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Potential Protective Role of Thymoquinone on Experimentally-induced Alzheimer Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MFZ designed the study, managed the literature searches and wrote the first draft of the manuscript. Authors MFZ and MAB managed the analyses of the study. Author MAB performed the statistical analysis, wrote the protocol and wrote the final draft of the manuscript. Author GSAEA prepared the histology plates and chose the AD model. Author ASA reviewed and prepared all the pharmaceutical aspects in the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the neuroprotective potential of thymoquinine (TQ) on the oxidative stress status of the brain in aluminum chloride ($AlCl_3$)-induced AD in rats.
Study Design: Animal research study.
Place and Duration of Study: King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Saudi Arabia, April 2018-June 2019.
Methodology: Thirty adult male Sprague Dawley albino rats were randomly divided into 3 groups. Group 1 (Control). Group 2 (AD): supplemented orally with $AlCl_3$ (17 mg/kg/day) for 4 weeks. Group 3 (TQ/AD): supplemented concomitantly with oral TQ (10 mg/kg/day) and $AlCl_3$ (17 mg/kg/day) for 4 weeks. At the end of the experiment, spatial working memory was assessed using the Y-maze

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spontaneous alternation test. Then, serum levels of malondialdehyde (MDA) and glutathione peroxidase enzyme (GPX) were assessed. Then, the rats were sacrificed, and the brain was isolated, and a light microscopic examination of the hippocampus was performed. Finally, the brain homogenate content of Aβ, tau protein and acetylcholine were biochemically determined.

Results: The AD group showed a significant decreased in the spontaneous alteration performance (SAP %) in Y-maze. Also, in the AD group, serum MDA, Aβ and tau protein were significantly increased with a significant decrease of serum GPX and acetylcholine. Examination of H&E-stained sections of the hippocampus of the AD group revealed decreased thickness and disorganization of the pyramidal cell layer of CA1 and CA3 where many pyramidal cells lost their triangular shape and appeared shrunken. The molecular and polymorphic layers showed increased glial cells and congested blood capillaries. The dentate gyrus showed marked disorganization with some cell loss. Co-administration of TQ with $AlCl_3$ in TQ/AD group, improved SAP % and significantly decreased serum MDA, Aβ, tau protein. It also increased serum GPX and acetylcholine levels. Also, TQ partially attenuated the histopathological changes in the hippocampus.

Conclusion: TQ could mitigate the oxidative stress markers, neurodegenerative indices and histopathological alteration encountered in AD that all reflected on improving the cognitive behavior. This may implement TQ as an adjuvant medical strategy in ameliorating AD.

Keywords: Alzheimer's disease; AlCl3, thymoquinone; oxidative stress; Aβ; tau protein; acetylcholine; Y-maze.

1. INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is a chronic neurodegenerative disorder characterized by a progressive loss of memory and impairment of cognitive functions in the elderly [1]. AD constitutes a global cognitive decline that involves memory, orientation, judgment, and reasoning which eventually affects daily living activities [2,3]. About 35.6 million people around the world were diagnosed as AD in 2010. This number is expected to be doubled by 2030 [4]. AD is the fifth leading cause of death above the age of 65 years [5].

The pathogenesis of AD is multi-factorial. It is linked to the deficiency of the neurotransmitter acetylcholine and loss of cholinergic function in the central nervous system, extracellular deposits of amyloid-beta peptides (Aβ) in senile plaques and intraneuronal neurofibrillary tangles (NFTs) [6,7]. Other mechanisms associated with AD include oxidative stress, inflammation and other possible less defined mechanisms [8,9].

Currently, there is no cure for AD and the available successful drugs can only provide symptomatic relief for a short period, producing modest symptomatic improvements in mild-to-moderate stages of AD [10]. These drugs have several adverse effects due to their nonselective action on a variety of tissues both centrally and peripherally [11]. Hence, the search for alternative or adjuvant anti-amnestic therapies is a priority.

There is an ongoing worldwide effort to find better methods to prevent AD, delay its onset and progression as well as treating it. Medicinal plants, particularly *Nigella sativa* (*N. Sativa*), have been used since old times in the prevention and/or treatment of various neurological disorders including memory and cognitive disorders. *N. Sativa* was reported to act on multi-level molecular mechanisms in such diseases. Most of the therapeutic properties of this plant are thought to be related to the phenolic major bioactive component thymoquinone (TQ) [12].

Several studies demonstrated anti-inflammatory [13] and antioxidant [14] effects of TQ. It might as well prevent amyloid-beta (Aβ) aggregation and decreases its neurotoxicity in hippocampal and cortical neurons. It was also found that TQ could prevent neuronal degeneration and slows the decline of cognitive ability [15].

This work aimed to investigate the potential protective effect of TQ on behavioral, biochemical and histological alterations in $AlCl_3$-induced Alzheimer's-like rat model.

2. MATERIALS AND METHODS

2.1 Chemicals

2.1.1 $AlCl_3$

(Sigma-Aldrich Co., USA) was provided as a bottle containing a powder of 500 mg. A daily fresh solution of $AlCl_3$ was prepared by

dissolving 100 mg powder in 100 ml distilled water.

2.1.2 TQ

(Sigma-Aldrich Co., USA) was provided as solid crystals (purity 99%) contained bottle (5 gm). A weekly stock solution was made by dissolving 250 mg in 100 ml of corn oil. The stock was stored at 8°C.

2.2 Experimental Animals

Thirty adult male Sprague Dawley albino rats of approximately 250±25-gram body weight (BW) were used in this study. The rats were obtained from the animal house of King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. The rats were kept acclimatizing for one week before the start of the experiments. The animals were housed in well-ventilated cages at a temperature of 23±2°C, under the natural 12-hour day/night cycle with free access to a standard commercial rodent chaw and water ad libitum throughout the whole study period.

2.3 Experimental Design

The rats were weighed and randomly divided into 3 groups of 10 rats each:

2.3.1 Group 1 (Control group)

The rats of this group were orally administered via gastric gavage with 1 ml/kg/day of corn oil (the chosen vehicle of TQ) for 4 weeks.

2.3.2 Group 2 (AD group)

In this group, the Alzheimer's-like rat model was experimentally induced by oral administration of $AlCl_3$, (17 mg/kg/day) dissolved in distilled water for 4 weeks [16]. It is to be noted that the chosen dose of $AlCl_3$ in this study was perpetually reported to be the minimal dose for producing a detrimental biological effect on the tissues of rodents in short term use [17].

2.3.3 Group 3 (TQ/AD group)

The rats of this group were supplemented concomitantly with both oral TQ at a dose of 10 mg/kg/day dissolved in corn oil [18] and oral $AlCl_3$ (17 mg/kg/day) dissolved in distilled water for 4 weeks.

2.4 Induction of AD in the Rats

In this study, the AD-like model was experimentally induced by oral gavage administration of $AlCl_3$ dissolved in distilled water (17 mg/kg/day) for 4 weeks [16]. The development of an AD-like state was considered when the spatial recognition and working memory was impaired upon eliciting the Y maze cognitive test [19].

2.5 Experimental Protocol

At the end of the schedule for the experimental design and following overnight fasting, blood samples were withdrawn from the retro-orbital venous sinuses in non-heparinized Eppendorf tubes. The blood samples were left to clot in a water bath at room temperature, followed by their centrifugation at 3000 p.m. for 15 minutes. The clear supernatant serum was separated, frozen and stored at -20°C till further biochemical analysis of serum malondialdehyde (MDA) and serum glutathione peroxidase enzyme (GPX).

Afterward, the rats were weighed and anesthetized using phenobarbital (40 mg/kg) then sacrificed by cervical dislocation followed by decapitation [20]. Then the whole brain was removed, and the hippocampus was isolated and impregnated in neutral buffered formalin. The harvested brain was immediately frozen at -80°C until homogenized for measuring Aβ, tau proteins and acetylcholine.

2.6 Behavioral Study

2.6.1 Y-maze spontaneous alternation test

The Y-maze test was performed as previously described by Wall and Messier [21]. The maze was made of painted wood (to eliminate the spatial orientation visual cues) and has 3 identical arms, 40 cm long, 35 cm high and 12 cm wide, positioned at equal angles and labeled A, B, and C. The rats were placed at the end of arm A and allowed to move freely through the maze during a 5-min timed session. Spontaneous alternation was visually monitored to observe the pattern of rat entry into the maze's arms. An arm entry was complete when the hind paws of the rat have been entirety inside that arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets (i.e., ABC, BCA…). The maze was thoroughly cleaned with alcohol between each behavior task.

2.6.2 Behavioral analysis

In this study, total arm entries and spontaneous alternation performance (SAP) scores were

monitored to calculate spontaneous alternation percentage (SAP %) (The percentage of the correct triad). These three spatial working memory-indicator parameters were assessed using the Y-maze spontaneous alternation test as described by Wall and Messier [21] and Paul et al. [19]. SAP% was calculated using the equation:

SAP (%) = [(number of alternations)/(total arm entries−2)] × 100

2.7 Biochemical Analysis

Serum MDA (nmol/ml): was measured by the thiobarbituric acid colorimetric method using the MDA assay kit (MDA, Cell Biolabs, USA, Catalog No., STA-330) [22].

Serum GpX (u/ml): Was measured colorimetrically using a commercial assay kit [23].

Aβ in the brain tissue (pg/mg): Was measured by RT.qPCR [24].

Tau protein in the brain tissue (ng/mg): Was measured by RT.qPCR [25].

Acetylcholine is the brain tissue (μmol/mg): Was measured by the colorimetric method using a choline/acetylcholine assay kit (Acetylcholine, BioVision Inc., California, USA) [26].

2.8 Histological Study

The formaldehyde-impregnated brain hemisphere was dehydrated using ascending grades of ethanol (70% for 4 h, 90% for 1 h and 100% for 1 h) then cleaned in 3 changes of xylene and embedded in paraffin wax. Thereafter, from the formed paraffin blocks, sagittal sections of 5 μm thicknesses of the brain were cut with a microtome (Leica RM 2025, Germany), then mounted on glass slides and stained with the Hematoxylin and Eosin (H&E) stain for routine histological examination of the hippocampus [27]. Finally, histological sections were viewed, and representative photo-micrographs were taken using Olympus BX41 research optical photomicroscope equipped with an Olympus DP25 digital camera, at Anatomy Department, Faculty of Medicine, King Abdulaziz University.

2.9 Statistical Study

In the present study, the data were analyzed using the statistical package for the social sciences program, version 23 (SPSS Inc., Chicago, Illinois, USA). The results were expressed as mean±standard deviation (Mean±SD). An unpaired t-test was used to compare means between every two independent groups. The significance of differences between groups was determined by one-way analysis of variance (ANOVA) followed by posthoc Tukey test. P-value ≤ 0.05 was considered statistically significant [28].

3. RESULTS AND DISCUSSION

3.1 BEHAVIORAL RESULTS

The behavioral study assessment showed the SAP % at the y maze in AD group was 54.00±7.00% which was statistically significant lower ($P<0.001$) than the corresponding mean value in the control group 72.00±10.00%. On the other hand, the mean value of SAP% in TQ/AD group was 71.00±10.00% which was statistically significant higher ($P<0.001$) than AD group (Fig. 1).

3.2 Biochemical Results

The mean value of serum MDA in AD group was 55.22±9.75 nmol/ml which was statistically significant higher ($P<0.0001$) than the corresponding mean value in the control group 8.77±2.00, On the other hand, the mean value of serum MDA in TQ/AD group was 21.28±4.00 nmol/ml which was statistically significant ($P<0.0001$) lower than AD group (Table. 1) & (Fig. 2).

The mean value of serum GPX in the AD group was 53.36±8.32 u/mg which was statistically significantly lower ($P<0.0001$) than the corresponding mean value in the control group 101.04±15.12 u/mg. On the other hand, the mean value of serum GPX in the TQ/AD group was 91.45±14.06 u/mg which was statistically significant ($P<0.0001$) higher than the AD group (Table. 1) & (Fig. 3).

The mean value of Aβ in the brain tissue in the AD group was 11.44±1.68 pg/mg which was statistically significant higher ($P<0.0001$) than the corresponding mean value in the control group 1.73±0.39 pg/mg. On the other hand, the mean value of Aβ in the TQ/AD group was 4.59±1.13 pg/mg which was statistically significant ($P<0.0001$) lower than the AD group (Table. 1) & (Fig. 4).

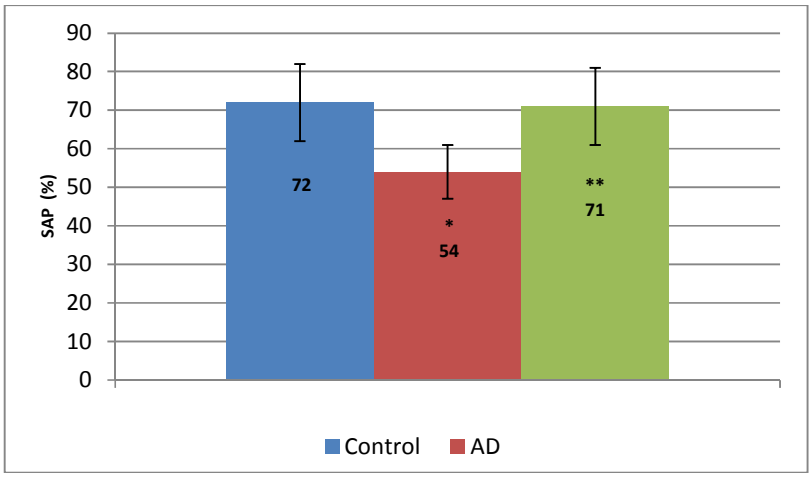

Fig. 1. Y-maze spontaneous alteration performance (SAP%) in all groups (Mean±SD)

*The number of rats in each group was 10. AD: Alzheimer's group. TQ/AD: thymoquinone/Alzheimer group. Mean±SD (mean±standard deviation). * Significant (P<0.05) compared with the control group. ** Significant (P<0.05) compared with the AD group.*

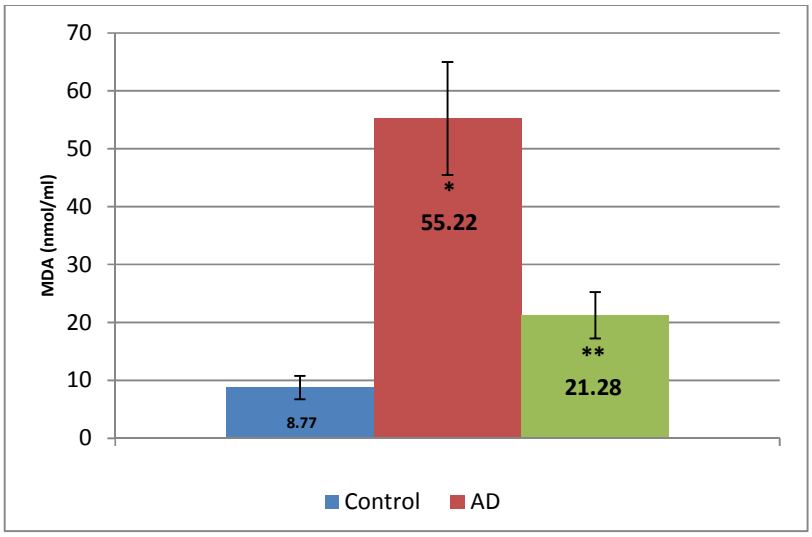

Fig. 2. Serum MDA in nmol/ml of all groups (Mean±SD)

*The number of rats in each group was 10. AD: Alzheimer's group. TQ/AD: thymoquinone/Alzheimer group. Mean±SD (mean±standard deviation). * Significant (P<0.05) compared with the control group. ** Significant (P<0.05) compared with the AD group.*

Table 1. Biochemical parameters in Control, AD and TQ/AD groups (Mean±SD)

Variable	Control	AD	TQ/AD	P1	P2
MDA (nmol/ml)	8.77±2.00	55.22±9.75	21.28±4.00	< 0.0001	< 0.0001
GPX (u/mg)	101.04±15.12	53.36±8.32	91.45±14.06	< 0.0001	< 0.0001
Aβ (pg/mg)	1.73±0.39	11.44±1.68	4.59±1.13	< 0.0001	< 0.001
Tau (ng/mg)	1.17±0.26	11.48±2.01	5.45±1.17	< 0.0001	< 0.0001
Acetylcholine (μmol/mg)	40.78±5.24	18.93±3.83	32.41±5.23	< 0.0001	< 0.0001

The number of rats in each group was 10. MDA: Serum malondialdehyde, GPX: Serum glutathione peroxidase, Aβ: amyloid beta, tau protein, Ach: acetylcholine, AD: Alzheimer group. TQ/AD: thymoquinone/Alzheimer group. Mean±SD (mean±standard deviation).P1: comparison between the control group and the AD group. P2: comparison between AD group and TQ/AD group.

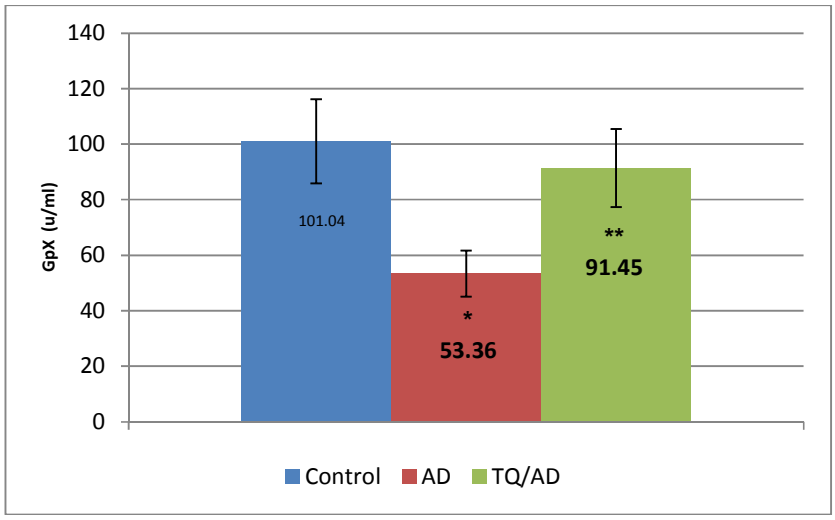

Fig. 3. Serum GPX in u/mg of all groups (Mean±SD)

The number of rats in each group was 10. AD: Alzheimer's group. TQ/AD: thymoquinone/Alzheimer group. Mean±SD (mean±standard deviation).
** Significant (P<0.05) compared with the control group.*
*** Significant (P<0.05) compared with the AD group.*

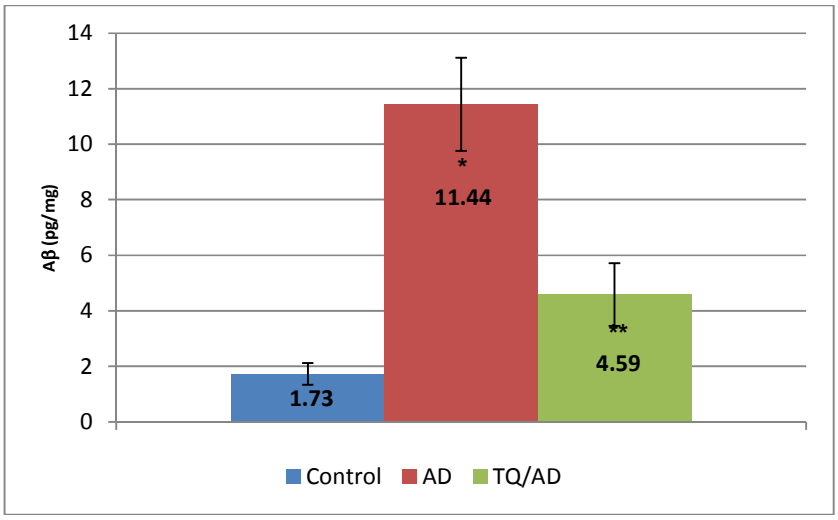

Fig. 4. Aβ in the brain homogenate in pg/mg of all groups (Mean±SD)

The number of rats in each group was 10. AD: Alzheimer's group. TQ/AD: thymoquinone/Alzheimer group. Mean±SD (mean±standard deviation). Significant (P<0.05) compared with the control group.*
*** Significant (P<0.05) compared with the AD group.*

The mean value of tau protein in the brain tissue in the AD group was 11.48±2.01 ng/mg which was statistically significantly higher (P<0.0001) than the corresponding mean value in the control group 1.17±0.26 ng/mg. On the other hand, the mean value of tau protein in the TQ/AD group was 5.45±1.17 ng/mg which was statistically significant (P<0.0001) lower than the AD group (Table. 1) & (Fig. 5).

The mean value of Ach in the brain tissue in the AD group was 18.93±3.83 μmol/mg which was statistically significantly lower (P<0.0001) than the corresponding mean value in the control group 40.78±5.24 μmol/mg. On the other hand, the mean value of Ach in the TQ/AD group was 32.41±5.23 μmol/mg which was statistically significant (P<0.0001) higher than the AD group (Table. 1) & (Fig. 6).

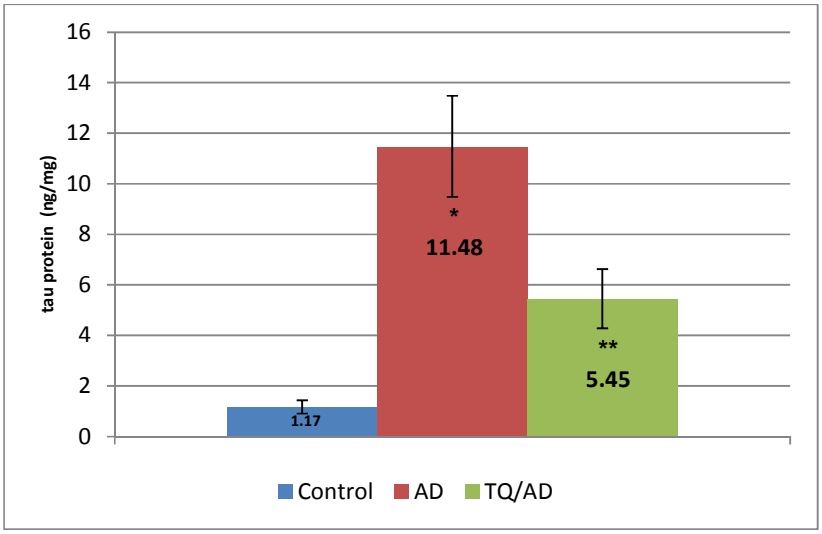

Fig. 5. Tau protein in the brain homogenate in ng/mg of all groups (Mean±SD)

The number of rats in each group was 10. AD: Alzheimer's group. TQ/AD: thymoquinone/Alzheimer group. Mean±SD (mean±standard deviation). Significant (P<0.05) compared with the control group. ** Significant (P<0.05) compared with the AD group.*

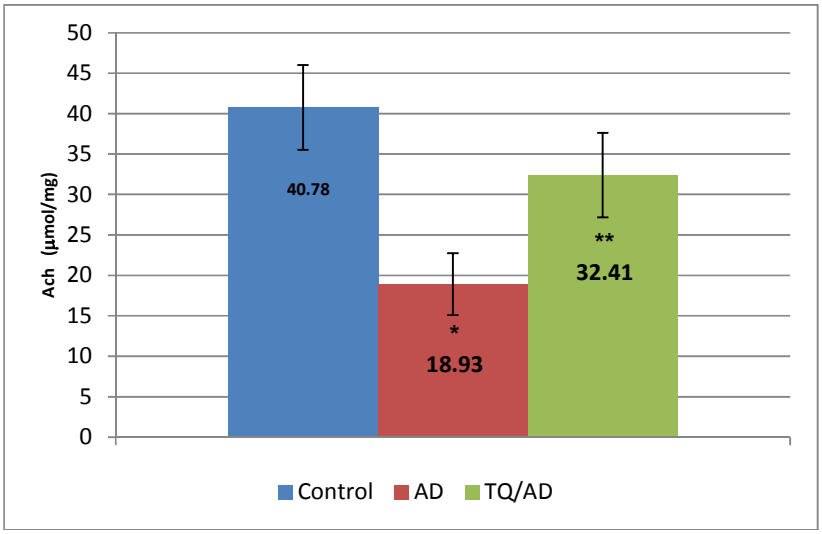

Fig. 6. Acetylcholine in the brain homogenate in µmol/mg of all groups (Mean±SD).

*The number of rats in each group was 10. AD: Alzheimer's group. TQ/AD: thymoquinone/Alzheimer group. Mean±SD (mean±standard deviation). * Significant (P<0.05) compared with the control group. ** Significant (P<0.05) compared with AD group*

3.3 Histological Results

3.3.1 Control group

Examination of H&E-stained sections of the hippocampus of this group revealed the normal histological structure of the hippocampus. It is formed of the hippocampus proper and the dentate gyrus (DG) that appeared as a dark stained C-shaped structure enclosing the end of the hippocampus proper (Fig. 7A). The hippocampus proper appeared as C-shaped formation Cornus Amomonis (CA) which is arranged in three regions: CA1, CA2, and CA3. Each of these regions is formed of three cellular layers: superficial molecular, middle pyramidal and inner polymorphic layers. The main cellular layer among them is the pyramidal layer (Fig. 7A). CA1 region is formed of the three usual layers in which the pyramidal layer appeared

most prominent and is composed of about 4-5 compact layers of small pyramidal cells that showed vesicular nuclei and pale basophilic cytoplasm (Fig. 7B). Also, CA3 showed the three usual layers, however, the pyramidal layer is composed of a zone of less densely packed large pyramidal cells with vesicular nuclei and pale basophilic cytoplasm (Fig. 7C). In CA1 and CA3, both the molecular and the polymorphic layers were relatively cell-free layers. They contained axons and dendrites (cell processes) of neuronal and glial cells, few scattered glial cells (astrocytes) and blood capillaries that all settled on the eosinophilic background (Fig. 7C). DG appeared formed of three layers: molecular, granular and polymorphic. The granular layer constituents the principle cellular layer and appeared having densely packed small granular cells. The molecular layer contained dendrites of the granular cells. The polymorphic layer showed few nongranular cells and the axons of the granular cell (Fig. 7D).

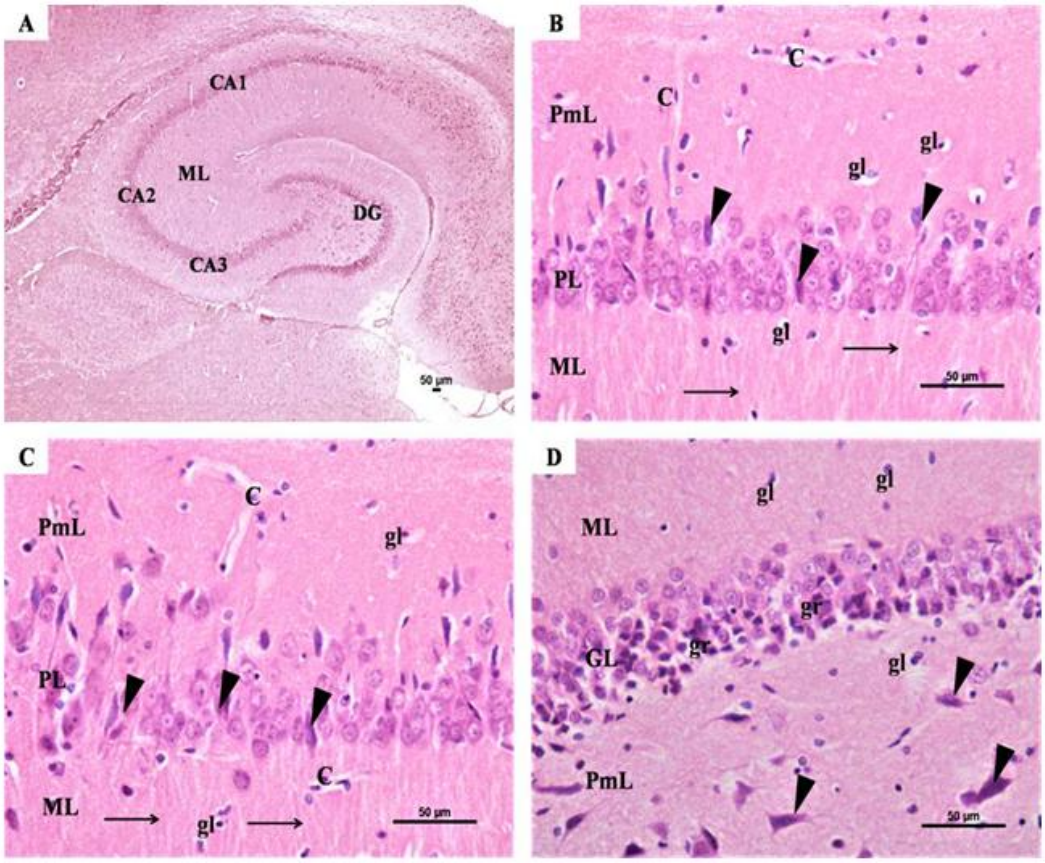

Fig. 7. Photomicrographs of the hippocampus from the control group: [A] Showing the C-shaped structure of the hippocampus in the form of the three regions of Cornu Ammonis (CA1, CA2, CA3) and the dentate gyrus. [B] Showing the three layers of CA1: the molecular layer (ML) with apparent processes of pyramidal cells (arrows), pyramidal layer (PL), which is formed of many compact layers of small pyramidal cells containing large vesicular nuclei and pale basophilic cytoplasm (arrowhead) and polymorphic layers (PmL) with few glial cells (gl) and some capillaries (C). [C] Showing the three layers of CA3: the molecular layer (ML) with neuronal processes of pyramidal cells (arrows), the pyramidal layer (PL) which is formed of numerous large pyramidal cells with large vesicular prominent nuclei and pale basophilic cytoplasm (arrowheads) and polymorphic layer (PmL) with glial cells (gl) and some capillaries (C). [D] Showing the three layers of the dentate gyrus: molecular layer (ML) with some glial cells (gl), granular layer (GL) that contained densely packed granular cells (gr) with dark nuclei and polymorphic layer (PmL) which contained some pyramidal cells (arrowhead).
A (H & E X 40) and B, C, D (H&E × 400).

3.3.2 AD group

Examination of H&E-stained sections of the hippocampus of this group demonstrated some obvious histopathological changes in the hippocampus proper and DG. In CA1 and CA3 the pyramidal cell layer appeared shrunken. Their pyramidal cells appeared disorganized with small condensed nuclei and dark cytoplasm. Some of these cells lost their pyramidal shape and some appeared shrunken with pericellular hollos around them (Fig. 8 A & 8B).

The molecular and polymorphic layers showed increased glial cells (astrocytes) and dilated congested blood capillaries (Fig. 8B). DG showed marked disorganization of their cell layer with some cell loss. The granular layer appeared shrunken with degenerated granular cells that exhibited vacuolation and dark condensed nuclei (Fig. 8C).

3.3.3 TQ/AD group

Examination of H&E-stained sections of the hippocampus of this group revealed less prominent histological changes when compared to an AD group. CA1 and CA3, displayed the relatively apparent normal thickness of the pyramidal layer (Fig. 9A&9B). Most of the small pyramidal cells of CA1 and large pyramidal cells of CA3 appeared preserved with vesicular nuclei and pale basophilic cytoplasm. However, there are some cells with dark condensed nuclei (Fig. 9B). The molecular and polymorphic layers contained scanty normal glial cells (astrocytes) and blood capillaries (Fig. 9B). The histological architecture of DG was mostly preserved, where its granular cells were compactly arranged with rounded pale vesicular nuclei (Fig. 9C).

4. DISCUSSION

AD is a major cognitive disease of the brain that, to date, has no settled underlying mechanism(s) and its successful treatment remains one of the big challenges in the field of neurology. Hence it continues to be associated with a high rate of morbidity and mortality.

The results of this study showed a significant decrease in SAP% in the AD group compared to the control group. This finding was in agreement with Pagnier et al. [29] who found a decrease in alteration behavior at Y- the maze in an AD mice model.

The above-mentioned decline in behavior alteration performance is mostly attributed to the neurological cell damage and synaptic dysfunction encountered in rats' brain with AD [30]. Also, this finding was in agreement with Abulfadl et al. [31] who found a decrease in the step-through latency in the passive avoidance test of an AD rat model. On the other hand, TQ/AD group showed a significant improvement in SAP% when compared to the AD group. Interestingly, this improvement of the behavioral performance reflected mostly preserved memory and learning in TQ/AD group. In agreement with our results, Bargi et al. [32] and Dalli et al. [33] reported that TQ could reduce memory impairment and improve learning in passive avoidance test in rat models of AD. They have attributed their results to the neuroprotective role of TQ in AD. They have correlated this neuroprotection to the ability of TQ in decreasing Aβ level in brain tissue and reducing Aβ-induced inflammatory mediators in the hippocampus of these animals and subsequently decreasing the cytokine-induced suppression of the long-term potentiation (LTP).

Biologically, the hippocampus is the most sensitive area in the brain to the deleterious effect of inflammatory mediators [34]. This is probably due to the enrichment of the hippocampus with cytokine receptors [32]. This beneficial role of TQ in improving behavioral performance in AD is presumably due to its potent anti-inflammatory effect [13].

The exact mechanism(s) behind AD is unsettled yet. Nevertheless, there is accumulating evidence that the pathogenesis may be related to the release of reactive oxygen species (ROS), with intermingling oxidative stress in the brain tissue, particularly in the hippocampal region [35]. Previous studies on the chronological events of AD showed the oxidative stress to be the earliest event in AD preceding Aβ aggregation. This oxidative stress is correlated to the incidence [36], duration [37], severity [35] and the mortality rate [38] of AD. Moreover, the oxidative stress is accused of direct neuronal apoptosis [39], enhancement of Aβ aggregation as well as tau phosphorylation [37,40].

The mechanism beyond the harmful effects of oxidative stress on the brain tissue in AD is due to the overproduction of ROS. These species are reported to cause peroxidation of polyunsaturated fatty acids in the cell membrane of the brain cells resulting in the formation of toxic byproduct metabolites such as MDA [41]. Consequently, in this study, MDA was measured in the brain tissue of rats and it displayed a high level in the AD group.

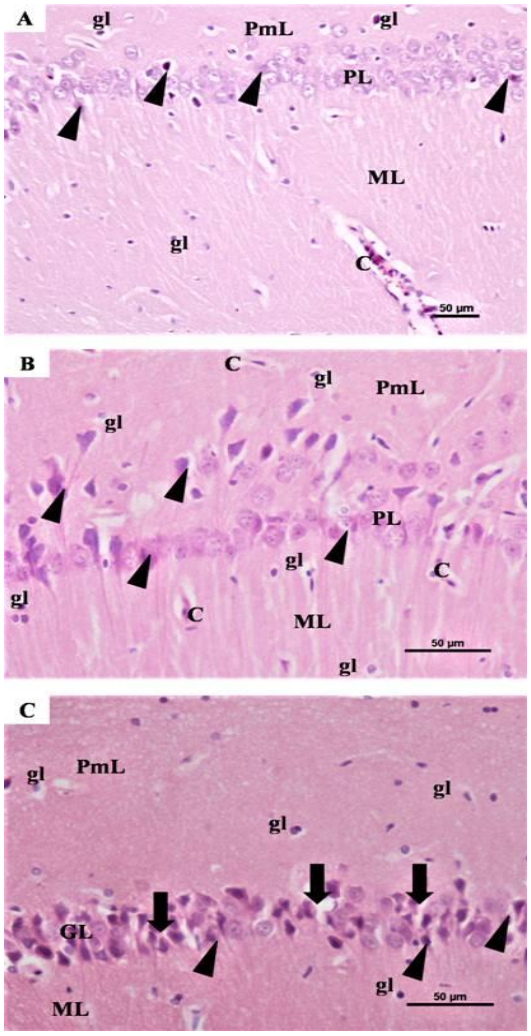

Fig. 8. Photomicrographs of the hippocampus from the AD group: [A] Showing CA1 region with the molecular layer (ML), pyramidal layer (PL), and polymorphic layers (PmL). There are dispersed degenerated pyramidal cells with eosinophilic cytoplasm and condensed nuclei in some cells having a halo around them (arrowhead). [B] Showing disorganization and degenerated pyramidal cells with dark cytoplasm and condensed nuclei (arrowhead) in the pyramidal layer (PL) of the CA3 region that reveals dilated congested capillaries (C). there are increased glial cells (gl) in molecular (ML) and polymorphic (PmL) layers in both CA1 and CA3. [C] Showing many degenerated granule cells in the dentate gyrus with vacuolated cytoplasm and condensed nucleus (thick arrow) in the granular layer (GL) also there are shrunken pyramidal cells with dark cytoplasm and small condensed nuclei (arrowheads). A, B, C (H&E × 400)

Redox imbalance between high oxidants and low antioxidant levels in the brain tissue represents a hallmark of AD [39]. This redox imbalance was evident in this work, was not only there was an increase of MDA level, but also there was a significant decrease in GPX level in the serum of AD group compared to the control group. Upon comparing TQ/AD and AD groups, the results revealed a decrease in the MDA accompanied by an increase in GPX level in the former group. Our results were concomitant with the findings of numerous previous studies that reported a significant improvement of oxidative stress, MDA, superoxide dismutase (SOD), catalase and reduced glutathione (GSH) when TQ was administrated [42,32]. Furthermore, Alhebshi et al. [43] found that TQ administration restored the redox balance in an in vitro study in which

cultured rat hippocampal and cortical neurons displayed Aβ aggregation similar to what is encountered in AD disease upon their treatment with human Aβ 1-42. The restoration of the redox balance upon TQ administration could be attributed to its antioxidant properties [44]. This crucial antioxidant role of TQ, encountered in our study, provided a promising prophylactic potential of this compound against AD.

Our results showed an increase in the Aβ level in the brain of the $AlCl_3$-induced AD group compared to the control one. This result was in agreement with Wang et al. [45] who conduct an experimental meta-analysis in which aluminum administration was found to increase Aβ levels in the brain of experimental animals.

For decades, it was reported that the extracellular accumulation of senile plaques in the brain is one of the hypotheses behind the development of AD. Aβ is the major component of these senile plaques which is made from β-plated sheet fibrils, neuritis of destructed synapses and dendrites, invading astrocytes and inflammatory cell infiltration. Biochemically,

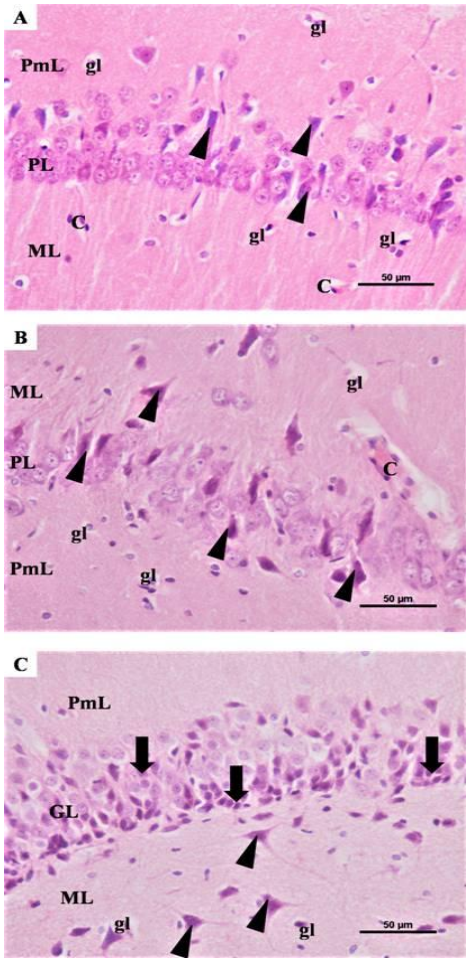

Fig. 9. Photomicrographs of the hippocampus from TQ/AD group: [A] Showing CA1 region with increased thickness of the pyramidal layer (PL) with preservation of most of the pyramidal cells (arrowheads) and fewer cells showing condensed nuclei as compared to the AD group. [B] Showing CA3 region with increased thickness of the pyramidal layer (PL) and preservation of large pyramidal cells (arrowheads). [C] Showing dentate gyrus with increased thickness of the granular cell layer (GL) with less vacuolated cytoplasm and less condensed nuclei (thick arrow) as compared to AD group with preservation of the glial cells (gl) and pyramidal cells (arrowheads) in the molecular layer (ML). PmL = polymorphic layer, A, B, C (H&E × 400)

Aβ is released from a sequential proteolytic cleavage of a large, ~700 amino acids, type I transmembrane glycoprotein called amyloid precursor protein (APP) which is abundant in the central neurons and to a lesser extent in the glial cells [46].

The proteolytic processing of APP has two different pathways. First, the non-amyloidogenic pathway that involves APP cleavage by α-secretase enzyme and results in the formation of extracellular soluble APP (sAPPα) and a transmembrane C83 protein residue (also called carboxyterminal fragments alpha (CTFα) part). CTFα is further cleaved by γ secretase, yielding a protein fragment called p3 and amino-terminal intracellular domain (AICD). sAPPα residue is a non-harmful protein that is thought to have a neuroprotective role through the suppression of abnormally elevated intracellular Ca2+ in neurons via reducing the glutamate-induced NMDA currents [47]. The second pathway is the amyloidogenic pathway in which APP is cleaved by β-secretase resulting in a sAPPβ and C99 protein residue (also called carboxyterminal fragments beta, CTFβ). The latter is further cleaved by γ secretase forming the non-soluble Aβ [48]. γ secretase has an imprecise cleavage point, yielding isoforms of Aβ of the different amino acid length of which Aβ40 and Aβ42 are the most relevant [46].

Numerous previous studies have adopted AlCl3-induced AD models in mice [49], rats [50], rabbits [51] as well as in cultured cells [52]. These studies documented an increase in Aβ level and conformational changes of Aβ that enhanced its aggregation in a fashion that mimics the picture of AD in humans.

In the clinical field, studies on the effect of Al on Aβ in humans are scanty in comparison to those in animals. Yet, there are several epidemiological studies correlated with the development of AD to the increase of environmental Al level [53-55]. Al is known to exert neurotoxic effects through vascular, inflammatory, oxidative, metabolic and many other proposed mechanisms that ultimately result in cellular brain damage [55,56].

The results of our study showed a significant decrease in the Aβ level in the brain homogenate of the TQ/AD group when compared to the AD group. These results were concomitant with Abulfadl et al. [31] who found decreased Aβ level in brain tissue upon histological examination after TQ supplementation.

Also, in vitro studies have found that TQ significantly decreased Aβ neurotoxicity and aggregation when it was applied simultaneously with this protein residue onto cultured hippocampal neuronal cells in a dose-dependent manner [57,43]. These in vitro studies have attributed this neuroprotective effect of TQ in inhibiting Aβ neurotoxicity to its antioxidant effect, where it could scavenge ROS released in the culture media.

Not only Aβ, but also tau protein is strongly related to the pathophysiology of AD. Functionally, tau protein gives support to the intraneuronal microtubules (MT), controls the spacing between them [58], prevents the disassembling of these MT and maintains the transport system of neurons [59]. This transport system is laboring mainly by two proteins. First, kinesin which is the motor protein responsible for the anterograde transport from the cell center to the periphery of different cellular vesicles inside the neurons. Second, dynein, which is the motor protein responsible for the opposite, retrograde, transport of these vesicles [60].

Pathologically, clinical presentation and severity of AD almost always correlate with tau protein and NFT's formation more than the correlation with Aβ plaque [61,62]. In AD, the high concentration of tau can inhibit kinesin action by competing for its binding site on MT [63]. This inhibition of kinesin leads to a marked slowing of the anterograde movement of the cellular components. Contrary, the retrograde movement is unaffected [64]. The net effect is redistribution and accumulation of essential cell components towards the MT organizing center leavening the cell periphery deprived of energy and vulnerable to oxidative stress [65].

Furthermore, Tau affinity to MT is affected by the rate and extent of its phosphorylation. The phosphorylated tau has a low affinity towards MT and it can sequester other tau proteins to form aggregation [66]. Tau phosphorylation is under the influence of phosphatases and kinases enzymes, wherein AD the later becomes more prominent [60]. This is because, in AD, several kinases are known to be increased by Aβ-induced oxidative stress [40]. Once the tau is hyperphosphorylated, it dissociates from the MT leaving them to collapse. Also, the hyperphosphorylated tau form cross-linking structures called NFTs, in which oxidative stress facilitates their aggregation [40] inside the neurons [67,55]. Besides, phosphorylated tau is

abnormally deposited intracellularly in the central neurons in AD and causes a disturbance in axonal transport and in MT assembly [62]. This is of great value since the oxidative stress exerts extravagant cellular damage in the presence of an abnormally increased tau level [65].

Here it is worth mentioning that the tau neurotoxicity is correlated with its phosphorylation rather than with NFTs formation [68]. This is based on previous experiments that showed suppressing tau expression preserved the neural brain cells despite the continued accumulation of NFTs [69].

In this study, there was a significant decrease in the tau level in TQ/AD group when compared with the AD group. This significant decline of the actual tau level eventually decreases the chances of tau phosphorylation. This encountered the neuroprotective role of TQ is probably due to its ability to reduce the oxidative stress that may indirectly decrease tau phosphorylation and in turn preserve MT from collapse.

In this study, Ach level was significantly decreased in the brain of AD group compared with the control group. This was in line with Yassin et al. [70] who found that Ach level decreased in rats upon AD induction using $AlCl_3$. Ach is one of the major excitatory neurotransmitters in the brain. Ach along with cholinergic receptors and cholinergic neurons altogether form the cholinergic system. This system has an important role in cerebral perfusion [71], vasodilatation [72] as well as the integrity of the blood-brain barrier [73].

It was reported that Ach is highly linked to AD. Cholinergic abnormalities are reported to exist as early as the prodromal phase of the disease [74]. At the late stage, up to 75% of cholinergic neurons are lost [75]. The deterioration of the cholinergic system is one of the hypotheses that is adopted to explain the pathophysiology of AD. Previous studies have found that there was a decrease in choline acetyltransferase activity [76], cholinergic neurons [77] acetylcholine [78], as well as cholinergic receptors [79] in the AD of humans and other species. It was also found that cholinergic depletion increased Aβ deposition [80], tau phosphorylation and pro-inflammatory cytokines formation [81].

Therefore, restoration of cholinergic functions in AD might improve the pathophysiological aspects and hence the clinical presentation [74]. This is the basis of the anti-cholinesterase inhibitor drugs like rivastigmine and donepezil in the management of AD which increases the Ach level. In line with this basis of management, our results revealed a significant increase in the Ach level in the brain tissue of the TQ/AD group compared with the AD group when compared to the AD group. In support of our results, Jukic et al. [82] have found that TQ possessed anticholinesterase activity.

In the current study, histological examination of the hippocampus of rats in the AD group revealed that the pyramidal layers in CA1 and CA3 regions of the hippocampus showed marked shrinkage in size of small and large pyramidal cells, respectively with some cell loss. These histological results were in agreement with Padurariu et al. [83] and Nirmala et al. [84]. They demonstrated in their study that the cytoplasm of neurons was shrunken, the nuclei were side-moved and dark-stained, neurofibrillary degeneration and neuron loss were observed in the hippocampus of rat received $AlCl_3$. Moreover, Yassin et al. [70] observed in sections of rat brains receiving $AlCl_3$ (17 mg/kg) for 4 weeks that the pyramidal cells lost their triangular shape, showed darkened nuclei and were surrounded with pericellular haloes together with brain necrosis, spongy appearance, and plaques. Similar results were reported by Aly et al. [85] who reported the presence of β-amyloid plaques in the cerebral cortex and the hippocampus. Besides, slight disorganization of the pyramidal cell layer, little degeneration of pyramidal cells and slight spongiosis were reported by a finding of the AD model studied by Abo El-Khair et al. [86].

As regards the granular layer it showed an apparent increase in the number of the granule cells with decreasing in their diameter. There was marked shrinkage in the size of granule cells with some cell loss and marked vacuolation. This indicated clear evidence of chronic inflammation and oxidative damage [87]. Inflammatory changes are features of AD. Several studies have reported this observation and have shown that a cluster of astrocytes and inflammatory cell infiltrate around Aβ-containing plaques [88]. Also, an increase in expression of inflammatory cytokines, interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) had been detected in the brain of AD animal models and IL-1-positive microglia present with Aβ-containing plaques, a similar event occurs in activated astrocytes [88].

In this study, there was a reduction in neuronal population in the hippocampus of rats of the AD group. On the other hand, for unknown reasons, the molecular & polymorphic layers revealed enlarged and excess astrocytes and widened blood capillaries. This neuronal loss coincides with the finding of Nobakht et al. [89] who reported that there was a reduction in neuronal population in the hippocampus of the rat model with AD. Serrano-Pozo et al. [90] mentioned that neuronal loss is the main pathological substrate of the cortex and hippocampus which is evident in sections stained with hematoxylin and eosin, it can be more readily shown with a Nissl staining or a NeuN immunohistochemistry. It is reported that the neuronal loss is a common feature in AD and can be triggered by various factors, such as β amyloid plaques, perturbed calcium regulation, glutamate, ischemia, inflammatory processes or oxidative stress [91]. The above-mentioned histopathological changes in the hippocampus of AD group was partly ameliorated on TQ administration.

5. CONCLUSION

TQ could mitigate the neurodegenerative markers and oxidative stress indices encountered in AD, presumably via its antioxidant and anti-inflammatory effects. This may implement TQ as an adjuvant alternative medical strategy in ameliorating this devastating disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that the experimental protocol of this study was revised and approved by the local ethical guidelines established by the Unit of Biomedical Ethics Research Committee (REC), Faculty of Medicine, King Abdulaziz University, Saudi Arabia (Reference NO; 452-16, 2017). The experimental procedures were performed following the international guiding principles for the care and use of the research animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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