Effect of Date Molasses on Levetiracetam Pharmacokinetics in Healthy Rats

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Research Article

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Abstract

Eighteen healthy eight-week-old male Wister rats weighing 200 g were used. Rats were chosen randomly, their tails were identified, and separated into cages/groups. The first group received 0.1 mL of levetiracetam intravenously. The second received an oral dose of 11.5 mg of levetiracetam in 5 mL of water, and the third was the control group. . One week of pre-administered date molasses significantly decreased levetiracetam pharmacokinetic parameters in rats, as C_{max} (72 vs. 14 ng/mL, p=0.01), T_{max} (1.78 vs. 0.44 hr, p<0.001), and AUC (880 vs. 258 ng.h/mL, p<0.001). This decrease in plasma levetiracetam levels caused by date molasses could be attributed to decreased levetiracetam absorption. On the other hand, the current study discovered that rats given date molasses for a week had a reduced rate and extent of absorption. Polysaccharides in date molasses could theoretically increase the risk of epileptic seizures. When taking levetiracetam to stop epileptic seizures, doctors and patients should be told not to overdose.

Introduction:

Epilepsy is a debilitating and widespread neurological disorder defined by aberrant electrical activity in the brain, which results in seizures or odd behavior, sensations, and even loss of consciousness [1]. Epilepsy affects an estimated 50 million people worldwide, with 75 percent of those living in resource-poor countries having limited or no access to medical care or treatment. Around 50 per 100,000 people in developed countries are estimated to have epilepsy each year. In countries with limited resources, the annual incidence of epilepsy ranges from 100 to 190 per 100,000 people [2]. Epilepsy patients often take the anticonvulsant drug levetiracetam (LEV). Partial-onset seizures (seizures affecting only a portion of the brain) in adults, children, and infants aged one month and older can be controlled with LEV, either independently or in combination with other medications. It calms the brain's overactive excitability [3, 4]. In conjunction with epileptic treatment, low-sugar diets like the ketogenic diet (KD) have improved epileptic seizure symptoms [5, 6]. KD is usually recommended if a child's seizures have not improved after taking several different seizure medications. To lose weight, people following the KD follow a low-calorie, low-protein, and low-carbohydrate diet. If you follow the "long-chain triglyceride diet," you will get about 3 to 4 grams of fat for every 1 gram of carbs and protein [7]. According to recent studies, refractory epilepsy in children and adults can be successfully treated with the KD [8–11].

Additionally, "date molasses" can refer to several different products. It has a dark ruby red hue and a thick, sticky sweetness to the liquid. By far the most common date-derived product is date syrup, which can be made in three ways: I as an accidental byproduct of storing bagged, humid dates (especially in the Gulf area), (ii) at the home or village level by extraction and boiling down of the juice, and on a semi- and full-industrial scale. It costs about 9.5 cents per kilogram of date molasses [12]. In Middle Eastern countries, it is a necessity. It is loaded with essential vitamins, minerals, and polysaccharides. It also contains a small number of proteins. In addition to the previously mentioned antioxidants and vitamins/minerals, these also contain iron, calcium, magnesium, vitamin B6, and selenium, all of which are essential nutrients for human health [13].

As a food supplement commonly found in Mediterranean households, we hypothesized that the polysaccharides in dates molasses might interact with LEV and reduce its pharmacokinetic parameters. However, date molasses has never been studied concerning the pharmacokinetics of LEV in vivo, and there are no studies on date molasses interaction. This is something we are going to investigate further. As a result, the study's goal is to determine the effects of date molasses oral administration on rat LEV pharmacokinetics.

Materials And Methods:

Animals and in vivo experimental design

The study used eighteen eight-week-old male Wister rats weighing 200 g. Before beginning the study, rats were chosen at random, had their tails identified, and were placed in separate cages/groups. A week before the study began, all rats were acclimatized, fed regular food, and water was *ad libitum*.

Rats were divided into three groups. Group 1 was administered 0.1 mL of LEV intravenously; group 2 was administered an oral dose of 11.5 mg of LEV in 5 mL of water, and group 3 was kept only drinking for seven days' date syrup (Dates Molasses; 250 g mixed with 250 mL water) and then on day seven an oral dose of 11.5 mg of LEV in 5 mL of water was administered.

Chemicals: LEV was supplied from Hikma, Jordan, with a potency of 99.8%, whereas the following reagents and chemicals were obtained from Merck (Darmstadt, Germany): orthophosphoric acid (AR grade 85%), acetonitrile (HPLC grade), and Milli-Q purified water.

High-performance liquid chromatography (HPLC)

The HPLC system used was Thermo Finnigan Surveyor UV-VIS with a detector, LC Pump (SRVYR-LPUMP), and autosampler (SRVYR-AS). The column used was ACE C18-AR (250mm*4.6mm) 5µm, an average particle size.

The mobile phase, internal standard, and the standard solutions

The buffer solution was prepared by adding 3.4g KH_2PO_4 to 1000 mL of HPLC- grade water, and pH adjusted to 5.2 with orthophosphoric acid. The mobile phase was prepared by mixing 830 mL of the buffer solution with 170 mL of acetonitrile and orthophosphoric acid. The mixture was then filtered using a 0.45 µm membrane filter and degassed by sonication. The internal standard (IS) was prepared by weighing 3 mg of pure Metformin hydrochloride and dissolving it in 100 mL of freshly prepared acetonitrile. Then 1 mL of it was diluted with 200 mL of acetonitrile.

Preparation of stock solutions and working solutions

Stock solutions of LEV were prepared by weighing and transferring 20 mg of each of the active ingredients into a 100 mL volumetric flask and diluted up to the mark with ACN.

Preparation of working standard solution:

Step Dilution 1:2.5 mL of LEV solution was pipetted into a 20 mL volumetric flask.

Concentration, then diluted to Seven concentrations 10, 25, 50, 75, 100, 500, 1000 ng.

For the sample solution for LEV preparation, a volume of plasma was pipetted in Eppendorf and diluted with IS, then vortex for 1 minute, and then centrifuged for 15 minutes. The supernatant was injected into the HPLC.

Wavelength selection

UV-VIS scan applied for the solution of LEV was within a range of 200–400 nm. Maximum absorbance of 205 nm was obtained.

Method development

The method of development of the study was to best chromatographic conditions for assaying LEV using metformin as an IS, such as having a short retention time with good resolution and symmetric peaks. Different factors such as pH, ion pair, the composition of mobile phase, and column were evaluated.

After three trials for chromatographic conditions, in which two were rejected, we found the best resolution at pH5.2, wavelength: 210nm, for 10 µl injection volume, flow rate 1mL / min, and oven temperature 25°C.

Samples were drawn at different times: initial ,0.33,0.66,1,2,4,8,24,36,72and 96 Hours.

Pharmacokinetic analysis

The maximum plasma LEV concentration (C_{max}) and time to reach the maximum concentration (T_{max}) were calculated by averaging the highest plasma concentration and its corresponding time of LEV in each rat. Besides, the area under the curve under the plasma concentration-time profile from time zero to time t (AUC_t), zero to infinity ($AUC_{0\longrightarrow\infty}$), and elimination half-life ($t_{1/2}$) were calculated based on non-compartmental pharmacokinetic calculations.

Furthermore, $AUC_{0\longrightarrow\infty}$ was calculated by adding AUC_t to the value of dividing the last measurable concentration at time T over the elimination rate constant. The $t_{1/2}$ was calculated from the slope of the semi-logarithmic of the last plasma concentration points vs. time.

Data Analysis

The PK parameters; C_{max}, T_{max}, AUC_t ($AUC_{0\longrightarrow\infty}$), and $t_{1/2}$ were presented as mean (± SD). One-way Analysis of Variance was used to calculate the significant differences of each parameter between the groups, followed by Tukey as a post hoc test to find out the differences between each group.

Results:

Pharmacokinetics of LEV in rats with and without taking of date molasses

Table 1 shows that rats given date molasses for a week had a reduced rate and extent of absorption. Compared to the control group, the oral pharmacokinetics of LEV, when combined with dates molasses, were altered. Although there was statistical significance ($P < 0.05$), the time it takes to reach the maximum plasma concentration (T_{max}) was reduced in the presence of Dates molasses.

Table 1
The pharmacokinetic parameters of LEV after oral administration to rats with and without date molasses (mean SD).

Route of Administration	AUC	T_{max}	C_{max}
	Mean ± SD	Mean ± SD	Mean ± SD
LEV i.v	522 ± 115*	0.50 ± 0.18**	55.9 ± 2.2
LEV Oral	880 ± 306	1.78 ± 0.55	72.1 ± 50.7
LEV Oral + Dates	258 ± 38**	0.44 ± 0.17**	14.1 ± 5.9*
*p < 0.05 less than LEV oral			
**p < 0.001 less than LEV oral			

Discussion:

Administering a combination of drugs and or food may alter their pharmacokinetics [14, 15]. This study investigated the combination of date fruit molasses on LEV Pharmacokinetics in healthy rats. Where one week of pre-administrating date molasses, significantly decreased LEV pharmacokinetic parameters in rats; C_{max} (72 vs. 14 ng/mL, $p = 0.01$), T_{max} (1.78 vs. 0.44 hr, $p < 0.001$), and AUC (880 vs. 258 ng.h/mL, $p < 0.001$). This decrease in plasma LEV levels caused by date molasses could be attributed to a decrease and delay in LEV absorption. However, a previous study found that food slows the rate of LEV absorption but not the extent of absorption [16]. The latter conclusion was reached because LEV AUC did not differ without and with food. On the contrary, the current study found that rats given date molasses for a week had a reduced rate and extent of absorption.

Furthermore, it has been demonstrated that combining a low sugar diet, such as the ketogenic diet, with epileptic treatment reduces epileptic seizures [17, 18]. Polysaccharides in dates molasses could theoretically increase the risk of epileptic seizures. As a result, more research is needed to verify this. When taking LEV to control epileptic seizures, doctors and patients should be advised to limit their intake

of sugar or dates molasses. Our significant findings also necessitate further human research to examine the potential consequences on patients where the drug's activity may decrease.

Declarations:

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Wael Abu Dayyih, Raghad Layth, Mohammad Hailat, Bayan Alkhawaja, Lina Al Tamimi, Zainab Zakaraya, Aseel Aburumman, Nisreen Al Dmour, Hisham Al-Matubsi. Wael Abu Dayyih wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethics approval

This research has been approved by SREC No. SREC-1/2022 Scientific Research & Ethics Committee, Faculty of Pharmacy, Mutah University.

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Figures

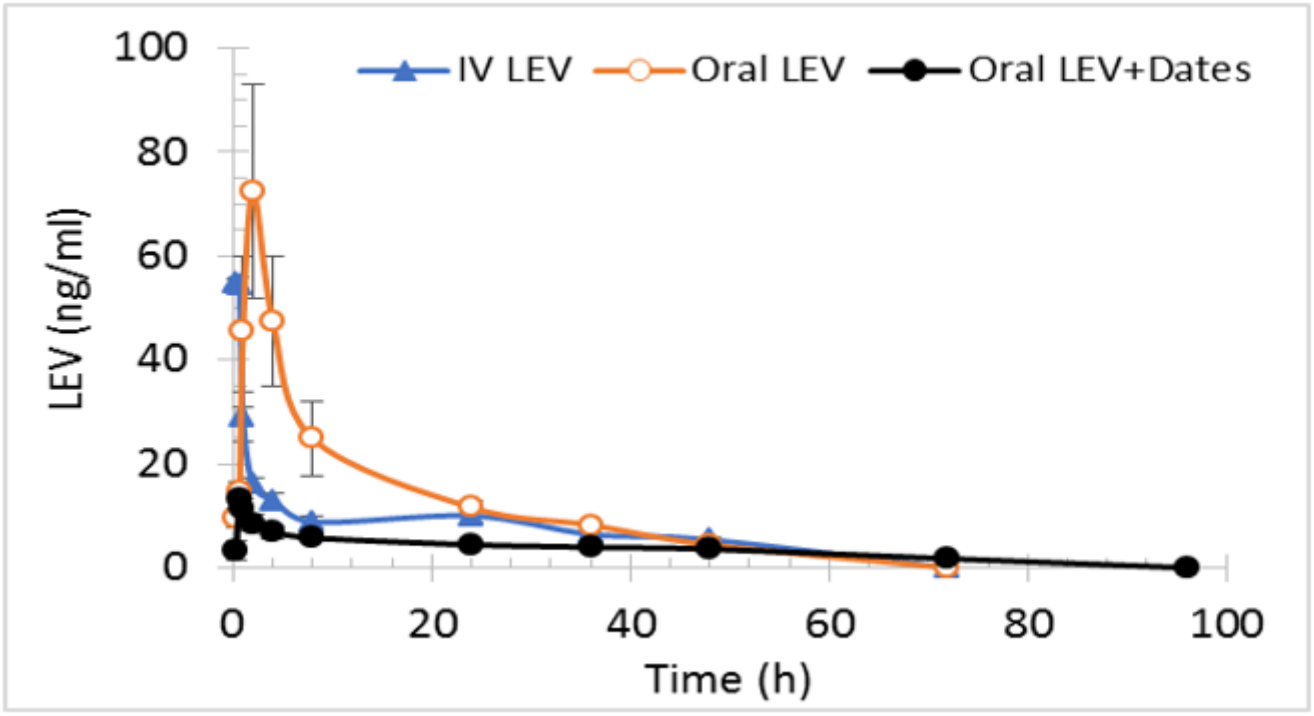

Figure 1

Pharmacokinetics profile of LEV administered either alone as I.V., orally, or orally together with date molasses (*n*=6).