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Pharmacokinetic and pharmacodynamic interactions of valproate, phenytoin, phenobarbitone and carbamazepine with curcumin in experimental models of epilepsy in rats

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ABSTRACT

The present study investigates the interaction of curcumin with four antiepileptic drugs (AEDs) in male Wistar rats. In the first protocol, seizures were induced using pentylenetetrazole (PTZ) and valproate was injected intraperitoneally (i.p.) in therapeutic and sub-therapeutic doses 30 min before PTZ administration. Curcumin was co-administered with sub-therapeutic dose of valproate 60 min before PTZ injection. In the second protocol, seizures were induced by maximal-electroshock. Phenytoin, phenobarbitone and carbamazepine were injected in their therapeutic and sub-therapeutic doses 120, 60 and 30 min, respectively, before seizure induction. Curcumin was administered along with sub-therapeutic doses of phenytoin, phenobarbitone and carbamazepine, 60 min before induction of seizures. Behavioral parameters were assessed using elevated plus maze test and passive avoidance paradigm. Rat brain oxidative stress parameters were assessed and the serum levels of the AEDs were estimated. The AEDs in their therapeutic doses produced complete protection against seizures. However, sub-therapeutic doses of these AEDs failed to completely protect against seizures. Co-administration of curcumin with sub-therapeutic dose of valproate significantly increased the latency to myoclonic jerks. The percentage protection against seizures with sub-therapeutic doses of valproate, phenytoin, phenobarbitone and carbamazepine was also enhanced by concomitant curcumin administration. Both PTZ and MES induced seizures caused significant impairment of cognitive functions. Co-administration of curcumin with these AEDs in their sub-therapeutic doses prevented the impairment of learning and memory due to seizures whereas no such improvement was observed in the groups administered the sub-therapeutic doses of the AEDs alone. Additionally, curcumin reversed the oxidative stress due to seizures. However, curcumin co-administration did not cause any significant alteration in the serum levels of the AEDs. The results thus suggest the potential of curcumin as an adjunct to these AEDs in epilepsy with the advantage of increasing the efficacy, reducing the dose and side effects of the AEDs.

1. Introduction

Despite the availability of a wide range of conventional as well as newer antiepileptic drugs (AEDs) and a remarkable progress in the understanding of pathophysiological processes underlying seizures, there are still approximately 30% of epileptic patients who remain refractory to the current therapies. These patients often require novel and more efficacious treatment regimen in order to prevent occurrence of seizure attacks (Luszczki et al., 2009). Another concern associated with epilepsy is the side effects associated with AEDs. Cognitive impairment is an important side effect and is observed in more than half of the epilepsy patients on AEDs (Hernandez et al., 2005; Shannon and Love, 2005). The risk of cognitive side effects increases with higher dosages of the AEDs (Jokeit et al., 2005; Naidech et al., 2005). Phenytoin, valproate, phenobarbitone and carbamazepine are the commonly used AEDs, effective against all types of partial and tonic–clonic, generalized tonic–clonic and absence seizures (Leppik, 2005; Mc Namara, 2006). However, patients often need therapy on high doses for a prolonged period to attain seizure control. Moreover, combination of two or more AEDs is often required to control seizures in these patients. Though several two-AED combinations are useful for patients with refractory epilepsy (Stephen and Brodie, 2002), approximately 14% of epileptic patients remain refractory to the AED combinations (Kwan and Brodie, 2000). Additionally, polytherapy can be associated with problematic pharmacokinetic interactions which can result in adverse effects (Patsalos et al., 2002; Patsalos and Perucca, 2003). Therefore, a need was felt for some novel combinations of AEDs or a combination of AEDs with natural products possessing anti-seizure activity for suppressing epileptic attacks. An improved treatment which will counteract the side effects and drawbacks of the existing AEDs is the need of the hour.

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The use of traditional, complementary and alternative medicine along with allopathic drugs is very common (Barnes, 2004; Eisenberg et al., 1998, 2001; WHO, 2002–05). The relatively fewer side effects of traditional medicines in comparison with modern drugs have led to exploration of the concomitant use of traditional medicines in clinical settings since time immemorial. Moreover, there is a renewed public interest in naturally occurring treatments and dietary factors related to health and disease. A study from China has reported the use of traditional medicines by 16.32% of patients either alone or along with the standard antiepileptic drugs (Chen et al., 2000). Herbal medicines are one of the most common forms of traditional, complementary and alternative medicine, since they are considered to be both safe and effective.

Curcumin is a major component of the spice turmeric and is responsible for most of the pharmacological activities. It is a complex molecule with multiple biological targets and different cellular effects. Traditional Indian medicine claims the use of *Curcuma longa* L. (Zingiberaceae) powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (Ammon and Wahl, 1991). Curcumin is well documented to be a powerful antioxidant (Biswas et al., 2005). Curcumin has been reported to scavenge superoxide anions, peroxynitrite radicals and quench singlet oxygen. Curcumin has also been shown to inhibit hydrogen-peroxide-induced cell damage (Biswas et al., 2005). Moreover, antioxidant, anti-inflammatory, cancer chemopreventive, antidepressant, immunomodulatory and anti-convulsant properties of curcumin have been reported (Aggarwal and Harikumar, 2009; Aggarwal and Sung, 2009; Mehla et al., 2010; Sharma et al., 2009). Curcumin has also been shown to possess neuroprotective activity (Aggarwal and Harikumar, 2009; Aggarwal and Sung, 2009; Pan et al., 2008; Zhao et al., 2008). Further, curcumin ameliorated cognitive impairment induced by the phenytoin, phenobarbitone and carbamazepine in rats (Reeta et al., 2009, 2010). These studies suggested a potential of curcumin as an adjuvant to the AEDs. However, since curcumin cannot be a standalone AED, it was felt important to study the interaction profile of curcumin with the conventional AEDs. Hence, this study was planned to evaluate the pharmacodynamic and pharmacokinetic interaction profiles of four commonly used AEDs namely valproate, phenytoin, phenobarbitone and carbamazepine with curcumin in rats.

2. Materials and methods

2.1. Animals

All experiments were performed in male Wistar rats weighing 200–250 g. The animals were obtained from the Central Animal Facility of All India Institute of Medical Sciences, New Delhi, India. The rats were kept in polyacrylic cages (38×23×10 cm) with 4 animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rat diet and tap water *ad libitum*. However, the rats were deprived of food 12 h before the behavioral testing, as this is known to enhance their motivation to perform the test. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures were reviewed and approved by the Institutional Animal Ethics Committee.

2.2. Experimental design

After 7 days of adaptation to the laboratory conditions, the animals were randomly assigned to the experimental groups consisting of six rats per group. Each rat was used only once. All the tests were performed between 9:00 and 15:00 h. In the present study, two protocols were designed. In the first protocol, interaction of sodium valproate with curcumin was evaluated in pentylenetetrazole (PTZ)-induced seizures whereas in the second protocol, pharmacokinetic and pharmacodynamic interactions of phenytoin, phenobarbitone and carbamazepine with curcumin was studied in maximal electroshock (MES)-induced seizures.

2.3. Protocol 1

The animals were divided into 6 groups and assigned the following treatments: group I was the control group, where the animals received the vehicles and no active treatment. Group II was the PTZ group, where the animals received PTZ along with vehicle. Animals in group III received valproate (300 mg/kg) before PTZ. In group IV, animals were administered sub-therapeutic dose of valproate (150 mg/kg) prior to PTZ injection. In group V, curcumin (300 mg/kg) was administered orally along with sub-therapeutic dose of valproate (150 mg/kg) before induction of seizures with PTZ. In group VI, curcumin (300 mg/kg) alone was administered prior to PTZ injection. PTZ and sodium valproate were dissolved in normal saline and injected intraperitoneally (i.p.) in different groups at a volume of 0.1 ml/100 g body weight. Curcumin dissolved in 50% dimethyl sulphoxide (DMSO) was administered orally (per oral, p.o.) by gavage at volumes not greater than 1.0 ml/100 g body weight. PTZ was injected in a dose of 60 mg/kg to induce seizures. Sodium valproate was administered 30 min before PTZ injection. Curcumin was administered 60 min before PTZ administration. The animals were observed for 30 min after PTZ challenge.

2.4. Protocol 2

There were 12 groups with 6 animals in each group. Group I — control, where the animals received no active treatment; group II — maximal electro-shock (MES) group wherein vehicle was administered before MES; group III — phenytoin (40 mg/kg, i.p.) was injected 120 min before MES; group IV — phenytoin (20 mg/kg, i.p.) was administered 120 min before MES; group V — curcumin (300 mg/kg, p.o.) was administered 60 min and phenytoin (20 mg/kg, i.p.) was given 120 min before MES; group VI — phenobarbitone (40 mg/kg, i.p.) was administered 60 min before MES; group VII — phenobarbitone (20 mg/kg, i.p.) was given 60 min before MES; group VIII — curcumin (300 mg/kg, p.o.) and phenobarbitone (20 mg/kg, i.p.) were administered 60 min before MES, group IX — carbamazepine (20 mg/kg, i.p.) was injected 30 min before MES; group X — carbamazepine (10 mg/kg, i.p.) was given 30 min before MES; group XI — curcumin (300 mg/kg, p.o.) was administered 60 min and carbamazepine (10 mg/kg, i.p.) was given 30 min prior to MES; group XII — curcumin (300 mg/kg, p.o.) was administered 60 min prior to MES. Phenytoin, phenobarbitone and carbamazepine, dissolved in 50% DMSO were administered intraperitoneally (i.p.) in different groups at a volume of 0.1 ml/100 g body weight. Curcumin dissolved in DMSO (50%) was administered orally by gavage at volumes not greater than 1.0 ml/100 g body weight.

2.5. PTZ induced seizures

PTZ was prepared freshly and dissolved in normal saline and administered in a dose of 60 mg/kg, i.p. This dose of PTZ has been standardized as 100% convulsant dose with minimal mortality in rats (Malhotra and Gupta, 1997). The latency to myoclonic jerks and incidence of generalized tonic clonic seizures (GTCS) with loss of righting reflex were noted. The animals were observed for 30 min.

2.6. Maximal electroshock induced seizures (MES)

Electroconvulsions were produced by a suprathreshold fixed current sinus wave stimulus (current intensity—70 mA, duration—0.2 s) delivered via ear clip electrodes using ECT unit (Ugo Basile, Italy). The animals were observed for occurrence of tonic hind limb

extension (THLE), i.e., the hind limbs of animals outstretched 180° to the plane of the body axis.

2.7. Behavioral tests

Behavioral parameters were performed before and after induction of seizures by PTZ and MES. During the behavioral experiments, only one animal was tested at a time.

2.7.1. Elevated plus maze

The elevated plus maze consists of two closed arms and two open arms forming a cross, with a quadrangular center. The maze is placed above the floor. Acquisition and retention of memory processes were assessed using elevated plus maze as described earlier (Reeta et al., 2009). On the 1st day, the initial transfer latency was measured. 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 arm (transfer latency) was recorded. The time that the rat took to enter the enclosed arm was taken as the initial transfer latency. In this experiment when the rat did not enter the enclosed arm for 60 s, it was gently pushed on the back into the enclosed arm and the initial transfer latency was assigned 60 s. The rat was allowed to move freely in the plus maze regardless of open and closed arms for 10 s after the measurement of initial transfer latency. The rat was then gently taken out of the plus maze and was returned to its home cage. Twenty four hours later, the retention transfer latency test was performed in the same way as in the initial transfer latency. If the rat did not enter the enclosed arm within 60 s on the second trial, the transfer latency was assigned 60 s.

2.7.2. One trial passive avoidance task

The effect of combination of curcumin with the conventional antiepileptic drugs on long term memory in rats was determined in the step-through passive avoidance task according to the method previously described (Reeta et al., 2009). The apparatus consists of two grid floor compartments separated by a guillotine door. One compartment was lit using a bulb, while the other was dark. The floor of both the compartments consisted of steel grids, used to deliver electric shocks. Each rat was placed in the lighted chamber. After 60 s of habituation, a guillotine door was opened, and the initial latency (IL) to enter the dark chamber was recorded. Rats exhibiting an initial latency time of more than 60 s were excluded from further experiments. Immediately after the rat enters the dark compartment, the guillotine door was closed and an electric foot shock (75 V, 0.2 mA, 50 Hz) was delivered to the floor grids for 3 s. The rat was removed from the dark compartment 5 s later and returned to its home cage. After 24 h, retention latency (RL) time was measured in the same way as in the acquisition trial, but foot shock was not delivered, and the latency time was recorded up to a maximum of 600 s. The step through passive avoidance task gives information about the ability of the animals to acquire the task (learning) and to recall the task (retrieval). Therefore, it may be regarded as a measure of long term memory (Luszczki et al., 2009).

2.8. Oxidative stress parameters

Lipid peroxidation and reduced glutathione were estimated in the whole brain of the rats at the end of the study period. After decapitation under ether anesthesia, the brains were quickly removed, cleaned with chilled saline and stored at −80 °C until biochemical analysis were carried out within the next 7 days.

2.8.1. Tissue preparation

10% (w/v) homogenate of the whole brain tissue samples was made with ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots were prepared to estimate lipid peroxidation and glutathione.

2.8.1.1. Measurement of lipid peroxidation. Malondialdehyde (indicator of lipid peroxidation) was estimated as described by Ohkawa et al. (1979). Acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue homogenate. The mixture was heated at 100 °C for 60 min. It was then cooled under tap water and 5 ml of n-butanol: pyridine (15:1, v/v) and 1 ml of distilled water were added. The mixture was then shaken vigorously and centrifuged at 4000 rpm for 10 min. The organic layer was withdrawn and absorbance was measured at 532 nm using a spectrophotometer.

2.8.1.2. Estimation of glutathione. Glutathione (GSH) was estimated by the method described by Ellman (1959). To precipitate out the proteins, brain homogenate was centrifuged with 5% trichloroacetic acid. The supernatant was separated and 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5′5 dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of distilled water were added to 0.1 ml of this supernatant. The mixture was vortexed and the absorbance was read at 412 nm within 15 min in a spectrophotometer.

2.9. Estimation of serum levels of AEDs

1.5 ml of blood was collected from the retro-orbital plexus in plain dry centrifuge tubes immediately after the observation time of seizures with PTZ or MES. The blood was allowed to clot in the room temperature for 30 min after collection. Blood was then centrifuged at 3000 rpm for 10 min and the serum was separated in Eppendorf tubes. The serum valproate, phenytoin, phenobarbitone and carbamazepine levels were estimated in an automated immunoassay analyzer (Axsym, Abbott Laboratories, IL, USA), using fluorescence polarization immunoassay technique.

2.10. Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) with Bonferroni post hoc statistical tests for behavioral and oxidative stress data and unpaired 't' test was used for serum levels of AEDs. All statistical analyses were performed using SPSS statistical software package version 13.0. A $p<0.05$ was taken as the level of significance.

3. Results

3.1. PTZ-induced seizures

3.1.1. Effect on seizures

There were no seizures with PTZ in the valproate 300 mg/kg treated group. However, the sub-therapeutic dose of valproate (150 mg/kg) failed to completely protect against PTZ-induced seizures. Though, this dose of valproate significantly increased myoclonic jerk latency from 41.3 ± 3.9 s in PTZ group to 85.2 ± 7.6 s ($p<0.05$) (Fig. 1A), it showed only 50% protection against GTCS in rats (Fig. 1B). When curcumin was co-administered with the sub-therapeutic dose of valproate, it significantly potentiated the anti-seizure effect of the sub-therapeutic dose of sodium valproate [$F(5,30)=50.724$, $p=0.0001$]. The latency to myoclonic jerks significantly increased from 41.3 ± 3.9 s in PTZ group to 113.3 ± 12.1 s in curcumin along with valproate (150 mg/kg, i.p.) group ($p<0.001$) (Fig. 1A). Myoclonic jerks latency increased significantly ($p<0.01$) in the curcumin along with valproate group as compared to valproate (150 mg/kg, i.p.) alone. Curcumin (300 mg/kg) alone, also significantly increased the myoclonic jerk latency as compared to PTZ group ($p<0.05$) though it produced only 50% protection against GTCS. However, 100% protection against GTCS was observed when curcumin was administered along with the sub-therapeutic dose of valproate (150 mg/kg, i.p.) (Fig. 1B).

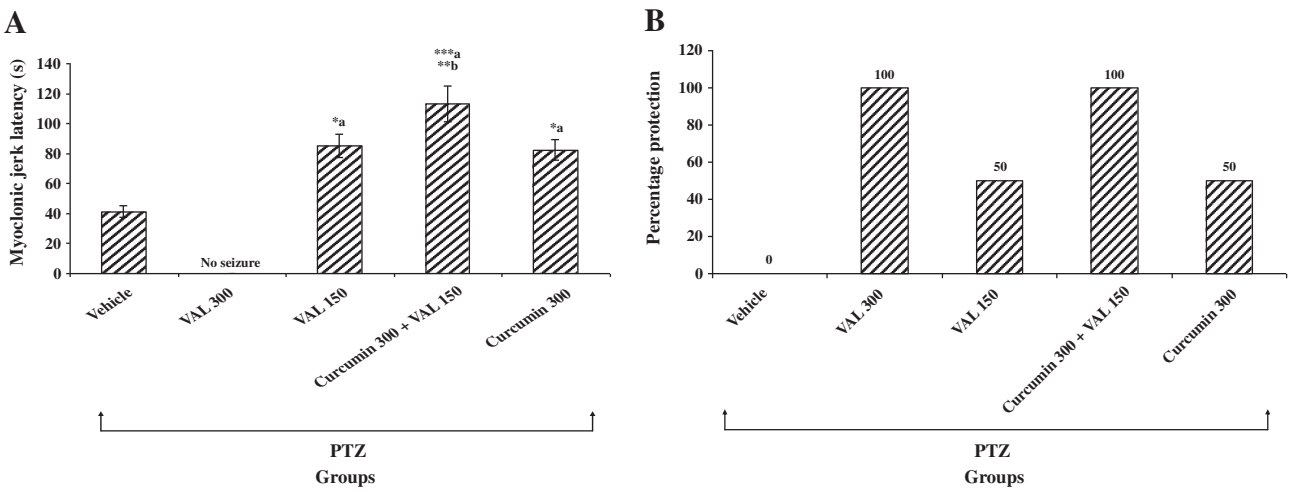

Fig. 1. (A) Effect of curcumin on the myoclonic jerk latency in PTZ-induced seizures in rats. Data are presented as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, a—as compared to PTZ; b—as compared to valproate 150 mg/kg. Val 300 — valproate 300 mg/kg; Val 150 — valproate 150 mg/kg. (B) Effect of curcumin on the percentage protection against generalized tonic clonic seizures (GTCS) in PTZ-induced seizures in rats.

3.1.2. Effect on elevated plus maze test

No significant differences were observed in the initial transfer latency between the different groups. However, there was a significant difference in the retention transfer latencies [$F(5,28) = 5.743$, $p = 0.001$]. PTZ caused a significant increase in the retention transfer latency from 15.8 ± 3.4 s in control group to 32.3 ± 1.8 s in the PTZ group ($p<0.01$). Valproate (150 mg/kg) did not show improvement in the memory retention in the animals as the retention transfer latency in this group was not lowered significantly as compared to PTZ group. As compared to the PTZ group, the retention transfer latencies were significantly ($p<0.01$) less in the valproate (300 mg/kg) and curcumin (300 mg/kg) along with valproate (150 mg/kg) groups, the values being 17.5 ± 3.1 s and 15.3 ± 2.3 s, respectively (Fig. 2A).

3.1.3. Effect in passive avoidance test

There was no significant difference between the initial latency of all the groups whereas significant difference was observed in the retention latency among the different groups [$F(5,28) = 5.111$, $p = 0.002$]. There was a significant decrease in retention latency from 296.1 ± 9.0 s in control group to 224.0 ± 6.7 s in PTZ group which indicated impairment of memory in PTZ group as compared to the control group ($p<0.01$). The retention latency in the valproate (300 mg/kg) and curcumin along with valproate (150 mg/kg) was significantly more (276.2 ± 14.2 s; $p<0.05$ and 284.8 ± 8.9 s; $p<0.01$, respectively) as compared to the PTZ group (Fig. 2B) thus indicating better retention in these groups. The sub-therapeutic dose of valproate (150 mg/kg) alone and curcumin (300 mg/kg) alone however did not show any significant improvement in passive avoidance test as compared to the control and PTZ groups (Fig. 2B).

3.1.4. Effect on the oxidative stress markers

Significant differences were observed in the brain malondialdehyde (MDA) [$F(5,28) = 14.034$, $p = 0.0001$] and glutathione (GSH) [$F(5,28) = 16.933$, $p = 0.0001$] levels among the groups. There was a significant increase in MDA levels ($p<0.001$) and a significant decrease in GSH levels ($p<0.001$) in the PTZ group as compared to control group. Valproate (150 and 300 mg/kg) and curcumin along with valproate (150 mg/kg) significantly reversed the increased level of MDA as well as the reduced the level of GSH as compared to PTZ

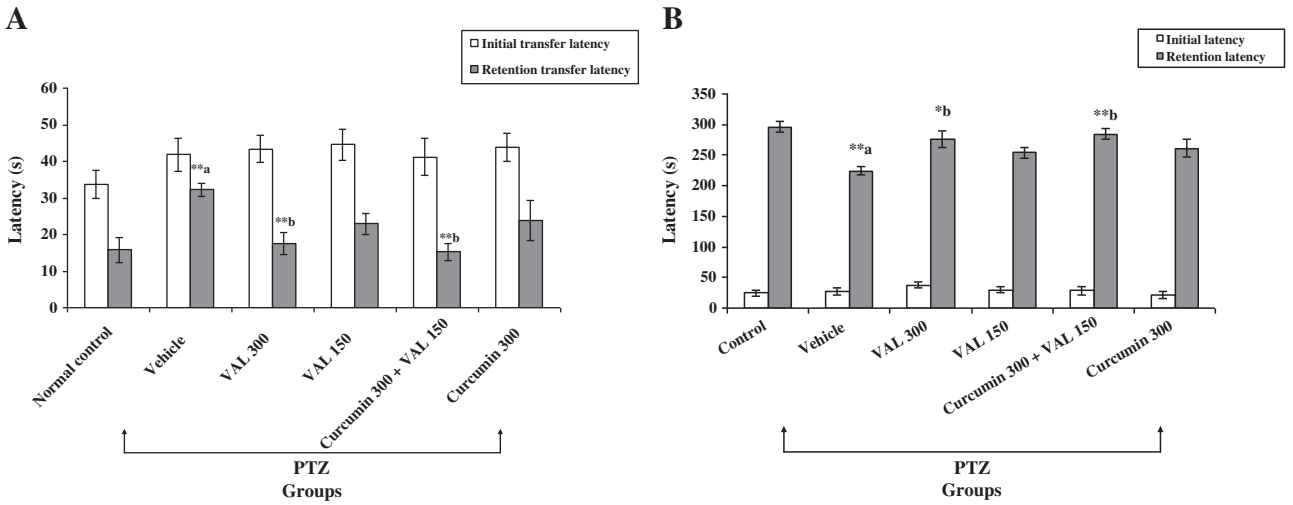

Fig. 2. (A) Effect of curcumin in elevated plus maze test in PTZ-induced seizures. (B) Effect of curcumin in passive avoidance test in PTZ-induced seizures. Data are presented as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, a—as compared to control; b—as compared to vehicle treated PTZ group.

group. Co-administration of curcumin significantly reduced the level of MDA ($p<0.05$) and increased the level of GSH ($p<0.01$) as compared to valproate (150 mg/kg) alone group (Fig. 3A and B). Curcumin (300 mg/kg) alone caused significant reversal of the increased MDA levels ($p<0.05$) and the decreased GSH levels ($p<0.05$) in comparison to the PTZ group, bringing the values towards the normal control group.

3.2. MES-induced seizures

3.2.1. Effect on seizures

Phenytoin (40 mg/kg), phenobarbitone (40 mg/kg) and carbamazepine (20 mg/kg) showed 100% protection in MES-induced seizures. There was 33.3%, 33.3% and 50% protection with the sub-therapeutic doses of phenytoin (20 mg/kg), phenobarbitone (20 mg/kg) and carbamazepine (10 mg/kg), respectively. Curcumin (300 mg/kg) alone showed 33.3% protection in MES-induced seizures. When curcumin (300 mg/kg, p.o.) was administered along with the sub-therapeutic doses of phenytoin (20 mg/kg) and phenobarbitone (20 mg/kg), 83.3% protection against seizures was observed whereas with the sub-therapeutic dose of carbamazepine (10 mg/kg), there was 66.6% protection. This suggests that curcumin potentiates the antiepileptic effect of these AEDs (Fig. 4).

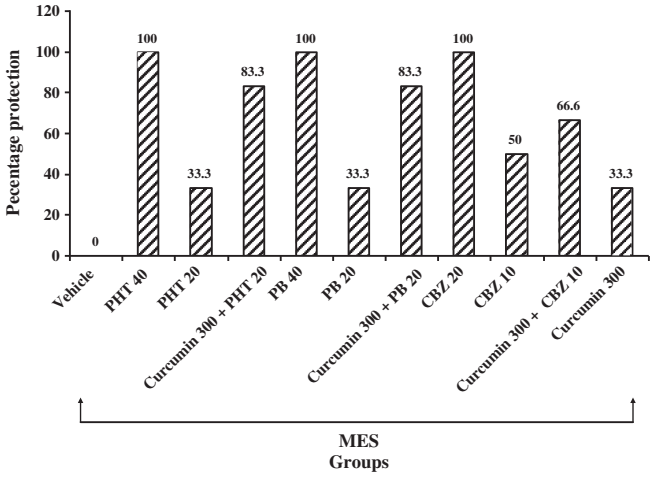

Fig. 4. Effect of curcumin on the percentage protection against tonic hind limb extension in MES-induced seizures in rats. PHT 40 — phenytoin 40 mg/kg; PHT 20 — phenytoin 20 mg/kg; PB 40 — phenobarbitone 40 mg/kg; PB 20 — phenobarbitone 20 mg/kg; CBZ 20 — carbamazepine 20 mg/kg; CBZ 10 — carbamazepine 10 mg/kg.

3.2.2. Effect on elevated plus maze test

Though there was no significant difference between the initial transfer latency of all the groups, retention transfer latency differed significantly among the groups [$F(11,40)=3.955$, $p=0.001$]. The retention transfer latency increased from 15.8 ± 3.4 s in control to 35.3 ± 5.8 s in MES group. The retention transfer latency decreased from 35.3 ± 5.8 s in MES group to 12.8 ± 2.0 s ($p<0.01$) in phenytoin (40 mg/kg), 11.8 ± 1.9 s ($p<0.01$) in phenobarbitone (40 mg/kg) and 12.3 ± 2.0 s ($p<0.01$) in carbamazepine (20 mg/kg) treated groups whereas no significant improvement was observed in the retention transfer latencies in the groups administered sub-therapeutic doses of phenytoin, phenobarbitone and carbamazepine. However, co-administration of curcumin (300 mg/kg) along with phenytoin (20 mg/kg), phenobarbitone (20 mg/kg) and carbamazepine (10 mg/kg) improved the cognitive functions as compared to MES group as shown by the significant decrease in the retention transfer latency as compared to MES group (Fig. 5A). Curcumin (300 mg/kg) alone did not cause any significant change in the retention transfer latency as compared to the control group.

3.2.3. Effect on passive avoidance test

Initial latency of all the groups did not show significant difference. However, a significant difference was observed in retention latency [$F(11,40)=2.917$, $p=0.006$]. There was a significant decrease in the retention latency from 296.1 ± 9.0 s in control group to 229.7 ± 6.2 s in the MES group ($p<0.05$). Though there was a significant increase in the retention latency of the groups administered the AEDs in their therapeutic doses, the retention latency in the phenytoin (20 mg/kg), phenobarbitone (20 mg/kg), carbamazepine (10 mg/kg) and curcumin (300 mg/kg) alone treated groups was not increased significantly as compared to the MES group. However, co-administration of curcumin along with the sub-therapeutic doses of these AEDs caused improvement in cognitive function as indicated by the increase in the retention latency as compared to the MES group ($p<0.05$) (Fig. 5B).

3.2.4. Effect on oxidative stress parameters

3.2.4.1. Effect on brain MDA levels. There was a significant difference in the brain MDA levels in all groups [$F(11, 40)=9.485$, $p=0.0001$].

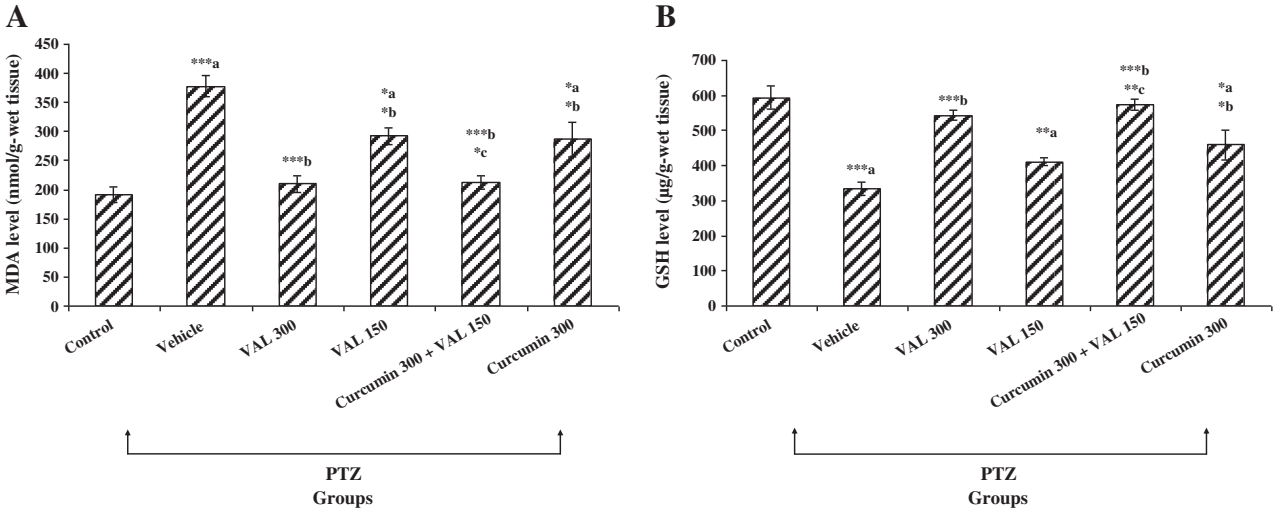

Fig. 3. (A) Effect of curcumin on the brain MDA levels in PTZ-induced seizures in rats. (B) Effect of curcumin on the brain GSH levels in PTZ-induced seizures in rats. Data are presented as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, a—as compared to control; b—as compared to vehicle treated PTZ group; c—as compared to valproate 150 mg/kg.

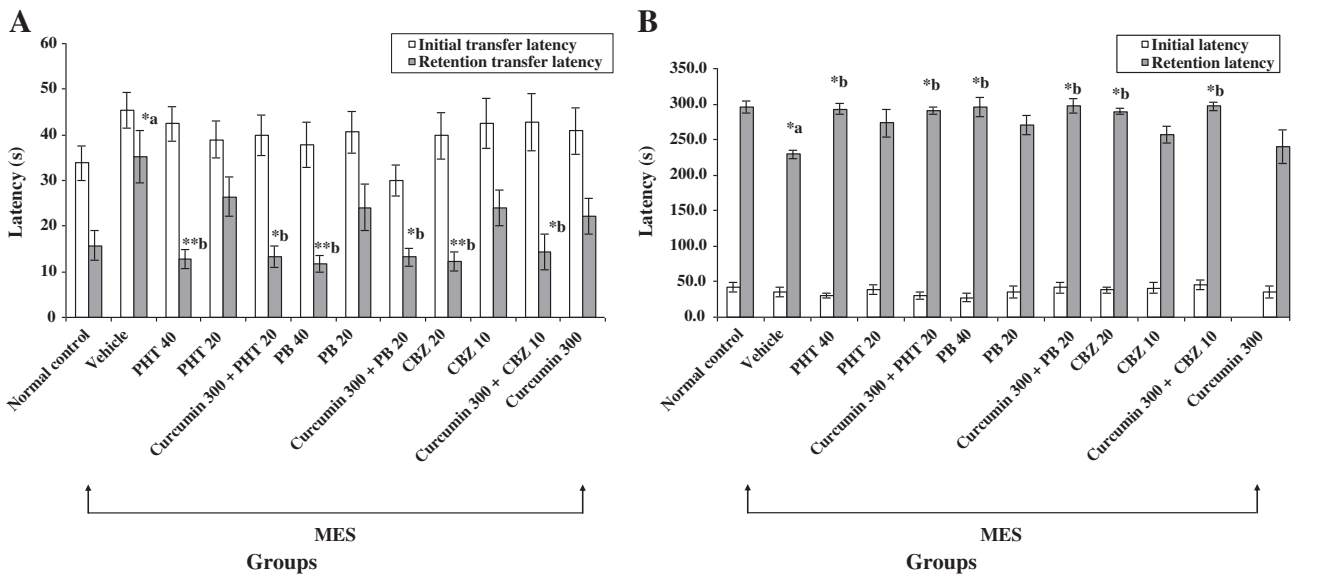

Fig. 5. (A) Effect of curcumin on elevated plus maze test in MES-induced seizures in rats. (B) Effect of curcumin on passive avoidance test in MES-induced seizures in rats. Data are presented as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, a—as compared to control; b—as compared to vehicle treated MES group.

A significantly ($p<0.001$) higher MDA level was observed in the MES group (302.8 ± 11.3 nmol/g wet tissue) as compared to the control group (192.0 ± 13.6 nmol/g wet tissue) thus showing more lipid peroxidation with MES. Though the higher doses of phenytoin (40 mg/kg), phenobarbitone (40 mg/kg) and carbamazepine (20 mg/kg) significantly ($p<0.01$) reduced the MDA level as compared to the MES group, the sub-therapeutic doses of phenytoin, phenobarbitone and carbamazepine did not reduce the MDA level significantly as compared to MES group. When curcumin was administered along with the sub-therapeutic doses of phenytoin, phenobarbitone and carbamazepine, there was a decrease in the oxidative stress as shown by the significantly less MDA level as compared to MES group as well as to the sub-therapeutic doses of these drugs alone. Curcumin alone also significantly ($p<0.01$) reduced the raised MDA level in comparison to MES group (Fig. 6A).

3.2.4.2. Effect on brain GSH levels. Brain GSH levels showed significant difference among the groups [$F(10,40)=8.230$, $p=0.0001$]. MES caused significant ($p<0.001$) decrease in GSH levels (405.8 ± 34.2 μg/g wet tissue) as compared to the control group (594.3 ± 32.0 μg/g wet tissue). As compared to the MES group, GSH levels were significantly more in the groups administered the higher doses of phenytoin, phenobarbitone and carbamazepine ($p<0.01$, $p<0.05$ and $p<0.01$, respectively) whereas the sub-therapeutic doses of phenytoin (20 mg/kg), phenobarbitone (20 mg/kg) and carbamazepine (10 mg/kg) did not significantly alter the GSH levels as compared to the MES group. When curcumin was administered along with phenytoin (20 mg/kg), phenobarbitone (20 mg/kg) and carbamazepine (10 mg/kg), there was a significant increase in the GSH levels as compared to the MES group as well as these drugs alone in their sub-therapeutic doses. Curcumin alone also caused a significant ($p<0.05$) increase in GSH level in comparison to the MES group (Fig. 6B).

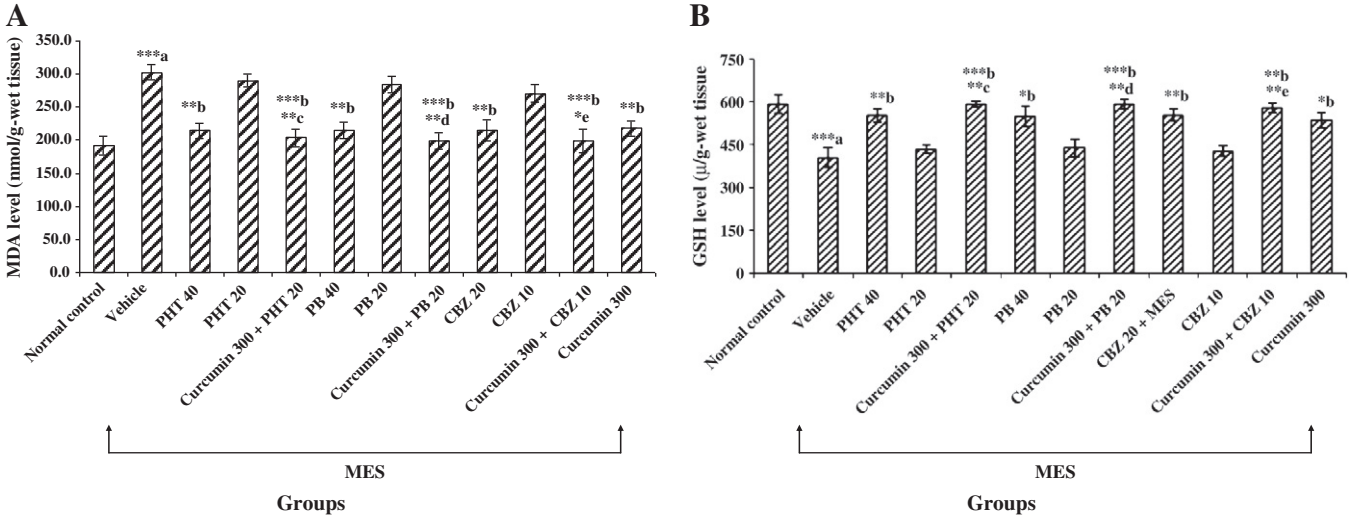

Fig. 6. (A) Effect of curcumin on the brain MDA levels in MES-induced seizures in rats. (B) Effect of curcumin on the brain GSH levels in MES-induced seizures in rats. Data are presented as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, a—as compared to control; b—as compared to vehicle treated MES group; c—as compared to phenytoin 20 mg/kg; d—as compared to phenobarbitone 20 mg/kg; e—as compared to carbamazepine 10 mg/kg.

3.3. Effect of curcumin on the serum levels of valproate, phenytoin, phenobarbitone and carbamazepine

When curcumin was administered along with sub-therapeutic doses of valproate, phenytoin, phenobarbitone and carbamazepine, there was no significant difference in serum levels of these AEDs as compared to those groups administered the sub-therapeutic doses of these AEDs alone (Table 1).

4. Discussion

Recurrent seizures in epileptic patients frequently produce psychiatric disorders such as cognitive deficits, behavioral abnormalities, emotional impairments and attention deficit hyperactivity disorder (Thome-Souza et al., 2004; Vingerhoets, 2006). In general, epileptic patients have three to six times higher risk for psychiatric disorders than that found in age-matched healthy persons. Several factors are responsible for cognitive deficit in epileptic patients, including the duration and frequency of the epilepsy, seizure patho-physiology and the patient's family history (Stafstrom and Sutula, 2005; Thome-Souza et al., 2004; Vingerhoets, 2006). In addition, AEDs are also responsible for impairment of cognitive function.

Curcumin has been reported to possess anti-oxidant, anti-inflammatory, pro-apoptotic, anti-bacterial and anti-cancer properties (Epstein et al., 2010). Though, curcumin has been studied in a wide range of doses in animals, however, neuroprotective effect has been reported with the higher dose. Curcumin (300 mg/kg) has been found to prevent memory impairment in streptozotocin, phenytoin, phenobarbitone and carbamazepine treated rats (Isik et al., 2009; Reeta et al., 2009, 2010) and to be neuroprotective in focal cerebral ischemia in rats (Zhao et al., 2008). In a previous study carried out in our laboratory, curcumin (100, 200 and 300 mg/kg, p.o.) showed dose dependent protection against the development of kindling, myoclonic jerks and GTCS in PTZ induced kindling in rats and also ameliorated the cognitive impairment in kindled rats (Mehla et al., 2010). Considering these results and the fact that curcumin has a wide safety margin, curcumin was used in a dose of 300 mg/kg in the present study. Serum concentrations of curcumin are reported to peak 1–2 h after oral dosing (Cheng et al., 2001) and therefore, curcumin was administered 60 min before inducing seizures either by PTZ or MES.

In the present study, interaction profile of curcumin (300 mg/kg, p.o.) with the four AEDs (phenytoin, phenobarbitone, valproate and carbamazepine) was evaluated. The therapeutic doses as well as sub-therapeutic doses of AEDs were used and curcumin was administered along with sub-therapeutic doses of AEDs. This study showed that curcumin administration along with sub-therapeutic dose of valproate produced 100% protection against PTZ induced seizure while valproate (150 mg/kg) produced only 50% protection. When curcumin was administered along with sub-therapeutic doses of phenytoin and phenobarbitone, 83.3% protection was observed in MES induced seizure model while only 33.3% protection was seen when the drugs were administered alone in their sub-therapeutic doses. The combination of curcumin and carbamazepine afforded 66.6% protection. These data indicate that curcumin enhances the action of sub-therapeutic doses of valproate, phenytoin, phenobarbitone and carbamazepine. These findings are in agreement with results of previous studies which have reported antiepileptic effect of curcumin in amygdala kindling, kainic acid and MES induced seizures in rats (Bharal et al., 2008; DU et al., 2009; Gupta et al., 2009). Curcumin has also been reported to attenuate the hippocampal cell death induced by kainic acid in rats by reducing the oxidative stress and immunoreactivity of caspase-3 (Shin et al., 2007). Although, isobolographic analysis of interactions is accepted as the gold standard for detection of interactions between drugs (Gessner, 1995), this analysis was not considered in the present study as the primary objective was not to find the most appropriate concentration/dose of the two drugs i.e., curcumin and the antiepileptic drugs. Since the side effects with the AEDs are reported to increase with an increase in dose and no side effects have been reported with curcumin even with very high doses, the objective of this study was to evaluate if 100% protection against seizures can be achieved with the reduced doses of AEDs when given in combination with a fixed neuroprotective dose (300 mg/kg) of curcumin.

In the present study, PTZ and MES induced seizures caused impairment of cognitive function in rats as evaluated by passive avoidance and elevated plus maze test. Memory function in this study was evaluated by elevated plus maze test as well as passive avoidance paradigm. Though primarily used for assessment of anxiety, elevated plus maze test has also been employed as a model for evaluation of memory in rodents (Blatt and Takahashi, 1998; Da Cunha et al., 2005; Itoh et al., 1990; Reeta et al., 2009). It is believed that the transfer latency (the time in which animal moves from the open arms to the enclosed arms) is shortened if the animal has previously experienced entering the enclosed arms and thus the shortened transfer latency is related to memory. Elevated plus maze has therefore been quoted as a useful tool for discovering nootropic effects (Itoh et al., 1990). Moreover, it has the advantages of being a simple procedure, not time-consuming and there is no need to manipulate appetitive behaviors or use aversive stimulus (Itoh et al., 1990). An increase in retention transfer latency in elevated plus maze test and a decrease in retention latency in passive avoidance test was observed in PTZ and MES groups indicating an impairment of memory function. Improvement in cognitive function with curcumin has been reported in previous studies in AED induced as well as seizures induced cognitive impairment (Mehla et al., 2010; Reeta et al., 2009, 2010). The observed cognitive impairment in the seizure groups may be attributed to the seizures itself as disturbances in memory and other psychomotor functions are well reported in patients of epilepsy (Black et al., 2010; Endermann, 2010; Taylor et al., 2010). AEDs at their sub-therapeutic doses did not show any significant improvement in cognitive functions probably due to their failure to completely protect against seizures. Co-administration of curcumin (300 mg/kg, p.o.) along with sub-therapeutic doses of AEDs caused significant reversal of cognitive impairment induced by PTZ and MES seizures. Curcumin along with sub-therapeutic doses of AEDs significantly increased the retention latency in passive avoidance test and decreased the retention transfer latency in elevated plus maze test in both models of seizures. There was, thus, an improvement in cognitive functions when curcumin was administered along with sub-therapeutic doses of AEDs.

Seizure activity and chronic AEDs treatment in epileptic patients have been associated with an increased level of free radicals and reduced activity of antioxidant defense mechanisms (Choi, 1993; Hamed et al., 2004; Ono et al., 2000; Sudha et al., 2001) which may

Table 1
Effect of curcumin on serum levels of valproate, phenytoin, phenobarbitone and carbamazepine in rats.

Groups	Serum level (μg/ml)
Valproate 300 mg/kg, i.p.	118.05 ± 15.05
Valproate 150 mg/kg, i.p.	90.88 ± 6.81
Valproate 150 + curcumin 300 mg/kg, p.o.	87.56 ± 14.34
Phenytoin 40 mg/kg, i.p.	13.02 ± 1.06
Phenytoin 20 mg/kg, i.p.	7.27 ± 2.16
Phenytoin 20 mg/kg, i.p. + curcumin 300 mg/kg, p.o.	9.86 ± 0.72
Phenobarbitone 40 mg/kg, i.p.	34.76 ± 4.70
Phenobarbitone 20 mg/kg, i.p.	28.93 ± 0.93
Phenobarbitone 20 mg/kg, i.p. + curcumin 300 mg/kg, p.o.	23.11 ± 1.61
Carbamazepine 20 mg/kg, i.p.	8.08 ± 0.96
Carbamazepine 10 mg/kg, i.p.	5.97 ± 0.99
Carbamazepine 10 mg/kg, i.p. + curcumin 300 mg/kg, p.o.	4.92 ± 0.74

Data are presented as mean ± SEM. (n = 6 in each group).

result into recurrent seizures and cognitive deficit. The results of this study showing an increased oxidative stress in the seizure groups support the findings of these previous studies. In the present study, PTZ and MES caused an oxidative stress probably due to free radical generation. MDA is an end product of free radical generation (Liu et al., 1997) and glutathione plays an important role in protecting cells against oxidative damage as a free radical scavenger (Ono et al., 2000). Both PTZ and MES caused an increase in the level of MDA and reduced the GSH levels in rat brain. Thus, these experimental seizures would have caused an imbalance between antioxidant and oxidant defense system which may be responsible for seizures as well as impairment of memory function.

In the present study, administration of valproate, phenytoin, phenobarbitone and carbamazepine in their therapeutic doses before the induction of seizures produced 100% protection against seizures and reversed the oxidative stress by maintaining the balance between the MDA and GSH levels in rat brain. However, the sub-therapeutic doses of AEDs did not reduce the oxidative stress. Curcumin along with the sub-therapeutic doses of the AEDs prevented the increase in MDA level and the decrease in GSH level as compared to sub-therapeutic dose of AEDs alone in both the seizure models. This decrease in brain MDA levels with concomitant curcumin indicates an attenuation of lipid peroxidation. Moreover, there was a significant increase in the brain GSH levels in the groups when curcumin was co-administered with sub-therapeutic doses of the AEDs. In the brain, glutathione is thought to play a central role in defense against reactive oxygen species. Glutathione can directly detoxify reactive oxygen species and can act as a substrate for several peroxidases. During scavenging of free radicals, glutathione disulfide is produced and GSH is reduced. In conditions of overproduction of free radicals or a deficiency of antioxidant systems, GSH is consumed and levels will fall (Xu and Stringer, 2008). Glutathione reacts with the free radicals and can protect cells from singlet oxygen, hydroxyl radical and superoxide radical damage. Curcumin has been reported to cause decrease in lipid peroxidation, mitochondrial dysfunction, and apoptotic indices in oxidative stress conditions (Wang et al., 2005). As oxidative stress is known to contribute to the deficits of cognitive function (Fukui et al., 2002; Keller et al., 2005), the observed increased oxidative stress by PTZ and MES may be one of the factors responsible for the cognitive deficit.

Curcumin did not cause any significant change in the serum levels of valproate, phenytoin, phenobarbitone and carbamazepine when administered along with the sub-therapeutic doses of these AEDs. These findings are in agreement with our previous results where curcumin did not cause any change in serum level of phenytoin, phenobarbitone and carbamazepine (Reeta et al., 2009, 2010), when curcumin was administered for 21 days along with these AEDs. This suggests the probable involvement of other mechanisms in the potentiation of the anti-seizure effect of the sub-therapeutic doses of these AEDs by curcumin.

The results of the present study thus show that curcumin potentiates the anti-convulsant activity of sub-therapeutic doses of valproate, phenytoin, phenobarbitone and carbamazepine in rats. This combination could be useful because of the reduced doses of AEDs without lowering the anti-seizure effects when combined with curcumin. Therefore, curcumin can be a potential adjunct to the conventional AEDs as curcumin helps in increasing the efficacy, reducing the dose and decreasing the side effects of these AEDs.

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