Original

Modulation of Synaptophysin Immunoreactivity, Oxidative Stress Markers, and Cerebral Cortex Toxicity: The Role of Magnesium Glycinate in Cyclophosphamide-Treated Rats

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ABSTRACT

Background: The usage of cyclophosphamide (CP) in chemotherapy is limited by neurotoxicity, anxiety-related behaviours, and cognitive dysfunction. Its shortcomings in this regard have prompted the search for substances that can ameliorate the adverse effects without compromising its usage in cancer management. This study investigated whether magnesium glycinate (MgGly) attenuates CP-induced anxiety-like behaviour, oxidative stress, and cortical degeneration in Wistar rats.

Methods: Forty-eight male rats (n=8/group) were divided into: control, low-dose MgGly (22.8 mg/kg), high-dose MgGly (32.8 mg/kg), CP + low-dose MgGly, CP + high-dose MgGly, and CP-only. CP (150 mg/kg, i.p.) was given on days 1, 3, and 5; MgGly was administered orally for 21 days. We evaluated neurobehaviour, cerebral oxidative stress (measured by MDA, TAC, SOD, and CAT), cortical cytoarchitecture, and synaptophysin immunoreactivity.

Results: CP intoxication resulted in severe anxiety and motor deficits, significantly reducing open-arm time and line crossing. Biochemically, CP elevated cerebral MDA levels and depleted SOD, TAC, and CAT activities, inducing profound cortical degeneration and synaptic stripping. High-dose MgGly (32.8 mg/kg) significantly reversed these impacts, restoring open-arm time, attenuating MDA (0.68±0.02 μM), and recovering SOD (0.38±0.02 U/ml), TAC (5.83±0.41 mM), and CAT (1.02±0.05 U/mg). It also robustly preserved cortical architecture and synaptophysin immunoreactivity. Protective effects were strictly dose-dependent, favouring the 32.8 mg/kg regimen. MgGly alone lacked toxicity.

Conclusion: This study demonstrated that magnesium glycinate confers robust, dose-dependent neuroprotection against CP-induced oxidative stress, neurotoxicity, cortical degeneration, and a mechanistically supported and clinically accessible adjunct to mitigate chemotherapy-related neurocognitive and neurodegenerative impacts.

Keywords: Oxidative Stress, Chemotherapy, Neurotoxicity, Synaptophysin immunoreactivity, Magnesium glycinate, Anxiety-like behaviours.

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INTRODUCTION

Cyclophosphamide (CP), a potent nitrogen mustard alkylating agent, remains an indispensable cornerstone of combination chemotherapy for diverse malignancies, including breast and ovarian cancers, as well as a critical immunosuppressant for severe autoimmune disorders [1]. However, its enduring clinical utility is significantly compromised by debilitating neurological sequelae. CP-containing regimens are primary contributors to chemotherapy induced cognitive impairment (colloquially termed "chemobrain") which is frequently accompanied by anxiety, depression, and profound fatigue, ultimately devastating the quality of life for a substantial subset of survivors [2,3]. Preclinical models robustly recapitulate these clinical phenotypes; repeated CP administration elicits anhedonia, anxiety-like behaviours, and spatial memory deficits in rodents. These behavioural alterations are intrinsically linked to impaired hippocampal neurogenesis and diminished brain derived neurotrophic factor (BDNF) expression [4].

Mechanistically, the pathogenesis of CP induced neurotoxicity is driven by a detrimental triad of oxidative stress, neuroinflammation, and apoptosis. CP initiates central neuroinflammation by activating Toll-like receptor 4 (TLR4) and the NLRP3 inflammasome, triggering NF-κB dependent cytokine storms and downstream caspase 1 and caspase 3 mediated neuronal apoptosis [5]. Concurrently, CP aggressively disrupts cerebral redox homeostasis. In rodent cortical and hippocampal tissues, it precipitates a surge in malondialdehyde (MDA) and lipid peroxidation while crippling endogenous antioxidant defenses, notably superoxide dismutase (SOD) and glutathione. These biochemical insults manifest morphologically as progressive cortical vacuolation, severe encephalopathic changes, and widespread neuronal loss [5].

Magnesium, a critical divalent cation, plays an essential neuromodulatory role in the central nervous system by regulating NMDA receptor gating, synaptic plasticity, hypothalamic–pituitary–adrenal (HPA) axis tone, and neuroinflammatory pathways [6,7]. Experimental magnesium depletion provokes profound neuroinflammation, HPA axis hyperactivity, and affective disorders, all of which are reversible via targeted repletion. Clinically, oral magnesium supplementation significantly ameliorates anxiety and depressive symptoms in stressed, hypomagnesemic cohorts [8]. Beyond foundational neuromodulation, targeted magnesium delivery (e.g., magnesium L threonate) provides robust neuroprotection against toxin induced brain injury, notably preventing oxaliplatin induced affective and cognitive deficits by normalizing TNF α and NF κB signaling and arresting oxidative stress [7].

Among oral formulations, organic chelates like magnesium glycinate (bisglycinate) uniquely offer superior gastrointestinal tolerability and enhanced bioavailability, leveraging partial dipeptide mediated transport to optimize intestinal absorption [9]. Despite these pharmacokinetic advantages and the recognized potential of magnesium-based neuroprotection, the specific efficacy of magnesium glycinate in mitigating CP induced oxidative brain injury and affective dysfunction remains entirely uncharacterized. To address this critical translational gap, the present study evaluates the hypothesis that magnesium glycinate ameliorates CP induced anxiety like behaviour, oxidative neurotoxicity, and cortical degeneration in Wistar rats. Utilizing established behavioural paradigms (OFT and EPM) alongside quantitative biochemical assessments of lipid peroxidation, antioxidant reserves, and high resolution, cortical histopathology, this research aims to establish magnesium glycinate as a viable, well tolerated neuroprotective adjunct in oncological care.

MATERIALS AND METHODS

Chemicals and Reagents: Magnesium glycinate (250 mg tablets; Vitacost, New York, USA) and cyclophosphamide for injection (xGen Pharmaceuticals DJB, Inc., USA) were obtained commercially. Colorimetric assay kits for malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), and catalase (CAT) were sourced from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

Experimental Animals and Ethical Approval

Forty-eight (48) adult male Wistar rats (eight weeks old; body weight 150–170 g) were obtained from certified animal breeders in Iwo, Osun State, Nigeria. Animals were housed in polypropylene cages (45 × 24 × 20 cm) under standard laboratory conditions: ambient temperature of 22 ± 2°C, relative humidity of 50 ± 5%, and a 12-hour light–dark cycle. Standard pelleted feed (Top Feeds®, Nigeria) and clean water were provided ad libitum throughout the study. Following a two-week acclimatization period, animals were randomly assigned to six experimental groups (n = 8 per group) using a computer-generated randomization sequence. The

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sample size of eight animals per group was determined a priori using G*Power software (version 3.1), based on a projected effect size of f = 0.40, α = 0.05, and a desired power of 0.80, yielding a minimum of 7 animals per group; one animal per group was added to account for potential attrition.

All experimental procedures were conducted in strict accordance with the European Council Directive 2010/63/EU on the protection of animals used for scientific purposes, and received approval from the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Ilesa, Ilesa, Osun State, Nigeria (Approval Code: UNILESA/FBMS/2025/04).

Experimental Design and Drug Administration: Animals were allocated into six groups (n = 8) and treated for 21 consecutive days as follows:

- **Group A (Control):** Received distilled water orally and normal saline intraperitoneally (i.p.)
- **Group B (Low-Dose MgGly):** Received magnesium glycinate 22.8 mg/kg orally daily
- **Group C (High-Dose MgGly):** Received magnesium glycinate 32.8 mg/kg orally daily
- **Group D (CP + Low-Dose MgGly):** Received cyclophosphamide 150 mg/kg i.p. on days 1, 3, and 5, and magnesium glycinate 22.8 mg/kg orally daily
- **Group E (CP + High-Dose MgGly):** Received cyclophosphamide 150 mg/kg i.p. on days 1, 3, and 5, and magnesium glycinate 32.8 mg/kg orally daily
- **Group F (CP only):** Received cyclophosphamide 150 mg/kg i.p. on days 1, 3, and 5

Magnesium glycinate dosing was adapted from Aniebo (2023) [10]. Cyclophosphamide was dissolved in normal saline and administered as three i.p. injections on alternate days (days 1, 3, and 5) to establish a cumulative neurotoxic insult model. Body weights were recorded weekly using a calibrated electronic balance throughout the experimental period.

Neurobehavioural Assessments

On days 20 and 21, all animals underwent neurobehavioural evaluation under standardized conditions: dim lighting, quiet environment, and consistent timing (09:00–13:00 h). Each apparatus was thoroughly cleaned with 10% ethanol between subjects to eliminate olfactory cues.

Open Field Test (OFT): Locomotor and anxiety-related behaviours were assessed using the open field test as previously described [11]. Each rat was individually placed at the centre of an open field arena (100 × 100 × 40 cm, floor divided into 25 equal squares) and observed for 5 minutes. The following parameters were scored: (i) **line crossing frequency** (number of grid lines crossed with all four paws, an index of locomotor activity); (ii) **rearing frequency** (number of times the animal stood on its hind limbs, reflecting exploratory drive); (iii) **grooming frequency** (number of self-grooming episodes, a stereotypic anxiety-related behaviour); and (iv) **defecation count** (number of faecal boluses deposited, a physiological correlate of stress and autonomic arousal).

Elevated Plus Maze (EPM): Anxiety-like behaviour was evaluated using the elevated plus maze, a validated paradigm exploiting the innate conflict between rodent exploratory drive and aversion to open, elevated spaces [12]. The apparatus comprised two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) arranged perpendicularly and elevated 50 cm above the floor. Each rat was placed at the central platform facing an open arm and observed for 5 minutes. The percentage of time spent in the open arms and closed arms was recorded as the primary indices of anxiety-like behaviour, with reduced open-arm time indicative of heightened anxiety.

Brain Tissue Collection and Preparation

On day 22, animals were sacrificed by cervical dislocation under light anaesthesia. The cranium was carefully dissected and the whole brain was excised. The right cerebral hemisphere was homogenised in ice-cold phosphate-buffered saline (PBS, pH 7.4) at a 1:9 (w/v) ratio using a glass homogeniser. The homogenate was centrifuged at 4,000 × g for 15 minutes at 4°C, and the resulting supernatant was collected and stored at −80°C for subsequent biochemical analyses. The left cerebral hemisphere was fixed in 10% neutral buffered formalin for histological and immunohistochemical processing.

Biochemical Assessment of Oxidative Stress

Malondialdehyde (MDA) Assay

Cerebral lipid peroxidation was quantified by measuring MDA concentration using the thiobarbituric acid reactive substances (TBARS) method [13]. Briefly, brain homogenate supernatant was reacted with thiobarbituric acid under acidic, high-temperature conditions to generate a pink chromogen, the absorbance of which

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was measured spectrophotometrically at 532 nm. MDA levels were expressed as μM.

Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using the FRAP (Ferric Reducing Antioxidant Power) colorimetric assay, with Trolox as standard [14]. Absorbance was read at 593 nm and results expressed as mM Trolox equivalents.

Superoxide Dismutase (SOD) Activity

SOD activity was assessed by measuring the inhibition of pyrogallol autoxidation according to the method of Misra and Fridovich (1972), as adapted for brain tissue [15]. Enzyme activity was expressed as units per millilitre (U/ml).

Catalase (CAT) Activity

Catalase activity was measured by monitoring the rate of hydrogen peroxide (H_2O_2) decomposition at 240 nm, as described by Aebi (1984) [16]. Results were expressed as units per milligram protein (U/mg protein). Total protein concentration in all samples was determined by the Bradford method using bovine serum albumin (BSA) as standard.

Histological Processing

The fixed left cerebral hemispheres were dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin wax. Coronal sections (5 μm) of the cerebral cortex were cut using a rotary microtome and mounted on positively charged glass slides.

Haematoxylin and Eosin (H&E) Staining

Sections were deparaffinised in xylene, rehydrated through graded ethanol, and stained with Mayer's haematoxylin for 5 minutes, followed by counterstaining with eosin for 2 minutes. Slides were dehydrated, cleared, and mounted with DPX for light microscopic examination of general cortical cytoarchitecture, including assessment of neuronal morphology, neuropil integrity, perineuronal oedema, and pyknotic nuclei.

Cresyl Fast Violet (Nissl) Staining

To evaluate neuronal Nissl substance integrity and chromatolytic changes, deparaffinised sections were stained with 1% Cresyl Fast Violet solution for 10 minutes at 60°C, differentiated in 70% ethanol, dehydrated, cleared in xylene, and coverslipped. Nissl body abundance and chromatolysis were assessed as indices of neuronal health and degeneration.

Synaptophysin Immunohistochemistry

Synaptophysin immunoreactivity, a presynaptic vesicle glycoprotein and reliable marker of synaptic density and integrity was evaluated by immunohistochemistry as described by Krenacs (2010) [17]. Paraffin-embedded cortical sections were dewaxed through two changes of xylene (5 minutes each) and rehydrated through graded ethanol (100%, 95%, 70%, 5 minutes each), followed by rinsing in distilled water. Heat-induced epitope retrieval (HIER) was performed in citrate buffer (pH 6.0) for 25 minutes using an automated stainer, after which slides were cooled to room temperature and rinsed in PBS/TBST.

Endogenous peroxidase activity was quenched with 3% H_2O_2 for 15 minutes. Non-specific binding was blocked by incubation in 10% normal goat serum in PBS for 30 minutes. Sections were incubated overnight at 4°C with anti-synaptophysin monoclonal primary antibody (clone IHC669), then rinsed thrice in PBS (5 minutes each). Sequential incubation with biotinylated secondary antibody (20 minutes) and HRP-streptavidin complex (20 minutes) was followed by visualisation with 3,3'-diaminobenzidine (**DAB**) chromogen for 10 minutes, producing a brown precipitate at sites of immunoreactivity. Sections were counterstained with haematoxylin (1–5 minutes), dehydrated, cleared in xylene, and mounted.

Photomicrography: All stained sections were examined using a Sellon-Olympus trinocular microscope (XSZ-107E, China) at ×400 magnification. Photomicrographs were acquired with a digital camera (Canon PowerShot 2500). Histopathological and immunohistochemical scoring was performed by a board-certified pathologist who was blinded to the experimental group allocations.

Statistical Analysis: All data are presented as mean ± standard error of the mean (SEM). Histopathological and immunohistochemical scoring was performed by a board-certified pathologist who was blinded to the experimental group allocations; investigators responsible for biochemical analyses were similarly blinded to group assignments throughout data acquisition and processing. Parametric data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test to identify statistically significant inter-group differences. All analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

RESULTS

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Effect of Magnesium Glycinate on Locomotor and Exploratory Behaviour (Open Field Test)

In the Open Field Test, cyclophosphamide intoxication (Group F) precipitated significant behavioural deficits, evidenced by a marked reduction in line crossing (10.20 ± 0.969), grooming (6.00 ± 0.836), and rearing (6.20 ± 0.735), alongside a significant increase in defecation count (4.40 ± 0.400) compared to the Control group (Group A). Magnesium glycinate administration alone (Groups B and C) produced no adverse behavioural effects, maintaining parameters comparable to the control. Co-administration of high-dose magnesium glycinate (Group E) significantly ameliorated CP-induced deficits, notably increasing rearing frequency to 12.00 ± 0.894 and line crossing to 16.80 ± 2.035, demonstrating a dose-dependent preservation of motor function and exploratory drive.

Effect of Magnesium Glycinate on Anxiety-Like Behaviour (Elevated Plus Maze)

The Elevated Plus Maze (EPM) evaluation revealed a pronounced anxiogenic effect following cyclophosphamide administration. Rats in Group F spent significantly less time in the open arms (13.99 ± 0.230) and more time in the closed arms (86.01 ± 0.220) relative to the control group (19.21 ± 0.090 and 73.76 ± 0.870, respectively). Conversely, treatment with both low and high doses of magnesium glycinate (Groups D and E) significantly increased open arm exploration time (25.99 ± 0.870 and 26.24 ± 1.010, respectively) and decreased closed arm confinement compared to the CP-only group. Notably, magnesium glycinate supplementation alone (Groups B and C) also significantly enhanced open arm time relative to the control, indicating inherent anxiolytic properties.

Effect of Magnesium Glycinate on Cerebral Oxidative Stress and Antioxidant Status

Biochemical analysis of the cerebral cortex demonstrated that cyclophosphamide (Group F) induced severe oxidative damage, significantly elevating malondialdehyde (MDA) levels (0.9107 ± 0.015 μM) compared to Group A (0.4383 ± 0.025 μM). Concurrently, CP significantly depleted endogenous antioxidant defenses, reducing Total Antioxidant Capacity (TAC) to 3.167 ± 0.4206 mM, Superoxide Dismutase (SOD) to 0.2247 ± 0.041 U/ml, and Catalase (CAT) to 0.836 ± 0.024 U/mg protein. Intervention with high-dose magnesium glycinate (Group E) robustly reversed this pathology, significantly attenuating MDA levels (0.6823 ± 0.023 μM) and restoring TAC (5.826 ± 0.4061 mM), SOD (0.3840 ± 0.019 U/ml), and CAT (1.019 ± 0.048 U/mg protein) activities compared to Group F.

Effect of Magnesium Glycinate on Synaptic Integrity (Synaptophysin Immunoreactivity)

Immunohistochemical evaluation of synaptophysin revealed dense and widespread presynaptic terminal expression in the cortical neuropil of the normal control and supplement-only groups (Groups A, B, and C). Cyclophosphamide intoxication (Group F) caused severe depletion of synaptophysin immunoreactivity and widespread loss of presynaptic terminals. While low-dose magnesium glycinate (Group D) demonstrated only moderate restoration, the high-dose intervention (Group E) illustrated a robust, dose-dependent preservation of synaptophysin immunoreactivity and intact neuronal architecture that closely resembled the healthy baseline.

Effect of Magnesium Glycinate on Cortical Cytoarchitecture and Nissl Substance

Histopathological assessment via H&E and Cresyl Fast Violet staining displayed normal cytoarchitecture with abundant Nissl bodies and pale vesicular nuclei in Groups A, B, and C. Group F exhibited severe neurodegeneration characterized by widespread shrunken, pyknotic nuclei, extensive neuropil vacuolation (spongiosis), perineuronal halos indicative of severe edema, and extensive chromatolysis with distinct "ghost" cells. Group D exhibited minimal protection, presenting with persistent chromatolysis and degenerating neurons. In contrast, Group E demonstrated marked, dose-dependent structural preservation; this included significantly reduced vacuolation, restored normal cellular morphology, and the notable reappearance of Nissl substance within surviving pyramidal neurons.

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Table 1: Effect of magnesium glycinate on Open Field Test parameters (line crossing, grooming, rearing, and defecation) in cyclophosphamide-intoxicated Wistar rats.

Group	Line Crossing	Grooming	Rearing	Defecation Count
A. Control	25.80 ± 1.158	13.20 ± 1.020	17.60 ± 1.435	1.40 ± 0.400
B. Low-Dose (MgGly)	27.40 ± 5.741	10.60 ± 0.927	15.80 ± 1.068	2.00 ± 0.548
C. High-Dose (MgGly)	28.40 ± 4.479	11.80 ± 1.200	15.80 ± 2.223	1.60 ± 0.678
D. CP + Low-Dose MgGly	14.20 ± 1.655 [c]	8.60 ± 0.979 [a]	8.60 ± 0.678 [a,b,c]	3.40 ± 0.678
E. CP + High-Dose MgGly	16.80 ± 2.035	9.40 ± 0.678 [a,b,c]	12.00 ± 0.894 [d]	2.80 ± 0.490
F. CP only	10.20 ± 0.969 [a,b,c]	6.00 ± 0.836 [a,b,c]	6.20 ± 0.735 [a,b,c]	4.40 ± 0.400 [a,b,c]

Values are expressed as Mean ± SEM (n = 8). Superscript letters indicate statistical significance at p < 0.05: (a) compared to the Control group (Group A); (b) compared to the Low-Dose Magnesium glycinate group (Group B); (c) compared to the High-Dose Magnesium glycinate group (Group C); (d) compared to the CP + Low-Dose Magnesium glycinate group (Group D); (e) compared to the CP + High-Dose Magnesium glycinate group (Group E)

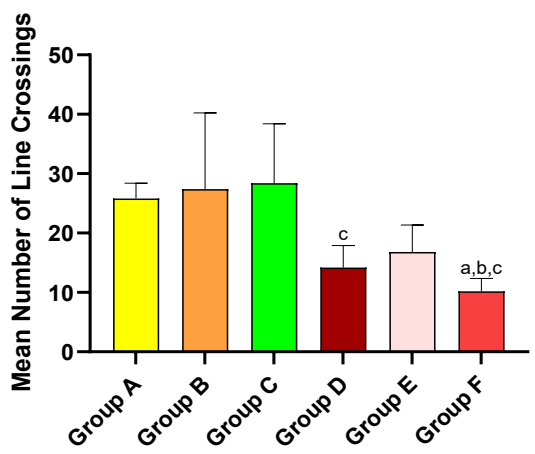

Figure 1: Effect of magnesium glycinate on locomotor activity (line crossing frequency) in the Open Field Test in cyclophosphamide-intoxicated Wistar rats.

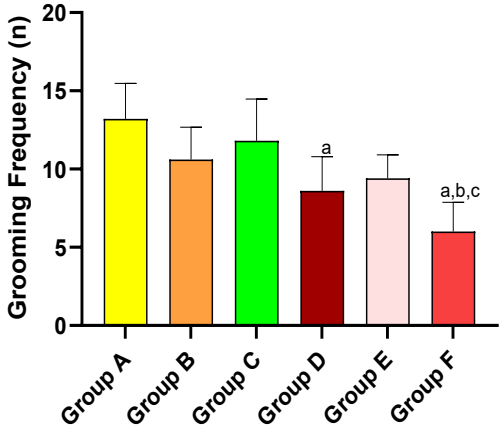

Figure 2: Effect of magnesium glycinate on grooming behaviour frequency in the Open Field Test in cyclophosphamide-intoxicated Wistar rats.

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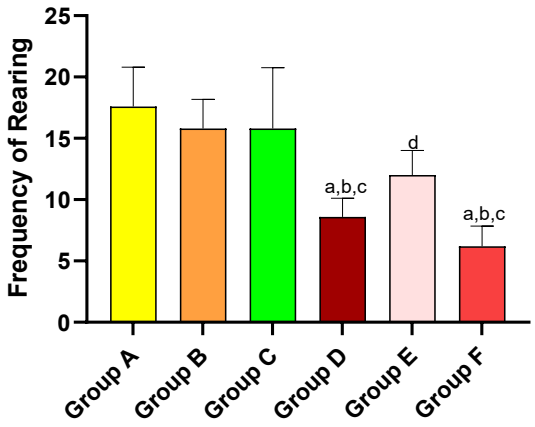

Figure 3: Effect of magnesium glycinate on rearing frequency in the Open Field Test in cyclophosphamide-intoxicated Wistar rats.

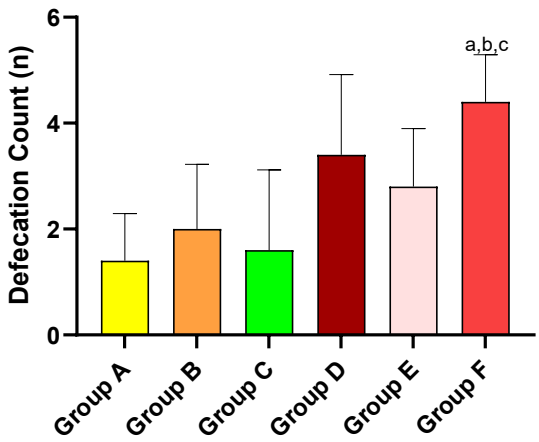

Figure 4: Effect of magnesium glycinate on physiological stress (defecation count) in the Open Field Test in cyclophosphamide-intoxicated Wistar rats.

Table 2: Effect of magnesium glycinate on anxiety-like behaviour (time spent in open and closed arms) in the Elevated Plus Maze in cyclophosphamide-intoxicated Wistar rats.

Group	Group	OPEN ARM TIME Mean ± SEM	Closed Arm time Mean ± SEM
A. Control	A	19.21 ± 0.090	73.76 ± 0.870
B. Low-Dose (MgGly)	B	28.99 ± 0.110 [a]	71.01 ± 0.230
C. High-Dose (MgGly)	C	34.42 ± 0.320 [a,b]	65.58 ± 1.010 [a,b]
D. CP + Low-Dose MgGly	D	25.99 ± 0.870 [a,b,c]	80.79 ± 0.100 [a,b,c]
E. CP + High-Dose MgGly	E	26.24 ± 1.010 [a,b,c,d]	74.01 ± 0.910 [a,b,c,d]
F. CP only	F	13.99 ± 0.230 [a,b,c,e]	86.01 ± 0.220 [a,b,c,d,e]

Values are expressed as Mean ± SEM (n = 8). Superscript letters indicate statistical significance at p < 0.05: (a) compared to the Control group (Group A); (b) compared to the Low-Dose Magnesium glycinate group (Group B); (c) compared to the High-Dose Magnesium glycinate group (Group C); (d) compared to the CP + Low-Dose Magnesium glycinate group (Group D); (e) compared to the CP + High-Dose Magnesium glycinate group (Group E)

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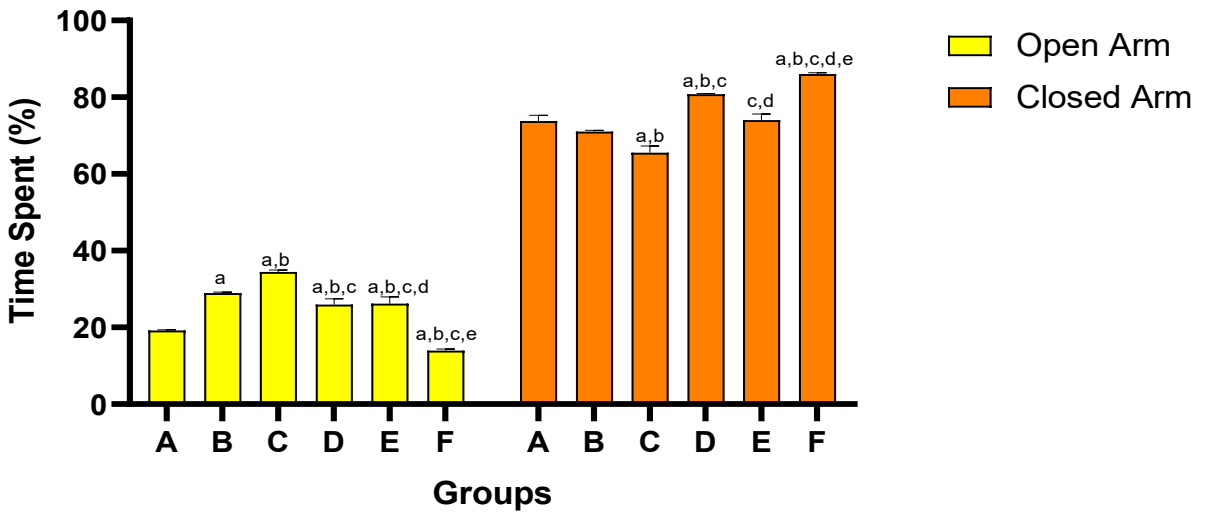

Figure 5: Effect of magnesium glycinate on the time spent in the open and closed arms of the Elevated Plus Maze in cyclophosphamide-intoxicated Wistar rats.

Table 3: Effect of magnesium glycinate on oxidative stress (MDA) and antioxidant status (TAC, SOD, CAT) in cyclophosphamide-intoxicated Wistar rats.

Group	MDA (µM)	TAC (mM Trolox Equivalence)	SOD (U/ml)	CAT (U/mg protein)
A. Control	0.4383 ± 0.025	6.967 ± 0.1043	0.5973 ± 0.023	1.309 ± 0.016
B. Low-Dose (MgGly)	0.4063 ± 0.028	7.097 ± 0.4285	0.6417 ± 0.017	1.431 ± 0.026
C. High-Dose (MgGly)	0.3670 ± 0.012	7.792 ± 0.1369	0.6267 ± 0.017	1.417 ± 0.034
D. CP + Low-Dose MgGly	0.9240 ± 0.034 ^{a,b,c}	4.003 ± 0.1137 ^{a,b,c}	0.2943 ± 0.006 ^{a,b,c}	0.916 ± 0.020 ^{a,b,c}
E. CP + High-Dose MgGly	0.6823 ± 0.023 ^{a,b,c,d}	5.826 ± 0.4061 ^{c,d}	0.3840 ± 0.019 ^{a,b,c}	1.019 ± 0.048 ^{a,b,c}
F. CP only	0.9107 ± 0.015 ^{a,b,c,e}	3.167 ± 0.4206 ^{a,b,c,e}	0.2247 ± 0.041 ^{a,b,c,e}	0.836 ± 0.024 ^{a,b,c,e}

Values are expressed as Mean ± SEM (n = 8). Superscript letters indicate statistical significance at p < 0.05: (a) compared to the Control group (Group A); (b) compared to the Low-Dose Magnesium glycinate group (Group B); (c) compared to the High-Dose Magnesium glycinate group (Group C); (d) compared to the CP + Low-Dose Magnesium glycinate group (Group D); (e) compared to the CP + High-Dose Magnesium glycinate group (Group E)

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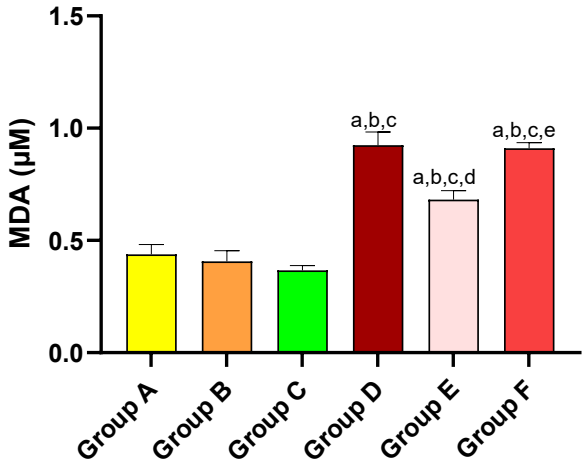

Figure 6: Effect of magnesium glycinate on cerebral malondialdehyde (MDA) levels, a biomarker of lipid peroxidation, in cyclophosphamide-intoxicated Wistar rats.

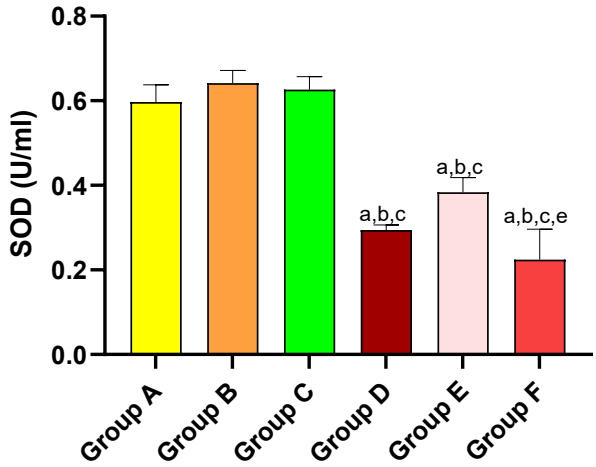

Figure 8: Effect of magnesium glycinate on cerebral Superoxide Dismutase (SOD) activity in cyclophosphamide-intoxicated Wistar rats.

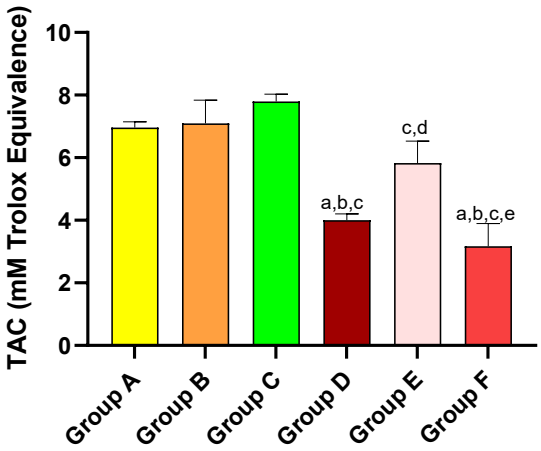

Figure 7: Effect of magnesium glycinate on cerebral Total Antioxidant Capacity (TAC) in cyclophosphamide-intoxicated Wistar rats.

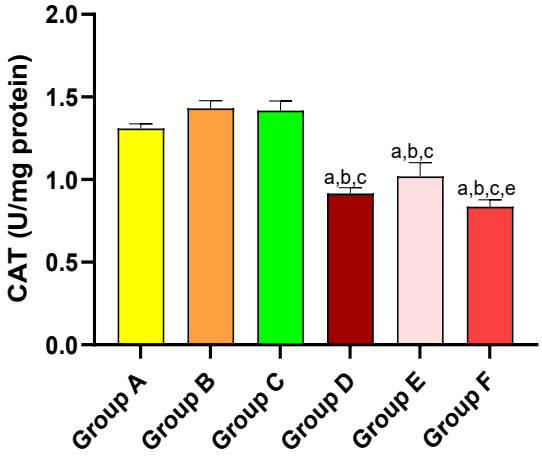

Figure 9: Effect of magnesium glycinate on cerebral Catalase (CAT) activity in cyclophosphamide-intoxicated Wistar rats.

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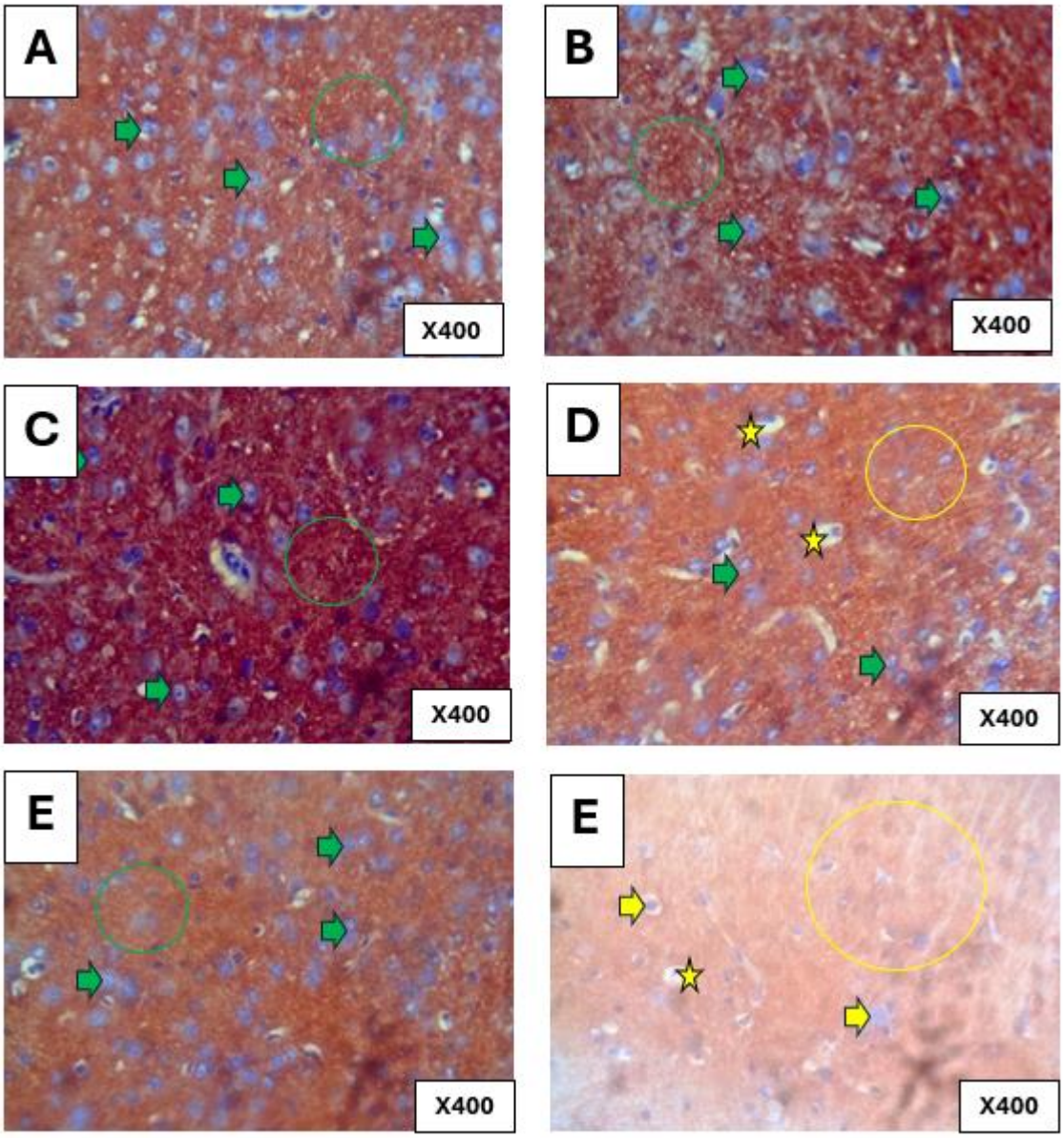

Figure 10: Photomicrographs of Synaptophysin immunoreactivity in the cerebral cortex of Wistar rats across experimental groups (Magnification X400).

(A) Control group; **(B)** Magnesium glycinate (22.8 mg/kg) group; and **(C)** Magnesium glycinate (32.8 mg/kg) group, all showing dense and widespread synaptophysin expression in the neuropil (green circles) with normal morphological nuclei (green arrows). **(F)** Cyclophosphamide (CP, 150 mg/kg) intoxicated group, showing severe depletion of synaptophysin immunoreactivity (yellow circle), widespread loss of presynaptic terminals (yellow stars), and altered cellular morphology (yellow arrows). **(D)** CP + Magnesium glycinate (22.8 mg/kg) group, demonstrating moderate restoration of synaptophysin expression with mixed areas of recovery (green arrows) and mild persistent depletion (yellow circle and star). **(E)** CP + Magnesium glycinate (32.8 mg/kg) group, illustrating a robust, dose-dependent preservation of synaptophysin immunoreactivity (green circle) and intact neuronal cellular architecture (green arrows), closely resembling the control.

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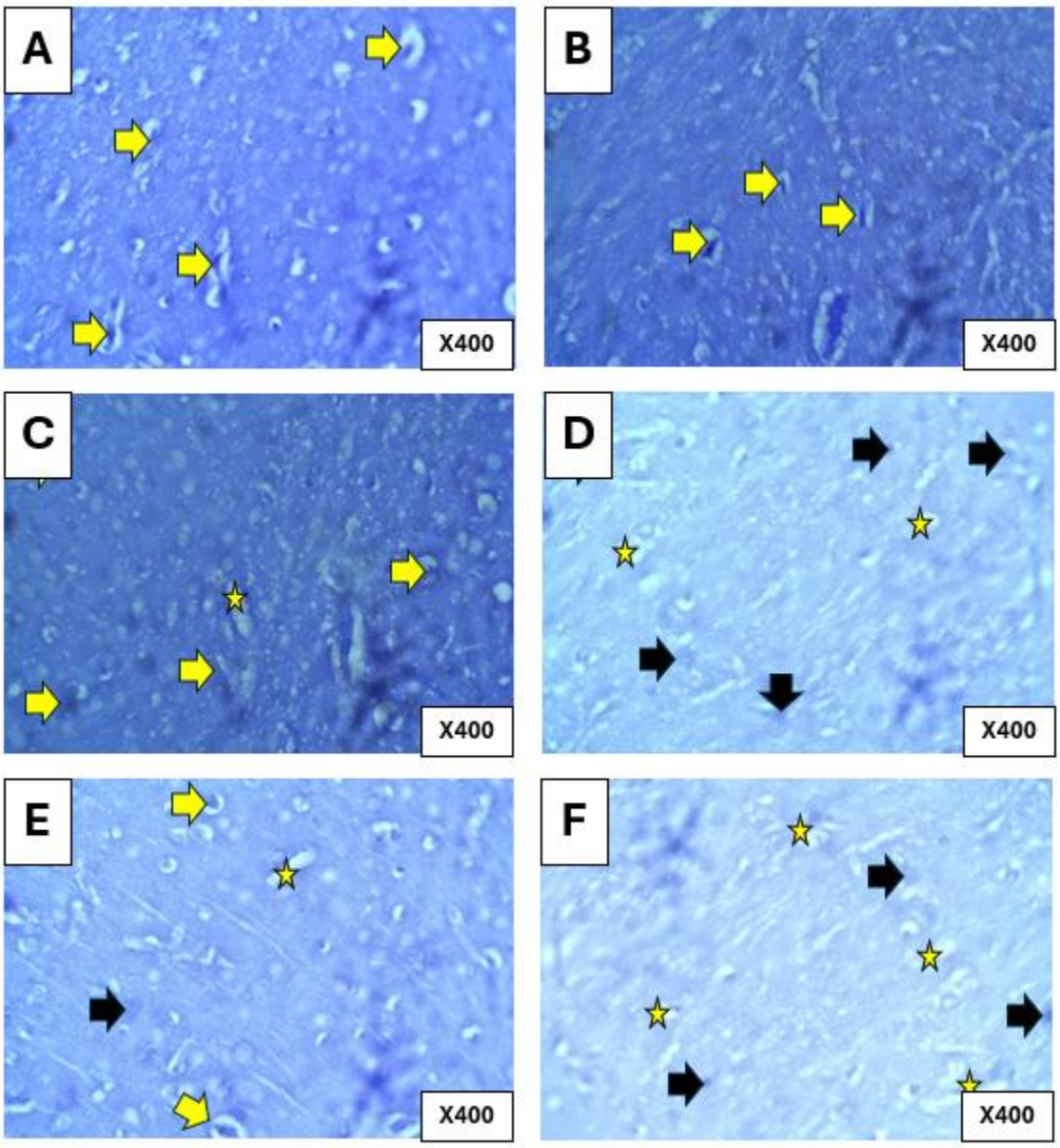

Figure 11: Photomicrographs of Cresyl Fast Violet-stained sections of the cerebral cortex across experimental groups (Magnification X400).
(A) Control group; **(B)** Magnesium glycinate (22.8 mg/kg) group; and **(C)** Magnesium glycinate (32.8 mg/kg) group, all demonstrating normal cytoarchitecture with abundant, intensely stained Nissl bodies in the cytoplasm of healthy pyramidal neurons (yellow arrows). **(F)** Cyclophosphamide (CP, 150 mg/kg) intoxicated group, showing severe neurodegeneration characterized by extensive chromatolysis (yellow stars) and numerous shrunken, pyknotic, or "ghost" cells lacking distinct Nissl substance (black arrows). **(D)** CP + Magnesium glycinate (22.8 mg/kg) group, exhibiting minimal protection with persistent, widespread chromatolysis (yellow stars) and degenerating neurons (black arrows). **(E)** CP + Magnesium glycinate (32.8 mg/kg) group, illustrating a marked, dose-dependent preservation of cortical architecture, showing a restoration of Nissl substance in surviving pyramidal neurons (yellow arrows) alongside only mild localized chromatolysis (yellow star) and fewer pyknotic cells (black arrow).

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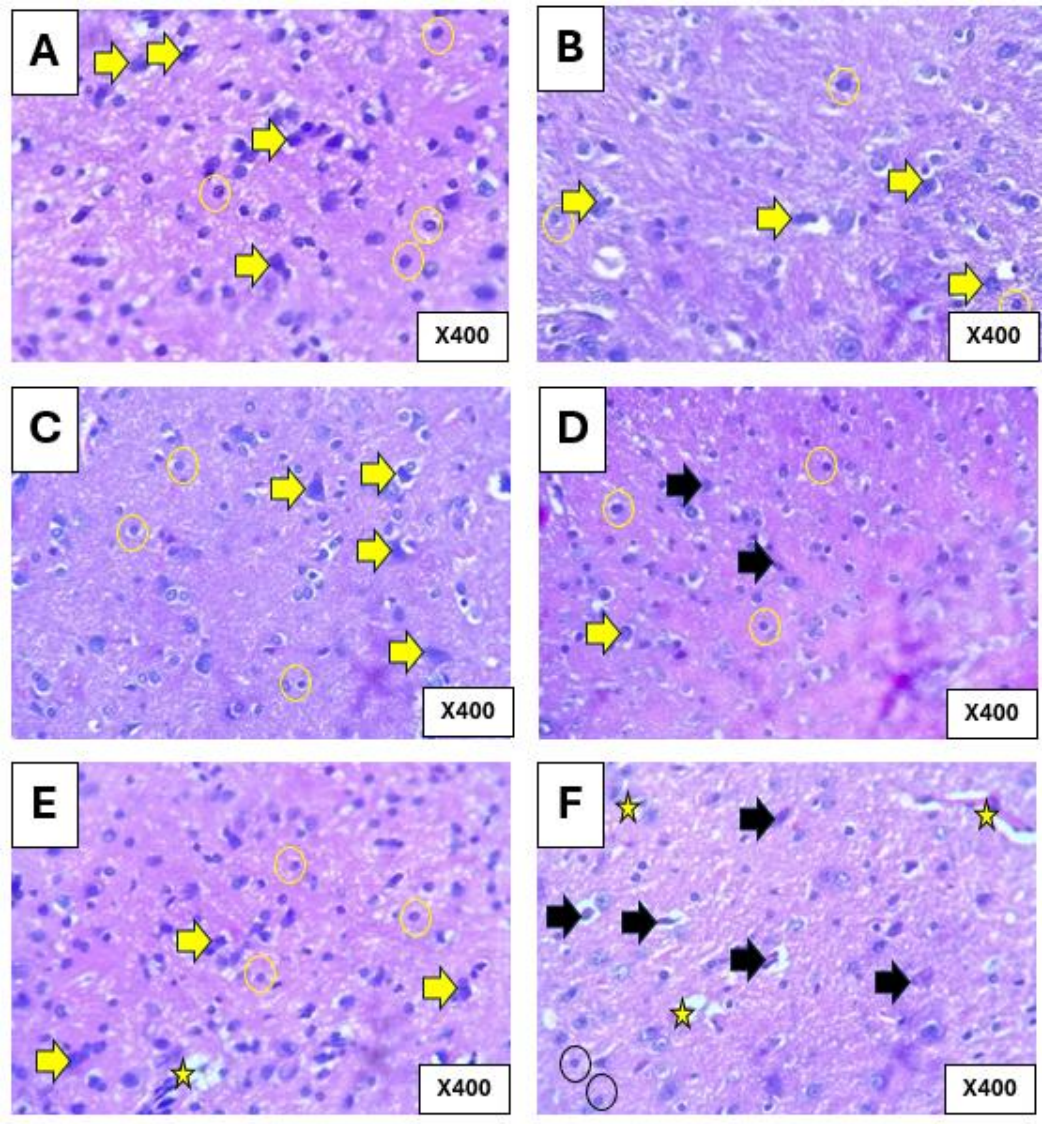

Figure 12: Photomicrographs of Hematoxylin and Eosin (H&E) stained sections of the cerebral cortex across experimental groups (Magnification X400).
(A) Control group; **(B)** Magnesium glycinate (22.8 mg/kg) group; and **(C)** Magnesium glycinate (32.8 mg/kg) group, all exhibiting normal cortical cytoarchitecture with a cohesive neuropil and abundant healthy pyramidal neurons (yellow arrows) containing prominent, pale vesicular nuclei (yellow circles). **(F)** Cyclophosphamide (CP, 150 mg/kg) intoxicated group, demonstrating severe neurodegeneration characterized by widespread shrunken, hyperchromatic pyknotic nuclei (black arrows), extensive neuropil vacuolation/spongiosis (yellow stars), and degenerating neurons surrounded by perineuronal halos indicative of severe edema (black circles). **(D)** CP + Magnesium glycinate (22.8 mg/kg) group, showing weak histological protection with a mixture of surviving neurons (yellow arrows/circles) and persistent pyknotic, degenerating cells (black arrows). **(E)** CP + Magnesium glycinate (32.8 mg/kg) group, illustrating a marked, dose-dependent amelioration of CP-induced damage, characterized by the restoration of normal cellular morphology (yellow arrows/circles) and significantly reduced vacuolation (yellow star), closely resembling the healthy control architecture.

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DISCUSSION

The present study indicates that cyclophosphamide (CP) produces an anxiety-like behaviour, severe oxidative neurotoxicity, and significant cortical degeneration in Wistar rats. However, co-administration of magnesium glycinate provides a dose dependent neuroprotective effect at behavioural, biochemical, and histological levels. Cyclophosphamide-administered animals (Group F) showed significant reductions in locomotor and exploratory activity (line crossings, grooming, and rearing) and increased defecation in the open field test (OFT), alongside reduced open arm time and increased closed arm occupancy in the elevated plus maze (EPM), indicating heightened anxiety and emotional distress (Tables 1 to 2; Figures 1 to 5). These findings align with previous reports that CP-based and CP combination regimens cause anxiety and depression like behaviour and cognitive deficits in rodents and humans [2,3,4]. Given that CP crosses the blood–brain barrier and generates reactive metabolites such as acrolein, which trigger mitochondrial dysfunction, microglial activation, and cytokine release, the observed anxiety-like profile likely reflects convergent injury to hippocampal, prefrontal, and amygdalar circuits that govern emotional reactivity and exploratory drive [5,18].

Behaviourally, CP alone reduced line crossings by more than half relative to controls and significantly decreased rearing and grooming while increasing defecation counts (Table 1; Figures 1 to 4), a pattern consistent with reduced exploratory drive and elevated autonomic arousal in the OFT [19]. Reductions in rearing are particularly indicative of impaired vertical exploration and environmental scanning, behaviours that rely heavily on intact hippocampal and cortical integration, whereas increased defecation is a classical autonomic index of heightened emotionality and stress reactivity in rodents [19,20]. The EPM data support an anxiogenic effect: CP treated rats spent the least time in the open arms and the most time in closed arms compared with all other groups (Table 2; Figure 5). Together, these outcomes are consistent with the approach avoidance conflict framework underlying the OFT and EPM, in which decreased central or open arm exploration is interpreted as increased anxiety-like behaviour [12,20]. Similar CP induced behavioural disturbances have been documented in models where CP or CP containing combinations impair hippocampal neurogenesis, synaptic plasticity, and cortical microstructure [4,5,21]. Collectively, these results suggest that the behavioural phenotype observed here is not merely a nonspecific sickness response, but rather reflects specific disruption of neural networks involved in risk assessment, novelty processing, and emotional regulation [4].

Magnesium glycinate demonstrated an anxiolytic-like and behaviour-stabilizing effect. In unintoxicated animals (Groups B and C), magnesium supplementation did not impair locomotion (line crossings comparable to controls) and modestly increased open arm time in the EPM, consistent with reports that chronic magnesium administration produces anxiolytic-like effects without motor sedation in rodents [22]. This finding aligns with magnesium's role as a physiological NMDA receptor blocker and modulator of GABAergic and serotonergic transmission, which can shift the balance of excitatory–inhibitory signalling toward reduced anxiety without compromising general activity [6,23]. In CP-intoxicated rats, coadministration of magnesium glycinate (Groups D and E) significantly improved OFT indices; line crossing, grooming, and rearing increased, while defecation decreased compared with CP alone, indicating partial normalization of exploratory and stress related behaviours (Table 1; Figures 1 to 4). High dose magnesium glycinate (32.8 mg/kg; Group E) provided greater behavioural improvement than the low dose, particularly for rearing and defecation, suggesting a dose-responsive anxiolytic and anti-stress effect under CP challenge. The dose dependence implies that a threshold increase in brain magnesium is required to counteract CP-induced dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and limbic network hyperexcitability, consistent with reports that magnesium supplementation normalizes stress-induced corticosterone elevations and anxiety-like behaviours [23]. A similar pattern was evident in the EPM: although CP-treated rats receiving magnesium (Groups D and E) did not fully reach the open arm exploration observed in magnesium only groups, they showed significantly longer open arm time and reduced closed arm occupancy compared with CP-only animals, reflecting a substantial reduction of CP-induced anxiety (Table 2; Figure 5).

The behavioural improvements correlated with changes in oxidative stress markers. CP-only rats exhibited approximately a twofold elevation of cerebral malondialdehyde (MDA) relative to controls, accompanied by significant reductions in total antioxidant capacity (TAC), superoxide dismutase (SOD), and catalase (CAT) activities (Table 3; Figures 6 to 9). This pattern, characterized by elevated lipid peroxidation with depletion of endogenous antioxidant

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defenses, is typical of CP induced neuronal injury and has been repeatedly implicated as a primary driver of chemotherapy-induced cognitive impairment, often termed chemobrain [2,5]. Increased MDA reflects ongoing peroxidative damage to polyunsaturated fatty acids in neuronal membranes, which compromises membrane fluidity, receptor function, and ion channel kinetics, while SOD and CAT depletion reduces the brain's ability to detoxify superoxide and hydrogen peroxide, thereby amplifying redox-sensitive signalling cascades such as NF-ϰB and p38 MAPK that promote neuroinflammation and apoptosis [5,18]. Previous studies using diverse antioxidants or phytochemicals, such as curcumin, gallic acid, nerolidol, edaravone, flavocoxid, and carvacrol, have shown that reducing MDA and restoring SOD, CAT, and glutathione activity attenuates CP-induced neuroinflammation, apoptosis, and behavioural deficits [5,21,24]. In this context, the significant reduction of MDA and concomitant recovery of TAC, SOD, and CAT by magnesium glycinate, again more pronounced at the higher dose (Group E), supports the interpretation that its neuroprotective actions are mediated via mitigation of oxidative and nitrosative stress. Because magnesium is a critical cofactor for numerous antioxidant enzymes and for ATP-dependent mitochondrial processes, replenishing brain magnesium may stabilize mitochondrial function, reduce electron-leak–driven ROS production, and restore antioxidant enzyme activity, thereby interrupting the vicious cycle of oxidative damage and neuronal dysfunction triggered by CP [7,22].

Histological and immunohistochemical data provide structural confirmation of this biochemical protection. In CP only brains (Group F), synaptophysin immunostaining in the cortex revealed depletion of presynaptic terminals with disrupted neuropil organization (figure 1F), while Cresyl Fast Violet and H&E sections demonstrated extensive chromatolysis, neuronal shrinkage, pyknotic ghost cells, neuropil vacuolation, and perineuronal edema (Figures 11F and 12F). These features are characteristic of CP-induced cortical and hippocampal degeneration described in other experimental models [5,24]. Loss of synaptophysin, a presynaptic vesicle protein, is widely regarded as a sensitive marker of synaptic degeneration and correlates with learning and memory deficits in diverse neurodegenerative and chemotherapeutic models, suggesting that CP-induced synaptic loss underlies at least part of the behavioural impairment [4,7,25]. Similarly, chromatolysis and Nissl body dispersion reflect failure of protein synthesis and axonal transport in injured neurons, while vacuolation and perineuronal halos indicate cytotoxic and vasogenic edema, consistent with severe metabolic and ionic imbalance [26]. In comparison, magnesium-only groups (B and C) preserved normal cytoarchitecture and dense synaptophysin expression comparable to controls (figure 10 to 12), indicating that magnesium glycinate is not inherently neurotoxic at the administered doses. Coadministration of magnesium glycinate, particularly at 32.8 mg/kg (Group E), produced a dose-dependent restoration of synaptophysin immunoreactivity, reconstitution of Nissl substance and pyramidal neuron morphology, and a reduction in vacuolation and pyknotic profiles. This preservation of synaptic and neuronal integrity is consistent with the normalization of antioxidant defenses and supports a causal link between oxidative stress reduction and structural neuroprotection. The concordance between synaptic preservation, restored Nissl architecture, and improved behavioural performance strengthens the argument that magnesium glycinate acts upstream on oxidative and inflammatory pathways rather than merely masking behavioural symptoms [27].

Mechanistically, these findings align with the known neuromodulatory and cytoprotective roles of magnesium. Magnesium acts as a voltage-dependent blocker of N-methyl-D-aspartate (NMDA) receptors, limiting calcium influx and downstream reactive oxygen species (ROS) generation and thereby reducing excitotoxic and oxidative injury [6,23]. Experimental magnesium deficiency in rodents produces anxiety and depression like behaviours, hypothalamic pituitary adrenal (HPA) axis hyperactivation, and increased proinflammatory cytokine expression, all of which are reversible with magnesium repletion [6,23]. In the context of CP toxicity, normalization of magnesium status may therefore act at multiple nodes: dampening glutamatergic overactivation, restraining HPA hyperactivity, and limiting microglial and astrocytic release of pro-inflammatory cytokines such as TNF-α and IL-1β, which are known to exacerbate synaptic dysfunction and neuronal death [5,28,29]. Chronic magnesium supplementation has also been shown to enhance brain antioxidant defenses and modulate neuroinflammatory pathways, including TNF alpha and NF kappa B signaling [22,7]. Magnesium L threonate and other brain penetrant magnesium formulations

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prevent oxaliplatin induced or vincristine induced memory, emotional, and pain phenotypes by normalizing TNF alpha and NF kappa B signaling, correcting magnesium deficiency in serum and cerebrospinal fluid, and restoring synaptic markers [7,29]. The present data extend this paradigm to CP and suggest that magnesium glycinate may similarly decrease TLR4 and NLRP3 driven inflammatory signaling and downstream caspase activation described in CP neurotoxicity, although these molecular endpoints were not directly measured here [5]. Future work incorporating direct assays of TLR4/NLRP3 activation, caspase-3 cleavage, and cytokine profiles will be essential to confirm whether these pathways are indeed normalized by magnesium glycinate in the CP model.

The selection of magnesium glycinate is mechanistically relevant. Organic chelates such as magnesium diglycinate or bisglycinate exhibit favorable gastrointestinal tolerability and, in patients with impaired intestinal absorption, demonstrate greater or earlier isotope uptake than magnesium oxide, consistent with partial dipeptide mediated transport [9]. Enhanced systemic bioavailability increases the likelihood of sufficient brain magnesium elevation to modulate NMDA receptors and antioxidant systems effectively, as has been demonstrated for magnesium L threonate and related preparations [7,29]. Because glycinate is a neutral amino acid that can engage peptide transporters in the gut and possibly influence blood–brain barrier transport, the glycinate chelate may confer both pharmacokinetic advantages (better absorption) and potential pharmacodynamic contributions (via glycine's modulatory effects at NMDA receptors), although the latter remains speculative and warrants direct evaluation [30]. The dose dependent gradient observed in the present work, where high dose magnesium glycinate produced superior behavioural normalization, stronger antioxidant restoration, and more complete histological preservation than the low dose, supports the hypothesis that tissue magnesium availability is a key determinant of neuroprotection under CP stress [31].

From a translational perspective, these results are relevant given the high prevalence of chemotherapy induced cognitive and emotional disturbances and the lack of safe, inexpensive, mechanistically rational adjuncts [2,32]. Magnesium deficiency is common in oncology patients, driven by inadequate intake, gastrointestinal losses, and drug induced renal wasting, and has been linked to more severe chemotherapy-induced neuropathy and poorer clinical outcomes [2]. Importantly, oral magnesium is inexpensive, widely available, and generally well tolerated, making it an attractive candidate for supportive care protocols if efficacy against chemobrain can be confirmed in clinical trials. Together with evidence that correcting hypomagnesemia improves mood and quality of life in stressed adults [8], our findings identify magnesium glycinate as a candidate for adjunctive therapy in patients receiving CP-based regimens.

CONCLUSION

The results from the current study indicate that cyclophosphamide exposure significantly disrupts neurobehavioural and cortical integrity, characterized by severe anxiety-like behaviour, pronounced oxidative stress, and extensive synaptic and neuronal degeneration. However, coadministration of magnesium glycinate mitigated these pathological alterations in a dose-dependent manner. By reducing lipid peroxidation, restoring endogenous antioxidant defenses, and preserving synaptic organization and cellular architecture, magnesium glycinate demonstrated a potent neuroprotective effect. The results of this study highlight magnesium glycinate as a mechanistically supported and clinically accessible adjunctive strategy to attenuate chemotherapy-related neurotoxicity. These findings may inform future translational and clinical research on the way forward in alleviating the debilitating effects of Cyclophosphamide in cancer management. However, further research is required to ascertain these effects in humans.

Declarations

Authors' contribution: Conceptualization (Ojo FO and Folorunso KP), Data curation (Olatundun BE, Oyeleke AA, Afolabi O, Ojo AK, Onaolapo TM, Olawale AA), Formal analysis (Olatundun BE, Hassan LA, Afolabi O), Funding acquisition (Tertiary Education Trust Fund -TETFUND), Methodology (Ojo FO), Resources (L.A Hassan, Lawal MB, Ogundiran RJ), Writing original draft (Ojo FO and Folorunso KP), Writing and editing of manuscript (Folorunso KP, Ojo FO, Olawale AA, Onaolapo TM), Approval of final manuscript (All the authors)

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