BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Evaluation of Memory Enhancing Activity of *Caralluma Fimbriata* Extracts

Somayeh Afsah Vakili[*1], Syed Fayazuddin[2], Ajay George [2]
1. Department of pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, Bangalore-560070, Karnataka, India.
2. Department of Pharmacology, St. Johns Pharmacy college, Bangalore-560104, Karnataka, India.

ABSTRACT

Caralluma fimbriata (*Asclepiadaceae*) grows wild all over India and is a traditional food plant. It is well known as a famine food, appetite suppressant and thirst quencher. The current investigation was designed to evaluate memory enhancing activity of aqueous and ethanol extracts of *Caralluma fimbriata* by using Cook'pole climbing apparatus and estimation of acetyl cholinesterase inhibitory activity. Scopolamine butyl bromide (1mg/kg bw, i.p) were administrated to animals thirty minutes before foot shock to produce amnesia. Animals were trained to leap on the pole to avoid electrical shock. They received 200 and 400 mg/kg dose of aqueous and ethanol extracts of *Caralluma fimbriata* orally one hour before the induction of foot shock daily. Acetyl cholinesterase activity was appraised by supplying an acetyl thiocholine as substrate which cause to release thiocholine as consequence of cleaving by acetyl cholinesterase. Thiocholine reduced Ellman reagent to thionitrobenzoic acid as yellow product which could absorb light at 412 nm. The groups treated with aqueous and ethanol extracts of *Caralluma fimbriata* were exhibited to enhance memory by restored mean percentage of conditional avoidance response towards normal. Acetyl cholinesterase inhibitory activities of extracts of *Caralluma fimbriata* were found to have the supportive memory enhancing activity by magnifying the cognitive function.

Keywords: *Caralluma fimbriata*, memory enhancing activity, cook'pole climbing apparatus.

*Corresponding Author Email: somayehafsah@yahoo.com
Received 25 September 2017, Accepted 05 October 2017

Please cite this article as: Vakili SA *et al.*, Evaluation of Memory Enhancing Activity of Caralluma Fimbriata Extracts. British Journal of Medical and Health Research 2017.

INTRODUCTION

Memory is a notable cognitive function of brain. Central cholinergic neurotransmitter has main regulatory cognitive functions. Major feature of Alzheimer' disease is cholinergic neuronal loss in hippocampal area. Nowadays, anti cholinesterase activity is the centrepiece of remedy of dementia in Alzheimer' disease by amplifying of central cholinergic activity [1,2] Generally anticholinesterase chemical compounds for instance heptylphysostigmine, physostigmine, galantamine and tacrine have non-selectivity efficacy, poor bioavailability and adverse cholinergic side effects in the periphery with narrow therapeutic ranges and hepatotoxicity [1]. Scopolamine as centrally acting anti-muscarinic drugs can impair learning and memory [3,4]. Several herbs have been identified to possess nootropic activity [5-8]. Plant extract may also provide a source of new compound by reason of having less side effect and low cost. There is a lack of scientific data regarding the effect of aqueous and ethanol extracts of *Caralluma fimbriata* on learning and memory enhancing activity. Hence, the current investigation was carried out to display nootropic effect of aqueous and ethanol extracts of *Caralluma fimbriata*.

MATERIALS AND METHOD

Plant material and Preparation of extracts

The leaves of *Caralluma fimbriata* were collected from Chennai, Tamil Nadu, India and authenticated by Green Chem of India, Doddinduvadi district, Bangalore, Karnataka, India. The fresh leaves of *Caralluma fimbriata* were washed with tap water and air dried for one hour. Then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature. The leaves powder was macerated in the solvents including ethanol 95% (v/v) and water at room temperature, undergoing mechanical shaking for 4 hours followed by filtration. The extracts acquired were concentrated in a rotary evaporator at 40°C and the residue was extracted twice again analogously, there by obtaining the crude solvent extracts.

Chemicals

Scopolamine butyl bromide and piracetam procured from Stride acrolabs Ltd, Bangalore, India. Other chemicals were analytical up grade and acquired from local store of Visveswarapura Institute of Pharmaceutical Sciences.

Animals

Female albino wistar rats (180-200gm) acquired from the NIMHANS animal house, Bangalore. The animals were kept under standard conditions in an animal house as per the guidelines of "Committee for the Purpose of Control and Supervision on Experiments on Animals" (CPCSEA) for at least one week prior to use. The rats had free access to standard

rat chow and water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore. (Registration No: 152/1999, renewed in 2012).

Estimation of acetyl cholinesterase activity by Ellman's method [9]

Rats were decapitated; brains were removed rapidly and kept in ice-cold saline. Frontal cortex, hippocampus and septum were quickly dissected out on a petri dish chilled on crushed ice. The tissues were homogenized in 0.1m Phosphate buffer. Added 0.4 ml of the homogenates to 2.6 ml phosphate buffer and 100 µl of DTNB. Absorbance was measured at 412 nm in a UV spectrophotometer. When absorbance reaches stable value, it was recorded as the basal reading. Added 20µl of acetyl thiocholine iodide and recorded the change in the absorbance for a period of 10 minutes. Change in the absorbance per minute was determined. The enzyme activity is calculated using the following formula:

$$R= 5.74 \times 10^{-4} \times A/CO$$

R= Rate in mole of substrate hydrolyzed/minute/gm tissue

A= Change in absorbance/ minute

CO= Original concentration of the tissue (mg/ml)

Conditioned avoidance response (Cook' pole climbing apparatus) [10]

Animals were subjected to a training schedule individually by placing inside the cook' pole climbing apparatus 60 minutes after oral administration. Buzzer was given followed by a shock through the grid floor. The rat had to jump on the pole to avoid foot shock. Jumping prior to the onset of the shock was considered as avoidance. The session was terminated after completion of 30 trials with an interval of 20–30 seconds given for each trial. This procedure was repeated at 24 h intervals until all groups reach 95 to 99% avoidance. After attaining complete training of a particular group, the animals were treated with a single dose of scopolamine butyl bromide (1 mg/kg body weight, i.p.), thirty minutes before the next day dosing. The training schedule was continued further with the daily doses of the aqueous and ethanol extracts of *Caralluma fimbriata* until they returned to normal level from scopolamine induced amnesia.

Treatment schedule

For estimation of acetyl cholinesterase activity by Ellman's method in rats, wistar albino rats were divided into 6 groups consisting of 6 animals in each group. Group 1 was vehicle group (Distilled water 2ml/100g bw). Group 2 received standard drug piracetam 200mg/kg i.p. Group 3,4 rats were administrated orally with aqueous extracts of *Caralluma fimbriata* at dose of 200 mg/kg and 400 mg/kg respectively. Group 5,6 rats received ethanol extracts of *Caralluma fimbriata* orally at dose of 200 mg/kg and 400 mg/kg respectively. For

conditioned avoidance response in rat, wistar albino rats were divided into 5 groups consisting of 6 animals in each group. Group 1 rats served as normal control and received 2ml/100g bw distilled water, group 2,3 rats received aqueous extracts of *Caralluma fimbriata* orally at dose of 200 mg/kg and 400 mg/kg respectively. Group 4,5 rats were administrated orally with ethanol extracts of *Caralluma fimbriata* at dose of 200 mg/kg and 400 mg/kg respectively.

Statistical analysis:

The data were performed as mean ± S.E.M. Results were statistically analysed by using one way ANOVA followed by Dunnett's test and $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Nootropic drugs refer as cognitive enhancers or smart drugs that improve cognitive function, particularly executive functions, memory, creativity or motivation in healthy individuals. Typically, the memory enhancing drugs are ostensible for providing neurochemicals and issuing oxygen supplement. The use of cognition-enhancing drugs by healthy individuals in the absence of a medical indication is one of the most debated topics among neuroscientists, psychiatrists, and physicians [11]. In the present investigation, administration of aqueous and ethanol extracts of *Caralluma fimbriata* in *wistar* rats indicated significant improvement in memory functions by attenuating amnesic effect which produced the scopolamine butyl bromide. ACh has a crucial role in the enhancement of sensory perceptions and inhibition of ACh hydrolysis may be achieved through the use of AChE inhibitors [12]. Results attained from current research revealed memory enhancing effect in rats treated with aqueous and ethanol extracts of *Caralluma fimbriata* and it showed dose-dependently inhibition of AChE enzyme in specific brain regions Frontal cortex, hippocampus, and septum. This clearly manifests that the mechanism of aqueous and ethanol extracts of *Caralluma fimbriata* involved in nootropic action of may be due to inhibition of AChE enzyme and hence elevation of acetylcholine levels which maintains the normal cognitive function in the brain. The previous phytochemical analysis of *Caralluma fimbriata* manifested the presence of phenolic compounds such as flavonoids which could be responsible for memory enhancing activity [13]. Flavonoids may mimic the actions of estrogens in the brain [14], or may influence the synthesis of acetylcholine and neurotropic factors such as BDNF and nerve growth factor in hippocampus and frontal cortex. Figure 1 manifests the effects of aqueous and ethanol extracts of *Caralluma fimbriata* on acetylcholinesterase (AChE) activity in rats' brain. The groups treated with aqueous and ethanol extract of *Caralluma fimbriata* had displayed reduction in AChE activity as compared to control group. Control group had exhibited 7.460

X 10^{-7} µmol/min/g tissue of acetylcholinesterase activity in rat brain. Prior administration of Piracetam as standard had manifested a significant decline in acetylcholinesterase activity 4.010 X 10^{-7} µmol/min/g. Prior administration of aqueous extract of *Caralluma fimbriata* at dose of 200 mg/kg p.o and 400 mg/kg p.o have showed significant decrease in acetylcholinesterase activity 4.880 X 10^{-7} µmol/min/g ($P<0.01$) and 4.608 X 10^{-7} µmol/min/g ($P<0.001$) respectively as compared to control group, while, the significant decline was observed in groups treated with ethanol extract of *Caralluma fimbriata* at dose of 200 mg/kg p.o and 400 mg/kg p.o with acetylcholinesterase activity 4.288 X 10^{-7} µmol/min/g ($P<0.001$) and 4.160 X 10^{-7} µmol/min/g ($P< 0.001$) respectively as compared to control group.

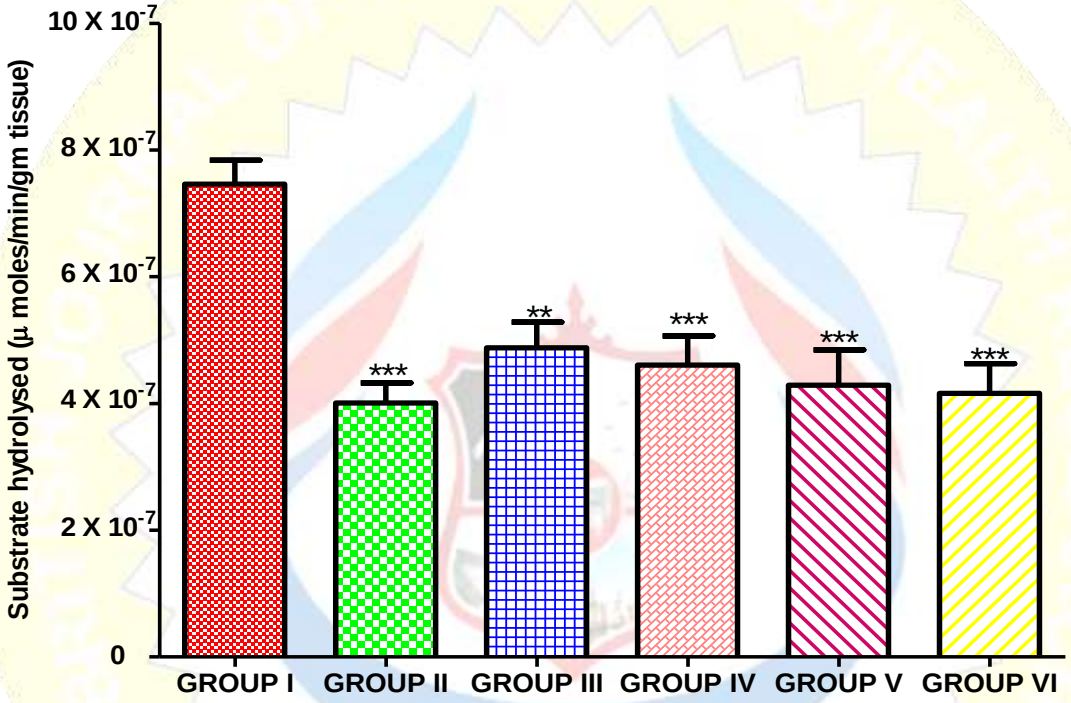

Figure 1: Effect of aqueous and ethanol extracts of *Caralluma fimbriata* on acetylcholinesterase (AChE) activity in rat's brain. Data is expressed as mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Dunnett's test. ***P*< 0.01 and ****P*< 0.001 were considered statistically significant.

Figure 2 indicates the effects of aqueous and ethanol extracts of *Caralluma fimbriata* on mean percentage of conditioned avoidance response after oral administration in rats. The CAR of rats administrated with the aqueous and ethanol extract of *Caralluma fimbriata* and vehicle ascended gradually to 95% over seven to ten days. The percentage avoidance was higher in the groups administered with aqueous and ethanol extract of *Caralluma fimbriata* compared to vehicle treated control group. The acquisition (time to achieve 95% CAR) for the groups treated with aqueous and ethanol extracts of *Caralluma fimbriata* was quicker and found to be dose dependent. Animal in group II and III administered with aqueous extract of

Caralluma fimbriata at a dose of 200 mg/kg p.o and 400 mg/kg p.o have taken ten days and nine days respectively to reach the point of acquisition, while, animals in group IV and V administered with ethanol extract of *Caralluma fimbriata* at a dose of 200 mg/kg p.o and 400 mg/kg p.o have taken eight days and seven days respectively to reach the point of acquisition. Administration of scopolamine produced amnesia as seen from reduction in the observed CAR. The amnesia was found to be greater in control group compared with the groups treated with aqueous and ethanol extract of *Caralluma fimbriata* and was also found to be dose dependent. Animals treated with aqueous extract of *Caralluma fimbriata* at a dose of 200 mg/kg and 400 mg/kg had taken five and four days whereas, group treated with ethanol extract of *Caralluma fimbriata* at a dose of 200 mg/kg and 400 mg/kg had taken three days each to reach the point of acquisition after administration of scopolamine butylbromide. The control group had taken eleven days for retention and recovery from scopolamine induced amnesia.

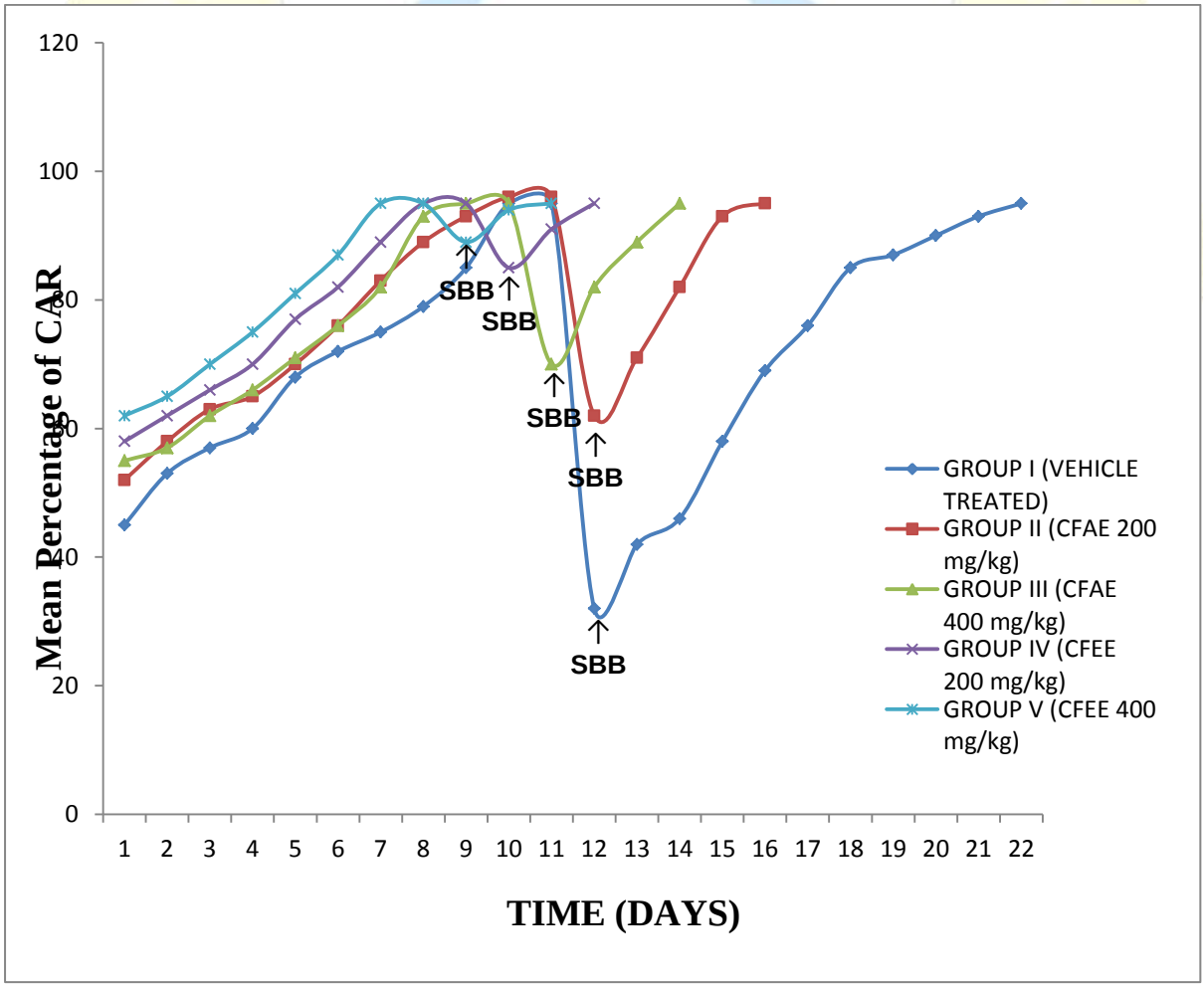

Figure 2: Effect of aqueous and ethanol extracts of *Caralluma fimbriata* on mean percentage of conditioned avoidance response after oral administration in rats. Scopolamine butylbromide (SBB) was administered 30 minutes before next day dosing with

the extracts after attaining complete acquisition. CFAE: aqueous extract of *Caralluma fimbriata*, CFEE: ethanol extract of *Caralluma fimbriata*.

CONCLUSION

This investigation divulges that aqueous and ethanol extracts of *Caralluma fimbriata* have memory enhancing activity so it can be appraised worthwhile as promising herb for the patients of Alzheimer's disease (AD) and other cognitive deficit states.

ACKNOWLEDGEMENT

The authors are grateful to Green Chem Company, Bangalore, Karnataka, India for issuing extracts for this investigation.

REFERENCES

1. Bores GM, Huger FP, Petko W, Mutlib AE, Camacho F, Rush DK, et al. Pharmacological evaluation of novel Alzheimer' disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. J Pharmacol Exp Ther. 1996; 277(2):728-738.
2. Nordberg, A., Svensson, A.L. Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology. Drug Safety. 1998; 19(6); 465-480.
3. Habbu PV, Mahadevan KM, Shastry RA, Chilakwad SR. Antiamnesic potentiality of Argyreia speciosa (Burm.F) Boj. In mice. Int Journal Green pharm. 2010; 4(2): 83-89.
4. Bennett BM, Reynolds JN, Prusky GT, Douglas RM, Sutherland RJ, Thatcher GR. Cognitive deficits in rat after forebrain cholinergic depletion are reversed by Novel NO mimetic ester. Neuropsychopharmacol. 2007; 32(3): 505-513.
5. Sujith K, Ronald Darwin C, Satish, Suba V. Memory-enhancing activity of *Anacyclus pyrethrum* in albino wistar rats. Asian Pac J Trop Dis. 2012; 2(4): 307-311.
6. Chauphan B, Choudhary AK. Memory enhancing activity of methanolic extract of *Pterocarpus marsupium.* Phytopharmacol. 2012; 2 (1): 72-80.
7. Rao MK, Rao MS, Rao GS. Treatment with *Centella asiatica* (Linn) fresh leaf extract enhances learning ability and memory retention power in rats. Neurosci. 2007; 12(3): 236-241.
8. Jyoti A, Sharma D. Neuroprotective role of *Bacopa monniera* extract against aluminium induced oxidative trace in the hippocampus of rat brain. Neorotoxicol. 2006; 27(4): 451-457.
9. Srikumar BN, Ramkumar K, Raju TR, Shankaranarayana Rao BS. Assay of acetylcholinesterase activity in the brain. Bangalore: National Institute of Mental

Health and Neuro Sciences [Internet] 2004 [cited 2017 June 8]. Available from: https://www.scribd.com/doc/7280614/Assay-of-Acetylcholinesterase-Activity-in-the-Brain.

10. Cook L, Weidley E. Behavioural effects of some psycho-pharmacological agents. Ann NY Acad Sci. 1957; 66:740-752.
11. Mohan M, Kaul N, Punekar A, Girnar R, Junnare P, Patil L. Nootropic activity of *Moringa oleifera* leaves. JNR. 2005; 5(1): 59-62.
12. Jagetia GC, Rao SK, Baliga MS, Babu K. The evaluation of nitric oxide scavenging activity of certain herbal formulations in vitro: a preliminary study. Phytother. Res.2004; 18(7):561-565.
13. Rajaram K, Suresh KP. Phytochemical studies and GC-MS analysis of *Caralluma fimbriata* wall. Int J Pharm Res Dev. 2011; 3(10), 105-110.
14. Jager AK, Saaby L. Flavonoids and the CNS. Molecules. 2011; 16(2):1471-1485.