

**ALPHA LIPOIC ACID POTENTIATE THE ANTIEPILEPTIC
ACTIVITY OF PHENOBARBITAL IN EPILEPTIC MICE.****R. S. Cheke², P. R. Todkari¹, N. P. Sawadadkar^{2*}, V. J. Chaware², K. R. Biyani²**¹Department of Quality Assurance, Anuradha College of Pharmacy, Chikhli.²Department of Pharmacology, Anuradha College of Pharmacy, Chikhli
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Author****Nilesh Prakashrao****Sawadadkar**Department of
Pharmacology, Anuradha
College of Pharmacy,
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India.**ABSTRACT**

Phenobarbital is most commonly used antiepileptic drug. But most of patient suffers from the side effects of Phenobarbital. For the safety and efficacy of this drug it is necessary to identify the adjuvant therapy that could increase the therapeutic effect. One potential therapy is Alpha Lipoic Acid. Alpha Lipoic Acid is a biologic antioxidant which also used to decrease the oxidative stress produced in epilepsy. 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 with the alpha lipoic acid which is used as adjuvant. The reduced dose of Phenobarbital could reduces the side effects. In these study all the animals were divided into sixteen groups & each groups consist of six

animals. All group received PTZ (65mg/kg i.p.). Mice were pre treated with Phenobarbital and alpha lipoic acid. Testing drugs doses were randomised within groups of animal such that each group was required during a given test series. The pharmacological screening shows that Phenobarbital, co-administration of Phenobarbital & alpha lipoic acid significantly increased latency to clonic convulsions & reduced mortality in PTZ treated. Also alpha lipoic acid, Phenobarbital combination treatment decreased serum MDA activity. It is concluded that alpha lipoic acid potentiates the anti epileptic activity of Phenobarbital in mice. Alpha lipoic acid, Phenobarbital in combination decreased oxidative stress and prevents epilepsy also the reduced dose of Phenobarbital causes reduction in side effects.

KEYWORDS: ALA, PTZ, PB, Seizures, MDA.

INTRODUCTION

Epilepsy is the condition of spontaneously recurrent seizures and is one of the major neurological disorders of the brain, affecting approximately 0.5-1.0% of the world population. Seizure is the characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain. Abnormal cellular discharge may be associated with a variety of causative factors such as trauma, oxygen deprivation, tumors, infection and metabolic derangements. However, no specific factors are found in about half of patients suffering from epilepsy.^[1]

Phenobarbital is the most commonly used drug for the seizures. Phenobarbital acts as an anticonvulsant by depressing monosynaptic and polysynaptic transmission in the CNS. However, it also increases the threshold for electrical stimulation of the motor cortex. But 30–40% of patients do not achieve seizure control with a single antiepileptic drug (AED). For that combinations are required.

Also Phenobarbital acts as a long acting non-selective depressant of the central nervous system with the ability to produce all levels of CNS mood changes ranging from excitation to mild sedation, hypnosis, and deep coma. Phenobarbital can also induce anaesthesia if given in high therapeutic doses. Because of above side effects it is necessary to reduce the dose of Phenobarbital in such way that the therapeutic effect of Phenobarbital can not affect. For that another drug is required which could be used to control the seizures although the dose of phenobarbital is reduced.^[7]

Alpha lipoic acid is a disulfide compound that is produced in small quantities in cells, and functions naturally as a co-enzyme in the pyruvate dehydrogenase and α -ketoglutarate dehydrogenase mitochondrial enzyme complexes. Alpha Lipoic acid is a potent anti-oxidant that has been widely used in food supplement preparations. Alpha Lipoic acid has been used for control oxidative stress produced in epileptic, diabetic patients.^[4]

The main aim of present study is to potentiate the antiepileptic activity of Phenobarbital and also reduce the dose of Phenobarbital in such a way that it could not affect its therapeutic benefit by combining with the alpha lipoic acid 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) and fed with commercial pellet diet (Hindustan Lever Kolkata, India) and water *ad libitum* 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), Alpha Lipoic Acid (wockhardt Pharma, Aurangabad).

Pentylenetetrazole (PTZ) induced convulsions

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 i.p.) were injected intraperitoneally 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.

Testing drugs doses were randomised within groups of animal such that each group was required during a given test series.^[9]

Estimation of Oxidative Stress

Oxidative stress can be measured by estimating the Lipid Peroxidation Level.^[3]

RESULTS

Pentylenetetrazole in Single dose induced seizure in mice. Intraperitoneal administration of PTZ (65mg/kg i.p) caused clonic convulsions as well as lethality in mice. Mice were pretreated with Phenobarbital and Alpha Lipoic Acid. Alpha Lipoic Acid and Phenobarbital showed most significant increase in latency to clonic convulsions and reduced mortality. Testing drugs doses were randomised within groups of animal such that each group was required during a given test series as shown in given table.

Table Number:-1 Effect of Alpha Lipoic Acid and PB against PTZ induced convulsions and oxidative stress.

PB(mg/kg)	ALA (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level nMol/ml	PB(mg/kg)	ALA (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level nMol/ml
0 mg/kg	0	15sec \pm 1.5	6/6(100%)	7.70 \pm 0.78	10 mg/kg	0	38 sec \pm 2.7	4/6(66%)	6.60 \pm 0.68
	100	45 sec \pm 4.8	6/6(100%)	7.50 \pm 0.77		100	47 sec \pm 3.8	0/6 (0%)	6.70 \pm 0.62
	200	47 sec \pm 3.9	6/6(100%)	6.05 \pm 0.68		200	59 sec \pm 4.7	0/6 (0%)	5.90 \pm 0.58
	400	59 sec \pm 5.7	6/6(100%)	5.30 \pm 0.47		400	60 sec \pm 5.9	0/6 (0%)	5.17 \pm 0.46

PB (mg/kg)	ALA (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level nMol/ml	PB(mg/kg)	ALA (mg/kg)	Latency to Clonic Convulsion	Mortality Rate	MDA Level nMol/ml
15 mg/kg	0	49 sec \pm 5.3	0/6 (0%)	6.50 \pm 0.71	20 mg/kg	0	-	0/6 (0%)	5.47 \pm 0.51
	100	58 sec \pm 6.1	0/6 (0%)	6.05 \pm 0.68		100	-	0/6 (0%)	5.21 \pm 0.37
	***200	----	0/6 (0%)	5.50 \pm 0.51		200	-	0/6 (0%)	5.00 \pm 0.29
	***400	----	0/6(0%)	4.90 \pm 0.46		400	-	0/6(0%)	4.80 \pm 0.38

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

*** Reduced Effective dose at which latency to clonic convulsion is abolished and mortality rate is 0.

DISCUSSION AND CONCLUSION

Alpha lipoic acid is a disulfide compound that is produced in small quantities in cells, and functions naturally as a co-enzyme in the pyruvate dehydrogenase and α -ketoglutarate dehydrogenase mitochondrial enzyme complexes. Alpha Lipoic acid is a potent anti-oxidant that has been widely used in food supplement preparations. Alpha Lipoic acid has been used for control oxidative stress produced in epileptic, diabetic patients.^[4] Antioxidant play an important role in anti seizure activity, it should be reduced the oxidative stress in epilepsy. 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 species.^[8] Moreover, the possible effect of Alpha Lipoic Acid, and Phenobarbital on PTZ-induced oxidative stress was investigated.

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. 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.^[5]

Alpha Lipoic Acid potentiate the antiepileptic activity of Phenobarbital and also dose of Phenobarbital is reduced in such a way that that could not affects its therapeutic benefit by combining with the alpha lipoic acid which is used as adjuvant. The reduced dose of Phenobarbital may reduced the side effects.

CONCLUSION

In conclusion this study has demonstrated that Phenobarbital has anticonvulsant action against PTZ induce seizure, while in combination with alpha lipoic acid 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. As their degree of anticonvulsant activity not render them useful as anticonvulsants per se, alpha lipoic acid 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 alpha lipoic acid, Phenobarbital decreased serum MDA activity, increased by administration of PTZ, thereby suggesting that these drug acts positively on lipid peroxidation. Combination of alpha lipoic acid with Phenobarbital may be promising for the treatment of epilepsy also the dose of Phenobarbital can be reduced. At one level we reduce the dose of Phenobarbital at that level we found the abolishment in convulsion and decreased oxidative stress. The reduced dose of Phenobarbital causes reduction in side effects. Hence we can conclude that the dose of Phenobarbital can be reduced for decreasing the side effects also this dose is effective to control epilepsy and oxidative stress.

Further studies of experimental epilepsy should determine whether the beneficial effects of such combinations persist over longer periods.

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REFERENCES

1. Ambavade S, Mhetre N, Muthal A and Bodhankar S. Pharmacological evaluation of anticonvulsant activity of root extract of *Saussurea Lappa* in of European integrative medicine. 2009; 131-137.
2. Celina M. Monzon, Mario R. Delfino (h), Maria del C. Sarno and Mario R. Delfino, Phenobarbital in pharmaceutical tablets by modified conductimetric analysis, 2008; 110-117.
3. Eqbal MA, Dauqan S, Aminah A. Antioxidant enzymes, including superoxide dismutase, catalase, and glutathione. *Adv. J. Food Sci. Technol*, 2011; 3(4): 308-316.
4. Joseph I. Evans, a-lipoic acid: a multifunctional antioxidant that Improves insulin sensitivity in patients with Type 2 diabetes, *diabetes technology & therapeutics*, 2000; 2(3): 410-414.
5. Liang LP, Beaudoin ME, Fritz MJ, Fulton R and Patel M. Kainite-induced seizures, oxidative stress and neuronal loss in aging rats. *Neuroscience*, 2007; 147: 1114–1118.

6. Macdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. *Epilepsia*, 1995; 36: 2-12.
7. Phenobarb and phenobarb elixir prescribing information, division of pharmascience, page no: 2-7.
8. Surekha T. Nemade, Melinkeri R R , Oxidative and Antioxidative Status in Epilepsy, 2010; 8-10.
9. Todkari P.R.,Sawadadkar N.P. Evaluation of anti-convulsant activity of methanolic extract of leaves of *Leonurus cardiaca* against pentylenetetrazole induced convulsions in Mice, *World J Pharm Sci*, 2014; 2(10): 1350-1352.