RESEARCH PAPER

Anti-Apoptotic Effect of Thymoquinone and Intermittent Fasting on the Hippocampus in A Rat Model of Alzheimer's Disease: A Histological and Immunohistochemical Study

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ABSTRACT

Alzheimer's disease (AD) is an age-dependent neurodegenerative condition. Aluminum chloride (AlCl3) is a worldwide neurotoxicant metal which is extensively used in daily life. Thymoquinone (TQ) is the active compound of Nigella Sativa seeds with anti-apoptotic and neuroprotective effects. Intermittent fasting (IF) is a dietary pattern possessing antioxidant and anti-apoptotic qualities that reduce neuroinflammation. The purpose of this study was to evaluate the hippocampal histological and immunohistochemical alterations in adult male albino rats in an experimental model of AlCl3 induced AD and to assess the possible neuroprotective effect of TQ, IF and their combination. In this study, sixty-four adult male albino rats were used. The rats were classified into seven groups: control group, TQ group (oral 50 mg/kg/day), IF group (underwent fasting for 14 hours/day), AL group (oral 300 mg/kg/day AlCl3), AL+TQ group received both AlCl3 and TQ, AL+IF group received AlCl3 and underwent fasting and AL+TQ+ IF group received AlCl3, TQ and underwent fasting. Hippocampal sections were stained by Congo red stain for assessment of amyloid and were immunostained using anti Bax antibodies for assessment of apoptosis. Morphometric and statistical studies were performed. The AL group showed marked hippocampal degenerative changes with amyloid deposition and significant increase in immune expression for Bax marker. These changes were ameliorated in protection groups, especially combination group where degenerative changes, amyloid deposition and Bax immune expression were reduced. In conclusion, aluminum chloride is neurotoxic, both TQ and IF provide potent neuroprotective and anti-apoptotic effects with the best outcome by their combination.

Keywords: Hippocampus, Alzheimer's disease, Thymoquinone, Intermittent fasting.

How to cite this article: El-Salam RAA, Sarhan NR, El-Hadidy AR, Omar NM. Anti-Apoptotic Effect of Thymoquinone and Intermittent Fasting on the Hippocampus in A Rat Model of Alzheimer's Disease: A Histological and Immunohistochemical Study. Int J Drug Deliv Technol. 2026;16(57s): 1975-1985. DOI: 10.25258/ijddt.16.57s.200

Source of support: None

Conflict of interest: None

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that exacerbates over time and results in behavioral changes, memory loss, and cognitive deterioration (Vaz and Silvestre, 2020). The histopathological features of AD were manifested in the hippocampus of AlCl3 treated animals (Offiong et al. 2024). The hippocampus consisted of the hippocampus proper (cornu ammonis), dentate gyrus and subicular complex. The cornu ammonis (CA) regions are CA1, CA2, CA3 and CA4 (Sumadevi, 2024). The primary causes of AD pathogenesis are extracellular amyloid β plaque accumulation and intracellular hyperphosphorylated tau aggregates that form neurofibrillary tangles (NFTs) (Ma et al., 2022). Other pathological features include neurons and synaptic loss, massive production of reactive oxygen species (ROS), damage to mitochondrial DNA, and alterations in neurotransmitter levels, mainly decreased acetylcholine (Amakiri et al., 2019). The animal model of AD induced by AlCl3 was commonly used in studying the mechanisms which cause the pathogenesis of the disease (Agrawal et al., 2024).

Exposure to Al is very common in daily life and from many sources including food, food additives, drinking water, Al wares, pharmaceutical and personal care products, some drugs and occupational exposure (Niu, 2023). Aluminum can cross the BBB and accumulate in all brain regions including the hippocampus. Al generates ROS that lead to lipid peroxidation and oxidative damage to DNA and proteins. It also modifies calcium signal pathways in the hippocampus that are crucial for neuronal plasticity and memory (Ogunlade et al., 2022).

Nigella sativa (black seed) is considered a promising medicinal plants that has many therapeutic benefits. Thymoquinone, its main active component, has marked anti-oxidant, anti-inflammatory, anti-apoptotic, anti-cancerous and neuroprotective effects (Gawas et al., 2023). Intermittent fasting (IF) is considered a kind of fasting which includes feeding and fasting in alternating cycles. It includes eight hours of normal eating and sixteen hours of fasting with total food deprivation (Mattson, 2025). Health benefits of intermittent fasting includes reduction of free-radical production, suppression of inflammatory cytokines, anti-apoptotic effect and enhancement of insulin sensitivity and stress resistance (Gudden et al., 2021).

Despite advances in medical science, current therapies for AD weren't sufficient to modify the progression of this disease which triggers the need to find alternative strategies such as herbal remedies and diet control that help delay the course of the disease. Thus, the current study's aim was to examine the histological and immunohistochemical

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alterations in the hippocampus of Al-induced AD as well as to evaluate possible protective benefits of thymoquinone, intermittent fasting, and their combination.

MATERIAL AND METHODS:

Materials:

Aluminum chloride (AlCl3): obtained from Sigma-Aldrich Chemical Company, USA (catalog number, 11019-500G). It was available in the form of stock that contains 500 gm of anhydrous aluminum chloride.

Thymoquinone (TQ): obtained from Sigma-Aldrich Chemical Company, USA (catalog number, 274666) as solid crystals (purity 99%) containing bottle (1 gm).

Study protocol and Experimental animals

The current experimental study was carried out in Mansoura University's Medical Experimental Research Center (MERC), Faculty of Medicine. The Institutional Review Board (IRB), Faculty of Medicine, Mansoura University, authorized the study's protocol (Code: MD.21.06. 482.R1). The MERC and international guidelines for the use of laboratory animals were followed when conducting the research. In our study, sixty-four adult male albino rats weighing between 180 and 200 grams were used. They were kept for acclimatization 2 weeks before the experiment in plastic cages under adequate ventilation and average temperature (25 ± 2 C˚) with regular 12 h light/12 h dark cycles. They were given unlimited access to food and water during the acclimatization period.

Experimental animals:

Rats were classified into seven groups:

Control group (16 rats):

Ia (8 rats): given oral gavage of distilled water at 9 p.m. every day for four weeks, with unrestricted access to food and water throughout the day.

Ib (8 rats): given 1 ml/kg/day of corn oil (vehicle for TQ) via oral gavage at 10 pm daily for 4 weeks and had unrestricted access to food and water throughout the day.

TQ group (8 rats): given 50 mg/kg/day TQ dissolved in corn oil via oral gavage at 10 pm daily for 4 weeks and permitted unrestricted access to food and water throughout the day (Liu et al., 2016).

IF group (8 rats): underwent fasting for 14 hours per day (from 5 am to 7 pm) and free access to food and water in the remaining time of the day for 4 weeks (Mindikoglu et al., 2020).

AL group (8 rats): given 300 mg/kg/day AlCl3 dissolved in distilled water by oral gavage at 9 pm daily for 4 weeks and permitted unrestricted access to food and water throughout the day (Gazia, 2019).

AL+TQ group (8 rats): given concomitantly both 300 mg/kg/day AlCl3 dissolved in distilled water at 9 pm daily and 50 mg/kg/day TQ dissolved in corn oil at 10 pm daily both by oral gavage for 4 weeks with unrestricted access to food and water throughout the day (Liu et al., 2016).

AL+IF group (8 rats): given 300 mg/kg/day AlCl3 dissolved in distilled water by oral gavage at 9 pm daily for 4 weeks and underwent fasting for 14 hours per day (from 5 am to 7 pm) (Abas and Sabry, 2020) with unrestricted access to food and water throughout the day.

AL+TQ+IF group (8 rats): underwent fasting for 14 hours per day (from 5 am to 7 pm) with free food and water access in the remaining time of the day for 4 weeks. During this period, they were supplemented concomitantly with both AlCl3 (300 mg/kg/day) dissolved in distilled water at 9 pm daily and TQ (50 mg/kg/day) dissolved in corn oil by oral gavage at 10 pm daily by oral gavage.

Specimen collection and processing:

Intraperitoneal injection of 40 mg/kg sodium pentobarbital was given to rats to induce anesthesia at the end of the experiment (Dutton et al., 2019). After that, the animals were euthanized by the process of decapitation. The hippocampus was obtained after quick brain dissection and processed for histological and immunohistochemical analysis.

Histological study:

Light Microscopic Study:

Hippocampal specimens were fixed in 10% neutral buffered formalin for examination by light microscopy. For histological examination, paraffin sections (4-5um) were stained with H&E stain (Bancroft and Layton, 2019) and for demonstration of amyloid accumulation, sections were stained with Congo red stain (Gilbertson, 2019).

Immunohistochemical study:

Deparaffinized paraffin sections (5 micrometers) were employed on coated slides for localization of apoptotic marker Bax (Bcl2-associated X protein) (Lopez et al., 2022) using Labeled Streptavidin–Biotin immunoperioxidase technique (Sanderson et al., 2019). Following a 15-minute incubation period in hydrogen peroxide to inhibit endogenous peroxidase, the slides were washed two times (5-minute each) in PBS solution. For 10 – 20 min, sections were subjected to boiling in citrate buffer (pH 6.0), cooling for 20 min at room temperature, and washing four times in PBS to achieve antigen retrieval (unmasking of the antigenic peptide). Incubation of the sections with normal goat serum (XO9O7; Dako, Carpinteria, USA) was performed to prevent non-specific background staining. The primary antibodies, anti-Bax, were incubated with the sections for 60 min at room temperature. The primary antibody used for Bax was rabbit polyclonal antibody, dilution 1:200, catalog No. bs-0127R, Bioss, Beijing, China. The next step is rinsing of the slides in PBS 3 times (5 minutes each). Secondary antibody (biotinylated goat antipolyvalent) (Dako-K0690; Dako Universal LSAB Kit) was applied to the slides and they were incubated for 10 minutes at room temperature in the humidity chamber, then rinsing the sections in PBS for 3 times, each for 5 minutes. Streptavidin-biotin- peroxidase solution (LSAB 2 Kit: Dako) was applied to the sections and the slides were incubated in the humidity chamber for 10 minutes at room temperature and then rinsed 2 times in PBS, each for 5 minutes and dried. Finally, slides were incubated with substrate chromogen DAB (3, 30 Diaminobenzidine) for five to ten minutes, resulting in brown precipitate at the reaction site. The counterstain used was Mayer's Hematoxylin. Negative control slides were included in Tris-buffered saline instead of the primary antibodies. Positive control sections were prepared from rat kidney

(Liang et al., 2023). A brown cytoplasmic immune reaction was considered positive.

Morphometric study:

Using a 40 X objective lens and an Olympus® digital camera (E420, China) installed on an Olympus® microscope (CX23LEDRF, Japan) with a 0.5 X picture adapter, slides were photographed. Six slides from each rat per group (8 rats) were examined. To estimate the morphometric data, ten non-overlapping fields were selected randomly from each slide. After that, the photos were examined using Video Test Morphology software on an Intel Core I3 computer (Russia, Saint-Petersburg). It was used to assess number of degenerated pyramidal cells with deep acidophilic cytoplasm and shrunken dark nuclei / HPF (X 400) using H&E-stained sections and the percentage area of Bax immune-expression / HPF (X 400) using anti-Bax stained sections both in CA3 region of the hippocampus

STATISTICAL ANALYSIS:

The morphometric data were coded, tabulated, and analyzed using SPSS program version 26. Descriptive statistics were estimated as mean ± Standard deviation (±SD). In order to statistically compare the different groups, the significance of difference was evaluated using ANOVA then post-hoc Tukey for multiple comparisons. Statistically significant differences were considered if P value was < 0.05 (Hazra and Gogtay, 2016).

RESULTS:

No animals died during this study.

The histological, immunohistochemical and morphometric results of the control subgroups were comparable to each other and were collectively presented as control group.

I) Light Microscopic Results:

1. Hematoxylin and Eosin (H&E) Stain:

The present work illustrated the results of CA3 region examination as the histological alterations were clearly detected in this region. Examination of CA3 region in H&E stained hippocampal sections from control, TQ & IF groups revealed four strata; oriens, pyramidale, lucidum and radiatum. Stratum pyramidale was the most prominent layer of CA3 region. It consisted of 4-5 loosely arranged layers of pyramidal cells with basophilic cytoplasm, vesicular nuclei, and prominent nucleoli. The stratum lucidum is a narrow acellular zone beneath the stratum pyramidale. Glia cells were present in stratum oriens, between pyramidal cells and in the perineural region. Stratum radiatum displayed the characteristic streaking pattern of fibers of apical dendrites of the pyramidal cells. AL group revealed evident degenerative changes. Disturbed cell arrangement was obvious in CA3. Most of pyramidal neurons appeared shrunken with deeply stained acidophilic cytoplasm, dark shrunken nuclei and pericellular halos. Numerous glia cells were observed. Dilated blood vessels surrounded by wide perivascular spaces and neuropil vacuolations were observed. AL+TQ, AL+IF & AL+TQ+IF groups showed preservation of the hippocampal architecture. This preservation was more evident in AL+TQ+IF group. The majority of pyramidal cells possessed basophilic cytoplasm, vesicular nuclei and prominent nucleoli. However, some shrunken pyramidal cells with dark shrunken nuclei and deeply stained acidophilic cytoplasm were still noted. Blood vessels and neuroglia cells in stratum oriens, radiatum and between the pyramidal neurons were also noticed (Figure 1).

The number of degenerated CA3 pyramidal cells showed non-significant changes between control & TQ group, However, IF group showed a significant reduction as compared to them ($p < 0.05$). AL group showed significant elevation ($p < 0.05$) in their number as compared to control, TQ & IF groups. AL+TQ, AL+IF & AL+TQ+IF groups showed significant decline ($p < 0.05$) in the number of degenerated cells compared to AL group, however, their number remained significantly higher compared to control, TQ & IF groups. AL+IF group had significantly lower number of degenerated cells as compared to AL+TQ group. AL+TQ+IF group exhibited significantly lower number of degenerated cells compared to AL+TQ & AL+IF groups (Figure 2).

2. Congo red stain:

Negative Congo red staining for amyloid was seen in the pyramidal cells of CA3 region and neuropil in control, TQ & IF groups. In AL group, most of the pyramidal cells were shrunken with positive Congo red staining for amyloid with the appearance of some positive amyloid deposits in the neuropil. AL+TQ & AL+IF groups showed positive Congo red staining in some shrunken pyramidal cells and in the neuropil. AL+TQ+IF group showed positive Congo red staining in few shrunken pyramidal cells and in the neuropil (Figure 3).

II) Immunohistochemical Results:

Anti-Bax stained sections of control, TQ & IF groups revealed weak positive cytoplasmic immune expression for Bax in few pyramidal cells of CA3 region. AL group showed strong positive cytoplasmic immune expression for Bax in many shrunken pyramidal cells and in the neuropil. Regression of Bax immune expression was observed in the treatment groups with moderate cytoplasmic immune expression for Bax in AL+TQ group, mild positive immune expression in AL+IF group and weak positive immune expression in AL+TQ+IF group (Figure 4).

Regarding percentage area of Bax immune expression in CA3 region, there was non- significant change between control, TQ & IF groups but it was significantly higher in AL group compared to them ($p < 0.05$). AL+TQ, AL+IF & AL+TQ+IF groups showed significantly lower percentage area than AL group. AL+TQ & AL+IF groups were comparable to each other. AL+TQ+IF group had significantly lower percentage area than AL+TQ & AL+IF groups. However, in AL+TQ & AL+IF & AL+TQ+IF groups, percentage area of Bax immune expression remained significantly higher than control, TQ & IF groups (Figure 5).

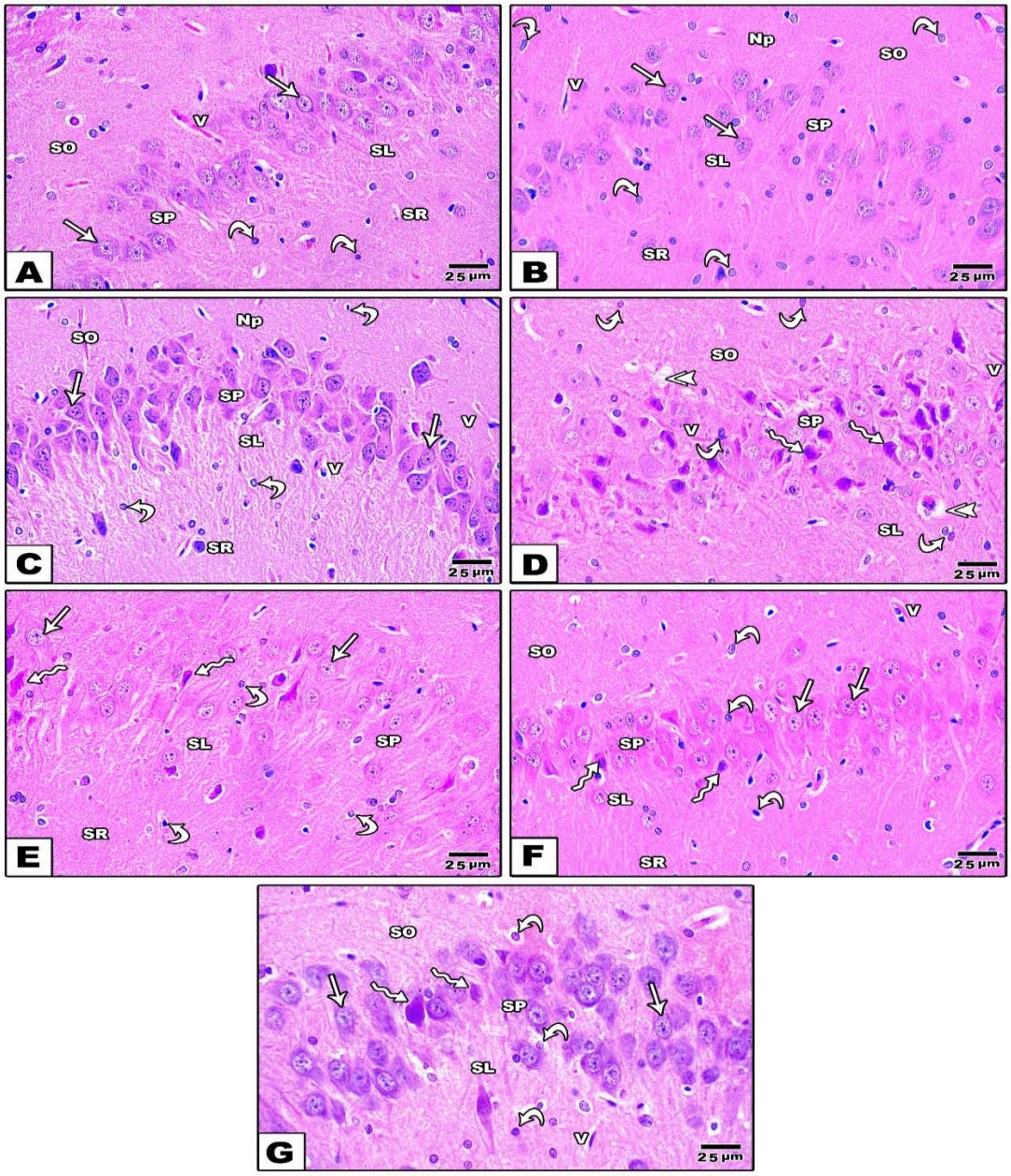

Figure 1. H & E-staining in the hippocampus of different study groups. (A, control group; B, TQ group; C, IF group; D, AL group; E, AL+TQ group; F, AL+IF group; G, AL+TQ+IF group). A, B & C: showing 4 layers of CA3 region; stratum oriens (SO), stratum pyramidale (SP), Stratum lucidum (SL) and stratum radiatum (SR). Stratum pyramidale (SP) is formed of loosely arranged pyramidal cells (arrows) with vesicular nuclei, prominent nucleoli and basophilic cytoplasm. Blood vessels (v) and neuroglia (curved arrows) in the perineural regions, SR and SO are evident. D: Most of pyramidal cells (zigzag arrows) are shrunken with dark shrunken nuclei, deeply stained acidophilic cytoplasm and pericellular halos. Neuropil vacuolations (double arrow heads), many neuroglia (curved arrows) and blood vessels (v) are observed. E, F & G: most pyramidal cells (arrows) have vesicular nuclei and pale basophilic cytoplasm. Some pyramidal cells (zigzag arrows) are shrunken with dark nuclei, dark acidophilic cytoplasm and pericellular halos. Blood vessels (v) and neuroglia (curved arrows) are seen (H&E x 400, scale bar = 25µm).

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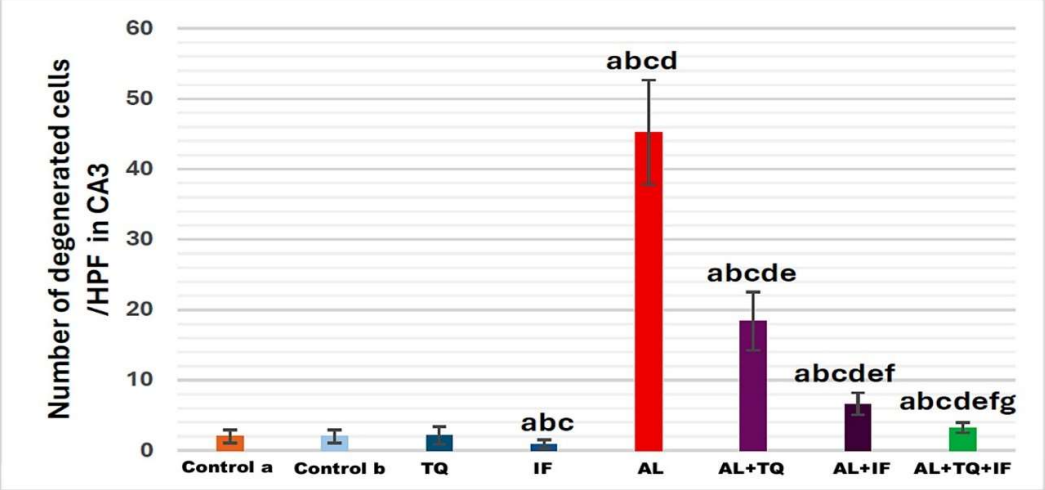

Figure 2. Number of degenerated cells in CA3 in different study groups. Data are represented as mean ± SD. One-way Anova test is used. a: significance against control a group; b: significance against control b group; c: significance against TQ group; d: significance against IF group; e: significance against AL group; f: significance against AL+TQ group; g: significance against AL+IF group. Significance: p value < 0.05 (n = 8 rats/ group).

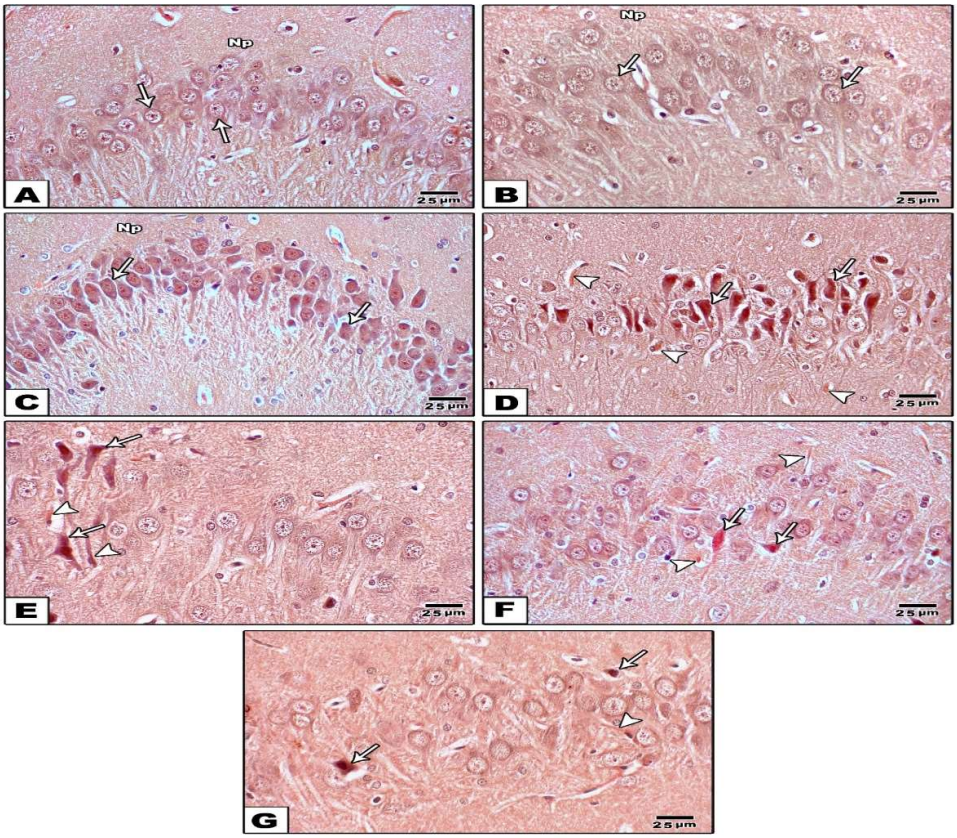

Figure 3. Congo red staining in the hippocampus of different study groups. (A, control group; B, TQ group; C, IF group; D, AL group; E, AL+TQ group; F, AL+IF group; G, AL+TQ+IF group). A, B & C: negative Congo red staining for amyloid in the pyramidal cells of CA3 and the neuropil (Np). D: Most of the pyramidal cells (arrows) appear shrunken with positive Congo red staining for amyloid. Positive deposits (arrow heads) in the neuropil and along blood vessels are observed. E&F: positive Congo red staining in some shrunken pyramidal cells (arrows) and in the neuropil (arrow heads). G: positive Congo red staining in few shrunken pyramidal cells (arrows) and in the neuropil (arrow heads) (Congo red x 400, scale bar = 25µm).

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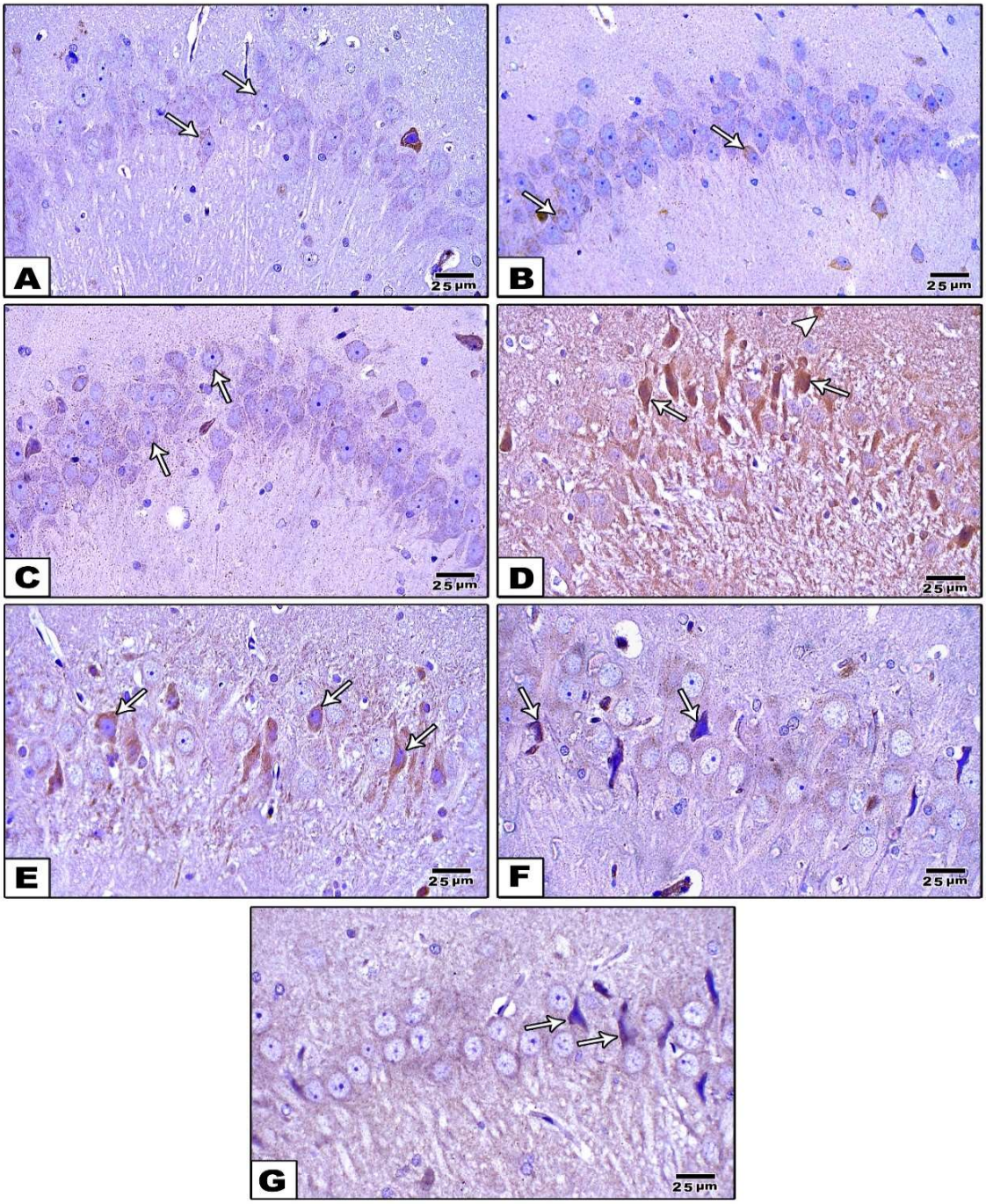

Figure 4. Bax immune expression in the hippocampus of different study groups. (A, control group; B, TQ group; C, IF group; D, AL group; E, AL+TQ group; F, AL+IF group; G, AL+TQ+IF group). A, B & C: weak positive cytoplasmic immunostaining for Bax in few pyramidal cells of CA3. D: strong positive cytoplasmic immunostaining for Bax in many shrunken pyramidal cells (arrows) and in neuroglia (arrowhead). E: moderate positive cytoplasmic immunostaining for Bax in some shrunken pyramidal cells (arrows) F: mild positive cytoplasmic immunostaining for Bax in some shrunken pyramidal cells (arrows). G: weak positive cytoplasmic immunostaining for Bax in few shrunken pyramidal cells (arrows) (Anti-Bax x 400, scale bar = 25µm).

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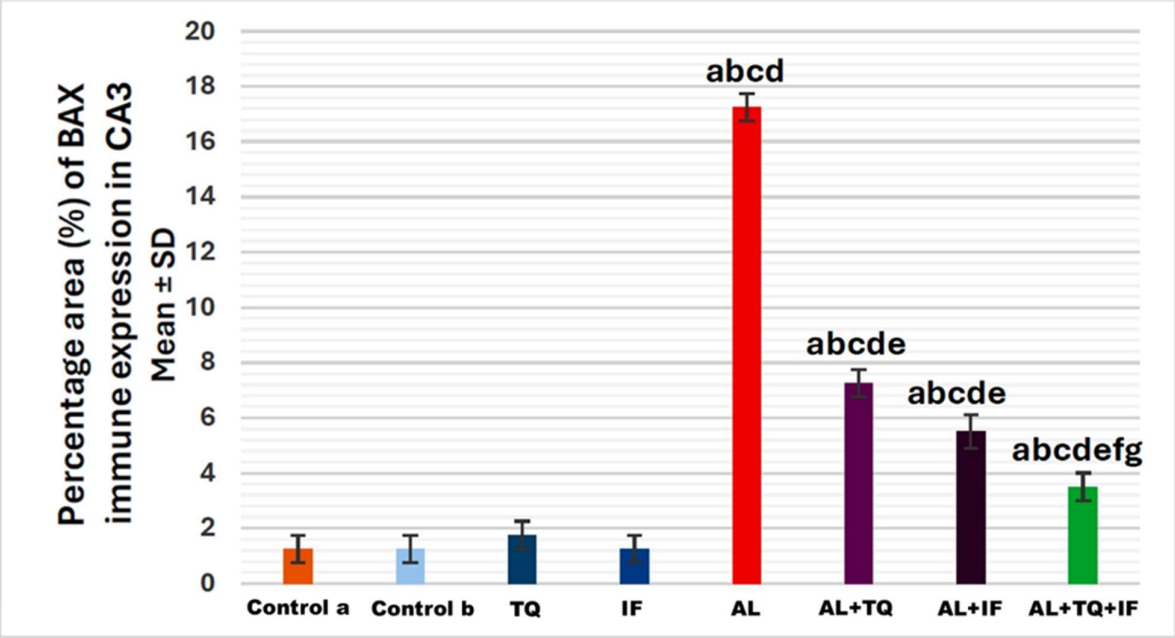

Figure 5. Percentage area (%) of Bax immune expression in CA3 in different study groups. Data are represented as mean ± SD. One-way Anova test is used. a: significance against control a group; b: significance against control b group; c: significance against TQ group; d: significance against IF group; e: significance against AL group; f: significance against AL+TQ group; g: significance against AL+IF group. Significance: p value < 0.05 (n = 8 rats/ group).

DISCUSSION

Alzheimer's disease (AD) is considered one of the most common neurological disorders with degenerative changes. It is associated with loss of synaptic and neuronal functions in a progressive manner that results in cognitive decline and memory loss (Kim et al., 2024). Many factors have been attributed to increasing incidence of AD mainly the accumulation of aluminum in the brain. Exposure to Al is very common and numerous studies have discovered that high Al levels are linked to neurodegeneration and cognitive impairment (Dey and Singh, 2022). Aluminum chloride has been used in many studies to create an animal model of AD neurotoxicity. In experiments, aluminum exposure results in oxidative brain damage, neuronal inflammation, neurodegenerative changes, and worsens biochemical changes. Aluminum can also cross the BBB and act as neurotoxin that leads to AD pathogenesis (Sajad et al., 2025).

Examination of CA3 region in AL group showed numerous shrunken pyramidal cells with deeply stained acidophilic cytoplasm, deeply stained shrunken nuclei and pericellular halos with high significant increase in their number in comparison to control, TQ & IF groups. Similar microscopic picture was reported by Alsemeh et al. (2020) in animal model of methotrexate neurotoxicity and by Ogunlade et al. (2022) and Jadhav and Kulkarni (2023) in animal model of AlCl3 neurotoxicity. On the other hand, El-Beltagi et al. (2022) reported focal neuropil hemorrhage and pyramidal cells with fragmented karyorrhexitic nuclei and nuclei with crescent shape and chromatin margination in AlCl3 induced AD rat model. Such degenerative changes observed in AL group could be attributed to oxidative stress induced by aluminum toxicity which provokes neuronal damage and subsequent apoptosis. Excessive oxidative stress is induced by aluminum toxicity with overproduction of ROS. Polyunsaturated fatty acids in brain cell membranes undergo peroxidation due to ROS. As a result, toxic byproduct metabolites are formed such as MDA and NO and endogenous antioxidants and SOD levels are decreased (Yisa et al., 2024). Aluminum accumulation in the brain of AD causes significant damage to neuronal cells, glia cells and brain vasculature cells (Song et al., 2024). The appearance of many glia cells in different zones could be explained as a response to elevated inflammatory markers. The elevation of pro-inflammatory cytokines like interleukin 1β induces microglia activation. The microglia are neuroprotective as they phagocyte Aβ, thereby preventing AD (Rao et al., 2022). The presence of neuropil vacuolations denotes degenerative changes related to ROS accumulation. These changes may refer to vacuolar changes of the neuronal processes within the neuropil and myelin sheaths vesiculations (Corey et al., 2023).

Congo red stained sections showed shrunken pyramidal cells with positive staining for amyloid indicating the deposition of amyloid β in the hippocampal tissue. Similar observations were reported by Anwar et al. (2021) in the hippocampus of AlCl3-induced AD model in rats. It has been reported that senile plaques accumulation in the brain is one of the causes behind AD development (Chen et al., 2023). Cleavage of amyloid precursor protein (APP) is induced by β- and γ-secretases, initiating the production of Aβ. Aβ can readily permeate the brain tissue and cause

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oxidative stress, neuroinflammation, and neuronal death in the hippocampus (Hamdan et al., 2022).

Anti-Bax stained sections showed strong positive cytoplasmic immune expression for Bax with significantly higher percentage area of Bax immune expression than control, TQ & IF groups. These data give explanation to the presence of many apoptotic shrunken neurons with dark shrunken nuclei in H & E-stained sections of this group. Such apoptotic cells exhibited morphological criteria of pyknosis including cell shrinkage, nuclear condensation and shrinkage (Zhang et al., 2018). In consistence with our findings, Hamdan et al., (2022) documented an increase in Bax expression in the brain of AlCl3-induced AD animal model.

Proteins related to the BCL-2 family including Bax are crucial for regulating apoptosis. Bax has a major function in apoptosis and can transform into oligomers embedded in the outer membrane of mitochondria, leading to mitochondrial outer membrane permeabilization (MOMP). Apoptotic signaling cascade is irreversibly executed by MOMP's cytoplasmic liberation of cytochrome c (Spitz et al., 2021).

H&E stained sections in AL+TQ group revealed that the majority of pyramidal cells had vesicular nuclei, prominent nucleoli and basophilic cytoplasm with some pyramidal cells still appeared shrunken. Statistically, there was significant decline in number of degenerated cells as compared to AL group. These results could be due to the anti-inflammatory, antioxidant, and neuroprotective effects of TQ. It has a role in lipid peroxidation inhibition, it crosses the BBB, scavenges free radicals and diminishes ROS-mediated reactions (Elsabbagh and Ellakwa, 2025). Examination of Congo red stained sections revealed regression in the Congo red positive cells. This is consistent with Anwar et al. (2021) who investigated the role of antioxidants on Aβ deposition in hippocampus of AD rats. Prior studies reported that TQ decreases the levels of Aβ in brain tissue. It also minimizes plaque formation and increases the neurons survival rate and protects pyramidal cells from neurotoxic Aβ effects (Zaher et al., 2020; Khan et al., 2022).

TQ also lowers Bax immune expression in AL+TQ group compared to AL group indicating its anti-apoptotic effect. This downregulation of Bax immune expression could explain the diminished number of shrunken degenerated pyramidal cells observed in H&E-stained sections. TQ has an anti-apoptotic effects by suppressing caspase 3, 8, 9 enzymes and cytochrome c (Pottoo et al., 2022). TQ also prevents mitochondrial dysfunction and restore MMP (mitochondrial membrane potential) by reduction of oxidative stress (Sadeghi et al., 2023).

Intermittent fasting is considered a dietary restriction treatment having anti-inflammatory, anti-apoptotic, antioxidant, and neuroprotective benefits (Xiong et al., 2023). IF shows alternating periods of regular eating with cycles of fasting for 12 to 24 hours. For humans, glucose is the main energy source. Blood glucose levels quickly drop after eating a meal high in carbohydrates. Glycogen levels will drop and fat metabolism will become the source of energy through the production of ketone bodies, depending on how much glycogen is stored in the liver and how much energy is consumed. This happens 12 to 36 hours after consuming carbohydrate meal (Albrahim et al., 2023). Ketone bodies are used by the brain and other organs to meet their energy needs. This is the most distinctive metabolic aspect of fasting and indicates a metabolic switch from glucose to ketone bodies (Chen et al., 2023). The intermittent fasting in AL+IF group ameliorated the histopathological change in the hippocampus. Examination of H&E-stained sections showed that most of pyramidal cells were more or less similar to control. However, some shrunken pyramidal cells were still found. Statistically, there was significant decline in the number of degenerated cells as compared to AL and AL+TQ groups.

Normal oxidative metabolism leads to the production of ROS. Intermittent fasting temporarily decreases metabolism and protein synthesis which results in attenuation of free radicals formation and reduction of oxidative stress (Pan et al., 2022; Elias et al., 2023). IF also decreases mitochondria-related ROS production (Albrahim et al., 2023). Moreover, studies showed that IF has anti-inflammatory effects, it decreases the concentrations of proinflammatory cytokines including iNOS, TNF-α and interleukins including 1α, 1β, 6 & 10 (Liu et al., 2023).

Congo red stained sections showed positive Congo red staining for amyloid in some shrunken pyramidal cells and in the neuropil. Similar finding was reported by Gregosa et al. (2019) in AD model following dietary restriction. IF reduces blood APP which decreases Aβ plaques accumulation and decreases cognitive affection. IF also creates physical barriers to protect neurons from neurotoxic plaques (Shippy et al., 2020).

Anti-Bax stained hippocampal sections of AL + IF group showed mild positive immune expression for Bax in CA3 pyramidal cells. There was significantly lower expression as compared to AL group, comparable to AL+TQ group and higher expression than control, TQ & IF groups. López-Domínguez et al. (2015) stated that IF has anti-apoptotic role and decreases the ratio of Bcl-2/Bax in mice following caloric restriction. IF promotes DNA repair, lowers levels of the pro-apoptotic factor Bax, and increases anti-apoptotic proteins synthesis (Xiong et al., 2023).

In our study, the combination of TQ & IF had a more protective effect than giving either of them alone. The majority of pyramidal cells were similar to control, few Congo red positive cells were noted and there was significant reduction in Bax immune-expression. Many trials used combination methods to ameliorate AD. Giri et al. (2024) and Srivastava et al. (2021) indicated that combination therapy may be effective to apply more synergetic effect and improve response to treatment.

CONCLUSION

Both TQ & IF have neuroprotective effects on Al induced hippocampal changes in animal model of AD. This effect was better in IF group and the best neuroprotection was achieved when IF was combined with TQ administration giving the hope for lessening of AD via modulating lifestyle and using antioxidant herbal supplements.

AUTHOR CONTRIBUTIONS:
Conceptualization, methodology, software validation, formal analysis, curation of resource data, writing, preparation of the initial draft, writing, review, and editing, Reem Ahmed Abd El-Salam, Nahla Reda Sarhan, Email: nahlaredasarhan@mans.edu.eg, Azza Radwan El-Hadidy, Email: azza@mans.edu.eg and Nesreen Moustafa Mohammed Omar, Email: nesrinemoustafa@mans.edu.eg The published version of the work has been reviewed and approved by all authors.

DATA AVAILABILITY STATEMENT: All data is contained within the article.

CONFLICTS OF INTEREST: There is no conflict of interest declared by the authors.

FUNDING: The study presented in this paper has no funding, according to the author(s).

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