Antiapoptotic and neuroprotective role of Curcumin in Pentylenetetrazole (PTZ) induced kindling model in rat

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Kindling, a sub threshold chemical or electrical stimulation, increases seizure duration and enhances accompanied behavior until it reaches a sort of equilibrium state. The present study aimed to explore the effect of curcumin on the development of kindling in PTZ kindled rats and its role in apoptosis and neuronal damage. In a PTZ kindled Wistar rat model, different doses of curcumin (100, 200 and 300 mg/kg) were administrated orally one hour before the PTZ injections on alternate day during the whole kindling days. The following parameters were compared between control and experimental groups: the course of kindling, stages of seizures, Histopathological scoring of hippocampus, antioxidant parameters in the hippocampus, DNA fragmentation and caspase-3 expression in hippocampus, and neuron-specific enolase in the blood. One way ANOVA followed by Bonferroni post hoc analysis and Fischer’s Exact test were used for statistical analyses.

PTZ, 30 mg/kg, induced kindling in rats after 32.0±1.4 days. Curcumin showed dose-dependent anti-seizure effect. Curcumin (300 mg/kg) significantly increased the latency to myoclonic jerks, clonic seizures as well as generalized tonic-clonic seizures, improved the seizure score and decreased the number of myoclonic jerks. PTZ kindling induced a significant neuronal injury, oxidative stress and apoptosis which were reversed by pretreatment with curcumin in a dose-dependent manner. Our study suggests that curcumin has a potential antiepileptogenic effect on kindling-induced epileptogenesis.

Keywords: Apoptosis, Caspase-3 expression, Enolase, Epileptogenesis, Oxidative stress, Neuronal damage, Seizures

Kindling is the process by which repeatedly induced seizures (sub threshold chemical or electrical stimulation) result in increased seizure duration and enhanced behavioral involvement of those induced seizures. The first stimulation produces a seizure short in duration and has only minimal behavioral accompaniment. With repeated stimulations, however, the duration lengthens and the behavior intensifies until these characteristics reach a plateau1. The behavioral progression in the kindling process suggests that it starts with a limited number of neural circuits and subsequently recruits additional circuits as the behavioral component of the seizure evolves to convulsions. The increasing duration of the seizures suggests that the existing resistance of the circuits to seizure activity is also weakened. It is clear that kindling results in changes in the ability of the brain to limit seizures. Over the years, kindling has become a tool to examine the effects of multiple seizures on the brain and whether these effects can be modified.

There are a number of reports that kindling can lead to the development of spontaneous seizures after enough stimulations. Investigators have examined the effect of multiple manipulations (pharmacological, counter stimulation, and lesioning) on the progression of kindling or on fully kindled seizures2,3.

As kindling is a chronic animal model of limbic epilepsy and also a model for epileptogenesis, there has been active research examining the underlying changes in anatomy and physiology that accompany kindling. Anatomically, rats with chronic limbic epilepsy have evidence for significant neuronal loss throughout the limbic system and related subcortical sites such as the middle thalamic nuclei4,5. Both prolonged and brief seizures can cause neuronal loss. Seizure-induced neuronal loss may occur both acutely, as the result of an initial insult, and chronically, due to subsequent progressive injury. In addition, there is significant sprouting of the mossy fibers in dentate hilus as well as widespread gliosis and glial activation6,7. Animal data also suggest that even a single seizure may be damaging and can cause DNA fragmentation which is the characteristic feature...
of apoptosis. Gamma-enolase, a neuron-specific enzyme critical in energy metabolism is elevated following anoxia, stroke, or status epilepticus. In animal studies, serum levels of neuron-specific enolase (sNSE) correlated with histological findings.

Reactive oxygen species have been implicated in the development of seizures under pathological conditions and linked to seizure-induced neurodegeneration. Oxidative stress/damage has significant role in neurodegenerative diseases apart from other disorders. Natural antioxidants like polyphenols provide neuroprotection through a variety of biological actions, such as interaction with transition metals, inactivation of free radicals, modulation in the activity of different enzymes, and effects on intracellular signaling pathways and gene expression. Several epidemiological studies suggest that diets rich in antioxidants play an important role in the protection against various pathologies. The main sources of these molecules are found in fruits and vegetables and are associated with lower risks of cancer, heart disease, hypertension, neurodegenerative diseases, and stroke. The most common dietary polyphenols in general are the flavonoids as well as nonflavonoid compounds resveratrol. Polyphenols, both flavonoids and non-flavonoids, have proven to be effective in alleviating and protecting against the general mechanisms of neurodegenerative diseases in various cell culture and animal models. Asuntha et al. have demonstrated the ability of ethanol extract of Argeome mexicana (L.) in reducing the severity of lithium-pilocarpine induced status epilepticus in Wistar rats as well as pentylenetetrazole (PTZ)-induced lipid peroxidation in the brain. Pancharagavya Ghrita, an ayurvedic formulation attenuates seizures, cognitive impairment and oxidative stress in PTZ-induced seizures in rats. Extracts of Alternanthera brasiliana (L.) Kuntze, Acorus calamus Linn. and Spinacia oleracea L. have been shown to possess anticonvulsant activity. Rao et al. has reviewed role of inflammatory cytokines and growth factors affecting seizure susceptibility, taking step forward in epilepsy research. Although herbal therapy for epilepsy and other neurological disorders has a long tradition in some cultures, the mechanisms of action of most of these treatments have remained unknown. Recent studies, however, have begun to elucidate potential neuroprotective and antiepileptogenic actions of substances of botanical origin.

Curcumin, a non-flavonoid polyphenol, is derived from turmeric, the powdered rhizome of the medicinal plant Curcuma longa Linn. and is the principal ingredient in the popular Indian spice, turmeric. Turmeric has been used for centuries in parts of India as an herbal therapy for a variety of symptoms and medical conditions, ranging from infections and inflammatory diseases to cancer; however, it also is used to treat neurological diseases, such as Alzheimer’s and epilepsy and also nematode infestation in poultry. Curcumin has been shown to inhibit acute seizures and neuronal death in the kainate model. Studies have also demonstrated antiseizure activity in amygdala and PTZ kindling models. But none of these studies had evaluated the underlying mechanisms of curcumin as an antiepileptic agent.

Therefore, the present study attempts evaluating the effect of curcumin on the process of kindling (induced by sub threshold PTZ injections) along with the mechanisms of action of curcumin.

Materials and Methods
Experimental animals
Young male Wistar rats of 15-20 wk old, weighing 200-250 g was used for the present study. The animals were maintained at 23±2°C with a relative humidity of 65±5% in 12 h light/dark cycle. Animals had free access to standard pellet chow and tap water ad libitum, and were acclimatized to laboratory conditions for at least 7 days prior to experimentation. Institutional Animal Ethics Committee (IAEC) approval (No.55/IAEC/267 dated 27.07.2011) was obtained before the start of the study and the study was carried out according to the ARRIVE guidelines.

Diets and treatments
Rats were weighed and divided into 6 groups of 8 rats each: Group I, Vehicle Control (Dimethyl Sulfoxide); and Group II, Negative control [Vehicle + Pentylenetetrazole (PTZ) 35 mg/kg]; Groups III received Sodium Valproate @200 mg/kg and IV-VI, Curcumin @100, 200 and 300 mg/kg, respectively. Groups III-VI received PTZ 35 mg/kg additionally. Group III served as positive control.
Drug preparation and dosing scheduled
PTZ was dissolved in 0.9% saline in strength of 0.31% and injected intraperitoneally (i.p.) in a volume not exceeding 10 mL/kg, at a subconvulsive dose of 35 mg/kg every alternate day up to 10 wk. Curcumin was dissolved in DMSO (1 mg/mL) and given orally at three different doses (100, 200, 300 mg/kg) 1 h before PTZ injection up to 10 wk Sodium Valporate (200 mg/kg) was dissolved in 0.9% saline and administered by intraperitoneal injection 30 min before PTZ administration up to 10 wk. Curcumin powder was purchased from Sigma-Aldrich, USA and its purity was ≥65%. PTZ was purchased from Sigma-Aldrich, USA. Sodium valproate powder was purchased from Sigma-Aldrich, USA and it was ≥98% pure.

Pentylenetetrazole (PTZ) induced kindling in rats
PTZ was injected i.p. as mentioned above. After each injection of PTZ, the rats were placed singly in isolated transparent Plexiglas cages and were observed for 2 h/day. For behavioral seizure rating, a modified Racine scale was employed as follows: 0 – No response; 1 – Ear and facial twitching; 2 – Myoclonic jerks without rearing; 3 – Myoclonic jerks with rearing; 4– Turn over into side position, clonic – tonic seizures; and 5 – Turn over into back position, generalized tonic – clonic convulsions. An animal was considered kindled when it exhibited stage 4 or 5 of seizure score on three consecutive trials.

Studies with Hippocampus
When the animals became kindled, on the same day, animals were sacrificed by decapitation under overdose of anaesthesia with pentobarbitone. Regarding the time between the last doses of PTZ and killing of animals, all the animals which became fully kindled in all the groups were sacrificed on the same day immediately after the seizure score and rest of the unkindled animals were treated with their respective treatment up to 10 wk and then sacrificed. Hippocampus was carefully dissected out of the brain obtained from the decapitated animals and the following parameters were evaluated.

Histopathology of Hippocampus
One half of each brain from every animals in each group (n=8) was fixed in 10% formalin and was subjected to histopathological studies using hemotoxylin and eosin (H&E) stain. Evaluation was based on various parameters like degenerative changes in neuron like cytoplasmic vacuolation, nuclear chromatin clumping and fragmentation, hypereosinophilia and condensed cytoplasm and fragmentation of the cells. A semiquantitative histopathologic score was used to determine the relative percentage of damaged neurons as followed earlier: Normal, no injury = 0; Rare neuronal injury (<5 clusters) = 1; Occasional neuronal injury (5–15 clusters) = 2; Frequent neuronal injury (>15 clusters) = 3; and Diffuse neuronal injury = 4.

Hippocampal Lipid Peroxidation and Antioxidant studies
Estimations of thiobarbituric acid-reactive substance (TBARS) were carried out in every animals in each group (n=8) as per Okhawa et al.; determination of catalase activity (CAT) was done by method of Luck and reduced glutathione (GSH) estimations was done by method of Griffith.

Hippocampal DNA fragmentation study
DNA was isolated from hippocampal brain specimens of 4 animals in each group using DNA isolation kits and was subjected to agarose gel electrophoresis.

Protein expression study of caspase 3 in hippocampus by western blot analysis
For protein expression study, hippocampus of 4 animals in each group was homogenized in lysis buffer. Insoluble materials were removed by centrifugation. Approximately, 50 µg of total protein extracts was subjected to SDS-polyacrylamide gel electrophoresis followed by electrophoretic transfer on nitrocellulose membrane. Bovine serum albumin in TBS20 mM (pH7.2)/ Tween-20 was used to block the membranes. Specific primary antibody against active caspase 3, and β-actin was added to the membrane separately followed by washing and addition of respective HRP-conjugated secondary antibody. The blots was developed using enhanced chemilumenesence plus detection reagents A and B (Amersham Biosciences, UK limited). The change in band intensity in case of each protein was quantified by scanning densitometry using scion image 4.3 software.

Estimation of Neuron-Specific Enolase (NSE) in the blood
About 0.2 mL of blood was collected from each anesthetized animal by cardiac puncture prior to decapitation. Blood sample was subjected to the
estimation of Neuron-Specific Enolase (NSE) by ELISA according to the manufacturer’s instructions.

Statistical analysis
Data are expressed as the mean ± SD. One way analysis of variance (ANOVA) using Bonferroni post hoc analyses and Fisher’s exact tests were performed using SPSS (20.0 version; LEAD Technologies, Chicago, IL, USA). Statistical significance was considered at \( P < 0.05 \).

Results
Pentylenetetrazole (PTZ) induced kindling in rats
Rats in the control group showed a seizure score of zero (0) throughout the study period (10 wk). In PTZ (35 mg/kg, i.p.) alone treated group, the number of kindled animals showed progressive increase in a time-dependent manner from 0/8 (0%) in 1\textsuperscript{st} wk to 4/8 (50%) in 4\textsuperscript{th} wk to 7/8 (87.5%) in 10\textsuperscript{th} wk (Table 2). Sodium valproate (Gr. III) pre-treatment significantly reduced the seizure score in the PTZ treated animals throughout the study period (Table 1). In this group, none of the animal developed kindling at any point of time.

Curcumin showed dose-dependent protection against PTZ-induced kindling in rats. All the three doses of curcumin showed decrease in seizure scoring and significantly less scoring was seen with both 200 (Gr. V) and 300 (Gr. VI) mg/kg doses compared with PTZ alone treated group (II) (Table 1). Regarding the number of animals developed kindling, significantly less number of animals developed kindling with all three doses of curcumin and at higher doses (200 and 300 mg) of curcumin only one animal in each developed kindling at 4 wk (Table 2). Sodium valproate on the other hand, showed significant anti-kindling effect from 1\textsuperscript{st} wk till the end of study (Tables 1 and 2).

Table 1—Effect of curcumin on the seizure score in PTZ kindled rats

<table>
<thead>
<tr>
<th>Time (wk)</th>
<th>Control (vehicle)</th>
<th>Vehicle+PTZ</th>
<th>Valproate (200 mg/kg)+PTZ</th>
<th>Curcumin (mg/kg)+PTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00±0.00</td>
<td>0.79±0.31(^a)</td>
<td>0.04(^b,c,d,e)</td>
<td>0.82±0.31(^a)</td>
</tr>
<tr>
<td>2</td>
<td>0.00±0.00</td>
<td>1.40±0.18(^a)</td>
<td>0.15±0.17(^b,c,d,e)</td>
<td>1.30±0.31(^a)</td>
</tr>
<tr>
<td>3</td>
<td>0.00±0.00</td>
<td>2.01±0.15(^a)</td>
<td>0.27±0.25(^b,c,d,e)</td>
<td>2.00±0.57(^a)</td>
</tr>
<tr>
<td>4</td>
<td>0.00±0.00</td>
<td>2.33±0.25(^a)</td>
<td>0.33±0.17(^b,c,d,e)</td>
<td>2.44±0.47(^a)</td>
</tr>
<tr>
<td>5</td>
<td>0.00±0.00</td>
<td>2.91±0.30(^a)</td>
<td>0.05±0.01(^b,c,d,e)</td>
<td>3.23±0.62(^b)</td>
</tr>
<tr>
<td>6</td>
<td>0.00±0.00</td>
<td>3.59±0.12(^a)</td>
<td>0.06±0.02(^b,c,d,e)</td>
<td>3.28±0.33(^a)</td>
</tr>
<tr>
<td>7</td>
<td>0.00±0.00</td>
<td>3.90±0.24(^a)</td>
<td>0.08±0.15(^b,c,d,e)</td>
<td>3.13±0.31(^a)</td>
</tr>
<tr>
<td>8</td>
<td>0.00±0.00</td>
<td>4.50±0.18(^a)</td>
<td>0.42±0.12(^b,c,d,e)</td>
<td>3.19±0.33(^a)</td>
</tr>
<tr>
<td>9</td>
<td>0.00±0.00</td>
<td>4.71±0.12(^a)</td>
<td>0.42±0.12(^b,c,d,e)</td>
<td>3.13±0.33(^a)</td>
</tr>
<tr>
<td>10</td>
<td>0.00±0.00</td>
<td>4.96±0.12(^a)</td>
<td>0.42±0.12(^b,c,d,e)</td>
<td>3.17±0.25(^a)</td>
</tr>
</tbody>
</table>
Histopathology of Hippocampus

A semi quantitative histopathological score was used to determine the relative percentage of damaged neurons. Hematoxylin and Eosin (H & E) stained sections from hippocampus of PTZ kindled rat showed diffuse neuronal injury with most of the neurons demonstrating nuclear chromatin clumping, hypereosinophilia, condensation of cytoplasm and fragmentation of the cells (overall HP score = 3.88 ± 0.34) in the PTZ+vehicle treated group (Gr. III). In contrast, in the curcumin 200 and 300 mg/kg pretreated groups (Gr. V & VI), most of the neurons exhibited a normal morphology with intact shape, vesicular nucleus, conspicuous nucleoli and amphiphilic cytoplasm. Only few neurons showed features of neurodegeneration (overall HP score = 2.13 ± 0.34) indicative of occasional neuronal insult. However, 100 mg/kg curcumin pretreated group (Gr. IV), failed to show a similar protective effect (overall HP score = 3.19 ± 0.40). The neurons in sodium valproate (200 mg/kg) pretreated group (35 mg/kg)+PTZ showed diffuse neuronal injury with most of the neurons demonstrating nuclear chromatin clumping, hypereosinophilia, condensation of cytoplasm and fragmentation of the cells (overall HP score = 3.88 ± 0.34) indicative of occasional neuronal insult. Pretreatment with sodium valproate (200 mg/kg) and three different doses of curcumin (100, 200, 300 mg/kg) significantly reduced the whole brain MDA level in PTZ treated animals and it was statistically less compared to PTZ alone treated groups (Table 4).

The catalase activity was significantly decreased in PTZ alone treated rats when compared to the control (overall HP score = 0.81 ± 0.40), exhibited near normal morphology, similar to that observed in control group (overall HP score = 0.0 ± 0.0) (P<0.05) (Table 3).

Hippocampal lipid peroxidation and antioxidant studies

Treatment with PTZ alone significantly reduced the GSH levels in rat brain when compared with normal control rat. Pre-treatment with sodium valproate (200 mg/kg) and three different doses of curcumin (100, 200, 300 mg/kg) significantly increased the level of GSH in brain towards the normal as compared to that of PTZ alone treated group (Table 4).

<table>
<thead>
<tr>
<th>Groups</th>
<th>CV</th>
<th>Nuclear chromatin clumping and fragmentation</th>
<th>Hypereosinophilia and condensed cytoplasm</th>
<th>Fragmentation of cells</th>
<th>HP score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>Pentylenetetrazole (PTZ)+vehicle</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>3.88±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(35mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valproate (200 mg/kg)+PTZ</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.81±0.40&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curcumin (100 mg/kg)+PTZ</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>3.19±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curcumin (200 mg/kg)+PTZ</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2.13±0.34&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curcumin (300 mg/kg)+PTZ</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>2.25±0.45&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

[CV, cytoplasmic vacuolation; NCCFr, Nuclear chromatin clumping and fragmentation; HECOCP, Hypereosinophilia and condensed cytoplasm; Fr, fragmentation of cells; HP score, Histopathological score (HP)] Data are expressed as Mean ± SD. <sup>a,b,c</sup>; vehicle + PTZ<sup>a</sup>; curcumin (100 mg/kg)+PTZ<sup>b</sup>; curcumin (200 mg/kg)+PTZ<sup>c</sup>; and curcumin (300 mg/kg)+PTZ<sup>d</sup>.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH levels (nM/mg protein)</th>
<th>MDA levels (µmol of H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; decomposed/min/mg protein)</th>
<th>Catalase activity (µmol of H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; decomposed/min/mg protein)</th>
<th>sNSE levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(vehic le)</td>
<td>0.0219±0.0012</td>
<td>201.45±1.05</td>
<td>9.16±0.05</td>
<td>5.03±0.27</td>
</tr>
<tr>
<td>Pentylenetetrazole (PTZ) (35 mg/Kg)+vehicle</td>
<td>0.0106±0.0005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>241.23±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.96±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.46±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Valproate (200 mg/Kg)+PTZ</td>
<td>0.0210±0.0006&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
<td>209.28±1.81&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
<td>8.43±0.11&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>6.12±0.28&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curcumin (100 mg/kg)+PTZ</td>
<td>0.0145±0.0013&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>233.16±1.17&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.38±0.29&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>13.37±1.00&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curcumin (200 mg/kg)+PTZ</td>
<td>0.0201±0.0008&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>215.72±0.50&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>7.69±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.85±0.21&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curcumin (300 mg/kg)+PTZ</td>
<td>0.0198±0.0007&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>222.67±0.37&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>8.21±0.08&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>6.94±0.73&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SD. n=8, One way ANOVA followed by Bonferroni post-hoc analysis. P<0.05 compared to control<sup>a</sup>; vehicle + PTZ<sup>b</sup>; curcumin (100 mg/kg)+PTZ<sup>c</sup>; curcumin (200 mg/kg)+PTZ<sup>d</sup>; and curcumin (300 mg/kg)+PTZ<sup>5</sup>
animals. In the sodium valproate and curcumin pre-treated groups, the catalase activity in rat brain were comparable with that of the control group but significantly higher as compared to that of the PTZ alone treated group of animals (Table 4).

**Hippocampal DNA fragmentation study**

All the brain samples from different groups were subjected to DNA fragmentation. The PTZ + vehicle treated and Curcumin 100 mg/kg pre-treated group showed laddering pattern with formation of large number of fragments ranging from 1517 base pairs to 100 base pair fragments indicating DNA fragmentation. In contrast, sodium valproate (200 mg/kg) and curcumin 200 and 300 mg/kg pre-treated group did not show formation of small fragments and hence no prominent laddering pattern (Fig. 1).

**Protein expression study of caspase 3 in hippocampus by western blot analysis**

Fig. 3 shows the protein expression of β actin (Fig. 3a) and Caspase 3 (Fig. 3b) in hippocampus of PTZ treated rats. Induction of kindling with PTZ caused a significant increase in expression of caspase 3 in the hippocampus as compared to control group in which the expression of caspase was very low. Treatment with curcumin (100-300 mg/kg) caused dose depended decreased in the expression of caspase 3 in the hippocampus in PTZ kindled rats and the expression was negligible with curcumin 300 mg/kg dose (Fig. 3). In the sodium valporate group also the expression of caspase 3 was negligible.

**Estimation of neuron-specific enolase (NSE) in the blood**

The level of sNSE in serum was significantly elevated in PTZ alone (17.46±1.06 ng/mL) treated group as compared to the control group of animals (5.03±0.28 ng/mL). In the sodium valproate pre-treated group (6.12±0.28 ng/mL), there was significant reduction in sNSE level as compared to that observed with PTZ alone treated group. All the three doses of curcumin also showed significant reduction in sNSE level as compared to that observed in PTZ alone treated group of animals. Curcumin at 200 and 300 mg/kg showed significant reduction in sNSE levels as compared to that with curcumin 100 mg/kg (Table 4).

**Discussion**

In the present study, we assessed the effect of chronic administration of curcumin in different doses (100, 200 and 300 mg/kg) on PTZ-induced kindling. We also assessed the effect of curcumin on PTZ-induced neuronal damage (measured by sNSE and histopathological scoring), oxidative stress and apoptosis (by measuring DNA fragmentation and expression of proapoptotic protein caspase 3 in the hippocampus) in rats. The result of the present study showed that sub-convulsant dose of PTZ (30 mg/kg, i.p.) has induced kindling in the rats in 10 wk. Administration of curcumin dose dependently protected the rats against kindling as indicated by decreased seizure score. The protection offered by sodium valproate on PTZ kindling is well established and our results came in parallel with previous studies which indicated that Sodium Valproate increased PTZ thresholds to different seizure types.8,48

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![Fig. 1—Effect of curcumin on DNA fragmentation in hippocampus in PTZ treated rats. [DL: DNA Ladder; 1-2: control; 3-4: sodium valproate (200 mg/kg)+PTZ group; 5-6: vehicle+PTZ group; 7-8, 9-10 and 11-12: Curcumin (100/200/300 mg/kg)+PTZ]

![Fig. 2— Expression of (a) β actin and (b) Caspase 3 in hippocampus in PTZ treated rats. [1: vehicle control, 2: vehicle+PTZ, 3-5: Curcumin (100/200/300 mg/kg)+PTZ; and 6: sodium valproate (200 mg/kg)+PTZ]
The antiepileptic effect of curcumin demonstrated in our study is consistent with previous studies where curcumin in dose ranging 100-200 mg/kg showed protection against kainic acid-induced, PTZ-induced and amygdaloid seizures. Freeradicals are normal products of cellular aerobic metabolism involved in the development of seizures. However, when the production of free radicals increases or defense mechanism of the body decreases, they cause cellular dysfunction by attacking at the polyunsaturated sites of the biological membranes causing lipid peroxidation. The increase in levels of malondialdehyde (MDA) is a marker of lipid peroxidation. In the present study, we have measured oxidative stress parameters viz. malondialdehyde (MDA), reduced glutathione and catalase activity in kindled brain tissues to ascertain the involvement of oxidative stress in epileptogenesis and its modulation by an antioxidant, curcumin. Repeated PTZ administration has significantly increased the free radical generation as indicated by increased MDA in the vehicle treated PTZ-kindled rats. Curcumin (100, 200 and 300 mg/kg, p.o.) dose dependently decreased the MDA levels in the brain tissue of PTZ-kindled rats consistent with the previous study. In the present study, glutathione and catalase activity were decreased in the vehicle treated PTZ-kindled rats while curcumin (100, 200 and 300 mg/kg, p.o.) treated groups showed increased levels/activity in kindled brain tissue. These results indicate that during kindling there was excessive oxidative stress persisting as a consequence glutathione levels and catalase activity were depleted while combating oxidative stress. However, curcumin treatment has restored the reduced glutathione level in the brain tissues of PTZ treated rats.

Serum neuron specific enolase (sNSE) is a sensitive marker of neuronal damage in several central nervous system (CNS) diseases including epilepsy. Studies have also demonstrated the increased level of sNSE in various animal models of epilepsy and also in patients with epilepsy. Elevation of sNSE after status epilepticus can be correlated with overall histologic evidence for damage. Our data also showed increase in sNSE in PTZ alone treated group and decrease in valproate and curcumin pretreated groups, accompanied by histological evidence of neuronal damage in hippocampus suggests that such elevations of sNSE appear to be roughly proportional to the extent of histological damage. Thus, we postulate that the increased sNSE here is a consequence of death in distinct neuronal populations in different brain structures and curcumin has been successful in preventing this excitotoxicity induced neuronal damage in PTZ treated rats. Wang et al. also demonstrated that there was significant decreased in serum NSE level with topiramate treatment in PTZ kindled rats.

To investigate the extent of neuronal cell damage caused by PTZ-induced epileptic seizures, we carried out nuclear DNA fragmentation studies. Animal data suggest that even a single seizure may be damaging and can cause DNA fragmentation which is the characteristic feature of apoptosis. Also, in our previous study, we demonstrated DNA fragmentation in the brain in PTZ-induced kindling in the rats. Here too, all the brain samples of the PTZ treated group showed laddering pattern with formation of large number of small fragments indicating DNA fragmentation. While the sodium valproate and curcumin group the brain samples did not show any major fragmentations. These findings suggest seizures cause an early production of oxidative damage to DNA bases before significant DNA strand breaks appear, indicating that reactive oxygen species may be a contributory factor in the mechanism by which seizures cause cell death.

To conclude, the present data support that curcumin offers protection against PTZ kindling in rats and it could be a promising candidate to control both development of seizure and oxidative stress induced neuronal injury and apoptosis during epilepsy. However, further biochemical, molecular and clinical studies are required to ascertain its effectiveness and mechanism of action during epilepsy.

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References


