toxin control group. The mean time spent on the pole was significantly higher in the "lightning extract" group than in the toxin control group *(Table 3)*.

The ratio of the maximum in three attempts to the maximum possible time spent on the pole *(Table 4)* in the "lightning extract" group was significantly higher than in the other groups. The same relation was significantly higher in the control group without toxin, as well as in the "cavitation extract" group, compared to the toxin control group. There were no significant differences in this parameter between the "cavitation extract" and toxin-free intact control groups. The relation of the mean exposure time on the pole to the maximal possible in three attempts in the "lightning extract" group was also significantly higher than in the other groups, and in the "cavitation extract" group compared with the toxin control group. There were no

significant differences in this parameter between the other groups.

In the midbrain samples of four animals from each the intact control, toxin control and two "extract" groups, the number of TH+ cells was counted. The number of TH+ cells in animals that received an injection of paraquat was significantly lower than in intact control animals, as well as in animals in the "cavitation extract" and the "lightning extract" groups (H3=12.794, P=0.005, Kruskal-Wallis test). At the same time, the number of TH+ cells in animals from the "cavitation extract" group was significantly lower than in control intact animals and animals from the "lightning extract" group *(Tables 5 and 6)*.

Representative slices of the SNM of the intact control mouse, the toxin control mouse and the "lightning extract" mouse, stained for TH, are shown in *Figure 3*. The number of TH+ neurons in the SNM and the VTA of the toxin control mouse (middle slice) is significantly less as compared to the intact control mouse (upper slice) and the animal from the "lightning extract" group (bottom slice). In the latter sample, the number of TH+ neurons in the SNM and the VTA is practically the same as in the intact control animal.

Evaluation of the protective effect of the "cavitation extract" of the *Ulomoides dermestoides* beetle against 0.5 mM neurotoxin MPP+ in a model experiment using SH-SY5Y neuroblastoma showed the dependence of the effect on the dose of the administered extract *(Figure 4)*. The best result was obtained using a 5% addition of the extract; the protection was 23%. Dilution of the extract did not cause reliable protection.

For the *Ulomoides dermestoides* "lightning extract", a protection of 26.5% was detected with the administration of 1% extract *(Figure 5)*.

Table 2: Intergroup comparisons of the duration of the pole exposure (in three attempts)

Timing parameters	Groups in comparison	Mean duration of the pole exposure (sec)
Maximal time	Intact control	28.9 ± 7.2
	Toxin control	18.1 ± 2.1
	"Cavitation extract"	32.4 ± 5.2
	"Lightning extract"	41.9 ± 10.1
Minimal time	Intact control	7.5 ± 0.6
	Toxin control	9.9 ± 0.9
	"Cavitation extract"	9.0 ± 0.7
	"Lightning extract"	12.1 ± 0.8
Mean time	Intact control	15.4 ± 2.5
	Toxin control	13.6 ± 1.4
	"Cavitation extract"	17.8 ± 2.0
	"Lightning extract"	24.0 ± 3.9

Table 3: Comparison of the groups

Timing parameter ratio to maximal possible time of pole exposure	Groups in comparison	Criterion statistics meanings (Hodges-Lehmann location shift)	Significance of intergroup differences (p)
Maximal time	"Cavitation extract"/Toxin control	10	0.02*
	"Lightning extract"/Toxin control	9.2	0.12
	"Cavitation extract"/Intact control	7.6	0.24
	"Lightning extract"/Intact control	6.5	0.5
	Intact control/Toxin control	1.7	0.74
	"Cavitation extract"/"Lightning extract"	-0.2	0.99
Mean time	"Cavitation extract"/Toxin control	4	0.08
	"Lightning extract"/Toxin control	6.4	0.04*
	"Cavitation extract"/Intact control	4.1	0.22
	"Lightning extract"/Intact control	6.5	0.1
	Intact control/Toxin control	0.1	1
	"Cavitation extract"/"Lightning extract"	2.6	0.79
*p<0.05, difference from the paraquat-control group			

Table 4: Pairwise intergroup comparisons of the maximum and mean ratio of exposure time on the pole versus maximum possible duration in three attempts (180 seconds) (Marasquilo's procedure at a significance level of α=0.05)

Timing parameters	Statistics and significance in χ^2	Groups in comparison	Meaning of criterion statistics	Critical statistical value (r)	Significance of intergroup differences
Maximal ratio	χ^2=207.4, p<0.000	"Cavitation extract"/Intact control	0.02	0.029	No
		"Cavitation extract"/Toxin control	0.08	0.027	Yes
		"Cavitation extract"/"Lightning extract"	0.053	0.035	Yes
		Intact control/Toxin control	0.06	0.021	Yes
		"Lightning extract"/Intact control	0.072	0.03	Yes
		"Lightning extract"/Toxin control	0.132	0.028	Yes
Mean ratio	χ^2=61.1, p<0.000	"Cavitation extract"/Intact control	0.013	0.023	No
		"Cavitation extract"/Toxin control	0.024	0.022	Yes
		"Cavitation extract"/"Lightning extract"	0.034	0.028	Yes
		Intact control/Toxin control	0.01	0.017	No
		"Lightning extract"/Intact control	0.048	0.024	Yes
		"Lightning extract"/Toxin control	0.058	0.023	Yes

Table 5: The mean number of TH+ cells on nine slices of SNM in sampled studied animals of all groups

Nos. of mice	Intact control	Toxin control	"Lightning extract"	"Cavitation extract"
1	3992	2416	4256	3728
2	4512	3408	5576	3448
3	4656	3184	4016	3536
4	4720	3232	4776	3712
Mean	4470	3060	4656	3606
S.E.M.	165	220	345	68

Table 6: Significance of differences in the average number of TH+ cells on nine slices of SNM between all studied groups of animals according to the Conover-Iman test

Compared groups	Statistical criterion value (q)	Statistical criterion critical value	Significance of intergroup difference (P)
Intact control/Toxin control	6.58	2.18	<0.000*
"Lightning extract"/Intact control	0.69	2.18	0.502
"Cavitation extract"/Intact control	3.81	2.18	0.003*
"Lightning extract"/Toxin control	7.27	2.18	<0.000*
"Cavitation extract"/Toxin control	2.77	2.18	0.017*
"Cavitation extract"/"Lightning extract"	4.5	2.18	0.001*
*p<0.05, difference from the paraquat-control group			
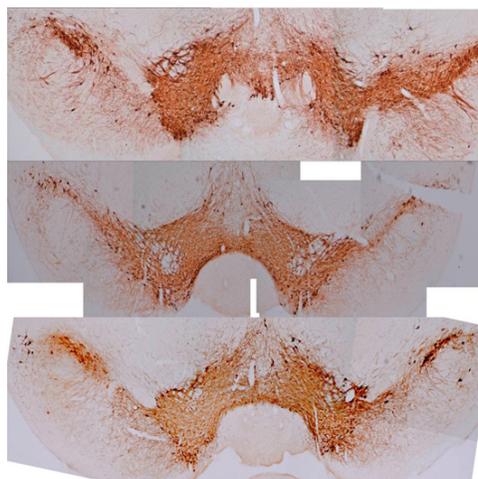
Figure 3: Representative slices of the SNM of the animals: Upper slice-intact control; middle slice-toxin control; bottom slice-"lightning extract" group. Stained for TH
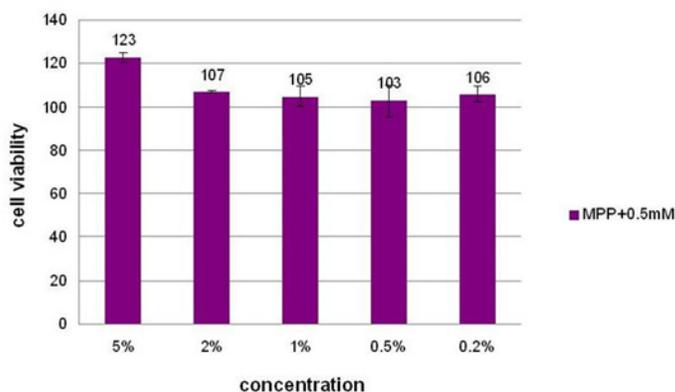
**Figure 4: Protective effect of *Ulomoides dermestoides* "cavitation extract"**
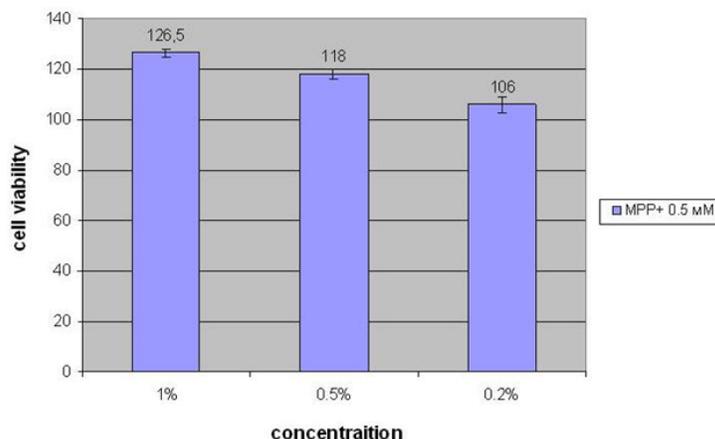
Figure 5: Protective effect of *Ulomoides dermestoides* "lightning extract" on SH-SY5Y cells in the presence of MPP+ neurotoxin (0.5 mM)

DISCUSSION

The use of methods for the extraction of biologically active substances from the raw biomass of the *Ulomoides dermestoides* darkling beetle using cavitation effects, both directly and by the electroexplosive method, made it possible to obtain extracts with protective properties in the used model of an early stage of Parkinson's disease. The presented experiments show that continuous administration of the "cavitation extract" with food had some positive effect on the motor skills of animals after intoxication with paraquat as compared to the control group also intoxicated with paraquat but avoided the antidote. Intake of the "lightning extract" preparation with food almost completely eliminated the toxic effect of paraquat in the rotarod test. In the vertical pole test, the mice from both "extract" groups could hold on to an upright pole longer without sliding or jumping off. For this reason, the proportion of the maximum and the mean (in three attempts) times spent on the pole (from the maximal possible) in the mice of these groups was higher than in mice from the toxin control group. This is probably due to a higher muscle tone and better coordination of movements, both during locomotion and maintaining a stationary position on the supporting surface of the vertical pole. The mice from the "lightning extract" group demonstrated the best results.

The behavioral tests results were consistent with the results of histochemical examination of mouse brain slices. The significant decrease in the degree of SNM dopaminergic neurons damage due to paraquat toxin was shown in mice when preparations from *Ulomoides dermestoides* extracts were added to the food. The best results were found in animals that received the "lightning extract".

The results of the neurotoxic model of Parkinson's disease in mice were confirmed *in vitro* using the SH-SY5Y neuroblastoma model in the presence of the MPP+ neurotoxin (0.5 mM). Both tested beetle extracts were able to maintain cell viability by 23-26%. However, this value was achieved with the introduction of 5% cavitation extract and 1% lightning extract, which correlated with the in vivo effects of the extracts in mice.

In our previous study (Ushakova NA, *et al.*, 2021), a biologically active extract from the *U. dermestoides* beetle biomass was obtained using the electro-pulse plasma dynamic extraction method. The beetle water extract contained a complex of antioxidant substances of both protein and non-protein nature. The antioxidant proteins include superoxide dismutase (SOD) and catalase, the main enzymes of the body's antioxidant system, as well as heat shock proteins with molecular weights of 60, 70 and 83 kDa, which protect biological systems from damaging effects under stress, including oxidative stress (Ekimova IV, *et al.*, 2018). The aqueous extract also includes non-protein substances with anti-infectionous and anti-inflammatory properties: various phenolic compounds and ethyl-p-hydroquinone. This determines the rather high antioxidant activity of the aqueous extract of the beetle, i.e., 1 mg of dry matter/mL of the extract has an equivalent antioxidant activity to 0.2 mM Trolox (a water-soluble analog of vitamin E). It was shown that the beetle extract can lead to a 25%-30% increase in the average lifespan of nematode *C. elegans* under normal conditions, and a 12%-17% increase under conditions of oxidative stress (with paraquat), and significantly inhibits the fructosylation reaction of serum albumin (Ushakova NA, *et al.*, 2021).

It is well known that the SNM is namely the most susceptible to the generation of free radicals of all cerebral structures (Nuzzo D, 2021; Weng M, *et al.*, 2018). The dopamine produced by SNM neurons undergoes enzymatic reactions, and one of the by-products of these transformations is hydrogen peroxide, which decomposes into highly toxic hydroxyl radicals. Normally, these radicals are inactivated by the glutathione system. However, it is known that the activity of antioxidant systems decreases with age. This may be one of the reasons for the age-related decrease in the resistance of dopaminergic nigrostriatal neurons to proparkinsonian factors and the progressive increase in patients with Parkinson's disease. The concept of the role of oxidative stress in the pathochemical mechanisms of neuronal damage in Parkinson's disease determines one of the ways of its therapy with antioxidants. A diet rich in antioxidant substances found in vegetables and fruits, and additional intake of antioxidants in the form of dietary supplements are important.

The search and development of antioxidant pharmacotherapy is carried out in three directions. The first is based on the use of well-known endogenous antioxidants, for example, vitamins E and C. Vitamin E has a mild effect, however, when introduced into the body, it quickly loses its activity. Another direction is the creation of synthetic analogs, which are characterized by a stronger effect penetrating the blood-brain barrier. Typically, these substances have severe side effects. The third direction is associated with the search for new natural antioxidants or natural antioxidant complexes. This group includes the extracts of darkling beetles of the Tenebrionidae family.

CONCLUSION

Therefore, the beetle aqueous extract shows promise as a biologically active complex exhibiting antioxidant activity. At the same time, the presence of relatively large amounts of phenol-containing substances indicates a possible toxic effect; therefore, the practical use of this extract requires caution and study of its toxicity.

DECLARATIONS

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