https://ijrps.com

ISSN: 0975-7538
Research Article

Ameliorative effect of *Cleome gynandra* Linn against scopolamine induced amnesia in mice

Manasa A*, Karimulla S.K, Gobinath M

Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh, India

ABSTRACT

The objective of this study was to evaluate various extracts of leaves of *Cleome gynandra* L for its memory enhancing ability. The exteroceptive behavioral models such as elevated plus maze, Morris water maze, Radial arm maze, Y maze and Open field test were used to evaluate the learning and memory, whereas scopolamine is the natural ageing inducing amnesia served as interoceptive models. Parameters like Escape Latency Time (ELT), Time Spent in Target Quadrant (TSTQ), Transfer latency (TL), Peripheral movements and Rearings alternation to estimate the short-term memory and determination of brain Acetyl cholinesterase and β-Amyloid protein levels. The activity was compared with standard drug Piracetam. Single dose of (200mg/kg/oral) various extracts were orally administered for eighteen successive days in separate groups of animals and the doses were selected according to the animal weight. The activity was compared with standard drug Piracetam. Among different extracts, ethanolic extract has significantly improved the learning and memory in mice. Furthermore, the single doseof ethanolic extract was significantly reversed the amnesia induced by scopolamine (0.4mg/kg I.P). The presence of steroids, Flavonoides and potent antioxidant property of ethanolic extract of leaf of *Cleome gynandra* L. may be contributing favorably to memory enhancement effect. Since scopolamine induced amnesia was reversed by *Cleome gynandra* L., it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic transmission and inhibition of β-Amyloid levels in mouse brain.

Keywords: Dementia; Alzheimer's; *Cleome gynandra;* Acetyl cholinesterase; β-Amyloid; Piracetam

INTRODUCTION

Alzeimer's disease (AD) is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behavior, personality changes and ultimately death (Jewart RD., et al 2005). AD is characterized by the presence of excessive amounts of neurotic plaques containing amyloid β protein loss of cholinergic markers in brain. Loss of cholinergic cells particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine (Selkoe DJ 1994). A decrease in acetyl choline in the brain of patients with AD appears to be a critical element in producing dementia (Becker R., et al 1998). The cause of AD is not known clearly. Recently, the mainstay treatments for the AD are acetylcholinesterase inhibitor (AchE) which increase the availability of acetylcholine at cholinergic synapses. AChE inhibitors from general chemical classes such as physostigmine, tacrine, galantamine and heptylphysostigmine have been tested for the symptomatic treatment of AD (Becker R et 1988). However, non-selectivity of these drugs, their limited efficacy, and poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity are among the sever limitations to their therapeutic success (Bores GM et al., 1996).

Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders (Silman I et al., 2005).

Indian systems of medicine emphasize use of herbs, nutraceuticals or life style changes for controlling age related neurodegenerative disorders. The *Cleome gynandra* Linn. (Ayurvedic Pharmacopoeia of India, Volume-1:13) Is traditionally used as anti-diabetic, antioxidant, anticancer, analgesic, and anti-malarial, antihelmintic, antidiabetic, anti-inflammatory. (Narendhirakannan RT et al., 2005, Asis Bala et al., 2010) The leaves and seeds are used as mustard, headache, and neuralgia externally counter irritant and roots are considered dicoction and fabrifuge. Based on the scientific evidences for leaves of *cleome gynandra* for its antioxidant activity (Anbazhagi T et al., 2009) congnitives studies have been evaluated. Hence in this protocol various leaf extracts of the plant were evaluated for learning and memory activity against scopolamine induced amnesia in mice.

* Corresponding Author
Email: amaluru.manasa@gmail.com
Contact: +91-9652945541
Received on: 05-11-2015
Revised on: 21.11.2017
Accepted on: 04.12.2017

MATERIALS AND METHODS

Plant material

Dried leaves of *cleome gynandra* Linn. Was collected locally in the month of December and authenticated by expert botanist Dr. Bhaskar, Principal/Lecturer in-charge, Department of botany, V.R. College, Nellore, Andhra Pradesh, India.

Equipment and Chemicals

Electronic balance, Morris water maze, Elevated plus maze, Y-maze, Radial arm maze, Syringes and needles.

Drugs: *Cleome gynandra* Linn. Dried leaves extract, dose: 200mg/kg oral, Piracetam in water for injection 200mg/kg (I.P), and scopolamine 0.4mg/kg (I.P), 5,5-dithionitrobenzoic acid (DTNB), Acetylcholine iodide were purchased from sigma Aldrich.(D S Reddy, 1997)

Animals

Swiss albino mice (20-25gm) of male sex and of approximately the same age were selected for the study. They were housed in polypropylene cages and fed with standard rodent pallet diet (Hindustan Level Limited, Bangalore) and water ad libitum. All experiments were performed in the morning according to current guidelines for care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. (Smith G, 1988)

Preparation of extract

The Leaves of *cleome gynandra* Linn was collected and dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve no.40 and stored in an airtight container for the extraction. (Trease and Evans, Pharmacognosy, 15th Edition).

Extraction of dried Leaves of *Cleome gynandra Linn.*

The dried powdered material was extracted with successive method of 99% ethanol, ethyl acetate and petroleum ether by using soxhlet apparatus. The collected extract is evaporated to remove ethanol, ethyl acetate and petroleum ether by using rotary vaccum evaporator. Dried powder of petroleum ether, ethyl acetate and ethanolic extracts of leaf of *Cleome gynandra* Linn (PEECG, EAECG, EECG) is mixed with 10% Tween80 and administered to the animal.

Preliminary Phytochemical Analysis

The extracts were subjected to preliminary Phytochemical screening of the leaf extract of *cleome gynandra* Linn. was carried out and showed the presence of Glycosides, Flavonoids, steroids, Tannins, Terpenoids And Amino acids.

Experimental design

Grouping and Treatment Protocol

The six groups of animals were made, each group consisting of six mice. The following were the groups.

Group I: It is served as normal group - normal food and distilled water is administered orally to mice for 18 days.

Group II: It is served as diseased group – administered distilled water orally for fifteen successive days and scopolamine (0.4 mg/kg b wt) was given from 15th to 18th day, after 45 minutes of administration.

Group III: It is served as standard group – Piracetam (200mg/kg//b w t/day) is administered intra peritoneally for fifteen successive days to mice. Scopolamine is injected after 45min of administration daily from 15th day to 18th day.

Group IV: It is served as test group 1- PEECG (200mg/kg/b.wt) is administered orally for fifteen successive days and scopolamine is injected (I.P) after 45min of administration daily from 15th day to 18th day.

Group V: It is served as test group 2 – EAECG (200mg/kg/b.wt) is administered orally for fifteen successive days and scopolamine is injected (I.P) after 45min of administration daily from 15th day to 18th day.

Group VI: It is served as test group 3- EECG (200mg/kg) is administered orally for fifteen successive days and scopolamine is injected (I.P) after 45min of administration daily from 15th day to 18th day.

PHARMACOLOGICAL STUDIES

Behavioral Studies

a) Radial Arm Maze

Construction of radial arm maze

A radial arm maze is used to evaluate working memory in the animals. Each arm (50 x 12cm.) of the eight-arm radial maze extends from an octagonal shaped central hub of 30cms diameter. The platform is elevated 40cms above the floor; small black metal cups (3cm in diameter & 1cm deep) are mounted at the end of each arm that serve as receptacles for reinforcing food. The Radial arm maze method serves as interceptive behavioral model to evaluate learning and memory in mice. The procedure and end point applied in present study for testing learning and memory have been described below.

Mechanical screening for memory in radial arm maze

The animals for the experiments were preselected by conducting at least one daily training trail. At the beginning of trail, a food pellet was placed in one receptacle. An overnight fasted mouse was placed in the central hub and allowed to choose the arm freely, to get the food. The trail was considered to be complete when mouse visited all eight arms. Entry into an arm that the mouse had not previously visited was recorded as a correct response & re-entry was counted as an error. A trail in which animals made no error or only

one error at the eight choices was recorded as a "successful" trail. The percentage of successful mice was calculated as the index of radial maze task performance. On the 18th day, 60 minutes after the last dose, animals of respective groups were subjected to scopolamine (0.4mg/kg I.P.) treatment for inducing amnesia. After 30 minutes each mouse was placed on central hub & tested again for successful trail. Results of Radial arm maze method were shown Table no.1.

b) Elevated plus maze

The apparatus consists of two open arms (35 X 6 cm) and two enclosed arms (35 X 6 X 15 cm). The arm was connected together with a central square of 5 X 5 cm. The maze was elevated to a height of 100 cm. The maze was placed inside a light and sound attenuated room. Mice were placed individually at the end of an open arm of elevated plus maze (EPM) facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms Transfer Latency (TL) was recorded (14).Transfer latency (TL) was taken as the time taken by animal move from the open to enclosed arm with in 90sec. The mouse was allowed to explore the maze for 10 sec and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day. On the 18th day, 90 min after the treatment of last dose first trial is given and after 24hr TL was noted for second time (i.e. on 19th day) (Hanumanthachar Joshi et al., 2006), (N Venkato Rao et al., 2008). The inflexion ratio was calculated by the formula (Jaiswal A K, et al., 1992).

$$IR = \frac{(L_o - L_1)}{L_o}$$

Where,

L_0 is the initial transfer latency (TL) in Sec on first time,

L_1 is the transfer latency (TL) in Sec on 2ndtime.

Decrease IR indicates the induction of amnesia, and increased IR indicates in improvement in cognition and memory impairment.

c) Y-maze task

Construction of Y-maze task

A variety of y maze task paradigms are available for the evaluation of spatial working and long term memory in rodents. Using food or sweetened water as an incentive to reach the goal, animals are either required to execute a specific search sequence or minimize time/errors in quest for a reward (starter et al., 1998). Temporal measurements and error scoring parameters are key parameters recorded for the evaluation of drug effects administered either or prior to or post training. The spontaneous alteration task par diagram is the simplest version of y maze task used to measure the spatial working memory in rats (Kelsey and waller, 2004).

Procedure

Spatial recognition memory was assessed by recording spontaneous alternation behavior in a single-session Y-maze on the 18th day (Rasoolijazi and Nobakht, 2007). The maze was made of black wood. Each arm was 40 cm long, 30 cm high and 15 cm wide. The arms converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was as follows: each rat, naive to the maze, was placed at the end of one arm and was allowed to move freely through the maze during an 8-min session. The series of arm entries were recorded visually. Entry was considered to be complete when the base of the animal's tail was entirely within the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The maximum number of possible spontaneous alternations was determined as the total number of arms entered minus 2, and the percentage was calculated as the ratio of actual to possible alternations × 100.

d) Morris Water Maze

Each animal was subjected to four consecutive trials each day with a gap of 5 min for four consecutive days, during which they were allowed to escape on to the hidden platform and to remain there for 20 seconds. If the mouse failed to find the platform within 120 sec, it was guided gently on to the platform and allowed to remain there for 20 sec. Escape latency time (ELT) is defined as the time taken by the animal to locate the hidden platform was choosen as parameter for learning and memory. ELT was noted as an index of learning. On 18th day the platform was removed. Mouse was placed in water maze and allowed to explore the maze for 120 sec. Each mouse was subjected to four such trials and each trial was started from a different quadrant. Mean time spent in all the three quadrants i.e. Q1, Q2 and Q3 was recorded and the time spent in target quadrants (TSTQ) in search of the missing platform provided as an index of retrieval. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory (Parle Milin et al., 2009).

e) Estimation of Brain AchE Activity

On the 18th day animals were euthanized by cervical dislocation carefully to avoid any injuries to the brain tissue. The whole brain AchE activity was measured by using the Ell man method (Ellman GL et al., 1961).The end point was the formation of the yellow color because of the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5, 5-dithionitrobenzoic acid (DTNB), and the optical density

(OD) of the yellow color compound formed during the reaction at 420 nm every minute for a period of 3 min was measured. Protein estimation was done using Auto analyzer (ELICO CL 380). AchE activity was calculated using the following formula (Ellman GL et al., 1961).

$$R = \frac{\delta\ 0.D. \times Volume\ of\ Assay(3ml)}{E\ \times\ mg\ of\ protein}$$

Where R is the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein. δ OD is the change in absorbance per minute and E is the extinction coefficient, which is 13 600 $m^{-1}cm^{-1}$.

STATISTICAL ANALYSIS

All the data was expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (graph pad prism 5.01). Statistical significance was set accordingly.

RESULT AND DISCUSSION

Alzheimer's disease related dementias are neurodegenerative conditions characterized by progressive brain dysfunction occurring in a step-wise biologic sequence: neuronal injury, synaptic failure and neuronal death. Neurofibrillary tangles, amyloid plaques and degeneration of cholinergic neurons are the pathological hallmarks of alzheimer's disease (Cummings JL, 2004). To improve cholinergic transmission, different strategies are adopted, including increase of Ach synthesis, the augmentation of pre synaptic Ach release, another stimulation of cholinergic post synaptic muscarinic and nicotinic receptors and the inhibition of Ach synaptic degradation by employing cholinesterase inhibitors (Izzo AA, 2007). Despite the availability of various treatment strategies, the severity and prevalence of this disease is not yet under control. Therefore, alternative and complementary medicines including herbal supplements are being utilized in the management of this disease (Dhingra D et al., 2003). The antiamnesic effect of *Cleome gynandra* Linn extracts were assessed by using *in vivo* model of Scopolamine induced amnesia in mice by Morris water maze, Radial arm maze, Elevated plus maze, Y-maze. Scopolamine produces amnesia by blocking the muscarinic acetylcholine receptors in the brain (Joshi Hanumanthachar et al., 2007).

Scopolamine induced amnesia is a well-accepted models of amnesia and it has been used by researcher for screening the drug candidates for the treatment of dementia or Alzheimer's disease. In this study we have evaluated well known phytomedicine *Cleome gynandra* Linn for possible application for the treatment of Alzheimer's disease.

Radial arm maze model for learning performance

Radial arm maze task performance for learning of Group I Animals treated with vehicle was found to take 12.93±0.44 seconds average for successful trails. Group II Animals treated with scopolamine 0.4mg/kg I.P was found to take 17.15±1.10 seconds average for successful trails. Group III Animals treated with piracetam200mg/kg I.P was found to take 11.24±0.62 seconds average for successful trails. Group IV Animals treated with PEECG- 200mg/kg P.O was found to take 13.25±0.81 seconds average for successful trails. Group V Animals treated with EAECG -200mg/kg, P.O was found to take 11.64±0.40 seconds average for successful trails. Group VI Animals treated with EECG-200mg/kg, P.O was found to take 10.24±0.54 seconds average for successful trails. Among the test extract tested at 200mg/kg P.O showed the value below to standard drug piracetam. This clearly indicates EECG (200mg/kg, P.O) significantly increased the learning memory performance. The result shown Table no.1.

Radial arm maze for memory

Radial arm maze task performance for learning of Group I Animals treated with vehicle was found to take 9.0±0.45 Errors average for successful trails. Group II Animals treated with scopolamine 0.4mg/kg I.P was found to take 17.0±0.77 Errors average for successful trails. Group III Animals treated with piracetam200mg/kg I.P was found to take 12.44±0.88 Errors average for successful trails. Group IV Animals treated with PEECG- 200mg/kg P.O was found to take 13.06±0.83 Errors average for successful trails. Group V Animals treated with EAECG- 200mg/kg P.O was found to take 12.17±0.74 Errors average for successful trails. Group VI Animals treated with EECG- 200mg/kg P.O was found to take 11.61±0.78 Errors average for successful trails. Among the test extract, EECG at 200mg/kg P.O showed the value near to standard drug piracetam. This clearly indicates ethanolic extract significantly increased the learning memory performance. The result shown Table no.2.

Elevated Plus Maze

The results are given in Table 3 and plotted graph is shown in Fig 1. The Inflexion ratio (IR) of the Group II (sleep deprived) animals were significantly decreased in comparison with the Group I (normal control) animals ($p< 0.001$). Plant extract (200 mg/kg) dependently increased IR in Group IV, Group V & Group VI, significantly ($p< 0.001$) and is comparable with Piracetam (200mg/kg) Group III compared with group II. Decrease IR (sleep deprived, Group II) indicates the induction of amnesia, and increased IR (Treatment groups) indicates protection from memory loss due to sleep deprivation and improved in cognition and memory impairment.

Table 1: Effect of cleome gynandra Linn. On learning performance by Radial arm maze maze based on duration to visit all the arms

S.NO	GROUP	Average time Taken
1	Normal (distilled water)	12.93±0.44
2	Diseased (Scopolamine0.4mg/kg/I.P)	17.15±0.10###
3	Standard (Piracetam200mg/kg/I.P + scopolamine0.4mg/kg/I.P)	11.24±0.62***
4	Test1 (PEECG200mg/kg/P.O + scopolamine0.4mg/kg/I.P)	13.95±0.81*
5	Test2 (EAECG200mg/kg/P.O + scopolamine0.4mg/kg/I.P)	12.81±0.54**
6	Test-3 (EECG 200mg/kg/P.O + scopolamine0.4mg/kg/I.P)	10.24±0.54***

Values are expressed as Mean±SEM,n=6. #-Diseased group compared with normal group; *-Std, test-1, test-2 and test-3 compared with diseased group; #/*-indicates p<0.5, ##/**-indicates p<0.01; ###/***-indicates p<0.001.

Table 2: Effect of Cleome gynandra Linn on memory retention against scopolamine induced amnesia by radial arm maze model

S.NO	GROUP	NO.OF. ERRORS
1	Normal (distilled water)	9±0.45
2	Diseased (Scopolamine0.4mg/kg/I.P)	17±0.77###
3	Positive control (piracetam200mg/kg/I.P +scopolamine0.4mg/kg/I.P)	12.44±0.88***
4	Test-1 (PEECG200mg/kg/P.O +scopolamine0.4mg/kg/I.P)	14.06±0.93*
5	Test2 (EAECG200mg/kg/P.O +scopolamine0.4mg/kg/I.P)	13.17±0.83**
6	Test-3 (EECG200mg/kg/P.O +scopolamine0.4mg/kg/I.P)	11.61±0.78***

Values are expressed as Mean ± SEM, n=6. #-Diseased group compared with normal group; *-Std, Test-1,Test-2 and test-3 compared with diseased group; #/*-indicates p<0.5, ##/**-indicates p<0.01; ###/***-indicates p<0.001.

Table 3: Inflexion on Elevated plus Maze

S.NO	GROUP	Inflexion ratio (IR);(Mean±SEM)
1	Normal (distilled water)	0.524±0.005
2	Diseased (Scopolamine0.4mg/kg/I.P)	0.211±0.010##
3	Positive control (piracetam200mg/kg/I.P +scopolamine0.4mg/kg/I.P)	0.405±0.014**
4	Test-1 (PEECG200mg/kg,P.O + scopolamine0.4mg/kg,I.P)	0.385±0.013ns
5	Test2 (EAECG200mg/kg/P.O + scopolamine0.4mg/kg/I.P)	0.455±0.015*
6	Test3 (EECG200mg/kg/P.O + scopolamine0.4mg/kg/I.P)	0.483±0.017**

Values are expressed as Mean±SEM, n=6. #-Diseased group compared with normal group; *-Std, test-1,test-2 and test-3 compared with diseased group; #/*-indicates p<0.5, ##/**-indicates p<0.01; ###/***-indicates p<0.001.

Y-maze Task

During 18 days treatment, the spatial alterations shown by normal group and control group on day 1 and day 9 was found to be similar. The results were shown in Table no: 4. However, on the day 18 scopolamine exposure control group shows decrease in spa-

Table 4: Effect of PEECG, EAECG, EECG on y maze task (mean ± S.E.M)

S.NO	GROUP	Spatial alterations		
		Day-1	Day-9	Day-18
1.	Normal (distilled water)	64.53±2.62	67.7±3.79	66.4±2.83
2.	Diseased (Scopolamine0.4mg/kg/I.p)	65.64±1.18	60.5±2.90	42.85±1.94###
3.	Positive control (piracetam200mg/kg/I.p + scopolamine0.4mg/kg/I.p)	64.31±2.30	61.48±2.56	65.35±2.57***
4.	Test-1 (PEECG200mg/kg,p.o + scopolamine0.4mg/kg,I.p)	60.12±1.80	59.1±2.17	63.53±3.68*
5.	Test-2 (EAECG200mg/kg/p.o + scopolamine0.4mg/kg/I.p)	59.90±1.67	58.10±2.16	62.4±3.65**
6.	Test-3 (EECG200mg/kg/p.o + scopolamine0.4mg/kg/I.p)	58.10±2.97	65.4±2.85	69.68±3.55***

Values are expressed as Mean ± SEM, n=6. #-Diseased group compared with normal group; *-Std, test-1, test-2 and test-3 compared with diseased group; #/*-indicates p<0.5, ##/**-indicates p<0.01; ###/***-indicates p<0.001.

Table 5: Effect ofplant extract and in combination with Piracetam on Scopolamine induced amnesia in mice using Morris Water Maze. (Escape Latency Time in Second)

S.NO	GROUP	TREATMENT	
		Before Scopolamine	After Scopolamine
1.	Normal (Distilled water)	35.5±1.35	24.43±0.95
2.	Diseased (Scopolamine0.4mg/kg/i.p)	34.5±0.95	31.53±1.12###
3.	Positive control (Piracetam200mg/kg/i.p + Scopolamine0.4mg/kg/i.p)	29.4±0.75	15.5±0.73***
4.	Test-1 (PEECG200mg/kg/p.o + scopolamine0.4mg/kg/i.p)	32.5±0.85	19.4±0.84**
5.	Test-2 (EAECG200mg/kg/p.o + scopolamine0.4mg/kg/i.p)	31.42±0.86	17.52±0.80**
6.	Test-3 (EECG 200mg/kg/p.o + scopolamine0.4mg/kg/i.p)	30.12±0.85	16.61±0.78***

Values are expressed as Mean±SEM, n=6. #-Diseased group compared with normal group; *-Std, test-1, test-2 and test-3 compared with diseased group; #/*-indicates p<0.5, ##/**-indicates p<0.01; ###/***-indicates p<0.001.

Table 6: Effect of plant extract and in combination with Piracetam on scopolamine induced amnesia in mice using Morris Water Maze. (Time Spent in Target Quadrant in Second)

S.NO	GROUP	Time Spent in Target Quadrant in Second (TSTQ in sec.)
1.	Normal (Distillrd water)	70.5±0.51
2.	Diseased (Scopolamine0.4mg/kg/i.p)	32.7±0.90###
3.	PositiveControl (Piracetam200mg/kg/i.p + scopolamine)	60.8±0.92***
4.	Test-1 (PEECG200mg/kg/p.o + scopolamine0.4mg/kg/i.p)	64.8 ±0.82**
5.	Test-2 (EAECG200mg/kg/p.o+scopolamine0.4mg/kg/i.p)	62.7±0.75**
6.	Test-3 (EECG 200mg/kg/p.o + scopolamine0.4mg/kg/i.p)	61.5 ±0.71***

Values are expressed as Mean±SEM, n=6. #-Diseased group compared with normal group; *-Std, Test-1, Test-2 and test-3 compared with diseased group; #/*-indicates p<0.5, ##/**-indicates p<0.01; ###/***-indicates p<0.001.

tial alterations which was significant (p<0.001) over normal

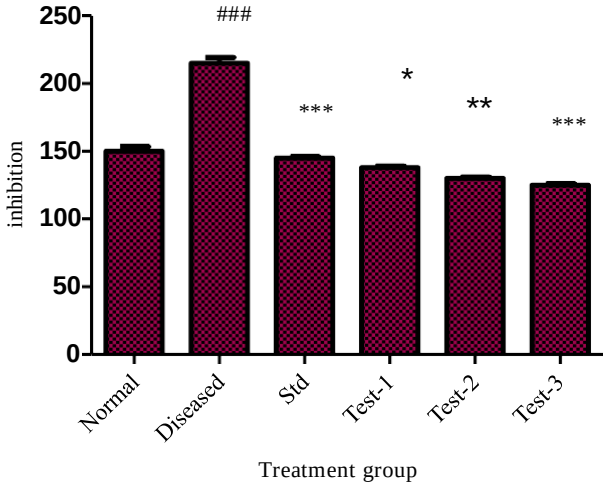

Figure 1: Effect of PEECG, EAECG, EECG on Acetylcholine levels

group. For 18 days treatment with PEECG, EAECG, EECG at 200mg/kg showed significant ($p<0.01$ and $p<0.001$) increases in spatial alterations respectively in y maze task. The treatment with standard drug Piracetam for 18 days also shows increase in spatial alterations which was significant ($p<0.01$) over control group.

Morris water maze

Morris water maze for Escape Latency Time in Seconds: (ELT)

The antiamnesic effects of plant extract are presented in Table 4, 5. It was observed that when Scopolamine administered, it has significantly ($P< 0.001$) increased ELT value (31.53±1.12) as compared to the normal group (Table 4). When the Piracetam was administered for fifteen days at the dose of 200mg/kg, it has significantly ($P< 0.001$) decreased ELT value (15.5±0.73) as compared to the Scopolamine treated group. It was observed that administration of EECG at the dose of 200 mg/kg resulted in a significant decreased ELT value (16.61±0.78) as compared to the Scopolamine treated group. It showed that, when the EECG administered with Piracetam, produces synergistic effect.

Morris water maze for Time Spent in Target Quadrant In Second: (**TSTQ**)

It was observed that administration of Scopolamine resulted in a significant ($P< 0.001$) decreased TSTQ value (32.5±0.90) as compared to the normal group (Fig 2). Chronic administration of Piracetam for fifteen days at the dose of 200mg/kg, has resulted in significant increased TSTQ value (60.8±0.92) as compared to the Scopolamine group. EECG resulted in a significant ($P< 0.001$) increase in TSTQ (61.5±0.71) as compared to the Scopolamine treated group (Table 5). This shows that, the EECG has potent antiamnesic activity near to that of Piracetam. EECG has synergism with Piracetam.

Estimation of AchE Activity

In this study we have determined the level of AchE in the whole brain homogenate of all group animals, which was used to assess the antiamnesic activity. It was observed that administration of Scopolamine resulted in a significantly ($P<0.001$) increased AchE values (215±4.29) as compared to the normal group. When the Piracetam was administered at the dose of 200mg/kg, it has significantly decreased AchE value (140±0.85) as compared to the Scopolamine treated group. The activity of AchE after administration of EECG at the dose of 200 mg/kg has resulted in a significantly decreased AchE value (125±0.91) as compared to the Scopolamine treated group. The activity was found to be more than the Piracetam treated animals; which shows the synergistic effect with Piracetam. EECG has potent antiamnesic activity as that of Piracetam on scopolamine induced amnesia in mice and also shows synergistic effect.

Normal
Distilled water
Control
Scopolamine (0.4 mg/kg/I.P)
Positive control
Piracetam (200mg/kg/I.P)+Scopolamine (0.4mg/kg/I.P)
Test-1
(PEECG 200mg/kg P.O) + Scopolamine (0.4 mg/kg/I.P)
Test-2
(EAECG 200mg/kg/P.O) + Scopolamine (0.4 mg/kg/I.P)
Test-3
(EECG200mg/kg/I.P) + Scopolamine (0.4mg/kg/I.P).

CONCLUSION

Cleome gynandra Linn. Is important plant of Indian Traditional systems of medicine.The plant is major ingredient of Ayuvedic formulations for the treatment of central nervous disorders. When the Plant Extract was given orally for fifteen successive days before the challenges of Scopolamine on 15th to 18th day, showed good Amnesia activity. *Cleome gynandra* Linn. Plant

extract is antidiabetic agent and can be exploited as Alzheimer's agent and also shows significant synergistic effect when ethonolic extract of three different extracts administered with Piracetam.

ACKNOWLEDGEMENT

The authers sincerely thank to Dr.SK.Karimulla, Head of the Department, and Dr.M.Gobinath, Principal, P.Naresh babu, Anupam Kanti Bag, Niveditha, RIP college of Pharmacy, Nellore, for support and encouragement. our gratitude goes to RIP college, Nellore, for providing all the necessary facilities. and sincerely thanks to Teaching and non-Teaching staff for the support of this research. And specially thanks to my friends, seniors and Juniors for support and encouragement in research study.

REFERENCES

Anbazhagi, T., Kadavul, K., and Suguna, G., Petrus, A.J.A. (2009). Studies on the pharmacognostical and in vitro antioxidant potential of *Cleome gynandra* linn leaves. Natural Product Radiance, 8(2), 151-157.

Asis Bala, Biswakanth Kar, Pallab K. Haldar, Upal K. Mazumder and Samit Bera. Evaluation of anticancer activity of Cleome gynandra on Ehrlich's Ascites Carcinoma treated mice.Journal of Ethnopharmacology. 2010; 129(1):131-134.

Becker R, Giacobini E, Mcllhany M, Sherman K. Potential pharmacotherapy of Alzheimer disease. A comparison of various forms of physostigmine administration. Acta Neurological Scandinavica 1998; 116: 19-32.

Becker R, Giacobini E. Acta Neurol.Scand. 1988; 116: 19-32.

Bores GM, Huger FP. J Pharmacol.Exp.Ther1996; 277:7 28-738.

Cummings JL. Alzheimer's disease. N Engl J Med 2004; 351:56-67.

D S Reddy, Assessment of Nootropic and amnesic activity of centrally acting drugs. Indian journal of pharmacology 1997, 29: 208-221.

Dhingra D, Parle M, Kulkarni SK. Comparative brain cholinesterase inhibiting activity of Glycerrhiza glabra, Myristica frangras, ascorbic acidand metrifonate in mice. J Med Food 2003; 9:281-289.

Ellman GL, Courtney KD, Valentino A, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem pharmacol 1961; 7:88–95.

Hanumanthachar Joshi, Milind Parle, Ingiber officinale: Evaluation of Its Nootropic Effect in Mice, Afr. J. Trad. Cam, 2006, 3 (1): 64 –74.

http://www.drugbank.ca/drugs/DB00747.

Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the evaluation of memory in mice: effects of noortropics, scopolamine and electroconvulsive shock. Psychopharmacology.1990; 101:27-33.44.

Izzo AA, Capasso F. Herbal medicines to treat Alzheimer's disease. Trends Pharmacol Sci 2007; 28:47-48.

Jaiswal A K, Bhattacharya S K, Effect of Shilajit on memory, anxiety and brain monoamines in rats, Indian journal of Pharmacology, 1992, 24, 12.

Jewart RD, Green J, Cognitive, Behavioral, and physiolo gical changes in Alzheimer's disease patients as a function of incontinence medication. Am J Geriatr Psychiatry 2005; 13:324-8

Joshi Hanumanthachar, kauvar navneet, Chauhan jyotibala. Evaluation of Nootropic effect of Argyreia speciosa in Mice. Journal of Health Science 2007; 53(4) 382-388.

N Venkato Rao, Basabaraj Pujara, S K Nimbal, S M Shantakumar, S Satyanarayan, Noortropic activity of tuber extract of Pueraria tuberosa (roxb), Indian Journal of Experimental Biology, 2008, 46, 591-598.

Narendhirakannan RT, Kandaswamy M and Subramanian S. Anti-inflammatoryactivity of Cleome gynandra L. on hematological and cellular constituents in adjuvant-induced arthritic rats. Indian. J Med Food. 2005; 8(1):93-9.

Parle Milind, Bansal Nitin. Antiamnesic activity of an Ayurvedic formulation Chyawanprash in mice. Evidence based Complementary and Alternative Medicine 2009; 9:1-28.

Pettenati C. Annicchiarico R, Caltagrione C. Clinical pharmacology of anti-Alzheimer's disease. Fundam Clin Pharmacol 2003; 17:659-672.

Selkoe DJ. Neuropathol.Exp.Neurol1994; 53:438-447

Silman I, Sussaman J. Curr. Opin. Pharmacol.2005; 5: 293-302.

Smith G. "Animal models of Alzheimer's disease: experimental cholinergic denervation". Brain resrev. 1988. 13:103-118.

The Ayurvedic Pharmacopoeia of India. part-1 Volume-1:13.

Trease and Evans, Pharmacognosy, 15th Edition, ELBS Publications, New Delhi.138.