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RESEARCH

Therapeutic Effects of Geranium Oil in MPTP-Induced Parkinsonian Mouse Model

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Abstract

Parkinson's disease (PD) is an incurable neurodegenerative disease characterized by motor and non-motor disabilities resulting from neuronal cell death in the substantia nigra and striatum. Microglial activation and oxidative stress are two of the primary mechanisms driving that neuronal death. Here, we evaluated the effects of geranium oil on 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) mouse model for PD, on microglial activation, and oxidative stress. We demonstrate that oral treatment with geranium oil improved motor performance in this model. The therapeutic effects of geranium oil were observed as a significant increase in rotarod latency and distance among the mice treated with geranium oil, as compared to vehicle-treated MPTP mice. Geranium oil also prevented dopaminergic neuron death in the substantia nigra of the treated mice. These therapeutic effects can be partially attributed to the antioxidant and anti-inflammatory properties of geranium oil, which were observed as attenuated accumulation of reactive oxygen species and inhibition of the secretion of proinflammatory cytokines from geranium oil-treated activated microglial cells. A repeated-dose oral toxicity study showed that geranium oil is not toxic to mice. In light of that finding and since geranium oil is defined by the FDA as generally recognized as safe (GRAS), we do not foresee any toxicity problems in the future and suggest that geranium oil may be a safe and effective oral treatment for PD. Since the MPTP model is only one of the preclinical models for PD, further studies are needed to confirm that geranium oil can be used to prevent or treat PD.

Keywords Geranium oil · Essential oil · MPTP model · Neuroinflammation · Parkinson's disease · *Pelargonium graveolens*

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, affecting approximately 2% of adults over the age of 60. Currently, there are no highly effective preventive or curative pharmacological or nutritional treatments for PD. PD is characterized by the selective loss of dopaminergic neurons in the striatum and substantia nigra pars compacta (SNpc). As dopamine is transported by dopaminergic projections from the substantia nigra to the striatum, this loss leads to dopamine deficiency in the striatum, which, in turn, causes prominent motor function disturbances [1, 2]. Multiple mechanisms contribute to neurodegeneration in PD, including oxidative stress, neuroinflammation, microglial activation and elevated levels of IL-1β and IL-6. Therefore, the inhibition of microglia-mediated neuroinflammation bears potential as a protective strategy against PD [3–9]. It has also been widely accepted that excessive reactive oxygen species (ROS) are key mediators

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of PD pathogenesis and dopaminergic neuron death, as they induce the oxidation of cell proteins, lipids and DNA [10, 11]. In recent years, there has been increased interest in the therapeutic effects of phytochemicals. At the same time, the increasing number of patients with PD has resulted in growing interest in phytochemicals that can support neuronal health and help to prevent PD [12–14]. Essential oils are multicomponent mixtures containing hundreds of low-MW phytochemicals. They are used as folk medicines to treat various kinds of inflammatory diseases, organ dysfunction and systemic disorders [15]. The advantage of plant essential oils for the treatment of multifactorial diseases such as PD lies in the variety of compounds that they include, which act synergistically and simultaneously on several targets [16–18]. Geranium oil is obtained by steam distillation of *Pelargonium graveolens* l'Heritier ex Aiton [19] and is defined by the FDA as a generally recognized as safe (GRAS) substance. The main constituents (≥ 10%) of geranium oil are citronellol (26%), citronellyl formate (16%) and linalool (10%) [20]. Geranium oil has been used for many years in traditional medicine for various purposes [21] and has been shown to exhibit anti-inflammatory activity in mice [20, 22–24]. The components of geranium oil are lipophilic and have even low molecular weights (~ 150 g/mol) which means that they are likely to be capable of crossing the blood–brain barrier and acting synergistically on various targets. However, the safety of geranium oil for brain cells and its effects in mice models of neurodegenerative diseases, such as PD, have not been studied previously. In a previous study, we showed that geranium oil down-regulates microglial activation [20]. In light of that finding and the fact that robust microglial activation has been observed in the SNpc in PD patients and animal models for PD [7, 9], we set out to determine whether daily consumption of geranium oil is safe and whether it can mitigate behavioral impairment and dopaminergic damage in a mouse model of PD. Among the various toxic models of PD, the MPTP model has become the most commonly used, since MPTP is the only known dopaminergic neurotoxin capable of inducing a clinical picture indistinguishable from PD in both humans and primates [25]. In the current study, we assessed the anti-inflammatory and antioxidant effects of geranium oil in primary cultures of microglial cells, as well as its therapeutic effect and the safety of daily consumption of geranium oil in the 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) mouse model for PD.

Materials and Methods

The Materials and Methods section is presented as supplementary material.

Results and Discussion

Geranium Oil Attenuates the Secretion of IL-1β and IL-6 From LPS-Stimulated Microglial Cells

Given the robust microglial activation that has been found in the SNpc in PD patients and animal PD models [7, 9], the potential inhibitory effect of geranium oil on the release of the inflammatory cytokines IL-1β and IL-6 was examined in activated microglial cells. We described the gas chromatography-mass spectrometry (GC-MS) profile of geranium oil in a previous publication [20]. Microglial cells were activated by LPS, in the presence or absence of the geranium oil and cytokine levels in the conditioned media were later determined by ELISA. In unstimulated microglial cells, levels of IL-1β and IL-6 were very low or undetectable (Fig. 1a, b). In contrast, stimulation of the cells with LPS resulted in a remarkable increase in the secretion of these cytokines, which were dose-dependently reduced by 83% (IL-1β) and 85% (IL-6), following treatment with 20 µg/mL of geranium oil (Fig. 1a, b). The anti-inflammatory steroid dexamethasone, which served as a positive control, inhibited the cytokines to similar levels, although lower concentrations of dexamethasone were needed to obtain the inhibitory effect. These effects were not the result of any cytotoxic activity of the oil, as was confirmed by a cell-viability assay (Fig. 1c).

Geranium Oil Reduces Levels of Peroxyl Radicals in Microglial Cells

Excessive ROS are key mediators of PD pathogenesis [10, 11]. To measure the ability of geranium oil to reduce intracellular ROS levels in microglial cells, we treated the cells with ABAP, which is a generator of peroxyl radicals [26]. Subsequent treatment of microglial cells with geranium oil reduced ABAP-induced ROS levels (Fig. 2a). We used a cell-viability assay to verify that the reduced levels of ROS were not due to any cytotoxic effect of the oil (Fig. 2b).

Effects of Geranium Oil on the Viability of SH-SY5Y Human Neuroblastoma Cells

We also examined whether geranium oil affects the viability of SH-SY5Y human neuroblastoma cells [27]. Our results demonstrate that geranium oil did not affect cell viability and no toxic effects were observed even at a concentration of 100 µg/mL, which was the highest concentration tested (Fig. 3).

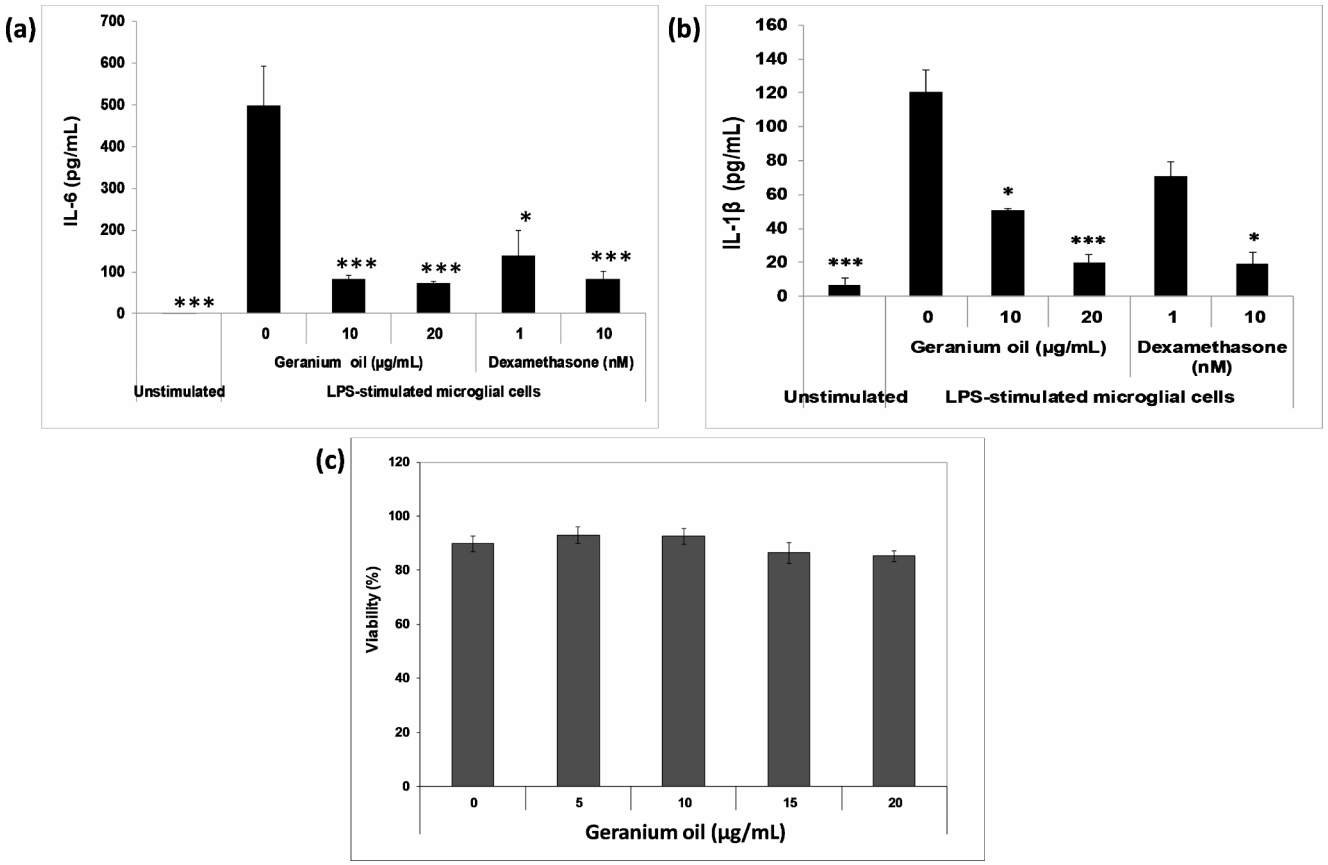

Fig. 1 Down-regulation of IL-1β and IL-6 secretion from LPS-stimulated microglial cells by geranium oil. Microglial cells were treated with the indicated concentrations of geranium oil and then stimulated with LPS (100 ng/mL) for 24 h. Conditioned medium was then collected and **(a)** IL-6 and **(b)** IL-1β levels were measured by ELISA. Data represent the means ± SEM of three (for IL-1β) or two (for IL-6) independent experiments ($n = 6$ for IL-1β; $n = 4$ for IL-6). * $p < 0.05$; *** $p < 0.001$ relative to LPS-treated cells. **(c)** Microglial cell viability was tested after 20 h of incubation with geranium oil using the crystal-violet assay. Each point on the graph represents the mean ± SEM of one experiment ($n = 4$). No statistical differences were observed between treatments

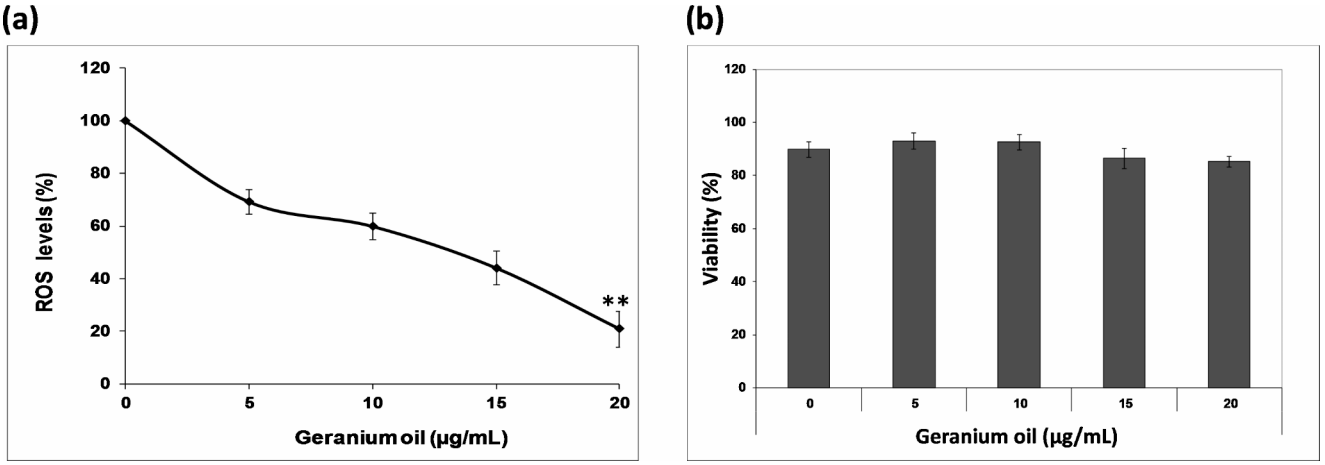

Fig. 2 Geranium oil reduced ROS levels in microglial cells. Cells were incubated for 1 h with various concentrations of geranium oil. **(a)** Cells were then loaded with the non-fluorescent cell-permeating compound, 2'7'- dichlorofluorescein diacetate (DCF-DA) for 30 min and washed with PBS. 2,2'-Azobis(amidinopropane) (ABAP, 0.6 mM) was added and fluorescence was measured 3 h later. **(b)** Viability was tested after 20 h using the crystal-violet method. No statistical differences were observed between treatments. Each point on the graph represents the mean ± SEM of two experiments ($n = 8$). ** $p < 0.01$ relative to cells treated with ABAP alone

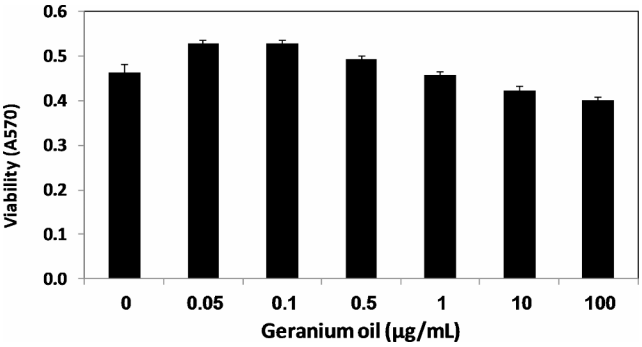

Fig. 3 Geranium oil did not affect the viability of SH-SY5Y cells. SH-SY5Y cells were treated with geranium oil for 24 h. Cell viability was measured by MTT. Data represent the means ± SEM of two experiments ($n = 12$). No statistical differences were observed between treatments

Effects of Geranium Oil on the Rotarod Performance of the Mice

Geranium oil has been used for many years in traditional medicine for various purposes [21] and has been shown to have anti-inflammatory activity in mice [22–24]. In an earlier study, we determined the components of this essential oil, which are lipophilic and have low molecular weights (~ 150 g/mol) and are, therefore, predicted to cross the blood-brain barrier [20]. Given our results regarding the lack of toxicity of geranium oil to microglial and neuronal cells, as well as our finding that geranium oil down-regulates microglial activation, we set out to determine whether daily consumption of geranium oil is safe and whether it can mitigate the behavioral impairments and dopaminergic damage caused by the administration of MPTP to mice. To that end, we studied the effect of 11 days of oral treatment with geranium oil on motor coordination by challenging MPTP-induced acute PD C57BL/6 mice with the rotarod task (Fig. 4).

As compared to the healthy control mice, the MPTP PD mice treated with vehicle alone traveled significantly shorter distances (Fig. 5a) and spent significantly less time (Fig. 5b) on the rotarod on Day 11 of the study (Fig. 5). Treatment with geranium oil significantly increased the distance the mice traveled on the rotarod (Fig. 5a) and the amount of time they spent on the bar before they fell (*i.e.*, latency; Fig. 5b), as compared to the values recorded for MPTP mice treated with vehicle alone. No statistically significant differences in body weights were found between the different treatment groups (Fig. 5c). In this study, the effective dose of geranium oil that was shown to mitigate the behavioral impairment of PD mice was 500 mg/kg. The human equivalent dose (HED) is estimated to be 40.5 mg/kg, calculated as described previously [28].

In previous studies, various plant extracts and phytochemicals, such as chlorogenic acid (MW 354.31 g/mol), were shown to improve neurobehavioral activity in Parkinsonian mice models [12, 13, 29]. Our work differs from that previous research in several important ways. First, the molecular weights of those compounds are greater than those of the components of geranium oil, which is an essential oil. Second, our study also differed from those earlier studies in that we exclusively studied the effects of oral administration of the substance of interest (i.e., geranium oil); whereas previous studies did not always examine that method of administration. Third, we have used the MPTP model and not other models (e.g. paraquat or rotenone). Finally, we applied our treatment concomitant with PD induction; whereas some of that previous work examined potential preventative effects (i.e., treatment before the induction of PD). It remains to be determined whether the effects of geranium oil are elicited by a single constituent or whether they are the outcome of the synergistic activities of several components. Some of the neuroprotective activities of the bioactive phytochemicals present in geranium oil might be attributable to citronellol, which is the main (~ 26%) low-MW (MW = 156.26 g/mol) substance in this oil [20].

	Pre-dosing							MPTP			
Day	-7	-6	-5	-4	-3	-2	-1	1-5	6-10	11	12
								Daily oral gavage with geranium oil			Mice were sacrificed; brains were immunostained for dopamine transporter
Rotarod test	V		V		V					V	

Fig. 4 Study design and time course of geranium oil and MPTP treatments, rotarod task and brain-section immunostaining

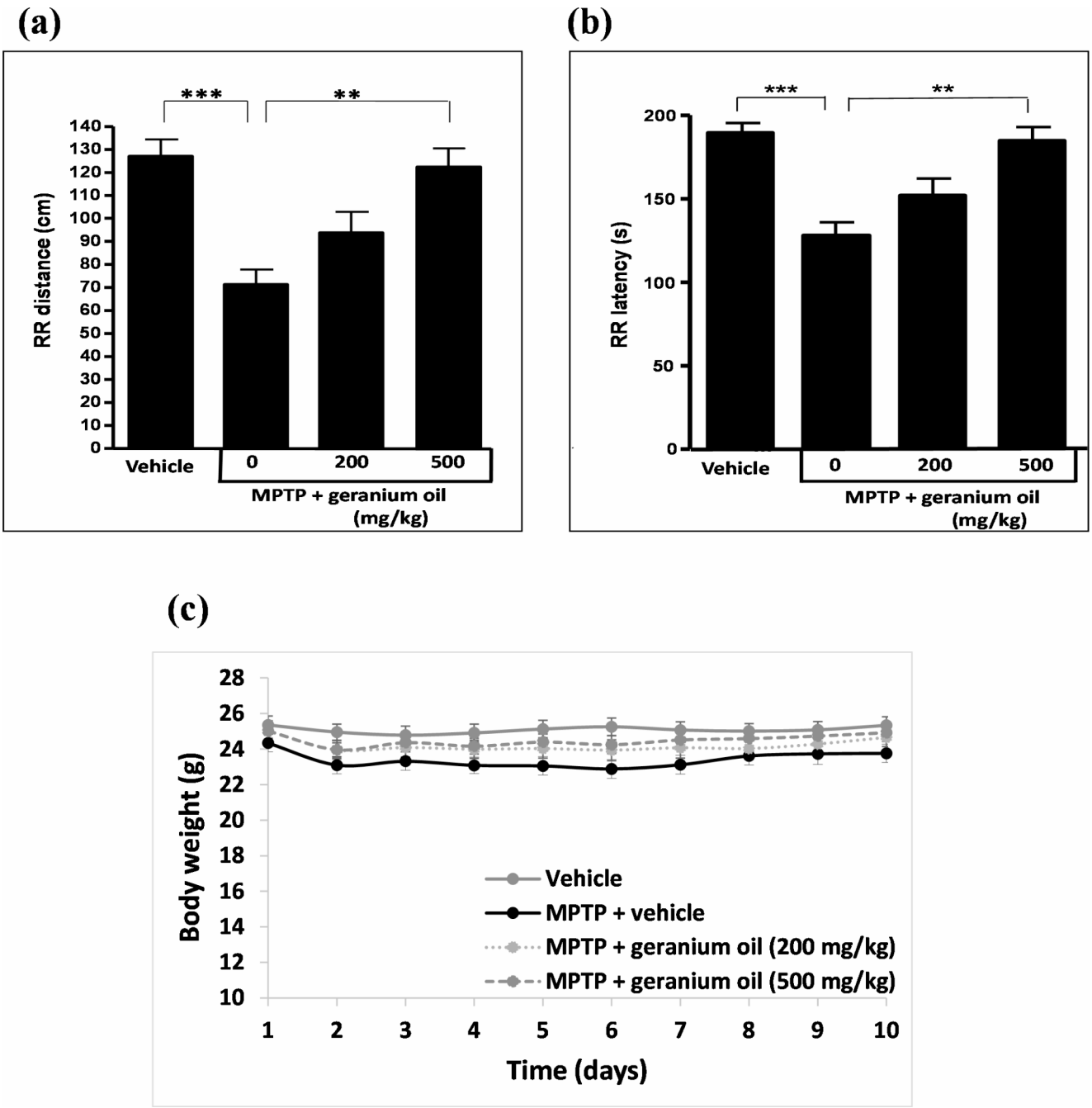

Fig. 5 Geranium oil improved motor performance in Parkinson's disease model mice. Mice (10–12 per group) were trained on the rotarod (RR), 3 trials/day, 7, 5, and 3 days before induction of PD by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). On Day 11, mice were placed on the rotarod to measure motor performance. Data are presented as **(a)** the mean time on the rotating bar until the first fall and **(b)** the distance traveled before the fall. Each mouse was tested three times. Data are expressed as the mean ± SD ($n = 12$ animals/group). Results were analyzed using two-way ANOVA repeated measurements and post hoc Bonferroni post-tests. *** $p < 0.001$; ** $p < 0.01$. **(c)** Body weight of experimental mice. No statistical differences were observed between treatments. Each point represents the mean ± SEM of 11–13 mice/group

Effect of Geranium-Oil Treatment on the Number of Dopaminergic Neurons in the Substantia Nigra

To determine whether geranium oil prevents neuronal mortality in the SNpc of MPTP mice, mice were sacrificed after 11 days of treatment with the geranium oil or vehicle and the brains of all of the sacrificed mice were fixed and sectioned. As there is a direct relationship between the reduction in DAT expression and nigral cell loss [30], samples were immunostained for DAT and the dopaminergic neurons in

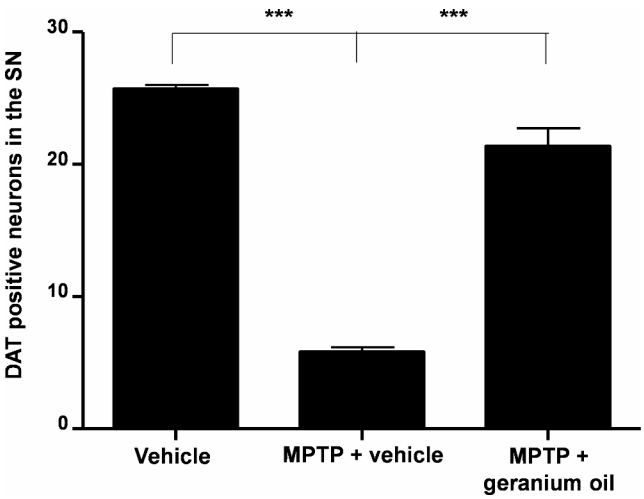

Fig. 6 Effect of the geranium-oil treatment on the number of dopamine transporter-positive neurons in the SNpc of PD model mice. At the end of the study, on Day 12, brains were immunostained for DAT. Three fields were sampled from each mouse. In each field, the dopaminergic neurons were counted and the area of SNc was measured. SNpc area was included in the analysis of variance as a covariable, to remove any possible contribution. Data are expressed as mean ± SD. $n = 33$. *** $p < 0.001$

the substantia nigra were counted. As shown in Fig. 6, the number of dopaminergic neurons in the SNc was markedly reduced in the brains of the MPTP PD mice, as compared to the control group of healthy mice. In contrast, daily consumption of geranium oil significantly enhanced the number of DAT-positive dopaminergic neuronal cells in the SNpc, relative to the SNpc of untreated MPTP mice.

Repeated-Dose Oral Toxicity in Mice

Neither the safety of geranium oil for brain cells nor its effects in mice models of PD or any other neurodegenerative diseases have been studied previously. Although the essential oil of geranium is defined by the FDA as GRAS, we specifically tested the safety of the oil we produced from our specific cultivar in a repeated oral toxicity experiment in mice. Geranium oil or vehicle was administered by oral gavage for 14 consecutive days. Mice from all groups showed stable food consumption during the study, with no significant differences between the treatment groups. No changes in body weight (Table 1) and no adverse clinical symptoms were observed among any of the tested mice in any of the tested groups.

We did not observe any changes in the skin, fur, eyes, mucous membranes, incidence of secretions and excretions (e.g., diarrhea), autonomic activity (e.g., lacrimation, salivation, piloerection, pupil size, unusual respiratory pattern), gait, posture or response to handling. We did not observe the presence of any bizarre behavior, tremors, convulsions, unusual sleep behavior or coma. No animal was found in a moribund or severely distressed condition. There were no observations of animals presenting severe pain. No gross pathological findings were observed in any of the mice at the end of the study. Hematology and clinical chemistry parameters at the end of the study are presented in Table 2. All of the data obtained were within the normal accepted range for mice and no statistically significant differences were observed between the group treated with geranium oil and the vehicle-treated group.

Conclusions

Our data show that daily consumption of geranium oil significantly and almost completely inhibited the impairment of motor performance and degeneration of dopaminergic neurons in the tested PD model. These therapeutic effects can be partially attributed to the antioxidant and anti-inflammatory properties of geranium oil in microglial cells, as demonstrated by the attenuated accumulation of ROS in microglial cells and the inhibition of the secretion of the proinflammatory cytokines IL-1β and IL-6 from microglial cells that had been treated with geranium oil. Neither the safety of geranium oil for brain cells nor its effects in mice models of PD or any other neurodegenerative diseases have been studied previously. Our findings provide proof of the safety and neuroprotective effects of geranium oil against MPTP-induced neurotoxicity. Further studies will be needed to confirm that geranium oil can be used to prevent or treat PD.

Table 1 No changes in body weight after 14 days of oral geranium-oil supplementation

Treatment	Day 1	Day 4	Day 8	Day 11	Day 15
Vehicle	23.1 ± 1.03	23.1 ± 0.59	23.5 ± 0.32	23.8 ± 0.35	23.5 ± 0.87
Geranium oil (500 mg/kg)	23.4 ± 1.64	23.7 ± 1.43	23.9 ± 1.54	24.4 ± 1.61	24.1 ± 1.69

Data are expressed as mean ± SD ($n = 3$ mice/group). No statistical differences were observed between treatments

Table 2 No changes in blood hematology or clinical chemistry after 14 days of oral geranium-oil supplementation

	Vehicle	Geranium oil (500 mg/kg)		Vehicle	Geranium oil (500 mg/kg)
WBC (10^3/μL)	11.5 ± 0.84	9.7 ± 0.71	Calcium (mg/dL)	9.7 ± 0.2	9.9 ± 0.2
RBC (10^6/μL)	9.7 ± 0.15	10.2 ± 0.15	Phosphate (mg/dL)	7.3 ± 0.5	9.4 ± 0.4
HGB (g/dL)	16.2 ± 0.23	16.8 ± 0.21	Glucose (mg/dL)	184 ± 10.6	156 ± 4.0
Hematocrit (%)	49.7 ± 0.46	51.7 ± 0.61	Urea (mg/dL)	47.2 ± 2.6	50.6 ± 1.1
MCV (fL)	51.2 ± 0.35	50.7 ± 0.25	Cholesterol (mg/dL)	146 ± 21	168 ± 16
MCH (pg)	16.7 ± 0	16.5 ± 0.17	TP (g/dL)	4.7 ± 0.5	5.5 ± 0.1
MCHC (g/dL)	32.7 ± 0.20	32.5 ± 0.42	Albumin (g/dL)	3.3 ± 0.1	3.8 ± 0.1
Neutrophils (%)	18 ± 2.3	16 ± 2.1	Glob (g/dL)	1.4 ± 0.6	1.76 ± 0.1
Bands (%)	0 ± 0	0 ± 0	T. bilirubin (mg/dL)	0.07 ± 0.0	0.03 ± 0.0
Lymphocytes (%)	82 ± 2.6	84 ± 1.7	Alk. phos. (IU/L)	179 ± 26	183 ± 3.1
Monocytes (%)	0.3 ± 0.58	0 ± 0	SGOT (IU/L)	63 ± 9.0	69 ± 8.3
Eosinophils (%)	0 ± 0	0.3 ± 0.58	SPGT (IU/L)	41 ± 12.9	71 ± 9.0
Basophils (%)	0 ± 0	0 ± 0	Na (mmol/L)	155 ± 3.1	158 ± 5.3
Platelets (10^3/μL)	1019 ± 56.1	975 ± 192.2	K (mmol/L)	7.2 ± 0.2	7.8 ± 0.0
			Cl (mmol/L)	96 ± 4.0	95 ± 4.2

Data are expressed as mean ± SD (n = 3 mice/group). No statistical differences were observed between treatments

Abbreviations

ABAP	2,2'-azobis(amidinopropane)
DAT	Dopamine transporter
DCF-DA	2'7'-dichlorofluorescein diacetate
IL-1β	Interleukin 1β
IL-6	Interleukin 6
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally recognized as safe
LPS	Lipopolysaccharide
MCT	Medium-chain triglyceride
MPTP	1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Molecular weight
PD	Parkinson's disease
ROS	Reactive oxygen species
SNc	Substantia nigra zona compacta

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11130-023-01112-3.

Author Contributions Anat Elmann: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. Alona Telerman: Formal analysis, Investigation. Nativ Dudai: Funding acquisition, Formal analysis, Supervision. Uzi Ravid: Funding acquisition, Formal analysis, Supervision.

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Data Availability Data will be made available upon request.

Declarations

Competing interests The authors declare no competing interests.

Ethics Approval 1. Preparation of primary cultures: The research was conducted in accordance with the US National Institute of Health (NIH) guidelines for the care and use of laboratory animals and was approved by the National Permit Committee for Animal Science (IL-135/07). 2. In vivo experiments: All animal experiments were carried out in accordance with the US National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and were approved by the National Permit Committee for Animal Science (IL-10-12-111 and IL-12-12-223).

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