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Eugenia Caryophyllata effect on Phenytoin metabolism in animal model

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Background: Phenytoin (PHT) is commonly prescribed antiepileptic drug which is widely used for management of all types of seizure disorders, except absence seizure. PHT is an inducer of cytochrome P450 (CYP450) CYP2C9 and CYP2C19. The present study is established to study the effect of Eugenia Caryophyllata (EUC) on PHT pharmacokinetics (PK) in healthy male rabbits.

Materials and Methods: An *in-vivo* parallel designed PK herb-drug interaction study between EUC and PHT was conducted in healthy male rabbits. The present study was conducted in two groups (N=6 for each). In the first group, animals were given orally 15 mg/kg PHT prepared in saline solution and blood samples (1.0 ml) were collected from ear marginal vein at different time intervals (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 12.0, 24.0, 36.0 and 48 hr) after dosing. The second group was pretreated with 70 mg/kg/day oily extract from EUC and were given to rabbits for 7 consecutive days. On the 8^{th} day, PHT was co-administered one hour after the last dose of the EUC oily extract and blood samples were collected at the predetermined intervals as in the first group. The analysis of PHT blood samples was performed by using PHT detection kit based on chemiluminescent enzyme immunoassay (CLEIA) and different PK parameters such as C_{max}, t_{max}, $AUC_{0-\infty}$, $t_{1/2}$, MRT and ke were determined for both groups.

Results: Our results showed statistically insignificant differences ($P \geq 0.05$) for the following PK parameters: C_{max}, t_{max}, $AUC_{0-\infty}$, $t_{½}$, MRT and ke of PHT when given alone or concurrently with EUC.

Conclusion: Our results showed that EUC may not likely to interfere the PK of PHT when co-administered with PHT. Further confirmation of our findings is required in humans before these results can be applied in patient care.

Keywords: Phenytoin, pharmacokinetic drug interaction, CYP450 2C9, eugenia caryophyllata

Introduction

Antiepileptic drugs (AEDs) are widely used for long-term adjunctive therapy or as monotherapy in epilepsy and other indications. Some of these drugs are highly susceptible to drug interactions (Landmak et al., 2010). PHT is a commonly prescribed antiepileptic drug and widely used for management of both generalized and partial epilepsy except absence seizures (Chua et al., 2000). PHT is an inducer of Cytochrome P450 (CYP450) CYP2C9 and CYP2C19 (Hwang et al., 2004) and has a narrow therapeutic window (Von Winckelmann et al., 2008). Clinically important, CYPs involve certain isoforms that appear to have therapeutic relevance. Only a few of these are important in terms of understanding the interactions of AEDs as CYP1A2, CYP2C9/10, CYP2C19, and CYP3A3/4. Knowledge of the isoenzymes involved in the metabolism of some established AEDs allows prediction of interactions with new drugs (Sirot et al., 2006; Williams et al., 2004; Zhou et al., 2007).

Essential oils have been used as anticonvulsants in traditional medicine in many cultures worldwide, especially in the Middle East, India, China and Brazil. Moreover, they are a particular class of natural medicines obtained by distillation of plant material to obtain a volatile extract having anxiolytic, sedative, neuroprotective and anticonvulsive properties (Abuhamdah et al., 2015; Debas et al., 2006; De Sousa et al., 2015; Dohare et al., 2008; Wang et al., 2018). Eugenia Caryophyllata (EUC) (*Syzygiumaromaticum*, Clove) belongs to *Myrtaceae* families grown naturally in Indonesia and cultivated in many parts of the world, including Brazil (Agra et al., 2008; Costa., 1994; Correa et al., 1998). It is used in cooking, food processing, pharmacy, perfumery and cosmetics and has 36 components. One of these components are essential oils which are a colorless or light yellowish fluid extract from dried flower buds with strong phenolic smell and sharp acrid taste. The highest concentration of EUC essential oils are eugenol (88.58%), eugenyl acetate (5.62%) and β-cariophyllene (1.38%). However, the differences in oil composition

are correlated with different regions or countries where the plant is cultivated (Oliveira et al., 2007). The eugenol is widely used and well known for its medicinal properties. Traditionally, EUC oil extract is used in dental care, as an antiseptic and as analgesic (Öztürket et al., 2005). *In vitro* studies demonstrated that EUC oils inhibit CYP2C9 and CYP2C19 (Foster et al., 2003). Unfortunately, an *in-vivo* study of EUC-PHT interaction is still not investigated. The present study aims to assess the potential of herb-drug interaction between EUC-PHT in healthy male rabbits.

Materials and Methods

A. Animals

Twelve randomly selected healthy male rabbits (Weighted between 3.2-3.4 kg, age: 7-9 months) were enrolled in this study. The rabbits were divided in to two groups and rabbits were monitored for general health, activity and weight. The study was approved by the Experimental Animal Care Center, College of Pharmacy, Al-Azhar University, Gaza, Palestine. The animals were maintained in accordance with the recommendations of the 'Guide for the Care and Use of Laboratory Animals. All rabbits were maintained under standard laboratory conditions of a 12-hour light/dark cycle at 25±2°C. The animals were given pellet diet with free access to water (ad libitum) and fasted overnight prior to the experiments. The study was carried out at the Faculty of Pharmacy, Al-Azhar University of Gaza (AUG), Gaza, Palestine.

B. Study design and blood sampling

An *in-vivo* parallel designed PK herb-drug interaction study between PHT-EUC was conducted in healthy male rabbits. The study was conducted in two groups (N=6 for each). In the first group (control group), animals were given PHT 15 mg/kg prepared in normal saline solution and blood samples (1.0 ml) were collected from ear marginal vein using sampling cannula (23G) (Parasuraman et al., 2010) at different time intervals (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 12.0, 24.0, 36.0

and 48 hr) after dosing. The second group (test group) was pretreated with 70 mg/kg/day oil extract of EUC given to rabbits for 7 consecutive days. On the 8^{th} day, PHT was co-administered one hour after the last dose of the EUC oil extract (The EUG oil extract has been purchased from local Pharmacy) and blood samples were collected at the predetermined intervals as in the first group. The animals were given the doses (P.O) by using oral gavage where the drug solution was placed in a corner of rabbit mouth and pushed down slowly to prevent choking. Physical tests were followed to assess clinical safety during this research. Plasma was separated by centrifugation and stored at -80°C until PHT analysis.

C. Analysis of Phenytoin serum samples

Blood samples were collected in labelled, heparinized test tubes and centrifuged at 3000 rpm for 10 minutes. Plasma was separated by centrifugation and stored at –80°C until its analytical assay. PHT blood concentrations were assayed by a chemiluminescent immunoassay CLIA using an ARCHITECT analyzer 1000 Abbott Laboratories, Abbott Park, IL, USA.

D. Pharmacokinetic analysis

The plasma concentrations were used to construct PK profiles by plotting drug concentration-time curves of the two groups. The PK parameters including: C_{max}, t_{max}, $AUC_{0-\infty}$, $t_{1/2}$, MRT and ke were determined for both groups. The C_{max} and t_{max} were directly determined from the plasma concentration versus time curves. The $AUC_{0-\infty}$was determined by the following equation: $AUC_{0-\infty} = AUC_{0-48} + Ct / k_e$, where, Ct is defined as the last measured serum PHT concentration at time t, and K_e is the elimination rate constant. The K_e was determined by the least squares regression of plasma concentration-time data points lying in the terminal region by using semi-logarithmic dependence that, corresponds to first-order kinetics. The $t_{1/2}$ was calculated as 0.693/Ke. Pharmacokinetic analysis was determined by means of model independent method (Non-Compartmental Approach) WinNonlin Professional Software (Version

6.3, Pharsight Corporation, Cary, NC) and (GraphPad Prism versión 4.00; San Diego, CA, USA).

E. Statistical analysis

The data is presented as mean with standard deviation (SD) for each time point in each group. Differences in PK parameters of PHT alone and with EUC were assessed by t-test using general linear model procedures. The statistical analysis was performed using SPSS program (version 16.0). A statistically significant differences was considered when $P \leq 0.05$.

Results and Discussion

In the present study, the effect of EUC on PK of PHT was investigated by using healthy male rabbits. The mean plasma concentrations of PHT when administered alone or in combination with EUC extract are shown in (Figure1). The concentration time profile obviously indicated that the two treatments were comparable. The mean PK parameters of PHT administered alone or in combination with EUC oil as well as the statistical significance following their comparison are given in (Table 1).

In the two periods of treatments, C_{max}, t_{max}, $AUC_{0-\infty}$, $t_{½}$, MRT and ke for PHT when administered alone and when co-administered with EUC oil were calculated. The mean peak plasma concentration C_{max} was 3.64 ± 26.04µg/mL in control group versus 3.52±8.60 µg/mL in test group, t_{max}, time taken to reach C_{max}, was 8.40±39.12 h versus 8.30±0.003 h, $AUC_{0-\infty}$ was 76.90±48.23 µg/ml.h versus 67.59±7.59 µg/ml.h, $t_{½}$ was 20.68±24.26 h versus 16.46±10.48 h, elimination rate constant was 0.035±25.19 h^{-1} versus 0.0419±39.2 h^{-1} and MRT was 14.60±11.59 versus 14.67±1.68 h respectively.

Table1: Different PK parameters after administration of PHT alone (control group, n=6) and in combination with EUC (test group, n=6) (Mean ± SD).

PK Parameters	Group (n=6)	Mean ± SD	P-Value
C_{max} (µg/ml)	PHT	3.64 ± 26.04	**0.799**
	PHT + EUC	3.52 ± 8.60	
t_{max} (hrs)	PHT	8.40 ± 39.12	**0.374**
	PHT + EUC	8.30 ± 0.003	
ke (hr^{-1})	PHT	0.035 ± 25.19	**0.206**
	PHT + EUC	0.042 ± 39.12	
$t_{1/2}$ (hrs)	PHT	20.68 ± 24.26	**0.184**
	PHT + EUC	16.64 ± 10.48	
MRT (h)	PHT	14.60 ± 11.59	**0.929**
	PHT + EUC	14.67 ± 1.68	
$AUC_{0-\infty}$ (µg/ml.h)	PHT	76.9 ± 48.23	**0.599**
	PHT + EUC	67.59 ± 7.59	

Abbreviations are C_{max}: Maximum serum concentration, t_{max}: Time to reach C_{max}: $AUC_{0-\infty}$: Area under the curve from 0 h to infinity, $t_{1/2}$: Terminal half-life, MRT: Minimum residence time and K_e: Terminal elimination constant: SD: Standard deviation: Statistical significance $p \leq 0.05$.

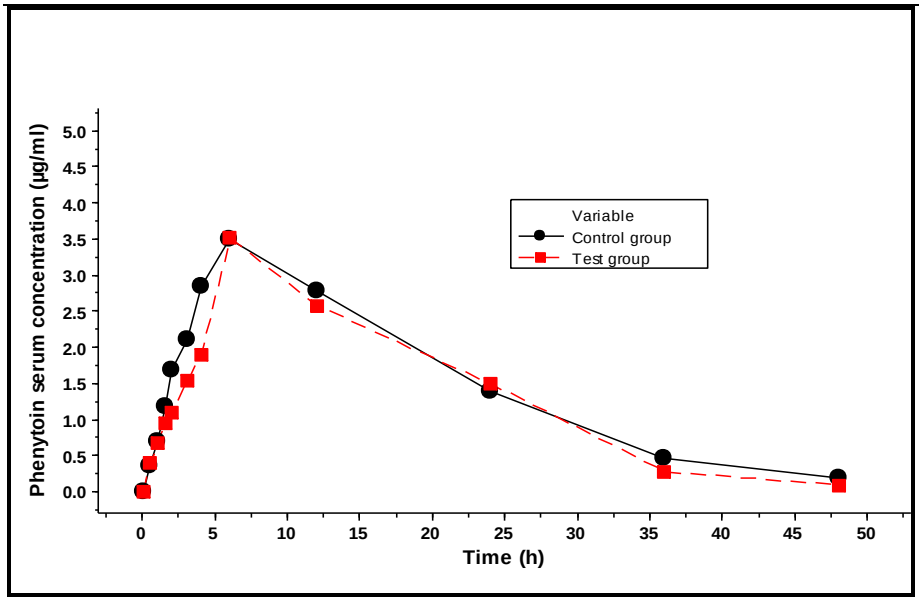

Figure 1: Mean rabbits serum concentration-time profile of PHT when administered alone and in combination with EUC (N=6).

Patients on a polypharmacy regimen would be expected to be at a higher risk of experiencing a clinically-significant event. Hence, it is necessary to consider the total xenobiotic loading of natural health products and pharmaceutical dosage forms in order to better predict the potential of interactions. As the duration of use is increased, the potential for an interaction would also be expected to increase (Foster et al., 2003). PHT is preferred drug among older antiepileptics due to low cost and efficacy against different types of seizures. Since, PHT has a narrow therapeutic index (Von Winckelmann et al., 2008), the monitoring of PHT level is a common practice in clinical set up for the intervention of patients. Due to its long list of drug-drug interactions, PHT has chances to interact with the other concomitant drugs. The patients suffering from epilepsy can have concurrent occurrence of some other diseases. PHT is given for long duration; hence its PK may be altered by drugs indicated in other disease conditions. This may lead to an increase or decrease in its plasma levels and subsequent deleterious effects either due to its toxicity or

loss of its effective seizure control (Mc-Namara., 2001; Perucca., 2005). PHT is metabolized by both CYP2C9 and CYP2C9 where CYP2C9 is responsible for 90% of its metabolism (Hung et al., 2004), whereas CYP2C19 metabolizes the remaining 10% (Tassaneeyakul et al., 2016).

The usage of herbal products in various (traditional) therapies around the world raises the question about their safety and evidence-based efficacy. Unexpected and significant herb–drug interactions may occur and put individuals at risk, particularly those people suffering from who use multiple medicines. The information about herb–drug interactions are scarce, derived mainly from *in vitro* studies that cannot be directly extrapolated to the *in vivo* conditions. That way, it is important to conduct *in vivo* non-clinical studies with enough potency for further examination in humans. (Samojlik et al., 2012; Samojlik et al., 2016).

The twelve male rabbits were completed the study and there were no death or replacement during the study. The utility of rabbit as a model to study drug–drug interactions is well documented. Clinical and physical examination during and post study indicated no abnormalities. The present study showed good tolerability for both groups. (Stargrove., 2008; Riviere., 2007). Despite the widespread use of herbal medicines, documented herb-drug interactions are sparse. PK herb drug interaction study demonstrated that the chronic intake of caraway essential oil influences the PK properties of both orally and intraperitoneally applied paracetamol (Samojlik et al., 2012).

Regarding our results, PK parameters of control and test groups did not differ significantly as shown in (Table 1). The group pretreated with EUC showed no statistically significant differences in the observed PK parameters after oral administration of PHT when compared with control group. However after oral administration of PHT in the treated group with EUC, our obtained results showed slightly decreasing in the following PK parameters as $C_{max,}$ t_{max}, $t_{1/2}$ and $AUC_{0-\infty}$ when compared with the control group and the differences between both groups were statistically insignificant

(P>0.05). In conclusion, our results showed that, EUC may not likely to interfere the PHT PK when co-administered with PHT, so it can be used safely without precautions or dose monitoring. The obtained results should merit further investigations to clarify their clinical relevance.

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