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Study of anticonvulsant activity of acetazolamide on albino rats and its influence on anticonvulsant activity of sodium valproate

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ABSTRACT

Background: Epilepsy is a common neurological disorder. 30-40% of patients will continue to have seizures despite the use of antiepileptic drugs either alone or in combination. The present study is undertaken to evaluate the anticonvulsant activity of Acetazolamide (ACZ) in albino rats and its influence on anticonvulsant activity of sodium valproate.

Methods: Albino rats (150-200gms) of male sex were randomly selected, from central animal facility, MMCRI, Mysore. They were divided into 6groups (per model) of 6 rats each, control group-normal saline 0.5ml, standard group-sodium valproate (300mg/kg), dose 1-ACZ (8.75mg/kg), dose 2-ACZ (17.5mg/kg) and dose 3-ACZ (35mg/kg), dose 4-ACZ (8.75mg/kg) with sodium valproate (150mg/kg). The anti-convulsant activity was screened using MES model and PTZ model.

Results: Results were analysed by ANOVA followed by post hoc Fisher's LSD test. The ACZ has shown anticonvulsant activity at the dose of 17.5mg/kg and 35mg/kg body weight and combination of ACZ 8.75mg/kg with sodium valproate 150mg/kg both in MES model and PTZ model. The anticonvulsant activity of ACZ was less when compared to Sodium Valproate in both MES model and PTZ model. The anticonvulsant activity of combination, ACZ 8.75mg/kg with Sodium valproate 150mg/kg was comparable and more significant when compared to standard drug alone in MES model and PTZ model.

Conclusions: The ACZ has shown anticonvulsant activity in MES model and PTZ induced seizure model of epilepsy. This study has shown that ACZ potentiated the effect of sodium valproate and can be used as add on drug with sodium valproate in epilepsy.

Keywords: Acetazolamide, Epilepsy, Maximal electroshock seizure model, Nephrotoxicity, Pentylene tetrazole, Tonic hind limb extension

INTRODUCTION

Epilepsy are socially handicapping disorders and even a single seizure occurring in certain circumstances may have disastrous effect. The impact of epilepsy on every aspect of both the lives of patient and family is significant.[1] Despite the massive scale of the problem and much research, epilepsy remains poorly understood. Despite more than 20 approved drugs and several non-pharmacological options, up to 30% of patients are still refractory to treatment.

Despite the introduction of several new therapeutic options in the 1990s, a significant fraction of the patients with epilepsy continue to live with uncontrolled seizures.[2] There is still a need for an ideal antiepileptic agent with properties like broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability and low costs.[3] Contemporary anticonvulsant therapy, however, is neither universally effective nor invariably safe. Their important adverse effects include central nervous system depression, ataxia, megaloblastic anaemia, cardiac arrhythmias, hepatic dysfunction and teratogenicity.[4]

Although most people with epilepsy become seizure free with appropriate therapy, 30-40% of patients will continue to have seizures despite the use of antiepileptic drugs either alone or in combination. Discovery and development of anti-epileptic drugs has been especially difficult, because of the regulatory issues of satisfactorily proving safety and efficacy, ethical constraints on placebo-controlled trial designs. For this reason known antiepileptic drugs are used in combination for the treatment.[5]

As the drug acetazolamide has got anti convulsive activity, this drug can be tried to use as a 2nd line of drug in the treatment of epilepsy. As this drug is cheap and easily available, this helps peoples of developing countries like India. Many physicians and studies believe that acetazolamide can be used as a 2nd line drug. But this drug is not receiving enough identification as anti-epileptic. As most cases of epilepsy is treated by multidrug regimens, acetazolamide can be used as add on drug with 1st line drugs. Hence this study has been taken up to evaluate anti convulsive activity of acetazolamide.

METHODS

Materials

Chemicals

- Sodium valproate-Obtained from Sun pharmaceuticals, dissolved in Normal saline, given at the dose of 300 mg/kg[11]body wt intraperitonially.
- Pentylene tetrazole-Obtained from Sigma Aldrich chemical corporation Bangalore, dissolved in Normal saline, given at the dose of 80 mg/kg[12] body wt intraperitonially.
- Acetazolamide-Obtained from Wyeth limited, dissolved in Normal saline. Dose used was 8.75mg/kg, 17.5mg/kg, 35mg/kg per body wt.[13]
- Normal saline.

Animals: Male Swiss Albino rats

Equipment: Electro-convulsiometer with accessories, 1ml Tuberculin syringe, Electronic weighing balance, Animal weighing balance.

Animals

Seventy-two adult healthy male albino rats of wistar strain of similar characteristics weighing 150-200gms were selected from the central animal facility, MMCRI, Mysore. The above test animals were divided into two groups MES and PTZ model.

Maximal electroshock seizure model (MES)

The most commonly used simple model for evaluation of drugs useful in generalized seizures is the electroshock model, which has been validated both clinically and electroencephalographically (1952).[6] MES model

evaluates the ability of drugs to prevent electrically induced tonic hind limb extension (THLE) in mice or rats. Efficacy of drugs in this model correlate with their ability to prevent partial and generalized tonic clonic seizures in man.

Procedure

Adult albino male rats (150-200gms) are used. Six animals per drug dose/ vehicle are normally used. All animals are maintained on an adequate diet and allowed free access to food and water, except during testing. Stimulation is carried out through transauricular electrodes (applied to the pinna with small crocodile clips covered with cotton wool and saline-moistened) are used. Maximal seizures are evoked by supramaximal electroshock stimulation of 150mA, 50HZ, for 0.2 seconds by using conventional electroconvulsiometer (Figure 1). The parameters studied in MES methods were

- Tonic hind limb flexion.
- Tonic hind limb extension (Figure 2).
- Clonus.
- Stupor ((Unconsciousness) from the end of clonus to regain consciousness)
- Post ictal depression (from the regain of consciousness till the animals starts walking) duration of each parameters was recorded in seconds.

Figure 1: Electroconvulsiometer.

Figure 2: Tonic hind limb extension phase.

Figure 3: Clonus phase

The abolition of the hind limb tonic extension is taken as an index of anticonvulsant activity.[7]

Pentylene tetrazole model

Threshold PTZ seizures in rats and mice have long been considered as a model for absence attacks because of its selective therapeutic response to anti absence seizure drugs.[8]

The PTZ model evaluates the ability of potential antiepileptic agents to prevent CLONIC seizures and may correlate with activity against absence seizures. Activity of a drug in this model may affect GABAergic brain systems, either by enhancing GABA levels or by altering sensitivity of postsynaptic GABA receptors.[9]

Proceure

In this study, PTZ at a dose of 80mg/kg IP (dissolved in Normal saline) was used. PTZ is given 30 minutes after the test drug.

The rats are observed for half an hour after drug administration. Prolongation of duration of seizure latency was taken as an index of protection and indicates efficacy of anticonvulsant activity of the test compound.[10] The parameters studied in PTZ methods were

- Seizure latency (time taken for onset of seizure)
- Myoclonic Jerk.
- Clonus (Figure 3).

Table 1: MES model.

Groups	Drug Administered	Method of evaluation	Observations
Group1 animals (Control group-1; C1)	0.35ml/100g of normal saline intraperitoneally	After an interval of 30 minutes they were subjected to MES stimulation of 150mA for 0.2 seconds through transauricular electrodes by using electroconvulsiometer	The duration of different parameters were noted.
Group 2 animals (Standard group-1; S1)	Six albino rats received 300mg/kg of Sodium Valproate in normal saline, intraperitoneally.[11]	After an interval of 30 minutes they were subjected to MES stimulation of 150mA for 0.2 seconds through transauricular electrodes by using electroconvulsiometer	The duration of different parameters were noted.
Group 3 animals (Test group-1; T1)	Six albino rats received 8.75mg/kg of Acetazolamide in normal saline, intraperitoneally.[13]	After an interval of 30 minutes they were subjected to MES stimulation of 150mA for 0.2 seconds through transauricular electrodes by using electroconvulsiometer	The duration of different parameters were noted.
Group 4 animals (Test group-2; T2)	Six albino rats received 17.5mg/kg of Acetazolamide in normal saline, intraperitoneally.[13]	After an interval of 30 minutes they were subjected to MES stimulation of 150mA for 0.2 seconds through transauricular electrodes by using electroconvulsiometer	The duration of different parameters were noted.
Group 5 animals (Test group-3; T3)	Six albino rats received 35mg/kg of Acetazolamide in normal saline, intraperitoneally.[13]	After an interval of 30 minutes they were subjected to MES stimulation of 150mA for 0.2 seconds through transauricular electrodes by using electroconvulsiometer	The duration of different parameters were noted.
Group 6 animals (Test group-4; T4)	Six albino rats received 8.75mg/kg of Acetazolamide and 150mg/kg of Sodium Valproate in normal saline, intraperitoneally.	After an interval of 30 minutes they were subjected to MES stimulation of 150mA for 0.2 seconds through transauricular electrodes by using electroconvulsiometer	The duration of different parameters were noted.

Table 2: Pentylenetetrazole (PTZ) model.

Groups	Drug Administered	Method of evaluation	Observations
Group 7 animals (Control group-2; C2)	Each rat of this group received 0.35ml/100g of normal saline intraperitoneally	After an interval of 30 minutes pentylenetetrazole (PTZ) 80 mg/kg[12] body weight was injected intraperitoneally	The duration of different parameters were noted.
Group 8 animals (Standard group-2; S2)	Six albino rats received 300mg/kg of Sodium Valproate in normal saline, intraperitoneally	After an interval of 30 minutes pentylenetetrazole (PTZ) 80 mg/kg[12] body weight was injected intraperitoneally	The duration of different parameters were noted.
Group 9 animals (Test group-5; T5)	Six albino rats received 8.75mg/kg of Acetazolamide in normal saline intraperitoneally	After an interval of 30 minutes pentylenetetrazole (PTZ) 80 mg/kg[12] body weight was injected intraperitoneally	The duration of different parameters were noted.
Group 10 animals (Test group-6; T6)	Six albino rats received 17.5mg/kg of Acetazolamide in normal saline, intraperitoneally	After an interval of 30 minutes pentylenetetrazole (PTZ) 80 mg/kg[12] body weight was injected intraperitoneally	The duration of different parameters were noted.
Group 11 animals (Test group-7; T7)	Six albino rats received 35mg/kg of Acetazolamide in normal saline, intraperitoneally	After an interval of 30 minutes pentylenetetrazole (PTZ) 80 mg/kg[12] body weight was injected intraperitoneally	The duration of different parameters were noted.
Group 12 animals (Test group-8; T8)	Six albino rats received 8.75mg/kg of Acetazolamide and 150mg/kg of Sodium Valproate in normal saline, intraperitoneally	After an interval of 30 minutes pentylenetetrazole (PTZ) 80 mg/kg[12] body weight was injected intraperitoneally	The duration of different parameters were noted.

RESULTS

Tonic hind limb flexion

Table 3 shows the comparison of Tonic Limb Flexion (THLF) duration in seconds among various groups in Maximal Electro Shock Seizure Model.

ANOVA analysis shows statistically significant ($p<0.0001$) difference between groups included in the study with respect to the variable (THLF duration) considered in the study.

Post hoc LSD test shows statistically significant difference between:

- Control group vs Standard group ($p < 0.0001$)
- Control group vs Test group1 (Not significant)
- Control group vs Test group 2 ($p < 0.0001$)
- Control group vs Test group 3 ($p < 0.0001$)
- Control group vs Test group 4 ($p < 0.0001$)
- Standard group vs Test group 4 ($p = 0.03$)
- Test group 1 vs Test group 4 ($p = 0.0001$).

Table 3: Comparison of tonic hind limb flexion (THLF) duration in seconds among various groups in maximal electro shock seizure model.

Sl. No. of rats	1	2	3	4	5	6	Mean	SE	P *
Control group	12.05	9.85	8.4	6.4	8.3	6.3	8.55	0.89	
Standard group	4.13	5.06	4.8	5.91	5.24	3.53	4.78	0.34	
Test group 1	6.03	6.74	7.85	4.9	6.9	5.16	6.26	0.46	<0.0001
Test group 2	4.63	2.62	4.85	3.75	5.04	6.37	4.54	0.52	
Test group 3	5.86	4.8	3.64	2.44	5.11	4.43	4.38	0.49	
Test group 4	2.01	1.92	1.74	2.99	3.09	1.36	2.19	0.29	

ANOVA

Table 4 depicts the mean duration of Tonic Hand Limb Flexion of various groups in Maximal Electroshock Seizure Model.

The mean duration of Tonic Hind Limb Flexion (THLF) in control group was 8.55 seconds. The Acetazolamide at doses of 8.75mg/kg reduced the duration of THLF to 6.26sec which was statistically not significant when

compared to control group. The Acetazolamide at doses of 17.5mg/kg and 35mg/kg reduced the duration of THLF to 4.54sec and 4.38sec respectively, which were statistically significant when compared to control group. The Acz at doses of 8.75mg/kg along with Sodium valproate at the dose of 150mg/kg reduced the duration of THLF to 2.19sec, which was statistically significant when compared

to control group. The standard drug, sodium valproate reduced the duration of THLF to 3.8sec. The Acetazolamide at dose of 8.75mg/kg along with Sodium valproate at dose of 150mg/kg reduced the duration of THLF to 2.19sec, which was statistically significant when compared to standard group.

Table 4: comparison of tonic hind limb extension (THLE) duration in seconds among various groups in maximal electro shock seizure model.

Sl. No. of rats	1	2	3	4	5	6	Mean	SE	P *
Control group	13.18	9.18	17.83	14.06	16.37	10.23	13.48	1.38	
Standard group	#	#	#	#	#	#			
Test group 1	11.43	10.18	14.55	11.68	12.79	12.11	12.12	0.60	0.005
Test group 2	8.33	#	#	#	7.62	3.39	3.22	1.60	
Test group 3	7.48	#	7.96	#	#	#	2.57	1.63	
Test group 4	#	#	#	#	#	#			

*ANOVA, Note: # - THLE completely abolished

Table 4 shows the comparison and mean duration of Tonic Hind Limb Extension (THLE) duration is seconds among various groups in Maximal Electro Seizure Model respectively. On post hoc test: Control group vs Test group 1(not significant).

The mean duration of Tonic Hind Limb Extension (THLE) in control group was 13.48seconds. The acetazolamide at doses of 8.75mg/kg reduced the duration of Tonic Hind Limb Extension to 12.12sec, which was statistically not significant. The acetazolamide at doses of 17.5mg/kg and 35mg/kg abolished the THLE phase in 50% and 66.66% of the animal respectively. The Acetazolamide dose of 8.75mg/kg along with Sodium valproate at dose of 150mg/kg abolished the THLE phase in 100% of animals. The standard drug, sodium valproate abolished the THLE phase in 100% of animals.

Clonus

Table 5 show the Comparison of Clonus duration and mean clonus duration in seconds among various groups in Maximal Electro Shock Seizure Model. Post hoc LSD test shows statistically significant difference between:

- Control group vs Standard group (p <0.0001)
- Control group vs Test group 1 (Not significant)
- Control group vs Test group 2 (p =0.005)
- Control group vs Test group 3 (<0.0001)
- Control group vs Test group 4 (<0.0001)
- Standard group vs Test group 4 (p = 0.04)
- Test group 1 vs Test group 4 (p <0.0001)
- Test group 2 vs Test group 4 (p =0.001)
- Test group 3 vs Test group 4 (p =0.02).

The mean Clonus duration in control group was 17.18seconds. The Acetazolamide at doses of 8.75mg/kg, reduced the Clonus duration to 12.77sec which was statistically not significant when compared to control group. The Acetazolamide at doses of 17.5mg/kg and 35mg/kg reduced the Clonus duration to 10.98sec and 9.49sec respectively, which was statistically significant when compared to control group. The standard drug, sodium valproate reduced the duration of clonus to 9.19sec. The Acetazolamide at dose of 8.75mg/kg along with Sodium Valproate at dose of 150mg/kg reduced the duration of clonus to 4.08sec, which was statistically significant when compared to standard group.

Table 5: Comparison of clonus duration in seconds among various groups in maximal electro shock seizure model.

Sl. No. of rats	1	2	3	4	5	6	Mean	SE	P *
Control group	23.02	13.85	17.07	10.78	17.11	21.26	17.18	1.85	
Standard group	10.15	8.23	9.83	6.61	10.72	9.61	9.19	0.62	
Test group 1	15.49	14.2	15.53	11.61	9.09	10.62	12.77	1.10	<0.0001
Test group 2	12.81	10.44	15.58	9.23	8.81	9.01	10.98	1.10	
Test group 3	9.88	10.43	12.33	7.05	8.42	8.82	9.49	.745	
Test group 4	2.11	4.09	4.34	3.01	5.23	5.67	4.08	0.55	

**ANOVA

Stupor

Comparison of Stupor duration and mean stupor duration in seconds among various groups in Maximal Electro Shock Seizure Model has been shows in Table 6 respectively. Post hoc LSD test shows statistically significant difference between

- Control group vs Standard group (p =0.007)
- Control group vs Test group 1 (Not significant)
- Control group vs Test group 2 (p =0.002)
- Control group vs Test group 3 (p =0.016)
- Control group vs Test group 4 (<0.0001)
- Standard group vs Test group 4(Not significant)
- Test group 1 vs Test group 4 (p =0.02).

The mean Stupor duration in control group was 125.12seconds. Acetazolamide at doses of 8.75mg/kg, reduced the stupor duration of to 97.90sec which was statistically not significant when compared to control group. The Acetazolamide at doses of 17.5mg/kg and 35mg/kg reduced the stupor duration to 56.54sec and 69.74sec respectively, which was statistically significant when compared to control group. The standard drug, sodium valproate reduced the duration of stupor to 65.22sec.

The Acetazolamide at dose of 8.75mg/kg along with Sodium Valproate at dose of 150mg/kg reduced the duration of stupor to 43.65sec, which was statistically not significant when compared to standard group.

Table 6: Comparison of stupor duration in seconds among various groups in maximal electro shock seizure model.

Sl. No. of rats	1	2	3	4	5	6	Mean	SE	P*
Control group	160.18	151.87	99.9	132.63	102.23	103.89	125.12	10.97	
Standard group	87.90	105.58	45.36	48.74	39.66	64.06	65.22	10.45	
Test group 1	104.74	166.41	62	84.90	71.19	98.17	97.90	15.18	<0.001
Test group 2	59.74	39.20	59.48	67.16	62.55	51.10	56.54	4.08	
Test group 3	52.03	82.88	67.29	119.57	50.55	46.1	69.74	11.41	
Test group 4	30.26	79.82	20.99	48.11	60.74	21.98	43.65	9.63	

*ANOVA

Table 7: Comparison of postictal depression duration in seconds among various groups in maximal electro shock seizure model.

Sl. No. of rats	1	2	3	4	5	6	Mean	SE	P*
Control group	232.35	100.97	177.87	67.02	83.61	96.48	126.38	26.31	
Standard group	27.07	55.23	42.73	28.85	52.71	49.27	42.64	4.96	
Test group 1	91.89	121.58	44.61	20.52	180.10	135.70	99.07	24.22	<0.001
Test group 2	93.60	79.41	60.23	32.03	41.88	43.55	50.11	7.20	
Test group 3	82.95	66	50.96	66.28	20.55	57.23	45.66	7.38	
Test group 4	20.78	27.33	36.95	19.78	40.31	32.39	29.59	3.44	

*ANOVA

Table 8: Showing percentage protection in tonic hind limb extension among various treatment groups in maximal electroshock seizure model.

Treatment Groups	Percentage protection
Standard Group	100%
Test Group 1	Nil
Test Group 2	50%
Test Group 3	66.66%
Test Group 4	100%

Postictal depression

Table 7 shows the comparison of Postictal depression duration in seconds shows the mean postictal depression in seconds among various groups in Maximal Electro Shock Seizure Model. ANOVA analysis shows statistically

significant (<0.001) difference between groups included in the study with respect to the variable (Postictal Depression duration) considered in the study. Post hoc LSD test shows statistically significant difference between.

- Control group vs Standard group (p =0.01)
- Control group vs Test group 1 (Not significant)
- Control group vs Test group 2 (p=0.02)
- Control group vs Test group 3(p=0.01)
- Control group vs Test group 4(p=0.002)
- Standard group vs Test group 4 (Not significant).

The mean Postictal Depression duration in control group was 126.38seconds. The Acetazolamide at doses of 8.75mg/kg, reduced the postictal depression duration to 99.07sec which was statistically not significant when compared to control group. The Acetazolamide at doses of 17.5 mg/kg and 35 mg/kg reduced the Postictal Depression

duration of to 50.11sec and 45.66sec respectively, which was statistically significant when compared to control group. The standard drug, sodium valproate reduced the duration of Postictal Depression to 42.64sec. The acetazolamide at dose of 8.75mg/kg along with sodium valproate at dose of 150mg/kg reduced the duration of Postictal Depression to 29.59sec, which was statistically not significant when compared to standard group. The percentage protection in Tonic Hand Limb Extension among various treatment groups is listed in Table 8.

Seizure latency

Comparison of seizure latency duration (seconds) of various groups in the pentylenetertazole model has been shows in Table 9. ANOVA analysis shows statistically significant (p <0.0001) difference between groups included in the study with respect to the variable (Seizure Latency duration) considered in the study. Post hoc LSD test of seizure latency duration in seconds among various groups in Pentylene Tetrazole Model (Table 9) shows statistically significant difference between

- Control group vs Standard group (p <0.001)
- Control group vs Test group 5 (Not significant)
- Control group vs Test group 6 (p =0.02)
- Control group vs Test group 7 (p =0.018)
- Control group vs Test group 8 (p <0.0001)
- Standard group vs Test group 8 (Not significant)
- Test group 5 vs Test group 8 (<0.0001)
- Test group 6 vs Test group 8 (p = 0.006)
- Test group 7 vs Test group 8 (p = 0.007).

In control group, there was a mean seizure latency of 142.33sec, following PTZ injection. At the dose of 8.75mg/kg, the seizure latency was 220.50sec, which was statistically not significant. Whereas, at the dose of 17.5mg/kg and 35 mg/kg the seizure latency was 277.33sec and 281.00sec respectively, which was statistically significant, when compared to control group. The standard, sodium valproate produced a mean seizure latency of 329.67sec. The Acetazolamide at dose of 8.75mg/kg along with Sodium Valproate at dose of 150mg/kg the seizure latency was 432.83 sec, which was statistically not significant when compared to standard group.

Table 9: Comparison of seizure latency duration in seconds among various groups in pentylene tetrazole model.

Sl. No. of rats	1	2	3	4	5	6	Mean	SE	P*
Control group	161	189	102	151	103	148	142.33	13.91	
Standard group	335	494	173	351	382	243	329.67	45.56	
Test group 5	193	247	201	251	174	257	220.50	14.44	<0.0001
Test group 6	287	306	357	249	263	202	277.33	21.56	
Test group 7	254	366	200	310	233	323	281.00	25.44	
Test group 8	390	525	423	493	451	315	432.83	30.69	

*ANOVA

Table 10: Comparison of clonus latency in seconds among various groups in pentylene tetrazole model.

Sl. No. of rats	1	2	3	4	5	6	Mean	SE	P*
Control group	249	225	116	267	157	276	215.00	26.36	
Standard group	#	#	#	#	#	#			
Test group 5	203	277	208	276	180	265	234.83	17.43	<0.0001
Test group 6	406	367	391	302	283	422	361.83	23.26	
Test group 7	473	502	436	398	307	451	427.83	28.07	
Test group 8	#	#	#	#	#	#			

*ANOVA, Note: # - Abolition of clonus

Clonus latency

Table 10 depicts the omparison of Clonus latency (seconds) and the mean clonus latency (seconds) among various groups in Pentylene Tetrazole Model respectively.

In control group, there was a mean delay in Clonus of 215sec, following PTZ injection. At the dose of 8.75mg/kg, the delay in Clonus was 234.83sec, which was statistically not significant, when compared to control group. Whereas, at the dose of 17.5mg/kg and 35 mg/kg the delay in Clonus was 361.83sec and 427.83sec respectively, which was statistically significant, when compared to control group.

The Acetazolamide at dose of 8.75mg/kg along with Sodium Valproate at dose of 150mg/kg abolished the clonus in 100% of animals. The standard drug, sodium valproate abolished the clonus in 100% of animals.

Percentage Protection in Seizure Latency among various treatment group in Pentylenetetrazole Model has been listed in Table 11.

Table 11: Showing percentage protection in seizure latency among various treatment group in pentylenetetrazole model.

Treatment Groups	Percentage protection
Standard Group	100%
Test Group 5	66.8%
Test Group 6	84.14%
Test Group 7	85.25%
Test Group 8	131.32%

DISCUSSION

Significant advances are being made in recent years to treat epilepsy using second-generation drugs.[14] Polypharmacy is often advocated to 30% of all epileptic patients for refractory partial or generalized tonic clonic seizures.[14] However, none of the new drugs fulfill the ultimate goal of drug treatment of epilepsy, i.e. complete control of seizures.[16] Despite the massive scale of the problem and much research, epilepsy remains poorly understood. Despite more than 20 approved drugs and several non-pharmacological options, up to 30% of patients are still refractory to treatment. Discovery and development of anti-epileptic drugs has been especially difficult, because of the regulatory issues of satisfactorily proving safety and efficacy, ethical constraints on placebo-controlled trial designs. For this reason, known antiepileptic drugs are used in combination for the treatment.[17]

In this study, the anticonvulsant activity of Acetazolamide was studied against MES and PTZ induced convulsions when it is given alone and upon combination with sodium valproate. The present study demonstrates that abolition of tonic hind limb extension shows that drug poses anticonvulsant activity for MES model and an increase in the seizure threshold, suggesting that the drug possess anticonvulsant property against the PTZ model.

Dose 1-8.75mg/kg body weight

Analysis of results of 3rd group animals in Maximal Electro Shock (MES) model (Table 3, 4, 5, 6, 7) that received 8.75mg/kg of test compound showed a reduction in all phases namely tonic hind limb flexion, tonic hind limb extension, clonus, stupor and postictal depression. Reduction in duration of all phases was statistically not significant when compared to control group. Analysis of results of 9th group animals in Pentylenetetrazole (PTZ) model (Table 9, 10) showed an increase in duration of seizure latency and increase in clonus latency which was statistically not significant, when compared to control group.

Dose 2-17.5mg/kg body weight

Analysis of results of 4th group animals in MES model (Table 3, 4, 5, 6, 7) that received 17.5mg/kg of test compound, showed a reduction in all phases. Reduction in duration of tonic hind limb flexion, clonus, stupor and postictal depression were statistically significant, when compared to control group. There was complete abolition of tonic hind limb extension in 3 animals out of 6 animals. 50% abolition in tonic hind limb extension at this dose was observed.

Analysis of results of 10th group animals in PTZ model (Table 9, 10) when compared to control group, showed an increase in duration of seizure latency and increase in clonus latency which was statistically significant.

Dose 3-35mg/kg body weight

Analysis of results of 5th group animals in MES model (Table 3, 4, 5, 6, 7) that received 35mg/kg of test compound, showed a reduction in all phases. Reduction in duration of tonic hind limb flexion, clonus, stupor and postictal depression were statistically significant, when compared to control group. There was complete abolition of tonic hind limb extension in 4 animals out of 6 animals. 66.66% abolition in tonic hind limb extension at this dose was observed.

Analysis of results of 11th group animals in PTZ model (Table 9, 10) when compared to control group, showed an increase in duration of seizure latency and increase in clonus latency which were statistically significant. Increase in clonus latency was statistically significant, when compared to group 9.

Dose 4-Acetazolamide 8.75 mg/kg + Sodium valproate 150 mg/kg body weight

Analysis of results of 6th group animals in MES model (Table 3, 4, 5, 6, 7) that received acetazolamide 8.75mg/kg body weight and sodium valproate 150mg/kg body weight (sub anticonvulsant dose), showed a reduction in all phases. Reduction in duration of tonic hind limb flexion, clonus, stupor and postictal depression were statistically significant, when compared to control group. Reduction in duration of tonic hind limb flexion and clonus was statistically significant, when compared to standard group whereas reduction in stupor and postictal depression was statistically not significant when compared to standard group. Reduction in duration of tonic hind limb flexion was statistically significant, when compared to test group 1. Reduction in duration of clonus was statistically significant, when compared to test group 1, 2 and 3. Reduction in duration of stupor was statistically significant, when compared to test group 1. There was complete abolition of tonic hind limb extension in all 6 animals. 100% abolition in tonic hind limb extension at this dose was observed.

Analysis of results of 12th group animals in PTZ model (Table 9, 10) when compared to control group, showed an increase in duration of seizure latency which was statistically significant, whereas increase in duration of seizure latency when compared to standard group was

statistically not significant. There was increase in duration of seizure latency in 12th group which was statistically significant when compared to group 9, 10 and 11. There was complete abolition of clonus in all 6 animals in this group. i.e. 100% abolition of the clonus.

Swinyard et al, have considered abolition of hind limb tonic extension as the protective end point against MES induced seizures.[18] At doses of 8.75mg/kg there was no significant anticonvulsant activity. At the dose of 17.5mg/kg and 35mg/kg tonic hind limb extension was abolished in 50% and 66.66% of animals respectively when compared to control. This indicates that the anticonvulsant activity of acetazolamide is less when compared to the standard drug, sodium valproate. The combination of Acetazolamide (8.75mg/kg) with sodium valproate (150mg/kg) showed significant reduction in all phases of MES model, when compared to the control and standard drug sodium valproate. Most importantly the combination of acetazolamide (8.75mg/kg) with sodium valproate (150mg/kg) showed complete abolition of tonic hind limb extension in all 6 animals.

According to this study, Sodium valproate 300mg/kg showed 100% protection against tonic hind limb extension. The combination of acetazolamide 8.75mg/kg with sodium valproate 150mg/kg showed 100% (Table 8) protection against tonic hind limb extension.

The anticonvulsant activity of acetazolamide 8.75mg/kg in combination with sodium valproate 150mg/kg is comparable and is more significant when compared to sodium valproate 300mg/kg. The above analysis gives us the result that

- Acetazolamide by itself has significant anticonvulsant activity in the dose of 17.5mg/kg. But anticonvulsant activity produced by it is not significant when compare to the standard drug sodium valproate in the dose of 300mg/kg.
- Acetazolamide when combined with sodium valproate showed significant anticonvulsant activity and the combination of these drugs in half of their anticonvulsant dose was found to be more significant when compared to the standard drug alone. This suggests that acetazolamide has potentiated the effect of sodium valproate.

Hence, the test compound may be useful in generalized tonic-clonic seizures (grand mal) or partial epilepsy and the combination drugs may be superior when compared to standard drug alone in generalized tonic-clonic seizures (grand mal) or partial epilepsy. In the Pentylenetetrazole Model, prolongation of seizure latency was taken as protective end point.[19]

At doses of 8.75 mg/kg of acetazolamide, there was no significant anticonvulsant activity, whereas at 17.5mg/kg and 35 mg/kg there was significant anticonvulsant activity.

Percentage protection offered by sodium Valproate was 100% against seizure latency. The percentage protection of Acetazolamide at the doses of 8.75mg/kg, 17.5mg/kg and 35mg/kg were 66.8%, 84.14% and 85.25% respectively (Table 11). The percentage protection offered by combination acetazolamide 8.75mg/kg with sodium valproate 150mg/kg was 131.32% against seizure latency.

This indicates that the anticonvulsant activity of acetazolamide is less when compared to the standard drug, sodium valproate and the anticonvulsant activity of acetazolamide 8.75mg/kg with sodium valproate 150mg/kg is comparable and more significant when compared to standard drug alone. The above analysis gives us the result that

- Acetazolamide by itself has significant anticonvulsant activity in the dose of 17.5mg/kg. But anticonvulsant activity produced by it is not significant when compared to the standard drug sodium valproate in the dose of 300mg/kg.
- Acetazolamide when combined with sodium valproate showed significant anticonvulsant activity and the combination of these drugs in half of their anticonvulsant dose was found to be more significant when compared to the standard drug alone. This suggests that acetazolamide has potentiated the effect of sodium valproate. Hence, the test compound may be useful in absence seizures (petit mal epilepsy) and the combination drugs may be superior when compared to standard drug alone in absence seizures (petit mal epilepsy).

All the rats recovered following MES test and PTZ model. No deaths were recorded. Control group animals (which received normal saline) were not protected against both MES and PTZ models.

REFERENCES

1. Daniel H. Lowenstein. Seizures and epilepsy. Harrisons Principles of Internal Medicine, 17th Ed. Mc Graw Hill; 2009:2498-2513.
2. Satoskar RS, Bhendarkar SD, Rege NN. Pharmacology and Pharmacotherapeutics. 19th Ed. Popular Prakashan; 2005:122-140.
3. Desai CK. Comparative evaluation of anticonvulsant activity of calcium channel blockers in experimental animals. Indian J Exp Biol. 1995;33:931-34.
4. La Roche SM, Helmers SL. The new antiepileptic drugs. JAMA. 2004;291(5):605.
5. White HS. Preclinical development of antiepileptic drugs: Past, present and future directions. Epilepsia. 2003;44(7):2-8.

6. Woodbury L.A and V.D Davenport. Archives International pharmacodynamic and therapie. 1952;92:97-107.
7. Castel-Branco MM, Alves GL, Figueiredo IV, Falcão A, Caramona MM. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. 2009;31(1):101-6.
8. Godman LS, Toman JEP, Swinyard EA. Comparison of Maximal seizures evoked by PTZ and electroshock in mice and their modification by anticonvulsant. J pharmacy pharmacology. 1953:168-76.
9. Michael A, Roger R, Porter J. Antiepileptic drugs: pharmacological Mechanisms and Clinical efficacy with consideration of promising developmental stage compounds. Pharmacol Rev. 1990;42(3):224.
10. James JEP, James EP, Grey ME. Anticonvulsants In evaluation of drug activities pharmacometrics. Volume I. Laurence DR, Bacharahah ARI (Eds.). London. Academic press;1964:287.
11. Brahmane RI, Wanmali VV, Pathak SS, Salwe KJ. Role of cinnarizine and nifedipine on anticonvulsant effect of sodium valproate and carbamazepine in maximal electroshock and pentylenetetrazole model of seizures in mice. J pharmacol Pharmacothera. 2010;1(2):78.
12. Rehni AK, Singh N. Reversal of pentylenetetrazole-induced seizure activity in mice by nickel chloride. Indian J Pharmacol. 2009;41(1):15.
13. Anderson RE, Chiu P, Woodbury DM. mechanism of tolerance to the anticonvulsant effects of acetazolamide in mice: relation to the activity and amount of carbonic anhydrase in brain. Epilepsia. 1989;30(2):208-16.
14. Sabers A, Gram L. Drug treatment of epilepsy in the 1990s-achievements and new developments. Drugs. 1996;52:483-93.
15. Pellock JM. Antiepileptic drug-therapy in the United-States-a review of clinical-studies and unmet needs. Neurology. 1995;45:S17-S24.
16. Loscher, W. New visions in the pharmacology of anticonvulsion. Euro J Pharmacol. 1998;342:1-13.
17. White HS. Preclinical development of antiepileptic drugs: Past, present and future directions. Epilepsia. 2003;44(7):2-8.
18. Swinyard EA, Brown WC,Goodman LSJ. Pharmacol Exp Ther. 1952;106:319-30.
19. Vogel HG, editor. Drug discovery and evaluation: pharmacological assays. 3rd Edi. Springer Science & Business Media; 2008:673.