Original Article

Nootropic Studies of Ethanolic Extract of *Mimosa pudica* Linn. in Albino Wistar Rats

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ABSTRACT

Aim: To evaluate the neuropharmacological parameters of the ethanolic extract of *Mimosa pudica (M.pudica)* Linn. (EEMP) in albino wistar rats. "Nootropics" are agents that enhance the cognitive skills. Learning and memory can be conceived as both a psychological process, as well as a change in synaptic neural connectivity. Hardly there are a few medicines for the treatment of neurodegenerative disease and are having harmful effects. So to overcome the harmful effect and to develop a cost effective method this study is selected. To overcome the unwanted effect of synthetic medicines and to develop a cost effective method for the treatment of neurodegenerative diseases.
Methodology: EEMP went for preliminary phytochemical screening. The nootropic studies in both acute & chronic models of amnesia, induced by scopolamine & $AlCl_3$ respectively with elevated plus maze & object recognition procedures were done. The Inflexion ratio (IR) specific for sustained memory and Discrimination index (DI), specific for selective memory were assessed respectively from the above tests. Observations were expressed in mean ± SEM and the statistical analysis was done by one way ANOVA followed by Dunnett's Multiple Comparison Test.
Results and Discussion: The preliminary phytochemical screening of EEMP confirmed the presence of flavanoids, phenolic compounds etc. which may be responsible for the antioxidant effect. The nootropic studies observed statistical significance in $AlCl_3$ model.
Conclusion: The study confirmed the nootropic (cognition enhancement) activity of EEMP, which may be due to the presence of flavanoids and the reported activity may be attributed to its antioxidant property,

Keywords: *Mimosa Pudica* Linn., Nootropic.

INTRODUCTION

The human brain is almost certainly the least understood of our organs. When facing the diseases affecting the brain, the medical sciences are in an unfortunate situation of studying and attempting to prevent/cure unknown pathological processes where even the normal conditions are poorly understood. On this back ground, numerous diseases are in a desperate need for an improved understanding and more adequate methods of intervention. Alzheimer's disease, primarily affects the elderly population, and is estimated to account for 50-60% of dementia cases in persons over 65 years of age.[5]

Memory is ability of an individual to record event, information and retains them over short or long periods of time and recalls the same whenever needed. Age, stress and emotion are conditions that may lead to memory loss, amnesia, anxiety, high blood pressure, dementia, more ominous threat like schizophrenia and Alzheimer's diseases. "Nootropics" are agents that enhance the cognitive skills. Learning and memory can be conceived as both a psychological process, as well as a change in synaptic neural connectivity. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative states.[9]

Before the development of modern medicine people relied on natural remedies for the treatment of CNS related maladies. It is on this basis that researchers keep on working on medicinal plants in order to produce/develop medicines from herbs, nutraceuticals or life style changes for controlling age related neurodegenerative disorders.

The plant used in the present study is *M.pudica* which was used in the Ayurveda, in piles, jaundice, leprosy, ulcers and snake bite.[13] Mimosa Pudica can be used for several homeopathic treatments including ailments related to the urinary tract.

MATERIALS AND METHODS

Plant material

The plant materials of *M.pudica* (whole plant) was collected from nearby places of University College of Pharmacy, Cheruvandoor, and authenticated by Joby Paul, Mahatma Gandhi university .The plant was collected in the months of January to March and shade dried at room temperature and subjected to extraction procedures.

Animals

Wistar albino rats weighing 150-200 *g* of either sex maintained under standard husbandry conditions (temp 23 ± 2 ^{o} *C*, relative humidity 55 ± 10% and 12 *hr* light dark cycle) were used for the screening which was obtained from the animal house of the University College of Pharmacy, Cheruvandoor. Animals were fed with standard laboratory food and *ad libitum* during the study period. The experiments were performed after getting the approval for experimental protocol from the institutional animal ethics committee, India 2012 under the IAEC no: *015 /MPH/UCP/CVR/12*.

Drugs & Chemicals

Piracetam, Scopolamine & Aluminium chloride, Ethanol 95%, Carboxy methyl cellulose.

Preparation of ethanolic extract

Ethanol is used for the extraction process because almost all the components of m.pudica is soluble in ethanol solvent. Plant materials shade dried and grounded with the help of an electrical grinder to get a free flowing powder. This powder was subjected to extraction with dehydrated alcohol at room temperature for 24 h using soxhlet

apparatus.[11] The extract obtained was filtered through Whatman filter paper and dried at 40-50°C to get a blackish green semisolid mass, which was taken for final use.

Experimental protocol for Nootropic Activity

Scopolamine induced model of dementia

Treatment protocol for inducing acute dementia using scopolamine.

Group I: Control; Treated with Scopolamine (0.3 mg/Kg) +CMC (0.5%w/v)

Group II: Standard; Treated with Scopolamine (0.3 mg/kg) +Piracetam (50mg/kg)

Group III: Test; Treated with Scopolamine (0.3 mg/kg) +EEMP (500mg/kg)

All the groups contained 6 animals and each animal was given the recommended treatment per orally 30' before conducting the experimental trials. Scopolamine, used for inducing acute dementia was given30, minutes prior to the recommended treatment.

Aluminium chloride induced model of dementia

Treatment protocol for inducing Chronic Dementia using $AlCl_3$.

Group I: Control; Treated with CMC (0.5%w/v)

Group II: Standard; Treated with $AlCl_3$ (50 mg/kg) +Piracetam (50 mg/kg)

Group III: Test; Treated with $AlCl_3$ (50 mg/kg) +EEMP (500mg/kg).

All the groups contained 6 animals and each animal was given the recommended treatment per orally. Aluminium chloride, used for inducing chronic dementia was given for 41 days prior to the recommended treatment. Recommended treatments were started on the 21st day.

Elevated Plus-Maze Test

Elevated plus maze consisted of wood with two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm) was used. The maze was elevated to the height of 40 cm. The rats should be placed individually at the end of one arm facing away from the center of the elevated plus maze and the time the rat took to move from open arm to either of the closed arms (Transfer latency, TL) was recorded. On the first day, the rats were allowed to explore the plus maze for 20 sec[14]**.** After the measurement of TL rats were returned to their home cages after the first trial. Twenty four hours later, the rats were placed on the elevated plus – maze individually as before and TL was recorded again. TL measured on 1st (**L_1)** and 2nd (**L0)** day served as parameters for acquisition and retrieval respectively. From these the inflexion ratio(IR) was calculated using the formula: **$IR=L_1/L_0$**.

Novel Object Recognition (NOR) Test

The open field apparatus consisted of white colored plywood (70x60x30 cm) with a grid floor that can be cleaned with hydrogen peroxide after each trial. The objects to be discriminated were placed at diagonally opposite corners of the box. On the day of test in the first trial (T1), two identical objects were presented in two opposite corners of the box and the amount of time taken by each rat to complete 20 s explorations was measured. Exploration meant directing the nose at a distance less than 2cm to the object and/or touching with the nose[16]. During the second trial (T2, 90 min after T1) a new object replaced one of the object presented T1 and time spent for exploring new (N) and familiar (F) objects was recorded. The Discrimination index (DI) was calculated as (N-F)/ (N+F). The animals were treated with the vehicle or drugs, 30 min before the first trial.

Statistical analysis

The results of the studies were expressed as mean ± SEM (standard error of mean). The difference between the control and treated means were analyzed using one-

way analysis of variance (ANOVA). P-values < 0.05 were taken to be statistically significant. Dunnets post hoc test was used for multiple comparisons. The statistical analysis was done using the software Graphpad prism version no: 5.0. Results were presented as Tables and Figures.

RESULTS & DISCUSSION

There is substantial evidence that oxidative stress is a causative or at least ancillary factor in the pathogenesis of major neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, etc. Evidence of increase in lipid peroxidation and oxidation of DNA and proteins had indeed been seen in the substantia nigra of patients affected with AD. **(Jain *et al*., 2008)**[7] pointed out that oxidative stress had either primary or secondary role in the etiologic progression. The high lipid content of nervous tissue, coupled with its high aerobic metabolic activity, makes it particularly susceptible to oxidative damage. Those brain regions that are rich in catecholamines are exceptionally vulnerable to free radical generation[2]. The catecholamine adrenaline, noradrenaline, and dopamine can spontaneously break down (auto-oxidize) to free radicals, or can be metabolized to radicals by the endogenous enzymes such as MAO (monoamine oxidases).

Due to their lower redox potential Fl-OH are thermodynamically able to reduce highly oxiding free radicals such as super oxide,peroxyl,alkoxyl,hydroxyl radicals by hydrogen atom donation.

Piracetam has an effect on NMDA glutamate receptors which are involved with learning and memory processes. Piracetam, which is a derivative of GABA, plays an important role in cognitive function.

Many neural system accommodations take place in the chronic neurological deficit situation which may not be present in the acute deficit. In chronic deficits, it is critical to test the chronic efficacy of the drug to determine possible tolerance development or induction of a persisting effect. In the present study the dementia models were induced using both scopolamine and $AlCl_3$. Both models were specifically tested for both sustained and selective attention with Elevated plus maze and Novel object recognition tests, respectively. The latency to reach the central platform of the elevated plus maze is indicative of the learning ability of the animals. The animal is said to have learnt if the latency to reach the central platform is reduced. The object recognition test is a simple and quick method to test short term memory in rats and mice. Rats can identify a familiar object more readily as compared to a new object.

In the present investigation, the increase in IR which was 0.16±0.0425**(Table:3.4)**,by the EMP(500 mg/Kg, p.o.) proved that *Mimosa pudica* possessed nootropic activity in $AlCl_3$ induced model of dementia as seen from the result of EPM test **(Figure:3.3)** and this was specific for sustained attention[4].

EEMP extract exhibited an increase in mean discrimination index of 0.564±0.039 (**Table: 3.5)**. The improvement in discrimination index proved from **Figure: 3.4** that EEMP met major criteria for nootropic activity, i.e. improvement of memory in cognitive deficit and this test was specific for selective attention[6].

According to Filho *et al.*, 2006, EEMP is AchE inactive which may be the reason for the nonsignificant results obtained from the scopolamine model of dementia, where the mechanism of action is through the muscarinic Ach receptor, after EPM and NOR tests.

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Table 3.1. Chemical constituents present in EEMP

Sr. No	Test	EEMP
1	Alkaloids	+
2	Glycosides	+
3	Terpenoids	-
4	Carbohydrates	+
5	Proteins	+
6	Steroids	+
7	Flavanoids	+
8	Phenols	+
9	Tannins	-
10	Quinones	-
11	Saponins	-

+ Indicates the presence, - Indicates the Absence

Scopolamine Induced Cognitive Impairment: Elevated plus maze test

Table 3.2. Effect of EEMP on mean Inflexion Ratio in Elevated Plusmaze test

Sr.No	Groups	Treatment	Mean IR
1	Control	CMC(0.5%w/v) + Scopolamine (0.3mg/kg) (p.o.)	1.03±0.178
2	Standard	Piracetam(50mg/kg)+ scopolamine (0.3mg/kg) (p.o.)	2.836±0.781*
3	Test	EEMP(500mg/kg) + scopolamine (0.3mg/kg)(p.o.)	1.583±0.202^{NS}

Novel Object Recognition Test

Table: 3.3. Effect of EEMP on mean Discrimination Index in Novel object recognition test

Sr.No.	Groups	Treatment	Mean D I
1	Control	CMC (0.5%w/v)+ Scopolamine (0.3mg/kg)(p.o.)	0.355±0.138
2	Standard	Piracetam (50mg/kg)+ scopolamine (0.3mg/kg) (p.o.)	0.808±0.065*
3	Test	EEMP(500mg/kg) + scopolamine (0.3mg/kg)(p.o.)	0.505±0.123 ^{NS}

Model for Chronic Dementia: Aluminium Chloride Induced Cognitive Impairment

Table: 3.4. Effect of EEMP on mean Inflexion Ratio in Elevated Plus maze test

Sr.No	Groups	Treatment	Mean IR
1	Control	CMC(0.5%w/v) + $AlCl_3$(50mg/kg) (p.o.)	0.019±0.00176
2	Standard	Piracetam(50mg/kg)+ $AlCl_3$(50mg/kg) (p.o.)	0.221±0.038**
3	Test	EEMP(500mg/kg) + $AlCl_3$(50mg/kg) (p.o.)	0.16±0.0425*

Novel Object Recognition Test

Table: 3.5. Effect of EEMP on mean Discrimination Index in Novel object recognition test

Sr.No	Groups	Treatment	Mean DI
1	Control	CMC(0.5%w/v) +$AlCl_3$ (50mg/kg)(p.o.)	0.27±0.037
2	Standard	Piracetam(50mg/kg)+ $AlCl_3$ (50mg/kg) (p.o.)	0.64±0.043 ***
3	Test	EEMP(500mg/kg) +$AlCl_3$ (50mg/kg)(p.o.)	0.564±0.039***

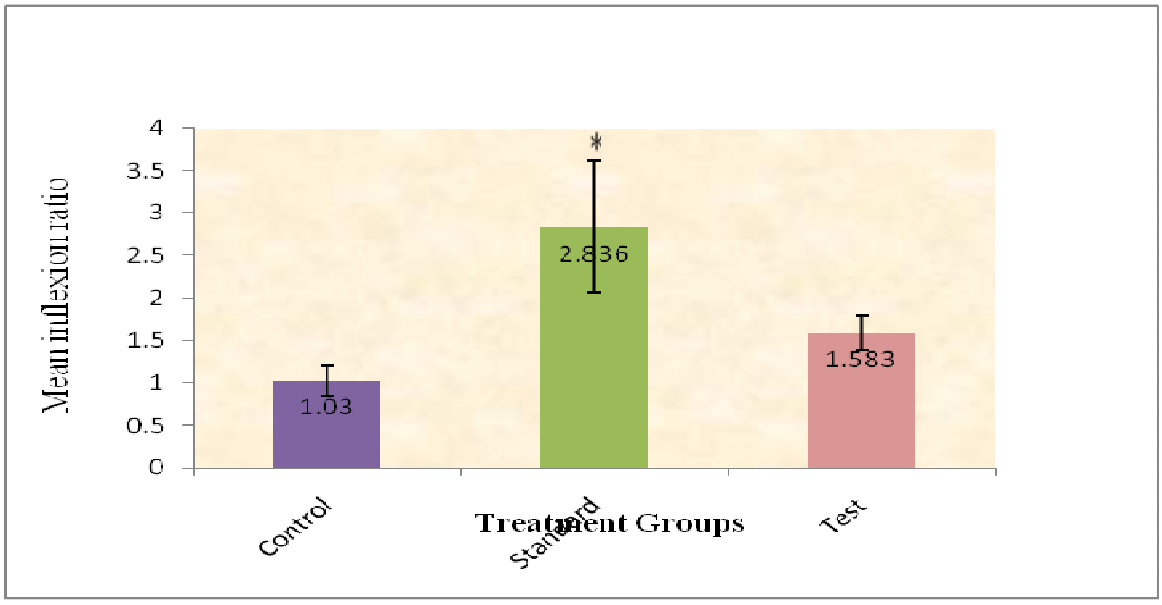

Figure.3.1. Effect of EEMP on sustained attention after Elevated Plusmaze test in acute model of dementia induced by scopolamine, expressed as mean±SEM, analysed by one way ANOVA followed by Dunnett's post hoc tests: F value: 3.76, DF : 2, 15, *P<0.05,**P<0.01, ***P<0.001.

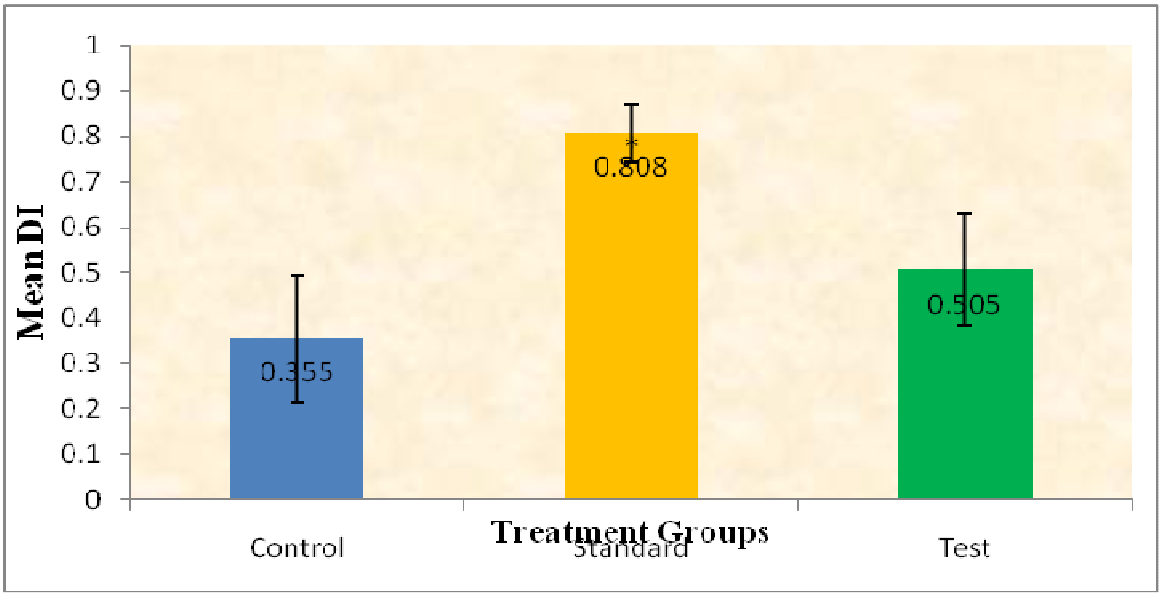

Figure.3.2. Effect of EEMP on selective attention in Novel object recognition test, in acute model of dementia induced by scopolamine, expressed as Mean±SEM, analysed by one way ANOVA followed by Dunnett's post hoc tests: F Value: 4.170; DF: 2, 15; *P<0.05,**P<0.01, ***P<0.001.

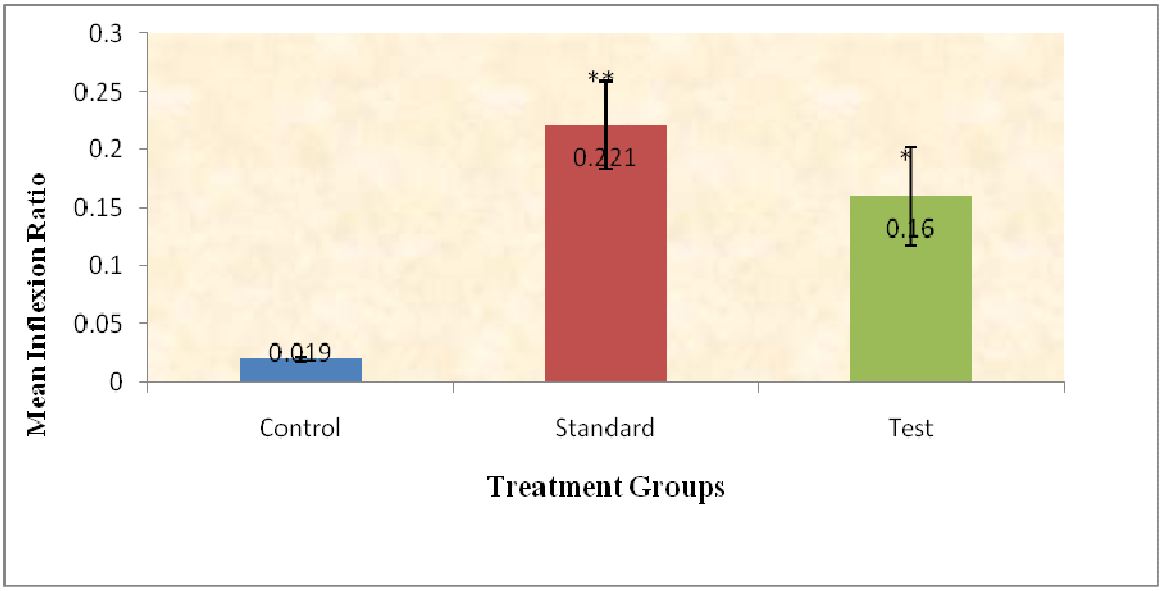

Figure.3.3. Effect of EEMP on sustained attention in Elevated Plusmaze test in chronic mode of dementia induced by $AlCl_3$, expressed as mean±SEM, analysed by one way ANOVA followed by Dunnett's post hoc tests: F: 4.170, DF:2, 15, *P<0.05,**P<0.01, ***P<0.001.

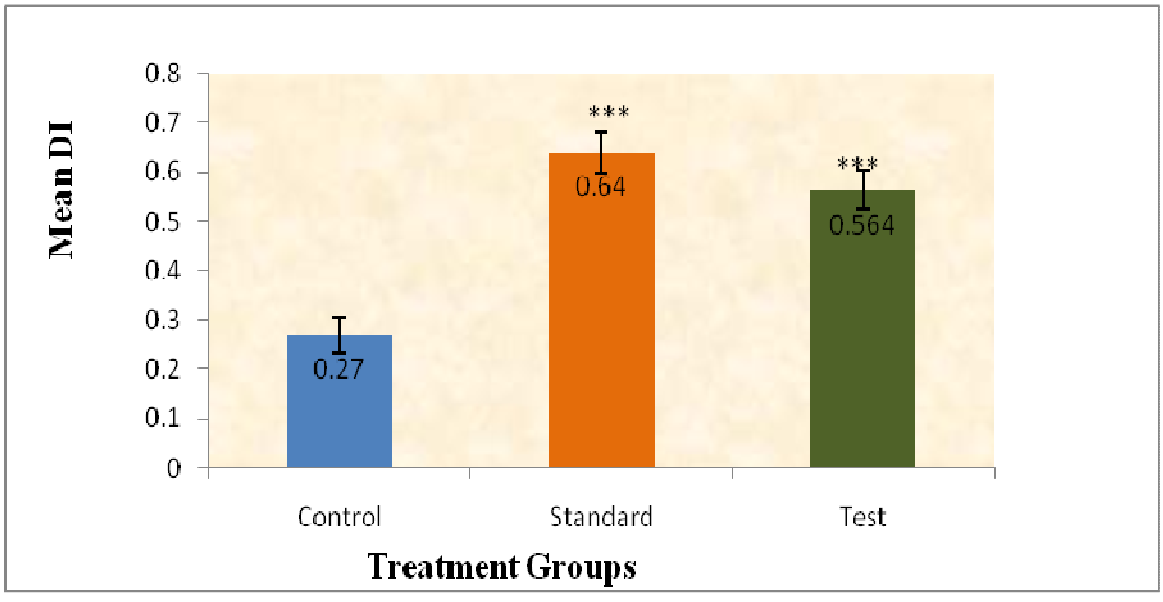

Figure.3.3. Effect of EEMP on selective attention in Novel object recognition test in chronic model of dementia induced by $AlCl_3$, expressed as mean±SEM, analysed by one way ANOVA followed by Dunnett's post hoc tests: F:20.10, DF:2,15, **P<0.05,**P<0.01, ***P<0.001.