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**EFFECT OF VITAMIN C SUPPLEMENTATION ON PHENYTOIN
INDUCED BEHAVIOURAL ABNORMALITIES AND REGIONAL LIPID
PEROXIDATION IN RATS**

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Abstract

Aim: The present study explores the effect of chronic vitamin C supplementation on phenytoin induced behavioural abnormalities.

Methods: Male Wistar rats were divided into 5 groups and each group received vehicle, Phenytoin (20mg/Kg), Vitamin C (50, 100, 200 mg/kg) for 45 days respectively. Motor coordination was evaluated by Rota Rod, exploratory behaviour was assessed by Hole Board, memory was evaluated using the Elevated Plus Maze and spontaneous motor activity was examined by Actophotometer. On day 45, plasma concentration of vitamin C, regional brain malondialdehyde (MDA) and histopathological studies were done.

Results: Chronic administration of phenytoin (20mg/kg, p.o.) significantly induced behavioural abnormalities, reduced significantly plasma vitamin C levels, elevated regional brain MDA and revealed damaged cells and congestion in different brain regions. Vitamin C (50, 100 and 200 mg/kg, orally), when administered with phenytoin, significantly prevented phenytoin-induced behavioral abnormalities, oxidative stress and improved the histopathological abnormalities in a dose-dependent manner.

Conclusion: The results of the present study indicate that vitamin C is effective in preventing phenytoin-induced oxidative stress and behavioral abnormalities in dose dependent manner. Thus vitamin C was suggested to improve the quality of epileptic patient under long-term phenytoin therapy.

Keywords: Antioxidant, oxidative stress, phenytoin, Vitamin C, behavioral abnormality.

Introduction

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 group of brain cells (neurons). Epilepsy is successfully treated with anti-epileptic drugs and the major aim of anti-epileptic drug (AED) therapy is relief from seizures, a huge population of epileptic patients is still not reaching this goal^{1,2}. Phenytoin is a most common and effective AED prescribed for a prolonged period to achieve seizure control in all types of partial and tonic-clonic seizures^{3,4,5}. AED treatment is associated with dose dependent^{6,7} cognitive deficit^{8,9}.

Phenytoin treatment is known to induce cognitive dysfunction¹⁰, cerebellar dysfunction and degeneration^{11,12,13}. Cerebellum maintains motor coordination, sensory-motor integration, motor learning and cognition¹⁴. The dose related adverse effects of phenytoin include ataxia (lack of coordination of muscle movements), nystagmus (involuntary eye movement), lethargy and slurred speech¹⁵.

The central nervous system (CNS) is especially susceptible to free radical as it is rich in lipids, consumes high oxygen and has scarcity of antioxidant enzymes compared to other tissues¹⁶. The entire nervous system including brain, spinal cord and the peripheral nerves are rich in poly unsaturated fatty acids and iron. The high level of iron is essential for brain development but iron ions form reactive oxygen species (ROS)¹⁷, which rearranges the double bonds of fatty acids and generates a number of degradation products like lipid alkoxyl, peroxy radicals and lipid hydroperoxides¹⁸. ROS increases the permeability of the blood brain barrier, inhibits the mitochondrial respiration, leading to lipid peroxidation¹⁹ and influences gene expression, subsequently affecting apoptosis and neuronal death²⁰. High oxidative metabolism especially in catecholamine rich areas such as basal ganglia makes neurons particularly vulnerable to membrane lipid peroxidation²¹. Oxidative stress is intimately linked to the degenerative processes in most neurological diseases, such as epilepsy, alzheimer's disease, parkinson's disease, stroke, cerebral ischaemia, multiple sclerosis, huntington's chorea, tardive dyskinesia, and amyotrophic lateral sclerosis etc²².

Although the mechanism(s) of phenytoin-initiated toxicity is unknown, phenytoin is enzymatically bioactivated to a reactive intermediate leading to increased formation of reactive oxygen species (ROS), which damages

essential macromolecules, including DNA²³. Lipid peroxidation adversely affects the membrane signal transduction systems relevant to cognition and produces neurodegenerative disorders²⁴ like epilepsy, Alzheimer's disease, Parkinson's disease, stroke, cerebral ischemia, multiple sclerosis, Huntington's chorea, tardive dyskinesia, and amyotrophic lateral sclerosis etc²². Free radical status is intimately linked to the degenerative processes in most neurological diseases.

In our earlier studies also we have proofs on phenytoin induced cognitive impairment, disturbance in motor coordination, exploration behaviour and locomotor activity²⁵ where spirulina attenuated phenytoin induced cognitive impairment, disturbance in motor coordination, exploration behaviour and locomotor activity²⁵.

Vitamin C (ascorbic acid), is a micro nutrient participates in several enzymatic reactions and also essential for the synthesis of catecholamines²⁶. There is considerable evidence that vitamins increase the levels of brain catecholamines, protect against oxidative damage, reduce the neuronal damage and slow the progression of Alzheimer's disease (AD)²⁷. Catecholamines inhibit lipid peroxidation and this counters the oxidative stress in neurodegenerative diseases, including Alzheimer's disease (AD)²⁸. Short and long-term supplementation with ascorbic acid has beneficiary effects on acquisition and retrieval processes of passive avoidance learning and memory in rats²⁹ and decreases the risk of AD³⁰. Vitamin C supplementation prevents cognitive impairment³¹ and was also proved to improve tardive dyskinesia induced by antipsychotic drugs³².

Behavioural toxicities of phenytoin are considered to be due to production of reactive oxygen species, it is proposed to study the influence of vitamin C on phenytoin induced oxidative stress and behavioral abnormalities.

Materials and Methods

Animals

Pathogen free adult male albino rats weighing 150-200 gm were used. Male rats were chosen in order to avoid fluctuations due to oestrous cycle. The rats were housed in polypropylene cages at room temperature ($25 \pm 3^{\circ}$ C) with 12/12 hours light and dark cycle and were fed with a balanced diet and tap water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee of M.S. Ramaiah College of Pharmacy, Register No. 220/abc/CPCSEA.

Study Protocol

The rats were divided into five groups each group consisted of nine animals. Six animals were used for behavioral and biochemical parameters, three animals were used for histopathological studies.

First group served as control and received drinking water orally daily by gavage for 45 days. Second group received 20mg/Kg phenytoin dissolved in water daily by oral gavage for 45 days between 11.00 hrs and 12.00 hrs. Third, fourth and fifth groups received orally 50, 100, 200 mg/kg of ascorbic acid respectively daily for 45 days 1 hr prior to administration of (between 10.00 hrs and 11.00 hrs) 20mg/Kg p.o phenytoin.

Only one behavioral parameter was assessed at a given time. The behavioral parameters were analyzed between 9.00 and 10.00 hrs i.e 22hrs after the administration of vitamin C and phenytoin. Memory was assessed using elevated plus maze, motor co-ordination was studied using Rota Rod, locomotor activity was explored with Actophotometer and alertness was evaluated using Hole Board apparatus on 0, 15th, 30th and 45th day. On 45th day after the behavioral test, blood samples were collected from retro orbital plexus under light ether anaesthesia for estimation of vitamin C. The animals were sacrificed and the brains were quickly removed, cleaned with chilled saline, differentiated into cortex, mid brain, medulla, pons and cerebellum.

Assessment of behavioral Parameters

Motor co-ordination test

Motor co-ordination test was conducted in rats using a Rota-Rod apparatus (Inco-Ambala, India). Each animal was placed on the rotating rod and the time it takes for falling down was noted. 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.

Test for Alertness (Exploratory Behavior)

This test was done using Hole Board, which consisted of a 0.5m³ wooden board with 16 holes (3cm in diameter). Each rat was placed singly 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 dippings performed within the next 4 minutes was noted for each animal.

Test for memory impairment

Elevated plus maze test was performed 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 was 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 s 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.

Test for Locomotor Activity

Spontaneous motor activity was monitored using Actophotometer. Each animal was subjected to adaptation for 2-5 minutes, because the first measure of animal's activity is the rate of habituation to a novel environment. Thus, during prolonged exposure to a new environment, animals typically spend progressively less time in movement and exploration, so the second measure was considered as the rate of spontaneous activity of the rats. The counting was started following 5 minutes of adaptation period. Increase in count was regarded as central nervous system stimulant activity. Decrease in count was regarded as central nervous system depressant activity.

Oxidative stress parameters

Blood sample was collected from retro orbital plexus under light ether anesthesia. Vitamin C in the blood and lipid peroxidation in regional brain was estimated after decapitation under ether anesthesia 24 h after the administration of last dose of phenytoin. The brains were quickly removed, cleaned with chilled saline, dissected into cortex, mid brain, medulla, pons and cerebellum according to the method of Glowinski and Iversen (1966)³³. The separated brain regions were stored at -80 °C and biochemical analysis was carried.

Vitamin C:

To 0.5 ml of plasma, 1.5ml of 6% TCA was added and centrifuged (3500rpm/ 20min). To 0.5 ml of the supernatant 0.5ml of DNPH reagent (2% DNPH and 4% thio urea in 9 N H₂SO₄) was added and developed color was read at 530nm after 30 min ³⁴.

Determination of MDA content (Lipid peroxidation):

Malondialdehyde, was measured spectrophotometrically by the method of Colado et al. (1997) 35, using 1, 1, 3, 3-tetraethoxypropane as standard. Malondialdehyde is expressed as nmol/g tissue. Brain regions were separately thawed and 10 % (w/v) homogenate was prepared with 0.1 M ice-cold phosphate buffer (pH 7.4). To 500 µl of tissue homogenate, 300 µl of 30% trichloroacetic acid, 150 µl of 5 N hydrochloric acid and 300 µl of 2% w/v 2-thiobarbituric acid were added and then the mixture was heated for 60 min at 90 °C, cooled to room temperature and was centrifuged at 12,000 g for 10 min. Pink colored supernatant was measured spectrophotometrically at 532 nm immediately.

Histopathological studies:

Histopathological study was conducted according to Li et al. (1998) 36. Rats were deeply anesthetized under ether anesthesia, brain tissue was dissected out carefully and was kept in 4% paraformaldehyde overnight for post-fixation. The tissue was dehydrated and embedded in paraffin for 4 h in infiltration unit. Block was prepared in block preparation unit (Shandon Histocenter-2) and coronal sections (10 µm) were cut with the help of a microtome (Leica RM 2255, Lab India) and were stained with hematoxylin and eosin on poly-l-lysine coated slides.

Statistical analysis

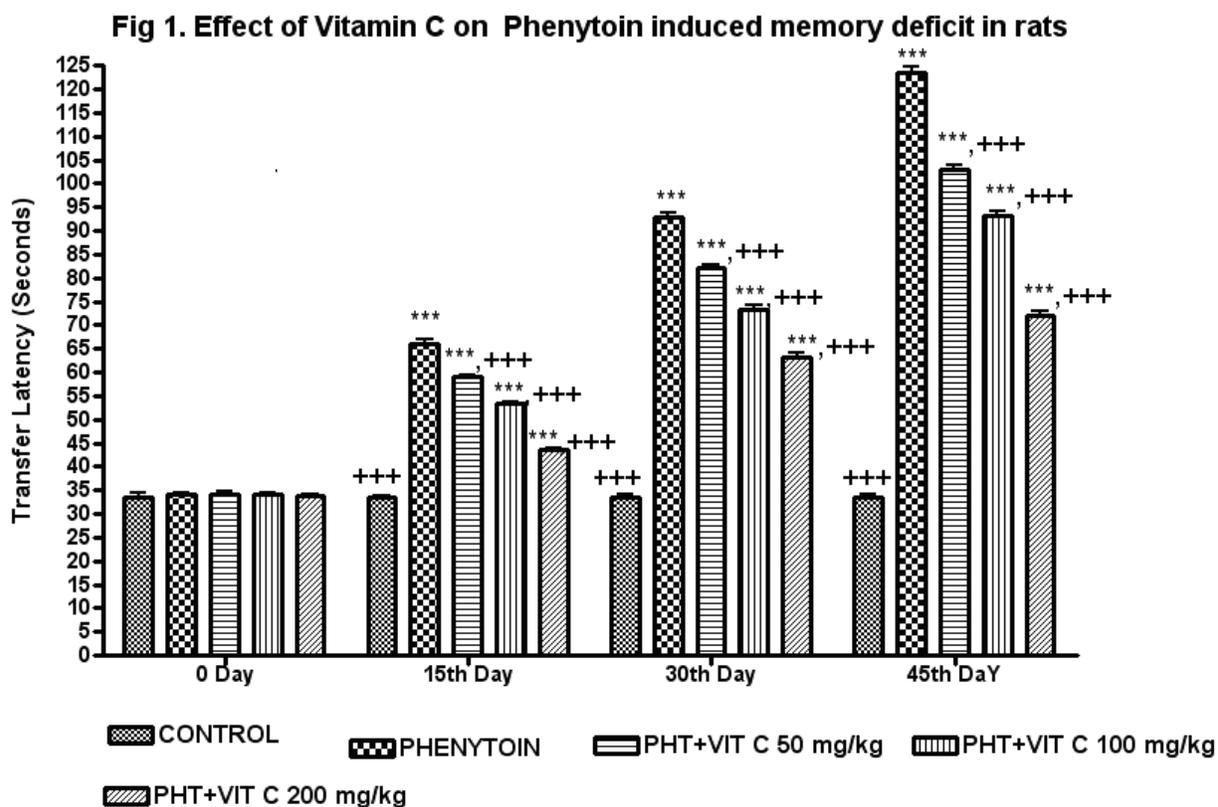
The results were expressed as mean±SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) with Tukey's post hoc statistical tests. $p < 0.05$ was considered significant.

Results

Effect of vitamin C on phenytoin induced memory impairment:

Fig.1. explains the effect of chronic treatment of phenytoin, phenytoin + vitamin C on memory. There was no significant difference in the transfer latency of the control, phenytoin, and phenytoin with vitamin C (50, 100, 200 mg/kg) pre treated 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 Vitamin C 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 103 ± 0.99 ($p < 0.001$), 93.3 ± 0.89 ($p < 0.001$), and 72.1 ± 0.94 ($p <$

0.001) in vitamin C 50, 100 and 200 mg/kg co-administered groups respectively on 45th day of the study. Vitamin C 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 values.



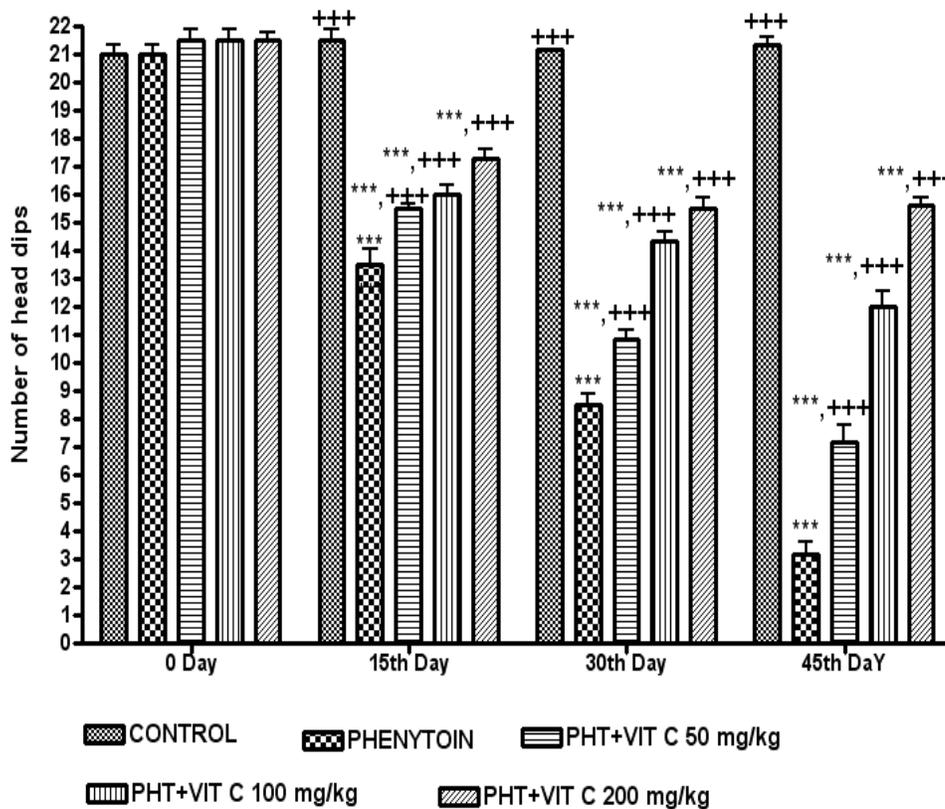
Values are expressed as mean \pm SEM of 6 animals ***p < 0.001 vs Control group; +++p < 0.001 vs Phenytoin group

Effect of vitamin C on phenytoin impaired exploratory activity

There was no significant difference in the exploratory activity of the control, phenytoin treated, phenytoin with vitamin C (50, 100, 200 mg/kg) pre treated groups on the 0 day of study. The exploratory activity was assessed by the number of head dipping into the holes of the hole board apparatus. The number of the head dipping decreased from 21 ± 0.32 (0 day) to 3.16 ± 0.47 (45th day) ($p < 0.001$) in phenytoin-treated animals. Co administration of Vitamin C in all the three doses significantly increased the exploratory movements from 15th day till 45th day. The number of head dipping increased from 3.16 ± 0.47 in the phenytoin treated group to 7.16 ± 0.6 ($p < 0.001$), 12 ± 0.57 ($p < 0.001$) and 15.6 ± 0.3 ($p < 0.001$) in vitamin C 50, 100 and 200 mg/kg co-

administered groups respectively on 45th day of the study. Vitamin C at all the three doses produced significant reversal of phenytoin impaired exploratory behavior in a dose dependent manner but the values did not reach the normal values (Fig.2).

Fig 2. Effect of Phenytoin and Phenytoin + Vitamin C on exploratory behaviour in rats

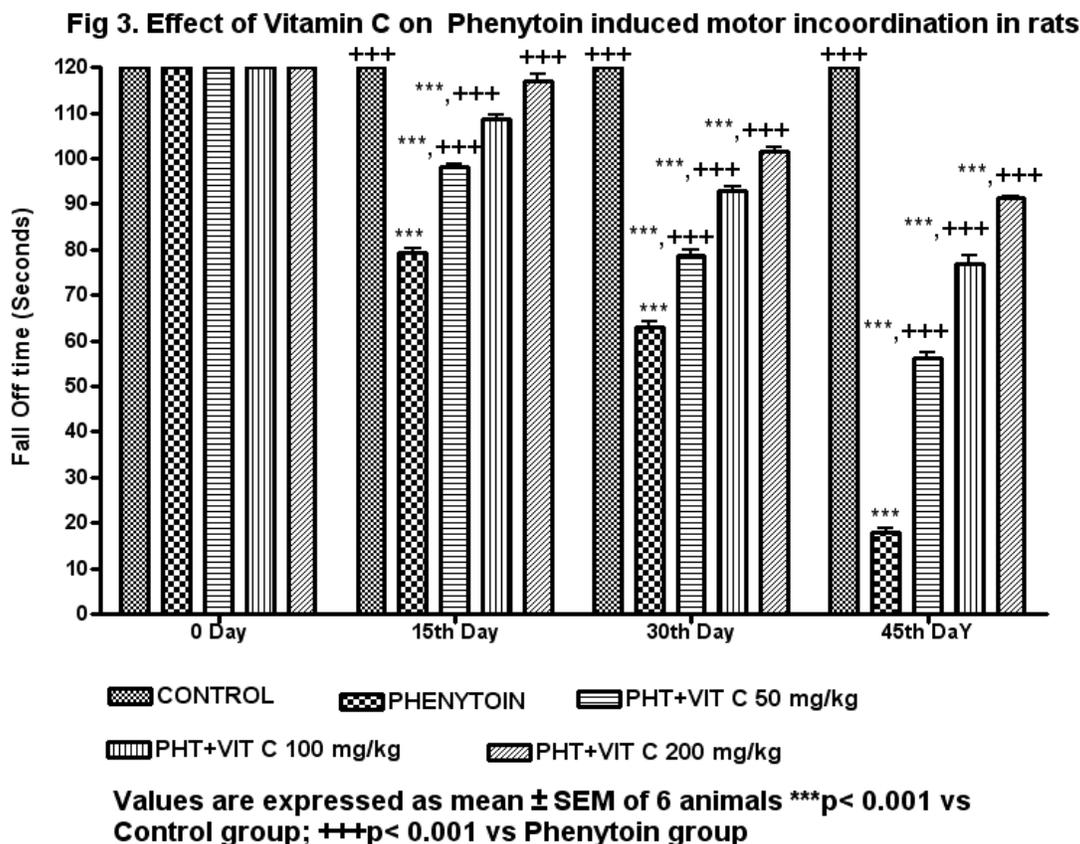


Values are expressed as mean \pm SEM of 6 animals ***p< 0.001 vs Control group; +++p< 0.001 vs Phenytoin group

Effect of vitamin C on phenytoin-induced motor inco-ordination

There was no significant difference in motor coordination of the control, phenytoin treated, phenytoin with vitamin C (50, 100, 200 mg/kg) pre treated groups on the 0 day of the study. Phenytoin (20 mg/kg, p.o.) significantly impaired Rota Rod performance of rats from the 120 sec (0 day) to 17.83 \pm 0.94 sec on 45th day (p< 0.001). Co-administration of Vitamin C in all the three doses significantly improved the motor coordination from 15th day till 45th day. The values increased from 17.83 \pm 0.94 sec in the phenytoin treated group to 56.16 \pm 1.4 (p< 0.001), 76.8 \pm 1.95 (p< 0.001) and 91.3 \pm 0.49 (p < 0.001) in vitamin C 50, 100 and 200 mg/kg co-

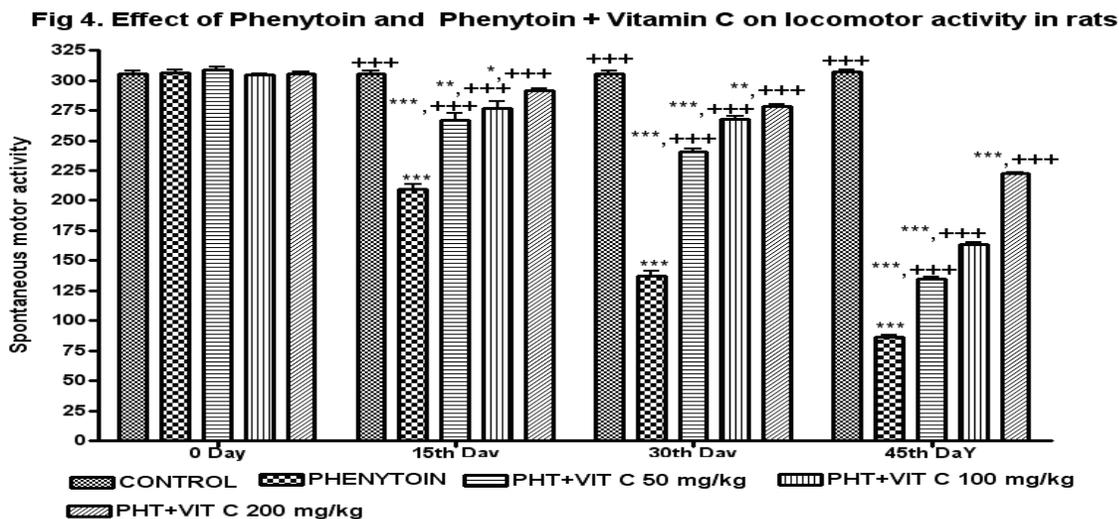
administered groups respectively on 45th day of the study. Vitamin C at all the three doses produced significant reversal of phenytoin induced impairment of motor co-ordination in a dose dependent fashion but the values did not reach the normal (Fig.3).



Effect of vitamin C on phenytoin impaired locomotor activity

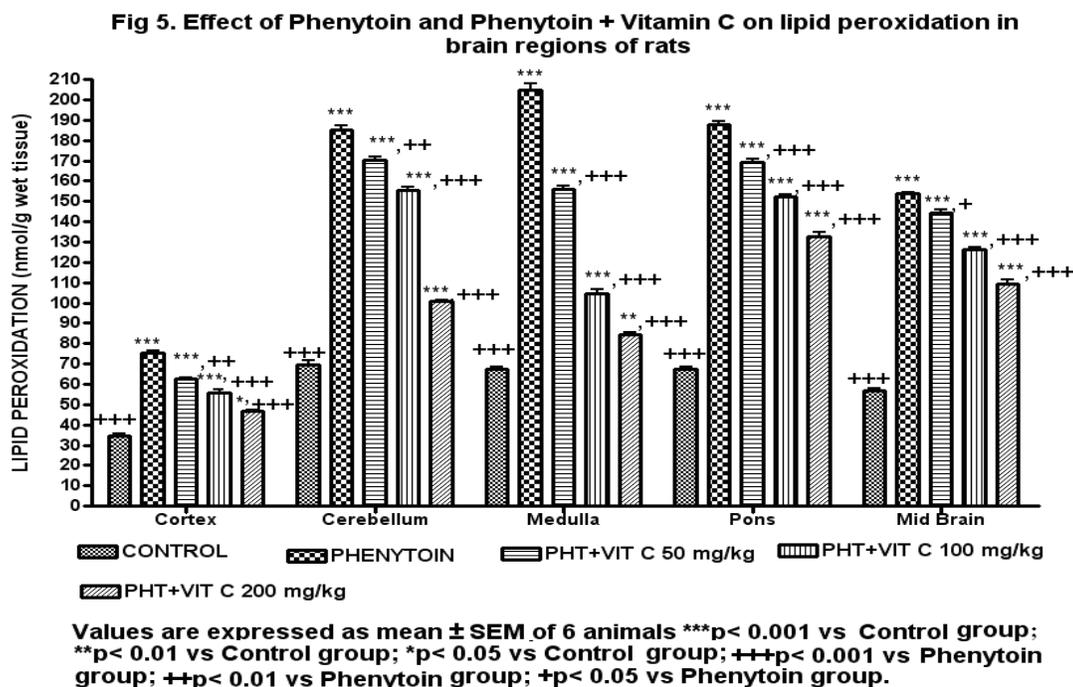
There was no significant difference in spontaneous motor activity of the control, phenytoin, and phenytoin with vitamin C (50, 100, 200 mg/kg) pre treated groups on the day of study. Phenytoin 20 mg/kg, p.o., significantly decreased the spontaneous motor activity by reducing the performance of the rats on Actophotometer count from 306.33 ± 2.4 (0 day) to 86.16 ± 1.49 (45th day) ($p < 0.001$). Co administration of Vitamin C 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 134.66 ± 1.54 ($p < 0.001$), 163.83 ± 1.3 ($p < 0.001$) and 222.5 ± 1.11 ($p < 0.001$) in vitamin C 50, 100 and 200 mg/kg co-administered groups respectively on 45th day of the study.

Vitamin C 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 values (Fig.4).



Effect of vitamin C on regional brain MDA levels

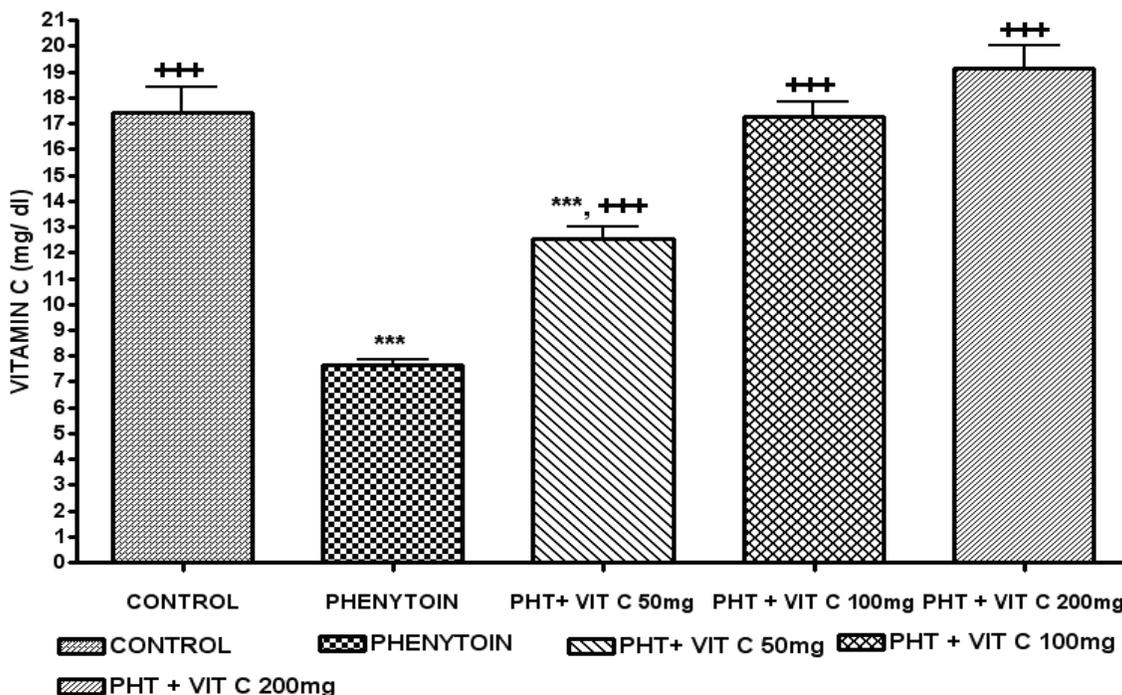
Phenytoin showed a significant rise in lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex. Vitamin C significantly reduced ($p < 0.001$) the lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex dose dependently but the values did not reach the normal values when compared with the control group (Fig. 5).



Effect of vitamin C on plasma vitamin C levels

Fig. 6. shows the effect of chronic treatment of phenytoin, phenytoin + vitamin C on plasma vitamin C levels. Chronic phenytoin treatment significantly decreased Vitamin C levels when compared to control animals. Vitamin C at the dose of 50, 100 and 200mg/kg significantly reversed the phenytoin decreased vitamin C levels when compared to phenytoin treated animals.

Fig 6. Effect of chronic treatment of phenytoin and phenytoin + vitamin C on plasma vitamin C



Values are expressed as mean± SEM of 6 animals; ***p < 0.001 vs control group
 ++p < 0.001 vs phenytoin group

Effect of vitamin C on phenytoin induced alterations in regional brain histopathology

Fig. 7 shows the effect of phenytoin and vitamin C on periventricular neurons. Control group exhibited normal periventricular region while phenytoin treated group illustrated severe congestion and remarkable degeneration of periventricular neurons. Phenytoin + 50mg/kg vitamin C treated group showed less congestion and periventricular neuronal degeneration than phenytoin group, whereas Phenytoin + 100mg/kg vitamin C treated group showed no congestion and negligible periventricular neuronal degeneration and Phenytoin + 200mg/kg vitamin C treated group showed no congestion whereas periventricular neuronal cells were intact.

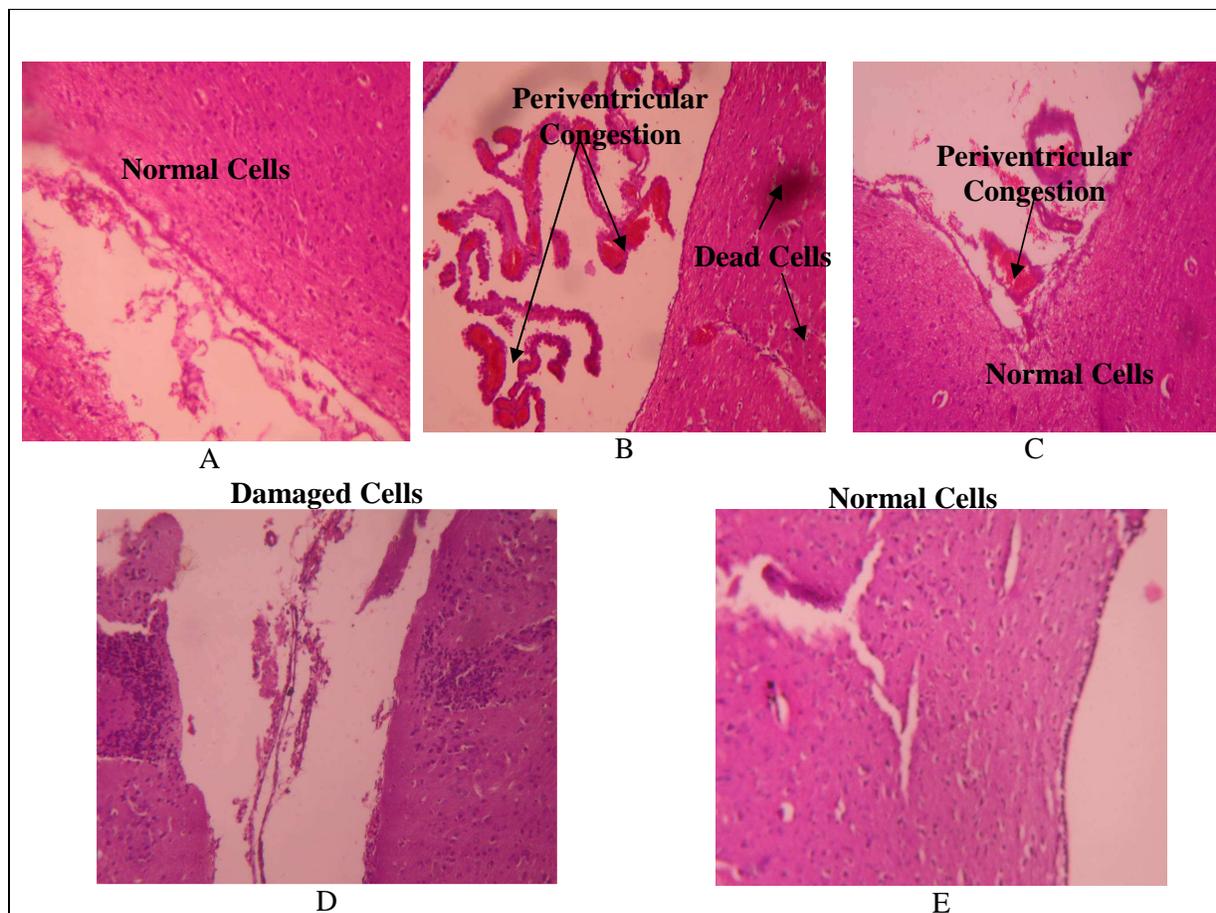
Histopathology:

Fig. 7. Micrograph showing effect of phenytoin and vitamin C on periventricular neurons. (A) Control showing normal periventricular region (B) Phenytoin treated group showing severe congestion and remarkable degeneration of periventricular neurons. (C) Phenytoin + 50mg/kg vitaminC treated group showed less congestion and periventricular neuronal degeneration than phenytoin group. (D) Phenytoin + 100mg/kg vitaminC treated group showed no congestion and negligible periventricular neuronal degeneration. (E) Phenytoin + 200mg/kg vitamin C treated group showed negligible congestion whereas periventricular neuronal cells are intact.

Fig. 8. shows the effect of vitamin C on phenytoin-induced histopathological changes in rat hippocampal neurons (all three layers). Normal Pyramidal neurons of hippocampus were observed in case of control. In phenytoin treated group there was an obvious decrease in the number and density of cells. Phenytoin + Vitamin C (50mg/kg) treated group showed a decrease in the number and density of cells and congestion. The hippocampal neurons of Phenytoin + Vitamin C (100 and 200mg/kg) treated group had normal density of cells resembling the control group.

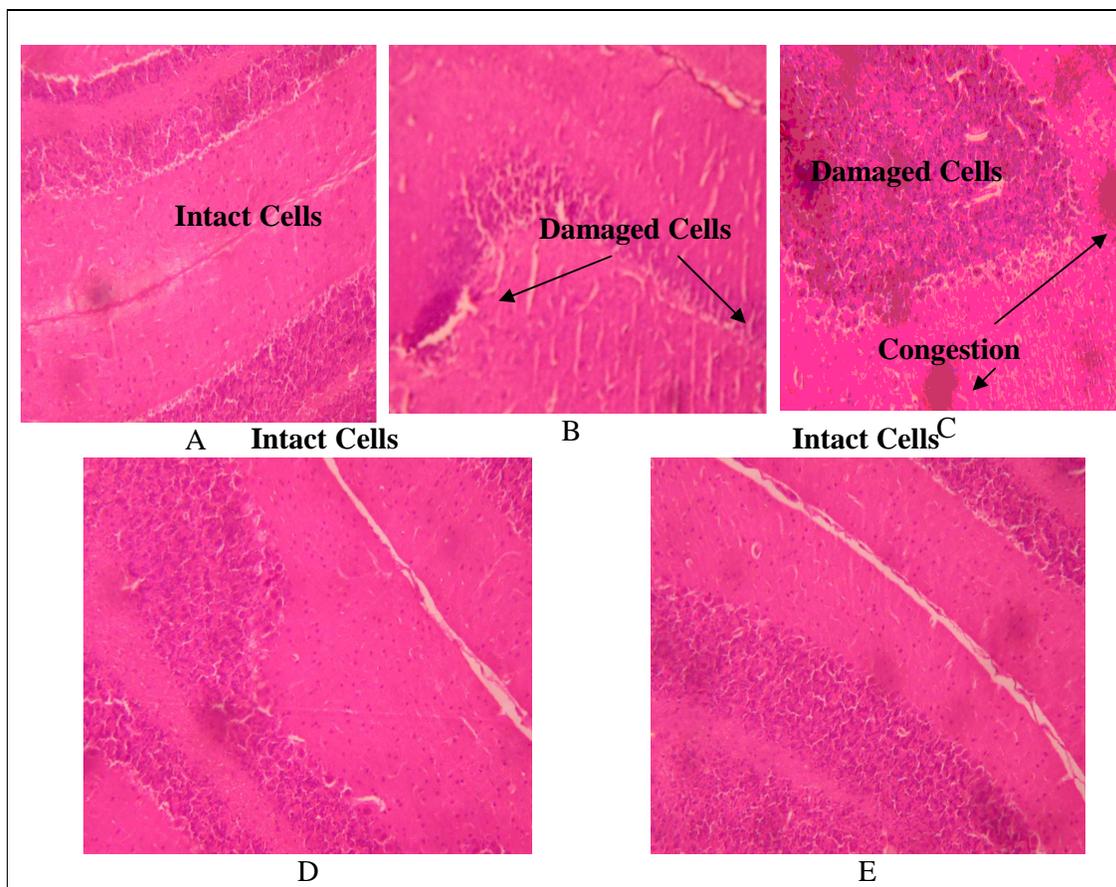


Fig. 8. Micrographs showing effect of vitamin C on phenytoin-induced histopathological changes in rat hippocampal neurons (all three layers). (A) Pyramidal neurons of hippocampus in a control case. (B) The same hippocampal subfield there is an obvious decrease in the number and density of cells in phenytoin treated rat. (C) The same hippocampal subfield in phenytoin + Vitamin C (50mg/kg) treated group showing a decrease in the number and density of cells and congestion (D) Hippocampal subfield in phenytoin + Vitamin C (100mg/kg) treated group appearing similar to that of control. (E) Hippocampal subfield in phenytoin + Vitamin C (200mg/kg) treated group appearing similar to that of control.

Discussion

The results of the present study indicate that phenytoin significantly impaired the motor coordination, memory, exploratory behavior and spontaneous motor activity. In addition to this it was also observed that phenytoin significantly decreased the levels of non enzymatic endogenous antioxidant vitamin C in blood and increased the lipid peroxidation in brain regions. Chronic phenytoin treatment also damaged the brain regions as evidenced by brain histopathological investigations.

Phenytoin impaired the Rota Rod performance of rats. This can be attributed to phenytoin induced ataxia which was also observed by many other previous investigators^{37,15,25}. Vitamin C 200mg/kg significantly reduced the ataxia induced by phenytoin on 15th day as the values reached nearer to normal values, though vitamin C in all

the three doses significantly reversed phenytoin induced ataxia but the values did not reach normal till the end of the study. This indicates free radical generation from the 15th day to 45th day which was not completely quenched by vitamin C leading to lipid peroxidation in vital brain regions regulating motor coordination. The significant increase in lipid peroxidation in cerebellum may be one of the contributing factors for the incidence of ataxia since cerebellum maintains motor coordination. Vitamin C significantly reduced the lipid peroxidation induced by phenytoin at a higher dose.

Hole board test helps in assessing the exploratory behaviour and alertness of the rats. Phenytoin significantly reduced the exploratory behaviour and alertness as observed by the decrease in number of head dippings in the holes of the hole board. Phenytoin induced decrease in exploratory behaviour³⁷ and dose related sedation¹⁵. Exploratory activity or alertness are the parameters of CNS activity, since phenytoin reduces alertness the drug causes sedation and decreases the wakeful state of the animals. Vitamin C significantly increased the phenytoin reduced head dippings in a dose dependent manner, values did not reach normal range. Sleep and wakeful cycle is maintained by reticular activating system in the mid brain and cortex. Vitamin C supplementation improved the alertness in phenytoin treated rats by significantly reducing phenytoin induced lipid peroxidation in mid brain and cortex.

Cognitive deficit is one of the major problems associated with epilepsy, underlying pathology and drug therapy leads to disturbance in cognitive function³⁸. Phenytoin is one of the cheapest and widely used anticonvulsant, but is known to affect the learning and memory^{25,39,40}. Cognition is adversely influenced by many factors especially in epileptic seizures, it is logical to evaluate the effect of AEDs on cognitive function in experimental animals with out any additional complexities of the disease state. In the present study memory function was assessed by elevated plus maze. Elevated plus maze was used initially for evaluation of anxiety but it is also be used as a model for assesment of learning and memory in rodents⁴¹⁻⁴⁶. It is hypothesized that if the transfer latency (the time taken by the animal to move from open arm to the enclosed arms) is prolonged it indicates the impairment of learning and memory. In the present study, chronic treatment with phenytoin 20 mg/kg appreciably caused memory impairment (prolonged the transfer latency) in non- epileptic rats in elevated plus maze paradigm, signifying the risk of this drug causing cognitive impairment even in healthy individuals as well.

Our results are online with previous studies wherein learning was impaired by most of the conventional anti epileptics in non-epileptic rats ^{25,47,48} , non epileptic pigeons ⁴⁹⁻⁵¹ and also in healthy volunteers ⁵²⁻⁵⁴ .

Chronic administration of phenytoin is likely to cause oxidative stress in experimental animals ⁵⁵⁻⁶⁰ and long-term treatment with phenytoin reduced activities of endogeneous antioxidant enzymes like superoxide dismutase, glutathione reductase, glutathione peroxidase, Vitamin C, Vitamin E and increased thiobarbituric acid reacting substances (TBARs) in epileptic patients. The results of the present study also illustrated an increase in oxidative stress in the phenytoin treated rats, as indicated by increase in MDA levels in different regions of brain. MDA is an end product of lipid peroxidation⁶¹ and also is a biochemical marker to measure lipid peroxidation which indicates the extent of neuronal damage in various brain regions²⁵. In the present study phenytoin showed an increased lipid peroxidation in different brain regions. Memory is maintained by various groups of neurons present in hippocampus, cerebral cortex, cerebellum and mid brain. The cerebral cortex is the part of the brain involved in many higher level tasks such as language, memory, and consciousness. Memory of learned motor sequences (motor subroutines) seems to be stored in the supplementary motor area which is called the "executive centre" of the brain. A portion of this area which has proven amenable to animal studies is so-called "working memory". Cerebellar learning critically involves the cerebellar cortex, while the cerebellar nuclei play a more critical role in long term memory storage ⁶²⁻⁶⁴ and memory is consolidated in the cerebellum ^{65,66}. In the present study phenytoin showed increased lipid peroxidation in cerebral cortex, cerebellum and mid brain, which was confirmed by histopathological studies. Increased lipid peroxidation in different brain regions causes peroxidative injury to the neuronal membranes, macromolecules, alters neurotransmitters, disrupts key neuronal functions and perturbs motor function as well ^{67,68}. Neuronal damage in hippocampus, cortex, cerebellum and mid brain by phenytoin may be in fact responsible for memory impairment.

Actophotometer was used to assess the spontaneous motor activity. Phenytoin significantly reduced the spontaneous motor activity, indicating the CNS depressant activity of phenytoin. Supplementation with vitamin C increased the spontaneous motor activity and significantly reversed phenytoin induced depression in a dose dependent fashion.

Long term administration of phenytoin (20mg/kg) significantly reduced the plasma vitamin C concentration whereas chronic co-administration of vitamin C (50, 100, 200 mg/kg) along with phenytoin significantly reversed phenytoin reduced decrease in the plasma vitamin C concentration reduced by phenytoin. Charlton et al. (2004) reported that the plasma vitamin C levels were lower in subjects suffering with dementia⁶⁹.

Ascorbic acid (vitamin C) is an essential micronutrient required for normal metabolic functioning of the body^{70,71}. It acts as an electron donor and therefore a reducing agent⁷². Also, it is a cofactor for several enzymes involved in the biosynthesis of some neurotransmitters⁷³⁻⁷⁵. In addition it has modulatory action on brain neurotransmitter like cholinergic, serotonergic and dopaminergic system⁷⁶⁻⁷⁸, important in learning and memory processes⁷⁹. Local application of ascorbic acid enhanced the response of neurons to dopamine⁸⁰⁻⁸³ and glutamate⁸⁴. Glutamate has a critical role in learning and memory processing⁸⁵. Ascorbic acid potentiates hippocampal evoked potential activity, one of the brain structures involved in many kinds of learning and memory⁸⁶.

Vitamin C protects against cognitive impairment⁸⁷ and Delwing et al. (2006) recommended that supplementation with vitamin C may be a novel therapeutic strategy for the cognitive dysfunction associated with hyperprolinemia type II⁸⁸. Cognitive impairment is prevented by the combination of vitamins E and C in post-menopausal women^{75,88-92}. Arzi et al. (2004) confirmed that chronic oral supplementation of vitamin C (300 mg/kg, 60 days) improved step-down avoidance learning of aged but not young mice⁹³. Ascorbic acid prevents memory impairment caused by scopolamine⁹¹, hyperprolinemia⁸⁸, ovariectomy⁹⁰, methylmalonic acid⁹⁴, homocystein⁹² and also reduces the incidence of dementia caused by aging^{75,91,93}. Online with these in the present study also chronic treatment with ascorbic acid (50, 100 and 200mg/kg) for 45 days showed an improvement in phenytoin induced memory impairment in rats.

Ascorbic acid is a potent antioxidant⁹⁵⁻⁹⁷ highly concentrated in the central nervous system⁷¹. Ascorbic acid prevents memory deficit by its antioxidant effect^{76,89,92}. In the present study also vitamin C (50, 100, 200 mg/kg) supplementation with phenytoin decreased the lipid peroxidation in different brain regions, also reversed the phenytoin induced decrease in non enzymatic antioxidant vitamin C. Vitamin C supplementation is proved to improve tardive dyskinesia induced by antipsychotic drugs³².

The histopathological changes in brain were examined by using HE stain in sequential brain sections to confirm the extent of damage induced by phenytoin. Brain sections of phenytoin treated rats stained by HE showed damaged cells and congestion in periventricular region, disorganization and down regulation of cells in the hippocampus which confirms phenytoin induced apoptosis in cortex, periventricular region and hippocampus. Vitamin C at the dose of 50mg/kg was not much effective in reversing the phenytoin induced alterations in the brain regions as they damaged cortical cells, periventricular congestion and hippocampal down regulation to an appreciable extent. The higher doses of Vitamin C (100, 200 mg/kg) were effective in reversing phenytoin induced damages in rat brain regions as they showed damage to a lesser extent.

Conclusion

In conclusion, the results of the present investigation suggest vitamin C decreased phenytoin induced oxidative stress, lipid peroxidation and behavioural disturbances. It is proved that long-term treatment with phenytoin causes serious behavioral abnormalities which may be due to increased oxidative stress induced by the drug. This is evidenced by increased regional brain lipid peroxidation and brain histopathology reports. Vitamin C at a dose of 100, 200 mg/kg appears to be effective against oxidative stress and thereby behavioral abnormalities caused by phenytoin. This investigation reports beneficial effect of Vitamin C on phenytoin induced behavioral abnormalities.

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