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Pharmacological evaluation of Khameera Marwarid Abdul Hameed and Piperine on Pentylenetetrazole induced kindled mice

[*1] Shamsuzzaman Md, [2] Behl T, [3] Pillai KK
[1, 2] Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India
[3] Department of Pharmacology, Jamia Hamdard, Delhi, India

Abstract
Epilepsy is one of the most widespread neurological disorders in the world. To elucidate the consequences of convulsion, we examined biochemically and electrophysiologically the brains of mice that had sustained two complete tonic clonic convulsions after administration of pentylenetetrazole (PTZ 75mg/kg intraperitoneally, i.p) Khameera Marwarid Abdul Hameed reduced the latency time. The high dose of Khameera Marwarid Abdul Hameed 500mg/kg induced the seizures frequency, but at low dose 50mg/kg reduce the seizure frequency. PTZ was given 45mg/kg on 1, 3, 5 and on 8 day, on 10th day 75mg/kg (I.P) and after that observe the seizure activity. At high dose 2mg/kg (Piperine) the seizure frequency was high, but at low dose 1mg/kg (Piperine) the seizure frequency was low. The sodium valproate 300mg/kg (p.o) was used as a standard drug. The test drugs was administered and compared with standard drug. Control mice were injected saline(0.9%) and PTZ 75mg/kg, after that observe the seizure activity for last 30 minutes, generally observe the tonic clonic convulsion at high dose of 75mg/kg, but at low dose 45mg/kg 50% animal shows the convulsion(tonic convulsion).The combined formulation (KAH(50 mg/kg + Piperine 1 mg/kg) was found to be more effective and worked as an antiepileptic drugs when compared to standard group (sodium valproate 300mg/kg). These result show that Khameera Marwarid Abdul Hameed at low dose(50mg/kg) was act with varied mechanism of action and it is clinically useful in the treatment of epilepsy and show less side effect as compared to other antiepileptic drugs.

Keywords: epilepsy; pentylenetetrazole; piperine, kindled; convulsion

Introduction
Epilepsy is a common chronic neurological disorder characterized by a recurrent unprovoked seizure. These seizures are transient sign and symptom of abnormal, excessive or synchronous normal brain activity. About 50 million people worldwide have epilepsy, with almost 90% people being in developing countries. 0.5% of all human beings suffer from epilepsy, which means that in the U.K. alone around 300000 to 600000 people are affected.
Epilepsy is one of the most common serious neurological conditions with an annual incidence of 50/100,000 per year. [1-2] Seizures are controlled in nearly 70% of patients with epilepsy, mostly through drug effects on membrane ion channels or on GABAergic or glutamatergic transmission. However, for the remaining 20-30% with intractable seizures, recent advances in systemic antiepileptic drug (AED) development have had little impact. Refractory epilepsy is associated with considerable medical, social, and psychiatric morbidity and enormous financial cost. Thus, novel approaches to the treatment of these patients are needed. [3-4] Abnormal synchronization of neuronal discharges is of recognized critical importance in seizure; however, the mechanism underlying this pathological synchrony remains uncertain. In this context, there is growing interest in electronic communication via gap junctions, and speculation, based largely on studies *in vitro* and on *ex vivo* brain tissue that gap junctions may be important in the generation and propagation of seizures. The pathogenesis of abnormal neuronal synchrony underlying seizures, formerly thought to be based mainly on the chemical synaptic transmission, now includes a role of gap junctional communication. This concept has been strengthened by evidence from several *in vitro* models, in which pharmacological manipulations of gap junctional communication predictably affect the generation of seizures, with blockers diminishing seizures. [5-7] Thus, it seems that gap junctions may represent a novel therapeutic target for the future.

Method
Animals: Male albino mice, Central Animal House Facility, Jamia Hamdard; age 7-8 weeks, weighing 18-25 g were used. They were housed in a group of eight animals/cage in poly-propylene cages, at constant temperature of 22 ± °C and relative humidity of 60-70%, air changes >12 h^{-1}, with automatically controlled 12/12 h light/dark cycle, lights on at 7.00h. All animals were provided with the pelleted standard diet and drinking water bottles. Experiments were conducted during the light phase between 9.00 a.m. and 4.00 p.m. All the experiments were performed in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC). After decapitation animals should be send to central animal house Jamia Hamdard for incineration.

Drugs and reagents: Pentyleneterazole, sodium valproate, Piperine was purchased from Sigma Aldrich, US. Khameera Marwarid Abdul Hameed (KAH) was purchased from Hamdard Wakf Laboratory, Ghaziabad (UP), it is in paste formulation given by orally

Drug administration: Khameera Marwarid Abdul Hameed (KAH) and PTZ solutions were freshly before administration. PTZ was dissolved in heparinized sterile 0.9% saline. The PTZ was given (I.P) 45mg/kg on 1, 3, 5, and on 8th day and on 10th day 75mg/kg. After drug administration observed the seizure type up to 30 min

Table 1: Treatment Schedule

Group No	Treatment	Dose, and Route of Administration
Group 1	Vehicle + PTZ	0.9%saline + PTZ 45mg/kg on day 1,3,5,8 and on day10 PTZ 75mg/kg i.p
Group 2	SV +PTZ	300mg/kg p.o daily for a period of 10 days. PTZ Dose same as in Control group
Group 3	KAH+PTZ	50mg/kg p.o daily for a period of 10 days. PTZ Dose same as in Control group
Group 4	KAH+PTZ	500mg/kg p.o daily for a period of 10 days. PTZ Dose same as in Control group
Group 5	P_1+PTZ	1 mg/kg orally daily for a period of 10 days. PTZ Dose same as in Control group
Group 6	P_1+PTZ	2 mg/kg orally daily for a period of 10 days PTZ Dose same as in Control group
Group 7	KAH+SV+PTZ	50 mg +150 mg oral, p.o. daily for a period of 10 days. PTZ Dose same as in Control group
Group 8	P1+SV+PTZ	Comp P_1 1mg p.o + sod valproate 150 mg p.o. daily for a period 10 days. PTZ Dose same as in Control group

KAH: Khameera Marwarid Abdul Hameed;P_1: Piperine; n=7 'n' is the number of animals per group. Sodium valproate (SV) was given daily for a period of 10 days.Test drugs were given daily for a period of 10days.

Results

Pentylenetetrazole (PTZ) induced kindling in mice: Repeated treatment with PTZ produced seizures in all mice. During convulsion excess salivation, defecation and urine secretion was observed in all mice. No mortality was observed at highest dose of PTZ (60 mg/kg i.p.). The average onset of action for PTZ to produce convulsion was 65.4 ± 0.01seconds. In the PTZ treated group (Group 1) the level of malionaldehyde (MDA) was found to be 9.64 ± 0.81 ($p<0.01$) nmol MDA/mg protein. The levels of reduced glutathione (GSH) were found to be significantly lower in the whole brain homogenate (0.06 ± 0.01µmol/mg protein) ($p<0.01$). The levels of Ca^{++}ATPase was found to be significantly increased (2.94 ± 0.46nmol Pi release/min/mg protein) as compared to sodium valproate treated group (1.23 ± 1.99nmol Pi release/min/mg protein) ($p<0.01$).

Effect of SVP (300mg/kg p.o.) on PTZ kindled mice: Sodium valproate was given daily (300mg/kg p.o.) for a period of ten days PTZ was administered on alternate days from lower to higher doses, at higher dose all mice showed convulsion but less severe than the group1. During convulsion saliva secretion, was observed in all mice. No mortality was observed at highest dose of PTZ (60mg/kg i.p.) The average onset of action for PTZ to produce convulsion was 83.4 ± 0.06seconds ($p<0.01$). Sodium valproate treated group (group 2) the levels of MDA was found to be 0.45 ± 0.02nmol MDA/mg protein when compared to PTZ treated group (group 1) ($p<0.01$). Similarly in sodium valproate treated group (group2), the levels of reduced glutathione (GSH) were found to be significantly more in the whole brain homogenate The levels of reduced glutathione (GSH) was found to be more when compared to group (1) (2.23± 0.10µmol/mg protein) ($p<0.01$). In sodium valproate treated group (group 2) the levels of Ca^{++} ATPase was found to be significantly decreased when compared to PTZ treated group (1.23 ± 1.99 nmol Pi release/min/mg protein) ($p<0.01$).

Table 2: Effect of sodium valproate and test compound on onset of seizure duration on mice

Group no	Treatment	Onset of seizure duration in second
Group 1	Vehicle + PTZ	65.4 ± 0.01
Group 2	SV+ PTZ	83.4 ± 0.06*
Group 3	KAH+ PTZ	$85.2 \pm 0.02^{**}$
Group 4	KAH+ PTZ	63.0 ± 0.12^{ns}
Group 5	P_1+ PTZ	82.2 ± 0.11**
Group 6	P_1+ PTZ	$72.6 \pm 0.06^{*}$
Group 7	KAH+SV+ PTZ	$90.0 \pm 0.05^{**}$
Group 8	P_1+SV+ PTZ	86.4 ± 0.04**

KAH: Khameera Marwarid Abdul Hameed, P_1: Piperine

All experimental results were expressed as the Mean ± SEM. Comparisons between experimental and control groups were performed by ANOVA followed by Dunnett's test for post hoc comparison, when appropriate. A value of $p<0.05$ was considered to be significant, while $p>0.05$ was non-significant *$P<0.05$, **$P<0.01$, ***$P<0.001$, when compared with normal control group (i.e group 1)

Effect of KAH (50,500mg/kg p.o.) on PTZ kindled mice: KAH1 was given daily (50mg/kg p.o.) for a period of ten days. PTZ was administered on alternate days from lower to higher doses, at higher dose all mice showed convulsion but less severe than the group (1) During convulsion excess saliva secretion was observed in all mice. Twitching of head, jerky movement of fore limb and hind limb tonic clonic seizure were observed. 50% mortality was observed at highest dose of KAH1 (500mg/kg). The average onset of action for PTZ to produce convulsion was found to be 85.2 ± 0.02seconds for KAH (50mg/kg) and 63.0 ± 0.12seconds for KAH1 500mg, when compared to PTZ group (1). KAH1 significantly reduced the MDA levels in PTZ induced kindled mice. MDA was found to be 0.46 ± 0.01nmol MDA/mg protein. KAH increased the levels of reduced glutathione (GSH) in the whole brain homogenate, the levels of reduced glutathione (GSH) was found to be 1.27± 0.03µmol/mg protein in PTZ induced kindled mice ($p<0.01$). KAH (50 mg/kg p.o.) decreased the levels of Ca^{++}- ATPase

significantly when compared to (PTZ treated group 1) (1.31± 0.16nmolPi release/min/mg protein), ($p<0.01$). High dose of KAH did not reduce Ca^{++}- ATPase levels.

Effect of Piperine (1,2mg/kg p.o.) on PTZ kindled mice: Compound P1 increase the latency to produce convulsion at 1mg/kg and 2mg/kg. However the lower dose increased the latency more as compared to higher dose. Compound P1 reduced the malionaldehyde (MDA) in PTZ kindled mice. The levels of MDA were found to be 0.49 ± 0.03nmol MDA/mg protein. The levels of reduced glutathione (GSH) were found to be significantly more in the whole brain homogenate of compound P1 treated group (2.22± 0.01µmol/mg protein) ($p<0.01$). The levels of Ca^{++}- ATPase was found to be significantly decreased in compound P1 treated groups (1.70 ± 1.15nmol Pi release/mg/protein) ($p<0.01$).

Effect of KAH and Sodium valproate on PTZ kindled mice: When KAH (50mg/kg p.o.) and Sodium valproate (150mg/kg p.o.) were given concurrently for a period of 10 days. The latency to convulsions produced was found to be 90.6 ± 0.05seconds ($P<0.01$). The level of MDA was found to be 0.41 ± 0.01nmol MDA/mg protein ($p<0.01$). The whole brain glutathione (GSH) levels were more in KAH1 treated group. The levels of reduced glutathione (GSH) was found to be 1.20± 0.05µmol/mg protein ($p<0.01$). The Ca^{++}-ATPase activity was significantly higher in PTZ treated group. KAH1 was found to decrease the levels of Ca^{++}-ATPase (1.12 ± 0.87nmol Pi release/min/mg protein).

Table 3: Effect of sodium valproate and test compound on GSH changes in the mice brain

Group no	Treatment	GSH (µmol /mg protein)
Group 1	Vehicle + PTZ	0.06 ± 0.01
Group 2	SV + PTZ	2.23 ± 0.10**
Group 3	KAH + PTZ	1.27 ± 0.03**
Group 4	KAH+ PTZ	1.18 ± 0.05**
Group 5	P_1+ PTZ	2.24 ± 0.09**
Group 6	P_1+ PTZ	2.22 ± 0.01**
Group 7	KAH+SV+ PTZ	1.20 ± 0.05**
Group 8	P_1+ SV +PTZ	2.01 ± 0.07**

KAH: Khameera Marwarid Abdul Hameed, P_1: Piperine
All experimental results were expressed as the Mean ± SEM. Comparisons between experimental and control groups were performed by ANOVA followed by Dennett's test for post hoc comparison, when appropriate. A value of $p<0.05$ was considered to be significant, while $p>0.05$ was non-significant *P<0.05, **P<0.01, ***P<0.001, when compared with normal control group (i.e. group 1)

Combined effect of Piperine and Sodium valproate on PTZ kindled mice: When Compound P1 (1mg/kg p.o.) and sodium valproate (150 mg/kg) were given concurrently to PTZ kindled mice, the latency to produce convulsion was 86.4 ± 0.04seconds. The level of MDA was found to be 0.46 ± 0.01nmol MDA/mg protein when compared to PTZ treated group (group 1) ($p<0.01$). The levels of reduced glutathione (GSH) was found to be more when compared to group (1) (2.01 ± 0.07µmol/mg protein) ($p<0.01$). The levels of Ca^{++}-ATPase was found to be significantly decreased when compared to control group (PTZ treated group 1) (1.97 ± 0.84 nmol Pi release/min/mg protein) ($p<0.01$).

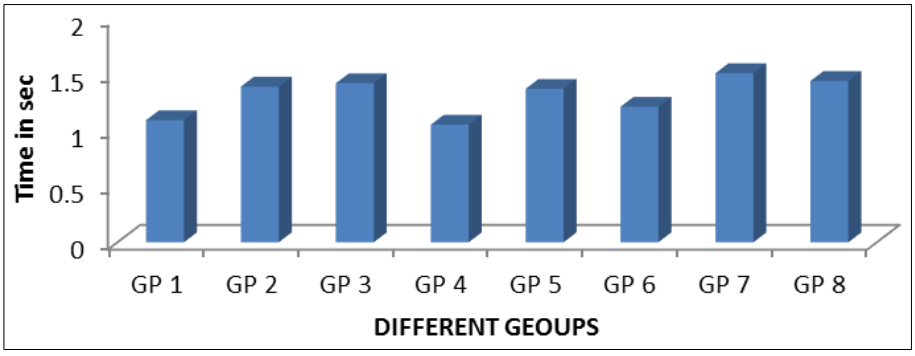

Fig 1: Effect of KAH and piperine on PTZ induced kindled mice (Onset of seizure)

I.P.PTZ: concentration of PTZ solution 45mg/kg on 1, 3 5 and on 8th day, 75mg/kg on 10th day. KAH (Khameera Marwarid Abdul Hameed), dose of KAH was 500mg/kg(4th gp) and 50mg/kg(5thgp), Sodium valproate 300mg/kg (2nd gp), Piperine 1mg/kg and 2mg/kg

Effect of Std/Test drugs on Ca^{++}- ATPase levels on PTZ kindled mice: Repeated treatment with PTZ increased the Ca^{++}- ATPase levels; the effect of standard drug sodium valproate (150mg/kg p.o.) significantly decreased the Ca^{++}-ATPase levels (1.23 ± 1.99 nmol Pi release /min/mg protein). Test drugs (KAH and Piperine) significantly decreased the levels of Ca^{++} ATPase levels when compared to control group (group 1)

Table 4: Effect of Na valproate and test compound on Ca^{++}-ATPase changes in mice brain

Group No	Treatment	Ca^{++}- ATP ase (nmol Pi release/min/mg protein)
Group 1	Vehicle + PTZ	2.94 ± 0.46
Group 2	SV + PTZ	1.23± 1.99**
Group 3	KAH+ PTZ	1.31±1.16**
Group 4	KAH+ PTZ	2.89 ± 0.26ns
Group 5	P_1+ PTZ	1.70 ±1.15**
Group 6	P_1+ PTZ	1.63 ± 2.89**
Group 7	KAH+SV+ PTZ	1.12 ± 0.87**
Group 8	P_1+SV+ PTZ	1.97 ± 0.84*

KAH: Khameera Marwarid Abdul Hameed, P_1: Piperine

All experimental results were expressed as the Mean ± SEM. Comparisons between experimental and control groups were performed by ANOVA followed by Dunnett's test for post hoc comparison, when appropriate. A value of $p<0.05$ was considered to be significant, while $p>0.05$ was non-significant *P<0.05, **P<0.01, ***P<0.001, when compared with normal control group (i.e group 1)

Discussion

Kindling is a model of epilepsy and epileptogenesis [8]. The repeated administration of a subconvulsant dose of pentylenetetrazole, a blocker of $GABA_A$ receptor mediated Cl^- channel, produced a progressive increase in convulsant activity culminating in generalized seizures (chemical kindling) in animals [9]. PTZ- induced kindling is an experimental model of epilepsy that shares many features in common with electrical limbic kindling.

The mechanism of the epileptogenic action of PTZ at the cellular neuronal level is still unclear. Electrophysiological studies have shown it acts at cell membrane level decreasing the recovery time between action potentials by increasing potassium permeability of the axon [10]. Other studies have implicated an increase in membrane currents of several other ions, such as sodium and calcium, leading to an overall increase in excitability of the neuron membrane [11]. Another study reported a decreased function of GABA-coupled chloride channel upon repeated administration of PTZ to be responsible for its mechanism of action [12].

Sodium valproate is a broad spectrum anticonvulsant, that blocks sustained high-frequency repetitive firing of neurons in culture at therapeutically relevant concentrations [13]. Its action against partial seizures may be a consequence of this effect on Na^+ currents. Reduction in NMDA-mediated signaling may also be important [14]. Much attention has been paid to the effects of valproate on GABA. Several studies have shown increased levels of GABA in the brain after administration of valproate, although the mechanism for this increase remains unclear. An effect of valproate to facilitate glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, and GABA transporters (GATs) has been described [15]. At very high concentrations valproate inhibits GABA transaminase in the brain, thus blocking degradation of GABA. However at relatively low doses of valproate needed to abolish pentylenetetrazole seizures, brain GABA levels may remain unchanged [16]. Valproate produces a reduction in the aspartate content of rodent brain, but the relevance of this effect to its anticonvulsant action is not known. Valproic acid is a potent inhibitor of histone deacetylase and through this mechanism changes the transcription of many genes [17].

Generally in epilepsy the levels of neurotransmitters in the CNS are altered, the levels of GABA (inhibitory neurotransmitter) are decreased, that play an important role in the epilepsy. [18]

The increase in latency time to convulsion produced by sodium valproate might be due its multiple mechanisms of action. The increase in latency time, produced by Khamira Marwarid Abdul Hameed and piperine also reveals the neuroprotective effect of these test drugs. The concurrent administration of test drugs with lower dose of sodium valproate also increased the latency time which suggests the possible additive effects of test drugs with sodium valproate.

The rise in the levels of MDA and reduction in the levels of GSH in pentylenetetrazole induced kindled mice reveal a role of oxidative stress in kindling. [19]

The attenuating effects of sodium valproate and test drugs on oxidative stress suggest antioxidant action of standard and test drugs.

An increase in Ca^{++}- ATPase activity in kindled mice has been reported. Similar observations in our lab further confirm the role of Ca^{++}- ATPase in kindled mice. The change in Ca^{++}- ATPase levels caused by sodium valproate and test drugs KAH and piperine also confirm the role of Ca^{++}-ATPase in seizure. [20]

The reduction in frequency of seizure observed with low dose of KAH and piperine and increased frequency observed with higher dose of test drugs reveal that these drugs might have a narrow margin of safety which needs further investigations.

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