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Effect Of Quercetin As Therapeutic And Protective Agent In Aluminum Chloride-Induced Alzheimer's Disease Rats

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease clinically characterized by progressive cognitive impairment. This work aimed to investigate the role of quercetin (Q) in the treatment and protection of $AlCl_3$-induced AD in rats through exploring the molecular mechanisms underlying its neuroprotective and therapeutic properties. In this study, male Wistar rats were allocated to two experiments: experiment I (therapeutic effect of quercetin) in which the rats were divided into: normal control group (C-Saline) which was induced with saline for 56 days, C-Q50 group, which was administered orally with Q (50 mg/kg) for 28 days after induction saline for 28 days, AD ($AlCl_3$-Saline) group received $AlCl_3$ (50 mg/kg) intraperitoneally for 28 days followed by saline for 28 days, and the $AlCl_3$-Q50 group received Q (50 mg/kg) orally for 28 days after induction with $AlCl_3$ for 28 days. In experiment II rats were divided into: normal control (NC) group, Q50 (50 mg/ kg) group, $AlCl_3$ (AD) group, and $AlCl_3$ + Q50 group which was co-administrated with $AlCl_3$ + Q50 for 56 successive days. Dopamine (DA) and acetylcholinesterase (AChE) levels were estimated for all rat groups. The results showed that post-treatment of the AD-induced rats with Q50 as well as co-administration of $AlCl_3$with Q50, significantly reduced the AChE level in serum, significantly increased the DA level, and significantly increased the body weight change compared to the $AlCl_3$group, but did not show any significant change in the brain weight. We concluded that quercetin significantly improved the cholinergic and dopaminergic dysfunctions by lowering the Acetylcholine esterase level and restoring the dopamine level

Keywords: Alzheimer disease; Quercetin; Acetyl choline esterase, Dopamine; $AlCl_3$; Body weight change.

1.Introduction

Neurodegenerative disorders are becoming more dominant in the developed countries among people over 65 years due to genetic and environmental factors. It is predicted that 1 among 85 individuals will be affected by AD in 2050[1]. AD is a complex progressive neurodegenerative and dementia disorder. It is the most prevalent type of dementia, and the cause is mostly unclear.

It accounts for almost 50 percent to 75 percent of all dementias and as stated by the world alzheimer report, 46.8 million individuals were suffering from dementia in 2015, that is predicted to become twofold every 20 years [2].Loss of short-term memory is the first clinical symptom, and as the disease progresses, signs such as forgetting names and words during speaking, mood swings, failure to calculate, and inability to utilize daily living objects and tools emerge[3].

Aluminum exposure has been identified as a significant environmental factors linked to an elevated risk of AD. Aluminum is a widespread chronic neurotoxin [4, 5], thus it's important to avoid it. Aluminum chloride exposure causes the formation of hazardous intermediates such as hydrogen peroxide and hydroxyl radicals, which may help to mediate aluminum toxicity[6]. aluminum, a well-established neurotoxicant, it has been linked to in the etiology of AD owing to its simple entry and retention in the central nervous system[7]. Several studies have revealed that AD is associated with a number of neurotransmitter disorders, with the acetylcholine (ACh)

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system being more assenting in relation to AD [8]. AChE has a strong catalytic activity and inactivates ACh by resolving it into choline and acetic acid, allowing for efficient cholinergic nerve transmission. The activity of AChE is a direct reflection of how well the cholinergic system is working. Changes in the cholinergic system produced by aluminum, particularly acetylcholine metabolism and AChE activation, may have an effect on rat cognitive ability [9, 10]. DA is a neurotransmitter that participate in a variety of brain activities, including locomotion, cognition, emotion, positive reinforcement, food intake, and endocrine homeostasis[11].Al has been demonstrated to affect a biochemical pathway in the rat brain that results in a decrease in dopamine levels. Quercetin (3, 5, 7, 3′, 4′-pentahydroxyflavone) is among the most popular powerful antioxidants founded in plants and one of the most widespread flavonoids in edible plants [12].Q may utilize multiple neuroprotection mechanistic targets for AD, including downstream modulation of oxidative stress and neuroinflammation, contributing to direct neuroprotection, AChE enzyme inhibition and boosting the level of acetylcholine and decreasing the Tau phosphorylation and Aβ aggregation. Q can alsoaffect tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), reactive oxygen species(ROS), nitric oxide (NO), acetyl cholinesterase (AChE), and amyloid beta protein (Aβ)[13].The goalof this study is to assess the neuroprotective and therapeutic effects of quercetin in $AlCl_3$-induced Alzheimer's disease by estimating thedopamine level, and measuring the acetylcholine esterase level.

2. Material and methods

2.1. Drugs and chemicals

Aluminum chloride ($AlCl_3$) hydrate (Cat. # 229393) and solid quercetin ≥ 95% (Cat. # Q4951, high-performance liquid chromatography) were purchased from Sigma-Aldrich Chemicals Co., St. Louis, USA

2.2. Animals

Sixty-four male wistar rats (8 weeks) weighing 140 ± 10 g were provided by the National Research Centre Animal House, Giza, Egypt. Before beginning the experiments, the rats were kept in polypropylene cages and acclimatized to pathogen-free environment for three days. Under a 12 hour light/dark cycle, the rats were kept at 24 1 °C and 55–65 percent humidity. The rats were given ad libitum water and a regular rodent diet (17.48 percent protein, 6.85 percent fat, 62.99 percent carbohydrates, 4.08 percent ash, and 2.16 percent minerals and vitamins). Animal handling protocols were carried out in compliance with the Ethical Committee of Medical Research of National Research Centre, with approval number "18,144," Giza, Egypt, and the National Institutes of Health's recommendations for laboratory animal care and use (NIH Publications No. 8023, revised 1978).

2.3. Experimental setting

After the adaptation period, the rats (n = 64) were weighed and allocated into two experiments**:** experiment I (therapeutic effect of quercetin) (n=32) and experiment II (co-administration of $AlCl_3$ with quercetin) (n=32). In the experiment I the rats were equally distributed into two major groups; group I (n = 16) received saline orally for 28 days, and group II (n = 16), which was used for AD model induction (intraperitonealy administered with $AlCl_3$ [14] (50 mg /kg) for 28 days. Following AD induction, the NC (n = 16) and $AlCl_3$ rats (n = 16) divided into four groups, each with eight rats, to receive Q50 treatment for 28 days as follows: (1) (C-Saline) group received saline via gastric intubation (2) (C-Q50) group administered Q orally dissolved in saline by gastric intubation (50 mg/kg bwt) [15]; (3) ($AlCl_3$-Saline) (AD) group administered orally with saline by gastric intubation; (4) ($AlCl_3$-Q50) group received Q dissolved in saline orally by gastric intubation (50 mg/kg bwt)[16].

While the experiment's two (n=32) rats were divided into four groups (each with eight rats) and given the following treatments for eight weeks: group (5) the normal control (NC) group, which received saline orally via gastric intubation, and group (6) Q50, which received Q dissolved in saline via gastric intubation (50 mg/kg).
[15], group (7) the $AlCl_3$ (AD) group which was used for AD model induction (intraperitoneally administered with $AlCl_3$(50 mg /kg)[17], group (8) ($AlCl_3$+ Q50) group co-administered with $AlCl_3$ (intraperitoneally administered with $AlCl_3$(50 mg /kg) followed by (after 10 –15 min) Q (orally administered with Q(50 mg/kg) dissolved in saline by gastric intubation).

2.4. Sample collection

The rats' final body weight and brain weight were recorded at the end of the experiment. After 14 hours of holding food, blood samples were taken from the retro-orbital plexus under light diethyl ether anesthesia, just before the rats were sacrificed by cervical decapitation. The blood was collected in dry clean centrifuge tubes and allowed to clot to separate the sera, and the serum samples were separated by centrifugation at 4000 r/min for 10 minutes at 4°C. Aliquots of serum were frozen and stored at -20 °C for the measurement of biochemical markers.

2.5. Assessment of dopamine (DA) and acetylcholinesterase levels (AChE)

The protein of each sample was determined by the Biuret Protein Content Assay Kit (Cat.MBS779655). Dopamine (Cat. #SG-20553) and AChE (Cat. #SG-20512) levels were measured by enzyme-linked immunosorbent assay (ELISA) kit for rats (Sino gene clon Co., Ltd, Hangzhou, China) according to the manufacturer's guidelines. With a precision of 10% inter-assay and 8% intra-assay, this test showed no cross-reactivity with other analogs.

3. Statistical analysis

SPSS version 25 software for Windows was used to conduct the analyses (SPSS, Chicago, IL). The data was presented as a mean ± SE. Differences between groups were tested for statistical significance using one-way analysis of variance (ANOVA), followed by tukey's test. $P < 0.05$ was considered significant for all data analyses.

4. Results

4.1. Experiment I (therapeutic effect of quercetin)

The initial body weight was similar in all the experimental groups. The body weight change in the AD-induced rats group was significantly lower than in the normal control group. In contrast, the post treatment of the AD group with Q50 significantly increased the body weight change compared to the untreated AD-induced rats group (fig1).

Otherwise, when compared to the $AlCl_3$-Saline group, the treated AD-induced rats with Q50 rats showed no significant difference in brain weight (fig1).

The levels of AChE and dopamine in the normal control and Q50 groups were roughly comparable. When compared to the normal control group, the ($AlCl_3$-Saline) group had a significant increase in AChE levels in serum (552.6580.812). However, when compared to the normal control group, dopamine levels were significantly lower in the ($AlCl_3$-Saline) (32.62501.89) group (fig 2).

Post-treatment of AD-induced rats with Q50 resulted in a significant decrease in serum AChE levels (147.8182.59) compared to the ($AlCl_3$-Saline) group. On the other side, post-treatment of the ($AlCl_3$-Saline) group with Q50 caused a significant elevation of DA level in serum (82.38±17.71) compared to the AD-induced rats group (fig 2).

4.2. Experiment II (co-administration of $AlCl_3$ with quercetin)

All of the experimental groups had roughly the same body weight at the start. When compared to the NC group, $AlCl_3$ caused a significant reduction in body weight change in AD-induced rats. In contrast to the AD group, co-administration of $AlCl_3$ with Q50 resulted in a considerable increase in body weight change (fig3).

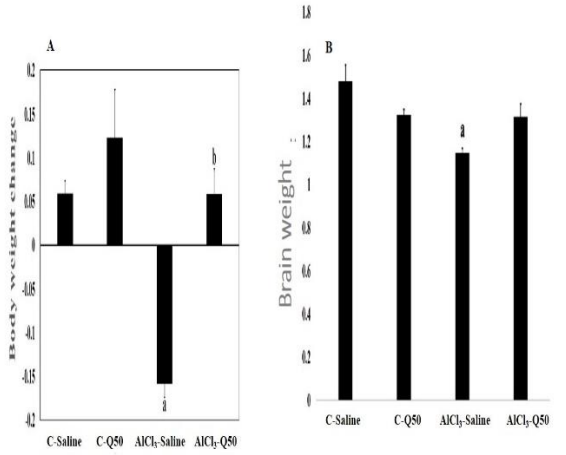

Fig. 1. The effect of post-treatment with Q on body weight change (A) and brain weight (B)in $AlCl_3$-induced AD rats. Data are represented as mean ± S.E (n = 5). a: Significant change at $P < 0.05$ in comparison with (C-Saline) group ; b: significant change at $P < 0.05$ in comparison with ($AlCl_3$-Saline) group, comparison between groups was done using Tukey's test

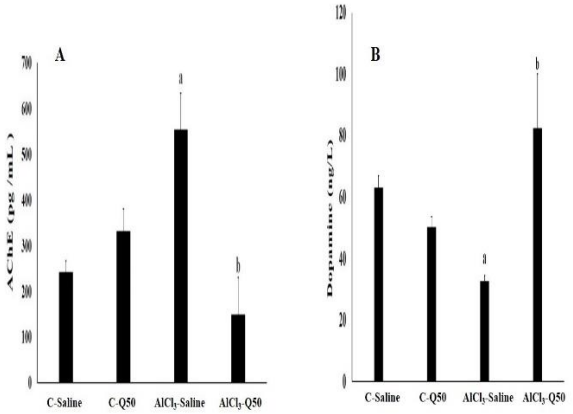

Fig. 2. The effect of Q post-treatment on serum AChE activity (A) and DA levels (B) in $AlCl_3$-induced AD rats. The data are presented as mean S.E. (n = 5). a: Significant difference at P 0.05 when compared to the normal control group (C-Saline); b: significant difference at P 0.05 when compared to the ($AlCl_3$-Saline) group, as determined by Tukey's test.

The brain weight of AD-induced rats was significantly lower when compared to the normal control group.

Otherwise, the co-administered ($AlCl_3$+Q50) group did not show any significant difference in brain weight when compared to the AD-induced rats group (Fig3).

There were no significant differences in AChE and Dopamine levels between the NC and Q50 groups.

When compared to the normal control group, the AChE level in serum was significantly higher in the AD-induced rats group (622.04±133.84), (table4). When compared to the AD-induced rats group, co-administration of $AlCl_3$ with Q50 resulted in a significant decrease of AChE levels in serum (250.65±57.72**)**. Even though, co-administration of $AlCl_3$ with Q50 caused a significant increase of DA level in serum (69.71±6.144) when compared to the AD-induced rats group (fig 4).

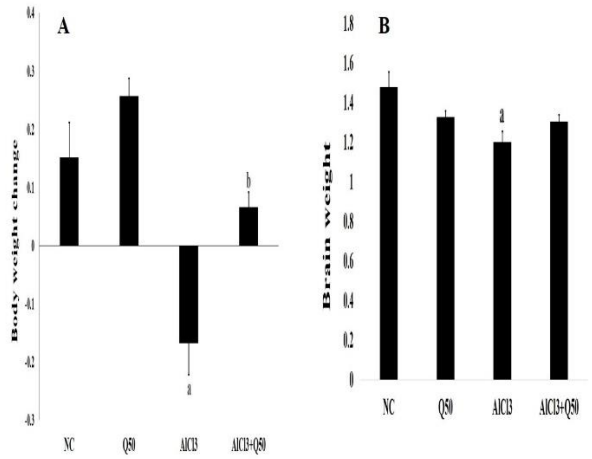

Fig. 3. The effect of co-administration of $AlCl_3$ with Q on body weight change (A) and brain weight (B)in $AlCl_3$-induced AD rats. Data are represented as mean ± S.E. (*n* = 5). a: Significant change at P < 0.05 in comparison with NC ; b: significant change at P < 0.05 in comparison with $AlCl_3$ group, comparison between groups was done using Tukey's test

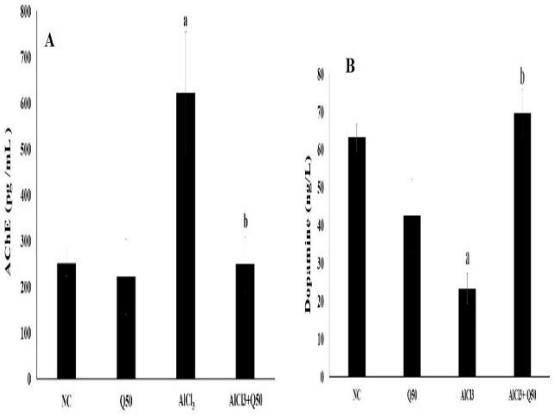

Fig. 4. The effect of co-administration of $AlCl_3$with Q on serum AChE activities (A) and DA levels (B)in $AlCl_3$-induced AD rats. Data are represented as mean ± S.E (*n* = 5). a: Significant change at P < 0.05 in comparison with NC; b: significant change at P < 0.05 in comparison with $AlCl_3$group, as determined by Tukey's test.

5. Discussion

AD is the most frequent cause of progressive dementia among the elderly, and the number of patients with AD is anticipated to triple by 2050 [1, 2]. Environmental factors are a major contributor to the progression of Alzheimer's disease[18]. Aluminum (Al) is a neurotoxic substance that generates neurofibrillary tangles and amyloid aggregates in the brain [19]. Moreover, aluminum has also been demonstrated to impair neurological function via Aβ degradation in brain tissue, neuron death, and Alzheimer's-like symptoms [20-22]. As a result, learning and memory are crucial aspects in identifying and treating AD [23].

Quercetin is a potent plant-derived antioxidants, and one of the most common flavonoids found in edible plants and belongs to flavonols subclass, which represents a big group of polyphenols [12, 24]. The Q neuroprotective benefits have been documented in several studies, including *in vitro* and *in vivo* conditions of neurodegenerative disorders, like cognitive impairment [25],ischemia, traumatic injury[26], Parkinson's disease (PD)[27], and Huntington's disease [28]. Numerous investigations are being conducted in order to develop a viable treatment for Alzheimer's disease. To date, no efficient results have been obtained. The objective of this work is to develop a powerful and safe natural protective agent and treatment for AD. This can be accomplished through investigating the role of Q in the treatment and protection of $AlCl_3$-induced AD rats by delving into the molecular processes behind its neuroprotective and therapeutic characteristics. In this study, the $AlCl_3$-induced AD rats showed a significant decrease in body weight change compared to normal control in both experiments (figs 1&3) which is consistent with the recent study [29] in which aluminum chloride was administered to female rats (10 mg/kg BW) intraperitoneally at 10 mg/kg to rats twice a week resulted in a significant reduction in body weight in the intoxicated group compared to control group because aluminum has an anorectic effect and impacts the neural pathways that control satiety[29]. Abdel-Aal *et al* [30] indicated that rats given $AlCl_3$ injections for sixty days did not increase weight compared to control rats, which could be attributed to a lack of appetite. Post treatment of Q50 to AD-induced ratsas well as co-administration of Q (50 mg/kg) and $AlCl_3$ rats showed improvement in bodyweight change (figs 1&3). These findings are in accordance with Hassan *et al.*[31] who found that significant increases in weight gain by around 70% in the Q group and approximately 50% when paired with imidacloprid, a neurotoxic compound, when weight gain in rats is used as a measure of growth rate..

In both of our experiments, the brain weight of the AD-induced rats was significantly lower than that of the normal control group (figs 1&3) which contradicts a study [29] which showed that aluminum chloride intoxication at 10 mg/kg administered by injection to the peritoneum of rats for two weeks, did not affect the brain weight. This disparity in results could be attributed to differences in $AlCl_3$ dose and duration.

Co-administration of $AlCl_3$+Q50 in addition to Post treatment of Q50 to AD-induced rats, on the other hand, did not result in any significant change in brain weight when compared to the AD-induced rats (figs 1&3). Mesram *et al.* [32] found that; the postnatal pups which received quercetin–NaF have shown significant ($p< 0.05$) recovery in brain weights in all age groups.

Acetylcholine (Ach) regulates neuronal activity in the hippocampus and neocortex, it is widely distributed along the synaptic cleft of the cholinergic synapse and is neurotransmitted to promote learning and memory. Ach is also an important marker that represents cholinergic nerve function and is associated with memory and cognition loss in Alzheimer's disease patients.[33].

AChE is involved in synapses and connections between neurons and muscles [34].The primary function of AChE is to stop cholinergic transmission by hydrolyzing Ach [35]. AChE is a promising therapeutic target for symptomatic relief because cholinergic neurotransmission decrease is a consistent and early indicator of Alzheimer's disease [36]. Al may impair rat cognitive functions via cholinergic system modifications such as acetylcholine metabolism and AChE activation [9, 10]. Consistent with previous findings, $AlCl_3$-induced AD rats had higher AChE activity than NC rats [9, 10]. In our data, the AD-induced rats group in both experiments showed a significant increase in AChE levels when compared to the normal control group (figs 2&4).

Our findings are consistent with previous studies [37-39] that found $AlCl_3$ to increase AChE activity in the brains of rats and mice, but they contradict the findings of the most recent study [3], which found that $AlCl_3$ exposure resulted in a significant decrease ($P<0.0001$) in AChE activity, when compared to normal control group. Al can interact with plasma membrane lipids affecting the structure and function of several proteins [40]. The activity of membrane-associated enzymes may be affected by these changes in the membrane. [41]. According to Gulya *et al.* [42], an increase in AChE activity after aluminum exposure is caused by an allosteric interaction between the cation in the enzyme molecule's peripheral anionic site.This effect was reversed in rats co-administrated with$AlCl_3$ and Q (50 mg/kg BW) to $AlCl_3$-induced AD as well as treated AD-induced rats with Q50; the AChE level was similar to the control group (figs 2&4)which is in line with previous studies [43] which have shown that Q and lipoic acid significantly reduced AChE activity in the brains of $AlCl_3$ treated rats. As well as, it was demonstrated that Q could prevent the increase in AChE activity when compared to Cd/ethanol group[44]. It is possible that Q can improve synaptic transmission by inhibiting membrane-associated AChE, resulting in less free ACh hydrolysis. Thus, the rise in ACh could have a neuroprotective effectowing to the manipulation of the nerve impulse in the central nervous system. In addition, a recent study [45] discovered that the negative effect of $AlCl_3$ was reversed in the $AlCl_3$ + QT and $AlCl_3$ + QT-SPION groups. It was found that Q at a dose of 50 mg/kg body weight prevent the increase in AChE activity caused by diabetes in the cerebral cortex and hippocampus [46]. AChE inhibitors are the most commonly used medicines in the treatment of Alzheimer's disease. AChE inhibition increases acetylcholine levels, improves hippocampus cholinergic neurons, and reduces cognitive impairments, memory impairment, and Aβ plaque aggregation.[47]. These could be related to strong hydrogen bonds between the hydroxyl groups of Q and the amino acid residues at the AChE active site, resulting in inhibition of acetylcholine hydrolysis and increased acetylcholine levels in synapses. [48].

The dopaminergic system has been extensively examined as a critical neurotransmitter system linked with emotion and cognition, and is one of the neurotransmitter disorders that has been investigated in AD[49]. Several changes occur in the dopaminergic system as a result of neuropathological aging[50]. Dopamine acts on neurons via dopamine receptors (DRs), which are categorized into two types based on pharmacological and biochemical properties: D1-like and D2-like.[11]. Neurotoxic effects of intracerebral injection or systemic exposure to Al include neurofibrillary degeneration in animal brains[51].Our results found that AD-induced rats group showed a substantial drop in DA level in both the experiments when compared with the normal control group (figs 2&4)that is compatible with the recent study [52]that referred to DA level was significantly decreased in AD-like model rats.

It has been reported that Al modifies the biochemical pathway involving dopamine-hydroxylase[53] and reduces the dopamine level in rat brain[54]. Al treatment lowered DRD1 and DRD2 densities in the cortex of mice in a dose-dependent way at all three bregma levels, particularly in the Al group with the highest dose [55].

Post treatment of the AD-induced rats with Q50 as well as co-administration of $AlCl_3$ with Q50 caused a

significant elevation in DA levels compared to the AD-induced rats group (figs 2&4)that is in line with the previous study [56] which demonstrated that oral administration of Q moderately but significantly attenuates striatal dopamine loss and behavioral deficit. [57, 58] showed that oral Q treatment has been proven to be useful in lowering striatal dopamine loss. A probable mechanism of Q's beneficial effect in AD rats was the recovery of the malfunctioning of different neurotransmitters. As a result, the impacts of Q as a neuroprotective and therapeutic agent may be linked to targeting the most prominent neurotransmitters damaged by Alzheimer disease.

Conclusion

From the current study can conclude that the exposure to Aluminum chloride is more severe as it leads to a disturbance in the cholinergic function as well as areduction in the brain weight and body weight change. Quercetin (50 mg/kg) may be a promising natural neuroprotective and therapeutic agent against the onset of Alzheimer's disease symptoms. Co-administration of quercetin and $AlCl_3$ as well as the post-treatment of AD-induced rats with Q50 significantly restored dopamine levels and body weight change. Furthermore, it significantly reduced AChE levels while having no discernible effect on brain weight.

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