

N-Acetylcysteine Attenuates Haloperidol-Induced Neurobehavioural, Oxidative, Inflammatory, and Cerebellar Histopathological Alterations in Wistar Rats

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Abstract

Haloperidol, a widely used first-generation antipsychotic, has been associated with neurotoxic effects within the central nervous system, including the cerebellum. However, the neuroprotective potential of N-acetylcysteine (NAC) against haloperidol-induced cerebellar toxicity remains insufficiently characterised. This study investigated the neurobehavioural, biochemical, and histomorphological effects of NAC in a rat model of haloperidol-induced cerebellar neurotoxicity. Sixty Wistar rats were randomly assigned to six groups ($n = 10$): control (distilled water), NAC-treated groups (500 and 1000 mg/kg), haloperidol (1 mg/kg), and haloperidol plus NAC (500 and 1000 mg/kg). Treatments were administered for 21 days, followed by behavioural assessment on day 22 using the open-field, catalepsy and elevated plus-maze tests. Animals were subsequently euthanised for biochemical and histological assessment of the cerebellar cortex. Haloperidol significantly reduced body weight, feed intake, and locomotor activity and increased catalepsy and anxiety-like behaviour. Haloperidol exposure was also associated with increased malondialdehyde, IL-6, IL-1 β , TNF- α , and acetylcholine levels, together with reduced total antioxidant capacity and IL-10. Histological assessment revealed marked disruption of cerebellar architecture, including Purkinje cell loss and reduced neuronal density. Co-administration of NAC attenuated the haloperidol-associated neurobehavioural, biochemical, and histomorphological alterations, with the 1000 mg/kg dose generally producing greater attenuation than the 500 mg/kg dose. These findings indicate that NAC may confer neuroprotective effects against haloperidol-associated cerebellar toxicity, potentially through modulation of oxidative and inflammatory disturbances. Further mechanistic studies are warranted to establish the pathways underlying these effects.

KEYWORDS: Antipsychotic, Neurobehavioural, Haloperidol, N-acetylcysteine, Antioxidant, Anti-Inflammatory

1. Introduction

Neurotoxicity refers to the capacity of physical, chemical, or biological agents to adversely affect the structure or function of the nervous system [1]. It occurs when the nervous system is exposed to neurotoxic agents, whether naturally occurring or synthetic, that interfere with normal neuronal function [2]. Neurotoxicity may result from exposure to environmental

pollutants, heavy metals, pharmaceutical agents, or endogenous toxins. Drug-induced neurotoxicity specifically describes adverse effects of pharmacological agents on the nervous system [3] and may manifest as cognitive impairment, motor dysfunction, sensory disturbances, or, in severe cases, persistent neuronal injury [4]. Among pharmacological agents implicated in neurological adverse effects, haloperidol has received

considerable attention because of its extensive clinical use and well-established association with extrapyramidal motor disturbances.

Haloperidol is a first-generation antipsychotic and potent dopamine D2 receptor antagonist widely used in the management of schizophrenia, Tourette's disorder, Huntington's chorea, and intractable hiccups [5]. Its strong D2 receptor antagonism underlies its therapeutic efficacy but also contributes to its relatively high propensity to induce extrapyramidal symptoms [6]. Clinical exposure to haloperidol has been associated with neurological adverse effects including parkinsonism, akathisia, dystonia, tardive dyskinesia, and other motor disturbances [6,7]. These effects are primarily attributable to dopamine receptor blockade. However, experimental evidence suggests that prolonged haloperidol exposure may additionally promote oxidative stress, neuronal alterations, and other forms of central nervous system injury [7]. It is therefore important to distinguish the established pharmacological consequences of D2 receptor antagonism from experimental evidence of potential structural and biochemical neurotoxicity.

The cerebellum warrants particular consideration because of its essential role in motor coordination, balance, posture, and movement, as well as its contribution to motor learning and aspects of cognitive processing [8]. Its highly organised cytoarchitecture, characterised by distinct Purkinje cell and granular layers, provides a useful anatomical framework for evaluating neuronal and tissue alterations [8]. Although the neurological effects of haloperidol have been extensively investigated, evidence specifically concerning its effects on cerebellar structure remains comparatively limited. Experimental reports of oxidative and neurodegenerative changes following chronic haloperidol exposure provide a rationale for investigating potential alterations in cerebellar structure and function [5,7]. Nevertheless, cerebellar neurotoxicity should be considered an experimental concern rather than an established clinical consequence of therapeutic haloperidol use.

N-acetylcysteine (NAC) is a thiol-containing compound and precursor of glutathione that has been used clinically since the 1960s [9]. It is included on the WHO Model List of Essential Medicines as an antidote for poisoning [10] and is widely used as a mucolytic agent and in the treatment of paracetamol overdose and respiratory disorders [9]. Its potential neuroprotective properties have been attributed principally to its capacity to replenish glutathione and strengthen endogenous antioxidant defences, modulate inflammatory responses, and protect cells against oxidative and toxic insults [11,12]. NAC may also access the central nervous system [13] and has been investigated in experimental models of neurological injury. In addition, its potential to preserve mitochondrial function may contribute to neuronal protection under conditions of oxidative stress [14]. These properties provide a plausible rationale for investigating

NAC as a potential attenuating agent against the biochemical and structural alterations associated with haloperidol exposure. However, evidence for NAC-mediated neuroprotection varies across experimental conditions, and its effects on haloperidol-associated cerebellar alterations remain insufficiently characterised.

Chronic haloperidol administration has been associated experimentally with oxidative stress and neurodegenerative alterations within the central nervous system, with potential consequences for motor and cognitive function [15]. Although both NAC and haloperidol have been investigated in relation to antioxidant, inflammatory, and neurological processes [16], limited studies have examined the effects of NAC on haloperidol-associated cerebellar alterations using an integrated assessment of neurobehavioural, biochemical, and histomorphological outcomes. Therefore, the present study investigated the effects of NAC on haloperidol-associated neurobehavioural, biochemical, and cerebellar histomorphological alterations in Wistar rats. We hypothesised that NAC administration would attenuate the behavioural, biochemical, and structural alterations associated with haloperidol exposure.

2. Materials and Methods

2.1. Chemicals and Drugs

Haloperidol (Janssen Pharmaceuticals, Belgium), N-acetylcysteine (Swanson Health Products, Fargo, North Dakota, USA), distilled water, and assay kits for lipid peroxidation (malondialdehyde), interleukin-10, interleukin-1 β , interleukin-6, tumour necrosis factor- α (TNF- α), and total antioxidant capacity (Biovision Inc., Milpitas, CA, USA) were used.

2.2 Animals

Sixty healthy male Wistar rats, weighing 100–150 g, were obtained from Empire Breeders, Osogbo, Osun State, Nigeria. Animals were housed in wooden cages (20 $\times$ 10 $\times$ 12 inches) at room temperature (25°C $\pm$ 2.5°C) lights on and off at 7:00 am at 7 pm, respectively at the animal house, Stadium area, Ogbomoso, and acclimatised for two weeks with free access to standard rodent chow (Top Feeds®, Nigeria; 29% protein, 11% fat, 58% carbohydrate) and water. All procedures complied with the European Council Directive (EU2010/63) on the care and use of animals for scientific purposes.

2.3 Experimental Methods

Animals were randomized after acclimatization and assigned to six groups (n = 10). Group A (control) received distilled water (5 ml/kg, i.p.) only for 21 days; Groups B and C received NAC (500 and 1000 mg/kg, orally, respectively) with distilled water (5ml/kg, i.p) for 21 days; Group D received haloperidol alone (1 mg/kg, i.p.) for 21 days, which is a Parkinsonism-like disease model [17]; Groups E and F received haloperidol (1 mg/kg, i.p.) combined with NAC (500 and 1000 mg/kg, orally, respectively) for 21 days. All animals received distilled water as vehicle, with volume equivalent to the control group. Haloperidol was

administered by intraperitoneal injection one hour before oral gavage administration of NAC, following previously described protocols [17, 18]. Animals were subjected to behavioural paradigms including the open field, elevated plus maze, and Y-maze following the treatment period. Rats were sacrificed by cervical dislocation twenty-four hours after behavioural testing, and blood taken via an intracardiac puncture was used to assess lipid peroxidation (by assaying malondialdehyde levels), inflammatory cytokines (interleukin (IL)- 6, 10 and 1 β), and Total antioxidant capacity. Rat brains were removed, fixed in 10% formol-calcium, and slices of the cerebellum were processed, embedded in paraffin, and stained with haematoxylin and eosin, and Cresyl fast violet for general histological study.

2.4 Body Weight and Feed Intake

Body weight and feed intake were assessed weekly and daily, respectively, using an electronic weighing balance (Mettler Toledo Type BD6000, Greifensee, Switzerland), as described by Erdenebayar et al [19].

$$\frac{\text{Final body weight (or feed intake)} - \text{Initial body weight (or feed intake)}}{\text{Initial body weight (or feed intake)}} (g)$$

2.5 Behavioural Testing

Behavioural assessments were conducted on day 22 under blinded conditions, with the observer unaware of the treatment-group assignments throughout the behavioural testing procedures as described by Onaolapo et al [20].

2.5.1 Open field test

The open field test was conducted according to the method described by Leite-Almeida et al [21]. The apparatus consisted of a square arena (36 × 36 × 26 cm) enclosed with transparent glass walls and a hard floor divided into 16 equal squares. Each rat was individually placed at the centre of the arena and allowed to explore freely for 10 minutes. During the observation period, total horizontal locomotion (number of squares crossed with all four limbs), rearing frequency (number of times the animal stood on its hind limbs), and grooming behaviour (number of body-cleaning episodes using the forepaws) were recorded. To avoid olfactory cues and prevent cross-contamination, the apparatus was thoroughly cleaned with 70% ethanol before the introduction of each subsequent rat.

2.5.2 Catalepsy Bar Test

The animal's forepaws were placed on a horizontal bar as high as 20-25cm, and the amount of time it took them to move out of this unusual conformation and put both paws on the ground was recorded [22]. A cataleptic rat was expected to continue holding onto the bar for a prolonged period of time as opposed to a normal rat [22].

2.5.3 Elevated plus maze. Time spent and entries into open versus closed arms were recorded over 5 minutes as indices of anxiety-related behaviour [23].

2.6 Biochemical Assays

On day 23, animals were sacrificed by cervical dislocation [24] and blood collected by intra-cardiac puncture. Serum was separated by centrifugation (3500 rpm, 10 minutes) for assay of inflammatory markers (TNF- α , IL-10, IL-6, IL-1 β), oxidative stress indices (MDA, TAC), and acetylcholine activity. Lipid peroxidation was assessed as MDA-equivalent TBARS, with absorbance read at 532 nm [25, 26]. Total antioxidant capacity was determined by the ABTS method, with results expressed as mEq Trolox®/L (27). Inflammatory cytokines (IL-1 β , IL-6, IL-10, TNF- α) were quantified by ELISA techniques using commercial kits (Enzo Life Sciences Inc., NY, USA) [27].

2.7 Tissue Histology

Brains were fixed in 10% buffered formol-calcium, and cerebellar sections were processed, embedded in paraffin, cut at 5 μ m, and stained with haematoxylin and eosin, and cresyl fast violet, following standard protocols [28- 30].

2.8 Photomicrography and Histomorphometric Analysis

Stained sections were examined and photographed using a trinocular microscope (Olympus XSZ-107E) with an attached digital camera, with evaluation performed blind to group assignment. Quantitative image analysis (cellular density, mean cellular area, stained area fraction, vacuolation index, optical density, and texture entropy) was performed using ImageJ following calibrated thresholding and segmentation, as described by Gao and Vali [31].

2.9 Statistical Analysis

Data were analysed by both one-way and two-way ANOVA using Chris Rorden's ezANOVA (version 0.98), with the two-way ANOVA used to derive the weekly change in body weight. Results were expressed as mean $\pm$ S.E.M., with $p < 0.05$ considered statistically significant.

3. Result

3.1 Effect of N-acetylcysteine (NAC) on Body Weight

Figure 1 shows the effect of NAC on relative change in body weight of rats with haloperidol-induced neurotoxicity. There was a significant ($p < 0.05$) increase in the body weight of rats administered NAC 500 mg/kg and NAC 1000 mg/kg, and a significant decrease in body weights of rats administered HAL, when compared to control group. Also, body weight increased significantly in rats administered HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg, when compared to the haloperidol control.

3.2 Effect of N-acetylcysteine (NAC) on Feed Intake

Figure 2 shows the effect of NAC on relative change in feed intake in rats with haloperidol-induced neurotoxicity. There was a significant ($p < 0.05$) increase in feed intake of rats administered NAC 1000MG, when compared to control group, and significant ($p < 0.05$) decrease in groups administered HAL, when compared to CTRL group. Also, feed intake increased significantly ($p < 0.05$) in both HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg, when compared to HAL group.

3.3 Effect of N-acetylcysteine (NAC) on Open-field Line-Crossing

Figure 3 displays the effect of NAC on horizontal locomotor activity in rats with haloperidol-induced neurotoxicity. There was a significant ($p < 0.05$) decrease in line crossing of the HAL group, when compared to control (CTRL). Also, the line crossing frequency increased significantly ($p < 0.05$) in HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg groups, when compared to HAL control.

3.4 Effect of N-acetylcysteine (NAC) on Rearing Activity in the Open field

Figure 4 shows the effect of NAC on rearing activity in rats with haloperidol-induced neurotoxicity. There was significant ($p < 0.05$) decrease in HAL and NAC 1000 mg/kg groups, when compared to CTRL group. There was also significant ($p < 0.05$) increase in HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg groups, when compared to HAL group.

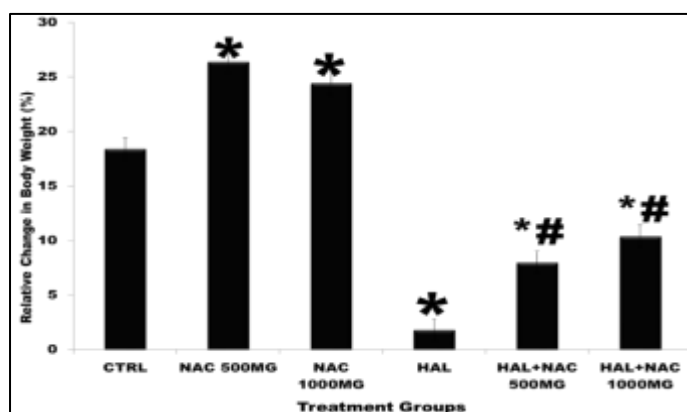


Figure 1: Effect of NAC on relative change in body weight of Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs. CTRL, # $p < 0.05$ significant difference from HAL, no. of rats per treatment group =10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

3.5 Effect of NAC on Open-field Self-Grooming Behaviour

Figure 5 shows the effect of NAC on self-grooming behaviour in rats with haloperidol-induced neurotoxicity. There was significant ($p < 0.05$) decrease in HAL when compared to CTRL group. Also, there was visible but no significant ($p < 0.05$) increase in the self-grooming of HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg groups, when compared to the HAL group.

3.6 Effect of N-acetylcysteine (NAC) on Catalepsy

Figure 6 shows the effect of NAC on catalepsy of haloperidol-induced rats using the catalepsy bar test. There was significant ($p < 0.05$) increase in HAL group, when compared to CTRL group. There was also significant ($p < 0.05$) decrease in HAL+NAC 1000 mg/kg, when compared to the HAL group.

3.7 Effect of N-acetylcysteine on Open- and Closed-Arm Time

Figure 7 shows the effect of NAC on the time spent in the open arm of the elevated plus maze apparatus by rats with haloperidol-induced neurotoxicity. There was a significant ($p < 0.05$) decrease in open arm times of HAL group, when compared to CTRL group. There was also significant ($p < 0.05$) increase in open arm times of HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg groups, when compared to the HAL group. Figure 8 shows the effect of NAC on the time spent in the closed arm of the elevated plus maze apparatus by rats with haloperidol-induced neurotoxicity. There was a significant ($p < 0.05$) increase in closed arm times of the HAL group, when compared to the CTRL group. There was also significant ($p < 0.05$) decrease in open arm times of HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg, when compared to the HAL group.

3.8 Effect of NAC on oxidative Stress markers and Acetylcholine levels

Table 1 shows the effects of NAC on malondialdehyde (MDA), total antioxidant capacity (TAC) and Acetylcholine levels in rats with haloperidol-induced neurotoxicity. Lipid peroxidation measured as MDA concentration increased significantly ($p < 0.05$) with HAL compared to CTRL group. However, the MDA concentration decreased significantly ($p < 0.05$) with HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg, when compared to the HAL group. Also, TAC levels measured significant ($p < 0.05$) decrease with HAL group compared to CTRL group. Compared to HAL, TAC levels increased significantly ($p < 0.05$) with both HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg.

Acetylcholine levels decreased significantly ($p < 0.05$) with NAC 500 mg/kg and NAC 1000 mg/kg groups, when compared to CTRL group. There was also significant ($p < 0.05$) decrease with HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg groups compared to the HAL group.

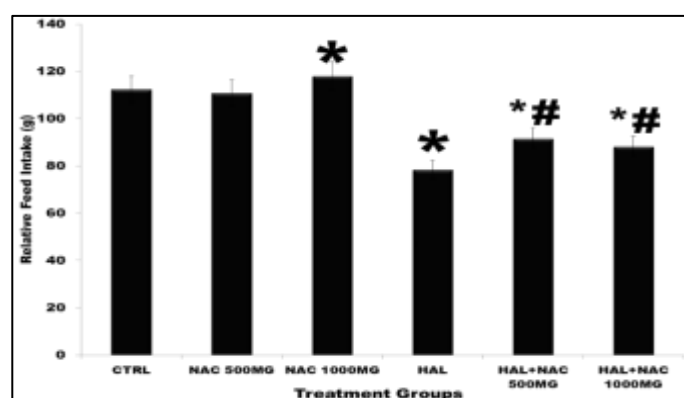


Figure 2: Effect of N-acetylcysteine (NAC) on relative change in feed intake of Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs. CTRL, # $p < 0.05$ significant difference from HAL, no. of rats per treatment group =10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

3.9 Effect of N-acetylcysteine (NAC) on inflammatory markers

Table 2 shows the effects of NAC on anti-inflammatory cytokine levels in rats with haloperidol-induced neurotoxicity. IL-10 level had a significant ($p < 0.05$) decrease in HAL group compared to CTRL group. It also had significant ($p < 0.05$) increase in HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg, when compared to the HAL group. For pro-inflammatory cytokines such as IL-6, IL-1 β and TNF- α levels, there was a significant ($p < 0.05$) increase with HAL compared to CTRL group. Also, there was significant ($p < 0.05$) decrease with HAL+NAC 500 mg/kg and HAL+NAC 1000 mg/kg compared to the HAL group.

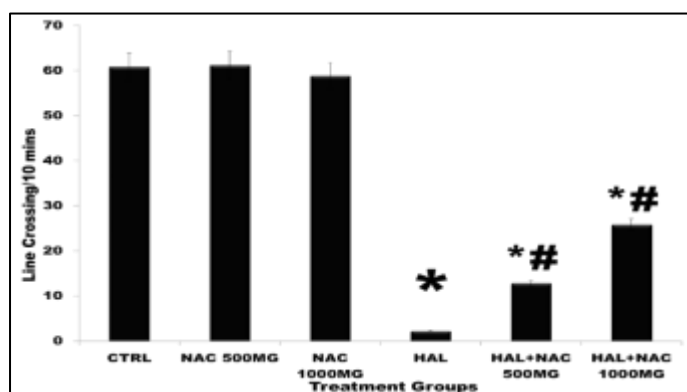


Figure 3: Effect of NAC on line crossing in Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs. CTRL, # $p < 0.05$ significant difference from HAL, no. of rats per treatment group =10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

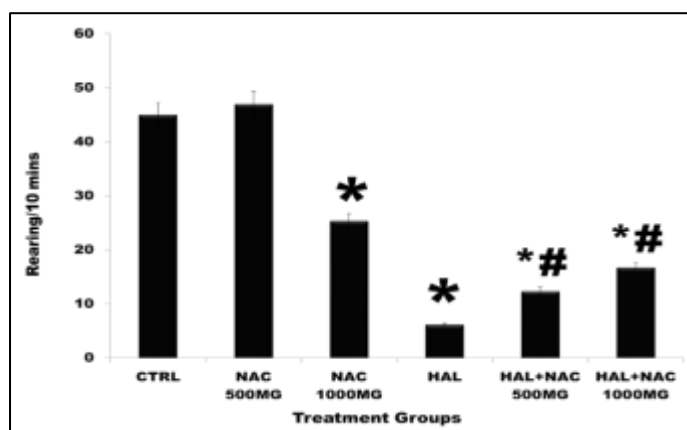


Figure 4: Effect of NAC on rearing in Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ vs. CTRL, # $p < 0.05$ significant difference from HAL, no. of rats per treatment group =10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

3.10 Effect of N-acetylcysteine (NAC) on Cerebellar Cortex Histomorphology

Plate 1 (A–F) shows the effect of NAC on the histomorphology of the cerebellar cortex following haloperidol administration. haematoxylin and eosin-stained sections of the control group (1A) revealed preserved cerebellar cytoarchitecture, with an intact molecular layer, Purkinje cell layer, and granular layer, well-aligned Purkinje cells, and compactly arranged granule

cells. NAC alone at 500 and 1000 mg/kg (1B, 1C) showed cortical organization comparable to control, with preserved Purkinje cell alignment and densely packed granule cells. The haloperidol group (1D) showed disrupted Purkinje cell arrangement, reduced neuronal compactness, mild vacuolar degeneration, and a less compact granular layer. In the HAL+NAC500 mg/kg group (1E), degenerative changes were more pronounced than in the haloperidol-only group, with marked loss of cellular compactness, increased vacuolation, and distorted Purkinje cell alignment. The HAL+NAC1000 group (1F), however, showed improved preservation of Purkinje cell distribution and cerebellar cortical lamination relative to group E. Cresyl fast violet-stained sections (Plate 2, A–F) showed a similar pattern. Control (2A) and NAC-alone groups (2B, 2C) demonstrated intact molecular, Purkinje, and granular layers, with well-defined Purkinje cells showing intense Nissl uptake and densely packed granule cells. The haloperidol group (2D) showed reduced neuronal compactness, altered Purkinje cell morphology, sparse Purkinje cell distribution, and disrupted Nissl substance integrity. Co-treatment with NAC attenuated these changes in a dose-dependent manner: the HAL+NAC500 group (2E) showed only partial preservation, with moderate restoration of Purkinje cell alignment and granular layer compactness, while the HAL+NAC1000 mg/kg group (2F) showed more marked preservation of neuronal organization, Purkinje cell integrity, and Nissl staining intensity than groups D and E. Overall, haloperidol induced cerebellar neurodegenerative changes that were mitigated by NAC in a dose-dependent manner, with the higher dose (1000 mg/kg) affording the greater protection of cerebellar cortical architecture.

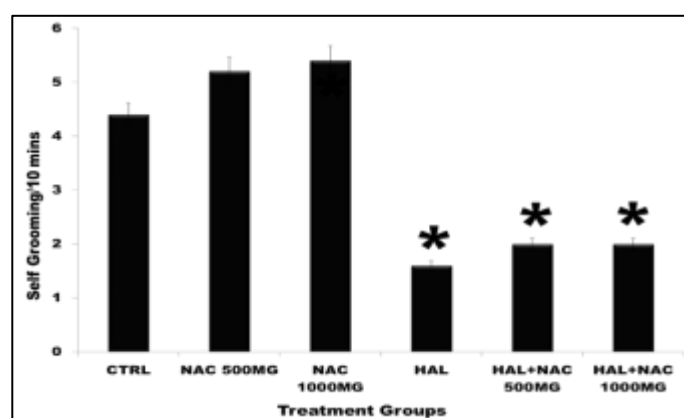


Figure 5: Effect of NAC on self-grooming behaviour of Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M, * $p < 0.05$ significant difference from CTRL, no. of rats per treatment group =10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

3.11 Advanced quantitative histomorphometric analyses of H&E-stained cerebellar cortex sections

Quantitative image analysis of H&E-stained cerebellar sections revealed significant alterations in several histomorphometric

parameters following treatment (Table 3). The control, NAC 500 mg/kg, and NAC 1000 mg/kg groups showed relatively comparable total cell counts of 109.0 ± 11.12 , 107.0 ± 7.85 , and 104.0 ± 10.22 , respectively. Compared with the control group, NAC 1000 mg/kg significantly increased vacuolation (9.50 ± 1.11), optical density (0.155 ± 0.01), and layer thickness ($290.27 \pm 4.12 \mu\text{m}$), while NAC 500 mg/kg significantly increased layer thickness to $285.00 \pm 3.10 \mu\text{m}$. Haloperidol administration produced a significant reduction in total cell count to 38.0 ± 6.21 , accompanied by increased vacuolation (15.60 ± 1.65), optical density (0.100 ± 0.01), and layer thickness ($336.64 \pm 5.11 \mu\text{m}$) compared with the control group, indicating marked alterations in cerebellar morphology.

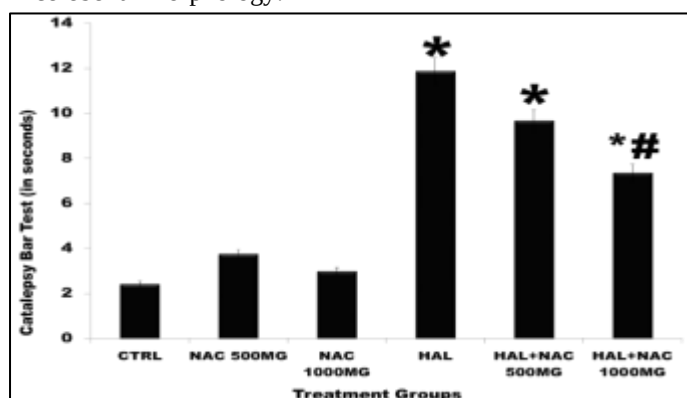


Figure 6: Effect of NAC on cataleptic behaviour of Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M., * $p < 0.05$ vs. CTRL, # $p < 0.05$ significant difference from HAL, no. of rats per treatment group = 10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

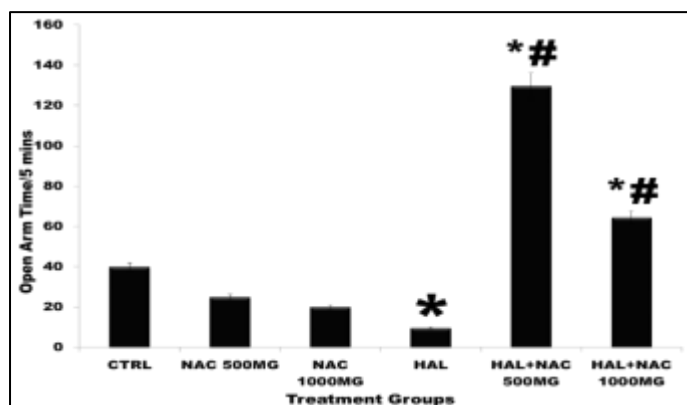


Figure 7: Effect of NAC on open arm time of Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M., * $p < 0.05$ vs. CTRL, # $p < 0.05$ significant difference from HAL, number of rats per treatment group = 10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

Co-treatment with NAC significantly attenuated several of the haloperidol-associated changes. The HAL + NAC 500 mg/kg group showed an increase in total cell count to 100.0 ± 9.23 and a reduction in layer thickness to $304.00 \pm 4.10 \mu\text{m}$ compared with the HAL group, although vacuolation remained significantly elevated at 13.20 ± 1.23 . Similarly, HAL + NAC 1000 mg/kg increased total cell count to 106.0 ± 10.11 and reduced layer thickness to $299.27 \pm 3.10 \mu\text{m}$ relative to HAL, with vacuolation also reduced to 11.76 ± 1.20 . The 1000 mg/kg

NAC co-treatment therefore produced greater restoration of total cell count and layer thickness toward control values, while both NAC doses attenuated the morphological alterations associated with haloperidol exposure.

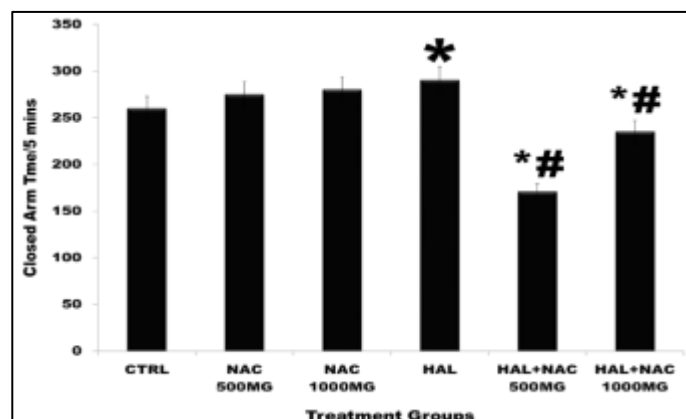


Figure 8: Effect of NAC on closed arm time of Wistar rats with haloperidol-induced neurotoxicity. Each bar represents Mean $\pm$ S.E.M., * $p < 0.05$ vs. CTRL, # $p < 0.05$ significant difference from HAL, no. of rats per treatment group = 10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

Table 1: Effects of N-acetylcysteine (NAC) on Oxidative Stress Markers

Groups	MDA (μM)	TAC (MM Trolox Equivalence)	ACH(mM)
CTRL	0.68 $\pm$ 0.01	13.98 $\pm$ 1.35	0.45 $\pm$ 0.01
NAC 500	0.67 $\pm$ 0.01	18.65 $\pm$ 2.62*	0.83 $\pm$ 0.05*
NAC 1000	0.65 $\pm$ 0.01	14.45 $\pm$ 1.35	1.01 $\pm$ 0.03*
HAL	0.82 $\pm$ 0.02*	8.76 $\pm$ 0.35*	2.42 $\pm$ 0.09*
HAL+NAC 500	0.77 $\pm$ 0.02*#	11.99 $\pm$ 0.87#	0.90 $\pm$ 0.01*#
HAL+NAC 1000	0.75 $\pm$ 0.02*#	13.94 $\pm$ 0.28#	0.85 $\pm$ 0.01*#

Values are presented as Mean $\pm$ S.E.M., * $p < 0.05$ significant difference from control. # $p < 0.05$ significant difference from HAL, number of rats per treatment group = 10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

Table 2. Effects of N-acetylcysteine (NAC) on inflammatory cytokines

GROUPS	IL-10 (pg/ml)	IL-6 (pg/ml)	IL-1 β (pg/ml)	TNF- α (pg/ml)
CTRL	15.12 $\pm$ 0.61	5.68 $\pm$ 0.09	4.56 $\pm$ 0.5	32.06 $\pm$ 0.61
NAC 500MG	24.49 $\pm$ 0.45*	4.83 $\pm$ 0.24	4.93 $\pm$ 0.26	31.18 $\pm$ 2.9
NAC 1000MG	24.75 $\pm$ 0.21*	4.09 $\pm$ 0.15*	3.99 $\pm$ 0.3	30.86 $\pm$ 0.08
HAL	1.12 $\pm$ 0.14*	12.78 $\pm$ 0.22*	11.78 $\pm$ 0.47*	48.36 $\pm$ 1.34*
HAL+NAC 500MG	8.54 $\pm$ 2.56*#	3.90 $\pm$ 0.75*#	5.38 $\pm$ 0.1*#	35.92 $\pm$ 0.24*#
HAL+NAC 1000MG	10.78 $\pm$ 0.41*#	3.06 $\pm$ 0.17*#	5.16 $\pm$ 0.02*#	33.21 $\pm$ 1.29#

Values are presented as Mean $\pm$ S.E.M., * $p < 0.05$ significant difference from control. # $p < 0.05$ significant difference from HAL, number of rats per treatment group = 10. CTRL: Control; HAL: Haloperidol; NAC: N-acetylcysteine.

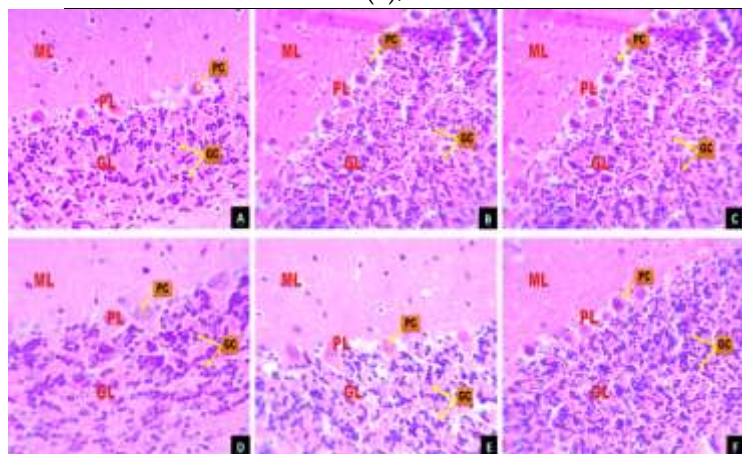


Plate 1: Photomicrograph of haematoxylin and eosin stained cerebellar cortex section of rats with haloperidol-induced neurotoxicity (x400 Magnification). ML: molecular layer; PL: Purkinje layer; PC: Purkinje cell; GL: granular layer; GC: granular cells.

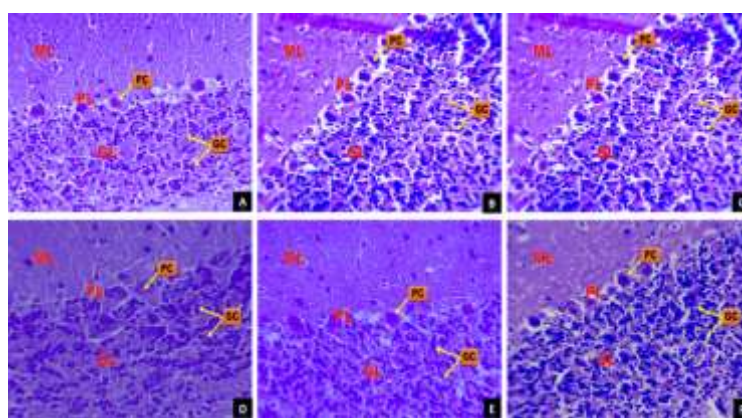


Plate 2: Photomicrograph of CFV-stained cerebellar cortex sections of rats with haloperidol-induced neurotoxicity (x400 magnification). ML: molecular layer; PL: Purkinje layer; PC: Purkinje cell; GL: granular layer; GC: granular cells

Discussion

This study assessed the neuroprotective effects of N-acetylcysteine (NAC) against haloperidol-induced neurotoxicity in Wistar rats, evaluating body weight, behaviour, oxidative and inflammatory markers, cholinergic activity, and cerebellar histomorphology. Overall, NAC attenuated haloperidol-induced deficits across all parameters, with greater protection consistently observed at the 1000 mg/kg dose.

Haloperidol significantly reduced body weight and feed intake, consistent with earlier reports [32, 33], likely reflecting dopamine D2 blockade-associated hyperphagia suppression [34], sedation and extrapyramidal effects limiting feed access [5], oxidative/mitochondrial impairment of energy metabolism [35], oro-pharyngeal dysphagia (36), and IL-6-mediated appetite suppression [37]. NAC alone modestly influenced feed intake and energy consumption in a dose-dependent manner, consistent with reports of NAC's role in regulating body weight and binge-related feeding [38, 39]. Co-administration with haloperidol partially restored body weight relative to the haloperidol group,

though not to control levels, suggesting a partial protective effect [40] likely mediated by NAC's antioxidant and anti-inflammatory actions and its influence on dopaminergic tone [41-43].

Haloperidol reduced locomotion, rearing, and self-grooming, and induced catalepsy and anxiogenic-like behaviour, consistent with its known pharmacology and prior findings [44-49]. These effects can be attributable to D2 receptor antagonism within the nigrostriatal pathway, which impairs motor initiation and exploratory drive [50]. Improved locomotor activity, reduced catalepsy (most notably at 1000 mg/kg), and increased open-arm exploration in the elevated plus maze, was observed with NAC, likely through glutathione-mediated antioxidant protection of dopaminergic neurons [51] and modulation of cortico-limbic glutamatergic transmission [40, 52, 53]. Self-grooming, however, remained suppressed across NAC-treated groups, indicating incomplete reversal of behavioural deficits, consistent with reports that NAC's benefits are more pronounced for locomotor than for more complex behaviours [42].

Haloperidol markedly disrupted redox and inflammatory homeostasis, increasing MDA and reducing total antioxidant capacity, consistent with its known generation of reactive oxygen species and lipid peroxidation [54, 55], alongside reduced IL-10 and elevated IL-6, IL-1 β , and TNF- α , indicative of NF- κ B-mediated neuroinflammation [56]. N-acetylcysteine reversed both patterns, consistent with previous studies, defining its role as a glutathione precursor that scavenges free radicals, stabilizes mitochondrial function, inhibits NF- κ B activation, and limits microglial activation [40, 57-59]. Administration of NAC also reduced haloperidol-elevated acetylcholine levels, suggesting restoration of dopamine-acetylcholine balance disrupted by D2 blockade [46], potentially via NAC's mitigation of oxidative interference with cholinergic signalling and acetylcholinesterase activity [60, 61].

Histologically, haloperidol induced marked cerebellar cytoarchitectural disruption distorted molecular, Purkinje, and granular layers, reduced Nissl affinity, chromatolysis, and Purkinje cell degeneration, consistent with reports linking antipsychotic exposure to oxidative stress, mitochondrial dysfunction, and neuronal degeneration [62-64]. N-acetyl cysteine treatment, particularly at 1000 mg/kg, preserved cortical architecture and restored Nissl substance, consistent with its antioxidant and glutathione-enhancing effects on neuronal survival [65]. Quantitative morphometry corroborated the observation that haloperidol reduced neuronal profile density and texture entropy, indicating neuronal loss and reduced tissue complexity [66], while altering stained area fraction and optical density, consistent with tissue rarefaction, neuronal swelling, or shrinkage-related condensation. Notably, relatively preserved Nissl positivity despite reduced neuronal density in haloperidol-treated tissue likely reflects diffuse basophilic staining of

degenerating elements rather than true metabolic preservation, underscoring the value of combining structural and metabolic stains when assessing neurodegeneration. Co-treatment with NAC attenuated these alterations dose-dependently, with the 1000 mg/kg group showing the greatest restoration of neuronal density, tissue organization, and cortical thickness relative to both the haloperidol and HAL+NAC500 groups, consistent with earlier reports of NAC's protective and antioxidant efficacy in models of neuronal injury [67,68].

Table 3: Advanced quantitative histomorphometric parameters of H&E-stained cerebellar cortex sections

GROUPS	Total Cell Count	Vacuolation %	Optical Density	Layer Thickness (µm)	Texture Entropy
CTRL	109.0 ±11.12	7.50 ±1.12	0.0048 ±0.0001	275.00 ± 2.20	6.66 ±0.21
NAC 500	107.0 ±7.85	9.00 ±1.32	0.0099 ±0.0001	285.00 ±3.10	6.97 ±0.13*
NAC 1000	104.0 ±10.22	9.50 ±1.11*	0.155 ±0.01*	290.27 ±4.12*	6.95 ±0.11*
HAL	38.0 ±6.21*	15.60 ±1.65*	0.100 ±0.01*	336.64 ±5.11*	6.09 ±0.10*
HAL+NAC 500	100.0 ±9.23#	13.20 ±1.23*#	0.135 ±0.01*	304.00 ±4.10*#	6.18 ±0.09*
HAL+NAC 1000	106.0 ±10.11#	11.76 ±1.20*#	0.143 ±0.01*	299.27 ±3.10*#	6.31 ±0.10*#

Values represent Mean ±S.E.M of histomorphometric parameters obtained from representative H&E-stained cerebellar cortex photomicrographs using calibrated digital segmentation analysis (20 µm/pixel). *p<0.05 significant difference from control. #p<0.05 significant difference from HAL, number of rats per treatment group = 10. NAC: N-acetylcysteine; HAL: Haloperidol.

Conclusion

This study demonstrates that chronic haloperidol administration is associated with cerebellar neurotoxicity in Wistar rats, characterised by altered neurobehavioural performance, disrupted redox and cholinergic homeostasis, elevated pro-inflammatory cytokines, and histoarchitectural degeneration, including Purkinje cell loss, chromatolysis, vacuolation, and reduced neuronal density. These findings support a potential contribution of oxidative stress and inflammatory dysregulation to haloperidol-associated cerebellar alterations. N-acetylcysteine, particularly at 1000 mg/kg, dose-dependently attenuated these alterations, restoring antioxidant capacity, suppressing pro-inflammatory cytokines, normalising cholinergic activity, and preserving cerebellar cytoarchitecture and Nissl integrity. The exacerbated histomorphometric changes observed at the lower NAC dose highlight the importance of dose optimisation in antioxidant-based neuroprotective strategies. Overall, these preclinical findings suggest that NAC warrants further investigation as a candidate of adjunct

neuroprotective agent against antipsychotic-induced cerebellar toxicity, therefore, clinical efficacy has not been established and cannot be inferred from this rat model.

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Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Declarations Ethics approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology (ERCFBMSLAUTECH:145/1/2026).

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