Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 4(2) March 2026
DOI: https://dx.doi.org/10.4314/jobasr.v4i2.17

Neuroprotective and Anti-Parkinsonian Effects of Micronized Purified Flavonoid Fraction (Daflon®) in a Haloperidol-Induced Mouse Model of Parkinsonism

Samaila, S.[1*], Danborno, A. M.[2], Toryila, J. E.[3], & Adelaiye, A. B.[4]
[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Federal University Wukari, Taraba State, Nigeria.
[2,3,4]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Bingham University, Karu, Nasarawa State, Nigeria.
[*]Corresponding Author Email: samailasaminu@fuwukari.edu.ng

ABSTRACT
The use of haloperidol to treat mental illnesses like schizophrenia, mania, hyperactivity, and agitation is limited by its ability to cause Parkinson-like symptoms. Daflon (micronised purified flavonoid fraction) is an oral phlebotropic medication with potent antioxidant and anti-inflammatory properties. This study investigates the anti-Parkinsonian potential of Daflon in a mouse model of haloperidol-induced Parkinsonism. Thirty male mice (n=6/group) received daily treatments for seven days: Group I/control (saline, 2 mL/kg, orally), Group II (haloperidol, 2 mg/kg, intraperitoneally), Group III-IV (haloperidol 2 mg/kg, intraperitoneally + Daflon 50/100 mg/kg orally), and Group V (haloperidol 2 mg/kg, intraperitoneally + levodopa/carbidopa 10 mg/kg orally). On day 7, motor function was assessed using the catalepsy bar test. Subsequently, animals were euthanised, and brain tissues were collected to evaluate dopamine concentrations, glutamate concentrations, acetylcholinesterase (AChE) activity, and striatal histopathology. Haloperidol significantly increased cataleptic descent latency ($p < 0.001$), decreased dopamine concentrations ($p < 0.05$), and increased AChE activity ($p < 0.05$), while causing neuronal distortions in the striatum; glutamate concentrations remained unaffected ($p > 0.05$). Daflon co-treatment, particularly at 100 mg/kg, significantly shortened descent latency ($p < 0.05$), improved dopamine concentrations ($p < 0.05$), lowered AChE activity ($p < 0.05$), but glutamate concentration remained unaffected ($p > 0.05$). Histological results showed preservation of striatal neurons. Daflon exhibited neuroprotective effects comparable to levodopa/carbidopa. The findings of this study show that Daflon possesses antiparkinsonian effects against haloperidol-induced Parkinsonism.

Keywords:
Anti-Parkinsonian,
Daflon,
Haloperidol,
Catalepsy,
Neuroprotection

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative condition that progresses slowly and primarily affects dopamine-producing neurons in the brain. It is the second most prevalent neurodegenerative disorder following Alzheimer's disease (Dong-Chen *et al*., 2023). As reported in recent epidemiological studies, Parkinson's disease represents the most rapidly spreading neurological disorder globally, with rising prevalence, mortality, and disability rates (Su *et al*., 2025; Li *et al*., 2025). People over the age of sixty make up most of the elderly population estimated to have Parkinson's disease (Bhidayasiri *et al*., 2024). The exact cause of Parkinson's disease is not well known; however, it is generally believed to arise from a combination of genetic factors, environmental influences, and changes that occur with ageing. The majority of cases are designated as "idiopathic," indicating that they occur without a known cause (Dong-Chen *et al*., 2023).

Parkinson's disease is a major cause of Parkinsonism, a term used to describe a group of conditions that show motor symptoms such as tremor, stiffness, slow movements, and difficulty with balance, similar to those seen in Parkinson's disease (Shrimanker *et al*., 2024). Drug-induced Parkinsonism (DIP) is a significant and increasingly recognised cause of secondary Parkinsonian symptoms, particularly among older individuals, making it the most common form of secondary Parkinsonism (Shin & Chung, 2012).

How to cite this article: Samaila, S., Danborno, A. M., Toryila, J. E., & Adelaiye, A. B. (2026). Neuroprotective and Anti-Parkinsonian Effects of Micronized Purified Flavonoid Fraction (Daflon®) in a Haloperidol-Induced Mouse Model of Parkinsonism. *Journal of Basics and Applied Sciences Research*, *4*(2), 163-175. https://dx.doi.org/10.4314/jobasr.v4i2.17

The older, first-generation antipsychotics, such as haloperidol and chlorpromazine, are classic examples of medications with a well-established high risk for causing DIP (Solmi *et al*., 2017).

Haloperidol is a neuroleptic drug used to treat mental illnesses like schizophrenia, acute mania, hyperactivity, and severe agitation. It is among the well-known causes of drug-induced Parkinsonism (Rajaram *et al.*, 2015; Rahman & Marwaha, 2023). The main therapeutic effect of haloperidol comes from blocking dopamine receptors, particularly the D2 subtype, in the brain (Krum *et al*., 2020; Rafiq *et al*., 2022). Haloperidol blockade of D2 receptors rapidly interferes with dopaminergic signalling and simultaneously leads to an increase in dopamine turnover. These increase in dopamine turnover leads to the generation of reactive oxygen species as by-products, and their build-up places extra stress on neurons. Over time, the resulting oxidative stress leads to disruption in mitochondrial activity and triggers inflammatory processes. The extrapyramidal side effects commonly seen in clinical practice due to haloperidol are explained by these interconnected changes (Perera *et al*., 2011; Kabra *et al*., 2020).

The haloperidol-induced animal model of Parkinsonism is widely utilised as a model of Parkinson's disease due to its ability to reproduce acute motor symptoms that patients with Parkinson's disease often manifest (Waku *et al.*, 2021). It is not only used to study haloperidol-related extrapyramidal effects but to also test potential therapies for Parkinson's disease (Vásquez-Builes *et al.*, 2021). The pathological relevance of this model is further supported by the metabolism of haloperidol into 4-(4-chlorophenyl)-1-pyridinium (HPP+), a neurotoxin that is structurally and mechanistically analogous to 1-methyl-4-phenylpyridinium (MPP+) the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Just like the active metabolite of MPTP, MPP+ is a well-established inducer of Parkinsonian-like neurodegeneration, thereby providing a clear biochemical link between haloperidol administration and the resulting motor deficits (Crowley *et al*., 2013).

Flavonoids have attracted growing interest in neuroscience because of their antioxidant and anti-inflammatory properties (Bellavite, 2023). Micronised purified flavonoid fraction (Daflon) is a commercially available flavonoid preparation widely used as an oral phlebotropic drug for venous disorders. It consists mainly of micronised diosmin (≈90%) and smaller amounts of flavonoids expressed as hesperidin (≈10%), compounds naturally derived from citrus fruits but micronised to improve absorption (Abdel-Rafei *et al*., 2016; Attia, 2018). Because Daflon improves venous tone, reduces venous stasis, and limits oedema formation. It is prescribed for a wide range of conditions where venous function is impaired, including varicose veins, venous ulcerations, lymphatic insufficiency, and haemorrhoids. Beyond its vascular effects, Daflon also exhibits antioxidant and anti-inflammatory activities by scavenging free radicals, enhancing endogenous antioxidant defences, and reducing pro-inflammatory cytokine release (Abdel-Salam *et al*., 2012; Kobo *et al*., 2014; Samaila *et al*., 2025). These pharmacological properties of Daflon make it a potential candidate that could be used to address the rapid and multifactorial neurodegenerative processes in Parkinson's disease. Previous studies have shown that Daflon or its individual components possess neuroprotective effects in several experimental models of neurotoxicity (Abdel-Salam *et al*., 2012; Abdel-Rafei *et al*., 2016; Shabani & Mirshekar, 2018). However, its potential effects in the haloperidol-induced mouse model of Parkinsonism remain poorly explored. The rationale behind this present study is to address this gap, in other to get novel insight into Daflon's potential as a therapeutic agent for Parkinson's disease.

MATERIALS AND METHODS

Experimental Animals

Thirty (30) male Swiss mice 8 to 12 weeks old and weighing between 22 and 30 grams were used in this study. The animals were obtained from the Animal Care Unit of Bingham University, Karu, Nigeria. They were housed under standard laboratory conditions of 12-hour light/dark cycle and room temperature. The animals were handled humanely, and their bedding was changed at regular intervals to minimise discomfort, standard laboratory chow and clean drinking water was provided *ad libitum*. Ethical approval for this study was obtained from the Ethics Review Committee of Bingham University, Karu (approval no. BHU/ERC/25/A003). All experimental protocols conformed to the National Institutes of Health guide for the care and use of laboratory animals (National Research Council, 2011).

Chemicals and Drug Preparation

Haldol® -Janssen (haloperidol injection BP) 5 mg/mL haloperidol injection BP (Annygod Pharma Co., LTD), Daflon® 500 mg /micronised purified flavonoid fraction (Servier Egypt Industries Limited) and Sinamet® levodopa/carbidopa 250mg/25mg tablets (Annygod Pharma Co., LTD) were all purchased from Alpha Pharmacy & Stores Ltd, Lagos, Nigeria. Dosing solutions were prepared by dissolving all the drugs in normal saline (0.9% w/v), which served as the vehicle. The stock solutions were prepared in advance and stored under appropriate conditions, and the appropriate doses were then calculated from this solution before administration.

Study Design

The mice were randomly allocated into five experimental groups of six animals each (n = 6). Treatments were

administered once daily for seven consecutive days. Group I served as the control and received normal saline (2 mL/kg, orally). Group II received haloperidol (2 mg/kg, intraperitoneally). Group III was treated with Daflon (50 mg/kg, orally), followed 30 min later by haloperidol (2 mg/kg, intraperitoneally). Group IV received Daflon (100 mg/kg, orally) and, after a 30-min interval, haloperidol (2 mg/kg, intraperitoneally). Group V received levodopa/carbidopa (10 mg/kg, orally), followed by haloperidol (2 mg/kg, intraperitoneally) after 30 min. The doses used in the present study were selected based on previous reports employing haloperidol at 2 mg/kg (*Salam & Nada, 2011; Waku et al., 2021*), Daflon at 50 and 100 mg/kg (*Abharian et al., 2025; Skhawy et al., 2024*), and levodopa/carbidopa at 10 mg/kg (*Shrestha et al., 2016*).

Neurobehavioral Assessment

Catalepsy Bar Test

Catalepsy is characterised by an inability or diminished capacity to correct an externally imposed posture or initiate voluntary movement, often reflecting motor deficits similar to those observed in Parkinson's disease (Saleem *et al.*, 2021). The catalepsy bar test was carried out by following the protocols previously described by Sanberg *et al.* (1988) and Ali & Rajini (2016). Each mouse was placed with its hind limbs on the bench and forelimbs resting on a horizontal bar (1 cm diameter, 4 cm above the surface), the mouse was observed for a period of 60 seconds and the time taken to remove the forepaws or initiate movement was recorded using a stopwatch. Assessments were conducted at 30, 60, 90, and 120 min time points after haloperidol administration. At each time point, two trials were performed with a 1-min interval, and the mean latency was calculated. The apparatus was cleaned with 70% ethanol between animals to eliminate olfactory cues.

Sample Collection for Biochemical Analysis and Histological Studies

Twenty-four hours after completion of the neurobehavioural studies, the animals were anaesthetized with ketamine (90 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally). Following confirmation of loss of reflexes, euthanasia was performed by exsanguination. For the biochemical analyses, the whole brains of the animals were rapidly excised, rinsed in ice-cold phosphate-buffered saline (pH 7.4), and maintained on ice for subsequent analysis. For histological studies, after anaesthesia, the thoracic cavity of the animals were cut open and they were perfused transcardially with normal saline (0.9% w/v), followed by 4% paraformaldehyde as previously described Wu *et al.* (2021). The brains were then harvested and fixed in 10% neutral-buffered formaldehyde (Adebayo *et al*., 2025).

Biochemical Analysis

Brain tissue homogenates were analysed for dopamine, glutamate, and acetylcholinesterase (AChE) activity. Dopamine concentration was measured using a commercial ELISA kit (Elabscience® Dopamine ELISA Kit, Catalog No. E-EL-0046; Elabscience Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. Glutamate concentration was determined using an enzymatic spectrophotometric method as previously described by Mitz and Giesy (1985). AChE activity was assessed using a colorimetric assay kit (Elabscience® Acetylcholinesterase Activity Assay Kit, Catalog No. E-BC-K174-M; Elabscience Biotechnology Co., Ltd., Wuhan, China) following the manufacturer's protocol.

Histological Analysis

The fixed brain tissues were processed for light microscopy. Serial sections of the striatum were obtained and stained with Hematoxylin and Eosin (H&E) to evaluate general histoarchitecture and cellular morphology. Additionally, Cresyl Fast Violet (CFV) staining was employed to visualize Nissl substance, allowing for the assessment of neuronal integrity within the striatum.

Data Analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 8 software (version 8.0.2 [263]; GraphPad Software, San Diego, USA). One-way or two-way analyses of variance (ANOVA) were conducted, followed by Tukey's post hoc multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of Daflon on Descent Latency in the Catalepsy Bar Test

Figure 1 shows the results of the catalepsy bar test recorded at 30, 60, 90, and 120 minutes. At 30 minutes, the group treated with haloperidol alone exhibited a significantly ($p < 0.01$) prolonged descent latency (48.03 ± 5.86 s) when compared with the control group (0.68 ± 0.66 s). Co-administration of Daflon at 50 mg/kg (45.75 ± 5.30 s) and 100 mg/kg (37.67 ± 6.51 s) did not significantly ($p = 0.9982$ and $p = 0.7600$, respectively) affect the descent latency when compared with the haloperidol group. In contrast, co-treatment with the standard drug levodopa/carbidopa significantly ($p < 0.05$) reduced the descent latency (20.95 ± 4.17 s) compared with the haloperidol group. At 60 minutes, the descent latency significantly ($p < 0.001$) increased in the haloperidol group (68.75 ± 6.04 s) compared with the control group (1.08 ± 0.55 s). Co-administration of

Daflon at both 50 mg/kg (55.00 ± 6.09 s) and 100 mg/kg (42.65 ± 7.21 s) showed a non-significant ($p = 0.5268$ and $p = 0.1125$, respectively) effect on the descent latency relative to the haloperidol group. In contrast, co-treatment with levodopa/carbidopa significantly ($p < 0.01$) reduced the descent latency (31.28 ± 4.56 s) compared with the haloperidol group. At 90 minutes, haloperidol administered alone resulted in a further significant ($p < 0.001$) increase in descent latency (88.57 ± 6.36 s) compared with the control group (0.55 ± 0.47 s). Co-administration of Daflon at 50 mg/kg showed no significant ($p = 0.4648$) effect on descent latency (73.00 ± 6.44 s) when compared with the haloperidol group, whereas at 100 mg/kg, the co-treatment produced a significant ($p < 0.05$) reduction in descent latency (57.97 ± 6.48 s) relative to the haloperidol group. The co-administration of the standard drug levodopa/carbidopa also significantly ($p < 0.01$) reduced the descent latency (43.02 ± 6.09 s) compared with the haloperidol group. At 120 minutes, the haloperidol-treated group maintained a significantly ($p < 0.001$) elevated descent latency (113.40 ± 4.96 s) compared with the control group (0.37 ± 0.17 s). The effect observed with co-administration of Daflon at 50 mg/kg (102.90 ± 5.51 s) did not differ significantly ($p = 0.6320$) from the haloperidol group, while co-administration with the 100 mg/kg dose of Daflon produced a significant ($p < 0.05$) reduction in descent latency (79.58 ± 7.95 s) compared with the haloperidol group. Similarly, co-treatment with levodopa/carbidopa significantly ($p < 0.05$) decreased descent latency (71.98 ± 8.60 s) when compared with the haloperidol group.

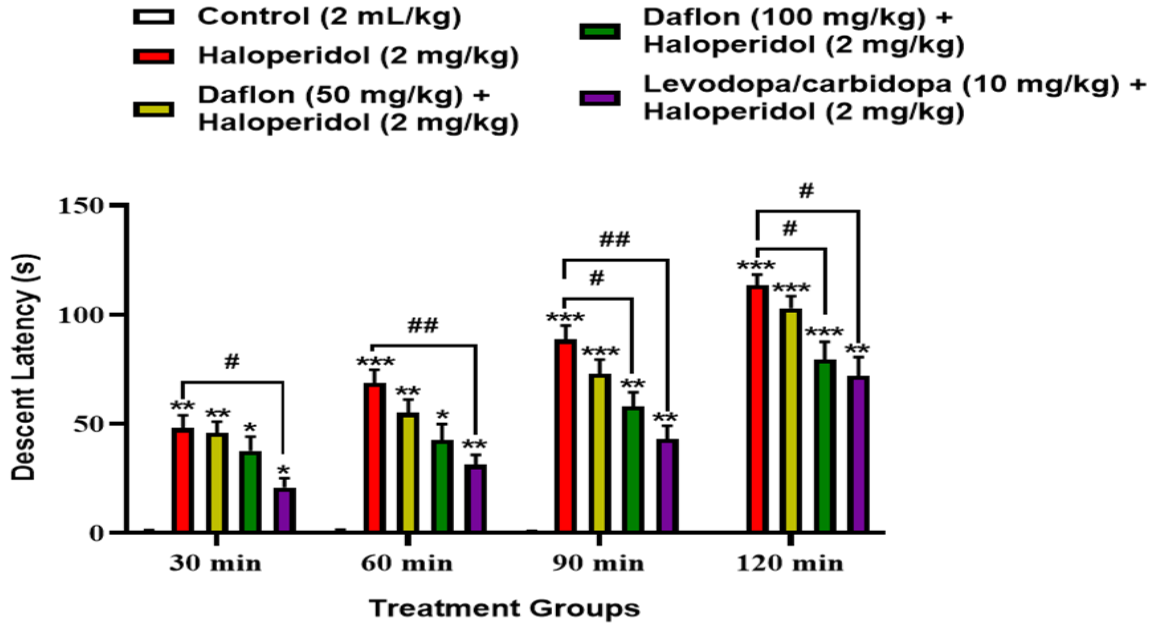

Figure 1: Effects of Daflon on haloperidol-induced catalepsy in mice. Data are presented as mean ± SEM (n = 6). * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$ vs control group; # - $p < 0.05$, ## - $p < 0.01$ vs haloperidol group.

Effect of Daflon on Dopamine Concentrations

As presented in **Figure 2**, mice administered haloperidol alone showed a significant ($p < 0.01$) reduction in dopamine concentrations (14.03 ± 0.38 pg/mL) compared with the control group (17.03 ± 0.57 pg/mL). Co-treatment with Daflon at 50 mg/kg showed a mild elevation in dopamine concentrations (15.57 ± 0.20 pg/mL) that was not significant ($p = 0.1958$) relative to the group that received haloperidol alone. In contrast, co-treatment with Daflon at 100 mg/kg significantly ($p < 0.05$) improved dopamine concentrations (16.23 ± 0.71 pg/mL) compared with the haloperidol group. Furthermore, co-treatment with the standard drug levodopa/carbidopa significantly ($p < 0.01$) improved dopamine concentrations (16.75 ± 0.37 pg/mL) compared with the haloperidol group.

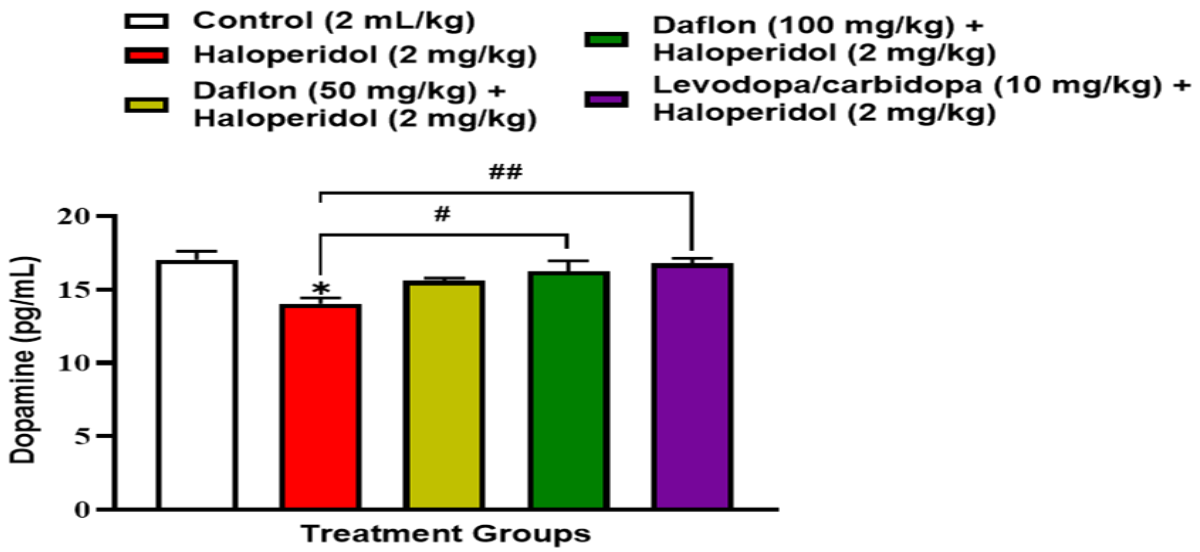

Figure 2: Effect of Daflon on dopamine concentrations in brain tissue homogenates of mice with haloperidol-induced Parkinsonism. Data are presented as mean ± SEM (n = 5). * - $p < 0.05$ vs control group; # - $p < 0.05$, ## - $p < 0.01$ vs haloperidol group.

Effect of Daflon on Glutamate Concentrations

Figure 3 shows that the glutamate concentrations in the group that received haloperidol alone (37.10 ± 1.57 mM) did not differ significantly ($p = 0.9998$) from the control group (36.70 ± 1.31 mM). Similarly, co-administration of Daflon at 50 mg/kg (37.70 ± 2.31 mM) and 100 mg/kg (31.70 ± 1.06 mM), as well as the standard drug levodopa/carbidopa (31.30 ± 1.50 mM), did not significantly ($p = 0.9988$, $p = 0.1624$, and $p = 0.1181$, respectively) affect glutamate concentrations compared with the haloperidol group.

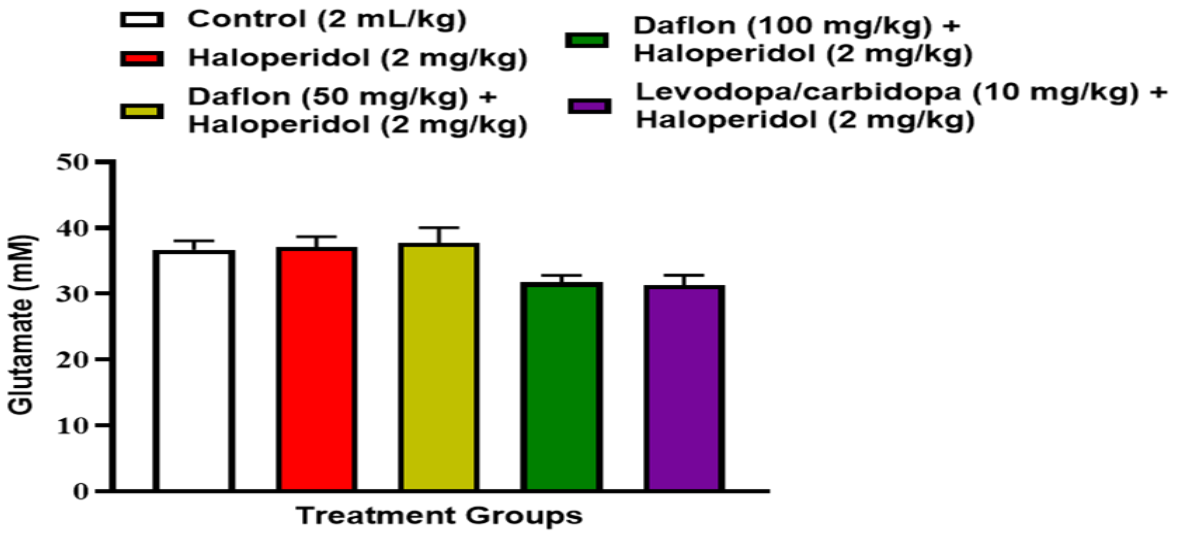

Figure 3: Effect of Daflon on glutamate concentrations in brain tissue homogenates of mice with haloperidol-induced Parkinsonism. Data are presented as mean ± SEM (n = 5).

Effect of Daflon on Acetylcholinesterase Enzyme (AChE) Activity

As depicted in **Figure 4**, mice in the group that were administered only haloperidol exhibited a significant ($p < 0.05$) elevation in AChE activity (0.03547 ± 0.00299 U/mg protein) relative to the control group (0.02419 ± 0.00120 U/mg protein). Co-treatment with Daflon at 50 mg/kg did not significantly ($p = 0.5468$) reduce AChE activity (0.03026 ± 0.00304 U/mg protein) relative to the haloperidol group. In contrast, co-treatment of Daflon at

100 mg/kg significantly ($p < 0.05$) lowered AChE activity (0.02530 ± 0.00204 U/mg protein) compared with the haloperidol group. Similarly, co-treatment of the standard drug levodopa/carbidopa resulted in a significant ($p < 0.05$) decrease in AChE activity (0.02416 ± 0.00217 U/mg protein) compared with the haloperidol group.

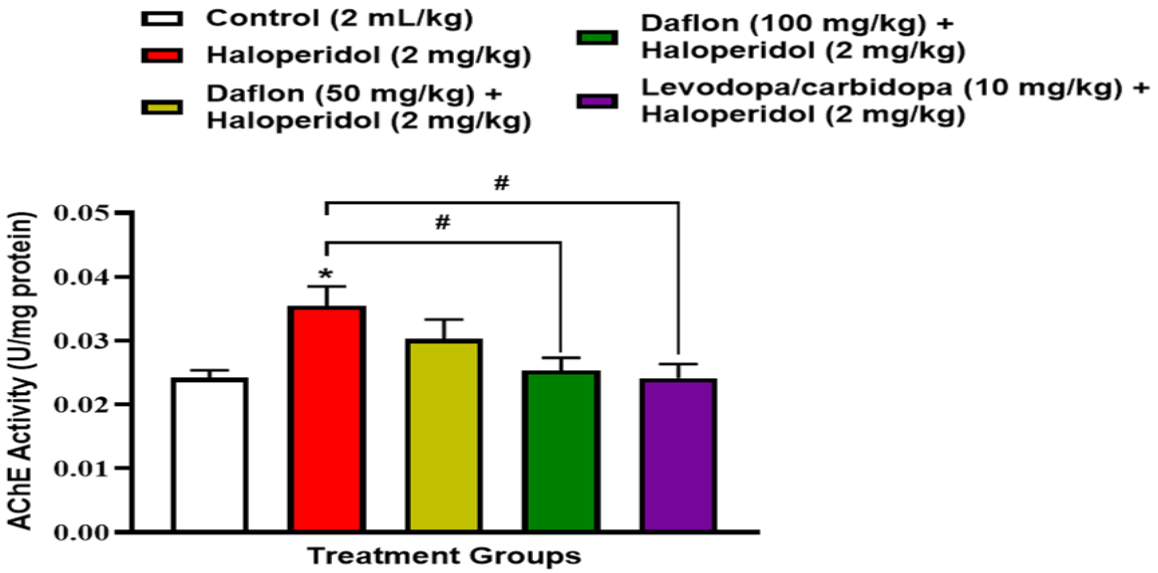

Figure 4: Effect of Daflon on acetylcholinesterase enzyme (AChE) activity in brain tissue homogenates of mice with haloperidol-induced Parkinsonism. Data are presented as mean ± SEM (n = 5). * - $p < 0.05$ vs control group; # - $p < 0.05$ vs haloperidol group.

Histological and Histochemical Evaluation of the Striatum

Histological (Hematoxylin and Eosin [H&E] stain) and histochemical (cresyl fast violet [CFV] stain**)** analyses of the control group revealed normal striatal histoarchitecture, characterized by well-defined pyramidal and stellate cells with intense Nissl staining (**Figures 5A and 6A**). In contrast, the haloperidol-only group (2 mg/kg) exhibited significant structural distortions, including pyknosis, perineuronal vacuolation, and gliosis, along with evidence of chromatolysis and reduced CFV staining intensity (**Figures 5B and 6B**). Co-administration of Daflon (50 and 100 mg/kg) mitigated these neurotoxic effects, showing only mild distortions such as cytoplasmic vacuolation and moderate staining intensity (**Figures 5C-D and 6C-D**). Similarly, the levodopa/carbidopa-treated group (10 mg/kg) maintained a preserved histoarchitecture and staining profile comparable to the control group (**Figures 5E and 6E**).

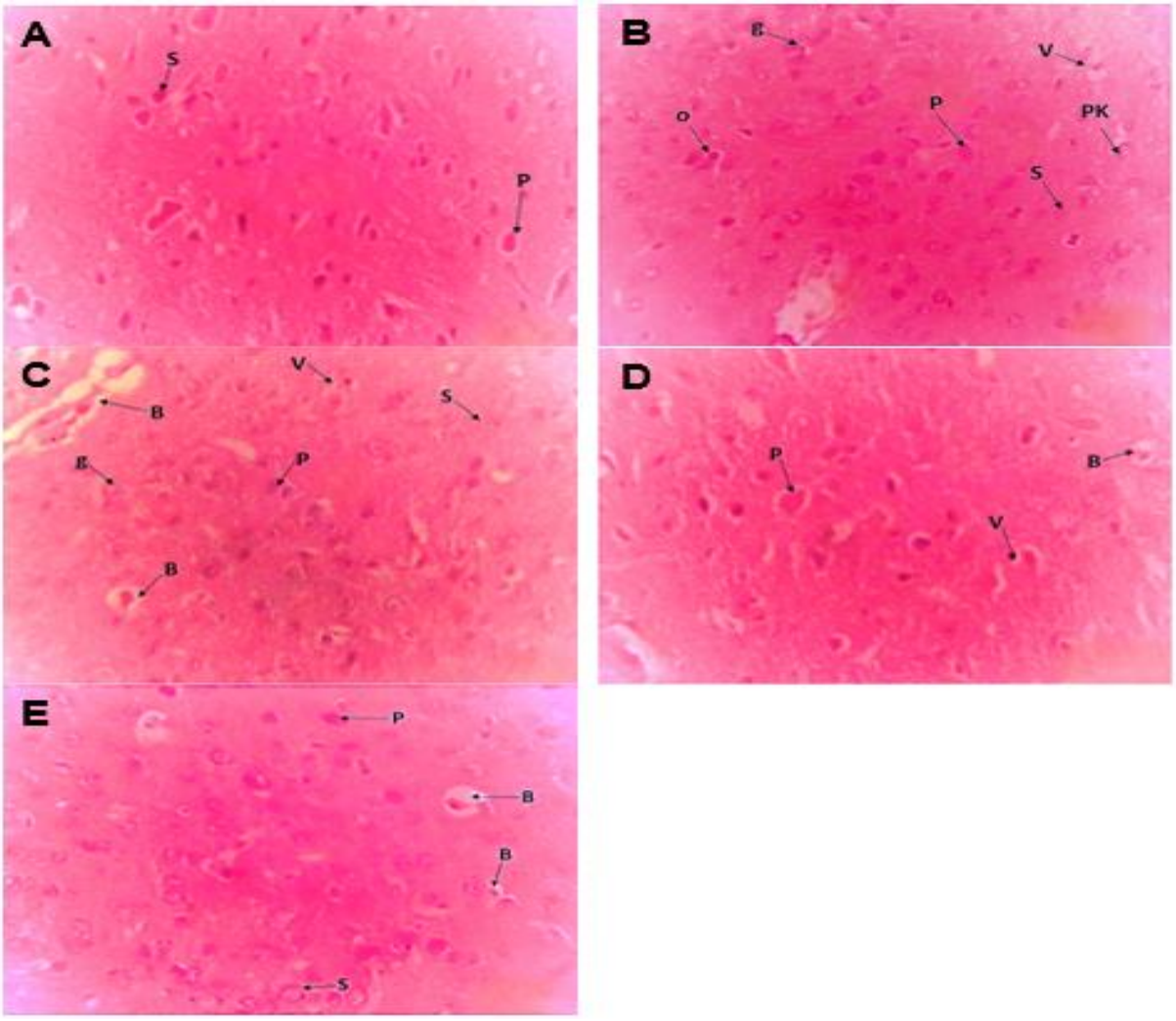

Fig. 5 (A-E): Photomicrographs of the striatum showing the cytoarchitecture. P = pyramidal cells; S = stellate cells; g = gliosis; O = oligodendrocytes; PK = pyknosis; V = perineuronal vacuolations; B = blood vessel. (Mag x 250 H&E)

(**A**) Control (normal saline, 2 mL/kg), showing normal histoarchitecture with pyramidal (P) and stellate (S) cells. (**B**) Haloperidol (2 mg/kg), showing histoarchitectural distortions such as pyknosis (PK), perineuronal vacuolation (V), and gliosis (g). (**C**) Daflon (50 mg/kg) + haloperidol 2 mg/kg), showing mild distortions of the histoarchitecture, such as perineuronal vacuolation (V) and gliosis (g). (**D**) Daflon (100 mg/kg) + haloperidol (2 mg/kg), showing mild distortions of the histoarchitecture, such as perineuronal vacuolation (V). (**E**) Levodopa/carbidopa (10 mg/kg) + haloperidol (2 mg/kg), showing a preserved histoarchitecture comparable to that of the control group.

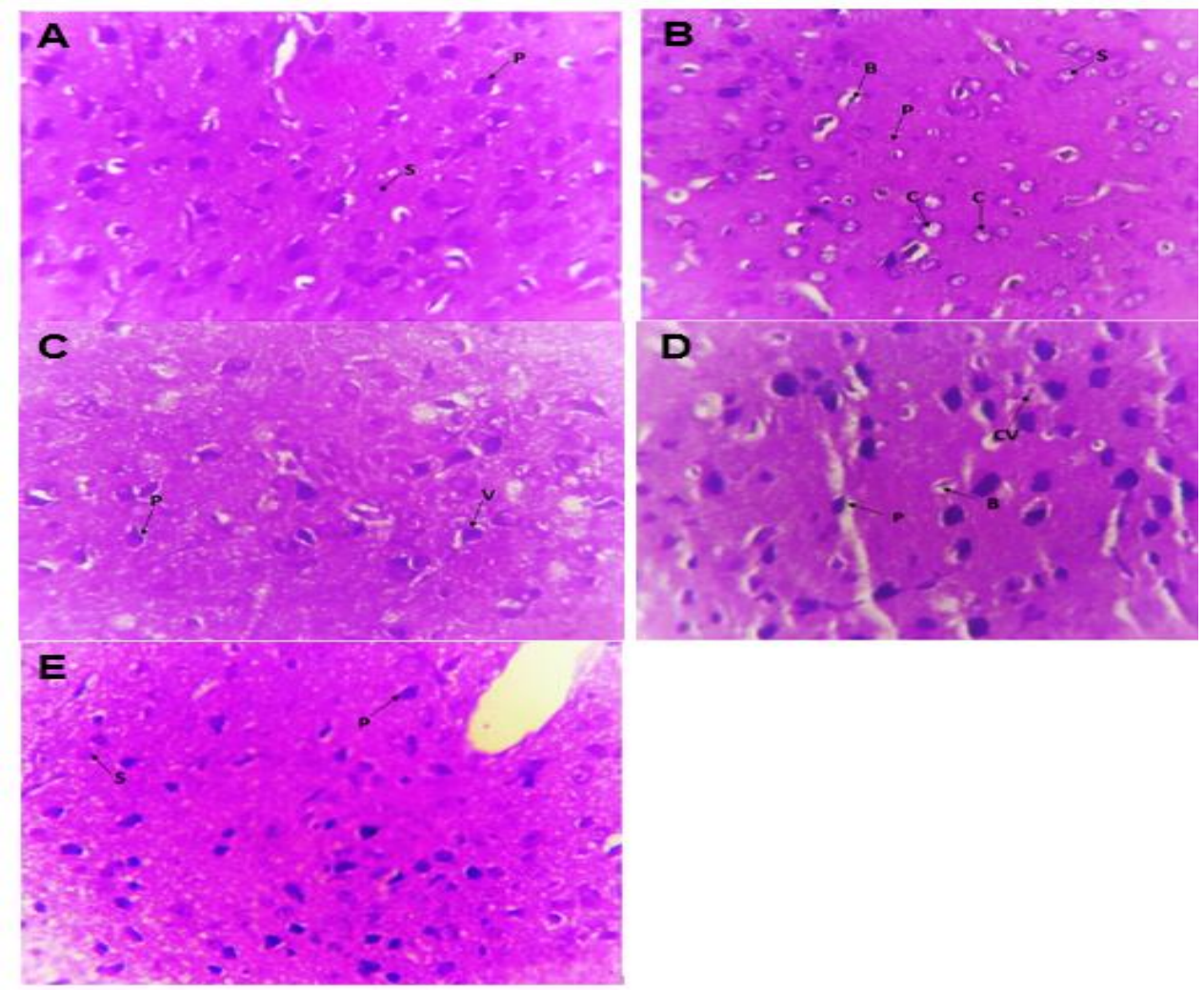

Fig. 6 (A-E): Photomicrographs of the striatum showing the cytoarchitecture. P = pyramidal cells; S = stellate cells; B = blood vessels; C = chromatolysis; perineuronal vacuolation (V); CV = cytoplasmic vacuolations. (Mag x 250 CFV)

(**A**) Control (normal saline, 2 mL/kg), showing normal histoarchitecture with pyramidal (P) and stellate (S) cells and intense staining. (**B**) Haloperidol (2 mg/kg), showing histoarchitectural distortions, such as chromatolysis (C) and reduced staining intensity. (**C**) Daflon (50 mg/kg) + haloperidol 2 mg/kg), showing mild distortions of the histoarchitecture, such as perineuronal vacuolation (V) and moderate staining intensity. (**D**) Daflon (100 mg/kg) + haloperidol (2 mg/kg), showing mild distortions of the histoarchitecture, such as cytoplasmic vacuolation (CV) and moderate staining intensity. (**E**) Levodopa/carbidopa (10 mg/kg) + haloperidol (2 mg/kg), showing various neuronal cells with staining intensity comparable to that of the control group.

The anti-Parkinsonian potential of the flavonoid-rich venoactive agent Daflon, a micronized purified flavonoid fraction (MPFF) made up of diosmin and hesperidin, was in this study investigated in a male mouse model of haloperidol-induced Parkinsonism. Haloperidol is a common antipsychotic that can cause a number of motor abnormalities which are often referred to as extrapyramidal symptoms (Del Agua Villa *et al*., 2024). It is a well-known experimental model for Parkinsonism due to its potent dopamine D2 receptor blocking effects. Blocking D2 receptors in the nigrostriatal pathway causes catalepsy, which is a state of immobility that is similar to the bradykinesia and akinesia seen in Parkinson's disease (Waku *et al*., 2021; Rahman & Marwaha, 2023).

The administration of haloperidol in this study caused a considerable motor deficit, as seen by a longer descent latency in the bar test, which is consistent with earlier research demonstrating that haloperidol can interfere with dopaminergic transmission to alter motor functions (Waku *et al*., 2022). Co-treatment with Daflon, especially at the higher dose of 100 mg/kg, significantly decreased

the descent latency at the 90 and 120-minute intervals, suggesting a restorative impact on the motor circuits impaired by haloperidol. This finding is aligns with the result of our previous studies indicating that Daflon possesses anti-cataleptic effects against haloperidol induced motor impairments in female wistar rats (Samaila *et al*., 2025). Daflon showed similar effectiveness to the standard drug levodopa/carbidopa, which suggests that it possesses the potential to improve motor functions. Daflon's ability to alleviate haloperidol-induced catalepsy may be due to the effects of its individual flavonoid components. Hesperetin, a principal metabolite of hesperidin present in Daflon, has demonstrated efficacy in inhibiting catalepsy and reversing locomotor deficits in animal models of haloperidol-induced catalepsy and neurotoxicity (Menze *et al*., 2012; Dhingra *et al*., 2018).

Dopamine and glutamate are very important neurotransmitters that help control movement in the nigrostriatal pathway. Reduction in striatal dopamine concentrations is the primary neuropathological hallmark of Parkinson's disease (Ramesh & Arachchige, 2023). Changes in glutamate homeostasis can also make neurodegeneration worse and cause a lot of motor impairments (Zhang *et al*., 2019; Iovino *et al*., 2020). Haloperidol-induced Parkinsonism is fundamentally driven by the reduction of striatal dopamine concentrations and the subsequent imbalance of neurotransmitters (Del Agua Villa *et al*., 2024). The results of this study revealed that haloperidol caused a significant reduction in dopamine concentrations, but co-treatment with Daflon at 100 mg/kg effectively antagonised the haloperidol-induced reduction in dopamine concentrations by significantly restoring dopamine concentrations, just like the standard drug levodopa/carbidopa. This finding is consistent with previous research, indicating Daflon can indirectly affect the level of dopamine by influencing the nuclear factor kappa B (NF-kB) and nitric oxide/cyclic GMP pathway (NO/cGMP) (Re *et al*., 2025). Also, because Daflon possesses antioxidant and anti-inflammatory properties, it might help protect the dopamine-producing neurons in the brain from the harmful impact of haloperidol, and in turn, this could support better dopamine production and release (Samaila *et al*., 2025).

Furthermore, the results of this study showed no significant changes in glutamate concentrations in all the treatment groups of the study. The absence of effect post-haloperidol administration aligns with findings from other studies indicating the variable impacts of antipsychotics on glutamate levels (Egerton *et al*., 2017; Zahid *et al*., 2022). The present result of this study suggests that the neurotoxic effect of haloperidol may likely not be mediated by glutamate excitotoxicity, a mechanism central to disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Arnold *et al*., 2024; Wu *et al*., 2025). Also, the lack of Daflon's effect on glutamate concentration suggests that its neuroprotective actions may likely be through other pathways, rather than modulation of glutamate excitatory neurotransmission (Kamal *et al*., 2024). However, due to the short duration of this study, there will be a need for studies of longer duration to confirm these assertions.

Cholinergic dysfunction is an important contributor to gait, balance, and cognitive impairment in Parkinson's disease (Crowley *et al*., 2024; Zhang *et al*., 2024). The enzyme acetylcholinesterase (AChE) that regulates cholinergic tone also plays an important role in motor control, cognition, and other functions of the central nervous system (Trang & Khandhar, 2023). In Parkinson's disease, the alterations that occur in AChE activity are often complex and region-specific (Shim *et al*., 2021). Additionally, research by Silva *et al.* (2024) and Schmitz *et al.* (2025) has shown that haloperidol's effects on cholinergic enzymes vary depending on the kind of brain structure assayed. This study showed a significant increase in AChE activity after haloperidol administration, which indicates a decrease in cholinergic tone, in keeping with the earlier report of Han *et al.* (2024). The rise in AChE activity observed in this study is likely attributable to influences across multiple brain regions, as the assay was conducted on the entire brain. Co-treatment with the higher dose of Daflon significantly lowered AChE activity, suggesting that Daflon's effects may be mediated by the stabilisation of cholinergic balance. These benefits may be attributed to Daflon's ability to enhance dopaminergic function while attenuating oxidative stress and neuroinflammation, as previously reported in the literature (Fidelis *et al*., 2025; Samaila *et al*., 2025).

Histological and histochemical analyses of the striatum from this study demonstrated that haloperidol administration resulted in distinct histopathological changes indicative of neuronal injury. In the control group, striatal sections showed normal histoarchitecture, including pyramidal, stellate, and glial cells with strong Nissl staining intensity. By contrast, haloperidol-treated animals exhibited distinct degenerative features such as pyknosis, gliosis, perineuronal vacuolation, and chromatolysis, along with a marked reduction in Nissl substance. These structural changes point toward impaired neuronal protein synthesis and ongoing neurodegeneration, which parallels earlier reports on haloperidol's capacity to cause oxidative stress and neuronal damage (Altunkaynak *et al*., 2011; Abdel-Salam *et al*., 2018). Treatment with Daflon reduced the severity of these pathological features in a dose-dependent manner. In animals administered 50 mg/kg Daflon in

conjunction with haloperidol, the striatal regions exhibited only mild vacuolation and a partial preservation of neuronal morphology. The higher dose (100 mg/kg) provided even more protection, with fewer signs of degeneration and better staining intensity. The striatum of the high-dose Daflon group was not exactly like the control, but it looked more normal, and the level of preservation was similar to that of the levodopa/carbidopa group. The persistent mild gliosis, vacuolation, and reduced staining intensity suggest that the neuroprotective effect was only partially effective. This may have occurred because the treatment didn't last long enough for the neurons to fully recover. The histological findings of this study demonstrate neuroprotective effects consistent with those reported by Fidelis *et al*. (2025). Despite these promising results, further research is necessary to evaluate the long-term impact of Daflon against haloperidol-induced Parkinsonism and to fully elucidate its underlying mechanisms of action.

CONCLUSION

In conclusion, this study demonstrates that Daflon possesses neuroprotective effects against haloperidol-induced behavioural, neurochemical, and striatal structural changes in mice. Daflon improved motor performance and dopamine concentrations, lowered AChE activity, and preserved neuronal architecture in the striatum. While these findings highlight the promising neuroprotective potential of Daflon against haloperidol-induced Parkinsonism, further research is required to explore its long-term effects and fully elucidate its underlying mechanisms.

REFERENCE

Abdel-Rafei, M., Amin, M., & Hasan, H. (2016). Novel effect of Daflon and low-dose γ-radiation in modulation of thioacetamide-induced hepatic encephalopathy in male albino rats. *Human & Experimental Toxicology*, *36*(1), 62–81. https://doi.org/10.1177/0960327116637657

Abdel-Salam, O. M. E., Sleem, A. A., Youness, E. R., Mohammed, N. A., & Omara, E. A. (2018). Bone marrow-derived protect against haloperidol-induced brain and liver damage in mice. *Biomedical and Pharmacology Journal*, *11*(1). http://biomedpharmajournal.org/?p=19546

Abdel-Salam, O. M., Youness, E. R., Mohammed, N. A., Abd-Elmoniem, M., Omara, E., & Sleem, A. A. (2012). Neuroprotective and hepatoprotective effects of micronized purified flavonoid fraction (Daflon®) in lipopolysaccharide-treated rats. *Drug discoveries & therapeutics*, *6*(6), 306–314. https://doi.org/10.5582/ddt.2012.v6.6.306

Abharian, N., Naderi, N., Nikray, N., Khoramjouy, M., Noori, S., Shafaroodi, H., & Faizi, M. (2025b). Effect of Micronized Purified Flavonoid Fraction Containing Hesperidin and Diosmin on Vincristine-Induced Neuropathy in Rats; the Role of Nitric Oxide Pathway. *Iranian Journal of Pharmaceutical Research. January*, *24*(1), e154455. https://doi.org/10.5812/ijpr-154455

Adebayo O. F., Egesie G. & Odeh O. S. (2025). Antioxidant-Mediated Hepatoprotective Effects of Crude Ethanolic Extract of Carica papayaLeaves in Streptozotocin-Induced Diabetic Wistar Rats.. *Journal of Basics and Applied Sciences Research*, 3(5), 31-40. https://dx.doi.org/10.4314/jobasr.v3i5.4

Ali, S.J. & Rajini, P.S. (2016). Effect of monocrotophos, an organophosphorus insecticide, on the striatal dopaminergic system in a mouse model of Parkinson's disease. *Toxicology and Industrial Health* 32(7): 1153-1165. https://doi.org/10.1177/0748233714547733

Altunkaynak, B. Z., Ozbek, E., Aydin, N., Aydin, M. D., Altunkaynak, M. E., Vuraler, O., & Unal, B. (2011). Effects of haloperidol on striatal neurons: relation to neuronal loss (a stereological study). *Folia neuropathologica*, *49*(1), 21–27.

Arnold, F. J., Putka, A. F., Raychaudhuri, U., Hsu, S., Bedlack, R. S., Bennett, C. L., & La Spada, A. R. (2024). Revisiting Glutamate Excitotoxicity in Amyotrophic Lateral Sclerosis and Age-Related Neurodegeneration. *International journal of molecular sciences*, *25*(11), 5587. https://doi.org/10.3390/ijms25115587

Attia, T. M. (2018). Efficacy and Safety of Daflon® in the Treatment of Idiopathic Epistaxis. *American Journal of Rhinology and Allergy*, *33*(1), 62–68. https://doi.org/10.1177/1945892418809237

Bellavite, P. (2023). Neuroprotective potentials of flavonoids: experimental studies and mechanisms of action. *Antioxidants*, *12*(2), 280. https://doi.org/10.3390/antiox12020280

Bhidayasiri, R., Sringean, J., Phumphid, S., Anan, C., Thanawattano, C., Deoisres, S., Panyakaew, P., Phokaewvarangkul, O., Maytharakcheep, S., Buranasrikul, V., Prasertpan, T., Khontong, R., Jagota, P., Chaisongkram, A., Jankate, W., Meesri, J., Chantadunga, A., Rattanajun, P., Sutaphan, P., . . . Bunnag, T. (2024). The rise of Parkinson's disease is a global challenge, but efforts to tackle this must begin at a national level: a

protocol for national digital screening and "eat, move, sleep" lifestyle interventions to prevent or slow the rise of non-communicable diseases in Thailand. *Frontiers in Neurology*, 15. https://doi.org/10.3389/fneur.2024.1386608

Crowley, J. J., Ashraf-Khorassani, M., Castagnoli, N., Jr, & Sullivan, P. F. (2013). Brain levels of the neurotoxic pyridinium metabolite HPP+ and extrapyramidal symptoms in haloperidol-treated mice. *Neurotoxicology*, *39*, 153–157. https://doi.org/10.1016/j.neuro.2013.09.005

Crowley, S. J., Kanel, P., Roytman, S., Bohnen, N. I., & Hampstead, B. M. (2024). Basal forebrain integrity, cholinergic innervation and cognition in idiopathic Parkinson's disease. *Brain*, *147*(5), 1799–1808. https://doi.org/10.1093/brain/awad420

Del Agua Villa, C., Atudorei, M., Siebner, H. R., & Rickhag, M. (2024). Pharmacological targeting of dopamine D1 or D2 receptors evokes a rapid-onset parkinsonian motor phenotype in mice. *European Journal of Neuroscience*, *60*(12), 7006–7024. https://doi.org/10.1111/ejn.16622

Dhingra, D., Goswami, S., & Gahalain, N. (2018). Protective effect of hesperetin against haloperidol-induced orofacial dyskinesia and catalepsy in rats. *Nutritional neuroscience*, *21*(9), 667–675. https://doi.org/10.1080/1028415X.2017.1338549

Dong-Chen, X., Yong, C., Yang, X., Chen-Yu, S., & Li-Hua, P. (2023). Signaling pathways in Parkinson's disease: molecular mechanisms and therapeutic interventions. *Signal Transduction and Targeted Therapy*, *8*(1). https://doi.org/10.1038/s41392-023-01353-3

Egerton, A., Bhachu, A., Merritt, K., McQueen, G., Szulc, A., & McGuire, P. (2017). Effects of Antipsychotic Administration on Brain Glutamate in Schizophrenia: A Systematic Review of Longitudinal ^{1}H-MRS Studies. *Frontiers in psychiatry*, *8*, 66. https://doi.org/10.3389/fpsyt.2017.00066

Fidelis, F. B., Akhigbe, T. M., Oladipo, A. A., Oyedokun, P. A., Lasisi-Sholola, A. S., Adepoju, O. P., Ajao, O., Adeleye, O. O., Ogundipe, O. O., & Akhigbe, R. E. (2025). Daflon attenuates cisplatin-induced cerebellar neurotoxicity, anxiety-like behavior, and motor dysfunction by downregulating TLR4/NF-kB signaling. *BMC pharmacology & toxicology*, 27(1), 13. https://doi.org/10.1186/s40360-025-01046-3

Han, J., Hao, X., Fatima, M., Chauhdary, Z., Jamshed, A., Abdur Rahman, H. M., Siddique, R., Asif, M., Rana, S., & Hussain, L. (2024). Pharmacological Assessment of Aqueous Ethanolic Extract of Thalictrum Foetidum Against Haloperidol-Induced Parkinson's Like Symptoms in Animal Model: A Dose-Dependent Study With Mechanistic Approach. Dose-response : a publication of *International Hormesis Society*, 22(3), 15593258241282020. https://doi.org/10.1177/15593258241282020

Iovino, L., Tremblay, M., & Civiero, L. (2020). Glutamate-induced excitotoxicity in Parkinson's disease: The role of glial cells. *Journal of Pharmacological Sciences*, *144*(3), 151–164. https://doi.org/10.1016/j.jphs.2020.07.011

Kabra, A., Baghel, U. S., Hano, C., Martins, N., Khalid, M., and Sharma, R. (2020). Neuroprotective potential of Myrica esulenta in Haloperidol induced Parkinson's disease. *Journal of Ayurveda and Integrative Medicine*, *11*(4), 448–454. https://doi.org/10.1016/j.jaim.2020.06.007

Kamal, N., Abdallah, M. S., Wahed, E. A., Sabri, N. A., & Fahmy, S. F. (2024). Evaluation of the Effect of Loratadine versus Diosmin/Hesperidin Combination on Vinca Alkaloids-Induced Neuropathy: A Randomized Controlled Clinical Trial. *Pharmaceuticals*, *17*(5), 609. https://doi.org/10.3390/ph17050609

Kobo, P. I., Ayo, J. O., Aluwong, T., Zezi, A. U., Maikai, V., and Ambali, S. F. (2014). Flavonoid mixture ameliorates increase in erythrocyte osmotic fragility and malondialdehyde concentration induced by Trypanosoma brucei brucei-infection in Wistar rats. *Research in Veterinary Science*, *96*(1), 139–142. https://doi.org/10.1016/j.rvsc.2013.10.005

Krum, B. N., Martins, A. C., Queirós, L., Ferrer, B., Milne, G. L., Soares, F. a. A., Fachinetto, R., and Aschner, M. (2020). Haloperidol Interactions with the dop-3 Receptor in Caenorhabditis elegans. *Molecular Neurobiology*, *58*(1), 304–316. https://doi.org/10.1007/s12035-020-02124-9

Li, M., Ye, X., Huang, Z., Ye, L., & Chen, C. (2025). Global burden of Parkinson's disease from 1990 to 2021: a population-based study. *BMJ open*, *15*(4), e095610. https://doi.org/10.1136/bmjopen-2024-095610

Menze, E. T., Tadros, M. G., Abdel-Tawab, A. M., & Khalifa, A. E. (2012). Potential neuroprotective effects of hesperidin on 3-nitropropionic acid-induced

neurotoxicity in rats. *Neurotoxicology*, *33*(5), 1265–1275. https://doi.org/10.1016/j.neuro.2012.07.007

National Research Council (2011). Guide for the care and use of Laboratory animals. In *National Academies Press eBooks*. https://doi.org/10.17226/12910

Perera, J., Tan, J. H., Jeevathayaparan, S., Chakravarthi, S., & Haleagrahara, N. (2011). Neuroprotective effects of alpha lipoic Acid on haloperidol-induced oxidative stress in the rat brain. *Cell & bioscience*, *1*(1), 12. https://doi.org/10.1186/2045-3701-1-12

Rafiq, H., Farhan, M., Rafi, H., Rehman, S., Arshad, M., and Shakeel, S. (2022). Inhibition of drug induced Parkinsonism by chronic supplementation of quercetin in haloperidol-treated wistars. *PubMed*, *35*(6), 1655–1662. https://pubmed.ncbi.nlm.nih.gov/36789825

Rahman, S., & Marwaha, R. (2023, September 1). *Haloperidol*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK560892/

Rajaram, C., Reddy, K. R., and Sekhar, K. B. C. (2015). Neuroprotective activity of *Tephrosia purpurea* against haloperidol-induced Parkinson disease model. *Pharmacologia*, 6(4), 125–130. https://scialert.net/abstract/?doi=pharmacologia.2015.125.130

Ramesh, S., & Arachchige, A. S. P. M. (2023). Depletion of dopamine in Parkinson's disease and relevant therapeutic options: A review of the literature. *AIMS Neuroscience*, *10*(3), 200–231. https://doi.org/10.3934/neuroscience.2023017

Re, A., Tm, A., & Ca, A. (2025). Micronized purified flavonoid fraction (daflon) improves sexual function by modulating NF-kB /NO/cGMP and steroidogenic signaling in cisplatin-treated male Wistar rats. *Biochemistry and Biophysics Reports*, *43*, 102144. https://doi.org/10.1016/j.bbrep.2025.102144

Salam, O. A., and Nada, S. (2011). Piracetam reverses haloperidol-induced catalepsy in mice. *Turkish Journal of Medical Sciences*: 41(4), 693-699. https://doi.org/10.3906/sag-1006-870

Saleem, U., Gull, Z., Saleem, A., Shah, M. A., Akhtar, M. F., Anwar, F., Ahmad, B., and Panichayupakaranant, P. (2021). Appraisal of anti-Parkinson activity of rhinacanthin-C in haloperidol-induced parkinsonism in mice: A mechanistic approach. *Journal of Food Biochemistry*, *45*(4). https://doi.org/10.1111/jfbc.13677

Samaila, S., Timothy, P. V., Kayode, A. O., Williams, I. K., Christopher, C. H., Michael, J. C., & Danborno, A. M. (2025). Ameliorative effects of micronised purified flavonoid fraction on haloperidol-induced motor impairments and oxidative stress in female Wistar rats. Nigerian Journal of Neuroscience, 16(4), 144–151. doi: 10.47081/njn2025.16.4/003

Sanberg, P.R., Bunsey, M.D., Giordano, M. & Norman, A.B. (1988). The catalepsy test: its ups and downs. *Behavioral Neuroscience* 102(5): 748-759. https://doi.org/10.1037/0735-7044.102.5.748

Schmitz, I., Bobermin, L. D., da Silva, A., Weber, F. B., Thomaz, N. K., Schmitz, F., Brondani, M., Fachinetto, R., Leipnitz, G., Wyse, A. T. S., Gonçalves, C. A., & Quincozes-Santos, A. (2025). A single dose of haloperidol decanoate induces short-term hippocampal neuroinflammation: focus on the glial response. *Pharmacological reports : PR*, *77*(3), 800–808. https://doi.org/10.1007/s43440-025-00706-9

Shabani, S., & Mirshekar, M. A. (2018). Diosmin is neuroprotective in a rat model of scopolamine-induced cognitive impairment. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *108*, 1376–1383. https://doi.org/10.1016/j.biopha.2018.09.127

Shim, K. H., Go, H. G., Bae, H., Jeong, D., Kim, D., Youn, Y. C., Kim, S., An, S. S. A., & Kang, M. J. (2021). Decreased exosomal acetylcholinesterase activity in the plasma of patients with Parkinson's disease. *Frontiers in Aging Neuroscience*, *13*. https://doi.org/10.3389/fnagi.2021.665400

Shin, H. W., & Chung, S. J. (2012). Drug-induced parkinsonism. *Journal of clinical neurology (Seoul, Korea)*, *8*(1), 15–21. https://doi.org/10.3988/jcn.2012.8.1.15

Shrestha, S., Phornchirasilp, S., & Thisayakorn, K. (2016). Comparative Analysis of Drug-Induced Parkinsonism Like Behaviors In C57bl6 Black Mice Using A Force Plate Actimeter. *International Journal of Pharmaceutical Sciences and Research*, 7(2), 873-81. https://doi:10.13040/IJPSR.0975-8232.7(2).873-81

Shrimanker, I., Tadi, P., Schoo, C., & Sánchez-Manso, J. C. (2024, March 13). *Parkinsonism*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK542224/

Silva, B. R. D., Lima, J. M. F. A., Echeverry, M. B., & Alberto-Silva, C. (2024). Haloperidol-Induced Catalepsy

and Its Correlations with Acetylcholinesterase Activity in Different Brain Structures of Mice. *Neurology international*, *16*(6), 1731–1741. https://doi.org/10.3390/neurolint16060125

Skhawy, N. E., Diab, H. E., Hassan, S. A., & Hassan, A. Y. (2024). Potential anti-schistosomal effect of Daflon, a repurposed drug targeting different stages of Schistosome maturity. *Parasitology Research*, 123(12). https://doi.org/10.1007/s00436-024-08418-4

Solmi, M., Murru, A., Pacchiarotti, I., Undurraga, J., Veronese, N., Fornaro, M., Stubbs, B., Monaco, F., Vieta, E., Seeman, M. V., Correll, C. U., & Carvalho, A. F. (2017). Safety, tolerability, and risks associated with first- and second-generation antipsychotics: A state-of-the-art clinical review. *Therapeutic and Clinical Risk Management*, *13*, 757–777. https://doi.org/10.2147/TCRM.S117321

Su, D., Cui, Y., He, C., Yin, P., Bai, R., Zhu, J., Lam, J. S. T., Zhang, J., Yan, R., Zheng, X., Wu, J., Zhao, D., Wang, A., Zhou, M., & Feng, T. (2025). Projections for prevalence of Parkinson's disease and its driving factors in 195 countries and territories to 2050: modelling study of Global Burden of Disease Study 2021. *BMJ (Clinical research ed.)*, *388*, e080952. https://doi.org/10.1136/bmj-2024-080952

Trang, A., & Khandhar, P. B. (2023, January 19). *Physiology, acetylcholinesterase*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK539735/?utm_source=chatgpt.com

Vásquez-Builes, S., Salazar-Duque, C., Tieck-Fernández, M. P., Rojas-Gallego, I. C., & Díaz-Silva, G. A. (2021). Drug-induced parkinsonism: what should a psychiatrist know? DELETED, 22(4). https://doi.org/10.24875/rmn.20000010

Waku, I., Magalhães, M. S., Alves, C. O., & de Oliveira, A. R. (2021). Haloperidol-induced catalepsy as an animal model for parkinsonism: A systematic review of experimental studies. *The European journal of neuroscience*, *53*(11), 3743–3767. https://doi.org/10.1111/ejn.15222

Waku, I., Reimer, A. E., & De Oliveira, A. R. (2022). Effects of immediate aversive stimulation on Haloperidol-Induced catalepsy in rats. *Frontiers in Behavioral Neuroscience*, *16*, 867180. https://doi.org/10.3389/fnbeh.2022.867180

Wu, J., Cai, Y., Wu, X., Ying, Y., Tai, Y., & He, M. (2021). Transcardiac Perfusion of the Mouse for Brain Tissue Dissection and Fixation. Bio-protocol, 11(5), e3988. https://doi.org/10.21769/BioProtoc.3988

Wu, W., Gong, X., Qin, Z., & Wang, Y. (2025). Molecular mechanisms of excitotoxicity and their relevance to the pathogenesis of neurodegenerative diseases-an update. *Acta Pharmacologica Sinica*. https://doi.org/10.1038/s41401-025-01576-w

Zahid, U., McCutcheon, R. A., Borgan, F., Jauhar, S., Pepper, F., Nour, M. M., Rogdaki, M., Osugo, M., Murray, G. K., Hathway, P., Murray, R. M., Egerton, A., & Howes, O. D. (2022). The effect of antipsychotics on glutamate levels in the anterior cingulate cortex and clinical response: A 1H-MRS study in first-episode psychosis patients. *Frontiers in Psychiatry*, *13*. https://doi.org/10.3389/fpsyt.2022.967941

Zhang, X., Wang, M., Lee, S. Y., Yue, Y., Chen, Z., Zhang, Y., Wang, L., Guan, Q., Fan, W., & Shen, T. (2024). Cholinergic nucleus degeneration and its association with gait impairment in Parkinson's disease. *Journal of neuroengineering and rehabilitation*, *21*(1), 120. https://doi.org/10.1186/s12984-024-01417-7

Zhang, Z., Zhang, S., Fu, P., Zhang, Z., Lin, K., Ko, J. K., & Yung, K. K. (2019). Roles of Glutamate Receptors in Parkinson's Disease. *International journal of molecular sciences*, *20*(18), 4391. https://doi.org/10.3390/ijms20184391