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AN EXPERIMENTAL EVALUATION OF ANTICONVULSIVE EFFECTS OF CURCUMIN IN WISTAR RATS

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ABSTRACT

Curcumin is the active coloring principle of Turmeric. In the present study, the anticonvulsive effect of Curcumin was studied in albino Wistar rats in two methods. First, Curcumin in a dose of 100mg/kg was tested in comparison to Phenytoin sodium by Maximal Electroshock Seizure (MES) method in which the Duration of Tonic hind limb extension was observed. Secondly, antiepileptic effect of Curcumin was compared to Diazepam in Pentylenetetrazol (PTZ) induced convulsions in which onset and severity of convulsions was observed. Results obtained were analyzed statistically by Kruskal Wallis test and Mann Whitney U test using SPSS 20.0.0 software. The results showed that Curcumin significantly reduced the duration of tonic hind limb extension with a $p < 0.05$ in MES model and In PTZ test, it increased the latency to the onset of seizures with $p< 0.01$. The anticonvulsive effect of Curcumin was thus comparable to the standard antiepileptic drugs.

KEYWORDS: **Diazepam, Maximal Electroshock seizures, Phenytoin, Pentylenetetrazol**

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INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent unprovoked seizures.[1] The incidence of epilepsy is ~ 0.3-0.5% in different populations throughout the world and its prevalence has been estimated to be 5-10 persons per 100 individuals.[2] Antiepileptic drug therapy is the mainstay of treatment for most patients with recurrent seizures. The antiepileptic drugs appear to act by blocking the initiation or spread of seizures. Despite regular treatment, 20-30% of the epileptic patients continue to have seizures. Approximately one-third of the patients develop refractory epilepsy requiring treatment with a combination of two or more antiepileptic drugs.[2] The problems of refractoriness and high toxicity profile of the current antiepileptic drugs have been focused on and research is on to develop highly efficacious and less toxic drugs. Herbal drugs are being increasingly explored because of their low adverse effect profile. Turmeric is the dried rhizome of Curcuma longa belonging to the family of Zingiberaceae, cultivated widely in the South East Asian countries.[3] Turmeric contains about 5% diaryl heptanoid coloring materials known as curcuminoids, the chief of which is diferuloylmethane (82%). The others are dimethoxymethane (15%) and bismethoxycurcumin (3%). The volatile oils in turmeric (about 5%) contain sesquiterpenes, sesquiterpene alcohols, ketones and monoterpenes. The rhizomes also contain arabinose (1%), fructose (12%) and glucose (2%).[3] Turmeric has a long history of use in food as a spice, mainly as an ingredient in many varieties of curry powders and sauces. It has also been used traditionally in Ayurvedic medicine for the treatment of certain diseases like arthritis, ulcers, jaundice, wounds, trauma and also in hepatitis. Most of the activity of turmeric in the treatment of various ailments was shown to be due to Curcumin, its principle coloring agent. Curcumin is the product obtained by solvent extraction of turmeric and purification of the extract by crystallization. It is an oil soluble orange yellow crystalline powder, practically insoluble in water and soluble in alkali. It is stable at high temperatures and in acids but unstable in alkaline conditions and in the presence of light.All the curcuminoids exhibit keto-enol tautomerization.[3] A number of properties of curcumin have been explored in research, which includes anti-inflammatory, anticancer, antioxidant, neuroprotective, hypoglycemic, antiobesity, antiprotein aggregatory and antiinfective properties.

Antiepileptic activity of curcumin was demonstrated in Increasing Current Electroshock Seizure (ICES) test[4], PTZ induced kindling[5,6], Kainic acid induced seizures[7]and Iron induced epileptogenesis[8]. All the previous studies showed that curcumin decreases the levels of oxidative markers like malondialdehyde and glutathione thereby proving that its anticonvulsant effect is due to its antioxidant property. Some studies also reported that co administration of curcumin with Antiepileptic drugs like valproic acid, phenytoin, phenobarbitone and carbamazepine in subtherapeutic doses increased latency to seizures and reduced oxidative stress without altering their pharmacokinetics.[9] None of the previously published studies evaluated the anticonvulsant activity of curcumin in comparison to standard antiepileptics like Phenytoin and Diazepam. Also, curcumin was not extensively tested in animal models for absence seizures namely PTZ induced convulsions. The present study was thus planned to evaluate the anticonvulsive effects of curcumin in comparison to standard antiepileptic drugs namely Phenytoin sodium and Diazepam in animal models representing Generalized Tonic Clonic seizures and Absence Seizures.

MATERIALS AND METHODS

The anticonvulsant effect of curcumin was evaluated in two methods after obtaining permission from the Institutional Animal Ethics Committee, Deccan College of Medical Sciences.

1. Maximal Electroshock seizure method
2. Pentylenetetrazol Induced Convulsions (PTZ Test)

Method1: Maximal Electroshock Seizure Method

Maximal Electroshock Seizure [MES] test is useful in studying the drugs effective against generalized tonic-clonic seizures. In this test, a current of 150mA at 60hz is given for 0.2 sec using two ear electrodes. The resultant seizure passes through the phase of tonic flexion for 1.5sec followed by a phase of tonic hind limb extension lasting for about 10 sec and finally followed by variable clonic phase. A reduction or complete abolition of tonic hind limb extensor phase is considered as anticonvulsant activity of drug.

Requirements for MES method:

- Curcumin –It was obtained as a 99.9% pure powder from Kancor Ingredients Ltd. 200mg of this curcumin powder was dissolved in 10ml of Methanol to make a stock solution of concentration 20mg/ml.
- Phenytoin sodium(Eptoin, Abbott) - To one ampoule of Phenytoin sodium containing 100 mg of phenytoin in 2ml, distilled water 3ml was added to prepare a stock solution of 20mg / ml concentration.
- Techno's Electroconvulsiometer
- 18 Wistar rats– animals were reared in central animal house, DCMS. Food and water was given ad libitum. They were screened one week prior and housed separately.

Procedure:

18 albino rats were taken and were divided into three groups-

Group 1– Control group – only electroshock

Group 2 – Phenytoin Sodium – 20 mg/kg intraperitoneally (i.p)

Group 3 – Curcumin 100 mg/kg i.p.

After 30 minutes of drug administration in Group B and C, the animals were subjected to MES test.

Method 2: Pentylenetetrazol Induced Convulsions (PTZ test)

Pentylenetetrazol (PTZ) is a tetrazol derivative believed to act by antagonizing the inhibitory GABAergic neurotransmission. PTZ test is used for screening of drugs effective in petit mal epilepsy or absence seizures. In this method PTZ is injected intraperitoneally 30 min after the test drugs are administered intraperitoneally. The onset of action is indicated by straubs tail, jerky movements of the whole body and convulsions. The latency in the onset of seizures and the severity of seizures is noted. A delay or complete abolition of convulsions was considered as positive anticonvulsant effect.

Requirements for PTZ test:

- 18 male wistar rats
- Pentylenetetrazol (Nimedia) - PTZ 60mg powder was taken and dissolved in 10ml of distilled water to make a stock solution of concentration 6mg/ml.
- Diazepam (Calmpose, Ranbaxy) - One vial of Calmpose contains 10mg diazepam in 2ml. To this, 3ml of distilled water was added to make a stock solution containing 2mg/ml.
- Curcumin - 200mg of curcumin powder was dissolved in 10ml of methanol to make a stock solution of concentration 20mg/ml.

Procedure:

18 albino mice were taken and divided into three groups-

Group 1 – Control – Only PTZ 80 mg/kg ip

Group 2 – Diazepam –4 mg/kg ip + PTZ 80 mg/kg ip

Group 3 – Curcumin – 100mg/kg ip + PTZ 80mg/kg
Drugs were injected intraperitoneally 30 min prior to administration of PTZ.
The severity of seizures was assessed using the following scale

Table No1
SEVERITY OF SEIZURE SCORE IN PTZ TEST

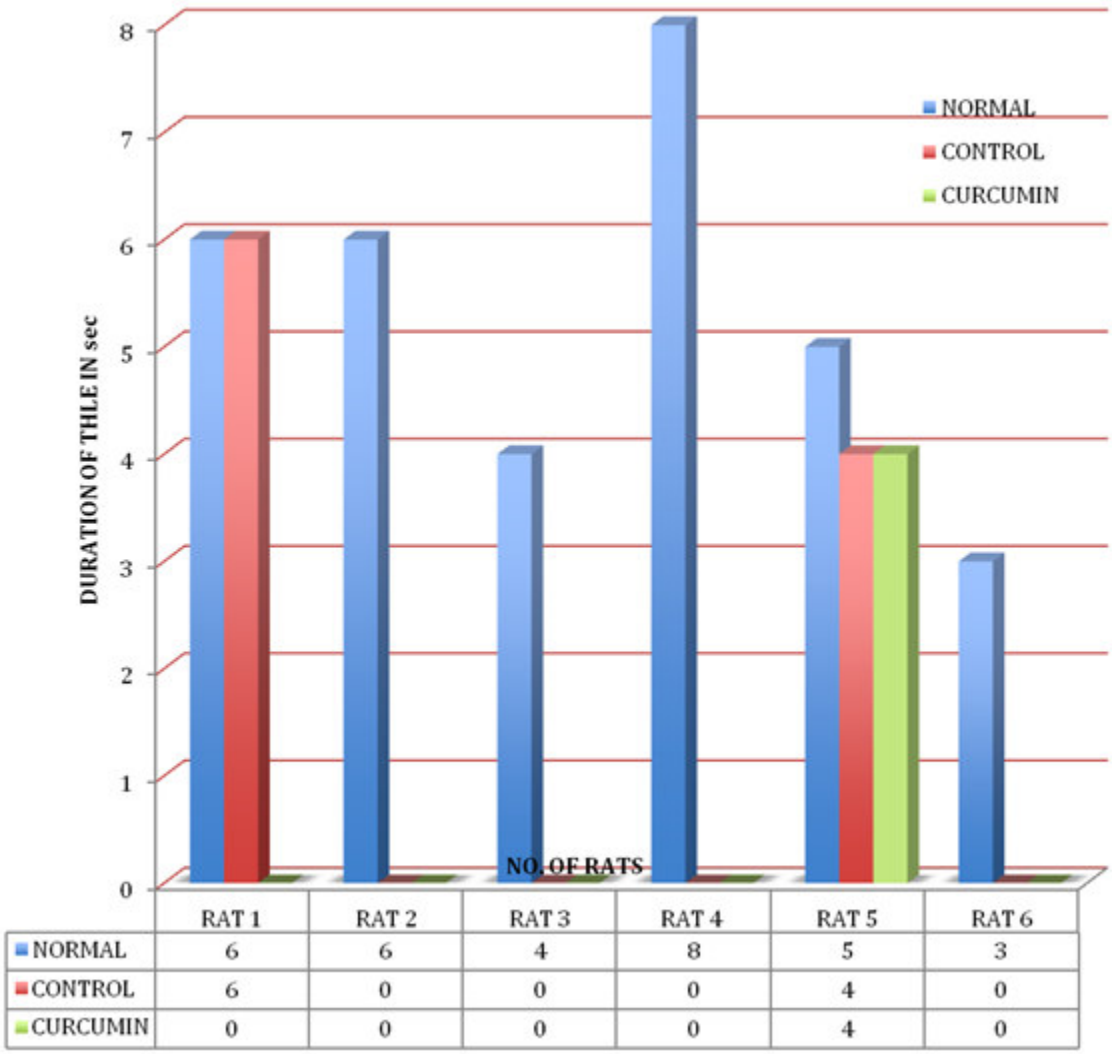
SCORE	CHANGES OBSERVED
0	NO BEHAVIOURAL ABNORMALITIES
0.5	ATYPICAL BEHAVIOUR
1	ISOLATED MYOCLONIC JERKS, EAR & FACIAL TWITCHINGS
2	ATYPICAL MINIMAL SEIZURES, CONVULSIVE WAVE THROUGHOUT THE BODY
3	FULLY DEVELOPED MINIMAL SEIZURES, CLONUS OF THE HEAD MUSCLES AND FORELIMB, RIGHTING REFLEX PRESENT
4	MAJOR SEIZURES, GENERALIZED WITHOUT TONIC PHASE
5	GTCS BEGINNING WITH RUNNING

RESULTS

Method 1: Maximal Electroshock Seizure Method
DURATION OF TONIC HIND LIMB EXTENSION (THLE) IN SECONDS IN VARIOUS GROUPS
Table No.2: DURATION OF THLE IN SEC IN VARIOUS GROUPS

S.NO.	GROUP 1 CONTROL	GROUP 2 PHENYTOIN	GROUP 3 CURCUMIN
1	6	6	0
2	6	0	0
3	4	0	0
4	8	0	0
5	5	4	4
6	3	0	0

Figure 1
DURATION OF TONIC HIND LIMB EXTENSION IN SEC



	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6
NORMAL	6	6	4	8	5	3
CONTROL	6	0	0	0	4	0
CURCUMIN	0	0	0	0	4	0

Results were analyzed using SPSS software 20.0.0. Non parametric Kruskal Wallis test and Mann Whitney U test were performed, as the data is small and variation was high (19- 65%).
On Kruskal Wallis test, $p<0.026$ was obtained which implies that there is statistically significant difference between the groups.

The results obtained on Mann – Whitney U test are as follows,

Table No. 3
p*VALUE OBTAINED ON MANN WHITNEY'S TEST IN MES MODEL*

Comparison Between	P value	Statistical inference
Groups 1& 2	<0.05	Significant
Groups 1& 3	< 0.01	Significant
Groups 2 & 3	>/= 0.05	Not significant

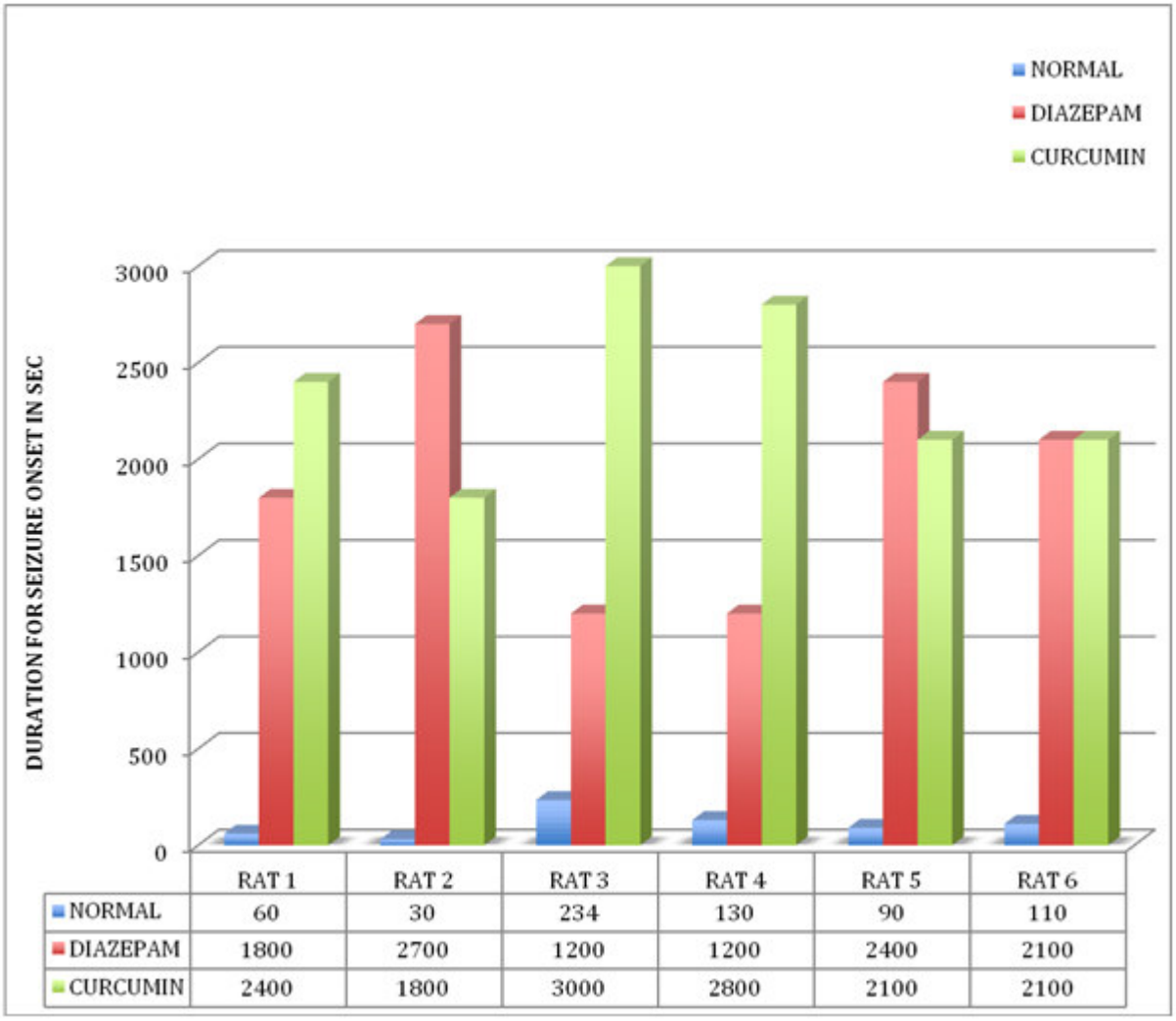
The results show that curcumin decreased the duration of tonic hind limb extension in MES model significantly comparable to that of Phenytoin sodium.

Method 2: Pentylenetetrazol Induced Convulsions (PTZ test)

Table No.4
RESULTS OBTAINED ON PTZ TEST

S.NO.	TREATMENT GROUP	CONVULSIONS		
		ONSET (sec)	NATURE & SEVERITY	DEATH/ RECOVERY
1	CONTROL	60	Stage 5	Died
2	CONTROL	30	Stage 5	Died
3	CONTROL	234	Stage 1	Recovered
4	CONTROL	130	Stage 5	Died
5	CONTROL	90	Stage 5	Died
6	CONTROL	110	Stage 5	Died
7	DIAZEPAM	1800	Stage 0	Recovered
8	DIAZEPAM	2700	Stage 0	Recovered
9	DIAZEPAM	1200	Stage 0.5	Recovered
10	DIAZEPAM	1200	Stage 0.5	Recovered
11	DIAZEPAM	2400	Stage 0.5	Recovered
12	DIAZEPAM	2100	Stage 0.5	Recovered
13	CURCUMIN	2400	Stage 0.5	Recovered
14	CURCUMIN	1800	Stage 0.5	Recovered
15	CURCUMIN	3000	Stage 0	Recovered
16	CURCUMIN	2800	Stage 0.5	Recovered
17	CURCUMIN	2100	Stage 0.5	Recovered
18	CURCUMIN	2100	Stage 0.5	Recovered

GRAPH 2
LATENCY TO THE ONSET OF SEIZURES



	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6
NORMAL	60	30	234	130	90	110
DIAZEPAM	1800	2700	1200	1200	2400	2100
CURCUMIN	2400	1800	3000	2800	2100	2100

Results were analyzed using SPSS software 20.0.0. Non parametric Kruskal Wallis test and Mann Whitney U test were performed as the data was small and variation was high (19- 65%).

On Kruskal Wallis test, $p < 0.002$ was obtained between the groups which implies that there is statistically significant difference between the groups

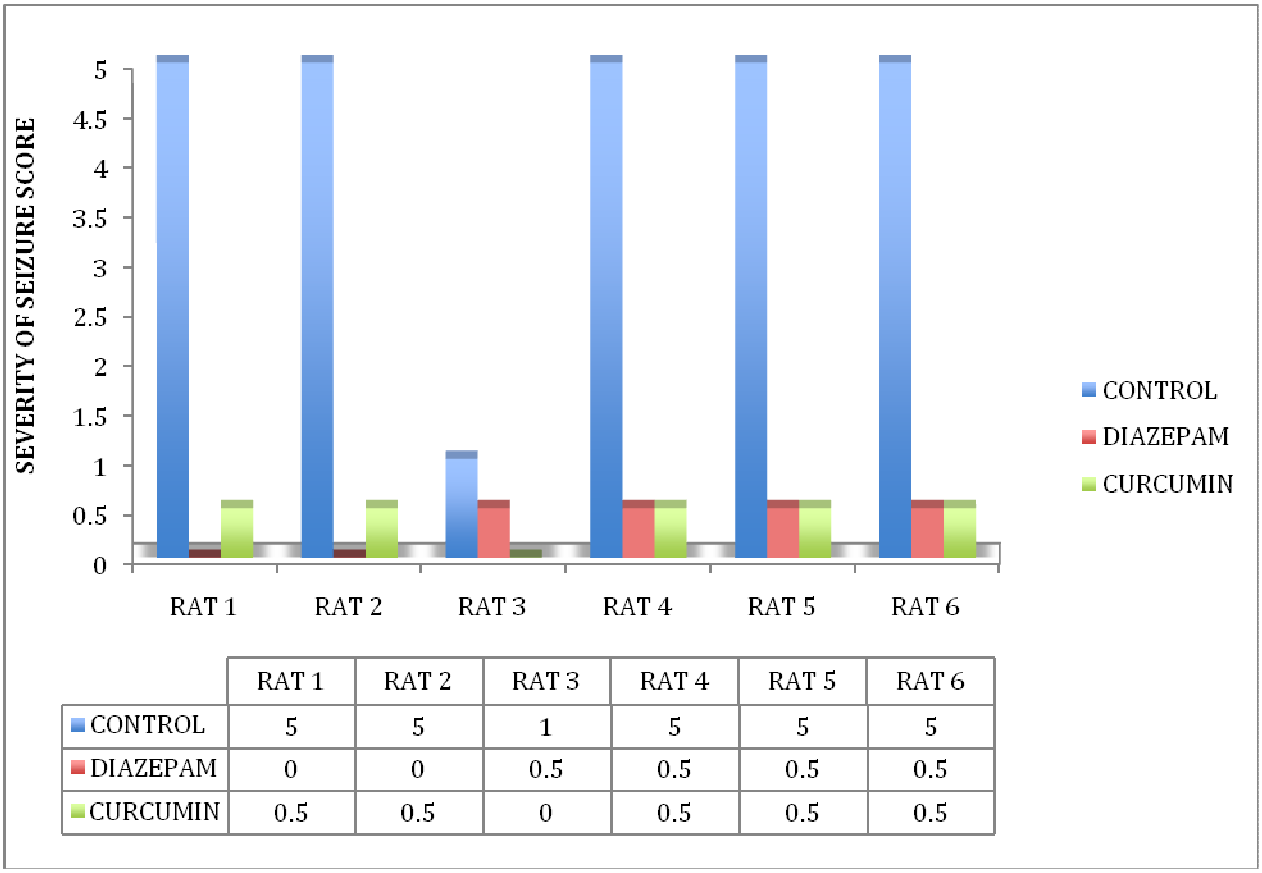
On Mann – Whitney U test, the following results were observed.

Table No. 5
P *VALUE OBTAINED ON MANN WHITNEY U TEST FOR LATENCY TO THE ONSET OF SEIZURES IN PTZ TEST*

Comparison Between	p value	Statistical inference
Groups 1& 2	<0.004	Significant
Groups 1& 3	< 0.004	Significant
Groups 2 & 3_	0.195	Not significant

The results show that the curcumin delayed the latency to the onset of seizures similar to diazepam.

GRAPH 3
SEVERITY OF SEIZURE SCORE



	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6
CONTROL	5	5	1	5	5	5
DIAZEPAM	0	0	0.5	0.5	0.5	0.5
CURCUMIN	0.5	0.5	0	0.5	0.5	0.5

The severity of seizures was found to be reduced with curcumin similar to that seen with Diazepam

DISCUSSION

The anticonvulsive activity of curcumin was proposed to be due to reduction of oxidative stress, Mammalian Target Of Rapamycin [mTOR] inhibition and through adenosine A1 receptor activation. Oxidative stress is one of the possible mechanisms in the pathogenesis of epilepsy. Intracellular calcium levels play a key role in regulating neuronal excitability and synaptic transmission. Oxidative stress disturbs this calcium homeostasis leading to neuronal loss in epilepsy. Also, the severity and recurrence of epileptic seizure is associated with high oxidative stress. Antioxidants therefore prevent epileptic seizure and are also neuroprotective. The antioxidant effect of curcumin and its seizure inhibitory activity was demonstrated in Pilocarpine induced model[10], Kainic acid induced model[7], Iron induced model of epilepsy[8] and in PTZ induced kindling.[5,6]

mTOR is a serine/threonine kinase belonging to phosphoinositide 3-kinase- related kinase family and is highly conserved in mammal. mTOR pathway has shown to play a pivotal role in neurodegeneration, neurogenesis and synaptic plasticity.Curcumin inhibits mTOR signaling at micromolar ranges and thus shows anticonvulsant effect.[11] The anticonvulsant activity of curcumin in PTZ induced convulsions was prevented by nonspecific adenosine receptor antagonist 8- phenyltheophylline and specific A1 receptor antagonist 8-cyclopentyl-1-3-diproxylxanthine but not by specific A2 antagonist 8-(3-cholorostryl) caffeine. This suggests that curcumin may activate adenosine A1 receptors to abolish convulsions.[12]

Based on the results of Maximal Electroshock Seizure and PTZ test, it is shown that curcumin has significant anticonvulsive

effects comparable to that of standard antiepileptic drugs – Phenytoin sodium and Diazepam respectively in animal models representing Generalized Tonic Clonic Seizures and Absence Seizures. Despite being highly effective in animal models of epilepsy, the clinical utility of curcumin is still restricted because of its unfavorable pharmacokinetic properties. The bioavailability of curcumin is low because of poor oral absorption, rapid metabolism and rapid systemic elimination. Maximum plasma concentrations are attained after 1-2_hrs of oral intake. Curcumin gets metabolized in liver by glucoronidation and sulfation to inactive metabolites. 75% of curcumin in the form of its metabolites gets excreted in feces and a negligible amount in urine.[13]

To improve the bioavailability of curcumin, the following methods are proposed:

1. Use of adjuvant like Piperine that interferes with its glucoronidation[14]
2. Use of liposomal curcumin[15]
3. Use of curcumin nanoparticles[13]
4. Use of curcumin phospholipid complex[13]
5. Use of structural analogues of curcumin like EF24[13]

Animal toxicity studies showed staining of fur, discoloration of feces, increase in liver weight and hyperplasia of mucosal epithelium in ceacum and colon of rats. No carcinogenicity and teratogenecity potential was demonstrated. A dose escalation study in healthy volunteers showed that curcumin caused diarrhea, headache, rash and yellow stool.[16] It can be concluded that exploring curcumin for its pleiotropic effects including epilepsy is limited by its poor pharmacokinetic profile. So development of curcumin with improved bioavailabilty may pave a way for its improved clinical utility.

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