



***In Vivo* Evaluation of anti- Alzheimer impact of *Asparagus sprengeri* and *Lactobacillus plantarum***

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**Abstract**

Alzheimer disease (AD), the most prevalent neurological disorder, is typified by cerebral neuron decline. In its earliest phases, AD triggers short-term memory fails, while in its later phases, it leads to long-term memory problems, fluctuations in mood, and withdrawal from society in elderly people. One highly neurotoxic material that aids in the deterioration of nerve cells is aluminum. Neurofilamentous defects and metabolic changes can also be brought on by long-term exposure to aluminum in the cerebral cortex. The current study evaluated Y maze learning assessments, acetylcholinesterase (AChE), oxidative enzymes, in homogenates of the cerebral cortex as well as histopathological assessment of cortex in normal, induced group by  $AlCl_3$  and treated by *Asparagus sprengeri* (*A.sprengeri*) and *Lactobacillus plantarum* (*L.plantarum*) extracts separately or in mixture. The use of either *A.sprengeri* or *L.plantarum* extracts was shown to substantially enhance the mental abilities of the induced animals. It also increased levels of oxidative enzymes, such as SOD and GSH, and decreased MDA enzymes, as well as essential neurotransmitter AChE enzyme in homogenates of the cerebral cortex. These findings were further supported by improvements in histological examination. Additionally, the effects of mixed therapies are more comparable to those of solo treatment. This study offered an evidence in using *A.sprengeri* or *L.plantarum* separately or together as herbal remedies to treat rats with aluminum chloride-induced Alzheimer's disease and improve cognitive function.

## Introduction

Alzheimer's disease (AD) is a progressive neurological problem that has significant socioeconomic and emotional implications for societies [1]. The first indication of AD is a decline in cognition, especially in short-term memory loss; long-term memory retention is intact. The ability to make managerial choices and perform routine tasks significantly deteriorates as the disease worsens and memory loss became evident [2]. Rather than these changes in the brain, the main cause of death for AD patients is usually one of their associated multiple medical conditions, which include disability, and inadequate nutrition due to intestinal issues [3]. AChE inhibitors like rivastigmine and tacrine have been approved by the US Food and Drug Administration to alleviate the signs and effects of AD [4]. Globally, AD is thought to impact about 45 million people, while until 2050, this number is expected to triple every 20 years [5]. Besides, according to Paula et al. [6], the neurotransmitter AChE, which was first identified as a synthetic material in 1867 and is utilized to transport impulses between nerve cells or via other muscle fibres, is found in reduced concentrations in AD patients,

Neurodegenerative illnesses such as AD are believed to be triggered by the buildup of aluminum in the brain [7, 8]. Studies on epidemiology have demonstrated a link among long-term aluminum intake and harm to the nervous system and cognitive decline [9]. Dialysis dementia was developed by long-term dialysis patients who were given dialysates containing aluminum. The prolonged contact of miners to aluminum powder has been highlighted as a

possible cause of cognitive impairment [10]. Aluminum-treated animals displayed signs similar to AD [11]. After extended exposure, aluminum (Al) has been shown to accumulate in all regions of the rat brain [12]. A successful therapy for AD is not yet available. The current pharmacological remedies are only able to marginally enhance mental abilities or reduce symptoms while having multiple bad consequences; they are unable to prevent the disease from advancing. There has to be a class of medications that can treat a greater variety of targets in order to treat not just the signs of AD but also the underlying disease with fewer adverse effects [13– 15].

*A. sprengeri* is the representative of the asparagus genus in Egypt, but it is not a widely grown plant. Asparagus is a perennial herbaceous plant with numerous biological properties that has been employed in food and medicinal product since earlier times. Asparagus contains phytochemical molecules, which have been demonstrated in many studies to many biomedical characteristics [16]. While, *L.plantarum* is a probiotic microbe that is a member of the Gram-positive lactic acid bacteria phylum Firmicutes [ 17]. Lactic acid from fermented foods derived from plants is frequently discovered to contain it [ 18, 19]. Additionally, it has been demonstrated that this specific strain can enhance quality of life and colonize the human gastrointestinal mucosa when taken regularly. The objective of the current investigation is to evaluate the role of *A. sprengeri* and *L.plantarum* extracts in an Animal Induced Alzheimer's disease.

## Materials and methods

### 1. *A. Sprengeri* Tuber

At harvest, fresh tubers of *A. sprengeri* were gathered from the Egyptian Plant school. The gathered tubers were cleansed, the leftover dirt washed away with tap water, the peels were manually removed, the tubers were chopped into little pieces, and the pulp was all mixed together in a food processor. After the homogenized plant was filtered, the filtrate spent a whole day being dehydrated in a cabinet dryer. Using an electric mill, the dried materials. *A. sprengeri* extract, were ground until they were small enough to fit through a 60 mesh filter [20].

### 2. Preparing the bacterial strain

A pure culture of *L. plantarum* 20174 was acquired from the Regional Centre for Mycology and Biotechnology at Al-Azhar University, Egypt. Using a 1% inoculum and a 24-hour incubation period at 37°C, the strain was cultivated three times in sterile nutrient broth prior to use. In vivo experiment 24 involved. The preparation of *L. plantarum* according to *in vivo* published study [21].

### 3. Design of Animal Experiments

After ten days of acclimatization, forty female albino Wister rats (120–140 g body weight) from the animal unit of Ain-shams University Faculty of Medicine were randomly split into five groups (eight rats apiece). Saline solution was given orally via gavage to the first group of animals. Group 2: received 100 mg/kg of  $\text{AlCl}_3$  orally for a period of eight weeks. Group 3: Animals were induced with  $\text{AlCl}_3$  and fed 200 mg/kg of *A. sprengeri* extract three times a day for eight weeks. Group 4: Animals were induced with  $\text{AlCl}_3$  and

administered 200 mg/kg of an *L. plantarum* (made as follows: 1 mg:1 ml distil water) three times a day for eight weeks. Group (5): Animals were induced using  $\text{AlCl}_3$  and fed 200 mg/kg extract of *A. sprengeri* and *L. plantarum* respectively three times a day for eight weeks [22, 23].

### 4. Assessment of Behavior (Y maze memory test)

The apparatus consisted of a three-armed, black plastic maze measuring 16 cm in width, 50 cm in length, and 32 cm in height. Rats from different groups were strapped to one arm and allowed to move through the maze at full speed for eight minutes without the aid of food or drink. For an entry to be deemed lawful, the animal had to have four paws within the arm; if it accessed three arms in a succession, it was said to have changed abruptly. The following formula was used to determine the amount of random change:  $[(\text{total count of arm entries} - 2) / (\text{number of variants})] \times 100$  [24].

### 5. Measurement of physiological parameters

After scarifying the rats, the cerebral cortex was removed from brains and homogenized. According to manufacturer instructions (Thermo-Fisher, CA, USA), malondialdehyde (MDA), acetylcholinesterase (AChE), glutathione (GSH), and superoxide dismutase (SOD) were assessed [25, 26].

### 6. Histopathological analyses

Section of cortex from several tested groups were stained with hematoxylin eosin (H&E), and investigated under a microscope (Nikon BZ23, Japan) [27].

## 7. Statistical testing

The outcome of experiments was represented as means  $\pm$  standard deviations and assessed using Graph pad prism V.5 CA, USA). Where one-way analysis of variance was applied and  $P < 0.05$  considered as significant.

## Results

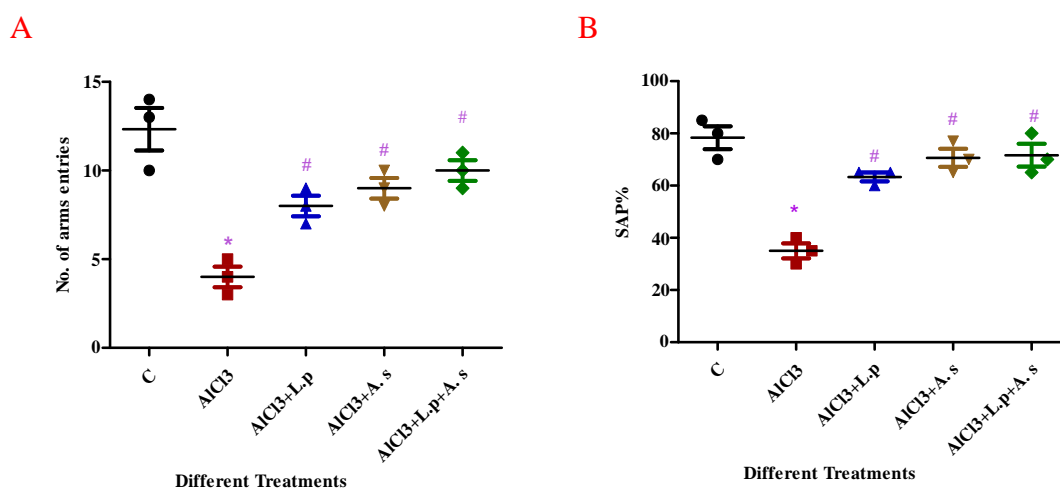
### Mazy assay outcome

The Y-maze test results are displayed in (Figure 1). There was a dramatic drop in the number of arm entries when comparing the treated group by *A. sprengeri*, *L. plantarum* and control groups. However, there was no appreciable difference between the control group and the treated groups. Treatment with *A. sprengeri* and *L. plantarum*, either separately or in combination, significantly raises the Spontaneous alternation percentage (SAP)% in comparison to the induced group.

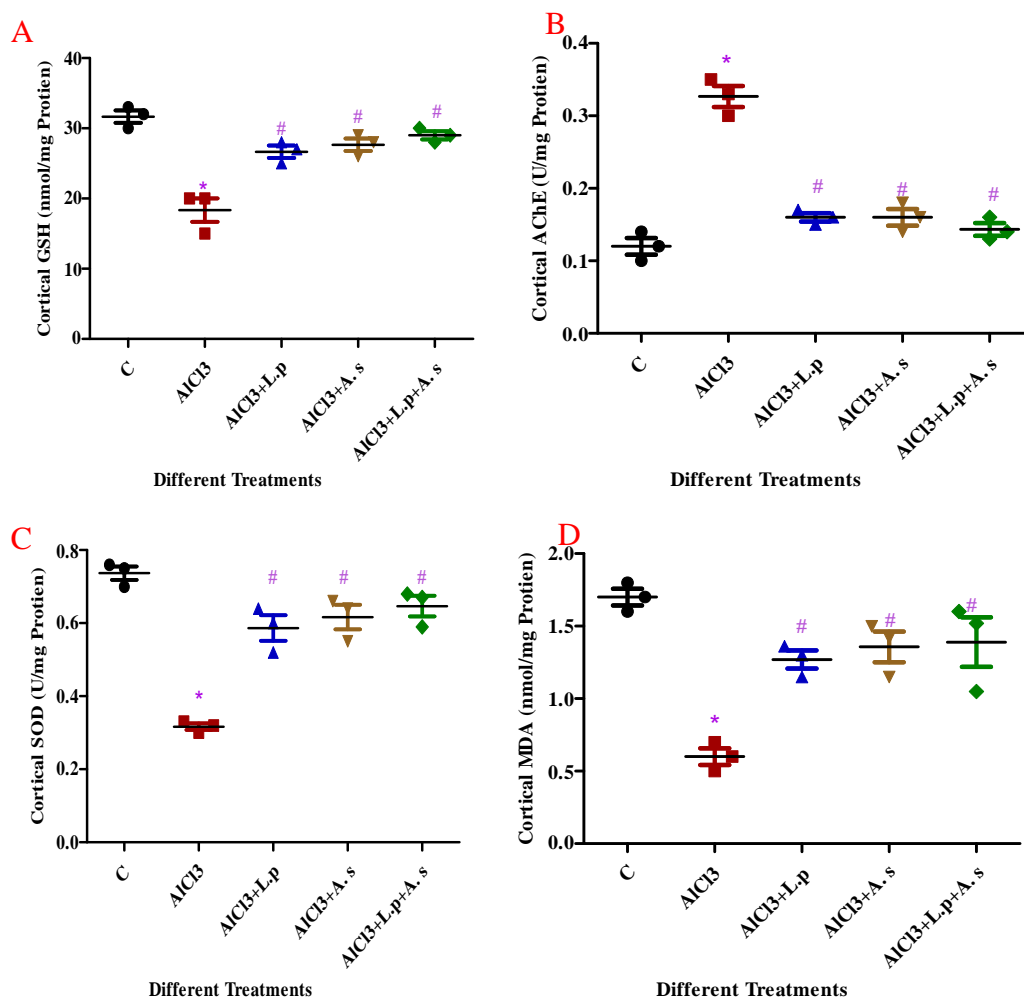
### Assessment of physiological parameters

Measurements of oxidative markers, such as GSH and SOD, in the cortical homogenates of

various animal groups helped to demonstrate the roles of *A. sprengeri* and *L. plantarum* when administered alone or in combination. Nevertheless, using *A. sprengeri* and *L. plantarum* separately or in combination was elevated the enzyme levels in cortical samples of treated animals where the combination therapy exhibited the highest level. It was shown that GSH, and SOD dramatically dropped in cortical samples on induced rats ( $p < 0.05$ ). Besides, assessment of neurotransmitter acetylcholinesterase enzyme (AChE) in various groups revealed that induction using  $AlCl_3$  led to dramatic elevation of AChE level ( $p < 0.05$ ) which significantly reduced upon using either with *A. sprengeri* or *L. plantarum* alone or in combination. Lastly, evaluation of MDA enzyme in various groups showed that a dramatic reduction of enzyme level ( $p < 0.05$ ) in the induced group which reversed by using *A. sprengeri* or *L. plantarum* and reached the maximal level upon using a mixture of *A. sprengeri* or *L. plantarum* as depicted in (Figure 2).



**Figure 1** Impact of the *A. sprengeri*, *L. plantarum* and *A. sprengeri*+ *L. plantarum*(1:1) on the (A) count of arm entries (B) and SAP%; spontaneous alternation percentage after induction using  $AlCl_3$  versus control group. Outcomes were represented means  $\pm$  SD (n = 10 rats/group). Statistical difference was calculated by one-way ANOVA—\* compared to control group, # compared to induced group ( $p < 0.05$ )

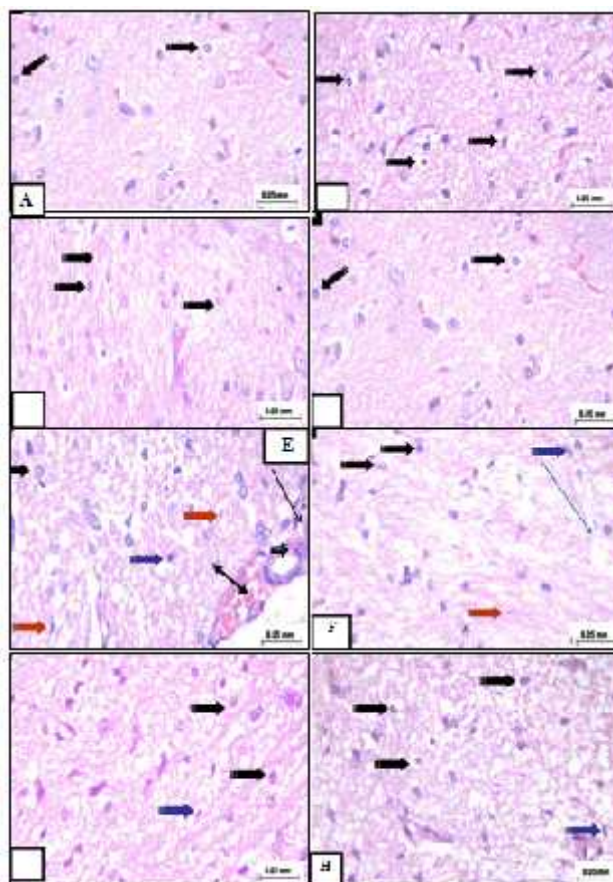


**Figure (2)** Impact of the *A. sprengeri*, *L. plantarum* and *A. sprengeri*+ *L. plantarum* (1:1) on the (A) GSH; (B) AchE; (C) SOD and (D) MDA after induction using AlCl<sub>3</sub> versus control group. Outcomes were represented means ± SD (n = 10 rats/group). Statistical differentiated was calculated by one-way ANOVA— \* compared to control group, # compared to induced group (p < 0.05)

### Histological Examination

Examination of H and E of cortex brain tissue sections from group 1, showed normal histological pattern of the cerebral cortex, classic monomorphic pattern of neurons with long dendrites, microglia cell and normal shape of pyramidal cells in between them normal width blood vessels were seen. The ground substance between the nerve cells is normally occupied with homogenous neuropil (Figure 3 A, B, C and D). However, In the group 2: displayed many

histopathological alterations in from of neuropil vacuolation, shrunken, and pyknosis with infiltrated inflammatory cells, dilated blood capillary of neurons. Hemorrhagic area was found (Figure 3 E). In the groups: 3,4: mild pathological alterations, in form of mild vacuolation in neuropil, many intact neurons, and little number of gliosis could be seen (Figures 3 F,G). In group 5: best results could be achieved where cerebral cortex neurons display normal appearance (Figure 3 H) and (Table 1).



**Figure 3**(A, B, C, D)Photomicrographs from cortex of normal brain tissue section from group 1, showing normal histological picture of brain tissue. (Arrow= granular cell) ;(E)Photomicrograph from cortex of brain tissue section from group 2, showing many histopathological alterations in form of neuropil vacuolation, shrunken, and pyknosis (red arrow) with infiltrated inflammatory cells (long arrow) and dilated blood capillary of neurons (head arrow). Hemorrhagic area was found (double head arrow); (F) Photomicrograph from cortex of brain tissue section from group 3, showing nearly normal histological picture of brain tissue; (G) Photomicrograph from cortex of brain tissue section from group 4, showing nearly normal histological picture of brain tissue; (H) Photomicrograph from cortex of brain tissue section from group 5, showing best results could be achieved where cerebral cortex neurons display normal appearance (H&E; X 400).

Groups	Shrunken, pyknotic neuron	Cortical Disorder
G1	-	-
G2	++	++
G3	+	+
G4	-	-
G5	-	-

**(Table 1)** Assessment of status of neurons and cortex sections from different groups; where (G1) Negative control group, (G2) Induced by  $AlCl_3$ ; (G3) Induced by  $AlCl_3$  and treated by *A. sprengeri*; (G4) Induced by  $AlCl_3$  and treated by *L. plantarum*; (G5) Induced by  $AlCl_3$  and treated by *A. sprengeri* + *L. plantarum* [(-) indicates normal, (+) indicates mild, (++) indicates moderate]

## Discussion

The main sources of Al for humans and animals are packaged foods and water for drinking. Al can enter the body by touching the skin, inhaling particles, or using oral composites

made of resin or immunization additives, among other medical purposes [28, 29]. Herbal remedies have long been utilized to improve cognitive function and cure other AD-related illnesses. Natural products antioxidant qualities have a wide

range of potential applications in human health [30, 31].

In the present work rats' behavioral health was evaluated using the Y-maze test as common test to examine memory functions of rats [32]. Oral treatment of *A. sprengeri* and *L. plantarum* may be able to alleviate memory loss and cognitive alterations in AD-model rats, as suggested by our findings on the maze test, which revealed substantial differences among the rats in the control, induced, and protected groups. Additionally, it has been reported that *L. plantarum* lessens the damage that ageing rats experience related to memory and understanding by lessening the disruption of the mitochondria caused by D-galactose [33-35]. Besides, the current results were consistent with those of other study groups [36, 37] that highlighted the involvement of substances from nature such coconut oil and *Pinushalepensis* in protecting the brain due to their antioxidant properties.

Investigators propose that by reducing ROS accumulation, bioactive substances in extracts maintain vulnerable neurons, improve cognition and blood circulation, enhance brain effectiveness, and promote neurogenesis [38-40]. In the present study using *A. sprengeri* and *L. plantarum* alone or in a mixture form regulate the level of enzymes SOD, GSH,MDA as well as the neurotransmitter AchE in the induced animals to same levels of normal groups.

Oxidative stress and oxidant-induced brain damage in aged rats are the main causes of the loss in brain function [41]. The present results in the same line with, Shekhar et al. [42] showed that *Syzygium aromaticum* could both increase the percentage of anti-oxidant mechanisms and

scavenge reactive oxygen species (ROS). Furthermore, *Boswelliasacra* is a crucial product medicinal relevance to numerous disorders, according to Miran et al. [43]. Furthermore, several of the molecular processes by which natural species protect neurons were highlighted by Amir Rawa et al. [44]. These pathways included the inhibition of pro-inflammatory molecules, the inhibition of microglial invasion, and the modification of  $\beta$ -cell released insulin.

Alzheimer's often causes the destruction of neurons and their neural networks in brain regions related to memories, such as the entorhinal cortex. Afterwards, it affects the areas of the cerebral cortex responsible for speech, thinking, and interacting with others. In contrast, the cortex quickly loses its structural integrity, which is associated with its functional isolation from other areas of the brain [45, 46]. According to the preliminary histological examination, using *A. sprengeri* and *L. plantarum* alone or in a mixture enhance the histological structure of examined cortex section in the examined groups. Natural products have an extended record of being secure and beneficial. There are range of components that make up the natural products and how these components interact with the body's different physiological objectives. The natural products have a number of medicinal benefits that show how well its many components work together, including treating Alzheimer's disease [47]. *A. sprengeri* and *L. plantarum* could be applied to treat Alzheimer's disease

### Conclusion

The current study proposes application of *A. sprengeri* and *L. plantarum*, either alone or in mixture, to treat Alzheimer's disease. To be

applied in potential large-scale following findings verification

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