

Anticonvulsant Effect of the Hydroalcoholic Extract of *Ajwa dates* (*Phoenix dactylifera*) in Animal Model

Hafsa Hashmi¹, Dr. Muzzafar Abbas¹, Dr G.A Miana¹, Dr Fahim Afridi¹

Department of Pharmacology, Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan. 1

Abstract— Epilepsy is one of the most common serious life threatening neurological disorder. The current modern antiepileptic drugs are associated with side effects, dependency, sedation, chronic toxicity, and teratogenic effects and in approximately 30% of the patients is ineffective. Ajwa dates are used as many traditional/classical medicines for the treatment of different diseases including convulsions and seizures. Ajwa dates use as an antioxidant, antiinflammatory, hepatoprotective, nephroprotective, hypolipidemic and cardioprotective. The current study evaluates the impact of anticonvulsant effect of Hydroalcoholic extract of ajwa dates in animal model and has anticonvulsant properties similar to commercially available drugs. In this study, anticonvulsant effects of hydroalcoholic extract of Ajwa date fruit were examined by using pentylentetrazole (PTZ) model in mice. Thirty minutes later to saline (10ml/kg) Ajwa date extract (200, 400 mg/kg) and diazepam (1mg/kg) treatment, an i.p. dose of PTZ (90 mg/Kg) were given to all animals and each animal were observed for onset time of myoclonic /seizures jerks and tonic-clonic seizures/jerks, as well as duration of tonic-clonic seizures for 30 min via digital video camera. The animals were also observed for mortality (% mortality = number of mice dead after convulsion/total number of mice used × 100). Ajwa date's extract at the doses of 200, 400 mg/kg prolonged the time of onset of seizure and decreased the duration of seizure compared to control (saline) group. It was proved that Ajwa dates have anticonvulsant effect and can be useful for the control and treatment of seizures and in these effects, opioid receptors/ benzodiazepine receptors might probably be involved. More studies are needed in order to investigate its exact mechanism. Hydro-alcoholic extract of Ajwa date has *in vivo* anticonvulsant activity in the pentylentetrazole induced seizures and its anticonvulsant activity is comparable with the Diazepam. More studies are needed to investigate its exact mechanism and to evaluate the efficacy of hydro-alcoholic extract of Ajwa date in other seizures. Clinical trials to be conducted in human beings to evaluate its anticonvulsant activity.

Index Terms— Ajwa dates, Seizure, Hydroalcoholic extract, pentylentetrazole, Diazepam, Male BALB/c mice, Normal saline



1. INTRODUCTION

Among many neurological disorders, epilepsy is the most serious and life threatening disorder. [1] Seizures developed due to the rapid excitation and periodic firing of brain neurotransmitter/cerebral neurons. [1] voltage gated sodium channels control the initiation of normal and seizure types action potential. [2] All age groups, genders and social groups are affected by epilepsy. roughly 50 million population have been diagnosed with epilepsy worldwide. [2] The overall patient's quality of life is predominantly affected by epilepsy. [3] Many drugs are available presently to inhibit the effect of epileptic seizures, but neither effective prophylaxis nor cure is available. [3] Recent clinical study shows that none of the newly introduced 3rd generation antiepileptic drugs exhibit refined efficacy over the traditional antiepileptic drugs such as carbamazepine and valproate. [4] Diverse group of anti-epileptic drugs are prescribed by the practitioners but these drugs have wide range of adverse effects including liver toxicity, ischemia, depression, cognitive disabilities and motor impairment. [9] Furthermore, many antiepileptic drugs are narrow therapeutic window drugs. [7] In spite of diverse discovery of current antiepileptic drugs into clinical practice, only 20-30% patients are managed by the available pharmacological therapy. [1] Due to this, there is a requirement of such advanced discovery of anti-epileptic drugs which have an up grade/effective efficacy and appropriate safety profile. [4] Herbal medicines are playing significant/foremost role in the invention of several new drugs including antiepileptic medicines. [8] Date fruits (Phoenix dactyifera L) widely cultivated as an economical and food crop in the middle east, Pakistan, India and many other arid regions of the world. There are different kinds of dates, among them Ajwa date is most popular for its significant therapeutic properties. It is cultivated specifically in the holy city of Al Madina Al-Munawwrah, KSA. [5] They are wonderful source of proteins, vitamins and energy. They also provide many useful and essential minerals, fats, high dietary fibers and sugars. They comprised numerous chemical substances like glycosides, flavonoids, sterols and polyphenols. [6] Ajwa dates use as an anti-oxidant, anti-inflammatory, hepatoprotective, nephroprotective, hypolipidemic and cardioprotective. [7] Moreover, Ajwa dates are very beneficial for nursing mothers, as they are helpful in enriching the breast

milk. Different studies showed that females who eat Ajwa dates on daily basis, their children are least sensitive to infections and diseases. They are rich source of iron, so are helpful in the treatment and prevention of anemia. [12] The current study evaluates the impact of anticonvulsant effect of Hydroalcoholic extract of Ajwa dates in animal model.

- Hafsa Hashmi has received degree of Doctor of pharmacy from Riphah Institute of Pharmaceutical Sciences Islamabad, Pakistan in 2017, Further interested in MPhil in clinical practice/pharmaceutics.
- E-mail: hafsa.hashmi22@gmail.com

2. PROCEDURE

2.1. Animal

Male BALB/c mice (30–47 g) were obtained from the NIH (National Institute of Health). The animals were individually placed in animal house with 12/12 h light/dark cycle at $21 \pm 2^\circ\text{C}$ and had free access to food and water.

2.2. PLANT MATERIAL

Ajwa dates used in the present study were fresh, ripe, medium sized, fleshy, soft, and have basal white lines on the black exocarp. Briefly, the edible part of date fruit was manually separated, and soaked in hydro-alcoholic solution i.e. 99.9% ethanol and purified water for 3 to 4 days. The mixture was then filtered and evaporated with the help of rotary evaporator to get the extract.

2.3. Chemicals

Drugs used in the study were as follows:
Pentylentetrazole (induces convulsion)
Normal saline (Control drug)
Diazepam injection (Standard drug)
All chemicals were dissolved in normal saline and prepared freshly each time and administered intraperitoneally.

3. Anticonvulsant Assay (PTZ-Induced Seizure)

3.1. Methodology

Animals were randomly divided into five groups of five each. It consisted of one control group, one standard group and two test groups.

- [1] Control group (C): Normal saline (10 mL/Kg)
- [2] Test 1 (T₁): Ajwa date's extract (200 mg/kg)
- [3] Test 2 (T₂): Ajwa date's extract (400 mg/kg)

[4] Standard group (S): Diazepam (1 mg/Kg)

The animals were injected (i.p) with control drug, Ajwa date's extract (test groups) and standard drug. Thirty minutes later, an i.p. dose of Pentylentetrazole (PTZ) (90 mg/Kg) was given to all animals and each animal was observed for 30 minutes for the occurrence of the seizure and timing was maintained using the digital clock.

The following parameters were studied

- Time of onset of the myoclonic jerks
- Time of onset of the tonic-clonic seizures (seizure latency)
- Duration of clonic phase of seizure for 30 minutes via digital video camera

At the end of the thirty minutes the animals were inspected for any injury or residual damage. The animals were also observed for mortality (% mortality = number of mice dead after convulsion/total number of mice used x 100).

3.2. Statistical Analysis

Statistical analyses were analyzed through one-way analysis of variance (ANOVA) using the statistical Package for Social Sciences followed by Post-hoc Turkey-Kramer's test. The p value <0.05 was considered as the level of significance. Data are presented as a line diagram for Seizure latency presentation and bar diagram was constructed using the Graph Pad Prism 5. All the values were expressed as mean \pm SEM (standard error of the mean). Asterisk (*) indicates the statistical significance, with the level significance for comparison.

4. Result

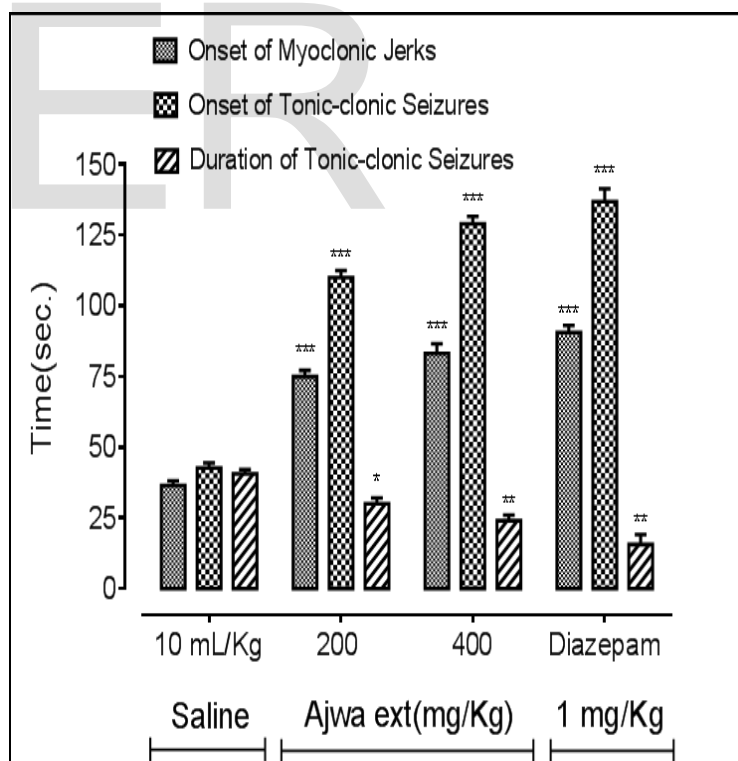
Test groups, that significant delayed the onset of myoclonic jerks, tonic-clonic seizure and shorten the duration of tonic-clonic seizure were considered to exhibit anticonvulsant activity.

Table 1: Time of onset of the myoclonic, tonic-clonic seizure and Duration of clonic phase of seizure (seizure latency).

| S. N o. | Group | Seizure Latency in Seconds (Mean \pm SEM) | | |
|---------|---------------------------------------|---|---|-------------------------------------|
| | | Time of onset of the Myoclonic Seizure | Time of onset of the Tonic-Clonic Seizure | Duration of Clonic Phase of Seizure |
| 1 | Normal saline (C) | 36.45 \pm 1.7 | 42.67 \pm 1.8 | 40.60 \pm 1.5 |
| 2 | Ajwa date's extract (T ₁) | 75.00 \pm 2.1 | 110.00 \pm 2.5 | 30.00 \pm 2.1 |
| 3 | Ajwa date's extract (T ₂) | 83.20 \pm 3.4 | 129.00 \pm 2.7 | 24.00 \pm 2.0 |
| 4 | Diazepam (S) | 90.60 \pm 2.5 | 136.80 \pm 4.6 | 15.60 \pm 3.6 |

5. Figures

Figure 1: Time of onset of the myoclonic, tonic-clonic seizure and Duration of clonic phase of seizure (seizure latency) by control group, standard group and two test groups (Ajwa date's extract at two doses)



6. DISCUSSION

Seizures were seen in all the groups. There was no statistically significant difference between the mean duration of clonic phase of the seizure, onset of myoclonic and tonic-clonic seizure of normal saline control group (36.45 ± 1.7 , 42.67 ± 1.8 , 40.60 ± 1.5).

Diazepam (standard drug) prolonged the onset of myoclonic and tonic-clonic seizure (90.60 ± 2.5 , 136.80 ± 4.6 sec respectively) in a statistically significant manner and this standard drug also shorten the duration of tonic-clonic seizure (15.60 ± 3.6) (Table 1, Figure 1).

Test groups (T_1 , T_2) prolonged the time of onset of seizure and decreased the duration of seizure, when compared to control (saline) group and these test groups showed the same effect when compared to standard drug (Diazepam) (Table 1, Figure 1).

Ajwa date's extract at the dose of 200 mg/kg (Test group T_1) prolonged the onset of myoclonic and tonic-clonic seizure (75.00 ± 2.1 , 110.00 ± 2.5 respectively) in a significant manner and decreased the duration of seizure (30.00 ± 2.1) compared to control (saline) group (Table 1, Figure 1).

Ajwa date's extract at the dose of 400 mg/kg (Test group T_2) also delayed the onset of myoclonic and tonic-clonic seizure (83.20 ± 3.4 , 129.00 ± 2.7 respectively) in a significant manner and reduced the duration of seizure (24.00 ± 2.0) compared to control (saline) group (Table 1, Figure 1).

When the two doses of Ajwa date's extract was compared among themselves, the test group T_2 showed better effect than test group T_1 . It was observed that prolongation of the onset of myoclonic and tonic-clonic seizure and reduction of the duration of seizure by Ajwa date's extract at the dose of 400 mg/kg was greater than the dose of 200 mg/kg.

7. CONCLUSION

Hydro-alcoholic extract of Ajwa date has *in vivo* anticonvulsant activity in the PTZ induced seizures and its anticonvulsant activity is comparable with the Diazepam. More studies are needed to investigate its exact mechanism and to evaluate the efficacy of hydro-alcoholic extract of Ajwa date in other seizures. Clinical trials to be conducted in

human beings to evaluate its anticonvulsant activity.

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