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The aqueous extract of *Ulomoides dermestoides* larvae counteract motor and cognitive disfunction in a neurotoxic model of Parkinson's disease

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Abstract
Insects are a potential source of novel bioactive compounds with pharmaceutical applications. Our previous study revealed the presence of antioxidant system proteins in the aqueous extract of *Ulomoides dermestoides* Fairmaire 1893 larvae, which exhibited high antioxidant activity in vitro and improved cognitive and motor functions in aging mice. To determine whether the extract of *Ulomoides dermestoides* larvae can enhance motor control and cognitive performance in a neurotoxic model of the initial stage of Parkinson's disease, adult male C57Bl/6 mice (n = 10/group) were injected intraperitoneally with paraquat neurotoxin, which penetrates the blood–brain barrier and causes oxidative stress that destroys dopaminergic neurons of the substantia nigra (more detail about protocol treatment and motor and cognitive impairments typical for the mice model of PD can be found in our previous publication – Kovalzon et al. [19]. Two groups, E0.2 and E0.4, received either 0.2 or 0.4 mg/kg of daily oral doses of the aqueous extract of *Ulomoides dermestoides* larvae, respectively, for 1 week before and during neurotoxin administration. The effects were compared to neurotoxin-only (Ctox) and intact control (Cin) groups. Motor coordination and cognitive performance were assessed using the Rotarod, vertical pole, and object recognition tests. Neurotoxin administration significantly impaired motor and cognitive functions in Ctox. The E0.4 but not E0.2 treatment significantly attenuated the behavioral effects of the neurotoxin (up to the 90% of Cin values). Oral administration of the aqueous extract of *Ulomoides dermestoides* larvae can significantly counteract the impairments of motor skills and cognitive performance induced by the neurotoxic model of Parkinson's disease. The neuroprotective effect is likely to reflect the earlier established antioxidant properties of the extract.

Keywords Antioxidants · Parkinson's disease · Experimental models · Entomotherapy

Introduction

In recent years, the recognition of oxidative stress as a crucial contributor to the pathochemical mechanisms of neuronal damage in Parkinson's disease has led to the possibility of antioxidant therapy [3, 11, 13, 33]. Free radical oxidation plays important role in both normal and pathological processes, including neuro-cognitive and inflammatory responses, vascular tone modulation, excitation propagation, cell growth regulation, neuroplasticity, neurotransmitter secretion, and the development of neurodegenerative and autoimmune diseases. Therefore, the regulation of free

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radical reactions by antioxidants is of utmost importance. The antioxidant system, primarily composed of enzymes, such as superoxide dismutase, catalase, and coenzyme glutathione, along with low molecular weight non-enzymatic antioxidants (such as vitamins A, C, D, E, K, F, ubiquinones, tryptophan, phenylalanine, flavonoids, carotenes, lipoic acid, cysteine, and methionine), modulates the activity of free radicals. Under pathological conditions, a disbalance between the pro- and antioxidant systems leads to the accumulation of free radicals, causing irreversible changes in membrane lipids, proteins, polysaccharides, and nucleic acids. This phenomenon is known as oxidative stress.

The compact part of the substantia nigra in the brain is highly susceptible to the generation of free radicals. Dopamine, synthesized by neurons in this region, undergoes enzymatic degradation. Some of the products of this degradation process can include hydrogen peroxide, which can further decompose into hydroxyl radicals, the highly reactive and potentially toxic species. Under normal conditions, the glutathione system is responsible for inactivating these radicals. However, with advancing age, the activity of antioxidant system decreases, and this can be one of the reasons for the reduced resistance of dopaminergic nigrostriatal neurons to pro-parkinsonian factors and the increase in the prevalence of Parkinson's disease in elderly patients [7, 37].

Advancements in understanding the pathogenesis of neurodegenerative disorders heavily rely on the development of suitable experimental models. Particularly, interest lies in models representing preclinical and early clinical stages of Parkinson's disease [39]. One of those is the neurotoxic model, which is induced by a pesticide paraquat (1,1'-dimethyl-4–4'-bipyridine dichloride) [2, 4, 21, 25, 26, 43]. Upon penetrating the blood–brain barrier, paraquat is taken up by the dopamine transporter protein, to its delivery to the neurons located in the compact part of the substantia nigra. In dopaminergic neurons, paraquat initiates a cycle of oxidative stress, impairing mitochondrial functions, promoting protein aggregation (such as the formation of Lewy bodies), and stimulating further production of pro-inflammatory mediators. This results in the death of these neurons, leading to reduced dopamine release and the manifestation of Parkinson's disease like symptoms in the experimental mice.

The search for natural antioxidants that can alleviate age-related disorders, including reduced resistance to pro-parkinsonian factors, is a pertinent research area [31]. Of particular interest are the antioxidants of natural origin and insects are one of the potential sources of such bioactive compounds for pharmaceutical applications [1, 9, 12, 35]. Tenebrionid beetles are traditionally used in Central and South America to treat *Asthma bronchiale* [10]. In the pathogenesis of this disease, oxidative stress caused by various inhalable pollutants plays an important role. At the same time, experimental studies, including those in our laboratory, have shown that darkling beetle extracts have potent antioxidant, anti-inflammatory and antimicrobial activity [16, 24, 40, 45]. Since oxidative stress (including our experimental model) is one of the main factors influencing the development of Parkinson's disease, we hypothesized that the antioxidant activity of the darkling beetle extract could counteract the death of brain neurons and the development of symptoms of this disease. As neuronal death caused by oxidative stress may not be limited to the substantia nigra only and is likely to affect higher brain functions related to memory and attention, we also tried to determine the effect of the extract in the object recognition memory test.

The tenebrionid *Ulomoides dermestoides* Fairmaire 1983 (Coleoptera: Terebrionidae) (synonyms: *Martianus dermestoides*; *Palembus dermestoides*) is a darkling beetle regarding a medical insect because its whole-body extracts and fats demonstrate broad spectrum pharmacological activity [5, 18, 23, 34, 44]. Earlier, we detected antioxidant proteins in an aqueous extract of *Ulomoides dermestoides* darking beetle larvae [42]. Out of a total of 169 proteins and protein groups documented in the extract, 104 proteins were reliably identified and included those with antioxidant activity, such as cytochrome C-2, nucleoside diphosphate kinase, calmodulin, superoxide dismutase, catalase, glutathione S-transferase, peroxiredoxin, glutathione synthetase, thioredoxin, 70 kDa and 60 kDa heat shock proteins, and chitinase complex. Collectively these represented 13.4% of the extract weight and could be responsible for the antioxidant effects of the extract observed. In an in vitro study, the catalase activity was 64.7 ± 10.1 μmol H_2O_2/min/mg protein, with the antioxidant activity of 1 mg of protein per ml of the extract being equivalent to 2.1 ± 0.15 mM trolox.

Importantly, unlike the extract from adult darkling beetle *Ulomoides dermestoides* that also has antioxidant activity [40], the larval extract did not include toxic phenolic compounds. Administration of 0.4 mg/kg of the larval extract with food twice a week for three months improved object recognition ability and coordination of movements in aging mice [42]. In this study, we aimed to investigate the effects of the same aqueous extract of *Ulomoides dermestoides* larvae on the motor control and cognitive performance of mice in a neurotoxic model of the initial stage of Parkinson's disease.

Materials and methods

Animals

We used adult male C57Bl/6jsto mice weighing 25–30 g from our local colony. The mice were housed individually in cages in a vivarium room with controlled environmental conditions, including a temperature of 22–24 °C and a 12-h light/dark cycle; lights on at 9:00 AM and off at 9:00 PM. Each mouse received 15 g of food per day, consisting of boiled oats and peas with vegetable oil, according to their standard diet. We based the amount of food on the average consumption of mice in our colony, and we observed that the mice typically consumed all of the food provided.

Extract preparation

In this study, we examined the aqueous extracts obtained from *Ulomoides dermestoides* larvae that were cultivated under controlled laboratory conditions on dry food substrates, consisting of a mixture of oatmeal and corn flour [42]. The larvae were separated from the feed substrate, and the biomass was washed and homogenized in cold distilled water, followed by a 2-day cold extraction with slow stirring. The extract was pre-filtered to remove solids, and then filtered using 0.1 μm antibacterial filter (Millipore membrane filter, Merck, Germany). The resulting extract was stored at -18 °C until further use. Prior to the experiment, the extract was immobilized onto a food sorbent, and the resulting mixture was lyophilized to obtain a dry powder containing the extract, which was subsequently added as experimental powder to the diet of the experimental animals.

Treatment and experimental design

Standard protocol for the induction of Parkinson's disease like symptoms using neurotoxin was used, as described earlier [19]. In brief, 10 mg/kg of paraquat (Paraquat-dichlorid, SIGMA) was dissolved in 0.3 ml of saline and injected intraperitoneally daily between 15.00 and 17.00 h for 3 days.

The mice were randomly assigned into four groups of 10 mice each: two experimental and two control groups. The controls included the intact group (Cin), which received no treatment, and the toxin-only group (Ctox). The experimental groups E0.2 and E0.4 received both the neurotoxin injections and the larval extract, immobilized onto a food sorbent mixed with food, 0.2 mg/kg of body weight (E0.2 group) or 0.4 mg/kg of body weight (E0.4 group). The choice of the experimental doses of the extract was based on the earlier findings using the adult beetle extract [19]. The extract treatment started one week prior to the first injection of the neurotoxin and continued throughout the experiment, until the end of the behavioral testing.

Four days after the last administration of the toxin, the effects of the treatment were evaluated for motor activity and coordination (the Rotarod and vertical pole tests) [15, 22, 29, 30] and cognitive performance (object recognition memory test) [17]. The whole testing period lasted eight days.

Behavioral testings

Rotarod test

Testing was conducted as described earlier [19, 41]. In brief, the experimental procedure involved placing mice on a Rotarod that was set to rotate at 6 rpm for a period of 600 s, followed by an automatic increase in the rotation speed by 1 rpm every 30 s until the speed of 20 rpm was reached. The retention time and the sustained maximum rotation speed were recorded. The test was considered successfully completed if the mouse retention time was 1050 s including 30 s at the sustained maximum rotation speed of 20 rpm.

Vertical pole test

Testing was conducted as previously reported [19, 41]. In brief, the test involved placing a mouse with its head upwards close to the top of a vertical 50 cm high pole with a diameter of 1 cm. Typically, a mouse would reorient itself to descend to the bottom of the pole. The mouse was then returned to its cage for at least 30 s, followed by 2 more trials with the 30-s intervals between them. The test was considered successful if the mouse completed its descent from the pole within 3 min from the start of testing. If more than 3 min elapsed, it was taken off the pole and the trial was not included in the statistical analysis. All experiments were video recorded for further behavioral analysis, using behavioral data analysis and presentation software BORIS 7.13. Since it is difficult for a mouse to stay on a pole and in the vast majority of cases it tends to descend from it, just what allows or, on the contrary, prevents the mouse from being on the pole longer may indicate the presence or impairment of motor control mechanisms (the longer the mouse is on the pole, the better motor control is). On the other hand, the presence of effective motor control mechanisms may allow the mouse to descend from the pole faster without wasting effort trying to stay on it. Based on this, to assess the effectiveness of motor control and coordination in mice of the control and experimental groups, we chose the parameter of the maximum time spent on the pole in three attempts.

Object recognition memory test

A cognitive performance test to evaluate the recognition of new objects, referred to as "response to novelty", was conducted as described earlier [14, 17, 41]. The recognition technique is based on the tendency of rodents to examine a new object longer than an already familiar one, the one with which they have already been in contact, which serves as an object for comparison. Mice were tested in standard cages (29.5 × 18.5 × 13 cm). Before testing, the mouse was placed in an empty cage for 10 min for habituation. It was then placed in a test cage with two identical small objects (~ 2 × 4 cm each) fixed 3 cm apart (to prevent their movement due to the animal's exploratory activity). The animal was allowed to examine the objects for the next 5 min. Examination of the object included sniffing the object from a distance of up to 3–4 cm, sniffing the object on direct contact, licking and attempting to gnaw the object. Video recording of mouse activity was performed using a Canon LEGRIA HF G60 camera. After five minutes of testing the mouse was placed in a habituation cage for 10 min. The animal was then returned to the cage for testing with the same objects and the procedure was repeated two more times with the 10-min interval between them, for a total of 3 presentations. This is the phase of memorization, or "familiarization" with the object, which was used as a reference to assess the ability to distinguish a novel object.
Test presentations were performed sequentially 3 and 24 h after the last presentation in the familiarization phase. The duration of the test presentations was 5 min. One of the reference objects during test presentations was replaced by a new one. The new object differed from the reference one in several ways—material, texture, shape and color. After each presentation, the cage and objects were thoroughly wiped with an alcohol solution, so as not to leave any olfactory signals of mouse presence in the cage. When analyzing the data, the average examination time of the new and the reference object in the two tests was taken into account. If the duration of the examination of one of the objects in the test (the first or second trial) did not exceed 0.5 s, the test was not considered passed; in this case the data obtained from this mouse were excluded from the statistical analysis (since such mouse demonstrated a weak exploratory activity and showed no interest in the objects). For this reason, the data from the two males (from groups E0.2 and E0.4) were not included in the statistical analysis.

Statistical analysis

A nonparametric Kruskal–Wallis test was used in the analysis of data obtained on the Rotarod and vertical pole. Post hoc pairwise comparisons were performed using the Conover-Iman test. In addition, when analyzing the vertical pole data, inter-group comparisons were made of the proportion of the actual maximum time spent by the mouse on the pole versus the maximum possible time on the pole under experimental conditions (N^x180 for all groups, where N is the number of individuals in the group, and 180 is the maximum possible duration on the pole, expressed in seconds). The analysis of the data obtained in the test for recognition of new objects was carried out using the Wilcoxon test. Intergroup comparisons were made between the proportions of the average actual time used by control and experimental groups mice to examine new and reference objects, according to the formula N^x300, where N is the number of individuals in the

group, and 300 is the maximum duration of the test, expressed in seconds. The proportions were compared using the χ^2 test. Adjusted Wald test was used for pairwise post hoc comparisons. If it was not possible to use the analysis of variance due to the wide variation in the data and insufficient *n* in each sample, hierarchical cluster analysis was used to determine the degree of correlation between the data. The proximity between variables was measured using Pearson's dissimilarity test. The complete linkage was used as agglomeration method. To define the truncation level automatically, the inertia method was used. During the course of the experiment, one male died in group Cin of an unknown cause. The data for this mouse were not included in the statistical analysis of object recognition memory test. Eventually, the statistical analysis of this test included data from 9 males in groups E0.2, E0.4, and Cin, and 10 males in group Ctox. The behavioral indices recorded in groups Ctox and Cin were used as the reference values. The deviation of these indices in groups E0.4 and E0.2 from those in group Ctox, and their proximity to those in group Cin, were used to evaluate the effect of the larval extract. The statistical analysis was performed using the software packages NCSS 21.0.4 and XLSTAT 2019.2.2.

Results

Rotarod test

Based on the findings presented in Fig. 1 and Table 1, it can be observed that the mice in group E0.4 exhibited performance that was most similar to that of group Cin, with E0.4 mice demonstrating 90–92% of the performance of Cin mice in both limiting sustained Rotarod speed (Fig. 1A) and maximum retention time on the Rotarod (Fig. 1B). The results of group E0.2, although showing a bias from Ctox to Cin, were lower than for group E0.4. This is also confirmed by the results of cluster analysis (Fig. 2A, B), which show that groups Cin and E0.4 are combined into one cluster, and Ctox and E0.2 into another.

Vertical pole test

The behavior of the mice on the pole was varied, but did not differ significantly in all experimental and control groups. Mice could come down from it, head down or sideways, slip off it or jump off it. As can be seen from Fig. 3A, the medians of maximum duration on the pole did not differ significantly, but there was a clear tendency to shift from the Ctox to the Cin values in the E0.4 and E0.2 groups. This trend is also evident from the cluster analysis (Fig. 3B): the data for maximum duration on the pole in mice of experimental and control groups are distributed in two clusters: data from groups Cin and E0.4 are combined into one cluster, and Ctox and E0.2 into another. The result of group E0.4 is the closest to that of group Cin and was 90% of it. At the same time, paired intergroup comparisons of the proportion of the actual maximum time spent

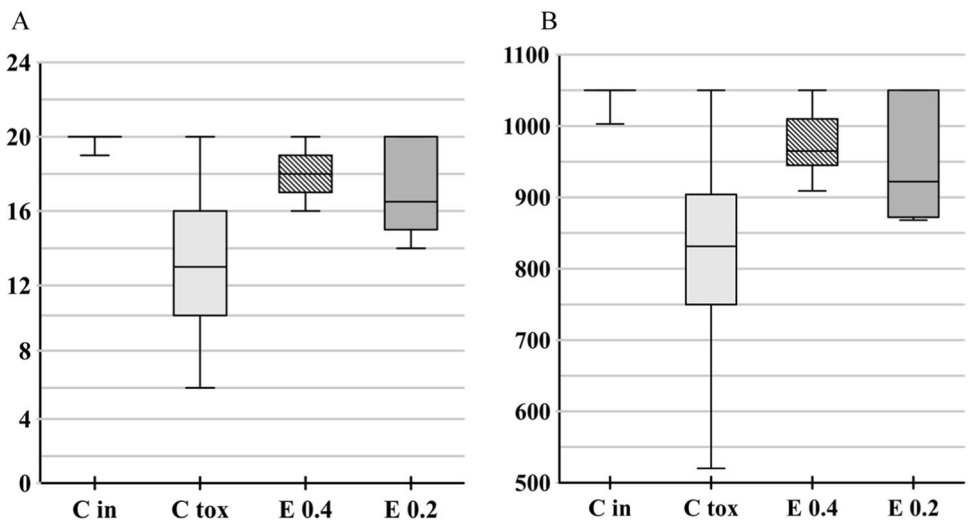

Fig. 1 Motor performance on the Rotarod test. **A**. The sustained maximum rotation speed (rpm). **B**. Retention time (seconds). In A and B, data presented as median (percentile: 10–90%)

Table 1 Motor performance on the Rotarod test. Results of the Conover-Iman test for pairwise comparisons of the retention time and the sustained maximum rotation speed of mice in the control and experimental groups. The Kruskal–Wallis test statistic and probability level (P) are also presented for the retention time (H3 = 13.9; P = 0.003) and sustained maximum rotation speed (H3 = 3.95; P = 0.003)

Groups compared	Retention time		The sustained maximum rotation speed	
	Differences of means of ranks	Probability level (P)	Differences of means of ranks	Probability level (P)
Ctox/ E0.4	9.3	**0.034***	8.7	**0.046***
Ctox/ E0.2	8.1	0.063	8.1	0.062
Ctox/ Cin	18.8	**< 0.001***	18.8	**< 0.001***
E0.4/ Cin	9.5	**0.031***	10.1	**0.022***
E0.2/ E0.4	1.2	0.778	0.6	0.888
E0.2/ Cin	10.7	**0.016***	10.7	**0.016***

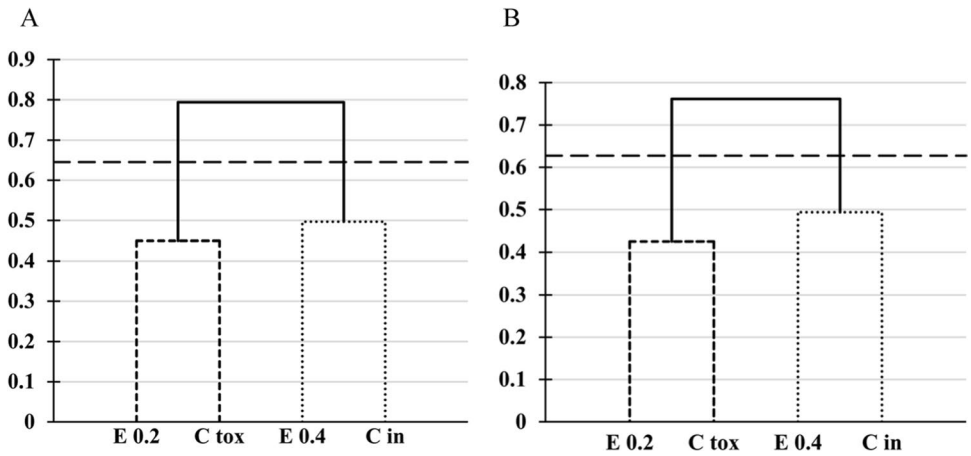

Fig. 2 Dendrograms of hierarchical cluster analysis of Rotarod test data: **A**. Retention time (seconds). **B**. The sustained maximum retention speed. The Y-axis in both graphs in Fig. 2 and graph B in Fig. 3 represents the Pearson's dissimilarity coefficient values. A dashed line in that figures indicates the level of truncation

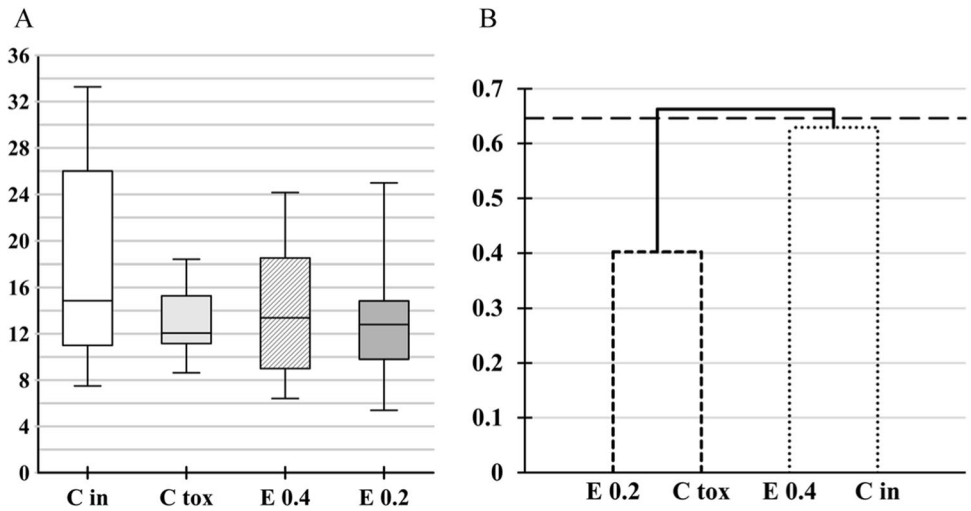

Fig. 3 Data and hierarchical cluster analysis of data of maximum duration of stay on the vertical pole for both experimental and control groups of mice. **A**. Median and standard deviation (σ) of the maximum duration (seconds). **B**. Dendrogram of hierarchical cluster analysis of data of the maximum duration

by mice on the pole, relative to the maximum possible duration according to the experimental conditions, showed (Table 2) that this proportion was significantly lower in group Ctox and in both experimental groups (E0.4 and E0.2) than in group Cin. It can be argued that the results of the vertical pole test were generally consistent with those of the Rotarod test. As the results showed, the features and characteristics of pole-descending behavior did not differ significantly between the mice in

Table 2 Wald's test results for paired intergroup comparisons of the proportion of actual maximum time spent by mice on the pole, relative to the maximum possible duration according to experimental conditions, in the control and experimental groups

Statistics and significance of the differences $\chi 2$	Paired comparisons of groups and proportion of actual maximum time spent by mice on the pole for each group (in brackets)	Wald test statistics (Student range Q)	Probability level (P)
$\chi^2 = 18.2$ $P < 0.001$	Ctox (0.075)/Cin (0.113)	5.565	**0.001***
	Cin (0.113)/E0.4 (0.085)	4.016	**0.023***
	Cin (0.113)/E0.2 (0.084)	4.100	**0.020***
	Ctox (0.075)/E0.4 (0.085)	1.559	0.684
	Ctox (0.075)/E0.2 (0.084)	1.475	0.717
	E0.4 (0.085)/E0.2 (0.084)	0.084	0.993

Table 3 Post hoc pairwise comparisons using the adjusted Wald test for the proportion of actual duration of mice examination of the reference and new object in the experimental and control groups from the maximum possible duration according to experimental conditions, analyzed using the chi-square test

Groups	$\chi 2$ test statistic and probability level (P)	Wald test statistics (Student range Q)	Proportions of the object's examination time		Probability level (P)
			New	Referent	
Ctox	$\chi^2 = 3.366$ P = 0.067	2.584	0.042	0.033	0.068
Cin	$\chi^2 = 9.017$ P = 0.003*	4.232	0.050	0.033	**0.003***
E0.4	$\chi^2 = 5.197$ P = 0.023*	3.207	0.036	0.026	**0.023***
E0.2	$\chi^2 = 0.559$ P = 0.455	1.051	0.029	0.026	0.458

Table 4 Comparison of the duration of examination of a reference and a new object by mice in the experimental and control groups (Wilcoxon test)

Groups	Wilcoxon test statistics (Z)	Probability level (P)
Ctox	1.478	0.139
Cin	2.073	**0.038***
E0.4	2.429	**0.015***
E0.2	0.652	0.515

the control and experimental groups. Since the pole-descending behavior of the Ctox and E.0.2 mice was not more complex and coordinated than that of the Cin and E0.4 mice, the faster pole-descending behavior of the mice from these groups cannot be explained by a more efficient motor control of the descending movements. From this we can conclude that the faster descent from the pole in Ctox and E.0.2 mice is due to weaker motor control and limited ability to stay on the pole.

Object recognition memory test

In group E0.4, similarly to group Cin, a significant "novelty response" was observed: the time spent examining a new object and the proportion of time spent on it were both significantly higher than those spent on the reference object (Tables 3 and 4). Furthermore, the median ratio of time spent examining a new object versus a reference object was higher in groups Cin and E0.4 compared to groups Ctox and E0.2, as shown in Fig. 4. Conversely, in groups Ctox and E0.2, there was no significant difference in the time spent examining a new versus a reference object. Therefore, the "novelty response" was attenuated by the neurotoxin, but the extract at a higher dose prevented this effect.

Discussion

The present study of the effect of *Ulomoides dermestoides* larvae extract on the motor skills and cognitive abilities of C57BL/6 mice in a neurotoxic model of the initial stage of Parkinson's disease demonstrates that the extract, when administered with food (0.4 mg/kg body weight test drug), to significant extent (up to 90%) eliminates the negative effects of paraquat injections. Specifically, the extract effectively prevents the development of motor skills and motor coordination impairments in intoxicated mice and preserves their exploratory activity towards the new object. It remains to be elucidated whether the effects observed are due to one or several substances contained in the extract. Although earlier established antioxidant properties of this extract [42] suggest that the effects observed can be related to those, future studies are warranted to explore the alternatives. Additionally, a decrease in the extract dosage results in a reduction in its effectiveness. The primary challenges in the study of neurodegenerative diseases are the creation of adequate experimental models and the search for early markers. In our previous study [19] it was shown that twice (one day apart) injection of paraquat neurotoxin at a dose of 10 mg/kg reduces the number of dopamine-synthesizing (tyrosine hydroxylase-immunopositive) cells in

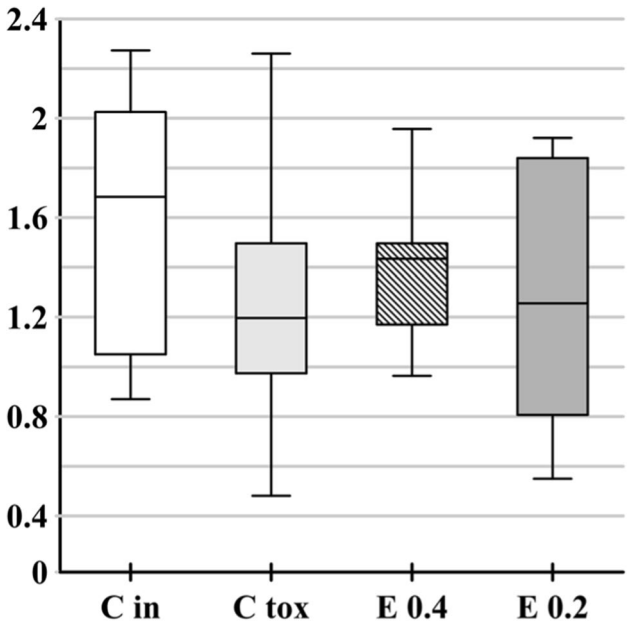

Fig. 4 The median (IQR factor = 1.5) ratio of the examination time of a new object to that of a reference object by mice in both experimental and control groups. The Y-axis represents the ratio of time spent examining a new object compared to the reference object

ventral substantia nigra by 31.5% compared to control. This pattern corresponds to the initial stage of Parkinson's disease, in which no obvious motor disturbances are observed yet [39]. However, testing of mice on a Rotarod and a vertical pole, performed in our previous and present studies, reveals an emerging impairment of motor control. Additionally, the present study revealed cognitive impairment for the first time for this neurotoxin, as demonstrated by a relative decrease in the time spent examining a new object (compared to the reference object) under the influence of the neurotoxin. Whether this suppression of the "novelty response" is due to perceptual, emotional, or mnemonic deficits in the experimental animals, it is premature to judge from the results of the present study. It is interesting to note, however, that apathy, a "loss of interest in novelty", is characteristic of Parkinson's disease patients and occurs early in the disease, presumably associated with impaired dopaminergic control of "emotionogenic" striatum structures [20, 28, 36, 38].

Insects have been extensively utilized in medicine; however, only a few substances derived from them have been thoroughly investigated using modern scientific techniques to confirm their therapeutic properties, and they have been utilized to produce medications. Such drugs have been used to treat various diseases, such as gastrointestinal, oncological, dermatological, respiratory, and urological diseases [6, 8, 27, 32]. Extracts obtained from *Ulomoides dermestoides* insects hold potential as medicinal agents, including for Parkinson's disease, as they contain potent natural antioxidant complexes with confirmed physiological activity.

Conclusion

Here we show that oral administration of the aqueous extract of *Ulomoides dermestoides* larvae can significantly attenuate the impairments of motor skills and cognitive performance induced in C57BL/6 mice neurotoxic model of the initial stage of Parkinson's disease. The effect of the extract is dose-dependent. These findings suggest that this extract larval may hold promise as a natural, powerful antioxidant with potential therapeutic applications in Parkinson's disease.

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Author contribution Conceptualization: Nina A. Ushakova, Alexander V. Ambaryan, Vladimir M. Kovalzon; Methodology: Nina A. Ushakova, Alexander V. Ambaryan; Writing—original draft: Vladimir M. Kovalzon; Writing—review & editing: Nina A. Ushakova, Alexander V. Ambaryan, Vladimir M. Kovalzon; Formal analysis and investigation: Alexander V. Ambaryan, Vladimir M. Kovalzon; Supervision: Nina A. Ushakova; Funding acquisition: Nina A. Ushakova; Resources: Nina A. Ushakova, Vladimir M. Kovalzon; Software: Alexander V. Ambaryan; Data curation: Nina A. Ushakova, Alexander V. Ambaryan, Vladimir M. Kovalzon.

Data availability Data will be made available upon request.

Declarations

Ethical approval Experiments on animals in vivo were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and supported by the Ethics Commission of the IPEE RAS (Protocol No. 56 of 03/22/2022).

Competing interest The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article. The sponsoring organization was not involved in the planning and execution of the experiments, the data processing and analysis, as well as the decision to publish them, the writing, discussion and editing of this manuscript.

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