

## NEUROPROTECTIVE ROLE OF ACORUS CALAMUS AND IT'S ACTIVE PRINCIPLE BETA ASARONE IN RETAINING THE MEMORY OF RATS USING 8 ARM RADIAL MAZE

C. Venkatramaniah <sup>\*1</sup>, A. Mary Antony Praba <sup>2</sup>, B. Mohammed Ismail <sup>3</sup>.

<sup>\*1,2</sup> Associate Professor, Department of Anatomy, Tagore Medical College, Chennai, Tamilnadu, India.

<sup>3</sup> Professor, Department of Anatomy, Anna Medical College, Mauritius.

### ABSTRACT

**Background:** The hippocampus is a part of the temporal lobe of our brain and is also a part of the larger medial temporal lobe memory system responsible for general declarative memory. It plays an important role in the consolidation of information from short-term memory to long-term memory with involvement in the detection of novel events, places and stimuli. Severe damage to the hippocampus results in profound difficulties in forming new memories and to some extent affects memories formed before the damage. As our memory plays a major role on our daily activities and memory loss becoming a threat to the society, we planned this study to find a way to prevent this situation by protecting the brain using a herb.

**Materials and Methods:** We used adult male Sprague Dawly rats for this study. Animals were divided into 8 groups and were given the drugs *Acorus calamus* extract or its active principle Beta Asarone in different concentration 10 days prior to lesion surgery and 10 post operative days intraperitoneally (IP). The neuroprotective role of the drug employed was analyzed by analyzing the memory of the animals in 8 arm radial maze.

**Results:** Those animals provided with drugs were performed well in 8 arm radial maze by retaining the memory against the lesion control group animals and proved the neuroprotective ability of the drug employed in this study but the LC animals were not at all interested in moving from the arm where they were placed because of anxiety and memory loss.

**Conclusion:** Based on the observations and results it was concluded that beta asarone was a powerful neuroprotector as it was retaining the memory of the animals by protecting the hippocampus even after kainic acid lesion. Not only beta asarone the ethanolic extract of *Acorus calamus* also has its efficacy in nervous system as it contains beta asarone as active principle.

**KEY WORDS:** Acorus Calamus, Beta Asarone, Retaining Memory, Ethanolic.

**Address for Correspondence:** Dr. C. Venkatramaniah, Associate Professor, Department of Anatomy, Tagore Medical College, Chennai, Tamil Nadu, India. **E-Mail:** [fio7rio@yahoo.co.in](mailto:fio7rio@yahoo.co.in)

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### INTRODUCTION

The hippocampus a part of our nervous system plays important roles in the consolidation of information from short-term memory to

long-term memory with involvement in the detection of novel events, places and stimuli according to Abrahams et al, 1999 [1]. Some researchers view the hippocampus as part of a

larger medial temporal lobe memory system responsible for general declarative memory.

Severe damage to the hippocampus results in profound difficulties in forming new memories (anterograde amnesia), and to some extent affects memories formed before the damage (retrograde amnesia). Although the retrograde effect normally extends some years before the brain damage, in some cases older memories remain-this sparing of older memories leads to the idea that consolidation over time involves the transfer of memories out of the hippocampus to other parts of the brain.

There is evidence that the hippocampus plays a role in finding shortcuts and new routes between familiar places. Brain images of computer stimulated virtual navigation task in human revealed people have more active hippocampi when they navigate correctly. A study at University College London by Maguire, et al., 2000 [2], showed that the hippocampus is larger in taxi drivers than in the general public, and that more experienced drivers have bigger posterior hippocampi.

Acorus calamus is commonly known as sweet flag in India. The leaves of Acorus calamus have a lemony scent as well as the roots have a sweet fragrance. Acorus calamus has long been known for its medicinal value, and has been cultivated in Asia for this reason. According Caraka and Susruta (600BC) Acorus Calamus is a drug for epilepsy, promote intellect in children, memory and used to boost up the activities of brain in the form of brain tonic. According to Khare, 2004 [3] the good olden medicinal ghee or the nervine tonic contains Acorus calamus, Baccopa moneira and Alpinia speciosa. Which is been used for mostly all the neuronal disability.

As the neurodegenerative disorders are increasing in an alarming pattern without any definite treatment and memory is the one which drive our day to day life without any hurdles, we designed this study to find out the protective role of the herb Acorus calamus in our nervous system and so our memory.

## MATERIALS AND METHODS

**Animals (Table -1):** Adult male Sprague Dawly rats (200–250gm) were housed under standard laboratory conditions and maintained in

compliance with strict institutional guidelines. The room environment was maintained at 20° C ± 2° C; alternating 12 h light–dark cycle with food and water *ad libitum*. Maximum effort was taken to minimize the unwanted stress to the animals and to reduce the number of animal to be used for this study.

**Table 1:** Showing the animal groups used for 8 arm radial maze study.

S.No	Animal groups	Lesion surgery	Ethanollic extract of Acorus calamus treatment before and after surgery	Beta Asarone treatment before and after surgery
1	Control (CO)	NO	NO	NO
2	Lesion control (LC)	YES	NO	NO
3	AC 15mg (AC 15)	YES	YES	NO
4	AC 25mg (AC 25)	YES	YES	NO
5	AC 35mg (AC 35)	YES	YES	NO
6	BA 10mg (BA 10)	YES	NO	YES
7	BA 15mg (BA 15)	YES	NO	YES
8	BA 20mg (BA 20)	YES	NO	YES

The animals were divided into 8 groups with 6 animals in each group. Group 1 contains the control animals. Group 2 was for lesion control. Group 3, 4 and 5 were for 15mg, 25mg and 35mg ethanolic extract of Acorus calamus treated group with lesion. Group 6, 7 and 8 were for 10mg, 15mg and 20mg of beta asarone treated group with lesion.

## DRUGS

### Ethanolic Extract Of Acorus Calamus (AC)

**Preparation:** The ethanolic extract of Acorus calamus was prepared by soxhletion method following Elayaraja et al., 2010 [4]. He proved the ethanolic extract expressed more antioxidant activity than other extracts of Acorus calamus.

**Beta Asarone (BA):** The drug beta asarone was purchased from Sigma Aldrich Ltd., St. Louis, USA. The IP dosages of the drug was started 10 days prior to lesion and also after the lesion so as to access the protective nature and treatment role of it in hippocampus and was given around 10' clock every day.

**Memory Study:** The memory of the animal was assessed by using the instrument 8 arm radial maze. All the animals including CO will be trained in the maze to collect the food kept in selective arms with visual cues, 10 days prior to lesion surgery. After 10 days of surgery the

animals will be checked for their memory in collecting the food by entering only in the particular arms with visual cues.

**8 Arm Radial Maze - (figure-1):** The radial 8 arm maze task is most extensively used to investigate specific aspects of spatial working and reference memory (5). This task is based upon the premise that animals have evolved an optimal strategy to explore their environment and obtain food with the minimum amount of effort.

**Fig. 1:** Showing the 8 arm radial maze.



### Training Rats

- Weigh each rat daily throughout the training to monitor the health and degree of food deprivation.
- Restrict food available to rats so that its body weight attains 85% of that prior to training.
- Give food reward in home cage for a few days prior to training, in order to acclimatize the rat to the reward in a familiar environment (A small piece -10 mg) of Kellogg's chocos was given as food reward).
- The plexiglass maze was set up 1 m above the floor for easy access to the rats and food cups. It is composed of a central octagonal platform with eight arms extending from it like the spokes of a wheel. Guillotine doors that can be opened and closed individually, separate the central platform from the arms. The maze should be placed in a room that contains various external cues that are visible to the rat while on the maze.
- Place a well-handled pair of rats (preferably cagemates) on the maze at the same time.
- Using two rats reduces the time it takes for each to acclimate to the maze.

- Spread food rewards around the entire maze to encourage exploration.
- Acclimation period should require only 1 or 2 days.
- On subsequent days, place the food cup with the food only on the selected arms (for this study 2,4,6 and 8<sup>th</sup> arm), and only at the ends of the arms and keep some visual cues at the selected arms only.
- Finally, place rat alone in the maze and keep food at the end of arms. Testing can begin when rat is comfortable while placed alone on the maze, explores without hesitation and without excessive defecation or urination.
- A typical rat will be ready for testing within 7 days. Rats should be trained in the maze once a day every day (including weekends, ideally) during training).
- A maximum of 5 minutes will be given to each rat to finish up the task successfully.
- Normal healthy young rats will perform this task almost perfectly every time. A healthy, happy, and motivated rat should learn this version of the task, such that working and reference memory errors are <15%, within 10 days.
- Begin study the effect of drug with lesions when performance is stable and choice accuracy is >85%.

### Testing the Rats

- Bait any four arms of maze with food, for this study the baited arms are 2,4,6 and 8.
- Place rat on central platform with all guillotine doors closed.
- Raise all doors simultaneously
- Allow the rats to freely explore the cage, collect the food rewards and finish the task.

### Parameters:

- Number of correct entries into baited arms.
- Number of entries into unbaited arms.
- Number of reentries into baited arms.
- Time elapsed between the beginning of the test session and the rat obtained all available food rewards.

### Scores:

- Entries into unbaited arms were considered as reference memory errors.



- Reentries into baited arms were considered as working memory errors.
- Less time- no fear and active
- Correct entries – good memory

**Hippocampal lesion surgery (Figure 2):** The animals were maintained in empty stomach 10h before the procedure and were anaesthetized using pentathol sodium. The hair in the head region was shaved using diluted savlon as sanitizer. The animale were fixed in the stereotaxic frame with the help of the tooth and nose bar.

**Fig. 2:** Showing the surgical procedure for making lesion.



A 2cm long incision was made along the scalp of the rats and the fascia was cleaned to point out the bregma. Necessary steps were taken to avoid infections at all levels. The hippocampus was marked in the scalp region by moving the manipulator from the bregma 3.6 cm posteriorly, 4cm right laterally and a small hole with 1mm diameter was made in the marked region.

0.5µl of kainic acid [6] was taken in a Hamilton syringe and was fixed in the frame. With the help of the manipulator the syringe was moved 3.5mm inferiorly from the dura to the hippocampus. The same chemical was injected in the rate of 1 µl per 1 minute following McGinty et al., 1983(7). The syringe was withdrawn and the scalp was sutured with proper care.

**Post operative care:** Proper antibiotic care was given post lesion with 2mg/kg/day gentamycin for 3 days.

## RESULTS AND DISCUSSION

**8 Arm Radial Maze for Working Memory [8]:** 8 arm radial maze was used to analyse the working and reference memory errors. As

hippocampus is the main organ for memory, neuroprotection of hippocampus by the given drug will retain the memory if not the animal will lose its memory. Normal healthy young rats will perform this task almost perfectly every time with a maximum of <15% working and reference memory errors in 10 days. Four parameters were studied under this technique.

**Number of Correct Entries into Baited Arm (Chart - 1):** This parameter tells about the level of neuroprotection in terms of retention of memory. All the animals were tested in the 8 arm radial maze for this study 10 days after the lesion.

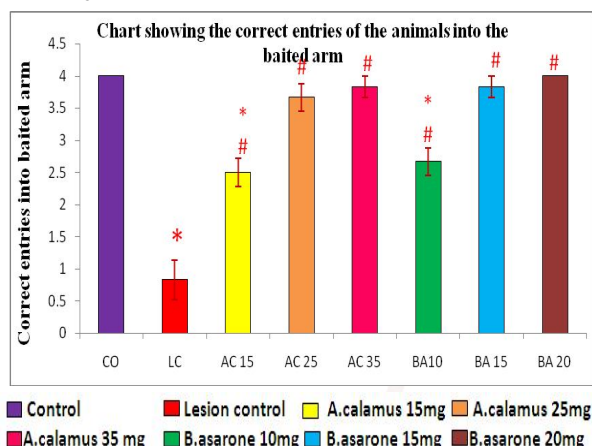
The CO group of animals performed normally and the LC group of animals were not performing well with restriction in their movements as they were in full stress, anxiety and lost their memory because they were not provided with any neuroprotective drug. The BC 20 animal group performed well in this parameter as their memory was equivalent to the CO animals and significantly higher than the LC group. The animal groups BC 15, AC 25 and AC 35 also performed significantly well in comparison with LC group with minimal memory errors. But the other groups the AC 15 and BC 10 were not performed well and can be concluded as low dosage groups as their errors were significantly low with LC group and significantly high with CO group.

The animal groups BC 20 performed well (figure-3) and so proved the effective neuroprotective role of the drug.

**Fig. 3:** Showing the BA20 animal performing in radial maze 10 days after lesion.



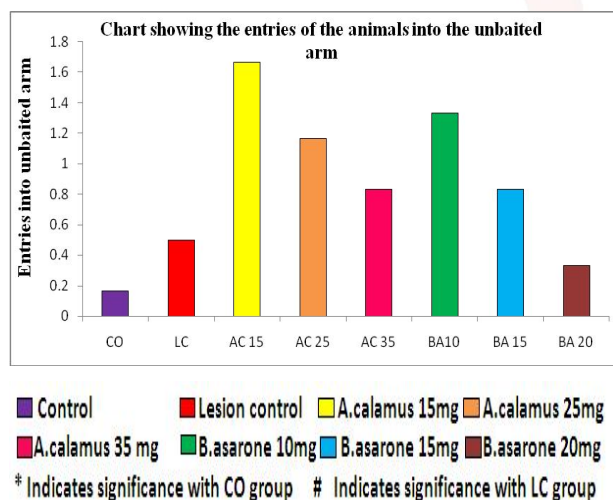
**Chart 1:** Bar diagram for the correct entries of the animals into the baited arm of 8 arm radial maze on 10th day of lesion.



**Entries into Unbaited Arm (Chart - 2):** 10 days after lesion when the animals were recovered completely, they were tested for reference memory errors in 8 arm radial maze. Entries into unbaited arm says about the reference memory errors, as the animals could not be able to recollect the old memory.

The CO group of animals performed normally. As the LC group of animals was in full anxiety, they restricted their movements and were not entering into all the arms. That made it difficult to discuss about the parameter with other groups. The animals of group BC 20 performed significantly low errors in avoiding the unbaited arms. That shows the drug was good enough to protect the animal's memory and so from epilepsy. The animal groups BC20 exhibits good performance and proved the neuroprotection.

**Chart 2:** Bar diagram for the entries of the animals into the unbaited arm of 8 arm radial maze on 10th day of lesion.

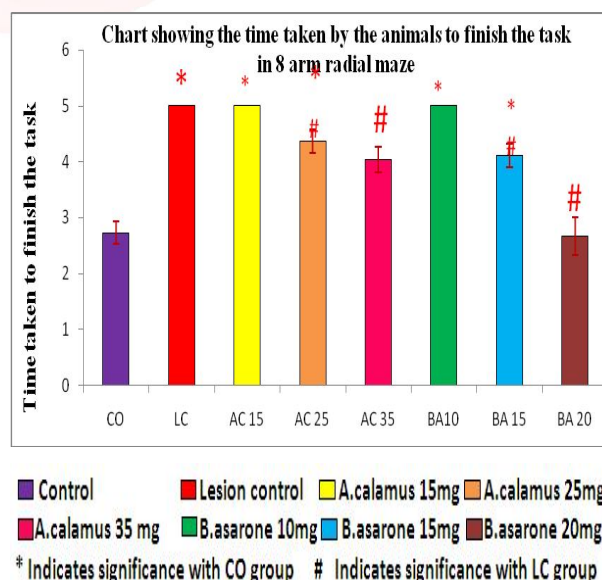


**Finishing time of 8 Arm Maze (Chart - 3):**

Animals were also tested for the finishing time of the task in 8 arm radial maze. This is the time taken by the animal to collect all the four food rewards. Collecting all the four food rewards within 300seconds or 5minutes. Showing good memory and stress free condition of the animal.

The CO group was performing normally. As like the other parameters the LC group animals were in full stress and so not finished the task till the end of the allotted time that is 5 minutes. As like the LC group the AC 15 and BA 10 performed significantly high errors in comparison with CO group. The animal groups AC 25, AC 35 and BA 15 have finished the task within the said but the finishing time was significantly higher than the CO group. The time taken by the animal group BC 20 was significantly less than the LC group and was equivalent with the control group so we can conclude the animals were protected well with the neuroprotector beta asarone. Other group animals were not faster in their performance as their drug dosage was very low. The active performance of the animals in this parameter is because of the effective dosage and the drug employed in this study and BA20 is the best dosage in neuroprotection.

**Chart 3:** Bar diagram for the finishing time of the animals in 8 arm radial maze on 10th day of lesion.



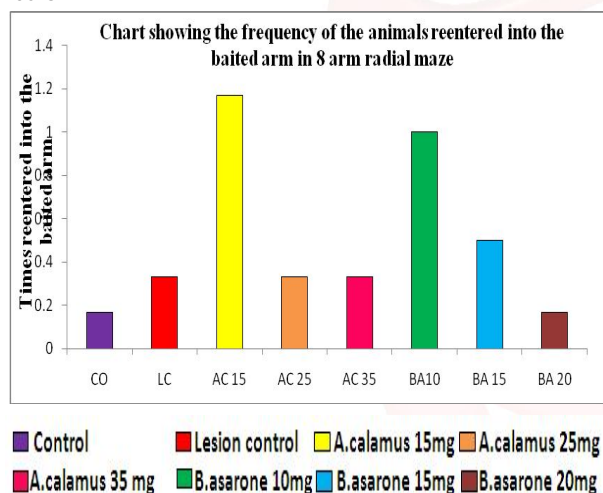
\* Indicates significance with CO group # Indicates significance with LC group

**Reentries into Baited Arm (Chart - 4):**

Reentries into baited arms of the 8 arm radial maze was done for working memory errors. These animals cannot memorize the arm already they visited and so enter the baited arm again to collect the food reward.

The CO group of animals performed normally by not re-entering into the baited arms in the 8 arm radial maze. As the lesion control group animals were in full anxiety, they restricted their movements, randomly entering into the arms and also have not entered into all the arms. The values on the bar diagram drawn on the basis of this observation was not looking reliable because of the high anxiety level of the LC group animals. That made the parameter difficult to compare with the other groups. The animal group BC 20 performed equal with the CO group that shown their working memory was good and proved the drug BC 20 was effective in boosting memory and other nervous system related problems.

**Chart 4:** Bar diagram for the reentries of the animals into the baited arm of 8 arm radial maze on 10th day of lesion.



## CONCLUSION

The hippocampus is located inside the medial temporal lobe and plays an important roles in short-term, long-term memory, spatial navigation, initial learning and also important for some spatial memory tasks that requires finding the way to a hidden goals (Burgess, 2002)(9). The popular Medhya Rasayana the Acorus calamus which is present in Memocare Plus, according to experimental and clinical studies could improve memory and reduce stress naturally (Singh et al., 2008) [10]. According to Geng et al., 2010 [11], Oral administration of beta-asarone improved cognitive function by suppressing neuronal apoptosis in rats injected with beta-amyloid in the hippocampus.

In this study the animals were allowed to perform in the 8 arm radial maze 10 days after the lesion to assay memory of the animals and so the neuroprotective ability of drug employed. As for conclusion in the present study the LC group animals performed very poor in comparison with the CO group. The AC 15 and BA10 group animals were showing a few correct entries and also had a few wrong entries, stating its poor protection in hippocampus and poor memory. The drug groups AC 25 and BA15 were showing a near equivalent level of correct entries and other parameters and proved the neuroprotective ability of the particular drugs that made the animals to remember the correct arms with the food reward and performed normally like the CO group.

This draws the conclusion that the drug chosen was effective in neuroprotection but the dosage was not enough in few animal groups but the drug group AC 35 and BA 20 were very active and finished the task in 8 arm radial maze very fast correctly in all the parameters, with an upper hand for the drug BA20, stating the protective nature and dosage of the drug.

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## Conflicts of Interests: None

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