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# Effect of Thymoquinone against Aluminum Chloride-Induced Alzheimer-Like Model in Rats

**Ahmed A. El Sayed, Samy A. Hussein Aziza, Mohammed K. Mahfouz, Afaf D. Abd El-Magid, Ahmed Ibrahim M. Ibrahim**

*Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt*

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**ABSTRACT**

Alzheimer Disease (AD) is a progressive neurodegenerative disorder characterized by oxidative stress, neuro-inflammation, and synaptic dysfunction. Senile plaques and neurofibrillary tangles, which form outside and inside of cerebral neurons, respectively, are partially to blame for this abnormal aggregation. The main bioactive component of Nigella sativa seed volatile oil is thymoquinone (TQ), which has anti-inflammatory and antioxidant actions in several neurological disorders. The purpose of the study is to assess TQ's ability to protect neurons against oxidative stress in rats with aluminum chloride (AlCl3)-induced AD. Materials and Techniques For this investigation, 30 mature male Sprague Dawley albino rats were employed. Three groups were created at random from them. Group 1 (control group) (control group). Group 2 (AD group): AlCl3 (100 mg/kg/day) was added orally for 8 weeks. For eight weeks, members of Group 3 (the TQ/AD group) received oral TQ (10 mg/kg/day) and AlCl3 (100 mg/kg/day) supplements concurrently. The Y-maze spontaneous alternation test was used to evaluate spatial working memory at the conclusion of the session. The serum concentrations of glutathione peroxidase enzyme (GPX) and malondialdehyde (MDA) were then measured. After the rats were killed, the concentrations of tau protein, AR, and acetylcholine in the brain tissue homogenate were measured. Results: The Spontaneous Alteration Performance (SAP%) at the Y-maze behavior test significantly decreased in the AD group, indicating a deficit in spatial working memory. Additionally, serum MDA and GPX dramatically decreased whereas serum AR, tau protein, brain acetylcholine, and serum MDA all significantly increased. TQ and AlCl3 treatment together significantly reduced acetylcholine, tau protein, and AR in the TQ/AD group. in brain and serum MDA and increased (SAP%) and serum GPX. Conclusion: TQ could mitigate the neurodegenerative markers and oxidative stress indices encountered in AD, presumably via its antioxidant and anti-inflammatory effects.

## 1. INTRODUCTION

The progressive neurodegenerative condition Alzheimer Disease (AD), which primarily affects senior people, causes changes in mood, behavior, and cumulative neurophysiatric changes, including a sharp reduction in cognitive function. (Babri et al., 2014). According to Van Cauwenberghe et al. (2016), AD is the most frequent cause of dementia, accounting for up to 70% of all cases. German physician Alois Alzheimer originally used the name AD to characterize this illness in 1906. (Korolev, 2014). Epidemiologically, AD is one of the primary health issues whose prevalence has grown recently throughout the world. Around 44 million people had AD worldwide in 2015, and by 2050, that number is anticipated to treble (Van cauwenberghe et al., 2016). According to studies, about 1% of people over 65 and close to 4% of people over 85 are known to have Alzheimer's disease. Clinical characteristics of AD The development and progression of AD are really caused by a number of changeable and non-modifiable risk factors. The likelihood of having AD increases exponentially with age, roughly doubling every five years beyond age 65, making age the biggest risk factor for the illness (Querfurth and Laferla, 2010). Although the specific cause(s) of AD is still unknown, it has been hypothesized that aberrant protein deposits in the cerebral cortex and other parts of the brain are responsible for the neuronal loss seen in AD, particularly in cholinergic neurons and neuronal synapses. These abnormal proteins include the microtubules-associated protein (tau protein), which becomes highly phosphorylated and subsequently aggregates inside the neurons to form intracellular "Neurofibrillary Tangles," and the insoluble amyloid beta (A) proteins that are deposited outside the cerebral neurons to form extracellular "senile plaques" (NFTs). These senile plaques and NFTs are thought to be two of the principal pathology symptoms of AD (Morrison and Lyketsos, 2005). The pathological features of the disease include oxidative stress, neuro-inflammation, and synaptic dysfunction. These pathological features may be a result of abnormal aggregation of senile plaques and NFTs, which results in deleterious synaptic and neuronal loss and frequently starts years before memory loss. Even though

* Correspondence to: mido_mido23180@yahoo.com

basic research into AD has advanced significantly over the past 20 years, current treatments like donepezil (Aricept), rivastigmine, galantamine, and memantine can only temporarily improve cognitive symptoms. No medication can stop, reverse, or even significantly slow the unavoidable neurodegenerative process. These synthetic medications have a number of negative side effects, are quite expensive, and are not used for all AD cases (Raschetti et al., 2007). As a result, there is a significant need for innovative natural therapies derived from medicinal plants that can be used to guard against or stop AD disease progression in its early stages. Thymoquinone (TQ), which is a key bioactive component of the volatile oil found in Nigella sativa seeds, was tested in this study (Bai et al., 2013). In various neurological illnesses, TQ has anti-inflammatory and antioxidant properties. Additionally, it is said to protect neuronal cells against the toxicity that is caused by AlCl3 (Ismail et al., 2013).

The current study aims to assess the neuroprotective potential of TQ on the oxidative stress status of the brain in rat models of AD caused by aluminum chloride.

## 2. MATERIAL AND METHODS

Experiment was conducted according to the guide for care of laboratory animals and approved by the ethical animal committee, Faculty of Veterinary Medicine, Benha University (Approval no. BUFVTM 01-5-22).

### *2.1. Chemicals*

The AlCl3 was purchased as powder from Sigma-Aldrich Co. Munich, Germany) and was dissolved in distilled water. Thymoquinone (TQ) was purchased from Sigma-Aldrich Co., Darmstadt, Germany) was dissolved in corn oil as a final concentration of 20mg/ml (w/v).

### *2.2. Experimental groups*

The present study was carried out on a total of 30 rats divided into 3 experimental groups of 10 rats each:

*Group 1 (Control group):* The rats of this group were orally administered via gastric gavage with 1 ml/kg/day of corn oil for 8weeks.

*Group 2 (AD group):* In this group, Alzheimer's like rat model was experimentally induced by oral administration of AlCl3 (100mg/kg/day) dissolved in distilled water for 8 weeks (Yassin et al., 2013)

*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 and oral AlCl3 (100 mg/kg/day) dissolved in distilled water for 8 weeks.

### *2.3. Experimental protocol*

Blood samples were taken from the retro-orbital venous sinuses in non-heparinized Eppendorf tubes at the conclusion of the experimental time period and after an overnight fast. The blood samples were centrifuged for 15 minutes at 3000 rpm after being allowed to coagulate at room temperature in a water bath. The clear serum supernatant was removed, frozen, and kept at -20°C until further biochemical testing for serum glutathione peroxidase enzyme and malondialdehyde (MDA), a marker of lipid peroxidation and oxidative stress (GPX, as a marker of antioxidant enzyme activity).

### *2.4. Behavioral study*

Y-maze spontaneous alternation test: The Y-maze test was carried out in accordance with Wall and Messier's earlier instructions (Wall and Messier, 2002). The labyrinth features three similar arms that are each 40 cm long, 35 cm high, and 12 cm broad. The arms are positioned at equal angles and are named A, B, and C. The maze was manufactured of painted wood (to minimize the spatial orientation visual signals). During a scheduled 5-minute session, the rats were positioned at the end of arm A and permitted to freely navigate the maze. To study the pattern of rat entry into the maze's arms, the spontaneous alternation was visually tracked. When the rat's rear paws were entirely inside the arm, admission was deemed to be complete. According to the definition of alternation, the three arms onon overlapping triplet set (i.e., ABC, BCA) assessment of spatial working memory: In this study, the following spatial working memory indicator parameters were assessed using the Y-maze spontaneous alternation test (Wall and Messier, 2002).

### *2.5. Biochemical analysis*

Aβ in the brain tissue (pg/mg protein) was measured by RT.qPCR. Tau protein in the brain tissue (ng/mg protein) was measured by RT.qPCR. Acetyl choline in the brain tissue (µmol/mg protein): Was measured by the colorimetric method using a choline/acetylcholine assay kit (Acetyl choline, Bio Vision Inc., California, USA). Serum GPX (U/ml) was measured calorimetrically using a commercial assay kit (Bio diagnostic, Egypt). Serum MDA (nmol/ml): (was measured by thiobarbituric acid colorimetric method using MDA assay kit (MDA, Cell Biolabs, USA).

### *2.6. Statistical analysis*

In this study, the statistical package for social sciences program me version 23, was used to analyze the data (SPSS Inc., Chicago, Illinois, USA). Mean ± SD was used to express the results. One-way ANOVA and the post-hoc Tukey test were used to examine the significance of differences between groups. Statistics were judged significant at $P < 0.05$.

## 3. RESULTS

The obtained data in (Table 1) of Aβ in the brain tissue in AD group was 11.4±2.8 pg/mg protein which was significantly higher ($p<0.05$) than the corresponding value in the control group (1.7 ±0.3).

The obtained data in (Table 1) of tau protein in the brain tissue in AD group was 11.5 ±2.2 ng/mg protein, which was significantly higher ($p<0.05$) than the corresponding value in the control group (1.1 ± 0.2).

The obtained data in (Table 1) of acetyl choline in the brain tissue in AD group was 18.9±5.5 µmol/mg protein which was significantly lower ($p<0.05$) than the corresponding value in the control group (40.7±5.2).

The obtained data in (Table 1) of serum GPX in the brain tissue in AD group was 53.3 ±13.8U/ml which was significantly lower ($p<0.05$) than the corresponding value in the control group (101.0 ± 21.3).

The obtained data in (Table 1) of serum MDA in the brain tissue in AD group was 55.2 ±9.5nmol/ml which was significantly higher ($p<0.05$) than the corresponding value in the control group (8.7 ± 1.8).

Table 1 Biochemical parameters in control, AD and TQ/AD groups (Mean± SD, n=10).

| Biochemical parameters | Experimental Groups | | |
|---|---|---|---|
| | Control | AD | TQ /AD |
| Aβ (pg/mg) | 1.7±0.3 | 11.4 ±2.8[a] | 4.5 ±1.4[ab] |
| Tau protein (ng/mg) | 1.1 ±0.2 | 11.5 ±2.2[a] | 5.4 ±1.2[ab] |
| Acetyl choline (µmol/mg) | 40.7 ±5.2 | 18.9 ±5.5[a] | 32.4 ±4.2[ab] |
| Serum GpX (U/ml) | 101.0 ±21.3 | 53.3 ±13.8[a] | 91.4 ±16.0[ab] |
| Serum MDA (nmol/ml) | 8.7 ± 1.8 | 55.2 ±9.5[a] | 21.2 ±4.9[ab] |

Values with different letters within the same raw differed significantly at P< 0.05

## 4- DISCUSSION

This study presents a new perspective on the beneficial effects of thymoquinone for Alzheimer's treatment in comparison with Aricept (Donepezil) as cholinesterase inhibitors drug. According to the study's findings, the AD group's spontaneous alternation percentage (SAP%) was significantly lower than that of the control group. This result was consistent with that of who discovered that an AD mouse model reduced modification behavior at the Y-maze. This result concurred with that reported by Abulfadl et al. (2018), who discovered a reduction in step-through latency in the passive avoidance test of the AD rat model.

According to Oakley et al. (2006), the neurological cell loss and synaptic dysfunction present in AD-affected rats' brains are mostly to blame for the drop in behavior alteration performance that was noted above. Our results showed an increase of Aβ level in the brain of Alcl3-induced AD group compared to the control one. This result was in agreement with who conduct an experimental meta-analysis in which aluminum administration was found to increase Aβ levels in the brain of experimental animals.

In this study, there was a significant decrease of the elevated tau level in TQ/AD group when compared with AD group. This encountered neuroprotection role of TQ is not only due to its ability to decrease tau level, but also due to reduction of oxidative stress as was confirmed in this work upon measuring GPx level. These antioxidant effects of TQ that may indirectly decreased tau phosphorylation and in turn preserve the microtubules from collapse.

In this study, acetylcholine level was significantly decreased in the brain of AD group compared with the control group. This was in line with Yassin et al. (2013) who found that acetyl choline level decreased in Alcl3-induced AD of rats. In addition, this decline of acetyl choline was reported by Pákáski and Kálmán (2008). Actually, acetylcholine is one of the major excitatory neurotransmitters in the brain. Acetylcholine, cholinergic receptors and cholinergic neurons forming the cholinergic system. This system has an important role in cerebral perfusion, vasodilatation as well as the integrity of the blood brain barrier (Van beek and Claassen, 2011)

It was reported that acetylcholine is highly linked to AD. Cholinergic abnormalities are reported to exist as early as the prodromal phase of this disease (Hampel et al., 2018). At late stage, up to 75% of cholinergic neurons are lost. The deterioration of the cholinergic system is one the hypothesis that is adopted to explain pathophysiology of AD. Previous studies have found that there was a decrease of choline acetyltransferase activity that is responsible for formation of Ach in the cytosol of the cholinergic nerve terminals, cholinergic neurons acetylcholine as well as cholinergic receptors in AD of humans and other species.

It was found that cholinergic depletion increased A R deposition and tau phosphorylation and pro-inflammatory cytokines formation (Field et al., 2012). Therefore, restoration of cholinergic functions in AD might improve the pathophysiological aspects and hence the clinical presentation as well (Hampel et al., 2018). This is the basis of the anti-cholinesterase enzyme inhibitors group of drugs like rivastigmine and donepezil in management of AD via increasing Ach level. In line with basis of management, our results revealed a significant increase in acetylcholine level in the brain tissue of TQ/AD group compared with AD group. In support to our results, Jukic et al. (2007) have found that TQ possessed anticholinesterase activity.

Actually, the exact mechanism(s) behind AD are unsettled yet. Nevertheless, there are accumulating evidence that the pathogenesis maybe related to the release of Reactive Oxygen Species (ROS), with intermingling oxidative stress in the brain tissue, particularly in the hippocampal region. Previous studies on the chronological events of AD showed that oxidative stress might be the earliest event in AD even preceding A R aggregation.

This oxidative stress is correlated to the incidence, duration, severity and the mortality rate of AD (Castellani et al., 2009). Moreover, the oxidative stress is accused for direct neuronal apoptosis, enhancement of AD aggregation as well as tau phosphorylation.

The mechanism of harmful effects of oxidative stress on the brain tissue in AD is due to over production of ROS. These species are reported to cause peroxidation of polyunsaturated fatty acids in the cell membrane of the brain cells resulting in formation of toxic byproduct metabolites such as MDA (Casado et al., 2008). Consequently, in this study, MDA was measured in brain tissue of rats and it displayed a high level in AD group.

Actually, redox imbalance between high oxidants and low antioxidant level in the brain tissue represents a hallmark of AD (Weydert and Cullen, 2010). This redox imbalance was evident in this work, where not only there was an increase of MDA level, but also there was a decrease in GPx level in the brain tissue of AD group compared to the control group. Upon comparing TQ/AD and AD groups, the results revealed a decrease in the MDA accompanied with 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) on TQ administration. Furthermore, (Delacourte et al., 2002) found that TQ administration increased cell survival rate of hippocampal cells and restored the redox balance in an invitro study in which cultured rat hippocampal and cortical neurons displayed A R aggregation similar to what is encountered in AD disease upon their incubation with human AR 1-42. This restoration of the redox balance upon TQ administration could be attributed to its anti-oxidant property (AL-Amjed et al., 2006). This crucial antioxidant role of TQ, encountered in our study, provided a promising prophylactic potential against AD.

## 5. CONCLUSION

It is possible to infer from the study's data that TQ plays a neuroprotective role in an experimental AD model. This is mainly because of its antioxidant properties and capacity to raise Ach in rat brain tissue. This could serve as motivation for additional clinical research

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