



OPEN Dietary elimination of gluten attenuates amyloid- β and tau pathology in Alzheimer's disease-like in aluminum chloride-induced rats: an experimental animal study

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This study evaluates the comparative neuroprotective efficacy of a multi-component dietary pattern (the Mediterranean-DASH Intervention for Neurodegenerative Delay) versus a single-component elimination strategy (gluten-free diet) in an AlCl_3 -induced rat model of Alzheimer's disease. Sprague-Dawley rats were randomly divided into six groups ($n=6$ each) and fed a balanced diet, the MIND diet, or a gluten-free diet, with or without AlCl_3 (100 mg/kg/day) administered orally for 8 weeks. AlCl_3 administration significantly elevated $\text{A}\beta$, total tau, and p-Tau S231 compared to non-induced controls ($P<0.001$ for all AD effects). Conversely, all three diets differed significantly from one another for total tau and p-Tau S231 ($P<0.05$ for all comparisons). Under AD-inducing conditions, the MIND diet significantly reduced cortical $\text{A}\beta$ and total tau by 41.9% and 22.7%, while the gluten-free diet significantly reduced them by 50.3% and 41.6%, relative to the AD disease-control group (all $P<.001$). Histopathologically, both diets preserved cortical and hippocampal architecture, but behaviorally, diet showed no significant effect on the novel object recognition discrimination index ($P=0.092$). Both the MIND and gluten-free diets successfully attenuated $\text{A}\beta$ accumulation, tau pathology, and neurodegeneration in AlCl_3 -induced AD models, with the gluten-free diet yielding greater numerical reductions.

Keywords Alzheimer's disease, MIND diet, Gluten-free diet, Amyloid-beta, Tau hyperphosphorylation, Neuroprotection

Abbreviations

AD	Alzheimer's disease
$\text{A}\beta$	Amyloid-beta
$\text{A}\beta_{42/40}$	Amyloid-beta 42/40
AIN-93G	American Institute of Nutrition 1993 Growth Diet
AlCl_3	Aluminum chloride
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	Aluminum chloride hexahydrate
ANOVA	Analysis of variance
ARRIVE	Animal Research: reporting of in vivo experiments
BCA	Bicinchoninic acid (protein assay)
CO_2	Carbon dioxide
CU-IACUC	Cairo University Institutional Animal Care and Use Committee
DI	Discrimination index
ELISA	Enzyme-linked immunosorbent assay
g	Gram

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H&E	Hematoxylin and eosin
h	Hour
IRats	Intelligent rodent analysis tracking software
kcal	Kilocalorie
kg	Kilogram
mL	Milliliter
mg	Milligram
min	Minute
MIND	Mediterranean-DASH intervention for neurodegenerative delay
μm	Micrometer
No.	Number
p	Probability value (statistical significance)
pg	Picogram
p-Tau	Phosphorylated tau
p-Tau S231	Tau phosphorylated at serine 231
RIPA	Radioimmunoprecipitation assay buffer
SD	Standard deviation
SPSS	Statistical package for the social sciences
USDA	United States Department of Agriculture
VACSERA	The Egyptian Holding Company for Biological Products and Vaccines
v.27	Version 27
w/v	Weight per volume

As the most prevalent cause of dementia¹, Alzheimer's disease (AD) is a major neurodegenerative disorder characterized by progressive deterioration in memory and cognition², resulting in profound clinical and socioeconomic consequences^{3–5}.

AD is distinguished by neuropathological features including extracellular amyloid- β deposition, intracellular accumulation of hyperphosphorylated tau, synaptic dysfunction, neuronal loss, and sustained neuroinflammatory activation⁶. Despite substantial advances in understanding AD physiology, effective AD-modifying therapies remain limited, highlighting the need to identify modifiable factors that may influence AD onset and progression².

Several genetic and environmental factors contribute to the development of AD⁷. Diet, in particular, has emerged as a crucial and modifiable environmental determinant of neurodegeneration^{2,8}. Nutritional factors, therefore, influence brain health through multiple interconnected pathways, including the modulation of inflammatory signaling, oxidative stress, metabolic homeostasis, synaptic plasticity, and gut-brain axis communication⁹. Meanwhile, chronic metabolic and inflammatory disturbances exacerbate neuropathological progression and cognitive decline associated with AD⁹.

In this context, comparative evaluation of distinct dietary interventions may provide critical insight into their differential effects on AD-related pathology¹⁰.

A balanced diet provides essential macro- and micronutrients to maintain physiological function rendering it an optimal control¹¹. Conversely, the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet has been specifically developed to target cognitive decline¹². The MIND diet is associated with reduced risk of AD due to its anti-inflammatory and antioxidant properties, as well as its potential to modulate amyloid and tau pathology¹². Alternatively, a gluten-free diet represents a distinct dietary approach focused on the exclusion of gluten-containing grains¹³. Current research suggests that, particularly under susceptible conditions, gluten exposure may increase intestinal permeability and trigger systemic immune activation, thereby aggravating neuroinflammation via microglial activation and cytokine signaling within the central nervous system¹⁴.

However, whether dietary gluten restriction can modify the progression of AD pathology remains poorly understood, particularly in controlled experimental models integrating molecular and behavioral outcomes¹⁴.

Accordingly, the current study aimed to investigate the effects of three different dietary interventions—balanced diet, MIND diet, and gluten-free diet—on experimentally induced AD in rats. It assessed histopathological alterations in the cortex and hippocampus, amyloid- β accumulation, total tau protein, phosphorylated tau protein, and tau ratio, alongside cognitive performance evaluated using the Y-maze and novel object recognition tests. By combining behavioral, histological, and molecular endpoints, this study seeks to determine how dietary interventions differentially modulate AD-associated neurodegeneration and cognitive impairment in vivo.

Materials and methods

Animals and study design

Male Sprague–Dawley rats (200–250 g) were obtained from the Egyptian Holding Company for Biological Products and Vaccines (VACSERA), Helwan, Egypt. All procedures were approved by the Institutional Animal Care and Use Committee, Cairo University (CU- IACUC; Approval No: CUIF225) and were conducted in accordance with the ARRIVE guidelines. All experiments were performed in accordance with the relevant guidelines and regulations of the Institutional Animal Care and Use Committee, Cairo University (CU-IACUC). Animals were housed under standard environmental conditions (22 ± 3 °C, $55 \pm 5\%$ humidity, and a 12-h light/dark cycle). The photoperiod was fixed with lights on from 07:00 to 19:00 and remained identical for all groups throughout the study, with no experimental manipulation. All rats were provided ad libitum access to water.

Rats were assigned to six groups (n = 6 per group):

1. Balanced Diet, no AD induction

Ingredient	Balanced (Control)	Gluten-Free	MIND diet
Dry pasta	350	0	0
Boiled rice (gluten-free)	0	350	0
Wheat germ (whole grain)	0	0	300
Apple (raw, chopped)	150	150	0
Strawberries (raw)	0	0	150
Carrots (raw, grated)	100	100	0
Arugula (raw, chopped)	0	0	150
Skimmed milk (long-life, UHT)	150	150	150
Egg white (boiled, mashed)	70	70	0
Beans (boiled, mashed)	0	0	100
Salmon (steamed, flaked; Atlantic salmon)	0	0	100
Walnuts (raw, chopped)	0	0	50
Corn oil	30	30	0
Olive oil	0	0	50
Total	1000	1000	1000

Table 1. Composition of experimental diets based on whole food ingredients (g/kg diet). Diets were provided as 60 kcal/rat/day rations. Skimmed milk (long-life, UHT) was used to enhance shelf stability while maintaining a low-fat profile. All diets were designed to maintain comparable macronutrient distribution across groups, differing primarily in ingredient composition.

Macronutrient	Amount (g)	Energy (kcal)	% Energy
Carbohydrates	11.05	44.2	77%
Protein	2.21	8.84	15%
Fat	0.533	4.8	8%
Total	—	~60	100%

Table 2. Macronutrient composition of daily rations (60 kcal/day). Macronutrient distribution (77% carbohydrate, 15% protein, 8% fat) was calculated based on total energy intake. Energy values were calculated using standard Atwater factors (4 kcal/g for carbohydrates and protein, 9 kcal/g for fat).

2. Balanced Diet + AD induction
3. MIND diet, no AD induction
4. MIND diet + AD induction
5. Gluten-free diet, no AD induction
6. Gluten-free diet + AD induction

The dietary interventions commenced immediately and continued for 8 weeks, with animal body weights recorded weekly. Investigators remained blinded to group allocation throughout all behavioral testing and outcome analyses.

Dietary interventions

The Diets were custom formulated to be isocaloric and isonitrogenous (Table 1).

- *Balanced diet*: Rodent-adapted balanced diet according to the Standard AIN-93G rodent formulation.
- *MIND diet*: Rodent-adapted Mediterranean-DASH Intervention for Neurodegenerative Delay diet, enriched in neuroprotective components and formulated to reflect key characteristics of the human MIND dietary pattern.
- *Gluten-free diet*: Macronutrient-matched to the balanced diet but excluding gluten-containing grains (wheat).

Diets were prepared fresh weekly, vacuum-sealed, and stored at 4 °C.

Detailed compositions are provided in Table 1.

The diets consisted of weighed whole-food ingredients that were freshly prepared weekly, vacuum-sealed, and stored at 4 °C to deliver precisely 60 kcal/rat/day (Table 1). Food components were prepared (chopped or mashed, as appropriate), individually weighed, and provided separately to ensure complete consumption and minimize selective feeding. Atlantic salmon was selected as a source of long-chain omega-3 fatty acids¹⁵. The macronutrient composition (Table 2) was derived from standard food composition databases (USDA FoodData Central) based on the ingredient quantities listed in Table 1¹⁶. An isocaloric dietary composition with a matched macronutrient distribution was utilized to isolate the effects of the specific ingredients, preventing general caloric variations from independently influencing the biological outcomes.

AD induction

Aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$; 97.84% purity; Lot No. L402622202; Loba Chemie Pvt. Ltd., Mumbai, India) was dissolved in distilled water and administered daily via oral gavage at a dose of 100 mg/kg body weight. Doses were adjusted daily based on body weight (10 mL/kg). Treatment commenced on day 1 of the dietary intervention and continued for 8 consecutive weeks. Control animals received an equivalent volume of distilled water.

This protocol is a well-established model of AD-like pathology, inducing amyloid- β accumulation, tau hyperphosphorylation, neuroinflammation, and cognitive impairment¹⁷.

Behavioral testing

Behavioral assessments were conducted during the light phase (09:00–13:00, i.e., during the light phase, weeks 7–8) under standardized conditions by blinded investigators.

The Y-maze test

Spatial working memory was assessed using spontaneous alternation behavior. The Y-maze consisted of three identical arms ($50 \times 10 \times 40$ cm) positioned at 120° angles. Each rat explored freely for 8 min. Arm entries and alternations were video-recorded and scored using IRats software¹⁸. The percentage of alternation was calculated¹⁹.

$$\text{Alternation}(\%) = \left(\frac{\text{Number of alternations}}{\text{Total arm entries} - 2} \right) \times 100$$

The novel object recognition test

Recognition memory was evaluated over three phases (habituation, familiarization, test) with a 24-h inter-trial interval in a $50 \times 50 \times 40$ cm arena. During the test phase, one familiar object was replaced with a novel object (5 min exploration each phase). Novel object exploration was video-recorded and scored using IRats software¹⁸. Discrimination index was calculated²⁰.

$$DI = \frac{\text{Time}_{\text{novel}} - \text{Time}_{\text{familiar}}}{\text{Time}_{\text{novel}} + \text{Time}_{\text{familiar}}} \times 100$$

The tissue collection

At week 8 following behavioral testing, rats were sedated by CO_2 inhalation followed by decapitation. Brain tissues were excised within 3 min, in the sagittal plane bisected on ice, and cortex/hippocampus dissected. One hemisphere was immersion-fixed in 10% neutral buffered formalin for histology; the contralateral was snap-frozen in liquid nitrogen and stored at -80°C .

Histopathology

Brain specimens were fixed in 10% neutral buffered formalin, trimmed, and subsequently dehydrated through a graded series of ethyl alcohol. Samples were cleared in xylene and embedded in paraffin blocks. Tissue Sects. ($4\text{--}6\ \mu\text{m}$ thickness) were prepared and stained with hematoxylin and eosin (H&E) and Masson's trichrome to facilitate histopathological assessment²¹.

Biochemical assays

Brain tissues were homogenized (1:10 w/v) in RIPA buffer supplemented with protease and phosphatase inhibitor cocktails. Total protein concentrations were determined using the BCA assay (Pierce, #23,225). Biomarker concentrations were quantified using commercially available sandwich ELISA kits in accordance with the manufacturer's instructions: A β 42/40 (Invitrogen, #KHB3481; sensitivity $<10\ \text{pg/mL}$), total tau (Invitrogen, #KHB0041), and phosphorylated tau Serine 231 (p-tau S231) (Invitrogen, #KHB0031). All samples were analyzed in duplicate, and results were normalized to total protein content and expressed as pg/mg protein. The p-tau/total tau ratio was calculated for each sample to determine the phosphorylation state²².

Statistics

Data are presented as mean $\pm$ SD. Normality (Shapiro–Wilk) and homogeneity of variance (Levene's) were verified. Two-way ANOVA assessed diet $\times$ AlCl_3 effects, followed by Tukey's post hoc test for significant interactions. Non-normal behavioral data were arcsine-transformed. Significance was set at $p < 0.05$ (SPSS v.27). A priori power calculation was performed using G*Power 3.1 (ANOVA: Fixed effects, special, main effects and interactions module) for the pre-specified Diet $\times$ AD-induction interaction (numerator df = 2, 6 groups), $\alpha = 0.05$, target power = 0.80. As no published AlCl_3 -induced rat model study employing a full factorial (Diet $\times$ AD-induction) design with a reported interaction effect size was identified in the literature, the anticipated effect size was estimated conservatively ($f = 0.65$) relative to the range of effect sizes reported for comparable behavioral and biochemical outcomes in single-factor AlCl_3 -induced rat AD models (Cohen's $f \approx 1.6\text{--}4.6$, derived from Haider et al.²³, including $f = 2.13$ for the discrimination index in novel object recognition). However, because interaction effects represent a second-order comparison (the difference between diet effects across induction status) and are generally smaller in magnitude than the main effects from which they are derived, we judged that applying this single-factor main-effect range directly to our interaction term would have overestimated our ability to detect it. We therefore used a more conservative anticipated effect size ($f = 0.65$) for the power calculation. This indicated a required total sample size of $N = 40$ ($n \approx 6.7/\text{group}$). To maintain equal group sizes and in accordance with the 3Rs principle of animal ethics (minimizing animal use), $n = 6$ per group ($N = 36$) was used, corresponding to an

achieved power of approximately 76% for the interaction term, slightly below the conventional 80% threshold. The overall experimental design, timeline, behavioral assessments, tissue collection, and laboratory analyses are summarized in Fig. 1.

Results

Behavioral outcomes reveal partial dissociation from molecular improvements

Y-maze performance

AlCl_3 -induced neurotoxicity impaired spatial working memory, as evidenced by reduced spontaneous alternation in the disease-control group compared with the non-induced balanced-diet group (63.43 ± 3.34 vs. 76.67 ± 2.89 ; overall effect of AD induction, $P < 0.001$). For the overall dietary effect, Tukey's multiple-comparisons test showed significant differences in spontaneous alternation between the balanced and MIND diet groups ($P < 0.001$) and between the MIND and gluten-free diet groups ($P < 0.001$), whereas the balanced and gluten-free diet groups did not differ significantly ($P = 0.594$). Thus, the numerical differences in alternation among dietary groups should be interpreted in the context of these statistically significant overall dietary comparisons rather than as evidence of a "pronounced" reduction attributable to either intervention. For total Y-maze arm entries, Tukey's multiple-comparisons test showed no significant differences between the balanced and MIND diet groups ($P = 0.054$), balanced and gluten-free diet groups ($P = 0.656$), or MIND and gluten-free diet groups ($P = 0.235$). Although the MIND group showed a numerically higher mean number of arm entries, this difference did not reach statistical significance. Arm entries and alternation behavior were quantified using IRats automated behavioral analysis software. Given that total arm entries did not show statistically significant dietary differences, the behavioral findings should be interpreted primarily in terms of spontaneous alternation, while acknowledging that the absence of an independent locomotor assessment limits definitive separation of working-memory effects from general activity.

Novel object recognition performance

Recognition memory, assessed using the discrimination index (DI), showed substantial variability across the dietary groups. The disease-control group had a DI of -0.48 ± 0.71 , whereas the MIND and gluten-free diet groups under AD-inducing conditions showed DI values of 0.41 ± 0.46 and 0.14 ± 0.79 , respectively. Although these values were numerically higher than that of the disease-control group, the overall effect of diet was not statistically significant ($P = 0.092$), and Tukey's multiple-comparisons test showed no significant differences between the balanced and MIND diet groups ($P = 0.160$), balanced and gluten-free diet groups ($P = 0.113$), or MIND and gluten-free diet groups ($P = 0.975$). Notably, the positive DI values observed in the AD-induced

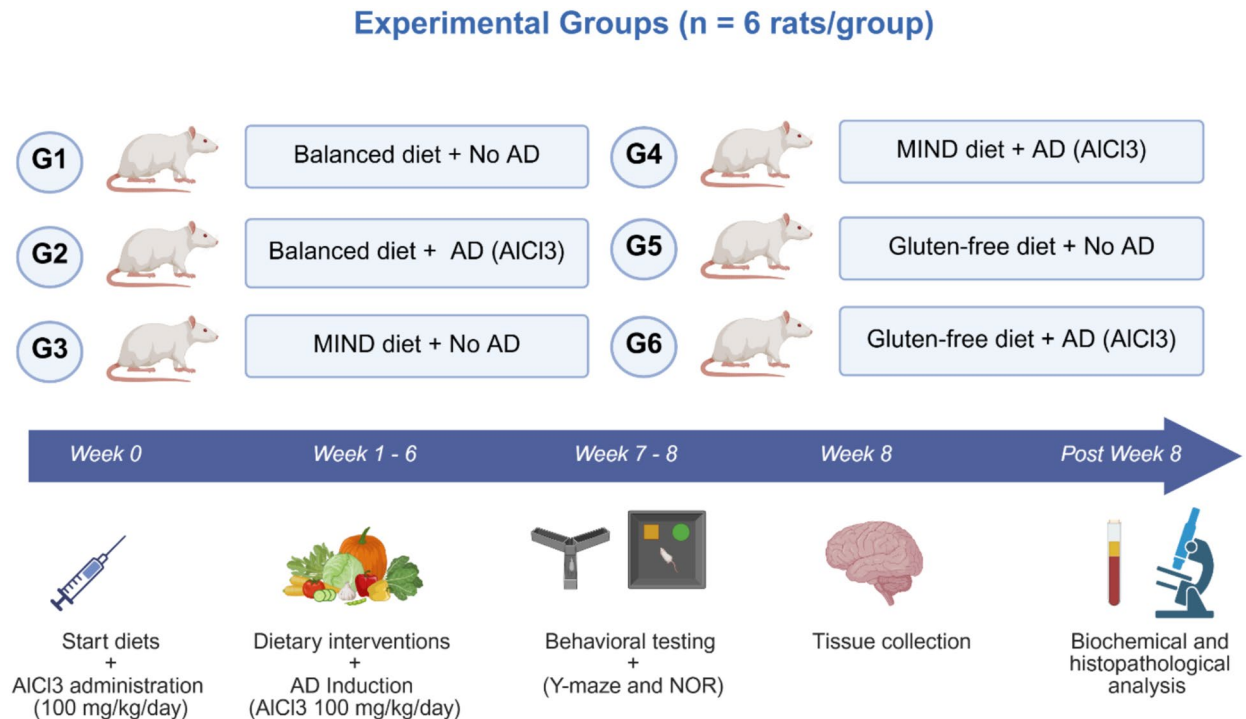


Fig. 1. Experimental design and study workflow. Thirty-six male Sprague–Dawley rats (n = 6 per group) were randomly assigned to six experimental groups receiving a balanced diet, MIND diet, or gluten-free diet, with or without aluminum chloride (AlCl_3)-induced Alzheimer's disease. Dietary interventions and AlCl_3 administration (100 mg/kg/day) were conducted concurrently for 8 weeks. Behavioral assessments, including the Y-maze and novel object recognition (NOR) tests, were performed before tissue collection. Brain tissues were subsequently collected for biochemical analyses and histopathological evaluation.

MIND and gluten-free groups were higher than those observed in their respective non-induced counterparts (Table 3), despite the expected direction being toward the non-induced values. This unexpected pattern, together with the substantial variability in DI values, makes the behavioral findings difficult to interpret as evidence of improved recognition memory. Therefore, the observed differences are presented as descriptive and ambiguous findings rather than evidence of a beneficial cognitive effect of either dietary intervention.

Dietary interventions suppress amyloid- β accumulation In AlCl_3 -induced neurodegeneration

AlCl_3 administration significantly increased amyloid- β ($\text{A}\beta$) levels in both hippocampal and cortical tissues compared with non-induced controls (overall AD induction effect, $P < 0.001$ for both tissues). In animals maintained on the balanced diet, AlCl_3 induction elevated $\text{A}\beta$ levels from 73.05 ± 6.62 to 165.70 ± 10.03 pg/mg protein in the hippocampus, and from 69.25 ± 3.16 to 195.61 ± 3.18 pg/mg protein in the cortex. Dietary intervention significantly impacted hippocampal $\text{A}\beta$ (overall diet effect, $P < 0.001$). Tukey's multiple-comparisons test revealed that the balanced-diet group had significantly higher hippocampal $\text{A}\beta$ than both the MIND ($P = 0.001$) and gluten-free ($P < 0.001$) diet groups, which did not differ significantly from one another ($P = 0.360$). Similarly, a significant overall diet effect was observed for cortical $\text{A}\beta$ ($P < 0.001$), with the balanced diet yielding significantly higher $\text{A}\beta$ levels than both the MIND and gluten-free diets (both $P < 0.001$), while the latter two again showed no significant difference ($P = 0.508$). Under AD-inducing conditions, simple-effects analysis (Tukey-adjusted pairwise comparisons using the pooled error term from the full two-way model) showed that the MIND diet significantly reduced hippocampal and cortical $\text{A}\beta$ by 24.8% and 41.9%, respectively, relative to the disease-control group (hippocampal: $t = 6.73$, $P < 0.001$; cortical: $t = 10.31$, $P < 0.001$), whereas the gluten-free diet reduced them by 28.3% and 50.3%, respectively (hippocampal: $t = 7.66$, $P < 0.001$; cortical: $t = 12.39$, $P < 0.001$). Unlike total tau and p-Tau S231, the gluten-free diet did not differ significantly from the MIND diet for either hippocampal ($t = 0.93$, $P = 0.632$) or cortical ($t = 2.08$, $P = 0.136$) $\text{A}\beta$ — so while the gluten-free diet produced numerically larger $\text{A}\beta$ reductions, this diet-specific advantage was not statistically confirmed for $\text{A}\beta$.

Modulation of tau pathology by dietary interventions

Consistent with the $\text{A}\beta$ findings, a two-way ANOVA revealed significant main effects of AD induction on both total tau ($F(1,30) = 538.77$, $P < 0.001$, $\eta^2 = 0.761$) and p-Tau S231 ($F(1,30) = 2559.31$, $P < 0.001$, $\eta^2 = 0.870$), together with significant main effects of diet (total tau: $F(2,30) = 33.56$, $P < 0.001$, $\eta^2 = 0.095$; p-Tau S231: $F(2,30) = 91.50$, $P < 0.001$, $\eta^2 = 0.062$). Critically, the Diet $\times$ AD-induction interaction was also significant for both markers (total tau: $F(2,30) = 35.94$, $P < 0.001$, $\eta^2 = 0.102$; p-Tau S231: $F(2,30) = 85.55$, $P < 0.001$, $\eta^2 = 0.058$), indicating that the effect of diet on tau pathology depended on AD-induction status. Given this interaction, dietary effects were examined separately within the AD-induced subgroup rather than relying on marginal comparisons averaged across induction status. In animals maintained on the balanced diet, total tau increased from 75.42 ± 10.55 to 239.25 ± 11.65 pg/mg protein, while p-Tau S231 increased from 31.48 ± 2.32 to 140.37 ± 4.87 pg/mg protein in non-induced and AlCl_3 -induced animals, respectively. Within the AD-induced subgroup, Tukey-adjusted pairwise comparisons showed that both diets significantly reduced tau pathology relative to the AlCl_3 -induced disease control (Balanced Diet + AD): the MIND diet reduced total tau to 184.92 ± 10.41 pg/mg protein (a 22.7% reduction; $t = 6.40$, $P < 0.001$) and p-Tau S231 to 117.96 ± 2.84 pg/mg protein (a 16.0% reduction; $t = 7.95$, $P < 0.001$), while the gluten-free diet produced larger reductions, lowering total tau to 139.63 ± 9.63 pg/mg protein (41.6% reduction; $t = 11.73$, $P < 0.001$) and p-Tau S231 to 87.84 ± 4.35 pg/mg protein (37.4% reduction; $t = 18.63$, $P < 0.001$). The gluten-free diet also outperformed the MIND diet directly within the AD-induced subgroup for both total tau ($t = 5.33$, $P < 0.001$) and p-Tau S231 ($t = 10.68$, $P < 0.001$). These comparisons directly support the reported percentage reductions, as they are restricted to the AD-induced groups rather than averaged across induction status. For the p-Tau/total tau ratio, the pattern differed: significant main effects of AD induction ($F(1,30) = 58.68$, $P < 0.001$, $\eta^2 = 0.569$) and diet ($F(2,30) = 4.98$, $P = 0.027$, $\eta^2 = 0.097$) were observed, but the Diet $\times$ AD interaction was not significant ($F(2,30) = 2.28$, $P = 0.145$, $\eta^2 = 0.044$), indicating that diet's effect on the ratio did not depend on induction status. Marginal Tukey comparisons were therefore appropriate for this outcome: the balanced and MIND diet groups differed significantly ($P = 0.030$), whereas the balanced-versus-gluten-free ($P = 0.860$) and MIND-versus-gluten-free ($P = 0.075$) comparisons did not reach significance. This suggests that, unlike total tau and p-Tau S231, the p-Tau/total tau ratio was altered by the MIND diet but not clearly by the gluten-free diet (Fig. 2).

Group	A β Hippo	A β Cortex	Total Tau	p-Tau S231	p-Tau/Tau	Y-maze Alt (%)	Entries	NOR DI
Balanced Diet (no AD)	73.05 $\pm$ 6.62	69.25 $\pm$ 3.16	75.42 $\pm$ 10.55	31.48 $\pm$ 2.32	0.42 $\pm$ 0.04	76.67 $\pm$ 2.89	10.33 $\pm$ 3.51	-0.72 $\pm$ 0.30
Balanced Diet + AD	165.70 $\pm$ 10.03	195.61 $\pm$ 3.18	239.25 $\pm$ 11.65	140.37 $\pm$ 4.87	0.59 $\pm$ 0.04	63.43 $\pm$ 3.34	11.33 $\pm$ 3.79	-0.48 $\pm$ 0.71
MIND Diet (no AD)	70.71 $\pm$ 7.81	63.68 $\pm$ 13.97	69.38 $\pm$ 8.03	36.52 $\pm$ 3.27	0.53 $\pm$ 0.03	33.18 $\pm$ 1.11	25.00 $\pm$ 16.09	-0.39 $\pm$ 0.53
MIND Diet + AD	124.57 $\pm$ 7.12	113.65 $\pm$ 9.12	184.92 $\pm$ 10.41	117.96 $\pm$ 2.84	0.64 $\pm$ 0.05	26.76 $\pm$ 8.37	30.00 $\pm$ 14.80	0.41 $\pm$ 0.46
Gluten-Free Diet(no AD)	64.09 $\pm$ 5.56	67.37 $\pm$ 15.25	77.58 $\pm$ 11.69	31.09 $\pm$ 2.19	0.41 $\pm$ 0.05	79.37 $\pm$ 9.84	11.00 $\pm$ 4.00	0.02 $\pm$ 0.03
Gluten-Free Diet + AD	118.87 $\pm$ 7.05	97.14 $\pm$ 6.11	139.63 $\pm$ 9.63	87.84 $\pm$ 4.35	0.63 $\pm$ 0.07	51.62 $\pm$ 13.79	22.00 $\pm$ 14.42	0.14 $\pm$ 0.79

Table 3. Mean $\pm$ SD of biochemical and behavioral outcomes by diet and AD condition. Values are mean $\pm$ SD. A β , amyloid-beta; AD, Alzheimer's disease induction; NOR DI, novel object recognition discrimination index; Hippo, hippocampus.

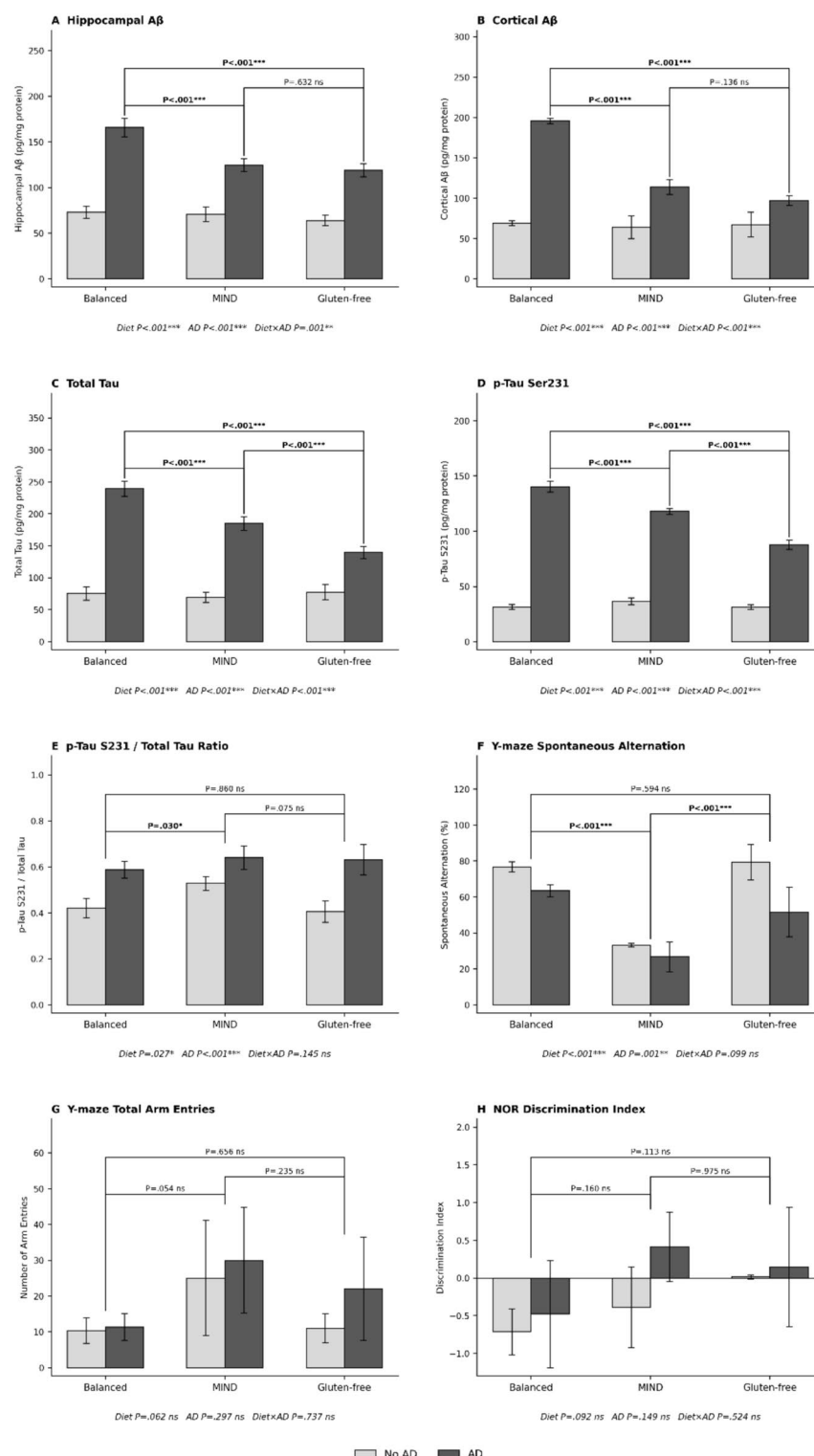


Fig. 2. Effects of dietary interventions on Alzheimer's disease biomarkers and cognitive performance. (A) Hippocampal amyloid- β (A β). (B) Cortical A β . (C) Total tau. (D) Phosphorylated tau (p-Tau Ser231). (E) Ratio of p-Tau Ser231 to total tau. (F) Y-maze spontaneous alternation. (G) Total number of arm entries in the Y-maze. (H) Novel object recognition (NOR) discrimination index. Male Sprague-Dawley rats were assigned to balanced, MIND, or gluten-free diet groups with or without aluminum chloride (AlCl₃)-induced Alzheimer's disease (AD). Data are presented as mean $\pm$ standard deviation (SD) (n = 6 per group). Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. $P < 0.05$ was considered statistically significant.

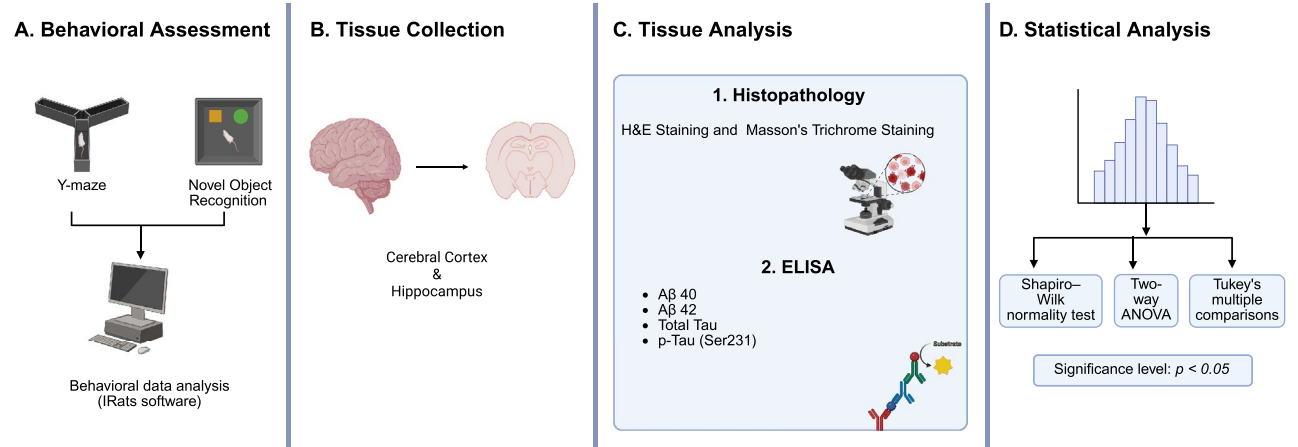


Fig. 3. Effects of dietary interventions on behavioral performance and Alzheimer’s disease-related biomarkers. (A) Hippocampal amyloid-β. (B) Cortical amyloid-β. (C) Total tau. (D) Phosphorylated tau (Ser231). (E) p-Tau/Total Tau ratio. (F) Y-maze spontaneous alternation. (G) Total Y-maze entries. (H) Novel object recognition discrimination index. Data are presented as mean ± SD. Statistical analysis was performed using two-way ANOVA followed by Tukey’s multiple comparisons test. $p < 0.05$ was considered statistically significant.

Group	Cortex degeneration	Striatum degeneration	Fascia dentata injury	Representative features
Balanced Diet (G1)	0–1 (Normal)	0–1	0–1	Normal morphology
Balanced Diet + AD (G2)	3 (Severe)	2 (Moderate)	3 (Severe)	High necrosis, nuclear pyknosis
MIND Diet (G3)	0–1 (Normal)	0–1	0–1	Normal morphology
MIND Diet + AD (G4)	1 (Mild)	1 (Mild)	1 (Mild)	Few necrotic neurons, mild pyknosis
Gluten-Free Diet (G5)	0–1 (Normal)	0–1	0–1	Normal morphology
Gluten-Free Diet + AD (G6)	1 (Mild)	1 (Mild)	2 (Moderate)	Degenerated neurons, pyknosis

Table 4. Histopathological assessment of neurodegeneration.

Histopathological evidence of neuroprotection

Histological examination revealed severe neurodegeneration in the disease control group, characterized by widespread neuronal loss, nuclear pyknosis, and tissue disorganization (score: 3, severe). Both dietary interventions conferred marked neuroprotection. The MIND diet reduced degeneration to mild levels (score: 1) across cortical and hippocampal regions. The gluten-free diet similarly reduced pathology, with mild degeneration in most regions and moderate injury confined to the dentate gyrus. Non-induced groups exhibited preserved cytoarchitecture, confirming the specificity of AlCl₃-induced damage. Neurodegeneration was scored using a semi-quantitative 0–3 severity scale developed for this study (0–1 = normal/mild, with normal structure and few degenerative changes; 2 = moderate, with moderate necrotic or shrunken neurons; 3 = severe, with a high number of necrotic, shrunken neurons and severe nuclear pyknosis). Scoring was performed by assessors blinded to group allocation Fig. 3. The histopathological assessment of neurodegeneration is summarized and scored using this scale in Table 4.

Scoring reference

- 0–1 (Normal/Mild): Normal structure, few degenerative changes.
- 2 (Moderate): Moderate necrotic or shrunken neurons.
- 3 (Severe): High number of necrotic, shrunken neurons with severe nuclear pyknosis.

Groups 1, 3, and 5 represent the respective control diets without AD induction, showing normal histology across all regions. Group 2 (Balanced + AD) represents the severe disease induction. Groups 4 and 6 represent the intervention diets (MIND and Gluten-Free) under AD induction, showing significant histological protection compared to the disease-control group (G2) Fig. 4. Representative photomicrographs of cortical, striatal, and hippocampal dentate gyrus histology are shown in Fig. 4.

The control group shows normal histoarchitecture with intact neuronal morphology. The disease model group shows marked neuronal degeneration, necrosis, and nuclear pyknosis. The MIND diet group shows preserved neuronal structure with mild histopathological alterations, whereas the gluten-free diet group shows reduced neuronal degeneration compared with the disease model group. Scale bar = 25 μm.

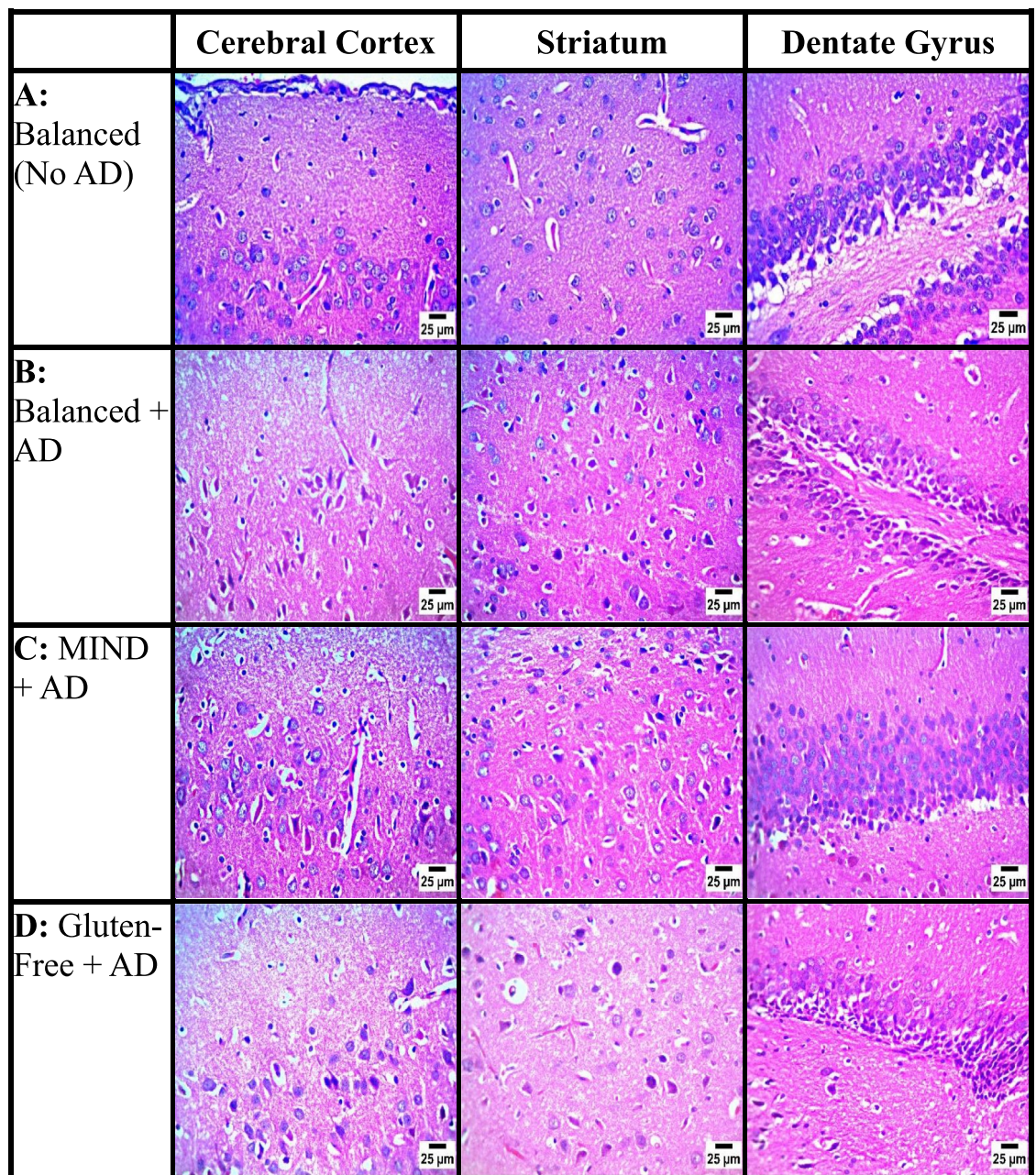


Fig. 4. Histopathological analysis of the cerebral cortex, striatum, and hippocampal dentate gyrus following dietary interventions. Representative haematoxylin and eosin (H&E)-stained sections from (A) balanced diet (control) group, (B) balanced diet + AD (disease model) group, (C) MIND diet + AD group, and (D) gluten-free diet + AD group. The control group shows normal histoarchitecture with intact neuronal morphology. The disease model group shows marked neuronal degeneration, necrosis, and nuclear pyknosis. The MIND diet group shows preserved neuronal structure with mild histopathological alterations, whereas the gluten-free diet group shows reduced neuronal degeneration compared with the disease model group. Scale bar = 25 μ m.

Integrated effects of diet on AD-related outcomes

Collectively, these findings demonstrate that both dietary interventions attenuate the core molecular features of AD-like pathology, including A β accumulation and tau dysregulation, with the gluten-free diet yielding numerically greater biochemical reductions. However, behavioral outcomes were less consistent, indicating a partial dissociation between molecular improvements and functional recovery. The biochemical and behavioral parameters are summarized in Tables 3 and 5, respectively.

Outcome	MIND + AD (%)	Gluten-Free + AD (%)
A β Hippo	– 24.8%* (P < 0.001)	– 28.3%* (P < 0.001)
A β Cortex	– 41.9%* (P < 0.001)	– 50.3%* (P < 0.001)
Total Tau	– 22.7%* (P < 0.001)	– 41.6%* (P < 0.001)
p-Tau S231	– 16.0%* (P < 0.001)	– 37.4%* (P < 0.001)
p-Tau/Tau Ratio	+ 8.5%	+ 6.8%
Y-maze Alternation	– 57.8%	– 18.6%
Y-maze Entries	+ 164.7%	+ 94.1%
NOR Discrimination Index	– 186.2%	– 130.1%

Table 5. Percent change of outcomes relative to disease control (balanced diet) + AD group. Hippo, hippocampus; A β Hippo, hippocampal amyloid-beta; A β Cortex, cortical amyloid-beta. *Tukey-adjusted P-value from simple-effects comparison vs. the Balanced Diet + AD (disease-control) group, using the pooled error term from the full Diet $\times$ AD two-way model, restricted to markers with a significant Diet $\times$ AD interaction (A β Hippo, A β Cortex, Total Tau, p-Tau S231). P-values are not reported for the remaining outcomes because their Diet $\times$ AD interaction was not significant, so marginal (overall) comparisons apply instead, as described in-text.

Percent change formula²⁴

$$\text{PercentChange(\%)} = \frac{\text{TreatmentADgroup} + \text{ADgroup} - \text{Balanced} - \text{dietADmodelgroup}}{\text{Balanced} - \text{dietADmodelgroup}} \times 100$$

- The *Gluten-Free Diet* showed the strongest improvement in the primary AD pathological biomarkers, achieving the greatest reductions in A β (Hippocampus/Cortex), Total Tau, and p-Tau S231.
- The *MIND Diet* also successfully reduced these pathological markers, but with a smaller magnitude of effect compared to the Gluten-Free Diet.
- Behavioral performance was more variable and did not align perfectly with the biochemical improvements:
 - The *Disease-Control (Balanced Diet + AD)* group performed best on Y-maze alternation.
 - Both intervention diets (MIND and Gluten-Free) significantly increased Y-maze entries compared to the Negative Control, suggesting an increase in overall activity.
 - The NOR discrimination index changes were variable, reflecting the complex nature of these cognitive assessments.

Discussion

The present study demonstrates that dietary interventions exert significant effects on Alzheimer's disease (AD)-like pathology in an AlCl₃-induced rat model. Both the MIND and gluten-free diets attenuated amyloid- β accumulation, reduced tau pathology, and improved histological integrity, although these molecular benefits were not uniformly reflected in behavioral outcomes.

Dietary modulation of amyloid and tau pathology

The observed reduction in amyloid- β (A β) and tau burden suggests that both dietary interventions influence upstream pathogenic processes, potentially including amyloid precursor protein processing, tau kinase activity, and protein clearance pathways²⁵. Relative to disease controls, the gluten-free diet produced numerically greater reductions in A β and tau than the MIND diet; however, because these two diets differ across multiple ingredients beyond gluten content (see [Materials and methods](#)), this comparison cannot definitively isolate gluten exclusion as the specific driver. In contrast, the gluten-free diet's improvement over the balanced diet—from which it differs solely through the substitution of gluten-free rice for wheat pasta—more directly implicates gluten restriction itself. This protective effect likely occurs through the suppression of systemic and neuroinflammatory drivers known to exacerbate amyloidogenesis and tau hyperphosphorylation²⁶. Future studies directly comparing gluten-containing and gluten-free variations of the MIND diet are required to isolate gluten's specific mechanistic contribution within that dietary pattern.

Chronic exposure to gluten has been associated with increased intestinal permeability and immune activation in susceptible individuals¹⁴. Even in non-celiac contexts, gluten-derived peptides may contribute to low-grade systemic inflammation, which can amplify central inflammatory signaling²⁷. Removal of gluten may therefore reduce peripheral inflammatory inputs that accelerate neurodegenerative processes¹⁴.

Role of neuroinflammation and oxidative stress

Neuroinflammation represents a central mechanism linking environmental and metabolic factors to AD progression²⁸. AlCl₃ exposure is known to induce oxidative stress and activate pro-inflammatory pathways, including microglial activation and cytokine release²⁹. Both dietary interventions likely exert their protective effects through the modulation of these pathways. The MIND diet, enriched in antioxidants, polyphenols,

and unsaturated fatty acids, has been widely associated with reduced oxidative stress and improved neuronal resilience³⁰; these components may suppress amyloidogenic processing and inhibit kinases involved in tau phosphorylation³¹. Conversely, the relatively stronger biochemical effects observed with the gluten-free diet may indicate a direct influence on systemic inflammatory tone, potentially mediated by a reduced antigenic load and improved metabolic homeostasis.

An important consideration when interpreting the numerically greater biochemical reductions of the gluten-free diet relates to the formulation of the experimental MIND diet. Although the MIND diet was enriched with neuroprotective components—including salmon, walnuts, berries, and olive oil—wheat germ constituted a major component of the dietary base. Wheat germ contains gluten-associated proteins and other antigenic constituents that may influence immune and inflammatory responses²⁷. Consequently, it is possible that the high wheat-derived antigenic load in this experimental formulation partially offset the beneficial effects of its neuroprotective ingredients. This may help explain why the gluten-free diet produced greater reductions in A β accumulation and tau pathology despite sharing several favorable nutritional characteristics with the MIND diet. Future studies should compare gluten-containing and gluten-free versions of the MIND diet to determine whether the observed differences are attributable to gluten exclusion itself or to broader differences in dietary composition.

Finally, the graded pattern of histological injury observed across the groups (normal cytoarchitecture in non-induced groups, severe neurodegeneration in the disease-control group, and mild-to-moderate injury in the MIND and gluten-free intervention groups) parallels the biochemical gradient reported for A β and tau. Groups with lower A β and tau burdens also demonstrated better-preserved histology, and the gluten-free diet—which produced the largest biochemical reductions—likewise showed the mildest residual injury outside the dentate gyrus. This concordance is consistent with a shared underlying protective mechanism linking reduced A β /tau accumulation to preserved neuronal and tissue architecture. However, this parallel is strictly correlative: no experiment in this study directly tested a mechanistic link between the biochemical and histological endpoints, and the histological scoring, while performed by blinded assessors, was semi-quantitative rather than based on unbiased stereological cell counts.

Gut-brain axis as a mechanistic link

The gut-brain axis has emerged as a critical pathway in the pathogenesis of neurodegenerative disorders³². Dietary composition directly shapes gut microbiota¹⁵. These micro-organisms, in turn, regulate immune responses, metabolite production, and blood–brain barrier integrity³³.

The gluten-free diet may promote a less pro-inflammatory gut environment, reducing circulating endotoxins and inflammatory mediators. This, in turn, influences central nervous system function. This mechanism may partially explain the reduction in A β and tau observed in this group.

Similarly, the MIND diet may exert beneficial effects through microbiota-derived metabolites³⁴. For instance, short-chain fatty acids have anti-inflammatory and neuroprotective properties³⁵. Differences in dietary composition and bioactive components between the two interventions likely contribute to their differential effects on disease pathology.

Dissociation between molecular and behavioral outcomes

Despite clear improvements in biochemical and histological markers, behavioral recovery was limited and variable. This dissociation highlights the complexity of linking molecular changes to functional outcomes, particularly within relatively short or dose-limited intervention periods^{36,37}.

Because locomotor activity was not independently assessed, we cannot rule out that differences in general activity level contributed to Y-maze performance, potentially masking or confounding effects on spatial memory. Moreover, behavioral assays such as the Novel Object Recognition Test are inherently sensitive to exploratory behavior and environmental variability³⁸. Previous studies have shown that AlCl₃ exposure can disrupt neurotransmitter systems, induce oxidative stress, and alter behavioral regulation, potentially producing hyperactivity, disorientation, or anxiety-like responses²⁹. Furthermore, differences in dietary fat composition, carbohydrate sources, and energy metabolism may influence spontaneous activity levels independently of memory function. Consequently, undetected differences in activity level may have reduced the sensitivity of the Y-maze spontaneous alternation test to detect subtle cognitive improvements, contributing to the apparent dissociation between molecular pathology and behavioral outcomes observed in the present study.

These findings suggest that while dietary interventions can effectively modulate core pathological processes, longer durations or multimodal strategies may be required to achieve consistent cognitive improvement³⁹.

Translational and practical considerations

Both the MIND and gluten-free diets demonstrated broadly comparable neuroprotective effects across molecular and histopathological outcomes. However, significant differences may develop when considering real-world applicability. The MIND diet requires sustained adherence to a structured dietary pattern involving multiple food groups and specific nutritional targets⁴⁰. That may lead to practical challenges in certain populations⁴¹.

In contrast, a gluten-free dietary approach is primarily based on the elimination of a defined set of components (gluten-containing grains), without necessitating a comprehensive restructuring of overall dietary patterns⁴². This relative simplicity may facilitate implementation and adherence, particularly in settings with limited access to specialized foods or nutritional guidance⁴³.

Importantly, these considerations were not directly evaluated in the present study and should therefore be interpreted with caution. Future studies incorporating measures of adherence, accessibility, and economic feasibility will be essential to determine the sustainability and translational potential of these dietary strategies.

Limitations

A limitation of the present study is that inflammatory mediators, gut microbiota composition, intestinal permeability, and markers of systemic immune activation were not directly measured. Therefore, proposed mechanisms linking gluten exclusion to reduced neurodegeneration remain speculative and require confirmation in future investigations. In addition, the formulation of the experimental MIND diet included wheat germ as a major dietary component, which may have influenced inflammatory signaling and contributed to the differential effects observed between dietary interventions. An a priori power calculation ($f = 0.65$, $\alpha = 0.05$, power = 0.80, based on the pre-specified Diet $\times$ AD-induction interaction) indicated a required sample size of $N = 40$; $n = 6$ per group ($N = 36$) was used for reasons of equal group allocation and the 3Rs principle, yielding an achieved power of approximately 76% — slightly below the conventional 80% threshold⁴⁴. Combined with the large standard deviations observed for some behavioral outcomes (the NOR discrimination index), the study may be underpowered to detect true effects on these endpoints, and the non-significant or variable behavioral findings should be interpreted with this in mind. Additionally, Levene's test indicated unequal error variances across groups for cortical A β ($P = 0.022$), violating the homogeneity-of-variance assumption underlying Tukey's HSD test; although the pattern of significant pairwise differences for this marker is consistent with the other AD-pathology outcomes, the exact P-values for cortical A β should be interpreted with some caution, and a variance-robust alternative (e.g., Games-Howell) may be warranted in future analyses. The AlCl₃ model reproduces AD-like pathology through aluminum neurotoxicity and does not capture the genetic/molecular etiology underlying sporadic or familial human AD. The study used exclusively young adult male rats, so findings may not generalize across sex or to aged animals, which are more representative of typical AD onset. Gluten-related mechanisms are best established in humans with coeliac disease or non-coeliac gluten/wheat sensitivity, conditions not modelled by AlCl₃ exposure; translating these findings into dietary recommendations for the general AD population, most of whom do not have a diagnosed gluten-related disorder, is therefore speculative. This study also did not include an independent measure of locomotor activity such as an open-field test; total Y-maze arm entries did not differ significantly between dietary groups ($P = 0.054$ – 0.656), but in the absence of a dedicated locomotor assay we cannot fully exclude undetected activity differences as a confound, limiting our ability to distinguish genuine working-memory effects from confounding exploratory activity in the Y-maze (see [Discussion](#), Dissociation between molecular and behavioral outcomes). Finally, the 8-week intervention duration may have been too short for behavioral and cognitive endpoints to converge with the molecular and histological findings.

Implications and future directions

These findings support the concept that dietary interventions can modulate key pathological features of AD⁴⁵. That provides a potentially accessible and non-pharmacological strategy for disease modification⁴⁵. The differential effects observed between diets highlight the importance of understanding specific mechanistic pathways, including inflammation, metabolism, and gut microbiota interactions.

Future studies should aim to

- Characterize inflammatory and microbiome profiles associated with each diet.
- Evaluate long-term cognitive outcomes with extended intervention durations.
- Investigate combined dietary and pharmacological approaches.
- Assess real-world feasibility, including adherence and cost-effectiveness.

Conclusion

In summary, both the MIND and gluten-free diets suppressed Alzheimer's-like pathology in an AlCl₃-induced model, significantly reducing amyloid- β and tau burden relative to disease controls. The gluten-free diet produced significantly greater reductions in tau pathology than the MIND diet, although the two diets did not differ significantly in their effect on amyloid- β . Although these molecular and histological improvements were evident, behavioral outcomes remained variable and did not reach statistical significance, underscoring the complexity of translating pathological modulation into functional recovery. These findings highlight the potential of dietary strategies in modulating neurodegenerative processes while emphasizing the need for further investigation into their long-term efficacy and practical implementation.

Data availability

Availability of data and materials The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 16 July 2026; Accepted: 10 September 2026

Published online: 16 October 2026

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Author contributions

G.A. conceived and designed the study, conducted the animal experiments, performed the biochemical and histopathological analyses, analyzed and interpreted the data, and drafted the manuscript. ARB.A. contributed to the study design and the interpretation of the findings. D.A. contributed to the interpretation of the findings and critically reviewed the manuscript. AMK.N. assisted with data analysis, and interpretation of the results. R.G. supervised the research and critically revised the manuscript. All authors read and approved of the final manuscript.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethics approval

All animal experimental procedures were reviewed and approved by the Cairo University Institutional Animal Care and Use Committee (CU-IACUC) (Approval No. CUIF225).

Additional information

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