

PROTECTIVE ROLE OF ACORUS CALAMUS AND BETA ASARONE IN EXPLORATION AND ANXIETY LEVELS ON EXPERIMENTAL EPILEPTIC RAT MODEL

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ABSTRACT

Background: The hippocampus is a major component of the brain. Damage to hippocampus results in profound difficulties in forming new memories and to some extent affects memories formed before the damage, anxiety and movement restriction. As our memory plays a major role on our daily activities and exploration plays a very big role in developing the society, we planned a study to analyze the difficulties of hippocampal lesion and to find a way to prevent this situation by using some neuroprotective herbal drugs.

Materials and Methods: We used adult male Sprague Dawley 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 studied by analyzing the level of special exploration and anxiety of the animals.

Result: The LC group animals restricted their movements due to anxiety. They also had a very high latency period. But the drug treated animals were showing a good level of exploration by moving freely on the field, also they scored less latency for their credit. Between the drug groups the Beta Asarone group brought into view an upper hand than *Acorus calamus*.

Conclusion: Based on the study it was concluded though both the drugs were smart enough to act and protect the nervous system, beta Asarone is more specific and the dosage BC20 was found more apt for the applied purpose.

KEY WORDS: Hippocampus, Memory, Hippocampal Lesion, *Acorus Calamus*, Beta Asarone.

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INTRODUCTION

It is running along the floor of the temporal horn of the lateral ventricle. In rat, the two hippocampi resemble a pair of bananas, joined at the stems by the hippocampal commissure that crosses the midline under the anterior corpus

callosum. In human or monkey brains, the portion of the hippocampus down at the bottom, near the base of the temporal lobe, is much broader than the part at the top.

The hippocampus is a major component of the brain of vertebrates and it belongs to the limbic

system. The German anatomist Duvernoy was the first to illustrate the structure of hippocampus, also wavered between “seahorse” and “silkworm.” “Ram’s horn” was proposed by the Danish anatomist Jacob Winsløw; and a decade later his fellow Parisian, the surgeon de Garengot, used “cornu Ammonis” - horn of Amun (the ancient Egyptian god) (Pearce, 2001[1]). Like the cerebral cortex, it is a paired structure, with mirror-image halves in the left and right sides of the brain. 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 is also important for some spatial memory tasks that requires finding the way to a hidden goals or exploration. Studies conducted on freely moving rats and mice have shown that many hippocampal neurons have “place fields”, that is, they fire bursts of action potentials when a rat passes through a particular part of the environment.

As an anxiety free life with exploration sounds great, so we took this as a study to explore the protective nature of the herb employed.

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 $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$; 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 for open field study.

S.No	Animal groups	Lesion surgery	Ethanollic extract of <i>Acorus calamus</i> 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 3,4and 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.

HIPPOCAMPAL LESION SURGERY – (figure-1):

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. 1: Lesion surgery.



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 3.6cm posteriorly, 4cm right laterally and a small hole with 1mm diameter was made in the marked region.

0.5µl of kainic acid (Longo and Mello, 1998[2]) 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[3]. 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 gentamysin for 3 days.

DRUG

Ethanollic extract of *acorus calamus* (ac) preparation: The ethanollic extract of *Acorus calamus* was prepared by soxhletion method following Elayaraja et al., 2010 [4]. He proved that ethanollic 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.

Study of anxiety and exploration

Open field test: It is an analytical tool to find out the level of exploration and anxiety in experimental laboratory animals. For this study we selected four parameters to analyse the animals. A high frequency of the parameters indicates high exploration and lower level of anxiety except the parameter latency period.

Apparatus (figure-2): The open field apparatus was constructed out of Plexiglas and measured 1m x 1m with 36 cm walls. All the walls and the floor were darkened to give an appropriate environment for rats to explore freely. Bright lines were drawn on the floor with a marker and the floor was divided into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field (Brown, Corey, & Moore, 1999 [5]). The central square is used because some rat strains have high explorative activity and cross the lines of the central chamber many times during a test session. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations (Carrey, McFadyen, & Brown, 2000[6]).

Fig. 2: Open field apparatus.



The field was lit by a 60-watt red lamp for background lighting. The open field maze was cleaned between each mouse using 70 % ethyl alcohol. Behavior was recorded for latter analysis manually.

Procedure: Rats were carried to the test room in their home cages and were handled by the base of their tails at all times.

Rats were placed into one of the four corners of the open field and allowed to explore the apparatus for 5 minutes.

After the 5 minute test, rats were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and permitted to dry between tests.

Parameters (Brown et al, 1999 [7])

1. Line Crossing: Frequency with which the rat crossed one of the grid lines with all four paws.
2. Center Square Entries: Frequency with which the rats crossed one of the red lines with all four paws into the central square.
3. Center Square Duration: Duration of time the rat spent in the central square.
4. Rearing: Frequency with which the rat stood on their hind legs in the maze.
5. Grooming: Duration of time the animal spent licking or scratching itself while stationary.
6. Urination: number of puddles of urine.
7. Defecation: number of fecal boli produced in the given time.

Parameters studied and scores

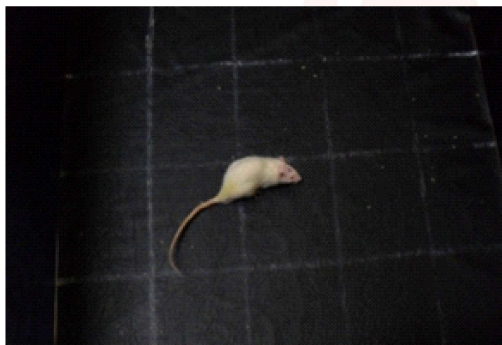
1. Line Crossing- is a measure that the rat crossed one of the grid lines with all four paws. A high frequency of these behaviours indicates increased exploration and lower level of anxiety
2. The number of central square entries and the duration of time spent in the central square are measures of exploratory behaviour and anxiety. A high frequency/duration of these behaviours indicates high exploratory behaviour and low anxiety levels.
3. Latency is the time taken by the animals to make its first movement in the open field apparatus. Increased latency period indicates high anxiety level and low exploration.
4. Rearing: Frequency with which the rat stood

on their hind legs in the maze. A high frequency/duration of these behaviours indicates high exploratory behaviour and low anxiety levels.

RESULT AND DISCUSSION (figure-3):

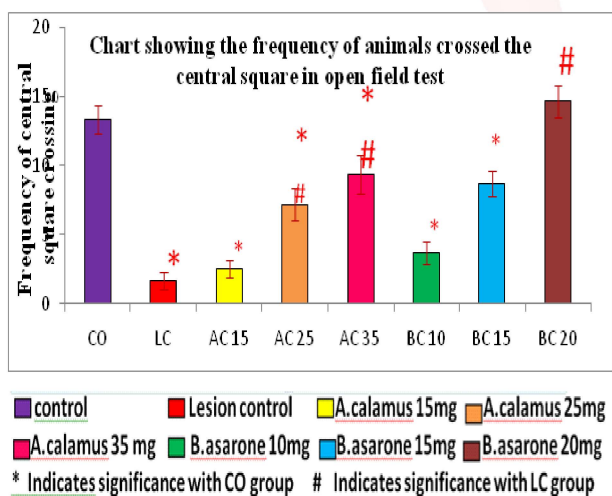
Open field test for spacial exploration (Malek *et al.*, 2003[8]): Open field test was performed to measure the exploration and anxiety of the animals in an open field. Four parameters were selected to analyse the animals. The frequency of parameters were taken as indicators for the level of exploration and anxiety and so indicates the efficacy of the drug given.

Fig. 3: showing the animal performing in open field apparatus 10 days after lesion.



Frequency of central square crossing (chart-1): The number of central square entries are, measures of, exploratory behaviour and anxiety. A high frequency of these behaviours indicates high exploratory behaviour and low anxiety levels. That indirectly shows the protection of the hippocampus by the given drug.

Chart-1: Bar diagram for frequency of animals crossed the central square in open field test on 10th day of lesion.

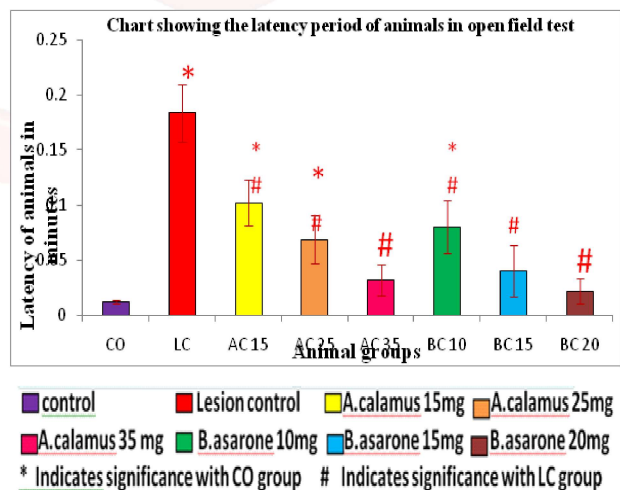


The frequency of central square crossing by the animal belongs to LC group was significantly low when compared with the CO group. The drug

groups AC 25, AC 35 and BC 15 were moving across the central squares but were significantly low in comparison to the CO group that shows the dosage of drug is not optimum. The groups AC 15 and BC 10 were not performed well as like the LC group. Their movements were equivalent with the LC group and concluded as showing poor exploratory activity and high level of anxiety. The drug group animals BC 20 were freely moving significantly more across the central square and were equivalent to the CO group of animals and so concluded as highly explorative and free from anxiety.

Latency (Chart-2): Latency is the time taken by the animals to make its first movement in the open field apparatus. If the hippocampus was affected the anxiety level of the animal will be more, and so the animal will take more time to make its first movement. That shows the drug was not neuroprotective or the dosage was not enough. So we can analyse the effect of the drug by watching the latency of the animals in the open field test.

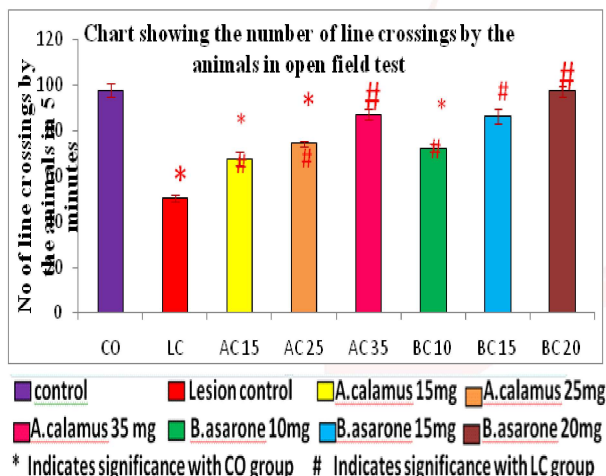
Chart 2: Bar diagram for latency period of animals in open field test on 10th day of lesion.



In the present study the animal group LC exhibited highest latency and so can be concluded as in full anxiety. AC 15, AC 25 and BC 10 shown significantly low latency compared with LC group and high latency compared with CO group shows the drug dosage was not enough. The drug dosages AC 35 and BC 20 were taken significantly low latency time when compared with LC group and equivalent latency time in comparison with CO group and can be taken as highly active and protected from neuronal degeneration.

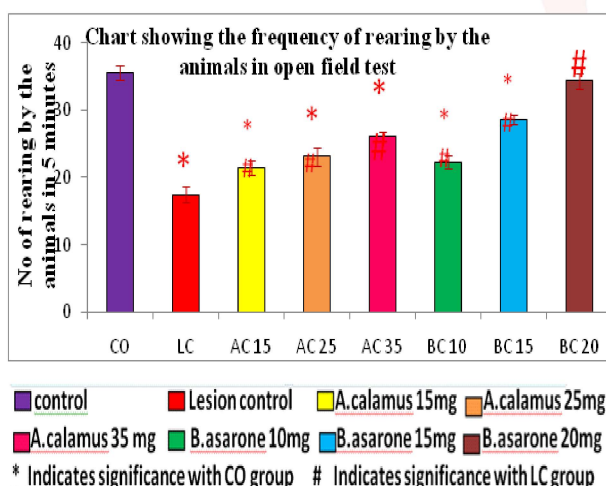
Number of line crossing (chart-3): Line Crossing is a measure that the rat crossed one of the grid lines with all four paws. The sum of line crossed measures the level of exploration and anxiety. A high frequency of these parameters indicates increased exploration and lower level of anxiety. That indirectly says the level of hippocampal protection.

Chart 3: Figure showing the bar diagram for no. of line crossed by the animal in open field test on 10th day of lesion.



The LC group of animals performed very poorly in this parameter when compared with CO group as they were in anxiety because of hippocampal damage. All the other animal groups the AC 15, AC 25 and BC 10 were crossed significantly more lines when compared with the LC group and less performed when compared with CO group. The animal groups BA 20, BA 15 and AC 35 exhibits significantly more line crossing in comparison with the LC group. The line crossing of BA 20 was equal with the CO group that proves good neuroprotection.

Chart 4: Bar diagram for frequency of rearing of animals in open field test on 10th day of lesion.



Rearing (chart-4): The parameter rearing explains the frequency with which the rats stood on their hind legs in the field. Rears are measures of exploration and anxiety. A high frequency of this parameter indicates increased exploration and lower level of anxiety.

As like the other parameters the LC group of animals performed very poorly compared with the CO group in this parameter as they were in anxiety because of hippocampal damage and without drug treatment. The animal groups BA 10, BA 15, AC 35, AC 25 and AC 15 exhibits high significance in rearing performance in comparison with the LC group but not equivalent with the CO group and shows the protection was not enough. The animals belongs to 20 was performing high rearing with the LC group and equivalent with the CO group draws a conclusion that the drug dosage exhibits effective results.

CONCLUSION

Effect of ethanolic extract of acorus calamus on spacial exploration in open field test:

Tripathi, 2010 [9] concluded, the exploratory activity of rat was restored and the behavioural deficit was prevented very well with Acorus calamus administration in comparison to stressed group in adopted model of depression. In this work the animals were tested in the open field on 10th day of the lesion. More number of line crossing and more rearing activities said about the high exploratory activities of the animals and so high neuroprotection in hippocampus. In this experiment the AC 15 and AC 25 group animals were found doing line crossing and rearing but was not equal with the CO group stating it's low exploratory activity. The AC 35 group animals did more line crossing equal with the CO group but rearing was not equivalent with the CO group. That draws a conclusion that the animals have exploratory activity but not equal with the CO group that shows the drug was effective but the dosage was not optimum.

Effect of beta asarone on spacial exploration in open field test:

Han, 2013[10] used open-field tests to evaluate the antidepressant-like effects of β -asarone and considered it as a new therapeutic agent for curing depression. For this work the number of line crossing and rearing were taken as parameters in open field

apparatus to access the exploratory activity of the animals. High exploratory activity directly says about the antiepileptic activity of the drug given. The BA 10 animals were poor in line crossing and rearing and can be concluded as less explorative. The drug groups BA 15 shown a good number of line crossing and rearing but the frequency was significantly less in comparison with the CO group and gave a conclusion that the animal groups were explorative but the frequency was not equal with the normal animals. The BA 20 group of animals in this study exhibited high number of line crossings and rearing and the frequency was equal when compared with the CO group. This proved the animals were highly explorative and the drug was acting on the hippocampus against the neurogenerative disorder.

As a whole both the drugs were acting on the hippocampus and were effective in protection but the degree of action varies for both the drugs within the given short period of time. So this work supports the neuroprotective role of both the drugs employed. As the herbal drugs are proved for any adverse effect it is advisable to take minimal amounts of the herb in either of the forms as a food supplement along with our routine energy drink, coffee or tea so as to protect our nervous system as it is always said prevention is better than cure.

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Conflicts of Interests: None

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