A STUDY OF ANXIETY ON EXPERIMENTAL EPILEPTIC RAT MODELS USING DARK LIGHT BOX.

ABSTRACT

The word neurodegeneration refers to defects in neuronal structure and consequently its function. The main characteristics of these disorders are relentless progression and cognitive declination. Epilepsy is one of the neurodegenerative disorders, around 50 million people in the world are affected with. Though it is one of the major health problems in the present society, there are several gaps in understanding the consequences related to neurological disorders. As research works related to neurodegeneration is very much limited in India we have planned one as an initiative.

We segregated 8 animal groups, each with 6 animals for this work. The animal groups are LC, CO, AC15, AC25, AC35, BA10, BA15 and BA20. This study was conducted on 10^{th} day after the lesion by considering the day of lesion as day '1' and the next day as day 2^{nd}. All the animals were recovered completely within these 10 days and were used to study the parameters.

KEY WORDS

LC – lesion control, CO – control, AC e– Acorus calamus crude extract, BA – Beta asarone, epilepsy.

INTRODUCTION

Anxiety and stress became the normal part of the life now a days, but leads to loss of concentration, muscle tension and irritability if persist for a long time. Epilepsy is one of the major neurodegenerative disorder related with anxiety that affects people of any gender, race and nation. Epilepsy is the fourth most common neurological disorder related with seizure, coexisting

Comment [AO1]: THE ABSTRACT
1.The introduction of the abstract did not capture the relationship between Anxiety and epilepsy
2. The objective was stated
3.The results and conclusion was not stated

health conditions, abnormal behavior and sudden unexpected death. About 65 million people around the world are affected by epilepsy. There are two forms of epilepsy and temporal lobe epilepsy (TLE) is the most common form of focal epilepsy. Focal epilepsy may be of simple type, may not affect the memory or behavior and complex type that affect the memory and behavior of the person (MedlinePlus). Drug-resistant TLE is even serious and associated with high risk for psychosocial impairment, cognitive decline and mortality. It is a chronic non-communicable and incurable disease of the brain, but 70% of people living with epilepsy could live seizure- free if properly diagnosed and treated (WHO).

Comment [AO2]: Kindly rephrase

Comment [AO3]: No references

The hippocampus was considered to be the generator of TLE. This view was due to the frequent observation of the histopathology of hippocampus of TLE patients. Surgical removal of the sclerotic hippocampus improved this epileptic condition, also the experimental neurophysiologists who work on normal hippocampi identified CA3 as the site of origin of discharge in a variety of models of experimental hippocampal epilepsy (Avoli, 2007). Because of strong interconnections, seizures beginning in either the medial or lateral temporal areas often spread to involve both areas and also to neighboring areas on the same side of the brain as well as the temporal lobe on the opposite side of the brain (Bartsch, 2012).

Anxiety is one of the symptoms of epilepsy (*Tuft etal.*,) and for most of the epileptic patients the major point of anxiety is the seizure that arise at any place, any time without warning that even worsen the condition. So epilepsy and anxiety goes hand in hand.

Anxiety and epilepsy have some underlying neurochemical features that involve GABA and serotonin in particular. GABAergic drugs such as valproate, phenobarbital and benzodiazepine have both seizure- and anxiety-reducing properties (Ochoa *et al*.,). Reduced serotonin receptor

binding has been shown both in patients with panic anxiety and in patients with epilepsy (Liu et al).

Manouze *et al.*?, studied the effects of individual housing as compared to conditions maintaining social contact on stress markers and epilepsy and concluded isolated pilo animals were very aggressive, social isolation constitutes a major stressful situation that can lead to a depression-like profile.

As a medicinal plant *Acorus calamus* and its oil were employed in a number of neuronal disorders in olden days. It has a number of active principles and beta asarone is one of the main active principle

Present days Memocare Plus is a noble herbal formulation that has blended these time-honored memory-enhancing herbs, which can enhance memory and reduce stress and anxiety. These herbs delay brain aging and stimulate regeneration of neurons (Singh *et al.*, 2008).

In order to confirm the action of *Acorus calamus* in neurodegeneration related anxiety condition we formulated this study, collected the data and analyzed it. The results were amazing.

Comment [AO4]: References did not contain the YEAR: TUFT ET AL….?

Comment [AO5]: Clearly give a statement to justify the use of the herb by an epileptic patient.

Comment [AO6]: Kindly find references to justify most the claims

MATERERIALS AND METHODS

Animal

We used adult male SD rats weighing 200-250gms for this study. The total study design was approved by the CPCSEA (IAEC/XIII/10/CLBMCP/2008-09).

Comment [AO7]: 1.How did you arrive at these doses, or give references to support the doses
2.Route of administration and why; not stated?
3.Extract preparation procedure not stated?
4.Why the use of only male rats, because you mentioned in the study background that it affects both gender ?

S.No	Groups of animals	Hippocampal Lesion with Kainic acid	Pre and post treatment of Acorus calamus	Pre and post treatment of beta asarone

1	CO (Control)	Not	Not	Not
2	LC (Lesion control)	Done	Not	Not
3	AC 15 (AC15mg)	Done	Done	Not
4	AC 25 (AC25mg)	Done	Done	Not
5the	AC 35 (AC35mg)	Done	Done	Not
6	BA 10 (BA10mg)	Done	Not	Done
7	BA 15 (BA15mg)	Done	Not	Done
8	BA 20 (BA20mg)S	Done	Not	Done

Table 1 showing animal groups used for enzyme study

The above is the animal groups used for the study. AC 15, 25 and 35 groups were given crude extract of 15, 25 and 35mg's of Acorus calamus per kg body weight. Whereas groups BA 10, 15 and 20 were given 10, 15 and 20 mg's of Beta asarone, the principle component of Acorus calamus per kg body weight before and after 10 days of lesion with Kainic acid.

DARK AND LIGHT FIELD TEST (ANXIETY)

APPARATUS

- This test takes advantage of the natural conflict of a rodent between the exploration of a novel environment and the aversive properties of a large, brightly lit open field.
- The test apparatus was a rectangular Plexiglas box was divided by a partition into two environments. One compartment was dark, and the other compartment was brightly illuminated.

- The compartments were connected by an opening located at the floor level in the center of the partition. The more time a mouse spends in the light compartment and the more transitions it makes the less it was considered anxious.
- This test was used to assess anxiety. The basic measure was the animal's preference for dark, enclosed places over bright, exposed places.

Fig-1

Figure 1 showing the dark and light box

PROCEDURE

- Each rat was taken from its home cage and placed into the dark chamber facing the end wall (parallel to the partition
- Activities and time in the light-dark box was video-recorded for 5 min.
- At the end of every light-dark box test, the number of fecal boli and urine puddles were recorded.
- Duration in each chamber and the number of light-dark transitions were also recorded.
- A single transition is counted when all the four paws entered a chamber.

PARAMETERS

1. Time in light field
2. Time in dark field

SCORE

The more a mouse spends in the light compartment and the more transitions it makes the less it was considered anxious

RESULTS &DISCUSSION:

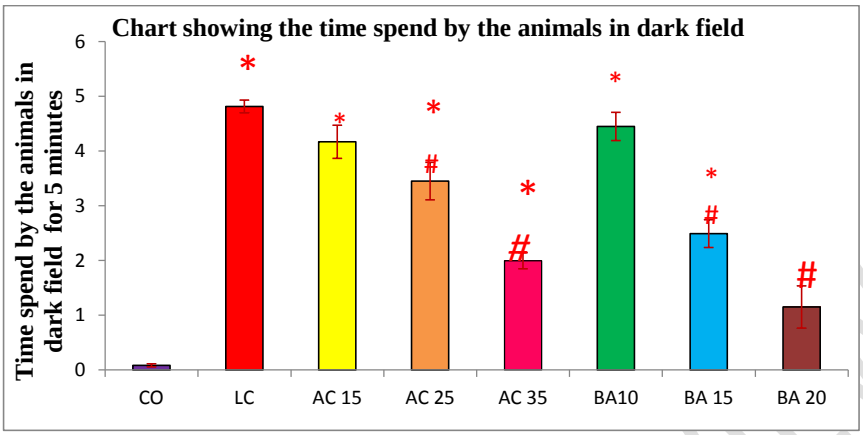
a. DARK AND LIGHT FIELD TEST FOR ANXIETY (Gorzó *et al.*, 1998)

This test is used to access anxiety. The basic measure of this test is the animal's preference for dark, enclosed places over bright exposed places. The more a mouse spends time in light compartment the less it is considered anxious. Arrant *et al.,* 2013 studied the anxiety level of adolescent and adult male rats by using dark light box and concluded this as a good technology in analyzing the anxiety of animals. Radhakrishnan A and Gulia KK 2018, analysed the anxiety level of different traits of rats by using Elevated Plus Maize and categorized them based on anxiety.

A. **TIME IN DARK FIELD**(Fig-2)

Fig-2

Comment [AO8]: You could have done hematoxylin and Eosin to show the general cyto-architecture of the Hippocampus. Especially the CA 3 region. To help buttress your results. (this may apply to further studies in the future)



ontrol **Lesion control** **Acalamus 15mg** **Acalamus 25mg**
A.calamus 35 mg **B.asarone 10mg** **B.asarone 15mg** **B.asarone 20mg**

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

Figure 2 showing the time spend by the animals in dark field of dark and light box 0n 10th day of lesion

This parameter shows the level of anxiety of the animals. The more the animal spend in the dark field the more the animal feels anxious that indirectly shows the ineffective nature of the drugs. The animal group LC was spending more time in the dark field in comparison with the CO group. The animal groups AC 14 and BA 10 were spending significantly more time in dark field in comparison with CO group and equal with LC group. The drug groups AC 25, AC 35 (df=3,20. F=56) and BA 15 spend significantly more time in comparison with the CO group and less with the LC group and concluded as poor neuroprotective. The drug groups BA 20 (df=3,20. F=51) spends significantly low time in the dark field with LC group, that shows the drug dosage was effective in preventing epilepsy.

B. **TIME IN LIGHT FIELD** (Fig-3)

Fig-3

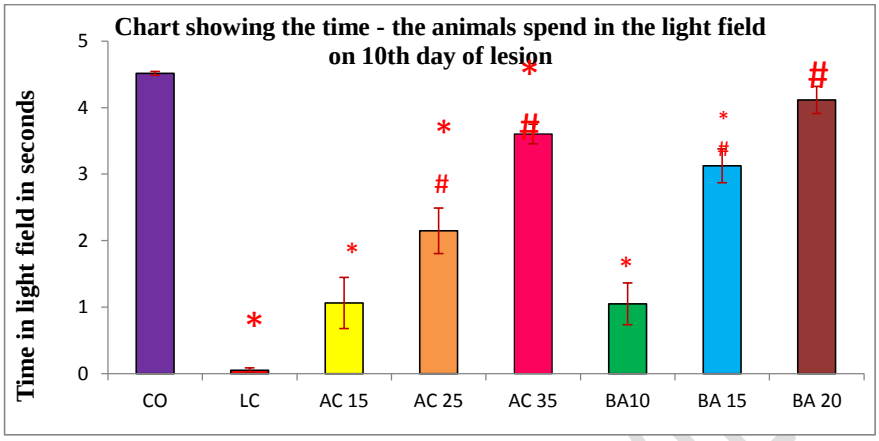


Figure 3 showing the time spend by the animals in light field of dark and light box on 10th day of lesion

The more time the animal spends in the light field shows the low anxiety level of the animal the high neuroprotection of the drug and dosage.

LC group of animals spend most of the time in dark box and so were not ready to spend their time in light field this shows high anxiety in them. The drug groups AC 15 and BC 10 also spend more time in the dark field than light field. The drug groups BC 15 (df=3,20. F=47), AC 25 and AC 35 (df=3,20. F=32) spend significantly more times in light field in comparison with LC group and less significant with the CO group shows poor protection. The animals belongs to BA 20 spend more time in light field in comparison with LC group and was equivalent with the CO animals shows good neuroprotection.

CONCLUSION

Acoruscalamus is commonly known as sweet flag in India. *Acorus calamus* is a drug of choice for epilepsy, promotes intellect in children, memory and used to boost up the activities of brain in the form of brain tonic.Esfandiari proved in his study that different fractions of *Acorus*

calamus are effectivein preventing stress development neuroinflammation. In this present study we also proved that *Acorus calamus* and its principle content, the Beta asarone has effective role in preventing epilepsy and neurodegeneration related anxiety in experimental epileptic rats and this work can be expanded in future for better results.

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