Received: 10 October 2025 | Accepted: 27 December 2025 Nig J Neurosci 16(4):144-151.2025

Nigerian Journal of Neuroscience

https://njn.neurosociety.org.ng

DOI: 10.47081/njn2025.16.4/003

Original Article https://ojshostng.com/index.php/NJN Open Access

Ameliorative Effects of Micronised Purified Flavonoid Fraction on Haloperidol-Induced Motor Impairments and Oxidative Stress in Female Wistar Rats

Saminu Samaila[1], Pennan V. Timothy[2], Oluwatobi A. Kayode[2], Iyomo K. Williams[2], Confidence H. Christopher[1], Joshua C. Michael[2], Angela M. Danborno[2]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Federal University Wukari. Nigeria.
[2]Department of Physiology, Faculty of Basic Medical Sciences, Bingham University, Karu, Nigeria.

ABSTRACT

Limited treatment options exist for drug-induced Parkinsonism, often linked to haloperidol. Micronised purified flavonoid fraction (MPFF) has antioxidant properties. However, its effects on haloperidol-induced Parkinsonism are not well established. This study examines MPFF's capacity to mitigate haloperidol-induced motor impairment and oxidative stress. Twenty female Wistar rats were randomly assigned to four groups: haloperidol (1 mg/kg, intraperitoneally), MPFF (100 mg/kg, orally), haloperidol + MPFF (1 mg/kg, intraperitoneally + 100 mg/kg, orally), and control (normal saline, 2 mL/kg, orally). The treatment lasted for 14 days, followed by tests for catalepsy and hang wire performance, as well as biochemical assays for superoxide dismutase (SOD), malondialdehyde (MDA), dopamine, and acetylcholinesterase (AChE). Haloperidol significantly ($p < 0.01$) increased catalepsy latency and reduced hang wire latency ($p < 0.01$). It also decreased SOD and dopamine levels ($p < 0.05$) and raised MDA levels ($p < 0.05$). Co-treatment with MPFF reduced catalepsy latency at 120 min ($p < 0.01$) and improved hang wire performance in both the MPFF-alone ($p < 0.01$) and co-treatment groups ($p < 0.05$). MPFF enhanced SOD activity in both the MPFF-alone ($p < 0.01$) and co-treatment groups ($p < 0.05$) and lowered MDA in the co-treatment group ($p < 0.05$). Dopamine levels increased in the MPFF-alone and co-treatment groups ($p < 0.05$). When compared to the haloperidol-only group, the co-treatment group had higher AChE activity ($p < 0.05$). In conclusion, MPFF reduced oxidative stress and haloperidol-induced motor impairments in female Wistar rats, supporting its potential as a protective agent against haloperidol-induced Parkinsonism.

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Keywords

Haloperidol, Micronszed purified flavonoid fraction, Catalepsy, Oxidative stress, Motor impairments

Correspondence: Saminu Samaila, MSc, Department of Human Physiology, Faculty of Basic Medical Sciences, Federal University Wukari, Nigeria. Email: samailasaminu@fuwukari.edu.ng; Phone Number -:+2348032812902; ORCID - 0000-0002-9548-2844
Pennan V. Timothy - timothyvictorious55@gmail.com, 0009-0008-9454-5605; Oluwatobi A. Kayode - oluwatobikayode2004@gmail.com, 0009-0005-6462-3017; Iyomo K. Williams - kayodeiyiomo@gmail.com, 0009-0009-3886-3278; Confidence H. Christopher - confidence@fuwukari.edu.ng, 0009-0004-5079-1856; Joshua C. Michael - joshuamichaelch@gmail.com, 0009-0003-1348-4913; Angela M. Danborno - angeladanborno@yahoo.com, 0000-0001-7868-7170

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

INTRODUCTION

Haloperidol, a first-generation antipsychotic drug, has long been prescribed for conditions such as schizophrenia, acute mania, hyperactivity, and severe agitation, owing to its potent antagonism of dopamine D_2 receptors (Rafiq *et al*., 2022; Rahman and Marwaha, 2023). Despite its therapeutic efficacy, long-term use is often complicated by extrapyramidal symptoms and tardive dyskinesia, which may become irreversible (Datta *et al*., 2016; Guzen *et al*., 2019). Drug-induced Parkinsonism (DIP) is another common adverse effect of dopamine-receptor-blocking agents like haloperidol, particularly in older adults (Shin and Chung, 2012). Clinically, DIP can closely resemble idiopathic Parkinson's disease and may perilously remain after withdrawal, necessitating immediate

prevention and care (Shin and Chung, 2012; Vaiman *et al*., 2022).

Haloperidol-induced motor impairment is not limited to the dopaminergic D_2-blockade. Oxidative stress, mitochondrial dysfunction and neuroinflammation are known to be the major contributors of neuronal injury and impaired motor functions associated with it (Xiao *et al*., 2022; Vaiman *et al*., 2022). Specifically, haloperidol has been found to weaken the body's natural antioxidant defences and cause lipid peroxidation in critical areas like the frontal cortex, hippocampus, and striatum (Parikh *et al*., 2003; Valvassori *et al*., 2021). Essentially, this oxidative imbalance does not just trigger the damage caused by haloperidol but actually speeds it up. This shows that oxidative imbalance acts within the disease process itself and contributes to neuronal vulnerability, which further aggravates antipsychotic-related neurotoxicity by sustaining dopaminergic dysfunction.

Antioxidant-based strategies have shown promise in alleviating haloperidol-related behavioural and biochemical disturbances in rodents (Perera *et al*., 2011; Valvassori *et al*., 2021). However, many of the agents tested so far show quite a bit of variability, and only a small number have been systematically examined for effects on both motor function and markers of oxidative stress (Forman and Zhang, 2021). This points to a need for multifunctional compounds with antioxidant, anti-inflammatory, and neuromodulatory properties.

Flavonoids, a diverse class of polyphenolic phytochemicals, have demonstrated such properties and are increasingly recognised for their neuroprotective potential across models of neurodegeneration and toxicity (Minocha *et al*., 2022; Bellavite *et al*., 2023). Micronised purified flavonoid fraction (MPFF), commonly referred to as Daflon, is an oral formulation that contains mostly micronised diosmin (~90%) and hesperidin (~10%). This formulation was developed to improve bioavailability, and in practice this is usually cited as the primary reason for micronisation (Abdel-Rafei *et al*., 2016; Attia, 2018). From a clinical perspective, MPFF is prescribed for a range of vascular-related conditions. Venous insufficiency is the most common indication, although haemorrhoids and lymphatic disorders are also frequently reported in routine clinical use (Abdel-Salam *et al*., 2012; Kobo *et al*., 2014). Beyond these indications, attention has increasingly focused on the biological effects of its main components (diosmin and hesperidin), which have shown protective effects across several pathological settings, including oxidative stress, vascular injury, diabetes, and neurodegeneration (Islam *et al*., 2020; Mirzaei *et al*., 2023). Experimental studies further demonstrate their ability to enhance antioxidant enzyme activity, reduce lipid peroxidation, and improve nerve function (Huwait and Mobashir, 2022; Wójciak *et al*., 2022; Roy *et al*., 2023; Gölboyu *et al*., 2024; Banaderi *et al*., 2024). Taken together, these findings raise the hypothesis that MPFF may counteract haloperidol-induced motor impairments through antioxidant-mediated preservation of dopaminergic function and neural integrity.

According to reports from the Institute of Medicine (US) Forum on Neuroscience and Nervous System Disorders (2011), sex differences play an important role in drug responses. Still, this factor is often acknowledged without being fully integrated into experimental design or interpretation. Oestrogen, for example, exerts neuroprotective effects on dopaminergic neurons, partly by modulating glial activity, reducing oxidative stress, and promoting neuroprotective gene expression. These mechanisms appear to shield female brains from nigrostriatal injury in preclinical models (Numakawa *et al*., 2011; Villa *et al*., 2016; Cattaneo and Pagonabarraga, 2025). Such findings like these draw attention to the use of female subjects in experimental studies. With this in mind, the present study focused on investigating the effects of MPFF on haloperidol-induced motor impairments and oxidative stress in female Wistar rats.

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MATERIALS AND METHODS

Experimental Animals

Twenty female Wistar rats (120–150 g) were used in this study. The animals were obtained from the Animal Care Unit of Bingham University, Nigeria, and maintained under standard laboratory conditions in plastic cages with sawdust bedding. They had free access to vital feed and water throughout the study. Ethical approval for this study was obtained from the Ethics Review Committee of Bingham University, Karu. The study received ethical approval number BHU/ERC/25/A003. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Drug Preparation

MPFF /Daflon® 500 mg (Servier Egypt Industries Limited) and Haldol®-Janssen 5 mg/mL haloperidol injection BP (Annygod Pharma Co., Ltd) were obtained from Alpha Pharmacy & Stores Ltd, Lagos, Nigeria. The two drugs were prepared separately and were not mixed. Each drug was first dissolved in normal saline (0.9% w/v) to make a stock solution, and the appropriate doses were then calculated from this solution before administration. Stock solutions were prepared in advance and stored under appropriate conditions, and aliquots were taken for daily administration.

Experimental Design

The rats were divided into four groups of five animals (n = 5). The control animals were given normal saline, 2 mL/kg, by oral route. Another group got haloperidol alone, 1 mg/kg, by intraperitoneal injection (Waku *et al*., 2021). A third group received only MPFF at 100 mg/kg, given orally (Abdel-Rafei *et al*., 2016). The fourth group received both haloperidol (1 mg/kg, intraperitoneally) and MPFF (100 mg/kg, orally). Treatments were given once a day for 14 days, with normal saline used as the vehicle.

Neurobehavioural Assessments

These tests were conducted 24 h after the last administration. Before the commencement of the behavioural

assessments, the rats were acclimatised to the test room for 30 min to minimise stress.

Catalepsy Bar Test

The catalepsy bar test was conducted following protocols previously described by Sanberg *et al*. (1988) and Ali and Rajini (2016). In this procedure, each rat was initially habituated to a rigid bar for 30 s by placing its front paws on the bar. Following this habituation period, haloperidol-induced catalepsy was tested and assessed for each rat at 30, 60, and 120-min intervals using the standard bar test. Each rat was positioned so that its hindquarters remained on the bench or work area while its forelimbs (forepaws) rested on a metallic horizontal bar, 1 cm in diameter, placed 6 cm above the bench or work area. A stopwatch was used to record how long the rats maintained this position (the total time the rats took to remove both forepaws from the bar or move their heads in an exploratory manner or climb over the bar using their hind limbs) was noted, and the average time was calculated from three consecutive trials with 1-min intervals between trials (by averaging the results of these trials, the variability in data induced by stress during handling was reduced compared to relying on a single trial for determination). A cutoff time of 120 s was applied. After the observation period, the rats were removed and returned to their housing cage. The bar and base where the rats were tested were cleaned using a cotton ball soaked in 70% ethanol and dried before introducing another animal, to eliminate possible bias due to odours left by the previous animal.

Hanging Wire Test

The hanging wire test was carried out essentially as described by Nishitani *et al*. (2020). The apparatus for this study was made of a metallic wire, about 2.5 mm in diameter and 50 cm long, stretched between two poles, each 30 cm high. The wire was clamped securely and hung roughly 50 cm above the base, positioned between two retort stands or poles. To cushion any fall, foam bedding was placed at the bottom of the setup. Each rat was gently grasped by the tail and allowed to firmly grip the wire at the centre with its forepaws, such that its body weight was suspended on the wire. The time (in seconds) before the rat falls was recorded (the latency to fall). A cutoff time of 120 s was applied. After each rat's trial, the wire was cleaned with 70% ethanol to maintain cleanliness and eliminate any potential confounding factors.

Animal Sacrifice and Sample Collection

Twenty-four hours after completing the neurobehavioural assessments, animals were anaesthetised with ketamine hydrochloride (90 mg/kg, intraperitoneally) and xylazine hydrochloride (10 mg/kg, intraperitoneally). Adequate anaesthesia was checked by confirming the absence of reflex responses, and once this was established, euthanasia was carried out via cervical dislocation. Brains were rapidly excised, rinsed in ice-cold phosphate-buffered saline (PBS; 0.01 M, pH 7.4), and kept on ice for subsequent biochemical analyses.

Tissue Preparation for Biochemical Assays

Brain tissues were first weighed and then homogenised in ice-cold phosphate buffer (0.01 M, pH 7.4) at a ratio of 1 g of tissue to 9 mL of buffer, making a 10% w/v suspension. The tissues were kept cold during homogenisation to help preserve enzyme activity. Then, the homogenates were spun at 1,000 rpm for 5 min at 4 °C. The supernatants that formed on top were collected for later study. Aliquots were stored at –20 °C until further use. The supernatant fractions were employed for assays of dopamine, acetylcholinesterase activity, and oxidative stress biomarkers.

Biochemical Assays

Superoxide dismutase (SOD) activity, malondialdehyde (MDA) concentrations, dopamine levels, and acetylcholinesterase (AChE) activity were determined using commercially available assay kits (Elabscience® and Oxford Biomedical Research) strictly according to the manufacturers' instructions. SOD activity was assayed with a WST-1-based colorimetric kit (Elabscience®, Cat. No: E-BC-K020-M), MDA levels were quantified using the TBARS assay kit (Oxford Biomedical Research, Cat. No: FR40), concentrations of dopamine were determined by a competitive ELISA kit (Elabscience®, Cat. No: E-EL-0046), and AChE activity was evaluated using a colorimetric kit (Elabscience®, Cat. No: E-BC-K174-M). All results were normalised to protein concentration, and absorbance readings were obtained with a microplate reader at the wavelengths recommended for each assay.

Statistical Analysis

All values are presented as mean ± SEM. Behavioural outcomes were analysed using one-way or two-way ANOVA as appropriate, followed by Tukey's post hoc test for multiple comparisons. Biochemical parameters were analysed using one-way ANOVA with Tukey's post hoc. Statistical significance was set at $p < 0.05$. Analyses were conducted using GraphPad Prism software version 10.

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RESULTS

Effect of MPFF on Haloperidol-Induced Catalepsy

As illustrated in Figure 1, haloperidol treatment produced a significant increase in descent latency across all time points compared with the control group. At 30 min, haloperidol-treated rats recorded a latency of 10.82 ± 1.66 s versus 0.53 ± 0.25 s in controls ($p < 0.01$). The effect persisted at 60 min (17.22 ± 1.84 s vs 0.60 ± 0.17 s, $p < 0.01$) and 120 min (19.86 ± 1.75 s vs 1.23 ± 0.41 s, $p < 0.01$). MPFF alone did not alter descent latency relative to controls, with values of 0.64 ± 0.25 s, 1.28 ± 0.25 s, and 3.84 ± 1.03 s at 30, 60, and 120 min, respectively. Co-administration of MPFF with haloperidol reduced descent latencies to 13.74 ± 0.98 s at 30 min, 13.84 ± 1.96 s at 60 min, and 12.53 ± 1.52 s at 120 min, with the reduction at 120 min reaching statistical significance compared with haloperidol alone ($p < 0.01$).

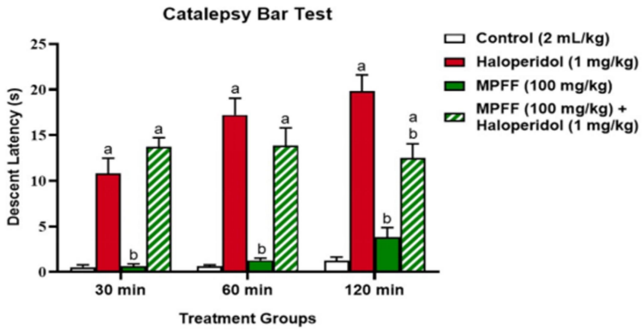

Fig. 1: Effect of MPFF on catalepsy in female Wistar rats with haloperidol-induced motor dysfunction and oxidative stress. Data are presented as mean ± SEM (n = 5). a - $p < 0.01$ vs control group; b - $p < 0.01$ vs haloperidol group.

Effect of MPFF on Neuromuscular Strength

As shown in Figure 2, haloperidol significantly reduced hanging time in the hang-wire test, with rats maintaining suspension for 41.40 ± 2.19 s compared with 56.04 ± 1.70 s in controls ($p < 0.01$). Rats treated with MPFF alone stayed on the bar for 57.18 ± 3.05 s, noticeably longer than the haloperidol group ($p < 0.01$) and roughly similar to controls. When MPFF was administered along with haloperidol, the hanging time improved to 52.61 ± 2.25 s. This was higher than haloperidol alone ($p < 0.05$), although it still fell a little short of the control group's performance.

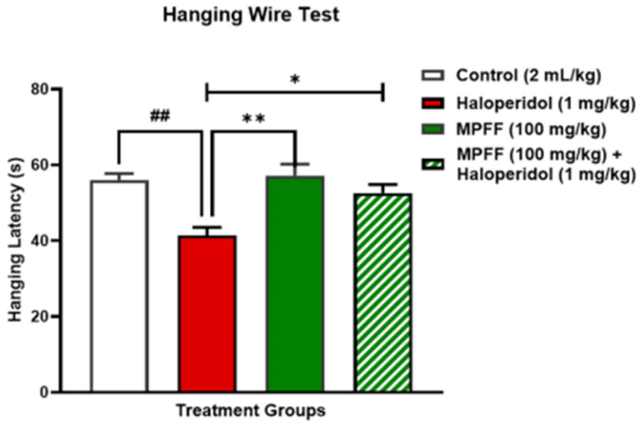

Fig. 2: Effect of MPFF on neuromuscular strength in female Wistar rats with haloperidol-induced motor dysfunction and oxidative stress. Data are presented as mean ± SEM (n = 5). ## - $p < 0.01$ vs control group; * - $p < 0.05$, ** - $p < 0.01$ vs haloperidol group.

Effect of MPFF on Oxidative Stress Markers

The effects on SOD activity and MDA levels are presented in Fig. 3A and 3B. Controls exhibited SOD activity of 0.027 ± 0.0023 U/mg protein. Haloperidol reduced this activity to 0.014 ± 0.0026 U/mg protein ($p < 0.05$). MPFF alone significantly increased SOD activity to 0.031 ± 0.0030 U/mg protein ($p < 0.01$ vs haloperidol, $p < 0.05$ vs control). Co-treatment also enhanced activity, reaching 0.028 ± 0.0038 U/mg protein, which was significantly greater than haloperidol ($p < 0.05$). For lipid peroxidation, MDA concentrations were 1.22 ± 0.10 µM in the control group. Haloperidol elevated MDA to 1.59 ± 0.08 µM ($p < 0.05$). MPFF alone produced 1.29 ± 0.07 µM, which did not differ significantly from controls. Co-treatment significantly reduced MDA to 1.24 ± 0.07 µM, lower than haloperidol ($p < 0.05$).

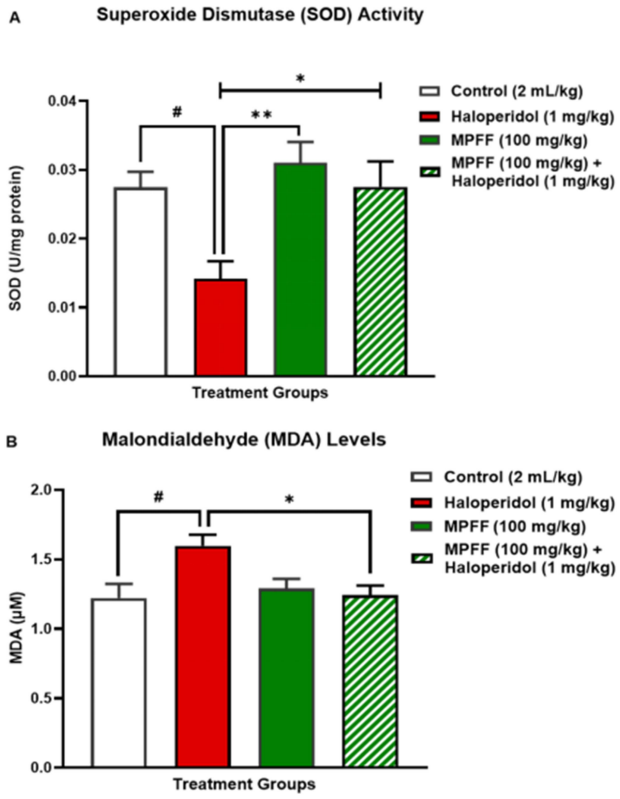

Fig. 3A and 3B: Effect of MPFF on oxidative stress biomarkers in brain tissue homogenates. (A) Superoxide dismutase (SOD) levels and (B) Malondialdehyde (MDA) levels. Data are presented as mean ± SEM (n = 5). # - $p < 0.05$ vs control group; * - $p < 0.05$, ** - $p < 0.01$ vs haloperidol group.

Effect of MPFF on Dopamine Concentrations

As depicted in Figure 4, dopamine concentrations in the control group were 92.36 ± 2.91 pg/mL. Haloperidol significantly reduced dopamine to 82.17 ± 2.79 pg/mL ($p < 0.05$). MPFF alone kept levels at 93.93 ± 2.27 pg/mL, which was substantially higher than haloperidol ($p < 0.05$) and about the same as the control. Dopamine levels rose to 94.68 ± 1.69 pg/mL when MPFF and haloperidol were administered together. This was a little higher than when haloperidol was given alone ($p < 0.05$).

Effect of MPFF on AChE Activity

The effect on AChE activity is shown Figure 5. Haloperidol reduced enzyme activity relative to saline (0.0022 ± 0.00033 vs 0.0033 ± 0.00028 U/mg protein). MPFF alone had no significant effect (0.0029 ± 0.00026 U/mg protein), whereas co-administration increased AChE activity

to 0.0035 ± 0.00027 U/mg protein, significantly higher than haloperidol alone ($p < 0.05$).

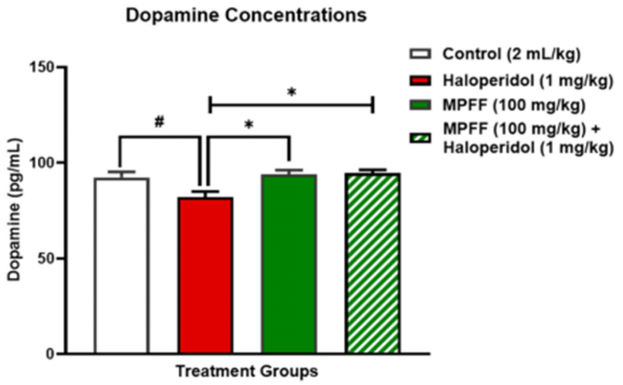

Fig. 4: Effect of MPFF on dopamine concentrations in brain tissue homogenates of female Wistar rats with haloperidol-induced motor dysfunction and oxidative stress. Data are presented as mean ± SEM (n = 5). # - $p < 0.05$ vs control group; * - $p < 0.05$ vs haloperidol group.

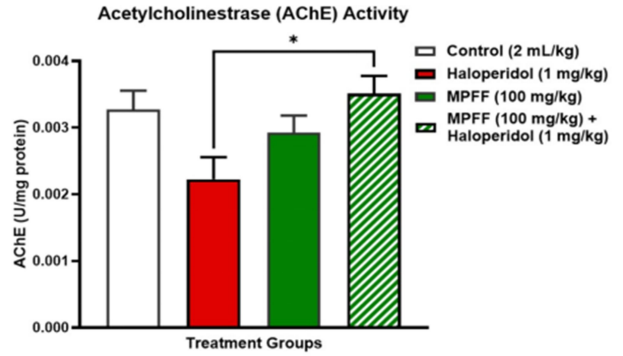

Fig. 5: Effect of MPFF on acetylcholinesterase (AChE) activity in brain tissue homogenates of female Wistar rats with haloperidol-induced motor dysfunction and oxidative stress. Data are presented as mean ± SEM (n = 5). * - $p < 0.05$ vs haloperidol group.

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DISCUSSION

The potential of MPFF, a combination of diosmin and hesperidin to counteract haloperidol-induced motor impairments and oxidative stress markers was investigated in this study. As shown in previous research studies, haloperidol is often used to model drug-induced Parkinsonism and catalepsy (Waku *et al*., 2021). It is well known to cause motor impairments in rodents through dopamine D_2 receptor blockade, and in this study, its administration led to the expected catalepsy and reduced neuromuscular performance. These behavioural changes were accompanied by decreased dopamine levels, reduced antioxidant activity, and elevated lipid peroxidation in brain tissue. The present findings are consistent with earlier reports showing that haloperidol induces oxidative stress and dopaminergic dysfunction, which together contribute to drug-induced Parkinsonism (Perera *et al*., 2011; Valvassori *et al*., 2021; da Silva *et al*., 2024).

Administration of MPFF was able to mitigate several of these effects. Rats that received MPFF alone performed at levels comparable to controls in both the catalepsy and hang-wire tests, implying that the flavonoid mixture on its own does not appear to compromise normal motor function. When MPFF was given together with haloperidol, a reduction in motor impairment was observed, reflected by shorter descent latency and better hang-wire performance compared to haloperidol treatment alone. The observed effects suggest a possible protective action of MPFF against haloperidol-related motor deficits. Similar patterns of improvement have been described for hesperidin, particularly in toxin-based models of Parkinson's disease (Antunes *et al*., 2021). This finding aligns with earlier observations that antioxidant compounds or agents that can preserve dopaminergic tone may also reduce haloperidol-driven immobility (Adedeji *et al*., 2014).

The biochemical findings offer some indication of what may be driving these effects. Exposure to haloperidol was associated with higher MDA concentrations alongside a reduction in SOD activity, changes that are generally taken to reflect an increase in oxidative stress. This pattern is consistent with previous reports showing that antipsychotic drugs, particularly typical agents such as haloperidol, can promote free radical generation and, at the same time, compromise endogenous antioxidant defences (Raudenska *et al*., 2013; Valvassori *et al*., 2021; Zamani *et al*., 2022). In contrast, administration of MPFF to the animals significantly elevated SOD activity and reduced MDA levels. Diosmin and hesperidin, the main components of MPFF, have been shown in several studies to restore antioxidant enzyme activity and decrease lipid peroxidation (Huwait and Mobashir, 2022; Wójciak *et al*., 2022). The same antioxidant mechanisms may likely have contributed to the improved behavioural outcomes observed in this study.

The MPFF-treated animals also had normal levels of dopamine. This result is particularly significant, as the depletion of dopamine is fundamental to the pathogenesis of extrapyramidal symptoms. Flavonoids, including hesperidin, have been demonstrated to inhibit dopaminergic neuronal degeneration and alleviate mitochondrial dysfunction and apoptosis in 6-OHDA models (Antunes *et al*., 2021). The findings from this study suggest that MPFF may exert a comparable protective effect, possibly by reducing oxidative stress and helping to preserve mitochondrial function. That said, the use of whole-brain homogenates limits regional interpretation, and further work focusing on vulnerable areas such as the striatum and substantia nigra would be needed to confirm this effect.

Haloperidol is known to affect cholinergic pathways in the basal ganglia, although the effects are complex. In certain instances, it can modify AChE activity, thereby influencing cholinergic tone and resulting in extrapyramidal symptoms (da Silva *et al*., 2024). In line with this, haloperidol treatment in the present study led to a reduction in AChE activity in brain homogenates, an effect that was only partially reversed with MPFF administration. Disturbances in the balance between cholinergic and

dopaminergic activity are thought to play an important role in the development of Parkinsonian symptoms. This is because cholinergic signalling is tightly coupled to motor control (Bohnen and Albin, 2011; da Silva *et al*., 2024). The partial restoration of AChE activity with MPFF may reflect either a secondary normalisation of cholinergic-dopaminergic balance after dopamine preservation or a direct modulatory action of flavonoids on cholinergic enzymes reported in other models (Antunes *et al*., 2016; Bellavite, 2023), supporting this interpretation. The modest recovery of AChE activity may indicate an improvement in cholinergic homeostasis within motor circuits, which could, at least in part, contribute to the observed behavioural improvements.

An additional consideration in interpreting these findings relates to sex as a biological variable. Systematic analyses document a persistent under-representation of females in preclinical studies and a continuing shortfall in sex-based analysis of results (Allegra *et al*., 2023). Most preclinical studies on haloperidol-induced motor dysfunction have been conducted in male Wistar rats, with female subjects under-represented (Waku *et al*., 2021). By contrast, the present study employed female Wistar rats, thereby addressing an important gap. Sex hormones, particularly oestrogen, are known to influence nigrostriatal dopamine pathways, oxidative stress responses, and cholinergic balance, which may alter the severity of Parkinsonian features and the efficacy of neuroprotective interventions (Gillies *et al*., 2014; Cattaneo and Pagonabarraga, 2025). Oestrogen has demonstrated neuroprotective effects via antioxidant mechanisms, modulation of dopaminergic transmission, and regulation of mitochondrial function (Numakawa *et al*., 2011; Villa *et al*., 2016). As a result, the female hormonal environment may influence both haloperidol-induced pathology and the preventive effects of MPFF.

Collectively, the results suggest that MPFF may provide some protection against haloperidol-induced motor and biochemical disturbances. The improvements in behaviour, preservation of dopamine, and modulation of oxidative stress markers point towards an antioxidant and neurotransmitter-preserving action of the flavonoid mixture.

Conclusion

In conclusion, haloperidol caused noticeable motor impairments and altered several biochemical markers in rats. Treatment with MPFF appeared to lessen these motor impairments and brought some of the biochemical changes, including oxidative stress markers, dopamine, and AChE activity, closer to normal levels. These suggest that MPFF has neuroprotective potential against drug-induced Parkinsonism, possibly through antioxidant actions and some preservation of neurotransmitter balance.

DECLARATION

Acknowledgement

We gratefully acknowledge the technical support of Mr Jude Stephen Sankara from the animal care unit and other laboratory technicians of the Department of Physiology, Bingham University, Karu, Nigeria.

Grants and Financial Support

Not Applicable.

Conflict of Interest

None declared.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Review Committee of Bingham University, Karu. The study received ethical approval number BHU/ERC/25/A003.

Authors' Contribution

SS: conceptualisation, methodology, supervision, data analysis and writing (original draft); PVT: resources, methodology and data acquisition; OAK: resources, methodology and data acquisition; IKW: supervision, validation, visualisation, writing (review and editing); CHC: methodology and data acquisition; JCM: methodology and data acquisition; AMD: conceptualisation, resources, supervision, validation, visualisation, methodology, writing (review and editing).

Consent to Participate and Publish Data

Not Applicable.

The Use of Generative Artificial Intelligence

Not Applicable.

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The research was presented at the 22nd Conference of the Neuroscience Society of Nigeria at Enugu

Published by Neuroscience Society of Nigeria