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Effect of tantu pashan on electrical and chemical induced seizures in mice

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ABSTRACT

In traditional Indian system of medicine Tantu Pashan is being used for the treatment of epilepsy. Tantu Pashan is a mixture of *Piper nigram*, *Piper chaba* and Asbestos. But the scientific data about the efficacy of Tantu Pashan is not available. Hence, the present study was undertaken to evaluate efficacy of Tantu Pashan against maximal electrical shock (MES) and Pentylenetetrazole (PTZ) induced seizures in mice. Inbred albino mice of either sex weighing between 25 to 30 g were divided into five groups, containing eight animals in each. In acute study, drugs / vehicle were administered one hour before the experiment. In chronic study, drugs /vehicle were administered once a day for 21days and the experiment was conducted one hour after the last dose. Tantu pashan on acute administration in MES induced seizures; only at the highest dose (160mg/kg) significantly reduced the duration of hind limb tonic extension, while in PTZ induced seizures, it did not alter the duration of seizures to any significant level when compared with control group. On chronic administration, the test drug significantly reduced the duration of tonic hind limb extension and also the clonus phase in MES induced seizures. But, in PTZ induced seizures, neither it reduced the duration of clonic convulsion nor protected the animals from death. Results indicates that Tantu Pashan has protective effect against MES, but not against PTZ induced seizures.

Key words: Tantu Pashan, maximal electro-shock, epilepsy, pentylenetetrazole

INTRODUCTION

Epilepsy is the second most common disorder of central nervous system. It was characterized by recurrent episodes of disturbance of movement, sensation and consciousness. Conventionally used antiepileptic drugs are associated with many adverse effects[1,2]. Therefore there is a need for finding more effective and safer anticonvulsants. In recent years considerable interest has been focused on the role of herbal drugs in the treatment of epileptic disorders. In traditional Indian system of medicine Tantu Pashan is being used for the treatment of epilepsy. Tantu Pashan is a mixture of *Piper nigrum*, *Piper longum* and nanoparticles of Asbestos[3,4].

Piperine (1-peperoyl piperidine) is a major alkaloid isolated from the plants *Piper nigrum* Linn. and *Piper longum*[5] that belong to the family piperaceae. In Ayurveda, pepper and its derivatives are used to treat digestive disorder, cold, cough, intermittent fever, improved appetite, inflammation and epileptic fits[3]. *Piper nigrum* is still used in Chinese medicine for the treatment of epilepsy and for many other disease conditions[6]. Experimental evidence indicate that Piperine has analgesic,diuretic, antioxidant[7,8],anti-inflammatory[9], blood pressure lowering[10] properties. Piperine also found to be effective in Alzheimers disease[11] and epilepsy[12]. But scientific data about the efficacy of Tantu Pashan is not available. Hence, the present study was undertaken to evaluate the effect of Tantu Pashan in two experimentally validated models of epilepsy like, (Maximal electrical shock) MES & (pentylenetetrazole) PTZ induced seizures in mice

MATERIALS AND METHODS

Animals

Inbred male albino mice (Swiss strain) weighing between 25-30g were used in the study. Animals were acclimatized for a period of seven days prior to screening/experimentation. They were housed in groups of six in clean polypropylene cages with 12 hour light/dark cycle at 25±2.0ºC and 65±5% humidity. They had access to food (standard pellet diet) and water *ad libitum* except during overnight fasting prior to the challenge (electrical stimulation or PTZ), during the test period in acute and chronic study. All experiments were carried out between 11.00 am and 4.00 pm. The study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. The study protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Mangalore.

Drugs

Tantu Pashan(TP) (M/s. Sagar Pharmaceuticals Pvt Ltd,India), sodium valproate (Cadila Laboratories, India) and PTZ (Sigma, USA) were dissolved in normal saline, which served as the vehicle. Animals were divided into five groups (n=8) for both, MES and PTZ induced seizures. After overnight fasting, group I received 10ml/kg vehicle and served as the control. Group II received sodium valproate (130mg/kg); Groups III,IV and V received TP (40,80 and 160mg/kg) 60 minutes prior to the test in acute study.Doses of TP and sodium valproate that were used in the study did not produce either gross changes in behaviour[13] or motor in-coordination in animals (results not shown) as assessed by the rota-rod and the traction tests[14]. For chronic study drugs/vehicle were administered once a day for 21 days and the convulsive challenge was given 60 minutes after administration of the last dose. Vehicle and drugs were administered orally.

MES induced seizures:

Forty eight hours before the test, each animal was exposed to the shock and those animals which showed all the phases of convulsion were chosen for the study. On the day of experimentation, 60 minutes after administration of drug/vehicle, seizure was induced by delivering an electrical shock (50mA at 50Hz for 0.2 sec) by means of a convulsiometer (Techno India) through a pair of ear clip electrodes. The duration of flexor and extensor phases was noted[15].

PTZ induced seizures:

Sixty minutes after administration of drug/vehicle, animals were challenged with a convulsive dose of pentylenetetrazole (80 mg/kg body weight

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Table 1. Effect of acute administration of Tantu Pashan on MES induced seizures in mice

Groups (n=8)	Tonic flexion (seconds)	Tonic hind limb extension (seconds)	Clonic convulsion (seconds)
Normal saline -10 ml/kg	2.2±0.2	11.7±1.7	15.5±3.8
Sodium valproate – 130 mg/kg	1.1±0.4*	1.2±1.2**	1.8±1.8**
TP -40mg/kg	2.7±0.8	9.2±2.3	20.0 ±3.5
TP- 80mg/kg	1.3±0.5	7.2±2.4	12.0±3.1
TP -160mg/kg	1.1±0.4*	2.2±1.7**	7.0±2.4*
F value	2.9	5.2	5.3

*(Values are the mean±SEM,*P<0.05, **P<0.01versus control; n= number of animals)*

Table 2. Effect of chronic administration of Tantu Pashan on MES induced seizures in mice

Groups (n=8)	Tonic flexion (seconds)	Tonic hind limb extension(seconds)	Clonic convulsion (seconds)
Normal saline -10 ml/kg	2.1±0.2	15.2±0.3	29.1±1.9
Sodium valproate –130 mg/kg	0.2±0.2**	0.2±0.2**	6.5±3.5**
TP - 40mg/kg	1.0±0.5*	5.2±2.2**	20.5±4.1*
TP-80mg/kg	0.2±0.2**	0.8±0.8**	5.7±3.3**
TP - 160mg/kg	0.3±0.2**	0.8±0.8**	7.1±3.8**
F value	6.0	28.6	9.3

*(Values are the mean±SEM,*P<0.05, **P<0.01versus control; n= number of animals)*

Table 3.Effect of acute administration of Tantu Pashan on PTZ induced seizures in mice

Groups (n=8)	Duration of clonic convulsion (seconds)	Mortality	Number of episodes
Normal saline - 10 ml/kg	71.6±21.6	5/8 (62.50%)	1.3±0.1
Sodium valproate – 130 mg/kg	1.1±1.1*	0/8(0%)	0.1±0.1**
TP - 40mg/kg	62.8±25.9	3/8(37.5%)	0.8 ±0.2
TP - 80mg/kg	45.2±13.8	2/8(25%)	1.3±0.1
TP - 160mg/kg	60.8±21.5	2/8(25%)	1.3±0.2
F value	2.1		7.3

*(Values are the mean±SEM,*P<0.05, **P<0.01versus control; n= number of animals)*

Table 4. Effect of chronic administration of Tantu Pashan on PTZ induced seizures in mice

Groups (n=8)	Duration of clonic Convulsion (seconds)	Mortality	Number of episodes
Normal saline - 10 ml/kg	75.1±15.3	8 (87.5%)	2.0±0.3
Sodium valproate – 130 mg/kg	3.5±2.3*	1/8 (12.5%)	0.2±0.1**
TP - 40mg/kg	85.3±25.8	6/8 (75%)	1.3±0.1
TP- 80mg/kg	74.0±10.7	7/8 (87.5%)	2.2±0.3
TP - 160mg/kg	71.0±15.4	7/8 (87.5%)	2.1±0.3
F value	4.31		8.05

*(Values are the mean±SEM,*P<0.05, **P<0.01versus control; n= number of animals)*

i.p.). Duration of convulsion and the mortality were noted[16].

Statistical analysis:

The difference between groups was analysed by One-way ANOVA followed by Dunnet's multiple comparison test. P value <0.05 was considered significant.

RESULTS

A) Maximal electro shock induced seizures (Table 1&2)

In the acute study (Table 1) TP,only at the highest dose (160mg/kg) significantly (P<0.01) reduced the duration of tonic flexion, tonic hind limb extension and clonic convulsion.

In the chronic study (Table 2), TP at all the doses tested (40.80 and 160mg/kg) significantly reduced the duration of tonic flexion, tonic hind limb extension and clonic convulsion.These results comparable to standard drug sodium valproate (130mg/kg).

B) Pentylenetetrazole induced seizures (Table 3&4)

In the acute study at all the doses studied TP failed to alter the duration of pentyleneterazol induced clonic convulsion, but mortality was reduced and it was not dose dependent.The test drug failed to significantly alter the duration of clonic convulsions in the chronic study also.It failed to protect the animals from PTZ induced death.. However, the standard drug Sodium valproate significantly reduced the PTZ induced convulsions and protected all animals from death.

DISCUSSION AND CONCLUSION

The present study suggests thatTantu Pashan on single and repeated administration is effective against MES induced seizures as indicated by the reduction in the duration of hind limb extension and clonic convulsions. The efficacy of TP against MES seizure is more pronounced on chronic administration suggesting that the drug has cumulative effect.These results are comparable to the effects produced by the standard drug sodium valproate.This indicates that, as TP is effective against MES seizures in animals it may be useful in generalized tonic clonic seizures/grandmal epilepsy in human beings.

In case of PTZ induced seizures, both acute and chronic administration of TP failed to alter the duration of clonic convulsion and did not protect the animals from death. But the standard antiepileptic drug sodium valporate offered complete protection against PTZ induced seiures and death.This suggests that TP is not effective against chemical induced convulsion in animals and not useful against absence seizure in human beings.

Liu et al.[17] 1979 and Yuang et al.[6] 1993 reported that the extracts of *Piper nigrum* have exhibited potent efficacy against electroshock, audiogenic seizures and chemically induced seizures. Piperine is a major alkaloid isolated form the plants *Piper nigrum* Linn. and *Piper longum*[5]. Ruo Qi Hu et al. 1997 reported that piperine has an antagonistic action at N-methyl-D-aspartate (NMDA) receptors[12]. NMDA receptors have been proposed to be involved in the generation and maintenance of epileptiform activity[18]. Earlier studies have incicated that NMDA receptor antagonists more effectively block/antagonize the maximal electro shock induced seizures and they are either ineffective or weak against pentylenetetrazole/picrotoxin induced seizures in animals[19,20]. This could be the reason why Tantu Pashan is effective against maximal electro shock induced seizures and not effective against PTZ induced seizures in mice.

MES induced seizures is the best validated model for evaluating drug for the treatment of generalized tonic-clonic seizures and the profile of anticonvulsant activity of TP against MES induced seizures in mice, suggests its potential utility in the management of generalized tonic-clonic seizures in human beings. But, further studies are needed to elucidate exact mechanism of action and its usefulness in human beings.

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