

EFFECT OF *CURCUMA AMADA* (MANGO GINGER) ROXB. ON SCOPOLAMINE INDUCED MEMORY DEFICIT IN RATS

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ABSTRACT

Alzheimer disease (AD) is the most prominent cause of dementia which is characterized by progressive deterioration of cognitive function. Memory loss referred to as dementia, is also commonly associated with number of diseases or disorders including multiple sclerosis, nutritional deficiencies, sleep disorders, Parkinson's disease, Huntington's disease. Earlier studies state that traditionally herbal drugs play a key role in the treatment of dementia. The present study was focused to evaluate the nootropic activity of *Curcuma amada* in rats by Y- maze & elevated plus maze task performance. Acetonic extract of *Curcuma amada* (100,300 mg/kg p.o), aqueous extract of *Bacopa monniera* were compared for its nootropic activity with amnesic control scopolamine. *Curcuma amada* 300 mg/kg had shown significant nootropic activity on par with 100 mg/kg when compared with standard *Bacopa monniera*. Curcuminoids possess antioxidant and Acetylcholinesterase (AChE) activity which was reported in earlier studies. Therefore curcuminoids might be responsible for nootropic activity of *Curcuma amada*.

Keywords: *Curcuma amada*, *Bacopa monniera*, memory, Y- maze, Elevated plus maze, Scopolamine.

INTRODUCTION

Memory loss (amnesia) is progressive neurodegenerative disorder symptoms include loss of memory (dementia), unusual forgetfulness, and personality change and finally it may leads to death. Memory loss can occur as the result of a number of diseases or disorders, including multiple sclerosis, Alzheimer's disease, head trauma, nutritional deficiencies, sleep disorders, Parkinson's disease, Huntington's disease, stroke or brain tumors. Nootropics are the drugs that improve cognition, memory, intelligence, motivation, attention, and concentration^{1,2}. C.E. Giurgea³ described nootropics as chemicals that can boost brain

performance. Synonyms of nootropics are smart drugs, cognitive/memory enhancers.

Learning and memory can be perceived as both a psychological process, as well as a change in synaptic neural connectivity^{4,5}. Parasympathetic nervous system plays an important role in learning and memory in humans. It is well known that impairment of cholinergic neuronal system produces cognitive impairment⁶. Thereby, cholinergic like acetylcholine plays an important role in memory formation⁷. So, the synthesis of nootropic agents like piracetam and its analogues oxiracetam, amiracetam⁸ and rivastigmine have been developed⁹.

The continuation in the quest of natural nootropics and the scanty information on their

utility apart from the synthetic drugs which have potential adverse effects like hepatotoxicity (tacrine)^{10, 11, 12}. Traditionally many of the indigenous medicinal plants are used for medicinal purposes¹³. The herbs like *Allium sativum*, *Bacopa monniera*, *Camellia sinensis*, *Curcuma longa*, *Crocus sativus*, *Embllica officinalis*, *Ginkgo biloba*, *Withania somnifera*, *Zingiber officinale* etc have been proved considerable memory enhancing activity by virtue of their chemical constituents¹⁴.

Curcuma amada having morphological resemblance with ginger (*Zingiber officinale*) but imparts a raw mango (*Mangifera indica*) flavour. The genus name *Curcuma* was coined by Linnaeus in 1753 in his Species Plantarum. *Curcuma amada* (mango ginger) have pharmacological activities like antioxidant activity¹⁵, anti fungal, anticarcinogenic, platelet aggregation inhibitory activity¹⁶, anti inflammatory¹⁷, anti microbial^{18,19}, cardiovascular effects, gastro intestinal effects, hypotriglyceridemic activity^{20,21}, antihyperglycemic activity²², anthelmintic activity²³ which are due to the presence of curcuminoids²⁴. Hence the present work is focused to evaluate the nootropic activity of acetonic extract of *Curcuma amada* belongs to Zingiberaceae family.

Materials

Curcuma amada rhizomes was obtained as a gift sample from BRAHMA PLANTS AND HERBALS, Vijayawada, A.P, India (in intact lump form) and was stored carefully since its receipt, in air-tight polypropylene jars under darkness. All other chemicals and reagents used were of analytical grade.

Extraction procedure of *Curcuma amada* (mango ginger)

Air dried (35-50°C) rhizomes of *Curcuma amada* were extracted by soxhlet apparatus for 12 hrs, acetone as a solvent. The extract was filtered and evaporated. Acetone was suitable for extraction. Experimental works reported that 0.16, 0.02, 0.01% of curcumin, deoxycurcumin, bis-demethoxy curcumin respectively²⁵.

Animals

Albino wistar rats of either sex weighing between 150 to 200 gm were procured from registered breeders (149/1999/CPCSEA, Mahavir Enterprises, Hyderabad.). The animals were housed under standard conditions of temperature (25°C) and relative humidity (30-

70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water ad libitum. The experimental protocol has been approved by the institutional animal ethics committee (Regd. No. 516/01/A/CPCSEA).

Method

Acute toxicity study

Curcuma amada at different doses (500-2000 mg/kg) was administered with oral feeding tube and were observed for gross behavioral, neurological, autonomic and toxic effects according to OECD guidelines. No mortality was observed within 24 h of dose of 2000 mg/kg. The doses selected were 100 mg/kg and 300 mg/kg.

Elevated Plus Maze

Elevated plus maze is used for the assessment of acquisition and retention memory processes²⁶. The rats were treated with *Curcuma amada* 100mg/kg, *Curcuma amada* 300mg/kg and *Bacopa monniera* 100mg/kg for 7 days continuously. Transfer latency and duration closed arm visits are measured on 7th day served as parameters for acquisition.

Y- Maze Apparatus

Spatial recognition memory was assessed by using Y-maze two-trial recognition paradigm consisted of three trials separated by an inter-trial interval (ITI)²⁷. The number of entries and time spent in each arm were to be analyzed.

Experimental Design

Rats were divided into 5 groups each of 6 rats.
Group1: Control received only vehicle (1% acacia suspension) orally
Group2: Scopolamine received i.p (1mg/kg)
Group3: *Bacopa monniera* 100mg/kg p.o received
Group4: *Curcuma amada* 100 mg/kg p.o. received
Group5: *Curcuma amada* 300 mg/kg p.o. received

The rats were trained for 1 week before the experiment during which they should not receive any drug. Well trained rats were chosen for the study. Each animal received four trails for the first day and followed by eight trails per day for eight days with 5 min interval between each trail. In the **elevated plus maze model**, animals were dosed once in a day with the respective drugs, sixty minutes prior to the trail for 7 days. Group I is kept as control (1% acacia suspension).

Groups III, IV & V received their doses respectively for seven days and were given scopolamine on eighth day 30min prior to the treatment with their daily doses. After one hour all the animals were subjected EPM. Where as in **Y-maze**, pretreatment with amnesic agent 30 min prior to trials induces a marked decrease in spontaneous alteration performance with a

concomitant increase in the total duration of arm entries in single day study.

Statistical analysis

Data represented as mean ± SEM and were analyzed by one way ANOVA method followed by post hoc test Tukey's test. P < 0.05 considered as significant.

RESULTS

Table 1: Effect of acetic extract of *Curcuma amada* 100 mg/kg and 300 mg/kg on spatial recognition and transfer latency in rats using elevated plus maze

| S.NO | Duration of closed arm visits | Transfer latency |
|-------------------|-------------------------------|-----------------------------|
| Control | 183.3 ± 9.21 | 19.29 ± 1.84 |
| Scopolamine | 110.3 ± 8.6 ^(a) | 34.84 ± 2.09 |
| B.M100 mg/kg | 201.8 ± 15.44 ^(b) | 8.095 ± 1.36 ^(b) |
| C.amada 100mg/kg | 179.8 ± 14.56 ^(b) | 12.02 ± 1.55 ^(b) |
| C.amada 300 mg/kg | 226.1 ± 18.73 ^(b) | 6.703 ± 1.38 ^(b) |

Data are expressed as MEAN±SEM [N=6]. *P< 0.05 considered as significant. (a)Compared to control, [b] compared to scopolamine, [c] compared to *B.monniiera*

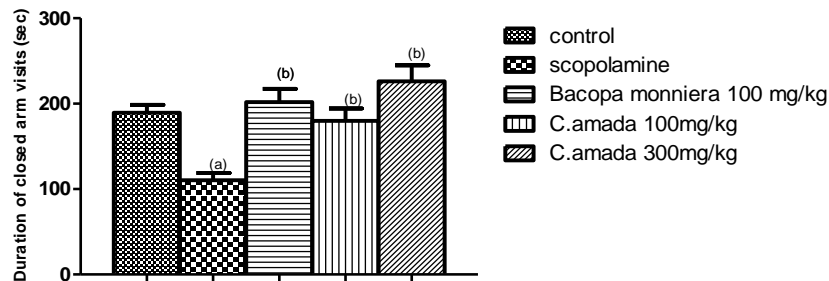


Fig. 1: Effect of acetic extract of *Curcuma amada* 100 mg/kg and 300 mg/kg on spatial recognition in rats using elevated plus maze

Data are expressed as MEAN±SEM (N=6). *P< 0.05 considered as significant. (a) compared to control, (b) compared to scopolamine ,(c) compared to *B.monniiera*

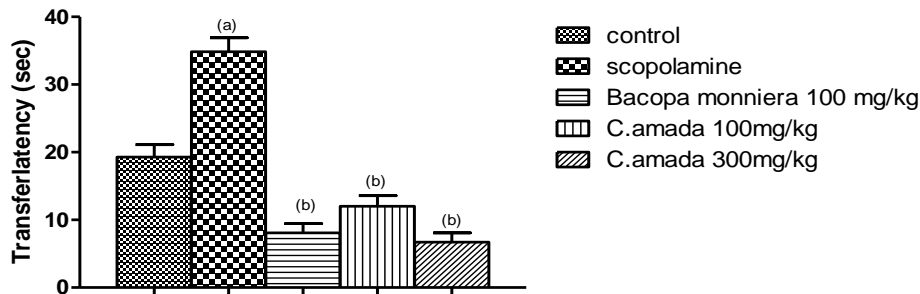


Fig. 2: Effect of acetic extract of *Curcuma amada* 100 mg/kg and 300 mg/kg on Transfer latency in rats using elevated plus maze

Data are expressed as MEAN±SEM (N=6). *P< 0.05 considered as significant. (a) compared to control, (b) compared to scopolamine ,(c) compared to *B.monniiera*

Table 2: Effect of acetonc extract of *Curcuma amada* 100 mg/kg and 300 mg/kg on recognition memory retrieval (duration of novel arm visits) and spontaneous alteration behaviour in rat using a Y-maze. a) 1hr b) 2hr and c) 4hr ITI

| S.NO | Recognition memory retrieval (Sec) | | | % Alteration | | |
|-------------------------|------------------------------------|---------------------------------|---------------------------------|-----------------------------|------------------------------|--------------|
| | 1hr | 2hr | 4hr | 1hr | 2hr | 4hr |
| Control | 135.5 ± 8.73 | 117.2 ± 7.06 | 99.33 ± 2.77 | 55.48 ± 4.68 | 53.98 ± 2.97 | 53.52 ± 3.61 |
| Scopolamine | 130.8 ± 10.44 | 85.83 ± 3.24 ^(a) | 105.7 ± 4.39 | 39.52 ± 4.01 | 31.05 ± 2.48 ^(a) | 50.36 ± 3.60 |
| B.monniere 100 mg/kg | 148.3 ± 10.66 | 151.5 ± 9.35 ^{(a) (b)} | 130.8 ± 5.34 ^{(a) (b)} | 57.84 ± 4.94 ^(b) | 64.02 ± 4.05 ^(b) | 57.76 ± 3.94 |
| C.amada 100mg/kg | 132.5 ± 8.92 | 121.3 ± 3.52 ^(b) | 106.3 ± 4.02 | 42.11 ± 3.57 | 47.19 ± 4.039 ^(b) | 50.49 ± 4.71 |
| C.amada 300 mg/kg | 145 ± 7.41 | 150.7 ± 8.16 ^(a) | 126.5 ± 8.15 ^(a) | 52.88 ± 2.84 | 62.08 ± 4.93 ^(b) | 59.62 ± 4.52 |

Data are expressed as MEAN±SEM [N=6]. *P< 0.05 considered as significant.
 (a)Compared to control, [b] compared to scopolamine, [c] compared to B.monniere

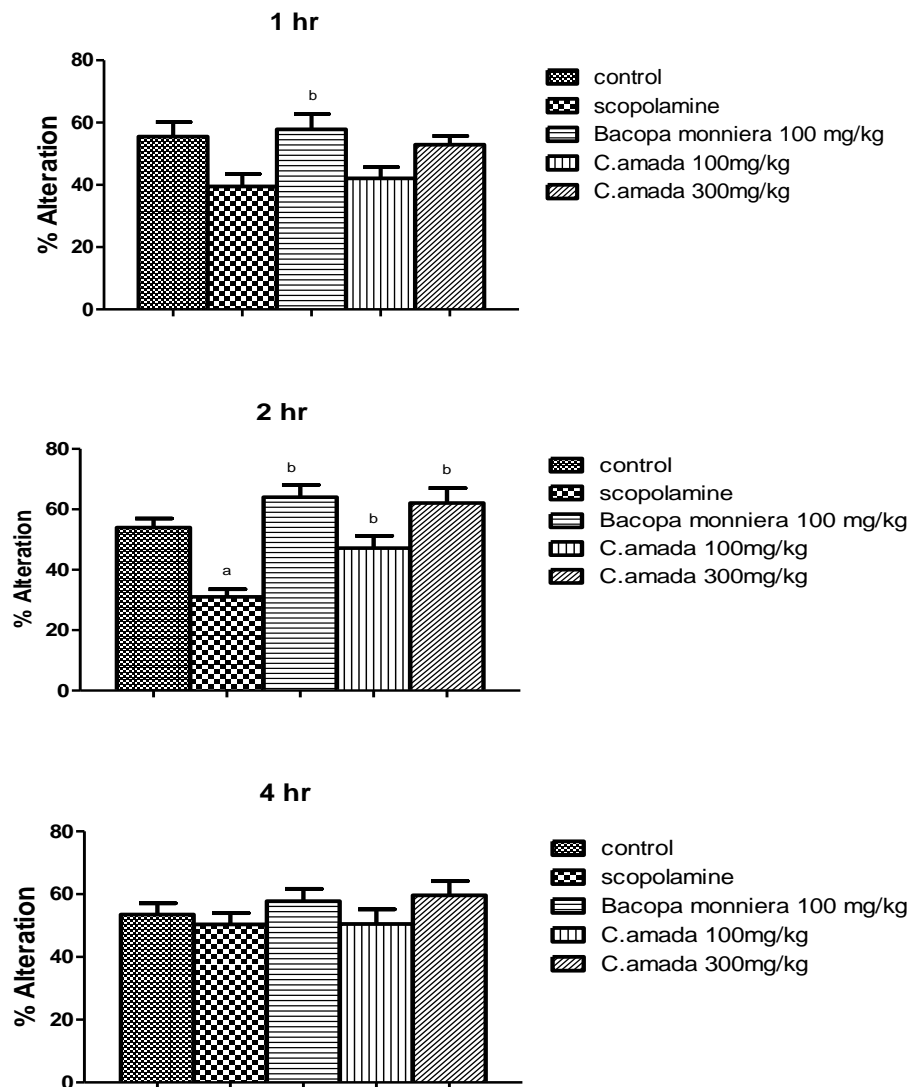


Fig. 3: Effect of acetonc extract of *Curcuma amada* 100 mg/kg and 300 mg/kg on spontaneous alteration behavior in rat using a Y-maze. a) 1hr b) 2hr and c) 4hr ITI

Data are expressed as MEAN±SEM (N=6). *P< 0.05 considered as significant.
 (a) compared to control, (b) compared to scopolamine ,(c) compared to B.monniere

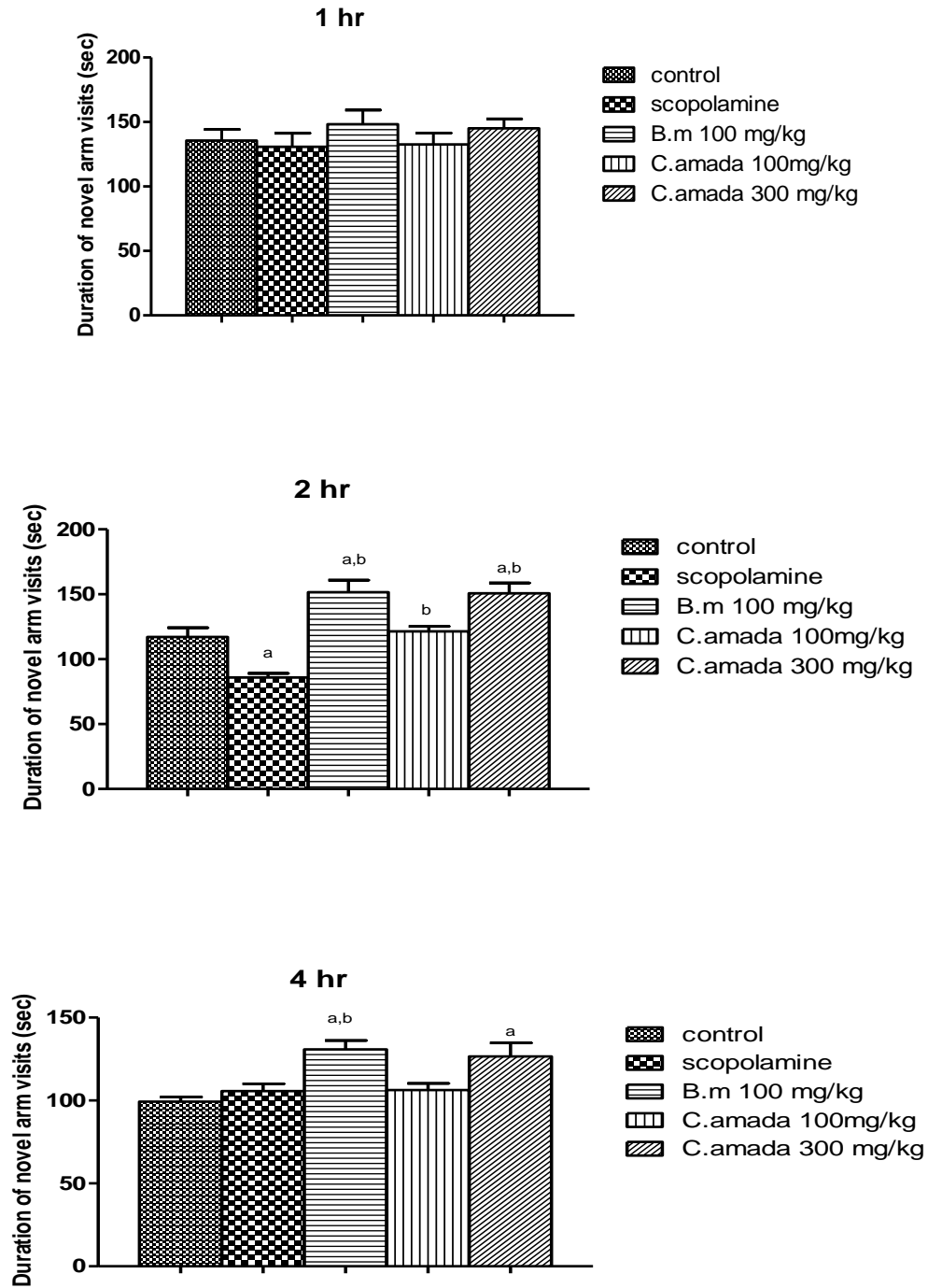


Fig. 4: Effect of acetonc extract of *Curcuma amada* 100 mg/kg and 300 mg/kg on recognition memory retrieval in mice with Y-maze. A) 1hr B) 2hr and C) 4hr ITI

Data are expressed as MEAN±SEM (N=6). *P< 0.05 considered as significant. (a) compared to control, (b) compared to scopolamine ,(c) compared to B.monniera

Preparation of extract and phytochemical study

Curcuma amada was extracted by continuous soxhlet apparatus. Phytochemical screening of acetonic extract showed positive result for volatile constituents, phenolic curcuminoids like curcumin, deoxycurcumin, bis-demethoxy curcumin.

Effect on transfer latency and spatial recognition in elevated plus maze

Fig. 1 shows the duration of time spent and Fig.2 shows transfer latencies of the different treatment groups with control. The latency to enter closed arm was significantly increased by scopolamine injected rats on first day and second day as compared to control (a) $P < 0.05$), indicating impairment of both learning and memory. Drug treated groups exhibited increased transfer latency (TL) on first and second day after 24 h compared to control group, indicating learning and memory ($P < 0.05$). Pretreatment with *B.monniiera* 100 mg/kg, *C.amada* 100 mg/kg and *C. amada* 300 mg/kg for 7 days showed significant difference in transfer latency for 1st and 7th day which was greater when compared to control (a) $P < 0.05$) and scopolamine (b) $P < 0.05$). The duration of time spent in the closed arm was similarly increased in pretreatment groups compared to control and scopolamine. Significant increase in duration of closed arm (protected arm) indicates spatial recognition learning and memory retrieval (table1).

Effect on spontaneous alteration and spatial recognition in y maze

The effect on alteration behavior was studied on two parameters, % alteration shown in Fig.3 and duration of arm visits shown in Fig.4. Animals were injected drugs and vehicle solutions memory recognition performances were evaluated by inter trial interval method immediately after the acquisition trial of the test. The duration of arm visit in the novel arm relative to that in the three arms of the Y-maze was called as retention. When arm differences for each group were analysed, *B.monniiera* 100 mg/kg, *C.amada* 100 mg/kg and *C. amada* 300 mg/kg showed significance when compared to scopolamine and control group, where as scopolamine showed significant decrease in spontaneous alteration, duration of novel arm visits and transfer latency compared to control at 2 hr ($P < 0.05$). However, no significant differences were found in any of the groups after

a 4 h ITI but *B.monniiera* 100 mg/kg, *C.amada* 300 mg/kg increased memory (table 2).

DISCUSSION

Curcuma spp. includes turmerin (a water soluble peptide), essential oils (such as turmerones, atlantones and zinziberone) and curcuminoids including curcumin²⁸. Curcumin has also been shown to endow with neuroprotective activity²⁹⁻³² and a promising role of curcumin in the treatment of Alzheimer's disease has been implicated. Further, curcumin supplementation counteracted the cognitive impairment caused by traumatic brain injury^{33,34}.

The important neurotransmitter involved in the regulation of cognitive functions is the central cholinergic system. These amnesic effects exerted by scopolamine can be evidenced by the decrease in Ach level³⁵. This decrease in Ach level with scopolamine was assumed to be due to its inhibitory action on Ach which is a main synaptic neurotransmitter in hippocampus³⁶. Hence decreased Ach levels have been resulted in the alteration of nootropic functions³⁷. The chemical and biological properties of curcuma species reported presence of curcuminoids, the pharmacological activity of it. Curcuminoids offer wide range of activities like antioxidant, antibacterial, anti-inflammatory and nootropic activity. AchE plays major role in memory, inhibitors of AchE were a thrust molecules for treatment of AD. *Curcuma amada* also possess antioxidant activity²³. Experimental works supported curcuminoids in *Curcuma longa* inhibit AchE. Touqeer Ahmed et al reported that curcuminoids were novel agents for the treatment of amnesia.

A recent literature report³⁸ with *Curcuma longa* extract indicated that it reduced brain acetylcholine esterase activity with improvement in cholinergic function in rats. Similar mechanism might be responsible for the observed improved activity of memory in the present study in rats due to the presence of curcuminoids. As mentioned above the antioxidant activity coupled with acetylcholine esterase inhibiting activity might be responsible for nootropic activity. *Bacopa monniiera* exhibits nootropic activity by its acetylcholine esterase inhibiting activity.

Curcuma amada at higher dose (300mg/kg) produce comparable effect with standard *Bacopa monniiera* (100mg/kg) as far as memory improvement activity is concerned.

The increase in the nootropic activity in *Curcuma amada* treated rats might be due to

increase in the Ach turnover in the hippocampus and cerebellum, the principle regions in the brain involved in the regulation of memory, cognition.

CONCLUSION

Nootropic effect of acetonic extract of *Curcuma amada* was evaluated by using Y-maze, elevated plus maze method. The results indicate that nootropic activity observed with acetonic extracts of *Curcuma amada* could be through improved learning and memory either by increasing the Ach levels by interfering with Acetylcholinesterase. Thus acetonic extract elicited significant nootropic effect in rats by interacting with cholinergic system. Phytoconstituents like flavonoids, curcuminoids have been reported (22) for their nootropic effect and these are present in acetonic extract of tubers of *Curcuma amada* (Roxb) and these active principles may be responsible for nootropic activity.

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