

Protective role of curcumin in maximal electroshock induced seizures, memory impairment and neurotransmitters in rat brain

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Abstract: Central nervous system disorders are of great concern in the present day world due to increasing stress and changing living conditions. Since epilepsy is one of the most prevalent CNS disorders, and as a number of side effects are associated with the present antiepileptic drug treatments, the presented research was designed to evaluate the effect of curcumin on generalized tonic clonic (GTC) seizures, together with its effect on memory retention in seizure induced rats, and the role of monoamines in protection from seizures and memory impairment. To investigate the effect on GTC, Maximal electro shock (150mA intensity for 0.2 sec) was administered using electro convulsimeter after 14 days of treatment with curcumin (5 and 10 mg/kg). Rats were trained for conditioned avoidance response before the initiation of treatment, and the effect on memory was studied after the induction of epilepsy using a conditioned avoidance response task. Fluorimetric estimations of norepinephrine, dopamine and serotonin in cerebral cortex, cerebellum, hippocampus, pons and hypothalamus made it evident that curcumin exerted a potential antiepileptic and memory retentive effects, with considerable influence on the brain monoamine levels.

Key words: epilepsy, memory, norepinephrine, dopamine, serotonin, curcumin

INTRODUCTION

Epilepsy affects approximately 50 million people of whom about 40 million people lack appropriate treatment [1] and is emerging fast as a hindrance to many lives. On the other hand, the abundant side effects of the existing antiepileptic drugs (AEDs) are of great concern for both patients and physicians. The long term use of traditional medicines for prophylaxis is ruled out in the treatment of epilepsy, since the unpredictability of seizure occurrence limits their therapeutic efficacy. Furthermore, cognitive defects pose a serious threat in epileptic patients [2] and the worsening effects of the existing AEDs are anchoring the cognitive deficits of the epileptic patients [3]. Although extensive research on the neurobiological bases was performed to understand the role of neurochemicals in regulation of epilepsy, studies showing the involvement of brain catecholamines and indoleamines in seizure reduction are meager. However, a few studies have reported the protective effect of norepinephrine (NE) against electroshock induced GTC seizures, and serotonin (5-HT) against pentylenetetrazole (PTZ) induced absence seizures [4], throw some light on the role of neurotransmitters in the treatment of seizures, involvement of brain monoamines, mainly the NE and dopamine (DA), in mediating the cognitive tasks in patients with Alzheimer's disease [5]. Additionally, several publications have provided information on seizures and their treatment, although very few attempts have been

made to study the effect of drugs on memory after seizure inductement, the role of monoamines in protection from seizure occurrence and memory impairment, and the effect of standard AED treatment on memory.

In view of these findings, we have chosen curcumin – the active constituent of *Curcuma longa* – which has been claimed to possess efficacy in the treatment of Alzheimer's disease [6], MAO-A & B inhibitory activity [7] enhancement of brain monoamine levels, and rhizomes being cited in the database for Indian medicinal plants in the treatment of epilepsy [8], for which no reported evidence was available, all these factors had made us to focus on the objectives of the present study, to evaluate: 1) the protective effect of curcumin on the Maximal electro shock (MES) induced seizures; 2) effect on memory retention after seizure induction; and 3) effect on the neurotransmitter levels in various regions of rat brain.

MATERIALS AND METHODS

Chemicals. Dopamine and norepinephrine were obtained from Sigma Chemical Co., USA. Serotonin was obtained from Qualigens, Mumbai, India, and other chemicals were obtained from SD Fine Chemicals, India.

Animals. Inbred adult male Wistar rats (200-250 g) were procured from the animal house of Bapatla College of Pharmacy, Bapatla, India, and were housed at a constant room temperature of $22 \pm 1^\circ\text{C}$, 40-50% relative humidity, and a 12-12 h light/dark cycle were maintained. Standard pellet feed (Rayan's Biotech, Hyderabad) and water were provided

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ad libitum throughout the period of the experiment. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The experimental design was approved by Institutional animal ethics committee (IAEC/XIII/3/2005-2006) and all the experiments involved in this work were performed in accordance with CPCSEA guidelines for the use and care of experimental animals.

In order to avoid variations in the results due to circadian rhythms of biogenic amine levels and their metabolism, all the experiments involving the measurement of the biogenic amines were conducted between 8.00 am and 10.00 am, after sub-acute treatment with curcumin, including the evaluation of antiepileptic activity and memory retention test.

Drugs and drug administration. Curcumin was obtained from Lailla Impex, Vijayawada, India, and was characterized by H⁺ NMR studies. For oral administration, Curcumin (5 and 10 mg/kg p.o.) was dissolved in peanut oil and diluted to the desired concentration with the same, on the day of administration. Phenytoin (PHT) (20mg/kg i.p.) was diluted with sterile saline for i.p. administration. The peanut oil and sterile saline were used as control treatments and the behavioural data did not differ between rats that received these vehicles, hence the results were compared with peanut oil treated control group.

Conditioned avoidance response: Training procedure [9]. Rats of all the groups were subjected to conditioned avoidance response before the induction of MES seizures. Each rat was placed individually in a pole climbing apparatus (Inco Co., Ambala, India) provided with a grid floor that can be electrified and conditioned with a buzzer. Rats were trained 3 times a day initially to avoid the electroshock (80 V, 5 pulses/sec) applied to the grid floor, intermittently preceded by the beep (conditioned stimulus). Rats that learned to avoid the shock by climbing the pole after training for 2 weeks, were selected for studying the effect on MES induced seizures, and later, the memory retention effect after induction of seizure and estimation of monoamine (5-HT, NE & DA) levels in various regions of brain.

Grouping of animals. Curcumin and PHT were administered for 14 days and 60 min prior to the induction of MES on the last day of treatment. Group I animals served as epileptic control. Group II and Group III animals were treated with curcumin 5 and 10 mg/kg p.o., respectively. Group IV animals were treated with standard AED, PHT 20 mg/kg i.p. In determining memory retention and monamines an additional control group not exposed to MES was used.

Induction of maximal electroshock seizures (MES). GTC seizures were induced, using an Electro convulsimeter (Inco Co., Ambala, India). Maximal seizures were elicited by 60 Hz alternating current of 150 mA intensity for 0.2 sec using corneal electrodes. A drop of electrolyte solution 0.9% sodium chloride with lignocaine was applied to the corneal electrodes [10], which ensures better contact and mortality rate to zero. Percentage protection of drugs from seizures was estimated by inhibition of complete hind limb tonic extension (HLTE) or HLTE not greater than a 90° angle with the plane of the body [10]. Various other parameters measured were: duration of 1) tonic flexion, 2) tonic extension, 3) clonic convulsions, and 4) righting reflex.

Conditioned avoidance response: Performance after seizure induction. After ensuring recovery from seizures, rats were placed individually in the pole climbing apparatus for determining the memory retention effect. A fixed number (i.e. 10) of shocks were applied to the electric grid floor, and the number of shocks avoided by each animal of a group were determined and tabulated.

Estimation of Rat Brain Catecholamines and Indoleamines. Rats were sacrificed by cervical dislocation and the brains immediately isolated. Anaesthesia was not used as it alters the brain amines [12]. After sacrificing, the brains were rapidly removed and the cerebral cortex, cerebellum, pons, hippocampus and hypothalamus were dissected on an ice-cold flat glass plate. Concentrations of NE, DA and 5-HT were measured by fluorimetry [13].

All brain regions were weighed and brain regions of 2 rats of the same group were pooled and homogenized with 6 ml of cold acidified Butanol at 800 x g. An aliquot from each homogenate pool served as a tissue sample for that group. The internal standards were prepared by the addition of known amounts of mixed standard (500 µg each NE, DA & 5-HT) to a portion of homogenate pools and processed parallelly with tissue samples. The reagent blanks and test samples for estimation were prepared following the same procedure described by Kari *et al.*, with slight modifications. NE, DA and 5-HT were read with an excitation and emission wavelength of 385 nm and 485 nm, 320 nm and 370 nm, and 360 nm and 470 nm, respectively, with a slit width of 10/10 nm.

STATISTICAL ANALYSIS

All the data obtained from the observations were statistically analyzed using one way ANOVA, when the F test ratio was significant, the inter group differences were evaluated using Dunnet's 't' test and P < 0.05 was considered to be significant.

RESULTS

Effects on various phases of GTC seizures. Treatment with curcumin at doses of 5 mg/kg and 10 mg/kg exhibited a percentage protection of 50 and 66, respectively. Whereas, PHT treated rats showed a 100% protection against the MES induced seizures by inhibiting HLTE. Neither of the 2 doses of curcumin nor the PHT treated rats had shown any significant changes in the duration of tonic flexion and clonic convulsions, but a significant reduction was observed in the time taken for the righting reflex in curcumin 10 mg/kg and PHT treated rats (P < 0.01, P < 0.001), respectively (Table 1).

Effect on the performance in conditioned avoidance response after induction of seizures. Rats that received MES (epileptic control) exhibited a significant decrease (P < 0.001) in memory retention when compared to the control animals (not exposed to MES). Whereas, a significant increase (P < 0.01 and P < 0.001) in the retention of memory was observed in both the dose levels of curcumin. PHT treated rats also had showed a significant performance impairment in the conditioned avoidance response (Table 2).

Table 1 Effect of curcumin on MES induced convulsions

Groups Treatment		Duration in different phases (sec)				
% of protection		Tonic Flexion	Tonic Extension	Clonic Convulsions	Righting Reflex	
I	Epileptic control	0%	5 ± 0.85	13.3 ± 0.86	13.4 ± 1.62	5.83 ± 1.014
II	Curcumin (5 mg/kg)	50%	4.33 ± 0.61ns	3.83 ± 2.27#	9.67 ± 0.53ns	4.16 ± 0.71ns
III	Curcumin (10 mg/kg)	66%	3.16 ± 0.47ns	3.16 ± 2.19#	9.63 ± 0.98ns	2 ± 0.57**
IV	PHT	100%	3.5 ± 0.56ns	0#	8.5 ± 1.66ns	1.16 ± 0.65#
d.f. 3, 20 F=		-	1.828	12.51	1.374	7.83

Values expressed are mean ± SEM of 6 observations. Values of curcumin (5 & 10 mg/kg) PHT treatments were compared with control individually for each phase. ANOVA was used to determine the significance between groups followed by dunnet's t test to determine the intergroup significance.
* p<0.05; ** p<0.01; # p<0.001; ns – non significant; PHT – Phenytoin, Control-Vehicle treated; MES was administered to all the groups

Table 2 Effect of curcumin on memory retention using conditioned avoidance response

Groups	I	II	III	IV	V
Treatment	Control	Epileptic control	Curcumin (5 mg/kg)	Curcumin (10 mg/kg)	PHT
No. of shocks avoided	8.2 ± 1.4	1.3 ± 0.41 ^{A#}	3.5 ± 0.61 ^{B**}	5 ± 1.2 ^{B#}	2.8 ± 0.43 ^{Bns}

d.f. = 4,25; F = 6.748
Data are expressed as mean ± SEM of 6 observations, representing the number of shocks avoided out of 10 applied.
A = Comparison of Group I Vs Group II; B = Comparison of Group II Vs III, IV and V; **p<0.01; #p<0.001; ns – non significant;
d.f. – degrees of freedom; PHT – Phenytoin, Control-Vehicle treated; MES was administered to all the groups except Control (vehicle treated).

Effect on Dopamine, Norepinephrine and Serotonin levels in different regions of rat brain. The monoamine levels of the groups II, III, IV and V, exposed to MES were compared with that of group I which served as control (no previous exposure to MES).

Effect on brain Dopamine. A significant decrease (p<0.001) in the DA levels were observed in cortex, pons, hippocampus and hypothalamus in rats exposed to MES. Rats treated with curcumin 5 and 10 mg/kg showed a significant increase (p<0.001) in DA levels in cortex, pons, hippocampus and hypothalamus. PHT treated rats showed a significant increase in cortex (p<0.01), hippocampus (p<0.001) and hypothalamus (p<0.01). Whereas, no significant change was observed in the cerebellum when compared to epileptic control (MES) rats Figure 1.

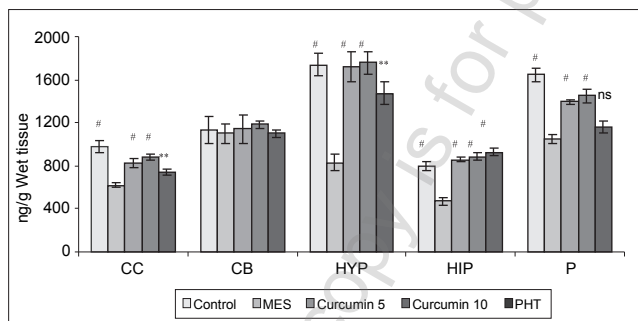


Figure 1 The effect of curcumin and PHT on dopamine levels (nanogram/gram of wet tissue) in various regions of rat brain (Cerebral Cortex (CC), Cerebellum (CB), Hypothalamus (HYP), Hippocampus (HIP) and Pons (P)) after sub-chronic treatment of rats with curcumin and exposure to MES on the 14th day of treatment, Each column represents the mean ± SEM of 3 samples (brain regions of 2 rats of same group were pooled). Values of Control, Curcumin 5 mg/kg, Curcumin 10 mg/kg and Phenytoin treated rats were compared with Epileptic control (MES). All the groups except Control were exposed to MES (significantly different from control, # p<0.001; ** p<0.01; * p<0.05; ns – non significant).

Effect on brain Norepinephrine. A significant decrease (p<0.001) in the NE levels of the hippocampus and hypothalamus was observed in rats exposed to MES. Curcumin at 5 and 10 mg/kg showed a significant (p<0.001) increase in the NE levels of cortex, hippocampus, hypothalamus (p<0.01) and pons (p<0.001), respectively, while PHT treated rats showed a significant increase (p<0.001) in NE levels of the cortex, pons and hippocampus. No significant changes were observed in the NE levels of cerebellum when compared to epileptic control (MES) rats Figure 2.

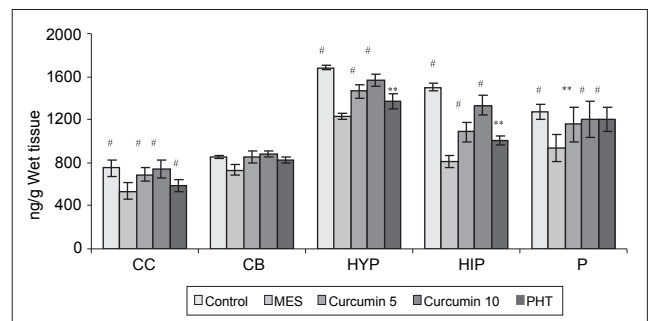


Figure 2 The effect of curcumin and PHT on norepinephrine levels (nanogram/gram of wet tissue) in various regions of rat brain (Cerebral Cortex (CC), Cerebellum (CB), Hypothalamus (HYP), Hippocampus (HIP) and Pons (P)) after sub-chronic treatment of rats with curcumin and exposure to MES on the 14th day of treatment, Each column represents the mean ± SEM of 3 samples (brain regions of 2 rats of same group were pooled). Values of Control, Curcumin 5mg/kg, Curcumin 10 mg/kg and Phenytoin treated rats were compared with Epileptic control (MES). All the groups except Control were exposed to MES (significantly different from control # p<0.001; ** p<0.01; * p<0.05; ns – non significant).

Effect on brain Serotonin. A significant decrease in brain serotonin levels were observed in cerebellum (p<0.05), hippocampus (p<0.001) and hypothalamus (p<0.001) of epileptic control rats (MES). Curcumin treatment at 5 mg/kg showed an increase in the levels of 5-HT in cerebellum (p<0.05), hippocampus (p<0.05) and hypothalamus

($p < 0.01$). Curcumin at 10 mg/kg showed a significant increase in cerebellum ($p < 0.05$), hippocampus ($p < 0.001$) and hypothalamus ($p < 0.001$). No significant changes were observed in the cortex and pons of all groups. PHT treatment did not show any significant changes when compared to Group II animals (Figure 3).

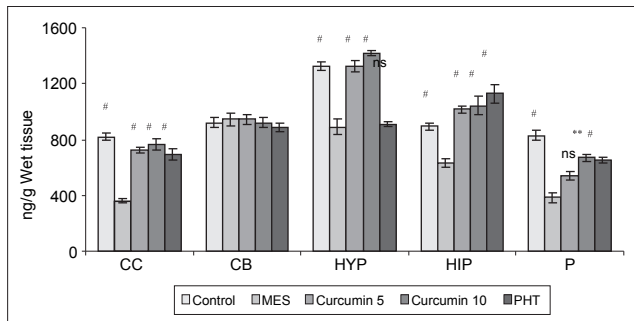


Figure 3 The effect of curcumin and PHT on serotonin levels (nanogram/gram of wet tissue) in various regions of rat brain (Cerebral Cortex (CC), Cerebellum (CB), Hypothalamus (HYP), Hippocampus (HIP) and Pons (P)) after sub-chronic treatment of rats with curcumin and exposure to MES on the 14th day of treatment. Each column represents the mean \pm SEM of 3 samples (brain regions of 2 rats of same group were pooled). Values of Control, Curcumin 5mg/kg, Curcumin 10 mg/kg and Phenytoin treated rats were compared with Epileptic control (MES). All the groups except Control were exposed to MES (significantly different from control # $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns – non significant).

DISCUSSION

Curcumin at doses of 5 mg and 10 mg/kg/p.o. exhibited a significant protection against the MES-induced GTC seizures, this might be due to the observed increase in the NE and DA levels in various parts of the brain substantiating reports that drug treatment which increases brain monoamine levels by inhibiting MAO tends to raise the seizure threshold [4], as Curcuma longa has been reported to increase the brain monoamine levels by inhibiting MAO-A and B in mice brain [7].

On the other hand, low concentrations of DA in cerebellum had been reported to show an inhibitory effect on glutamate, causing blockade of calcium channels [14], indicating a possible mechanism involved in the antiepileptic effect of curcumin, i.e. a decreased influx of calcium ions into the neuronal cells, thereby inhibiting neurotransmitter re-uptake. This could be effective in the treatment of CNS disorders as epileptic depolarization in single motor cortical and hippocampal neurons and focal epileptic discharges in neuronal cortical preparations have been reported to be decreased by calcium channel blockers [15]. As theoretical considerations suggest that calcium channel blockers possess anticonvulsant activity, the protective effect of curcumin against MES induced convulsions might be due to the decreased calcium influx, resulting in a decrease in NE mediated glutamate inhibition.

At both the dose levels of curcumin, a good memory retentive effect in seizure induced animals was observed when compared to the epileptic control and PHT treated rats. This increase in memory retention can be interpreted to the increased NE and DA concentrations in brain regions as drugs like L-Dopa have shown an improvement in cognitive test of patients of Alzheimer's disease by increasing DA levels. Other possible mechanism for the memory retentive effect of curcumin can be attributed to the MAO-B inhibitory activity of curcumin [16], as selegelline a MAO-B inhibitor has demonstrated mild

but significant improvement in cognitive tasks in a double blind placebo controlled trial in addition to its capacity to increase brain NE and DA concentrations [17] and in the treatment of Alzheimer's disease. Pathological abnormalities in serotonergic and noradrenergic innervations are known to exist in addition to cholinergic innervations in the brain of Alzheimer's patients [18]. This also indicates the rationality of a combination therapy of cholinergic and monoaminergic drugs in Alzheimer's disease, as forebrain dopaminergic system is related to cognitive functions [19].

However the role of NE and DA on memory retention is also controversial as psycho stimulants such as methyl phenidate and amphetamine, which are known to increase cerebral catecholamine turnover, have proven to be of little value in Alzheimer's disease [17]. Our studies and the data obtained allowed us to substantiate the use of curcumin in protection from GTC seizures and enhancing memory. Of particular interest are the effects of curcumin on the brain monoaminergic system, since it has been suggested that monoamines are of great clinical significance in the treatment of seizures and memory disorders [4, 20].

CONCLUSION

The results of the presented study, that the brain monoamines (NE, DA & 5 HT) play an important role in protection from epilepsy and memory impairment. The effect of curcumin on chemically-induced absence seizures and the effect on the threshold for generalized and localized seizures have to be interpreted for understanding the anti-epileptic activity of curcumin, and the role of monoamines in chemically-induced seizures are to be performed for meaningful extrapolations of anti-epileptic activity of curcumin.

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