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Experimental Research Article

Neuroprotective effects of Caffeic acid phenethyl ester administered with levodopa and benserazide in a rat model of Parkinson's disease

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ABSTRACT

Introduction: Parkinson's disease (PD) is a neurological disorder caused by the pathological destruction of dopaminergic neurons. Although it is commonly associated with motor symptoms, most patients also experience a range of non-motor symptoms, including mental health issues such as anxiety, depression, and memory loss. In this study, we investigated the effect of caffeic acid phenethyl ester (CAPE) on improving PD and reducing the side effects of levodopa.

Methods: Forty-nine male rats were randomly divided into seven groups. A PD model was induced by unilateral injection of 6-OHDA. A combination therapy involving three doses of CAPE (10, 20, and 40 µmol/kg) was administered, along with levodopa and benserazide. The animals were assessed during the study using various behavioral tests, such as tail suspension swing, apomorphine-induced rotation, elevated plus-maze, and open field. Additionally, histological tests, including Nissl staining and tyrosine hydroxylase (TH) immunohistochemistry, were performed to evaluate the animals further.

Results: Our research demonstrates that CAPE effectively reduces side effects associated with levodopa. Moreover, at higher doses, CAPE significantly improves non-motor symptoms, including anxiety and depression, in addition to enhancing motor function. Histological analysis also suggests a protective effect of CAPE on dopaminergic neurons in the substantia nigra.

Conclusion: The findings of this study suggest that co-administration of CAPE can help prevent L-DOPA-induced anxiety-like behaviors through its neuroprotective properties. Therefore, CAPE may have the potential as an adjunct therapy for the management of PD.

Keywords:
Parkinson's disease
Caffeic acid phenethyl ester
Motor activity
Levodopa
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Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder, characterized by clinical hallmarks such as bradykinesia, muscle rigidity, tremors, and impaired balance and coordination (DeMaagd and Philip 2015; Tolosa et al., 2021). Global statistics indicate a significant rise in the incidence and prevalence of PD, as well as an increase in years lived with disability, particularly among the elderly population, with those aged over 65 and the numbers soaring for individuals over 80 years old (Ou et al., 2021). Over the past 26 years, the prevalence of PD has more than doubled, going from 2.5 million patients in 1990 to 6.1 million patients in 2016 (Rocca 2018).

Oxidative stress is a pivotal factor contributing to the destruction of dopaminergic neurons in PD, with the production of reactive oxygen species (ROS) identified as a key contributor to this process. ROS-induced damage to cellular macromolecules leads to mitochondrial dysfunction and neuroinflammation (Chang and Chen 2020; Guo et al., 2018). Moreover, Parkinsonian patients exhibit a pathophysiologic decrease in the number of dopaminergic neurons within the substantia nigra pars compacta (SNc), which leads to motor dysfunctions (Gómez-Benito et al., 2020; Puspita et al., 2017). Although PD is classified as a movement disorder, almost all patients experience a range of non-motor symptoms, including anxiety, depression, memory loss, and sleep disturbances (Tolosa et al., 2021).

Levodopa (L-DOPA), a dopamine precursor, is the gold standard in PD treatment as it effectively slows disease progression (Goshima et al., 2019). However, long-term administration of L-DOPA causes motor complications in most patients, in addition to dyskinesia resulting from excess dopaminergic tone and anxiety (Ahlskog and Ahlskog 2013; Haddad et al., 2017). To mitigate these adverse effects, some studies focused on combining L-DOPA with benserazide to reduce L-DOPA-induced adverse events (Baba et al., 2022; Iwaki et al., 2015; Jiang et al., 2021). This combination not only reduces L-DOPA-induced dyskinesia but also enhances its availability in the central nervous system (CNS) by inhibiting peripheral DOPA decarboxylase. This prevents its conversion to dopamine outside the brain and facilitates its penetration through the blood-brain barrier (Iwaki et al., 2015; Yang et al., 2012).

The use of propolis has shown promising results as a potential strategy for PD modification, providing benefits for both heart and brain protection in animal studies (C. Gonçalves et al., 2020). Caffeic acid phenethyl ester (CAPE), a bioactive compound derived from propolis, is an antioxidant with a wide range of activities against PD, including inflammatory response adjustment, ROS, and nitric oxide synthase inhibition. Additionally, CAPE exhibits an anti-apoptotic function by suppressing the caspase 3 expression and preventing the release of apoptosis-inducing factor and cytochrome C into the cytoplasm (Kulkarni et al., 2021).

Several studies have explored the neuroprotective effects of CAPE on dopaminergic neurons. For instance, Kurauchi et al. demonstrated that CAPE helps protect nigral dopaminergic neurons from lipopolysaccharide-induced injury, thanks to its role in activating heme oxygenase-1 and brain-derived neurotrophic factor (Kurauchi et al., 2012). Similarly, research by Soner et al. showed that intrastriatal administration of CAPE improved motor performance and prevented the loss of TH-positive neurons in a 6-hydroxydopamine-induced rat model of PD (Soner et al., 2021). Despite these promising findings, no direct evidence currently supports CAPE's ability to reduce the side effects of L-DOPA. While CAPE has demonstrated strong neuroprotective potential, further research is needed to understand whether it can help mitigate L-DOPA-induced complications.

The potential of CAPE in mitigating the side effects of L-DOPA, especially those related to motor functions, is an intriguing area of research. Given its antioxidant capabilities, CAPE might not only contribute to managing oxidative stress in PD but also help in reducing the adverse effects associated with long-term L-DOPA therapy (Wang et al., 2010).

A recent study has also suggested a potential link between specific strains of Desulfovibrio bacteria and PD, indicating that environmental factors might play a role in the disease's etiology (Murros et al., 2021).

Antioxidants, including CAPE, have been identified as potential agents in mitigating oxidative stress induced by bacterial pathogens (Celik et al., 2007)Given the emerging evidence linking certain bacterial species to the pathogenesis of PD (Murros et al., 2021), the application of CAPE as part of a comprehensive therapeutic approach could substantially improve PD management strategies.

Our research aimed to determine whether CAPE, when co-administered with L-DOPA and benserazide, could alleviate L-DOPA's psychological side effects, including anxiety and motor disturbances. Additionally, we administered three different doses of CAPE to evaluate the impact of various concentrations on the desired outcomes.

Materials and Methods

Animals and Ethics

This study involved forty-nine adult male Wistar rats, each weighing between 200 and 250 grams, obtained from the Physiology Research Center. The rats were kept in a controlled environment with a temperature set at approximately 25±2 °C, constant humidity (40%–70%), and experienced a consistent 12-hour light/dark cycle. They had unrestricted access to both food and water. The research was approved by the Ethics and Research Committee of Semnan University of Medical Sciences (IR.SEMUMS.REC.1399.198), and procedures involving these animals, including any surgical interventions, were conducted in strict adherence to the guidelines provided by the National Institutes of Health (NIH).

Animal groups and experimental design

The animals were randomly assigned to seven experimental groups (n=7). Control group: received no intervention. Sham group: only stereotaxy was performed, and there was no intervention. PD group: received normal saline intraperitoneally after confirming the induction of PD. The treatment groups: PD+L+B group: received L-DOPA, and benserazide at a dose of 10 and 2.5 (mg/kg. IP. daily), respectively, for two weeks after confirming the induction of PD. PD+CAPE 10, 20, 40 groups: After confirming PD induction, they received the CAPE at concentrations of 10, 20, and 40 μmol/kg. IP. daily, respectively, and received the same treatment as the PD+L+B group for two weeks.

Induction of the PD Model Using 6-OHDA Injection

To induce the PD model in rats, unilateral injection of the neurotoxin 6-OHDA into the SNc was performed following the methodology described by Ghahari et al (Ghahari et al., 2020). Briefly, the rats were anesthetized with ketamine and xylazine at dosages of 100 mg/kg and 20 mg/kg, respectively (Sigma-Aldrich, USA). They were then positioned in a stereotaxic apparatus. The coordinates for injection, derived from the Paxinus and Watson atlas, focused on the left side of the brain (anterior: AP = - 5.04 mm, lateral: ML = - 2 mm, ventral: DV = - 8.1 mm). The 6-OHDA was administered over 5 minutes at a rate of 1 μl/min using a 5-μl Hamilton syringe. One week later, behavioral tests confirmed successful PD model induction, and treatment was started.

Behavioral Testing

The motor imbalance was evaluated using the tail suspension swing test (TSST) and the apomorphine-induced rotation test (AIRT), following previous studies (Ghahari et al., 2020; Rosa et al., 2020; Safari et al., 2016). Additionally, the Elevated plus-maze test (EPM) and Open field test (OFT) were conducted to assess motor activities, anxiety, and depression. All tests were performed daily between 9:00 AM and 2:00 PM. Behavioral assessments were conducted by an observer blinded to group allocations.

Tail Suspension Swing Test (TSST)

The TSST was employed to evaluate asymmetric motor behavior in rats (Rosa et al., 2020). In this test, rats were suspended by their tails approximately 5 centimeters above a flat surface in a vertical position. The frequency of their rotational movements was recorded over a one-minute duration. This test was administered at various stages: Step 1 (before any stereotaxic surgery or other invasive procedures), Step 2 (to confirm the PD model, a week post-surgery), and Steps 3 and 4 (to evaluate treatment effects at 7- and 14-days post-treatment, respectively).

Apomorphine-Induced Rotation Test (AIRT)

The AIRT was conducted 7 and 14 days following the stereotaxic surgery to evaluate the functional impact of 6-OHDA lesions, similar to the TSST. In summary, rats received an intraperitoneal injection of apomorphine hydrochloride (2.5 mg/kg, Sigma-Aldrich). Over a 30-minute observation period, the total number of rotations towards the unaffected side was recorded. These rotations were then quantified as net contralateral turns (Ghahari et al., 2020; Safari et al., 2016).

Open Field Test (OFT)

The OFT was conducted using a black wooden box, dimensions 72 cm x 72 cm, surrounded by walls 50 cm

in height. The floor was divided into a 25-square grid (18 cm × 18 cm each), creating two distinct zones: the center and the periphery. For the test, each rat was initially placed in the central area, and following a 30-second adjustment period, their activity was tracked for 5 minutes. The movements and behaviors of the rats were monitored and analyzed using Ethovision XT-7 software from Noldus, Netherlands (Sedaghat et al., 2019).

Elevated Plus-Maze Test (EPM)

"Given that anxiety and depression are major challenges in PD, and L-DOPA can make it worse by perturbing serotonin (5-HT) and norepinephrine (NE) systems (Eskow Jaunarajs et al., 2011), the effects of CAPE on anxiety-like behaviors were evaluated using an EPM task. As described in previous studies (Shafia et al., 2017), the EPM consists of a wooden, plus-shaped (+) apparatus with four arms (50 cm × 10 cm each) elevated 50 cm above the ground. The EPM includes a central platform (10 cm × 10 cm), connects two open arms with a 0.5 cm high glass edge, and two closed corridors with 40 cm high walls.

Histological Assay

Six rats were selected for the histological examination- three for Nissl staining and three for immunohistochemical staining. Briefly, the animals were deeply anesthetized using ketamine and xylazine (100 mg/kg and 15 mg/kg, respectively). They were then perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH=7.4). The brains were carefully extracted, rinsed with saline, and post-fixed in 4% paraformaldehyde in 0.1 M PBS for 72 hours at room temperature. The brain tissues were subsequently processed by dehydration in a series of ethanol solutions (70%, 80%, 95%, and 100% ethanol) and then cleared using xylazine. Following that, the brain tissues were embedded in paraffin and sectioned using a rotary microtome into slices of 6-7 μm thickness. The coronal serial sections were prepared for histological analysis from the region between -4.8 mm to -6.0 mm relative to the bregma based on the Paxinos and Watson atlas. From each brain, two slices were selected for analysis, with an approximate interval of 175 μm between them. This method allowed for spatial sampling across the region of interest while avoiding redundant or closely adjacent slices. The selected slices in different groups were taken from the same brain region. Cell counting was performed on both hemispheres of each slice to ensure representative data (Shafia et al., 2017).

Nissl Staining

For the identification of Nissl bodies in neurons, we employed cresyl fast violet staining, as described in previous studies (Liu et al., 2023; Omotoso et al., 2020). In summary, brain slices were initially prepared and then subjected to staining with a 0.5% solution of cresyl violet (at 24 °C for 10 minutes). The sections were subsequently washed and underwent a dehydration process through a graded series of ethanol solutions (50%, 70%, 95%, and 100%). They were then cleared in xylene and mounted onto glass slides for further histopathological examination. Slides from six different animals were analyzed using a Japanese Olympus microscope, with images captured at 40× magnification. Neuron counting was performed using ImageJ software.

Tyrosine Hydroxylase (TH) Immunohistochemistry

The procedure for TH immunohistochemistry began with deparaffinizing and rehydrating the brain sections. The 6-7 μm sections were first incubated in 10% methanol and 0.3% hydrogen peroxide in darkness for 10 min. Following this, the sections were washed three times with Tris buffer (pH: 7.4) and then placed in a citrate buffer (0.1 M, pH 5.8) for 15 minutes at a temperature between 90–95 °C. Background staining was blocked by 10% goat serum, 0.3% Triton X-100, and 1% bovine serum albumin (BSA) for 2 h at room temperature. The sections were then incubated with a primary antibody (dilution 1:500; Abcam 6211, USA) for one night at 4 °C. After washing with TBS, the sections were exposed to a secondary FITC-conjugated antibody (dilution 1:200; Abcam 214879, USA) for 2 h at room temperature. Following PBS washes, DAPI (4, 6-diamidino-2-phenylindole) staining was applied at room temperature. Finally, the sections were dehydrated, cleared with xylene, and cover-slipped. TH-positive cells were counted in six high-magnification fields (400x) using a fluorescent microscope (Liu et al., 2023; Yuan et al., 2005).

Data Analysis

The statistical analysis was carried out using SPSS software version 22.0. To compare different groups, one-way ANOVA was used, followed by Tukey's post

TABLE 1: Behavioral results of the Open Field Test. *: P<0.05

Group	Number	Time spent in center	Time spent in corner	Enter to the center (n)	Speed of the animal (m/s)	Distance traveled (cm)
Control	6	13.40±3.04	286.64±3.04	13.17±2.2	14.28±2.08	4040.18±325.75
Sham	6	13.18±1.82	286.86±1.82	14.67±3. 2	12.07±2.52	3254.13±354.24
PD	6	6.98±1.99	293.05±1.99	7.83±1.4	9.17±1.7	2707.67±210.35*
PD + L+B	6	6.53±1.02	293.50±1.02	6.83±1.8	7.62±0.7	2260.62±84.91*
PD + CAPE 10	6	8.90±3.37	291.13±3.37	10.50±2.6	11.17±1.28	3274.21±177.40
PD + CAPE 20	6	21.21 ±5.47 *	282.31±5.47 *	18.83±1.4	10.22±2.44	3004.09±283.76
PD + CAPE 40	6	12.56±2.46	287.47±2.46	22.83±3.6*	12.98±1.11	3727.68±130.48

hoc test. A p-value of less than 0.05 was considered statistically significant.

Results

Behavioral testing

Tail Suspension Swing Test

Before any surgical procedures, the first TSST showed no significant differences among groups (P> 0.001). The second TSST was performed 7 days after stereotaxic surgery and showed significant differences in behavior between the control and experimental groups (P< 0.001). These results indicate successful induction of the PD model using 6-OHDA (Figure 1B).

The third TSST was performed one week after the start of treatment. The findings revealed some behavioral improvement in treated rats. The PD+CAPE 10 group showed the most significant decrease in right-biased swings (mean: 56.33±1.5, P< 0.01). However, no significant differences were observed between PD+L+B and higher CAPE dose groups (CAPE 20 and CAPE 40, P=1 and P=0.8, respectively) (Figure 1B).

The fourth TSST was performed two weeks after the start of treatment. The results confirmed the long-term treatment effects of CAPE. PD+CAPE 10, 20, and 40 groups showed significant behavioral recovery, comparable to control and sham groups (P<0.01). The PD+L+B group showed only slight improvement, and the untreated PD group had minimal recovery (Figure 1B).

Apomorphine-Induced Rotation Test (AIRT)

AIRT has been performed to evaluate the motor impairment resulting from a 6-OHDA lesion and assess whether or not dopaminergic neuron function is maintained. The results of our study showed that all rats treated with 6-OHDA exhibited signs and symptoms consistent with PD seven days after induction of the PD model. Additionally, a significant decrease in the rat contralateral net turn on the 14th day of CAPE 10, PD+-CAPE 20, and PD+CAPE 40 groups compared to PD and PD+ L groups (96.29±7.63, 95.29±6.1, 94.86±5.64 vs 177.9±9.51 and 124.6±7.72 respectively, P<0.001) (Figure 1C). No significant difference was found between the CAPE-treated groups (P>0.05). Furthermore, while the PD+L+B group showed a significant decrease in contralateral net turn compared to the PD group (124.6±7.72 vs 177.9±9.51, P<0.001), it did not differ significantly from the three CAPE-treated groups. In addition, there was no significant change in the contralateral net turn of the control and Sham groups during the AIRT (Figure 1C).

Open field test (OFT)

The Open Field Test (OFT) results indicate that the PD+CAPE 20 group spent significantly more time in the center of the open field compared to other groups, while the PD+L+B and PD groups spent the least time in the center. The PD+CAPE 40 group showed a significantly higher number of center entries than other groups, but there was no significant difference between PD+CAPE 20 and PD+CAPE 40 groups (18.83±1.4 vs 22.83±3.6, P=0.05). Movement speed did not significantly differ among CAPE-treated groups (P<0.05). However, PD+L+B rats had lower central entries, movement speed, and distance traveled than PD rats, though these reductions were not statistically significant. Notably, the CAPE-treated groups traveled significantly greater distances than the PD and PD+L+B groups, with the PD+-

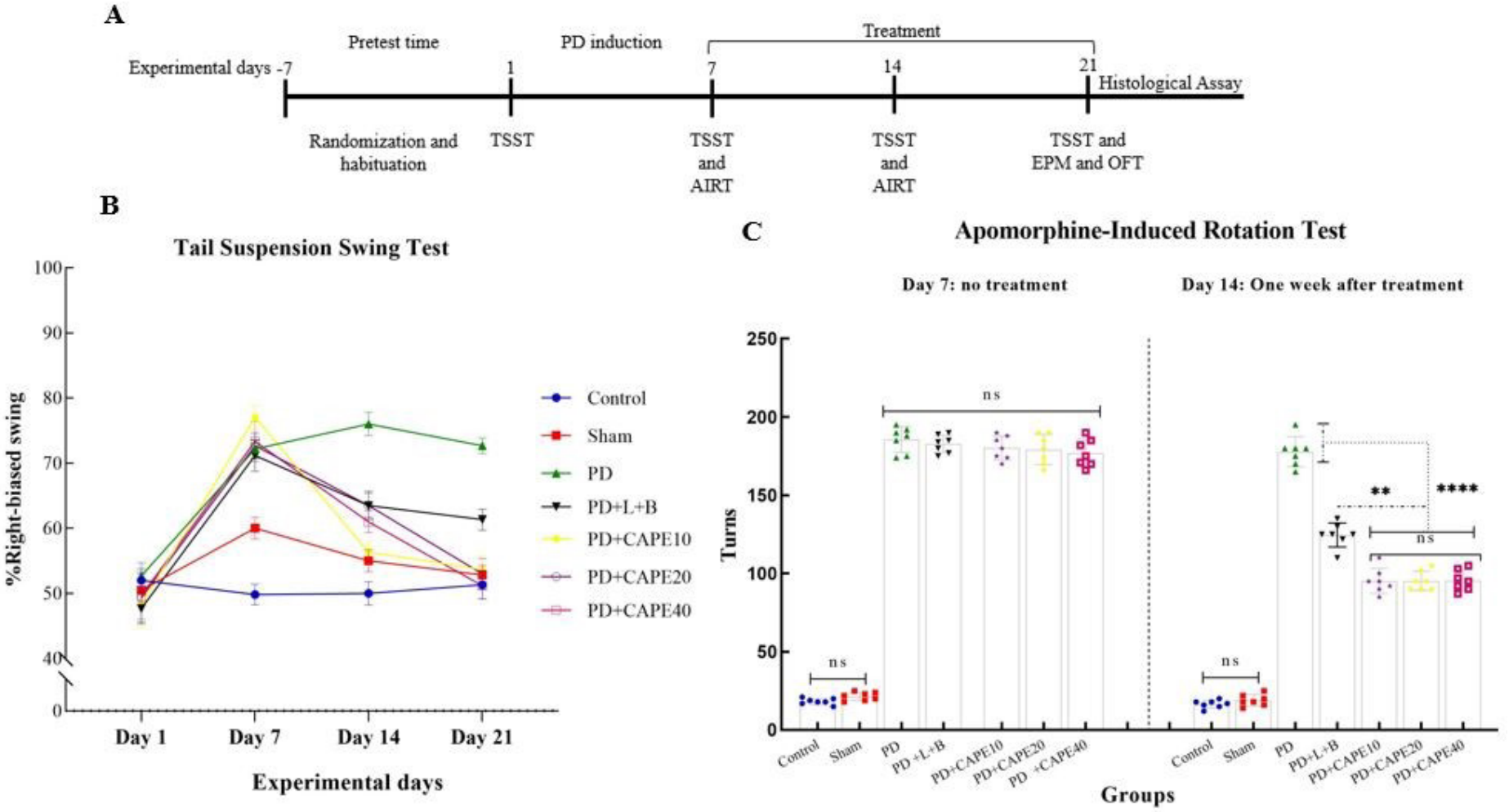

FIGURE 1. A: Schematic diagram of the experimental design of the study. PD: Parkinson's disease, TSST: Tail Suspension Swing Test, AIRT: Apomorphine-Induced Rotation Test, EPM: Elevated Plus-Maze Test, OFT: Open field test. B: Illustrates the % Right-biased swing assessed by the TSST. Day 1 reports the pre-surgical testing. Day 7 represents the % Right-biased swing seven days after stereotaxic surgery to validate the PD model. Days 14 and 21 represent the % Right-biased swing 7 and 14 days after the treatments. C: This figure illustrates the number of rat turns during the 30 minutes assessed by the AIRT. ns: $P>0.05$, **: $P<0.001$, ****: $P<0.0001$.

TABLE 2: Behavioral results of the Elevated plus-maze test. *: *P*<0.05

Group	Number	Time spent in open arms (sec)	Entries into the open arms (n)
Control	6	87.50±4.2	8.6±0.67
Sham	6	66.17±3.92	5.67±0.66
PD	6	27.33±4.23	2.83±0.47
PD + L+B	6	39.67±2.61	3.50±0.22
PD + CAPE 10	6	90.83±6.6	7.40±0.5
PD + CAPE 20	6	130.50 ±16.33 *	9.00±0.73 *
PD + CAPE 40	6	183.20±14.70*	9.33±0.98*

CAPE 40 group showing the highest increase. These findings suggest that L-DOPA treatment may contribute to anxiety-like behavior, while CAPE improves locomotor function in PD rats in a dose-dependent manner. (Table 1, and Figure S1).

EPM test

Next, the EPM test was used to evaluate whether CAPE could attenuate anxiety-like behaviors in PD rats. The results showed a significant difference in the time spent in the open arms between different groups (F: 34.64, DFn: 6, DFd: 35, P <0.0001). Rats in the PD+CAPE 20 (n = 6) and the PD+CAPE 40 (n = 6) groups spent significantly more time in the open arms compared with other groups. Surprisingly, rats in the CAPE-treated groups spent more time in the open arm than the control group (90.83±6.6, 130.5±16.33, and 183.20±14.70 vs 87.50±4.2). While the PD+L+B group showed some improvement in activity compared to the PD group, it did not show as much improvement as the CAPE-treated groups (Table 2, and Figure S2). In addition, the PD+CAPE 20 (9.00±0.73) and PD+CAPE 40 (9.33±0.98) groups had more entries into the open arm than other groups. Similar to the time spent in the open arm, the number of entries into the open arm did not differ significantly between the PD and PD+L+B groups (2.83±0.47 vs 3.50±0.22, P>0.05) (Table 2, and Figure S2).

Histological assay

Nissl staining

Our findings indicate that rats receiving a combination of CAPE, L-DOPA, and benserazide experienced the neuroprotective effect over the experiment period. Statistical analysis showed that the PD+CAPE 20 and the PD+CAPE 40 groups had the best neuroprotective effect compared with other groups (P < 0.01). Furthermore, while there was no significant difference in the number of neurons between the PD+CAPE 10 and PD+L+B groups, both had considerably more neurons than the PD group (P < 0.05) (Figure 2).

Tyrosine hydroxylase (TH) Immunohistochemistry

TH-immunohistochemistry staining was performed to evaluate TH expression in the SNc, as dopaminergic neurons are the main secreting neurons for producing TH. Results showed that the control group had the highest (n=6, 25.14±0.9) and the PD group had the lowest (n=6, 4.43±1.7) number of TH-positive neurons. Although treatment with L-DOPA and benserazide increased the number of dopaminergic neurons compared to PD, the increase was not significant (6.14±0.9 vs 4.43±1.7, P=0.09). However, the combined treatment with different doses of CAPE significantly protected dopaminergic neurons from damage by the 6-OHDA toxin. Compared to the PD and PD+L+B groups, the PD+-CAPE 10 group showed a significant increase in the number of TH-positive neurons (11.14±0.9 vs 4.43±1.7, P<0.0001, and 11.14±0.9 vs 6.14±0.9, P<0.0001). The results also showed that most TH-positive neurons were observed in the PD+CAPE 20 and PD+CAPE 40 groups. The number of TH-positive neurons was higher in the PD+CAPE 40 group, but there was no significant difference between them (16.14±1.3 vs 17.71±0.7, P=0.15). In conclusion, the findings suggest that CAPE effectively protects dopaminergic neurons in a dose-dependent manner (Figure 3).

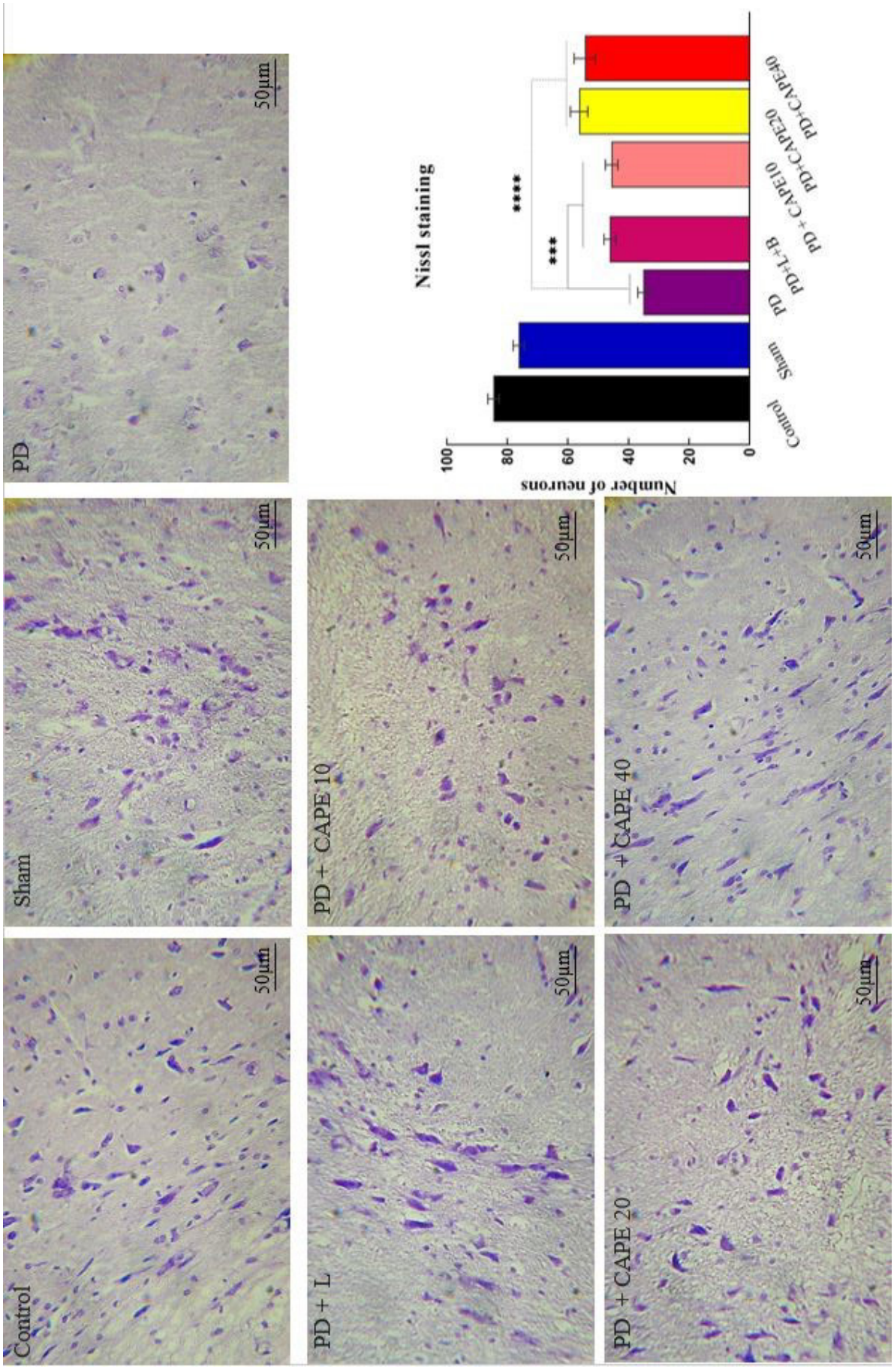

FIGURE 2. This figure illustrates the Nissl stain analysis to assess SNc neuron damage. There was no significant difference between the PD+CAPE 20 and PD+CAPE 40 groups, as well as between the PD+CAPE 10 and PD+L groups. ***: $P<0.001$, ****: $P<0.0001$.

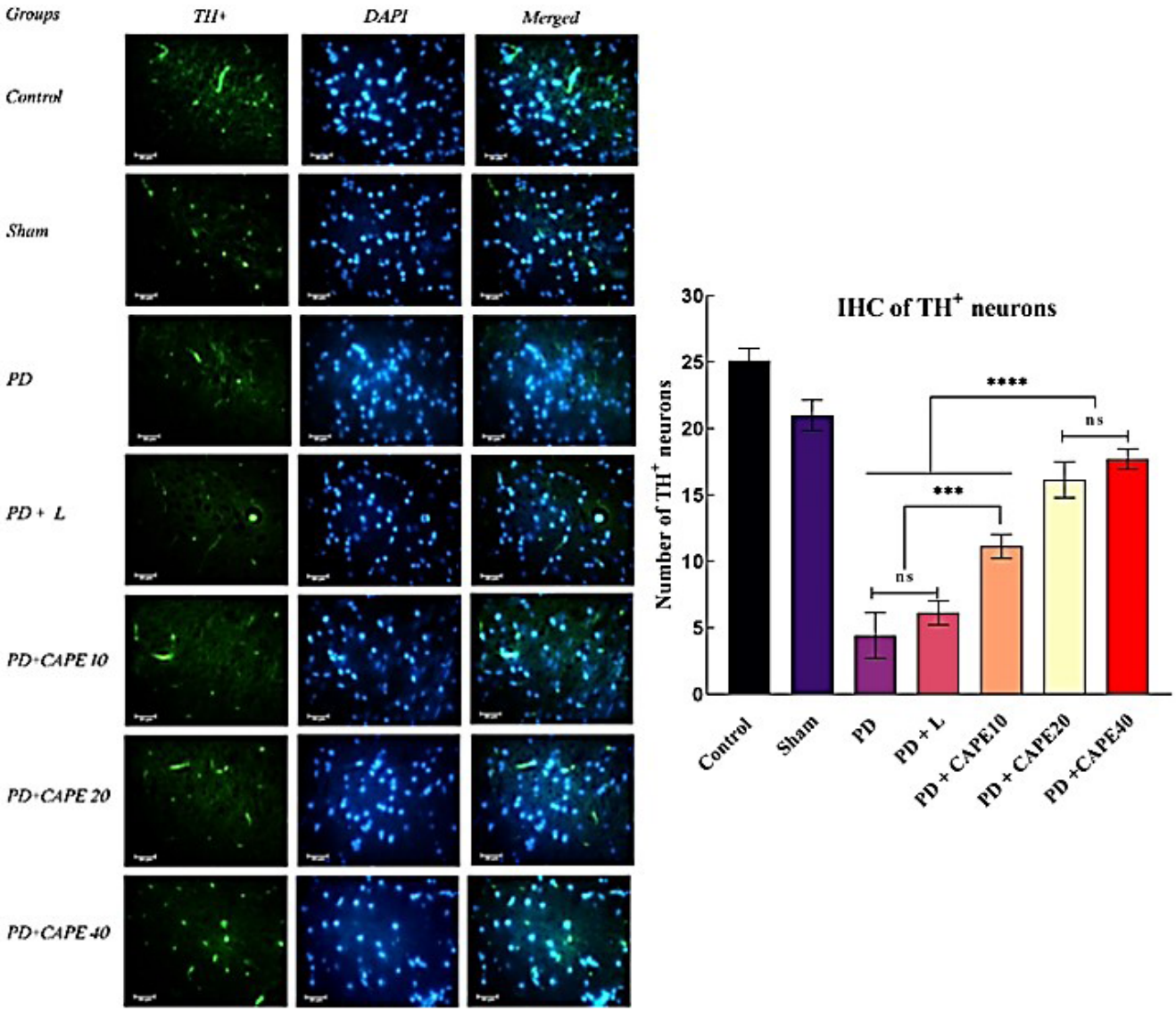

FIGURE 3. This figure illustrates an immunohistochemical staining analysis to detect TH expression in the SNc neurons. There was no significant difference between the PD+CAPE 20 and PD+CAPE 40 groups, as well as between the PD and PD+L+B groups. ns: $P>0.05$. CAPE facilitates an increase in the quantity of cells that exhibit dopamine positivity in the SNc. ***: $P<0.001$, ****: $P<0.0001$.

Discussion

PD is the second most prevalent neurological disorder after Alzheimer's disease, characterized by the degeneration of dopaminergic neurons in the SNc region of the midbrain. Traditionally, the standard treatment for PD has been a combination of L-DOPA and benserazide. This study investigates the effect of CAPE at three different doses combined with L-DOPA and benserazide in the rat model of PD.

As mentioned above, our study confirmed that one week after the unilateral injection of the neurotoxin 6-OHDA into the SNc, motor imbalance behavioral symptoms associated with PD were observed in both the TSST and AIRT. Although treatment with L-DOPA and benserazide did not significantly improve movement symptoms in PD rats, our results demonstrated that combination treatment with CAPE significantly improved behavioral activity. Previous studies have also confirmed the neuroprotective effect of CAPE on 6-OHDA-induced nigrostriatal damage, as evidenced by improvements in locomotor parameters assessed through AIRT and OFT (Soner et al., 2021).

The stability and improvement of the motor behavior in the CAPE-treated groups throughout the study sug-

gest effective compensation for dopamine deficiency in the affected hemisphere. CAPE appears to mitigate dopamine deficiency by enhancing the function of remaining dopaminergic neurons in SNc-associated areas, increasing their activity, or protecting them from further degeneration. Additionally, our previous study demonstrated that the CAPE improved motor performance in MPTP-induced PD rats by inhibiting the destruction of dopaminergic neurons through antioxidant, anti-apoptotic, and anti-inflammatory functions (Rahimi Jaberi et al., 2022). The activation of glial cells caused by inflammatory conditions leads to the release of ROS, which can cause damage to neurons sensitive to ROS, including dopaminergic neurons. As an antioxidant, CAPE exerts an anti-inflammatory effect that helps neutralize ROS and eliminate inflammatory agents, thereby contributing to the alleviation of PD symptoms (Rahimi Jaberi et al., 2022).

OFT showed that the movement speed of treated rats increased compared to the PD group, but the PD+L+B group indicated a lower speed than the PD group, which may be attributed to the side effects of treatment with the L-DOPA alone. Further evaluations showed an increase in duration spent at the center/the number of entries to the center in the treated groups, except the PD+L+B group, compared to the PD group, which indicates the anti-anxiety function of CAPE combination therapy.

Studies have shown that CAPE does not affect the pharmacokinetic parameters of L-dopa, including its absorption rate or availability. However, CAPE influences the conversion of L-DOPA to 3-O-methyldopa (3-OMD) and subsequently reduces the proportion of 3-OMD formed in a dose-dependent manner. This reduction in 3-OMD formation increases dopamine availability and mitigates dyskinesia and movement disorders (Wang et al., 2010).

The EPM test results were consistent with those of the OFT, showing a significantly greater time spent and number of entries in the open arms in the CAPE-treated groups compared to the PD group. Based on the OFT and EPM results, treatment with CAPE antioxidant appears to have led to an increased exploratory behavior in rats and significantly reduced anxiety.

Zaitone et al. confirmed that CAPE improves locomotor activity, reduces microglia expression and inflammatory mediators, and preserves SNc TH-positive neurons in a mouse model of PD (Zaitone et al., 2019). Furthermore, CAPE could have a protective effect on paraoxonase activity and levels of lipid profile, total sialic acid, total oxidant capacity, and total antioxidant capacity in plasma and brain tissue and prevent neurodegenerations in PD (Deveci and Karapehlivan 2018).

Our histopathological evaluations through nissl staining and TH immunohistochemistry revealed an increased number of neurons in the SNc area compared to the PD group. This increase was also confirmed in the number of dopaminergic neurons. Additionally, our histopathological and behavioral findings were consistent with other studies (Deveci and Karapehlivan 2018; Soner et al., 2021; Zaitone et al., 2019).

In summary, CAPE may protect dopamine-secreting neurons in the SNc and help mitigate the side effects of L-DOPA.

Conclusion

Examination of the motor functions of PD rats showed significant improvement in the treatment group that received the antioxidant CAPE with L-DOPA and benserazide compared to the PD group. These findings were further supported by histological studies regarding dopaminergic neurons. CAPE appears to improve motor performance by protecting dopaminergic neurons, along with mechanisms such as reducing ROS, preventing apoptosis in damaged neurons, increasing the activity of dopaminergic neurons, and protecting the remaining neurons against further destruction. In addition, the lack of behavioral improvement in the PD+L+B group compared to the PD group indicates the side effects of using L-DOPA, which were mitigated by combining it with CAPE.

Authors' contributions

M.S and HR.S: Conception and design, and project administration. H.P, M.M, M.E, and Z.MV: Behavioral tests, Stereotaxic surgery, and Histological assay. All authors read and approved the final manuscript. A.GH: Stereotaxic surgery. AA.V: Behavioral tests, data collection, software, and analysis.

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Conflict of interest

The authors declare no conflict of interest.

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Declarations

Data availability

Upon a reasonable request, the corresponding author will provide available data collected and analyzed during the current study.

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