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Protective Effect of Alpha Lipoic Acid against Phenytoin Induced Behavioral Abnormalities in Rats

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Abstract

Background: Long term administration of antiepileptic drug phenytoin is reported to cause behavioral abnormalities mediated via oxidative stress. The effect of an antioxidant alpha lipoic acid (ALA) against phenytoin induced behavioral abnormalities was investigated.

Methods: The study was carried out in albino wistar rats. The rats were divided into five groups of six animals each. Group 1 received 0.2% carboxy methyl cellulose (CMC, p.o), group 2 received 20 mg/kg phenytoin (p.o), groups 3,4 and 5 received 50, 100 and 200 mg/kg (p.o) of ALA in 0.2% CMC, respectively 1 h prior to phenytoin for 45 days. Motor coordination, exploratory behavior, memory and spontaneous motor activity were evaluated by Rota rod, Hole board, Elevated plus maze and Actophotometer respectively. On day 45, regional brain lipid peroxidation and acetylcholinesterase (ACh E) activity along with brain histopathological investigation were performed after euthanasia. In addition, pharmacokinetic and pharmacodynamic drug interactions between phenytoin and ALA were also studied. Results: Long term administration of phenytoin showed behavioral abnormalities, increased regional brain malondialdehyde (MDA) and ACh E activity. The histopathological investigation showed congested and damaged cells in brain regions. ALA substantially reversed phenytoin induced behavioral abnormalities, oxidative stress and alleviated the histopathological abnormalities. There were no significant differences in the serum levels of phenytoin and the degree of protection offered by phenytoin in ALA supplemented groups revealing that there were no pharmacokinetic and pharmacodynamic interactions between phenytoin and ALA.

Conclusion: This study reports the effectiveness of ALA against phenytoin induced behavioral abnormalities and oxidative stress in rats without altering the bioavailability of phenytoin and its therapeutic effect.

Introduction

Epileptic disorders affect approximately 0.5-1% of human population [1]. The main goal in the treatment of epilepsy is to attain a complete control over seizures without potential side effects to improve the quality of life. Phenytoin is a most common and effective antiepileptic drug (AED) prescribed for a prolonged period to achieve seizure control in all types of generalized as well as partial seizures and status epilepticus [2]. Phenytoin causes several serious side effects notably neurotoxicity [3]. This drug generates reactive oxygen species during its metabolism leading to severe oxidative stress which in turn results in neuro-degeneration. Long term phenytoin therapy causes cerebellar degeneration [4] resulting in cognitive impairment [5], ataxia, nystagmus and slurred speech [6].

Alpha-lipoic acid (ALA) also known as thioctic acid (TA), is essential for the function of different enzymes of oxidative metabolism [7,8]. ALA was initially used in the treatment of acute poisoning with amanita phalloides, deadly poison followed by its application in treating neuropathic complaints [9]. It is believed that ALA or its reduced form, dihydrolipoic acid (DHLA) possess a number of biochemical functions acting as biological antioxidants, as metal chelators, reducing the oxidized forms of other antioxidant agents such as vitamin C and E and glutathione (GSH). ALA has also shown to improve endothelial dysfunction [10] and to reduce oxidative stress post exercise training [11]. It also protects against the development of atherosclerosis and inhibits the progression of an already established atherosclerosis plaque [12,13]. These above-mentioned benefits have insisted the use of ALA as a potential therapeutic agent for many chronic diseases with great epidemiological as well as economic and social impact such as diabetes mellitus (DM) and its complications [14,15], hypertension [16], Alzheimer's disease (AD) [17], Down syndrome [18], cognitive dysfunction and some types of cancer [19]. ALA is recommended as a dietary supplement in medical and nutritional management of patients.

The therapeutic or toxic effects of phenytoin depend on its serum concentration. The serum levels of phenytoin were estimated at the end of the study period after the steady state of the drug was achieved to investigate if there were any pharmacokinetic interactions between phenytoin and ALA. Pharmacodynamic interference of ALA over antiepileptic protection offered by phenytoin was also studied.

Our work is a preliminary study to assess the ameliorative effect of ALA against phenytoin induced behavioral abnormalities. Phenytoin and its metabolites are reported to induce oxidative stress in brain regions leading to behavioral abnormalities. Hence, we explored the ameliorative effect of ALA against phenytoin induced behavioral abnormalities like impaired cognition, exploratory behavior, spontaneous motor activity and locomotor activity in addition to the estimation of regional brain lipid peroxidation and acetyl cholinesterase

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(ACh E) activity. Antioxidant supplementation is expected to offer a better antiepileptic therapy with minimal or no toxicity, which may improve the quality of life in patients under phenytoin treatment.

Materials and Methods

Animals

Adult male albino rats weighing 150-200 g were maintained at room temperature (25 ± 3°C), fed with a rodent lab diet and tap water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee of M.S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India, Ref. No.220/abc/CPCSEA.

Study protocol

The rats were divided into five groups consisting of six animals each. First group served as control and received 0.2% carboxy methyl cellulose (CMC) (p.o) for 45 days. Second group received 20 mg/Kg phenytoin dissolved in 0.2% CMC (p.o) for 45 days. Third, fourth and fifth group received 50, 100 and 200 mg/kg of ALA in 0.2% CMC (p.o) respectively 1 h prior to administration of 20 mg/Kg phenytoin for 45 days.

Evaluation of behavioral abnormalities

The behavioral parameters were analyzed 2 hrs after the administration of ALA and phenytoin. Memory, motor co-ordination, locomotor activity and exploratory behavior were assessed on 0, 15th, 30th and 45th day. On 45th day, behavioral tests were carried out, 3 h after phenytoin administration (steady state concentration) and phenytoin with ALA supplementation the animals were subjected to maximal electro shock (MES) induced convulsions to compare the degree of protection offered by phenytoin in phenytoin treated group and groups subjected to combination of phenytoin and antioxidants. Immediately after MES the animals were decapitated under ether anesthesia, blood was collected from retro-orbital plexus to estimate the serum levels of phenytoin. The brains were quickly removed and differentiated into cortex, mid brain, medulla, pons and cerebellum and were subjected to assessment of the extent of lipid peroxidation and acetylcholinesterase (ACh E) activity.

Motor co-ordination test

Motor co-ordination test was conducted in rats using a Rota-Rod apparatus (Inco-Ambala, India). The animals were screened for motor co-ordination and the animals which stayed on the rotating rod without falling for 120 sec were chosen for the study. Each animal was placed on the Rota rod and the time taken by the animal to fall down was noted [20].

Test for locomotor activity

Spontaneous motor activity was monitored using Actophotometer. Each animal was subjected to an adaptation period of 2-5 minutes after which their locomotor activity was assessed for 5 minutes. Increase in count was regarded as CNS stimulant activity. Decrease in count was considered as CNS depressant activity [20].

Test for memory impairment

Elevated plus maze test was used for the assessment of memory. The elevated plus maze consists of two closed arms and two open arms forming a cross, with a quadrangular center and has a height of 50 cm. The rats were placed individually at the end of one open arm facing away from central platform and the time it took to move from the open arm to either of the enclosed arms (transfer latency) was recorded on the day of acquisition trial. Transfer latency is the time taken by the rats

to move from one end of the open arm to enclosed arm. The rat was allowed to move freely in the plus maze regardless of open and closed arms for 10 sec after the measurement of transfer latency. The rat was then gently taken out of the plus maze and was returned to its home cage. On the test day, the transfer latency test was performed in the same manner as in the acquisition trial [21].

Test for alertness (Exploratory Behavior)

0.5 m³ wooden board with 16 holes (3 cm in diameter) was employed for the study. Each rat was placed individually on the board for a period of 6 minutes. In first 2 minutes the animal was allowed for acclimatization and then the number of head dipping performed in the next 4 minutes was noted for each animal [22].

Assessment of oxidative stress in brain tissues

The brain samples were quickly removed, cleaned with chilled saline, dissected into cortex, midbrain, medulla, pons and cerebellum [23], were stored at −40°C.

Estimation of lipid peroxidation in brain regions

The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde (MDA) according to the method of Ohkawa, et al. Briefly, 1 ml (10%) tissue homogenate was added to the reaction mixture containing 1 ml of trichloro acetic acid (15%) and 2 ml of thiobarbituric acid (0.38%). The reaction mixture was heated for 60 min at 90°C, cooled and centrifuged at 6900 rpm for 15 min. The absorbance of supernatant was measured at 532 nm against blank, which contained all reagents except homogenate. MDA was quantified and expressed as μmol of MDA per mg of wet tissue [24].

Estimation of acetylcholine esterase activity in brain regions

Acetylthiocholine iodide was used as a synthetic substrate for the assay of ACh E, replacing the natural substrate acetylcholine (ACh). This enzyme hydrolyses the substrate to yield acetate and thiocholine. The free thiol group of thiocholine reacts with 5,5'-dithio-bis-nitrobenzoic acid (DTNB) (Ellman's reagent) included in the assay mixture, producing the yellow 4-nitrothiolate anion. The release of this yellow anion is measured at 412 nm. The reaction mixture (2 mL final volume) contained 100mM potassium phosphate buffer, pH 7.5 and 1 mM DTNB. The method is based on the formation of the yellow anion, 5,5'-dithio-bis-acid nitrobenzoic, measured by absorbance at 412 nm during 2-min incubation at 25 C. The enzyme was pre-incubated for 2 min. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide [25].

Histopathological investigation on brain tissues

Brain tissues were dissected out carefully and were kept in 10% formalin solution prepared with normal saline. Tissues were stained using Hematoxylin and Eosin stain [26].

Maximal electroshock induced seizures (MES)

Electroconvulsions were induced by ear electrodes (current intensity-150 mA, duration - 0.2 sec). The animals were observed for tonic hind limb extension i.e., the hind limbs of animals outstretched 180° to the plane of the body axis [21].

Estimation of plasma phenytoin concentration by HPLC method

Chromatographic conditions: Mobile phase consisting of methanol: water: glacial acetic acid (67: 33: 1 v/v/v) was prepared and

mixed thoroughly, degassed and was used for the HPLC analysis. 1.0 ml per minute flow rate was maintained throughout the analysis. The eluent was monitored using a UV-VIS detector set at 230 nm and sensitivity was set at 0.001 a.u.f.s.

Preparation of standard graph

Standard solutions: Stock solution of 100 µg/ml of phenytoin was prepared in methanol and diluted with methanol to the required concentration. The solutions were stored at –4°C. For standard graph 2, 4, 6, 8, 10, 12, 14, 16, 18 20 µg/ml of pure phenytoin was used

Plasma extraction

To each 100 µl of plasma sample, 25 µl of internal standard (100 µg/ml carbamazepine solution) was added and extracted with 1.7 ml of ethyl acetate, vortexed for 1 min and centrifuged at 13,000 rpm for 8 min. The supernatant was evaporated to dryness, the residue was reconstituted with 100 µl of mobile phase, vortexed for 1 min. and 20 µl was injected onto C18 column. The retention times were 4.49 min. and 5.15 min. for phenytoin and carbamazepine respectively. The peak area obtained at different concentrations of the drug was plotted against the concentrations of the drug [27].

Statistical analysis

The results were expressed as mean ± SEM of each group. One way analysis of variance (ANOVA) followed by the Tukey's post hoc test was used to assess the differences among treatment groups. Statistical analysis was performed using GraphPad Instat software. $p < 0.05$ was considered significant.

Results

There were no significant differences in transfer latency, exploratory activity, motor coordination and spontaneous motor activity between control, phenytoin alone and phenytoin with ALA pretreated groups on 0 day of the study.

Effect of Alpha Lipoic Acid on phenytoin induced memory impairment

The effect of chronic treatment of phenytoin and phenytoin along with ALA on memory is shown in Figure 1. There was no significant difference in the transfer latency of the control, phenytoin and phenytoin with ALA (50, 100, 200 mg/Kg) pretreated groups on the 0 day of the study. The retention transfer latencies increased from 34 ± 0.36 sec (0 day) to 123.6 ± 1.22 sec (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of ALA in all the three doses significantly reduced the transfer latency from 15th day till 45th day. The values decreased from 123.6 ± 1.22 sec in the phenytoin treated group to 93.66 ± 0.55 sec (p< 0.001), 80.66 ± 1.49 sec (p< 0.001) and 73.66 ± 0.76 sec (p< 0.001) in ALA 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant reversal of phenytoin induced memory impairment in a dose dependent fashion but the values did not reach the normal.

Effect of Alpha Lipoic Acid on phenytoin impaired exploratory activity

There was no significant difference in the exploratory activity of the control, phenytoin treated and phenytoin with ALA (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of study. The exploratory activity was assessed by the number of head dippings into the holes of the hole board apparatus. The number of the head dippings decreased from 21 ± 0.32 (0 day) to 3.16 ± 0.47 (45th day) (p< 0.001) in phenytoin treated animals. Co-administration of ALA in all the three doses significantly increased the exploratory movements from 15th day till 45th day. The number of head dippings increased from 3.16 ± 0.47 in the phenytoin
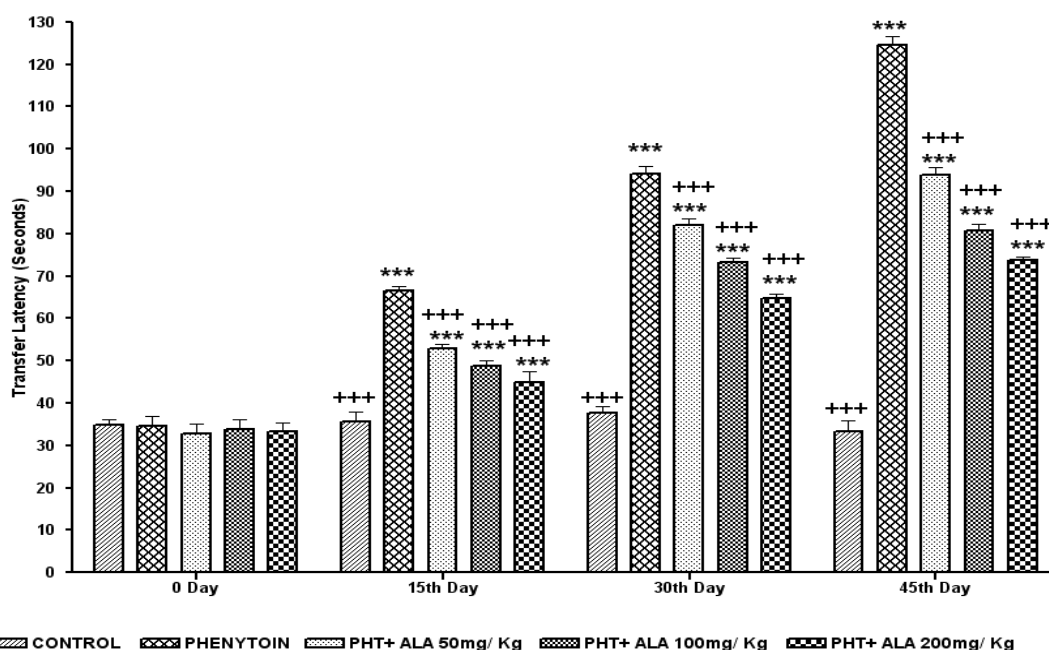
Values are expressed as mean± SEM of 6 animals.
***(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group

Figure 1: Effect of ALA on phenytoin induced memory impairment.

treated group to 8.83 ± 0.3 (p<0.001), 12.16 ± 0.47 (p< 0.001) and 13.5 ± 0.42 (p < 0.001) in ALA 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant reversal of phenytoin impaired exploratory behaviour in a dose dependent manner but the values did not reach the normal values (Figure 2).

Effect of Alpha Lipoic Acid on phenytoin induced motor in co-ordination

There was no significant difference in motor coordination of the control, phenytoin treated and phenytoin with ALA (50, 100, 200 mg/Kg) pre-treated groups on the 0 day of the study. Phenytoin (20 mg/Kg, p.o.) significantly impaired the Rota Rod performance of rats from the 120 sec (0 day) to 17.83 ± 0.94 sec on 45th day (p< 0.001). Co-administration of ALA in all the three doses significantly improved the motor coordination from 15th day till 45th day. The values increased from 17.83 ± 0.94 sec in the phenytoin treated group to 54.16 ± 1.24 sec (p< 0.001), 87.5 ± 1.11 sec (p< 0.001) and 93.163 ± 0.9 sec (p< 0.001) in ALA 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant reversal of phenytoin impaired muscle grip in a dose dependent fashion but the values did not reach the normal (Figure 3).

Effect of Alpha Lipoic Acid on phenytoin impaired locomotor activity

There was no significant difference in spontaneous motor activity of the control, phenytoin and phenytoin with ALA (50, 100, 200 mg/Kg) pre-treated groups on zero day of study. Phenytoin 20 mg/Kg, p.o., significantly decreased the spontaneous motor activity by reducing the performance of the rats on Actophotometer. The count reduced from 306.33 ± 2.4 (0 day) to 86.16 ± 1.49 (45th day) (p< 0.001). Co-administration of ALA in all the three doses significantly improved the

spontaneous activity from 15th day till 45th day. The values increased from 86.16 ±1.49 in the phenytoin treated group to 112 ± 1.94 (p< 0.001), 160.8 ±3.0 (p< 0.001) and 209.165 ± 2.27 (p < 0.001) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. ALA at all the three doses produced significant reversal of phenytoin impaired locomotor activity in a dose dependent fashion but the values did not reach the normal values (Figure 4).

Effect of Alpha lipoic Acid on phenytoin induced alterations in regional brain lipid peroxidation

Phenytoin significantly elevated the lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex. ALA significantly reduced (p< 0.001) the phenytoin induced lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex dose dependently but the values did not reach the normal values when compared with the control group (Figure 5).

## Effect of Alpha Lipoic Acid on phenytoin induced alterations in regional brain AchE activity

Phenytoin exhibited a significant increase in AchE activity in medulla, pons, midbrain, cerebellum and cortex. ALA significantly reduced (p< 0.001) the activity of AchE in medulla, pons, midbrain, cerebellum and cortex dose dependently and brought back the values near to the normal when compared with the control group (Figure 6).

Effect of phenytoin on regional brain histopathology

Figure 7 Illustrates the effect of phenytoin on brain. Control group showed normal brain architecture (Figure 7a). Phenytoin treated group revealed severe necrosis in cortex (Figure 7b).

Effect of Alpha Lipoic Acid on phenytoin induced alterations in regional brain histopathology

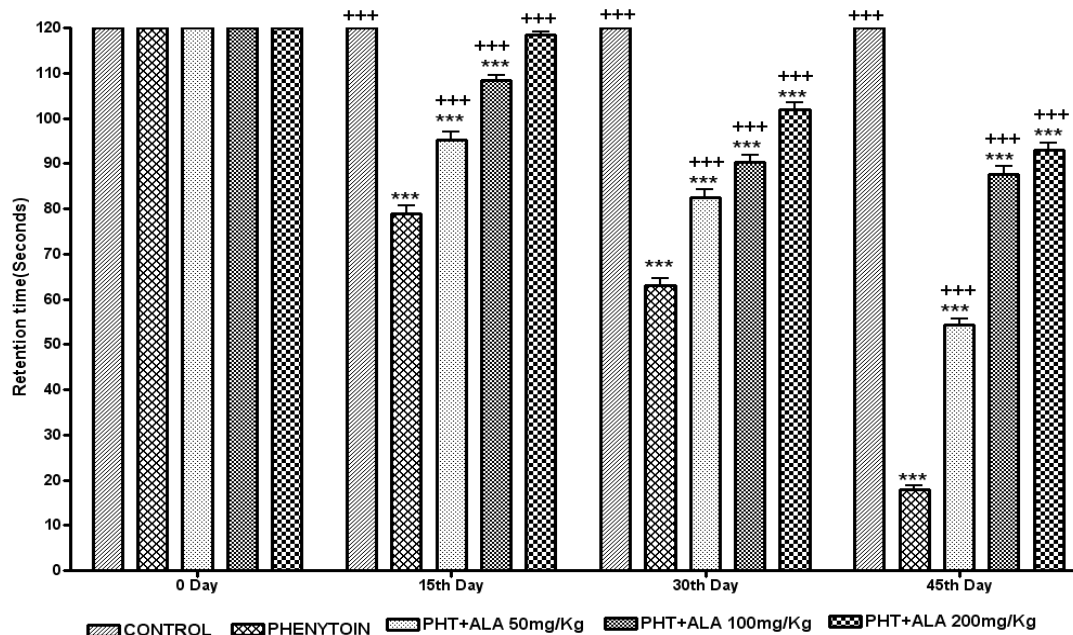Figure 8 shows the influence of ALA on phenytoin induced
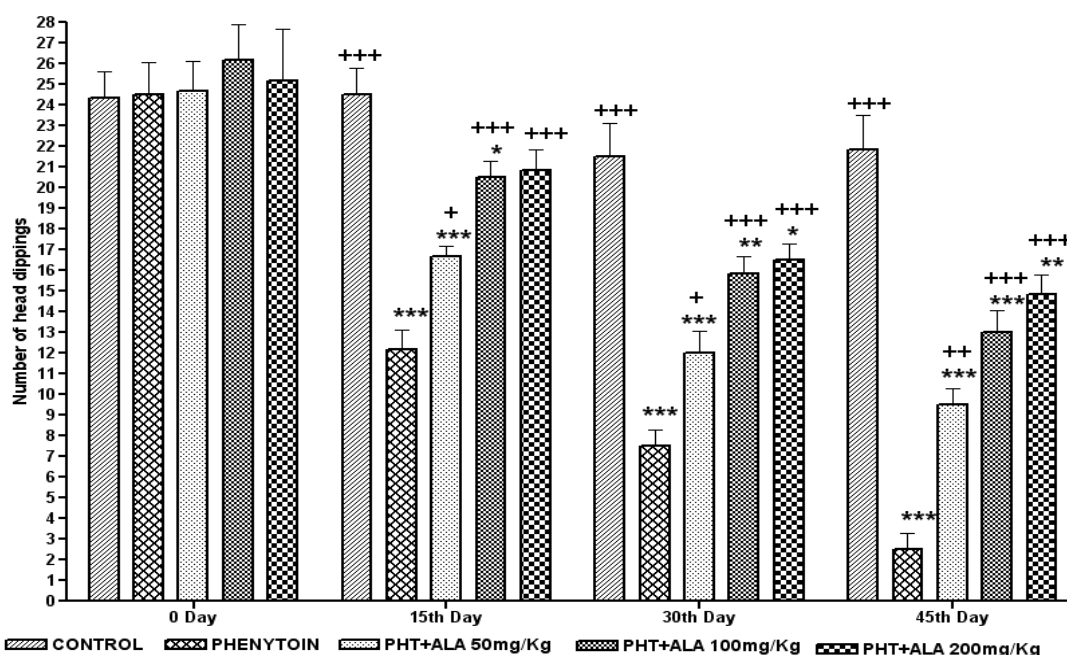
Values are expressed as mean± SEM of 6 animals.
***(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group
+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group

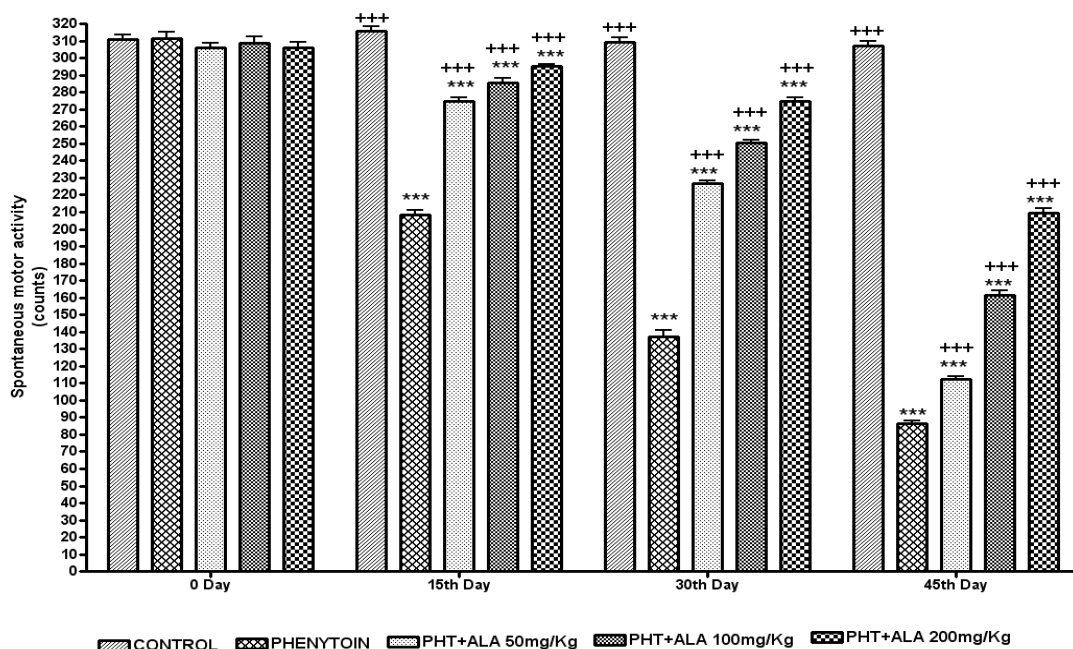**Figure 2:** Effect of ALA on phenytoin impaired exploratory behaviour.

Values are expressed as mean± SEM of 6 animals.

***(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group

Figure 3: Effect of ALA on phenytoin induced motor in-coordination.
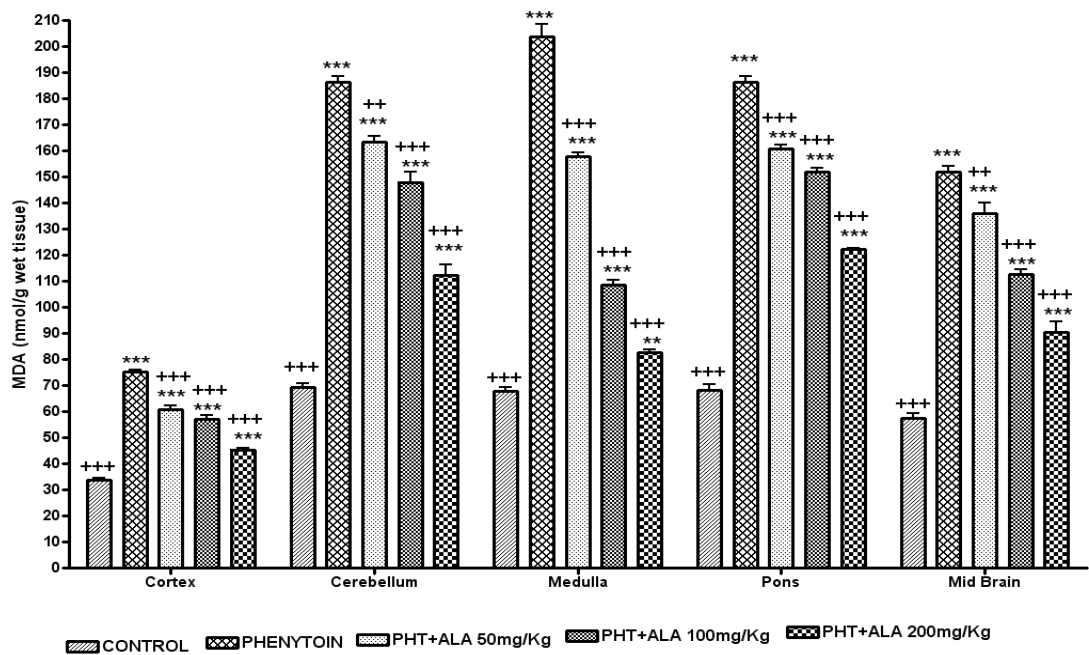

Values are expressed as mean± SEM of 6 animals.

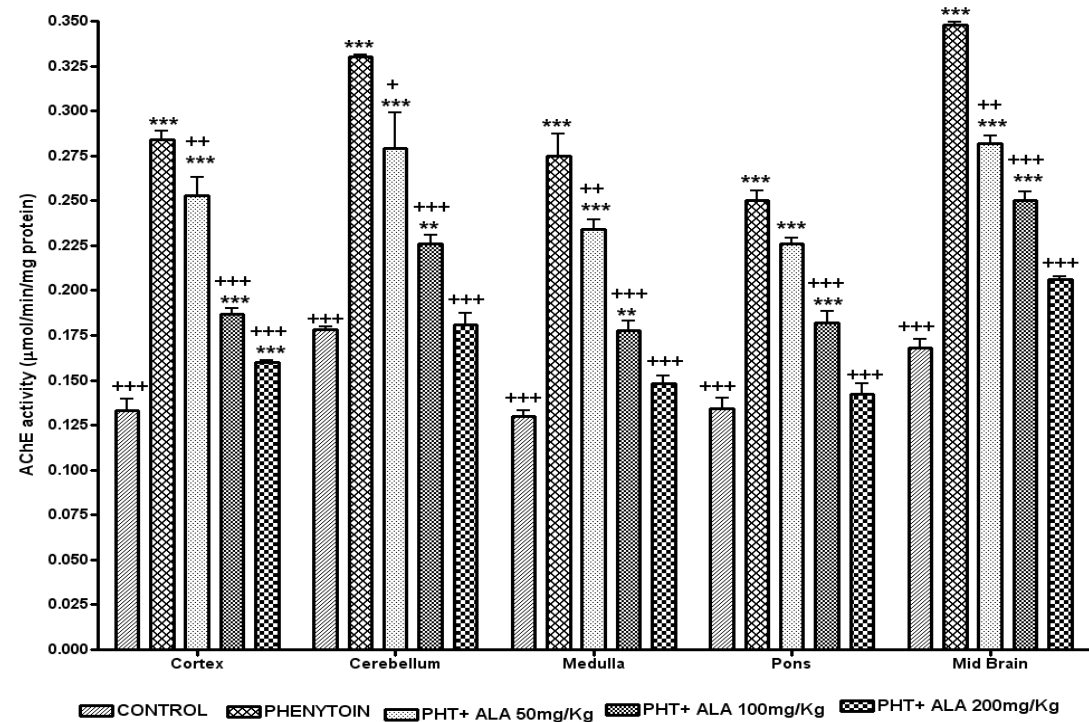***(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group

+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group

Figure 4: Effect of ALA on phenytoin impaired locomotor activity.

Values are expressed as mean± SEM of 6 animals.
***(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group
+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group

Figure 5: Effect of ALA on phenytoin induced alterations in regional brain lipid peroxidation.

Values are expressed as mean± SEM of 6 animals.
***(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group
+++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Phenytoin group

Figure 6: Effect of ALA on phenytoin induced alterations in regional brain acetyl cholinesterase activity.
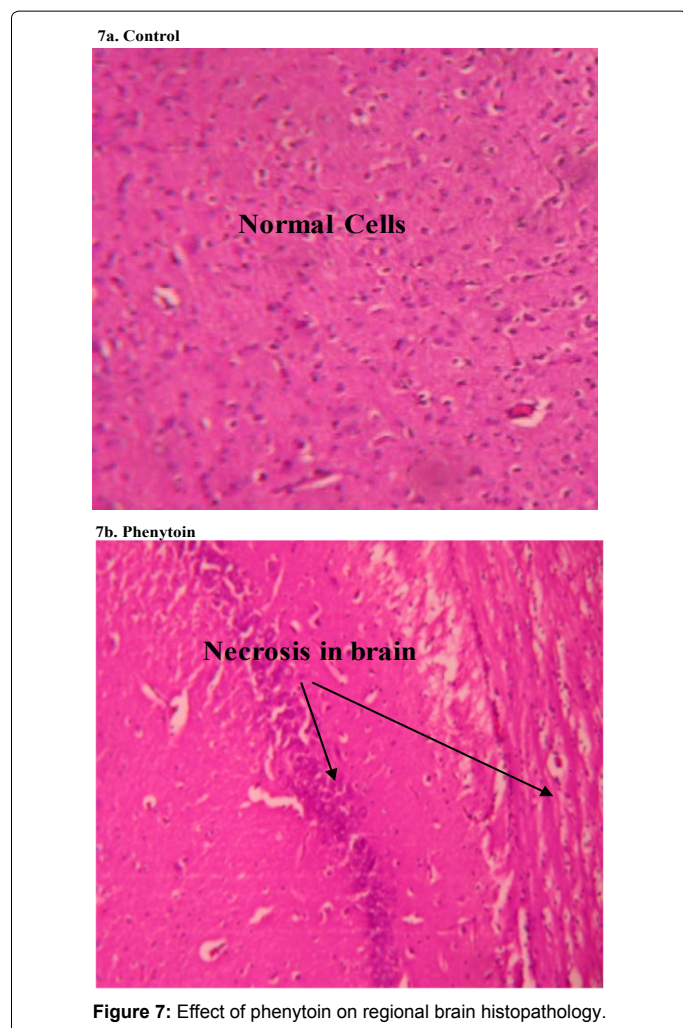
Figure 7: Effect of phenytoin on regional brain histopathology.

histopathological changes in rat brain. Phenytoin in combination with 50 mg/Kg ALA showed gliosis and congestion in brain (Figure 8a), 100 mg/Kg and 200 mg/Kg ALA showed normal brain parenchyma (Figure 8b and 8c).

Influence of ALA on pharmacodynamic effect of phenytoin

Phenytoin as well as phenytoin supplemented with ALA (50, 100 and 200 mg/kg) offered same degree of protection (100%) against MES induced convulsions in rats.

Effect of ALA on serum phenytoin levels

There was no significant difference in the serum concentration of phenytoin treated group as compared to the groups co-administered ALA 50, 100 and 200 mg/kg along with phenytoin. The serum phenytoin levels were 15.740 ± 1.8, 14.480 ± 1.6, 15.340 ± 2.2 and 15. 650 ± 1.2 µg/ml in the groups treated with phenytoin and ALA 50, 100 and 200 mg/kg along with phenytoin respectively. All these values were within the normal therapeutic range (10-20 µg/ml) of phenytoin.

Discussion

Phenytoin adversely affected motor-coordination, cognition, exploratory behavior and spontaneous motor activity. The drug also significantly raised the regional brain lipid peroxidation and ACh E
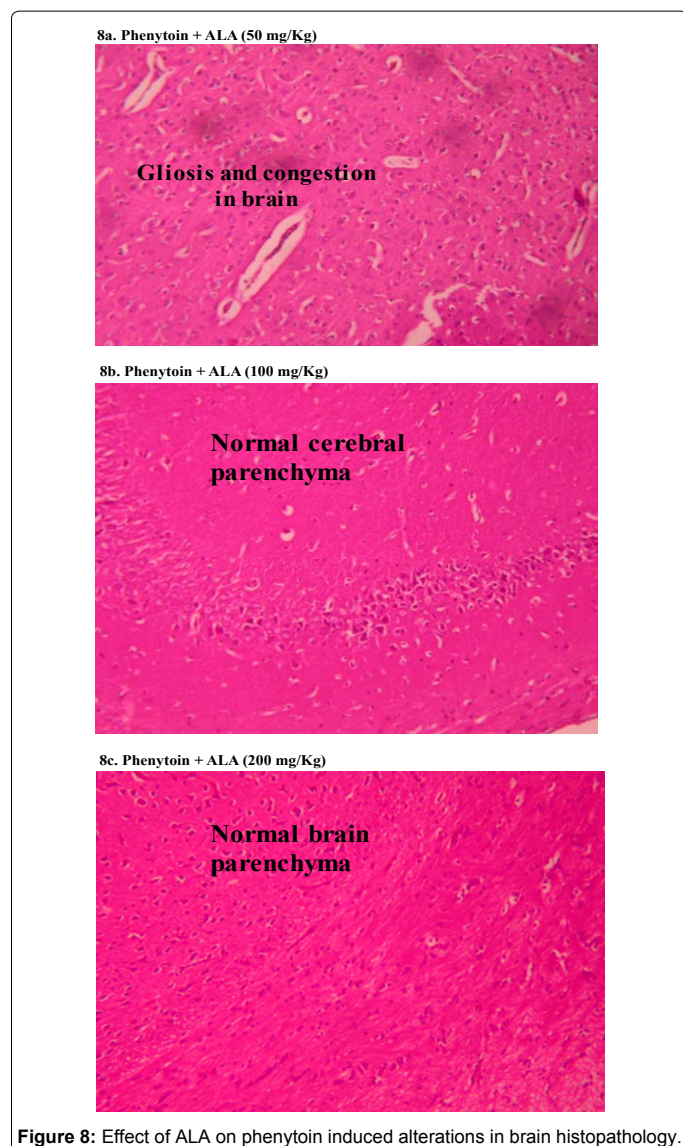
activity along with severe degeneration in the brain regions which was revealed from the histopathological investigations.

Cognitive impairment is observed in epileptic patients and the degree of impairment depends on the number of factors in epileptic patients including the underlying pathology. Therefore, it is rational to assess the extent of AEDs induced memory and cognitive function in experimental animals without any additional complexities of the disease. Phenytoin significantly impairs the process of learning and memory consolidation [28]. Phenytoin (20 mg/Kg) was reported to substantially prolong the transfer latency of rats in elevated plus maze test, indicating the risk of this drug in impairing cognition even in healthy individuals also. Our results are online with the above finding in which, learning and memory was impaired by phenytoin in rats. Phenytoin was reported to affect the exploratory behavior [29], induce sedation and decrease the wakeful state of the rats. Phenytoin significantly decreased the exploratory behavior as there was a decrease in the number of head dippings in the holes of the hole board. Phenytoin impaired the Rota rod performance of rats indicating muscle weakness and motor in co-ordination induced by the drug. Phenytoin significantly reduced the spontaneous motor activity, indicating the CNS depressant effect of the drug.

The cerebral cortex is involved in many higher level tasks such as language, memory and consciousness. Cerebellar cortex play a critical role in long term memory storage [30] and consolidation of memory [31]. In the present study, phenytoin increased the lipid peroxidation in cerebral cortex, cerebellum, mid brain, pons and medulla oblongata. Increased lipid peroxidation in different brain regions causes peroxidative injury to the neuronal membranes and macromolecules, alter neurotransmitters, disturb neuronal functions and motor function [32]. Neuronal damage induced by phenytoin in brain regions was considered to be responsible for memory impairment, motor in co-ordination, sedation, ataxia and loss of exploratory drive.

Cholinergic activity in the CNS is essential for learning, memory, cortical organization of movement and cerebral blood flow [33] and is regulated by acetylcholine Esterase (ACh E), which hydrolyses the neurotransmitter acetylcholine (ACh) in the synaptic cleft of cholinergic synapse and neuromuscular junctions [34]. The effects of cholinergic antagonists and lesions of cholinergic nuclei are related to cognitive deficits similar to those observed in aging and dementia [35,36]. The antimuscarinic drug scopolamine is the most widely used to induce amnesia in experimental subjects [37]. ACh E inhibitors enhance the availability of ACh in the synaptic cleft and reverse the scopolamine induced memory deficit. Many studies have shown that there is a relation between the decrease in cognitive functions and markers of the cholinergic system in senile dementia [38]. Cognitive functions dependent on central cholinergic neurotransmission. Although other neurotransmitters were known to be involved in learning and memory performance, acetylcholine plays a vital role in in storage and retrieval of memory. Decline in the cholinergic system underlies the cognitive deficits of dementia [39] and ACh E levels are reported to be high in AD. Melo, et al., (2003) studied the involvement of oxidative stress in the enhancement of ACh E activity and reported that amyloid beta-peptide enhanced ACh E activity mediated via oxidative stress [40].

Epileptic patients receiving phenytoin showed poor performance on memory tasks than untreated controls [41]. Investigations on the effect of phenytoin on learning, memory and psychomotor functions revealed that both acute and chronic administration of phenytoin considerably impaired learning and memory. It was reported that phenytoin decreased brain ACh levels [42]. Phenytoin's impairing

8a. Phenytoin + ALA (50 mg/Kg)

Gliosis and congestion in brain

8b. Phenytoin + ALA (100 mg/Kg)

Normal cerebral parenchyma

8c. Phenytoin + ALA (200 mg/Kg)

Normal brain parenchyma

Figure 8: Effect of ALA on phenytoin induced alterations in brain histopathology.

effects on learning and memory are attributed to enhanced ACh E activity in brain. Since central cholinergic system plays an important role in learning and memory and as phenytoin reduced ACh concentration in brain regions, the drug was reported to induce serious memory impairment [42]. In the present study ACh E activity in different brain regions was measured and our results were online with the previous reports which also revealed that phenytoin at therapeutic doses increased ACh E activity in the brain regions of the rats. The rats showed poor performance in the elevated plus maze test indicating memory impairment. It was believed that phenytoin via oxidative stress enhanced the ACh E activity and thereby depleted the levels of ACh in brain regions resulting in subsequent memory impairment.

Histopathological analysis of brain sections of phenytoin treated rats showed damaged cells and congestion in periventricular region and cortex, which substantiates phenytoin induced apoptosis in cortex and periventricular region. Phenytoin via oxidative stress induced the damage in rat brain, which in turn resulted in adverse behavioral abnormalities.

Since phenytoin induced oxidative stress was considered to be responsible for deterioration of behavioral parameters, the present investigation is aimed at evaluating the antioxidant potential of ALA to reverse phenytoin induced oxidative damage and behavioral abnormalities.

Effect of Alpha Lipoic Acid on phenytoin induced behavioural abnormalities

Oxidative stress was found to play a crucial role in age related neurodegenerative disorders. ALA supplementation was observed to significantly decrease haloperidol induced tardive dyskinesia and catalepsy. The protective effect of ALA against tardive dyskinesia was attributed to its ability to reduce haloperidol induced lipid peroxidation, by scavenging ROS and reactive nitrogen species. In the present study also it was found that ALA significantly increased the muscular coordination and muscle strength which was considerably reduced by phenytoin. This improvement in muscle grip was believed to be due to the antioxidant property of ALA and its protective action against oxidative stress in brain regions responsible for muscular coordination. ALA is proved to increase insulin sensitivity and activity which in turn plays a role in serotonergic activity by increasing the influx of tryptophan into the brain. This increased influx of tryptophan results in an increase in serotonin synthesis. In accordance with the serotonin theory of depression, it is possible to treat depression by increasing insulin activity. Therefore, ALA supplementation is thought to possess anti depressant activity. In the present study also it was found that ALA significantly increased the locomotor activity which was considerably reduced by phenytoin [43].

ALA derives its antioxidant capability from its ability to act as a scavenger of ROS, chelate metals and recycle endogenous antioxidants [44]. ALA scavenges singlet oxygen, H_2O_2, OH^-, NO and ONOO. ALA and its reduced form DHLA, further scavenge oxygen and peroxyl radicals [45]. ALA also chelates several divalent cations, e.g. Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}. Therefore, ALA inhibits ascorbate induced production of H_2O_2 by Cu^{2+} [46]. ALA recycles endogenous antioxidants, such as GSH [46] and Vit C [47], which in turn regenerates Vit E. GSH, Vit C and Vit E protect the brain from oxidative stress [47].

ROS species are thought to be involved in acute and chronic pathological conditions in the brain and neuronal tissue. The metabolic antioxidant ALA is a low molecular weight substance which crosses the blood brain barrier. ALA is taken up and reduced in cells and tissues to dihydro lipoic acid (DHLA), which is also exported to the extracellular medium; hence, protection is offered to both intracellular and extracellular environments. Both ALA and DHLA were considered to be potent antioxidants to regenerate other antioxidants like Vit C and Vit E and GSH through redox cycling. Thus, it would seem an ideal substance in the treatment of oxidative brain and neuronal disorders involving free radical processes. ALA reveals protection against cerebral ischemic reperfusion, excitotoxic amino acid brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism and other causes of acute or chronic damage to brain or neuronal tissue. Antioxidant properties of ALA achieve its possible therapeutic roles in a variety of brain and neuronal tissue pathologies [48].

Acute dose of lindane causes significant reduction in butyrylcholinesterase (BChE) activity both in olfactory lobe and cerebrum of mice along with reduction in catalase, total protein and elevation in cholesterol contents. Pre-treatment by a combination of antioxidants such as Vit E, Vit C, ALA and stilbene resveratrol (125 mg/

Kg, ip) significantly augmented the altered level of butyrylcholinesterase and protected the other parameters in both the brain regions. The study suggested the neuroprotective efficacy of combination of antioxidants against lindane induced neurotoxicity [49].

ALA modulated AchE activity, increased choline acetyl transferase activity, improved cholinergic transmission and thus reversed the memory impairment induced by many factors such as aging, epilepsy etc. [50]. Antioxidant property of ALA was believed to combat oxidative stress induced memory impairments by improving the cholinergic transmission. The present study showed that ALA along with its antioxidant property, brought down the phenytoin elevated AchE activity, thus preserved the cholinergic transmission and improved memory.

In the present study, ALA decreased phenytoin induced lipid peroxidation and thus reversed the behavioural abnormalities induced by long term phenytoin administration. ALA (50 mg/Kg) supplementation showed Gliosis and congestion in brain, whereas ALA (100 and 200 mg/Kg) treated group showed normal brain parenchyma. Thus ALA produced a dose dependent protective effect on phenytoin induced behavioural abnormalities and neurotoxicity.

Pharmacodynamic study was carried out to evaluate whether ALA supplementation hinders the therapeutic efficacy of phenytoin. In the present study, administration of phenytoin (20 mg/Kg for 45 days) produced 100% protection against MES induced seizures. Co-administration of ALA with phenytoin also offered the same degree of protection against MES induced convulsions. This finding suggests that antioxidant supplementation with phenytoin did not reduce the therapeutic effect of phenytoin, revealing that there was no pharmacodynamic interaction between phenytoin and the selected antioxidants.

The serum levels of phenytoin were estimated at the end of the study period well after the steady state (3 h after administration of phenytoin) of the drug was achieved. The serum levels relate to the therapeutic or toxic effects of phenytoin. It was evident from the findings of the present study that the serum levels of phenytoin were not different in the groups supplemented with ALA as compared to phenytoin alone treated group. This suggests that ALA did not alter the serum phenytoin concentration. This finding revealed that ALA did not alleviate the behavioral abnormalities by reducing serum phenytoin levels.

ALA offered protection against phenytoin induced behavioral abnormalities. The neuroprotective potential of ALA is due to its antioxidant property. Though the behavioral abnormalities were reversed, the values did not reach normal even with higher dose of ALA, indicating the involvement of other mechanisms in addition to oxidative stress in phenytoin induced behavioral abnormalities.

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