Research Article

Effect of Vitamin C on Behavioral Abnormalities and Regional Brain Lipid Peroxidation

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ABSTRACT

The present study is proposed to explore the protective effect of vitamin C against carbamazepine induced behavioral abnormalities via oxidative stress in brain tissue. Administration of carbamazepine (50 mg/kg) for 45 days in rats caused memory impairment along with decrease in locomotor activity, muscular coordination and spontaneous motor activity. Carbamazepine was also observed to increase the lipid peroxidation in cortex, midbrain, medulla, pons and cerebellum. Histopathological studies on brain regions showed necrosis and congestion in carbamazepine group. Supplementation of vitamin C (50, 100 and 200 mg/kg) along with carbamazepine for 45 days decreased the lipid peroxidation, improved cognition, muscle coordination, exploratory behavior and locomotor activity that were adversely affected by carbamazepine. Histopathological analysis also supported the protective effect of vitamin C supplementation against carbamazepine induced degeneration in brain. The results of the present study indicated that vitamin C is effective in preventing carbamazepine induced behavioral abnormalities and oxidative stress in a dose dependent manner.

Keywords: Carbamazepine, vitamin C, antioxidant, oxidative stress, behavioral abnormality.

INTRODUCTION

Epilepsy is a common chronic neurological disorder characterized by seizures (Blume et al., 2001), which are transient signs and symptoms of abnormal, excessive or hypersynchronous neuronal activity in the brain (Fisher et al., 2005). Epileptic disorders affect approximately 0.5-1% of human population. Over 2/3rd of cases are idiopathic, the rest being symptomatic, owing to alcohol withdrawal, head trauma, genetic predisposition or epileptogenic drugs (Delorenzo et al., 2005). It is estimated that there are 55,00,000 epileptic patients in India, 20,00,000 in USA and 3,00,000 in UK. Three to five percent of the population have a seizure sometime in their life and half to one percent of the population have 'active epilepsy' (Sridharan, 2002).

The ultimate goal in the treatment of epilepsy is to get rid of seizure without potential side effects and provide optimal quality of life. The conventional antiepileptic drug like carbamazepine causes several serious side effects notably neurotoxicity (Gupta and Malhotra, 1997). Carbamazepine in moderate dose adversely affects memory (Forsythe, 1991). Carbamazepine was observed to impair learning and memory in a dose dependent fashion and produces cognitive dysfunction (O'Dougherty, 1987) which was associated with oxidative stress (Arora et al., 2010). The cultured hippocampal neurons exposed to carbamazepine showed degeneration and apoptosis (Araújo et al., 2004). Metabolic breakdown of carbamazepine by the cytochrome P_{450} pathway generates toxic drug intermediates, reactive oxygen species (Opladen et al., 2010) and free radicals (Reiter

et al., 2002), which induce lipid peroxidation in brain, neurodegeneration (Singh et al., 2004) and dementia (Clarkson, 1995).

Anticonvulsant medications such as carbamazepine are cerebellar toxins (Autti-Ramo et al., 2002) producing cerebellar dysfunction and ataxia (Brust, 2006). The cerebellum plays an important role in motor control and cognitive functions (Wolf et al., 2009). Though the cerebellum is not involved in initiation of movements, it contributes to coordination, precision and accurate timing. It receives input from sensory systems, other parts of brain and spinal cord and fine tunes the motor activity (Fine et al., 2002). Since carbamazepine causes cerebellar dysfunction it produces disturbances in fine movement, equilibrium, posture and motor learning (Boyden, 2004). It has been reported that brain has a relatively low antioxidant defense system (Mates, 2000; Zhang et al., 2008). Brain is particularly susceptible to peroxidation due to simultaneous presence of high levels of poly unsaturated fatty acids and iron, (Halliwell and Gutteridge, 1989) which is the target of free radical damage. Excessive production of free radicals and oxidative stress (Lehtinen and Bonni, 2006) is implicated in the pathogenesis of neurological disorders, including epilepsy. Free radical induced oxidative stress is the common factor observed in the brain regions of epileptic patients (Liao et al., 2004).

The high rate of oxidative metabolism coupled with the low antioxidant defenses and abundant polyunsaturated fatty acids make the brain highly vulnerable to free radical damage (Devi et al., 2008; Bauer and Bauer, 1999; Andorn et al., 1990). Though iron is essential for normal neurological function in the synthesis of neurotransmitters and myelin and is heterogeneously distributed among the different regions and cells of the brain. However, it is the most important inducer of reactive oxygen species causing neurodegeneration and generates highly reactive hydroxyl, alkoxyl and peroxyl radicals from hydrogen peroxide and lipid peroxides (Halliwell, 2001). By redox cycling reactions iron produces reactive radicals such as superoxide anion, nitric oxide and reactive nitrogen species (Jomova, 2010) in biological systems. Reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation leading to cancer, cardiovascular disease, diabetes, atherosclerosis and neurological disorders. The mechanism of formation of free radicals is highly influenced by the action of cellular antioxidants such as vitamin C. Our earlier studies have proofs on phenytoin induced cognitive impairment, disturbance in motor coordination, exploration behaviour and locomotor activity, where spirulina and vitamin C attenuated phenytoin induced cognitive impairment, disturbance in motor coordination, exploratory behaviour and locomotor activity (Santhrani and Puspha, 2008; Saraswathy et al., 2011).

Vitamin C, an antioxidant, protects the cell membrane of vital organs from oxidative damage (Madan and Madan, 2009). It also plays a major role in improving attention, cognition, mood and motor control. Treatment with vitamin C improves neurobehavioral deficits and caused a significant reduction in MDA concentration (Ambali et al., 2010). Vitamin C is an important chain-breaking antioxidant responsible for scavenging free radicals and suppression of oxidative stress (Niki, 1987; Mann and Newton, 1975). Behavioural toxicity of carbamazepine is considered to be due to production of reactive oxygen species. The study is proposed to explore the effect of vitamin C on carbamazepine 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±3^{o} 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 six animals. The control group received drinking water orally daily by gavage. The carbamazepine group received 50 mg/Kg carbamazepine dissolved in water daily by oral gavage for 45 days between 11.00 hrs and 12.00 hrs. The group I received 50 mg/kg of ascorbic acid orally 1 hr prior to administration of 50 mg/Kg carbamazepine dissolved in water daily by oral gavage for 45 days between 11.00 hrs and 12.00 hrs. The group II received 100 mg/kg of ascorbic acid orally 1 hr prior to administration of 50 mg/Kg carbamazepine dissolved in water daily by oral gavage for 45 days between 11.00 hrs and 12.00 hrs. The group III received 200 mg/kg of ascorbic acid orally 1 hr prior to

administration of 50 mg/Kg carbamazepine dissolved in water daily by oral gavage for 45 days between 11.00 hrs and 12.00 hrs. The animals were subjected to behavioral test and are sacrificed on the 45th day of drug administration. Regional brain lipid peroxidation was estimated at the end of the study period on 45th day.

Behavioral test

Elevated plus maze test was carried out on 1st, 15th, 30th and 45th day of the drug administration. Only one animal was tested at a time. The memory test was performed 24 hrs after administration of the carbamazepine.

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 alertness

This test was done using hole board, which consisted of a 0.5 m^3 wooden board with 16 holes (3 cm 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.

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 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 retroorbital plexus under light ether anaesthesia. 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 carbamazepine. The brains were quickly removed, cleaned with chilled saline, dissected into cortex, mid brain, medulla, pons and cerebellum according to the method of Glownski and Iversen (Glownski, 1966). The separated brain regions were stored at -80°C and biochemical analysis was carried out within the next 7 days.

Estimation of non enzymatic antioxidants

Estimation of vitamin C

To 0.5 ml of plasma, 1.5ml of 6% TCA was added and centrifuged (3500 rpm/ 20 min). To 0.5 ml of the supernatant 0.5ml of DNPH reagent (2% DNPH and 4% thiourea in 9 N H2SO4) was added and developed color was read at 530 nm after 30 min (Omaye et al., 1979).

Tissue preparation

Brain regions were separately thawed and 10 % (w/v) homogenate was made with ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots were prepared to determine lipid peroxidation.

Determination of MDA content (Lipid peroxidation)

Malondialdehyde, was measured spectrophotometrically by the method of Colado et al., (1997), using 1, 1, 3, 3-tetraethoxypropane as standard. Malondialdehyde is expressed as nmol/g tissue. To 500 µl of tissue homogenate in phosphate buffer (pH 7.4), 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 15 min at 90° C cooled to room temperature and was centrifuged at 12,000 g for 10 min. Pink colored supernatant was obtained, which was measured spectrophotometrically at 532 nm immediately.

Histopathological studies

A histopathological study in brain tissue was conducted according to Li et al., (1998). Rats were deeply anesthetized under ether anesthesia. The brain was fixed by transcardial perfusion, first with 50 ml of phosphate-buffered saline (0.02 M, pH 7.4), then with 220 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline, pH 7.4 for pre-fixation of the tissue. Then the brain tissue was dissected out carefully and was kept in 4% paraformaldehyde overnight for post-fixation. After 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 picked up on poly-l-lysine coated slides and were stained with hematoxylin and eosin (HE).

Statistical analysis

The results were expressed as mean±SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey Kramers multiple comparison statistical test. $p< 0.001$was considered significant.

RESULTS

Memory impairment

Fig. 1. explains the effect of chronic treatment of carbamazepine, carbamazepine supplemented with vitamin C on learnt memory. There was no significant difference in the transfer latency of the control, carbamazepine treated, carbamazepine and vitamin C (50, 100, 200 mg/kg) treated groups on 0 day of the study. The transfer latencies increased from 33.83±1.85 sec (0 day) to 127±1.59 sec (45^{th} day) ($p< 0.001$) in carbamazepine treated animals. This shows the ill effect of carbamazepine on memory. Coadministration of vitamin C in all the three doses significantly reduced the transfer latency from 15^{th} day till 45^{th} day. The values decreased from 127±1.59 sec in the carbamazepine treated group to 97±1.78 sec ($p< 0.001$), 94.16±1.53 sec ($p< 0.001$) and 74±1.43 sec ($p < 0.001$) in vitamin C 50, 100 and 200 mg/kg co-administered groups respectively on 45^{th} day of the study. Vitamin C at all the three doses produced significant reversal of carbamazepine induced memory impairment in a dose dependent fashion but the values did not reach the normal values from 15^{th} day onwards.

Test for Alertness

There was no significant difference in the exploratory activity of the control, carbamazepine treated, carbamazepine and vitamin C (50, 100, 200 mg/kg) treated groups on 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 24.83±1.37 (0 day) to 2±0.57 (45^{th} day) ($p< 0.001$) in carbamazepine treated animals. Coadministration of vitamin C in all the three doses significantly increased the exploratory movements from 15^{th} day till 45^{th} day. The number of head dippings increased from 2±0.57 in the CBZ treated group to 6.83±0.79, 12.16±0.94 ($p< 0.001$) and 15.55±0.99 ($p< 0.001$) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45^{th} day of the study. Vit C at higher doses (100, 200 mg/Kg) produced significant reversal of CBZ impaired exploratory behaviour in a dose dependent manner but the values did not reach the normal level (Fig. 2).

Motor incoordination test

There was no significant difference in motor coordination of the control, CBZ treated and CBZ with Vit C (50, 100, 200 mg/Kg) pre-treated groups on 0 day of the study. CBZ (50 mg/Kg, p.o.) significantly impaired the Rota Rod performance of rats from 120 sec (0 day) to 74.33±4.047, 58.66±3.63 and 16.5±1.47 sec on 15^{th}, 30^{th} and 45^{th} day ($p< 0.001$) respectively. The impairment was significant on 15^{th} day of treatment itself. Co-administration of Vit C in all the three doses significantly improved the motor coordination from 15^{th} day to 45^{th} day. The values increased from 16.5±1.47 sec in the CBZ treated group to 57.16±1.85 sec ($p< 0.001$), 79±2.03 sec ($p< 0.001$) and 92.83±1.35 sec ($p< 0.001$) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45^{th} day of the study. Vit C at all the three doses produced significant reversal of CBZ impaired motor co-ordination in a dose dependent fashion but the values did not reach normal (Fig. 3).

Test for Locomotor Activity

There was no significant difference in the spontaneous motor activity of the control, CBZ and CBZ with Vit C (50, 100, 200 mg/Kg)

pretreated groups on the initial day of the study. CBZ (50 mg/Kg, p.o.) significantly decreased the spontaneous motor activity count from 318.5±4.43 (0 day) to 84±1.29 (45th day) ($p< 0.001$). Co-administration of Vit C in all the three doses significantly improved the spontaneous activity from 15th day till 45th day. The values increased from 84±1.29 in the CBZ treated group to 137.66±1.4 ($p< 0.001$), 165.83±2.72 ($p< 0.001$) and 226.83±2.34 ($p< 0.001$) in Vit C 50, 100 and 200 mg/Kg co-administered groups respectively on 45th day of the study. Vit C at all the three doses produced significant reversal of CBZ impaired locomotor activity in a dose dependent fashion but the values did not reach the normal values (Fig. 4).

MDA levels of brain regions

CBZ showed a significant rise in lipid peroxidation in medulla, pons, midbrain, cerebellum and cortex. Vit 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 when compared with the control group (Fig. 5).

Plasma Vitamin C levels

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

Histopathology

Fig.7. shows the effect of vitamin C on carbamazepine induced histopathological changes in rat brain. (A) The control group showed normal cortex. (B) The carbamazepine treated group showed obvious brain necrosis. (C) The carbamazepine plus vitamin C (50 mg/kg) treated group showed gliosis and congestion in brain. (D) The carbamazepine plus vitamin C (100 mg/kg) treated group showed normal ventricles. (E) The carbamazepine plus vitamin C (200 mg/kg) treated group showed normal cerebral parenchyma resembling the control group.

DISCUSSION

In the present study it was observed that carbamazepine significantly impaired the motor coordination, memory, exploratory behavior and spontaneous motor activity while supplementation with vitamin C improved cognitive performance and motor functions. Besides, carbamazepine significantly diminished the levels of non enzymatic endogenous antioxidant vitamin C in blood and increased the lipid peroxidation in brain regions which was reversed by vitamin C. In addition to this long term carbamazepine treatment also damaged the brain regions as confirmed by brain histopathological examinations.

Carbamazepine impaired the rota rod performance of rats. In a study by Delcker it was confirmed that carbamazepine exhibits dizziness, ataxia, drowsiness and reduction of alertness (Delcker, 1997). Carbamazepine induces neurophysiologic (Wesnes, 2009; Meador, 2007) changes and motor slowing (Mecarelli, 2004). The processing speed and attention after treatment with carbamazepine deteriorated along with cognitive impairment (Kaussner, 2010). Long term administration of carbamazepine (50 mg/kg, p.o.) significantly impaired the rota rod performance of rats. Vitamin C (50, 100 and 200 mg/kg) significantly improved the motor coordination in a dose dependent fashion. The mechanisms by which vitamin C normalized the cerebellar damage and prevented impairment of motor coordination may be by free radical scavenging effect.

Hole board test helps in assessing the exploratory behavior and alertness of the rats. Carbamazepine exposure induces reduction of alertness (Delcker, 1997). In the present study also, carbamazepine administration declined the exploratory activity by decreasing the number of head dippings. Coadministration of vitamin C in all the three doses significantly increased the exploratory movements in a dose dependent manner. The reticular activating system is responsible for mental wakefulness and alertness (Steriade, 1996). The reticular activating system also helps mediate transitions from relaxed wakefulness to periods of high attention (Kinomura, 1996). Vitamin C improved the exploratory activity and alertness by offering protection to reticular activating system against oxidative stress.

The transfer latency on elevated plus-maze apparatus is an experimental paradigm which is used as the model for assessment of memory function in animals (Sharma, 1992). Epilepsy as well as antiepileptic drugs are known to induce cognitive impairment (van Rijckevorsel-Harmant, 1991). Administration of carbamazepine caused a significant impairment of learning and memory which may be due to oxidative stress **(**Reeta, 2010**).** Carbamazepine exerts decline in cognitive and

motor functions in epileptic patients, which was exacerbated at higher concentrations (Massagli, 1991). In the present study administration of carbamazepine (50 mg/kg) showed increased lipid peroxidation in cerebral cortex, cerebellum and mid brain, which was also confirmed by histopathological studies.

Long term use of certain antiepileptic drugs increases oxidative stress (Maertens, 1995; Duncan, 2003) and affects the quality of life of epileptic patients. It was reported that generation of free radicals is observed in disease process of epilepsy and during administration of antiepileptic drugs (Gupta, 2006). The additive increase in reactive oxygen species because of the disease process (Torbati, 1992) combined with carbamazepine may be hypothesized for the net increase in reactive oxygen species (Gupta, 2006). Vitamin C neutralizes free radicals, by working both inside and outside the cells to combat free radical damage. The free radicals will seek out an electron to regain their stability. Vitamin C is an excellent source of electrons therefore, it can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity (Bendich, 1990; Bindhumol et al., 2003). In an *in vitro* study, ascorbic acid behaves as an efficient antioxidant by scavenging free radicals produced by drugs, by reducing lipid peroxidation and by scavenging peroxyl, thiyl, sulphenyl, urate, nitric oxide and other radicals (Halliwell, 1996). The supplementation of carbamazepine with vitamin C may counteract oxidative stress (Dib, 2002). The results of the present study illustrated an increase in oxidative stress in the carbamazepine treated rats, as indicated by increase in malondialdehyde levels in different regions of brain which on administration of vitamin C diminished as evidenced by histopathological examination. The free radicals generated due to oxidative stress cause a cascade of neurochemical events leading to neurodegeneration. Ironically, though some antiepileptic drugs inhibit free radical generation, some antiepileptic drugs like carbamazepine generates free radicals (Reiter, 2002). Carbamazepine administration decreased the levels of endogenous antioxidants resulting in accumulation of reactive oxygen species (Arora, 2010). Various studies have shown that antiepileptic drug (Gillham, 1990; Kalviainen, 1996) such as carbamazepine caused cognitive dysfunction in rats, epileptic patients (Duncan, 2003) and normal healthy volunteers (Aldenkamp, 1995; Prevey, 1996).

The locomotor activity was measured using an actophotometer (Turner, 1965; Reddy, 1998; Shah, 2011; Sumanth, 2010). Carbamazepine mainly affects motor function (Trimble, 1990; Gao, 1993) and decreases the spontaneous motor activity, indicating its central nervous system depressant activity (Braathen, 1997). In the present study, supplementation with vitamin C improved the spontaneous motor activity and significantly reversed carbamazepine induced depression in a dose dependent fashion.

Frontal cortex is associated with working memory whereas the posterior dorsolateral region is crucial for visual associative learning (Petrides, 1993a; 1993b). The frontal lobe of the cerebral cortex, especially the prefrontal area is involved in the mediation of executive functions and motor coordination (Dames, 1993; Lepage, 1999; Stuss, 1986; Swartz, 1996). Executive functions represent a cognitive construct (Duncan, 1986; Shallice, 1982; Welsh, 1988) which include planning, self-monitoring, organized search, concept formation, attention and impulse control. The action of vitamin C in the prefrontal area of the cortex may be the probable mechanism by which spontaneous motor activity was increased. Carbamazepine and carbamazepine-10,11-epoxide, the metabolite of carbamazepine are equipotent in producing neurotoxicity (Bourgeouis and Wad, 1984) which may affect the motor function. Carbamazepine provokes cerebellar dysfunction (Diener and Dichgans, 1988) and induces a significant decrease of postural stability (Delcker, 1997). Carbamazepine generates adverse effect such as psychomotor dysfunction ((Lesser, 1984; McPhee, 1986; Wildin et al., 1993) sedation, lack of concentration, ataxia probably by its action in the cerebellum. The effect of vitamin C on carbamazepine induced histopathological changes in rat showed normal cortex in control group. Obvious brain necrosis in carbamazepine treated group support the fact that the oxidative stress is involved in carbamazepine administration due to increase in free radical formation. In carbamazepine + Vitamin C (50 mg/kg) treated group it showed gliosis and congestion in brain indicating slight improvement by ascorbic acid. This gives the evidence of neuroprotective activity of ascorbic acid by the removal of free radicals produced during carbamazepine administration. The carbamazepine + Vitamin C (100 mg/kg) treated group showed normal ventricles and carbamazepine + Vitamin C (200 mg/kg) treated group showed normal cerebral parenchyma resembling the control group.

Thus, the results suggest that oxidative stress mediated by carbamazepine exerts its pathologic effects during carbamazepine administration and the neuroprotective role of ascorbic acid can be mediated by a reduction in lipid peroxidation levels. Possibly, this reduction is due to the modulatory activity of ascorbic acid in the antioxidant enzymes such as superoxide dismutase and catalase in the brain.

CONCLUSION

Long term treatment with carbamazepine causes serious behavioral abnormalities which may be due to increased oxidative stress. This is confirmed by disturbed behavioral pattern, increased regional brain lipid peroxidation and brain histopathological reports. Vitamin C at a dose of 50, 100 and 200 mg/kg appears to be effective against oxidative stress and thereby behavioral abnormalities caused by carbamazepine. The result of the current investigation suggests that vitamin C diminished carbamazepine induced oxidative stress, lipid peroxidation and behavioral disturbances.

ACKNOWLEDGEMENT

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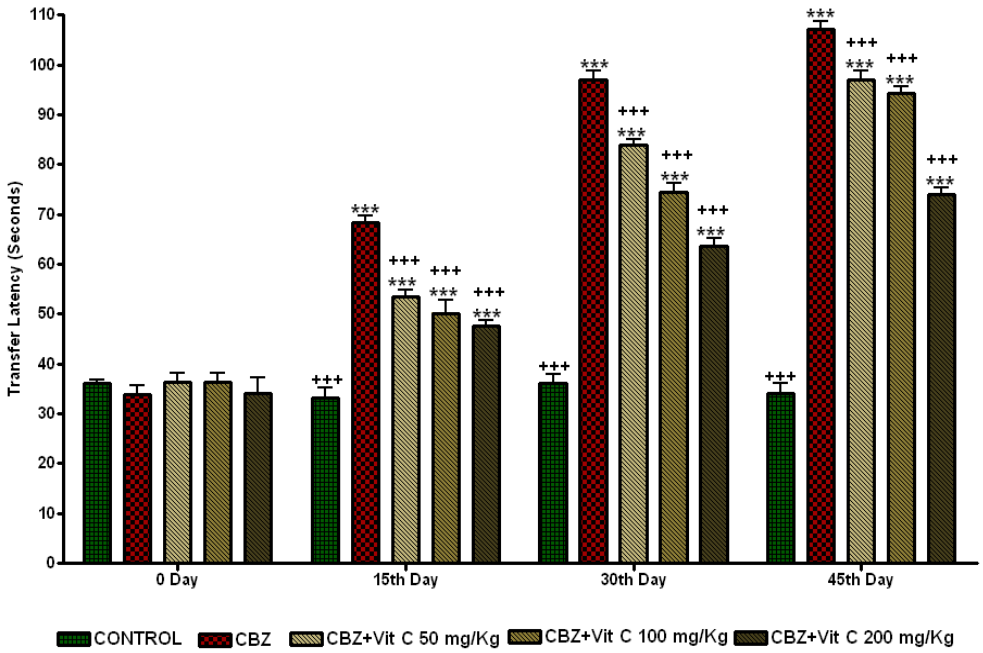
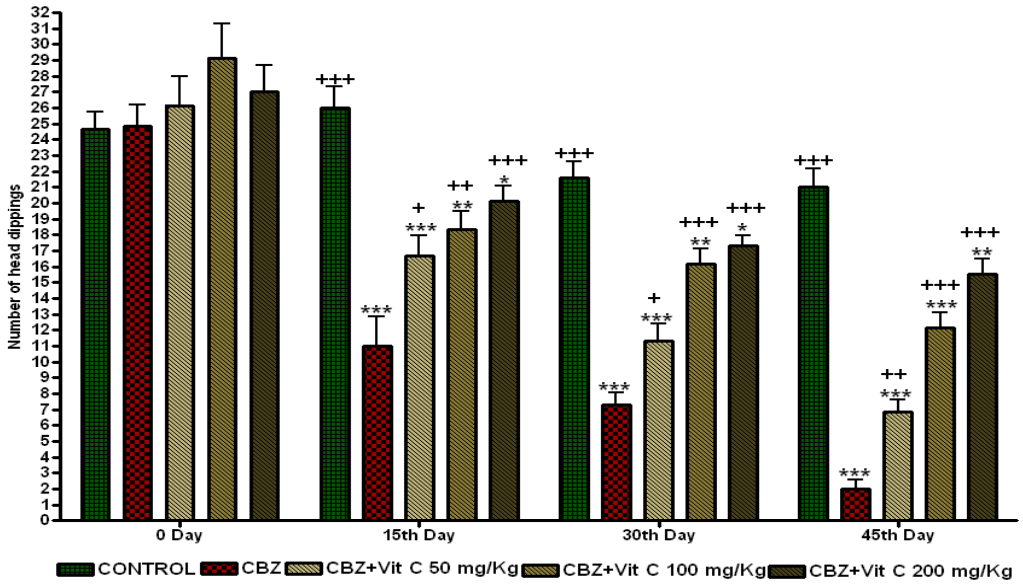
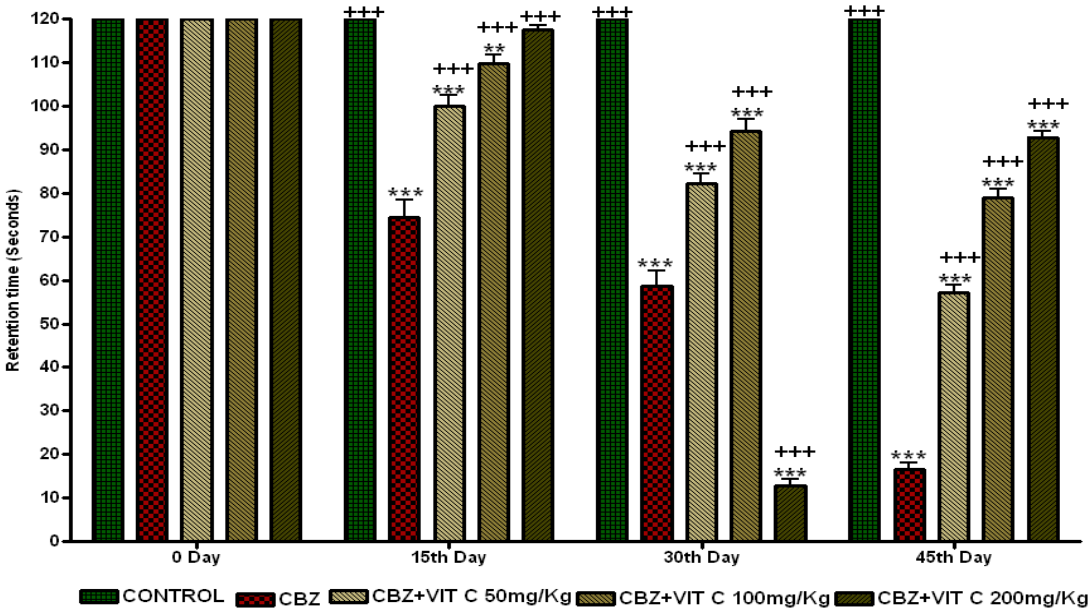


Values are expressed as mean± SEM of 6 animals. *(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group. +++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs CBZ group.**

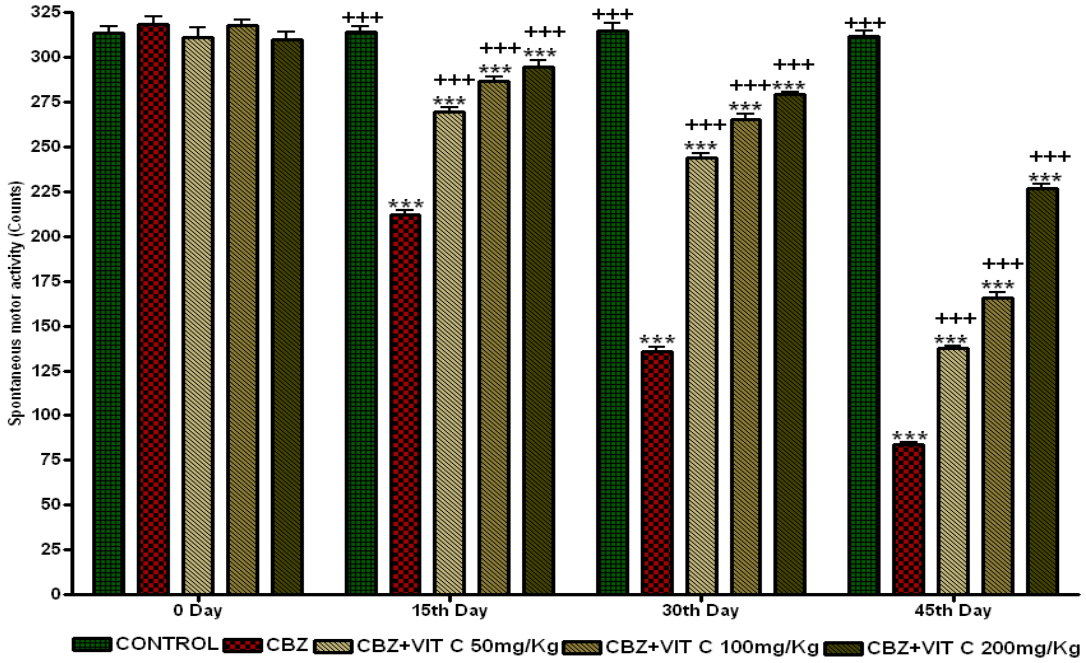
Fig. 1: Effect of Vitamin C on carbamazepine induced memory impairment



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 CBZ group.**
Fig. 2: Effect of Vitamin C on carbamazepine impaired exploratory activity

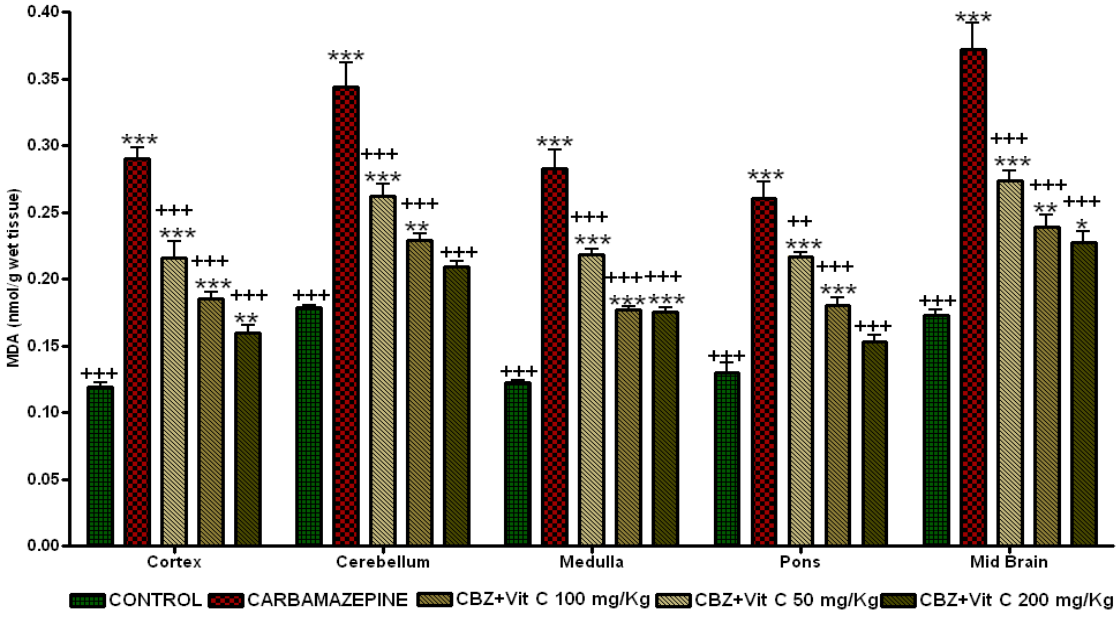


Values are expressed as mean± SEM of 6 animals. *(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group. +++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs CBZ group.**
Fig. 3: Effect of Vitamin C on carbamazepine induced motor in-coordination



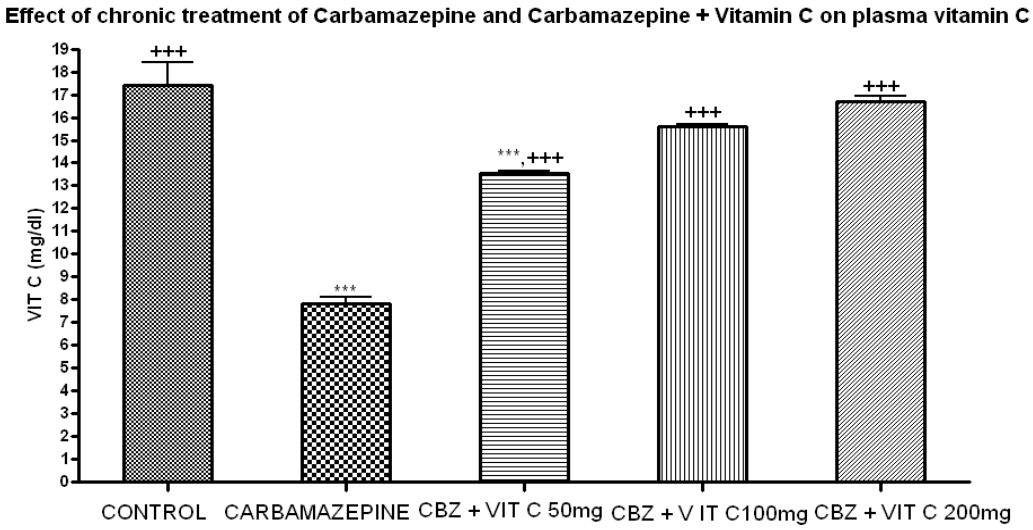
Values are expressed as mean± SEM of 6 animals. *(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group. +++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs CBZ group.**

Fig. 4: Effect of Vitamin C on carbamazepine impaired locomotor activity



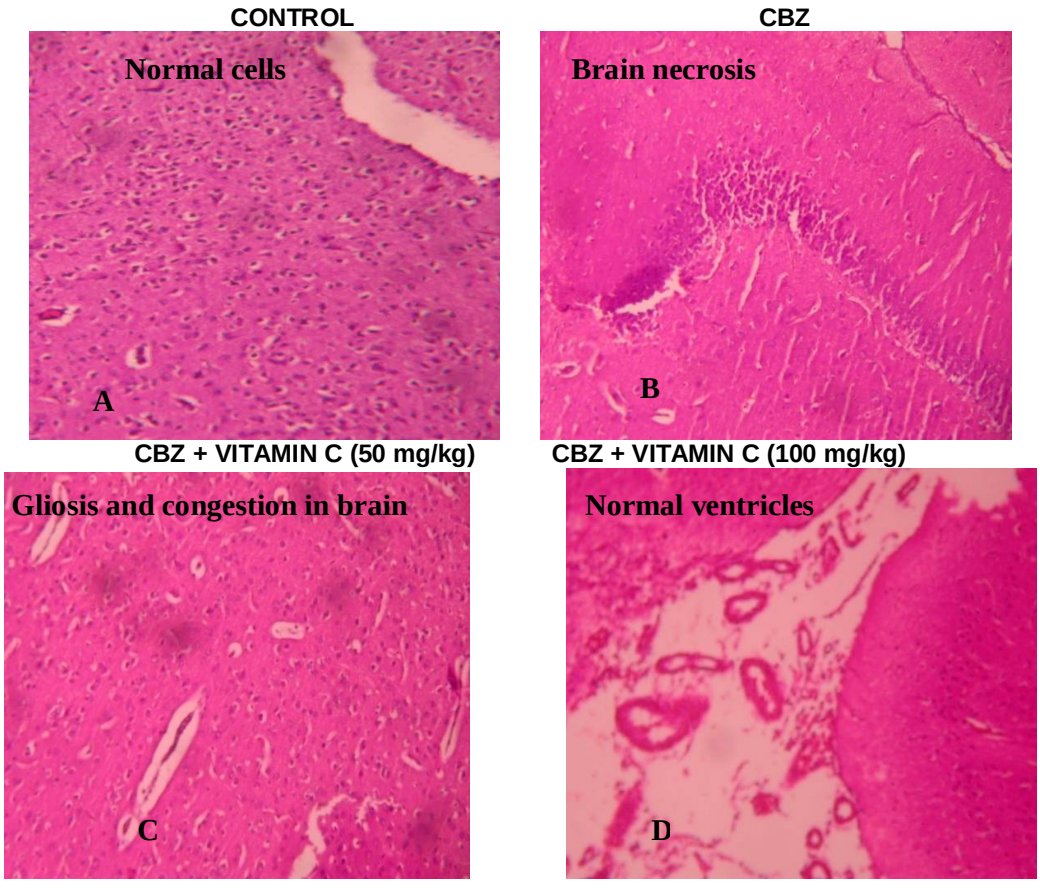
Values are expressed as mean± SEM of 6 animals. *(p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group. +++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs CBZ group.**

Fig. 5: Effect of Vitamin C on carbamazepine induced alterations in regional brain lipid peroxidation



Values are expressed as mean ± SEM of 6 animals ***p< 0.001 vs Control group; **p< 0.01 vs Control group; +++p< 0.001 vs Carbamazepine group; ++p< 0.01 vs Carbamazepine group.

Fig. 6:



CBZ + VITAMIN C (200 mg/kg)

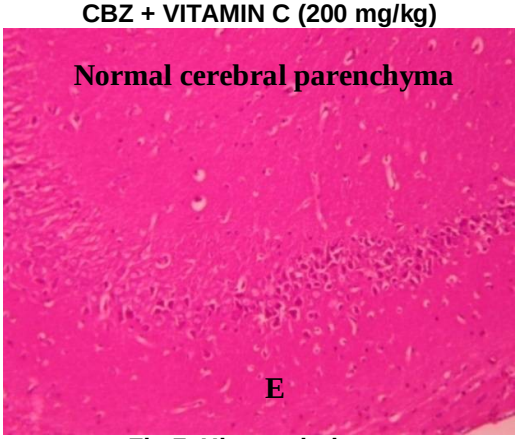


Fig.7. Histopathology:
The images illustrates the histopathological condition of control brain, carbamazepine (CBZ), CBZ + vitamin C 50, 100 and 200 mg/kg. (A) The control group showed normal cortex. (B) The carbamazepine treated group showed obvious brain necrosis. (C) Carbamazepine plus vitamin C (50 mg/kg) treated group showed gliosis and congestion in brain. (D) The carbamazepine plus vitamin C (100 mg/kg) treated group showed normal ventricles. (E) The carbamazepine plus vitamin C (200 mg/kg) treated group showed normal cerebral parenchyma resembling the control group

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