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Saminu Samaila

samailasaminu@fuwukari.edu.ng

Federal University Wukari

Confidence H. Christopher

Federal University Wukari

Iyomo K. Williams

Bingham University

Joshua C. Michael

Bingham University

Lawrence D. Adedayo

Federal University Wukari

Angela M. Danborno

Bingham University

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Protective Effects of Daflon Against Haloperidol-Induced Neurobehavioral Alterations, Oxidative Stress, Neuroinflammation, and Cerebellar Histomorphological Changes in Mice

Saminu Samaila [a,] *, Confidence H. Christopher [a], Iyomo K. Williams [b], Joshua C. Michael [b], Lawrence D. Adedayo [a], Angela M. Danborno [b]

[a] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Federal University Wukari, Wukari, Taraba State, Nigeria.

[b] Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Bingham University, Karu, Nasarawa State, Nigeria.

***Corresponding Author Email:** saminusamaila@gmail.com; samailasaminu@fuwukari.edu.ng

ORCID: 0000-0002-9548-2844

ABSTRACT

Background: Haloperidol is widely used to manage mental disorders, but its clinical use is limited by its extrapyramidal and neurotoxic side effects. This study investigates the potential of Daflon to attenuate haloperidol-induced neurobehavioral, biochemical, and cerebellar alterations in mice.

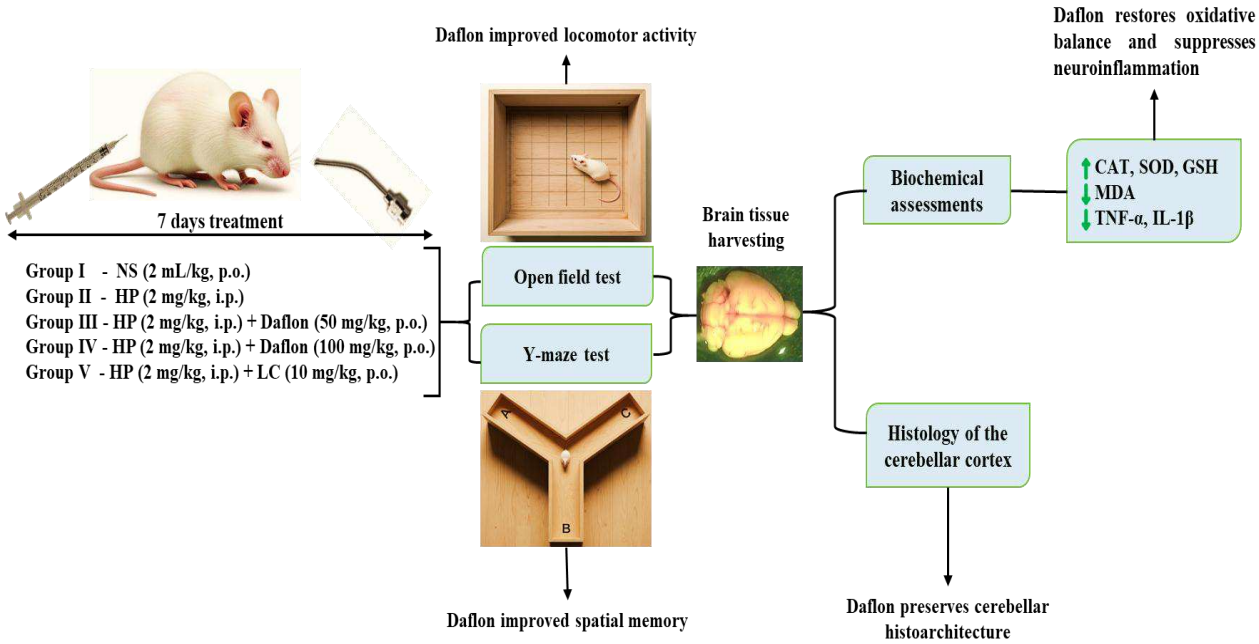
Method: Twenty-five male Swiss mice were randomly divided into five groups (n = 5/group): a saline control group (2 mL/kg), a haloperidol-only group (2 mg/kg), haloperidol plus Daflon groups (50 and 100 mg/kg), and a haloperidol plus levodopa/carbidopa (10 mg/kg) group. Following seven consecutive days of co-treatment, neurobehavioral performance was evaluated using open field and Y-maze tests. Brain tissues were subsequently harvested for histomorphological examination of the cerebellum and brain tissue biochemical assessments of the levels of CAT, SOD, GSH, MDA, TNF-α, and IL-1β.

Results: The group that received haloperidol alone exhibited significant behavioral impairments (reduced open-field locomotion and Y-maze spontaneous alternation, indicating short-term spatial working memory deficits), depleted antioxidants (CAT, SOD, GSH), and elevated lipid peroxidation (MDA) and proinflammatory markers (TNF-α, IL-1β) alongside localized cerebellar structural distortions. Groups co-treated with Daflon and the standard drug levodopa-carbidopa exhibited significant improvement in motor activity and short-term spatial working memory. This co-treatment also improved the antioxidant levels of CAT, SOD, and GSH, reduced MDA, TNF-α, and IL-1β levels, and preserved target cerebellar histoarchitecture

Conclusion: These outcomes suggest that Daflon possesses neuroprotective properties against haloperidol-induced neurobehavioral, biochemical, and cerebellar alterations. These therapeutic effects are likely mediated through Daflon's capacity to modulate oxidative stress and neuroinflammatory pathways, highlighting its potential as a promising adjunct therapy to mitigate antipsychotic-induced neurotoxicity.

KEYWORDS: Daflon, Haloperidol, Cerebellar cortex, Oxidative stress, Neuroinflammation, Motor activity, Spatial memory.

Graphical Abstract



Key: NS = Normal Saline; HP = Haloperidol; LC = Levodopa/Carbidopa; CAT = Catalase; SOD = Superoxide Dismutase; GSH = Reduced Glutathione; MDA = Malondialdehyde; TNF-α = Tumor Necrosis Factor-alpha; IL-1β = Interleukin-1 beta.

Introduction

Schizophrenia and related psychotic disorders remain major contributors to global neurological and psychiatric disability, affecting cognitive function, motor coordination, social interaction, and quality of life [1-3]. Typical antipsychotic agents such as haloperidol continue to be widely prescribed because of their efficacy in controlling positive psychotic symptoms and acute manic episodes [4, 5]. However, the clinical use of haloperidol is frequently limited by severe extrapyramidal symptoms, motor dysfunction, cognitive impairment, and progressive neurotoxicity associated with dopaminergic blockade [6-8]. Experimental studies have consistently demonstrated that haloperidol impairs locomotor activity, working memory, and learning performance while simultaneously disrupting brain histoarchitecture, particularly within regions involved in motor and cognitive

regulation [9-12]. These deficits are a result of haloperidol's potent antagonism of dopamine D2 receptors [9, 13]. In addition to receptor antagonism, haloperidol exacerbates neuronal damage by inducing oxidative stress and neuroinflammation, a pathological cascade driven by accelerated dopamine turnover and the subsequent generation of reactive oxygen species [8, 14, 15].

Several therapeutic approaches have been explored to reduce haloperidol-induced behavioral and neurochemical alterations, with growing attention directed toward naturally derived antioxidant and anti-inflammatory compounds. Experimental studies have shown that alpha-lipoic acid, vinpocetine, cinnarizine, cannabidiol, metformin, and oleanolic acid can attenuate the oxidative and behavioral consequences associated with haloperidol administration by modulating redox pathways and inflammatory signaling [6, 9, 11, 16, 17]. Nevertheless, many of these interventions remain limited by inconsistent behavioral outcomes, restricted mechanistic characterization, or inadequate histomorphological evaluation. Additionally, few studies have simultaneously examined behavioral performance, oxidative stress biomarkers, inflammatory mediators, and cerebellar histoarchitecture within the same experimental framework. The cerebellum is increasingly recognized as a vital contributor not only to motor coordination but also to cognition and emotional regulation [18, 19]. However, cerebellar involvement in antipsychotic-induced neurotoxicity remains insufficiently explored.

Micronised purified flavonoid fraction (MPFF; Daflon®), a flavonoid-based formulation composed primarily of diosmin and hesperidin, possesses established antioxidant, anti-inflammatory, endothelial-protective, and microcirculatory benefits [20-23]. Evidence from research studies indicates that Daflon can reduce oxidative stress and inflammatory injury in experimental models, suggesting its therapeutic relevance in neurodegenerative and neuroinflammatory conditions [24-28]. Although Daflon has demonstrated antioxidant and anti-inflammatory effects in several experimental

models and has been extensively investigated in chronic venous disease and inflammatory vascular disorders [20, 21], evidence regarding its ability to attenuate antipsychotic-induced neurobehavioral deficits and cerebellar injury remains insufficiently characterized. In particular, there is paucity of study that have comprehensively evaluated the effects of Daflon on behavioral performance, oxidative stress biomarkers, inflammatory cytokines, and cerebellar histomorphology within a single model of haloperidol-induced neurotoxicity. It is based on these considerations that this present study was conducted to investigate the neuroprotective effects of Daflon against haloperidol-induced neurotoxicity in mice.

Materials and Methods

Animals

For this investigation, we utilized twenty-five male Swiss mice weighing between 22 and 30 grams (aged 2-3 months) procured from the animal facility at Bingham University, Karu, Nigeria. The animals were housed in standard polypropylene cages under standard laboratory conditions, specifically a 12-hour diurnal light cycle, a temperature of 25 ± 2°C, and a relative humidity range of 50-60%. Prior to initiating treatments, the mice underwent a 7-day laboratory environment acclimation process and had unrestricted access to laboratory chow and clean drinking water throughout the study duration. All experimental procedures were conducted under the approval of the Bingham University Research Ethics Review Committee (approval number BHU/ERC/25/A003), ensuring that all procedures were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and ARRIVE 2.0 reporting recommendations [29, 30].

Drug preparation

Daflon® 500 mg tablets (Servier Egypt Industries Ltd.), Haloperidol 5 mg/mL injection BP (Haldol®, Janssen), and levodopa/carbidopa tablets (250/25 mg; Sinemet®) were purchased from a licensed

pharmacy vendor. Fresh stock solutions/suspensions were prepared daily using 0.9% physiological saline as the common vehicle. Dosing volumes were calculated according to body weight and administered in equivalent volumes across all the groups.

Experimental design

The twenty-five Swiss mice were distributed randomly into five distinct groups with five animals each and treatments were administered once every day for seven consecutive days. Group I served as control and received oral normal saline (2 mL/kg), while Group II was given haloperidol alone (2 mg/kg, i.p.). Groups III and IV were orally pre-treated with Daflon at 50 and 100 mg/kg, respectively, 30 minutes before receiving haloperidol (2 mg/kg, i.p.). Group V received oral levodopa/carbidopa (10 mg/kg) 30 minutes prior to haloperidol injection to serve as the reference treatment group. The chosen dosages were determined from prior experimental work and published pharmacological data. Haloperidol (2 mg/kg) was used to induce parkinsonian-like motor and cognitive deficits, consistent with validated experimental models of haloperidol-induced neurotoxicity and Parkinsonism [31]. The specific doses of Daflon (50 and 100 mg/kg) were selected based on literature demonstrating their safety profile and neuroprotective properties in rodent studies [32, 33]. Levodopa/carbidopa (10 mg/kg) served as the positive control and the dosage was derived from previous studies investigating drug-induced Parkinsonism in mice [34].

Neurobehavioral assessments

Behavioral assessments were conducted on day seven following the final treatment. In order to minimize variability associated with stress, mice were habituated to the testing environment for one hour prior to the commencement of the assessments. Open-field testing was conducted before Y-maze assessment on the same day.

Open field test

Locomotor activity was evaluated using the open field test apparatus. The testing procedure and behavioral scoring followed the established methodology described by Kraeuter *et al*. [35]. The open field apparatus used in this study consisted of a wooden square arena measuring 66 cm × 66 cm that was open at the top and enclosed by walls 30 cm high. The floor of the apparatus was demarcated using blue lines into sixteen equal squares, each measuring 16.5 cm × 16.5 cm. A central square measuring 16.5 cm × 16.5 cm was also drawn in red lines at the central zone of the arena. During the experiment, individual mice were positioned in the centre of the open field and given 1 minute to habituate, after which the animals were recorded for 5 minutes using an overhead video camera as they freely explored the arena. After each observation period, the animal was returned to its cage, and the testing enclosure was sanitized with 70% ethanol solution and allowed to dry completely prior to testing subsequent animals. At the end, the obtained video recordings were analysed offline by an observer to determine locomotor activity by counting the number of lines crossed with all four limbs. Locomotor activity, measured as the number of line crossings, was selected as the primary outcome measure because it directly reflects haloperidol-induced hypolocomotion and motor impairment.

Y-maze test

To evaluate short-term spatial working memory, we employed the Y-maze spontaneous alternation paradigm as described by Kraeuter *et al*. [36]. The maze apparatus used for this test was constructed from wooden opaque material to prevent external visual distractions. It had three identical arms that were set up at 120° to each other and were labelled A, B, and C. During the test, individual mice were positioned in the central zone of the Y-maze and left to explore all three arms for 5 minutes. A valid arm entry was counted only when the animal's four paws completely crossed into a given arm. Sequential entry into each of the three distinct arms on consecutive choices (such as triads A-B-C or B-C-A) was scored as a spontaneous alternation. Short term memory was determined by expressing

total actual alternations as a percentage of maximum possible alternations, computed using the formula: (Number of alternations / [Total number of arm entries − 2]) × 100. After each observation period, the animal was returned to its home cage, and the testing enclosure was sanitized with a 70% ethanol solution and air-dried to eliminate residual olfactory cues before testing subsequent animals.

Collection of samples for biochemical and histological assessments

Twenty-four hours after completing the neurobehavioral assessments, the animals were anaesthetized via intraperitoneal administration of ketamine (90 mg/kg) combined with xylazine (10 mg/kg). Upon confirming the suppression of reflex responses, the mice were humanely euthanized by exsanguination. Brains were quickly harvested on ice, after which the cerebellum was excised and placed in a 10% formalin solution for histological processing. The remaining brain tissue was immediately cleared of residual blood traces using cold PBS (pH 7.4) and stored frozen until subsequent biochemical assessments. Samples for biochemical analyses were coded prior to processing to minimize observer bias.

Biochemical analyses

Brain tissue samples were homogenized on ice in cold phosphate-buffered saline (0.01 M, pH 7.4; 1:9 w/v) and centrifuged (10,000 × g, 10 min, 4 °C) to collect clarified supernatants. Total protein concentration was quantified via bicinchoninic acid (BCA) assay to normalize biomarker data where applicable. Commercial colorimetric kits were employed to quantify oxidative status according to supplier protocols. Catalase (CAT; Elabscience®, E-BC-K031-S; 405 nm) activity was determined by H_2O_2 decomposition and expressed as U/mg protein. Total superoxide dismutase (SOD; Elabscience®, E-BC-K020-M; 450 nm) activity was measured via WST-1 reduction inhibition and expressed as (U/mg protein). Non-enzymatic antioxidant defenses were evaluated by quantifying

reduced glutathione (GSH; Elabscience®, E-BC-K030-M; 405 nm) concentrations within the tissue supernatant fraction, with final values expressed in millimolar (mM). Lipid peroxidation was assessed via the malondialdehyde (MDA; Oxford Biomedical Research, FR40; 532 nm) reaction with thiobarbituric acid, with concentrations interpolated from standard curves and reported as micromolar (μM) within the final supernatant volume. For pro-inflammatory cytokine determination, Concentrations of TNF-α (Elabscience® E-EL-M3063) and IL-1β (Elabscience® E-EL-M0037) were quantified using sandwich ELISA protocols at 450 nm and interpolated from standard curves and expressed as pg/mL according to the manufacturers' protocols.

Histological and histochemical analysis

The post-fixed cerebella were subjected to standard processing for light microscopy. Cerebellar tissue sections (5 μm thick) were microtomed and processed for histological analysis. To evaluate general cytoarchitecture and structural integrity, sections were stained with hematoxylin and eosin (H&E). In addition, parallel sections were stained with cresyl fast violet (CFV) to visualize perikaryal Nissl bodies in neurons. Slides were inspected via light microscopy by evaluators blinded to treatment allocation. Morphological features including Purkinje cell integrity, cellular organization, gliosis, chromatolysis, vacuolation, and neuronal loss were qualitatively assessed and compared across groups.

Statistical analyses

Statistical processing was performed using GraphPad Prism 8 (version 8.0.2; GraphPad Software, San Diego, CA, USA). Prior to analysis, standard verification of underlying parametric assumptions (normality of distribution and equality of variances) was conducted. Results are expressed as mean ± standard error of the mean (SEM). Inter-group variation was determined through one-way analysis of

variance (ANOVA), with subsequent pair-wise evaluations performed using Tukey's post hoc multiple comparisons test. Differences were considered significant at a value of $p < 0.05$.

Results

Effects of Daflon on motor and cognitive function

Open field test

Figure 1 illustrates a significant ($p < 0.001$) impairment in locomotor activity following haloperidol administration, reflected by a reduction in line crossings in the haloperidol-treated group (15.60 ± 1.44) compared with the control group (57.33 ± 4.38). The administration of Daflon at 50 mg/kg (30.60 ± 1.78) and 100 mg/kg (32.40 ± 1.78) along with haloperidol significantly ($p < 0.01$ for both doses) improved locomotor performance, as shown by an increased frequency of line crossings relative to the haloperidol-alone group. Similarly, the group that was co-treated with levodopa/carbidopa (32.20 ± 2.15) displayed a significant ($p < 0.01$) elevation in line crossings relative to the haloperidol-alone group. However, line crossings in all co-treated groups (Daflon and levodopa/carbidopa) remained significantly ($p < 0.001$) reduced relative to the control group.

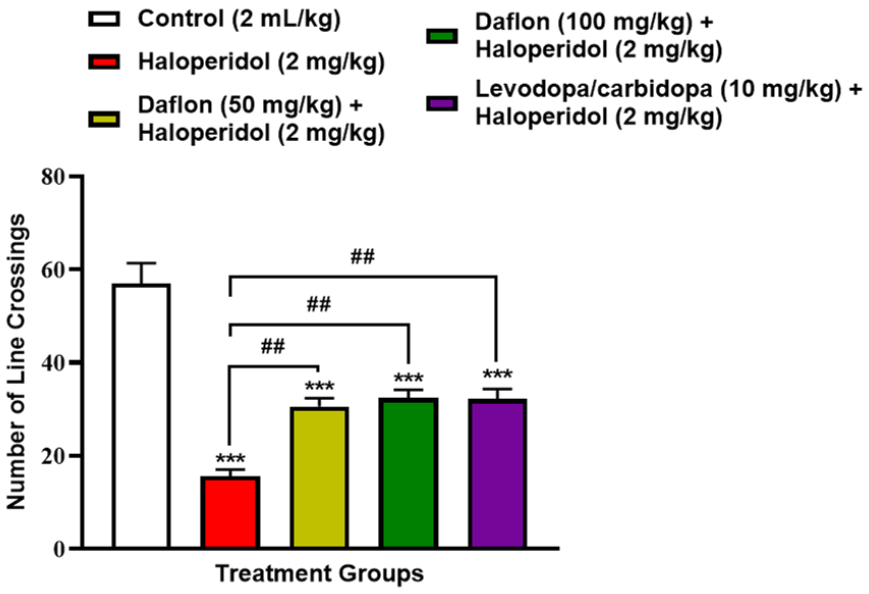


Figure 1. Effects of Daflon on locomotor activity (line crossings). Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: *** $p < 0.001$ in comparison to control; $^{\#\#}$ $p < 0.01$ in comparison to haloperidol alone.

Y-maze test

As shown in Figure 2, haloperidol administration significantly impaired spatial short-term memory, as reflected by the substantial ($p < 0.001$) decline in spontaneous alternation among haloperidol-treated animals (12.80 ± 1.16%) relative to the controls (31.70 ± 0.67%). Treatment with Daflon at 50 mg/kg (26.08 ± 0.81%) significantly ($p < 0.001$) improved spontaneous alternation performance relative to haloperidol group, although values remained significantly ($p < 0.01$) lower than the control baseline. Treatment with Daflon at 100 mg/kg (31.60 ± 0.87%) as well as the standard reference drug levodopa/carbidopa (31.90 ± 0.63%) significantly ($p < 0.001$, for both) improved spontaneous alternation performance relative to the haloperidol alone group and also improved alternation performance back to control levels.

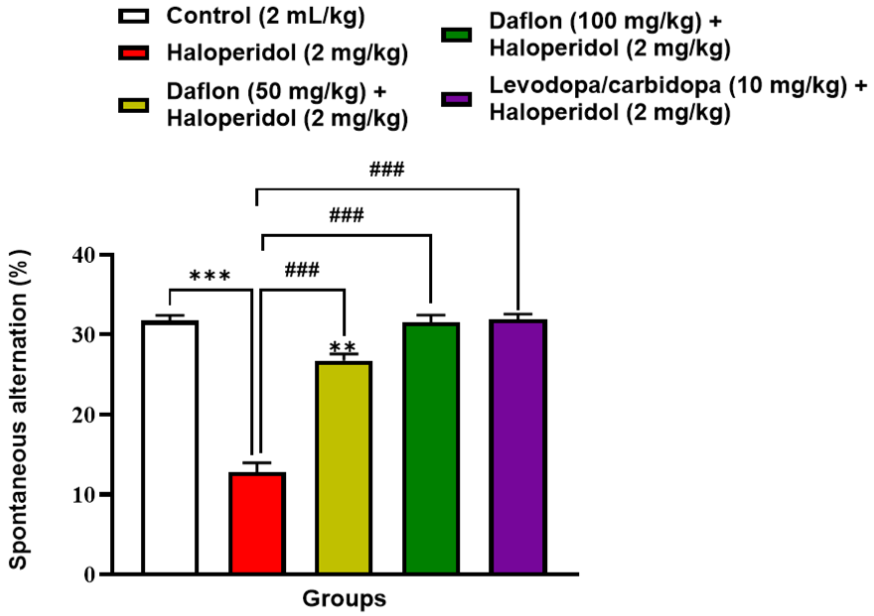


Figure 2. Effect of Daflon on spontaneous alternation in the Y-maze test. Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: ** $p < 0.01$, *** $p < 0.001$ in comparison to control; ### $p < 0.001$ in comparison to haloperidol alone.

Effects of Daflon on haloperidol-induced oxidative stress in brain tissue homogenates of mice

Impact of Daflon on catalase (CAT) enzymatic activity

As illustrated in Figure 3, the group that received haloperidol alone exhibited a significant ($p < 0.001$) depletion in levels of catalase (6.20 ± 0.80 U/mg protein) relative to control values (12.44 ± 0.66 U/mg protein). Co-treatment with Daflon 50 mg/kg (10.87 ± 0.95 U/mg protein) and 100 mg/kg (11.65 ± 0.43 U/mg protein) significantly ($p < 0.001$ for both doses) elevated catalase activity compared with haloperidol-treated animals. Similarly, co-treatment with the reference medication levodopa/carbidopa markedly ($p < 0.001$) improved catalase activity (12.76 ± 0.34 U/mg protein) relative to the haloperidol-alone group.

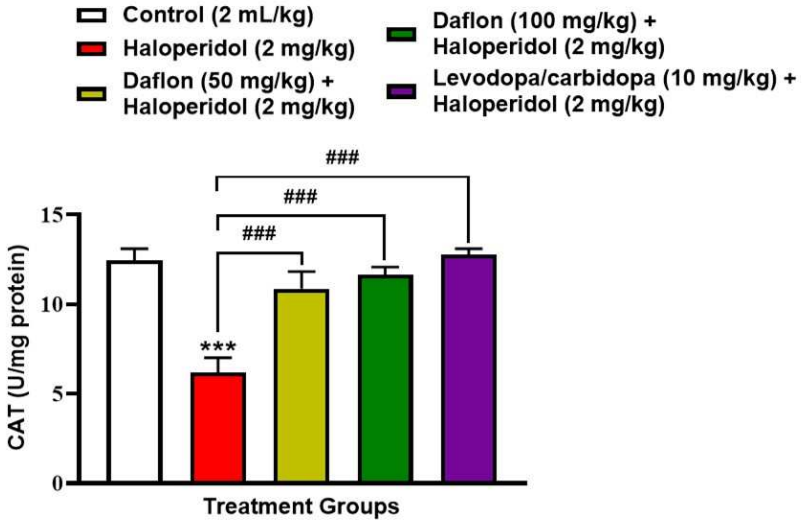


Figure 3. Impact of Daflon on catalase (CAT) enzymatic activity. Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: *** $p < 0.001$ in comparison to control; ### $p < 0.001$ in comparison to haloperidol alone.

Impact of Daflon on superoxide dismutase (SOD) activity

As presented in Figure 4, the administration of haloperidol alone significantly ($p < 0.05$) depleted the levels of SOD (0.0365 ± 0.0019 U/mg protein) in comparison with control values (0.0620 ± 0.0045 U/mg protein). Conversely, the concurrent administration of 50 mg/kg of Daflon significantly ($p < 0.01$) increased SOD levels (0.0683 ± 0.0091 U/mg protein) relative to haloperidol-alone group. This outcome was even more pronounced in the 100 mg/kg Daflon group, which demonstrated a highly significant ($p < 0.001$) elevation in enzyme levels (0.0839 ± 0.0035 U/mg protein) relative to haloperidol-alone group. The reference medication levodopa/carbidopa significantly ($p < 0.001$) elevated SOD levels (0.0919 ± 0.0053 U/mg protein) compared to the haloperidol-alone group, achieving a level that was also significantly higher ($p < 0.01$) than control levels.

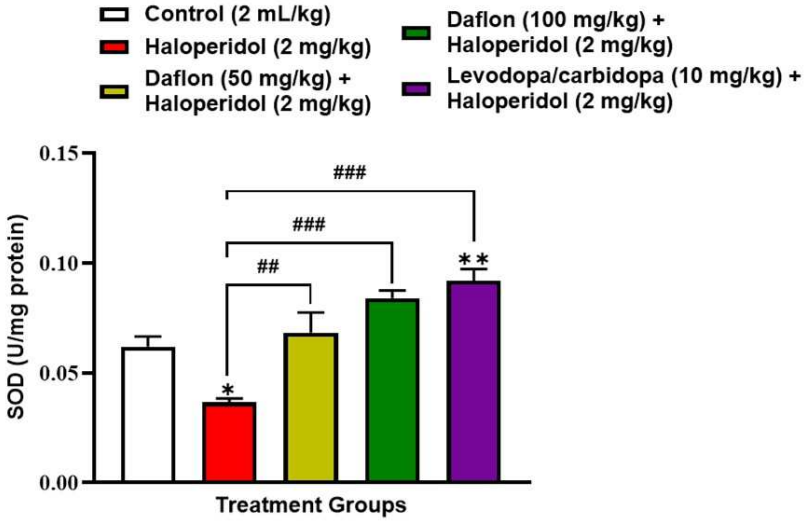


Figure 4. Impact of Daflon on superoxide dismutase (SOD) activity. Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: * $p < 0.05$, ** $p < 0.01$ in comparison to control; $^{\#\#}$ $p < 0.0$, $^{\#\#\#}$ $p < 0.001$ in comparison to haloperidol alone.

Effect of Daflon on reduced glutathione (GSH) levels

As shown in Figure 5, mice receiving only haloperidol demonstrated a marked ($p < 0.01$) depletion of GSH concentrations (0.1363 ± 0.0059 mM) relative to control group (0.1971 ± 0.0169 mM). Co-treatment of 50 mg/kg dose of Daflon along with haloperidol significantly ($p < 0.05$) improved GSH levels (0.1864 ± 0.0116 mM) relative to the haloperidol-treated group. Furthermore, pre-treatment with the 100 mg/kg dose of Daflon resulted in an even more substantial and significant ($p < 0.001$) elevation of central GSH levels (0.2291 ± 0.0105 mM) when compared with the group treated with haloperidol alone. In a similar trend, the reference medication, levodopa/carbidopa, markedly ($p < 0.001$) elevated the levels of GSH (0.2157 ± 0.0068 mM) relative to haloperidol-treated group.

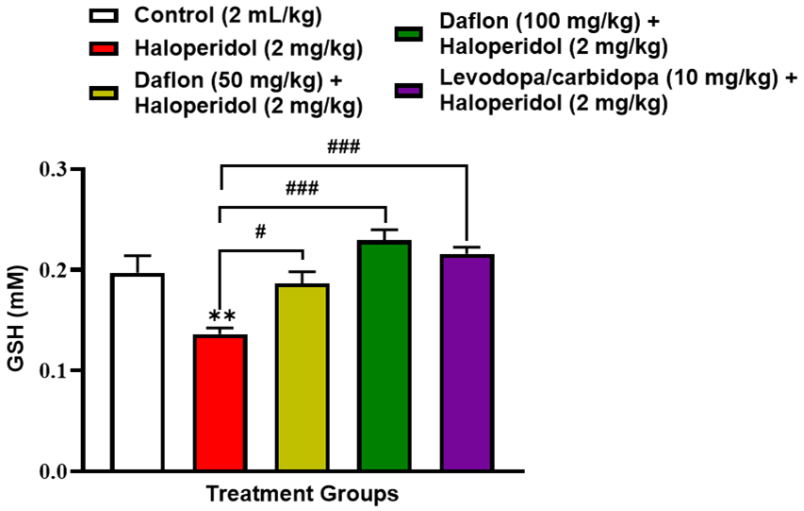


Figure 5. Effect of Daflon on reduced glutathione (GSH) levels. Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: ** $p < 0.01$ in comparison to control, $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ in comparison to haloperidol alone.

Effect of Daflon on levels of malondialdehyde (MDA)

As presented in Figure 6, the group of mice that received only haloperidol showed significant ($p < 0.01$) elevated levels of MDA (9.99 ± 0.20 µM) in comparison to the control (8.08 ± 0.26 µM). The group that received Daflon at the dose of 50 mg/kg along with haloperidol showed significantly ($p <$

0.01) reduced MDA levels (7.82 ± 0.50 µM) relative to the haloperidol group, while the group co-treated with Daflon at 100 mg/kg showed a more significant ($p < 0.01$) decrease in MDA levels (7.61 ± 0.41 µM) relative to the haloperidol-alone group. In a similar manner, MDA levels significantly ($p < 0.001$) reduced (6.62 ± 0.32 µM) in the group co-treated with the reference medication levodopa/carbidopa, relative to the group that received only haloperidol.

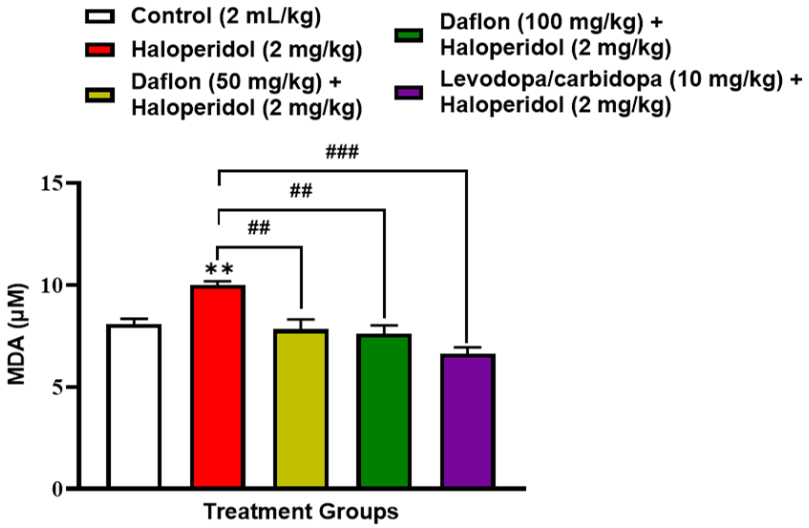


Figure 6. Effect of Daflon on levels of malondialdehyde (MDA). Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: ** $p < 0.01$ in comparison to control; $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ in comparison to haloperidol alone.

Effects of Daflon on haloperidol-induced neuroinflammation in brain tissue homogenates of mice

Daflon's effect on levels of tumour necrosis factor-alpha (TNF-α)

As shown in Figure 7, the group of mice that received haloperidol alone showed a significantly ($p < 0.05$) higher TNF-α levels (77.08 ± 2.68 pg/mL) compared with the control group (62.70 ± 3.03 pg/mL). Conversely, concurrent treatment with Daflon at both 50 mg/kg (60.65 ± 2.59 pg/mL) and 100 mg/kg (61.64 ± 4.43 pg/mL) led to a significant ($p < 0.05$ for both doses) attenuation of TNF-α levels relative to the haloperidol-alone group. In a similar trend, the standard reference drug

levodopa/carbidopa produced an even more pronounced and significant ($p < 0.001$) reduction in TNF-α levels (54.88 ± 3.15 pg/mL) when compared to the group receiving haloperidol alone.

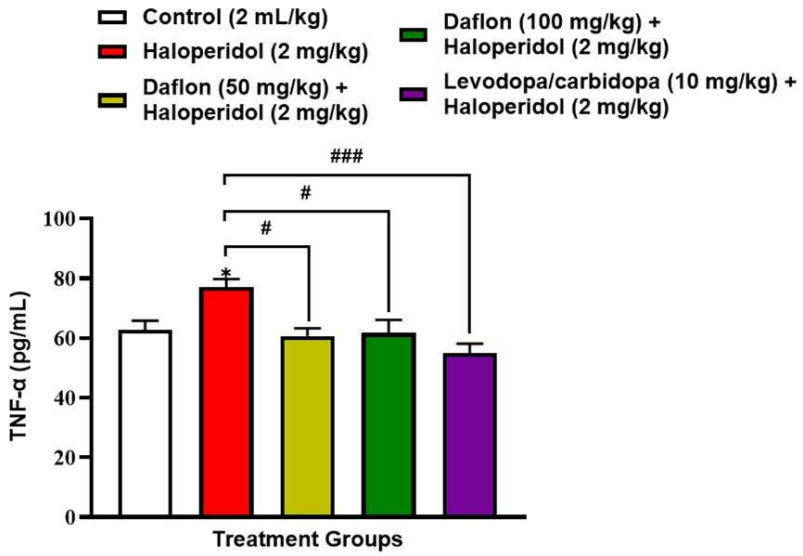


Figure 7. Daflon's effect on levels of tumour necrosis factor-alpha (TNF-α). Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: * $p < 0.05$ in comparison to control; $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ in comparison to haloperidol alone.

Impact of Daflon on the levels of interleukin-1beta (IL-1β)

As shown in Figure 8, the exclusive administration of haloperidol produced a marked ($p < 0.001$) elevation of IL-1β levels (163.4 ± 3.58 pg/mL) compared to the levels observed in the control group (128.1 ± 4.33 pg/mL). While the group that received Daflon at 50 mg/kg had a slight reduction of IL-1β levels (151.0 ± 3.81 pg/mL), statistically not significant ($p > 0.05$) relative to the group that received haloperidol alone; however, the values remained markedly ($p < 0.05$) elevated in comparison to control values. Conversely, Daflon 100 mg/kg co-treated group demonstrated a significant ($p < 0.05$) reduction of IL-1β levels (139.8 ± 4.83 pg/mL) relative to haloperidol alone treated group. In a similar trend, levodopa/carbidopa co-treated group, demonstrated a highly significant ($p < 0.001$) reduction in IL-1β concentrations (126.4 ± 7.90 pg/mL) relative to haloperidol-alone group.

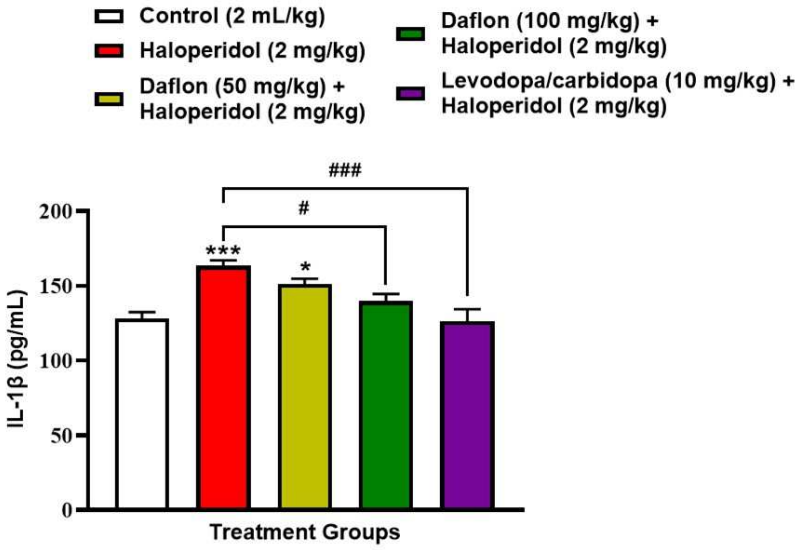


Figure 8. Impact of Daflon on the levels of interleukin-1beta (IL-1β). Values are depicted as mean ± SEM (n = 5 per group). Statistical annotations: * $p < 0.05$, *** $p < 0.001$ in comparison to control; $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ in comparison to haloperidol alone.

Histological and histochemical effects of Daflon on the cerebellar cortex of haloperidol-treated mice

The effects of Daflon on the histological and histochemical features of the cerebellar cortex following haloperidol administration are shown in Figure 9 and Figure 10, respectively. Administration of haloperidol resulted in distortion of the histoarchitecture of the cerebellar cortex characterized by gliosis and distortion of the Purkinje cell layer. Histochemical studies revealed that haloperidol also caused reduced Nissl substance staining intensity accompanied by mild neuronal distortion. However, co-administration of Daflon (50 or 100 mg/kg) or levodopa/carbidopa (10 mg/kg) ameliorated the histological and histochemical alterations induced by haloperidol.

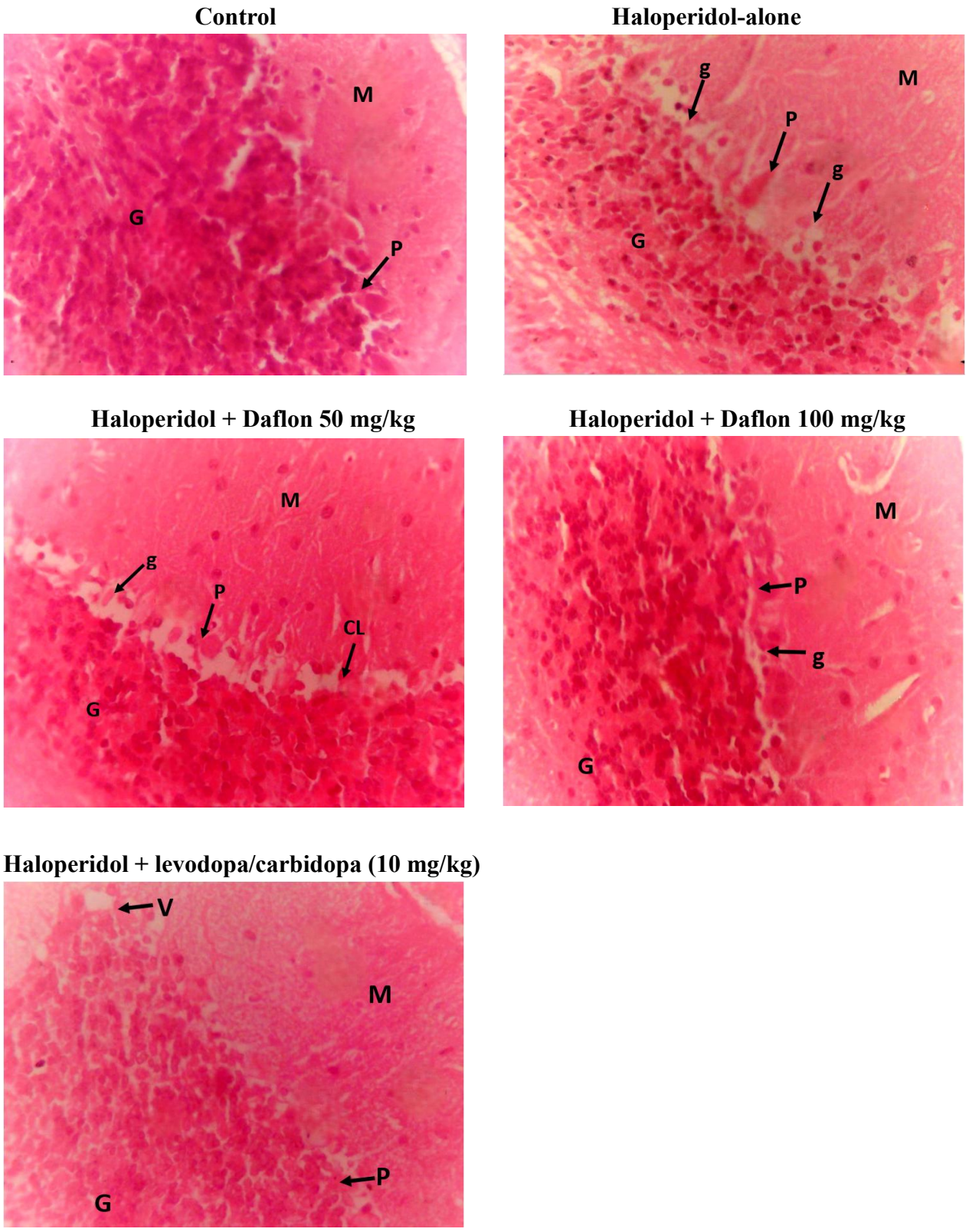
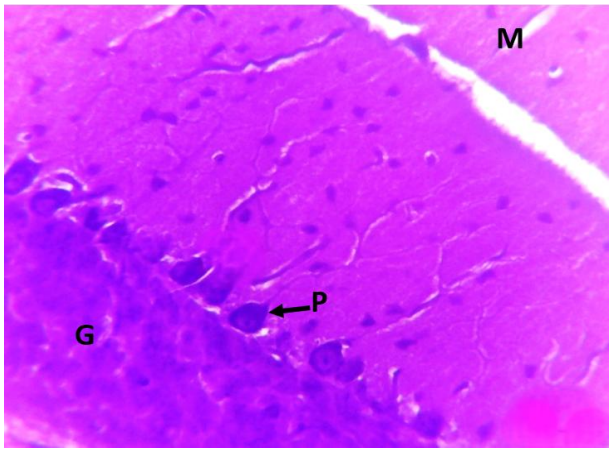


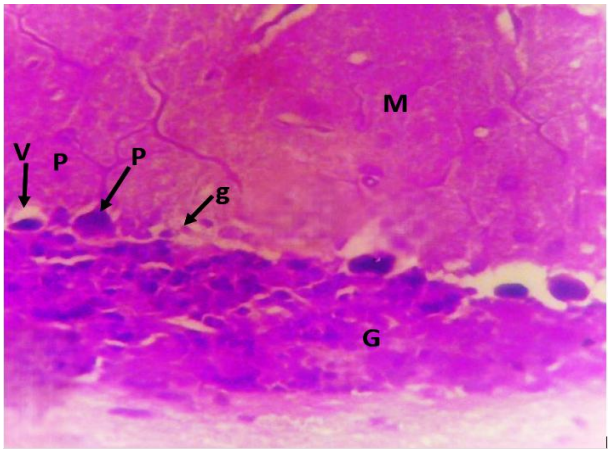
Figure 9. Representative photomicrographs showing the histopathological appearance of the cerebellar cortex among experimental groups. M: molecular layer; P: Purkinje cell layer; G: granular

layer; g: gliosis; CL: cell loss; V: perineuronal vacuolation. Hematoxylin and Eosin (H&E) stain, magnification × 250; calibration bar = 0.01 mm (10 µm) for all photomicrographs. The Control group showed a normal cerebellar cytoarchitecture characterized by distinct molecular, Purkinje cell, and granular layers. The Haloperidol-alone group exhibited distortion of the cerebellar cytoarchitecture characterized by gliosis and disruption of the Purkinje cell layer. The Haloperidol + Daflon (50 mg/kg) group showed mild distortion of the Purkinje cell layer accompanied by gliosis and cell loss. The Haloperidol + Daflon (100 mg/kg) group demonstrated mild structural alterations of the Purkinje cell layer with localized gliosis. The Haloperidol + levodopa/carbidopa (10 mg/kg) group showed mild distortion of the Purkinje cell layer characterized by minor perineuronal vacuolation.

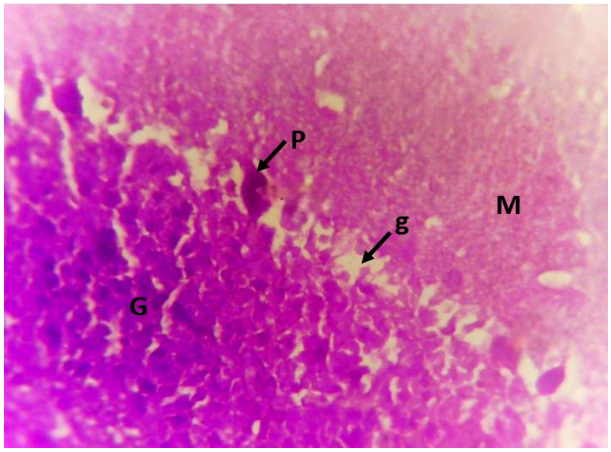
Control



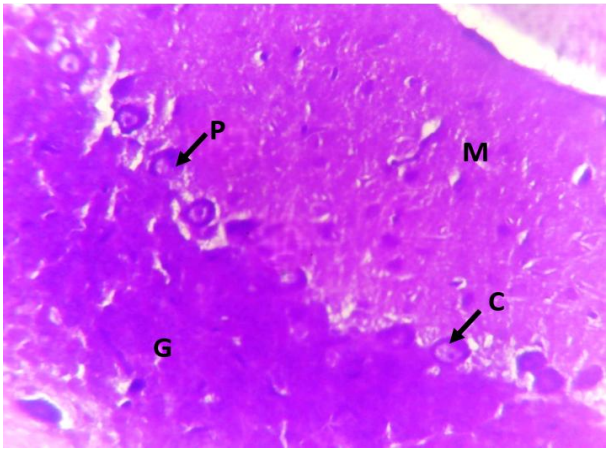
Haloperidol-alone



Haloperidol + Daflon 50 mg/kg



Haloperidol + Daflon 100 mg/kg



Haloperidol + levodopa/carbidopa (10 mg/kg)

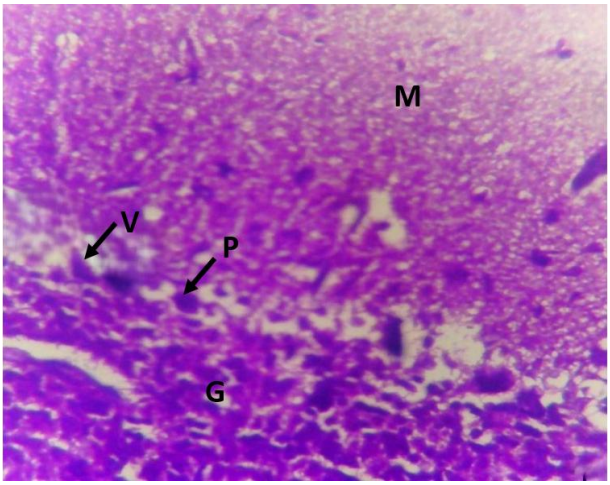


Figure 10. Representative photomicrographs showing the histochemical appearance of the cerebellar cortex among experimental groups. M: molecular layer; P: Purkinje cell layer; G: granular layer; g: gliosis; C: chromatolysis; V: perineuronal vacuolation. Cresyl Fast Violet (CFV) stain, magnification × 250; calibration bar = 0.01 mm (10 μm) for all photomicrographs. The Control group showed a normal cerebellar cytoarchitecture with intensely stained neurons indicative of preserved Nissl substance. The Haloperidol-alone group exhibited reduced Nissl substance staining intensity accompanied by mild distortion of the Purkinje cell layer, gliosis, and perineuronal vacuolation. The Haloperidol + Daflon (50 mg/kg) group showed moderate Nissl substance staining intensity with mild distortion of the Purkinje cell layer and gliosis. The Haloperidol + Daflon (100 mg/kg) group demonstrated increased Nissl substance staining intensity accompanied by mild distortion of the Purkinje cell layer and chromatolysis. The Haloperidol + levodopa/carbidopa (10 mg/kg) group showed intense Nissl substance staining comparable to the control group with mild distortion of the Purkinje cell layer characterized by perineuronal vacuolation.

Discussion

This investigation evaluated the neuroprotective potential of Daflon in mitigating haloperidol-mediated motor and cognitive impairments in mice with particular emphasis on oxidative stress,

neuroinflammatory alterations, and cerebellar histomorphology. The findings demonstrated that haloperidol challenge elicited pronounced behavioral dysfunction, evidenced by suppressed exploratory movement and compromised spontaneous alternation performance, alongside significant depletion in intrinsic antioxidant reserves, augmented membrane lipid damage, elevated pro-inflammatory cytokines, and disruption of cerebellar histoarchitecture. Daflon co-treatment substantially ameliorated these alterations, suggesting that its neuroprotective effects may stem from the attenuation of oxidative injury along with neuroinflammation.

In the present study, mice treated with haloperidol displayed significant reduction in motor performance and impairment in spatial working memory. This was demonstrated by the marked reduction in line crossings and spontaneous alternation in the open-field and Y-maze tests respectively. The observed motor and cognitive deficits are consistent with haloperidol's side effects associated with dopamine D2 receptor blockade, particularly in regions such as the substantia nigra, striatum, caudate nucleus, and hippocampus [9, 13, 37]. Similar behavioural impairments have been reported in experimental studies investigating haloperidol-induced neurotoxicity, where it resulted in catalepsy, hypolocomotion, motor incoordination and cognitive dysfunction [9, 11, 38].

The significant improvement in locomotor activity and spatial working memory observed following Daflon administration suggests that the flavonoid formulation possess protective influence on motor and cognitive functions, by possibly preserving neuronal integrity and synaptic function. The improvements observed following treatment with Daflon agrees with previous reports indicating Daflon or its constituent could reverse motor impairments in animal models of haloperidol-induced motor impairments and neurotoxicity [28, 39, 40]. The improvements may also be attributed to Daflon's capacity to enhance microcirculation, consistent with its pharmacological roles in stabilizing microvessels, limiting capillary permeability, and improving tissue perfusion [41-43]. It may as well

be due to its antioxidant and anti-inflammatory properties [20-23], in line with growing evidence that flavonoid-based therapies may reduce motor and cognitive deficits. Flavonoids have been shown to improve cognitive performance through modulation of neuroinflammatory signalling, enhancement of cerebral blood flow, and suppression of oxidative stress-mediated neuronal injury [44]. The present findings extend these observations by demonstrating that Daflon may attenuate haloperidol-induced cognitive dysfunction within a combined behavioural and biochemical framework.

Oxidative stress appears to represent a major mechanistic pathway underlying haloperidol neurotoxic effects [8, 14, 15]. In this study, haloperidol significantly reduced endogenous activity of catalase (CAT), superoxide dismutase (SOD), alongside reduced glutathione (GSH) concentrations, accompanied by a parallel rise in malondialdehyde (MDA) concentration, indicating excessive lipid peroxidation and impaired antioxidant defense capacity. Such observations align with previous literature demonstrating how haloperidol metabolism promotes free radical generation and oxidative neuronal injury through increased dopamine turnover as well as impaired mitochondrial function [6, 8]. This marked restoration of antioxidant enzyme activity and reduction in MDA levels following Daflon co-treatment are consistent with antioxidant-mediated mechanisms. Previous investigations have shown that micronised purified flavonoid fraction can suppress reactive oxygen species generation and improve endogenous antioxidant status in inflammatory conditions [26, 28, 45]. Daflon's ability to improve oxidative balance supports its potential as a neuroprotective agent because it addresses one of the central mechanisms driving haloperidol-induced motor and cognitive impairments as well as its neurotoxic effects. Although the data support oxidative modulation, contributions from pharmacokinetic interactions cannot be excluded.

In addition to oxidative stress, neuroinflammation likely contributed substantially to the behavioural and histological alterations observed in this study. Mice treated with only haloperidol exhibited

significantly elevated Tumor Necrosis Factor Alpha and Interleukin 1 Beta levels, signifying stimulation of pro-inflammatory pathways. Persistent elevation of these cytokines has been implicated in neuronal degeneration, synaptic dysfunction, and impairment of neuroplasticity [46-48]. Experimental evidence suggests that oxidative stress and neuroinflammation often interact synergistically, with reactive oxygen species promoting cytokine release while inflammatory mediators further exacerbate oxidative injury [49, 50]. The decline in levels of TNF-α and IL-1β following Daflon administration suggests suppression of inflammatory signalling pathways, possibly through inhibition of microglial activation and cytokine-mediated neuronal damage. These outcomes agree with previous reports indicating the actions of Daflon's flavonoid constituents, diosmin and hesperidin, which have shown capacity to inhibit signalling pathways involved in lowering the production of proinflammatory cytokines [51, 52]. The simultaneous improvement in behavioural performance and the reduction in cytokines levels observed in this study suggest that Daflon may contribute to observed behavioral improvements associated with haloperidol exposure. The findings further emphasizes the close relationship between neuroinflammation and neurobehavioral dysfunction.

This widespread neurochemical disruption observed in this study correlates directly with the localized tissue damage observed in our histological assessments. Histomorphological evaluation revealed distorted cerebellar architecture in mice treated with haloperidol alone, consistent with an earlier report indicating that haloperidol induces cell death and structural alterations such as gliosis in the cerebral cortex of rats [10]. Although the cerebellum has traditionally been associated with motor coordination, emerging evidence indicates its significant contribution to cognitive and emotional processing [18, 19, 53, 54]. The increased oxidative stress and elevations in the levels of pro-inflammatory cytokines detected in the whole-brain homogenates observed in our findings provides the metabolic background that possibly explains the distorted cerebellar architecture, gliosis, and

Purkinje cell layer disruption observed under light microscopy. The oxidative stress and inflammatory injury within cerebellar circuits may therefore contribute to both motor and cognitive impairments associated with antipsychotic-induced neurotoxicity.

Daflon's ability to maintain intense CFV staining and reduce perineuronal vacuolation suggests preservation of neuronal Nissl substance of the Purkinje cells of the cerebellar cortex, which is essential for smooth motor execution. Our findings aligns with the recent report of Fidelis *et al*. [55], indicating that Daflon can attenuate cerebellar neurotoxicity. The preservation of cerebellar histoarchitecture observed following Daflon treatment supports the biochemical and behavioural findings obtained in this study and suggests that the flavonoid formulation may protect against structural neuronal degeneration. Relatively few studies have examined cerebellar involvement in haloperidol-induced neurotoxicity; thus, the present findings contribute additional evidence suggesting the cerebellum as a vulnerable target of antipsychotic-associated oxidative injury.

Long-term antipsychotic therapy remains essential in the management of schizophrenia and related psychotic disorders, yet neurological adverse effects frequently compromise treatment adherence and quality of life. The present study suggests that flavonoid-based interventions such as Daflon may provide adjunctive neuroprotective benefits capable of minimizing oxidative and inflammatory complications associated with haloperidol exposure. Given the established peripheral clinical safety profile of Daflon in vascular disorders, it may warrant further preclinical investigation for potential adjunctive use. The study also reinforces the growing therapeutic relevance of antioxidants and anti-inflammatory strategies in mitigating drug-induced neurotoxicity.

Though the findings of this study provide evidence of the neuroprotective capacity of Daflon, certain methodological boundaries must be acknowledged. The experimental design utilized a relatively acute treatment duration and did not assess long-term neurobehavioral outcomes following prolonged

haloperidol exposure. Molecular cascades associated with apoptosis, mitochondrial dysfunction, and microglial activation were not directly investigated via Western blotting or real-time PCR. Histological assessment was qualitative, and the absence of a quantitative morphometric evaluation or digital image scoring is a notable study limitation that introduces potential subjectivity. Also, only male mice were included in the experimental design, making it difficult to determine possible sex-dependent differences in the neuroprotective response. Future studies should expand upon these initial findings by incorporating chronic exposure models, quantitative immunohistochemical profiling, and explicit mechanistic analyses across both sexes to fully map the molecular signaling pathways associated with Daflon-mediated neuroprotection.

Conclusion

This study demonstrates the neuroprotective capacity of Daflon against haloperidol-induced neurobehavioral, biochemical, and cerebellar alterations. While haloperidol exposure caused significant motor and cognitive impairment, oxidative stress, neuroinflammation, and localized cerebellar structural distortions, co-treatment with Daflon significantly attenuated these behavioral, biochemical, and histological alterations. Notably, the 100 mg/kg dose exhibited the most pronounced protective effects. These findings suggest that Daflon's neuroprotective effects are driven by its ability to modulate key pathways involved in oxidative stress and neuroinflammation, while also demonstrating that Daflon is a promising candidate that could help attenuate antipsychotic-induced neural injury. However, further molecular investigations are warranted to fully elucidate its underlying mechanisms.

Author contributions

SS.: Conceptualisation, Writing-review & editing, writing-original draft, visualisation, methodology, resources, formal analysis, data curation, conceptualisation. **CHC:** Methodology and data curation.

IKW: Methodology and data curation. **JCM:** Methodology and data curation. **LDA:** Review & editing, methodology and data curation. **AMD**: Conceptualisation, review & editing, supervision, methodology & validation.

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Data availability

The datasets that support the findings of this study are accessible from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All animal procedures were approved by the Research Ethics Review Committee of Bingham University, Karu, Nigeria (approval number. BHU/ERC/25/A003). All procedures complied with institutional animal care requirements and the National Research Council Guide for the Care and Use of Laboratory Animals and ARRIVE 2.0 reporting recommendations.

Competing interests

The authors declare no competing interests.

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