

Effects of a crude extract of *Echinops mosulensis* on an induced Parkinson's disease model in mice

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Received 8 July 2024 ♦ Accepted 16 August 2024 ♦ Published 11 September 2024

Citation: Ahmed AA, Kadhim HM (2024) Effects of a crude extract of *Echinops mosulensis* on an induced Parkinson's disease model in mice. Pharmacia 71: 1–14. <https://doi.org/10.3897/pharmacia.71.e131570>

Abstract

Parkinson's disease (PD) is the second most widespread chronic, progressive neurodegenerative disease after Alzheimer's disease. Key mechanisms contributing to PD development are oxidative stress, inflammation, protein misfolding and aggregation, apoptotic cell death, excitotoxicity, mitochondrial dysfunction, and loss of trophic support. We aimed to investigate the neuroprotective effect of a crude extract of Iraqi *Echinops mosulensis* on an induced PD model in mice. Forty male Swiss Albino mice were divided into four groups of ten mice; the negative control received distilled water orally, and the induction group received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (30 mg/kg/day) intraperitoneally (IP). The positive control group was administered pramipexole orally (1 mg/kg/day), and the final group received a crude extract of *E. mosulensis* orally (250 mg/kg/day). The experimental phase lasted for 25 days for all studied groups. On day 26, the behavior test was done, and on day 27, all animals were sacrificed. Homogenized brain tissue was prepared for analysis. Treatment with crude extract significantly reduced MDA, IL-6, IL-1 beta, caspase 3, cytochrome C, and alpha-synuclein levels compared to the induction group, while dopamine (DA) significantly increased. In addition, the catalepsy test was significantly reduced in the *E. mosulensis* group compared to the induction group. In conclusion, the crude extract of *E. mosulensis* exerts neuroprotective effects through multiple mechanisms, and it offers opportunities for developing a novel therapeutic method for treating PD.

Keywords

Parkinson's disease, MPTP, α -synuclein, *Echinops mosulensis*, phenolic compound

Introduction

Parkinson's disease (PD) is the second-most widespread chronic, progressive neurodegenerative disorder after Alzheimer's disease, affecting 1% of those over 60 years which is characterized by the degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNpc) and consequently suppression of the dopamine

neurotransmitter resulted from multiple mechanisms including; intraneuronal aggregation of α -synuclein in the form of Lewy bodies (Saeed et al. 2017; Alizzi et al. 2018; Koziorowski et al. 2021; Lorente-Picón and Laguna 2021). PD is clinically marked by a motor symptom that includes bradykinesia, rest tremor, rigidity, and abnormalities in posture and gait, as well as non-motor symptoms that frequently precede the motor symptoms, such as hyposmia,

urinary dysfunction, depression, orthostatic hypotension, constipation, memory loss, pain, and sleep disturbances (Nimmo et al. 2020; Tolosa et al. 2021). The exact cause of PD is still mostly unknown, but research indicates that various factors, including aging, genetics, and environmental agents, may be involved (Chia et al. 2020).

Despite decades of intensive research, no practical medicines are available to prevent or delay the disease's course. The existing treatments for PD only deal with symptoms; they do not reverse the gradual degeneration of dopaminergic neurons in the brain's substantia nigra (Thadathil et al. 2021). Thus, natural compounds derived from medicinal plants are an attractive choice for managing and preventing PD models (Atiq et al. 2023).

One of the most popular neurotoxins that cause animal parkinsonism is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Heng et al. 2022). MPTP functions as a pro-toxin that can pass across the blood-brain barrier when given systemically because of its lipophilicity. Next, astrocytes take it up and convert it to 1-methyl-4-phenylpyridinium (MPP⁺), which is released and eventually taken up by the dopaminergic neurons via dopamine transporters. Once within the cell, MPP⁺ disturbs the mitochondrial electron chain, which ultimately causes neuronal death (Cuenca-Bermejo et al. 2021; Aktas 2023).

Alpha-synuclein is a neuronal protein found in high concentrations in presynaptic nerve terminals (Burré 2015). Although its exact role remains unclear, evidence suggests that it controls neurotransmitter release, synaptic plasticity, and synaptic function (Koga et al. 2021). Throughout development, the levels of α -synuclein protein rise and stay elevated during adulthood (Burré 2015). So far, under pathological conditions, α -synuclein is accumulated in intracellular proteinaceous aggregates called Lewy bodies, a histopathological hallmark that defines PD (Daniels et al. 2019; Mor et al. 2019).

The genus *Echinops*, which contains numerous plants known as globe thistles, contains more than 120 species of annuals, biennials, and perennials. The genus is a member of the daisy family *Asteraceae*, and species of the genus spread throughout central Asia, the Mediterranean Basin, and north and tropical Africa (Khadim et al. 2014; Bitew and Hymete 2019). Several literature reviews have shown that the *Echinops* plant has various pharmacological properties, including antioxidant, antibacterial, and antifungal properties, mitigating the prostatic hyperplasia brought on by testosterone, anti-ulcerogenic activity, and hepatoprotective. Several chemicals from several classes, including lipids, steroids, alkaloids, flavonoids, and terpenoids, have been reported in the *echinops* plant (Mahmood and Khadeem 2013). Terpenes, flavonoids, and other phenolic substances have hepatoprotective, anti-inflammatory, and antioxidant properties (Zheleva-Dimitrova et al. 2023). The plant's root is a significant source of thiophenes, although most of its flavonoids and terpenes are separated from the aerial portion or the entire plant. Phenolic chemicals are among the many secondary metabolites that have a wide range of beneficial effects on people, including

treating neurodegenerative diseases and cancer. Numerous flavonoids, including rutin (*Echinops heterophyllus*) and luteolin (*Echinops niveus*), were identified (Khan et al. 2023).

Phenolic compounds have strong antioxidant properties that are achieved by scavenging free radicals such as reactive oxygen species (ROS), inhibiting the formation of ROS by blocking certain enzymes or chelating trace minerals needed for their production, and ultimately protecting or enhancing the antioxidant defense system (Hassan and Kadhim 2022). In glial cells, polyphenols can downregulate nuclear factor kappa B (NF- κ B), which is linked to the production of inducible nitric oxide synthase (iNOS) (Yahfoufi et al. 2018). NF- κ B is a potent modulator of gene expression that promotes inflammation. Tumor necrosis factor (TNF- α), interleukin (IL)-1 β , IL6, and IL8 are among the cytokines that are produced in response to activation of NF- κ B (Mohammed et al. 2022).

To the best of our knowledge, no prior research has been done on the chemical composition or biological impacts of Iraqi *Echinops mosulensis*. Numerous studies examining the biological effects of species belonging to the same genus, such as *Echinops heterophyllus*, *Echinops echinatus*, and *Echinops spinosissimus*, among others, were found. The current investigation examines the therapeutic potential of *Echinops mosulensis* crude extract to treat PD. By assessing *Echinops mosulensis* crude extract's ameliorative effect on the main pathological characteristics of Parkinsonism, including inflammation, oxidative stress, apoptosis, α -synuclein increment, and dopamine reduction, the use of *Echinops mosulensis* crude extract is a novel approach to treating Parkinsonism.

Methods

Study design

Male (Swiss Albino mice) weighing 22–29 g, aged 8–10 weeks, were randomly divided into four groups of ten animals (40 mice). The mice were sourced from the National Laboratory for Drug Control, Baghdad, Iraq, housed in polypropylene cages under a temperature-controlled environment (15–21 °C), with an inverted light-dark cycle (12/12 h), and acclimated for one week before starting the experiment at the Animal Facility of the Al-Nahrain University—Biotechnology Research Center, Baghdad, Iraq. The mice were maintained on a standard pellet diet and had free access to water and libitum. Before starting the experiment, only apparently healthy mice were included in the study after all mice were checked for lesions; the experiment lasted 25 days from the starting day.

Regarding the allocation of mice, Group I (the negative control group) consisted of ten remaining in the control group who received distilled water (DW) orally via a gavage tube daily for 25. Group II (induction group, MPTP) consisted of ten mice that received MPTP to achieve the model of PD by a dose (30 mg/kg/day) IP for five

consecutive days from day 15 to the end of day 19 (Zhang et al. 2017), then continued with distilled water orally via a gavage tube till day 25.

Group III (positive control, standard) consisted of ten mice that received pramipexole orally at a dose of 1 mg/kg/day for 25 days (Elhak et al. 2010), and MPTP (30 mg/kg/day) (IP) was given between day 15 and day 19, which was given 1 hour post-pramipexole (Hu et al. 2018). Group IV (the treatment group) consists of ten mice that received a crude extract of *E. mosulensis* orally (250 mg/kg/day) for 25 days (Abdulmohsin et al. 2019). And MPTP (30 mg/kg/day) (IP) was given between day 15 and day 19, which was given 1-hour post-pramipexole (Hu et al. 2018), as seen in Fig. 1.

Induction of Parkinson's disease

MPTP crystalline powder was ground, then weighed (60 mg) and dissolved in 40 ml of 0.9% normal saline (Nikokalam Nazif et al. 2020). MPTP was given to mice at 30 mg/kg/day for five consecutive days, with IP injection once daily (Zhang et al. 2017). A clear solution was used immediately after preparation, as it was stable only for 24 hours at 25 °C.

Materials

The herbal plant (Species: *Echinops mosulensis* Rech. f., Family: Asteraceae) was collected from Iraq, Erbil (36°11'28.0068"N, 44°0'33.0012"E) in April (Voucher number: 1170, date: 31-5-2023, Department of Biology, College of Science, University of Baghdad, by Assistance Professor Sukeyna Abaas Aliwy). The toxin, MPTP crystalline powder, was supplied from (Meryre, China), and pramipexole tablets were supplied from (Boehringer Ingelheim, Germany). Buffer phosphate saline from (Reagent World, USA), and formaldehyde solution 37% from (Fluka Chemical, UK).

Extraction of *E. mosulensis*

Aerial parts of the plant that had been shade-dried and coarsely powdered were extracted in a reflex apparatus using 400 ml of 85% ethanol until total exhaustion. A dark greenish-yellow residue, known as the crude fraction, was obtained by evaporating the alcoholic extract under reduced pressure at a temperature lower than 40 °C (Abdulmohsin et al. 2019). The extraction rate was (35 gm/270 gm), which means a total plant weight of (270 gm) was extracted in a volume of 400 ml of 85% ethanol to yield (35 gm) of dry crude *Echinops* extract.

Phytochemical qualitative analysis

Chemical tests were performed using the powdered specimens and/or ethanolic plant extracts, with standard protocols to identify the active ingredients (Harborne 1998); these tests involve tests for alkaloids (Wagner's reagents

& Mayer's) (Mahmood and Khadeem 2013), tests for flavonoids (Lead acetate test & NaOH test) (Shah and Hosain 2014), tests for steroids (Liebermann-Burchard test & H₂SO₄ test) (Nath et al. 1946), tests for tannins (FeCl₃ solution test) (Mailoa et al. 2013), tests of saponins (foam test) (Chen et al. 2010), and tests for terpenoids (NaOH test) (Harborne and Harborne 1973).

HPLC analysis of the plant extract

Quantification of individual phenolic compounds was performed by reversed-phase HPLC analysis using a SYKAMN HPLC chromatographic system equipped with a UV detector, Chemstation, and a Zorbax Eclipse Plus-C18-OSD, 25 cm, 4.6 mm column. The column temperature was 30 °C. The gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v), was performed as follows: initial 0–4 min, 40% B; 4–10 min, 50% B; and flow rate of 0.7 mL/min. The injected volume of samples was 100 µL, and the standard was 100 µL, and it was done automatically using an autosampler. The spectra were acquired at 280 nm (Radovanović et al. 2015).

Behavioral test

The catalepsy test was conducted on day 26 for all groups in the same room where the mice were kept, and mice were trained individually multiple times just before recording. The horizontal bar method was used to test mice for catalepsy. This procedure involved placing the mouse's forepaws on a horizontal wooden bar that had a diameter of 0.6 cm and was placed 5 cm above the floor. Time measurement was taken when the forepaws were moved off the bar. Three minutes was the latency cutoff for shifting paws away from the bar (Zhao-Shea et al. 2010; Romero-Fernandez et al. 2022).

Outcome measures

The end of day 25 marked the end of the experimental period, and the outcomes were evaluated the next day after the day of behavioral testing; all mice were anesthetized with ketamine and xylazine (150 and 15 mg·Kg⁻¹, respectively, IP) (Dantas et al. 2022; Hussein et al. 2024b; Obaid and Fawzi 2024). The brain tissue was then removed and prepared for biochemical analyses to measure the essay of dopamine (DA), IL-6, IL-1beta, malondialdehyde (MDA), caspase-3 (Cas-3), cytochrome c (Cty-c), and alpha-synuclein (α-synuclein) and histopathological analysis.

In brief, the brain was rapidly extracted, and any extra blood was rinsed and cleaned with phosphate-buffered saline (PBS) (7.4, 4 °C). After that, let it dry on filter paper before chopping it into little pieces. Brain tissue homogenate was prepared for each mouse by putting PBS and chopped tissue into a tube. Then, homogenization was accomplished for one minute using a tissue homogenizer (Karl Kalb, Germany), and all procedures listed above require the storage

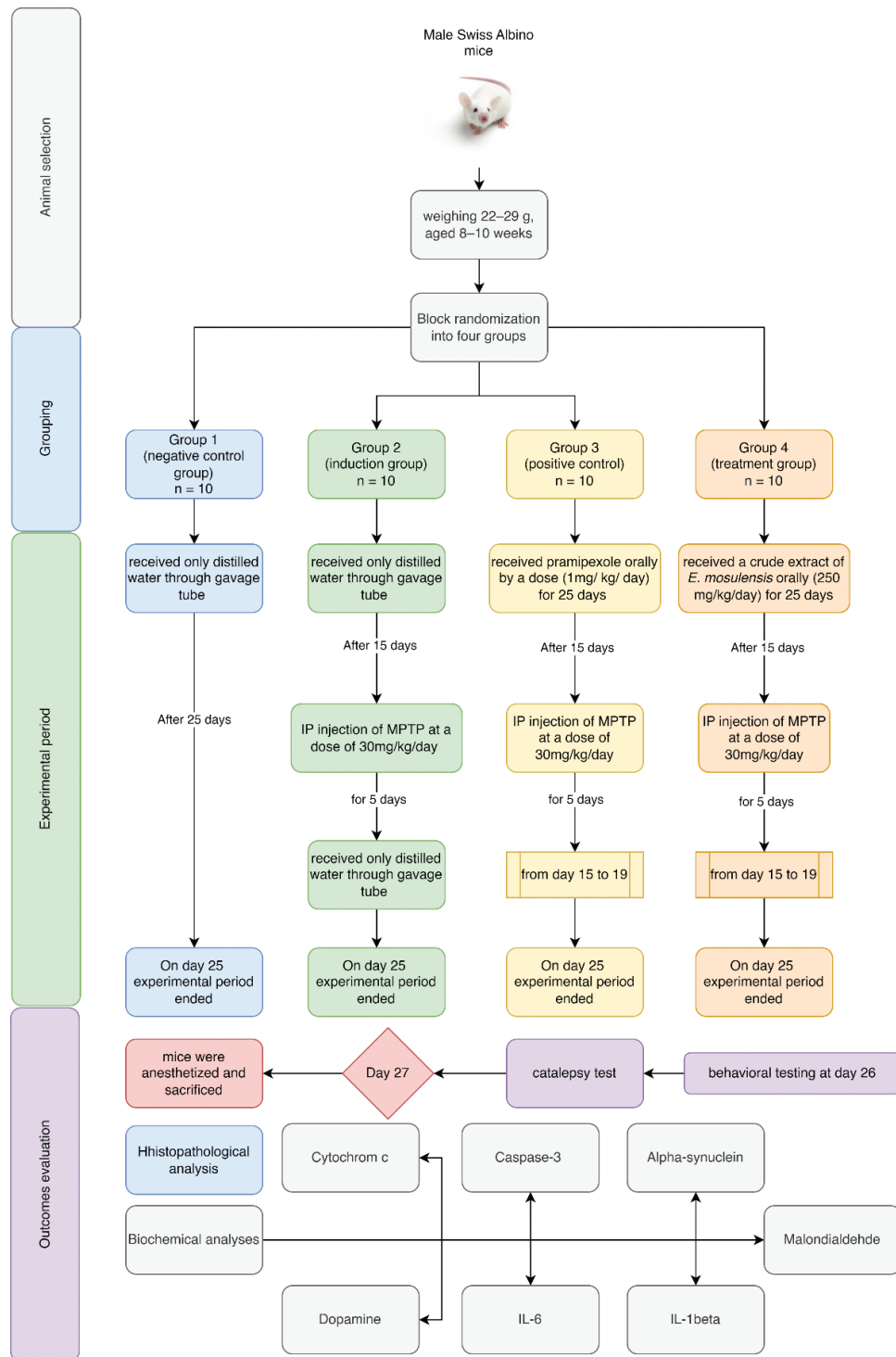


Figure 1. Flow chart of the study.

of samples on ice (Hassan and Kadhim 2022). The supernatant was separated by centrifugation (Cypress Diagnostics, Belgium), and the remaining material was allowed to settle and freeze before being used for the biomarker measurement procedures. The tissue was homogenized following the manufacturer's instructions using a glass homogenizer on ice and phosphate buffer solution in the following ratio (tissue weight (gm): PBS (ml) volume = 1:9) based on the weight of the tissue sample. The homogenate was centrifuged for five minutes at 4°C at 5000× gm to yield the

supernatant. Which was stored at ≤ -20 C until used for analysis by the Sandwich ELISA technique. To check the histological alterations, small slices of the midbrains from the treated and normal control animals were preserved in a 10% formaldehyde solution using the paraffin section procedure described previously (Alkhazragy and Alshawi 2023). After fixation and wax embedding, brain tissues were dehydrated in ascending alcohol grades. Paraffin slices with a 5–7 μ m thickness were cut, and hematoxylin-eosin staining was done (Mbiydenyuy et al. 2018).

Analytical procedure

The stored samples were thawed and used for the analysis by sandwich enzyme-linked immunosorbent assay (ELISA) (Elabscience, USA) and (Mybiosource, USA) techniques using the ELISA Reader (ELISA reader, Diagnostic Worldwide/Human Reader HS, Germany).

Ethical approval

The study was approved by the Research Ethical Committee of the College of Medicine, Al-Nahrain University, approval number (UNCOMIRB20240512), and data (11 November 2022), following the American Veterinary Association Guidelines (AVMA) (Underwood and Anthony 2020).

Statistical analysis

The Kolmogorov-Smirnova test of normality was performed, and some of the variables in each parameter did not follow the normal distribution; non-parametric statistical analysis was used in the current study. After comparing the four groups, the Kruskal-Wallis test was used to assess the overall significance. The two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli (correct for multiple comparisons by controlling the false discovery rate (FDR) was used for pair-wise comparison. The significance level was defined by a p-value ≤ 0.05 (alpha level). All analyses used GraphPad Prism version 10.2.0 for Windows, GraphPad Software, and Boston, Massachusetts, USA (Hussein et al. 2024a).

Results

Phytochemical analysis of echinops extract

Results of the qualitative phytochemical screening of the *Echinops* extract are given in Table 1.

HPLC analysis

The quantitative analysis of the *Echinops* extract is illustrated in Table 2 and Figs 2, 3.

Table 1. Phytochemical analysis of echinops extract.

Active constituents	Result
Flavonoids	+
Alkaloids	-
Steroids	+
Saponin	+
Tannins	+
Terpenoids	+

(+, -) represent the presence and absence of phytoactive constituents, respectively.

Table 2. The component of the *Echinops* extract using HPLC analysis.

Number	Component name	Concentration (ppm)
1	Apigenin	45.9
2	Gallic acid	20.5
3	Hesperetin	35.9
4	Kaempferol	74.9
5	Quercetin	80.4
6	Rutin	66.9
7	Luteolin	59.9

PPM: part per million.

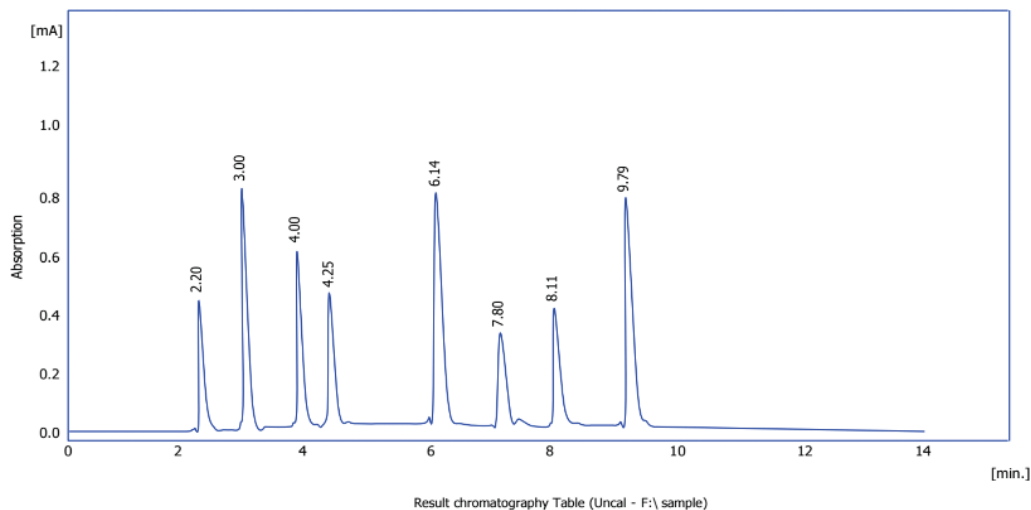


Figure 2. HPLC analysis of *Echinops* extract.

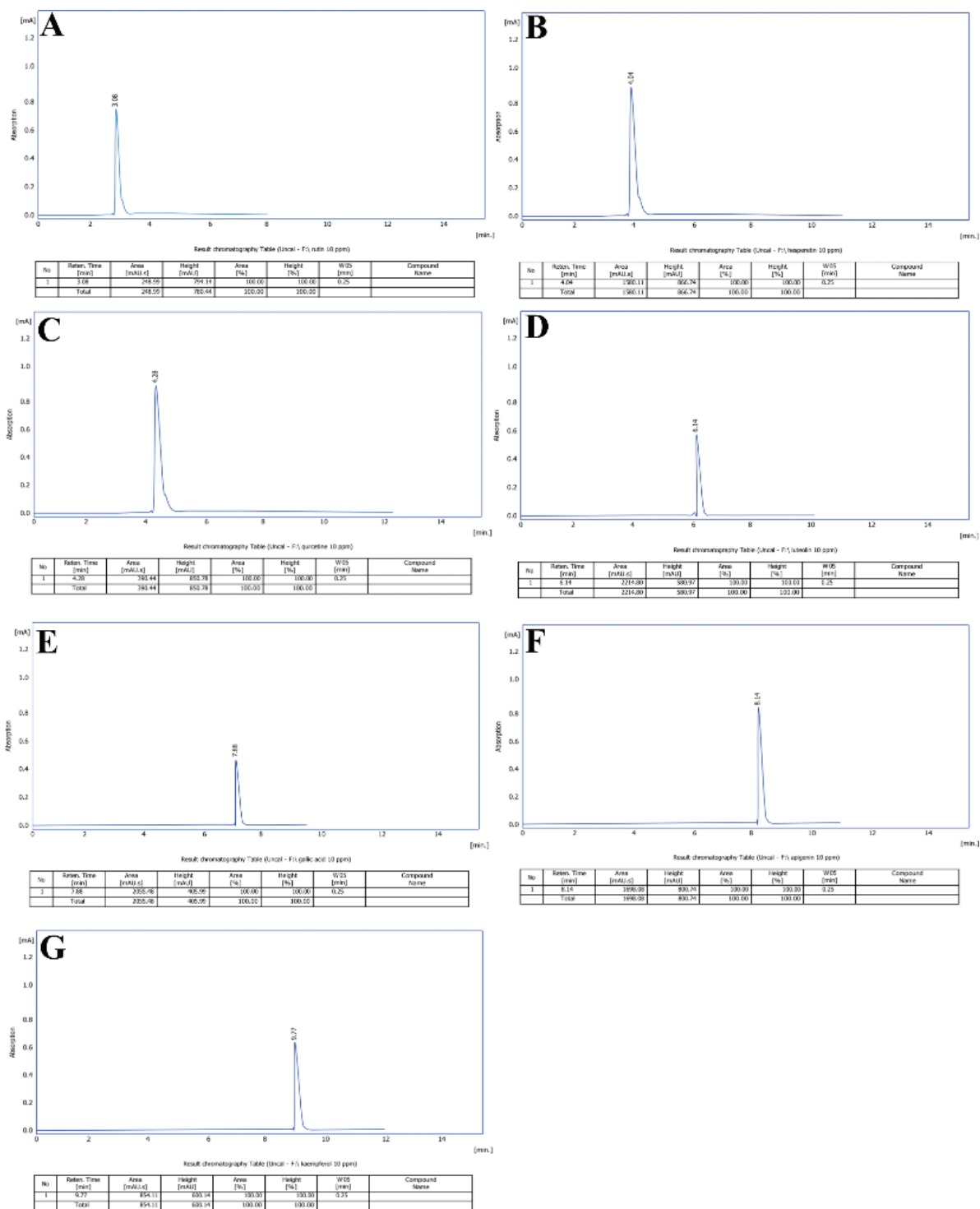


Figure 3. HPLC analysis of reference compounds. **A.** Rutin; **B.** Hesperetin; **C.** Quercetin; **D.** Luteolin; **E.** Gallic acid; **F.** Apigenin; **G.** Kaempferol

Biomarker

Neurobehavioral analysis, including the catalepsy test, was significantly higher in group II (longer duration to correct the mice's position (seconds) than in group I. In contrast, group IV was significantly lower than group II. DA was significantly lower in group II compared to group I. While in group IV, it was significantly higher than in group II. IL-6 and IL-1 beta were significantly higher in group II

compared to group I. While group IV was significantly lower than group II, MDA was significantly higher in group II compared to group I. While in group IV, it was significantly lower than in group II. Apoptotic markers Cyt c and Cas 3 were significantly higher in group II compared to group I. While group IV was significantly lower than group II, alpha-synuclein, a diagnostic protein, was significantly higher in group II compared to group I. Group IV was significantly lower than Group II, as illustrated in Fig. 4.

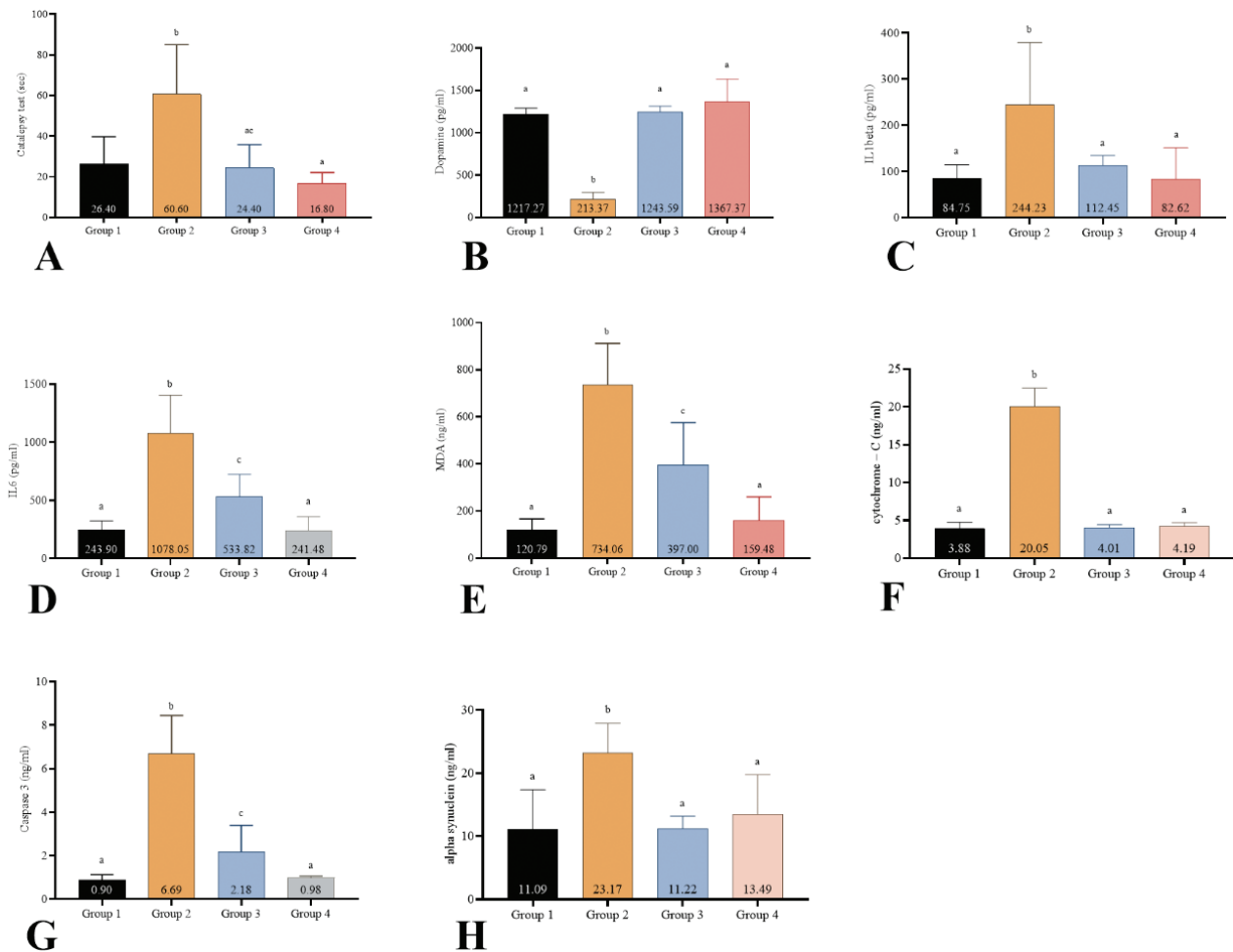


Figure 4. Assessment of biomarkers in the studied group. **A.** Catalepsy test; **B.** Dopamine levels; **C.** IL-1 beta; **D.** IL-6; **E.** MDA; **F.** Cyt C; **G.** Cas 3; and **H.** α-Synuclein (Columns with similar letters indicate no significant difference (p -value >0.05), while different letters indicate a significant difference (p -value ≤ 0.05)). Kruskal-Wallis's test (posthoc test of the two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli) (a vs. b p -value ≤ 0.05 , b vs. c ≤ 0.05) Each value represents the mean $\pm$ standard deviation.

Histopathological examination

The histopathologic section of the mice's midbrain in the negative control group showed normal Substantia Nigra (SN), no loss of neurons, no vacuolated space, no Lewy bodies, and melanin-containing neurons. The induction group shows distinctive mid-brain lesions, including severe vacuolated space, severe neuronal loss, severe pyknotic nuclei, and a lewy body. The positive control group shows a mild midbrain improvement, including mild vacuolated space, mild neuronal loss, few pyknotic nuclei, and no Lewy bodies. Finally, the crude extract of *E. mosulensis* showed good improvement compared to the induction group represented by mild neuronal loss, moderate vacuolated space, few pyknotic nuclei, and no Lewy bodies, as illustrated in Fig. 5.

Discussion

PD is a neurological disease that gradually reduces motor function. The current generation of PD therapies can help control symptoms, but they cannot halt or reverse the

progression of the illness. PD drugs may result in a variety of side effects or indications of drug resistance when used in long-term therapy. To reduce side effects and increase patient-specific care, new, safe drugs with a range of therapeutic approaches are needed; natural medicines created from medicinal plants are therefore a desirable option for handling and avoiding Parkinson's disease models (Pariyar et al. 2022; Atiq et al. 2023).

MPTP-stimulated Parkinson's disease models are categorized into disease models with various disease courses according to varied doses, intervals, times, and durations of administration. Though the consistent standard dose and the times are unknown, according to some authors, disease models fall into one of the following categories: Models that are acute, subacute, and chronic (Ma and Rong 2022). The current study administers MPTP (30 mg·kg⁻¹·d⁻¹, IP) subacutely to the animals for five consecutive days (Zhang et al. 2017). Consistent with earlier studies, MPTP causes a marked rise in catalepsy scores and a drop in striatal dopamine levels (Elbassouni and Ahmed 2019). This behavior assesses nigrostriatal function and how different neurotransmitter systems

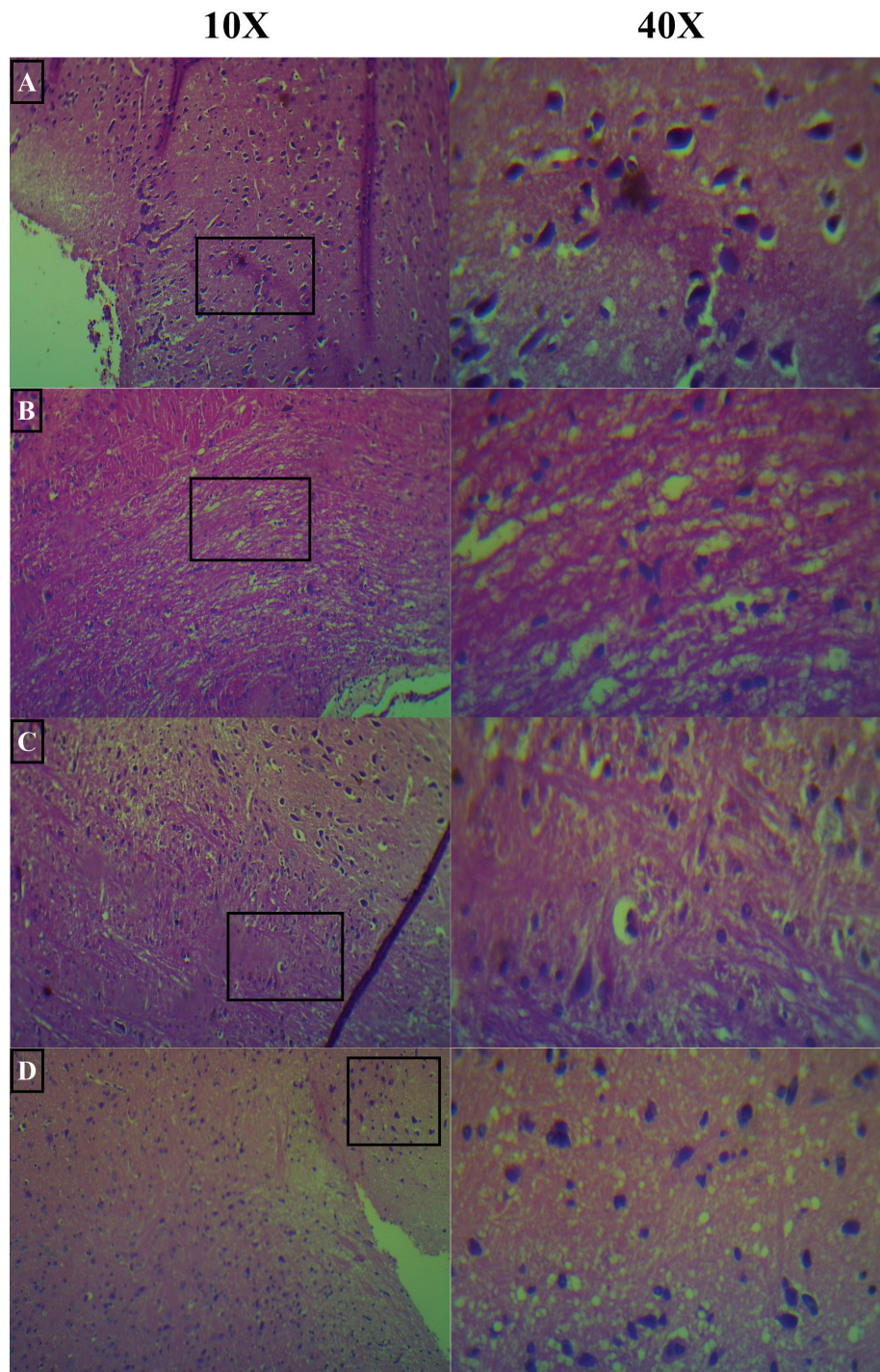


Figure 5. Histopathological examination of the mid-brain sections. **A.** Group I showed a normal picture of the midbrain; **B.** Group II showed severe vacuolated space, severe neuronal loss, severe pyknotic nuclei, and a Lewy body; **C.** Group III showed mild midbrain improvement, including mild vacuolated space, mild neuronal loss, few pyknotic nuclei, and no Lewy bodies; and **D.** Group IV showed mild neuronal loss, moderate vacuolated space, few pyknotic nuclei, and no Lewy bodies. H and E stain (10× and 40× magnification).

control it. It can also imitate the akinesia and rigidity associated with PD, and animals with dopaminergic lesions exhibit pronounced catalepsy (Vajdi-Hokmabad et al. 2017). Consistent with certain studies, lesioning by MPTP results in increased glutamate release in the striatum, excitotoxicity, and hyper-glutamatergic activity in the subthalamic nucleus (STN). These effects might be connected to a lack of dopamine production and,

consequently, loss of striatal dopamine secondary to the degeneration of nigrostriatal dopaminergic neurons seen in PD brain tissue (Airavaara et al. 2012; Hsieh et al. 2012; Moraes et al. 2016).

The current study is in line with previous studies regarding the effect of MPTP on MDA markers, which indicated that it is well-known that MPTP inhibits Complex 1 in the mitochondrial electron transport chain, which

reduces energy generation and results in neuronal death and brain damage. Problems with energy generation that cause dysfunction in a neuron's metabolism and, ultimately, production of ROS, reduced glutathione (GSH) levels, reduced activity of the antioxidant enzyme catalase, and increased lipid peroxidation levels that result in MDA synthesis. This result reveals an unequilibrium between the antioxidant defense system and MPTP-induced oxidative damage (Liu et al. 2015; Rai et al. 2017; Ardah et al. 2020).

Regarding the effect of MPTP on apoptotic proteins, cytochrome-C, and caspase-3, MPP⁺ is a toxic derivative of MPTP. After being released from astrocytes, MPP⁺ is absorbed by dopaminergic neurons, which disrupts mitochondrial respiratory activity, causes ATP synthesis to be reduced, and causes the production of excessive intracellular ROS, all of which ultimately result in the eventual death of dopaminergic neurons (Liu et al. 2015). ROS are produced inside the mitochondria, which causes the membrane to become hyperpolarized and permeability transition pores to open. Following the breakdown of the mitochondrial membrane, the pro-apoptotic protein cytochrome C is released into the cytoplasm (Shen et al. 2017). So far, the accumulation of cytochrome C creates an apoptosome complex with pro-caspase-9 and Apf, triggering caspase-9 activation and further activating the caspase cascade (Liu et al. 2015). Furthermore, cytosolic cytochrome C activates the Bax proteins, which encourage apoptosis through interactions with the Bcl-2 family proteins (Liu et al. 2015).

According to earlier research, MPTP treatment increases the expression of Bax and lowers the expression level of Bcl-2 in the SNpc and striatum of mice. It was discovered that these alterations were associated with the dopaminergic neurodegeneration brought on by MPTP (Haque et al. 2021). Additionally, the hallmarks of MPTP-induced apoptotic cell death include an increase in the Bax/Bcl-2 ratio, cytochrome C release, and activation of caspase 3, a last factor in caspase-dependent apoptosis that cleaves PARP and causes apoptotic cell death (Haque et al. 2021).

Regarding the effect of MPTP on inflammatory markers like IL-1 β and IL-6, the current study showed an increase in these inflammatory markers in accordance with a previous study, indicating that MPTP has a strong effect on microglia, activating them and causing changes in their morphology and phenotype (microgliosis), which results in the discharge of high concentrations of ROS (San Miguel et al. 2019). It is commonly recognized that ROS causes the NF- κ B pathway to be activated, phosphorylating a Kappa inhibitor and increasing the production of pro-inflammatory cytokines such as TNF, IL-1, and IL-6 (San Miguel et al. 2019).

The current study aligns with a previous study regarding the effect of MPTP on α -synuclein, causing a marked increase in this protein. When MPTP is administered, it causes neuronal degeneration, which is associated with the atypically high α -synuclein, leading to an increase in its toxicity by self-agglomeration or modification, which in turn may induce glial cells to produce oxidative stress and inflammation, which in turn may promote the production

of aberrant α -synuclein (Campolo et al. 2017; Zhang et al. 2017). Alpha-synuclein moves from its normal synaptic site to aggregates in degenerating neuronal cell bodies, which are the first stages of the formation of Lewy bodies (Campolo et al. 2017; Zhang et al. 2017). So far, in pathological conditions, α -synuclein transforms from monomers to inclusions, generating soluble oligomer species detrimental to neuronal cells (Campolo et al. 2017; Zhang et al. 2017).

Histopathological images dealing with the impact of MPTP on dopaminergic neurons revealed that the mid-brain displayed a loss of dopaminergic neurons, which in turn results in a suppression of the dopamine levels produced by these neurons. This effect is consistent with certain studies indicating that lesioning by MPTP results in increased glutamate release in the striatum, excitotoxicity, and hyper-glutamatergic activity in the subthalamic nucleus (STN). These effects might be connected to a lack of dopamine production and, consequently, loss of striatal dopamine secondary to the degeneration of nigrostriatal dopaminergic neurons in PD brain tissue (Airavaara et al. 2012; Hsieh et al. 2012; Moraes et al. 2016). Another histopathological image revealed an increase in Lewy bodies composed of alpha-synuclein, ultimately increasing these proteins. These results are consistent with earlier research, which revealed that when MPTP is administered, it causes neuronal degeneration, which is associated with the atypically high α -synuclein, leading to an increase in its toxicity by self-agglomeration or modification, which in turn may induce glial cells to produce oxidative stress and inflammation, which in turn may promote the production of α -synuclein. Alpha-synuclein moves from its normal synaptic site to aggregates in degenerating neuronal cell bodies, which are the first stages of the formation of Lewy bodies. So far, in pathological conditions, α -synuclein transforms from monomers to inclusions, generating soluble oligomer species detrimental to neuronal cells (Campolo et al. 2017; Zhang et al. 2017). It was also noticed that there was severe vacuolated space and severed pyknotic nuclei.

To the best of our knowledge, no prior research has been done on the chemical composition or biological impacts of Iraqi *Echinops*. Numerous studies examining the biological effects of species belonging to the same genus, such as *Echinops heterophyllus*, *Echinops echinatus*, and *Echinops spinosissimus*, among others, were found. A variety of phytochemical components present in *E. heterophyllus*, such as flavonoids, terpenoids, quinoline, and alkaloids, are responsible for the hepatoprotective and antioxidant qualities of the crude extract (Abdalmohsin et al. 2019). Many phytochemical and pharmacological studies of *E. echinatus* Roxb have been shown to explain the plant's effects, including hepatoprotective, diuretic, analgesic, antioxidant, antifungal, anti-inflammatory, antibacterial, and antipyretic ones (Maurya et al. 2015). The methanolic extract of the root of the western Algerian native *Echinops spinosissimus* has antibacterial and antioxidant qualities that successfully fight Gram-negative bacteria, especially *P. aeruginosa* (Zitouni-Nourine et al. 2022).

The phytochemical analysis of the *Echinops* extract used in the current study also showed notable amounts

of terpenoids, steroids, tannins, saponins, and flavonoids, an important component of the extract. Many compounds found in nature that have secondary metabolites, such as terpenoids, lignans, alkaloids, and flavonoids, have anti-inflammatory and anti-TNF- α activity *in vitro* by blocking upstream signaling at low micromolar doses comparable to those of drugs like adalimumab, infliximab, and etanercept, as well as the ability to suppress PGE2 production and COX expression. These metabolites also prevent the production of pro-inflammatory cytokines (IL-1, IL-6, and TNF) as well as the expression of vascular cell adhesion molecule-1 and intracellular adhesion molecule (Zahedipour et al. 2022; Arega et al. 2023).

Terpenoids inhibit several inflammatory processes, including PLA2 activity, TNF- α production, iNOS expression, COX-2 expression, and NF- κ B activation. Polyphenols also suppress the manufacture of inflammatory cytokines and chemokines, which further reduces inflammation by lowering the activities of COX and iNOS, which lowers the creation of reactive oxygen and nitrogen species (Yimer et al. 2020).

Tannins are another phytochemical (also called tannic acid) (Chung et al. 1998). Tannins encapsulated in lipids may offer a different approach by avoiding certain pathological characteristics of neurodegenerative illnesses. Since they effectively reduced inflammation and neuronal oxidative stress, two important characteristics of these pathogenic states (Díaz et al. 2022). As a result, tannins and flavonoids can dismutate ROS by chelating free radicals such as hydroxyl radicals (Rezaei and Alirezaei 2014). Tannic acid's neuroprotective impact was shown by a decrease in inflammatory mediators as well as a decrease in astrocyte and microglia activation. Protection of dopaminergic neurons, inhibition of oxidative stress and lipid peroxidation, and lowered synuclein expression, a pathologic characteristic of PD, lowering apoptosis and raising autophagy (Azimullah et al. 2023).

Saponins scavenge ROS, decreasing lipid peroxidation (Eram et al. 2013). Moreover, it is believed that saponins sequentially reduce inflammation by disrupting iNOS expression, which in turn suppresses COX-2 production and generates PGE2 (Yimer et al. 2020).

Most of the published studies have demonstrated that flavonoids only exhibit neuroprotective effects when prooxidants or neurotoxins are present, not in healthy physiological conditions. As these studies clearly explain, the oxidative damage generated by free radicals, a major contributor to many degenerative conditions, including PD, is prevented by flavonoids' antioxidant qualities. Numerous beneficial properties of flavonoids, such as their anti-inflammatory, antioxidant, antiapoptotic, antiviral, and antibacterial qualities, have been demonstrated (Magalingam et al. 2015). However, regarding neuroprotection, flavonoids' antiapoptotic and anti-inflammatory characteristics appear to stop the progressive loss of neurons in neurodegenerative diseases such as Parkinson's disease (Magalingam et al. 2015). Furthermore, certain chemicals, such as phenolic and flavonoid compounds, have been shown in earlier studies to have antiparkinson activity by raising SOD and neuron counts in rat brains while

decreasing TNF- α , pro-inflammatory cytokines, and MDA levels (Saadullah et al. 2022).

HPLC analysis of the flavonoid content of *Echinops* in the present study revealed the following active constituents: luteolin, rutin, hesperetin, quercetin, gallic acid, apigenin, and kaempferol.

In different animal models of Parkinson's disease studies, all these phenolic compounds, luteolin, rutin, quercetin, and apigenin, exhibited beneficial effects on inflammatory markers in which they restored and decreased the levels of these markers in their treated groups in comparison to induced PD groups. These findings agree with and strongly support our study findings regarding inflammatory markers, which reflect neuroprotection against PD models (Khan et al. 2012; Jackson Seukep et al. 2020; Ait Lhaj et al. 2023; Frederick and Patel 2023).

The results of the present study revealed that phenolic compounds of *E. mosulensis*, including luteolin, rutin, quercetin, gallic acid, hesperidin, apigenin, and kaempferol, have antioxidant effects as compared to the induction group following previous studies that showed that phenolic compounds have neuroprotective effects against MPTP-induced Parkinson's disease by scavenging reactive oxygen species (ROS) (Khan et al. 2012; Kouhestani et al. 2018; Ogunraku et al. 2019; Jackson Seukep et al. 2020; Behl et al. 2022; Ait Lhaj et al. 2023; Frederick and Patel 2023).

The current study's results demonstrated that phenolic compounds luteolin and gallic acid decreased apoptosis markedly compared to induction animals, in line with the previous study, which indicated that these compounds (luteolin) inhibit the 6-OHDA-stimulated Bax/Bcl-2 ratio and p53 expression in the cells (Hu et al. 2014). Gallic acid and its derivatives also suppress mitochondrial apoptotic signaling molecules (Ait Lhaj et al. 2023).

The results of the present study indicated that the phenolic compound of *E. mosulensis*, like apigenin, decreased α -synuclein levels as compared to the induction group, which is consistent with previous studies that showed that the phenolic compound downregulated α -synuclein expression (Ait Lhaj et al. 2023).

The results of the present study indicated that the phenolic compounds of *E. mosulensis*, including apigenin and kaempferol, increased dopamine levels as compared to the induction group, which is consistent with previous studies, which showed that the phenolic compounds raised dopamine levels in the brain and prevented the death of dopaminergic neurons (Ait Lhaj et al. 2023).

Apigenin has antiviral, antibacterial, anti-inflammatory, and antioxidant effects (Jackson Seukep et al. 2020). Apigenin has been observed to alter dopaminergic neurotransmission by downregulating α -synuclein expression and upregulating tyrosine hydroxylase and the dopamine D2 receptor protein expression, which increases dopamine synthesis (Ait Lhaj et al. 2023).

It has recently been shown that quercetin, a flavonoid produced from plant flavanol, has neuroprotective effects against neurodegenerative illnesses. Furthermore, because of its capacity to pass the blood-brain barrier, it has been

demonstrated to have a wide range of biological effects that support health in several conditions, such as cancer, cardiovascular disease, cataracts, inflammation, diabetes, and nervous system diseases. Quercetin's anti-inflammatory effects are attributed to its vital function in inhibiting the nuclear factor NF- κ B release and reducing the quantities of proinflammatory cytokines such as TNF- α , IL-1 β , IL-6, and IL-8. The latter function keeps cytokines from entering the nucleus. By scavenging reactive oxygen species (ROS), quercetin is a powerful antioxidant to combat oxidative stress. Additionally, this process maintains the survival of cells by neutralizing free radicals by either accepting or donating electrons to remove the radical's unpaired state, possibly transforming them into new, less damaging, and less active free radicals. To replenish the antioxidants and boost their ability to scavenge radicals, they can also neutralize the radical form of other antioxidant molecules, such as glutathione radicals (Ogunraku et al. 2019; Wang et al. 2021; Saleem et al. 2022). It can also suppress lipid peroxidation and provide membrane-stabilizing potential (Ogunraku et al. 2019).

It has been demonstrated that kaempferol (Kmp), an important flavonoid, increases cells' ability to defend against free radical damage. Moreover, Kmp scavenges hydroxyl radicals, and peroxynitrite enhances antioxidant enzyme activity and inhibits lipid peroxidation (MDA) (Kouhestani et al. 2018). Kaempferol increases dopamine and DOPAC levels, prevents the death of dopaminergic neurons, and enhances dopamine turnover. In addition, kaempferol improved performance in tests of spontaneous motor activity, indicating a positive impact on motor behavior (Ait Lhaj et al. 2023).

The many health advantages of luteolin have been well studied in various cell types and animal models, with particular attention paid to its anti-inflammatory, anti-microbial, anti-cancer, antioxidant, and anti-diabetic effects. Furthermore, luteolin has been demonstrated to have neuroprotective effects due to its permeability to the blood-brain barrier (Frederick and Patel 2023). Additionally, luteolin suppresses 6-OHDA-induced apoptosis by inhibiting the 6-OHDA-stimulated Bax/Bcl-2 ratio and p53 expression in the cells (Hu et al. 2014).

Gallic acid and its derivatives suppress mitochondrial apoptotic signaling molecules, which may prevent harm to neurons by scavenging reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals (Ait Lhaj et al. 2023).

Rutin seems to have strong anti-inflammatory and antioxidant properties because of its ability to protect neurons. Rutin's main effects on oxidative damage and inflammation are thus directly related to its ability to scavenge free radicals and suppress lipid peroxidation. As evidenced by its inhibition of cytokine production, iNOS expression, and microglia cell activation, rutin demonstrates anti-inflammatory effects (Khan et al. 2012).

Moreover, preclinical research has shown that flavonoids, such as hesperidin, rutin, silibinin, and baicalin, are effective therapeutic agents that decrease ROS,

decrease NOS expression, lower oxidative potential in the striatum, inhibit neuronal death, and improve dopaminergic (DA) survival (Behl et al. 2022).

In the current study, the histopathological picture demonstrated a good improvement of the crude extract of *E. mosulensis* on the midbrain, as evidenced by the absence of Lewy bodies, which consist of alpha-synuclein, and mild neuronal loss; these neurons produce dopamine, leading to an increase in dopamine levels, moderate vacuolated space, and few pyknotic nuclei.

A common dopamine agonist used to treat PD is pramipexole (PPX). In PD, improved motor functions are achieved by activating the D2 and/or D3 receptors. It was discovered that in animal models, PPX and additional D3 receptor-preferred agonists were neuroprotective agents against MPTP-induced neurotoxicity in mice, primates, and rats with 6-OHDA lesioning (Winner et al. 2009). Prior research indicated that PPX might have protective benefits through multiple mechanisms that exist beyond dopamine receptor interaction, including growth factor-like actions, antiapoptotic, anti-inflammatory, antioxidant properties, mitochondrial protection, and catastrophic effects, as well as decreased phosphorylation of α -synuclein, which could offer a mechanistic understanding for the proteasome impairment model (Chau et al. 2013; Zhang et al. 2017). Histopathological picture demonstrated a good improvement of pramipexole on the midbrain, as evidenced by the absence of Lewy bodies, which consist of alpha-synuclein, and mild neuronal loss; these neurons, which produce dopamine, led to an increase in dopamine levels, moderate vacuolated space, and few pyknotic nuclei.

Limitations of this study

It is worth noting that the current study utilized single doses; it would be optimal if multiple doses were used; however, to reduce the cost and minimize the number of animals used, a single dose was chosen as a preliminary study for future work. Additionally, our findings focus on studying the catalepsy test on mouse models; we suggest using different neurobehavioral tests, such as the rotarod test or cylinder test, among many others.

Conclusion

The crude extract of *E. mosulensis* exerts its neuroprotective effect through a multi-faceted mechanism of action, the significant reduction in the inflammatory, oxidative stress, apoptotic, and α -synuclein markers, along with a significant increase in dopamine marker and the enhanced histopathological features, collectively contribute to the amelioration of PD observed in crude extract of *E. mosulensis* treated group and supports the ameliorative potential of *E. mosulensis* and its antiparkinson effect is comparable to that of pramipexole treated group.

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