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Evaluating the Effect of Injectable Use of Hydroalcoholic Extract of date (Phoenix Dactylifera) on Pentylene Tetrazole Induced Seizure in White Mice

Abstract

Based on traditional medicine, dates have significant neurological and antioxidant properties. The present study was conducted to evaluate the therapeutic effects of hydroalcoholic extract of dates on pentylenetetrazole-induced seizures in Balb / c mice. In this experimental study, pentylenetetrazole (90 mg/kg) was used to induce a seizure. Mice were divided into eight groups. The case groups received the extract at concentrations of 30, 100, and 300 mg/kg, 45 minutes before injection, the control groups were divided into PTZ, PTZ and distilled water, PTZ and phenobarbital, and non-intervention groups. After PTZ injection, the onset, termination, and severity of seizure, as well as mortality of mice in different groups, were recorded and compared by the observer. The levels of sodium, potassium, calcium, phosphorus, and blood sugar in different groups were also assessed. At the end of the study, there was a significant difference between groups 1 and 3 ($p < 0.001$), groups 3 and 4 ($p < 0.01$), and groups 3 and 6 ($p < 0.05$) in terms of seizure onset. The duration of seizures between groups 3 and 4 as well as groups 3 and 5 was also reported to be significant ($p < 0.05$) and ($p < 0.001$). In serum factors, the difference between sodium levels of groups 7 and 8 ($p < 0.05$) and potassium levels of groups 2 and 5 ($p < 0.01$) and 5 and 6 ($p < 0.01$) was also significant. The results of this study showed that the hydroalcoholic extract of dates reduced the severity and duration of seizures in the study groups.

Keywords: *Seizure, Pentylene tetrazole, Dates, Balb / c mice*

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Introduction

Epilepsy is one of the most common neurological diseases in the world and is the second leading cause of neurological diseases after stroke. The number of people with epilepsy in the world is estimated at 40 million (1). Recurrent seizures not only disrupt the normal course of life of the affected person and physical-mental complications but also cause many problems for his or her family. If the seizure is not controlled, the physician will have to add anti-seizure drugs and the multiplicity of anti-seizure drugs' interactions with each other and the accumulation of complications will cause other problems (2).

Also, in some patients, despite taking all anti-seizure drugs alone or in combination and at a tolerable dose for the patient, debilitating seizures continue. In these cases, if a treatable cause is not discovered and degenerative neurological disease is not present, evaluation is considered for possible surgical treatment (3). The date or date palm, scientifically named Phoenix dactylifera, belongs to the genus of Palmaceae. It is a plant with multiple or single stems, sometimes reaching a height of 30 meters. The leaves of the plant are green to gray, hard and brittle, 3 meters or more in length. The fruit of the plant is about 4-7 cm long and 2-3 cm in diameter. This plant grows in the central, southern, and southeastern regions of Iran (4). Each 100 g of date fruit has 248 to 252 calories. Comparing the calories produced by this fruit with other fresh fruits shows its high potential so that it can be compared with very sweet and dry fruits such as currants. The minerals in the meat part of dates mainly include potassium, manganese, iron, calcium, silica, etc., the ratio and amount of which vary depending on different cultivars. Dates contain 25% sucrose, 50% glucose, albumin substances, pectin, water, vitamins A, B, C, and E, and some minerals (5).

Epilepsy is a disease that occurs in all ages, all races, and both genders (6). All anti-seizure drugs cause dyscrasias and sometimes hepatotoxicity (3). Due to the high prevalence, numerous complications, and problems of epilepsy, it seems helpful to find a way to reduce the prevalence, treat or prevent complications of this disease. The traditional use of herbs as part of the treatment of many disorders and diseases has yielded promising results. In recent years, a wide range of central nervous system activities has been reported for a methanolic extract of dates (8). However, no study has been conducted on the effect of this plant on seizures. Therefore, the present study was conducted to evaluate the effect of injectable use of a hydro-alcoholic extract of the date on pentylenetetrazole-induced seizure in Balb / c mice.

Methods

In this experimental study, 80 Balb / c male mice were selected in the weight range of 25-30 g. The animals were kept in equal conditions of light and darkness with a cycle of 12/12 and a temperature of 21 ± 2 ° C for two weeks. They were randomly divided into 8 groups of 10. Experimental groups 1 to 3 received 30, 100, and 300 mg/kg of the extract, respectively, and control groups 4 to 7 received PTZ, PTZ, distilled water, PTZ, and phenobarbital (2.6 mg/kg), extract of 30 mg/kg, respectively. Control group 8 received no treatment. After 45 minutes, all groups were injected with PTZ

(pentylenetetrazole) at a dose of 90 mg/kg intraperitoneally (8).

After PTZ injection, seizure onset time, seizure severity, seizure duration, and mortality of mice in groups were recorded by one observer and compared with each other. The scoring method was as follows: normal behavior received a score of zero, head tic received a score of 1, recurrent seizures and sudden movement of the anterior limb received a score of 2, seizures of the whole body and standing on three legs received score 3, tonic-clonic seizures received score 4, repeatedly rotation and jump of animal received score 4, and death of animal received score 6 (9). To more accurately evaluate the effect of the extract, the serum levels of calcium, sodium, potassium, phosphorus, and blood sugar of mice were compared in different groups. Finally, the data were analyzed using SPSS-15 software, Dunn post hoc test, and Kruskal-Wallis test. P <0.05 was considered statistically significant.

Results

The table presents the results of injection of hydroalcoholic extract of dates on seizure-related reactions, including onset time, duration, and severity of seizures in 8 groups, as well as serum levels of blood factors. Table 2 presents the animal mortality rate. According to the nonparametric analysis of variance or Kruskal-Wallis, no significant difference was observed between the groups at the onset of seizures, but the post hoc test showed a significant difference between the groups 1 and 3 (p <0.001), groups 3 and 4 (p <0.01), and groups 3 and 5 (p <0.05) in terms of seizure severity. This test also showed that groups 3 and 4 (p <0.05) and groups 3 and 5 (p <0.001) had a significant difference in seizure duration compared to other groups.

Kruskal-Wallis test showed a significant difference in the levels of sodium and potassium in the study groups, but the differences in calcium, phosphorus, and sugar levels of the groups were not significant. Based on the post hoc test, the sodium levels of the groups receiving date extract were significantly different from the control group (p <0.015), and also the potassium levels of groups 2 and 5 and groups 5 and 6 were significantly different (p <0.001). According to the Fisher's exact test, there was a significant difference between group 3 and other groups in terms of the number of deaths (p-value = 0.001).

Table 1 Statistical analysis of seizure onset, duration and severity with blood sugar, potassium, sodium, Phosphorus and Calcium levels in the studied groups after pentylene tetrazo injection

	Case Groups			Control Groups					
	Data 30 g	Date 100 g	Date 300 g	PTZ	PTZ and distilled water	PTZ and Phenobarbital	Date 30 g	control	sig
severity	75.10±3.15	2.50±5.9	42.90±2.44	50.90±4.14	14.2±50.12	9.14±5	-	-	001.0
Duration(seconds)	2.30±1.3	85.30±0.3	30±0/82.2	5.20±1.4	43.8±00.4	97.50±0.3	-	-	001.0
Onset(seconds)	20±0/92.2	63.80±0.1	05.2±1	53.50±0.1	5.70±0.1	6.10±0.2	-	-	298.0
Sugar(mg/dL)	1.30±85.139	9.30±40.140	3.100±17	4.122±40	2.1±36.151	5±48.149	134±41/6	6.1±23.111	027.0
Sodium(meq/L)	5.2±8.140	3.8±37.137	5.6±14.168	5.1±5.150	7±16.141	9.4±24.131	1±7.137	7.5±6.135	015.0
Potassium(meq/L)	02.7±1.8	01.2±1.9	01.72±1.9	6.13±0.8	64.5±02.7	9.4±2.10	7.9±2.9	88.2±02.8	001.0
Calcium(meq/L)	78.76±1.8	97.9±1.6	75.94±1.7	23.02±1.9	02.1±16.9	21.1±2.8	19.59±2.7	94.9±0.8	06.0

Phosphorus(meq /L)	41.18±3.9	55.9±2.5	92.68±9.5	19.17±2.5	33.6±3	26.48±5.9	86.36±3.7	53.9±4.5	067.0

Table 2 The number of deaths in the studied groups

group	6	5	4	3	2	1	sum
Number of died mice	3	8	3	0	3	6	33
Non-death	7	2	7	10	7	4	27

Discussion

The results of the study revealed a decrease in severity and duration of seizures in the groups receiving high concentrations of the extract compared to the control group. Also, the mortality of mice in Group 3 was significantly reduced compared to the other groups and the control group. Dates have antibacterial, antioxidant, immune system strengthening, and anti-mutagenic properties (10). Research suggests that methanolic extract of dates causes a wide range of activities of the central nervous system (8). Also, the optimal dose of aqueous extract of the plant can inhibit the damage induced by cerebral ischemia, which is most likely due to the antioxidant properties of the extract (11). It has also been shown that methanolic and aqueous extracts of dates show a hydroxyl radical inhibiting effect and the level of flavonoid and proanthocyanidin compounds in both extracts is significant (12).

In terms of cell biology, seizures are caused by overstimulation of brain neurons, impaired neuronal stimulation inhibition, and generally any loss of control over the membrane's resting potential, whatever the cause of epileptic seizures. Therefore, the inefficiency of any of the factors that play a role in these pathways can be a factor in causing epileptic seizures (13). PTZ has been proven to have a detrimental effect on neuronal membrane properties and to affect calcium and potassium channels (14). In a study, PTZ was found to increase the concentration of sodium ions within cells (15, 16).

Histochemical studies indicated that the number of GABAA and B receptor subunits decreased in patients' hippocampus and degeneration of GABAergic neurons was observed in patients' hippocampus. Sometimes the activity of GABA receptors is not reduced, but the expression of these receptors is seen as incomplete and immature, which can cause seizures (17). Antiepileptic drugs control seizures by various mechanisms such as inhibition of voltage-dependent sodium channels, exacerbation of the inhibitory effects of GABA and sodium channel inhibitors, and inhibition of glutamate receptors (18). In our study, no significant difference was observed in calcium concentration compared to the control group, but a significant difference was observed between the experimental and control groups in terms of the extracellular concentration of sodium and potassium ions. Therefore, the extract might reduce the severity of seizures by acting on sodium and potassium channels. It has been well proven that flavonoids can affect benzodiazepine receptors, and due to the presence of these compounds in dates, they may have an anti-seizure effect by acting on benzodiazepine receptors that bind to GABA receptors (18, 19). It is recommended that studies with higher concentrations and a more detailed investigation of serum factors be performed to further examine the effects of the extract.

Conclusion:

The results of this study showed that the hydroalcoholic extract of dates reduced the duration and severity of seizures and also reduced the seizure-induced mortality in Balb / c mice.

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Conflict of interest

None.

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References

1- Fauci A, Longo DL, Jameson JL, Hauser SL, Kasper DL, Loscalzo J. Harrison's principles internal medicine. 18rd ed. USA: Mc Graw Hill; 2011. pp: 2499-2514.

2- Goldenberg MM. Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. P T [Internet]. 2010 Jul;35(7):392–415. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20689626

3- "Seizures & Syncope." *Clinical Neurology, 11e* Eds. David A. Greenberg, et al. McGraw Hill, 2021, https://neurology.mhmedical.com/content.aspx?bookid=2975&sectionid=251840200.
4- Valiollah mozaffarian. Trees and shrubs of Iran. Tehran. Farhang moaaser; 1388. P: 484. Persian.
5- "Epilepsy and Other Seizure Disorders." *Adams and Victor's Principles of Neurology, 11e* Eds. Allan H. Ropper, et al. McGraw Hill, 2019, https://accessmedicine.mhmedical.com/content.aspx?bookid=1477&sectionid=138318583.
6- Newman, Nancy J MD. Merritt's Neurology, 11th Edition. Journal of Neuro-Ophthalmology 26(4):p 300, December 2006. | DOI: 10.1097/01.wno.0000249316.81367.74.
7- Mir hears H. Plants science, plants used in in the prevention and treatment of disease. P: 1-6. Persian.
8- Vyawahare NS, Pujari RR, Rajendran R, Khsirsagar AD, Ingawale DK, Patil MN. Neurobehavioral effects of Phoenix dactylafera in mice. PHARMACOLOGY, 2009; 1(3): 225- 232.
9- Barkia E, Grossman Y, Gutnick M. Long term changes in neocortical activity after chemical kindling with systemic pentylenetetrazole: An in vitro study. J Neurophysiol 72.1994; p: 72-83.
10- Shekaari MA, Panahi M, Dabiri S, Safi Khani Z Kalantaripour Taj P. Neuroprotective effects of Aqueous Date Fruit extract on focal cerebral ischemia in rats. Pakistan Journal of Medical Sciences. 2008; 24(5): 661-665. Persian.
11- Amir Siahpoosh, Rezvan Yazdanparast, Afsaneh Jaberkhalafian, Sara Alikazemi. Phenolic Compounds and Hydroxyl Radical Scavenging Activities of Aqueous and Methanolic Extracts of Phoenix Dactylifera L Cultivar Dayri Fruits. Scientific Medical Journal of Ahwaz University of Medical Sciences 2012; 11(77): 185-192. Persian.
12- Siddiqui KA, Siddiqui L, Ali R, Beamer H. Genes and epilepsies. Pak J Neurol Sci. 2007; 2(2):106-110.
13- Romoli M, Mazzocchetti P, D'Alonzo R, Siliquini S, Rinaldi VE, Verrotti A, et al. Valproic Acid and Epilepsy: From Molecular Mechanisms to Clinical Evidences. Curr Neuropharmacol [Internet]. 2019 Sep 13;17(10):926–46. Available from: http://www.eurekaselect.com/168625/article
14- Lister RQ. The use of a plus-maze to measure anxiety in the mouse. J Pscycharmacology.1987; 92: 180-185.
15- Rogawski, Michael A. "Antiseizure Drugs." *Basic & Clinical Pharmacology, 15e* Eds. Bertram G. Katzung, and Todd W. Vanderah. McGraw Hill, 2021, https://accessmedicine.mhmedical.com/content.aspx?bookid=2988&sectionid=250597700
16- Avoli M, Louvel J, Pumain R, Kohling R. Cellular and molecular mechanisms of epilepsy in the human brain. Prog Neurobiol. 2005; (77): 166-200.
17- Salah SM, Jager AK. Screening of traditionally used Lebanese herbs for neurological activities. J. Ethnopharmacol. 2005; 97(1): 145-149.