

# Pharmacological Studies on Khamira Marwarid: Effect on Pentylenetetrazole-Induced Seizures, Cognition and Biochemical Markers in Mice

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## ABSTRACT

Cognitive impairment in epileptics may be a consequence of the epileptogenic process as well as antiepileptic medication. Thus, there is need for drugs, which can suppress epileptogenesis as well as prevent cognitive impairment. In the present study, the effect of Khamira Marwarid (KAH<sub>1</sub>), a formulation based on Indian system of Unani medicine, was evaluated on the course of pentylenetetrazole (PTZ)-induced seizures, learning deficit and oxidative stress markers in mice. Male albino mice were injected PTZ (65 mg/kg sc) on the 5<sup>th</sup> day of the treatment for the development of seizures. Spontaneous Alternation Behaviour was carried out on the 1<sup>st</sup> and the 5<sup>th</sup> day of the treatment after PTZ administration, while the oxidative stress parameters (malondialdehyde and glutathione) were measured in the whole brain upon the completion of the behavioural assessment. The administration of Khamira Marwarid (KAH<sub>1</sub>), 50 mg/kg significantly decreased the PTZ-induced seizures and showed improvement in the learning deficit induced by PTZ as evidenced by the increased latency time and frequency of jerks and improvement in SAB. The findings suggest the potential of Khamira Marwarid (KAH<sub>1</sub>) as adjuvant to antiepileptic drugs with an added advantage of preventing cognitive impairment.

**Keywords:** *Pentylenetetrazole, Khamira Marwarid, Cognitive function, Epilepsy*

Epilepsy is one of the oldest conditions known to mankind and still the most common neurological condition affecting individual of all ages. At any given time, it is estimated that 50 million individuals worldwide have a diagnosis of epilepsy. The prevalence is much higher in developing countries than in developed countries owing to low economic status, limited access to health care [1]. Our understanding of the pathophysiology of the epilepsies has advanced dramatically in the last 30 years, especially in terms of their cellular physiology and genetics. Drug treatment of epilepsy has made remarkable strides, with the introduction of many new antiepileptic drugs (AEDs) since 1978. Improvement in terms of clinical outcome however, has fallen short of expectations; with up to one third of patients continuing to experience seizures or unacceptable side medication related side effects in

spite of efforts to identify optimal treatment regimens with one or more drugs [2]. There is an urgent need to identify the problems associated with drug therapy in epilepsy. Antiepileptic drug treatment may last a lifetime in many patients so the objective of treatment should be such so as to attain the best compromise between maximum seizure control and minimum side effects. The current vastly improved understanding of the molecular targets, coupled with advances in the pathophysiology of epilepsy which include a succession of breakthroughs in genetics will lead to improved therapies for epilepsy.

Cognitive impairments are commonly seen in patients undergoing chronic antiepileptic drug therapy. For many patients, they may be more debilitating than the actual seizures themselves and thus, contribute to a worse quality of life. Common cognitive deficits in

Table 1. Treatment Schedule

Group	Treatment	Dosage, Route of administration & Duration
A	NS	0.9% NaCl p.o., single dose for 5 days
B	PTZ	0.9% NaCl p.o., single dose for 4 days
C	SVP	0.9% NaCl p.o.+ 65 mg/kg PTZ s.c. on 5 <sup>th</sup> day 300 mg/kg S.V.P p.o., single dose for 4 days
D	KAH <sub>1</sub>	300 mg/kg S.V.P p.o.+ 65 mg/kg PTZ s.c. on 5 <sup>th</sup> day 50 mg/kg p.o. single dose for 4 days
E	KAH <sub>1</sub>	50 mg/kg p.o.+ 65 mg/kg PTZ s.c. on 5 <sup>th</sup> day 250 mg/kg p.o. single dose for 4 days
F	SVP + KAH <sub>1</sub>	250 mg/kg p.o.+ 65 mg/kg PTZ s.c. on 5 <sup>th</sup> day 50 mg/kg Compound P <sub>1</sub> + 300 mg/kg S.V.P p.o. single dose for 4 days 50 mg/kg Compound P <sub>1</sub> +300 mg/kg S.V.P p.o.+ 65 mg/kg PTZ s.c. on 5 <sup>th</sup> day

NS: Normal saline; PTZ: Pentylenetetrazole; SVP: Sodium valproate; KAH<sub>1</sub>: Khamira Marwarid

people with epilepsy are intellectual decline, reduced information processing speed, reduced reaction time, attention deficits and memory impairments [3,4]. The origin of such cognitive impairments has been attributed to several factors: a) the underlying etiology of epilepsy, b) the central side effects of AEDs, c) the effects of the seizures themselves and d) mood [5,6].

Thus, while the underlying brain pathology, type, frequency and severity of seizures and psychological factors play an important role, ironically the therapy used also add to the problem [7]. Most often, these factors are related and contributed in varying degrees to the cognitive profile of the individual patients. Of these factors, side effects associated with AED therapy may be one of the few potentially-preventable tolerability issues, so it is worthwhile to further explore ways to prevent or minimize them. Therefore, induced cognitive impairments need pharmacological intervention.

Modern antiepileptic therapy is neither universally effective nor invariably safe. Advancement in understanding pathophysiology of epilepsies in term of cellular physiology and genetics would allow for more judicious therapeutic approaches to this complex neurological disorder [8]. Current practice suggests that combining drug with different mechanisms is likely to optimize the therapeutic response. Monotherapy may not be effective in all cases; therefore the need of the hour is to search for combinations of AEDs with other drugs so as to achieve supra-additive efficacy and infra-additive toxicity. Combinations of AEDs with nootropic agents appear to be promising directions for research in this area for maximal seizure control with minimal cognitive deficits. The adjuncts chosen for reducing cognitive impairments should be free of additional risk of side effects.

On the background of these observations present study was conducted to investigate the effect of Khamira Marwarid (KAH<sub>1</sub>) on pentylenetetrazole induced seizures, cognition and oxidative stress in mice. Khamira Marwarid, a pearl based formulation of Indian system of Unani medicine, is extensively used as neuroprotective, memory enhancer, immunomodulator

and antistress [9,10]. Its effect on model of experimentally-induced seizures has not been evaluated scientifically. Our study made an attempt to evaluate its effect on memory, seizures and oxidative stress in the PTZ model with anticipation that it can be used as an adjunct with AEDs, so an effort was made to study its effect per se and in combination of sodium valproate (SVP) on PTZ-induced seizures. Sodium valproate was used as a reference drug for comparison and combination studies. This study was designed to develop a combination therapy for epilepsy that may help to achieve maximal efficacy with minimal side effects.

## MATERIALS AND METHODS

### Animals

Swiss strain adult male albino mice weighing between 18-25 g, raised at the Central Animal House Facility of Jamia Hamdard were used. The animals were housed in polypropylene cages in groups of 8 mice per cage and were kept under controlled environmental conditions (Temperature: 22-28°C, Natural light-dark cycle). The mice were maintained on a standard pellet diet (Amrut Laboratory rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd., Pune) and water ad libitum. Only active and apparently healthy animals with no visible lesions or gross abnormalities were selected for the experiments. All studies were conducted during the day time. The project was approved by Jamia Hamdard Animal Ethics Committee. Animal Ethics Number: 585.

### Drugs and dosing schedule

Pentylenetetrazole powder (Sigma, USA), Sodium Valproate powder (Sigma, USA) and Khamira Marwarid (KAH<sub>1</sub>) (Hamdard Laboratories) were used in the study. All the drugs were dissolved in distilled water. The dose of sodium vaproate (300 mg/kg) was selected on the basis of pilot experiments in our lab. This dose exhibited less than 50% protection against the

**Table 2.** Effect of Sodium valproate (SVP), Khamira Marwarid (KAH<sub>1</sub>) and their combination on PTZ-induced seizures in mice

Group	Treatment	Dose (mg/kg)	Latency (sec)		Frequency of jerks within 30 min
			Jerks	Myoclonus & clonic generalised seizures	
A	PTZ	65	333.14 ± 13.468	584.50 ± 60.713	11.50 ± 0.428
B	SVP	300	1184.4 ± 275.34**	1475.1 ± 160.58**	3.166 ± 1.327**
C	KAH <sub>1</sub>	50	502.80 ± 19.873**	905.30 ± 68.250*	8.166 ± 0.477*
D	KAH <sub>1</sub>	250	661.45 ± 20.083**	931.11 ± 20.028**	8.430 ± 0.856*
E	SVP+KAH <sub>1</sub>	300 + 50	1120.6 ± 214.86**	1300.3 ± 170.89**	2.833 ± 0.909**

Values are represented as Mean ± SEM. Number of animals: 6; PTZ: Pentylenetetrazole; SVP: Sodium Valproate; KAH<sub>1</sub>: Khamira Marwarid. Animals not showing seizures in 30 minutes were assigned a latency of 1800 seconds. Dose of PTZ: 65 mg/kg s.c. PTZ given on 5<sup>th</sup> day of treatment. The vehicle, standard drug and test drugs were given by oral route of administration. Treatment duration: 5 days. \**p* < 0.05 and \*\**p* < 0.01 versus Group A. Significant by Mann-Whitney test (latencies) and one-way ANOVA followed by Dunnett's *t* test (frequency of jerks).

chemoshock caused by PTZ (65 mg/kg, s.c.). Two doses of Khamira Marwarid, KAH<sub>1</sub> (50 mg/kg and 250 mg/kg) were used. All observations were made 90 minutes after sodium valproate and 60 minutes Khamira Marwarid (KAH<sub>1</sub>) treatment. All drugs were given in a volume of 10 mL/kg. Control animals received the appropriate vehicle. The treatment schedule is given in Table 1. There were 6 groups, each having 6 mice. The animals were treated as per the given schedule.

#### Pentylenetetrazole-induced seizures

Pilot experiments were carried out to ascertain the dose of PTZ that produced convulsions in 100% of animals without mortality. This was found to be 65 mg/kg, s.c. The animals were observed immediately after PTZ injection for a period of 30 minutes. The assessment was done following the method of Osonoe et al [11]. The latency to jerks, myoclonus and clonic generalized seizures with the loss of righting reflex was observed. In the absence of seizures within 30 minutes, the latency was taken as 1800 seconds.

#### Assessment of cognitive function

##### Spontaneous alternation behavior (SAB) on a cross maze

The method described by Ragozzino et al. was followed [12]. A wooden cross maze was used. Mice were placed individually on the central platform of the maze and were allowed to traverse the maze freely. The number and sequence of entries was noted during an observation period of 6 min. An alternation was defined as entry into four different arms on overlapping quintuple sets. Five consecutive arm choices within the total set of arm choices made up a quintuple set. A quintuple set consisting of arm choices B, A, C, B, D comprised an alternation while the set with B, A, D, B, A did not.

Percent alternation was calculated as follows:

$$\text{Alternation (\%)} = \left( \frac{\text{Actual number of alternations}}{\text{Possible number of alternations}} \right) \times 100$$

Where, possible alternations = number of arm entries minus 4.

Memory was assessed on the cross maze before (1<sup>st</sup> day) and after (5<sup>th</sup> day) of the drug treatment

#### Assessment of oxidative stress

At the end of the drug treatment schedule, the animals were killed under deep ether anaesthesia. Whole brain was removed and 10% tissue homogenates were prepared by separately homogenizing sufficient amounts of brain tissues in 0.15 M solution of potassium chloride (KCl). Homogenate was separated and used to determine protein content, malondialdehyde and glutathione. Protein content was estimated the method as described by Lowry and co-workers [13]. Malondialdehyde, an indicator of lipid peroxidation was estimated as described by Ohkawa and co-workers [14] and glutathione was assessed by the method as described by Ellman [15].

#### Statistical analysis

The data were expressed as mean ± standard error of mean (SEM). The results were analysed by a one-way analysis of variance (ANOVA) followed by a Dunnett's *t* test or Mann-Whitney test, wherever appropriate.

## RESULTS

#### Pentylenetetrazole-induced seizures

SVP (300 mg/kg p.o.) pretreatment for 5 days significantly increased the latency for the onset of jerks and myoclonus & clonic generalised seizures. It also significantly decreased the frequency of jerks. Khamira Marwarid, KAH<sub>1</sub> (50 and 250 mg/kg p.o.) significantly increased the latency for the onset of jerks and myoclonus & clonic generalised seizures. There was also a significant decrease in the frequency of jerks with

**Table 3.** Effect of Sodium valproate (SVP), Khamira Marwarid (KAH<sub>1</sub>) and their combination in the presence of PTZ on Spontaneous Alternation Behavior in mice

Group	Treatment	Dose (mg/kg)	% Alternation
A	NS	10 ml/kg	65.236 ± 1.666**
B	PTZ	65	49.296 ± 3.008
C	SVP	300	43.026 ± 1.737
D	KAH <sub>1</sub>	50	60.448 ± 1.437**
E	KAH <sub>1</sub>	250	68.273 ± 2.306**
F	SVP + KAH <sub>1</sub>	300 + 50	70.566 ± 1.059**

Values are represented as Mean ± SEM. Number of animals: 6; NS: Normal Saline (0.9% NaCl); PTZ: Pentylentetrazole; SVP: Sodium Valproate; KAH<sub>1</sub>: Khamira Marwarid; Dose of PTZ: 65 mg/kg s.c. PTZ given on 5<sup>th</sup> day of treatment except in Group A. Treatment duration: 5 days. The vehicle, standard drug and test drugs were given by oral route of administration. \**p* < 0.05 and \*\**p* < 0.01 versus Group B. Significant by one-way ANOVA followed by Dunnett's t test.

these two doses of Khamira Marwarid. Concurrent administration of Khamira Marwarid, KAH<sub>1</sub> (50 mg/kg p.o.) with SVP (300 mg/kg p.o.) significantly prolonged the latency to jerks and myoclonus & clonic generalised seizures. The frequency of jerks was also significantly decreased with this combination. All the comparisons were made with respect to the PTZ group (Table 2).

#### Cognitive function

##### Spontaneous alternation behavior (SAB) on a cross maze

The concomitant administration of KAH<sub>1</sub> (50 mg/kg p.o.) and SVP (300 mg/kg p.o.) significantly increased the % alternation as compared to the toxic control group. Pre-treatment with KAH<sub>1</sub> (50 and 250 mg/kg p.o.) significantly increased the % alternation. However, % alternation with SVP (300 mg/kg p.o.) was found to be insignificant (Table 3).

##### Assessment of oxidative stress

##### Malondialdehyde estimation

A significant reduction in the whole brain MDA

level by SVP (300 mg/kg p.o.) and Khamira Marwarid, KAH<sub>1</sub> (50 and 250 mg/kg p.o.) was observed. The combination of SVP (300 mg/kg p.o.) with Khamira Marwarid, KAH<sub>1</sub> (50 mg/kg p.o.) also reduced the MDA levels significantly (Table 4).

##### Glutathione estimation

A significant change in brain GSH level was observed. A significant increase in brain GSH level by SVP (300 mg/kg p.o.) and Khamira Marwarid KAH<sub>1</sub> (50 and 250 mg/kg p.o.) was observed. The combination of SVP (300 mg/kg p.o.) with Khamira Marwarid, KAH<sub>1</sub> (50 mg/kg p.o.) significantly increased the GSH levels (Table 5).

## DISCUSSION

Epilepsy continues to be a neurological disorder awaiting safer drugs with improved anticonvulsant and anti-epileptogenic effectiveness. Drug treatment of epilepsy has made remarkable strides, with the

**Table 4.** Effect of Sodium valproate (SVP), Khamira Marwarid (KAH<sub>1</sub>) and their combination in PTZ induced changes of brain MDA levels in mice

Group	Treatment	Dose (mg/kg)	MDA (nmoles/mg protein)
A	NS	10 ml/kg	0.706 ± 0.023**
B	PTZ	65	0.998 ± 0.074
C	SVP	300	0.716 ± 0.041**
D	KAH <sub>1</sub>	50	0.823 ± 0.041*
E	KAH <sub>1</sub>	250	0.678 ± 0.030**
F	SVP+KAH <sub>1</sub>	300 + 50	0.618 ± 0.021**

Values are represented as Mean ± SEM. Number of animals: 6; NS: Normal Saline (0.9% NaCl); PTZ: Pentylentetrazole; SVP: Sodium Valproate; KAH<sub>1</sub>: Khamira Marwarid; MDA: Malondialdehyde; Dose of PTZ: 65 mg/kg s.c. PTZ given on 5<sup>th</sup> day of treatment except in Group A. Treatment duration: 5 days. The vehicle, standard drug and test drugs were given by oral route of administration. \**p* < 0.05 and \*\**p* < 0.01 versus Group B. Significant by one-way ANOVA followed by Dunnett's t test.

**Table 5.** Effect of Sodium valproate (SVP), Khamira Marwarid (KAH<sub>1</sub>) and their combination in PTZ induced changes of brain GSH levels in mice

Group	Treatment	Dose (mg/kg)	GSH (µg/mg protein)
A	NS	10 ml/kg	0.408 ± 0.034**
B	PTZ	65	0.125 ± 0.024
C	SVP	300	0.298 ± 0.037*
D	KAH <sub>1</sub>	50	0.301 ± 0.022*
E	KAH <sub>1</sub>	250	0.378 ± 0.030**
F	SVP + KAH <sub>1</sub>	300 + 50	0.395 ± 0.030**

Values are represented as Mean ± SEM. Number of animals: 6; NS: Normal Saline (0.9% NaCl); PTZ: Pentylentetrazole; SVP: Sodium Valproate; KAH<sub>1</sub>: Khamira Marwarid; GSH: Glutathione; Dose of PTZ: 65 mg/kg s.c. PTZ given on 5<sup>th</sup> day of treatment except in Group A. Treatment duration: 5 days. The vehicle, standard drug and test drugs were given by oral route of administration. \**p* < 0.05 and \*\**p* < 0.01 versus Group B. Significant by one- way ANOVA followed by Dunnett's t test.

introduction of many new antiepileptic drugs since 1980s. Improvement in terms of clinical outcomes however, has fallen short of expectations, with up to one third of patients continuing to experience seizures or unacceptable medi

cation-related side effects in spite of efforts to identify optimal treatment regimens with one or more drugs.

Epilepsy is associated with the alternation in psychological, emotional and educational parameters. More than half of the epileptics had some sort of cognitive problems with abnormal behavioural manifestations [16]. These abnormalities are related to multiple factors including seizure type, age of onset, location of the focus, seizure frequency and the type of EEG pattern [17]. Another factor that affects cognition is antiepileptic drug therapy. Although, it is understood that the beneficial results of seizure suppression are of great clinical importance, there are indications of cognitive side effects of the drugs, administered at therapeutic doses, especially with polytherapy. Thus, there is a need for drugs which can suppress epileptogenesis and contain cognitive improving property.

Many laboratory models simulate human epilepsy as well as provide a system for studying epileptogenesis [18]. In the present study, we used the pentylentetrazole (PTZ) model, as it is the most widely employed technique for studying seizure mechanisms and considered to be a useful experimental model for human epilepsy [19]. Sodium valproate (SVP) was used in the present study since it is broad spectrum, first line drug used in the management of diverse seizure types [20,21]. It has been categorized as a drug with a narrow margin of safety and with well reported adverse effects on memory. Therefore, there is need for a drug combination which could bring supra-additive beneficial effects and infra-additive toxicity.

Khamira Marwarid, a formulation based on Indian system of Unani medicine, is extensively used as neuroprotective, memory enhancer, immunomodulator and antistress agent [9,10]. Our study made an attempt

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to evaluate its effect on memory, seizures and oxidative stress in the PTZ model with anticipation that it can be used as an adjunct with AEDs. The appearance of jerks and myoclonus & clonic seizures in mice after PTZ administration indicate that PTZ produced the above effects by multiple mechanisms of action on the central nervous system. PTZ is reported to inhibit hyperpolarisation process at post synaptic sites [22,23]. The ability of sodium valproate (SVP) to produce an increase in latency time to jerks and myoclonus & clonic generalized seizures might be due to its multiple mechanisms of anticonvulsant action [20,21]. The increase in latency time and decrease in frequency of jerks caused by Khamira Marwarid (KAH<sub>1</sub>) might be due to its neuroprotective effects.

The concurrent administration of sodium valproate (SVP) with lower dose of Khamira Marwarid (KAH<sub>1</sub>) also increases the latency time and decrease the frequency of jerks suggesting the possible additive effects of the test drugs with sodium valproate. The increase in the levels of brain MDA and reduction in the levels of GSH following PTZ-induced seizures reveal that PTZ produced oxidative stress in the brain. The reduction in the levels of MDA and the increase in the levels of GSH caused by the test drugs suggest that these drugs have antioxidant property. Becker et al. reported that PTZ caused learning impairment in rats. The change in SAB caused by PTZ might be due to its neurotoxic effect [24]. The improvement in SAB caused by Khamira Marwarid (KAH<sub>1</sub>) might be due to its neuroprotective effects. However, this needs further investigations in different models. Khamira Marwarid (KAH<sub>1</sub>) is known to have neuroprotective and memory enhancing properties as per unani system of medicine [9,10]. The present study substantiated the neuroprotective and memory enhancing action of Khamira Marwarid.

In conclusion, the present study demonstrates that Khamira Marwarid significantly prevented the cognitive impairment and attenuated the oxidative stress induced

by the PTZ model of epilepsy. Therefore, it could be useful support to the basic antiepileptic therapy in preventing the development of cognitive impairment reported with several AEDs.

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