Evaluation of Anticonvulsant Activity of Aqer Qerha (*Anacyclus pyrethrum* DC.) Root in Experimental Animals

[1]*M.A. Kalam,
[2]Md. Shafat Karim,
[3]Ghulamuddin Sofi and
[4]Ghufran Ahmad

[1]Regional Research Institute of Unani Medicine, 79 Chittaranjan Avenue, Kolkata-700073

[2]Department of Forensic Medicine & Toxicology, Govt. Tibbi College, Patna-80003

[3]National Institute of Unani Medicine, Kottigapalya, Magadi Main Road, Bangalore-560091

[4]Department of Ilmul Advia, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh-202002 (U.P.)

Abstract

The present study was carried out to evaluate the anticonvulsant activity of hydro alcoholic extract of Aqer Qerha (*Anacyclus pyrethrum* DC.) root and validate its use as antiepileptic drug as claimed in Unani system of medicine.

Two experimental models of epilepsy viz. PTZ induced seizure test and maximal electroshock seizure test (MES) were used in the study. The rats of wistar strain were divided into four groups of six animals each. Group I served as plain control and was given distilled water 2 ml/kg orally; Group II was given diazepam 5 mg/kg i.p. and served as standard control; Animals in Group III and IV were treated with hydro alcoholic extract of Aqer Qerha in the dose of 65 mg/kg and 130 mg/kg, respectively. The hind leg extension in MES test and onset of the first seizure, clonic and tonic seizure, total number of convulsions and duration of tonic and clonic convulsion were assessed in PTZ induced seizure test. The parameters were analyzed and compared statistically for different groups.

It was found that AQ significantly ($p<0.05$) reduced tonic hind leg extensor stage in MES induced epilepsy. In PTZ induced seizures, it delayed the onset of the first seizure, clonic and tonic seizure; and decreased the total number of convulsions and duration of tonic and clonic convulsion significantly ($p<0.05$). The drug at higher dose protected all the animals from death, while the percentage of protection from death at lower dose was 33%.

The study demonstrated that the test drug possesses significant anticonvulsant activity against both PTZ and maximal electroshock induced seizures. The study validated the claim of Unani physicians of using Aqer Qerha root in epileptic patients.

Keywords: Unani Medicine, PTZ, Aqer Qarha, Epilepsy, Seizure, *Anacyclus pyrethrum* DC, Antiepileptic drug.

Introduction

The most common serious disorder of the brain in the world according to the World Health Organization is Epilepsy (Anonymous, 2005). It be falls in all parts of the world and in every country; approximately 50 million people are affected by epilepsy globally (Reddy et al., 2005). In almost all parts of the word and in every civilization attempts have been reported to be made to cure epilepsy with plant drugs and other natural products. The review of Greco Arab, Indian and Chinese medicine etc testify the fact. Epilepsy is synonymous with *Sara* or *Mirgi*, described in almost all classical books of Unani medicine (Majusi, 1889; Razi,

[1]*Author for correspondence

1997). There are several plant preparations used by Unani physicians that are taken orally to control the seizures. Although herbs and their preparations are used since hundreds of years to control epilepsy by the physicians of Unani system of medicine, but evidence from the experimental and clinical models to determine the efficacy and safety of the described drugs is lacking. Many attempts have been made in the past to obtain anticonvulsant agents from plant drugs, and these efforts will continue till a satisfactory treatment is available (Sonavane *et al*., 2005). A number of drugs are available in allopathic medicine to treat the epileptic patients. These drugs have been reported to produce significant therapeutic effect, but various side effects consistent with these agents often render treatment difficult; so the demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes (White *et al.,* 1995). Classical Unani literature and some of the recent reports suggested that the roots of Aqer Qerha (*Anacyclus pyrethrum* DC.) from the Astraceae family possess antiepileptic properties and Unani physicians are using this drug to treat the epileptic patients since scores of years (Kalam *et al.,* 2015). Therefore present study was designed to study the anticonvulsant activity of hydro alcoholic extract of Aqer Qerha root in acute seizures induced by intraperitoneal administration of PTZ and maximal electroshock by electroconvulsive meter.

Material and Methods

Collection and identification of plant materials

AQ (the root of *Anacyclus pyrethrum* D C) was procured from local market of Bangalore. The drug was identified by the Regional Research Institute (RRI) of Ayurveda, Bangalore under reference No.-SMPU/NADRI/BNG/Drug, Authentication /2009-10/942. The specimen of the plant material studied was retained in the RRI for reference purpose. A similar voucher specimen of has also been submitted to the Dept of Ilmul Advia, NIUM, Bangalore for records and future reference.

Preparation of extract

The drug was pulverized in electric grinder in the form of coarse powder. The hydro alcoholic extract of the test drug was prepared with the help of Soxhlet apparatus. For this purpose 100 gm of powdered drug was extracted in 1:1 ethanol and distilled water (200 ml each) at the temperature of 70-80 ^{o}C for 7 hours. The liquid extract was cooled and filtered by Whatman filter paper 44. The filtrate was placed on a water bath until the entire solvent evaporated. The

extract was weighed and the yield percentage was calculated with reference to the crude drug. The yield percentage of the extract was found to be 16 % w/w.

Dose of the drugs and chemicals

The dose of AQ extract for rats was calculated by the method of Freirich *et al*. (1966) and was found to be 65 mg/kg. A second dose (double dose/130 mg/kg) was also taken for the study to assess the dose dependent effect. The test drug was administered by oral route with the help of gastric canula given in the form of suspension freshly prepared at the time of administration to the animals.

Pentylenetetrazole (60 mg/kg) and diazepam (5 mg/kg) were purchased from Sigma Nicolas Pharma India Ltd. The drugs were dissolved in water for injection and administered in a volume of 5 ml/kg.

Animals

Wistar rats of either sex aged between 2-3 months and weighing 150-200g, were used for the study. They were procured from Central Animal Research Facility (CARF) of National Institute of Mental Health and Neurosciences (NIMANS), Bangalore, India, and housed in Animal house Facility at NIUM, Bangalore. They were acclimatized to the laboratory condition for 5 days before experimental studies. Animals were maintained in a standard environmental condition. Food and water were provided *ad libitum*. The Institutional Animal Ethics Committee (IAEC), NIUM, Bangalore, approved the experimental protocol vide Reg. No 953/C/06/CPCSEA.

Methodology

Pentylenetetrazole (PTZ) clonic seizure test

The test was carried out by the method of Gupta (1997). Wistar rats were divided into four groups of six animals each and treated as follows:

Group I (control group): Distilled water, orally

Group II (standard control): Diazepam in a dose of 5 mg/kg i.p (Dharmesh *et al.,* 2010) Group III (test group A): Hydro alcoholic extract of AQ (65 mg/kg/1day)

Group IV (test group B): Hydro alcoholic extract of AQ (130 mg/kg/ 1 day)

Acute seizures were induced by intraperitoneal administration of PTZ. After 60 minutes of the treatment with the test drugs and 30 minutes of the standard drug, rats were injected PTZ (60 mg/kg i.p. after dissolving in normal saline). Total number of convulsion, onset of first seizure; onset of clonic convulsions, duration

of clonic convulsion, onset of tonic convulsion, duration of tonic convulsion, number of death within 30 min duration, and % age protection from death was also calculated. Just after administration of PTZ, animals were placed individually in cages and assessed for above mentioned parameters. ANOVA one way with Tuckey Kramer pair comparison test was used to analyse the data. Statistical differences was considered significant at $p<0.05$.

Maximal Electroshocks seizures test

The test was carried out by the method described by Branco *et al.* (2009). The rats were divided into four groups of six animals each and treated in similar way as in previous test.

After 30 minutes of the administration of standard drug in group II and after 60 minutes of administration of distilled water in group I and test drugs in group III and IV, seizures were induced by applying 150 mA electric shock for 0.2 second using an Electroconvulsometer in animals of all the groups. The animals observed for various components and duration of seizures specially the extensor phase of the seizure. The mean time duration of seizures in the all group were analysed statistically and comparison was made with the mean values of the control and standard groups by ANOVA one way test.

Observations and Results

Effect of AQ extract in PTZ induced seizures

Mean total number of convulsions was found to be 804.33 ± 103.74 in plain control; 686±35.15 in test group A and 247±126.96 in test group B. No convulsion appeared in animals of standard group. Mean total number of convulsion in test A and test B were significantly ($p < 0.01$) reduced when compared with mean score of plain control. Test group B showed significant ($p < 0.01$) reduction in mean number of convulsion when compared with test group A.

Mean onset of first seizure in plain control was 33.83 ± 1.76, while it increased to 57.5±4.83 and 64 ± 3.81 in test group A and B, respectively showing a significant ($p< 0.01$) delayed in the mean onset of first seizure. The effect of the two doses however was not found statistically different.

The mean onset of clonic convulsion was found to be 50.5 ± 5.59 in plain control, 68.16 ± 3.63 in test group A and 81 ± 4, in test group B ($p<0.05$).

The mean duration of clonic seizures in plain control was recorded as 11.5 ± 1.39. It decreased to 9.84 ± 0.95 and 9 ± 0.86 in test group A and respectively ($p< 0.05$).

The mean onset time of tonic convulsion was found to be 109.17 ± 8 sec in plain control which amounted to 350.33 ± 66.14 sec ($p<0.05$) and 795.66 ± 68.62 sec ($p<0.001$), respectively. A significant difference between the findings of group A and B was also recorded ($p< 0.01$).

Mean duration of tonic convulsion of control group was 18.5 ± 1.18 sec, while it was found decreased in test group A (15±1.18 sec) and B (3.33 ± 2.47sec), respectively. Group B showed significant reduction ($p<0.001$) as compared to plain control. A significant difference was also recorded between the two test drugs ($p<0.01$).

Protection from death was also assessed in all the groups. In plain control group all the animals died where as 100% protection was observed in the animals of standard and test group B. In test group A 33.33 % protection was recorded (Table 1 and Figure 2 & 3).

Effect of AQ in Maximal Electroshock Seizure (MES)

The mean duration of tonic hind limb extension in rats of group I treated with distilled water was 8.67 ± 2.20 sec, rats of group II treated with standard drug (diazepam) showed mean duration of 4.83±2.20 sec, whereas rats of group III

Table 1: Effect of AQ on PTZ induced seizures in rats

Groups	**Group I Distilled water**	**Group II Diazepam 5mg/kg i.p.**	**Group III AQ 65 mg/kg**	**Group IV AQ 130 mg/kg**
Parameters				
No. of Convulsions	804.33±103.74	0.00	686±35.5	247±126.96 [a,c]
Onset of 1st seizure (s)	33.83±1.76	0.00	57.5±4.83 [b]	64±3.80 [a]
Onset of Clonic convulsion (s)	50.5±5.58	0.00	68.17±3.63 [b]	81±3.99 [a]
Duration of Clonic convulsion (s)	11.5±1.38	0.00	9.83±0.95	9±0.86
Onset of tonic convulsion (s)	109.17±8.00	0.00	350.33±66.14	795.66±68.62 [a, c]
Duration of tonic convulsion (s)	18.5±1.18	0.00	15±1.18	3.33±2.5 [a, c]
Number of death	6	0	4	0
% of protection	0	100	33.33%	100

N=6

a– $p<0.01$ with respect to plain control, b –$p<0.05$ with respect to test A, c – $p<0.01$ with respect to test B.

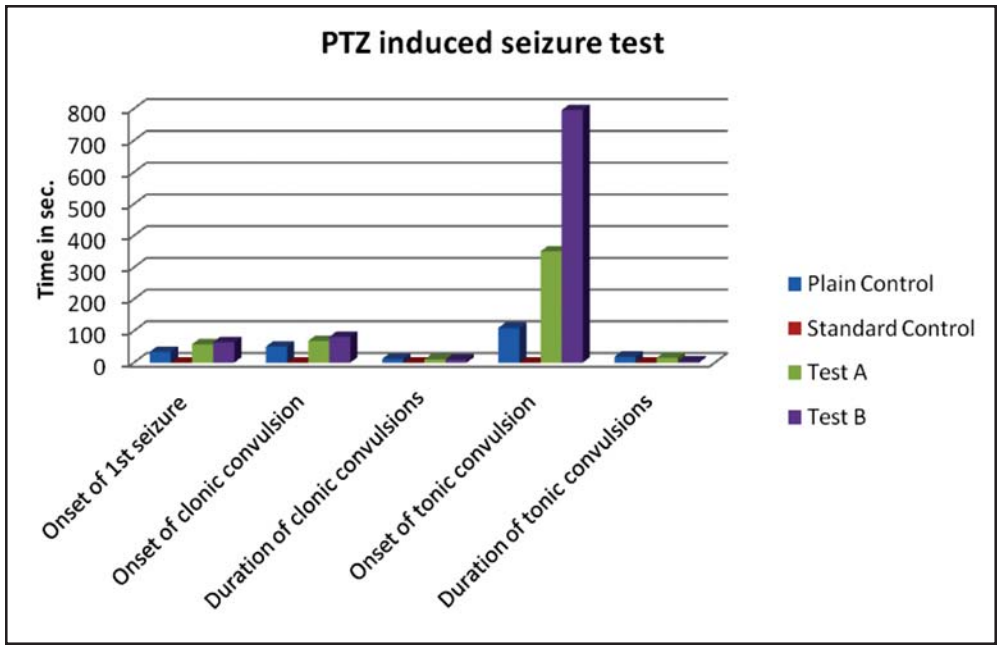

Figure 1: Effect of hydro alcoholic extract of AQ on PTZ induced seizures in rats

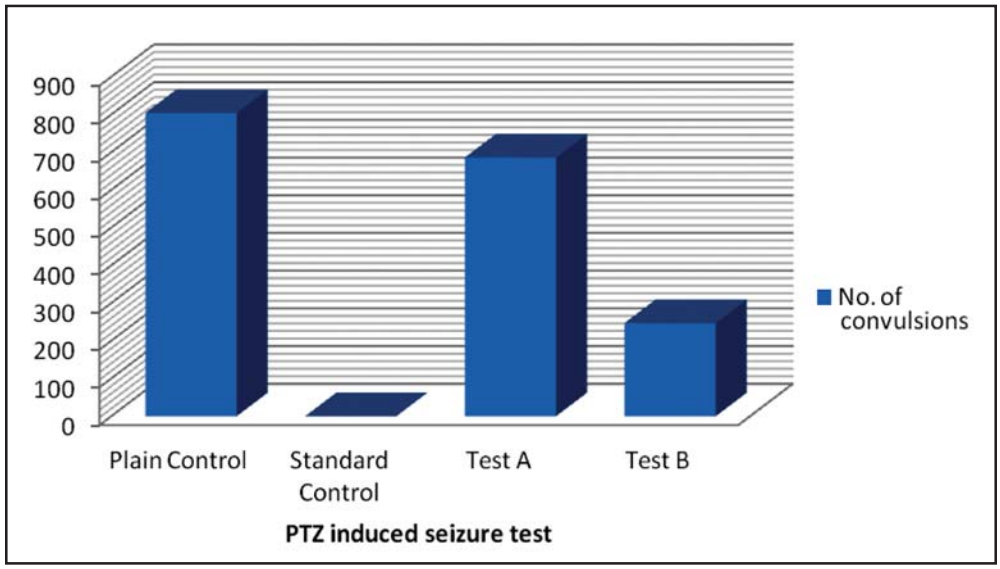

Figure 2: Effect AQ on no. of convulsion in PTZ induced seizure test in rats

and group IV treated with the AQ (65, and 130 mg/kg) exhibited hind leg extension of 5.5±.72 and 4.83±2.20 sec, respectively. When the mean hind leg extension of each group was compared with plain control group, it was found that the standard group and test group A and B significantly reduced the duration of the hind leg extension. The results are summarised in table 2 and figure 3.

Table 2: Effect of hydro alcoholic extract AQ on MES induced seizures in rats

Groups	Duration of hind leg extension (S) Mean ±SEM
Group I (Plain Control) Distilled water (2ml) p.o.	8.67 ± 2.20
Group II (Standard control) Diazepam 5mg /kg i.p.	3.67 ± .34*
Group III (Test A) HEAQ 65mg/kg p.o	5.5 ± .72*
Group IV (Test B) HEAQ 130mg/kg p.o	4.83 ± 1.20*

N=6

a – p<0.01 with respect to plain control, b –p<0.05 with respect to test A, c – p<0.01 with respect to test B.

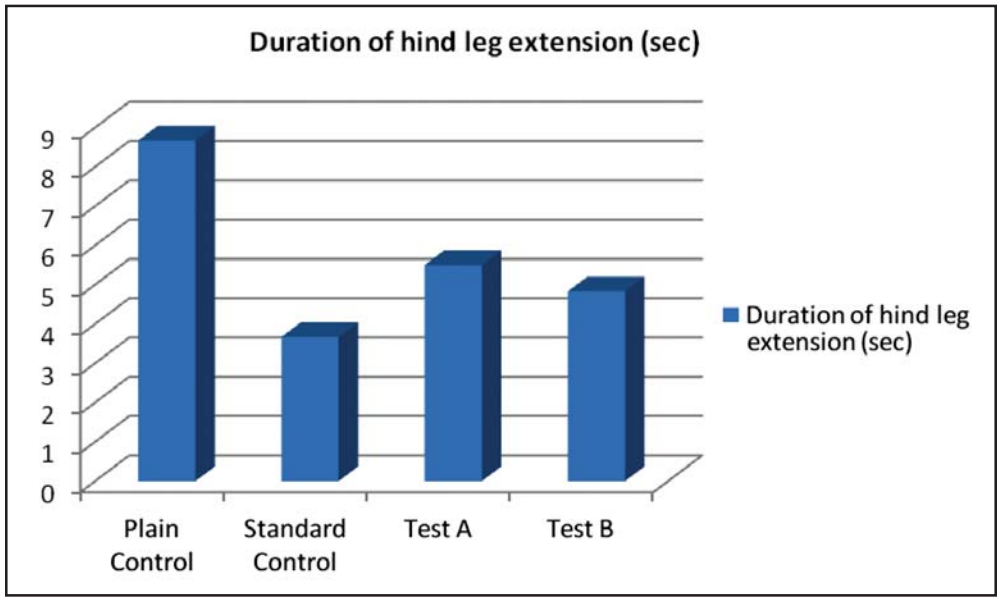

Figure 3: Effect of hydro alcoholic extract of AQ on MES induced seizures in rats

Discussion

PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures" (Oliveira *et al.*, 2001), and also a valid model for human generalized myoclonic seizures and generalized seizures of petit mal type (Loscher *et al.*, 1988). The MES test is the most frequently used animal

model for identification of anticonvulsant activity of drugs for the generalized ("grand mal") tonic-clonic seizures (Oliveira *et al.*, 2001, Loscher *et al.*, 1988). This model based on observation of the stimulation by repeated electrical pulses induced in different neuronal structures is one of the characteristic standards of epileptic activity (Quintans et al., 2002). In Pentylenetetrazole (PTZ) induced seizure test parameters like onset of first seizure, onset of tonic convulsions, clonic convulsions and duration of tonic and clonic seizures, percent protection were observed. Test group A and test group B significantly ($p< 0.01$) increased the mean onset of first seizure when compared with plain control, but no statistical difference was found between the effects produced by the two test drugs. Bothe the test groups showed significant ($p<0.05$) increase in mean onset of clonic convulsion, in comparison to plain control. In inter group comparison no difference was found between the two groups. In the mean onset time of tonic convulsion, on the comparison of test drugs with the plain control, the test drug A ($p<0.05$) and B ($p<0.001$) both showed significant effect. The test drug B was found to produce better response ($p< 0.01$) as compared to group A. The mean duration of tonic convulsion on the comparison of control with test drugs was found to be decreased only by the test drug B ($p<0.001$). Protection from death was also assessed in all the groups. In plain control group all the animals died where as 100% protection was observed in the animals of standard and test groups B. In test group A there was 33.33 % protection from death. Thus, onset of first seizure, clonic and tonic seizure in the test groups was significantly increased ($p<0.01$); the findings were comparable with that of the standard drug. The duration of tonic and clonic seizure was decreased in both the test groups and the reduction was significant when compared with the plain control group. The observations showed strong antiepileptic effect produced by the low and high doses (test drug A and B). However, test drug B was more effective than drug A.

The clinical aspect of certain generalized seizures especially absence seizures are highly correlated with experimental seizures produced in animals by the administration of PTZ (Anthony and Walter, 1998). It is proposed that PTZ induces convulsion either by inhibiting gamma amino butyric acid (GABA) pathway in CNS (Corda *et al.*, 1990) or by increasing the central noradrenergic activity (De Potter, 1980). The effect of the extract in this model, therefore suggests its involvement in GABA-ergic or noradrenergic pathways and its efficacy against generalized tonic clonic and partial seizures in rat.

Since PTZ is reported to induce convulsion by antagonizing the ã-aminobutyric acid (GABAA) receptor chloride Cl-channel complex to attenuate GABA-depending inhibition therefore the drugs protecting against tonic clonic seizures induced by PTZ are considered useful in controlling myoclonic and absence seizure in humans. Thus, demonstration of activity in this model suggested that

the test drug possesses anticonvulsant activity, which may underline its traditional use in the treatment of epilepsy in Unani system of Medicine. An earlier study on antiepileptic effect of the butanolic extract of *Anacyclus pyrethrum* (Fakir *et al.*, 2010) also strengthens its effect as anticonvulsant agent. It was found that treatment with AQ on PTZ induced epileptic rats significantly reduced the duration of convulsion and delayed the onset of clonic and tonic convulsion and also reduced the total number of convulsion, and protected treated rats from mortality. One generally accepted mechanism by which pentylenetetrazole exerts its action is by acting as an antagonist at the picrotoxin sensitive site of the GABAA receptor complex (Ramanjaneyulu *et al.*, 1984). Since PTZ has been shown to interact with the GABA neurotransmission and PTZ induced seizures can be prevented by drugs that enhance gamma amino butyric acid type A (GABAA) receptor mediated inhibitory neurotransmission such as benzodiazepines and Phenobarbital (Coulter *et al.*, 1989; Macdonald *et al.*, 1995) therefore the antagonism of PTZ- induced seizures suggests the interaction of the AQ with the GABA-ergic neurotransmission corresponding to generalized tonic-clonic seizures in humans (Kupferberg, 1989; Stables, 1995). Pentylenetetrazole is a selective blocker of the chloride ionophore complex to the GABAA receptor, and after repeated or single dose administration leads to a decrease in GABA-ergic function and to the stimulation and modification of density or sensitivity of different glutamate receptor subtype in many brain regions. It may also trigger a variety of biochemical processes including the activation of the membrane phospholipase, proteases and nucleases. Alteration in membrane phospholipids metabolism causes liberation of free fatty acids, diacylglycerols, eicosanoids, lipid peroxidase and free radicals. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure (Ihan *et al.*, 2006). It is likely that AQ may contain constituents that may be involved in this action. The screening for such constituents needs to be carried out as these compounds may be able to modulate the function of GABA or glutamate receptors.

In maximal electroshock induce seizure test, the mean hind leg extension of each group was compared with plain control group, it was found that standard and two doses of the test drug significantly reduced the duration of the hind leg extension. When the test drugs A and B were compared with standard control group, no significant difference in mean score was observed suggesting that the test drugs are as effective as the standard drug. The convulsion in MES method is due to the disturbed activity of GABA in the brain. Electroshock causes the inhibition of GABA release, and this in turn inhibits GABA synthesis (Sermet *et al.,* 1998). It is therefore, possible that AQ may have increase the release of GABA --(Jobe *et al.*, 1974). It is also possible that AQ may have some connection in the cascade of events in neurohumoral transmission.

It can be concluded that the administration of hydro alcoholic extract of *Anacyclus pyrethrum* root shows promising anti seizure activity in PTZ induced seizures and maximal electroshock induced seizure tests. The study thus validated the claim of Unani physicians of using Aqer Qerha root in epileptic patients. However, further studies are needed to evaluate the exact chemical ingredients and their mechanism of action responsible for antiepileptic effect, as several anticonvulsant drugs in current clinical use facilitate GABA neurotransmission by different mechanisms.

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