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Nootropic Effect of *Celastrus Paniculatus* Seed Extract on Phenytoin Induced Cognition Impairment in Male Albino Mice

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Abstract

Background: Several studies have reported that cognitive impairment leads to flawed spatial learning and impaired working memory and an enhanced anxiety-like behaviour. *Celastrus paniculatus*(CP), a conventional ayurvedic medicine, has been used to manage cognitive deficits in mentally disabled children. CP oil has also reported to possess neuroprotective and antioxidant properties. However, there are no sufficient studies that have analyzed the ways of reversing phenytoin-induced deficits. In this study, we intend to analyze the nootropic effects of Celastrus Paniculatus oil on phenytoin induced cognitive impairment.

Methods: This study was conducted at animal house by department of Pharmacology, Dhanalakshmi Srinivasan Medical College and hospital during the month of November 2016 to October 2019. A total of 72 male albino mice after acclimatization were divided into 12 groups. Group 1 assigned as control. Group II, III and IV received phenytoin in doses 8mg/kg, 12mg/kg and 22mg/kg respectively. Groups V, VI and VII received piracetam in doses 125mg/kg, 250mg/kg and 500mg/kg respectively. Groups VIII, IX and X received celastrus paniculatus oil in doses 100mg/kg, 200mg/kg and 400mg/kg respectively. Group XI received Phenytoin (12 mg/kg) and Piracetam (250 mg/kg) Group XII received Phenytoin (12 mg/kg) and Celastrus paniculatus (200 mg/kg). Both acute and chronic studies were done using radial arm maze test and cook's pole climbing apparatus. Data was entered in Microsoft excel and data analysis was done using SPSS version 17.

Results: Animals treated with phenytoin showed decreased number of right entries, increased number of re-entries, increased time in completing radial maze test and an increased number of escape response in pole climbing apparatus compared to normal animals. In contrast, CP oil treated mice showed improved performance in both radial maze test and pole climbing apparatus.

Conclusion: Long term treatment with CP oil showed enhanced cognitive abilities in phenytoin induced cognitive impaired mice. This study provides a new outlook on useful effects of chronic therapy of celastrus paniculatus oil on phenytoin induced cognitive dysfunctions.

Keywords:Celastrus paniculatus, phenytoin, radial arm maze test, pole climbing apparatus.

INTRODUCTION

The word 'nootropic' originates from the Greek term nóos meaning 'mind', and tropé meaning 'to bend or turn'. Romanian psychologist Dr. Corneliu E. Giurgea, first coined this term while investigating a brain-enhancing compound called piracetam. Nootropics are classified under a new group of psychotropic agents which have selective stimulatory effect on the central nervous system, particularly on intellectual

performance, learning ability and memory. [1]

Previously medicinal plants were used in the treatment of cognitive disorder, insomnia, and epilepsy. One popular plant is Celastrus paniculatus Wild. (Celastraceae), is well known for its ability to enhance memory. Ayurveda, the conventional traditional system of medicine, has utilized this plant extract for prevention and management of various diseases

Various studies elaborate that the oil obtained from the seeds of C. paniculatus possesses sedative and anticonvulsant properties. Analgesic property and anti-inflammatory effect of a C. paniculatus seed extract has been reported in rodents. [2] C. paniculatus seed has been known to improve memory function beneficial to psychiatric patients, and improve the intelligence quotient (IQ) of mentally disabled children. [3]

More recently, animals treated with C. paniculatus seed oil for 15 days elicited a significant fall in the levels of noradrenaline, dopamine, serotonin and their respective metabolites in brain and urine. Chronic treatment with C. paniculatus seed oil overturned scopolamine-induced memory deficit. A methanol extract of C. paniculatus seed oil elicited free radical scavenging effects. [10,11]

The 8 radial arm maze was introduced in 1976 to study hippocampal dependent learning and memory by Olten and Samuelson, 1976. The radial arm maze test has been used in behavioral neuroscience and pharmacology. Thus, RAM tests are helpful in evaluating the effect of drugs on stress and other environmental factors like learning and memory [5][6][7]. Reference and working memory are the two important variables that report the physiological status of the brain. Cooks Pole Climbing Apparatus is used to study cognitive function, which is a response to conditioned stimuli while learning and its retention. The current study was done to assess the nootropic property of celastrus paniculatus on phenytoin induced cognition impairment in albino rats. [1]

Objectives:

To evaluate the cognition enhancement property of *Celastrus paniculatus* seed oil on phenytoin induced cognition impairment in male albino mice.

Methods:

This study was conducted at animal house by department of Pharmacology, Dhanalakshmi Srinivasan Medical College and hospital during the month of November 2016 to October 2019.

Chemicals:

The inducer phenytoin (Dilantin) suspension product was purchased from Pfizer Inc. The standard piracetam (Nootropil syrup) product was purchased from UCB laboratories. The other chemicals used in the study were of analytical grade which were purchased partly from SRL Diagnostics, Chennai and the remaining from Department of Biochemistry, Dhanalakshmi Srinivasan Medical College, Perambalur.

Test drug:

The Celastrus paniculatus seed oil (Pure Malkangani oil) 50 ml bottle was bought from Deve Herbes pharmaceuticals, New Delhi. It was emulsified with 1% Tween-20 (solubilising agent) and Dimethyl sulphoxide (solvent).

Experimental animals:

Male Swiss albino mice weighing 30-45 grams were obtained from King's Institute, Guindy, Chennai. All the animals were received in the quarantine facility at Animal House, DSMCH, Perambalur and acclimatized for a week in clean polypropylene cages and maintained under standard environmental conditions (12 h dark/ 12 h light cycles; Temp 25±2ºC; Humidity 50-55%; air ventilation). The animals were fed on standard pellet diet (King's Institute, Guindy) and water *ad libitum*.

The experimental procedure was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institutional Animal Ethics Committee (IAEC/DSMCH/21) dated 19/10/2016.

Experimental design:

The animals to be evaluated were trained for 5 minutes a day for 3 days with interval led trials for each experimental methods and were allowed free access to standard pellet diet and tap water.

A total of 72 animals were divided into 12 groups with six animals in each group.

- Group I served as control.

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- Group II, III, and IV received Phenytoin (inducer) in dose of 8mg/kg, 12mg/kg and 22 mg/kg.
- Group V, VI, and VII received Piracetam (standard) in dose of 125mg/kg, 250mg/kg, and 500mg/kg.
- Group VIII, IX, and X received Celastrus paniculatus oil (test drug) in the dose of 100mg/kg, 200mg/kg and 400 mg/kg.
- Group XI received Phenytoin (12 mg/kg) and Piracetam (250 mg/kg)
- Group XII received Phenytoin (12 mg/kg) and Celastrus paniculatus (200 mg/kg).

Acute study:

On the 1st day the drugs were given orally 1hr before observation which was followed by experimental methods and findings were validated accordingly.

Chronic study:

This study starts on the 2nd day after the acute study and drugs Phenytoin, Piracetam and Celastrus paniculatus was given daily in the dose which is mentioned above and was allowed for free access to a standard pellet diet and tap water, if any restriction of diet is needed will be followed.The drugs were given till 22nd day, for a period of 21days and the various methods were performed. All observations were made on the day 22.

Experimental procedure:

1) Radial arm maze apparatus test:

The eight-arm radial maze apparatus for mice consisted of an equally spaced arms (30 x 6 x 15 cm) originating from an octagonal central platform**.** Two types of memory assessed are reference memory and working memory. The animals were kept in restricted diet to maintain a bodyweight of 85%. The animals are subjected to trials individually for 5 minutes per day for two days to explore the maze. Before the commencement of the behavioral assessment, all groups of mice were semi-starved over a period of 48 hrs in order to motivate them towards food reward to acclimatize the mice.

Partially baited task: Four arms were baited and the mice were trained to enter only the baited arms. This task helps us to assess the reference memory and working memory components of spatial memory.

Animals entering an unbaited arm was regarded as a reference memory error and any re-entry either to an unbaited or baited arm was regarded as working memory error. The maze should be cleaned with 70% ethanol and the arms (2, 3, 6 and 8) were then baited with food reinforcement. The mice were placed in the centre of the octagon. An arm choice was noted when a mice eats a bait or got to the end of an arm.

The arms were not baited again, therefore the first entry to the baited arm only was recorded as a correct choice. The procedure continued until the mice entered all four baited arms or elapse of five minutes. After the completion of trial, the second trial was initiated after an inter-trial interval of l hour. Training was done till the mice attained the eligibility criteria of 80% correct choice

Data taken from 4 trials was recorded and represented as blocks and then analyzed for number of correct choices along with reference and working memory errors. Percent correct choice was determined by dividing number of correct entries by total number of entries and then multiplying by 100. An entry into an unbaited arm was regarded as reference memory error (RME) and any re-entry was regarded as a working memory error (WME).

2) Cook's pole climbing apparatus

This Apparatus is used to study the cognitive function, which is a response to conditioned stimuli during learning and its retention. The apparatus consists of an experimental chamber (25 × 25 × 25 cm) with the floor grid in a sound proof environment

Scrambled shock (6mA) is administered to the grid floor composed of stainless-steel rods. A poll of 2.5 cm in diameter, hangs in the chamber with the help of a hole in the upper center portion of the chamber.The study mouse was kept in the chamber and allowed to explore for 45 seconds. Conditioned stimulus (CS) i.e., buzzer signal was switched on and unconditioned stimulus (US) i.e. electric shock given through the grid floor for 2 Seconds.

Animals learned to coordinate the buzzer with the imminent foot shock and was able to avoid the foot shock by climbing the pole after hearing the buzzer

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signal. Avoidance response will be defined as climbing reaction time 10 Seconds.

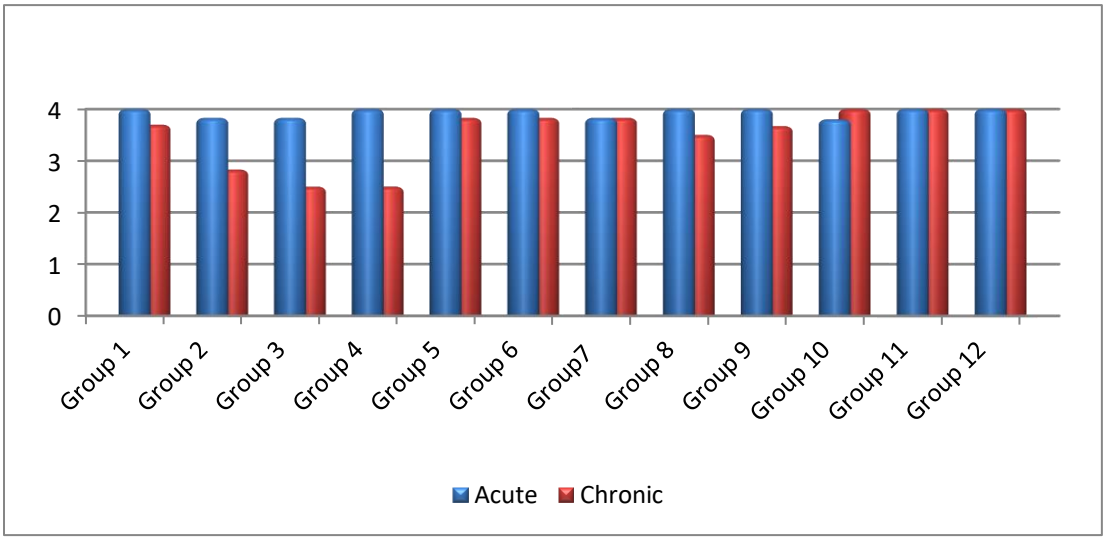
Every mouse was subjected to maximum 5 trials on 1st day, and 24 hrs later, mouse was subjected to relearning trials (2nd day 3 trials and on 3rd day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and Escape response (ER). Animals were screened by using this model and those which showed at least one escape response on day 1 or 2 were included in the study. The values were observed accordingly

Statistical analysis:

Data were analysed for statistical significance using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test and results were expressed as mean ± standard deviation (SD) using SPSS software.

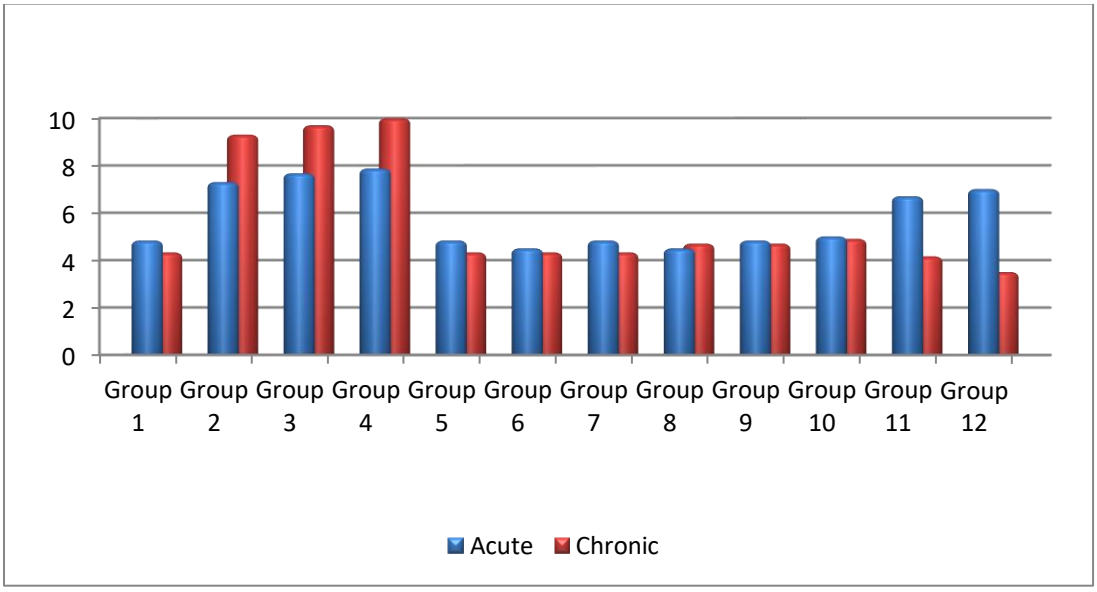
RESULTS:

GRAPH 1: COMPARISON OF RIGHT ENTRIES IN RADIAL MAZE



In group II, III, IV chronic administration of phenytoin significantly reduced the right entries. In group XI & XII the right entries have improved compared to phenytoin group (group III)

GRAPH 2: COMPARISON OF WRONG ENTRIES IN RADIAL MAZE TEST



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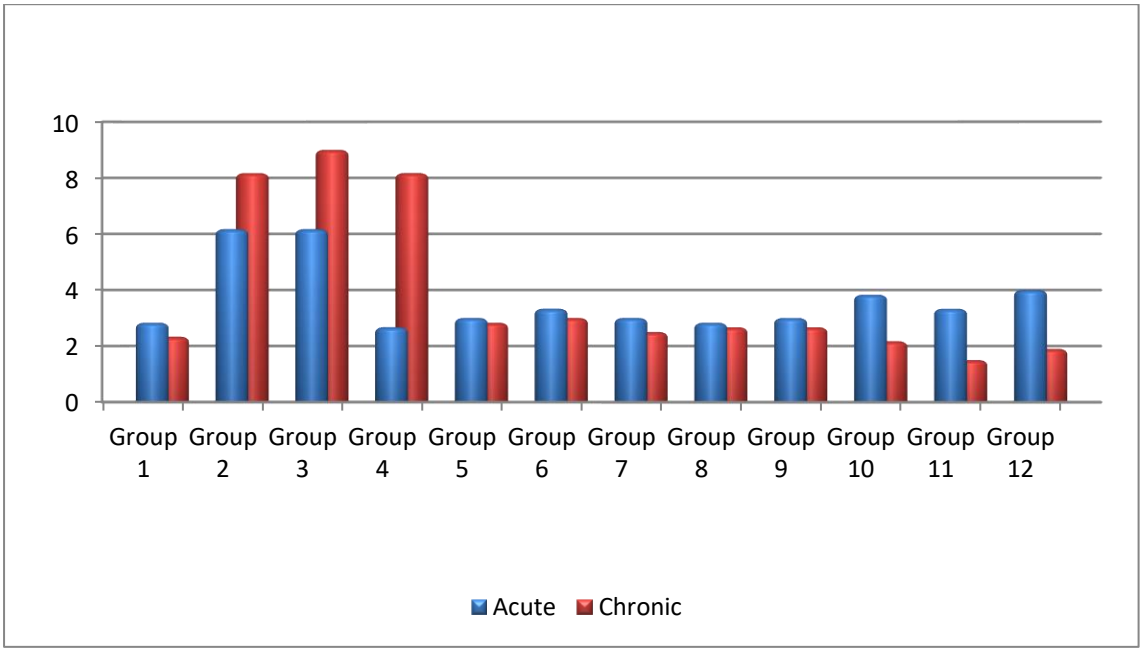
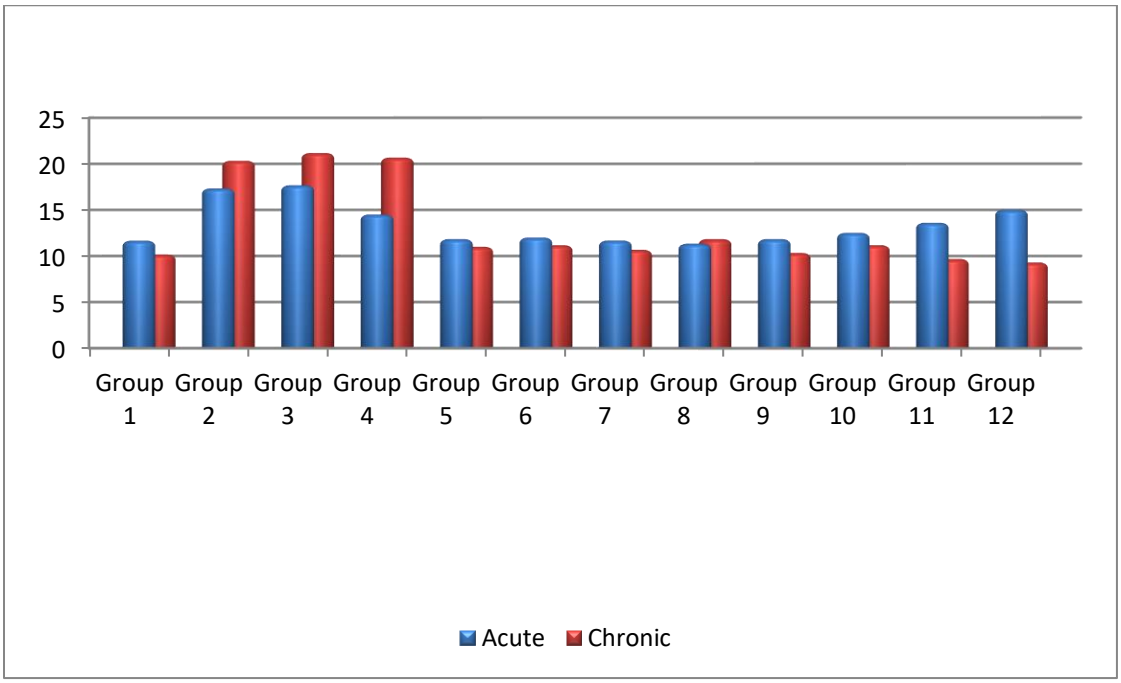
In group II, III, IV chronic administration of phenytoin significantly increased the wrong entries. In group XI and XII there is a reduction in wrong entries compared to phenytoin group (group III)

GRAPH 3: RE-ENTRY IN RADIAL MAZE TEST



In group II, III, IV chronic administration of phenytoin significantly Increased the Re-entries. In group XI & XII the re-entries are reduced compared to phenytoin group (group III)

GRAPH 4: TOTAL ENTRIES IN RADIAL MAZE TEST



In group II, III, IV chronic administration of phenytoin significantly increases total entries. In group XI & XII there is a decrease in total entries compared to phenytoin group (group III)

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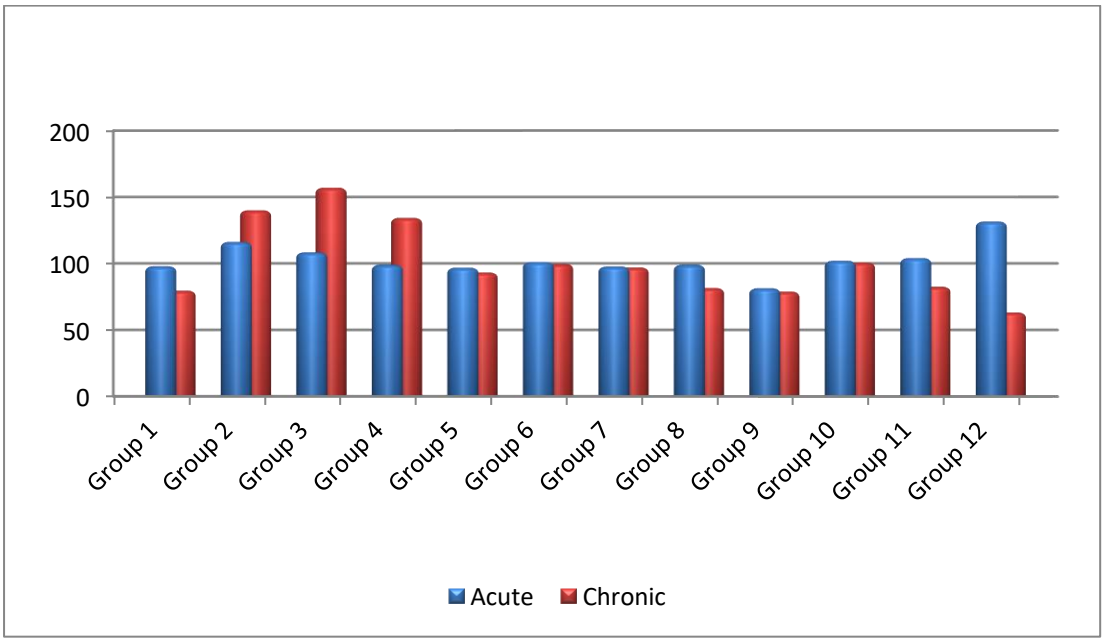
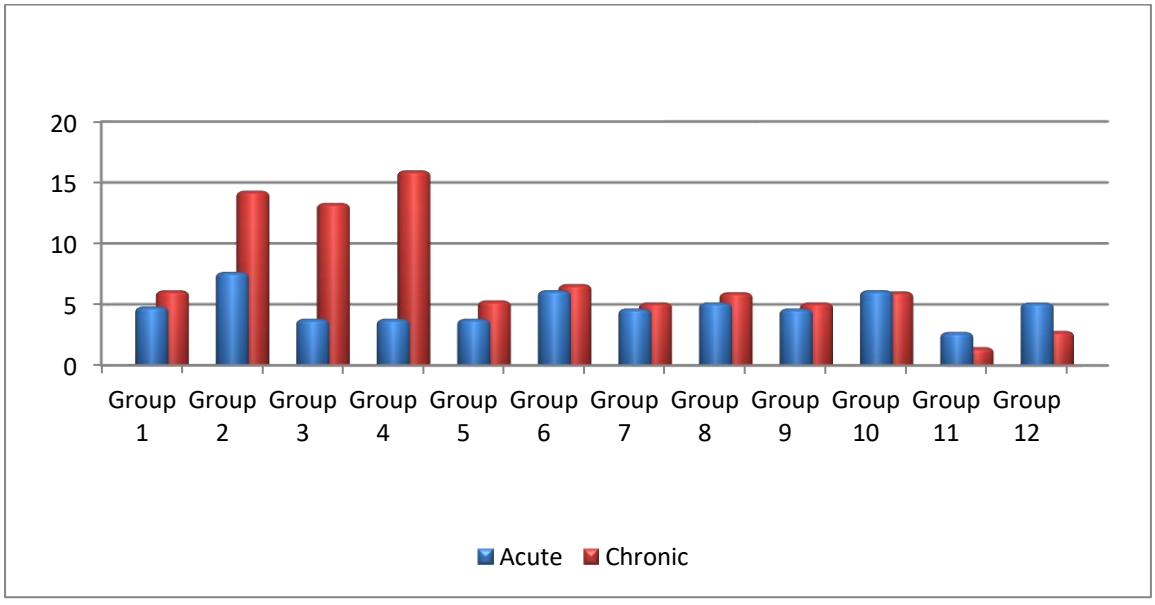
GRAPH 5: TIME TAKEN TO COMPLETE A RADIAL MAZE TEST



In group II, III, IV chronic administration of phenytoin in significantly increases the time taken to complete the radial arm maze. Time taken is decreased in group XI and XII compared to phenytoin group (group III).

GRAPH 6: LATENCY TIME IN POLE CLIMBING APPARATUS TEST



In group II, III, IV chronic administration of phenytoin significantly raised the latency time. Latency time is reduced in group XI and XII compared to phenytoin group (group III)

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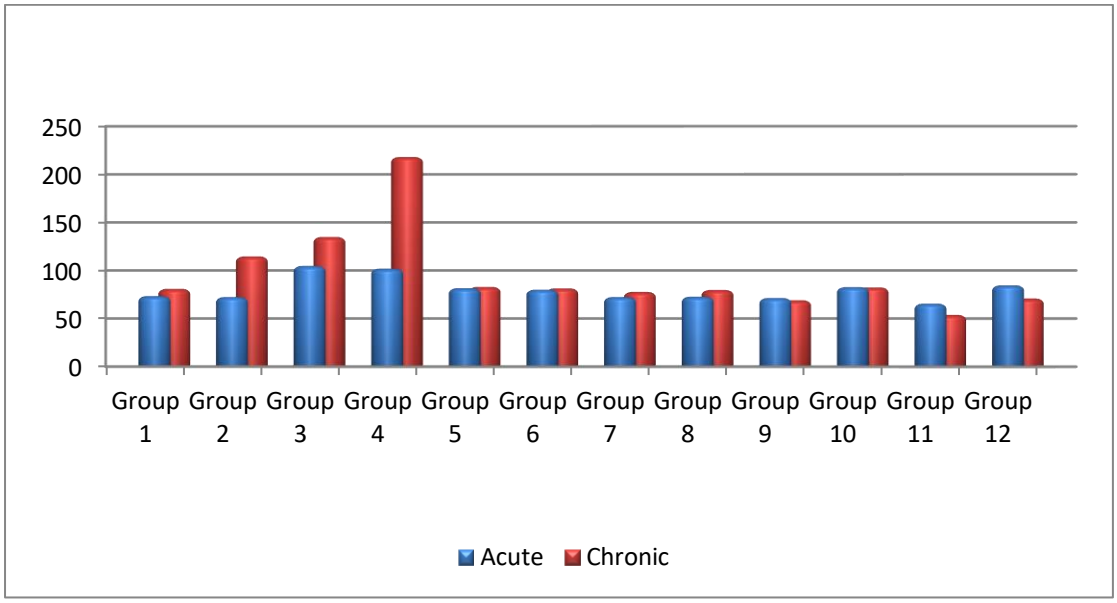
GRAPH 7: DURATION OF REACTION TIME IN POLE CLIMBING APPARATUS



In group II, III, IV chronic administration of phenytoin significantly raised the duration.The reaction time has significantly reduced in group XI and XII compared to phenytoin group (group III).

TABLE 1: CONDITIONED AVOIDANCE RESPONSE (CAR) / ESCAPE RESPONSE (ER) IN POLE CLIMBING APPPARATUS

GROUP	ACUTE	CHRONIC
I	3-ER/3-CAR	3ER/3CAR
II	4ER/2CAR	6ER
III	4ER/2CAR	5ER/1CAR
IV	3ER/3CAR	5ER/1CAR
V	3ER/3CAR	3ER/3CAR
VI	3ER/3CAR	3ER/3CAR
VII	3ER/3CAR	4ER/2CAR
VIII	3ER/3CAR	4ER/2CAR
IX	2ER/2CAR	3ER/3CAR
X	3ER/3CAR	3ER/3CAR
XI	6ER	4ER/2CAR
XII	3ER/3CAR	3ER/2CAR

In group I, IX, X is almost equal (i.e) In group IX, X signifies result similar to the control group- 3ER/3 CAR. And in group XII is almost similar 3 ER/2CAR.

Discussion:

In the present study we found out that administration of celastrus paniculatus along with phenytoin has

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improved the right entries in radial maze test when compared to phenytoin group alone. In a study done by Bhagya et al[(4)], it was observed that chronic daily treatment of Celastrus paniculatus oil for 14 days produced a significant increase in percentage correct choices i.e. right entries, a finding similar to our study.

In this study, it was observed that mice treated with celastrus paniculatus oil showed reduction in wrong entries in compared to piracetam and also a significant reduction compared to phenytoin. Vishnu P. V et al[(8)] found that malkangani extract exhibited statistically significant enhancement in cognitive ability when compared to piracetam. C. paniculatus showed the least number of wrong entries and maximum reduction in the number of wrong entries throughout treatment.

C.paniculatus seed oil exhibits a significant rise in spatial learning and spatial working memory, which are crucial parameters for cognitive analysis. Interestingly, chronic treatment with Celastrus Paniculatus oil for 14 days overturned spatial learning and memory impairment induced by stress in RAM task elicited by enhanced percentage correct choices and decreased RMEs. Working memory is also restored to normal after Celastrus Paniculatus oil treatment.

The Cooks pole climbing apparatus, using the conditioned avoidance responses, assesses the ability to acquire, retain, and retrieve the learned responses from memory. The word "passive avoidance" or "conditioned avoidance response" is used to describe experiments in which an animal learns to avoid a painful event by suppressing a particular behavior.

Learning the memory involve mechanisms like acquisition, storage, consolidation and recall. The data of the present experiments suggest that the drug induced changes could be interpreted as modification in the retrieval or recall phenomenon. The capability of an animal to identify the conditioned stimuli (buzzer) followed by unconditioned stimulus (shock) shows recall of task which explains long term memory. [(9)]

In our study we observed that the antiepileptic agent phenytoin clearly influenced recall or retrieval, in that the animals waited for the unconditioned stimulus (shock) to climb the pole. The escape response was more than conditioned avoidance response. Thus, phenytoin presumably influenced the memory process. Groups XI and XII exhibited more conditioned avoidance response and fewer escape responses were seen, thus indicating improved memory and cognition behavior in response to administration of celastrus paniculatus.

Conclusion:

Chronic treatment with CP oil showed enhanced cognitive abilities in phenytoin induced cognitive impaired mice. This study provides a new outlook on useful effects of chronic therapy of celastrus paniculatus oil on phenytoin induced cognitive dysfunctions.

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