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EFFECTS OF VERAPAMIL AND FERULIC ACID AGAINST CHEMICALS INDUCED CONVULSIONS IN ALBINO MICE

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ABSTRACT

Background: The currently available antiseizure drugs have a low therapeutic index and provided emerge satisfactory seizure control in only 60-70% of patients. Calcium channel blocker have shown potentials of a useful add-on drug for the available antiepileptic drugs. Role of oxidative stress in epileptogenic process has been supported in various studies. **Objectives:** To study Potentiation effect of verapamil and Ferulic acid against pentylenetetrazole and picrotoxin induced convulsions in mice. M**ethods:** For this study, swiss albino mice were used. Effects of verapamil and ferulic acid alone and in combination with diazepam (4mg/kg) were studied. Onset of convulsions and duration of convulsions, percentage protection was considered as the index for antiepileptic activity. **Result:** Verapamil (20mg/kg) produced non significant antiseizure effect and ferulic acid at dose (75mg/kg) reduced the convulsions and myoclonic jerk but verapamil and ferulic acid in combination with diazepam potentiate the antiepileptic effect. **Conclusion:** Verapamil and ferulic acid potentiated the antiepileptic effect of diazepam. Dose of diazepam can be reduced in epileptic patient receiving verapamil and ferulic acid

KEYWORD: Verapamil, Diazepam, Ferulic acid, Picrotoxin induced seizures, Pentylenetetrazole induced seizures.

1. INTRODUCTION

Epilepsy a chronic disorder of heterogeneous symptoms characterized by recurrent seizures,

of cerebral brain.[1-3] It is the second most common chronic neurological condition observed in worldwide.[4] Seizures that can happens spontaneously and repeatedly are knows as outward signs of epilepsy.[5] Epileptic seizures are characterized by increasing excitability in brain structures (such as within the cortex and subcortical area).[6]

Approximately 1 % of to the world population has epilepsy[7,8] The therapeutic objectives of the treatment of epilepsy is complete seizures control without excessive side effect. Uncontrolled epilepsy can result neuropsychiatric and social impairment, lower quality of life and higher risk of death.[9] Many of the existent ACD produce a host of undesirable side effects including teratogenesis, drowsiness, mental dullness, nausea, ataxia, hematologic changes, hirsutism, weight gain. For these reasons, new ACD are needed to improve seizure control and reduce the side-effect profile (Gasior et al., 1997).[10,11]

The history of hypertension appears to be an independent risk factor for new onset unprovoked seizures.[12] Overwhelming evidence indicate that calcium ions plays an essential role in the pathophysiology of epilepsy. During seizures one can observe a decrease in extracellular calcium concentration prior to onset of seizures activity followed by increase in the intracellular calcium concentration. An important characteristics all CCBs is their ability to inhibit the inward flow of calcium[13] CCBs have several advantages over the existing antiepileptic drugs,such as no effect on hepatic microsomal enzymes ,devoid sedation and wide therapeutic range.[14]

Some conventional antiepileptic drugs induced oxidative stress which limit their clinical condition. Ferulic acid is phenolic phytochemical with antioxidant and neuroprotective properties that prompted to evaluate its therapeutic potential in epilepsy. Which is usually associated with oxidative stress.[15]

2. MATERIALS AND METHODS

2.1 Animals

Swiss Albino mice of body weight 20-30 g were procured from Animal House of Yashoda Technical Campus, Faculty of Pharmacy, satara (Dist-satara) 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, Yashoda Technical Campus faculty of Pharmacy, Satara. Dist.– Satara (Maharashatra)

2.2 Drugs and Chemicals

P C Chem provided Picrotoxin and pentylenetetrazole drug, Diazepam (valium tablet) was purchased from Abbott healthcare Pvt, Ltd and Verapamil (Calaptin) was purchased from Abbott Healthcare Pvt, Ltd, Sakshi corporation navi Mumbai provided ferulic acid.

2.3 Inclusion criteria

A majority of mice showed tonic clonic seizure, clonic seizure, myoclonic twitches, hind limb extentions & recovery. Only those rats. showing the convulsive responses were used for experiment.

2.4 Animal care

Swiss albino mice (18-22 g) were selected. Animals were housed under an alternative 12 h light/dark cycle in polypropylene cages with softwood granulate bedding. Three animals were housed in a single cage. Pelleted food and water were made available ad libitum. Animals used in these studies were maintained in facilities fully accredited by the CPCSEA and all experiments were performed under protocols approved by the Institutional Animal Ethics Committee (YSPM/YTC/Pharma /2021-2022/IAEC/003)

2.5 Induction of convulsion by picrotoxin

Picrotoxin (10mg/kg) was administrated and the animals were observed until occurrence of extension -flexion of forelimb and hind limb with falling on back sometimes with spasm of neck muscles (clonic tonic seizures). Latency period of seizure and number of convulsed/ all number of animals in each group were recorded.

2.6 Induction of convulsions by pentylenetetrazole

Pentylenetetrazole (PTZ) –induced seizures in mice is an accepted in-vivo model for the screening of antiepileptic drugs. Seizures are induced by the administration of 80 mg/kg, i.p PTZ and the mice are then observed for a 120 minute period. Mice were administered drugs for days and on experimental day, PTZ 80mg/kg was injected intraperitoneally to mice 45min after vehicles or drugs and 30 min after the standard drug. Immediately after PTZ administration mice were observed.

2.7 Experimental groups

Anticonvulsant studies

2.7.1 Picrotoxin induced convulsion

In toxicant control (n=6), mice were injected with picrotoxin (10mg/kg), Standard group (n=6) mice were injected with Diazepam(4mg/kg) ,Test I -received picrotoxin(10mg/kg),Test II- received standard diazepam(4mg/kg),Test group III–received verapamil (20mg/kg) intraperitoneally; Test group IV– received Ferulic acid (75mg/kg) intraperitoneally, Test group V-received verapamil + Diazepam (20mg/kg+ 2mg/kg), Test group VI- received ferulic acid +Diazepam (75mg/kg+2mg/kg).Thirty minutes after pretreatment,10mg/kg of picrotoxin was administered to each mice. They were then observed for tonic hind limb seizures for 30 min period.

2.7.2 Pentylenetetrazole induced convulsion

Animal were divided into VI groups, (n=6 mice of either sex in one group). Group I received pentylenetetrazole (80mg/kg),group II was allotted for Diazepam (4mg/kg) and Group III received verapamil (20mg/kg) intraperitoneally; Group IV– received Ferulic acid (75mg/kg) intraperitoneally, Group V-received verapamil + Diazepam (20mg/kg+ 2mg/kg), Group VI-received ferulic acid +Diazepam (75mg/kg+2mg/kg). mice were administered drugs for seven days and on experimental day, PTZ 80mg/kg was injected intraperitoneally to mice 45min after vehicles or drugs and 30 min after the standard drug. Immediately after PTZ administration mice were observed for (1) onset of convulsions, (2)incidence (number of mice showing convulsions and (3) mortality for the duration of 30 minutes.

2.7.3 Statistical analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunneet's multiple Comparison Test by Graph pad prism software (GraphPad software inc.., Version 5.0.0). The mean values ± SEM were calculated for each parameter. Level of significances was kept at $p< 0.05$.

3. RESULTS

In Pentylenetetrazole induced convulsions the parameters like onset of convulsions, duration of convulsions and percentage protection were recorded and result obtained in different groups represented in table No 1.

As seen in table No 1, group II (diazepam) showed complete abolition of convulsions, highly

significant decrease in duration of convulsions, increase in seizure protection.

In group V (verapamil + diazepam) and group VI (ferulic acid + diazepam) showed significant delay onset of convulsions and reduced duration of convulsions and decreased mortality percentage when compared to normal control group ($p < 0.05$)

Table no. 1: Effect of Verapamil and Ferulic acid alone and its combination with diazepam on pentylenetetrazole induced convulsions.

Experimental group	Dose	Onset of convulsions (min)	Duration of convulsions (min)	No of Animals survived	Percentage protection (%)
Group I Toxicant (PTZ)	80 mg/kg i.P	1.08± 0.10	3.10±0.23	0/6	0%
Group II Standard (Diazepam)	4mg/kg i.p	-	-	6/6	100%
Group III (Verapamil)	20mg/kg i.p	2.10±0.1	5.20±0.26	4/6	66%
Group IV (Ferulic acid)	75mg/kg i.p	1.20±0.10	13.10±1.10	2/6	33%
Group V (Diazepam +verapamil)	2mg/kg+20 mg/kg i.p	2.68±0.20	6.10 ±0.32	6/6	100%
Group VI (Diazepam +ferulic acid)	2mg/kg+75 mg/kg i.p	2.10±0.16	7.18±0.31	6/6	100%

All Values expressed as mean ± SEM; n=6 mice in each group, by one -way ANOVA followed by Dunneet's Multiple Comparison Test (Compared with toxical control) $p<0.05$.

In Picrotoxin induced convulsions the parameters like onset of convulsions, duration of convulsions and percentage protection were recorded and result obtained in different groups represented in table No 2.

As seen in table No 2, Group II (diazepam) showed complete abolition of convulsions, highly significant decrease in duration of convulsions, increase in seizure protection.

In group V (verapamil + diazepam) and group VI (Ferulic acid + diazepam) showed significant delay onset of convulsions and reduced duration of convulsions and decrease mortality percentage when compared to normal control group ($p < 0.05$).

Table no. 2: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on picrotoxin induced convulsions.

Experimental group	Dose	Onset of convulsions (min)	Duration of convulsions (min)	No of animals survived	Percentage protection
Group I Toxicant (Picrotoxin)	10mg/kg i.p	2.07± 0.10	10.5 ±0.88	0/6	0%
Group II Standard (Diazepam)	4mg/kg i.p	-	-	6/6	100%
Group III (Verapamil)	20mg/kg i.p	12.35±1.10	11.05±0.78	3/6	50%
Group IV (Ferulic acid)	75mg/kg i.p	18.17±1.50	13.73 ±1.01	0/6	0%
Group V (Diazepam +verapamil	2mg/kg+ 20mg/kg i.p	9.10±0.48	8.15±0.42	6/6	100%
Group VI (Diazepam +ferulic acid)	2mg/kg+ 75mg/kg i.p	10.07±0.54	9.34 ±0.31	6/6	100%

All Values expressed as mean ± SEM; n=6 mice in each group, by one -way ANOVA followed by Dunneet's Multiple Comparison Test (Compared with toxical control) $p<0.05$.

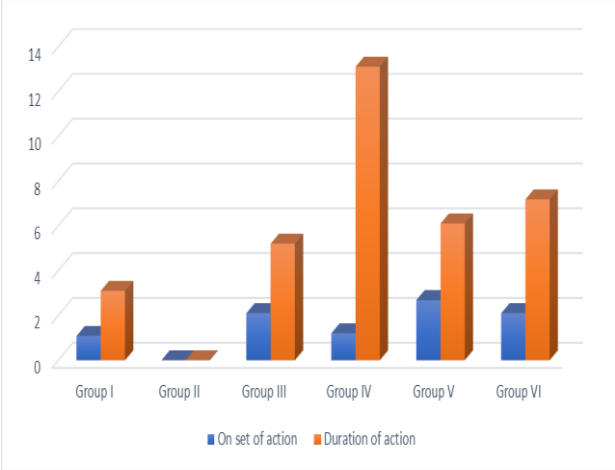

Fig. no. 1: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on pentylenetetrazole induced convulsions.

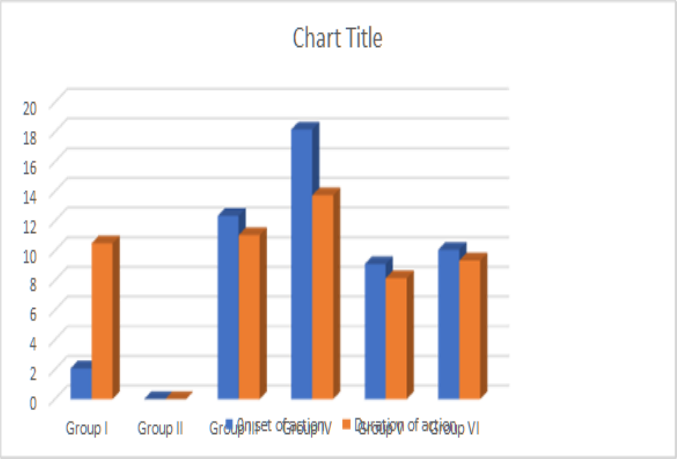

Fig. no. 2: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on picrotoxin induced convulsions.

4. DISCUSSION

Epilepsy is characterized by spontaneous recurrent seizures in which electrical activity in particular brain regions becomes over-excitable. Epilepsy is managed mainly with drugs; however, antiepileptic drugs currently in use neither provides a cure nor prevent relapse and are associated with many side effects such as fatigue, allergies, sedation, blood dyscrasias and teratogenesis, changes in mood and memory problems As a result of this, development of new, affordable and accessible pharmacological agents that can overcome these limitations has become a major goal in epilepsy research.[16]

PTZ may be exerting convulsant effect by inhibiting the activity of GABA receptors. GABA is a major inhibitory neurotransmitter in the brain and the inhibition of its neurotransmission promotes seizures.[17]

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 Patho physiological 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.[18]

Ferulic acid [(E)-3-(4 hydroxy-3-methoxy-phenyl)prop-2-enoic acid) is an active phenolic constituent of many plant species which has shown a wide range of pharmacological

properties including the effects against the inflammation, oxidative stress, cancer, hepatotoxicity, diabetes, thrombosis. Moreover, isopentyl ferulate (an ester derivative of ferulic acid) has shown. anticonvulsant effect in mice.[19]

During seizures one can observe a decrease in the extracellular calcium concentrations prior to onset of seizure activity followed by an increase in the intracellular calcium concentrations. An important characteristic of all CCBs is their ability to inhibit the inward flow of calcium ions. CCBs depress the epileptic depolarization of neurons has shown the presence of specific binding sites of CCBs that enable them to cross the blood brain barrier. This gives important evidence for the presence of central effects of CCB.[20,21]

In our investigation, verapamil and ferulic acid in combination with diazepam was evaluated in behavioral study that gives good indication of protection and reduced latency of convulsions induced by pentylenetetrazole and picrotoxin. Verapamil and ferulic acid alone shows anticonvulsant effect also but verapamil and ferulic acid in combination with diazepam shows more significant effect than alone drugs treatment. Verapamil and ferulic acid potentiated antiepileptic effects of diazepam.

5. CONCLUSION

It was concluded that verapamil alone at the dose 75mg/kg i.p could significantly delayed onset of convulsion and increased percentage protection of animals from death. Ferulic acid did not possess anticonvulsant activity.

Diazepam at the dose 4mg/kg significantly abolish onset of convulsion and duration of convulsion at compare to toxicant control animals.

Verapamil and ferulic acid potentiated the antiepileptic effect of diazepam. Dose of diazepam can be reduced in epileptic patient receiving verapamil and ferulic acid. However it need further confirmation to establish clinical utility of verapamil and ferulic acid.

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