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Research Article

# MEMORY STRENGTHENING ACTIVITY ON SEEDS OF *ABELMOSCHUS MOSCHATUS*

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**ABSTRACT**

Alzheimer's disease is a progressive neurodegenerative disorder characterized by gradual decline in memory. The present study was undertaken to investigate the memory strengthening effect of *Abelmoschusmoschatus Medic*. Ethanolic extract of seeds (100,200mg/kg. p.o) was administered for 7 successive days to young mice. Exteroceptivebehavioral model such as elevated plus maze was employed to evaluate learning and memory. To delineate the mechanism by which AM exerts memory strengthening activity, the effect of AM on whole brain AchE, Brain Malondialdehyde content , Reduced Glutathione were also assessed. Piracetam (200mg/kg, i.p) was used as a standard drug. Pretreatment with AM (100,200mg, p.o) for seven successive days significantly improved learning and memory in mice and reversed the amnesia induced by diazepam (1mg/kg, i.p). AM also decreased whole brain AchE and Malondialdehyde content and increase the brain reduced glutathione. Hence *Abelmoschusmoschatus Medic.*appears to be a promising candidate for improving memory, Anti cholinesterase activity and Anti oxidant property and it would be worthwhile to explore the potential of this plant in the management of dementia and Alzheimer's disease.

**Keywords**: *Abelmoschusmoschatus*, Alzheimer's disease, elevated plus maze model, Anti cholinergic.

## INTRODUCTION

Memory is the ability of an individual to record sensory stimuli, events, information etc., retain them over short or long periods of time and recall the same at a later date when needed. A set of memory related diseases affects the performance of the human brain. One of the diseases is the Alzheimer's disease (AD) that is much like the dementia is characterized by the development of senile plaques and neurofibrillary tangles, which are associated with neuronal loss affecting to a greater extent cholinergic neurons in brain[1]. Around 35 Million patients suffered from Alzheimer's disease all over the world and this number is increasing with each passing second. An epidemiological study reveals that dementia is largely hidden problem, especially in rapidly developing and heavily populated regions suchas India, China and Latin America. Dementia associated with Alzheimer's disease is the most common cause of memory impairment or cognitive disability in elderly people[2-4].

Reducing oxidative stress by antioxidants, protecting brain inflammatory lesions using anti inflammatory drugs and facilitation of brain cholinergic neurotransmission with anti cholinesterase are some positive approach to management of AD. Due to increase incidence of side effects of allopathic medicine (both nootropic and cholinesterase inhibitors) more research will be manifested towards the use of natural resources like medicinal plants for the management of various cognitive disorders.

*Abelmoschusmoschatus Medic.*ofMalvaceae familyis a medicinal herb and the seeds have

been traditionally used for Neurodegenerative disorders, Anti hysteric, Diuretic, Rheumatism, anti spasmodic, nervous debility and cystitis. Various Phytochemical investigations on the seeds reveals the presence of flavanoids, phenols and steroids may contribute for its hepato protective, diuretic, anti oxidant and anti proliferative activity. The present study was undertaken to investigate the anti Alzheimer's activity of *Abelmoschus moschatus* seeds on mice.

## MATERIALS AND METHODS

### Plant collection and Authentication

The fresh seeds of the plant *Abelmoschus moschatus Medic.,*was collected from Palayamkottai in Tirunelvelidist, Tamilnadu. The plant was identified and authenticated by V.Chelladurai, Research Officer, Botany (Scientist-c), Govt council for research in Ayurveda and siddha, Govt of India (Retired).

### Animals

All the experiments were carried out usingSwiss Albino mice procured from the disease free animal house of MMC, Chennai. Adult (4-6 months old) mice weighing around 25gm were used in present study. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. The experimental protocol was approved by the Institutional Ethics Committee (IAEC) and the care of laboratory animals was taken asper the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

### Drugs and Chemicals

The chemicals used in this study were obtained from drug houses. Diazepam Injection (Calmpose, Ranbaxy,India), 5,5' dithiobis-2-nitrobenzoic acid (DTNB), Acetylthio choline iodide, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium lauryl sulpahte, Tris buffer hydrochloride, ThioBarbituric acid.

### Acute toxicity studies

The acute oral toxicity study was carried out for ethanolic extract by using OECD guidelines 423 (organization of economic co-operation and development). A single dose of 2000mg/kg p.o was given and this was used as a starting dose. After oral administration, the animals were observed every 1 hour for 24 hrs to assess the general behavior and mortality. They were further observed for 72 hrs for toxic symptoms and mortality.

### Grouping of animals

**Group I:** Vehicle control- Distilled water administered orally for 7days, after 90min of administration transfer latency was recorded. Retention of learned task was examined after 24hrs.

**Group II:** Diazepam (1mg/kg) was injected before training. TL was recorded after 45min of injection. Retention was examined after 24hrs.

**Group III:**Piracetam (200mg/kg) was injected for 7days and on the 7$^{th}$ day after 90min of drug administration, diazepam (1mg/kg) was given i.p. TL was recorded after 45min of injection and after 24hrs.

**Group IV:** Test drug I (200mg/kg) was given orally for 7 days and on 7$^{th}$ day after 90min of drug administration, diazepam (1mg/kg) was given i.p. TL was recorded after 45min of injection and after 24hrs.

**Group V:** Test drug II (400mg/kg) was given orally for 7 days and on 7$^{th}$ day after 90min of drug administration, diazepam (1mg/kg) was given i.p. TL was recorded after 45min of injection and after 24hrs.

### Exteroceptive Behavioural Model

### Elevated plus Maze model[5,6]

### Procedure

The elevated plus-maze consisting of two open arms (16×5cm) and two enclosed arms (16×5×12 cm) was used. The maze was elevated to height of 25cm. mice were placed individually at the end of an open arm facing away from central platform and the time taken to move from the end of the open arm to either of closed arm (Transfer Latency, TL) was recorded. If the animal did not enter into one to the enclosed arms within 90sec, it was gently pushed into one of the two enclosed arms the TL was assigned as 90sec. The mice was allowed to explore the maze for another 10sec and then returned to its home cage. Retention of this learned task was examined after 24hrs, after the first day trial (i.e. on 8$^{th}$ day). On the 9$^{th}$ day animals in all the groups were euthanized by cervical dislocation and the brains were removed for the estimation of AchE, Malondialdehyde and Reduced Glutathione.

## PREPARATION OF BRAIN HOMOGENATE

Swiss albino mice were used for the experiments. The mice were decapitated; brains were removed quickly and placed in ice cold saline. The tissues were weighed and homogenized in 0.1M Phosphate buffer (PH-8)

and the brain homogenate was used for further studies.

1. Estimation of Brain AchE level.
2. In-vivo Anti-oxidant Activity.
   - Estimation of Brain Malondialdehyde.
   - Estimation of Brain Reduced Glutathione.

**Estimation of Brain AchE level[7, 8]**

Estimation of brain AchE activity provides a relatively easy and valuable assessment of cholinergic function. The method of AchE activity estimation is popularly known as Elman's method named after George Ellman who developed this method in 1961(Ellman et al., 1961).

The esterase activity was measured by providing an artificial substrate, acetylthiocholine (ATC). Thio choline released because of the cleavage of ATC by AchE was allowed to react with the –SH reagent 5,5'-dithiobis nitro benzoic acid), which is reduced to thio nitro benzoic acid, a yellow coloured anion with an absorption maxima at 412nm.

**ASSAY PROCEDURE**

1. 0.4ml of aliquot of brain homogenate was added to a cuvette containing 2.6ml of phosphate buffer (0.1M) and to this add 100µl of DTNB.
2. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance was measured at 412nm in spectrophotometer, when absorbance reaches a stable value was recorded as the basal reading.
3. 100µl of substrate (ATC) was added and change in absorbance was recorded for a period of 10mins at intervals of 2mins. Change in the absorbance per minute was determined.

The enzyme activity was calculated using the following formula

$$R = 5.74 (10^{-4}) \Delta A/Co$$

R = Rate in moles substrate hydrolyzed per min per gm of tissue,

$\Delta A$ = Change in absorbance per min,

Co = Original concentration of tissue (mg/ml).

**IN-VIVO ANTI OXIDANT ACTIVITIES**

**ESTIMATION OF BRAIN MALONDIALDEHYDE[9,10]**

Malondialdehyde, indicator of lipid per oxidation was determined as described by Ohkawa et al. The reaction mixture consist of 0.2ml of 8.1% sodium lauryl sulphate, 1.5ml of 20%acetic acid (PH 3.5) and 1.5ml of 0.8% aqueous solution of thiobarbituric acid was added to the 0.2ml of processed brain homogenate. The mixture was made up to 4ml with distilled water and heated at 95ºc for 60 minutes. After cooling with tap water, 5ml of n-butanol and pyridine (15:1v/v) and 1ml of distilled water was added and centrifuged. The organic layer was separated out and its absorbance was measured at 532nm using UV-Visible spectrophotometer.

**ESTIMATION OF BRAIN REDUCED GLUTATHIONE[9]**

GSH estimation in brain homogenate was measured according to the Ellman method. This method is based on the development of a yellow colour when 5, 5' dithio bis nitro benzoic acid (DTNB) is added to the compound containing the sulfhydryl groups. To the 0.5ml of brain homogenate was mixed with 1.5ml of 0.2ml of Tris buffer (PH-8.2) and 0.1ml of 0.01M DTNB and this mixture was brought to 10ml with 7.9ml of methanol. The above reaction mixture is centrifuged at approximately 300gm at room temperature for 15minutes. The absorbance of supernatant was read in a spectrophotometer against reagent blank (without sample) at 412nm.

**RESULTS**

**Acute Toxicity Study**

No mortality was observed following oral administration of Plant extracts even with the highest dose 2000mg/kg body weight. So, 1/10$^{th}$ and 1/5$^{th}$ of this dose (200 and 400mg/kg) were considered as a safety dose for this study.

**Effect on TL (Using Elevated Plus maze)**

Transfer Latency (TL) of first day (on seventh day of drug treatment) reflected acquisition or learning behavior of animals, Whereas, TL of next day reflected retention of information or memory. The animals treated orally with 100mg/kg and 200mg/kg showed remarkable reduction in ($P<0.01$) TL of 7$^{th}$ day as well as 8$^{th}$ day, indicating significant improvement in memory. Diazepam (1mg/kg) injected before training significantly increased ($p<0.01$) TL on days 7$^{th}$ and 8$^{th}$ day indicating impairment in learning and memory.

The AM at higher dose level (200mg/kg, p.o for 7successive days) successfully reversed memory deficits induced by Diazepam ($p<0.01$), Piracetam (used as the positive control) at a dose of 200mg/kg i.p also improved learning and

memory in mice and reversed the memory impairment produced by Diazepam as expected.

**Estimation of Brain AchE level**

*Abelmoschusmoschatus Medic* at dose of 100mg/kg and 200mg/kg p.o significantly ($p<0.01$) reduced the levels of acetyl cholinesterase as compared to Diazepam treated group by Elman's method, which is considered as indicator of inhibition of Acetyl cholinesterase activity in mice brain after 8 days of treatment. Piracetam (200mg/kg) i.p significantly ($p<0.01$) reduced the levels of Acetyl cholinesterase and indicated in table (2)

***IN-VIVO* ANTI OXIDANT ACTIVITY**

**Estimation of Brain Malondialdehyde content**

*Abelmoschusmoschatus Medic*at dose of 100mg/kg and 200mg/kg p.o significantly ($p<0.01$) reduced the levels of Malondialdehyde content as compared to Diazepam treated group, which is considered as indicator of inhibition of lipid per oxidase activity in mice brain after 8 days of treatment. Piracetam (200mg/kg) i.p significantly ($p<0.01$) reduced the levels of Malondialdehyde content and indicated in table (3)

**Estimation of Brain Reduced Glutathione Level**

*Abelmoschusmoschatus Medics*how remarkable increase in brain reduced glutathione level in both Piracetam and AM 200 treated groups. The percent decline in the reduced Glutathione level were 24.88% ($p<0.01$) and 27.47% ($p<0.01$) at AM concentration 200mg.

**DISCUSSION**

In the present study *Abelmoschus moschatus Medic.,* (100,200mg), when fed with normal diet for 7days improves the memory of mice reflected by diminished TL values as when compared to control group (1). Furthermore *Abelmoschus moschatus Medic.,* administration protected the mice from the development of memory deficits observed after diazepam treatment.

Biochemical estimation of different parameter as mentioned above show the elevation of acetylcholine level by significant reduction of acetyl cholinesterase activity in brain. Furthermore plant extract decreased the increase potential of MDA level, an indicator of lipidper oxidation index and increased level of reduced glutathione a potential element of free radical scavenging cycle in the brain as compared to control as well as disease control group. Therefore, it appears that *Abelmoschus moschatus* Seeds may possesses the memory enhancing capacity or useful in the treatment of Alzheimer's disease, in the view of its (i) AchE Inhibitory activity (ii) on the basis of its anti oxidant property a significant decreased in MDA level and sharp increase in anti-oxidant process by increase in reduced glutathione level in mice brain.

**Effect on TL: (Elevated plus Maze model)**

| S. No. | GROUPS | TL ON 7th DAY | TL ON 8th DAY |
|---|---|---|---|
| 1. | Control (Vehicle p.o) | 46.83± 2.71 | 30.83±2.04 |
| 2. | Disease control | 65.17±3.92** | 68.50±6.12** |
| 3. | Piracetam control(200mg/kg i.p) + Diazepam | 34.83±4.49** | 18.00±4.56** |
| 4. | AM (100mg/kg p.o) + Diazepam | 45.17±2.85** | 39.83±1.60** |
| 5. | AM (200mg/kg p.o) + Diazepam | 40.83±3.48* | 23.66±3.74* |

Values are expressed in mean ± SEM (n=6) ** denotes $p<0.01$ as compared to control group of young mice,
* denotes $p<0.05$ as compared to control group.(One way ANOVA followed by Dunnett's test)

**Biochemical estimations**

**Effect on Brain Cholinesterase Activity**

| Treatment Groups | Acetyl cholinesterase level (µmole/min/gm of tissue) |
|---|---|
| Control (Vehicle p.o) | 3.59± 0.12×10-7 |
| Disease control(Diazepam 1mg/kg i.p) | 6.47± 0.14×10-7 ** |
| Piracetam control(200mg/kg i.p) + Diazepam | 2.69 ± 0.09×10-7 ** |
| AM (100mg/kg p.o) + Diazepam | 4.75± 0.06×10-7 ** |
| AM (200mg/kg p.o) + Diazepam | 3.12 ± 0.02×10-7 ** |

Values are expressed as Mean ± SEM, (n=6) and **denotes $p < 0.01$ when compared to control group of young mice.
(One way ANOVA followed by Dunnett's test.)

**Effect on Brain Malondialdehyde Level**

| Treatment Groups | Brain Malondialdehyde content (unit/mg of wet tissue) |
|---|---|
| Control (Vehicle p.o) | 48.55± 0.12 |
| Disease control(Diazepam 1mg/kg i.p) | 66.57 ± 0.13** |
| Piracetam control(200mg/kg i.p) + Diazepam | 44.46 ± 0.10** |
| AM (100mg/kg p.o) + Diazepam | 50.82 ± 0.05** |
| AM (200mg/kg p.o) + Diazepam | 45.37 ± 0.11** |

Values are expressed as Mean ± SEM, (n=6) and **denotes p < 0.01 when compared to control group of young mice. (One way ANOVA followed by Dunnett's test.)

**Effect on Brain reduced Glutathione level**

| Treatment Groups | Brain Reduced Glutathione (unit/mg of wet tissue) |
|---|---|
| Control (Vehicle p.o) | 23.31 ± 0.17 |
| Disease control(Diazepam 1mg/kg i.p) | 17.23 ± 0.04** |
| Piracetam control(200mg/kg i.p) + Diazepam | 28.40 ± 0.12** |
| AM (100mg/kg p.o) + Diazepam | 24.87 ± 0.11** |
| AM (200mg/kg p.o) + Diazepam | 27.46 ± 0.21** |

Values are expressed as Mean ± SEM, (n=6) and denotes p < 0.01 when compared to control group of young mice. (One way ANOVA followed by Dunnett's test.)

## CONCLUSION

*AbelmoschusmoschatusMedic,* was administered orally for seven days showed a dose dependent improvement in memory of young mice and it also successfully reversed the memory deficits induced by diazepam. Furthermore *Abelmoschus moschatus* leads a significant decreased in cholinergic transmission, lipid per oxidation and increase in brain reduced glutathione level in mice brain accounts for its multifarious beneficial effects such as memory enhancing property, anti cholinesterase and antioxidant property.

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