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EVALUATION OF NOOTROPIC ACTIVITY OF HYDROXYCITRIC ACID IN PHENYTOIN TREATED RATS

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ABSTRACT: The present study investigates neuroprotective effect of hydroxy citric acid (50%) on phenytoin induced cognitive impairment and oxidative stress in rats.Male Wistar rats were divided into 6 groups and each group received vehicle, phenytoin (25mg/kg, i.p) and hydroxy citric acid (400 and 800mg/kg, orally) for 14 days respectively. Memory and learning was evaluated by using the Rectangular maze, Morris water maze and Locomotor activity were evaluated. On day 14, animals were sacrificed, brain tissue was dissected. The Acetylcholine Esterase (AChE) levels, antioxidants levels were estimated in the brain homogenate.The histopathological studies were done to check for the gross changes in histology. Phenytoin (25mg/kg, i.p) produced significant deficits in learning and memory as indicated by significant increase transfer latency in rectangular maze, Morris water maze and decrease number of counts in Actophotometer. It also produced significant elevation in brain AChE, MDA levels along with reduction in Catalase and DPPH levels. Hyrdroxy citric acid (400 and 800mg/kg) orally, when co-administered with phenytoin, significantly prevented phenytoin induced cognitive impairment, oxidative stress and improved the histopathological abnormalities in a dose dependant manner.

INTRODUCTION: Dementia, which is the most common cause of cognitive impairment, is defined as significant memory impairment; loss of intellectual functions which interfere with patient's working capabilities and usual social activities or relationship with others [1]. Dementia is a multisystem related neurodegenerative disorder.

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The essential feature of dementia is impairment in short and long-term memory, associated with impairment in abstract thinking, impaired judgment, other disturbances of higher cortical function and personality change [2, 3] .

Cognitive deficit is the one of the main problem associated with epilepsy [4]. Epilepsy is a chronic disorder characterized by recurrent seizures, which varies from a brief lapse of attention or muscle jerks, to severe and prolonged convulsions. The seizures are caused by sudden, excessive electrical discharges in a certain areas of brain cells. Phenytoin is a most common and effective anti - epileptic drug prescribed for a prolonged period to

achieve seizure control in all types of partial and tonic – clonic seizures [5]. In a normal cell there is balance between formation and removal of free radicals. However this balance can be shifted towards more formation of free radicals or when levels of antioxidants are diminished leading to 'oxidative stress. The oxidative stress can result is serious cell damage if the stress is massive and prolonged and it can play major role in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [6].

Phenytoin is enzymatically bioactivated to a reactive intermediate leading to increased formation of reactive oxygen species (ROS), which damages essential macromolecules such as DNA [7]. (-)-Hydroxycitric acid (HCA) is purported to be one of the active components in various over the counter weight loss formulations, appetite suppressor products. It is an active ingredient extracted from the rind of the Indian fruit *Garcinia cambogia* (Clusiaceae).

The fruit rinds of *Garcinia cambogia*, commonly known as brindal berry, kodampuli have been used in the Indian systems of the medicine for hundreds of years for their hypolipidemic effect. The fruit rinds of *Garcinia cambogia* mentioned in Sidha, have been used to treat central nervous system disorders. Some *Garcinia* species have been used commercially in the food industry to prevent or delay spoilage of foods.

Extracts of the *Garcinia* genus such as *Garcinia mangostana* and *Garcinia kola* usually contain hydroxyl citric acid as main active component. These extracts exhibit diverse biological activities such as anti-HIV, antimicrobial, antioxidant and anti-inflammatory activities [18]. Another genus available in Malaysia, *Garcinia atroviridis* or commonly known as 'asam gelugor' by the Malays were used as a condiment in Southeast Asia cuisine. Recent studies show that, the extracts of the fruit rinds of *Garcinia cambogia* contain hepatoprotective compounds [8].

In view of these, the present study was planned to investigate the antioxidant activity and anticholinesterase activity of hydroxycitric acid.

MATERIALS AND METHODS:

Drugs and Chemicals: Phenytoin (gift sample from Aurobindo Pharma, Hyderabad, India), 50%*Hydroxy citric acid* (pure powder) and chemicals like Thiobarbituric acid, Acetylcholinethioiodide, Trichloroacetic acid, DPPH (1, 1-diphenyl-2-picrylhydrazyl) reagent, DTNB (5, 5-Dithiobis (2-nitrobenzoic acid) reagent (Himedia India Ltd) and Hydrogen peroxide (SD Fine Chemicals Ltd) and Formalin 10% (SD Fine chemicals Ltd).

Animals: Pathogen free adult male albino rats weighing 200-250 gm were used. Male rats were chosen in order to avoid fluctuations due to oestrous cycle. The rats were housed in polypropylene cages, maintained at (24 ± 1°C) with relative humidity 45-55%, 12/12 hours light and dark cycle. The animals were fed with a balanced diet (standard chew pellets) and tap water *ad libitum*. The study protocol was approved by Institutional Animal Ethical Committee of Vaagdevi College of pharmacy, Hanamkonda, Warangal Register No. (IAEC NO: 1047/ac/07/CPCSEA). Animals (30) were weighed and kept in cages accordingly and randomly divided in to 6 groups (n=6). Drugs were prepared freshly and given daily for 14 days. On day 1, the training sessions for all the animals were given.

Acute Toxicity Test: Acute toxicity of *Garcinia* was carried out as per the OECD guideline 425. A limit test was performed using healthy albino mice of either sex (25-30g) maintained under standard dietary conditions. Prior to dosing, animals were fasted for 3-4 hours and the dose for each animal was determined based on body weight. Initially *Garcinia* was administered to one animal in a single dose of 2000 mg/kg by using an oral gavage. After the administration, food was withheld for a further 1-2 hours, then the animals were observed for first 24 hrs and further 14 days for any signs of behavioural changes, toxicity, changes in body weight and mortality [9].

Drug Study Protocol: The animals were divided in to 6 groups, each group contain 6 animals. Group-1 served as control and received vehicle only, Group- 2 received phenytoin (25mg/kg i.p) for 14 days, Group -3 received 50% Hydroxy citric

acid alone (400mg/kg p.o) for 14 days, Group - 4 received 50% Hydroxy citric acid alone (800mg/kg p.o) for 14 days, Group - 5 received phenytoin (25mg/kg i.p) and 50% Hydroxy citric acid (400mg/kg p.o) for 14 days, Group-6 received phenytoin (25mg/kg i.p) and Hydroxy citric acid (800mg/kg p.o) for 14 days.

Rectangular Maze Test: Assessment of learning and memory can be effectively done by this method. The maze consists of completely enclosed rectangular box with an entry and reward chamber appended at opposite ends. The box is partitioned with wooden slots into blind passages leaving just twisting corridor leading from the entry to the reward chamber. Animals were trained prior to the experiment by familiarizing with the rectangular maze for a period of 10 min for 2 h. Well-trained animals were taken for the experiment. Transfer latency (time taken to reach the reward chamber) was recorded. For each animal, four readings were taken and the average is taken as learning score (transfer latency) for that animal. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. The time taken by the animals to reach the reward chamber from the entry chamber was recorded on day 1, 3, 5, 7, and 9 [10].

Locomotor activity test: In order to detect the association of decreased activity in Actophotometer with changes in motor activity, the locomotor activity was recorded for a period of 5 min using actophotometer on 1th, 3th, 5th, 7th and 9th day. Each animal was observed in a square (30 × 30 cm) closed arena equipped with infrared light sensitive photocells using digital Actophotometer and locomotor activity was then expressed in terms of total photo beam counts for 5 min per animal.

Animals were placed individually in the activity chamber for a 3 min habituation period before actual start of activity tasks. The apparatus was placed in a dark, light, sound attenuated and ventilated testing room; the activity is carried out after 30min of the drug administration. Increase in count was regarded as central nervous system stimulant activity. Decrease in count was regarded as central nervous system depressant activity [12].

Morris water maze test: Method was carried out in a circular pool (90cm in diameter and 50cm in height) of water with a featureless inner surface [11]. The first day of experiment was dedicated to swimming training for 60 sec in the absence of platform. During the 4 consecutive days the rats were given the trial session with the platform in place. Once the rat located the platform, it was permitted to remain on it for 10 sec. If the rat did not locate the platform with in 120 sec, it was placed on the platform for 10 sec and then removed from the pool. One day after the final training trial sessions (on day5), rats were individually subjected to a probe trial session in which the platform was removed from the pool, and rats were allowed to swim for 20 sec to search for it and the latency time was determined [11, 13].

***In - vitro* Methods:** On day 14th following the behavioural testing, animals were sacrificed and the brain tissues were quickly removed cleaned with ice-cold saline and stored at -20°C for biochemical estimation.

1. **Preparation of Brain Homogenate:** Brain tissue samples were thawed and homogenized 10 times (w/v) with ice cold 0.1M phosphate buffer (pH 7.4). Aliquots of homogenates from rat brains were separated and were centrifuged at 3,000 rpm for 30 min and the supernatant was then used for biochemical estimation[14].

Biochemical tests:

1. **Acetylcholine Esterase (AchE) Estimation:** The cholinergic marker, acetylcholinesterase, was estimated in the whole brain according to the method of Ellman method. Ellman's reagent is 5, 5-dithiobis (2-nitrobenzoate) and it is also abbreviated as DTNB. Brain tissue homogenate was incubated for 5 min with 2.7ml of phosphate buffer and 0.1ml of DTNB. Then, 0.1ml of freshly prepared acetylthiocholine iodide (pH 8) substrate was added. The resulting yellow colour was due to the reduction of DTNB by certain substances in brain homogenate and due to non-enzymatic hydrolysis of substrate. The absorbance was read at 412nm. Acetyl cholinesterase concentration was spectro-photometrically determined [15].

2. **DPPH radical scavenging assay:** The free radical scavenging activity of the test drug was measured in vitro by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. About 0.3mM solution of DPPH was dissolved in 100ml ethanol and 1ml of this solution was added to 3ml of brain tissue homogenate supernatant was dissolved in ethanol. The mixture was shaken and allowed to keep at room temperature for 30 min and the absorbance was measured at 517nm using a spectrophotometer. The percentage of scavenging activity was determined using the formula given below[12].

Radical scavenging activity (%) = $[(A_0 – A_1 / A_0) \times 100]$

Where A_0 = absorbance of the control (blank, without compound); A_1 = Absorbance of compound.

3. **Catalase activity:** Hydrogen peroxide (H_2O_2) solution (2mM/L) was prepared with standard phosphate buffer (pH 7.4). The brain tissue homogenate supernatant was added to 0.6ml of H_2O_2 solution. Absorbance was determined at 230nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity was determined using the formula given below [16, 17].

Percentage scavenging of hydrogen peroxide = $(A_0 - A_1 / A_0) \times 100$

Here, A_0 = absorbance of the control, A_1 = absorbance of the presence of samples.

4. **MDA Levels Estimation:** To 0.5ml of the tissue homogenate 0.5ml of phosphate buffer was added and then with 1ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 3000rpm at 4°C for 10 min. The supernatants of the tissue homogenates were incubated with 1ml of 0.8% w/v of the thiobarbituric acid at 100°C for 15 min. After a cooling period, TBARS concentration was spectrophotometrically determined at 532nm. The levels of lipid peroxides were expressed as nano moles of TBARS. Standard graph was plotted using 1, 1, 3, 3-Tetra Ethoxy Propane (TEP) [16].

Statistical Analysis: The statistical analysis of data was done by the one way analysis of variance (ANOVA) followed by the Dunnett's test. The probability level less than 0.05 was considered as significant. Results were expressed as mean SD.

RESULTS AND DISCUSSION:

Acute Toxicity Test (OECD guideline no. 425): The LD_{50} value of the hydroxy citric acid was found to be greater than 2000mg/kg of body weight. Animals treated with hydroxy citric acid were free from signs toxicity and no mortality was found up to 2000 mg/kg. Hence two doses 400 and 800mg/kg were selected for present study.

Behavioral tests:

1. **Rectangular maze test:** Phenytoin (25 mg/kg i. p) increased transfer latency (time in seconds) this indicating the signs of cognitive impairment. Hydroxy citric acid at a dose of 400mg/kg and 800 mg/kg of the extract orally administered for 14 days markedly ($P<0.001$) decreased transfer latency time significantly as shown in **Fig. 1**.

2. **Locomotor activity:** Phenytoin (25 mg/kg i. p) decreased spontaneous motor activity (counts/ 10mins) indicating the signs of cognitive impairment. Hydroxy citric acid at a dose of 400mg/kg and 800 mg/kg of the extract orally administered for 14 days markedly ($P<0.001$) increased motor activity significantly as shown in **Fig. 2**.

3. **Morris water maze test:** Phenytoin (25 mg/kg i. p) increased transfer latency (time in seconds) indicates cognitive signs of cognitive impairment. Hydroxy citric acid at a dose of 400mg/kg and 800 mg/kg of the extract orally administered for 14 days markedly ($P<0.001$) decreased transfer latency significantly as shown in **Fig. 3**.

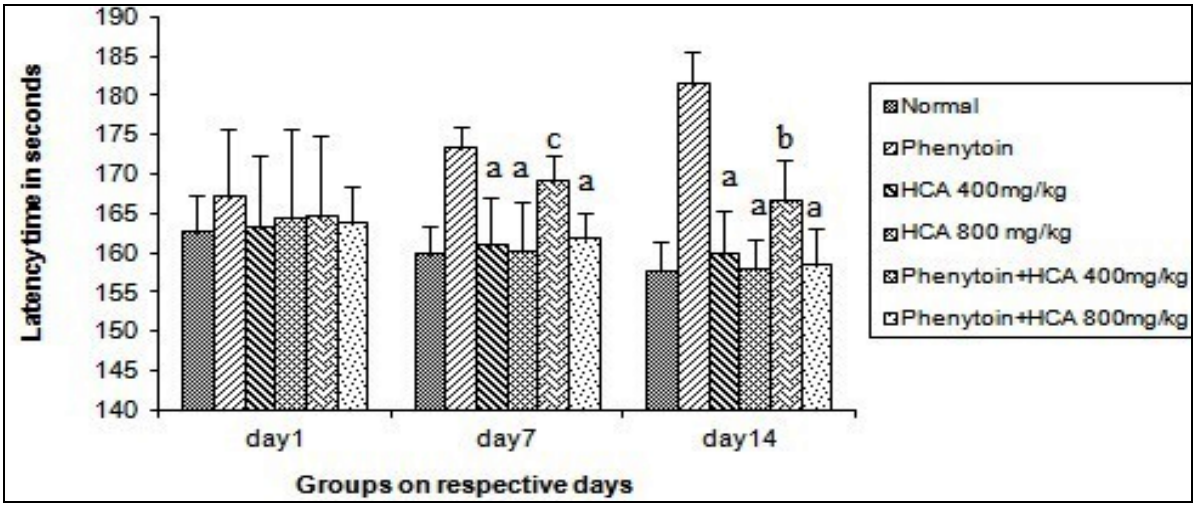

FIG. 1: EFFECT OF PHENYTOIN AND HYDROXY CITRIC ACID ON COGNITIVE FUNCTION. Values were expressed as Mean ± SD of transfer latency time in seconds; c*p<0.05, b**p<0.01, a***p<0.001 as compared to phenytoin treated group. Data was analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons (n=6 in each group).

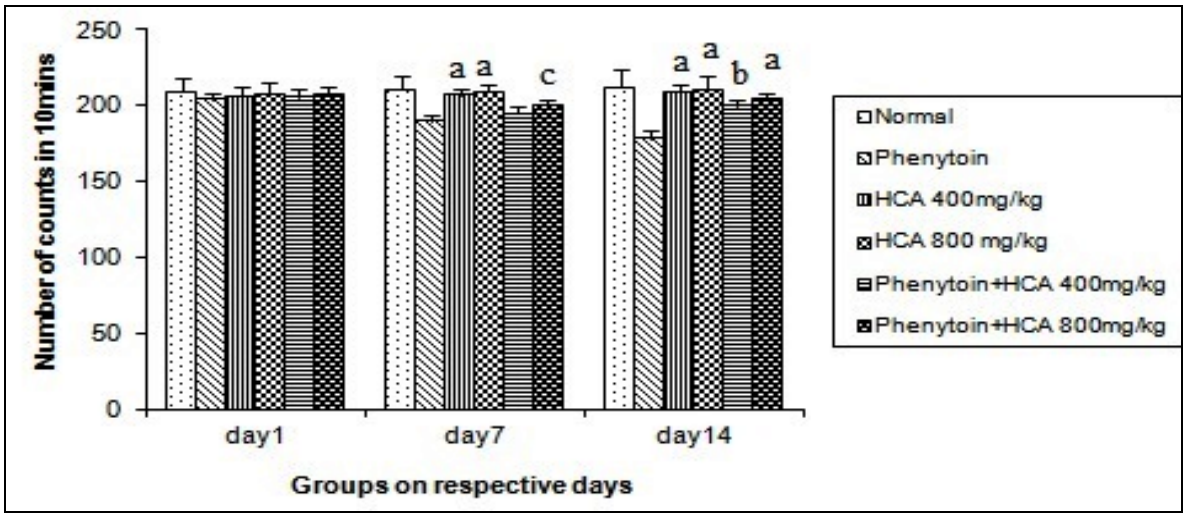

FIG. 2: EFFECT OF PHENYTION AND HYDROXY CITRIC ACID ON LOCOMOTOR ACTIVITY. Values were expressed as Mean ± SD of counts in 10 mins; c*p<0.05, b**p<0.01, a***p<0.001 as compared to phenytoin treated group. Data was analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons (n=6 in each group).

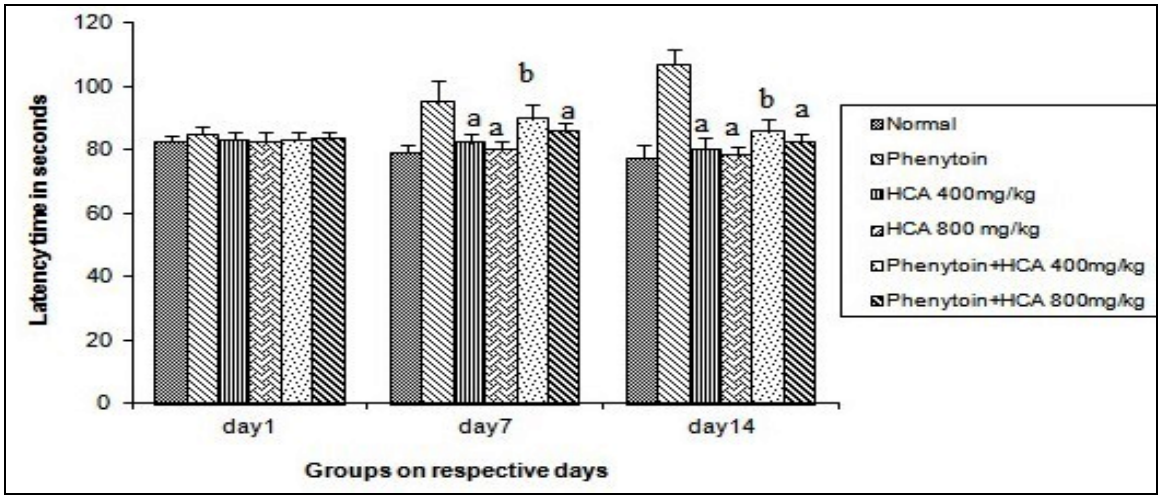

FIG. 3: EFFECT OF PHENYTOIN AND HYDROXY CITRIC ACID ON TRANSFER LATENCY IN MORRIS WATER MAZE. Values were expressed as Mean ± SD of transfer latency time in seconds; b**p<0.01, a***p<0.001 as compared to phenytoin treated group. Data was analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons (n=6 in each group).

Biochemical tests:

1. **DPPH Assay:** Antioxidant activity has been expressed in percentage inhibition of DPPH activity. Phenytoin treatment decreased DPPH activity significantly. Improvement of DPPH activity was observed in test drug treated groups compared to phenytoin treated group ($p<0.001$) as shown in **Fig. 4**.

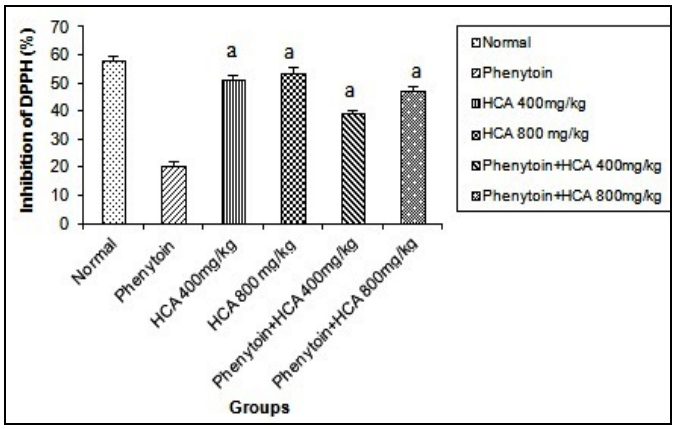

FIG. 4: EFFECT OF PHENYTOIN AND HCA ON DPPH ACTIVITY. Values were expressed as Mean ± SD; a***p<0.001 as compared to phenytoin treated group. Data was analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons (n=6 in each group).

2. **Catalase Activity:** Antioxidant activity has been expressed in percentage H_2O_2 scavenging activity. Phenytoin treatment decreased antioxidant activity significantly. Improvement of antioxidant activity was observed in test drug treated groups compared to phenytoin treated group ($p<0.001$) as shown in **Fig. 5**.

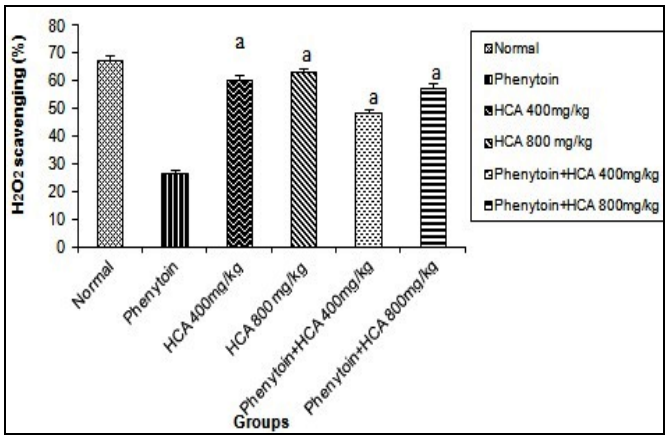

FIG. 5: EFFECT OF PHENYTOIN AND HCA ON CATALASE ACTIVITY. Values were expressed as Mean ± SD;a ***p<0.001 as compared to phenytoin treated group. Data was analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons (n=6 in each group).

3. **Effect of Hydroxy citric acid on brain MDA levels:** Antioxidant activity has been expressed in brain MDA levels (n moles/mg of tissue). Phenytoin treatment increased brain antioxidant activity significantly. Improvement of antioxidant activity was observed in test drug treated groups compared to phenytoin treated group ($p<0.001$) as shown in **Fig. 6**.

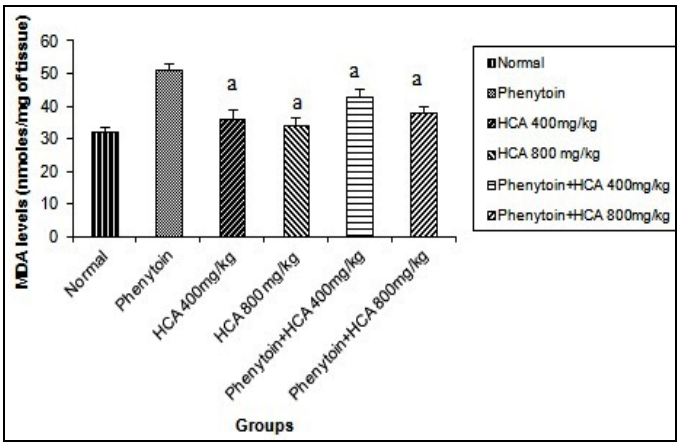

FIG. 6: EFFECT OF PHENYTOIN AND HCA ON RAT BRAIN MDA LEVELS. Values were expressed as Mean ± SD; a***p<0.001 as compared to phenytoin treated group. Data was analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons (n=6 in each group).

4. **AChE Enzyme Estimation (Ellman's Method):** Phenytoin treatment increased brain Acetylcholinesterase (AChE) significantly. Test drug treatment significantly decreased brain AChE levels compared to phenytoin treated group ($p<0.001$) as shown in **Fig. 7**.

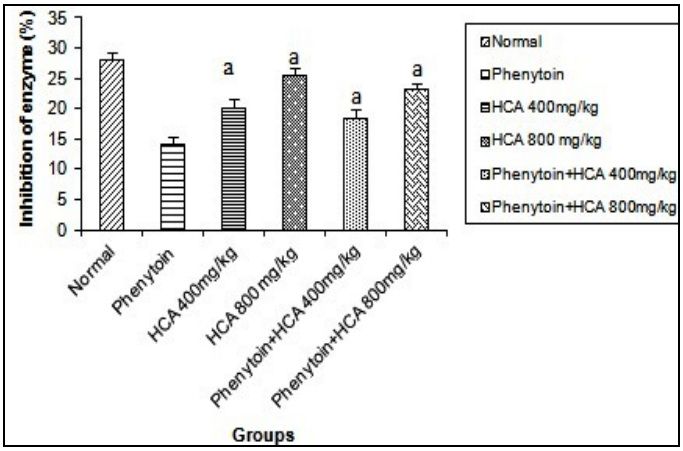

FIG. 7: EFFECT OF PHENYTOIN AND HCA on AChE ACTIVITY. Values were expressed as Mean ± SD; a***p<0.001 as compared to phenytoin treated group. Data was analyzed by one way ANOVA followed by Dunnet's test for multiple comparisons (n=6 in each group).

Histopathological Studies: After 14 days treatment, the brains of different groups were perfusion-fixed with 4% para formaldehyde in 0.1 M phosphate buffer. The brains were removed and post fixed in the same fixative overnight at 48°C. The brains were then routinely embedded in paraffin and stained with Hematoxylin-Eosin (HE). The hippocampal lesions were assessed microscopically a 40X magnification.

The phenytoin (25mg/kg, i.p) treated rats showed damaged cells in the hippocampus. Hydroxy citric acid (400mg/kg, oral) was not that much effective in reversing the phenytoin induced alterations in the brain. Hydroxy citric acid (800mg/kg) was effective in reversing the phenytoin induced damages in rat brain regions as shown in **Fig. 8**.

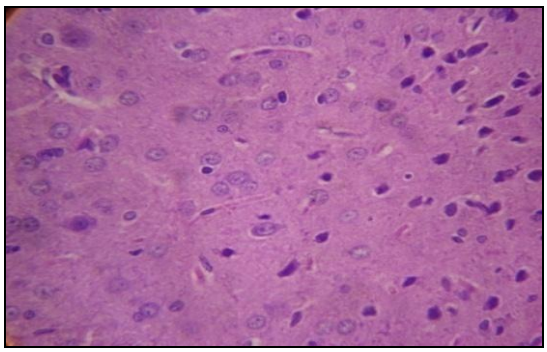

FIG 8.1: NORMAL CONTROL

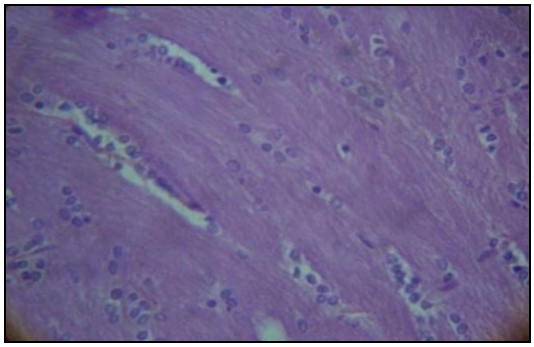

FIG 8.2: PHENYTOIN ALONE (25 mg/kg)

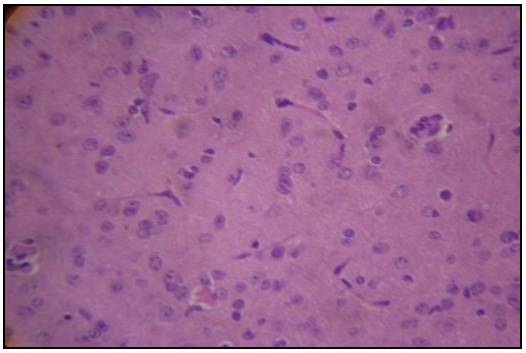

FIG 8.3: HCA (400 mg/kg)

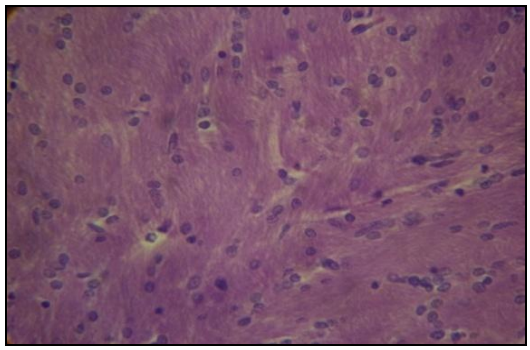

FIG 8.4: PHENYTOIN+ HCA (400 mg/kg)

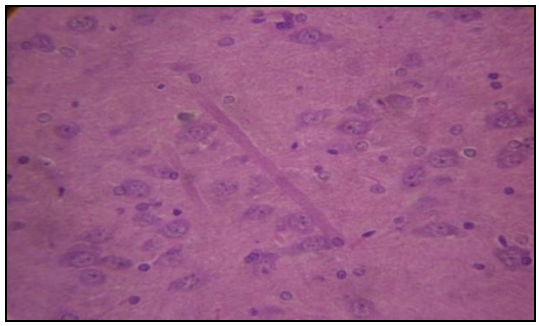

FIG 8.5: PHENYTOIN + HCA (800 mg/kg)

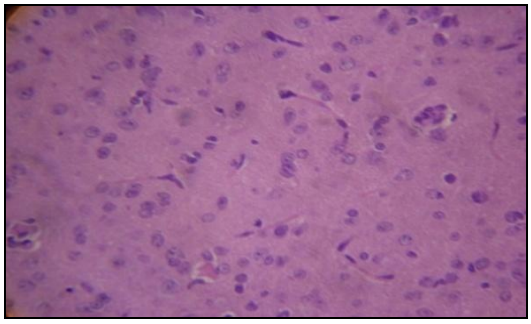

FIG 8.6: HCA ALONE (800 mg/kg)

FIG. 8: HISTOPATHOLOGICAL STUDIES OF PHENYTOIN AND HCA ON RAT BRAIN

The results of the present study show that phenytoin (25mg/kg i.p), when administered for 14 days significantly impaired the memory and spontaneous motor activity. It was also observed that phenytoin significantly decreased the levels of antioxidants like DPPH, Catalase and increased the brain MDA levels. Chronic phenytoin treatment also damaged the brain regions as evidenced by brain histopathological investigations.

Hydroxy citric acid (400 and 800mg/kg) orally, when administered with phenytoin, significantly prevented phenytoin induced cognitive impairment, oxidative stress and improved the histopathological abnormalities in a dose dependant manner.

Cognitive deficit is one of the major problems associated with epilepsy, underlying pathology and drug therapy leads to disturbances in cognitive function [4]. Phenytoin (PHT) is one of the cheapest and widely used anticonvulsants. But, as with many other antiepileptic drugs (Smith, 1991), it is known to adversely affect learning and memory [19, 20]. In the present study memory function was evaluated by Morris water maze and rectangular maze. Phenytoin (25 mg/kg i.p) increased transfer latency (time in seconds) this indicates cognitive impairment.

Hydroxy citric acid at a dose of 400mg/kg and 800 mg/kg of the extract orally administered for 14 days markedly ($P<0.001$) decreased transfer latency and reversed phenytoin induced cognitive impairment. Locomotor activity was evaluated by actophotometer. Phenytoin significantly reduced the spontaneous motor activity that indicates CNS depressant activity of phenytoin. Hydroxy citric acid at a dose of 400mg/kg and 800 mg/kg of the extract markedly ($P<0.001$) increased the spontaneous motor activity in a dose dependant manner. Phenytoin treatment decreased brain DPPH, Catalase activity significantly. Improvement of antioxidant activity was observed in test drug treated groups compared to phenytoin treated group.

The results of the present study also illustrated an increase in oxidative stress in the phenytoin treated rats, as indicated by increase in malondialdehyde (MDA) levels in different regions of brain. MDA is an end product of lipid peroxidation and also is a biochemical marker to measure lipid peroxidation which indicates the extent of neuronal damage in various brain regions [6].

In the present study, phenytoin showed an increased lipid peroxidation in brain. Studies performed to find natural antioxidants indicated that many *Garcinia* species and some of their constituents have shown significant antioxidant activity. Some *Garcinia* species have been used commercially in the food industry to prevent or delay spoilage of foods.

Recent studies showed that, the extracts of the fruit rinds of *Garcinia combogia*, also contain hepatoprotective compounds [5].

Hydroxy citric acid (400 and 800mg/kg, orally), when administered with phenytoin, significantly increased antioxidants levels (DPPH and Catalase) and also decreased brain MDA levels. So it suggests that potential hydroxy citric acid administration prevented cognitive impairment caused by chronic phenytoin therapy.

The histopathological changes in the brain were examined by using Hematoxylin-Eosin (HE) stain to confirm the extent of damage induced by phenytoin.

Brain sections of the phenytoin treated rats showed damaged cells in the hippocampus. Hydroxy citric acid at the dose of 400mg/kg was not effective in reversing the phenytoin induced alterations in the brain. The higher dose of Hydroxy citric acid (800mg/kg) was effective in reversing the phenytoin induced damages in rat brain regions.

CONCLUSION: In conclusion, the present study demonstrates that Hydroxy citric acid reduces the phenytoin induced cognitive deficit and oxidative stress. So it suggests that potential Hydroxy citric acid therapy prevented cognitive impairment caused by chronic phenytoin therapy. It also decreases acetyl cholinesterase (AChE) activity in brain.

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REFERENCES:

1. Marcela GG, Ascension M, Klaus P: Nutrition and cognitive impairment in the elderly: a review. British Journal of Nutrition 2001; 86:313-321.
2. Knopman DS, Dekosky ST, and Cummings JL: Practice parameter: Diagnosis of dementia (an evidence-based review): report of the quality standards subcommittee of the American academy of neurology. Neurology 2001; 56 (9):1143–1153.
3. Hyman BT, Phelps CH, Beach TG: National institute on aging-alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimer's & Dementia 2012; 8 (1):1–13.
4. Vermeulen J, Aldenkamp AP: Cognitive side effects of chronic anti-epileptic drug treatment: a review. Epilepsy Research 1995; 22:65-95.
5. Mc Namara, JO: Pharmacotherapy of the epilepsies, Goodman and Gilman's The pharmacological basis of therapeutics, Mc Graw-Hill, New York, 11 Edition, 2006.

6. Ashok S, Jayashree G, Pankaja N: Effect of free radicals and antioxidants on oxidative stress, A review. Journal of Dental and Allied Sciences 2012; 1 (2):63-66.
7. Saraswathy GR, Maheswari E, Santhrani T: Effect of vitamin C supplementation on phenytoin induced behavioural abnormalities and regional lipid peroxidation in rats. International Journal of Pharmacy and Technology 2011; 3(2):2248-2269.
8. Subashini N, Nagarajan G, Kavimani S: *In-vitro* antioxidant and anticholinesterase activities of *Garcinia cambogia*. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(3):129-132.
9. The Organization for Economic Co-operation Development (OECD). The OECD guideline for Testing of Chemicals 425, adopted 3rd October (2008).
10. Indumathy S, Kavimani S and. Raman K. V: Role of angiotensin antagonists in memory enhancement. International Journal of Pharma and Bio Sciences 2010; 1(3):1-4.
11. Morris R: Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods 1984; 11(1):47–60.
12. Vogel H. G, Schlkens B. A, Sandow J: Drug effects on learning and memory, in Drug Discovery and Evaluation: Pharmacological Assays, Springer, Berlin, Germany, 2nd edition, 2002: 595-643.
13. Manish KS, Prabhakar S, Lal KK and Anand A: Bacopa monniera Attenuates Scopolamine-Induced Impairment of Spatial Memory in Mice. Evidence-Based Complementary and Alternative Medicine 2011; 1-10.
14. Agarwal R, Tyagi E, Saxena G and Chandishwar N: Cholinergic Influence on Memory Stages: A Study on Scopolamine Amnesic Mice. Indian Journal of. Pharmacology 2009; 41 (4):192-196.
15. Abhinav K, Jogender M, Madhusudana K, Naidu VGM, Gupta YK, Ramakrishna S: Anti-amnesic activity of Vitex negundo in scopolamine induced amnesia in rats. Journal of Pharmacology & Pharmacy 2010; 1(1): 1–8.
16. Kaur I.P and Geetha T: Screening methods for antioxidants-a review, Mini-Reviews in Medicinal Chemistry 2006; 6(3):305–312.
17. Luck H and Bergmeyer HU: Catalase in Methods of Enzymatic Analysis, Academic Press, NewYork, NY, USA, 1971.
18. Mackeen MM, Ali AM, Lajis NH, Kawazu K, Hassan Z, Amran M, Habsah M, Mooi LY, Mohamed SM: Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* griff. ex T. anders. Journal of Ethnopharmacology 2000; 72(3):395-402.
19. Aldenkamp AP, Alpherts WCJ, Diepman L, Vantslot B, Overweg J, Vermeulen J: Cognitive side-effects of phenytoin compared with carbamazepine in patients with localization-related epilepsy. Epilepsy Research 1994; 19:37–43.
20. Sudha S, Lakshmana MK, Pradhan N: Chronic phenytoin-induced impairment of learning and memory with associated changes in brain ACh Esterase activity and monoamine levels. Pharmacology Biochemistry Behaviour 1995; 119–124.

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