(Article)

Mitigation of Aluminum Chloride-Induced Alzheimer's disease by blueberry in rat model

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ABSTRACT

Alzheimer's disease (AD) is a global chronic neuro-disorder disease categorized by the lack of cognition and memory. Here, the present exploration was intended to reveal the ameliorative properties of Blueberry (BB) against aluminum chloride ($AlCl_3$)-provoked neurobehavioral and pathological changes in AD animals. Eighteen male rats were allocated into three groups. Control group; rats received saline, $AlCl_3$ group; rats received $AlCl_3$ (70 mg/kg/day orally) for 28 days and $AlCl_3$ + BB group, rats treated with BB (0.4 mg/kg/day, orally) for 28 days, 1 h after the injection of $AlCl_3$. At the end of the experiment, the behavioral tests (open field, Morris water maze, and novel object) were done as well as brain was separated for biochemical, and histological measurements. Treatment with BB amended changes in body weights and biochemical and histopathological alterations induced by $AlCl_3$. Moreover, BB significantly improved the behavioral impairments caused by $AlCl_3$. The current study revealed that BB may contribute to protecting the brain from Alzheimer's disease caused by aluminum intoxication.

Keywords: Alzheimer's disease; blueberry; Cognitive impairment; oxidative stress; inflammation.

1-Introduction

Alzheimer's disorder (AD) is a neuronal defect disease that severely impairs behaviors, memory, and cognitive function [1], [2]. It is characterized by degeneration in the cholinergic neurons found in the amygdala, hippocampus, cerebral cortex, and basal ganglia of the brain, which leads to reduced

synthesis and secretion of the neurotransmitter acetylcholine (ACh) deposition of amyloid beta (Aβ) and intracellular accumulation of hyperphosphorylated tau proteins form neurofibrillary tangles (NFTs) causing dementia [1], [3]. About 36 million persons globally in 2010 were influenced by AD with estimations to increase to 66 million by 2030 and 115 million by 2050 [4].

Aluminum (Al) has been reported as a major risk factor for the cause and development of neurodegenerative diseases such as AD [5]. Cosmetics, antacids, and inhaled fumes can insert aluminum in the hippocampus, cortex [6, 7], and cerebellum [8] of the brain which affects memory and cognition. Although synthetic drugs such as donepezil, galantamine, and memantine are able to ameliorate cognitive symptoms which are associated with many side effects, take over much economic burden, and are not applied for all cases of AD [9]. Therefore, there is a request for detecting new medication of natural origin obtained from medicinal plants and with fewer side effects for the prevention and treatment of AD.

Blueberry is a small fruit derived from plants of the genus Vaccinium, belonging to the family Ericaceae [10]. It is among the edible fruits that are considered best for their potential health benefits. BB is an excellent source of bioactive compounds and has a high percentage of antioxidants represented as polyphenols [11]. The polyphenol content and the antioxidant activity are high in the leaves of the blueberry plant[12]. BB has been reported with a wide range of therapeutic activities, such as antioxidant [11], anti-inflammatory [13], and neuroprotective activities [14]. It has been indicated to ameliorate the risk of diabetes [15], obesity [16], aging [14], urinary tract infections [17] and cancer [18]. The present study was carried out to investigate the possible therapeutic effects of blueberry in Alzheimer's disease induced in rats by using $AlCl_3$.

2. Experimental
2.1 Chemicals

Blueberry was bought from Powbab, USA, and $AlCl_3$, and other chemicals were purchased from Sigma-Aldrich, USA. All the examination kits for biochemical analysis were almost of high analytical rank.

2.2. Experimental animals

Male Wister rats weighing 150–170 g were applied in this current research and were obtained from the animal facility of the faculty of pharmacy, Egyptian Russian University (Cairo, Egypt). All rats

were placed in clean cages with temperatures of 25 °C and 12 h light/dark series. Rats were provided with a free approach to water and food throughout the experiment and were adapted to the surrounding environment for one week before the experimental procedures. Experimental protocol was performed following the international guide for the care and use of research animals and approved by the institutional Ethics Committee of the Faculty of Pharmacy, Egyptian Russian University, Egypt, (approval number is: ERUFP-PO-23-001).

2.3. Experimental design

Eighteen male rats were weighed (body weights (BW) were recorded) and randomly alienated into three groups (each containing 6 rats) as group I-III. Group I is the control group, rats administrated saline (orally) for 28 days. Group II is the $AlCl_3$ group, rats administrated $AlCl_3$ (70 mg/kg, orally) dissolved in saline [19] for 28 days. Group III is $AlCl_3$ +BB treatment group, rats received 0.4 gm/kg blueberry powder dissolved in saline (orally) [20], 1 hour after injection of $AlCl_3$ (70 mg/kg, orally) for 28 days.

At the end of the experiment, rats were weighed (final body weights (BW) were recorded) and anesthetized using phenobarbital (40mg/kg) [21] then sacrificed by cervical dislocation followed by decapitation. The brain was dissected and the cortex and hippocampus were excised, washed with isotonic saline, and immediately frozen at –80 until homogenized for measuring the brain tissue content. Brains from the normal control and treated animals were separated from 2 random rats from each group for histopathological examination.

2.4. Behavioral tests

Open-field test

Open field test usually identifies the alterations in the locomotor activity. The experiment was performed on a square wooden box with $80 \times 80 \times 40$ cm measurements with black floor and red walls disconnected by white lines with 16 similar squares at 4×4 cm measurements. Animals were allocated separately in the apparatus center and cautiously monitored for 5 min in each trial. The number of squares passed (floor units that rats passed with both feet) was considered a measure of locomotor activity. Vertical activity, which was enumerated by the number of rearing movements (the animal stood on its hind limbs), was used to detect exploratory behavior. The ambulation frequency

calculation and the investigative rearing numbers were measured and utilized to determine the changes in the investigative capacity. After each tested animal, the wooden floor was cleaned.

Morris water maze (MWM) test

MWM test was used to assess the memory and spatial learning of the rats. A dark color pool with 180 cm diameter and 50cm deepness was used for this experiment. The apparatus is a large circular pool with dimensions 1.5 m in diameter, and 0.6 m in height. It was divided into 4 equal quadrants using white threads affixed to the rim of the apparatus. The level of water was kept at 35 cm height in the apparatus and the temperature was kept constant at 25 ± 2 °C. In addition, a movable round platform of 9 cm in diameter was introduced in the middle of one of the four quadrants (target quadrants) 1 cm below the water surface for the training phase. In the training phase, rats were trained 3 days before the test day, where the animals swam freely from various starting positions to find the platform for 120 seconds. In the test phase, a soluble dark blue non-toxic dye was used to make the water opaque and the platform was removed from the apparatus. The rats were left to swim starting from the quadrant facing the selective quadrant for 1 min. The time consumed by the rats in the selective quadrant was measured.

Novel object test

A novel object test was carried out to determine the recognition memory of the rats. It is dependent on the native ability of the rats to explore new objects over the familiar ones. The apparatus is made of a black open field box with dimensions 50 cm wide $\times$ 60 cm long $\times$ 80 cm high. The test consisted of three phases. In the habituation phase, rats were placed in the empty box for 10 minutes for exploration. In, Phase Two which considered the training phase, rats were placed in the test box with two identical objects placed opposite to each other in two opposite corners, 6cm from the walls. The rats were allowed to explore both objects for 3 mins. In the test phase, rats were placed into the box with one of the familiar objects replaced with a novel one that was different in shape, size, and color. The test ran for 3 minutes, and an overhead camera was used to determine the time spent by the rats to discover both objects during test sessions. Discrimination index, the variation in time spent discovering familiar and novel objects over the total time spent discovering both objects was calculated. The results can vary between +1 and -1, where a positive value shows more time spent exploring the novel object, a negative value indicates more time spent with the familiar object, and a zero score specifies a null preference.

2.5. Measurement of acetylcholine esterase (AChE) activity

The activity of AChE in the brain tissues of control and treated animals was measured according to the manufacturer's procedures of commercially available kits (Cat no. MBS2709297 MyBioSource, sunny Southern California, San Diego, USA). The results were stated as ng/mg/protein.

2.6. Detection of oxidative stress and antioxidant markers

The oxidative stress marker malondialdehyde (MDA) and level of the antioxidant glutathione (GSH)) of control and treated rats were detected in the brain tissues according to the manufacturer's protocol of commercially available kits (Cat no. MD 25 29 and cat no. GR 25 11 Bio diagnostic, Giza, Egypt) respectively. The values were expressed as nmol/mg protein.

2.7. Quantification of inflammatory markers

ELISA assay kit (Cat no. SEA079Ra Cloud-Clone Corp, Houston, USA), was used for the determination of (IL)-6 of control and treated animals in the brain tissues. The values were expressed as pg/mg/protein.

2.8. Quantification of Glutamate content

ELISA assay kit (Cat no. MBS756400 My BioSource, sunny Southern California, San Diego, USA), was used for determining the glutamate of control and treated animals in the hippocampus. The values were expressed as ng/mg/protein.

2.9. Histopathological study

About 10 ml of 10% formalin was added to the brains of different groups for 72h with the replacement of formalin solution every day. Test samples were washed, dried out in pure grades of ethanol, cleaned in xylene, and put in paraffin. Sections of the cerebral cortex and hippocampus were colored with hematoxylin and eosin for histopathological examination.

2.10. Statistical analysis

All data were expressed as mean ± S.D. Comparison among groups was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test. Data for ambulation and rearing frequency were stated as median and analyzed using the Kruskal–Wallis test followed by Dunn's multiple

comparison test. P < 0.05 was considered statistically significant. All statistical analysis and graphs were carried out using GraphPad Prism version 8.0.2 software.

3-Results

3.1. Body weight

Regarding the body weights of the animals, chronic exposure to $AlCl_3$ only caused a significant reduction of weight by 15.29% at the end of the model period. Such a decrease in body weight in the $AlCl_3$-induced AD was significant compared to the control group and BB + $AlCl_3$ group after 28 days. While the control group didn't show any decrease in body weight by the end of the period, oppositely, there was a significant increase in the animal's weight by 15.38%. Moreover, such an increase was also significant when correlated to the BB + $AlCl_3$ group. Interestingly, the weight of rats in the blueberry-treated group elevated by only 6.54% when weighed after 28 days (Table 1).

Table 1. Effect of blueberry on $AlCl_3$-induced body weight changes in AD rat model.

Control		Induction		Blueberry	
Before	After 28 days	Before	After 28 days	Before	After 28 days
176 ± 5.96	203.08 ± 12.63	168.83 ± 6.70	143.03 ± 9.73[#]	172.17 ± 10.55	184.22 ± 11.29[#*]

Data are illustrated as mean ± SD (n=6), # or *: are the significant from the control or $AlCl_3$ induced AD group, respectively at P < 0.05 by one-way analysis of variance ANOVA followed by Tukey-Kramer as a post-hoc test.

3.2. Behavioral tests

The results from the behavioral tests revealed that there was a notable disruption in the recognition, locomotor activity, memory, and spatial learning in the induction group compared to the control and treatment groups. For instance, in the open field test, there was a reduction in the locomotion of the induction group when compared to the control and treatment groups. It was observed by the decrease in the ambulation and rearing frequency by 63.37% and 77.65%, respectively when compared to a control group. Interestingly, compared to the induction group, pretreatment with BB revealed marked improvement in the locomotor activity, where the ambulation was enhanced by 1.98 folds while the rearing frequency increased by 3.35 folds [Figure 1]. On the other hand, the results showed that the memory and learning processes in the $AlCl_3$-induced AD rats in the MWM test were noticeably altered. The time taken in the target quadrant was significantly reduced in the induction group by

53.65%. However, the pretreatment with blueberry significantly increased the time spent by the rats in the target quadrant by 83.94% when correlated to the induction group [Figure 2]. Figure 3 shows that by using the novel object recognition test, $AlCl_3$-treated rats displayed a significant decrease in the recognition activity, based on the ability to explore the novel object. In which, they have taken less time discovering the novel object than the familiar one (17.74%). However, in the control and blueberry-treated groups, the rats spent more time exploring the novel object than the familiar object (1.96 folds and 1.67 folds) respectively. In addition, there was a significant decrease in the discrimination index when comparing the $AlCl_3$-induced group with the control and treatment groups. The discrimination index of the $AlCl_3$-induced group was decreased by 2.85 and 2.2 folds when compared with the control and treatment groups, respectively. In accordance, the total time exploring both objects was significantly reduced in the induction group by 2.26 folds when compared with the control group and 1.68 folds when compared with the treatment group [Figure 3].
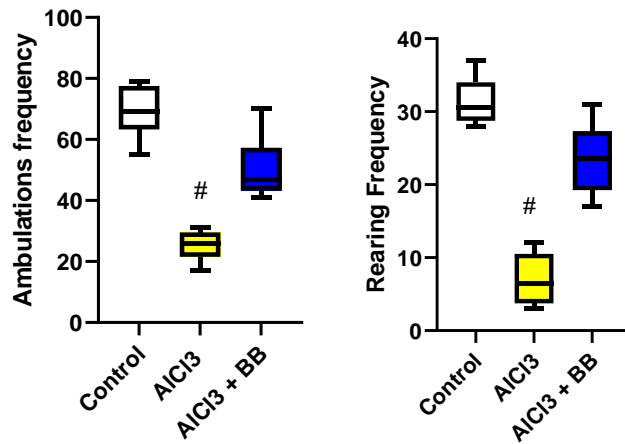
Figure 1: The Effect of Blueberry on $AlCl_3$-induced behavioral changes in AD rat model by open field test. Data of the results are performed as median (n=6), # or *: are the significant from the control or $AlCl_3$ induced AD group, respectively used at $P < 0.05$ by Kruskel–Wallis test followed by Dunn's as a post-hoc test.
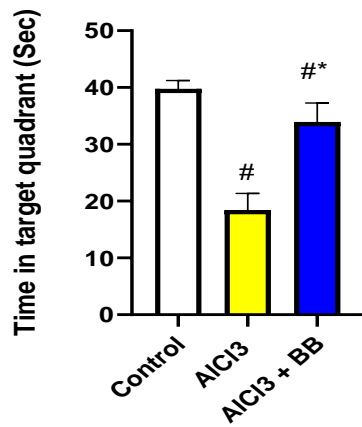
Figure 2: Effect of Blueberry on $AlCl_3$-induced behavioral changes in AD rat model by Morris maze test. Data of the results are performed as mean ± SD (n=6), # or *: are the significant from the control or $AlCl_3$ induced AD group, respectively used at $P < 0.05$ by one-way analysis of variance ANOVA followed by Tukey-Kramer as a post-hoc test.
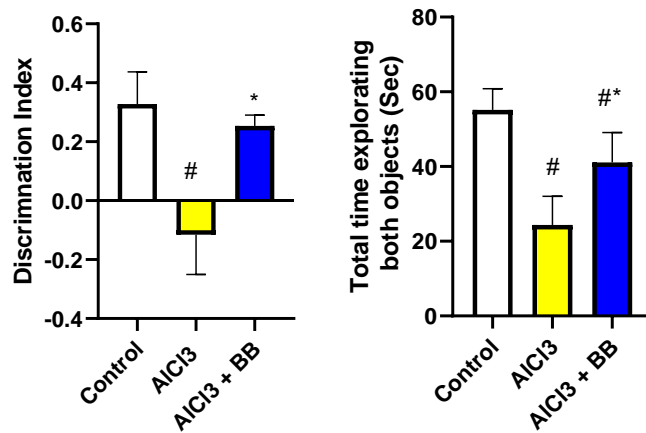
Figure 3: Effect of Blueberry on AlCl₃-induced behavioral changes in AD rat model by novel object recognition test. Data of the results are performed as mean ± SD (n=6), # or *: are significant from the control or AlCl₃ induced AD group, respectively used at P < 0.05 by one-way analysis of variance ANOVA followed by Tukey-Kramer as a post-hoc test.

3.3 Oxidative stress and antioxidant markers

There was an obvious change in the levels of oxidative stress and antioxidant markers in the rat's hippocampus. In the AlCl₃-induced AD group, the MDA level was significantly increased, while the status of GSH was significantly decreased. Compared to the induction group, pretreatment with blueberry significantly reduced MDA and elevated GSH brain contents but did not reach the control level [Figure 4].
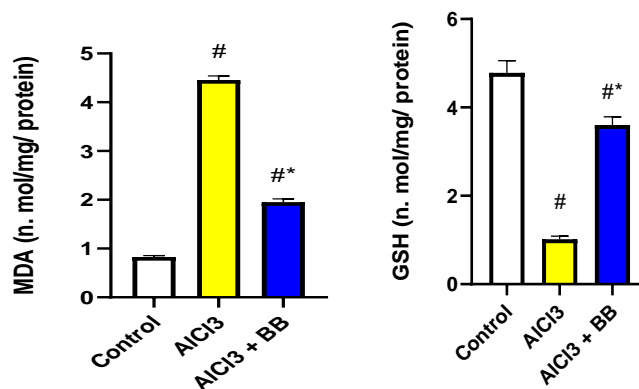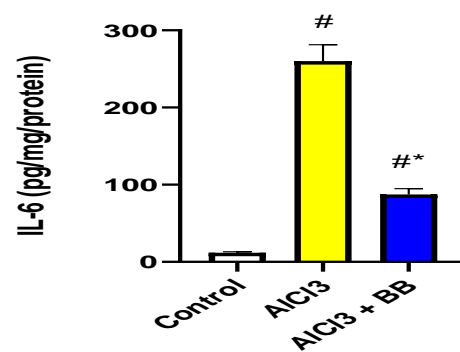

Figure 4: Effect of blueberry on the MDA and GSH contents in the hippocampus of AD rat model. Data of the results are performed as mean ± SD (n=6), # or *: are the significant from the control or AlCl3 induced AD group, respectively used at P < 0.05 by one-way analysis of

3.4. Inflammatory marker

The amount of the inflammatory mediator IL-6 was quantified to clarify the impact of AlCl₃ and subsequent treatment using blueberry. Figure 5, indicates that the content of the inflammatory mediator IL-6 was significantly increased in the AlCl₃ provoked AD group. However, the administration of BB significantly reduced the level of IL-6 compared with the induction group.

Figure 5: Effect of blueberry on the IL-6 levels in the hippocampus of AD rat model. Data of the results are performed as mean ± SD (n=6), # or *: are the significant from the control or AlCl3 induced AD group, respectively used at P < 0.05 by one-way analysis of variance ANOVA followed by Tukey-Kramer as a post-hoc test.

3.5. Glutamate

One of the main causes of AD is the imbalance between the levels of inhibitory and excitatory neurotransmitters such as glutamate. AD induction using AlCl$_3$ caused a significant upregulation of the glutamate content. On the other hand, treatment using blueberry attenuated this effect and significantly decreased the glutamate level in the rat's hippocampus compared to the induction group. Nevertheless, the glutamate content in the control group was significantly lower than in both groups [Figure 6].
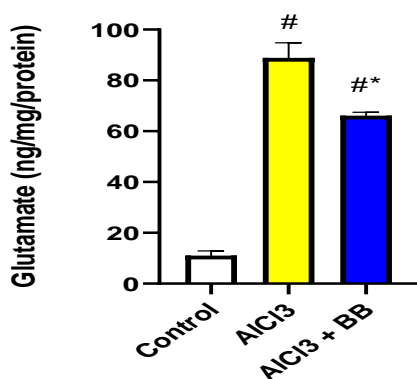
Figure 6: Effect of blueberry on the glutamate levels in the hippocampus of AD rat model. Data of the results are performed as mean ± SD (n=6), # or *:are the significant from the control or AlCl3 induced AD group, respectively used at P < 0.05 by one-way analysis of variance ANOVA followed by Tukey-Kramer as a post-hoc test.

3.6. AChE

The status of AChE activity was markedly elevated in the hippocampus of the AlCl$_3$-provoked AD [Figure 7]. It's interesting to note that adding blueberry to the rats exposed to aluminum chloride significantly improved AChE activity. Comparing the outcomes in the AChE activity between the control and the treated group, there was a significant difference between the normal content of AChE in the control group and that of the treated group.
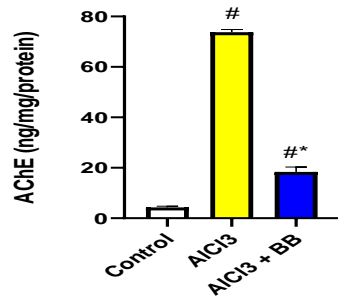
Figure 7: Effect of blueberry on the AChE activity in the hippocampus of AD rat model. Data of the results are performed as mean ± SD (n=6), # or *: are significant from the control or AlCl₃ induced AD group, respectively used at P < 0.05 by one-way analysis of variance ANOVA followed by Tukey-Kramer as a post-hoc test.

3.7. Histopathology

Histopathological examination of the control group showed an ideal cerebral cortex and ideal hippocampus. On the contrary, the AlCl₃-induced AD group exhibited variable histopathological changes in both sections.
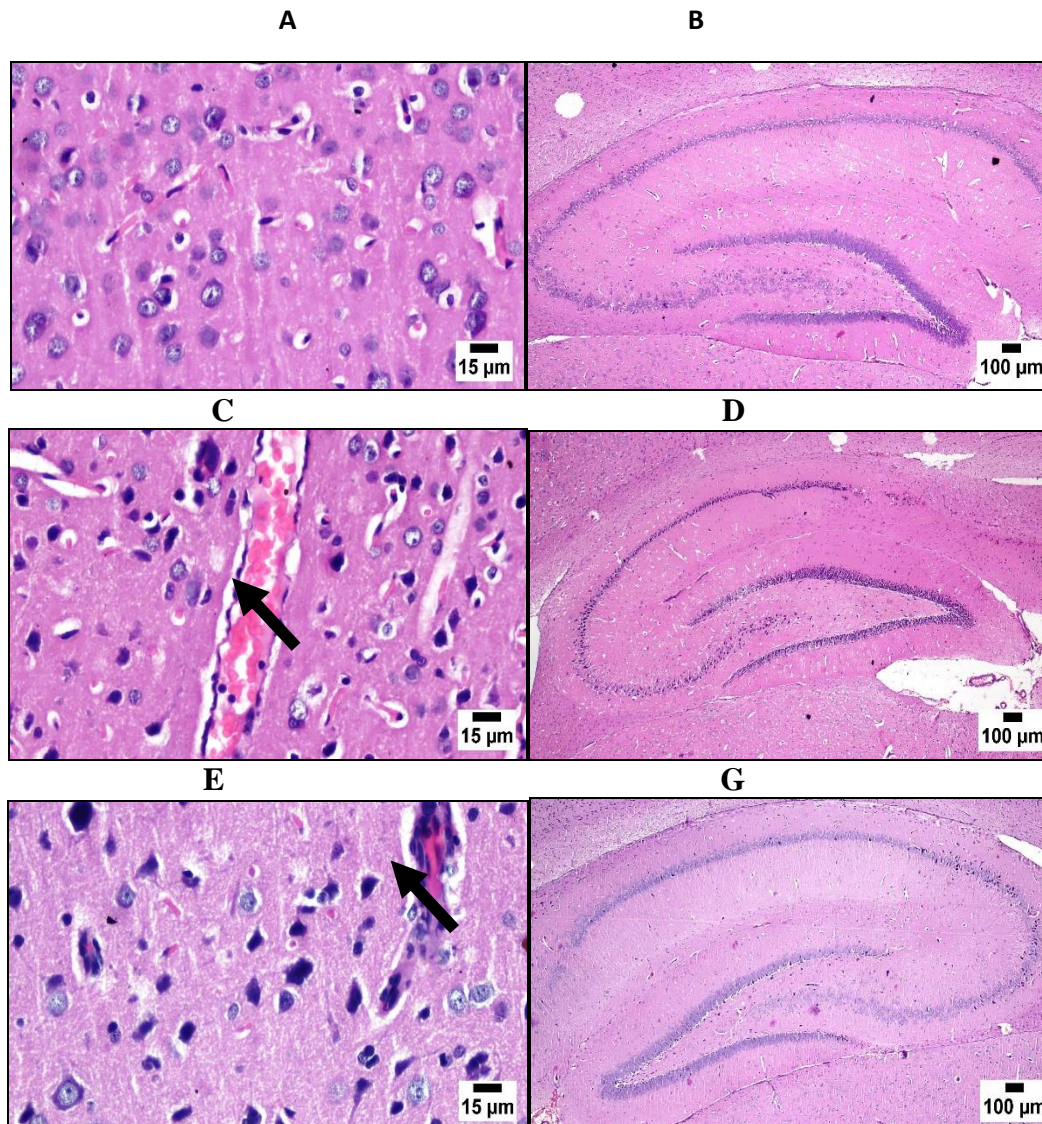
Figure 8: Effect of blueberry on cerebral cortex and hippocampus histopathology in AD rats. Control rats revealed normal histology of both cerebral cortex and hippocampus (A and B), respectively. In the AlCl$_3$-induced AD group, the cerebral cortex showed numerous dark degenerated neurons scattered in the cerebral cortex with congested blood vessels (C) and the hippocampus demonstrated numerous dark degenerated neurons (D). Marked improvement was noticed in BB BB-treated group, the cerebral cortex showed limited vasculitis in the cerebral cortex with a moderate number of degenerated neurons (E), while the hippocampus showed normal neurons (F).

The cerebral cortex showed diffuse gliosis accompanied by mild vasculitis. While the region of the hippocampus revealed dark degenerating neurons. Moreover, for the blueberry-treated group, the cerebral cortex indicated a moderate number of dark degenerated neurons with limited vasculitis, while the hippocampus showed normal neurons in all regions. [Figure 8].

4. Discussion

Aluminum (Al) is reported as a potent neurotoxin that has a role in dementia [22] and AD [23]. It can cross the blood-brain barrier and stimulate free radical production, which causes brain oxidative damage and neuronal inflammation leading to brain damage in areas responsible for memory and learning [5], [24]. In the same context, AlCl$_3$ intoxication stimulated severe oxidative stress by elevating MDA in the brain and reducing the antioxidant GSH in the current study. On the other hand, BB results in a significant decrease in MDA and a significant increase in GSH compared to the AlCl$_3$ group. It was reported that BB contains anthocyanin polyphenol which was previously used for the treatment of different type of neurodegenerative diseases including AD [25], [26]. Those results were confirmed by the histopathological examination where AlCl$_3$ administration showed a deviation from the normal picture meanwhile BB markedly alleviated the damage in the cerebral cortex and the dark degeneration in neurons of the hippocampus.

The present study shows that using AlCl$_3$ showed a significant increase in the levels of IL-6 and BB administration ameliorated this effect. Al crosses the blood-brain barrier and deposits in various regions of the brain tissues, leading to the deposition of Aβ, an abnormal extracellular protein [27], which is upstream to increase IL-6 production [28]. In addition, the deposition of Aβ induces microglial chemotaxis and provides a chronic stimulus to microglial cells contributing to the ongoing inflammatory process [29]. IL-6 has been negatively involved in memory formation, as prevention of IL-6 enhances long-term memory in a hippocampus-dependent task [28]. BB polyphenols can suppress oxidative stress and counteract the cytotoxic effect of Aβ in mice [30]. Extracts of BB apply

neuroprotective effects against Aβ neurotoxicity in hippocampal cell cultures of rats, with a lower average of neuronal loss [31].

Glutamate toxicity has been indicated to induce the pathogenesis of AD [32], [33]. Excitatory amino acid transporters (EAATs) such as Glutamate transporter-1 (GLT-1) [34], [35], which separate glutamate from the extracellular space, are required to prevent the toxic effects related to extracellular glutamate accumulation. Reduction in GLT-1 expression can decline glutamate clearance and stimulate excitotoxic cell death which is often considered to be the overactivation of postsynaptic NMDA receptors [36], [37]. In the current results, $AlCl_3$ administration was found to elevate brain glutamate concentration. This finding was in line with the results of Abdel-Zaher et al [38]. Moreover, the activation of NMDA receptors by glutamate was found to increase oxidative stress [39] and inflammatory cytokine liberation in rat brains [40]. On the other hand, coadministration of blueberry with $AlCl_3$ in rats significantly decreased glutamate compared to $AlCl_3$-treated rats. BB anthocyanins exert powerful neuroprotective activity which might be due to a direct scavenging activity and enhance endogenous antioxidants [41].

There is a correlation between increased brain glutamate levels and cognitive impairments in experimental animals [42], [43]. Also, several observations support the hypothesis that oxidative stress might contribute to the development of cognitive dysfunction [44], [45]. Thus, the increase of glutamate contents in the brain plays an important role in learning and memory dysfunction induced by $AlCl_3$ administration. Our results clearly showed that $AlCl_3$ administration induced behavioral dysfunction. Open field, Morris water maze, and novel object recognition tests were used to evaluate motor, behavioral, and cognitive alteration induced by $AlCl_3$ exposure. In the open field test, there was a decrease in the locomotive capacity of the $AlCl_3$-induced AD group, when compared with a control group, by the lowering in the ambulation and rearing frequency. This observation was in line with the findings of a previous study [46]. On the other hand, the results of the MWM test showed disturbances of the memory and learning processes in the $AlCl_3$-provoked AD rats, through decrease in the time spent at the target quadrant. Moreover, $AlCl_3$ treated rats displayed a significant decrease in the recognition activity, as well as a significant decrease in discrimination index (DI) was observed in the novel object recognition test. Oxidative stress induced by Al decreases the production of neurotransmitters and the sensitivity to different neurochemical systems, including the cholinergic system, that affects the regulation of cognitive functions [47]. These results are in accordance with studies that demonstrated behavioral impairment induced by $AlCl_3$ [46]. Blueberry-supplemented rats

showed a better performance concerning spatial learning and working memory with a faster learning rate and improvement of locomotion activity and exploratory behavior. These results are in agreement with those of Ramirez et al. [48] and Rendeiro et al. [49].

Acetylcholine is one of the major excitatory neurotransmitters in the brain and is highly linked to AD. Previous studies reported that at the early phase of Alzheimer's, cholinergic abnormalities were observed, while most cholinergic neurons are lost in the late phase [50]. Our results indicated that $AlCl_3$ administration significantly elevated AChE activity which is the key enzyme involved in the hydrolysis of ACh. These results are corroborated with the results of Zheng et al. [51]. Al can interact with the peripheral sites of AChE alter its secondary structure and enhance its activity [52]. Treatment with blueberry provides neuroprotection by reducing the activity of AChE. It is possible that the anthocyanins contained in blueberries together have synergistic effects in neuroprotection by exhibiting considerable acetylcholinesterase inhibitory activity [53].

5. Conclusions

The current study highlighted the therapeutic effects of BB supplementation against $AlCl_3$ exposure, indicated by the improvement of behavioral function, and amelioration of oxidative stress, IL6, glutamate, and ACHE levels in $AlCl_3$-treated rat brain, thereby preventing $AlCl_3$ brain toxicity. Clinical studies are required to evaluate the potential neuroprotective effect of blueberry supplementation in AD patients.

• Acknowledgment

• Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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