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ORIGINAL ARTICLE

# Effect of Plain and Fortified Amla Fruit Powder on Aluminum-induced Alzheimer's Disease in Wistar Rats

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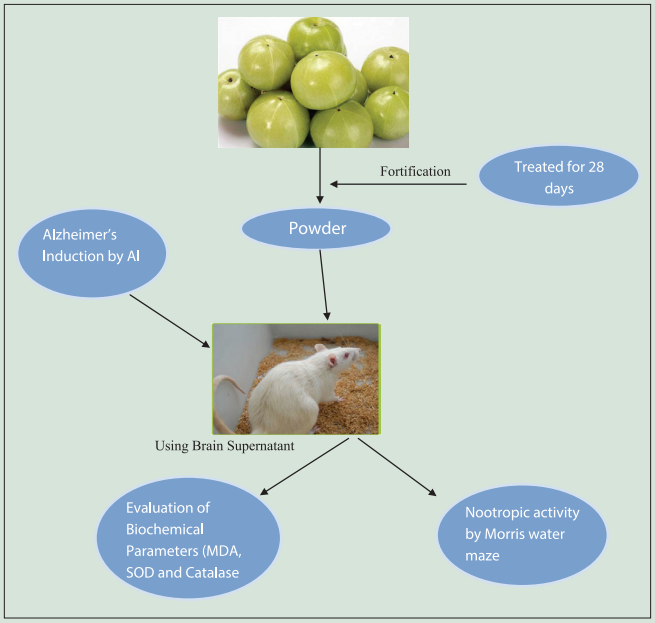
## ABSTRACT

**Objectives:** The present study was designed to evaluate the antioxidant and Nootropic activities of *Emblica officinalis* against aluminum-induced Alzheimer's disease (AD) in Wistar rats. **Materials and Methods:** Alzheimer's was induced using aluminum chloride at a dose of 4.2 mg/kg by intraperitoneal route for 28 days. In prophylactic studies, the plain amla powder (500 mg/kg) and fortified amla powder (300 mg/kg) were administered along with the aluminum chloride. In the curative study, the fortified amla powder (500 mg/kg) was administered to Alzheimer's-induced rats. Memory impairment was confirmed by measuring the nootropic activity using Morris water maize. At the end of the treatment, the oxidative stress parameters such as malondialdehyde (MDA), superoxide dismutase (SOD), and catalase were evaluated. **Results:** The present study showed statistically significant increase in nootropic activity in terms of decrease in latency period in treatment groups when compared with the controlled Alzheimer's-induced group. The administration of amla fruit powder (plain and fortified) decreased the MDA levels and increased the SOD and catalase levels. **Conclusion:** The present study proved that *E. officinalis* is a promising tool in antioxidant and neuroprotective activity against aluminum chloride-induced AD.

**Key words:** Aluminum chloride, Alzheimer's, Amla fruit powder, antioxidant, fortification, nootropic

## SUMMARY

- Nutraceuticals are the functional foods which has immense use in various diseases such as diabetes, cancer, Alzheimer's, and Parkinsonism
- Fortification is the technique, in which the concentration of active constituents is increased to produce a maximum efficacy with minimum dose
- *Emblica officinalis* consists of various active ingredients such as flavonoids, phenols, tannins, Vitamin C, and ascorbic acid which are natural known antioxidants
- The plain and fortified powders showed a significant percentage variation in increased nootropic activity in terms of decrease in latency period when compared with aluminum control group
- The powders significantly ($P < 0.0001$) depleted the lipid peroxidation (malondialdehyde) levels and elevated the superoxide dismutase and catalase levels.




**Abbreviations Used:** AD: Alzheimer's disease, Al: Aluminum, ROS: Reactive oxygen species, SCMC: Sodium carboxymethyl cellulose, p.o: Per oral route, MDA: Malondialdehyde; SOD: Superoxide dismutase.

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## INTRODUCTION

Alzheimer's disease (AD) is a chronic irreversible neurodegenerative disorder associated with age-related dementia, which is believed to be affecting about 35 million people worldwide every year. The prevalence of AD is found to be >5% in people aged 65–74 years, and it accounts up to 50% in the age group above 85 years.[1] The basic cause for AD remains unclear, but it is said to be based on various hypothesis and pathophysiological changes observed in brain, it is understood that reduced the synthesis of neurotransmitter acetylcholine, disruption of neuronal connections, accumulation of amyloid plaques, and neurofibrillary tangles of hyperphosphorylated tau proteins in the basal forebrain, which is the center for learning and memory, together leads to Alzheimer's.[2,3] During aging, the synaptic plasticity and neuronal integrity are decreased leading to age-related AD; however, the recent studies suggested that the Aβ and Aβ precursor proteins play a crucial role in the pathogenesis of early Alzheimer's.[4,5]

Brain is the special organ that consists of several metal ions such as Cu, Fe, Zn, Mn in the gray matter, which catalyzes various reactions. The alterations in the homeostasis of metal ions balance result in



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the disruption of various pathways, which ultimately culminate for the generation of reactive oxygen species (ROS).[6] The ROS increase the oxidative stress which further leads to neurodegeneration.[7] The metal ions have greater affinity toward Aβ proteins, the abnormal concentrations of ions lead to the accumulation of Aβ oligomer proteins.[8]

The important toxic metal involved in pathogenesis of AD is aluminum which potentiates the production of free radicals which are involved in inflammatory reactions leading to the tissue damage.[9,10] The exact treatment for AD is not available as it follows multiple pathways; hence, various classes of drugs such as antioxidants, anti-inflammatory, calcium blockers, and anticholinergics are being used. Multiple classes of drugs taken by a single person cause various side effects; hence, alternative natural therapies are gaining importance in the treatment of chronic diseases such as diabetes, cancer, arthritis, and Alzheimer's.

Nutraceuticals are the natural functional foods or bioactive phytochemicals that benefit us either by preventing or involving in the treatment of diseases.[11] Nowadays, nutraceuticals are gaining attention over pharmaceuticals because of their assumed safety, potential nutritional, therapeutic effect, availability, and the cost effectiveness.[12] *Emblica officinalis* (Amla) belongs to the family of Phyllanthaceae, which is an important medicinal plant in Indian traditional system consisting of various active ingredients such as Tannins, Alkaloids, Phenols, Flavonoids, Amino acids, carbohydrates, minerals, and Vitamin C.[13] In Ayurvedic preparations, amla is used alone or in combination for the treatment of various diseases such as cancer, diabetes, cardiac problems, liver ailments, and ulceritis. It also acts as immunomodulating agent, antitussive, mutagenic, antioxidant, memory enhancing, antipyretic, analgesic, and dyslipidemia.[14] Fortification is the technique employed for addition/doubling of nutrients to potentiate the active constituents used in the treatment of diseases.[15] As AD is a multifaceted disease; hence, the nutraceuticals with multifunctional properties are required for the treatment of AD. Hence, the present study made an attempt to evaluate the antioxidant and memory enhancing properties of plain and fortified amla powders in the treatment of aluminum-induced AD.

# MATERIALS AND METHODS

## Chemicals used in the study

- Aluminum chloride (CDH, India)
- 1,1,3,3-tetraethoxy propane (Sigma chemical Co., USA)
- Thiobarbituric acid (National Chemicals, Vadodara)
- Phenazinemethosulphate (Sigma Chemical Co., USA)
- Nitrobluetetrazolium (Sisco Research Laboratories Pvt Ltd., Mumbai)
- NADH (Sisco Research Laboratories Pvt Ltd., Mumbai)
- Plain Amla fruit powder (Self-prepared)
- Fortified Amla fruit powder (Self-prepared)
- Milk (from local suppliers).

## Preparation of extract

The amla fruits were purchased from the local market. They were washed, deseeded, grinded, dried at 45°C, made into fine powder. The fortified amla fruit powder was prepared using self-fortification technique where the concentration of ingredients was doubled. The powders were authenticated by the Botany Department of Andhra University, Visakhapatnam, and the voucher specimen no: 22205. Both the plain and the fortified amla powders were suspended in purified water, tested for acute and sub-acute toxicity studies and eventually found to be non-toxic.

## Animals

Wistar albino rats of either sex weighing 150–200 g were procured from NIN Laboratories, Hyderabad. The animals were acclimatized for 1 week before the experiment and maintained at 12 h light and dark cycles, respectively. They were fed with standard diet (Nutrimix Std. 1020, Nutritive Life Sciences, Hyderabad) and water *ad libitum*. The animal housing and handling were in accordance with CPCSEA guidelines. The prior permission for the study was obtained from the Institutional Animal Ethics Committee (IAEC), Andhra University, Visakhapatnam, bearing the registered No. 516/PO/c/01/IAEC.

## Experimental protocol

### *Alzheimer's induction*

The animals were acclimatized for laboratory conditions for a week. The aluminum chloride was used as a toxic agent to induce Alzheimer's. The aluminum chloride was dissolved in normal saline at a dose of 4.2 mg/kg body weight and injected through intraperitoneal (i.p.) route for 28 days. The induction of Alzheimer's was confirmed by measuring the nootropic activity at the end of the 28$^{th}$ day, and the animals were sacrificed for the evaluation of *in vivo* biochemical parameters.

### *Treatment schedule*

The animals were randomly divided into five groups consisting of six animals each ($n = 6$).

- Group I - normal control
- Group II - diseased control received aluminum chloride (4.2 mg/kg, i.p.)
- Group III - received plain amla powder (500 mg/kg) along with aluminum chloride (4.2 mg/kg) for 28 days
- Group IV - received fortified amla powder (300 mg/kg) along with aluminum chloride (4.2 mg/kg) for 28 days
- Group V - (curative study) Alzheimer's induced rats were treated with fortified amla powder (500 mg/kg) for 28 days.

### *Evaluation of nootropic activity/spatial memory enhancing activity*

The acquisition and retention of a spatial navigation task were examined using a Morris Water Maze. It is the most frequently used experimental method for testing the spatial memory and learning in rodents. The major advantage of Morris Water Maze is that the rats do not need to be deprived of food.

Coming to the task, it consisted of a watertight pool with a hidden platform at a height of 2 cm below the level of water surface. The water was made opaque by adding the milk; the rats were released in the pool with a maximum allowable time of 90 s after training for a specified period. The time taken by the rats to reach the hidden platform was noted in terms of the latency period.

### *Evaluation of in vivo antioxidant parameters (Malondialdehyde, superoxide dismutase, and catalase)*

After evaluating the spatial memory, the animals were sacrificed, and their brains were isolated, homogenized, and the homogenate was used for the evaluation of antioxidant parameters. These parameters were used as an index for measuring the tissue damage/neuronal degeneration induced by oxidative stress. Malondialdehyde (MDA) levels were measured as per the descriptions by Ohkawa *et al.*, 1979.[16] The superoxide dismutase (SOD) levels were measured as described by Kakkar *et al.*, 1994.[17] The catalase activity was measured as per the guidelines of Aebi 1974.[18]

## Statistical analysis

The results were expressed as mean ± standard error of mean. The values were analyzed by one-way ANOVA followed by Dunnett's test where $P < 0.001$, which were considered as statistically significant. Statistical analysis was performed by GraphPad Prism software (Version 5.0) (GraphPad Software, Inc., California.).

# RESULTS

The aluminum controlled group showed decreased nootropic activity in the terms of increased latency period when compared to the normal controlled group. The plain amla powder (500 mg/kg) and fortified amla powder (300 mg/kg) when administered along with aluminum chloride showed decrease in the latency period with a percentage variation of both at 89.2%, respectively when compared to the aluminum controlled group, whereas the curative study group also showed a remarkable percentage variation of 87.5% in latency period when compared to the aluminum controlled group. The results are clearly demonstrated in Table 1.

The aluminum-treated animals showed a significant increase in the lipid peroxidation levels (MDA) and decrease in the oxidative stress markers such as SOD and catalase levels when compared with the normal control group. The animals receiving fortified amla (300 mg/kg) showed statistically more significant decrease in brain MDA levels and increase in SOD and catalase levels when compared with the animals receiving plain amla powder (500 mg/kg). In curative studies, the fortified amla powder (500 mg/kg) showed a significant potential activity when compared with aluminum control and plain amla powder. The results are given in the Tables 2-4.

# DISCUSSION

AD is mainly characterized by two structures. They are extracellular plaques and intracellular neurofibrillary tangles. These protein aggregates are coupled with the loss of specific neuronal cells and degeneration of synapses and synaptic connections.[19] The living organisms consist of various transition metals which act as catalysts in cellular and metabolic reactions in the body. Hence, the maintenance of metal homeostasis is essential, and any alterations in the homeostasis lead to abnormalities.[20] Al is the important metal in the earth's crust which is neurotoxic. Chronic exposure to Al leads to the accumulation of metal ions in hippocampus and leads to generation of free radicals. These free radicals further damage the cholinergic neurons, which involve in the spatial memory which was evidenced by evaluating in the form of escape latency period using Morris water maze.[21]

Morris water maze is a test for spatial memory which is useful in evaluating the learning tasks of experimental animals. The generation of free radicals, accumulation of amyloidal plaques, and neurofibrillary tangles in the forebrain decreases the learning abilities of the animals; hence in the present study, the learning abilities were evaluated using Morris water maze.[22] In the present scenario, the pharmaceuticals available for the treatment of AD are used only to reduce the symptoms, and the cost burden is increasing day-by–day; hence, the nutraceutical with natural antioxidant properties is selected and evaluated in the study. The plain and fortified amla decreased the escape latency period. The fortified amla is more potent that the plain amla powder.

**Table 1:** Effect of amla powder on nootropic activity in terms of latency period

| Time period | Normal control | Aluminum control | Aluminum + plain amla (500 mg/kg) | Aluminum + fortified amla (300 mg/kg) | Curative studies (500 mg/kg) |
|---|---|---|---|---|---|
| Week 1 | 4.0±0.8 | 15.1±2.4 | 13.3±1.7 | 18.6±1.8 | 15.6±6.5 |
| Week 2 | 3.8±1.1 | 18.0±2.1 | 7.1±1.9 | 7.0±1.6 | 9.6±4.5 |
| Week 3 | 3.0±1.6 | 23.8±2.1 | 3.8±1.4 | 5.0±1.3 | 4.6±1.6 |
| Week 4 | 3.3±0.8 | 28.0±3.3 | 3.0±0.6 (89.2% ↓) | 3.0±0.6 (89.2% ↓) | 3.5±1.0 (87.5% ↓) |

The values are expressed as mean±SEM ($n$=6). The values in the parentheses represent the percentage decrease in terms of latency period when compared with aluminum control group. SEM: Standard error of mean

**Table 2:** Effect of amla powder on brain malondialdehyde levels (nmol/g wet tissue)

| Serial number | Normal control | Aluminum control | Aluminum + plain amla (500 mg/kg) | Aluminum + fortified amla (300 mg/kg) | Curative studies (500 mg/kg) |
|---|---|---|---|---|---|
| 1 | 1.90 | 3.86 | 1.8 | 1.5 | 1.38 |
| 2 | 1.76 | 3.74 | 1.58 | 1.4 | 1.70 |
| 3 | 1.67 | 4.0 | 1.64 | 1.43 | 1.58 |
| 4 | 1.62 | 3.92 | 1.76 | 1.59 | 1.85 |
| 5 | 1.8 | 3.85 | 1.4 | 1.6 | 1.51 |
| 6 | 1.78 | 3.9 | 1.54 | 1.6 | 1.3 |
| Mean±SEM | 1.755±0.09 | 3.878±0.08 | 1.51±0.08*** | 1.63±0.14*** | 1.55±0.20*** |

The values are expressed as mean±SEM ($n$=6) and the ***$P$<0.001 is considered as statistically significant. SEM: Standard error of mean

**Table 3:** Effect of amla powder on brain superoxide dismutase levels (units/mg protein)

| Serial number | Normal control | Aluminum control | Aluminum + plain amlan (500 mg/kg) | Aluminum + fortified amla (300 mg/kg) | Curativestudies (500 mg/kg) |
|---|---|---|---|---|---|
| 1 | 28.3 | 15.0 | 23.6 | 24.0 | 23.1 |
| 2 | 26.8 | 13.6 | 21.0 | 28.1 | 21.4 |
| 3 | 29.2 | 14.2 | 21.8 | 25.2 | 20.2 |
| 4 | 28.0 | 13.0 | 22.0 | 27.0 | 20.8 |
| 5 | 30.0 | 12.0 | 20.5 | 27.7 | 21.0 |
| 6 | 28.5 | 15.3 | 23.4 | 29.1 | 22.4 |
| Mean±SEM | 28.466±1.08 | 13.85±1.245 | 22.05±1.248*** | 26.85±1.908*** | 21.483±1.077*** |

The values are expressed as mean±SEM ($n$=6) and the ***$P$<0.001 is considered as statistically significant. SEM: Standard error of mean

**Table 4:** Effect of amla powder on brain catalase levels (μmoles/min per mg protein)

| Serial number | Normal control | Aluminum control | Aluminum + plain amla (500 mg/kg) | Aluminum + fortified amla (300 mg/kg) | Curative studies (500 mg/kg) |
|---|---|---|---|---|---|
| 1 | 22.7 | 12.5 | 18.6 | 23.9 | 22.5 |
| 2 | 23.5 | 12.7 | 20.1 | 25.4 | 20.4 |
| 3 | 27.0 | 14.09 | 17.9 | 25.0 | 21.6 |
| 4 | 26.1 | 13.7 | 19.1 | 26.8 | 20.1 |
| 5 | 25.3 | 15.0 | 18.8 | 24.7 | 18.8 |
| 6 | 24.2 | 14.5 | 19.3 | 26.3 | 22.1 |
| Mean±SEM | 24.8±1.627 | 13.74±0.99 | 18.96±0.73*** | 25.35±1.06*** | 20.91±1.39*** |

The values are expressed as mean±SEM ($n$=6) and the ***$P$<0.001 is considered as statistically significant. SEM: Standard error of mean

Various cellular mechanisms are involved in maintaining the balance between the productions of ROS and antioxidant mechanisms, any alterations in the balance lead to various neurological diseases like Alzheimer's.[23] Hence, the natural foods or nutraceuticals with antioxidant properties are suggested either directly or as an adjuvant in the treatment of various diseases. *E. officinalis* consists of various active ingredients such as flavonoids, phenols, tannins, Vitamin C, and ascorbic acid which are known as antioxidants.[24] The previous studies have reported that amla contains a high amount of Vitamin C, which acts as a natural antioxidant by minimizing the production of free radicals.[13] Other studies have reported that the presence of emblicanins A and B, gallic acid, ellagic acids leads to free radical scavenging activity and due to the presence of geranin, corilagin, and furosin help in nitric oxide scavenging properties.[25]

In the present study, the plain and fortified amla powders showed a significant rise in the antioxidant enzymes such as SOD and catalase in both prophylactic and curative studies due to the presence of flavonoids, phenols, tannins, and Vitamin C. The results are in concordance with the previously reported studies. The amla also significantly reduced the lipid peroxidation levels (MDA) which is in concordance with the previous studies.[25] The fortified powder is more potent than the plain powder due to the increased concentration of active constituents in low dose. The present study provides the basic knowledge regarding the fortification of foods/nutraceuticals which can be used in the treatment of diseases at a low dose or as an adjuvant along with pharmaceuticals to reduce the dose of pharmaceuticals and also minimizing the toxic effects.

## CONCLUSION

The *E. officinalis* is a rich source of Vitamin C, which is a natural antioxidant. In the present study, it is proved that the nutraceuticals can be used in the treatment of various ailments. The fortification technique, indeed, can be a useful method in drug development for treating diseases with minimal dose. From the observed results, it can be concluded that the neuroprotective potential of amla is mediated through antioxidant and radical scavenging activity. It also enhances the spatial memory. Further studies are to be conducted for other possible mechanisms involved in the treatment of Alzheimer's.


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### Conflicts of interest

There are no conflicts of interest.

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