

**VITAMIN E POTENTIATE THE ANTIEPILEPTIC
ACTIVITY OF PHENOBARBITAL**

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ABSTRACT

Phenobarbital is the mostly used drug for the seizures. However question regarding safety and efficacy of this drug make it particularly compelling to identify adjunct therapy that could boost therapeutic benefit. Study found that one adjunct therapy is vitamin e. The main aim of present study was to evaluate potentiation of anticonvulsant activity of Phenobarbital by vitamin e in mice using PTZ induced convulsions. In these methods all the animals were divided into sixteen groups & each groups consist of six animals. All group received PTZ. Testing drugs doses were randomised within groups of animal such that each group was required during a given test series. The results revealed that vitamin e significantly potentiated efficacy of phenobarbital but did not exert antiepileptic effect on its own. Concurrent administration of phenobarbital and vitamin e also decreased serum MDA activity. It is concluded that vitamin e potentiates the antiepileptic activity of phenobarbital.

Key Words:- Vitamin E, PTZ, Seizures, PB.

INTRODUCTION

Epilepsy is a common and frequently devastating disorder affecting an estimated 7 million people in India and 50 million people worldwide. Approximately 40% of them are women. The estimated incidence rate ranges from 40 to 60 per 1,000,000 population/year. The WHO estimated that approximately 80% patients with epilepsy live in developing countries and most of them do not get adequate medical treatment. Unfortunately, currently used antiepileptic drugs cause side effects which vary in severity from minimal impairment of the central nervous system to aplastic anemia or hepatic failure. Moreover, approximately 30% of people with epilepsy have “intractable seizures” that do not respond to even the best

available treatment. Approximately 30% of the patients continue to have seizures with current antiepileptic therapy. This fact demands new safer antiepileptic drugs for better and effective control of epilepsy¹ to identify adjunct therapies that could boost therapeutic benefit¹.

The nervous system for a number of biochemical, physiological & anatomical reason is more vulnerable to reactive oxygen species (ROS) in addition to the other organs of the body. Extensive lipid per oxidation caused due to generation of reactive oxygen species in biological membrane causes loss of fluidity, falls in membrane potential & increased permeability to H ions, leading to tissue damage. ROS are responsible for the induction of peroxidation of unsaturated fatty acids that are components of

neuronal membrane which results in depolarization. ROS also accelerate production of neurotoxic guanidine compounds (e.g. methylguanidine, guanidine) which are known to be convulsants in brain. Such reactions may be followed by excitatory and inhibitory neurotransmitter changes especially increased release of excitatory amino acids such as aspartic acid & decreased release of inhibitory amino acid such as GABA. These transmitter changes may be directly related to epileptogenicity by generation of ROS. These ROS in turn will accelerate production of neurotoxic guanidino compound in the pattern of a vicious cycle. This shows that ROS are responsible for generation of convulsions⁸. Vitamin E refers to a group of ten lipid-soluble compounds. It can be found in corn oil, soybean oil, margarine, and dressings. Also found most abundantly in wheat germ oil, sunflower, and safflower oils. As a fat-soluble antioxidant, it stops the production of reactive oxygen species formed when fat undergoes oxidation⁴. Phenobarbital is a long-acting barbiturate and the most widely used anti-seizure medication globally. As with other barbiturates, benzodiazepines are more commonly used for this purpose⁴. But PB has a variety of side effects restlessness, slow heartbeat, CNS depression, drowsiness, dizziness, teratogenicity⁶. The main aim of present study to potentiate the antiepileptic activity of Phenobarbital and also reduced the dose of Phenobarbital in such a way that that could not affects its therapeutic benefit by combining with the vitamin e which is used as adjuvant. The reduced dose of Phenobarbital may reduce the side effects.

MATERIALS AND METHODS

Animals: Swiss Albino mice of body weight 20-30 g were procured from Anuradha College of Pharmacy, Chikhli (Dist- Buldhana) & were used in this study. All procedures described were reviewed and approved by the IAEC, Anuradha College of Pharmacy, Chikhli. Dist.- Buldhana (Maharashtra).

Drugs and chemicals: All the drugs and chemicals used in this experiment were of

analytical grade. The Drugs and other additives used were:- Pentylenetetrazole (Dolphin chemicals, Mumbai), Phenobarbital(Sigma Aldrich), Vitamin E(wockhardt Pharma, Aurangabad).

Pentylenetetrazole (PTZ) induced convulsion:

Animal were divided into sixteen groups and each group consist of six animals. All group received PTZ (65mg/kg i.p) .PTZ (65 mg/kg) were injected intra peritoneally to mice 30 min after drug treatment. Immediately after PTZ administration mice were observed for (1) latency of clonic convulsions (elapsed time from PTZ injection until convulsion occurred),(2) incidence (number of mice showing convulsions) and (3) mortality for the duration of 30min⁵. Testing drugs doses were randomised within groups of animal such that each group was required during a given test series.

Estimation of Oxidative Stress: To 0.5 ml serum, 2.5 ml of 20 mg/dl trichloacetic acid was added and the tube was left to stand for 10 min at room temperature. After centrifugation at 3500 rev./min for 10 min, the supernatant was decanted and the precipitate was washed once with 0.05 mM sodium ACD. Then 2.5 ml of 0.05 M sulphuric acid and 3.0 ml of 0.2 mg/dl TBA in 2 M sodium sulfate are added to this precipitate and the coupling of lipid peroxide with TBA was carried out by the heating in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen was extracted with 4.0 ml of n-butyl alcohol by vigorous shaking. Separation of the organic phase was fascilated by centrifugation at 3000 rev/min for 10 min and its absorbance was determined at the wavelength of 530 nm³.

RESULTS AND DISCUSSION

Our present findings reveal dose dependent potentiation of anticonvulsant effects of phenobarbital by beta vitamin e in mice. Vitamin e when administered in the absence of phenobarbital failed to alter duration of convulsion and mortality rate, however vitamin e significantly potentiated the effect of phenobarbital as evidenced by decrease of duration of convulsion and mortality rate., in

particular it was found that combination of 200mg/kg vitamin e and 10mg/kg phenobarbital completely abolished duration of convulsion and mortality rate. , an effect that was equivalent to that seen after treatment with 20 mg /kg

phenobarbital alone. These data showing neuroprotective effect of vitamin e combined with phenobarbital may provide rationale for the examination of vitamin e as an adjunct therapy for the treatment of epilepsy.

Table Number:-1 Effect of vitamin e and PB against PTZ induced convulsions and oxidative stress.

PB(mg/kg)	Vitamin E (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level (nmol/ml)	PB(mg/kg)	Vitamin E (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level (nmol/ml)
0 mg/kg	0	15 sec ± 1.67	6/6(100%)	6.94 ± 0.67	10 mg/kg	0	49 sec ± 0.51	4/6(66%)	6.92 ± 0.68
	100	56 sec ± 4.28	6/6(100%)	5.84 ± 0.41		100	47 sec ± 0.45	0/6 (0%)	5.30 ± 0.58
	200	58 sec ± 6.17	6/6(100%)	5.38 ± 0.38		200	42 sec ± 0.29	0/6 (0%)	5.10 ± 0.47
	400	42 sec ± 7.10	6/6(100%)	5.32 ± 0.49		400	35 sec ± 0.28	0/6 (0%)	4.90 ± 0.37

PB (mg/kg)	Vitamin E (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level (nmol/ml)	PB(mg/kg)	Vitamin E (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level (nmol/ml)
15 mg/kg	0	56 Sec ± 0.21	0/6 (0%)	6.80 ± 0.71	20 mg/kg	0	-	0/6 (0%)	5.88 ± 0.71
	100	47 sec ± 0.46	0/6 (0%)	5.92 ± 0.39		100	-	0/6 (0%)	5.10 ± 0.47
	200	46 sec ± 0.33	0/6 (0%)	5.01 ± 0.55		200	-	0/6 (0%)	4.98 ± 0.44
	400	-	0/6(0%)	4.10 ± 0.42		400	-	0/6(0%)	4.28 ± 0.33

Values are mean ± SEM (n=6). P**< 0.1 were considered to be statistically significant.

The exact mechanism of action of vitamin e is not clearly known but it was suggested that it has been identified as an ideal antioxidant found naturally in our diets, vitamin e was found to be capable of regenerating endogenous antioxidants in the body including vitamin c, intracellular reduced glutathione (GSH). Vitamin e offer protection to cell membrane and prevent oxidative stress to the tissue of the body by neutralising toxic oxygen molecules that slow or prevent the oxidation of other chemical. Antioxidant play an important role in antiseizure activity, it should be reduced the oxidative stress in epilepsy². Epilepsy is one of the most common neurological disorders. However, the Pathophysiological mechanisms of epilepsy are not yet fully understood. Recent years have focused on the role of oxidative stress in seizures. There is emerging evidence that focuses on the role of oxidative stress and

mitochondrial dysfunction both as a consequence and a cause of epileptic seizure. Experimental seizures are known to be associated with a massive release of reactive oxygen species⁴. Moreover, the possible effect of vitamin e, Phenobarbital pre-treatment on PTZ-induced oxidative stress was investigated.

CONCLUSION

In conclusion this study has demonstrated that Phenobarbital has anticonvulsant action against PTZ induce seizure, while in combination with vitamin e produced a potent action as compared to Phenobarbital alone. The border implication of this report suggests a role for antioxidant for adjunctive therapy for epilepsy. Vitamin e may be used as an add on therapy with Phenobarbital and combination of both drugs may provide a greater effectiveness against epilepsy. PTZ

administration produced an increased lipid Peroxidation in serum of the mice, and therefore, demonstrated and confirmed the possible involvement of free radical oxygen in the PTZ induced seizures. Treatment with both vitamin e and Phenobarbital decrease serum MDA level, increased by administration of PTZ, thereby suggesting that these drug acts positively on lipid peroxidation. Combination of Vitamin e with Phenobarbital may be promising for the treatment of epilepsy. It is concluded that

vitamin e potentiate the antiepileptic activity of Phenobarbital.

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