

ORIGINAL RESEARCH ARTICLE

Effects of exercise and *D*-ribose-*L*-cysteine supplements on neuroinflammation and oxidative stress in an aluminum chloride-induced rat model of Alzheimer's diseaseOluwafemi Abidemi Adedotun^{1,2*}, Babatunde Ogunlade¹,
Kingsley Afoke Iteire³, and Adebunke Faith Balogun¹¹Department of Human Anatomy, School of Basic Medical Sciences, Federal University of Technology, Akure, Ondo, Nigeria²Department of Human Anatomy, Faculty of Allied Health Sciences, Elizade University, Ilara-Mokin, Ondo, Nigeria³Department of Anatomy, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo, Nigeria

Abstract

Alzheimer's disease (AD) pathology involves several pathways, with oxidative stress and inflammation among the major drivers. This study employs an animal model to evaluate the effects of treadmill exercise and *D*-ribose-*L*-cysteine (DRLC) on aluminum chloride (AlCl₃)-induced AD. Seventy adult male Wistar rats (150–200 g) were randomized into seven groups ($n=10$), including a control and six experimental groups: (i) AlCl₃-only; (ii) AlCl₃ + DRLC; (iii) AlCl₃ + exercise; (iv) AlCl₃ + DRLC + exercise; (v) DRLC-only; and (vi) exercise-only. A modified rodent treadmill apparatus was used for the exercise regimen, and AlCl₃ and DRLC (100 mg/kg each) were prepared freshly for every administration. Y-maze and open field tests were used to evaluate the neurotoxic effects of AlCl₃ on working and spatial memory. Oxidative stress markers, neurotransmitter levels, neuroinflammatory markers, and immunohistochemical analysis were also conducted to assess the impact of AlCl₃ and the treatments on the hippocampus and prefrontal cortex. Results indicate that AlCl₃ administration led to a decrease in neurobehavioral performance, monoamine neurotransmitter levels, and antioxidant parameters, with a corresponding increase in oxidative stress and neuroinflammatory markers. The deleterious effects of AlCl₃ were also evident in the hippocampus and prefrontal cortex of the rats, contributing to AD-like pathology, as indicated by neurodegeneration. However, combined DRLC supplementation and treadmill exercise attenuated AlCl₃-induced behavioral, biochemical, and histopathological alterations, restoring several outcomes toward control levels. In conclusion, the combined approach of exercise and DRLC proved to possess multiple therapeutic benefits in relieving AD symptoms due to their neuroprotective properties.

Keywords: Alzheimer's disease; *D*-ribose-*L*-cysteine; Aluminum chloride; Treadmill exercise; Hippocampus; Prefrontal cortex

***Corresponding author:**Oluwafemi Abidemi Adedotun
(oluwafemi.adedotun@
elizadeuniversity.edu.ng)

Citation: Adedotun OA, Ogunlade B, Iteire KA, Balogun AF. Effects of exercise and *D*-ribose-*L*-cysteine supplements on neuroinflammation and oxidative stress in an aluminum chloride-induced rat model of Alzheimer's disease. *Innov Med Omics*. 2026;3(1):48-65. doi: 10.36922/IMO025460060

Received: November 12, 2025**Revised:** December 8, 2025**Accepted:** January 26, 2026**Published online:** February 12, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia and is characterized by cognitive decline, loss of independence in activities of daily living, and neurodegeneration.¹ The condition is linked with an increased risk of adverse events and death in older adults and is the primary cause of memory loss in the elderly.² According to the Alzheimer's Association, 33% of people over 65 years old will die from dementia or AD.³ Consequently, AD is a significant health concern as people age, with 81% of AD patients being 75 years of age or older. Based on the estimates made by the Alzheimer's Association,⁴ by 2050, there will be around one million new cases of AD annually, or one new diagnosis every 33 s. Depending on the stage of disease, symptoms, such as depression, apathy, difficulties in social interaction, poor decision-making, disorientation, motor and swallowing difficulties, and other behavioral changes can worsen, making daily activities increasingly challenging.⁵ Factors, such as heredity, age, and sex influence the time course over which many of these symptoms manifest.⁶

The amyloid and cholinergic hypotheses are the two primary theories proposed as causes of AD, making it a multifactorial disease. Risk factors for AD include head trauma, vascular disorders, genetic susceptibility, infections, aging, and environmental exposures.⁷ The accumulation of tau and amyloid-beta ($\text{A}\beta$) proteins is associated with the gradual cognitive deterioration observed in AD.¹ Beta- and gamma-secretases successively cleave the amyloid pre-cursor protein to create $\text{A}\beta$. Thus, when $\text{A}\beta$ aggregates, harmful oligomers that impair neurons are formed.^{8,9} Conversely, tau originates from the microtubule-associated protein tau gene by alternative splicing, which produces soluble protein isoforms.⁹ $\text{A}\beta$ and tau have been found to have several functional interactions in the damage of neuronal circuits and cognitive impairment associated with AD.⁸ Unfortunately, there is presently no recognized treatment for the condition; however, present research focuses on understanding AD pathology by addressing several mechanisms, including aberrant tau protein metabolism, inflammatory responses, β -amyloid, cholinergic damage, and free radicals, to create prominent therapies that can put an end to or alter the course of the disease.⁹

Several studies have shown a strong correlation between heavy metals and neurodegenerative diseases, such as AD.^{10,11} The onset of AD was directly linked to exposure to certain metal toxicants, such as aluminum (Al), which is absorbed by the body through occupational exposure, contaminated food, contaminated drinking water, and food cooked in Al cookware.^{12,13} Given that Al directly

affects many nervous system metabolic pathways, it is a key heavy metal linked with the onset and progression of neurodegenerative disorders.¹³ Aluminum chloride (AlCl_3), found in many commercially made products, including food, toothpaste, medications, and packaged drinking water, has a highly diverse usage.¹⁴ It ultimately accumulates in the brain and can alter the blood-brain barrier.¹⁵ Consequently, Al brain poisoning is considered a contributing factor to neurological disorders and may impair the functioning of antioxidant enzymes, potentially harming neuronal DNA and altering brain chemistry.¹⁵ In addition, Al has been demonstrated to cause oxidative stress, $\text{A}\beta$ oligomer cross-linking and accumulation, as well as plaque development in the hippocampus and cortex, areas involved with memory and executive functions.^{11,16} Consequently, the neuroprotective properties of different chemical substances and plant-based compounds against AD can be thoroughly investigated using the AlCl_3 -induced AD rat model.^{13,17}

According to Mahalakshmi *et al.*,¹⁸ neuroprotection is the general term for preventing the death of neuronal cells through intervention and inhibition of the pathogenic process that results in cellular malfunction and death. In the quest for innovative treatments that may preserve neural tissue while improving the general outcome, the scientific community has shown great interest in the prospect of neuroprotection.¹⁹ Kumar and Singh²⁰ stated several theories around AD pathogenesis, including increased phosphorylated tau and amyloid beta levels caused by age, a positive family history, $\text{A}\beta$ deposition, genetic mutation in amyloid pre-cursor protein, decreased neuronal synaptic connections, and social and cognitive involvement. In addition, hippocampal degeneration, microglial activation, cerebral energy failure, neuroinflammation, and neuronal apoptosis appear to be associated with the pathogenesis of AD.²¹ Conversely, research has indicated that there are preventable risk factors for AD, such as diabetes, high blood pressure, depression, smoking, obesity, and a sedentary lifestyle.²² Given this, physical exercise might indirectly impact other disease-related characteristics and could be one of the most effective ways to combat various neurodegenerative diseases.²³ Regular exercise improves the health and function of the brain. Human and animal studies have revealed that exercise enhances cognitive function and speeds up the healing process after an injury.²⁴ The central noradrenergic, serotonergic, and dopaminergic systems are also impacted by physical exercise.²⁵ Exercise specifically causes the brain to release certain neurotransmitters that reduce physical and mental stress. It is also one of the few techniques for generating new neurons.²⁶ Although running has attracted a lot of attention in this area of study, all forms of aerobic exercise

have also shown neuroprotective advantages.²⁷ Based on a meta-analysis by Lin *et al.*,²⁸ exercise lowered dementia risk by 28% and AD risk by 45%, and increased daily physical activity is linked to a reduced risk of AD. Furthermore, recent research indicates that one of the major prominent risk factors for individuals with AD is inactivity;²² further stating that exercise helps to improve neurogenesis, boosts cerebral blood flow, and enhances brain hippocampal and mitochondrial volumes. Therefore, exercise could improve daily living activities, minimize neuropsychiatric symptoms, and enhance cognitive performance in AD patients.² Compared to pharmacological interventions, exercise has also been shown to present fewer side effects and improve adherence.²² Consequently, exercise appears to serve as a potential non-pharmacological approach to delay or slow down AD onset.²⁹ Lu *et al.*³⁰ and Tang *et al.*³¹ reported that treadmill exercise increased angiogenesis and reduced hippocampal region neuronal apoptosis, protecting rats from cerebral ischemia.

The etiology of AD is also dependent on oxidative stress-mediated cellular damage.³² The notion that oxidative stress is instrumental in AD pathophysiology is supported by the observed upsurge in lipid peroxidation and decrease in the endogenous level of antioxidants, which are present in the disease.^{32,33} Reactive nitrogen and reactive oxygen species are typical oxidant molecules that contribute to lipid peroxidative tissue damage. These oxidant molecules then trigger a neuroinflammatory cycle, leading to the progressive degeneration of the neural pathways that control learning and memory.³⁴ However, Moreira *et al.*³³ and Mandal *et al.*³⁵ reported that glutathione (GSH) is the mammalian tissues' most significant intracellular antioxidant defense molecule, particularly in the brain, and the degree of alterations in its redox cycle appears to be correlated with the severity of any diseased condition. The neural circuits involved in the pathogenesis of AD exhibit substantial degeneration in GSH-deficient animals.³⁶ Accordingly, it has been suggested that increasing GSH levels in the brain might help protect the integrity of neurons and stop the gradual memory deterioration expressed in AD patients.³⁷ Riboceine, a synthetic antioxidant, helps cells generate glutathione when needed. Ogunlade *et al.*³⁸ describe *D*-ribose-*L*-cysteine (DRLC) as a potent antioxidant that helps cells produce glutathione when needed in the body. When cells require glutathione, ribose, which is a component of riboceine, effectively supplies and protects the delicate cysteine molecule.³⁹ DRLC is the active constituent of riboceine. Because it is derived from *L*-cysteine, a semi-essential amino acid produced from methionine, DRLC serves as a pre-cursor for GSH synthesis.⁴⁰ DRLC is a nutritional supplement designed

to provide cysteine to the cells, increasing the GSH levels within them.⁴¹ GSH exists throughout the body and plays significant antioxidative roles, but it can be readily depleted due to oxidative stress.⁴² According to reports, people with AD may have modest cognitive improvement by elevating GSH levels.^{27,37}

The present research aims to uncover the protective properties of treadmill exercise and DRLC supplementation on AlCl_3 -induced AD in rats through the suppression of neuroinflammation, oxidative stress, and other neurodegeneration markers.

2. Materials and methods

2.1. Chemicals

DRLC and AlCl_3 were procured from Sigma Chemical Corporation (United States) and dissolved in distilled water. All other chemicals used in the study were of analytical grade.

2.2. Animal care and management

Seventy adult male Wistar rats (weighing 150–200 g) were obtained from the breeding stock at the Faculty of Agriculture and Agricultural Technology, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. The rats were kept in the Animal House Laboratory, Department of Human Anatomy, FUTA. They were housed in a wire gauze cage appropriately divided into four sections and large enough to allow sufficient airflow and free movement, promoting complete aeration, with a continuous 12-h light and dark schedule at room temperature ($22 \pm 3^\circ\text{C}$) for the duration of the study. The cage's floors were cushioned by coarse sawdust and lined with pieces of carpet. The rats were acclimatized for 7 days after being fed Growers Marsh (pellets), which were bought from a feed store at Agro Feeds, FUTA. The experimental animals were used in protocols that were approved by the Center for Research and Development, FUTA, and followed the Guide for the Care and Use of Laboratory Animals (FUTA/ETH/24/147).

2.3. Experimental design

The animals were randomized into seven groups ($n=10$). AlCl_3 (100 mg/kg body weight [BW])¹¹ and DRLC (100 mg/kg BW)³⁸ were freshly prepared before each administration. A treadmill apparatus modified for rodent exercise was used for the training regimen. The treadmill utilized shock grids to motivate the animals to keep running, and the speed was set at 0.5 m/s for 10 min/day.⁴³ This protocol was selected to provide a mild, non-exhaustive aerobic exercise regimen designed to minimize stress.^{44,45} The animals were grouped as follows:

- (i) Group A was the control group.
- (ii) Group B was given 100 mg/kg BW of AlCl_3 only.
- (iii) Group C was given 100 mg/kg BW of AlCl_3 and 100 mg/kg BW of DRLC.
- (iv) Group D was given 100 mg/kg BW of AlCl_3 and 10 min of treadmill exercise at 0.5 m/s.
- (v) Group E was given 100 mg/kg BW of AlCl_3 , 100 mg/kg BW of DRLC, and 10 min of treadmill exercise at 0.5 m/s.
- (vi) Group F was given 100 mg/kg BW of DRLC only.
- (vii) Group G was given 10 min of treadmill exercise at 0.5 m/s only.

For groups receiving more than one of the compounds and/or exercise regimen, administrations were performed sequentially with a 30-min interval. All animal-related procedures in this study were approved by the Departmental Committee on the Use and Care of Animals and conducted in accordance with the *Guide to the Care and Use of Laboratory Animals* (8th Edition).⁴⁶ All pharmacological administration was done through oral gavage once daily, and the experiment spanned 45 days. After the experiment, animals were sacrificed using cervical dislocation. The brain tissues of the rats were removed, purified, and rinsed with saline (0.9% sodium chloride).

2.4. Treadmill exercise protocol

An electric treadmill machine (JDB 1370, 6.0 HP, 12 km/h; SixBros® Electric Treadmill, United Kingdom) was used as the exercise apparatus, with some modifications⁴³ using a box of acrylic boards with eight compartments for the rats to run in, and a lid to keep them from escaping. In addition, copper wires were used to line the box, serving as panel terminals. To make contact with the rat's exposed rear end, the wires were bent into the shape of a hook, passing an electric shock with a tolerable voltage (132 V, 1.2 mA) through the wires to stop the rats from hanging back during the exercise session.⁴⁷ Before the exercise, all rats were acclimated to running on a treadmill for 10 min/day at a speed of 0.5 m/s for 1 week.⁴³ The rats in Group A were used as the control, and the animals in Groups D, E, and G were routinely exercised during the experiment.⁴⁸

2.5. Behavioral parameters

Open field and Y-maze tests were performed to assess AlCl_3 -induced neurobehavioral alterations, including locomotor/exploratory activity and spatial working memory, respectively, and to determine whether treadmill exercise combined with DRLC attenuates these deficits.

2.5.1. Open field test

The open field test was conducted according to the procedures outlined by Gould *et al.*⁴⁹ A wooden box

measuring 100 cm × 100 cm with walls that are 50 cm high (open from the top) was used as the open field apparatus.^{38,49} It was housed in a separate room with standard temperature and lighting conditions. The floor was marked with square grids, each 25 cm long, using a blue marker, and a central square of the same size was outlined with a red marker. An angled video recording device (EZVIZ H9c Dual, Hikvision, China) was set up to record the rats' movements inside the box arena. On day 0, following the acclimatization period before the experiment's commencement, the rats underwent neurobehavioral training, and after the final administration, they also went through behavioral testing. To prevent stress on the rats, each group was brought to the open field arena in its cage, with care taken to ensure no disturbance. Every rat was softly taken and placed in the center square, where it could explore for 5 min while the overhead camera recorded the video. For rats in each group, this process was repeated. To eliminate the smell of the previously tested rat, methylated spirit was used to wipe the apparatus before each test. In addition, during each test, a neutral observer steered clear of the apparatus.

Upon completing the test, the observer reviewed the video and tallied the number of squares each rat had explored. Two behavioral patterns (the frequency of rearing and the total lines crossed) were recorded. The number of times the rat crossed any of the grid lines with all four paws was recorded as the number of lines crossed; the times it crossed a red line into the central square with all four paws was noted as the center square entry; the total duration spent in the center square was labeled as the center square duration; the occurrences of the rat standing on its hind legs were counted as the rearing frequency; and the frequency of the rat showing forward extension of the head and shoulders before returning to the starting position was noted as the stretch attend posture.

2.5.2. Y-maze test

The Y-maze test was carried out following the methods outlined by Kraeuter *et al.*⁵⁰ to test the rats' working and cognitive memory. On day 0, following the acclimatization period before the experiment's commencement, the rats went through neurobehavioral training, and after the final administration, they also went through behavioral testing. The Y-maze was conducted from 9 a.m. to 3 p.m. in a calm, dimly lit room. An independent observer without knowledge of the experimental protocol scored each behavioral test after it was videotaped. A 10% ethanol solution was used to clean all test equipment to eliminate any potential bias from the previous animal's odor. The animals were placed in a Y-maze with arms that were 75 cm long, 15 cm wide, and angled at 120° from one another. The rats were given 5 min to explore the maze after being

placed on a pre-determined start arm. Arm entry was scored when the hind limbs were fully in the arm. When an animal successfully explored each of the maze's three arms per exploration triad (i.e., entering all three arms in the overlapping triplet sets is defined as correct alternation; for example, XYZ, ZXY, or YZX), the animal received a score. An alternation was deemed incorrect once two arms were explored per exploration triad (e.g., XYX, ZXZ, and YXY). The percentage of spontaneous alternation was calculated using Equation (1):

$$\text{Correct alternation}/(\text{total arm entries} - 2) \times 100 \quad (1)$$

2.6. Biochemical analysis

2.6.1. Tissue homogenization

Following the methods of Sun and Zigman,⁵¹ the brain tissues (hippocampus and prefrontal cortex) were homogenized (Bead Ruptor Elite, OMNI International, United States of America [USA]) in ice-cold 0.25 M sucrose solution. The homogenates were transferred to 5-mL tubes and centrifuged (Model 90-1; Jiangsu Jinyi Instrument Tech, China) at 3,000 rpm for 15 min. Pasteur pipettes were used to collect the supernatant, which was then placed into sample bottles and kept in a freezer at -4°C until further use.

2.6.2. Superoxide dismutase (SOD) assay

SOD activity was measured using a spectrophotometric (NanoDrop, Thermo Fisher Scientific, USA) method. A reaction mixture (3 mL), including 0.02 mL of tissue homogenate, 2.95 mL of carbonate buffer, and 0.03 mL of 2 mM SOD substrate in 0.005 M hydrochloric acid was used to initiate the reaction. The reference cuvette contained 2.95 mL of buffer, 0.02 mL of water, and 0.03 mL of substrate. The absorbance was measured at 480 nm at regular intervals of 1 min for 5 min. Values were expressed as units per milligram of protein (U/mg protein).

2.6.3. Catalase (CAT) assay

CAT activity was determined according to the method described by Clairborne,⁵² with slight modifications. Briefly, the reaction mixture contained 50 mM phosphate buffer (pH 7.0), tissue homogenate (100 μL), and 19 mM hydrogen peroxide in a final volume of 3 mL. The reaction was initiated by adding hydrogen peroxide and allowed to react for 1 min at room temperature. The decomposition of hydrogen peroxide was monitored by measuring the decrease in absorbance at 240 nm using the spectrophotometer. The reaction was terminated by adding 1 mL of a dichromate-acetic acid reagent (a mixture of 5% potassium dichromate in glacial acetic acid in a 1:3 ratio), followed by heating the tubes in a boiling water

bath for 10 min to develop a stable color. CAT activity was expressed as μmoles of hydrogen peroxide decomposed per minute per mg of protein using the molar extinction coefficient for hydrogen peroxide (43.6/M/cm at 240 nm).

2.6.4. Reduced glutathione level

Reduced GSH levels were determined according to the method described by Jollow *et al.*,⁵³ with minor modifications. Briefly, 0.5 mL of tissue homogenate was mixed with 0.5 mL of 4% sulfosalicylic acid and centrifuged at $10,000 \times g$ for 10 min at 4°C to precipitate proteins. The resulting supernatant (0.5 mL) was then added to 2.0 mL of 0.1 M phosphate buffer (pH 7.4) and 0.25 mL of 0.01 M 5,5'-dithiobis-(2-nitrobenzoic acid). The formation of the yellow-colored chromophore was measured spectrophotometrically at 412 nm against a reagent blank. GSH levels were calculated using a standard curve generated with known concentrations of reduced GSH and expressed as nmol of GSH per mg of protein.

2.6.5. Lipid peroxidation level

Lipid peroxidation was assessed by measuring the malondialdehyde (MDA) content in tissue homogenates, following the method of Farombi *et al.*,⁵⁴ with slight modifications. Briefly, 0.5 mL of tissue homogenate was mixed with 1.5 mL of 10% trichloroacetic acid, 1.5 mL of 0.75% thiobarbituric acid, and 0.5 mL of 5% (w/v) butylated hydroxytoluene in 0.1 M hydrochloric acid. The mixture was vortexed and then incubated in a boiling water bath at 95°C for 60 min. After cooling to room temperature, the tubes were centrifuged at $3,000 \times g$ for 10 min, and the absorbance of the pink-colored supernatant was measured at 532 nm using a spectrophotometer. The concentration of MDA was calculated using an extinction coefficient (ϵ) of 1.56×10^5 L/mol/cm, and the results were expressed as nmol MDA per mg protein.

2.6.6. Determination of nitric oxide (NO) level

NO levels were determined as nitrite using the Griess reagent, following the method of Moshage *et al.*,⁵⁵ with slight modifications. Briefly, 100 μL of the tissue supernatant was mixed with 100 μL of freshly prepared Griess reagent, consisting of 1% sulfanilamide and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% phosphoric acid. The reaction mixture was incubated at room temperature for 20 min in the dark to allow color development. The absorbance was measured at 550 nm using an ultraviolet-visible spectrophotometer. A standard curve was generated using sodium nitrite solutions (0–100 μM), and the nitrite concentration in the samples was calculated accordingly. Results were expressed as μM nitrite per gram of tissue ($\mu\text{M/g}$).

2.7. Tissue collection and processing

A few hours after the behavioral tests concluded, the animals were sacrificed using cervical dislocation. Subsequently, the brain tissues were washed with cold phosphate-buffered saline and fixed in 4% paraformaldehyde for 24 h. After being removed from the fixed brain, the hippocampus and prefrontal cortex were dehydrated using ascending alcohol concentrations (50%, 70%, 90%, and 100%). The tissues were then cleared twice for 15 min each in xylene. Tissues were subsequently embedded in paraffin wax at similar orientations after infiltration, and embedding was completed using paraffin wax in a Leica (Germany) hot air oven at 56°C. A rotary microtome (Leica RM2245, Germany) was used to obtain tissue sections serially, which were subsequently mounted on glass slides.

For histology, 3 μm sections were used. Pearse⁵⁶ method, as amended by Fischer *et al.*,⁵⁷ was adopted in staining the brain sections. Hematoxylin and eosin staining was used to show the prefrontal cortex and hippocampal general cytoarchitecture. Immunohistochemistry was performed using primary antibodies against brain-derived neurotrophic factor (BDNF) and $\text{A}\beta$ to assess BDNF expression and amyloid plaque burden, respectively. Sections underwent heat-induced antigen retrieval in 10 mM citrate buffer (pH 6.0) at 95°C for 15 min.⁵⁸ Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for 10 min. To prevent non-specific binding, sections were incubated in 5% normal goat serum for 30 min at room temperature.⁵⁹ The following primary antibodies were used: Anti-BDNF (rabbit polyclonal, ab108319; Abcam, United Kingdom) at 1:200 dilution and anti- $\text{A}\beta$ (mouse monoclonal, ab11132; Abcam, United Kingdom) at 1:250 dilution. Sections were incubated with the primary antibodies overnight at 4°C. After phosphate-buffered saline washes, they were treated with biotinylated secondary antibodies. The secondary antibodies used were goat anti-rabbit immunoglobulin G (IgG) for BDNF (NB7160; Novus Biologicals, USA) and goat anti-mouse IgG for $\text{A}\beta$ (11115-S; IBL-America, USA) at a 1:500 dilution for 1 h at room temperature. Immunoreactivity was visualized using an avidin-biotin complex method and 3,3'-diaminobenzidine as the chromogen.⁶⁰ Counterstaining was performed with Mayer's hematoxylin. Images were captured using a Leica® (Germany) DM5000B microscope and a Leica (Germany) EC3 digital camera.

2.8. Photomicrography and quantification

Immunostained slides were digitized with the Panoramic 250 Flash II slide scanner (3D Histech, Hungary). A complementary digital microscopy software, Caseviewer

(version 2.5), was employed in the random capturing of 8–10 parallel photomicrographic fields of the hippocampus and prefrontal cortex regions. Thereafter, analysis of photomicrographs was conducted using Immunoratio and cell count plugins of the ImageJ software (version 1.54p). The ImmunoRatio plugin estimates the ratio of brown 3,3'-diaminobenzidine (positive immunoreactivity) to the hematoxylin counterstain by digital color deconvolution. The number of manually selected cell types was recorded using the cell counter plugin.⁶¹ Data analysis was performed using the average scores of the analyzed photomicrographs.

2.9. Neurotransmitter analysis

2.9.1. Brain monoamine neurotransmitter analysis

Wet tissue was weighed and homogenized for about 1 min in 5 mL of hydrochloric acid in butanol to quantify the amount of monoamine neurotransmitters (serotonin, dopamine, and norepinephrine). The product was then centrifuged at 2,000 rpm for 10 min. A 1 mL aliquot of the supernatant phase was removed and placed in a centrifuge tube along with 2.5 mL of heptane and 0.31 mL of 0.1 M hydrochloric acid. Following 10 min of vigorous shaking, the tube was centrifuged under similar conditions to separate the two phases, and the organic layer on top was discarded. The aqueous phase (0.2 mL) was then extracted for the serotonin, dopamine, and norepinephrine assays. Every step was completed at 0°C.⁶² To analyze dopamine and norepinephrine, 0.05 mL of 0.4 M hydrochloric acid and 0.1 mL of ethylenediaminetetraacetic acid/sodium acetate buffer (pH 6.9) were added to 0.2 mL of aqueous phase. For oxidation, 0.1 mL of iodine solution (0.1 M in ethanol) was added. After 2 min, the reaction was ended by adding 0.1 mL of sodium sulfite solution, followed by 0.1 mL of acetic acid after another 1.5 min. After the sample had recovered to ambient temperature, the solution was heated to 100°C for 6 min. The spectrofluorometer (FP-6500, JASCO Inc., Japan) was used to measure the excitation and emission spectra for norepinephrine and dopamine analysis. To analyze serotonin, 0.2 mL of aqueous extract was mixed with 0.25 mL of *o*-phthalaldehyde reagent and heated to 100°C for 10 min to allow fluorophore formation. Measurements were then taken using the spectrofluorometer once the samples had cooled to room temperature.

2.9.2. Determination of acetylcholinesterase (AChE) content

Using Ellman's method,⁶³ the level of AChE in the hippocampus and prefrontal cortex was determined by mixing 0.05 mL of supernatant with 0.1 mL of 5-dithio-bis-(2-nitrobenzoic acid) (Ellman's reagent), 3 mL of sodium phosphate buffer (pH 8), and 0.1 mL of acetylthiocholine iodide. Using the PerkinElmer (United States) Lambda 20

spectrophotometer, the absorbance change was measured at 30-s intervals for 2 min at 412 nm. The results were expressed in μmol of acetylthiocholine iodide hydrolyzed per minute per mg of protein.

2.10. Quantification of inflammatory markers

The levels of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) in the supernatants of the hippocampus and prefrontal cortex of control and treated animals were determined using the appropriate assay kits as per the manufacturer's instructions (Mybiosource, USA).⁶⁴

2.11. Statistical analysis

Using GraphPad Prism® 8 Windows (GraphPad Software, USA), a one-way analysis of variance with Tukey's multiple comparisons test was used for the statistical analysis. Differences between means at $p < 0.05$ were considered significant, and the results were expressed as mean $\pm$ standard error of mean.

3. Results

3.1. Effect of DRLC and treadmill exercise on the neurobehavior of rats

Locomotor/exploratory activity and spatial working memory were evaluated using the open field and Y-maze tests, respectively. In the open field test, activity was quantified as the number of lines crossed during a 5-min session (Figure 1A). In the Y-maze test, working memory was assessed using the percent correct alternation (Figure 1B). The result revealed that rats in Group B showed a significant decrease ($p < 0.05$) in the number of lines crossed per 5 min and percentage correct alternation when compared to Group A (Figure 1). In addition, the rats in Groups C, D, and E showed a significant increase ($p < 0.05$) in the number of lines crossed per 5 min and percentage correct alternation when compared with Group B. There was also no significant difference in the number of lines crossed per 5 min and

percentage correct alternation when Group E was compared to the control group. In addition, no significant difference was observed in the number of lines crossed per 5 min and percentage correct alternation between Groups A, F, and G when compared to one another.

3.2. Effects of DRLC and treadmill exercise on antioxidant enzyme activities and oxidative stress markers

To evaluate the antioxidant activity in the brain (hippocampus and prefrontal cortex) of rats treated with DRLC and treadmill exercise against AlCl_3 -induced neurodegeneration, the levels of SOD, CAT, and GSH were examined. MDA and NO, two indicators of oxidative stress, were also investigated (Figure 2). The findings demonstrated that the antioxidant enzyme activities (SOD, CAT, and GSH) of rats in Group B significantly decreased ($p < 0.05$), accompanied by a corresponding increase ($p < 0.05$) in oxidative stress markers (MDA and NO) when compared to Group A. Furthermore, when compared to Group B, Groups C, D, and E demonstrated a significant increase ($p < 0.05$) in antioxidant enzyme activities (SOD, CAT, and GSH) and a consequent decrease ($p < 0.05$) in oxidative stress markers (MDA and NO). Moreover, when Group E was compared to the control group, there was no significant difference in the levels of antioxidant enzyme activities (SOD, CAT, and GSH) or oxidative stress markers (MDA and NO). In addition, when compared to each other, there was no significant difference between Groups A, F, and G.

3.3. Effects of DRLC and treadmill exercise on brain monoamine neurotransmitters and acetylcholinesterase levels

This study examined the hippocampus and prefrontal cortex for the monoamine neurotransmitters (serotonin, dopamine, and norepinephrine). When compared to Group A, Group B had significantly lower ($p < 0.05$) dopamine and serotonin levels and higher norepinephrine

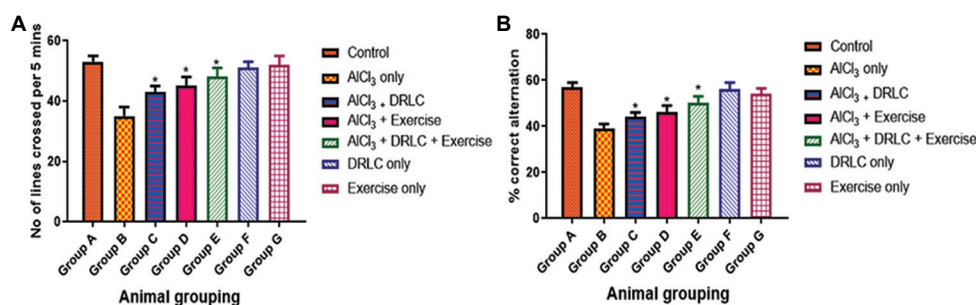


Figure 1. Effects of *D*-ribose-*L*-cysteine (DRLC) and treadmill exercise on neurobehavioral tests in aluminum chloride (AlCl_3)-induced neurodegeneration in normal and experimental rats. (A) Open field test. (B) Y-maze test. All data were analyzed using one-way analysis of variance followed by Tukey's multiple comparisons test. Data are expressed as mean $\pm$ standard error of mean ($n=10$). Group B differed significantly from Group A in all panels, indicating successful AD model induction. * $p < 0.05$ versus Group B (AlCl_3 -induced Alzheimer's disease model group).

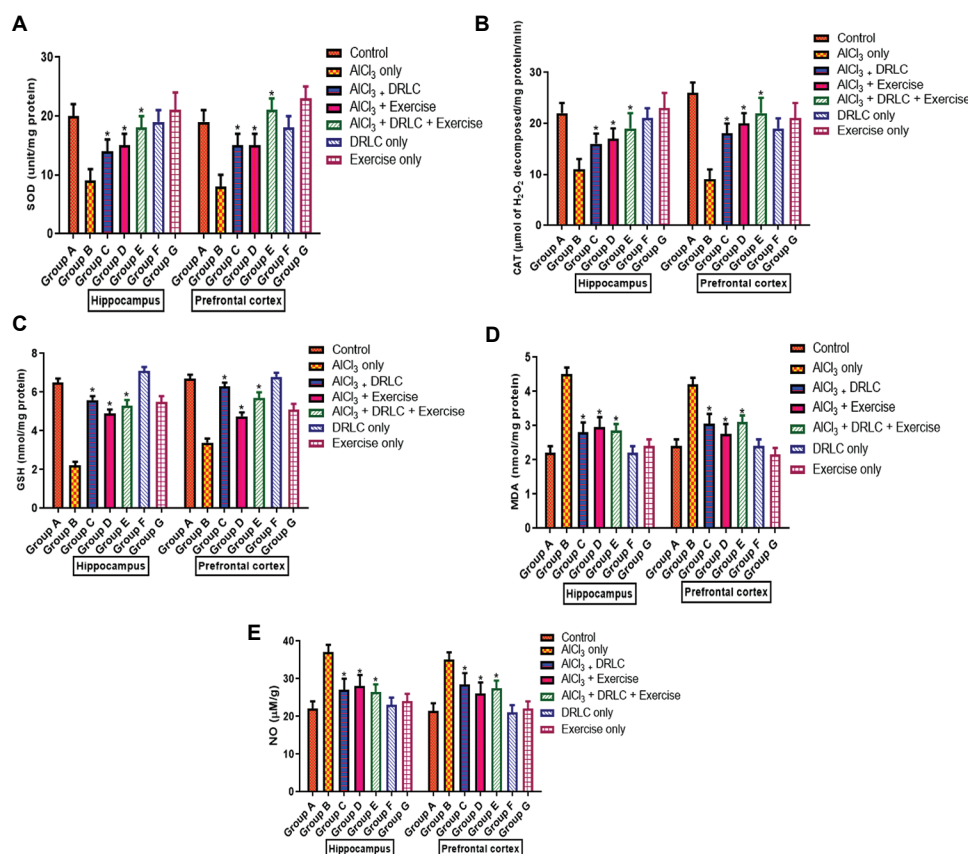


Figure 2. Effects of *D*-ribose-*L*-cysteine (DRLC) and treadmill exercise on antioxidant enzyme activities and oxidative stress markers in aluminum chloride (AlCl_3)-induced neurodegeneration in normal and experimental rats. (A) Superoxide dismutase (SOD), (B) catalase (CAT), (C) glutathione (GSH), (D) malondialdehyde (MDA), and (E) nitric oxide (NO). All data were estimated using one-way analysis of variance followed by Tukey's multiple comparisons test. Data are expressed as mean $\pm$ standard error of mean ($n=10$). Group B differed significantly from Group A in all panels, indicating successful AD model induction. * $p < 0.05$ versus Group B (AlCl_3 -induced Alzheimer's disease model group).

levels (Figure 3). However, when compared to Group B, Groups C, D, and E exhibited a significant increase ($p < 0.05$) in dopamine and serotonin levels with a corresponding decrease in norepinephrine levels. Furthermore, when comparing Group B to Group A, a significant decrease ($p < 0.05$) in AChE levels was observed in Group A. Similarly, Groups C, D, and E demonstrated a significant decrease ($p < 0.05$) in the brain AChE levels as compared to Group B. However, when Group E was compared to the control group, there was no significant difference in the levels of monoamine neurotransmitters (serotonin, dopamine, and norepinephrine) or AChE in the brain. In addition, when compared to each other, there was no significant difference in these parameters between the control Groups A, F, and G.

3.4. Effects of DRLC and treadmill exercise on brain inflammatory markers

Inflammatory markers (TNF- α and IL-6) in the brain tissues (hippocampus and prefrontal cortex) were quantified in

this study. Group B showed a significant increase ($p < 0.05$) in brain inflammatory marker levels (IL-6 and TNF- α) when compared with Group A (Figure 4). Conversely, Groups C, D, and E showed a significant decrease ($p < 0.05$) in the brain inflammatory marker levels (TNF- α and IL-6) when compared to Group B. However, there was no significant difference in the brain inflammatory marker levels (TNF- α and IL-6) in Group E when compared to the control group. Moreover, no significant difference was observed between Groups A, F, and G when compared to one another.

3.5. Effect of DRLC and treadmill exercise on hippocampal and prefrontal cortex histomorphology

The results of the histological assessment in this study showed normal hippocampal morphology in Group A (Figure 5A). Group B showed few pyramidal cells with vacuolated neuropil, distorted glial cells between the outer marginal layer and inner pyramidal layer (Figure 5B) when

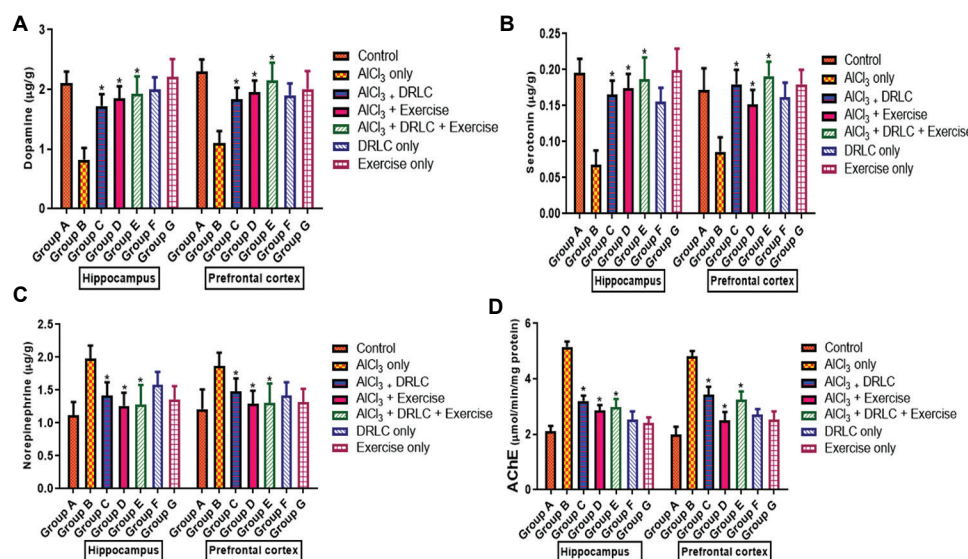


Figure 3. Effects of *D*-ribose-*L*-cysteine (DRLC) and treadmill exercise on brain monoamine neurotransmitters and acetylcholinesterase (AChE) levels in aluminum chloride (AlCl_3)-induced neurodegeneration in normal and experimental rats. (A) Dopamine, (B) serotonin, (C) norepinephrine, and (D) AChE. All data were estimated using one-way analysis of variance followed by Tukey's multiple comparisons test. Data are expressed as mean $\pm$ standard error of mean ($n=10$). Group B differed significantly from Group A in all panels, indicating successful AD model induction. * $p<0.05$ versus Group B (AlCl_3 -induced Alzheimer's disease model group).

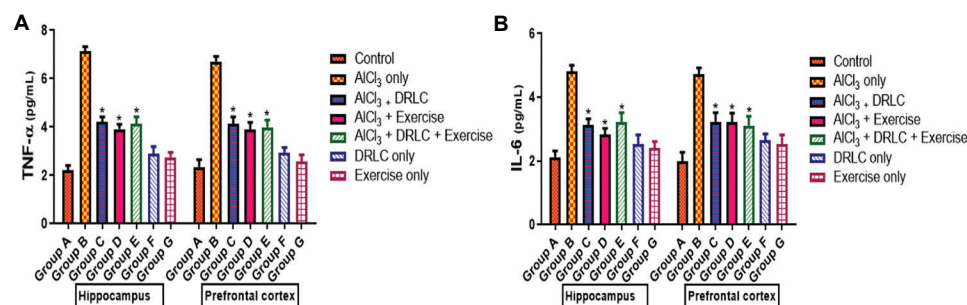


Figure 4. Effects of *D*-ribose-*L*-cysteine (DRLC) and treadmill exercise response on brain inflammatory markers in aluminum chloride (AlCl_3)-induced neurodegeneration in normal and experimental rats. (A) Tumor necrosis factor-alpha ($\text{TNF-}\alpha$). (B) Interleukin-6 (IL-6). All data were estimated using one-way analysis of variance followed by Tukey's multiple comparisons test. Data are expressed as mean $\pm$ standard error of mean ($n=10$). Group B differed significantly from Group A in all panels, indicating successful AD model induction. * $p<0.05$ versus Group B (AlCl_3 -induced Alzheimer's disease model group).

compared to the control group. Groups C (Figure 5C) and D (Figure 5D) showed a reduced glial proliferation between the outer marginal layer and inner pyramidal layer and fewer vacuolated neuropil areas than Group B. In addition, Group E (Figure 5E) showed a significant reduction in distorted glial cells in all hippocampal regions, as well as a low rate of neurodegeneration that is comparable to Group A. In Groups F (Figure 5F) and G (Figure 5G), there was normal orientation of glial cells similar to the control.

The photomicrograph representation of the prefrontal cortex in Group B showed distorted neuronal cells and vacuolated neuropils, indicating signs of neurodegeneration

when compared to Group A, which displayed normal cortical architecture with intact neurons and glial cells (Figure 6A). Group B exhibited significant atrophy and cellular disorganization (Figure 6B). In addition, Group C demonstrated a noticeable reduction in neuronal distortion and vacuolation (Figure 6C) compared to Group B. A similar trend was observed in Group D, which showed improved neuronal morphology and reduced signs of neurodegeneration (Figure 6D). Groups E, F, and G exhibited cortical structures comparable to the control group, further confirming the protective effects of these treatments against AlCl_3 -induced damage (Figure 6E-G, respectively).

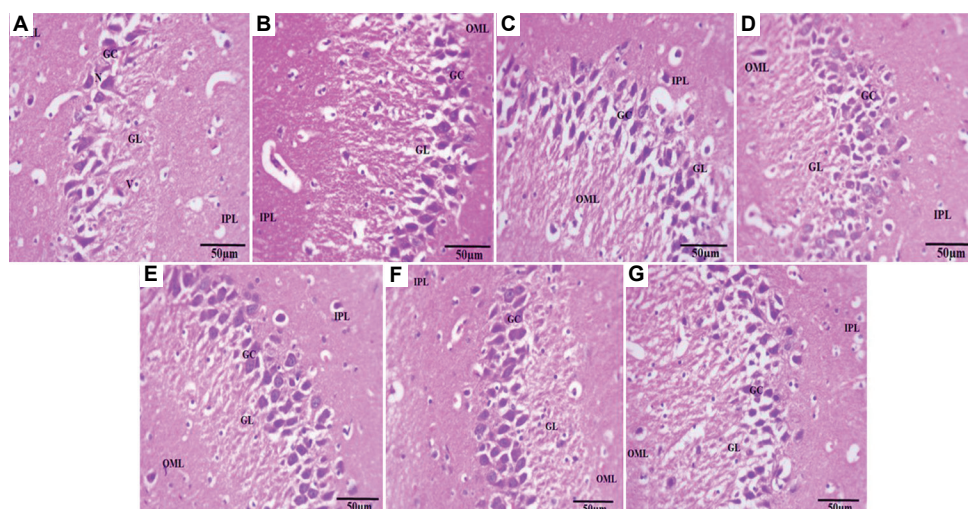


Figure 5. Photomicrographs of the CA3 region of the hippocampus showing the effect of *D*-ribose-*L*-cysteine (DRLC) and treadmill exercise against aluminum chloride (AlCl_3)-induced neurodegeneration in normal and experimental rats. (A) Control group; (B) AlCl_3 -only group; (C) AlCl_3 + DRLC group; (D) AlCl_3 + exercise group; (E) AlCl_3 + DRLC + exercise group; (F) DRLC-only group; and (G) exercise-only group. Scale bar: 50 μm ; magnification: 400 $\times$.

Abbreviations: GC: Glial cells; GL: Glial layer; IPL: Inner pyramidal layer; OML: Outer marginal layer; V: Vacuolated neuropil.

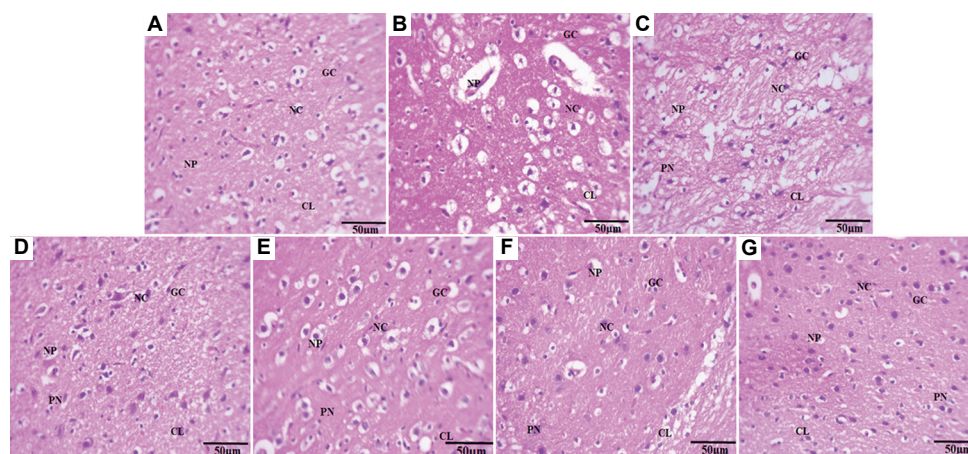


Figure 6. Photomicrographs of the prefrontal cortex showing the effect of *D*-ribose-*L*-cysteine (DRLC) and treadmill exercise against aluminum chloride (AlCl_3)-induced neurodegeneration in normal and experimental rats. (A) Control group; (B) AlCl_3 -only group; (C) AlCl_3 + DRLC group; (D) AlCl_3 + exercise group; (E) AlCl_3 + DRLC + exercise group; (F) DRLC-only group; and (G) exercise-only group. Scale bar: 50 μm ; magnification: 400 $\times$.

Abbreviations: CL: Cortical layer; GC: Glial cells; NC: Neuronal cells; NP: Neuropils; PC: Pyramidal cells.

3.6. Effects of DRLC and treadmill exercise on the immunohistochemical expressions and immunoreactivity of brain-derived neurotrophic factor and amyloid beta

The immunohistochemical expressions of BDNF and $\text{A}\beta$ in the hippocampus of the rats in Group A revealed that the structural architecture of the hippocampus's pyramidal cells included normal neuronal cells devoid of any pathological features (Figure 7A). In the external pyramidal layer, the pyramidal neurons were arranged in a typically normal and structurally well-defined manner, with appropriate delineation and a normal cytoarchitectural

assortment of the pyramidal cells. In contrast, Group B showed $\text{A}\beta$ plaque deposition and neuronal degeneration, exhibiting characteristic pathological alterations indicative of hippocampal degeneration, including chromatolysis, distortion, and pyknotic changes in the pyramidal cells of the CA3 region and the external pyramidal layer of the hippocampus (Figure 7B). However, Group E showed normal immunohistochemistry and normal neuronal cells with notable improvement and restoration in the morphological integrity and cytoarchitectural appearance of the hippocampus, along with the absence of amyloid deposit (Figure 7C and D), as similarly observed in Groups

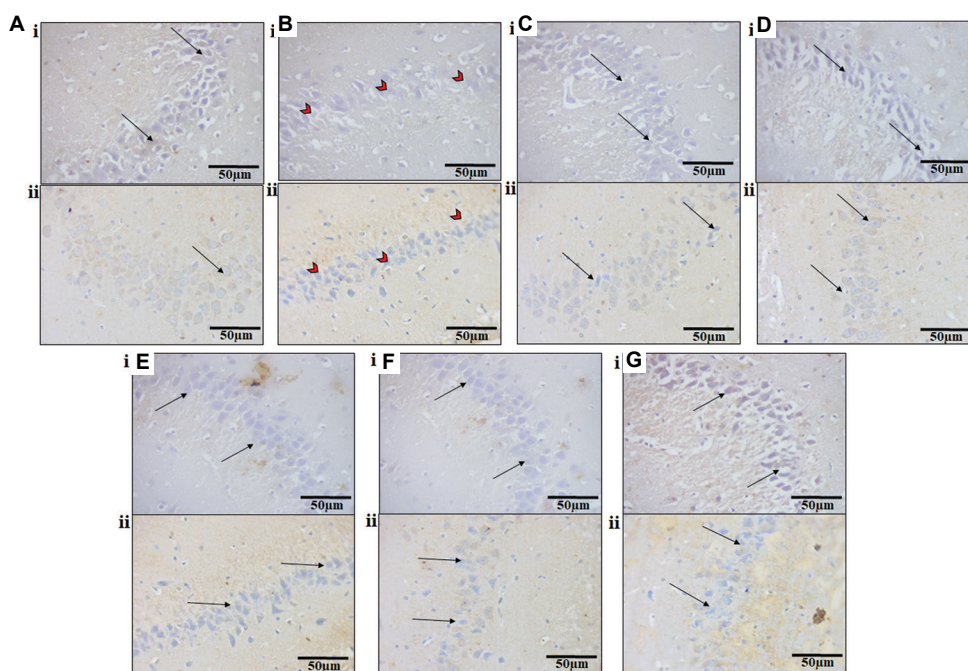


Figure 7. Immunohistochemical and immunoreactivity expressions of brain-derived neurotrophic factor (BDNF) and amyloid beta ($\text{A}\beta$) in the hippocampus. (A) Control group; (B) aluminum chloride (AlCl_3)-only group; (C) AlCl_3 + *D*-ribose-*L*-cysteine (DRLC) group; (D) AlCl_3 + exercise group; (E) AlCl_3 + DRLC + exercise group; (F) DRLC-only group; and (G) exercise-only group. Arrows indicate neuronal cells, and red arrowheads indicate neuronal degeneration with deposition of $\text{A}\beta$ oligomers. Images labeled (i) show BDNF immunostaining, and images labeled (ii) show $\text{A}\beta$ immunostaining. Scale bar: 50 μm ; magnification: 400 $\times$.

E, F and G (Figure 7E to G).

The immunohistochemical evaluation of BDNF and $\text{A}\beta$ expressions across the experimental groups demonstrated varying degrees of astrocytic activity in the prefrontal cortex. Group A displayed the baseline expression of BDNF and $\text{A}\beta$, with sparsely distributed astrocytes and faint processes (Figure 8A). In contrast, Group B showed a very low BDNF immunoreactivity, with a correspondingly high $\text{A}\beta$ immunoreactivity, characterized by numerous astrocytes with elongated, densely stained processes, reflecting heightened neuroinflammation and neurodegeneration due to AlCl_3 exposure (Figure 8B). Groups C, D, and E exhibited an increase in BDNF, with a similar decrease in $\text{A}\beta$ expressions compared to Group B, showing fewer astrocytes and less prominent processes, suggesting the treatment's potential role in attenuating the inflammatory response (Figure 8C-E). Groups F and G showed a moderate level of BDNF and $\text{A}\beta$ expressions, indicating controlled astrocytic activation under experimental conditions (Figure 8F and G). In addition, the immunoreactivity and quantification of BDNF- and $\text{A}\beta$ -positive cells, as well as the percentage of BDNF and $\text{A}\beta$ immunoreactivities, were conducted in this study, and the results showed varying degrees of expression in the hippocampus and prefrontal cortex between the control

and experimental groups (Figures 9 and 10).

4. Discussion

This study investigated the neuroprotective effects of exercise and DRLC supplementation on AlCl_3 -induced AD pathology in rats. Our findings support the hypothesis that combined intervention can mitigate cognitive decline, oxidative stress, neuroinflammation, and neurodegeneration caused by AlCl_3 exposure.

In terms of behavior, rats treated with AlCl_3 exhibited significant impairments in working memory and locomotor activity, as demonstrated by reduced spontaneous alternation in the Y-maze test and fewer lines crossed in the open field test. These outcomes align with previous research showing the detrimental effects of Al on cognitive performance and anxiety-like behavior.^{65,66} Exercise and DRLC treatments significantly improved these measures, supporting their role in enhancing hippocampal-dependent memory and exploratory behavior, likely through increased neurogenesis and synaptic plasticity.^{67,68} This functional recovery suggests that interventions targeting oxidative damage and neuroinflammation can restore behavioral deficits associated with AD.

Oxidative stress is a key contributor to AD pathology,

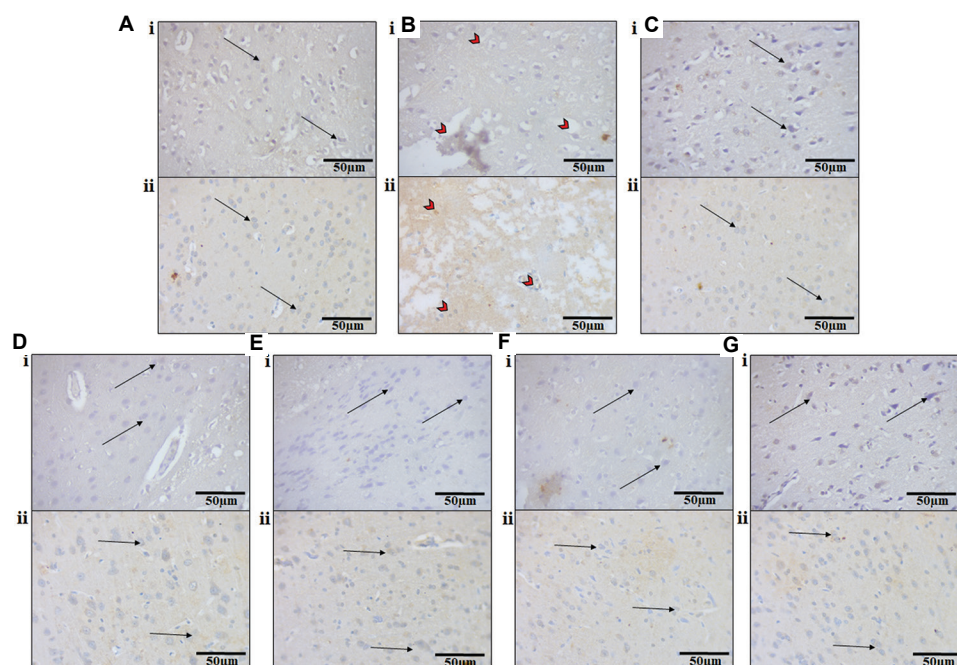


Figure 8. Immunohistochemical and immunoreactivity expressions of brain-derived neurotrophic factor (BDNF) and amyloid beta ($\text{A}\beta$) in the prefrontal cortex. (A) Control group; (B) aluminum chloride (AlCl_3)-only group; (C) AlCl_3 + *D*-ribose-*L*-cysteine (DRLC) group; (D) AlCl_3 + exercise group; (E) AlCl_3 + DRLC + exercise group; (F) DRLC-only group; and (G) exercise-only group. Arrows indicate cortical neurons, and red arrowheads indicate pathological changes, evident in neuronal degeneration and deposition of $\text{A}\beta$ plaques. Images labeled (i) show BDNF immunostaining, and images labeled (ii) show $\text{A}\beta$ immunostaining. Scale bar: 50 μm ; magnification: 400 $\times$.

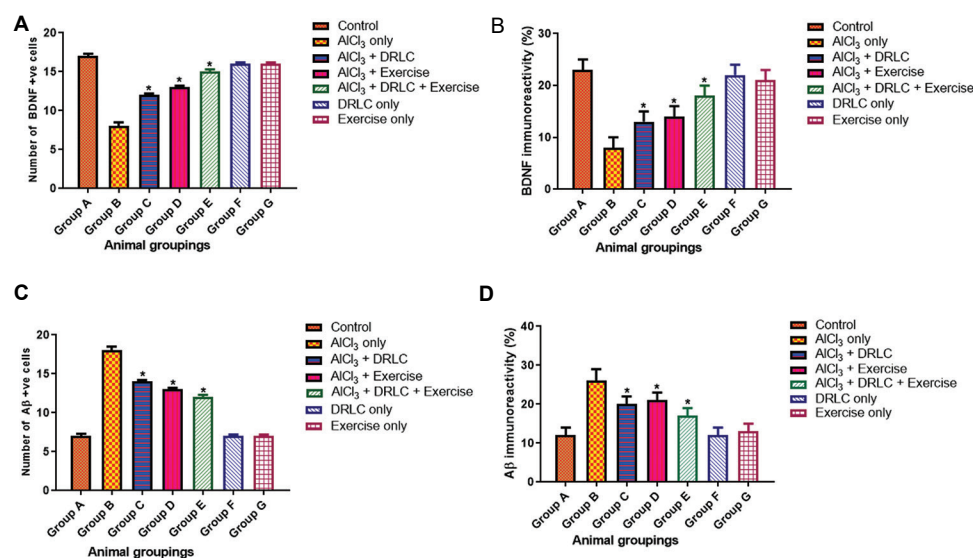


Figure 9. Immunohistochemical and immunoreactivity quantification of brain-derived neurotrophic factor (BDNF) and amyloid beta ($\text{A}\beta$) in the hippocampus. (A) Number of BDNF-positive cells and (B) BDNF immunoreactivity. (C) Number of $\text{A}\beta$ -positive cells. (D) $\text{A}\beta$ immunoreactivity. All data were estimated using one-way analysis of variance followed by Tukey's multiple comparisons test. Data are expressed as mean $\pm$ standard error of mean (n=10). Group B differed significantly from Group A in all panels, indicating successful AD model induction. * $p < 0.05$ vs. Group B (AlCl_3 -induced Alzheimer's disease model group).

driven by an imbalance between reactive oxygen species production and antioxidant defenses. Our data showed increased MDA and NO levels alongside decreased

activities of key antioxidant enzymes (CAT, SOD, and reduced GSH) in the brains of AlCl_3 -treated rats. These findings confirm earlier studies linking oxidative damage

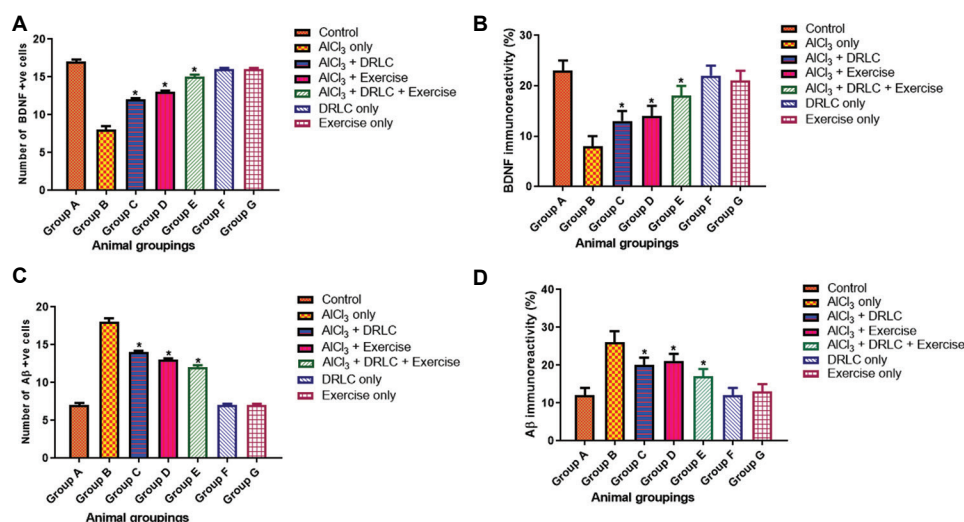


Figure 10. Immunohistochemical and immunoreactivity quantification of brain-derived neurotrophic factor (BDNF) and amyloid beta ($\text{A}\beta$) in the prefrontal cortex. (A) Number of BDNF-positive cells and (B) BDNF immunoreactivity. (C) Number of $\text{A}\beta$ -positive cells. (D) $\text{A}\beta$ immunoreactivity. All data were estimated using one-way analysis of variance followed by Tukey's multiple comparisons test. Data are expressed as mean $\pm$ standard error of mean ($n=10$). Group B differed significantly from Group A in all panels, indicating successful AD model induction. * $p<0.05$ vs. Group B (AlCl_3 -induced Alzheimer's disease model group).

to neuronal dysfunction in AD.^{69,70} Importantly, DRLC supplementation, which serves as a cysteine donor for GSH synthesis, alongside exercise, significantly restored antioxidant enzyme activities and reduced oxidative stress markers. This dual intervention likely enhanced endogenous antioxidant capacity, preventing oxidative damage to neural tissue and supporting cognitive function.^{71,72}

This study also highlighted disruptions in key neurotransmitter systems. AlCl_3 exposure led to decreased dopamine and serotonin, alongside increased norepinephrine and AChE levels. These alterations reflect cholinergic dysfunction and monoaminergic imbalance characteristic of AD, which impair cognition and memory.^{73,74} Combined DRLC and exercise treatment normalized these neurotransmitter levels, consistent with reports that physical activity modulates central dopaminergic and serotonergic pathways and that glutathione pre-cursors provide neuroprotection.^{13,75} These neurochemical improvements likely contributed to the observed behavioral enhancements.

Neuroinflammation is another pathological hallmark of AD, with pro-inflammatory cytokines, such as $\text{TNF-}\alpha$ and IL-6 driving neuronal damage and cognitive decline.^{13,76} Our findings revealed elevated $\text{TNF-}\alpha$ and IL-6 levels in AlCl_3 -treated rats, indicative of heightened neuroinflammatory response, consistent with reports linking chronic inflammation to AD progression.^{77,78} Notably, DRLC and exercise co-administration significantly suppressed these cytokines, suggesting anti-inflammatory effects, possibly

mediated by antioxidant activity and exercise-induced modulation of the immune system.^{79,80} This reduction in neuroinflammation likely plays a critical role in protecting neural tissues from ongoing degeneration.

Histological and immunohistochemical analyses further substantiated the biochemical and behavioral data. AlCl_3 exposure induced neuronal degeneration, $\text{A}\beta$ plaque deposition, reduced BDNF expression, and astrocytic activation in the hippocampus and prefrontal cortex. These pathological changes mirror AD neuropathology and confirm Al 's neurotoxic effects.^{81,82} Conversely, DRLC and exercise treatment preserved neuronal architecture, prevented $\text{A}\beta$ accumulation, enhanced BDNF expression, and reduced astrocytic activation, indicating attenuation of neurodegeneration and promotion of neuroplasticity. Exercise-induced increases of BDNF support neuronal survival and synaptic repair, which are critical mechanisms for cognitive recovery.^{22,83}

5. Conclusion

The findings from this study provide compelling evidence that combined exercise and DRLC supplementation protect against AlCl_3 -induced neurodegeneration by attenuating oxidative stress, neuroinflammation, and neurotransmitter imbalances while enhancing neurotrophic support. These effects culminate in improved cognitive and behavioral outcomes, supporting the therapeutic potential of integrating antioxidant supplementation with physical activity in AD management. Future studies should further explore the molecular mechanisms underlying these

benefits and their translational potential in clinical settings.

Acknowledgments

Special appreciation goes to the Department of Human Anatomy, Federal University of Technology, Akure, Ondo State, Nigeria, for providing the facilities and equipment needed to conduct this research seamlessly. A notable mention goes to Dr. Omamuyovwi Ijomone from the Laboratory of Experimental and Translational Neuroscience, whose lab access was monumental for the immunohistochemical findings in this study.

Funding

None.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Babatunde Ogunlade

Data curation: Adebunke Faith Balogun

Formal analysis: Oluwafemi Abidemi Adedotun

Investigation: Babatunde Ogunlade

Methodology: Oluwafemi Abidemi Adedotun

Project administration: Adebunke Faith Balogun

Software: Oluwafemi Abidemi Adedotun

Supervision: Babatunde Ogunlade

Validation: Babatunde Ogunlade

Writing – original draft: Oluwafemi Abidemi Adedotun

Writing – review & editing: Kingsley Afoke Itiere

Ethics approval and consent to participate

Ethical approval was obtained from the Centre for Research and Development (CERAD) of The Federal University of Technology, Akure (FUTA/ETH/24/147).

Consent for publication

Not applicable.

Availability of data

Data will be made available upon request to the corresponding author.

Further disclosure

Part of the findings from this study have been presented at a 1st FUTA SBMS Conference of Biomedical Research “From Bench to Bedside: Translating Basic Science Research into Clinical Impact”, under the title: “Neuroinflammatory and Oxidative Stress Responses of Exercise and DRLC Supplements on Aluminium Chloride-Induced Rat Model

of Alzheimer’s Disease,” held on June 25–27, 2025, at the Federal University of Technology Akure, Ondo, Nigeria.

References

- Breijyeh Z, Karaman R. Comprehensive review on Alzheimer’s disease: Causes and treatment. *Molecules*. 2020;25:5789.
doi: 10.3390/molecules25245789
- Cass SP. Alzheimer’s disease and exercise: A literature review. *Curr Sports Med Rep*. 2017;16:19-22.
doi: 10.1249/JSR.0000000000000332
- Barnes JN. Exercise, cognitive function, and aging. *Adv Physiol Educ*. 2015;39:55-62.
doi: 10.1152/advan.00101.2014
- Alzheimer’s Association. 2015 Alzheimer’s disease facts and figures. *Alzheimers Dement*. 2015;11:332-384.
doi: 10.1016/j.jalz.2015.02.003
- Alzheimer’s Association. 2021 Alzheimer’s disease facts and figures. *Alzheimers Dement*. 2021;17:327-406.
doi: 10.1002/alz.12328
- Vermunt L, Sikkes SA, Van Den Hout A, *et al*. Duration of preclinical, prodromal, and dementia stages of Alzheimer’s disease in relation to age, sex, and APOE genotype. *Alzheimers Dement*. 2019;15:888-898.
doi: 10.1016/j.jalz.2019.04.001
- Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer’s disease at 25 years. *EMBO Mol Med*. 2016;8:595-608.
doi: 10.15252/emmm.201606210
- Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer’s amyloid beta-peptide. *Nat Rev Mol Cell Biol*. 2007;8:101-112.
doi: 10.1038/nrm2101
- Abubakar MB, Sanusi KO, Uguzman A, *et al*. Alzheimer’s disease: An update and insights into pathophysiology. *Front Aging Neurosci*. 2022;14:742408.
doi: 10.3389/fnagi.2022.742408
- Hussien HM, Abd-Elmegied A, Ghareeb DA, Hafez HS, Ahmed HE, El-moneam NA. Neuroprotective effect of berberine against environmental heavy metals-induced neurotoxicity and Alzheimer’s-like disease in rats. *Food Chem Toxicol*. 2018;111:432-444.
doi: 10.1016/j.fct.2017.11.025
- Ogunlade B, Adelakun SA, Fidelis OP. Sulforaphane attenuates neuroinflammation, oxidative stress and cognitive impairment on aluminium-induced Alzheimer’s disease in wistar rats. *J Neurosci Neuropharmacol*. 2020;6(2):13-22.
- Exley C, Vickers T. Elevated brain aluminium and early onset

- alzheimer's disease in an individual occupationally exposed to aluminium: A case report. *J Med Case Rep.* 2014;8(1):41.
doi: 10.1186/1752-1947-8-41
13. Chen SX, Zhang M, Ahmed M, Surapaneni KM, Veeraraghavan VP, Arulselvan P. Neuroprotective effects of ononin against the aluminium chloride-induced Alzheimer's disease in rats. *Saudi J Biol Sci.* 2021;28(8):4232-4239.
doi: 10.1016/j.sjbs.2021.06.031
14. Cao Z, Wang F, Xiu C, Zhang J, Li Y. Hypericum perforatum extract attenuates behavioral, biochemical, and neurochemical abnormalities in Aluminium chloride-induced Alzheimer's disease rats. *Biomed Pharmacother.* 2017;91:931-937.
doi: 10.1016/j.biopha.2017.05.022
15. Mirza A, King A, Troakes C, Exley C. Aluminium in brain tissue in familial Alzheimer's disease. *J Trace Elem Med Biol.* 2017;40:30-36.
doi: 10.1016/j.jtemb.2016.12.001
16. Hamdan AM, Alharthi FH, Alanazi AH, et al. Neuroprotective effects of phytochemicals against aluminium chloride-induced Alzheimer's disease through ApoE4/LRP1, Wnt3/ β -catenin/GSK3 β , and TLR4/NLRP3 pathways with physical and mental activities in a rat model. *Pharmaceuticals.* 2022;15(8):1008.
doi: 10.3390/ph15081008
17. Aalikhani M, Safdari Y, Jahanshahi M, Alikhani M, Khalili M. Comparison between hesperidin, coumarin, and deferoxamine iron chelation and antioxidant activity against excessive iron in the iron overloaded mice. *Front. Neurosci.* 2022;15:811080.
doi: 10.3389/fnins.2021.811080
18. Mahalakshmi B, Maurya N, Lee S, Bharath Kumar V. Possible neuroprotective mechanisms of physical exercise in neurodegeneration. *Int J Mol Sci.* 2020;21(16):5895.
doi: 10.3390/ijms21165895
19. Majid A. Neuroprotection in stroke: Past, present, and future. *ISRN Neurol.* 2014;2:515716.
doi: 10.1155/2014/515716
20. Kumar A, Singh A, Ekavali. A review on Alzheimer's disease pathophysiology and its management: An update. *Pharmacol Rep.* 2015;67(2):195-203.
doi: 10.1016/j.pharep.2014.09.004
21. Jin Y, Sumsuzzman D, Choi J, Kang H, Lee S, Hong Y. Molecular and functional interaction of the myokine irisin with physical exercise and Alzheimer's disease. *Molecules.* 2018;23(12):3229.
doi: 10.3390/molecules23123229
22. Pahlavani HA. Exercise therapy to prevent and treat Alzheimer's disease. *Front Aging Neurosci.* 2023;15(124):38-69.
doi: 10.3389/fnagi.2023.1243869
23. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol.* 2011;10(9):819-828.
doi: 10.1016/S1474-4422(11)70072-2
24. Sutoo D, Akiyama K. Regulation of brain function by exercise. *Neurobiol Dis.* 2013;13(1):1-14.
doi: 10.1016/S0969-9961(03)00030-5
25. Schinder AF, Poo M. The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci.* 2020;23(12):639-645.
doi: 10.1016/S0166-2236(00)01672-6
26. Hosseini M, Alaei HA, Naderi A, Sharif MR, Zahed R. Treadmill exercise reduces self-administration of morphine in male rats. *Pathophysiology.* 2019;16(1):3-7.
doi: 10.1016/j.pathophys.2008.11.001
27. Lu B, Chow A. Neurotrophins and hippocampal synaptic transmission and plasticity. *J Neurosci Res.* 2019;58(1):76-87.
28. Lin TW, Tsai SF, Kuo YM. Physical exercise enhances neuroplasticity and delays Alzheimer's disease. *Brain Plast.* 2018;4:95-110.
doi: 10.3233/BPL-180073
29. Ryan SM, Kelly AM. Exercise as a pro-cognitive, pro-neurogenic and anti-inflammatory intervention in transgenic mouse models of Alzheimer's disease. *Ageing Res Rev.* 2016;27:77-92.
doi: 10.1016/j.arr.2016.03.007
30. Lu Y, Dong Y, Tucker D, et al. Treadmill exercise exerts neuroprotection and regulates microglial polarization and oxidative stress in a streptozotocin-induced rat model of sporadic Alzheimer's Disease. *J Alzheimer Dis.* 2017;56(4):1469-1484.
doi: 10.3233/JAD-160869
31. Tang Y, Zhang Y, Zheng M, Chen J, Chen H, Liu N. Effects of treadmill exercise on cerebral angiogenesis and MT1-MMP expression after cerebral ischemia in rats. *Brain Behav.* 2018;8:e01079.
doi: 10.1002/brb3.1079
32. Kamat PK, Kalani A, Rai S, et al. Mechanism of oxidative stress and synapse dysfunction in the pathogenesis of Alzheimer's disease: Understanding the therapeutics strategies. *Mol Neurobiol.* 2016;53(1):648-661.
doi: 10.1007/s12035-014-9053-6
33. Moreira PI, Santos MS, Oliveira CR, et al. Alzheimer disease and the role of free radicals in the pathogenesis of the disease. *CNS Neurol Disord Drug Targets.* 2008;7:3-10.
doi: 10.2174/187152708783885156

34. Wojsiat J, Zoltowska KM, Laskowska-Kaszub K, Wojda U. Oxidant/antioxidant imbalance in Alzheimer's disease: Therapeutic and diagnostic prospects. *Oxid Med Cell Longev*. 2018;2018(1):1-16.
doi: 10.1155/2018/6435861
35. Mandal PK, Saharan S, Tripathi M, Murari G. Brain glutathione levels--a novel biomarker for mild cognitive impairment and Alzheimer's disease. *Biol Psychiatry*. 2015;78(10):702-710.
doi: 10.1016/j.biopsych.2015.04.005
36. Czerniawski J, Miyashita T, Lewandowski G, Guzowski JF. Systemic lipopolysaccharide administration impairs retrieval of context-object discrimination, but not spatial, memory: Evidence for selective disruption of specific hippocampus dependent memory functions during acute neuroinflammation. *Brain Behav Immun*. 2015;44:159-166.
doi: 10.1016/j.bbi.2014.09.014
37. Adair JC, Knoefel JE, Morgan N. Controlled trial of n-acetylcysteine for patients with probable Alzheimer's disease. *Neurology*. 2001;57(8):1515-1517.
doi: 10.1212/WNL.57.8.1515
38. Ogunlade B, Fidelis OP, Afolayan OO, Agie JA. Neurotherapeutic and antioxidant response of D-ribose-L-Cysteine nutritional dietary supplements on Alzheimer-type hippocampal neurodegeneration induced by cuprizone in adult male wistar rat model. *Food Chem Toxicol*. 2021;147:111862.
doi: 10.1016/j.fct.2020.111862
39. Falana B, Adeleke O, Orenolu M, Osinubi A, Oyewopo A. Effect of D-ribose-L-cysteine on aluminium induced testicular damage in male sprague-dawley rats. *JBRA Assist Reprod*. 2017;21(2):94.
doi: 10.5935/1518-0557.20170023
40. Adelakun SA, Ogunlade B, Fidelis OP, Ajao AA. Nutritional supplementation of D-Ribose-L-Cysteine suppresses oxidative stress, spermatogenesis and steroidogenesis recovery in rats exposed to mercury chloride: Histomorphometry and biochemical evidence. *Endocr Metab Sci*. 2021;4:100105.
doi: 10.1016/j.endmts.2021.100105
41. Ojetola AA, Adedeji TG, Fasanmade AA. Changes in antioxidants status, atherogenic index and cardiovascular variables after prolonged doses of D-ribose-L-cysteine in male Wistar rats. *Heliyon*. 2021;7(2):e06287.
doi: 10.1016/j.heliyon.2021.e06287
42. Adedotun OA, Chukwunye CC, Balogun AF, Olawale MA, Babatunde JO, Ogunlade B. Histomorphological response of D-ribose-L-cysteine to ketamine-induced testicular toxicity in adult male Wistar rats. *Redox Experiment Medicine*. 2023;1:20-23.
43. Ogunsuyi OB, Aro PO, Umar HI, Oboh G. Administration of curcumin plus treadmill exercise modulate some neuronal enzyme activities, redox markers and BDNF mRNA expression in pilocarpine-induced epileptic seizure in rats. *Neurochem J*. 2023;17(3):467-476.
doi: 10.1134/S1819712423030145
44. Alomari MA, Khabour OF, Alzoubi KH, Alzubi MA. Forced and voluntary exercises equally improve spatial learning and memory and hippocampal BDNF levels. *Behav Brain Res*. 2013;247:34-39.
doi: 10.1016/j.bbr.2013.03.007
45. Bayod S, Del Valle J, Lalanza JF, et al. Long-term treadmill exercise induces neuroprotective molecular changes in rat brain. *J Appl Physiol*. 2011;111(5):1380-1390.
doi: 10.1152/japplphysiol.00425.2011
46. National Research Council. *Guide to the Care and Use of Laboratory Animals*. 8th ed. Washington, DC: The National Academies Press; 2011. p. 246.
47. Bali A, Jaggi AS. Electric foot shock stress: A useful tool in neuropsychiatric studies. *Rev Neurosci*. 2015;26(6):655-677.
doi: 10.1515/revneuro-2015-0015
48. Bedford TG, Tipton CM, Wilson NC, Oppliger RA, Gisolfi CV. Maximum oxygen consumption of rats and its changes with various experimental procedures. *Appl Physiol Respir Environ Exerc Physiol*. 1979;47(6):1278-1283.
doi: 10.1152/jappl.1979.47.6.1278
49. Gould TD, Dao DT, Kovacsics CE. The open field test. In: *Mood and Anxiety Related Phenotypes in Mice*. Vol. 42. Totowa, NJ: Humana Press; 2009. p. 1-20.
doi: 10.1007/978-1-60761-303-9_1
50. Kraeuter AK, Guest PC, Sarnyai Z. The Y-maze for assessment of spatial working and reference memory in mice. *Methods Mol Biol*. 2019;1916:105-111.
doi: 10.1007/978-1-4939-8994-2_10
51. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autooxidation. *Anal Biochem*. 1978;90(1):81-89.
doi: 10.1016/0003-2697(78)90010-6
52. Clairborne A. Catalase activity. In: Greewald AR, editor. *Handbook of Methods for Oxygen Radical Research*. United States: CRC Press; 1995. p. 237-242.
53. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*. 1974;11(3):151-169.
doi: 10.1159/000136485
54. Farombi E, Tahnteng J, Agboola A, Nwankwo J, Emerole G. Chemoprevention of 2-acetylaminofluorene-

- induced hepatotoxicity and lipid peroxidation in rats by kolaviron--a *Garcinia kola* seed extract. *Food Chem Toxicol.* 2000;38(6):535-541.
doi: 10.1016/S0278-6915(00)00039-9
55. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: A critical evaluation. *Clin Chem.* 1995;41(6):892-896.
doi: 10.1093/clinchem/41.6.892
56. Pearse AG. The role of histochemistry in increasing objectivity in histopathology. *Postgrad Med J.* 1975;51(600):708-710.
doi: 10.1136/pgmj.51.600.708
57. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. *CSH Protoc.* 2008;2008(5):pdb.prot4986.
doi: 10.1101/pdb.prot4986
58. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: An enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem.* 1991;39(6):741-748.
doi: 10.1177/39.6.1709656
59. Burry RW. *Immunocytochemistry: A Practical Guide for Biomedical Research.* Berlin: Springer Science and Business Media; 2011.
60. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem.* 1981;29(4):577-580.
doi: 10.1177/29.4.6166661
61. Erukainure OL, Ijomone OM, Sanni O, Aschner M, Islam MS. Type 2 diabetes induced oxidative brain injury involves altered cerebellar neuronal integrity and elemental distribution, and exacerbated Nrf2 expression: Therapeutic potential of raffia palm (*Raphia hookeri*) wine. *Metab Brain Dis.* 2019;34:1385-1399.
doi: 10.1007/s11011-019-00444-x
62. Pagel P, Blome J, Wolf HW. High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. *J Chromatogr.* 2000;746(2):297-304.
doi: 10.1016/S0378-4347(00)00348-0.
63. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959;82(1):70-77.
doi: 10.1016/0003-9861(59)90090-6
64. Jain A, Jain R, Jain S. Quantitative determination of TNF alpha and interleukin-6. In: *Springer Protocols Handbooks.* United States: Springer; 2020. p. 87-89.
doi: 10.1007/978-1-4939-9861-6_27
65. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology.* 1998;94(2):147-160.
doi: 10.1007/BF00176837
66. Vlasak T, Dujlovic T, Barth A. Aluminum exposure and cognitive performance: A meta-analysis. *Sci Total Environ.* 2024;906:167453.
doi: 10.1016/j.scitotenv.2023.167453
67. Kandola A, Hendrikse J, Lucassen PJ, Yücel M. Aerobic exercise as a tool to improve hippocampal plasticity and function in humans: Practical implications for mental health treatment. *Front Hum Neurosci.* 2016;10:373.
doi: 10.3389/fnhum.2016.00373
68. De Sousa Fernandes MS, Ordônio TF, Santos GCJ, et al. Effects of physical exercise on neuroplasticity and brain function: A systematic review in human and animal studies. *Neural Plast.* 2020;2020:8856621.
doi: 10.1155/2020/8856621
69. Lovell MA, Ehmman WD, Butler SM, Markesbery WR. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology.* 1995;45(8):1594-1601.
doi: 10.1212/WNL.45.8.1594
70. Tiwari SC, Soni RM. Alzheimer's disease pathology and oxidative stress: Possible therapeutic options. *J Alzheimers Dis Park.* 2014;04(05):162.
doi: 10.4172/2161-0460.1000162
71. Slitt AM, Dominick PK, Roberts JC, Cohen SD. Effect of ribose cysteine pretreatment on hepatic and renal acetaminophen metabolite formation and glutathione depletion. *Basic Clin Pharmacol Toxicol.* 2015;96(6):487-494.
doi: 10.1111/j.1742-7843.2005.pto_96613.x
72. Mattson MP. Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. *Cell Metab.* 2022;16(6):706-722.
doi: 10.1016/j.cmet.2012.08.012
73. Zatta P, Ibn-Lkhatay-Idrissi M, Zambenedetti P, Kilyen M, Kiss T. *In vivo* and *in vitro* effects of aluminium on the activity of mouse brain acetylcholinesterase. *Brain Res Bull.* 2012;59(1):41-45.
doi: 10.1016/S0361-9230(02)00836-5
74. Amberla K, Nordberg A, Viitanen M, Winblad B. Longterm treatment with tacrine (THA) in Alzheimer's disease--evaluation of neuropsychological data. *Acta Neurol Scand Appl.* 2013;88(S149):55-57.
doi: 10.1111/j.1600-0404.1993.tb04257.x
75. Meeusen R, De Meirleir K. Exercise and brain

- neurotransmission. *Sports Med.* 2005;20(3):160-188.
doi: 10.2165/00007256-199520030-00004
76. Cheng XJ, Gu JX, Pang YP, *et al.* Tacrine-hydrogen sulfide donor hybrid ameliorates cognitive impairment in the aluminium chloride mouse model of Alzheimer's disease. *ACS Chem Neurosci.* 2019;10(8):3500-3509.
doi: 10.1021/acscchemneuro.9b00120
77. Ghosh S, Lertwattanak R, Garduño JD, *et al.* Elevated muscle TLR4 expression and metabolic endotoxemia in human aging. *J Gerontol A Biol Sci Med Sci.* 2015;70(2):232-246.
doi: 10.1093/gerona/flu067
78. Uyar B, Palmer D, Kowald A, Murua-Escobar H, Barrantes I, Möller S. Single-cell analyses of aging, inflammation and senescence. *Ageing Res Rev.* 2020;64:101156.
doi: 10.1016/j.arr.2020.101156
79. Zagrodzki P, Joniec A, Gawlik M, *et al.* High fructose model of oxidative stress and metabolic disturbances in rats. Part I. Antioxidant status of rats' tissues. *Bull Vet Inst Pulawy.* 2019;51(3):407-412.
80. Valenzuela PL, Maffioletti NA, Joyner MJ, Lucia A, Lepers R. Lifelong endurance exercise as a countermeasure against age-related $\text{VO}_{2\text{max}}$ decline: Physiological overview and insights from masters athletes. *Sports Med.* 2020;50(4):703-716.
doi: 10.1007/s40279-019-01252-0
81. Cao Z, Yang X, Zhang H, *et al.* Aluminum chloride induces neuroinflammation, loss of neuronal dendritic spine and cognition impairment in developing rats. *Chemosphere.* 2016;151:289-295.
doi: 10.1016/j.chemosphere.2016.02.092
82. Zhang X, He Q, Huang T, *et al.* Treadmill exercise decreases deposition and counteracts cognitive decline in APP/PS1 mice, Possibly via hippocampal microglia modifications. *Front Aging Neurosci.* 2019;11:78.
doi: 10.3389/fnagi.2019.00078
83. Hosseini M, Alaei H, Reisi P, Radahmadi M. The effect of treadmill running on memory before and after the NBM-lesion in rats. *J Bodyw Mov Ther.* 2013;17(4):423-429.
doi: 10.1016/j.jbmt.2012.12.005