Protective Effect of Some Foods Rich in Choline, Selenium and Flavonoids from Alzheimer's in Mice

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Abstract

Background: Choline, selenium and flavonoids are identified as potential antioxidants that can detoxify different Reactive Oxygen Species (ROS) in neurological diseases.

Aim: This study aimed to evaluate the possible protective effects of some foods rich in choline, selenium and flavonoids from Alzheimer's in mice.

Methods: Memory impairment was induced by aluminum chloride $\text{AlCl}_3$ 40 mg/kg (BW) Intra peritoneal (IP) daily for six weeks. To study the activity of foods rich in choline, selenium and flavonoids with $\text{AlCl}_3$. Using some behavioral tests.

Results: The results showed a significant increase in Acetyl cholinesterase (ACHE) as well as a Significant decrease in Superoxide dismutase (SOD) and catalase (CAT) in the positive control group. In addition to the increase in the time of learning and the number of trials in behavioral tests. hippocampal nerve degeneration was also detected. Limit foods rich in choline, selenium, and flavonoids effects of degraded aluminum on biochemical analyzes as well as on memory and learning impairment. Moreover, a healthy hippocampus was also shown in the treated groups compared control groups.

Conclusion: Foods rich in choline, selenium, flavonoids and the mixture were more effective in minimizing the hazards of Alzheimer’s disease (AD). However, the mixture treatment has more pronounced protective effects than each of them alone.

Keywords: Alzheimer's disease, Aluminum Chloride, Choline, Selenium, Flavonoids and Behavioral Tests.
Introduction

Alzheimer’s disease is a progressive neurodegenerative disease most often associated with memory deficits and cognitive decline (DeTure and Dickson, 2019). That leads to nerve cell death and tissue loss throughout the brain. Over time, the brain shrinks dramatically, affecting nearly all its functions (Adlard et al., 2009).

Aluminum (Al) is the third most abundant element with the global industrialization and consequent pollution. Excessive Al intake might lead to deposition of amyloid beta (Aβ) in central nerve cells and overexpression of its precursor protein (Campbell et al., 2004 and Exely, 2005).

Promotes many studies of antioxidants in the prevention of Alzheimer’s disease (Viña et al., 2011). Choline is a micro and essential nutrient that shares a key role in multiple metabolic pathways related to the nervous system’s structural and functional integrity (Bonetti et al., 2017).

Soy milk is a rich source of choline (Zeisel et al., 2003). Selenium (Se) compounds have been reported to have great potential in the prevention and treatment of Alzheimer’s disease (Zhang and Song, 2021). Selenium (Se), a nutritionally essential trace element with known antioxidant potential, protects the brain from oxidative damage in various models of neurodegeneration (Ishrat et al., 2009).

Bananas and coconuts are among the foods rich in selenium (Șlencu et al., 2012).

Moreover, the interest in the consumption of flavonoids has been addressed due to their potential neuroprotective effects to prevent different diseases related to the nervous system such as Alzheimer, Parkinson and slow down cognitive decline, which can generate dementia (Grassi et al., 2016). Cocoa is a rich source of dietary polyphenols mostly from the subclass of flavonoids called flavanols (Lee et al., 2003).

Fortunately, the body can ingest choline and selenium from the natural source better than another chemical sources (Rodríguez et al., 2019 and Patterson et al., 2008). In addition diets rich in flavonoids (at low concentration) were shown beneficial effect to maintaining human cognitive functions to promote improvements in memory and protect vulnerable neurons (Huntley, 2009). So, the aim of this study, evaluate the possible protective effects of some foods rich in choline, selenium and flavonoids from Alzheimer’s in mice.
Materials and Methods

Materials:

Basal diet includes: casein, vitamins, minerals, cellulose and choline chloride were obtained from El-Gomhorya Company, Cairo, Egypt. And the food items used in the experiment are soybeans, dried coconut, banana and dark cocoa were purchased from the local market, in Cairo, Egypt. While, the chemicals such as aluminum chloride hydrated (ALCL₃) was obtained from the international Company, in Cairo, Egypt. And kits for biochemical analysis obtained from Alkane for pharmaceutical and chemical in Dokki, Egypt. In addition to male mice (albino mice) weighting 25 ± 5 g were obtained from the National Research Center in Giza, Egypt.

Methods:

Has been calculated the needs of mice from choline, selenium and flavonoids from natural source as soymilk, yellow bananas + dried coconuts and dark cocoa, respectively.

Table (1): Ratios of materials used in treatment.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Documented Ratio</th>
<th>Source</th>
<th>Food Content of Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline</td>
<td>2.5 mg/kg BW</td>
<td>Soymilk</td>
<td>(100g ➔ 23.5 mg)</td>
</tr>
<tr>
<td></td>
<td>(Chan et al., 2010)</td>
<td></td>
<td>(Gossell-Williams et al., 2005)</td>
</tr>
<tr>
<td>Selenium</td>
<td>(1 mg/kg BW)</td>
<td>Dried coconuts and Bananas</td>
<td>(1g ➔ 93 ng / 1g ➔ 24 ng )</td>
</tr>
<tr>
<td></td>
<td>(Lakshmi et al., 2015)</td>
<td></td>
<td>($lencu et al., 2012)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>(22.9 mg/kg BW /day)</td>
<td>Dark cocoa</td>
<td>(100g ➔ 52.73 mg )</td>
</tr>
<tr>
<td></td>
<td>(Sokolov et al., 2013)</td>
<td></td>
<td>(Kozlowska and Szostak-Wegierek, 2014)</td>
</tr>
</tbody>
</table>

Preparation of the injection material for Alzheimer's disease:

Mice were induced to develop Alzheimer's disease by intraperitoneal injection of daily freshly prepared (ALCL₃) (40 mg/kg body weight) (Abdulmalek et al., 2015) dissolved in normal saline (0.9% NaCl) (4 mg/ml) for 6 weeks (Saba et al., 2017).
Bilological Experiment:

Thirty six male albino mice, weighing about (25±5g) were purchased from National Research Center. These mice were divided into two main groups as follow:

First main group  kept as negative control group (6 mice) were fed on basal diet.

The second main group (30 mouse) kept as positive group, were divided into 5 subgroups as follows:

Subgroup 1 positive control group (6 mice) were fed on basal diet + ALCL3 (IP) 40 mg /kg BW.

Subgroup 2 (6 mice) were fed on basal diet and given orally Soymilk 0.32 ml /kg BW + ALCL3 (IP).

Subgroup 3 (6 mice) were fed on basal diet supplemented with Banana pulp 1.63g /kg BW + Dried coconut 2.5g / kg BW + ALCL3 (IP).

Subgroup 4 (6 mice) were fed on basal diet supplemented with Dark cacao 1g /kg BW + ALCL3 (IP).

Subgroup 5 (6 mice) were fed on basal diet supplemented with Mixture of natural foods 0.16 ml, 0.815mg, 1.25g and 500mg / kg BW+ ALCL3 (IP).

During the experiment in the fifth week of the experiment behavioral tests were performed on mice using the following behavioral tests (Y-Maze – Novel Object Recognition- Morris Water Maze) to measure the level of learning and reference memory. At the end of the experimental period (6 weeks). After performing the behavioral tests and the end of the experimental period, collection of blood samples and removed body organs were done.

Evaluation of Behavioral Parameters:

-Y-maze test
The test was performed according to (Arai et al., 2001).

- Novel object recognition test
The experiment was carried out, according to (Bevins and Besheer, 2006).

-Morris water maze test
The MWM test was performed according to (Nunez, 2008).
Biological Evaluation:
At the end of the mice experimental period, Biological evaluation of Relative organs weight (ROW) for brain, liver and kidney calculated according to Drury and Walligton, (1980) using the following formulas:

Biochemical analysis of tissue:
Determination of neurotransmitter activity in brain tissue
- Determination of Acetyl cholinesterase (ACHE) activity was assayed by ELISA method using acetyl cholinesterase activity assay kit purchased from Bio source CO.

Determination of enzymatic antioxidants activity in brain tissue:
- Determination of Catalase (CAT) activity was assayed using CAT activity assay kit purchased from bio diagnostic CO. Tissue CAT activity was determined according to the Colorimetric method described by Aebi, (1984) and Fossati et al., (1980).
- Determination of Superoxide dismutase (SOD) activity Assay Kit was using made by biodiagnostic CO. Tissue SOD activit was determined according to the Colorimetric method described by Nishikimi et al., (1972).

Histopathology Study:
Histopathological were performed for brain according to (Banchroft et al., 1996).

Statistical Analysis:
Results of biological evaluation of each group were statistically analyzed (mean ± standard deviation and one-way ANOVA test) by using SPSS (Version 26). package and compared with each other using the suitable test (least significant differences at P< 0.05) according to (Sendecor and Cochran, 1979).

Results and Discussion:
Relative Organs Weight (ROW) % Brain, Liver and Kidney:
The results illustrated in table (2) showed that the protective effect of some foods rich in choline, selenium and flavonoids on relative organs weight (ROW) % in Alzheimer's mice model. The results showed that the mean value of relative brain weight % for positive control group was lower than negative control group. Even, there were statistical significant differences observed between them at P<0.05. May be due to, presence of nuclear penetration and degeneration of neurons and The neurons showed intracytoplasmic vacuolization with diffuse gliosis in areas (Hippocampus and Striatum) of the brain, which was explained by
histopathology examination of brain tissue. It was consistent with the fact that the brain atrophy or shrinkage occurs due to the loss of cells as a result of inflammation. *(Gordon et al., 2018).* Moreover, these results were agreed with *(Almuhayawi et al., 2020)* whom reported that, Brain weight/body weight percent of the AD rat model revealed a statistically significant decrease (0.429 ± 0.015) *(P < 0.05)* in comparison to the control (0.658 ± 0.02).

On the other hand, all treated groups showed improvement in relative brain weight as compared to the positive control groups. But, the best result was recorded for the group that ate a mixture of natural foods. Accordingly, there were no statistical differences as compared to the negative control group. These results were agreement with *(Kwan et al., 2021)* whom showed that, in males, neither PAE nor choline significantly affected the absolute body and brain weights. Thus reducing brain-to-body weight ratio.

As regard, the result showed that the mean value of relative Liver weight % for positive control group was lower than negative control group. Even, there were statistical significant differences observed between them at P<0.05 level. While, the mean value of relative kidney weight % for positive control group approximately similar result compared to negative control group. Even, there were no statistical significant differences between them. This result agreed with *(Hosseini et al., 2020)* whom reported that, the lowest liver weight was observed in the ALCL₃ treatment group *(P < 0.05)* as compared to the other group. Moreover, *(Mahmoud and Elsoadaa, 2013)* whom reported that, Treated rats with ALCL₃ for 8 weeks increased the relative weight of kidney significantly.

**Table (2): Protective Effect of Some Foods Rich in Choline, Selenium and Flavonoids on relative organs weight (ROW %) (Brain, Liver and kidney) in Alzheimer's mice model.**
Result are expressed as mean ±SD. Values in each column have different letters are significantly differenced at (p<0.05).

All treated groups for relative Liver weight % were showed improvement compared to the positive control group. While treated groups for Relative Kidney weight % were lower than control groups. The best result for Relative Liver and kidney weights % was recorded for group treated with food rich choline compared to the negative control group. Accordingly, there were statistical no significant difference. These results agreement with Steane et al., (2021) whom reported that, Choline supplementation resulted also increased fetal liver weight and decreased fetal brain independent of alcohol exposure. Moreover, Sengul et al., (2021) whom reported that, during the experiment, the liver weights of the acrylamide (ACR) and Selenium 0.5 + ACR groups were decreased significantly as compared to the control.

**SOD, CAT and ACHE levels in Brain Tissue:**

Factors of lifestyle affect the risk of an individual developing AD, including dietary habits and exposition to environmental and occupational threats (Newcombe et al., 2018). Antioxidant enzymes such as SOD and CAT are the primary defense system that prevents biological Large molecules of oxidative stress (Deepa et al., 2017). The data in Table (3)
showed that protective effect of some foods rich choline, selenium and flavonoids on catalase (CAT), superoxide dismutase (SOD) and acetyl cholinesterase (ACHE) in Alzheimer's mice model. The results illustrated the levels of CAT and SOD for positive control group much lower than negative control group. While, level of ACHE was much higher than negative control group. Even, there were statistical significant differences observed between them at P<0.05 level. These results agreed with Boussadia et al., (2020) whom reported that, the effect of chronic ALCL$_3$ administration induced a significant reduction of Catalase (CAT) activity. Moreover, these results agreed with Rather et al., (2018) whom reported that, treated with ALCL$_3$ for 42 days showed a significant increase in the levels and activities of the ACHE.

On the other hand, all treated groups have CAT, SOD and Ache levels showed improvement compared to the positive and negative control groups. Even, there were statistical significant difference observed between them at P<0.05 level. The best result was recorded for group treated with mixture of natural foods as compared with the negative control group. Accordingly, there were statistical significant difference observed between them at P<0.05 level. These results were consistent with the fact that antioxidants had their protective roles in food against oxidative deterioration processes (Gulcin, 2020). Also, Doungue et al., (2018) whom reported that, the activities of catalase .

Table (3): Protective Effect of some Foods Rich in Choline, Selenium and Flavonoids on catalase (CAT), Superoxide dismutase (SOD) and Acetyl cholinesterase (ACHE) in Alzheimer's mice model.
Result are expressed as mean ±SD. Values in each column have different letters are significantly differenced at (p<0.05).

superoxide dismutase (SOD) level were significantly higher in the treated than that in the untreated than that in the untreated Alzheimer’s rats positive control (PC) groups (P<0.05), following the administration of juice and flavonoid fraction (both doses).Moreover, Nkpaa et al., (2021) whom reported that, control-treatment with Selenium at 0.2 and 0.4 mg/kg significantly (p<0.05) decreased the ACHE activity in the brain sections when compared to control group.

**The Behavioral Tests for Memory:**

The first test was the (Y- maze test), it was performed to test the spatial memory of the mice based on their working memory. The second test was the (Novel object recognition test), which was performed to measure the non-spatial memory of the mice based on their recognition memory.
Finally, the (Morris water Maze test) was performed to test the spatial memory of the mice based on their working and reference memory.

The data in Table (4) showed that the Protective effect of some foods rich in choline, selenium and flavonoids on y-maze, novel object recognition and morris water maze tests in Alzheimer’s disease mice model. The results showed that the learned rate of spatial working memory (Y-maze) as represented by the significant decrease in discrimination, and alternation percentage) and non-spatial recognition memory (Object Test) the positive control group was lowest than negative control group. Accordingly, there were statistical significant differences observed between them at P<0.05 level. While, spatial memory in AD and case of working (reference) memory, the number of entries in the quadrant where the platform was previously placed was evaluated (MWM). The positive control group was lower than negative control group. Even, there were statistical significant differences observed between them at P<0.05 level. These results agreed with Abdel-Aal et al., (2021) whom reported that, the time spent exploring a novel object was significantly longer than that consumed for the familiar one in the Control group. Moreover, Khalifa et al., (2020) whom reported that, rats treated with ALCL₃ stayed less time in target quarter when compared to normal rats.

Result are expressed as mean ±SD. Values in each column have different letters are significantly differed at (p<0.05).

On contrast, all treated groups for y-maze test, novel object recognition and morris water maze showed improvement as compared to the positive and negative control groups. The best result was recorded for group treated with mixture of natural foods as compared with the negative control group. Accordingly, there were statistical significant difference observed between them at P<0.05 level. But, no statistical significant difference as compared to negative control group in Y-maze test. It reflected that, foods rich choline, selenium and flavonoids have positive protective effect for behavioral y-maze test (spatial recognition and behavioral object tests (non-spatial recognition) of the memory also the reference memory in Morris's water maze of mice induced to Alzheimer's disease. These results agreed with Samad et al., (2021) whom showed that, Morris Water Maze (MWM) Activity showed the outcome of Selenium on the memory function compared control group.

<table>
<thead>
<tr>
<th>Behavioral tests</th>
<th>Parameters</th>
<th>Y- MAZE (%)</th>
<th>Novel Object recognition (%)</th>
<th>Test MWM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (-ve)</td>
<td>67.42 ± 13.53</td>
<td>0.73 ± 0.09</td>
<td>22 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>48.01 ± 5.29</td>
<td>0.33 ± 0.20</td>
<td>9.50 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Food Rich in Choline Soy milk</td>
<td>80.68 ± 6.08</td>
<td>0.73 ± 0.05</td>
<td>48 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Foods Rich in Selenium Banana Pulp + Dried Coconut</td>
<td>76.64 ± 6.20</td>
<td>0.70 ± 0.07</td>
<td>38 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Food Rich in Flavonoids Dark cacao</td>
<td>70.38 ± 9.87</td>
<td>0.72 ± 0.10</td>
<td>28 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Mixture Of Natural Foods</td>
<td>70.53 ± 10.08</td>
<td>0.93 ± 0.06</td>
<td>49 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>
Moreover, these results agreed with Ma et al., (2018) whom showed that, The Y-maze was used to assay spatial working memory in mice Flavonoid-rich-treated mice at both 40 and 80 mg/kg (and, respectively), indicating that Flavonoid-Rich Ethanol Extract from the Leaves of Diospyros kaki Attenuates (FELDK) improves working memory in D-gal-aged mice.

**Histopathological Examination:**

Next, histopathological examinations of the different brain regions namely hippocampus and striatum Microscopically, brain of mice from negative control group which was fed on basal diet showed normal histological structure of the neurons were recorded in (figures: 1, & 2).

On contrast, brain mice from positive control group which was treated ALCL3 showed Nuclear pyknosis and degeneration of the neurons in subiculum in hippocampus area (figure: 3). Also, it showed intracytoplasmic vacuolization in the neuronal cells, focal eosinophilic plagues formation with diffuse gliosis and the eosinophilic plagues formation and gliosis in Striatum (figures: 4 & 5). These results agreed with fact that The presence of toxic beta-amyloid activates immune system cells in the brain called microglia. Microglia try to clear the toxic proteins as well as widespread debris from dead and dying cells. Chronic inflammation is believed to set in when the microglia can’t keep up with all that needs to be cleared. Atrophy, or shrinkage (Gordon et al., 2018). Beta-amyloid plaques may contribute to cell death by interfering with neuron-to-neuron communication at synapses (National Institutes of Health, 2018). So, amyloid β is considered to be neurotoxic, driving the progression of neuronal damage and resulting in neuro degeneration by promoting neuro inflammation and disrupting neurogenesis, glial (microglial and astrocytic) related inflammatory response hyperphosphorylation (Cedernaes et al., 2017).

On the other hand, all treated groups showed Nuclear pyknosis and degeneration of the neurons in subiculum with hippocampus and intracellular oedema of the neurons in Striatum but lower than positive control group (figures. 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15). May be due
to, foods rich choline, selenium and flavonoids have a positive protective effect against aluminum-induced damage in brain tissues of male mice with Alzheimer's disease. This results agreed with Samad et al., (2022) whom reported that, As+Se (0.175 and 0.35 mg/ml/kg) administered rats showed rare Purkinje cells degeneration in the hippocampus indicating the slight degenerative changes through the onset of pyknosis. Moreover, Velazquez et al., (2019) reported that, Lifelong choline supplementation significantly reduces Aβ pathology.

Fig 1: Brain (Subiculum in hippocampus) of mice control (-ve) showing normal histological structure of the neurons (H&E, X 40). Arrow

Fig 2: Brain (Striatum) of mice control (-ve) showing normal histological structure of the neurons (H&E, X 40). Arrow

Fig 3: Brain (subiculum) of mice control (+ve) showing nuclear pyknosis and degeneration of the neurons (H&E, X 40). Arrow

Fig 4: Brain (Striatum) of mice control (+ve) showing intracytoplasmic vacuolization in the neuronal cells (H&E, X 40). Arrow
Fig 5: Brain (Striatum) of mice control (+ve) showing the eosinophilic plagues formation and gliosis (H&E, X 80). Arrow

Fig 6: Brain (subiculum) of mice in treated group with food rich in choline (Soy milk) showing normal histological structure of the neurons (H&E, X 40). Arrow.

Fig 7: Brain (Striatum) of mice in treated group with food rich in choline (Soy milk) showing Nuclear pyknosis and degeneration of the neurons (H&E, X 40) Arrow.

Fig 8: Brain (Subiculum) of mice in treated group with foods rich in selenium (Banana+ Dried coconut) showing Nuclear pyknosis and degeneration of the neurons (H&E, X 40). Arrow.
Fig 9: Brain (Striatum) of mice in treated group with foods rich in selenium (Banana + Dried coconut) showing intracellular oedema of the neurons (H&E, X 40). Arrow

Fig 10: Brain (Subiculum) of mice in treated group with food rich in flavonoids (Dark cocoa) showing nuclear pyknosis and degeneration in the neurons (H&E, X 40). Arrow

Fig 11: Brain (Fascia dentata and hilus) of mice in treated group with food rich in flavonoids (Dark cocoa) showing normal histological structure (H&E, X 40). Arrow

Fig 12: Brain (Striatum) of mice in treated group with food rich in food rich flavonoids (Dark cocoa) showing nuclear pyknosis and degeneration of the neurons (H&E, X 40). Arrow
Fig 13: Brain (Subiculum) of mice in group treated with mixture foods rich in choline, selenium and flavonoids showing Intra cellular oedema of the neurons (H&E, X 40). Arrow

Fig 14: Brain (Fascia dentata and hilus) of mice in group treated with mixture foods rich in choline, selenium and flavonoids showing Intra cellular oedema of the neurons (H&E, X 40). Arrow

Fig 15: Brain (Striatum) of mice in group treated with mixture foods rich in choline, selenium and flavonoids showing normal histological structure (H&E, X 40). Arrow
Conclusion:

Foods rich in choline, selenium and flavonoids have a positive protective effect in reducing aluminum risks during the induction and progression of Alzheimer's disease. This may be attributed to their additional anti-inflammatory effect as well as their ability to antagonize the aggregation of Aβ in The hippocampus. This was also confirmed by biochemical and behavioral examinations. The effect of the combination therapy was more obvious in reducing the risks of aluminum and thus preventing Alzheimer's.
References:


الفحص

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المتخصّص

الخلفية: تم تحديد الكولين والسيلينيوم والفلاقونويد كمضادات أكسدة محتملة يمكنها إزالة السموم من أنواع الأكسجين التفاعلي المختلفة (ROS) في الأماكن العصبية.

الهدف: تهدف الرسالة إلى تقييم التأثيرات الوقائية المحتملة لبعض الأطعمة الغنية بالكولين والسيلينيوم والفلاقونويد من مرض الزهايمر في الفئران.

الطريقة: تم إحداث ضعف في ذاكرة الرؤيةرصد، قام بشق بشرة وقم بجمع الدفع الصبي والفلاقونويد مع كلوريد الألومينيوم باستخدام بعض اختبارات السلوكيات.

النتائج: أظهرت النتائج زيادة معنوية في الاستجابة كولين بالسبيلات في مجموعة التحكم الموجبة، بالإضافة إلى زيادة وقت الالتصام، وقد تم الكشف عن تأثيرات مصدرية مهمة في التجربة في الاختبارات السلوكيات. كما تم الكشف عن تأثيرات مصدرية مهمة في اختبارات الألومينيوم من تأثيرات الألومينيوم المحتملة على التحليلات الكيميائية الحيوية وكذلك على النزعة، وضعف التعلم. علاوة على ذلك، ظهرت الكلمات السلبية أيضاً في المجموعات المعملية مقاومة بمجموعات التحكم.

الخلاصة: الأطعمة الغنية بالكولين والسيلينيوم والفلاقونويد أكثر فعالية في التقليل من خطر الانحلالات التي يسببها الألومينيوم. ومع ذلك، فإن العلاج المخلل بتأثيرات واقعية أكثر وضوحًا من كل واحد منهم على حدٍٓ.

الكلمات المفتاحية: مرض الزهايمر، كولوريد الألومينيوم، مضادات الأكسدة، السيليكون، السيلينيوم

الفلاكونويد والاختبارات السلوكيات.