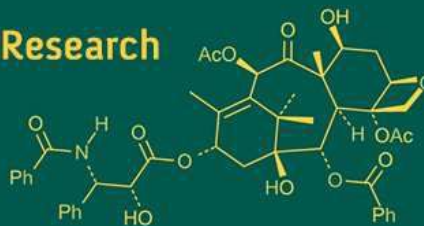


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Effect of Quercetin on Ferroportin-1 Mediated Ferroptosis in Experimentally induced Alzheimer's Like Disease

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Abstract

Background: Alzheimer's disease (AD) is a pervasive neurodegenerative disorder characterized by progressive cognitive decline and complex pathophysiology that remains incompletely elucidated. Ferroptosis, an iron-dependent regulated cell death pathway, has recently been implicated in AD-associated neurodegeneration. Accordingly, the present study aimed to investigate the potential protective role of quercetin against ferroportin-1 (FPN1)-mediated ferroptosis in experimentally induced Alzheimer's like disease induced by aluminium chloride ($AlCl_3$) in male rats.

Methods: Forty male rats were randomly assigned into 4 groups: control, quercetin-treated, $AlCl_3$ -induced AD, and $AlCl_3$ + quercetin-treated (quercetin administered one hour following $AlCl_3$). The cognitive performance was assessed using Morris Water Maze (MWM). On day 42, hippocampal and cortical tissues were harvested for molecular, biochemical, and histopathological analyses. FPN1 gene expression was evaluated by real-time PCR, while glutathione peroxidase 4 (GPX4) was measured by ELISA. Iron content, reduced glutathione (GSH), and malondialdehyde (MDA) levels were determined colorimetrically. Histopathological examination was performed using hematoxylin and eosin and Congo red staining.

Results: The $AlCl_3$ -induced AD group exhibited significant cognitive impairment, elevated iron and MDA levels, and marked suppression of FPN1, GPX4, and GSH, indicating enhanced ferroptotic activity. Histopathological analysis revealed neuronal degeneration and amyloid deposition. Quercetin administration significantly improved behavioral performance, restored FPN1 expression and antioxidant status, reduced iron overload and lipid peroxidation, and ameliorated histopathological alterations.

Conclusion: Overall, our findings suggest that quercetin exerts a neuroprotective effect against $AlCl_3$ -induced AD-like pathology through FPN1-mediated ferroptosis modulation and iron-driven oxidative stress attenuation.

Keywords: Alzheimer's disease, Ferroptosis, $AlCl_3$, Quercetin, FPN1, GPX4, GSH, MDA

Introduction

Alzheimer's disease (AD) was initially identified by Dr. Alois Alzheimer in 1907 [1]. It is the most common form of dementia, accounting for 60 to 70% of dementia cases in elderly patients. It is an irreversible, progressive, and chronic neurodegenerative disorder [2]. Clinically, AD is considered by progressive memory loss and cognitive dysfunction, ultimately dementia, and deterioration of thinking skills that interfere with daily life activities [3].

One of the most well-known neurotoxins is aluminum (Al). The neurotoxicity induced by $AlCl_3$ is comparable to the progressive neurodegenerative alterations that are observed in AD. As a result, the administration of $AlCl_3$ has been extensively employed to provoke an AD model [4].

The neuropathological hallmarks of AD are the extracellular amyloid beta ($A\beta$) accumulation and intracellular neurofibrillary tangles (NFTs) deposition of hyperphosphorylated tau protein [5]. However, the understanding of the pathogenesis of this disease is still incomplete. Improving our current knowledge on the pathogenesis of dementia helps to identify more effective diagnostic and therapeutic strategies [6].

Recently, ferroptosis has been identified as a regulated cell death form distinct from necrosis and apoptosis, characterized by iron dependency and excessive lipid peroxidation. It is triggered by intracellular iron accumulation, which promotes reactive oxygen species (ROS) generation and membrane lipids are subjected to oxidative damage. The glutathione-glutathione peroxidase 4 (GPX4) axis plays a central role in preventing ferroptosis by detoxifying lipid peroxides. Increasing evidence suggests that ferroptosis contributes significantly to neuronal loss and cognitive impairment in AD [7].

There are numerous physiological processes in the brain that require iron, an essential trace element, including neurotransmitter synthesis, mitochondrial respiration, and myelination. The Fenton reaction's capacity to catalyze the formation of ROS can result in oxidative damage to proteins, lipids, and nucleic acids, rendering excess intracellular iron highly toxic. Abnormal iron accumulation has been consistently reported in the cerebral cortex and hippocampus of patients with AD and is closely associated with A β deposition and tau pathology [8].

Ferroportin-1 is the sole known transmembrane iron exporter and a critical regulator of cellular and neuronal iron homeostasis. In the central nervous system, FPN1 facilitates iron efflux from neurons and glial cells, thereby preventing pathological iron accumulation and limiting iron-driven oxidative stress. Alterations in FPN1 expression disrupt iron balance, leading to intracellular iron overload, ROS generation, and lipid peroxidation [9].

Currently, there is no effective therapy that can significantly delay or stop the progressive brain damage in AD. Pharmacological treatments currently in use have limited efficacy and only provide symptomatic relief slowing the progression of cognitive decline without long-term cure. Accordingly, there is an urgent need to find alternative therapeutic ways to treat AD [10].

Quercetin is in the flavonoids group of plant pigments, which are responsible for the colors of numerous fruits, florals, and vegetables. It is identified as 3, 3', 4', 5, 7-pentahydroxyflavone [11]. It is well recognized for its anti-inflammatory, antioxidant activity, and iron-chelating properties. It exerts protective effects against several chronic diseases, including cancer and cardiovascular disorders. Quercetin is naturally abundant in various dietary sources, including vegetables (onions, tomatoes, and broccoli), fruits (apples, berries, and grapes) and some herbs like tea [12].

Materials and Methods

The Tanta University Faculty of Medicine's Ethical Committee granted authorization for the research to be carried out (Approval Code Number: 36264MD40/2/23).

Drug and chemicals

Aluminum chloride was obtained from El-Gomhoria Company for Trading Pharmaceutical Chemicals and Medical Appliances, Tanta, Egypt. It was obtained in the powder form.

Quercetin was obtained from Biovea Egypt as Quercetin 500 mg (60 vegan capsules). The purest and extremely bioavailable form is produced by an extract of the *Dimorphandra mollis* (Fava D'Anta pods) plant in Brazil (QU995).

Most chemical applied, unless otherwise mentioned, have been ordered from Sigma-Aldrich Chemical Co. (St.Loui, MO, US).

Experimental design

After acclimatization for one week, rats were randomly assigned to four groups (n = 10) as follows: Group1 (Normal control): rats received standard chow diet and distilled water throughout the experimental period; Group 2 (Quercetin-treated group): rats received quercetin at a dose of 50 mg/kg/day dissolved in 1 mL distilled water and administered orally by gavage daily for 42 consecutive days [13]; Group 3 (AlCl₃-induced AD group): rats received Al chloride (AlCl₃) at a dose of 100 mg/kg/day dissolved in 1 mL distilled water and administered orally by gavage daily for 42 days [14]; Group 4 (AlCl₃ + quercetin-treated group): rats received AlCl₃ in the same dose and regimen as Group 3, followed one hour later by quercetin at the same dose and route as Group 2 for 42 consecutive days.

At end of experiment, Memory performance was evaluated using MWMT to assess spatial learning and memory retention by recording acquisition and retention latencies.

Morris Water Maze Test [15]

The Morris Water Maze Test, a standardized assay for hippocampal-dependent spatial learning and memory, was employed to evaluate all experimental groups. The test is frequently employed to validate rodent models of neurocognitive disorders, including AD, as it differentiates between spatial (hidden platform) and non-spatial (visible platform) conditions. The unit was composed of a circular water tank (100 cm in diameter and 35 cm in height) that was filled with opaque water (18-23°C, depth 15.5 cm) and a submerged platform (4.5 cm in diameter, 1 cm below the surface). Each rat underwent four consecutive trials with 5-minute intervals, being allowed 120 seconds to locate the hidden platform and 20 seconds on the platform upon success. Acquisition latency was recorded during training, while retention latency was measured 24 hours later from a randomized starting point to assess memory consolidation. This protocol provides an integrated evaluation of spatial learning and memory retention across experimental groups.

Brain tissue preparation

At the end of the experimental period, rats that had been fasted overnight for 12 hours were anesthetized with thiopental sodium (20 mg/kg, i.p.) and then decapitated. The brains were rapidly excised, rinsed with ice-cold isotonic saline to remove residual blood, and blotted dry after the skulls were delicately opened. In order to prevent mechanical injury, the hippocampus and cerebral cortex were promptly dissected. A single portion of each tissue was divided into three sections: the first was wrapped in Al foil and stored at -80 °C for molecular analysis, the second was homogenized in cold phosphate buffer saline (50 mM, pH 7.4) for biochemical assays, and the third was fixed in 10% neutral buffered formalin for histopathological examination.

Biochemical and molecular assays

The brain tissue homogenates were used for assessment of oxidative stress and ferroptosis-related parameters.

Total iron level was determined spectrophotometrically using a commercially available colorimetric kit according to

the manufacturer's instructions supplied by BIOMED (Egypt).

For molecular assessment, total RNA was extracted from frozen brain tissues using a commercial RNA extraction kit, and RNA purity and concentration were evaluated spectrophotometrically.

The optical density at 260 nanometers and the 260/280 nanometers ratio were the two parameters utilized to assess the purity and concentration of RNA with a Nano-Drop spectrophotometer (Nano Drop Technologies, Inc., Wilmington). Subsequently, the RNA has been stored at

minus degree Celsius. Whole RNA was reverse transcribed to generate cDNA utilizing Revert Aid H Minus Reverse Transcriptase (Thermo Fisher Scientific, Waltham, Massachusetts, the United States of America) Prior to utilization, the cDNA has been stored at minus twenty degree Celsius. The relative gene expression of FPN1 has been assessed utilizing cDNA as a template in Step One Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). The reference gene used was β -Actin. The primers for both genes are recorded in Table 1.

Table 1: The primers used for qRT-PCR

Gene	Primer sequence	GenBank Accession number
<i>FPN1</i>	F: 5'- AATGGGAAGTGTGGCCTTCA -3' R: 5'- AGTTCATGGAGTCTGCACAC -3'	NM_65181
β -Actin	F: 5'- ACATCCGTAAAGACCTCTATGCC -3' R: 5'- TACTCTGCTTGCTGATCCAC -3'	NM_031144.3

FPN1: ferroportin

The $2^{-\Delta\Delta Ct}$ technique has been utilized to ascertain relative gene expression following the determination of cycle threshold (Ct) values for both housekeeping and target genes [16].

Malondialdehyde, thiobarbituric acid reactive substances (TBARS) colorimetric assay was employed to quantify lipid peroxidation. The assays supplied by Biodiagnostic; Birmingham, UK, (Cat. No: MD 25-29) were employed to determine the level in brain tissue homogenate samples.

Glutathione peroxidase 4 level in brain tissue homogenate was determined by ELISA using a commercially available kit (Catalogue number #: SRB-T-80533) obtained from SunRed Biological Technology Co., Ltd, Shanghai, China.

Reduced glutathione level was quantified using a colorimetric method based on DTNB reduction the GSH level in brain tissue homogenate was involved by Spectrophotometric assay using a commercially available kit supplied by BIOMED (Egypt).

Total protein concentration determined in tissue homogenates using bovine serum albumin as a standard by the Bradford method.

Histopathological examination

The brain specimens were fixed in 10% neutral buffered formalin, dehydrated in ethanol, clarified in xylene, and embedded in paraffin wax. A rotary microtome was employed to produce serial coronal sections with a thickness of 5 μ m. Hematoxylin and eosin (H&E) was used to evaluate the general histological architecture of the sections, while Congo red was used to detect amyloid deposition [17].

Statistical analysis

The means and standard deviations (SD) of all data were used for expression. A one-way analysis of variance (ANOVA) was run using SPSS software (version 18.0, 2011) for statistical analysis. We used Tukey's post hoc test to do numerous comparisons between groups after the fact. Statistical significance was determined when the p-value was less than 0.05 [18].

Results

Regarding the effect of Al chloride ($AlCl_3$) and quercetin on memory performance, assessment by the Morris Water Maze test showed that there were statistically significant

differences among the studied groups in both acquisition and retention latencies. A highly significant difference was detected in acquisition latency ($F = 59.79$, $p < 0.001$). The $AlCl_3$ -administered group found a significant ($p < 0.001$) rise in escape latency than the normal control group, indicating impaired learning ability. However, treatment with quercetin significantly ($p < 0.001$) reduced the escape latency than the $AlCl_3$ group. Insignificant differences were observed between the control and quercetin-treated groups. Similarly, retention latency was significantly prolonged ($p < 0.001$) in the $AlCl_3$ group than the control group, reflecting memory impairment. Quercetin co-administration significantly ($p < 0.001$) reduced retention latency than the $AlCl_3$ group, with insignificant difference than the control or quercetin-only groups.

Concerning brain iron content, there was a significant ($p < 0.001$) increase in iron levels in the $AlCl_3$ -administered group compared with the control group. The treatment with quercetin significantly ($p < 0.001$) reduced brain iron accumulation compared with the $AlCl_3$ group. Insignificant difference was detected between the control and quercetin-only groups (Fig.1), (table 2).

Regarding FPN1 mRNA expression, the relative expression level was significantly ($p < 0.001$) downregulated in the $AlCl_3$ group compared with the control group. Quercetin treatment significantly ($p < 0.001$) upregulated FPN1 expression compared with the $AlCl_3$ group, restoring its level close to that of the control group. Insignificant difference was observed between the control and quercetin-treated groups (Fig.1), (table 2).

Assessment of oxidative stress markers showed that MDA levels were significantly ($p < 0.001$) elevated in the $AlCl_3$ group than the control group. Quercetin administration significantly ($p < 0.001$) decreased MDA levels compared with the $AlCl_3$ group (Fig.1), (table 2).

Moreover, antioxidant parameters including GPX4 and GSH levels were significantly ($p < 0.001$) decreased in the $AlCl_3$ -administered group compared with the control group. Treatment with quercetin significantly ($p < 0.001$) increased GPX4 and GSH levels compared with the $AlCl_3$ group, indicating improvement of antioxidant defense status. Insignificant differences were detected between the control and quercetin-only groups (Fig.2), (table 2).

Correlation analysis revealed that brain iron content showed a significant positive correlation with MDA levels and both acquisition and retention latencies ($p < 0.001$), while it found a significant negative correlation with FPN1 expression, GPX4, and GSH levels ($p < 0.001$). Additionally, FPN1 expression demonstrated significant negative correlations with both acquisition and retention latencies and MDA levels, and significant positive correlations with GPX4 and GSH levels (table 3).

Histopathological results

Sections of hippocampus and cerebral cortex of control group I stained by hematoxylin, eosin, and Congo red stain. (A & B) showed normal histological architecture of neocortex; Intact pia mater (thin arrow), normal neuropil (*) and blood capillary. Normal granular cells with rounded pale nuclei (yellow arrow), pyramidal cells (red arrow), neuroglial cells with small dark nuclei (blue arrow) were seen. (C) revealed negative Congo red stain for amyloid plaque. (D & E) showed normal histological architecture of hippocampus formed of well-defined three layers; molecular, granule cell and polymorphic layers of cornu Ammonis. (F) hippocampus showed negative Congo red stain for amyloid plaque (Fig.3).

Sections of cerebral cortex and hippocampus of treatment only group II stained by hematoxylin, eosin, and Congo red stain. (A & B) showed normal histological architecture of neocortex; normal neuropil (*) and blood capillary. Normal granular cells with rounded pale nuclei (yellow arrow), pyramidal cells (red arrow), neuroglial cells with small dark nuclei (blue arrow) were seen. (C) revealed negative Congo red stain for amyloid plaque. (D & E) showed normal histological architecture of hippocampus formed of well-defined three layers; molecular, granule cell and polymorphic layers of cornu Ammonis. (F) hippocampus

showed negative Congo red stain for amyloid plaque (Fig.4).

Sections of cerebral cortex and hippocampus of diseased group III stained by hematoxylin, eosin, and Congo red stain. (A & B) showed small deeply stained (pyknotic) nuclei of pyramidal cells (red arrow) and neuroglial cells (blue arrow) with perinuclear halo surrounded by vacuolated neuropil (*) and dilated blood capillary in certain sites. Granular cells were normal with rounded pale-stained vesicular nuclei (yellow arrow). Homogenous eosinophilic area was seen (white arrow) and neuropil (*). (C & F) cerebral cortex revealed positive Congo red stain with distinct amyloid deposits (white arrow). (D & E) showed A photomicrograph of a section of cornu Ammonis 3 region of hippocampus of diseased group III showing well-defined three layers; polymorphic layer, decreased number of cell layers pyramidal cell and molecular layer. Most of the pyramidal neurons with shrunken deeply stained (pyknotic) nuclei (bifid white arrow) together with hemorrhage between pyramidal neurons (H) (Fig.5).

Sections of cerebral cortex and hippocampus of combined group IV stained by hematoxylin, eosin, and Congo red stain. (A & B) showed marked improvement of histological architecture of neocortex; normal neuropil (*) and blood capillary. Normal granular cells with rounded pale nuclei (yellow arrow), pyramidal cells (red arrow), neuroglial cells with small dark nuclei (blue arrow) were seen. Few glial cells had deeply stained nuclei with perinuclear halo (arrowhead). (C) revealed negative Congo red stain for amyloid plaque. (D & E) showed normal histological architecture of hippocampus formed of well-defined three layers; molecular, granule cell and polymorphic layers of cornu Ammonis. (F) Hippocampus showed negative Congo red stain for amyloid plaque (Fig.6).

Table 2: MWM performance test, Total iron, MDA, GPX4, GSH levels, and FPN 1 expression.

Parameter	group I	group II	group III	Group IV	P-value
Acquisition latency of MWM	37.9 ± 6.36	34.3 ± 3.3	87.5 ± 16 ^{ab}	43 ± 10.2 ^c	<0.001*
Retention latency of MWM	15.9 ± 3.51	15.4 ± 3.4	77.1 ± 15.5 ^{ab}	19.8 ± 4.56 ^c	<0.001*
Total iron level	7.041±0.935	8.025±1.38	16.007±1.674 ^{ab}	8.133±1.127 ^c	<0.001*
FPN 1 expression	1.05±0.093	1.09±0.057	0.49±0.06 ^{ab}	1.14±0.073 ^c	<0.001*
MDA	119.6 ± 14.49	115.1 ± 15.87	376.6 ± 31.4 ^{ab}	130.8 ± 17.51 ^c	<0.001*
GPX4	1146 ± 169.8	1255 ± 174.6	751 ± 103.8 ^{ab}	1143 ± 134.84 ^c	<0.001*
GSH	193.4 ± 14.2	207.4 ± 23.69	68.8 ± 8.76 ^{ab}	188.2 ± 14.17 ^c	<0.001*

*Statistically significant at $p \leq 0.05$. Values are expressed as mean ± SD. a: Statistically significant compared to Group I, b: Statistically significant compared to Group II, c: Statistically significant compared to Group III. Group I: (Control group), Group II: (Quercetin-treated group), Group III: (AlCl₃-administered group), Group IV: (AlCl₃ + Quercetin treated group).

Table 3: Pearson's Correlation matrix of different studied parameters among the diseased and treated groups

		Iron content (µg/gm tissue)	FPN1 mRNA (fold change)	MDA (nmol/gm tissue)	GPX4 (pg/mg tissue protein)	GSH (mg /gm tissue)	Morris Water Maze (acquisition latency)(sec)
FPN1 mRNA (fold change)	R	-0.909					
	p-value	<0.001*					
MDA (nmol/gm tissue)	R	0.956	-0.954				
	p-value	<0.001*	<0.001*				
GPX4 (pg/mg tissue protein)	R	-0.821	0.837	-0.855			
	p-value	<0.001*	<0.001*	<0.001*			
GSH (mg /gm tissue)	R	-0.930	0.961	-0.964	0.836		
	p-value	<0.001*	<0.001*	<0.001*	<0.001*		
Morris Water Maze (acquisition latency) (sec)	R	0.827	-0.855	0.896	-0.8	-0.873	
	p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	
Morris Water Maze (retention latency) (sec)	R	0.923	-0.893	0.946	-0.849	-0.938	0.906
	p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Statistically significant at $p \leq 0.05$.

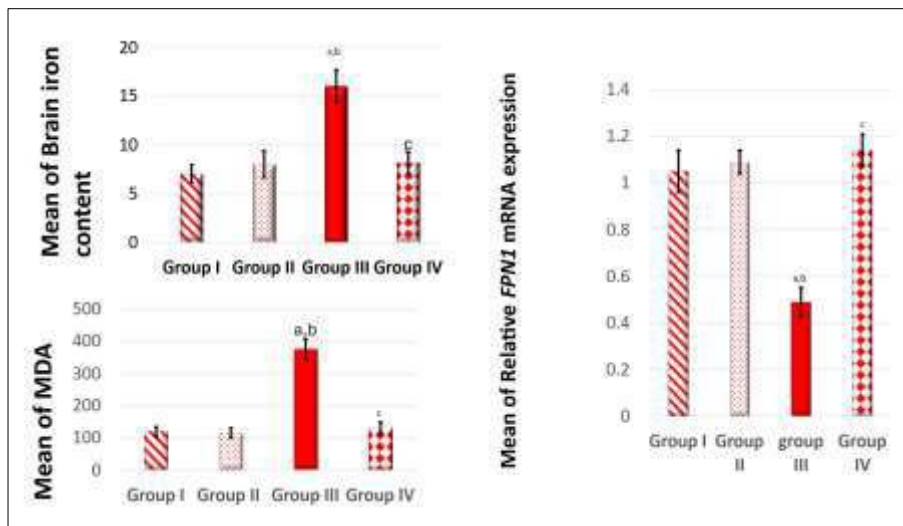


Fig 1: comparison among the studied groups as regards relative *FPN1* mRNA expression (fold change), brain iron content ($\mu\text{g/gm}$ tissue), MDA level (nmol/gm tissue) in brain tissue.

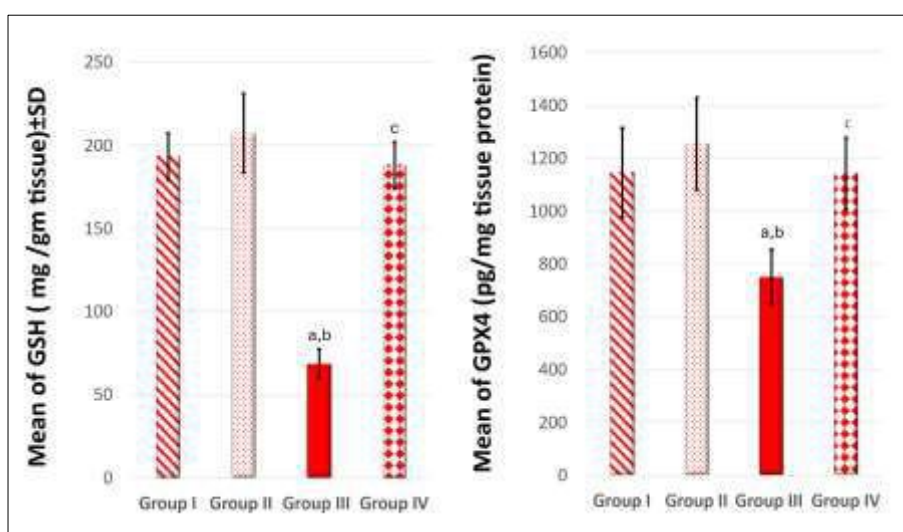


Fig 2: comparison among the studied groups as regards GPX4 level (pg/mg tissue protein) and GSH level (mg/gm tissue) in brain tissue

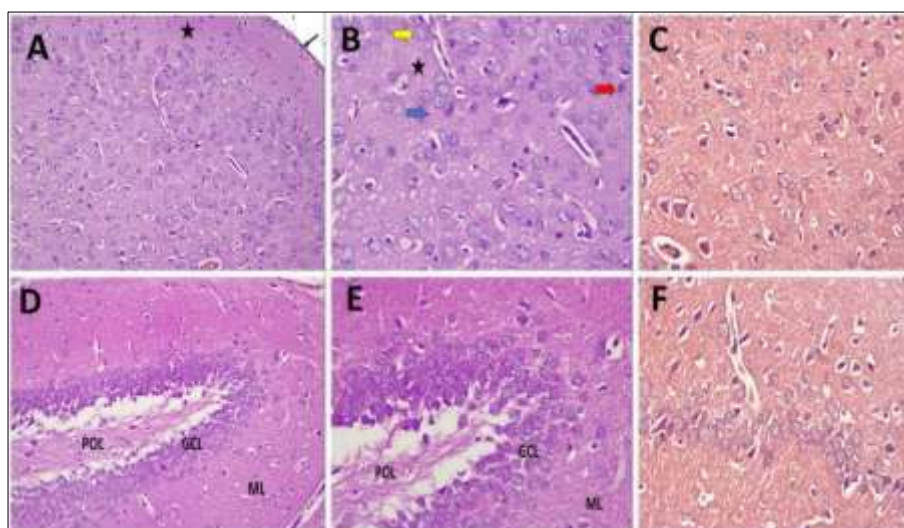


Fig 3: Histopathological sections of control group (Group 1)

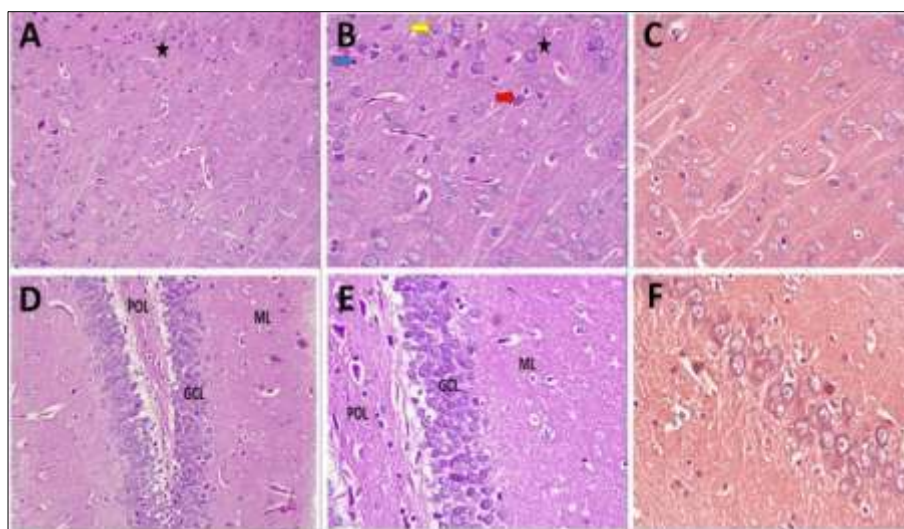


Fig 4: Histopathological sections of the quercetin-treated group (Group II)

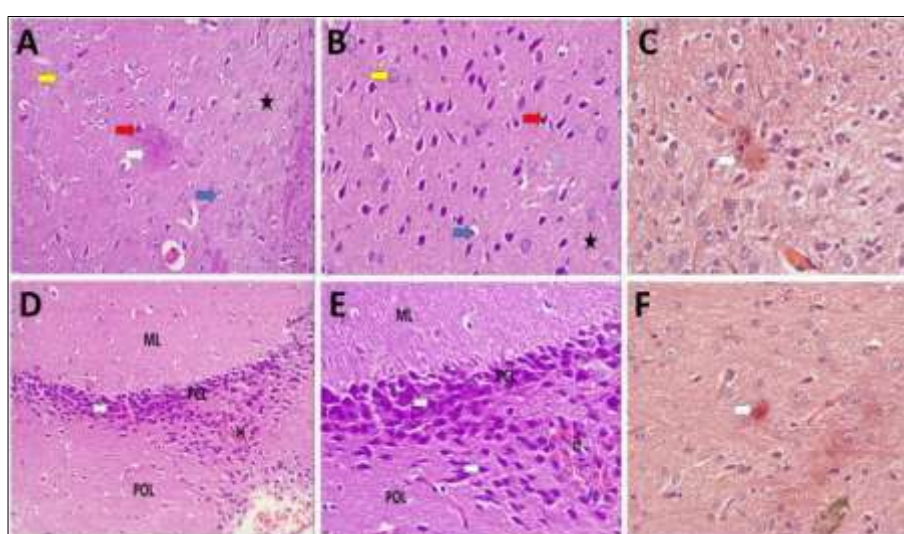


Fig 5: Histopathological sections of the AlCl_3 administered group (Group III)

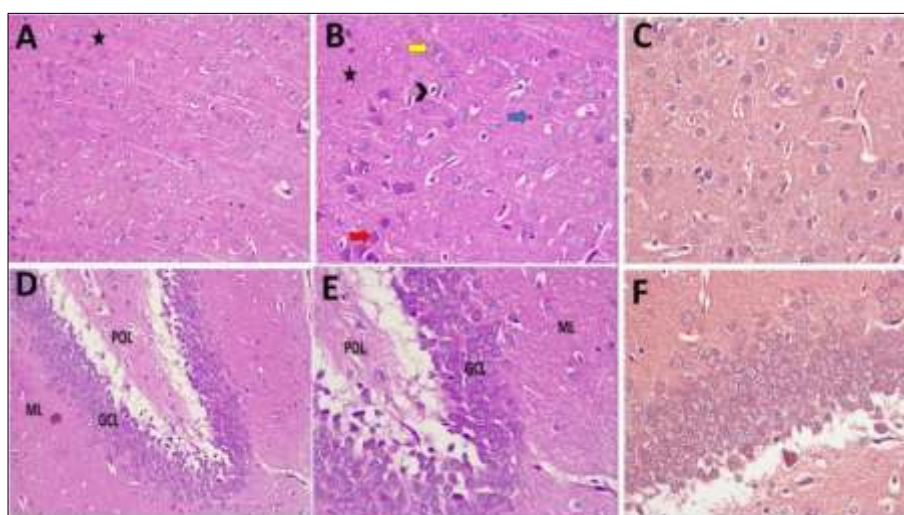


Fig 6: Histopathological sections of the combined treatment group (Group IV)

Discussion

AD is a neurodegenerative disorder that predominantly affects elderly individuals. And is characterized by gradual impairment in memory, cognitive function, and behavioral abilities [19]. The precise mechanisms and cause of AD have yet to be determined, as indicated by the data collected from

various studies. It has been proposed that the pathogenesis of AD is significantly influenced by the imbalance of iron metabolism and the increase in brain iron that is associated with increased ROS generation [20-22].

The ferroptosis is a novel form of programmed cell death that is distinguished by the accumulation of ROS, iron

excess, and the aggregation of lipid peroxides. The primary mechanisms of ferroptosis development are the accumulation of lipid peroxides, iron metabolism disorders, and an imbalance in antioxidant capacity. Disrupted function of the iron homeostasis-related protein expression such as FPN1 protein, GSH and GPX4 signaling increases the progression of ROS production and induces ferroptosis. The accumulation of MDA, a marker of lipid peroxidation, further confirms this oxidative injury. It has been revealed that oxidative stress is a prominent feature of ferroptosis induced by heavy metal exposure [23].

Therefore, the aim of the present study is to determine the effect of quercetin on FPN1-mediated ferroptosis in experimentally induced Alzheimer's like disease.

AD can be experimentally demonstrated using various animal models. Among these, the AlCl_3 -induced model is widely recognized for its ability to replicate key features of AD. Chronic exposure to Al, a known neurotoxin, has been effectively used in various studies as well-established model. This is due to the fact that AlCl_3 is highly bioavailable and can be readily administered orally [24-26].

Neurobehavioral assessment using the MWM test was employed to evaluate the cognitive effects of chronic AlCl_3 administration on memory and spatial learning [27]. The MWM test is a behavioral task that is frequently employed to evaluate hippocampal spatial memory abnormalities [28].

In the present study, AlCl_3 administration significantly increased escape latency time in the AlCl_3 -treated group than controls, indicating impaired cognitive function. These results are involved by Agrawal *et al.*, 2025 [29] who utilized the MWM test to demonstrate hippocampal-dependent memory deficits characteristic of AD pathology. The cognitive impairment observed can be attributed to AlCl_3 -induced neurotoxicity, which promotes ferroptosis, oxidative stress, neuronal apoptosis, and cholinergic dysfunction, all contributing to learning and memory decline [30].

Interestingly in the present study, co-treatment with quercetin significantly reduced escape latency than the AlCl_3 -only group, suggesting a neuroprotective role of quercetin against AlCl_3 -induced cognitive deficits. This finding aligns with the results from Abu-Elfotuh *et al.*, 2025 [30], who reported improved spatial memory in flavonoid-treated neurodegenerative models. The underlying biological mechanism may involve quercetin's potent antioxidant activity, which mitigates oxidative damage and preserves mitochondrial integrity.

Building upon the existing evidence that genes such as FPN1 (the sole known iron exporter) regulate iron metabolism [31], our study observed significant downregulation of the *FPN1* gene in the hippocampal tissue of the AD model group. This downregulation in gene expression led to decreased FPN1 protein levels in hippocampal cells and subsequently increased the intracellular LIP. The potential iron excess could facilitate lipid peroxidation by generating ROS through the Fenton reaction, which in turn would stimulate hippocampal neurodegeneration. This is in harmony with previous finding of Yang, *et al.*, 2022 [32] who stated that *FPN1* down-regulation played a pivotal role in iron dyshomeostasis in AD. Their findings showed that reduced *FPN1* expression led to impaired neuronal iron efflux, resulting in intracellular iron accumulation, oxidative injury, and enhanced $\text{A}\beta$ production and tau hyperphosphorylation.

They further demonstrated that excess iron promotes lipid peroxidation and ferroptosis, accelerating neuronal degeneration.

In this study, treatment with quercetin upregulated *FPN1* gene expression than the AD model group. This might be due to alleviation of iron accumulation, inhibition of ferroptosis and control neurodegenerative disease.

The present results also supported by Hammad *et al.*, 2023 [33] who reported that quercetin modulates iron homeostasis by upregulating the expression of *FPN1*. It also enhances *FPN1* mRNA and protein levels in various cell types, including macrophages and hepatocytes, leading to increased iron efflux and reduced intracellular iron accumulation. This regulation appears to be mediated, at least in part, through activation of the NRF2 signaling pathway. NRF2 promotes the transcription of target genes, such as SLC40A1 (encoding FPN1), by binding to the antioxidant response elements (AREs) in the promoter region.

Additionally, quercetin's iron-chelating capacity contributes to the reduction of LIP, which may further support *FPN1* upregulation as part of the cellular adaptive response to restore iron balance. Together, these mechanisms highlight the potential therapeutic relevance of quercetin in the management of disorders associated with iron overload and oxidative stress [33].

In the present study, it was observed that the administration of AlCl_3 resulted in a state of oxidative stress in the hippocampus and cortical tissues. This was demonstrated by the imbalanced significant accumulation of MDA levels and the depletion of the antioxidant GSH and GPX4 levels in AlCl_3 -induced AD rats compared to the normal control rats. These processes generate a pro-oxidant environment that predisposes to neuronal cell demise.

Our study also revealed that treatment with quercetin significantly reduced MDA levels and restored GSH and GPX4 levels. Cruz-Gregorio & Aranda-Rivera, 2023 [34] highlighted the potential effect of quercetin as an anti-ferroptotic agent through its modulatory effects on the key oxidative stress markers. Quercetin has been shown to significantly reduce MDA levels while concurrently increasing the levels of GSH and GPX4, both of which are essential in maintaining redox balance and preventing ferroptotic cell death. These findings support the role of quercetin as a therapeutic agent targeting ferroptosis pathways in AD as a neuroprotective agent.

Interestingly, our study demonstrated that brain iron content was significantly positively correlated with MDA levels and both acquisition and retention latencies, while significantly negatively correlated with *FPN1* mRNA expression, GPX4, and GSH levels, indicating that iron overload promotes oxidative stress and ferroptosis-mediated cognitive impairment. *FPN1* expression showed significant positive correlations with GPX4 and GSH and significant negative correlations with MDA and behavioral latencies, supporting its neuroprotective role. These findings are supported by Ge *et al.* 2026 [35], who reported that iron accumulation drives lipid peroxidation and increases MDA, while depletion of GSH impairs GPX4 activity, enhancing ferroptosis and neuronal damage. Thus, both our data and previous study highlight that maintaining iron homeostasis and antioxidant defenses is critical for protecting against ferroptosis-induced cognitive decline.

To clarify the changes occurred at the cellular level by AlCl_3 administration, the histopathological pictures obtained in the current study either by H&E or Congo red stains were examined under light microscope and polarized light; respectively revealed that AD dementia was successfully induced by AlCl_3 administration in both the hippocampal and the cortical tissues. This was demonstrated by the extracellular accumulation of senile plaques constituted of $\text{A}\beta$ in both the hippocampal and cortical tissues of the AlCl_3 -induced AD group, which was in contrast to all other groups.

The cerebral cortex of AlCl_3 -induced AD rats showed numerous neuropathological alterations. Neurons appeared shrunken, atrophied, and degenerated, exhibiting pyknotic nuclei, perinuclear halos, and the presence of NFTs, all of which indicate cellular necrosis and advanced neurodegeneration. Similarly, neuronal degeneration was evident in the hippocampus, accompanied by amyloid deposition and tangles, highlighting structural damage in regions essential for memory and learning. The Congo red stain was employed to evaluate the extent of $\text{A}\beta$ plaque deposition in the brain. The results of the study indicated that the cortex and hippocampus of disease control animals exhibited multifocal moderate $\text{A}\beta$ deposition, which was not present in the normal control group.

On the other side, we noticed that the toxic effect of AlCl_3 on brain cells was amended by the ingestion of quercetin. Our findings demonstrated that the degenerative and structural changes that happened in the brain by AlCl_3 were improved after quercetin use except for some changes that have been noticed. Notably, neuroglial cells with small, dark nuclei and a few glial cells exhibiting deeply stained nuclei surrounded by a perinuclear halo were detected. These findings are consistent with Micheal *et al.*, 2025^[36] that declared that AlCl_3 causes neuronal loss in the hippocampal regions while quercetin restores normal histoarchitecture and improves neuronal integrity.

Moreover, histopathological examination in the present study revealed that quercetin treatment was associated with a marked preservation of normal neuronal architecture in both hippocampal and cerebral tissues, as evidenced by an increased proportion of morphologically intact neurons and the absence of $\text{A}\beta$ plaque deposition or NFTs formation.

Based on all previous data, AlCl_3 posed risks to brain health by inducing several harmful histopathological and biochemical changes in the adult male albino rats brains. However, most of these changes were alleviated with the concurrent administration of quercetin.

Conclusion

The present study demonstrates that ferroptosis plays a central pathogenic role in AlCl_3 -induced neurotoxicity, characterized by iron overload, enhanced lipid peroxidation, and impaired antioxidant defenses. Treatment with quercetin markedly mitigated these effects, improving biochemical indices and brain histopathology through coordinated mechanisms, including FPN1-mediated iron regulation, reinforcement of the GPX4-GSH antioxidant system, suppression of oxidative stress and neuroinflammation, and iron-chelating activity. These findings highlight the therapeutic potential of targeting ferroptosis and position quercetin as a promising neuroprotective agent against iron-driven neurodegenerative processes.

Hypothesis for further work and recommendation

Excessive intake of AlCl_3 -containing products should be avoided, and consumption of quercetin-rich foods could be considered as a protective measure. Further studies with quercetin administered prior to AlCl_3 exposure are needed to evaluate its prophylactic role against neurotoxicity. Additional research is required to elucidate the molecular mechanisms of quercetin in ferroptosis, including its interactions with FPN1, GPX4, and related antioxidant pathways. Further exploration of ferroptosis-related pathways is warranted to clarify their contribution to the pathogenesis of AD. Clinical studies are required to assess the quercetin potential application as a therapeutic agent for AD in humans. Further investigations are needed to evaluate other therapeutic strategies for AD.

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