https://doi.org/10.31351/vol33iss(4SI)pp249-260

Effects of Flavonoid Fraction of *Echinops Mosulensis* on Induced Parkinson's Disease Model in Mice

Asmaa Abdulwahab Ahmed*, [1] and Haitham Mahmood Kadhim[2]

[1]Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq.
[2]Department of Pharmacology and Toxicology, College of Pharmacy, Al-Nahrain University,Baghdad, Iraq.
***Corresponding Author.**

Received 1/5/2024, Accepted 11/8/2024, Published 15/2/2025

Abstract

Toxins, especially 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), are among the most commonly utilized models of Parkinson's disease (PD) based on the method utilized, MPTP causes different changes like striatal dopamine depletion, and behavioral abnormality. Phytochemical analysis of *Echinops mosulensis* extract indicated that there was a significant amount of flavonoid present in the extract. Flavonoids are belonging to several kinds of plant polyphenols exert a potent antioxidant, neuroprotective, and anti-inflammatory properties. The aim of the present study was to investigate the neuroprotective effect of flavonoid fractions of Iraqi *Echinops mosulensis* on induced PD model in mice. Forty male mice were randomly arrayed in 4 groups (10 mice in each group). Group 1: healthy normal control group received distilled water orally via a gavage tube for 25 days. Group 2: induction group received MPTP (30mg/kg/day) intraperitoneally for 5 consecutive days to achieved model of PD then continue with distilled water orally via gavage tube for 25 days. Group 3: the positive control group administered pramipexole orally (1mg/ kg/ day) for 25 days then the induction was done with MPTP (30mg/kg/day) intraperitoneally 1 hour post-pramipexole from day 15 to 19. Group 4: received flavonoid fraction orally (250 mg/kg/day) for 25 days then the induction was done with MPTP (30mg/kg/ day) intraperitoneally 1 hour post- flavonoid fraction form the day 15 to day 19. At day 26, the behavior test was done and at the day 27 all animals were sacrificed. Homogenized brain tissue was prepared for analysis. In the present study there was significant decreased in Malondialdehyde, interleukin -1beta, cytochrome C, and α-synuclein levels of flavonoid fraction accompanied with significant increase in tyrosine hydroxylase level of flavonoid fraction as compared to induction ($p \leq 0.05$), as well as significant increase in distance traveled in treated group as compared to induction ($p \leq 0.05$). Additionally, good improvement in histopathological analysis of treated group as compared to induction. In conclusion, the flavonoid fraction of *E. mosulensis* has a neuroprotective effect on MPTP induced PD Model in Mice.

Keywords: parkinson's disease, α-synuclein , MPTP, *Echinops mosulensis*, flavonoid.

Introduction

Parkinson's disease (PD) is considered an age-dependent disorder, and ranks second among all common neurodegenerative disorders. This condition is defined by the progressive and selective kill of dopaminergic neurons in the substantia nigra pars compacta (SNpc), with consequent reduction of dopamine in the striatum [1,2]. Before, the disease was largely related to begin as movement illness, classically associated with tetrad of deficits in motor skills, such as postural instability, bradykinesia, rigidity, and resting tremor, besides, motor symptom, another symptoms frequently observed in PD are non-motor symptoms including cognitive deficits, olfactory deficits, autonomic dysfunction, depression, and sleep disorders. The aetiologies of PD are still unclear. Although, environmental and genetic factors can influence the onset and progression of PD [3].

Herbicide or pesticide exposure, prior head injury, agricultural occupation, rural living, well-water drinking are including as an environmental factors that effect on PD [4], as well as the genetic variation is accounts for 25% of the overall chance of having PD such as SNCA point mutations were the first to be related to PD [5,6] . Also, it was well known that several medications can cause Parkinsonism, including calcium-channel blockers, antiepileptic, gastrointestinal motility medications, and typical and atypical antipsychotics [7]. Its pathophysiology has been connected to numerous processes like mitochondrial failure, α-synuclein aggregation, neuroinflammation, oxidative stress, altered iron metabolism, inadequate support for neurotrophic factors, and cell apoptosis [8]. There is no known cure and symptom control is the main therapeutic goal, treatment may not be fully effective or result in secondary complications [9].

Thus, natural compounds derived from medicinal plants are a popular choice for managing and preventing PD models [10]. Alpha- synuclein is an intracellular protein that is mostly found in the terminals of presynaptic terminals [11].

Under normal physiological circumstances, α-synuclein is involved in the release, storage, and circulation of neurotransmitters in neurons as well as transport of vesicles [12]. The levels of α-synuclein protein rise during development and stay elevated throughout adulthood [13]. Remarkably, α-synuclein is identified as key player and its connected to fundamental pathological features that occure in PD, such as neuroinflammation and dysfunction of metabolic and physiological systems [14,15]. When pathological circumstances arise, α-synuclein creates lewy bodies (LBs) in the soma of affected neurons [11]. The commonly used PD model is MPTP model [16]. MPTP, a dopaminergic pyridine toxin, was accidentally found during the production of analogues of meperidine [17]. MPTP is non-toxic to neurons and extremely lipophilic and easily passes through blood-brain barrier (BBB). In astrocytes quickly transforms it into the toxic metabolite 1-methyl-4-phenylpyridinium-iodide (MPP+) by monoamine oxidase [18,19]. Then is picked up by DA neurons through DA transporters, and selectively damaging the neurons [20,21]. By suppression complex I of the mitochondrial electron transport chain and cause Parkinsonism in experimental animals, including primates and rodents [22]. The genus Echinops is a member of the Asteraceae family composed of over 130 species [23]. The genus *Echinops*, sometimes known as "Shekar tighal," which can be distributed all over the world primarily in Africa, Central Asia, and Mediterranean [24]. The genus *Echinops* has many species of annual, biennial, and perennial plants that are all individually known as globe thistles. The primary phytochemicals found in medicinal herbs like *Echinops* fall into many classifications, including, glycosides, lipids, steriods alkaloids, triterpenoids, saponins, and polyphenols, which are thought to function collectively and have variety of medicinal applications to treat different disorders [25,26]. Hepatoprotective, antioxidant, and anti-inflammatory properties are attributed to terpenes, flavonoids, and other phenolic substances [27]. Phenolic chemicals, among other secondary metabolites, have many beneficial impacts on humans to treat a variety of disorders, including neurological disorders and cancer because they have been shown to possess anti-inflammatory, antioxidant, and anti-angiogenesis properties [28,29]. *E. mosulensis* which was chosen for the study "Figure1." contained significant amount of flavonoids. Flavonoids are naturally occurring polyphenolic metabolites present in vegetables, fruits, and herbs which have numerous medical and pharmacological uses [30]. Following intake, they undergo fast metabolism, and these metabolites have the ability to pass through BBB and their penetration depend on how the flavonoid interact with certain efflux transporters found in the BBB, like the P-glycoprotein which determine their ability to enter the brain as well as degree of lipophilicity (less polar flavonoids capable of greater brain uptake than the more polar) to exert a variety of effects, including reducing inflammation, oxidative stress, and atherosclerosis [31,32]. Flavonoids have protectective effect against age-related neurodegenerative illnesses, specifically Alzheimer's, PD, and dementia [33]. A study reveals the numerous benefits of flavonoids, including their neuroprotective action as well as their antioxidant, anti-inflammatory, anti-proliferative, analgesic, anti-angiogenic, anti-cancer, anti-viral, anti-microbial, and anti-malarial properties [34]. Flavonoids are thought to be safe and well-tolerated when derived from dietary sources and herbal medicine; nevertheless, as they say, "Too much of anything is bad," therefore taking huge doses of flavonoids can damage rather than protect your body. Four common flavonoids—luteolin, apigenin, quercetin, and genistein—were investigated for possible toxicity by some researcher where concerns regarding possible developmental toxicity, endocrine disruption, and mutagenicity [35].

Figure1. Iraqi *Echinops mosulensis*

Materials and Methods

Drugs, herbal, and chemicals

Herbal plant of *E. mosulensis* was collected from Iraq, Erbil/ Shaklawa and soran district in spring on April. Toxin, MPTP hydrochloride crystalline powder was purchashed from (Meryre, china), pramipexole tablets was purchased from (Boehringer Ingelheim Pharma GmbH & Co KG, Germany). Mouse Eliza kits of (MDA, IL-1Beta, TH and cytochrome C) were purchased from (Elabscience, USA), while mouse Eliza α- synuclein kit was purchased from (Mybiosource, USA). Buffer phosphate saline from (Reagent world, USA) and Formaldehyde solution 37% from (Fluka chemical,UK).

Extraction and fractionation of E. mosulensis

400ml of 85% ethanol was used in soxhlet apparatus device to extract 270g of coarsely powdered, shade-dried aerial parts until it was completely exhausted. The extract of alcohol was evaporated at temperature below 40°C under reduced pressure to yield a dark yellow-greenish residue designated as crude fraction 1(F1). A portion of the crude extracts was suspended in D.W then undergone three partitions using an equal volume of ethyl acetate after being acidified with hydrochloric acid (5%) to pH 2. A reduced pressure was used to evaporate the ethyl acetate layer until it was completely dry then it was basified with 300 ml of 5% sodium hydroxide to pH of 10, and it was evaporated using chloroform. The aqueous basic layer was separated, allowed to dry out, acidified to pH of 2 using 5% hydrochloric acid, and then extracted using ethyl acetate to provide the fraction known as fraction 2 (F2), which includes the flavonoids [36].

Animals

Forty adult Swiss albino male mice, weighing 22-29gm were obtained from Al-Razi Center /Ministry of Industry and Minerals and kept in animal house at Iraqi Center for Cancer Research and Medical Genetics / College of Medicine/Al-Mustansiriya University under controlled environments of temperature, humidity, and 12-12hr light-dark cycle with unlimited access to water and regular food. All the mice were accommodated for one week before starting the experiment which lasted for 25 days. Experimental work was carried out in accordance with the protocol that was approved by the scientific committee of the Department of Pharmacology at the College of Medicine/Al-Nahrain University and reviewed by the Institutional Review Board of the same institution.

Experimental protocol

Healthy experimental mice were arbitrarily divided into 4 groups (10 animals /group) as follows:

Group 1: Mice orally administered distilled water (D.W) via oral gavage once daily for 25 days; this group represents the normal control group.

Group 2: Mice intraperitoneally (IP) injected MPTP (30mg/ kg/ day) for 5 consecutive days to achieved PD models [37], then continue with D.W via oral gavage for 25 days and represents the negative control group or induction group.

Group 3: Mice orally administered pramipexole at the dose (1mg/ kg/ day) via oral gavage for 25 days [38], and from day 15 to 19, injected with MPTP (IP) at the dose (30mg/kg/day) – 1hour post pramipexole and represents positive control group [39].

Group 4: Mice orally administered flavonoid fraction of *E. mosulensis* at the dose (250mg/ kg/ day) via oral gavage for 25 days [36], and from day 15 to 19, injected with MPTP (IP) at the dose (30mg/kg/day) – 1hour post flavonoid [39].

Behavioral test

Open field test (OFT) for all groups was done in day 26 at the same room where the mice were housed, at a time between 10 a.m and 2 p.m, and the mice were trained separately several times before being recorded. OFT is often used to assess movement, exploration, and anxiety [40]. Every mouse was put in the middle on an open area composed of (450 x 450 x 400 mm) white acrylic panels as shown in "Figure 2". Ten minutes were spent recording the movements of the mice using a camera placed one meter above their activity field and the number of crossed squares (with its four paws) was manually calculated [41]. The degree of activity can also be strongly impacted by tiny environmental changes. These consist of human activity, noise, odor, temperature, humidity, and lighting. It is crucial, then, that testing be done in a room with controlled humidity and temperature and indirect lighting at the same time. The test chambers need to be positioned uniformly throughout the facility, away from bright lights and dim areas. To lessen the effects of different environmental variables across the room, animals should also be given 10 to 30 minutes to acclimate to the testing room before any data is collected. To further lessen noise and distractions in the space during data collecting, everyone ought to exit the room [42]. After each mouse was tested, then the equipment was cleaned to get rid of any remaining smells [43]. Data analyzed by calculate the mean of total distance traveled (cm), per group. For the data that is not normally distributed, using a Kruskal-Wallis test with resulting p-values for multiple comparisons [42].

Figure 2. Open field test

Brain tissue processing

After behavioral analysis, and under diethyl ether anesthesia, all mice were killed by cervical dislocation, and then brain tissue was extracted and ready for examination. Briefly, the brain was removed quickly, washed and rinsed with PBS (7.4, 4´C) to eliminate any remaining blood. After that, dried on filter paper and cut into small pieces. For each mouse, brain tissue homogenate was made by filling minced tissue and PBS into a tube. After that, homogenization was achieved with the use of tissue homogenizer (Karl kalb, Germany) for one minute. All of the above mentioned steps involved keeping samples on ice [(44)]. Centrifuged (Cypress diagnostics, Belgium) by using refrigerated centrifuges to separate the supernatant, then left to settle and freeze on which the tests were performed to measure the biomarkers by using Enzyme-Linked Immunosorbent Assay (ELISA), (competitive-ELISA principle for MDA and Sandwich- ELISA principle for TH, Cyt-c, IL-1β, and alpha-synuclein) .Tissue homogenization was done according to the manufacturer's instructions on the basis of tissue sample weight using phosphate buffer solution in this ratio (tissue weight (gm): PBS (ml)= 1:9), with a glass homogenizer on ice and the supernatant was obtained by centrifuging the homogenate for five minutes at 4°C at 5000Xg [(45)].

Histopathological examination

Small slices of the midbrains for each of the treated and normal control animals were fixed in 10% formaldehyde solution with the method of paraffin sections as stated by Junqueira LC. et al. (1995) to evaluate for histological changes [(46)]. After fixation, brain tissues had been embedded in wax after being dehydrated in ascending grades of alcohol concentration: 70%, 80%, 95% and 100% . Paraffin slices with a thickness of 5-7 µm were cut, and hematoxylin–eosin staining was applied thereafter [(47,48)]. The histopathological changes were done depending on the following criteria. Sections were evaluated in accordance with the morphological alterations which are charchterized by precence of vacuolated spaces, pyknotic nuclei, and neuronal loss [(49),] intra-neuronal inclusion bodies, Lewy bodies (LBs) and Lewy neurites (LNs) [(50).]

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. Since comparing 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) was used for pair-wise comparison. The significance level was defined by p-value ≤0.05 (alpha level). All analyses used GraphPad Prism version 10.0.0 for Windows, GraphPad Software, and Boston, Massachusetts USA [(51)].

Results

Neurobehavioral analysis including open field test showed a significant decrease (p-value ≤0.05) in distance travelled of group 2 (induction or negative control) as compared to group 1(normal control). Interestingly, co-administration of flavonoid fraction (group 4) indicated significant rise (p- value ≤0.05) in distance travelled of open field test in comparison with the group 2 (induction group) as illustrated in "Table 1" and "Figure 3"

Table1. Effect of flavonoid fraction on distance travelled in brain tissue.

Parameters	Groups			
	Group 1 (Normal control)	**Group 2 (Induction) or (Negative control)**	**Group 3 (Positive control) or Pramipexole**	**Group 4 (Flavonoid fraction of *E. mosulensis*)**
Distance travelled in cm	1,443.20±772.28[a]	633.20±213.23[b]	1,772.90±934.20[a]	1,488.00±498.40[a]

Data are expressed as Mean ± STD, n=10.
a vs b = different letters indicate significant differences (p-value ≤0.05)
a vs a = Similar letters indicate no significant differences (p-value >0.05)

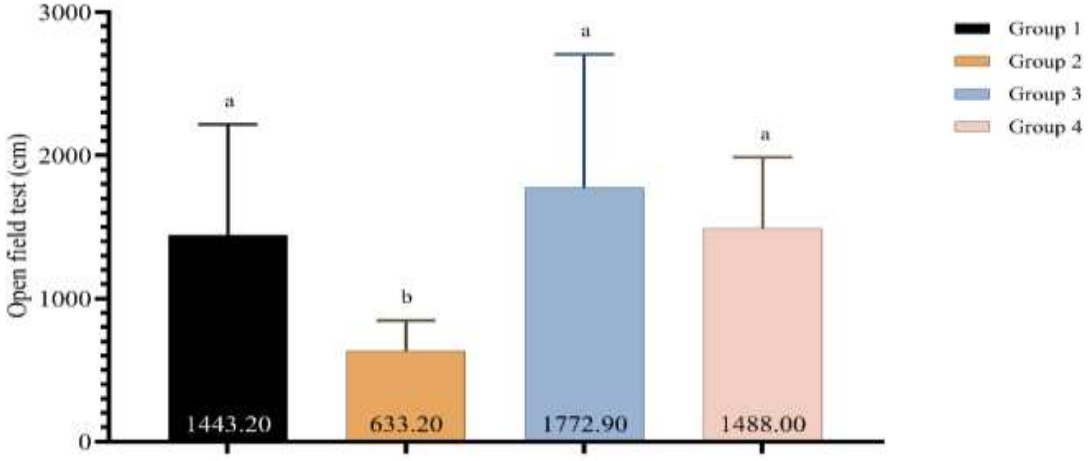

Figure 3 Distance travelled

Regarding biochemical parameters, there was a significant rise (p- value ≤0.05) in MDA, IL-1beta, α –synuclein, and Cyt c level in group 2 as compared to group 1. However, there was significant decrease (p- value ≤0.05) in the levels of these parameters in group 4 as compared with group 2. On other hand, there was a significant decline (p- value ≤0.05) in TH level in group 2 as compared to group 1. A significant rise (p- value ≤0.05) in TH level in group 4 as compared to group 2. as illustrated in "Table 2" and Figure "4, 5, 6, 7 and 8".

Table 2. Effect of flavonoid fraction on different parameters in brain tissue

Parameters	Groups			
	Group 1 (Normal control)	**Group 2 (Induction) or (Negative control)**	**Group 3 (Positive control) or Pramipexole**	**Group 4 (Flavonoid fraction of *E. mosulensis***
MDA (ng/ml)	120.79±45.70[a]	734.06±177.65[b]	397.00±178.20[c]	159.48±100.63[a]
IL-1beta (pg/ml)	84.75±29.56[a]	244.23±134.92[b]	112.45±21.61[a]	82.62±68.27[a]
Cyt c (ng/ ml)	3.88±0.87[a]	20.05±2.41[b]	4.01±0.43[a]	4.19±0.52[a]
TH (ng/ml)	8.31±6.75[a]	0.77±0.43[b]	2.46±1.06[c]	3.34±0.30[c]
α–synuclein(ng/ml)	11.09±6.30[a]	23.17±4.71[b]	11.22±1.97[a]	13.49±6.26[a]

Data are expressed as Mean ± STD, n=10.
Letter a vs b and c = different letters indicate significant differences (p-value ≤0.05)
Letter a vs a = Similar letters indicate no significant differences (p-value >0.05)

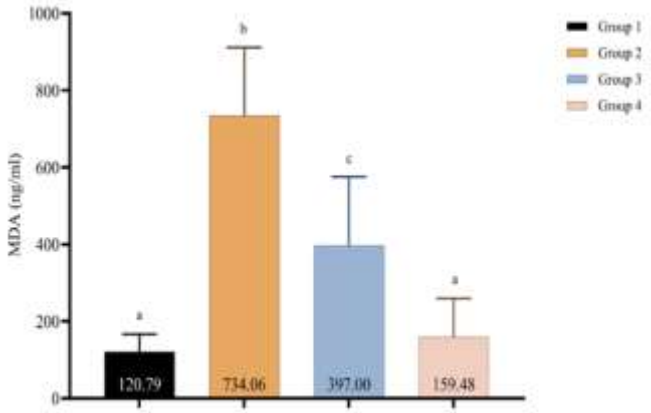

Figure 4. MDA level

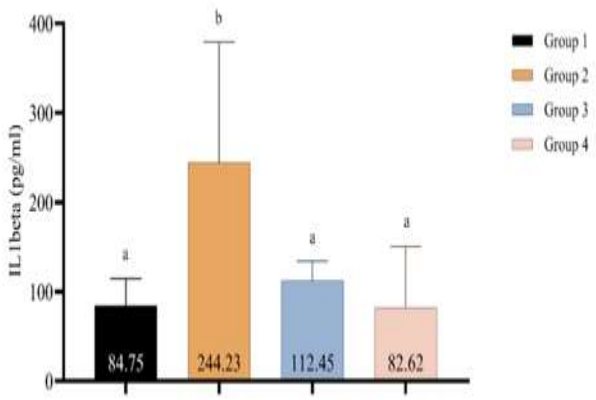

Figure 5. IL-1beta level

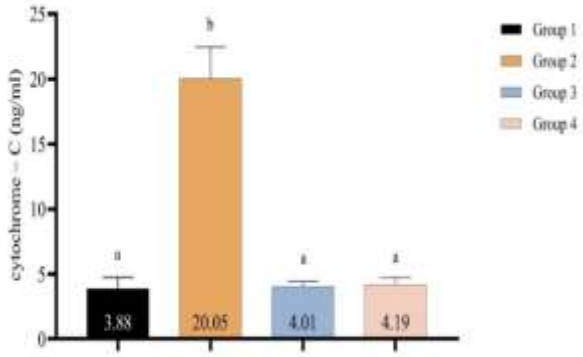

Figure 6. Cyt c level

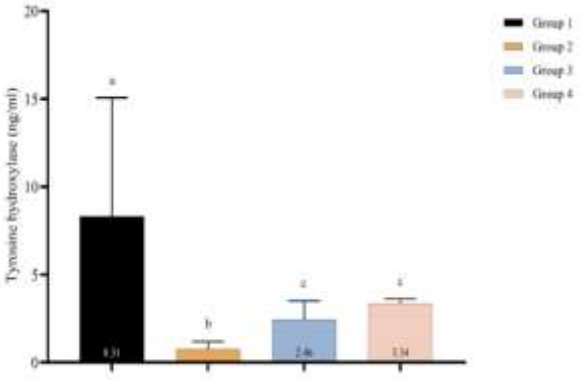

Figure 7. TH level

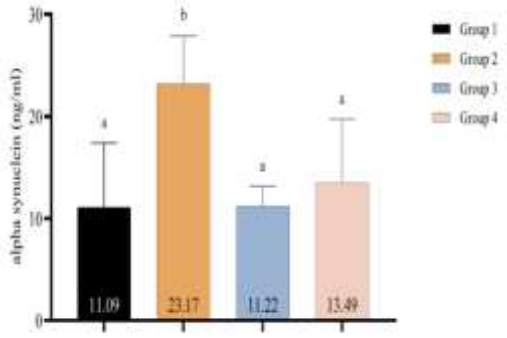

Figure 8. α-synuclein level

Histological examination

Microscopic examination of the mid brain of normal control mice (Group 1) showed normal histological picture of the brain tissue, that is composed of normal Substantia Nigra (SN), no loss of neuron, no vaculated space, no LBs and melanin containing neuron. In (Group 2) of male mice that administered MPTP, there was various histopathological alterations in mid brain tissue represented by sever vacuolated space, sever neuronal loss, sever pyknotic nuclei and the presence of LBs. While (group 3) showed mild vaculated space, mild neuronal loss, few pyknoted neucli, no LBs. Finally, flavonoid fraction of *E. mosulensis* showing good improvement as compared to (group 2) represented by mild neuronal loss, mild vaculated space, few pykonated neucli, no LBs as illustrated in "Figure 9".

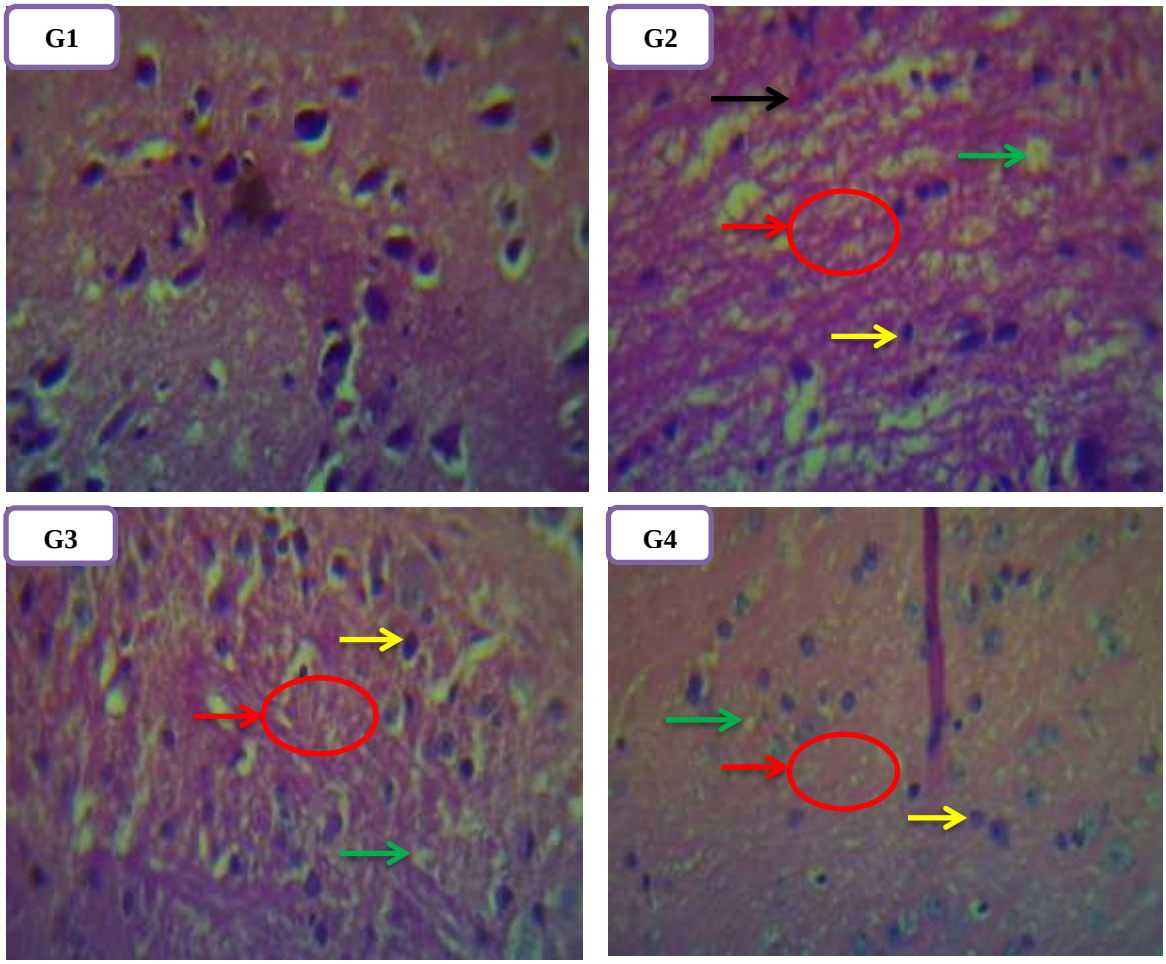

Figure 9. Histopathological examination of mid brain (H&E). Histological cross section of mid brain of mice in group 1 showing normal structure, while histological cross section of mid brain of mice in group 2,3, and 4 showing various alterations. Black line (—) represent lewy bodies, red line (—) and circle represent neuronal loss, yallow line (—) represented pyknotic nuclei, green line (—) represent vaculated space.

Identification of flavonoids in the plant extract by HPLC

The identification analysis by HPLC for plant extract detect the presence of luteolin, hesperetin ,rutin, apigenin ,quercetin , kaempferol, and gallic acid. In HPLC, qualitative identifications had been made by comparison of retention times gained at identical chromatographic circumstances of samples analyzed and authentic standards as shown in "Table 3"

Table 3. Retention time of standard phenolic and sample phenolic compound in minutes by HPLC

Standards	Standard retention time	Sample retention time
Rutin	3.08	3.00
Hesperetin	4.04	4.00
Qurcetine	4.28	4.25
Luteolin	6.14	6.14
Gallic acid	7.88	7.80
Apigenin	8.14	8.11
Kaempferol	9.77	9.79

Discussion

There are three categories of PD-like disease produced by MPTP: acute, subacute, and chronic. The subacute model of MPTP is frequently used for examining the effect of therapies because of its benefits and resemblance to the behavioral manifestation and neurochemical mechanisms of PD, including oxidative stress, apoptosis,

mitochondrial dysfunction, and, it is used to specifically and selectively ablate the dopaminergic neurons in the nigrostriatal pathway [52,53]. However, the use of the MPTP mouse model has led to a better knowledge of the underlying causes of cell death, the role of mitochondria in the disease, and the advancement of neuroprotective and neurorestorative methods [18].

In the present study, flavonoid fraction of *E. mosulensis* showed various improvements in behavioural test, biochemical parameters, and histopathological examinations as compared to group 2. The specific behavioral tests used in this study is OFT and biochemical parameters are MDA, IL-1beta, α –synuclein, Cyt c, and TH.

One of the largest classes of naturally occurring polyphenols is believed to be flavonoids [54]. Flavonoid can delay neurodegeneration, enhance learning, memory, and cognitive function because they change intracellular signaling pathways that improve cell survival and age-related neuronal activity [55]. It has been demonstrated by nearly all published research which indicated that flavonoids have neuroprotective properties only in pathological circumstances that is, when neurotoxins are present and not in normal physiological settings. The current study is in a line with previous study indicated that numerous protective properties of flavonoids, such as anti-inflammatory, antioxidant that increases locomotor activity, anti-apoptotic, inhibiting the aggregation of α-synuclein, antiviral, and antibacterial have been demonstrated. However, in terms of neuroprotection effect of flavonoids appear to stop the progressive neuronal loss in neurodegenerative diseases, such as PD [56,57].

In regard to the effect of flavonoid on OFT, the present study showed that a significant increase in locomotor activity is consistent with the previous study which indicated that flavonoids like hesperidin, rutin, silibilin, and baicalein have been demonstrated in pre-clinical studies to be strong therapeutic agents that reduce ROS, NOS expression, oxidative potential in the striatum, block neuronal death, and enhance dopaminergic (DA) survival and consequently improved locomotor activity [57]. According to our findings, flavonoid (kaempferol) enhanced TH expression, which is in line with prior research showing kaempferol has a protective impact against TH loss. One significant enzyme that is crucial to the synthesis of L-DOPA is TH. This first, rate-limiting stage of dopamine biosynthesis shows the clear link between PD and TH. L-DOPA serves as a precursor for the synthesis of dopamine, which in turn serves as a precursor for the synthesis of norepinephrine and adrenaline [58]. Any impairment in L-DOPA synthesis caused by decrease in TH in PD lead to reduced neuronal dopamine reuptake, which in turn causes dopaminergic dysfunction and the related motor deficits [59] . Regarding the effect of flavonoid on MDA and IL-1beta, the current study demonstrated significant improvement of these parameters are consistent with the previous study which suggested that phenolic and flavonoid compounds may have anti-Parkinson activity by raising SOD and neuron counts in rat brains while decreasing TNF-α, pro-inflammatory cytokines, and MDA levels [55]. Flavonoids greatly prevent diseases linked to oxidative stress by directly scavenging ROS through a number of mechanisms, such as the stimulation of antioxidant enzymes, the inhibition of nitric oxide-induced oxidative stress, and metal-chelating activity [60]. Flavonoid (quercetin) is thought to have anti-inflammatory effects due to its vital function in inhibiting the release of NF-κB nuclear factor and reducing the amounts of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8, the second function operates by blocking cytokine entrance into the nucleus [61]. Here, regarding the effect of flavonoid on apoptotic markers like cytochrome c, the current study suggested significant improvement is in a line with previous study which indicating that flavonoids (gallic acid) obstruct mitochondrial apoptotic signaling molecules, they may provide protection against neuronal injury [62]. Furthermore, hesperidin, another flavonoid inhibits both the caspase apoptotic cascade (which involves caspase-9 and caspase-3) and inhibit the following release of cytochrome c from mitochondria [63]. Regarding the effect of flavonoid on α-synuclein, the present study showed that a significant decrease in α-synuclein is consistent with previous study which indicated evidently that a number of flavonoids directly block the cytotoxic effects of α-synuclein aggregation inside a cellular setting [64] .

Pramipexole (PPX) is considered as a dopamine receptor agonist that is non-ergoline, and it's a typical first-line drug for treating PD, does not cause dyskinesias. Previous research demonstrated that PPX attenuated DA losses by avoiding the MPTP injury to the nigrostriatal dopaminergic neurons [37]. However, some studies have shown that PPX attaches to the inner side of the mitochondrial membrane and influences mitochondrial permeability transition pores, which suppresses mitochondrial permeability. This suggests that PPX affects mitochondrial functioning in addition to its dopaminergic agonistic effects. Even at modest dosages, PPX exhibits antioxidant, antiapoptotic, and neuroprotective qualities that have been linked to mitochondrial activities [65].

Flavonoids are thought to be safe and well-tolerated when derived from dietary sources and herbal medicine [35], It is now clear that flavonoids can protect neurons at low concentrations by interacting with vital intracellular signaling pathways in neurons that regulate neuronal survival and differentiation [66]. It is imperative to take into account the possible adverse effect and toxicity

linked to their ingestion, there have been reports of morbidity and mortality in patients due to overconsumption of flavonoids. The mutagenic potential of flavonoids is a crucial factor to take into account. Increased doses of flavonoids may disrupt essential enzymes involved in hormone metabolism, and have mutagenic effects [(35)] . While, dopamine agonist has been linked to an increased chance of experiencing dopaminergic side effects, such as edema, hallucinations, and problems with impulse control [(67)]. Non-ergoline dopamine agonists have been demonstrated to have a higher safety profile in terms of cardiac issue when evaluating the risk-benefit ratio of ergoline derivatives [(68)] .

Conclusion

According to the present findings, treatment with flavonoid fraction of *E. mosulensis* significantly ameliorates MPTP- induced PD; however, these results suggest that flavonoid fraction has most potential neuroprotective effect by decreasing oxidative stress, inflammation, apoptosis, α-synuclein overexpression. On other hand, marked increase in TH, as well as positive impact on histopathological change in PD mice models. Thus, may provide opportunities to develop novel therapeutic for treatment of PD.

Acknowledgment

This study was supported by university of Nahrain/College of Medicine and it was abstracted from PhD thesis. Authors thank Al-Nahrain University for supporting the project.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Funding

The authors received no financial support for the research, authorship and/or publication of this article.

Ethics Statements

This study was approved by the research ethical committees of College of Medicine, University of Al-Nahrain according to approval no. UNCOMIRB07042024 on 11/11/ 2022.

Author Contribution

Asmaa AbdulWahab Ahmed; contributed to data gathering, analysis, practical (follow the procedure) and written parts of the study. Haitham Mahmood Kadhim gave final approval and agreement for all aspects of the study, supervision, revision and rearrangement.

References

1. Santoro M, Fadda P, Klephan KJ, Hull C, Teismann P, Platt B, Riedel G. Neurochemical, histological, and behavioral profiling of the acute, sub-acute, and chronic MPTP mouse model of Parkinson's disease. J Neurochem. 2023; 164(2):121-142.
2. Van Nuland AJM, den Ouden HEM, Zach H, Dirkx MFM, van Asten JJA, Scheenen TWJ, Toni I, Cools R, Helmich RC. GABAergic changes in the thalamocortical circuit in Parkinson's disease. Hum Brain Mapp. 2020; 41(4):1017-1029.
3. Xue X, Zhang W, Zhu J, Chen X, Zhou S, Xu Z, Hu G, Su C. Aquaporin-4 deficiency reduces TGF-β1 in mouse midbrains and exacerbates pathology in experimental Parkinson's disease. J Cell Mol Med. 2019; 23(4):2568-2582.
4. Osborne JA, Botkin R, Colon-Semenza C, DeAngelis TR, Gallardo OG, Kosakowski H, Martello J, Pradhan S, Rafferty M, Readinger JL, Whitt AL, Ellis TD. Physical Therapist Management of Parkinson Disease: A Clinical Practice Guideline From the American Physical Therapy Association. Phys Ther. 2022; 102(4):pzab302.
5. Day JO, Mullin S. The Genetics of Parkinson's Disease and Implications for Clinical Practice. Genes (Basel). 2021;12(7):1006.
6. Koziorowski D, Figura M, Milanowski ŁM, Szlufik S, Alster P, Madetko N, Friedman A. Mechanisms of Neurodegeneration in Various Forms of Parkinsonism-Similarities and Differences. Cells. 2021;10(3):656.
7. Shin HW, Hong SW, Youn YC. Clinical Aspects of the Differential Diagnosis of Parkinson's Disease and Parkinsonism. J Clin Neurol. 2022; 18(3):259-270.
8. Costas C, Faro LRF. Do Naturally Occurring Antioxidants Protect Against Neurodegeneration of the Dopaminergic System? A Systematic Revision in Animal Models of Parkinson's Disease. Curr Neuropharmacol. 2022; 20(2):432-459.
9. Bock MA, Brown EG, Zhang L, Tanner C. Association of Motor and Nonmotor Symptoms With Health-Related Quality of Life in a Large Online Cohort of People With Parkinson Disease. Neurology. 2022; 98(22):e2194-e2203.
10. Atiq A, Lee HJ, Khan A, Kang MH, Rehman IU, Ahmad R, Tahir M, Ali J, Choe K, Park JS, Kim MO. Vitamin E Analog Trolox Attenuates MPTP-Induced Parkinson's Disease in Mice, Mitigating Oxidative Stress, Neuroinflammation, and Motor Impairment. Int J Mol Sci. 2023; 24(12):9942.
11. Henderson MX, Trojanowski JQ, Lee VM. α-Synuclein pathology in Parkinson's disease and related α-synucleinopathies. Neurosci Lett. 2019; 709:134316.
12. Gao H, Sun H, Yan N, Zhao P, Xu H, Zheng W, Zhang X, Wang T, Guo C, Zhong M. ATP13A2 Declines Zinc-Induced Accumulation of α-Synuclein in a Parkinson's Disease Model. Int J Mol Sci. 2022; 23(14):8035.
13. Burré J. The Synaptic Function of α-Synuclein. J Parkinsons Dis. 2015; 5(4):699-713.

14. Sian-Hulsmann J, Riederer P. The Nigral Coup in Parkinson's Disease by α-Synuclein and Its Associated Rebels. Cells. 2021; 10(3):598.
15. Mor DE, Daniels MJ, Ischiropoulos H. The usual suspects, dopamine and alpha-synuclein, conspire to cause neurodegeneration. Mov Disord. 2019; 34(2):167-179.
16. Park H, Chang KA. Therapeutic Potential of Repeated Intravenous Transplantation of Human Adipose-Derived Stem Cells in Subchronic MPTP-Induced Parkinson's Disease Mouse Model. Int J Mol Sci. 2020; 21(21):8129.
17. Chang HC, Liu KF, Teng CJ, Lai SC, Yang SE, Ching H, Wu CR. *Sophora Tomentosa* Extract Prevents MPTP-Induced Parkinsonism in C57BL/6 Mice Via the Inhibition of GSK-3β Phosphorylation and Oxidative Stress. Nutrients. 2019; 11(2):252.
18. Meredith GE, Rademacher DJ. MPTP mouse models of Parkinson's disease: an update. J Parkinsons Dis. 2011; 1(1):19-33.
19. Zhang J, Sun B, Yang J, Chen Z, Li Z, Zhang N, Li H, Shen L. Comparison of the effect of rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on inducing chronic Parkinson's disease in mouse models. Mol Med Rep. 2022; 25(3):91.
20. Razali K, Mohd Nasir MH, Othman N, Doolaanea AA, Kumar J, Nabeel Ibrahim W, Mohamed WMY. Characterization of neurobehavioral pattern in a zebrafish 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced model: A 96-hour behavioral study. PLoS One. 2022; 17(10):e0274844.
21. Campolo M, Casili G, Biundo F, Crupi R, Cordaro M, Cuzzocrea S, Esposito E. The Neuroprotective Effect of Dimethyl Fumarate in an MPTP-Mouse Model of Parkinson's Disease: Involvement of Reactive Oxygen Species/Nuclear Factor-κB/Nuclear Transcription Factor Related to NF-E2. Antioxid Redox Signal. 2017; 27(8):453-471.
22. Ardah MT, Bharathan G, Kitada T, Haque ME. Ellagic Acid Prevents Dopamine Neuron Degeneration from Oxidative Stress and Neuroinflammation in MPTP Model of Parkinson's Disease. Biomolecules. 2020; 10(11):1519.
23. Bitew H, Hymete A. The Genus *Echinops*: Phytochemistry and Biological Activities: A Review. Front Pharmacol. 2019; 10:1234.
24. Falah, Fereshteh, et al. In vitro screening of phytochemicals, antioxidant, antimicrobial, and cytotoxic activity of Echinops setifer extract. Biocatalysis and Agricultural Biotechnology, 2021, 35: 102102.
25. Mahmood, Ammar A. Razzak; Khadeem, Enas J. Phytochemical investigation of flavonoids glycoside in the Iraqi Echinops heterophyllus (Compositae). Pharmacie Globale, 2013, 4.9: 1.
26. Oubaid, E. N., Abu-Raghif, A. R., & Al-Sudani, I. M Phytochemical Screening and Antioxidant Activity of Uncaria tomentosa Extract: In Vitro: and: In Vivo: Studies. Medical Journal of Babylon. 2023; *20*(1), 136-142.
27. Zheleva-Dimitrova D, Simeonova R, Kondeva-Burdina M, Savov Y, Balabanova V, Zengin G, Petrova A, Gevrenova R. Antioxidant and Hepatoprotective Potential of *Echinops ritro* L. Extracts on Induced Oxidative Stress In Vitro/In Vivo. Int J Mol Sci. 2023; 24(12):9999.
28. Khan S, Al-Qurainy F, Al-Hashimi A, Nadeem M, Tarroum M, Shaikhaldein HO, Salih AM. Effect of Green Synthesized ZnO-NPs on Growth, Antioxidant System Response and Bioactive Compound Accumulation in *Echinops macrochaetus*, a Potential Medicinal Plant, and Assessment of Genome Size (2C DNA Content). Plants (Basel). 2023; 12(8):1669.
29. Hassan, R. F., & Kadhim, H. M. Exploring the role of phenolic extract as an ointment dosage form in inducing wound healing in mice. Journal of Pharmaceutical Negative Results. 2022; *13*(3), 186-193.
30. Evans JA, Mendonca P, Soliman KFA. Neuroprotective Effects and Therapeutic Potential of the Citrus Flavonoid Hesperetin in Neurodegenerative Diseases. Nutrients. 2022; 14(11):2228.
31. Zhang X, Molsberry SA, Yeh TS, Cassidy A, Schwarzschild MA, Ascherio A, Gao X. Intake of Flavonoids and Flavonoid-Rich Foods and Mortality Risk Among Individuals With Parkinson Disease: A Prospective Cohort Study. Neurology. 2022; 98(10):e1064-e1076.
32. Corona, G., Vauzour, D., Amini, A., & Spencer, J. P. The impact of gastrointestinal modifications, blood-brain barrier transport, and intracellular metabolism on polyphenol bioavailability: an overview. Polyphenols in human health and disease, 2014; 591-604.
33. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules. 2020; 25(22):5243.
34. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022; 27(9):2901.
35. Hasan S, Khatri N, Rahman ZN, Menezes AA, Martini J, Shehjar F, Mujeeb N, Shah ZA. Neuroprotective Potential of Flavonoids in Brain Disorders. Brain Sci. 2023; 13(9):1258.
36. Abdulmohsin, H. E. B. A., Raghif, A. A., & Manna, M. J. The protective effects of echinops heterophyllus extract against methotrexate-induced hepatotoxicity in rabbits. Asian. J. Pharm. Clin. Res. 2019; *12*, 384-390.

37. Zhang QS, Heng Y, Mou Z, Huang JY, Yuan YH, Chen NH. Reassessment of subacute MPTP-treated mice as animal model of Parkinson's disease. Acta Pharmacol Sin. 2017; 38(10):1317-1328.

38. Elhak SG, Ghanem AA, Abdelghaffar H, Eldakroury S, Eltantawy D, Eldosouky S, Salama M. The role of pramipexole in a severe Parkinson's disease model in mice. Ther Adv Neurol Disord. 2010; 3(6):333-7.

39. Hu M, Li F, Wang W. Vitexin protects dopaminergic neurons in MPTP-induced Parkinson's disease through PI3K/Akt signaling pathway. Drug Des Devel Ther. 2018; 12:565-573.

40. Nordquist, Rebecca E., et al. "Pigs as model species to investigate effects of early life events on later behavioral and neurological functions." Animal models for the study of human disease. Academic Press, 2017; 1003-1030.

41. Sáenz, J. C. B., Villagra, O. R., & Trías, J. F. Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. Behavioural brain research, 2006; 169(1), 57-65.

42. Tatem KS, Quinn JL, Phadke A, Yu Q, Gordish-Dressman H, Nagaraju K. Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases. J Vis Exp. 2014; (91):51785.

43. Sun C, Wang Y, Mo M, Song C, Wang X, Chen S, Liu Y. Minocycline Protects against Rotenone-Induced Neurotoxicity Correlating with Upregulation of Nurr1 in a Parkinson's Disease Rat Model. Biomed Res Int. 2019; 2019: 6843265.

44. Fadhel, Mohammed H.; Hassan, Ali F. Protective Effect of Omega-7 against Doxorubicin-Induced Cardiotoxicity in Male Rats. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512), 2023, 32.3: 35-40.

45. https://www.elabscience.com/List-detail-259.html

46. Alkhazragy, Baidaa; Alshawi, Nada N. Protective Effect of Cranberry Extract against Cisplatin-Induced Nephrotoxicity by Improving Oxidative Stress in Mice. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512), 2023, 32.2: 112-119.

47. Mbiydzenyuy NE, Ninsiima HI, Valladares MB, Pieme CA. Zinc and linoleic acid pre-treatment attenuates biochemical and histological changes in the midbrain of rats with rotenone-induced Parkinsonism. BMC Neurosci. 2018;19(1):29.

48. Bancroft, J.D. and Stevens, A. Theory and Practice of Histological Techniques. 3rd ed., Churchill Livingstone. 1990; P: 25-60.

49. Rasheed, M. Z., Andrabi, S. S., Salman, M., Tabassum, H., Shaquiquzzaman, M., Parveen, S., & Parvez, S. Melatonin improves behavioral and biochemical outcomes in a rotenone-induced rat model of Parkinson's disease. Journal of Environmental Pathology, Toxicology and Oncology, 2018; 37(2).

50. Bloomingdale P, Karelina T, Ramakrishnan V, Bakshi S, Véronneau-Veilleux F, Moye M, Sekiguchi K, Meno-Tetang G, Mohan A, Maithreye R, Thomas VA, Gibbons F, Cabal A, Bouteiller JM, Geerts H. Hallmarks of neurodegenerative disease: A systems pharmacology perspective. CPT Pharmacometrics Syst Pharmacol. 2022 ;11(11):1399-1429.

51. Ugalde-Muñiz P, Fetter-Pruneda I, Navarro L, García E, Chavarría A. Chronic Systemic Inflammation Exacerbates Neurotoxicity in a Parkinson's Disease Model. Oxid Med Cell Longev. 2020; 2020:4807179.

52. Chonpathompikunlert P, Boonruamkaew P, Sukketsiri W, Hutamekalin P, Sroyraya M. The antioxidant and neurochemical activity of Apium graveolens L. and its ameliorative effect on MPTP-induced Parkinson-like symptoms in mice. BMC Complement Altern Med. 2018; 18(1):103.

53. Wang LY, Yu X, Li XX, Zhao YN, Wang CY, Wang ZY, He ZY. Catalpol Exerts a Neuroprotective Effect in the MPTP Mouse Model of Parkinson's Disease. Front Aging Neurosci. 2019; 11:316.

54. Jackson Seukep A, Zhang YL, Xu YB, Guo MQ. In Vitro Antibacterial and Antiproliferative Potential of *Echinops lanceolatus* Mattf. (Asteraceae) and Identification of Potential Bioactive Compounds. Pharmaceuticals (Basel). 2020; 13(4):59.

55. Saadullah M, Arif S, Hussain L, Asif M, Khurshid U. Dose Dependent Effects of *Breynia cernua* Against the Paraquat Induced Parkinsonism like Symptoms in Animals' Model: *In Vitro*, *In Vivo* and Mechanistic Studies. Dose Response. 2022; 20(3):15593258221125478.

56. Magalingam KB, Radhakrishnan AK, Haleagrahara N. Protective Mechanisms of Flavonoids in Parkinson's Disease. Oxid Med Cell Longev. 2015; 2015:314560.

57. Behl T, Kaur G, Sehgal A, Zengin G, Singh S, Ahmadi A, Bungau S. Flavonoids, the Family of Plant-Derived Antioxidants Making Inroads into Novel Therapeutic Design Against Ionizing Radiation-Induced Oxidative Stress in Parkinson's Disease. Curr Neuropharmacol. 2022; 20(2):324-343.

58. Pan, X., Liu, X., Zhao, H., Wu, B., & Liu, G. Antioxidant, anti-inflammatory and neuroprotective effect of kaempferol on

rotenone-induced Parkinson's disease model of rats and SH-S5Y5 cells by preventing loss of tyrosine hydroxylase. Journal of Functional Foods, 2020; 74, 104140.

59. Nikokalam Nazif N, Khosravi M, Ahmadi R, Bananej M, Majd A. Effect of treadmill exercise on catalepsy and the expression of the BDNF gene in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine -induced Parkinson in male NMRI mice. Iran J Basic Med Sci. 2020; 23(4):483-493.

60. Benchikha N, Messaoudi M, Larkem I, Ouakouak H, Rebiai A, Boubekeur S, Ferhat MA, Benarfa A, Begaa S, Benmohamed M, Almasri DM, Hareeri RH, Youssef FS. Evaluation of Possible Antioxidant, Anti-Hyperglycaemic, Anti-Alzheimer and Anti-Inflammatory Effects of Teucrium polium Aerial Parts (Lamiaceae). Life (Basel). 2022; (10):1579.

61. Wang WW, Han R, He HJ, Li J, Chen SY, Gu Y, Xie C. Administration of quercetin improves mitochondria quality control and protects the neurons in 6-OHDA-lesioned Parkinson's disease models. Aging (Albany NY). 2021; 13(8):11738-11751.

62. Ait Lhaj Z, Ibork H, El Idrissi S, Ait Lhaj F, Sobeh M, Mohamed WMY, Alamy M, Taghzouti K, Abboussi O. Bioactive strawberry fruit (Arbutus unedo L.) extract remedies paraquat-induced neurotoxicity in the offspring prenatally exposed rats. Front Neurosci. 2023; 17:1244603.

63. Wang, D. M., Li, S. Q., Zhu, X. Y., Wang, Y., Wu, W. L., & Zhang, X. J. Protective effects of hesperidin against amyloid-β (Aβ) induced neurotoxicity through the voltage dependent anion channel 1 (VDAC1)-mediated mitochondrial apoptotic pathway in PC12 cells. *Neurochemical research*, 2013; *38*, 1034-1044.

64. Meng, X., Munishkina, L. A., Fink, A. L., & Uversky, V. N. Effects of various flavonoids on the α-synuclein fibrillation process. Parkinson's Disease, 2010; 2010(1), 650794.

65. Kosmowska B, Ossowska K, Wardas J. (2020). Pramipexole Reduces zif-268 mRNA Expression in Brain Structures involved in the Generation of Harmaline-Induced Tremor. Neurochem Res. 45(7):1518-1525.

66. Spencer JP. Flavonoids and brain health: multiple effects underpinned by common mechanisms. Genes Nutr. 2009 ;4(4):243-50.

67. Castro ES, Santos-García D, de Deus Fonticoba T, Expósito Ruíz I, Tuñas Gesto C, Arribí MM. Causes and factors related to dopamine agonist withdrawal in Parkinson's disease. Brain Behav.2016; 6(7):e00453.

68. Borovac JA. Side effects of a dopamine agonist therapy for Parkinson's disease: a mini-review of clinical pharmacology. Yale J Biol Med. 2016;89(1):37-47.

تاثيرات جزء فلافونويد الكره الشائكة على نموذج مرض الباركنسون المستحث في الفئران

اسماء عبدالوهاب احمد*[١] و هيثم محمود كاظم[٢]

[١]قسم الادوية، كلية الطب، جامعة النهرين، بغداد، العراق

[٢]قسم الادويه، كليه الصيدله، جامعة النهرين، بغداد، العراق

الخلاصة

تعد السموم وخاصة ١- ميثيل-٤- فينيل-١،٢،٣،٦ - تتراهيدروبيريدين (MPTP) من بين النماذج الاكثر شيوعا لمرض الشلل الرعاشي تبعا للأسلوب المستخدم، وتتسبب MPTP في تغيرات مختلفة مثل نضوب الستيرياتال دوبامين، والشذوذ السلوكي. وأشار التحليل الكيميائي النباتي لاستخراج شوك الجمل إلى وجود كمية كبيرة من الفلافونويد في المستخلص. وينتمي الفلافونويدات إلى عدة أنواع من البوليفينولات النباتية التي تمارس خواصاً قوية مضادة للأكسدة، ووقائية عصبية، ومضادة للإلتهاب. وكان الهدف من هذه الدراسة هو التحقيق في التاثير الوقائي العصبي لجزء الفلافونويد في نبات شوك الجمل العراقي على نموذج مرض الباركنسون المستحث في الفئران. أربعون من ذكور الفئران تم تصفيفها عشوائياً في أربع مجموعات (عشره في كل كروب). المجموعةالاولى: تلقت المجموعه الطبيعيه السليمه ماء مقطرعن طريق انبوب التجريع الفموي لمدة ٢٥ يوما. المجموعة الثانيه: تلقت المجموعه المحفزه للمرض MPTP (٣٠ ملغم/كغم/يوم) حقن داخل الصفاق لمدة ٥ أيام متتالية لتحقيق نموذج مرض الباركنسون ثم يواصل بعد ذلك مع ماء مقطر عن طريق انبوب التجريع الفموي لمدة ٢٥ يوماً. المجموعة الثالثه: تلقت مجموعه التحكم الايجابي براميبكسيول عن طريق الفم (١ ملغم/كغم/اليوم) لمدة ٢٥ يوماً ثم تم التحفيز مع MPTP (٣٠ ملغم/كغم/اليوم) حقن داخل الصفاق ساعة واحدة بعد براميبكسيول من اليوم ١٥ إلى ١٩. المجموعة الرابعه: تلقت جزء الفلافونويد عن طريق الفم (٢٥٠ ملغم/كغم/يوم) لمدة ٢٥ يوماً ثم تم االتحفيز مع MPTP(٣٠ ملغم/كغم/يوم) داخل الصفاق ساعة واحدة بعد الفلافونويد في اليوم ١٥ إلى اليوم ١٩. في اليوم ٢٦، تم إجراء اختبار السلوك وفي اليوم ٢٧ تم التضحية بجميع الحيوانات. وقد أُعِدَّت أنسجة المخ المتجانسة للتحليل. وفي هذه الدراسة، حدث انخفاض معنوي في مستويات جزء الفلافونويد في كل مالون داي الديهايد، انترليوكين- ١ بيتا، سايتوكروم c، الفا- سانيوكلين مع زيادة معنويه في مستوى هيدروكسيلايز التيروسين في جزء الفلافونويد بالمقارنة مع النحفيز (P≤٠,٠٥) ،

فضلاً عن زيادة معنويه في المسافة المقطوعه في المجموعة المعالجة بالمقارنة مع التحفيز (P ≥٠,٠٥) وبالإضافة إلى ذلك، تحسن جيد في تحليل النسيجي للمجموعة المعالجة مقارنة بالتحفيز. وفي الختام، جزء فلافونويد الكرة الشائكة له تاثير في الحماية العصبية على نموذج مرض الشلل الرعاشي الناجم عن تحفيز MPTP في الفئران.

الكلمات المفتاحية: مرض الباركنسون، الفا-سنيوكلين، ١ميثيل-٤- فنيل-١،٢،٣،٦ – رباعي هايدروبريدين، الكره الشائكة اوشوك الجمل، فلافونويد