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The Synergistic Effects of *Zingiber officinale* Extract and Vitamin E on Alzheimer Disease

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Abstract

Ginger, scientifically known as Zingiber officinale and belonging to the Zingiberaceae family, is a spice widely used in traditional medicine. It has been recognized for its antioxidant and anti-inflammatory properties, as well as its ability to enhance memory and improve blood circulation. This study aimed to investigate the synergistic effects of methanolic extract from Zingiber *officinale* and vitamin E as a potential treatment for Alzheimer's disease The study involved extracting *Zingiber officinale* using methanol and determining the maximum tolerated dose in rats. Alzheimer's disease was induced in rats by administering aluminum chloride for 30 days. The rats were divided into different groups: Group I received the extract, Group II received vitamin E, Group III received a combination of the extract and vitamin E, and Group IV received only the extract vehicle Dimethyl sulfoxide (DMSO). To assess the rats' cognitive and behavioral functions, a maze test was conducted before and after the treatment once a week. Acetylcholinesterase levels, a diagnostic marker for Alzheimer's disease, were measured in the blood serum. Finally, immunohistochemistry analysis was performed on brain tissue samples. The maze test results revealed that rats with induced Alzheimer's disease showed improved recognition of food within five minutes after 15 days of treatment. Acetylcholinesterase levels were significantly higher in Group I compared to Groups II and III, where cholinesterase levels were significantly lower. In conclusion, this study demonstrated that the combination of Zingiber officinale extract and vitamin E had a positive synergistic effect.

Keywords: *Zingiber officinale*, vitamin E, Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is a degenerative disorder of the nervous system that progresses over time. It falls under the category of dementia, which refers to a range of symptoms related to memory and cognitive functions. AD is characterized by a gradual decline in memory, learning ability, behavior, and cognitive functions. It primarily affects specific regions of the brain such as the hippocampus and entorhinal cortex, and later impacts the cerebral cortex responsible for memory, language, and behavior. The key features of AD include the accumulation of plaques made of beta-amyloid protein (Aβ) and neurofibrillary tangles (NFTs) composed of tau protein. These pathological changes result in weakened connections between neurons and eventual brain shrinkage over time (1). There are two types of drugs that have been approved by the FDA, cholinesterase inhibitors and memantine which treat the cognitive symptoms of Alzheimer's disease, although these medications cannot cure Alzheimer's disease or stop its progression but might help in reducing the symptoms (2) .Cholinesterase inhibitors are type of drugs that are known to suppress

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symptoms related to memory loss, language, and other cognitive processes, they work by preventing the breakdown of acetylcholine. Tacrine was the first approved drug by the FDA but it was then limited due to hepatotoxic side effects and it was then followed by donepezil (Aricept), rivastigmine, and galantamine. They all have similar efficacy but differ in the cost and the patient tolerance to each medication. The problem with these medications is that they could make adverse side effects and make dementia worse (3).

Medicinal plants have been known to decrease the symptoms and progression of many diseases such as AD. Many studies have been directed to examine the effects of medicinal plant extracts on AD in addition to isolating and identifying the active compounds (4). Several compounds such as alkaloids polyphenols, tannins, sterols, triterpenes,lignans, and flavonoids, have shown various beneficial pharmacological activities on the progression of the disease, such as anti-amyloidogenic, anti-inflammatory, antioxidant, and anticholinesterase effects. Some compounds, such as melatonin, garlic extract, curcumin, Ginkgo biloba extract, *zingiber officinale* extract, and vitamins E and C, have been used in patients with AD and showed positive results (5). Ginger (*Zingiber officinal*) belongs to the family of Zingiberaceae and Zingiber genus, It was widely used over the years as a herbal medicine in the treatment of numerous diseases, such as nausea, headaches, and colds (6). Ginger has many bioactive components that have been identified, such as phenolic and terpene compounds. The phenolic compounds include shogaols, gingerols, and paradols. Ginger also includes a lot of terpene components, such as α-curcumene, β-bisabolene, α-farnesene, and zingiberene and they are considered as the main components of ginger essential oils. Lately, ginger was found to hold several biological activities, such as anticancer, antimicrobial, anti-inflammatory, and antioxidant activities. Moreover, studies showed that ginger can prevent numerous diseases such as obesity, cardiovascular diseases, diabetes mellitus, and neurodegenerative diseases (7).

Vitamin E is known for its antioxidant and neuroprotective properties, and it also has anti-inflammatory and hypocholesterolemic effects, making it important for brain health (8). In elderly individuals, vitamin E supplementation can enhance the immune response. Aging and certain neurodegenerative diseases, such as Alzheimer's disease (AD), can lead to immune system dysfunction and subsequent inflammatory responses. Studies have demonstrated the anti-inflammatory activity of vitamin E when taken as a dietary supplement by elderly individuals (9).

Research on neuronal cultures of rats has shown that vitamin E application can prevent the formation of reactive oxygen species (ROS) and reduce oxidative stress markers. Certain forms of vitamin E also exhibit strong anti-inflammatory properties. In patients with AD, increased levels of proinflammatory cytokines such as IL-1, IL-6, and TNF-alpha have been detected in brain tissue. TNF-alpha and IL-1 may contribute to the expression of amyloid precursor protein and Aβ peptide, which are associated with AD. Therefore, it is possible that anti-inflammatory agents like vitamin E could potentially slow down or even prevent the development of AD. Additionally, low levels of alpha-tocopherol in the brain can down regulate genes involved in synaptogenesis, myelination, and glial functions(10) .Recent research on vitamin E deficiencies has identified a mechanism by which vitamin E inhibits the phosphorylation of tau protein, a process involving mitogen-activated protein kinase (p38MAPK) activation(11) .

Recent studies have linked vitamin E supplementation to enhanced memory performance. Specifically, low plasma levels of vitamin E have been associated with poorer memory function. Furthermore, in a study assessing the effects of vitamin E on cognitive function, participants with less than 50% of the recommended intake of vitamin E demonstrated poorer cognitive function compared to those with higher intake. High levels of tocotrienol and tocopherol forms of vitamin E have also been found to reduce the risk of cognitive impairment (12).

The objective of this research is to conduct an in vivo study to evaluate the antioxidant and anti-inflammatory effects of Zingiberofficinale (ginger) and vitamin E on the progression of AD. The study will also assess the cognitive and behavioral functions of rats with induced AD through the Y-maze test, measurement of acetylcholinesterase (ACHE) levels, and histological analysis.

2. Materials and Methods

2.1. Plant material and preparation of the extract

The extraction process involved the use of a percolator that was first washed with methanol. A

layer of cotton was placed at the bottom of the percolator, and a collecting jar was positioned underneath. Zingiber officinale, obtained as a powdered form from a local market (Haraz), was used for the extraction. A quantity of 150 grams of the powder was added to the percolator, followed by the addition of 900 ml of HPLC grade methanol. The percolator was adjusted to allow the extract to pass through drop by drop. It was then covered and left undisturbed for several days until the extraction process was complete. The percolation rate was maintained at a moderate pace of approximately 6 drops per minute until the extraction was finished (13). After extraction, the methanol was evaporated by placing the extract in a rotary evaporator equipped with vacuum control and a temperature-controlled water bath. Subsequently, the extract was transferred to a fume cupboard and left to dry.

2.2. Animals

2.2.1. Determining the maximum tolerated (MTD) dose of Zingiber officinale extract

A group of six male albino mice, weighing between 20 and 40 grams, were kept in standard cages at the NRC host institute following standard conditions. The experimental protocol received approval from the Ethics Committee. Two grams of the extract prepared earlier were combined with 2.5 ml of DMSO for oral administration at a dose of 5000 mg/kg. The mice were closely monitored and observed for 24 hours, and the LD_{10} (lethal dose for 10% of the mice) was subsequently calculated.

2.2.2. Induction of AD and experimental design

Aluminum chloride ($AlCl_3$) was prepared for induction by mixing 2.8 grams of $AlCl_3$ with 70 ml of water. The mixture was covered with aluminum foil paper and made ready for use.

A total of thirty-six male Wistar rats, weighing between 120 and 180 grams, were divided into five groups, with each group containing six rats. Except for the control group, all groups received a daily injection of 70 mg/kg of $AlCl_3$ for a duration of 30 days. Following the determination of the maximum tolerated dose of the extract, which was found to be 5000 mg/kg, the LD_{10} was calculated. Subsequently, the previously prepared extract and vitamin E were administered orally to the groups according to the experimental design for a period of two weeks.

Group I received treatment with Zingiber officinale.

Group II received treatment with vitamin E.

Group III received 86 µl of the Zingiber officinale extract and 340 µl of vitamin E.

Group IV served as the negative control and was induced with aluminum chloride only.

Group V served as the positive control and received Aricept.At the end of the treatment period, a Y-maze test was conducted to observe the behavioral and cognitive functions of the rats.

2.2.3. Determination of cognitive function:

Y-maze Test

Behavioral and cognitive functions of all groups were measured at the end of the treatment by performing Y-maze test for the rats. Y-maze is used to assess short term memory in rats, it is designed in the form of three arms and rats are allowed to explore the arms hence, rats with intact cognitive functions will remember the previously visited arms (14).

2.3. Biochemical assay

Acetylcholinesterase (ACHE) test

Blood was collected from rats using 100 µl blood capillaries tubes from the retro-orbital sinus, The maze was wiped and cleaned with 70% ethanol, the arms were labeled A, B and C, food was placed in one of the three arms and the rat was introduced to one of the arms of the maze and allowed to explore the maze. The rat was observed if it reaches the food within 5 minutes and the time duration was recorded. The blood was collected in EDTA collection tube under sterile conditions and the blood sample centrifuged for 5 minutes at 3000 RPM. Plasma was then isolated into Eppendorf tube and stored in refrigerator for acetylcholinesterase test which was performed using biochemical enterprise (BEN) kit PCB280.

2.4. Histological analysis

Rats were sacrificed by decapitation, and brain was then cut in half. The healthy part of the brain was placed in the 10% formalin for histological analysis, the other half was placed inside an aluminum foil and stored in the refrigerator for further histological studies. Obtained brain samples from rats in different groups were fixed in 10% formalin saline for 24 hours, washed using tap water then imbedded in serial dilution of alcohol (methyl, ethyl, and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin & eosin stain for examination through the light electric microscope.

2.5. Statistical analysis:

For statistical analysis, we used one-way ANOVA multiple comparisons (graph pad prism 9) to determine the significance between different groups.

3. Results

3.1. Maximum tolerated dose determination (MTD)

The maximum tolerated dose of the extract was determined to be 5000 mg/kg and LD_{10} was calculated accordingly.

3.2. Y-maze test

The findings suggest that *Zingiber officinale*, vitamin E, and the combination treatment have an impact on the time taken by rats to reach food Figure 1.

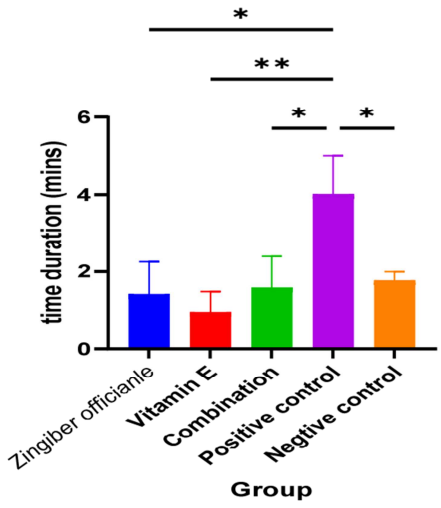

Figure 1: Y-maze test for different groups : the figure displays the results of the statistical analysis representing the time the rats took to reach food. The treatments include *Zingiber officinale*, vitamin E, a combination of *Zingiberofficinale*and vitaminE and a negative control (NC).

3.3. Histological analysis

3.3.1. Negative control (Group VI)

The figure represents histological analysis of the negative control group. The analysis shows normal structure of the neurons in figures A, B, C and D. fig. A represent cerebral cortex of the brain .Figure B is the subiculum hippocampus of the brain. Figure C is the brain fascia dentate hippocampus. Figure D is histology for the brain

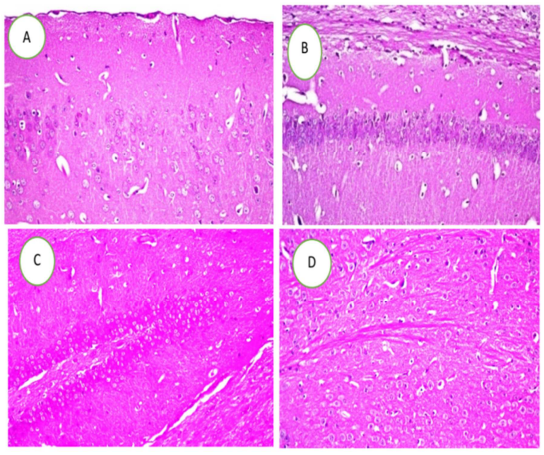

Figure 2: Histological analysis of negative control brain samples (H&E 400)

3.3.2. $AlCl_3$ control (group V)

The figure represents histological analysis of $AlCl_3$ group Figure A shows focal neuronal degeneration in the cerebral cortex. Figure B shows pyknosis and degeneration in most of the neurons in the subiculum hippocampus in the brain. Figure C shows degeneration and nuclear pyknosis in some of the neurons of brain fascia dentate hippocampus. Finally, figure D shows focal neuronal damage and eosinophilic plaques formation in the brain.

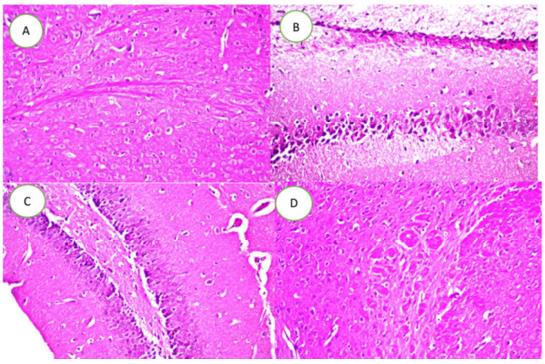

Figure 3: Histological analysis of $Alcl_3$ group brain samples (H&E 400)

3.3.3. Vitamin E group

The figure represents histological analysis of vitamin E group fig. (A) Shows nuclear pyknosis and degeneration in most of the neurons in the brain cerebral cortex. Fig. (B) Shows normal histological structure in the brain subiculum hippocampus. Fig. (C) Shows normal histological analysis in the brain fascia dentate hippocampus. Fig. (D) Shows nuclear pyknosis and degeneration of the neurons in the brain structure.

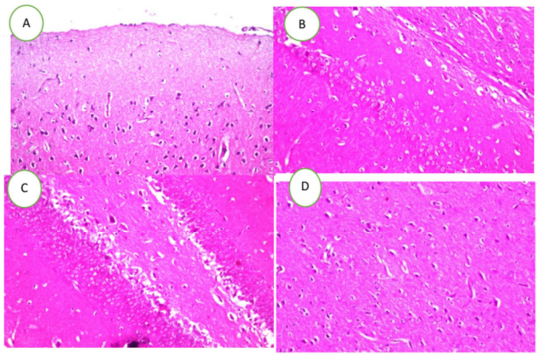

Figure 4: Histological analysis of vitamin E group (H&E 400)

3.3.4. Zingiber officinale group

The figure represents histological analysis of *zingiber officinal* group. Fig (A) shows nuclear pyknosis and

degeneration in few neurons in brain cerebral cortex. Fig. (B) Shows normal histological structure of brain subiculum hippocampus. Fig. (C) Shows normal histological structure in the brain fascia dentate hippocampus. Fig.(D) shows normal histological structure of the brain.

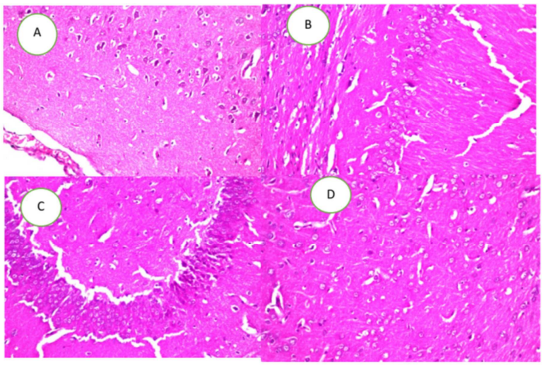

Figure 5: Histological analysis of zingiberofficinale group(H&E 400)

3.3.5. Combination group

The figure represents histological analysis of combination group. Fig. (A) Shows nuclear pyknosis and degeneration in most of the neurons in the cerebral cortex. Fig. (B) Shows normal histological structure in brain subiculum hippocampus. Fig. (C) shows normal histological analysis in brain fascia dentate hippocampus. Fig. (D) Shows normal histological analysis in brain structure.

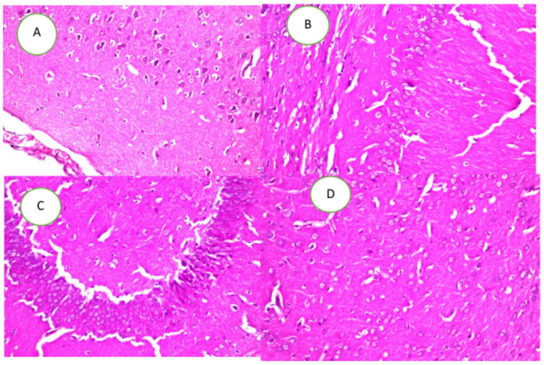

Figure 6: Histological analysis of combination group (H&E 400)

3.4. Acetylcholinesterase (ACHE) test

The statistical analysis represents ACHE levels in AD-induced rat brain. The results show a significance between *zingiber officnal* and vitamin E and a high significance value between combination and positive control, vitamin E and positive control, *zingiber officinal* and positive control, positive and negative control and finally, a significance between vitamin E and negative control.

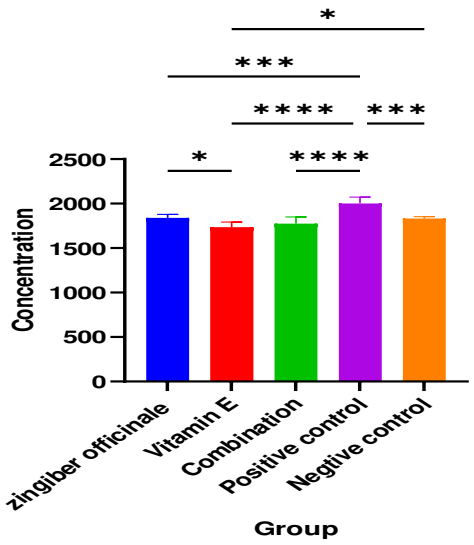

Figure 7: ACHE statistical analysis for different groups

4. Discussion

In this study, the combined effect of *zingiberofficinale* and vitamin E was investigatedon rats with Alzheimer's disease (AD). *Zingiber officinale* is an FDA-approved food additive considered safe. Previous studies showed no toxicity or mortality with ginger extracts at tested doses. The maximum tolerated dose was 5000 mg/kg per day (15). The Y-maze test was conducted to evaluate the cognitive and behavioral functions of rats with Alzheimer's disease (AD). The results demonstrated that *zingiber officinale*, vitamin E, and their combination had a positive impact on food searching behavior compared to the AD-induced alone group and the normal control group. This suggests that these treatments improved memory and cognitive functions in the AD-induced rats.

C. Y. Kim et al. demonstrated that 6-gingerol, a compound found in *zingiber officinale,* improved memory and cognitive impairment in AD-induced mice based on the Y-maze test (16). Another study found that ginger extract improved memory impairment in Wistar rats with focal cerebral ischemia by reducing oxidative stress and enhancing antioxidant enzymes (17). Ginger, along with Schizosaccharomycespombe, also showed positive effects on novel object exploration and spontaneous alterations in the Y-maze test in a mouse model of AD induced by Aβ1-42 plaque (16). A study involving middle-aged women with AD showed that *zingiber officinale* enhanced cognitive and memory functions. (18) Furthermore, *zingiber officinale* is considered a supplement that improves neurodegeneration and memory impairment by enhancing oxidative stress status and cognitive functions (19) .

In an experiment conducted by Kiasalari, Zahra et al., the effect of vitamin E on Alzheimer's disease was investigated. It was found that vitamin E significantly improved spatial memory deficits in the Y-maze test by reducing hippocampal oxidative stress (20) .Additionally, vitamin E inhibits oxidation under strong oxidative conditions of free radicals production(21) . Furthermore, it decreased the toxic effects of β-amyloid and improved cognitive functions in AD-induced rats. It was observed that AD patients who included vitamin E in their diet had better survival rates, and decreased levels of vitamin E were found in some AD patients (22). However, it is important to note that while our study showed the effectiveness of vitamin E, previous studies have reported conflicting findings. For instance, Nicolas Farina et al. found no improvement in cognitive functions among individuals with AD or dementia who consumed vitamin E. Therefore, the effectiveness of vitamin E against AD is still a matter of debate (12).Statistical analysis of the Y-maze test indicated that the combination group (vitamin E and *zingiber officinale*) exhibited the highest effectiveness compared to the normal control and positive control groups. Both vitamin E and *zingiber officinale* have antioxidant and anti-inflammatory effects on the brain, leading to improvements in cognitive and behavioral functions in AD-induced rats.

To better visualize the results of our study, histological analysis was performed for all five groups. In the $Alcl_3$ induced rats, there were areas with neuronal damage in the cerebral cortex, pykonsis and degeneration of neurons in subiculum and fascia dentate areas and plaques were detected in striatum of hippocampus in comparison to the normal control group that showed no histopathological alterations. In a recent study, rats were induced to develop AD, the rats developed many AD related features such as behavioral changes and memory defects due to the phosphorylation of tau protein in cerebral cortex leading neuronal damage (23). According to Singh et al., histopathological analysis of $Alcl_3$ induced rat brain showed high levels of neurofibrillary tangles and plaques in the hippocampus area (24).

. In contrast, the normal control group exhibited no histopathological alterations. According to Yao, 2004 , vitamin E administration resulted in decreased amount of amyloid plaques in hippocampus of AD induced rat's brain (25). Moreover, studies performed on vitamin E demonstrated that expression of genes responsible for the progression of AD in hippocampus, was shown to be responsive to vitamin E administration, also the hippocampus of rats that have deficiency in vitamin E showed decreased expression of APP (26). In our findings it was shown that vitamin E caused pykonsis and degeneration of neurons in cerebral cortex. However, In a previous study, vitamin E-deficient mice were found to have increased levels of lipid peroxidation products in the cerebral cortex, cerebellum and hippocampus (27).

Histological analysis of the group treated with extract showed no histopathological alterations in all of the hippocampus areas which indicates attenuation of AD symptoms and progression in this area. Previous studies demonstrated that *Zingiber officinale* could increase the density of neurons in hippocampus and improve the spatial memory. Moreover, *Zingiber officinale* could decrease oxidative stress in cerebral cortex and hippocampus areas (28). However, our findings demonstrated that there was pyknosis and neurodegeneration observed in cerebral cortex indicating that the extract didn't show an effect on the progression of the disease in this area.

The histological analysis of the combination group revealed no histopathological alterations in all areas of the hippocampus, indicating that the combination treatment attenuated the disease symptoms and progression in this region, leading to improved cognitive functions. However, nuclear pyknosis and neurodegeneration were observed in the cerebral cortex. Previous studies have shown that both vitamin E and *zingiber officinale* have anti-inflammatory and antioxidant effects, which contribute to the improvement of cognitive functions(29).

To validate our findings, we measured the levels of acetylcholinesterase (AChE) in all groups. Ginger exhibited the highest significance compared to the positive control and normal control groups. Talebi, İlgün, et al., determined that the methanolic extract of ginger could diminish AChE levels. 6-gingerol, methoxy-6-gingerol, 8-shogaol, diacetoxy-6-gingerol,and 6-shogaol, which are all compounds found in *zingiber officinal* were confirmed to decrease AchE activity (30). Moreover, Ginger and its components have improved cognitive function through attenuation of AChE levels and the improvement of the behavioral functions (31).

In a study, it was found that administering vitamin E to rats with Alzheimer's disease (AD) effectively reduced the elevated levels of acetylcholinesterase (AChE). The study concluded that vitamin E holds promise as a potential therapeutic agent for such diseases (32). Additionally, Damazio et al. conducted research showing that vitamin E can decrease AChE levels in an animal model of schizophrenia, suggesting its potential therapeutic use in psychiatric and neurodegenerative disorders (33).

Although the combination group demonstrated significant effects compared to the positive and negative control groups, it did not exhibit the highest impact on AChE levels. In contrast, *zingiber officinale* alone exhibited the greatest effect in decreasing AChE levels. This suggests that there may be conflicting effects of vitamin E when used alongside other drugs or substances.

5. Conclusion

In conclusion, the results demonstrated that using the combination of *Zingiber officinale* with vitamin E supplement was found to be effective in the improvement of behavioral and cognitive functions of AD- induced rats more than using each one alone. Finally, more studies on vitamin E is needed to determine possible side effects on other organs.

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