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Protective Effect of Curcumin Alone and in Combination with Isradipine against Chemicals Induced Seizures and Oxidative Stress in Mice

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Abstract

The isradipine and curcumin have long been used to treat Epilepsy and oxidative stress. In this study the Protective effects of curcumin drugs and antiepileptic activity of isradipine and in combination were examined. The extract of turmeric to reduces the oxidative stress which increased during epileptic seizures and in combination with isradipine which is a calcium channel blocker to treat Seizures. In addition there is effects on increased the time of clonus activity and reduces the oxidative stress were recorded.

Keywords: Isradipine, Curcumin, Turmeric, Stress.

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Introduction

Epilepsy is a common neurological condition associated with the alteration in psychological, emotional and educational parameters or epilepsy is due to abnormal recurrent and spontaneous electrical discharge of a group of neurons in the brain and exhibits itself as a seizure occurrence. Drugs used for the management of convulsive disorders are called anticonvulsants. (Khalili M et.al, 2011).

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start

chain reactions that damage cells. (Silva et al2009).

The excessive production of free radicals in the human body is involved in the pathogenesis of various diseases including atherosclerosis, diabetes mellitus, stroke, inflammatory diseases, cancer and epilepsy. (Obay BD et al. 2008).

Materials and Methods

Chemicals and reagents

Pentylenetetrazole, Picrotoxin, thiobarbituric acid, trichloro acetic acid, n-butanol¸ Tween 80, were purchased from (Ozone Chemicals, Mumbai), Isradipine (Apollo life science, Mumbai),normal saline, malonaldehyde standard (Cayman Chemicals). Standard drug Isradipine were purchased from Apollo Life Science, Mumbai. Drug like Curcumin were procured from well known organization and of analytical grade from the local market.

Animals

Eight to ten week old albino mice weighing 25-30 g will be procured from Anuradha College of Pharmacy Chikhli, Dist-Buldhana and will be used in this study. With the exception of the short time that the animals were removed from their cages for testing, all animals were maintained on an adequate normal laboratory pellet diet and allowed free access to food and water. The experimental design will be approved by Institutional Animal Ethical Committee and the study will be performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines for the use and care of animals.

Induction of epilepsy and oxidative stress in mice

Epilepsy and oxidative stress were induced by using pentyleletetrazole and picrotoxin.

Induction of seizures by Pentylenetetrazole (PTZ)

Convulsions were induced by a method similar to that described by Kulkarni SK. The convulsive dose of PTZ that causes minimum mortality in mice was determined in laboratory.PTZ was dissolved in water for injection to make 6.5 mg/ml PTZ solution. Mice were injected with PTZ in a dose of 65 mg/kg, intraperitonial (i.p.), following the injection, mice were put individually into open polypropylene cages and observed for 30 min for the occurrence of seizures. In test group i.e. Group II, III to group IV, PTZ was administered after suitable latency (1 hour after Curcumin and Isradipine respectively) corresponding to the time peak effect after oral administration of the test drug (Kulkarni SK 2008).

Anticonvulsant activity in mice seizures induced by PTZ

Mice were divided into four groups. Each group contained six animals.

Group I: (Toxic control) received PTZ (65 mg/kg;ip),

Group II: Received Curcumin(300mg/kg;p.o) and PTZ(65 mg/kg;ip)

Group III: Received Isradipine (5mg/kg;p.o) and PTZ(65 mg/kg;ip)

Group IV: Received Curcumin (300mg/kg;p.o) + Isradipine and PTZ(65 mg/kg;ip)

Picrotoxin induced convulsion

Healthy and convulsion albino mice weighing 25 – 30g were used. The animals were grouped containing six in each labeled I-IV. Convulsion was induced by Picrotoxin (2 mg/kg; ip). Time for onset of action (clonic and tonic seizures) and death was recorded. Group I was served as control. Group II, III & IV were treated with Curcumin (300mg/kg;p.o) Isradipine (5mg/kg; ip) and Curcumin (300mg/kg; p.o) + Isradipine (5mg/kg; ip) respectively one hour before the treatment of Picrotoxin. Delay in onset of action and death of animals were considered as anti-convulsant property.

Anticonvulsant activity in mice seizures induced by picrotoxin

Mice were divided into four groups. Each group contained six animals.

Group I: (Normal control) received Picrotoxin (2mg/kg,i.p)

Group II: Received Curcumin (300mg/kg;p.o) and Picrotoxin (2mg/kg,i.p)

Group III: Received Isradipine (5mg/kg; ip) and Picrotoxin(2mg/kg,i.p)

Group IV: Received Curcumin (300mg/kg;p.o) + Isradipine and Picrotoxin (2mg/kg,i.p)

After the observation period blood was collected immediately by retro orbital method in eppendrops tubes and centrifuged at 3000 rpm for 15 min and stored at 0-4 ºC for the determination of lipid peroxidation.

Biological evaluation

Lipid peroxidation assay

The lipid peroxidation assay was carried out by using the Modified Slater's method. The MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90–100 ºC . In the TBA test reaction, MDA or MDA-like substances and TBA react together for production of a pink pigment having an absorption maximum at 530 nm. The reaction was performed at 90 ºC for 30 min. Concentration of TBARS was determined by standard graphic, which was prepared with

serial dilutions of standard sulfuric acid to prepare a 10 µM solution.

In this method, 2.5 ml of 20% trichloroacetic acid and 1.0 ml of 0.67% TBA were added to 0.5 ml of serum, then the mixture was heated in a boiling water bath for 30 min. The resulting chromogen was extracted with 4.0 ml of n-butyl alcohol and the absorbance of the organic phase was determined at the wavelength of 530 nm. The determined values were expressed in terms of malondialdehyde (nmol/ml) used as reference standard (Satoh K. 1978).

Statistical analysis

Statistical analysis of data was carried out by applying one-way ANOVA followed by Tukey's multiple comparison tests. P value < 0.001 was considered significant. Results are expressed as mean ± SEM.

Results and Discussion

Pentylenetetrazole (PTZ) induced seizure in mice

As tabulated in Table 1, single dose, intraperitonial administration of PTZ (65mg/kg i.p) caused clonic convulsions 71.33±3.46 as well as 5/6(83.33%) mortality rate in mice. Mice were pretreated with Cucumin (300mg/kg p.o) showed significant (P<0.001) increase in latency to clonic convulsion 113.83±4.14 & significantly reduced mortality 2/6(33.33%) and Isradipine(5 mg/kg;i.p) showed significant (P<0.001) increase in latency to clonic convulsion 151.66±3.00 & significantly reduced mortality 1/6(16.66%) . However combination of Cucumin (300mg/kg,p.o) and Isradipine(5 mg/kg;i.p) showed most significant increase in latency to clonic convulsions 191.33±3.10 and reduced mortality 0/6(0%).

Picrotoxin induced seizure in mice

As tabulated in Table 1, single dose, intraperitonial administration of picrotoxin (2mg/kg i.p) caused clonic convulsions 78±3.7 as well as lethality 06/06(100%) in mice. Mice were pretreated with Cucumin (300mg/kg p.o) showed significant (P<0.001) increase in latency to clonic convulsion 98.66±3.91 & significantly reduced mortality 02/06(33.33%) and Isradipine(5 mg/kg;i.p) showed significant (P<0.001) increase in latency to clonic convulsion 133.5±2.9 & significantly reduced mortality 03/06(50%). However combination of Cucumin (300mg/kg,p.o) and Isradipine(5 mg/kg;i.p) showed most significant increase in latency to clonic convulsions 202.5±2.7 and reduced mortality 00/06(0%).

Lipid peroxidation level in PTZ induced oxidative stress

TBARS level, the indicator of lipid peroxidation, were significantly increased in serum of PTZ- induced epileptic seizures in

mice 5.7±0.13 when compared with controls 1.33±0.07. Curcumin 4.0±0.10 and Isradipine 3.8±0.12significantly potentiated this effect and reduce the oxidative stress in mice. While the combination of Curcumin and Isradipine significantly reduces the level of lipid peroxidation 2.3±0.10 ultimately reduces the oxidative stress in mice (Table 3 and Fig. 3).

Table1: PTZ Induced Convulsion in Mice

Groups	Treatments (mg/kg; ip)	Latency to Clonic Convulsions (sec)		Mortality Rate/Used (%)	
(I) Toxic control	Pentylenetetrazole (65mg/kg;ip)	85	Mean 71.33±3.46	01	Mean 5/6(83.33%)
		66		01	
		68		01	
		78		01	
		60		00	
		71		01	
(II) Test(1)	Curcumin (300mg/kg;p.o)	121	Mean 113.83±4.14	00	Mean 2/6(33.33%)
		110		00	
		126		01	
		119		01	
		98		00	
		109		00	
(III) Test(2)	Isradipine (5mg/kg;ip)	147	Mean 151.66±3.00	00	Mean 1/6(16.66%)
		153		00	
		142		00	
		161		01	
		148		00	
		159		00	
(IV) Test(3)	Curcumin (300mg/kg;po) + Isradipine (5mg/kg;ip)	193	Mean 191.33±3.10	00	Mean 0/6(0%)
		181		00	
		201		00	
		190		00	
		198		00	
		185		00	

Lipid peroxidation level in Picrotoxin induced oxidative stress

TBARS level, the indicator of lipid peroxidation, were significantly increased in serum of Picrotoxin- induced epileptic seizures in mice 5.23±0.26 when compared with controls 1.08±0.04. Curcumin 3.63±0.21 and Isradipine 3.9±0.17 significantly potentiated this effect and reduces the oxidative stress in mice. While the combination of Curcumin and Isradipine significantly reduces the level of lipid peroxidation 2.38±0.15 ultimately reduces

the oxidative stress in mice (Table 4 and Fig. 4).

The epilepsy is one of the most common neurological disorders. However, the physiopathology mechanisms of epilepsy are not yet fully understood. Recent years have focused on the role of oxidative stress in seizures (Sudha K. et.al.2001). Calcium ions play a central role in the control of neuronal excitability.

Table 2: Picrotoxin Induced Convulsion in Mice

Groups	Treatments (mg/kg; ip)	Latency to seizures (sec)		Mortality Rate/Used (%)	
(I) Toxic control	Picrotoxin(2mg/kg;ip)	71	Mean 78±3.7	01	Mean 06/06(100%)
		82		01	
		89		01	
		64		01	
		84		01	
		78		01	
(II) Test(1)	Curcumin (300mg/kg;p.o)	92	Mean 98.66±3.91	01	Mean 2/6(33.33%)
		94		00	
		112		01	
		86		00	
		102		00	
		106		00	
(III) Test(2)	Isradipine (5mg/kg;ip)	134	Mean 133.5±2.9	00	Mean 03/06(50%)
		132		01	
		144		01	
		139		01	
		125		00	
		127		00	
(IV) Test(3)	Curcumin (300mg/kg;po) + Isradipine (5mg/kg;ip)	203	Mean 202.5±2.7	00	Mean 0/6(0%)
		211		00	
		204		00	
		198		00	
		207		00	
		192		00	

Therefore this study was designed to find out the effect of Isradipine on PTZ and Picrotoxin induced convulsion (Devi.K.et.al.2010). Experimental seizures are known to be associated with a massive release of reactive oxygen species Moreover, the possible effect of Isradipine, Curcumin and their combination pretreatment on PTZ and Picrotoxxin -induced oxidative stress was investigated in this study.

Table 3: Effect of Curcumin, Isradipine and Combination on Serum Lipid Peroxidation Level in PTZ induced Oxidative Stress in Mice

Treatment	Concentration of MDA nMol/ml						Mean
	1	2	3	4	5	6	
Saline	1.6	1.3	1.1	1.2	1.4	1.4	1.33±0.07
Pentylenetetrazole (65mg/kg i.p.)	5.4	5.4	6.1	5.8	6.1	5.6	5.7±0.13
Curcumin (300mg/kg i.p) + PTZ (65mg/kg i.p.)	3.6	3.8	4.1	4.2	4.0	4.3	4.0±0.10
Isradipine (5mg/kg i.p) + PTZ (65mg/kg i.p)	3.8	3.9	4.2	3.3	4.0	3.7	3.8±0.12
Curcumin (300mg/kg,p.o) + Isradipine (5mg/kg i.p)+PTZ(65mg/kg i.p)	2.7	2.4	2.0	2.1	2.2	2.4	2.3±0.10

Table 4: Effect of Curcumin, Isradipine and Combination on Serum Lipid Peroxidation Level in Picrotoxin induced Oxidative Stress in Mice

Treatment	Concentration of MDA nMol/ml						Mean
	1	2	3	4	5	6	
Saline	1.0	1.2	1.1	1.2	1.1	0.9	1.08±0.04
Picrotoxin (2mg/kg i.p.)	5.3	4.4	6.1	5.2	5.8	4.6	5.23±0.26
Curcumin (300mg/kg p.o) + Picrotoxin (2mg/kg i.p.)	3.8	3.4	4.1	3.2	3.0	4.3	3.63±0.21
Isradipine (5mg/kg p.o) + Picrotoxin (2mg/kg i.p)	3.4	4.5	4.1	3.9	4.2	3.5	3.9±0.17
Curcumin(300mg/kg p.o) + Isradipine (5mg/kg i.p) + Picrotoxin(2mg/kg i.p)	1.9	2.3	3.0	2.1	2.4	2.6	2.38±0.15

PTZ may trigger a variety of biochemical processes including the activation of membrane phospholipases, proteases and nucleases. Marked alterations in membrane phospholipid metabolism result in the liberation of lipid peroxides and free radicals (Basara et al 2008). Therefore, free radical involvement in pathological conditions has generally been inferred from the measurement of indirect markers of oxidative stress, suggesting the onset of lipid and protein oxidation. Similarly, the present study showed that acute PTZ-induced epileptic seizures lead to an increase in oxidative stress, an indicator of lipid peroxidation in serum. Pretreatment of mice with fixed doses of isradipine and antioxidants like Curcumin significantly prevented PTZ induced elevations in lipid peroxidation. (Sudha et.al. 2001). Picrotoxin, anantagonist of GABAA-receptors, produces seizures by blocking the chloride ion channels, which are linked to GABAA-receptors to prevent the

influx of chloride ions into the brain neuron. Isradipine antagonize picrotoxin -elicited seizures because they are thought to produce their anticonvulsant effect by increasing chloride flux through chloride channels at GABAA receptor sites to enhance GABAergic system. It is, therefore, possible that Isradipine may be antagonising seizures induced by picrotoxin by enhancing GABAergic systems.

Calcium channel blockers such as verapamil, nifedipine, nicardipine, nimodipine and flunarizine were reported for their anti-convulsant. Keeping this in view, the present study was focused to establish Isradipine, a new calcium channel blocker belongs to the dihydropyridine class of calcium channel blockers. Isradipine binds to calcium channels with high affinity and specificity and inhibits calcium flux into cardiac and arterial smooth muscle cells. It exhibits greater selectivity towards arterial smooth muscle cells owing to alternative splicing of the alpha-1 subunit of the channel and increased prevalence of inactive channels in smooth muscle cells. The potentiation of anticonvulsant activity could be related to central blocked to calcium ion entry. L-channel is modulated by dihydropyridine agonists and antagonists. Therefore the anti convulsant effects may be due to antagonism of calcium ion influx through dihydropyridine L-channel. It is currently accepted that GABA neurons are inhibitory through modulation of the L-channel, in addition to enhancing an inward chloride ion conductance and also provide a site at which the endogenous calcium ion antagonist could exert its anticonvulsant effects (zapter et. el. 1998). Since calcium ions play an important role in seizure activity, the use of calcium channel inhibitors for treatment of epilepsy seems to be rational. Indeed, several reports revealed beneficial effects of calcium channel antagonists as an add-on treatment of epilepsy. (Czuczwar et.al. 1996).

Conclusion

This study has demonstrated that the Curcumin shows protective effect alone and in combination with Isradipne against PTZ and Picrotoxin induced seizures and oxidative stress. The border implication of this report suggests a role for calcium channel blockers for adjunctive therapy for epilepsy. Isradipine is an calcium channel blocker which blocks the calcium ion influx to stop the epileptic discharge. PTZ and Picrotoxin administration produced an increased lipid peroxidation in serum of the mice treated with PTZ and Picrotoxin, and therefore, demonstrated and confirmed the possible involvement of free radical oxygen in the PTZ and Picrotoxin induced seizures.

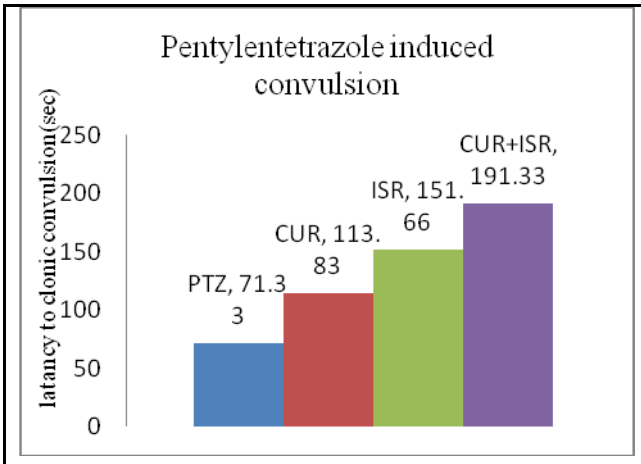

Fig.1: Effect of Curcumin, Isradipine and Combination on Latency to Clonic Convulsion Induced by PTZ in Mice

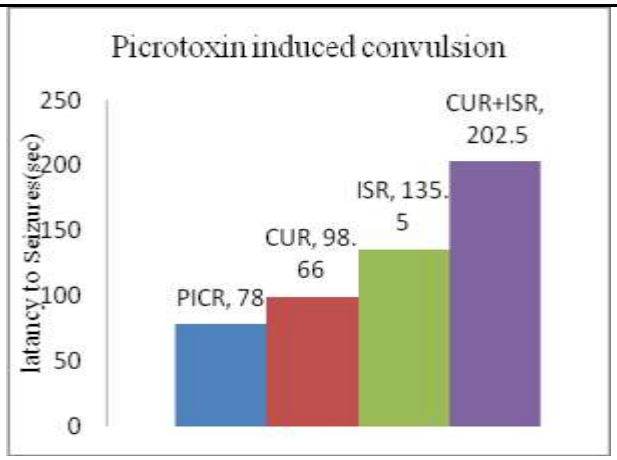

Fig. 2: Effect of Curcumin, Isradipine and Combination on Latency to Clonic Convulsion Induced by Picrotoxin in Mice

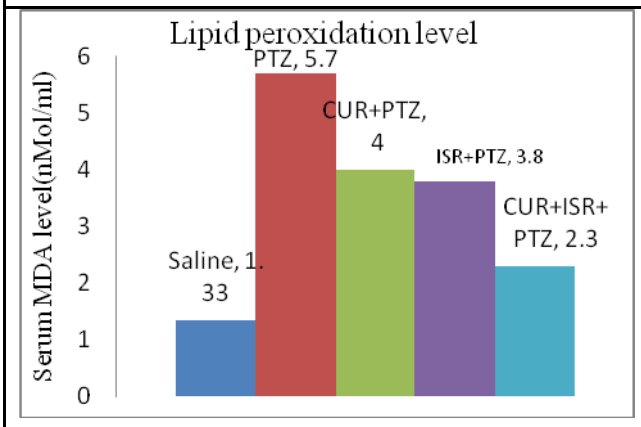

Fig.3: Effect of Curcumin, Isradipine and Combination on Serum Lipid Peroxidation Level in PTZ Induced Oxidative Stress in Mice

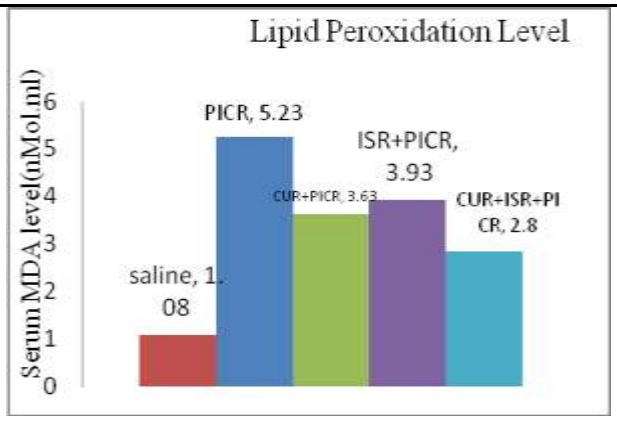

Fig.4: Effect of Curcumin, Isradipine and Combination on Serum Lipid Peroxidation Level in Picrotoxin Induced Oxidative Stress in Mice

Treatment with both Curcumin and Isradipine individually and their combined treatment decreased serum MDA activity, increased by administration of PTZ and Picrotoxin, thereby suggesting that these drug acts positively on lipid peroxidation. Curcumin is antioxidants may be used with AED's to reduced the oxidative stress during epileptic seizures and Isradipine is an calcium channel blocker shows the antiepileptic activity and combination of both drugs may provide a greater effectiveness against epilepsy as well as oxidative stress. Further studies of experimental epilepsy should

determine whether the beneficial effects of such combinations persist over longer periods.

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