Food and Chemical Toxicology 128 (2019) 61–67

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short-term effects of *Garcinia cambogia* extract on the pharmacokinetics of lamotrigine given as a single-dose in Wistar rats

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ARTICLE INFO

Keywords:
Herbal supplements
Herb-drug interaction
Pharmacokinetics
Garcinia cambogia
Lamotrigine
Rats

ABSTRACT

Garcinia cambogia supplements are widely used for weight loss. Knowing that epilepsy patients are at greater risk of developing overweight/obesity, the investigation of herb-drug interactions involving antiepileptic drugs of narrow therapeutic index is fully justified. This work was planned to assess potential pharmacokinetic-based interactions between *G. cambogia* extract and lamotrigine (LTG) through two independent pharmacokinetic studies. In the first study (co-administration study), rats were orally co-administered with a single-dose of *G. cambogia* extract (821 mg/kg) and LTG (10 mg/kg). In the second study (pre-treatment study), rats were orally pre-treated for 14 days with *G. cambogia* extract (821 mg/kg/day), being LTG administered (10 mg/kg) on the 15th day. Rats of the control groups received water instead of the extract. Following LTG administration, blood samples were collected until 96 h post-dose, and plasma LTG concentrations were determined and submitted to a non-compartmental analysis. Globally, no statistically significant effects were identified in the co-administration study of *G. cambogia* extract and LTG. In the 14-day pre-treatment study, a statistically significant decrease in the rate of systemic exposure to LTG and an increase of apparent volume of distribution were found. Even so, a minor or no clinical impact is expected from the administration of *G. cambogia* dietary supplements and LTG.

1. Introduction

Garcinia cambogia, also known as Malabar tamarind, has been traditionally used in rheumatic and bowel complaints and is now popularly used as an ingredient of dietary supplements for weight loss (Márquez et al., 2012; Semwal et al., 2015). Biological effects of *G. cambogia* are closely related to its phytochemical constituents. The fruits of *G. cambogia* contain organic acids, such as hydroxycitric acid (HCA), along with xanthones (e.g. oxy-guttiferones I, K, K2 and M), benzophenones (guttiferones I, N, J, K and M) and amino acids as glutamine, glycine and γ-aminobutyric acid (Semwal et al., 2015). In fact, the major bioactive constituent of *G. cambogia* fruits is the stereoisomer (−)-HCA, which is present in amounts of 10–30% in the free form and/or as a mineral salt or a stable lactone form (Márquez et al., 2012).

Marketed supplements of *G. cambogia* extract usually contain until 50–60% of (−)-HCA (Bakhiya et al., 2017; Márquez et al., 2012), which are widely used for weight loss and obesity management mainly due to appetite-suppressant, anti-obesity and hypolipidemic activities (Fassina et al., 2015; Semwal et al., 2015). Indeed, several clinical trials have reported potent inhibitory effects of HCA isolated from *G. cambogia* on lipogenesis and on the adenosine triphosphate (ATP) citrate lyase, a key enzyme in the biosynthesis of fatty acids, as well as a reduction in serum triglyceride levels in obese women (Márquez et al., 2012; Mopuri and Islam, 2017; Vasques et al., 2013). Additionally, enhanced gluconeogenesis and glycogenesis have also been ascribed to HCA in rats and mice (Jena et al., 2002). Moreover, in some non-clinical studies in rodents, *G. cambogia* fruit extracts have also been associated with weight loss and appetite suppression activity, probably as a result of the

Abbreviations: AED, antiepileptic drug; AUC, area under the concentration-time curve; $AUC_{0\text{-}24}$, AUC from time zero to 24 h; $AUC_{0\text{-}t}$, AUC from time zero to the last measurable concentration; $AUC_{0\text{-}\infty}$, AUC from time zero to infinity; $AUMC_{0\text{-}\infty}$, area under the first moment of the concentration-time curve from zero to infinity; CL/F, apparent clearance; C_{max}, peak concentration; HPLC-DAD, high-performance liquid chromatography–diode array detection; IS, internal standard; k_{el}, apparent elimination rate constant; LTG, lamotrigine; MEPS, microextraction by packed sorbent; MRT, mean residence time; p.o., per os; $t_{1/2el}$, elimination half-life; t_{max}, time to reach peak concentration; V_d/F, apparent volume of distribution

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https://doi.org/10.1016/j.fct.2019.03.051
Received 15 December 2018; Received in revised form 25 March 2019; Accepted 27 March 2019
Available online 30 March 2019
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increase in brain serotonin levels, reduction in plasma insulin levels and inhibition of the enteral absorption of glucose (Hayamizu et al., 2003; Ohia et al., 2001; Wielinga et al., 2005). Although most studies have shown efficacy on weight loss, in some human studies no significant weight or fat reduction was observed even when high doses of *G. cambogia* or HCA supplements were used for long periods of time (Kim et al., 2011; Onakpoya et al., 2011; Semwal et al., 2015; Yonei et al., 2008). Therefore, in this context the available results are somewhat controversial.

Natural food supplements are gaining popularity as an attractive alternative to counteract obesity, preventing obesity-related physiopathologic events. Since obesity and obesity-related chronic diseases are growing at an alarming rate (Esteghamati et al., 2015), conventional pharmacological approaches for the treatment of obesity seem to be overtaken by the use of herbal bioactive components with anti-obesity properties. Actually, epilepsy patients present a growing risk of developing obesity in comparison with general population (Arya et al., 2016; Janousek et al., 2013; Ladino et al., 2014). In particular, a higher prevalence of obesity has been observed in patients with refractory epilepsy and in those treated with antiepileptic drugs (AEDs) polytherapy regimens (Baxendale et al., 2015; Chukwu et al., 2014; Janousek et al., 2013). The long-term use of AEDs has already been associated with changes in some metabolic pathways, thus determining changes in body weight (Hamed, 2015). On the other hand, evidence from some experimental studies has suggested that peripheral hormones, such as leptin, ghrelin and adiponectin, which are altered in obesity state, may modulate seizure threshold, epilepsy and/or seizure-related damage (Lee and Mattson, 2014).

Hence, considering the increasing use of weight-loss herbal medicines/supplements worldwide, including among patients with epilepsy, it is essential to ensure the absence of important herb-drug interactions between herbal preparations and AEDs to avoid potential deleterious effects in terms of efficacy and safety. Actually, as some constituents of herbal extracts can be substrates, inducers and/or inhibitors of transporters and/or enzymes responsible for AEDs biodisposition (Oga et al., 2015; Roe et al., 2016; Tarirai et al., 2010; Wu et al., 2015), it is urgent to evaluate the potential risk for herb-drug interactions between weight-loss herbal extracts and AEDs.

Bearing in mind that lamotrigine (LTG) is a commonly prescribed AED with unique pharmacokinetic and pharmacodynamic properties, which make it a first-line option for several types of epileptic seizures and also in bipolar disorder (Nevitt et al., 2017; Vajda et al., 2013), it is fully justified to assess the effects of *G. cambogia* extract on the pharmacokinetics of LTG, which is primarily metabolized by *N*-glucuronidation mediated by UGT1A4 and UGT2B7 (Argikar and Remmel, 2009; Rowland et al., 2006). Indeed, despite its broad spectrum of efficacy, LTG presents some pharmacological disadvantages such as a narrow therapeutic range (3–15 μg/mL) and a considerable inter-individual variability in its pharmacokinetics, having also some propensity to interact with other drugs (Patsalos, 2013b; Patsalos et al., 2017), which raises additional concerns that support the need to investigate the potential for pharmacokinetic-based interactions between *G. cambogia* extract and LTG in *in vivo* conditions.

2. Materials and methods

2.1. G. cambogia extract and drugs

The *G. cambogia* fruit rind aqueous extract containing 60% of HCA, was purchased from Bio Serae Laboratories (Bram, France). The certificate of analysis of the extract was received and preserved (ref.410069; batch 1001341). LTG dispersible tablets (Lamictal® 25 mg, GSK), pentobarbital (Eutasil®, 200 mg/mL, Ceva Saúde Animal), sodium chloride 0.9% solution (Labesfal, Portugal), heparin sodium 5000 I.U./mL (B. Braun Medical, Portugal) were commercially acquired from referenced laboratories.

2.2. Animals

Adult male Wistar rats weighing 220 ± 22 g were obtained from local certified animal facilities (Faculty of Health Sciences of the University of Beira Interior, Covilhã, Portugal). Animals were housed at 12 h light/dark cycle under controlled environmental conditions (temperature 20 ± 2 °C; relative humidity 55 ± 5%) and were allowed free access to a standard rodent diet (Mucedola 4RF21 Settimo Milanese, Milan, Italy) and water *ad libitum*.

The experimental procedures were approved by the Portuguese National Authority for Animal Health, Phytosanitation and Food Safety (DGAV – Direção Geral de Alimentação e Veterinária) and all the animal experiments were conducted in accordance with the European Directive (2010/63/EU) for animal experiments.

2.3. Preparation of G. cambogia extract and LTG solutions

The solution of *G. cambogia* extract was daily prepared by dissolution of the powdered extract in distilled water to be administrated at a dose of 821 mg/kg (p.o.) considering the administration volume of 10 mL/kg of rat body weight. This dose was defined based on the human dose recommendation from the extract supplier, which was converted to rat species following a Food and Drug Administration (FDA) Guidance for Industry; this FDA guidance allows the conversion of animal doses to human equivalent doses based on body surface area (FDA, 2005). Additionally, a 10-fold potentiation factor of interaction was employed to avoid false negative results.

The LTG solution was obtained after dissolution of the dispersible tablets in the proper volume of distilled water to obtain the required drug solution to be administrated to animals. Each animal received a LTG dose of 10 mg/kg (p.o.) administered in a volume of 4 mL/kg of rat body weight. The LTG dose employed in these studies was defined based on previous experiments performed in the rat (Ventura et al., 2016, 2018).

2.4. Pharmacokinetic studies

Two independent pharmacokinetic studies were designed to investigate the potential of interaction between *G. cambogia* extract and LTG in Wistar rats. Twelve animals were used in each pharmacokinetic study, which were balanced and randomly allocated to the control and experimental groups. In the first pharmacokinetic study, rats of the experimental group ($n = 6$) were concomitantly treated with a single-oral dose of *G. cambogia* extract (821 mg/kg, p.o.) and LTG (10 mg/kg, p.o.). In the second study, rats of the experimental group ($n = 6$) were orally pre-treated during 14 days with *G. cambogia* extract (821 mg/kg/day, p.o.) followed by a single dose of LTG (10 mg/kg, p.o.) administrated on the 15th day. A 14-day period of time was considered for the repeated administration of *G. cambogia* extract according to the international guidelines and scientific data available on this scope (ICH, 2009; Ma and Ma, 2016). Rats of each control group ($n = 6$) received the corresponding volume of the vehicle of the herbal extract (water) and were similarly treated with LTG.

Briefly, each animal of both experimental and control groups was anesthetized on the night before LTG administration for insertion of a polyurethane cannula in a lateral tail vein (Introcan® Certo IV indwelling cannula 22G, 0.9 × 2.5 mm; B. Braun Melsungen AG, Germany) to be used for serial blood sampling. Anesthesia was performed by intraperitoneal injection of pentobarbital (60 mg/kg). Rats completely recovered from anesthesia, and they were submitted to an overnight fasting period, with free access to water, before LTG administration. To avoid the effect of food on LTG absorption and disposition, the fasting period was also maintained for 4 h after drug administration.

In each study, LTG and *G. cambogia* extract (or vehicle, in the control groups) were orally administrated by gavage in the morning. After treatment with LTG, blood samples were obtained from each

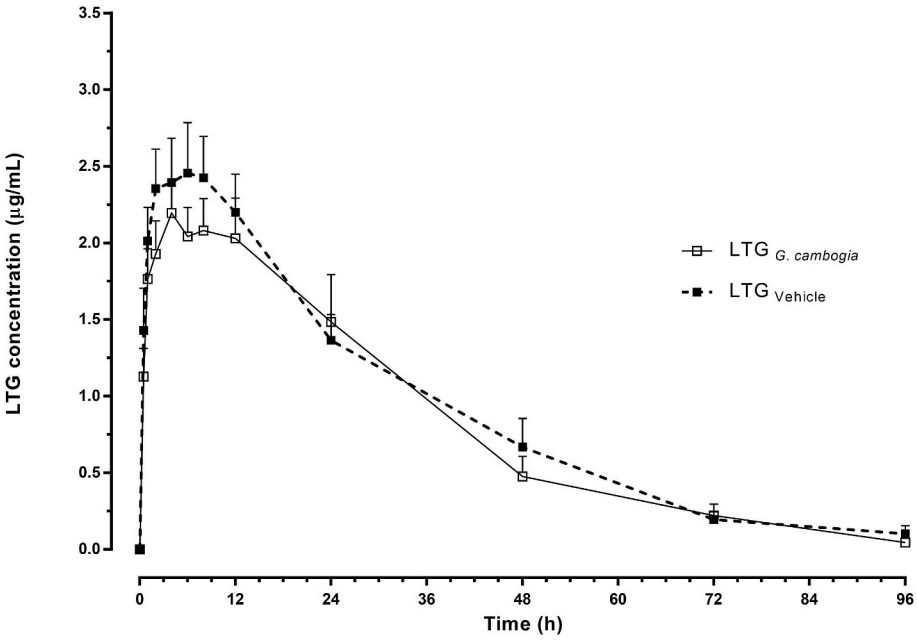

Fig. 1. Mean plasma concentration-time profiles of lamotrigine (LTG) obtained, over a period of 96 h, from rats co-administered with a single-dose of *Garcinia cambogia* extract (821 mg/kg, p.o.) or vehicle of the extract (water) and LTG (10 mg/kg, p.o.) by oral gavage. Symbols represent the mean values ± standard error of the mean (SEM) of six determinations per time point ($n = 6$).

animal at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h post-dose. Each blood sample (approximately 0.3 mL) was collected into EDTA tubes and then centrifuged at 3551 × *g* for 10 min (4 °C) to separate the plasma which was stored at −20 °C until analysis.

2.5. LTG quantification

The quantification of LTG in each plasma sample was achieved using a microextraction by packed sorbent (MEPS) technique coupled to a high-performance liquid chromatography–diode array detection (HPLC-DAD) method, previously developed and validated (Ventura et al., 2016).

2.6. Pharmacokinetic analysis

The peak plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were directly obtained from the experimental data. The individual plasma concentration-time profiles were submitted to a non-compartmental pharmacokinetic analysis using WinNonlin version 5.2 (Pharsight Co, Mountain View, CA, USA) to estimate a set of relevant pharmacokinetic parameters, including the area under the concentration-time curve (AUC) from time zero to 24 h ($AUC_{0\text{-}24}$); the AUC from time zero to last measurable concentration ($AUC_{0\text{-}t}$), which was calculated by the linear trapezoidal rule; the AUC from time zero to infinity ($AUC_{0\text{-}\infty}$), which was determined from $AUC_{0\text{-}t} + (C_{last}/k_{el})$, where C_{last} is the quantifiable concentration at the time of the last measurable drug concentration (t_{last}) and k_{el} is the apparent elimination rate constant calculated by log-linear regression of the terminal segment of the concentration-time profile; the elimination half-life ($t_{1/2el}$); the apparent clearance (CL/F); the apparent volume of distribution (V_d/F); the area under the first moment of the concentration-time curve from zero to infinity ($AUMC_{0\text{-}\infty}$); and the mean residence time (MRT). The drug concentrations below the lower limit of quantification of the assay were taken as zero for all calculations.

2.7. Effects of repeated-dose administration of G. cambogia extract on body weight

In addition to the pharmacokinetic studies, the effects of the repeated administration of *G. cambogia* extract on the body weight of rats, over the 14-day treatment period, were also investigated. So, the body weight of the animals of the experimental (*G. cambogia*) and control (vehicle) groups was evaluated and then compared between the first and the last day (14th) of the *G. cambogia* pre-treatment study.

2.8. Statistical analysis

The Shapiro–Wilk test of normality of data showed a non-normal distribution of pharmacokinetic data and therefore, all further statistical comparisons between groups were performed using the non-parametric Mann-Whitney test. The Wilcoxon signed-rank test was used for comparisons of body weight changes within the same group (day 1 vs. day 14) and the non-parametric Mann-Whitney test for comparisons between groups. A difference was considered to be statistically significant for a *p*-value lower than 0.05 ($p < 0.05$). Descriptive statistics of results is presented as median plus percentiles (25th percentile; 75th percentile) or as mean ± standard error of the mean (SEM) values as appropriate.

3. Results

3.1. Effects of G. cambogia extract on LTG pharmacokinetics after co-administration

The mean plasma concentration-time profiles of LTG obtained in rats ($n = 6$) following the simultaneous administration of a single-oral dose of *G. cambogia* extract (821 mg/kg) or vehicle and LTG (10 mg/kg) are shown in Fig. 1. The corresponding pharmacokinetic parameters directly obtained from experimental data and estimated by non-compartmental analysis are summarized in Table 1. As observed, a similar pattern of plasma concentration-time profiles is observed in both experimental (*G. cambogia*) and control (vehicle) groups. Although a slight trend towards lower LTG concentrations is observed in the experimental group (*G. cambogia*) between 1.0 and 12.0 h post-dose, no statistically significant differences were found ($p > 0.05$) between both groups (Fig. 1). Mean C_{max} was also slightly lower in the experimental group (1.2-fold) compared to the control group, but without statistical significance ($p > 0.05$). The median LTG t_{max} was 10.0 h in the experimental group and 5.0 h in the control group. Despite the longer median t_{max} value estimated for LTG in the experimental group no statistically significant differences were detected (Table 1). Regarding the extent of systemic exposure of LTG (as assessed by AUC

Table 1
Pharmacokinetic parameters estimated by non-compartmental analysis of the plasma concentration-time profiles of lamotrigine (LTG) obtained in rats after the co-administration with a single-dose of *G. cambogia* extract (821 mg/kg, p.o.) or vehicle of the extract (water) and LTG (10 mg/kg, p.o.) by oral gavage (n = 6). Data are presented as median plus percentiles (25th percentile; 75th percentile).

Parameter	Experimental Group LTG *G. cambogia*	Control Group LTG $_{Vehicle}$
C_{max} (μg/mL)	2.583 (1.806; 2.745)	2.638 (2.318; 3.386)
t_{max} (h)	10.0 (4.0; 15.0)	5.0 (3.3; 8.0)
AUC_{0-24} (μg.h/mL)	46.560 (33.800; 54.725)	45.570 (38.038; 61.625)
AUC_{0-t} (μg.h/mL)	84.917 (55.325; 101.852)	94.799 (61.100; 101.061)
$AUC_{0-\infty}$ (μg.h/mL)	89.822 (61.259; 109.747)	97.176 (63.731; 111.538)
k_{el} (1/h)	0.0383 (0.0263; 0.0442)	0.0429 (0.0348; 0.0515)
$t_{1/2el}$ (h)	18.1 (16.2; 26.5)	16.2 (13.5; 20.6)
CL/F (mL/h/kg)	113.500 (84.632; 163.493)	102.916 (89.622; 157.207)
V_d/F (mL/kg)	3149.138 (2657.290; 5412.517)	2845.619 (1990.817; 3938.587)
$AUMC_{0-\infty}$ (h.h.μg/mL)	2811.478 (2018.665; 4410.474)	2012.430 (1659.448; 4268.824)
MRT (h)	30.3 (24.7; 38.0)	25.0 (20.8; 37.5)

AUC, area under the concentration-time curve; AUC_{0-24}, AUC from time zero to 24 h; AUC_{0-t}, AUC from time zero to the last measurable concentration; $AUC_{0-\infty}$, AUC from time zero to infinite; $AUMC_{0-\infty}$, area under the first moment of the concentration-time curve from zero to infinity; CL/F, apparent clearance; C_{max}, peak concentration; k_{el}, apparent elimination rate constant; MRT, mean residence time; $t_{1/2el}$, elimination half-life; t_{max}, time to reach C_{max}; V_d/F, apparent volume of distribution.

values) quite similar values were obtained in both experimental and control groups. The mean values of $AUMC_{0-\infty}$ and V_d/F pharmacokinetic parameters calculated for both groups were also comparable and, as expected, the mean values estimated for the elimination pharmacokinetic parameters ($k_{el,}$ CL/F and $t_{1/2el}$) and MRT of LTG were also similar in both groups (*G. cambogia* extract *versus* vehicle).

3.2. Effects of repeated-dose pre-treatment with G. cambogia extract on LTG pharmacokinetics

The effects of the repeated administration of *G. cambogia* extract (821 mg/kg) during 14 days followed by a single-oral administration of LTG (10 mg/kg) on the 15th day can be observed from the mean plasma concentration-time profiles obtained in rats (n = 6), which are depicted in Fig. 2. The corresponding pharmacokinetic parameters either directly obtained from the experimental data or estimated by non-compartmental analysis are shown in Table 2. From the mean plasma concentration-time profiles, it is clear that substantially lower concentrations of LTG were obtained in the group of rats pre-treated with *G. cambogia* (experimental group) compared with the vehicle (control) group; nevertheless, the differences observed over time in the pharmacokinetic profiles of LTG were not statistically significant at any time point ($p > 0.05$) (Fig. 2). Analyzing the pharmacokinetic parameters, it is evident that the repeated administration of *G. cambogia* extract produced a marked reduction of the C_{max} of LTG, which was reduced by 1.5-fold ($p < 0.01$); differences were also found in the median t_{max} values estimated for the experimental (3.0 h) and control groups (3.0 h *versus* 16.0 h, respectively) reflecting a 5-fold change but without statistical significance ($p = 0.0758$). The extent of systemic exposure of LTG was diminished by 1.3-fold (AUC_{0-24}), 1.5-fold (AUC_{0-t}) and 1.5-fold ($AUC_{0-\infty}$) in the group of rats pre-treated with *G. cambogia*; however, no statistical differences were detected between the experimental and control groups ($p = 0.0931$ for AUC_{0-24}; $p = 0.0649$ for AUC_{0-t} and for $AUC_{0-\infty}$). Relatively to the other pharmacokinetic parameters, a statistically significant increase in apparent volume of distribution expressed as V_d/F ($p < 0.05$) was observed. Additionally,

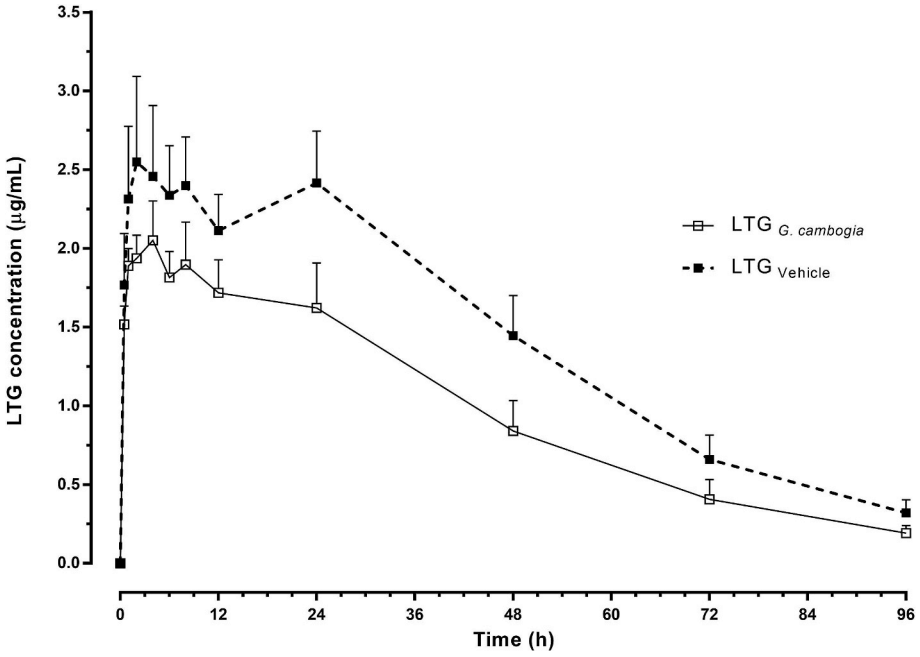

Fig. 2. Mean plasma concentration-time profiles of lamotrigine (LTG) obtained, over a period of 96 h, from rats submitted to a 14-day pre-treatment period with *Garcinia cambogia* extract (821 mg/kg/day, p.o) or vehicle of the extract (water) and treated on the 15th day with a single-dose of LTG (10 mg/kg, p.o) by oral gavage. Symbols represent the mean values ± standard error of the mean (SEM) of six determinations per time point (n = 6).

Table 2
Pharmacokinetic parameters estimated by non-compartmental analysis of the plasma concentration-time profiles of lamotrigine (LTG) obtained in rats submitted to a 14-day pre-treatment period with *G. cambogia* extract (821 mg/kg, p.o.) or vehicle of the extract (water) and treated on the 15th day with a single-dose of LTG (10 mg/kg, p.o.) by oral gavage ($n = 6$). Data are presented as median plus percentiles (25th percentile; 75th percentile).

Parameter	Experimental Group LTG $_{G.\ cambogia}$	Control Group LTG $_{Vehicle}$
C_{max} (μg/mL)	2.202 (1.853; 2.720) **	3.083 (2.999; 3.788)
t_{max} (h)	3.0 (1.0; 5.0)	16.0 (3.5; 24.0)
$AUC_{0\text{-}24}$ (μg.h/mL)	38.990 (33.975; 53.023)	55.725 (45.513; 62.258)
$AUC_{0\text{-}t}$ (μg.h/mL)	99.000 (60.488; 117.386)	140.596 (109.422; 170.568)
$AUC_{0\text{-}\infty}$ (μg.h/mL)	104.244 (66.260; 127.561)	149.888 (118.981; 186.724)
k_{el} (1/h)	0.031 (0.025; 0.044)	0.029 (0.027; 0.048)
$t_{1/2el}$ (h)	22.4 (15.9; 28.1)	24.1 (14.5; 25.5)
CL/F (mL/h/kg)	96.706 (79.515; 150.628)	67.251 (53.832; 88.203)
V_d/F (mL/kg)	3122.612 (2304.103; 4548.422) *	2008.346 (1745.135; 2483.958)
$AUMC_{0\text{-}\infty}$ (h.h.μg/mL)	3918.110 (2797.497; 5486.563)	6232.320 (4148.321; 8045.681)
MRT (h)	35.6 (31.2; 49.2)	40.9 (33.2; 43.5)

AUC, area under the concentration-time curve; $AUC_{0\text{-}24}$, AUC from time zero to 24 h; $AUC_{0\text{-}t}$, AUC from time zero to the last measurable concentration; $AUC_{0\text{-}\infty}$, AUC from time zero to infinite; $AUMC_{0\text{-}\infty}$, area under the first moment of the concentration-time curve from zero to infinity; CL/F, apparent clearance; C_{max}, peak concentration; k_{el}, apparent elimination rate constant; MRT, mean residence time; $t_{1/2el}$, elimination half-life; t_{max}, time to reach C_{max}; V_d/F, apparent volume of distribution. *$p < 0.05$ and **$p < 0.01$, significantly different from the control (vehicle) group.

no statistically significant differences were found in $AUMC_{0\text{-}\infty}$ neither in the other elimination phase parameters (k_{el}, CL/F, $t_{1/2el}$ and MRT) between both groups.

3.3. Effects of repeated-dose administration of G. cambogia extract on body weight

The effects of *G. cambogia* extract on the body weight of rats treated during 14 consecutive days are presented in Fig. 3. The rats of both control and experimental groups had a similar body weight at the beginning of the study (day 1). From the analysis of the results, it was observed a statistically significant increase in the body weight of the rats between day 1 and day 14 in both experimental (*G. cambogia*) and control (vehicle) groups ($p < 0.005$); however, there were no statistically significant differences in the body weight gain between both groups.

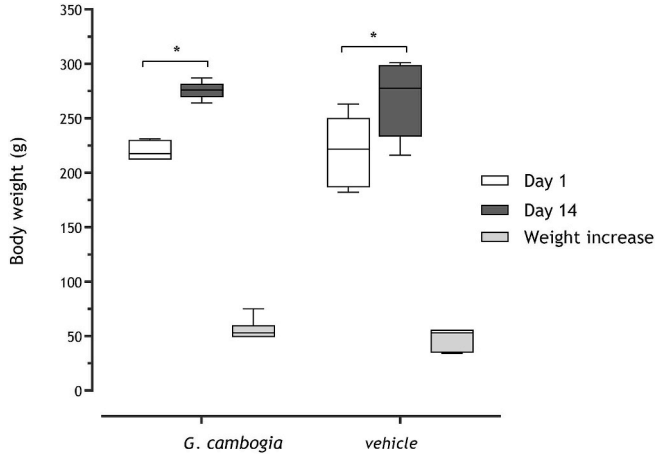

Fig. 3. Effects of *Garcinia cambogia* extract on the body weight of rats after a 14-day treatment period with *Garcinia cambogia* extract (821 mg/kg/day, p.o.) or vehicle (water) by oral gavage. Data are presented as box format representing the median plus percentiles (25th percentile; 75th percentile), with whiskers representing the lowest and highest value of six determinations ($n = 6$). *$p < 0.05$, day 1 *versus* day 14.

4. Discussion

Safety assessment of herbal supplements is of utmost importance particularly when such supplements are administrated with conventional drugs. In fact, some authors argue that herb-drug interactions are theoretically more prone to occur than drug-drug interactions due to its more complex phytochemical composition (Izzo et al., 2016). However, the lack of rigorous scientific information available regarding the clinical significance of herb-drug interactions entails difficulties for health professionals and consumers in making rational decisions about the safety of the combination of herbal medicinal supplements and drugs (Zhang et al., 2017). In the particular case of *G. cambogia* supplements, data on their safety have been controversial. On the one hand, there are several case reports in the literature describing episodes of severe toxicity associated with the consumption of *G. cambogia* supplements. For instance, Lopez et al. (2014) reported a case of suspected serotonin toxicity in a patient under stable therapeutic dosing of escitalopram (a serotonin reuptake inhibitor) after the addition of a nutritional supplement containing *G. cambogia* (Lopez et al., 2014). In addition, several case-reports of (hypo)mania and/or psychosis following the administration of *G. cambogia*-containing products have been published (Cotovio and Oliveira-Maia, 2016; Nguyen et al., 2017). On the other hand, Chuah et al. (2012) reviewed the results of seventeen clinical studies in which the safety of HCA and related supplements for human consumption was demonstrated, inclusively no adverse effects were observed at levels up to 2800 mg/day of HCA.

LTG interactions with other AEDs or/and co-prescribed drugs have been documented (Johannessen and Landmark, 2010; Patsalos, 2013a, b; Zaccara and Perucca, 2014). However, scarce information is available about LTG interactions with herbs. In particular, an herb-drug interaction between ginseng and LTG was reported, suggesting that the inhibition of UGT2B7 by ginseng constituents predisposed the patient to a drug hypersensitivity reaction (Myers et al., 2015). Another herb-drug interaction involving LTG was recently identified by our research group, where the simultaneous co-administration of *Paullinia cupana* and LTG resulted in a significant decrease of C_{max} and $AUC_{0\text{-}24}$ of LTG (Ventura et al., 2018).

Overall, the data obtained in the current work regarding the effects of *G. cambogia* extract on the pharmacokinetics of LTG did not raise major concerns related to the occurrence of important herb-drug interactions. Indeed, we have designed the co-administration study to

investigate the potential effects of *G. cambogia* extract on the gastrointestinal absorption and consequently on the extent of systemic bioavailability of drug, and no statistically significant differences were observed. Despite this, the co-administration of *G. cambogia* and LTG showed a slight tendency for a decrease of the C_{max} values and for a delay in the t_{max}, which are not expected to compromise the efficacy of LTG, and so, the co-administration *G. cambogia* extract and LTG is unlikely to be clinically relevant. Additionally, no significant changes have been observed in the extent of systemic exposure (as assessed by AUCs) and in the elimination pharmacokinetic parameters. On the other hand, the results of the study of the repeated administration of *G. cambogia* extract for 14 days showed a higher impact on median C_{max} values of LTG, which were lower in the experimental group comparatively to the control group. Additionally, although a decrease in the extent of systemic drug exposure had been observed following the repeated treatment with *G. cambogia* extract, no statistically significant differences were found between both groups. Also, no differences were observed in the elimination pharmacokinetic parameters. Considering that LTG undergoes hepatic elimination susceptible to enzyme modulation and knowing that induction mechanisms are time-dependent, the results observed in this specific study suggest that *G. cambogia* has no marked inducing effects on the LTG metabolism. Even so, it was indeed observed a tendency of reduction of the AUC values (which are closely related to the extent of systemic exposure and so to the efficacy of the drug) and a significant decrease in the C_{max} (which in turn is closely related to the rate of absorption). Thus, although no toxic effects are expected from the administration of LTG and *G. cambogia* extracts or supplements, in terms of efficacy the potential impact of the reduction of the C_{max} of LTG should not be completely neglected.

The 14-day treatment period with *G. cambogia* extract did not show a significant effect on the body weight of rats, which was somewhat unexpected given the uses claimed for *G. cambogia*-containing supplements. However, other non-clinical studies conducted in mice also found no significant effects on the body weight of animals after *G. cambogia* administration for four (Hayamizu et al., 2003) and sixteen weeks (Kim et al., 2013). On the contrary, in the study of Sripradha and Magadi (2015), *G. cambogia* administered at 400 mg/kg during ten weeks significantly decreased the body weight gain in male Wistar rats fed with high-fat diet. Similarly, some clinical studies have demonstrated that *G. cambogia* has significant effects on body weight management when administered for periods longer than two weeks (Chuah et al., 2013).

Furthermore, considering a critical discussion of the experimental protocol defined in these studies, the possible interference of pentobarbital (anesthetic agent) was tested in selectivity assays during the validation of the bioanalytical method and no interference in the analysis of LTG was observed. In addition, the pentobarbital was administered on both groups of animals (experimental and control groups) only on the night before LTG administration for insertion of a polyurethane cannula in a lateral tail vein of rats and, therefore, from a pharmacokinetic perspective, it is expected that pentobarbital has been mostly eliminated overnight because in the next morning all animals had normal activity moving freely and without any sign of being anesthetized. Regarding the influence of body fat on systemic distribution, this effect, if present, will certainly have a negligible influence because the animals had a similar baseline weight and the weight change was similar in both groups throughout the pre-treatment study. In terms of nutritional status, no especial monitoring was done, and the rats had free access to a standard rodent diet (Mucedola 4RF21 Settimo Milanese, Milan, Italy), with only few restrictions: an overnight fasting period, with free access to water, before LTG administration, and to avoid the effect of food on LTG absorption and disposition, the fasting period was also maintained for 4 h after drug administration.

At this point, it should be also highlighted that in future herb-drug interaction studies with *G. cambogia*-containing products, the quantification of HCA in blood could be useful in order to understand its pharmacokinetic properties and its influence on drug biodisposition as this phytochemical is the main constituent of *G. cambogia* extracts.

5. Conclusion

Based on the findings achieved in this non-clinical work, no significant changes were observed on the pharmacokinetics of LTG in Wistar rats after the co-administration of the drug and the *G. cambogia* extract, and so no clinically relevant pharmacokinetic-based herb-drug interactions are expected. When considering the repeated administration of the herbal extract and LTG, and considering the effects observed on LTG absorption, the therapeutic drug monitoring of LTG may be important to ensure its efficacy. Nevertheless, in order to generate more robust and reliable clinical evidence, it would be useful to perform a clinical trial specifically designed to assess the safety of the administration of *G. cambogia* extract and LTG.

Conflicts of interest

The authors have declared no conflict of interests.

Acknowledgements

The authors acknowledge the support provided by FEDER funds through the POCI - COMPETE 2020 - Operational Programme Competitiveness and Internationalisation in Axis I - Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT - Foundation for Science and Technology (Project UID/Multi/00709/2013).

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.03.051.

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