Cognitive and Anti Oxidant Property of *Mimusops elengi* Linn. in the Experimental Model of Alzhiemer’s Disease in Rats

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Abstract: Objective: *Mimusops elengi* Linn, is one such important medicinal plant whose bark, leaves and flowers have proved to have several pharmacological properties. In the present study, hydroalcoholic extract of flowers is used to study the protective role in Alzhiemer’s disease.

Materials and Methods: Rats were divided into 5 groups and treated with Colchicine (15μg/rat) through Intracerebroventricular route(I.C.V). *Mimusops elengi* flower extract(ME) was administered at doses of 100 and 200mg/kg(p.o route) once a day for 28 days. The standard group received Donepezil(img/kg,p.o) and the *Invivo* pharmacological tests and biochemical assessments were made on 7th, 14th and 28th day of administration.

Results: ME significantly (P<0.05,P<0.01) improved the behavioural parameters in *Invivo* pharmacological tests, Acetylcholinestarase levels and antioxidant in a dose dependent manner and significantly(P<0.05) reduced MDA levels.

Conclusion: ME possesses significant antioxidant activity and shows neuroprotection in Alzheimer’s disease.

Key Words: Alzheimer’s disease, *Mimusops elengi* Linn., Colchicine.

Introduction

Alzheimer’s disease(AD), also called Senile Dementia of the Alzheimer type(SDAT) or simply Alzheimer’s, is the most common form of dementia. Alzheimer’s disease(AD) is a progressive neurodegenerative disorder primarily manifesting as loss of memory, senile dementia, intraneuronal neurofibrillary tangle formation and cerebral parenchyma deposition and the β-amyloid protein in the form of amyloid plaques. The earliest striking symptom is the loss of memory (Amnesia). AD is the most common form of dementia, accounting for approximately 70% of cases in most industrialized countries and affecting an estimated 17 to 25 million people worldwide. Since no cure for AD is currently available, symptomatic treatment for AD focuses on the restoration of cholinergic function. Reports indicate that Donepezil and Memantine are more effective in improving cognitive impairment in patients with AD. In view of the above...
shortcomings in the drugs used for the treatment of AD there has been an increased interest in herbal products as a source of treatment.

The cause and progression of AD are not well understood. Research indicates that the disease is associated with plaques and tangles in the brain. The three major hypotheses exist to explain the causes of the disease are-Cholinergic hypothesis, Amyloid hypothesis and Tau hypothesis. A relatively selective loss of cholinergic neurons in the forebrain nuclei is the characteristic measure on postmortem AD brain tissue. Choline acetyl tranferase (CAT) activity in the cortex and hippocampus is reduced considerably in AD. Oxidative stress is one of the critical determinates in the stimulation of neuronal death and plays a key role in AD-associated degenerative neuronal changes. Several herbal drugs like Withania somnifera, Ginkgo biloba have been proved to be effective in treating AD.

*Mimusops elengi* Linn. is one such important medicinal plant whose bark, leaves and flowers have proved to have several pharmacological properties. The bark is proved to have anti ulcer, anti hypertensive and anthelminthic activities; leaves have anti pyretic action; fruits have proved to have anti microbial and antioxidant activities; and flowers are used as nervine tonic. Because of the nonavailability of proper curative therapy for AD, the present study has been undertaken to evaluate the possible role of *Mimusops elengi* Linn. flowers in experimental AD in rats.

**Materials and Methods**

**Animals**

Thirty male Wistar rats, weighing 150-200 g were procured from King’s Institute, Guindy. The animals were maintained in the animal house under standard laboratory conditions with natural dark and light cycle (approximately 12 h light / 12 h dark cycle) and room temperature (27±1°C) and constant humidity (60%) in accordance with Institutional Ethical Committee rules and regulations. They were fed on a standard balanced diet and provided with water ad libitum. The project proposal was approved by Institutional Animal Ethical committee (IAEC 75/2009).

**Chemicals**

Colchicine, THio barbituric acid, Nicotinamide dinucleotide sodium salt (NADH), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) were purchased from Sigma, USA and all other chemicals and reagents were purchased locally and were of the highest analytical grade. Donepezil (DOZ) was purchased from Sun Pharma, India.

**Collection of flowers and standardisation of extract**

The flowers of *Mimusops elengi* Linn. were collected from Nilgiri hills, Ooty, Tamilnadu and authentication (Voucherspecimennumber-PARC/2010/499) was done by Prof. P. Jayaraman, Ph.D., Plant Anatomy Research Centre, Medicinal plant Research Unit, Tambaram, Chennai-45. The collected flowers were cleaned, air dried at room temperature and ground to a coarse powder with an automixblender, passed through the sieve no:16 and stored in a deep freezer until the time for use. The powder was defatted with petroleum ether for 24 hours. Then, it was dried and cold macerated by using hydroalcoholic solvent (70% Ethanol and 30% Water) for about 5 days. The obtained extract (ME) was concentrated under reduced pressure and controlled temperature by Rotary evaporator at 40°C and stored in cool place. Preliminary Phytochemical investigation was carried out on the extract and the presence of alkaloids and saponins was proved.

**Experimental design**

The thirty animals were divided into five groups consisting of six animals in each group. The first group is the Normal –control group which recived distilled water (p.o). The second group is Colchicine-control group which recoived colchicine (15 μg/rat. I.C.V). The third and fourth groups received *Mimusops elengi* Linn. flower extract(ME) at doses of 00 and 200 mg/kg(p.o) respectively and the fifth group is the standard group which received Donepezil(img/kg.p.o). The ME treatment was given on the 1st day 30min prior to intracerebroventricular injection of colchicine and for another twenty seven days of the experimental period. Donepezil was also given to the standard group of animals for 28 days.
During the 7th, 14th and 28th days of the experimental period, all the animals in the five groups were studied for the behavioural activities and two animals from each group were sacrificed for biochemical assessments. Histopathological studies were also performed after 28 days of treatment.

**Behavioural Assessments**

**Elevated plus maze**

The Plus-maze apparatus consists of two open (50×10 cm) and two side-closed arms (50×10×40 cm) without roof, elevated 50 cm from the floor. The pretreated animals were placed individually for 5 minutes at the centre of the elevated plus-maze facing the head towards an open arm. The number of entries into the open and closed arm and the time spent in each arm were recorded. The percentage of number of entries (against the total number of entries both in open and closed arms) and time spent in the open and closed arms were calculated for each group.

**Radial Y-maze**

Radial Y-arm maze study was used to assess cognitive function. The apparatus is a three arm connected together in which the animals were trained to perform a standard radial arm maze (RAM) task. Rats were given 7 days habituation trials in which food pellets were scattered throughout the maze and the rats were allowed to freely explore inside it for 5 minutes. Following habituation sessions, the animals were trained for 10 daily trials on RAM task (10 trails/day). In this task, an animal was placed in the centre and was allowed to visit each of the 3 arms, which were baited with single food pellet. Entry into the arm previously visited within any daily trail was scored as an error. Animals not reaching this criterion were discarded from the study.

**Learned helplessness test: Conditioned Avoidance Training**

Sidman Jumping box was used for this purpose. This box was divided into two chambers and a gate is present. The animals were placed in one chamber of the box and were allowed to habituate to the test environment for 5 minutes and then were subjected to 30 avoidance trials. During the first 3 seconds of each trial, a light signal was present, allowing animals to avoid shocks. If a response did not occur during this period, a 0.8 mA shock and a “light conditioned stimulus” will be terminated. Avoidance sessions will be performed in the morning for 3 consecutive days (day 3, 4 and 5) in the morning, and the number of escape failures, referred to as “no crossing response during shock delivery” will be recorded.

**Water maze**

Spatial learning and memory was tested in a water maze. The maze consists of a black circular pool filled to a depth of 44 cm with water (25°C). Two each rat was placed in the water facing the wall at the start location and will be allowed 90 seconds to find the hidden platform. The animals were allowed a 20 second rest on the platform. The latency to reach the platform was recorded. If the rat was unable to locate the hidden platform, it will be lifted out and placed on the platform for 20 seconds. The procedure was repeated for all the four start locations. Sessions of 4 trials each will be conducted on the first day of testing separated by 4 hours, and one session of 4 trials were conducted on the next day. Each rat was placed in the pool at the same, randomly selected starting pole; the swimming path was observed and time spent in the quadrant of the pool, will be measured.

**Brightness Discrimination Test**

The rats were trained for a foot shock motivated in a brightness discrimination chamber. The rats were placed in the non-illuminated chamber for 10 minutes for adaptation. The chamber is so programmed that the alley on the left of this start box is illuminated. A foot shock (10 mA) causes the animals to leave the start box. Current flows to leave the floor grid in all parts of the chamber except the illuminated terminal box, so that the rat should finally enter this shock – free alley to escape punishment. In a positive trial, the animal immediately runs in to the illuminated alley; escape into the non-illuminated alley is a negative trial.

The light in the illuminated alley was switched off 25 sec after the entry of the rat. This was followed by a stochastic time interval before the next foot-shock was given. The terminal box then became the start box. The average interval between 2 successive foot-shocks was 57 seconds. The direction of alley illumination was
changed after every 3 trials to avoid position discrimination. A response was considered to be positive when the rat ran immediately into a lightened box in the last run prior to, and in the first run after, the change in the direction of alley illumination. The retention of learned behavior was assessed by re-learning. The re-learning test was done 24 hours after the completion of training and adaptation time was reduced from 10 minutes to 1 minute.

The result obtained from a re-learning test were analysed on the basis of increase in positive responses during relearning (DR), i.e., the difference between RR (positive responses during re-learning) and TR (positive responses during training) and the re-learning index (R.I), which was calculated from the formula:

\[ R.I = \frac{T_S - R_S}{T_S} \times 100(\%) \]

Biochemical Analysis

Estimation of Acetylcholinesterase activity

The anticholinesterase activity will be measured on the seventh, fourteenth, and twenty eight days after AD induced by colchicine. 20 mg of brain tissue per ml of phosphate buffer (pH 8.0, 0.1 ml) will be homogenized in a homogenizer. A 0.4 ml aliquot of brain homogenate will be added to a cuvette containing 2.6 ml of 0.1 M phosphate buffer (pH 8). 100 µl of DTNB reagent was added to the photo cell. The absorbance was measured at 412 nm. 20 µl of the acetylcholine iodide will be added. Changes in absorbance will be recorded and the change in the absorbance per minute will be calculated. The enzyme activity is expressed as µmoles/minute/mg tissue.

Estimation of Lipid Peroxidation in Brain homogenate

One milliliter of suspension medium was taken from 10% of the tissue homogenate. To this; 1 ml of 30% TCA was added, followed by 1 ml of 0.8% TBA reagent. The tubes were covered with aluminum foil and kept in a shaking bath at 30°C for 30 minutes at 80°C. These were then centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 535 nm at room temperature against the blank.

The content of MDA, expressed as n moles formed per milligram of protein in the tissue.

Assay of Superoxide dismutase (SOD)

Superoxide dismutase was assessed by the inhibition of formation of NADH-phenazinemethosulphalenitroblue tetrazolium formazan. The reaction was initiated by the addition of NADH after incubation for 90 s and stopped by the addition of glacial acetic acid. The colour formed at the end of the reaction was extracted into the butanol layer and measured at 520 nm.

Assay of Glutathione peroxidase (GPx)

The reaction mixture consisting of 0.2 ml of each EDTA, sodiumazide and H2O2, 0.1 ml of suitably diluted tissue was incubated at 37°C at different time intervals. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes will be centrifuged at 2000 rpm. To 0.5 ml of supernatant, 4 ml of disodium hydrogen phosphate and 0.5 ml DTNB was added and the colour developed was read out at 420 nm spectrophotometrically.

Activity is expressed as µ moles of glutathione oxidized/minutes/mg protein.

Assay of Reduced glutathione (GSH)

To 2 ml of the homogenate, prepared in KCl solution, 2.5 ml of 0.02 M EDTA was added and shaken. To 2 ml of the mixture, 4 ml of cold distilled water and 1 ml of 50% TCA were added and shaken for 10 minutes. The contents were centrifuged at 3000 rpm for 15 minutes. 2 ml of the supernatant was mixed with 4 ml of 0.4 M tris buffer (pH 8.9). The whole solution was mixed well and 0.1 ml of 0.01 M DTNB was added, the absorbance was read within 5 minutes of addition of DTNB at 412 nm against reagent blank with no homogenated.
Statistical Analysis

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet’s test. P values <0.05 were considered as significant, <0.01 is more significant.

Results

[Figure 1] depicts the results of Elevated plus maze which is used to evaluate the anxiety level, ME treatment significantly (P<0.05) reversed the decrease in open arm to closed arm ratio induced by colchicine indicating anxiolytic activity.

[Figure 2] depicts the results of Y-maze which is used to assess the cognitive behavior. The colchicine treated group animals undergo a significant (P<0.05) impairment indicating by a decrease of spontaneous alteration percentage. This percentage was found to be mainly improved in the 200mg/kg treated rats which were tested on 7th, 14th and 28th days of the experiment.

[Figure 3] shows the results of Conditioned Avoidance Response. It was proved that ME also improves memory deficits in the active avoidance task in Conditioned avoidance response test. ME treatment reverses the increased escape latencies with colchicine treatment, indicating improvement of memory with regard to negative reinforcement to electric shock. Hence, ME influence the psychological parameters associated with learning disabilities.

[Figure 4] shows the results of Watermaze. The rats treated with ME showed shorter swimming latencies to the goal performed which indicates improved spatial memory performance. ME treated rats also showed enhanced working performance in probe trials which indicates consolidation of memory. The ability to find the new platform kept in a different opposite quadrant was significantly compromised in col-treated rats. Water maze is more sensitive to spatial learning, dependent on the extra maze cues.

[Figure 5] shows the results of Brightness Discrimination test. In this test, ME shows an improvement in learning and memory behavior as there is an increase in reference time and re-learning index values.

[Figure 6] shows the results of Acetylcholinesterase levels which were proved to be increased by colchicine treatment when compared to Normal group and this levels were significantly (P<0.05) decreased on ME treatment.

[Figure 7] depicts the results of Lipid peroxidation which shows that col increased their levels when compared to control and was significantly (P<0.05) decreased by ME after 28 days of treatment.

![Figure 1](image_url)  
**Figure 1** Time spent in open arm in Elevated plus maze
Figure 2  Latency period (sec) in Radial Y-maze

Figure 3  Number of foot slips in Conditioned Avoidance Response

Figure 4  Escape latency in Water maze

Figure 5  Re-learning index % in Brightness discrimination test
Discussion

The present study demonstrates the beneficial effects of the standardized flower extract of *Mimusops elengi* Linn. in Colchicine induced AD in rats and proved that the extract (ME) significantly ameliorated the cognitive deficits induced by the neurotoxin in rats. Intracerebral infusion of colchicine causes it to bind with tubulin which is the structural and functional protein of microtubule and thereby generates more and more reactive oxygen species (ROS) leading to neurodegeneration and ultimately produces a condition like AD or produces experimental AD which is characterised by the extracellular deposition of senile plaques and the intracellular deposition of neurofibrillary tangles.

In the behavioural assessment tests, the ME treatment showed significant improvement in the anti-anxiety activity, spatial learning and memory.

The cholinergic loss in AD is a major component of neuropathy, which has been strongly demonstrated by the fact that cholinesterase inhibitors are effective in alleviating the symptoms of AD. Cholinergic neurotransmission with acetylcholinesterase inhibitor, physostigmine, reverses scopolamine–induced deficits in nondemented subjects and has been reported to improve the performance of AD patients that require long-term memory. Treatment with colchicines significantly enhances the acetylcholinesterase levels, especially after 28 days of administration.

Oxidative stress is a critical determinant in the stimulation of neuronal cell death and the toxicity results in an increase in the reactive oxygen species (ROS) and superoxide radicals, which results in oxidative damage within the cell. Free radicals play a crucial role in the pathogenesis of AD. Lipid peroxidation can be used as an index for measuring the damage that occurs in membranes of tissues as a result of free radical generation. In the present study, infusion of colchicine significantly increased the LPO level. The results of significant elevation of LPO level in cochicine treated group is due to the generation of free radicals via auto oxidation or through metal ion or superoxide catalysed oxidation process. In the present study, ME significantly decreased LPO level in a dose dependent manner compared to other groups. So, from the result of LPO levels it may be concluded that the protection by ME may be due to alkaloids and saponins which are present in *Mimusops elengi* Linn. flower extract.
Endogenous antioxidant status in colchicine induced experimental Alzheimer’s rat model was evaluated here by noting the activities of SOD, GPx and GSH as these are the important biomarkers for scavenging the free radicals[25]. Colchicine induced oxidative stress is further supported here by the study of antioxidant scavenger enzyme activities. The destruction of superoxide radicals is catalyzed by SOD, is an important defence system against oxidative damage. From our experimental results of the foresaid antioxidant enzyme activities in brain tissue, colchicine significant decreased SOD,GSH activities in colchicine treated experimental Alzheimer’s groups. ME flowers containing saponins and alkaloids significantly increased SOD,GSH levels.

Glutathione is an endogenous antioxidant, which is present majorly in the reduced form within the cells. It prevents the hydroxyl radical generation by interacting with free radicals. During this defensive process, reduced glutathione is converted to oxidized form under the influence of the enzyme glutathione peroxidase(GPX). The decreased level of reduced glutathione in colchicine treated experimental group seen in our study indicates that there was an increased generation of free radicals and the reduced glutathione was depleted during the process of oxidative stress[28,29]. ME treatment significantly increased the SOD,GPX and GSH levels due to the presence of alkaloids and saponins. Previous literature showed that Galantamine, Montanine are the alkaloid which acts by inhibiting the acetylcholine esterase activity[30] and Huperzine is another alkaloid with cognitive and neurorotective properties[31].Ginseng saponins are proved to have anti depressant effect which also helps in AD treatment[32]. Further study have to be done regarding the presence of the specific alkaloids with AChE inhibiting action and saponins in the extract of Mimusops elengi flowers.

In view of the above facts, Mimusops elengi flower extract can be said to play a protective role in Alzheimer’s disease.

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