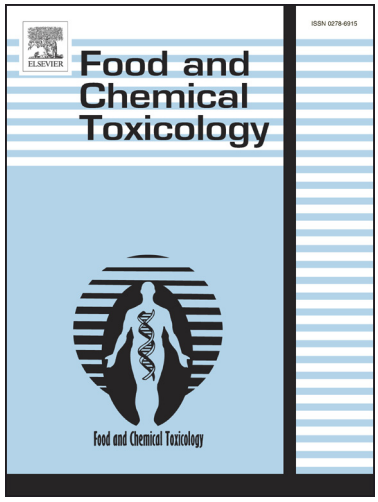

Investigating herb–drug interactions: the effect of Citrus aurantium fruit extract on the pharmacokinetics of amiodarone in rats

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Abstract

Citrus aurantium extract has been largely used in weight loss and sports performance dietary supplements. However, the safety of *C. aurantium*-containing products has been questioned mainly due to the association of its use with adverse events in the cardiovascular system. Therefore, this work aimed to assess the potential for herb-drug interactions among a standardized *C. aurantium* extract (GMP certificate) and amiodarone (narrow therapeutic index drug) in rats. In a first pharmacokinetic study, rats were simultaneously co-administered with a single-dose of *C. aurantium* (164 mg/kg, p.o.) and amiodarone (50 mg/kg, p.o.); in a second study, rats were pre-treated during 14 days with *C. aurantium* (164 mg/kg/day, p.o.) and received amiodarone (50 mg/kg, p.o.) on the 15th day. Rats of the control groups received the corresponding volume of vehicle. Overall, after analysis of the pharmacokinetic data, it deserves to be highlighted the significant increase of the peak plasma concentration of amiodarone in rats pre-treated with *C. aurantium* extract, while the extent of systemic exposure was comparable between both groups. This paper reports, for the first time, data on the potential of herb-drug interaction between *C. aurantium* extract and amiodarone. However, specific clinical trials should be performed to confirm these results in humans.

Keywords: Amiodarone; *Citrus aurantium*; Herb-drug interaction; Pharmacokinetics; Rats.

Abbreviations

AM, amiodarone; **AUC**, area under the concentration-time curve; **AUC_{0-t}**, AUC from time zero to the last sampling time; **$AUC_{0-\infty}$**, AUC from time zero to infinite; **C_{last}**, last quantifiable concentration; **C_{max}**, peak concentration; **CYP**, cytochrome P450; **FDA**, Food and Drug Administration; **i.p.**, intraperitoneal; **k_{el}**, apparent terminal rate constant; **LOQ**, limit of quantification; **MDEA**, mono-*N*-desethylamiodarone; **MRT**, mean residence time; **P-gp**, P-glycoprotein; **SD**, standard deviation; **$t_{1/2el}$**, apparent terminal elimination half-life; **t_{max}**, time to reach C_{max}; UV, ultraviolet detection.

1. Introduction

Citrus aurantium, previously called as *Fructus aurantii*, is the botanical name of a plant commonly known as bitter orange, sour orange, green orange, Seville orange or zhi shi (Bouchard et al., 2005; Haaz et al., 2006). The extract of the immature fruit or peel of *C. aurantium* has been widely used in weight loss dietary supplements and in sports performance products (Stohs et al., 2011a). In particular, after the ban of the sale of all ephedra-containing supplements by the Food and Drug Administration (FDA) in 2004, *C. aurantium* has gained an additional popularity as a safe alternative to *Ephedra* in herbal weight loss products (FDA and HHS, 2004; Hansen et al., 2012; Hansen et al., 2013). *C. aurantium* has been used as an ingredient of the dietary supplements marketed for weight loss aid due to its claimed effects on metabolism, increasing the basal metabolic rate and lipolysis, and also as appetite suppressant (Stohs et al., 2012). However, *C. aurantium* has not been traditionally employed for weight loss (Haaz et al., 2006). Historically, this herb has been mainly used in traditional Chinese medicine to treat gastrointestinal disorders like abdominal distension, dysentery and constipation (Mattoli et al., 2005; Stohs et al., 2011a).

Synephrine, also called *p*-synephrine or oxedrine, is considered to be the main pharmacologically active protoalkaloid present in the extracts of immature fruit or peel of *C. aurantium*, which comprises more than 85% of the total protoalkaloid content. Additionally, other minor protoalkaloids constituents include the biogenic amines octopamine, hordenine, tyramine and *N*-methyltyramine (Fugh-Berman and Myers, 2004; Stohs et al., 2011a; Hansen et al., 2012). Structurally, synephrine is closely related to ephedrine (one of the main active constituents found in the genus *Ephedra*) (Hansen et al., 2012; Hansen et al., 2013). However, despite their great structural similarities, synephrine contrary to ephedrine seems to exhibit little or no stimulant activity on the cardiovascular and central nervous system; the small chemical differences between synephrine and ephedrine also appear to significantly change their pharmacokinetic properties, particularly their ability to cross the blood-brain barrier (Stohs et al., 2011b). Based on receptor binding studies, synephrine exhibited poor affinity for β-1, β-2 and α-adrenoreceptors, which are usually associated with cardiovascular effects (particularly β-1 and α-adrenoreceptors); instead, synephrine showed β-3 adrenergic activity which is responsible for

increased thermogenesis and lipolysis (Stohs et al., 2011a). Even so, the safety and efficacy of supplements containing *C. aurantium* have been questioned (Bent et al., 2004; Fugh-Berman and Myers, 2004).

A large number of case reports have emerged over the last years associating the use of *C. aurantium*-containing products with serious clinical adverse events, most of them involving the cardiovascular system, such as syncope and prolongation of the QT interval (Nasir et al., 2004), myocardial infarction (Nykamp et al., 2004; Thomas et al., 2009), ischemic stroke (Bouchard et al., 2005), angina (Gange et al., 2006) and tachycardia (Firenzuoli et al., 2005), bradycardia and hypotension (Gray and Woolf, 2005), vasospasm and stroke (Holmes Jr. and Tavee, 2008), ventricular fibrillation (Stephensen and Sarlay Jr., 2009) and ischemic colitis (Sultan et al., 2006). Furthermore, a pharmacokinetic herb-drug interaction involving a decoction of *C. aurantium* and cyclosporine was also reported (Hou et al., 2000).

Hence, considering that *C. aurantium* has been extensively used as a replacement of *Ephedra* in the composition of weight loss dietary supplements and considering that the obesity and overweight are increasing at an alarming rate (Pittler et al., 2005), representing major independent risk factors for cardiovascular diseases (Scaglione et al., 2004; Bodary et al., 2007; Zalesin et al., 2011), an increase on the consumption of herbal supplements containing *C. aurantium* is still expected. Thus, as the concurrent use of these herbal products and conventional drugs may lead to significant clinical herb-drug interactions, it is therefore absolutely pertinent to investigate the potential for pharmacokinetic interactions between *C. aurantium* and narrow therapeutic index drugs, as is the case of amiodarone.

Amiodarone [2-*n*-butyl-3-(3,5-diiodo-4-diethylaminoethoxy-benzoyl)-benzofuran; (Fig. 1)] still remains one of the most widely prescribed antiarrhythmic drugs for the treatment of atrial fibrillation and ventricular arrhythmias despite the availability of novel antiarrhythmic agents (Papiris et al., 2008). From a pharmacological viewpoint amiodarone has unusual and complex pharmacokinetic properties (Shayeganpour et al., 2008; van Herendael and Dorian, 2010). Amiodarone has a variable oral bioavailability (20-80%) and the great lipophilicity of amiodarone and its main metabolite [mono-*N*-desethylamiodarone (MDEA)] (Fig. 1) leads to a high volume of distribution and a variable accumulation into tissues (van Herendael and Dorian, 2010).

MDEA is the product of the most predominant metabolic route of amiodarone, the *N*-deethylation which is catalyzed by cytochrome P450 (CYP) isoenzymes (Trivier et al., 1993; Soyama et al., 2002). Moreover, amiodarone is recognised as a drug of narrow therapeutic window (0.5–2.0 μg/mL) (Pérez-Ruiz et al., 2002; Shayeganpour et al., 2008) and it has also been associated to important clinical drug interactions (Siddoway, 2003; Edwin et al., 2010; Karimi et al., 2010; Roughead et al., 2010).

Taking into account all the reasons previously referred and bearing in mind the potential for co-administration of *C. aurantium* medicinal products and amiodarone, this work was planned to investigate if a standardized extract of the green fruit of *C. aurantium* may influence the pharmacokinetics of amiodarone in rats, following their simultaneous oral co-administration, and after a 14-day *C. aurantium* pre-treatment period.

2. Materials and methods

2.1. Drugs and materials

C. aurantium hidroalcoholic extract 10% synephrine (11.1% synephrine by high-performance liquid chromatography-ultraviolet detection batch analysis; approximately 94% of the total content in potent amines consists of synephrine) obtained from green fruit was purchased from Bio Serae Laboratories (Bram, France); the corresponding certificate of analysis ref. 410039 (batch 0907799) is provided as *Supplementary data*. Carboxymethylcellulose sodium salt for preparation of extract suspension was obtained from Sigma (St. Louis, MO, USA). A commercial formulation (ampoules) of amiodarone hydrochloride 50 mg/mL solution for intravenous injection was used for oral administration to rats after appropriate dilution with 5% glucose intravenous solution for infusion (B. Braun Medical, Portugal). Other compounds used were sodium chloride 0.9% solution for injection (Labesfal, Portugal); heparin sodium 5000 U.I./mL for injection (B. Braun Medical, Portugal); ketamine for injection (Imalgene 1000) and xylazine for injection (Vetaxilaze 20). Introcan® Certo IV indwelling cannula (22G; 0.9 x 2.5 mm) made of polyurethane from B. Braun Melsungen AG (Melsungen, Germany).

2.2. Animals

Adult male Wistar rats (361 ± 26 g) of approximately 10 weeks old were obtained from local animal facilities (Faculty of Health Sciences of the University of Beira Interior, Covilhã, Portugal). The rats were maintained under controlled environmental conditions (temperature 20 ± 2 ºC; relative humidity 55 ± 5%; 12-h light/dark cycle). The animals were allowed free access to a standard rodent diet (4RF21, Mucedola, Italy) during almost all experimental procedures and tap water was available *ad libitum*. At night on the day before dosing with amiodarone, a lateral tail vein of each rat was cannulated, under anaesthesia [ketamine (90 mg/kg)/xylazine (10 mg/kg); i.p. injection], by insertion of an Introcan® Certo IV indwelling cannula (22G; 0.9 x 2.5 mm) used for serial blood sampling. The rats fully recovered from anaesthesia overnight and were fasted for 12-14 h before amiodarone administration and maintained with free access to water; in order to avoid the effect of food on the oral bioavailability of amiodarone an additional fasting period was considered (4 h post-dose). Oral treatments of the rats with *C. aurantium* extract and amiodarone were performed by gavage. Blood sampling was conducted in conscious and freely moving rats, which were appropriately restrained only at the moment of blood collection, except for the last blood sampling that was taken by a terminal procedure (decapitation and exsanguination under anaesthesia). All the animal experiments were conducted in accordance with the European Directive (2010/63/EU) for the accommodation and care of laboratory animals and the experimental procedures were reviewed and approved by the Portuguese Veterinary General Division.

2.3. Experimental design and pharmacokinetic studies

Two separate and independent pharmacokinetic studies were designed to investigate the effects of *C. aurantium* on the kinetics of amiodarone: (1) a single oral co-administration study of *C. aurantium* extract and amiodarone; and (2) a 14-day repeated oral pre-treatment study with *C. aurantium* extract and on the 15th day a single oral dose of amiodarone was given. The dose of *C. aurantium* was selected based on the dose recommended to humans by the supplier of the extract (Bio Serae Laboratories) and taking into account the FDA Guidance for Industry on conversion of animal doses to human equivalent doses, which considers the body surface area (US DHHS, FDA, CDER, 2005); additionally, a 10-fold potentiating interaction factor was

applied. The experimental dose of herbal extract selected is greater than the typical human daily dose to avoid false negative results and considering potential differences in the extrapolation between species (rat *versus* human). On the other hand, the single oral dose of amiodarone (50 mg/kg) was established because it has provided plasma concentrations of amiodarone in rats within the plasma therapeutic range (Shayeganpour et al., 2005). In each day of the experiments *C. aurantium* extract was suspended in 0.5% carboxymethylcellulose aqueous solution affording a suspension of herbal extract at 16.4 mg/mL. Amiodarone commercial injectable solution (50 mg/mL) was also appropriately diluted with 5% glucose solution to extemporaneously prepare an amiodarone solution at 12.5 mg/mL. Appropriate volumes of *C. aurantium* extract suspension (10 mL/kg of body weight) and of amiodarone solution (4 mL/kg of body weight) were orally administered to rats by oral gavage.

In the first pharmacokinetic study, twelve Wistar rats were randomly divided into two groups (experimental and control groups). The rats of the experimental group (n = 6) were concomitantly treated with a single-dose of *C. aurantium* extract (164 mg/kg, p.o.) and a single-dose of amiodarone (50 mg/kg, p.o.); the extract suspension was administered just before amiodarone. The rats of the control group (n = 6) received, instead of the *C. aurantium* extract suspension, the corresponding volume of 0.5% carboxymethylcellulose aqueous solution (vehicle of the extract).

In the second pharmacokinetic study, twelve Wistar rats were also randomly divided into two groups. The rats assigned to the experimental group (n = 6) were orally pre-treated with *C. aurantium* extract (164 mg/kg, p.o.) once daily for 14 consecutive days (short-term repeated dose pre-treatment study), whereas the rats allocated to the control group (n = 6) were administered with an equivalent volume of vehicle for the same period of time. During the pre-treatment period, the rats were kept in 12-h light/dark cycle animal room with controlled temperature and humidity, as indicated above (see *Section 2.2.*); free access to a standard rodent diet and tap water was allowed. On 15th day, the rats of both groups (experimental and control) were gavaged with a single-dose of amiodarone (50 mg/kg, p.o.).

In both pharmacokinetic studies the treatments with *C. aurantium* extract (or vehicle) and/or amiodarone were always carried out on the morning between 9:00 am and 11:45 am. At night

on the day before amiodarone administration, the rats were anaesthetized for cannulation of a lateral tail vein and were fasted overnight as described above (see *Section 2.2.*). On the day after, multiple serial blood samples (approximately 0.3 mL) were collected through the cannula into heparinized tubes before dosing and at 0.25, 0.5, 1, 2, 4, 6, 8 and 12 h following amiodarone administration; the blood samples were collected from all 6 rats in each treatment group at each time-point. Then, at 24 h post-dose, blood and tissues (heart, liver, kidneys and lungs) were also harvested after decapitation of the rats. The blood samples were centrifuged at 4000 rpm for 10 min (4 ºC) to separate the plasma which was stored at –20 ºC until analysis. After exsanguination, liver, kidneys, heart and lungs were excised and stored at –20 ºC; the organs were weighed and homogenized in distilled water (3 mL of water per gram of tissue) before analysis of tissue homogenates samples.

2.4. Drug analysis

Plasma and tissue concentrations of amiodarone and its main metabolite MDEA were determined by using a liquid-liquid extraction procedure coupled to the high-performance liquid chromatography-diode array detection assay previously developed and validated (Rodrigues et al., 2013a).

2.5. Pharmacokinetic analysis

The plasma concentration *versus* time data for amiodarone and MDEA obtained from each individual rat were submitted to a non-compartmental pharmacokinetic analysis using the WinNonlin® version 4.1 (Pharsight Co, Mountain View, CA, USA). The peak concentrations of amiodarone and MDEA in plasma (C_{max}) and the time to reach C_{max} (t_{max}) were directly obtained from the experimental data. Other pharmacokinetic parameters estimated from the individual plasma concentration-time profiles were: area under the concentration-time curve (AUC) from time zero to the last sampling time at which concentrations were at or above the limit of quantification (LOQ; 0.100 µg/mL) of the method (AUC_{0-t}), calculated by the linear trapezoidal rule; AUC from time zero to infinite ($AUC_{0-\infty}$), calculated from $AUC_{0-t} + (C_{last}/k_{el})$, where C_{last} is the last quantifiable concentration and k_{el} is the apparent terminal elimination rate constant

calculated by log-linear regression of the terminal segment of the concentration-time profile; apparent terminal elimination half-life ($t_{1/2el}$) and mean residence time (MRT). The concentrations lower than the LOQ of the assay were taken as zero for all calculations.

2.6. Short-term repeated dose effect of C. aurantium on body weight

In the pre-treatment study (a 14-day *C. aurantium* treatment period), the body weight of the rats administered with *C. aurantium* extract (164 mg/kg/day, p.o.; experimental group) or vehicle (control group) was adequately registered on the first day and on the last day (14^{th}) of the treatments in order to evaluate the effect of *C. aurantium* extract on body weight changes.

2.7. Statistical analysis

Data were reported as the mean ± standard deviation (SD). Comparisons between two groups were usually performed using unpaired two-tailed Student's *t*-test; for body weight comparisons within the same group the paired Student's *t*-test was employed. A difference was considered to be statistically significant for a *p*-value lower than 0.05 ($p < 0.05$).

3. Results

3.1. Simultaneous co-administration of C. aurantium and amiodarone

The mean plasma concentration-time profiles ($n = 6$) of amiodarone and its main metabolite (MDEA) obtained after the intragastric simultaneous co-administration of rats with a single-dose of *C. aurantium* extract (164 mg/kg, p.o.) or vehicle (control group) and a single-dose of amiodarone (50 mg/kg, p.o.) are shown in Figure 2. Overall, the mean plasma concentrations of amiodarone were found to be statistically different only at 24 h post-dose ($p < 0.001$). In the case of MDEA, the plasma concentrations were similar in both groups, with values near or below the LOQ (0.100 µg/mL) of the method. The mean plasma pharmacokinetic parameters estimated for amiodarone and MDEA after non-compartmental analysis of each individual concentration-time profile are summarized in Table 1. From the observation of mean plasma concentration-time profiles of amiodarone (Fig. 2), it is evident that the time to reach C_{max} (t_{max}) was attained later in the experimental (*C. aurantium*) group than in the control (vehicle) group.

However, no statistically significant differences in the mean pharmacokinetic parameters for amiodarone were detected among the two groups (*C. aurantium versus* vehicle) (Table 1). These data show that the treatment of rats with *C. aurantium* extract does not significantly affect the extent of systemic exposure to amiodarone (as assessed by AUC), but the maximum exposure to the drug is delayed. Considering the scarcity of quantifiable plasma concentrations obtained for MDEA in both groups, only the C_{max} and t_{max} pharmacokinetic parameters are presented in Table 1.

To examine the biodistribution of amiodarone and MDEA in rats, either in the presence or absence of the co-administration with *C. aurantium*, at 24 h after dosing all animals were sacrificed and several organs were excised and analysed. The mean concentrations of amiodarone and MDEA determined in heart, lung, liver and kidney tissues, and also the plasma concentrations at the same time (24 h), are shown in Figure 3. The tissue concentrations of amiodarone and MDEA were markedly higher than those measured in plasma, and the concentration levels found for both compounds (amiodarone and MDEA) in the lung tissue were absolutely noteworthy. However, despite the significant differences detected at 24 h post-dose for amiodarone plasma concentrations ($p < 0.001$), at the same time only for heart tissue were also found statistically significant differences ($p < 0.05$), and the concentrations of amiodarone were higher in the group of rats treated with the extract.

3.2. Short-term repeated dose pre-treatment study with C. aurantium followed by amiodarone administration

The rats were administered for 14 days with *C. aurantium* extract (164 mg/kg/day, p.o.; experimental group) or vehicle (control group) in order to investigate a possible interference of the short-term repeated dose pre-treatment with the extract on the pharmacokinetics of amiodarone. The day after the last treatment with *C. aurantium* extract or vehicle, all animals received a single-oral dose of 50 mg/kg amiodarone and the mean plasma concentration-time profiles ($n = 6$) of the drug and its main metabolite (MDEA) are depicted in Figure 4. Comparing the mean plasma concentrations for amiodarone in both groups, they were found to be statistically different only at 2 h post-dose ($p < 0.05$), and they were higher in the group treated

with *C. aurantium*. The mean plasma pharmacokinetic parameters for amiodarone and MDEA determined by applying non-compartmental analysis to each individual concentration-time profile are listed in Table 2. The pre-treatment with *C. aurantium* extract determined a significantly higher C_{max} value for amiodarone ($p < 0.01$), while for the other pharmacokinetic parameters no significant differences were found between both groups. Hence, from the data shown in Table 2, it is evident that the degree of systemic exposure to amiodarone (as assessed by C_{max}) is higher in the rats pre-treated with *C. aurantium* extract comparatively with those of the control group, whereas the extent of systemic exposure to amiodarone is similar among experimental and control groups (ratios near to unity; as assessed by AUC_{0-t} and $AUC_{0-\infty}$). The plasma concentrations of MDEA were near or below the LOQ (0.100 µg/mL) of the method in both groups.

To investigate the influence of a 14-day pre-treatment period with *C. aurantium* extract (experimental group) or vehicle (control group) on the distribution and metabolism of amiodarone in rats, the concentrations of amiodarone and its major metabolite (MDEA) were also determined in various tissues (additionally to plasma) at 24 h post-dose and the results are shown in Figure 5. The concentrations of both compounds (amiodarone and MDEA) in tissues were manifestly greater than those measured in plasma, and the concentration levels found in lung tissue were extremely high in both experimental (*C. aurantium*) and control (vehicle) groups. However, at 24 h post-dose, no significant differences were observed for amiodarone and MDEA concentrations between both groups (AM_{Citrus} *versus* $AM_{Vehicle}$).

3.3. Short-term repeated dose effect of C. aurantium on body weight

In the rats submitted to a 14-day pre-treatment period with *C. aurantium* extract (164 mg/kg/day, p.o) or vehicle was found a statistically significant increase in body weight in both groups. In the group of rats treated with *C. aurantium* extract was observed an increase of the body weight from 366.17 ± 18.76 g to 379.00 ± 21.93 g ($p < 0.05$) and in the control (vehicle) group the body weight of the rats increased from 355.33 ± 28.45 g to 367.67 ± 23.51 g ($p < 0.01$) between the 1st and 14th day of the experiments. Furthermore, the increase in body weight of the rats of both groups (*C. aurantium versus* vehicle) was comparable (12.83 ± 8.13 g *versus* 12.33 ± 6.41 g, *p*

> 0.05). Hence, under these experimental conditions, the *C. aurantium* extract was shown to be ineffective to control the body weight gain in rats.

4. Discussion

Over the last years several interactions have been reported in literature describing the interference of herbal products and other compounds on the pharmacokinetics of amiodarone. For instance, the grapefruit juice dramatically inhibited the metabolism of amiodarone (Libersa et al., 2000), the co-administration of orlistat and amiodarone significantly reduced the systemic exposure to amiodarone and its main metabolite MDEA (Zhi et al., 2003), the exposure of rats to *β*-naphthoflavone (a polycyclic aromatic hydrocarbon) was found to increase the formation of MDEA probably through CYP induction (Elsherbiny et al., 2010) and, more recently, our research group documented the occurrence of herb-drug interactions between *Fucus vesiculosus* extract or *Paullinia cupana* extract and amiodarone in rats (Rodrigues et al., 2012; Rodrigues et al., 2013b).

Accordingly, the present work was delineated to investigate the potential of interaction between *C. aurantium* extract and amiodarone *in vivo*, using adult male Wistar rats. The pharmacokinetic studies herein reported were designed to examine the interference of *C. aurantium* extract on the gastrointestinal absorption (simultaneous co-administration study) and on the metabolism of amiodarone (14-day *C. aurantium* pre-treatment study). In fact, drug-drug or herb-drug interactions mainly occur at the level of absorption process and/or metabolic (inhibition or induction) pathways.

Overall, our results show that the simultaneous co-administration of a single-dose of *C. aurantium* extract and amiodarone caused an apparent delay to reach the peak plasma concentration of the drug, but it did not change significantly the level and the extent of systemic exposure to amiodarone (as assessed by C_{max} and $AUC_{0\text{-}t,}$ respectively). This increase in the time to achieve C_{max} is not expected to alter the efficacy of amiodarone and it is unlikely to be clinically important. At this point, it deserves to be mentioned that after the co-administration of amiodarone with other herbal extracts (*Fucus vesiculosus* extract and *Paullinia cupana* extract), which are also claimed to be useful for weight loss, a delay in the time to reach C_{max} was also

observed; however, in these studies a significant decrease in the systemic exposure to amiodarone was clearly evident in the rats treated with herbal extracts (Rodrigues et al., 2012; Rodrigues et al., 2013b).

Moreover, because of the central role that the induction of CYPs and P-glycoprotein (P-gp) plays on drug-drug and herb-drug interactions, and bearing in mind that the induction mechanisms are time-dependent, the interference of *C. aurantium* extract on the pharmacokinetics of amiodarone was also investigated by administering the extract for 14 consecutive days (164 mg/kg/day, p.o.) until 24 h before applying amiodarone. In fact, amiodarone is metabolized by several CYP isoenzymes including CYP1A1/2, CYP2C8, CYP2C19, CYP2D6 and CYP3A4 (Ohyama et al., 2000; Elsherbiny et al., 2010) and is a substrate of P-gp (Shapiro and Shear, 2002; Kalitsky-Szirtes et al., 2004). The only significant change was an increase in the level of systemic exposure to amiodarone (as assessed by C_{max}) in the rats pre-treated during 14 days with *C. aurantium* extract (*versus* vehicle). No significant differences were found in the extent of systemic drug exposure (as assessed by AUC_{0-t} and $AUC_{0-\infty}$). Therefore, to explain the increase of the C_{max} in the group of rats pre-treated with *C. aurantium* we hypothesise that the extract or some of its constituents could change (increase) the gastrointestinal motility, thus increasing the rate at which amiodarone passes to the intestine. Actually, the absorption of amiodarone across the intestinal membrane occurs by passive diffusion, which is a non-saturable process (Martín-Algarra et al., 1997). According to the studies of Jinzhao et al. (2005), the *C. aurantium* may improve the gastric emptying in rats with functional dyspepsia. Furthermore, extracts of *C. aurantium* also increased the rate of gastrointestinal motility enhancing, therefore, the absorption function of gastrointestinal tract (Li et al., 2007). In addition, *C. aurantium* can also antagonise the inhibition of the intestinal advance induced by atropine (Xue-Bao et al., 2005). In a study conducted by Fang et al. (2009) the effects of constituents of *C. aurantium* on the gastrointestinal movement were evaluated; the hesperidin had a stimulatory effect on the gastrointestinal muscle contraction while synephrine had an inhibitory effect.

Another possibility to explain the higher C_{max} values for amiodarone in the group of rats pre-treated with *C. aurantium* extract could be related to a time-dependent inhibitory effects induced

by *C. aurantium* or some of its phytochemicals on the CYPs and/or P-gp–mediated efflux activity. Actually, in a non-clinical study was reported that a decoction of *C. aurantium* increased the bioavailability of cyclosporine (Hou et al., 2000).

Based on the herb-drug interaction data obtained in the present work involving *C. aurantium* and amiodarone, it is suggested that the tested *C. aurantium* extract has no significant impact on the pharmacokinetic of amiodarone, even using an experimental dose of extract in rats higher than the typical recommended dose in humans. Even so, it should be taken in account that results from animal experiments cannot be directly extrapolated to humans; however, bearing in mind the studies of Shayeganpour et al. (2005) and Meng et al. (2001), the rat appears to be an appropriate animal model for man in this case. Nevertheless, to reliably assess the safety of the administration of *C. aurantium* extract and amiodarone specific clinical trials are required.

Additionally, in our study the increase of body weight of the rats pre-treated with *C. aurantium* extract or vehicle was comparable. These data are in accordance with other results found in the literature for *C. aurantium* extract. Arbo et al. (2008) also reported that the body weight gain did not change significantly comparing with the control group in rats treated during 28 days with 400, 2000 and 4000 mg/kg of *C. aurantium* extract. Only was observed a reduction in the gain of body weight for rats treated with 30 and 300 mg/kg of synephrine during 28 days (Arbo et al., 2008).

5. Conclusions

In conclusion, no significant effects were observed on the pharmacokinetics of amiodarone in rats after the simultaneous co-administration in single-dose of *C. aurantium* extract and amiodarone. On the other hand, following a 14-day pre-treatment period with *C. aurantium* extract a marked increase in the peak plasma concentrations of amiodarone was observed, but this did not show any significant impact in the extent of systemic exposure to amiodarone. Therefore, *C. aurantium* extract seems to have only minor effects on the pharmacokinetics of amiodarone in rats. However, it is important to be aware that this work only provides a non-

clinical proof of the effects of *C. aurantium* fruit extract on the pharmacokinetics of amiodarone. Thus, aiming at confirming these results in humans a thorough clinical trial is required.

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Conflicts of interest

The authors have declared no conflicts of interest.

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Figure captions

Fig. 1. Chemical structures of amiodarone (AM) and its major metabolite mono-*N*-desethylamiodarone (MDEA).

Fig. 2. Mean plasma concentration-time profiles of amiodarone (AM) and mono-*N*-desethylamiodarone (MDEA) obtained, over a period of 24 h, from rats simultaneously treated in single-dose with *Citrus aurantium* extract (164 mg/kg, p.o.), or vehicle (0.5% carboxymethylcellulose aqueous solution), and AM (50 mg/kg, p.o.) by oral gavage. Symbols represent the mean values ± standard deviation (SD) of six determinations per time point (n = 6). $^{\#}p < 0.001$ compared to control (vehicle).

Fig. 3. Mean plasma and tissue (heart, lung, liver and kidney) concentrations of amiodarone (AM) and mono-*N*-desethylamiodarone (MDEA) obtained, at 24 h post-dose, from rats simultaneously treated in single-dose with *Citrus aurantium* extract (164 mg/kg, p.o.), or vehicle (0.5% carboxymethylcellulose aqueous solution), and AM (50 mg/kg, p.o.) by oral gavage. Data are expressed as the mean values ± standard deviation (SD) of six determinations (n = 6). $^{*}p <$ 0.05 and $^{\#}p < 0.001$ compared to control (vehicle).

Fig. 4. Mean plasma concentration-time profiles of amiodarone (AM) and mono-*N*-desethylamiodarone (MDEA) obtained, over a period of 24 h, from rats submitted to a 14-day pre-treatment period with *Citrus aurantium* extract (164 mg/kg/day, p.o.), or vehicle (0.5% carboxymethylcellulose aqueous solution), and treated on the 15th day with a single-dose of AM (50 mg/kg, p.o.) by oral gavage (n = 6). Symbols represent the mean values ± standard deviation (SD) of six determinations per time point (n = 6). $^{*}p<0.05$ compared to control (vehicle).

Fig. 5. Mean plasma and tissue (heart, lung, liver and kidney) concentrations of amiodarone (AM) and mono-*N*-desethylamiodarone (MDEA) obtained, at 24 h post-dose, from rats submitted to a 14-day pre-treatment period with *Citrus aurantium* extract (164 mg/kg/day, p.o.),

or vehicle (0.5% carboxymethylcellulose aqueous solution), and treated on the 15th day with a single-dose of AM (50 mg/kg, p.o.) by oral gavage. Data are expressed as the mean values ± standard deviation (SD) of six determinations (n = 6).

Table 1 – Mean pharmacokinetic parameters estimated by non-compartmental analysis of the plasma concentration-time profiles of amiodarone (AM) and mono-*N*-desethylamiodarone (MDEA, major metabolite of AM) obtained in rats after the simultaneous co-administration in single-dose of *Citrus aurantium* extract (164 mg/kg, p.o.), or vehicle (0.5% carboxymethylcellulose aqueous solution), with AM (50 mg/kg, p.o.) by oral gavage (n = 6, unless otherwise noted).

Parameter	AM_{Citrus}		$AM_{Vehicle}$	
	AM	MDEA	AM	MDEA
t_{max} (h)	3.17 ± 1.83	12.00 ± 0.00[a]	1.83 ± 1.17	7.20 ± 3.03[b]
C_{max} (µg/mL)	1.405 ± 0.425	0.109 ± 0.008[a]	1.378 ± 0.439	0.125 ± 0.027[b]
AUC_{0-t} (µg.h/mL)	16.417 ± 3.805	ND	12.774 ± 1.685	ND
$AUC_{0-\infty}$ (µg.h/mL)	25.797 ± 2.903	ND	21.431 ± 5.088	ND
k_{el} (h^{-1})	0.0472 ± 0.0168	ND	0.0433 ± 0.0201	ND
$t_{1/2el}$ (h)	16.57 ± 6.82	ND	20.73 ± 14.05	ND
MRT (h)	24.63 ± 10.59	ND	28.64 ± 18.95	ND

ND, not determined.

[a]n = 2; [b]n = 5

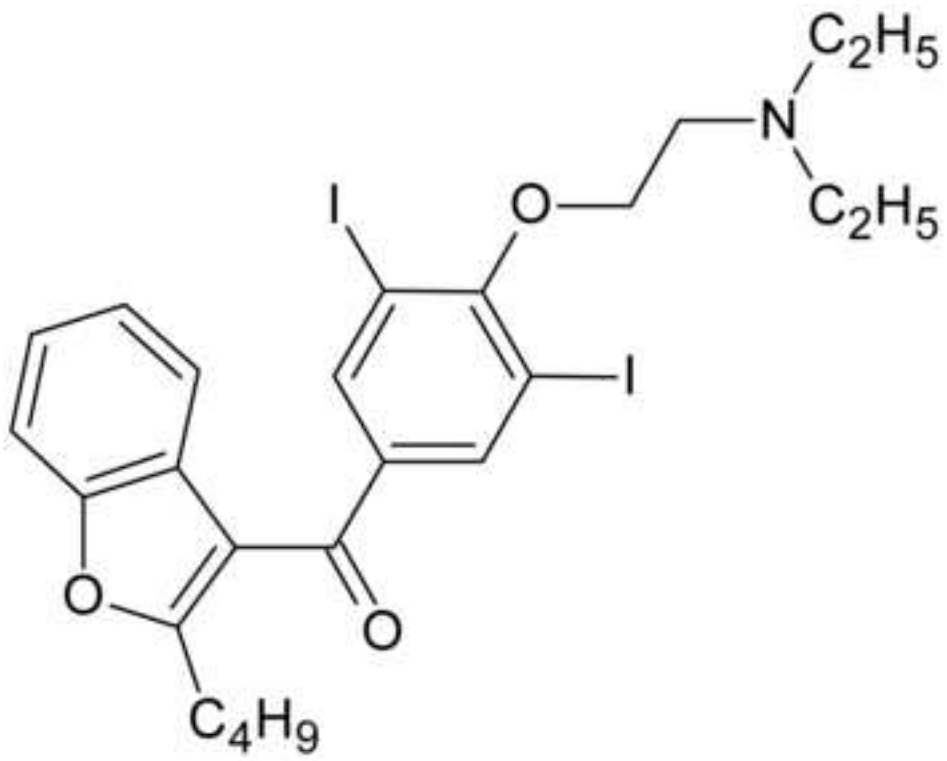
Table 2 – Mean pharmacokinetic parameters estimated by non-compartmental analysis of the plasma concentration-time profiles of amiodarone (AM) and mono-*N*-desethylamiodarone (MDEA, major metabolite of AM) obtained in rats submitted to a 14-day pre-treatment period with *Citrus aurantium* extract (164 mg/kg/day, p.o.), or vehicle (0.5% carboxymethylcellulose aqueous solution), and treated on the 15th day with a single-dose of AM (50 mg/kg, p.o.) by oral gavage (n = 6, unless otherwise noted).

Parameter	AM $_{Citrus}$		AM $_{Vehicle}$	
	AM	MDEA	AM	MDEA
t_{max} (h)	2.67 ± 1.51	7.00 ± 1.41[a]	2.00 ± 1.55	9.00 ± 4.24[b]
C_{max} (µg/mL)	1.654 ± 0.253*	0.107 ± 0.004[a]	1.046 ± 0.371	0.106 ± 0.008[b]
AUC_{0-t} (µg.h/mL)	14.318 ± 2.837	ND	12.282 ± 2.564	ND
$AUC_{0-\infty}$ (µg.h/mL)	23.901 ± 9.914	ND	22.057 ± 7.115	ND
K_{el} (h^{-1})	0.0485 ± 0.0182	ND	0.0442 ± 0.0265	ND
$t_{1/2el}$ (h)	16.38 ± 7.18	ND	21.62 ± 14.34	ND
MRT (h)	23.97 ± 9.52	ND	31.02 ± 20.31	ND

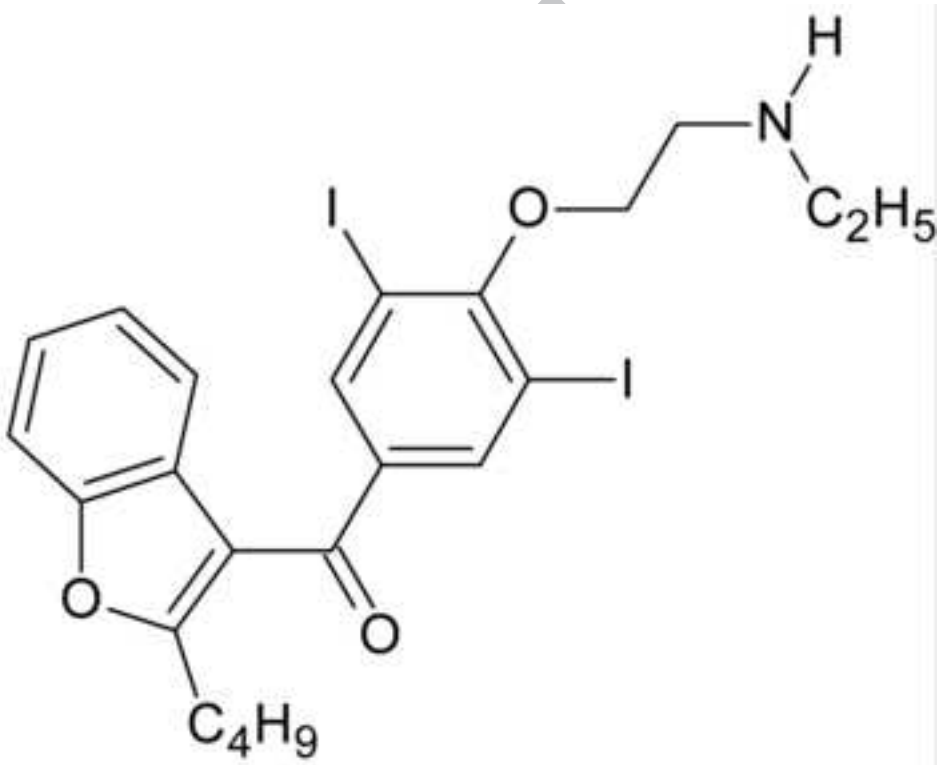
ND, not determined.

[a]n = 2; [b]n = 4.

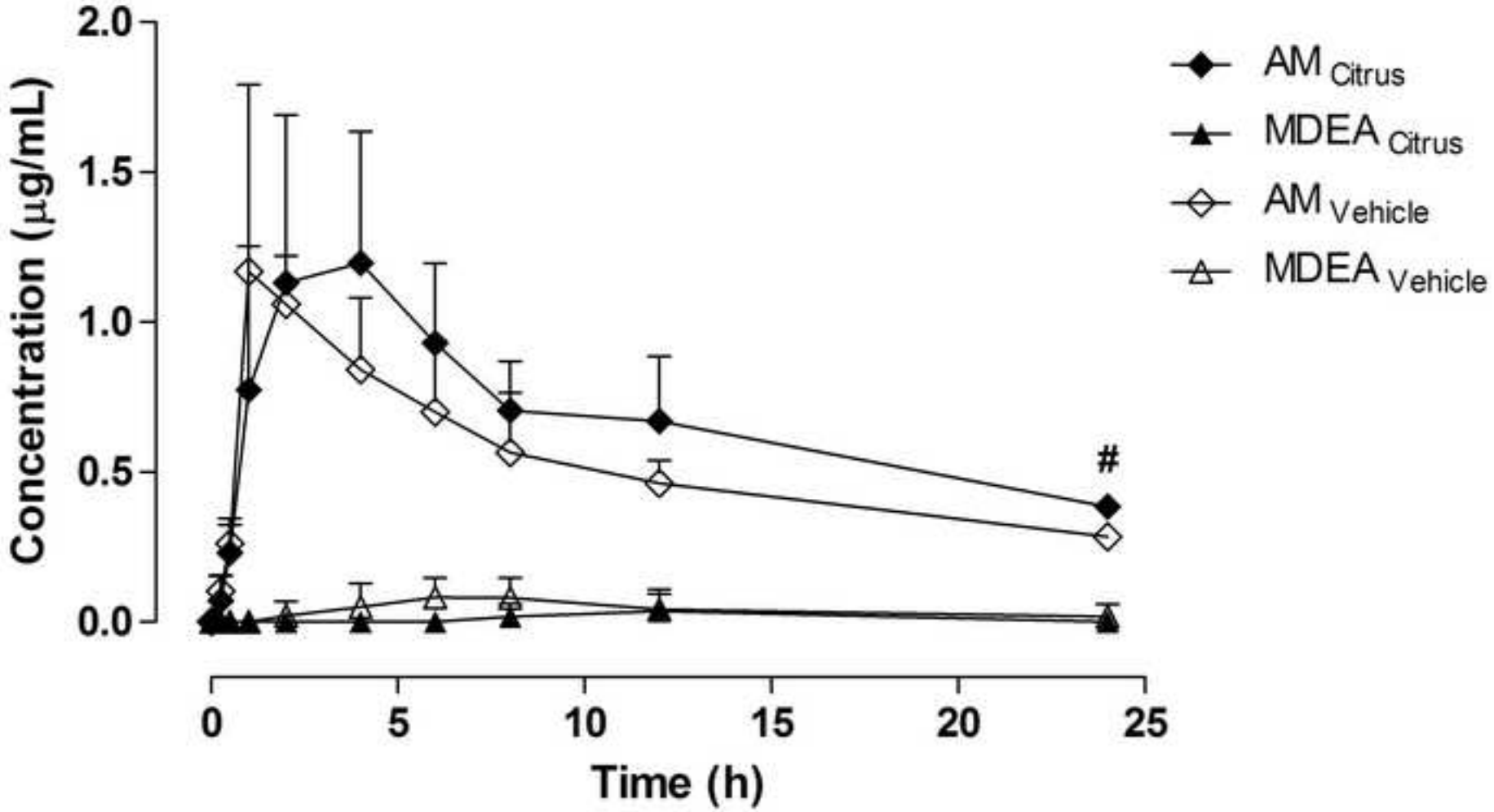
* $p < 0.01$, significantly different from the control group.



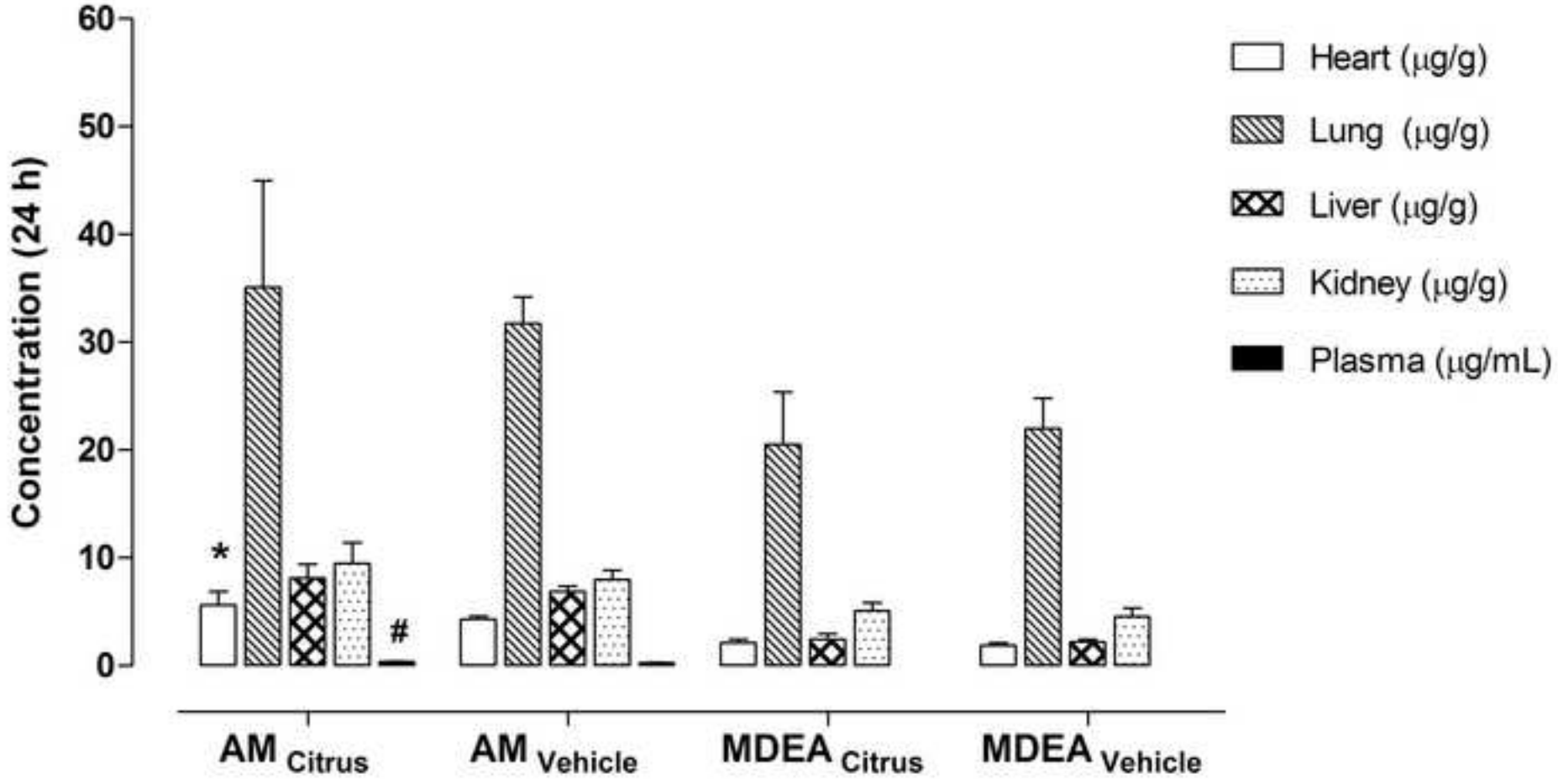
AM

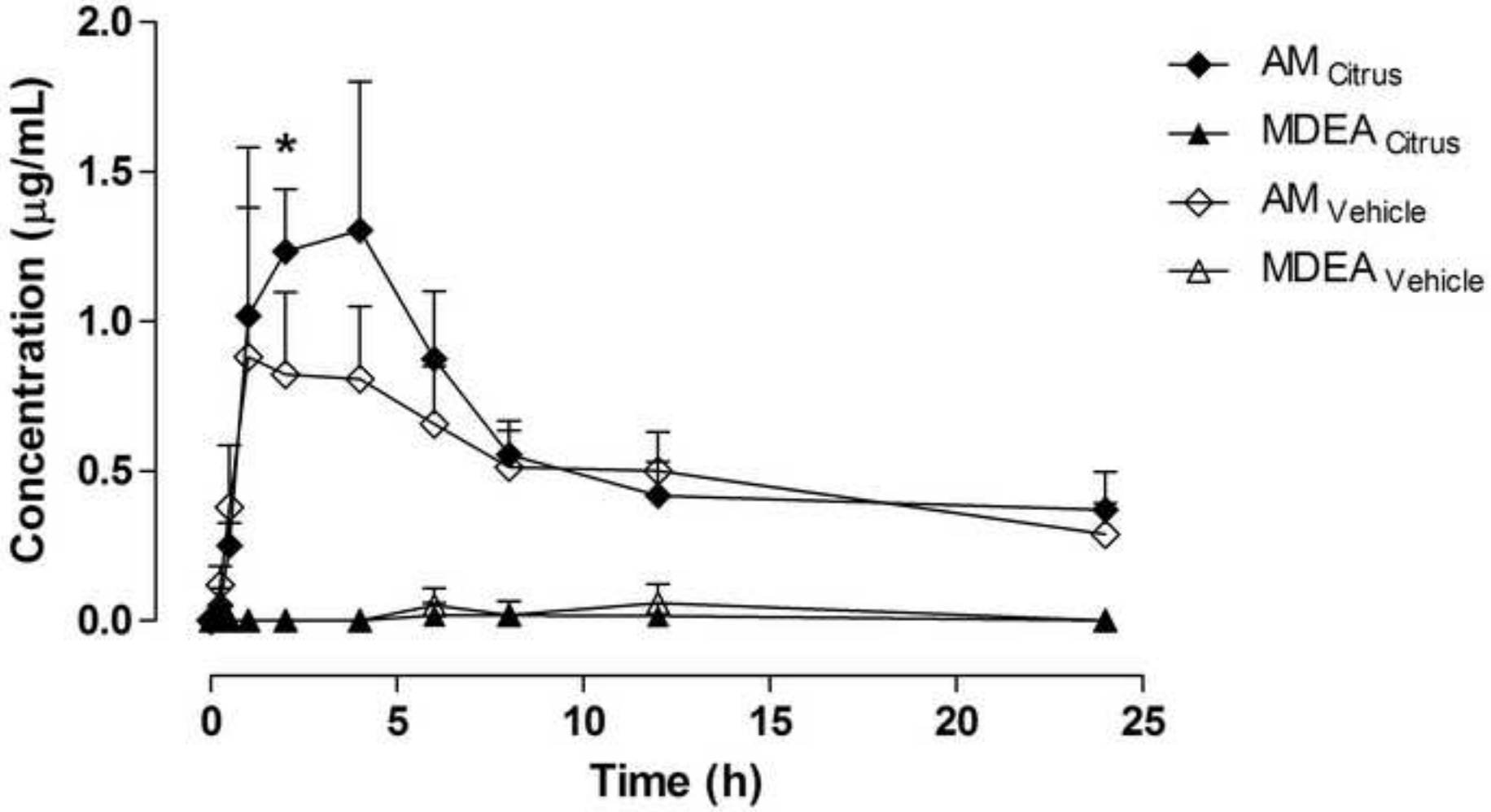


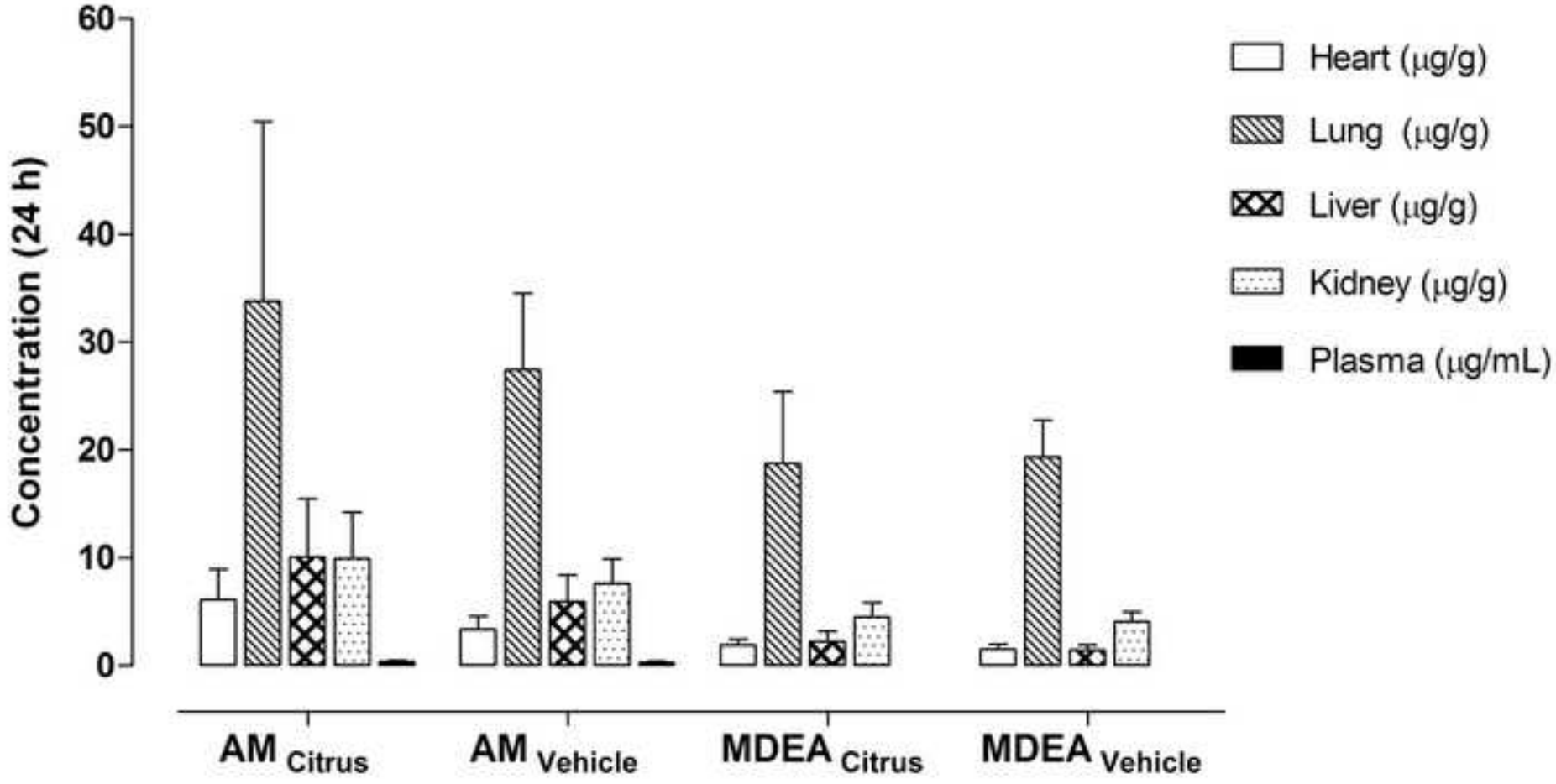
MDEA



Figure(s)







Highlights

► For the first time data on the potential of interaction between *C. aurantium* extract and amiodarone is reported.

► The simultaneous oral co-administration of *C. aurantium* extract and amiodarone induced a delay in the t_{max} of the drug.

► A 14-day oral pre-treatment with *C. aurantium* extract induced a significant increase in the C_{max} of amiodarone.

► The extent of systemic exposure to amiodarone was not affected by the co-administration or pre-treatment with *C. aurantium*.