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Alteration of the Pharmacokinetics of Theophylline by *Paullinia cupana* Kunth in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Paullinia cupana* Kunth has been popularly used to prepare different beverages by the Amazonian inhabitants for a long time ago mainly due to its stimulant properties. Although the utilization of this herbal drug has been increasing lately, little is known regarding the possibility of drug interactions. Therefore, this research tried to investigate the effects of the aqueous extract of *P. cupana* on the pharmacokinetics of theophylline (TPH), a CYP1A marker in rats.
Methodology: The extract was prepared according to the popular recipe and subjects received different once daily doses of extract (vehicle, 82.1 mg/Kg and 821 mg/Kg) by oral gavage during two weeks. Non-compartimental analysis was carried out to obtain the pharmacokinetic parameters.
Results: Animals treated with *P. cupana* (AUC: 1,197.2 ± 284.4 and 346.6 ± 37.0 µg.h/mL for 82.1 and 821 mg/Kg, respectively) had lower exposition to TPH than controls (3,539.48 ± 278.4

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µg.h/mL). On the other hand, drug clearance was higher in treated subjects (2.44 ± 0.4 and 7.27 ± 0.7 L/h/kg for 82.1 and 821 mg/Kg, respectively) than controls (0.71 ± 0.0 L/h/kg).
Conclusion: Therefore, the multiple oral administration of an aqueous extract of *P. cupana* caused a significant effect on the pharmacokinetics of TPH in rats.

Keywords: Pharmacokinetics; herbal drug interactions; Paullinia; rats.

1. INTRODUCTION

Guaraná, a natural product obtained from the seeds of *P. cupana* Kunth, is native to the Amazon region and widely used in Brazil due its stimulant properties [1]. Several studies have demonstrated its anti-inflammatory, anti-obesity and antiproliferative activities both *in vitro* and *in vivo* [2-4]. Due to these excellent results, this herb has gained popularity and has been used worldwide. Therefore, guaraná is the third native species most registered in the Brazilian National Sanitary Surveillance Agency (ANVISA) as a phytotherapeutic agent and was recently included in the top-15 rank of herbal nutraceutical sales in the United States [5,6].

Despite that, few reports have focused on its toxicological profile and this plant was one of the more frequently involved in adverse effects in a survey carried out in the European Union [7]. Considering this, a great number of case reports have been found relating the simultaneous use of natural products with the loss of therapeutic efficacy of some drugs [8]. Moreover, little is known about the safety of herbal medicines consumed in Brazil, especially pharmacokinetic information, in spite of its utilization [9].

On the other hand, the occurrence of medical interaction prompted by herbal medicines has been an issue investigated by the scientific community lately. This situation may happen because the utilization of natural products has been growing in the last years as an alternative to conventional clinical practice [10]. Considering this, assays to identify potential herb-drug interactions should be carried out as early as possible during drug development [11]. Herb-drug interactions may primarily occur through pharmacokinetic and pharmacodynamic pathways. The former phenomenon is generally associated to alterations in the activity of metabolizing enzymes and xenobiotic transporters, such induction or inhibition [10]. Therefore, the possibility of herbal drug interactions prompted by guaraná was highlighted recently [12]. In this context, the effect of *P. cupana* on the kinetic disposition of amiodarone and lamotrigine was reported in rats [13,14]. Therefore, the aim of this research was to investigate the effects of the aqueous extract of *P. cupana* on the pharmacokinetics of theophylline, a drug with low therapeutic index, in rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Theophylline (TPH) and caffeine (internal standard – IS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade) and other solvents used were purchased from Tedia (Fairfield, CA, USA). Formic acid, used for the preparation of the mobile phases, was acquired from Merck (Darmstadt, Germany). Purified water was obtained from a Milli-Q-system (Millipore, Milford, MA, USA). All solvents were filtered through 0.45 µm filter membranes before injecting into HPLC.

2.2 Preparation of *P. cupana* Extract

The seeds of guaraná were collected in the city of Maués, Brazil and the specimen was identified and authenticated by a taxonomist. After harvesting, samples were roasted in an oven and finally grounded in a blender and grinder. Conventional sieves with a mesh of approximately 1 mm were used to obtain homogeneous flour. The aqueous extract of guaraná was prepared mimicking the traditional approach and according to the Brazilian Pharmacopeia. Briefly, 200 g of the powder was added to 1000 mL of distilled water and immersed for 20 min at room temperature, yielding a 20% (w/v) extract. A fresh preparation was used to administer to the animals using the dose protocol reported by Rodrigues et al. [13] and Ventura et al. [14]. The plant name *Paullinia cupana* Kunth has been checked with http://www.theplantlist.org and the levels of TPH and caffeine in the plant material assessed by HPLC were 1.9 and 20.0%, respectively.

2.3 Animal Experiment

Young male Wistar rats weighing approximately 200 g had access *ad libitum* to feed and tap water and were acclimatized in a temperature- and humidity-controlled room under a 12 h light/dark cycle for one day before the beginning of the experiment. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care and the experimental protocol was approved by the Ethics Committee on the Use of Animals (Process number 073/2012-CEUA-UFAM).

Animals were randomly divided into three groups containing six subjects according to the following: Group 1, constituting the control group, were treated by gastrogavage with water (10 mL/kg, once per day) for 14 days; Groups 2 and 3, were treated by gastrogavage with guaraná preparations (82.1 mg/kg and 821.0 mg/kg, once per day) for 14 days, respectively.

One day after the last treatment, all subjects were anesthetized with i.p. injection of ketamine (90 mg/kg)/xylazine (10 mg/kg) and subjected to a surgical procedure to install a P10 polyethylene cannula (Intramedic, Becton Dickinson) in the left carotid artery to enable serial blood drawings up to 24 hours. The last administration of vehicle and guaraná preparations and the dosing of TPH were separated by three days in order to allow animal recovery from surgery and to completely wash out possible remaining methylxanthines from the herbal product.

After this period, an aqueous solution of TPH was administered orally to each animal at a dose of 10 mg/kg, and blood samples (approximately 0.3 mL) were collected through the cannulated carotid artery at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after TPH-treatment. Samples in heparinized tubes were centrifuged at 2000 x g for 10 min in order to obtain plasma for the determination of TPH. Thereafter, processed samples were stored at -70 ºC until analysis.

2.4 Analysis of Plasma Samples

The assay of TPH was conducted by means of the HPLC-UV method previously reported and validated by Tang et al. [15]. A calibration curve having seven different concentrations were obtained by analyzing spiked plasma samples in triplicate over the range of 0.1-50 µg/mL for TPH, which had the coefficient of determination (r^2) of 0.99. The sample (100 µl) was thawed at room temperature and placed in a glass tube (10 ml), and then 250 µl of IS solution (20 µg/ml), and 3 ml of ethyl ether:dichloromethane (3:2, v/v) were added. After vortex mixing for 120 s, the mixture was centrifuged at 3,000 g for 10 min. Then, the organic phase was evaporated to dryness at room temperature under a stream of compressed air. The residue was completely reconstituted with 100 µl of mobile phase and 70 µl was injected into an HPLC apparatus. The conditions for analysis were as follows: column size, 15 cm x 4.6 mm i.d.; packing, 5 µm C18 (Phenomenex, Torrance, CA, USA); mobile phase, methanol:water plus 1% formic acid (28:72, v/v); flow rate, 0.8 ml/min; wavelength, 275 nm.

2.5 Determination of Pharmacokinetic Parameters

Descriptive pharmacokinetic parameters were obtained through non-compartmental analysis performed by using Kinetica software (Thermo Electron Corporation, Waltham, MA). The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. The area under the curve to infinity (AUC0–∞) was calculated as $AUC_{0-\infty} = AUC_{0-t} + Ct/K_{el}$, where Ct is the last measurable concentration. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed values from the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($t_{½}$) of the drug was obtained by $0.693/K_{el}$. Total plasma clearance (CL/F) was calculated by Dose/AUC.

2.6 Statistical Analysis

Data are expressed as the mean ± standard deviation (S.D.). Comparisons between control group and guaraná pretreated groups were performed using the one-way analysis of variance (ANOVA), with Minitab. Differences were considered statistically significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

The results found in the present study indicate that the aqueous solution of *P. cupana* caused a marked alteration in the pharmacokinetics of TPH in rats (Table 1 and Figs. 1 and 2). The lower values of C_{max}, and especially of AUC, that is, of systemic exposure to TPH in the treated groups, might be the result of the greater Cl of

the drug, according to the values obtained. Thus, the efficacy of TPH when used simultaneously with this plant extract may be decreased due to a significant reduction of AUC (~74%).

Other two studies reported the alteration of the pharmacokinetics of therapeutic agents by *P. cupana* in rats. First, Rodrigues et al. [13] described that a significant reduction in the peak plasma concentration (73.2%) and in the extent of systemic exposure (57.8%) to amiodarone was found in rats simultaneously treated with guaraná. Latter, Ventura et al. [14] found that the co-administration of *P. cupana* and lamotrigine resulted in a significant reduction of C_{max} and AUC_{0-24} and prolonged the mean residence time of the antiepileptic agent. The same authors also stated that no significant effects were observed on lamotrigine pharmacokinetics following a 14-day pre-treatment period with the extract.

The Vd values found for the animals treated with the two doses of *P. cupana* were statistically higher than the values found for the group without treatment (Table 1). A single administration of guaraná extract promoted the reduction of amiodarone concentrations in several tissues of rats treated with this plant material [13]. Erythrocytes and plasma proteins are the major components responsible for binding of drugs to plasma. TPH is a drug with a moderate binding to plasma proteins in humans (~ 40%). Gao et al. [16] reported that intravenous administration of flavone baicalin promoted the increase of TPH free plasma fraction in Sprague-Dawley rats and, consequently, resulted in higher values of Vd. Additionally, Oliveira et al. [17] reported that the guaraná extract decreased the in vitro Tc-99m radiopharmaceutical binding to erythrocytes and plasma proteins of Wistar rats. Thus, the effects of the constituents of this plant material on protein binding of TPH deserve careful evaluation in an attempt to clarify the increase in Vd values observed in this study.

The mean C_{max} obtained from low and high guaraná doses (14.79 ± 1.6 and 12.79 ± 1.5 µg/ml, respectively) were statistically lower than control group (29.36 ± 5.3 µg/ml). Besides that, both doses resulted in noticeable reductions in AUC compared to control group (Table 1 and Fig. 2). According to Kennedy and Seely [18], the significant change caused in the kinetic disposition of TPH may be related to the great diversity of components present in the extract of *P. cupana*, especially methylxanthines (caffeine) and large amount of tannins. Caffeine and TPH found in this herb have been implicated in the up-regulation of several genes [19]. Additionally, some tannins also promote this effect according to a previous report [20].

Among CYP enzymes, CYP1A is one of the most studied through *in vivo* assays using drug probes, such as TPH, as seen in other reports [15,21,22]. However, this hypothesis must be investigated accordingly, in order to identify whether the high concentration of caffeine and other substances present in guaraná may be influencing the CYP1A expression of the animals.

As such, further studies to elucidate the observed phenomena should be performed, including evaluation of the protein content and expression of hepatic CYP1A messenger RNA after subchronic treatment using molecular biology techniques and evaluation of the effect on O-dealkylation of 7-ethoxyresorufine, which represents the enzymatic activity of this *in vitro* isoform [22].

Table 1. Pharmacokinetic parameters of theophylline obtained after administration of *P. cupana* to rats

Parameter	Control	*Paullinia cupana* (82.1 mg/kg)	*Paullinia cupana* (821 mg/kg)
AUC_{0-24h} (µg.h/mL)	439.03±36.6	169.9±30*	110.34±12.09*
$AUC_{0-\infty}$ (µg.h/mL)	3,539.48±278.4	1,197.2±284.4*	346.69±37.04*
$t_{1/2}$ (h)	12.85±0.58	14±2.4	7.014±0.93*
Vd (L)	13.13±0.74	43.54±10.55*	73.11±8.07*
Cl (L/h/kg)	0.71±0.05	2.44±0.47*	7.27±0.75*
K_{el} (h^{-1})	0.054±0.002	0.05±0.09	0.1±0.01*
C_{max} (µg/mL)	29.36±5.37	14.79±1.6*	12.79±1.5*
T_{max} (h)	2	2	1*

*Values are represented as means ± standard error of the mean of six determinations per time point (n = 6). * P<0.05.*

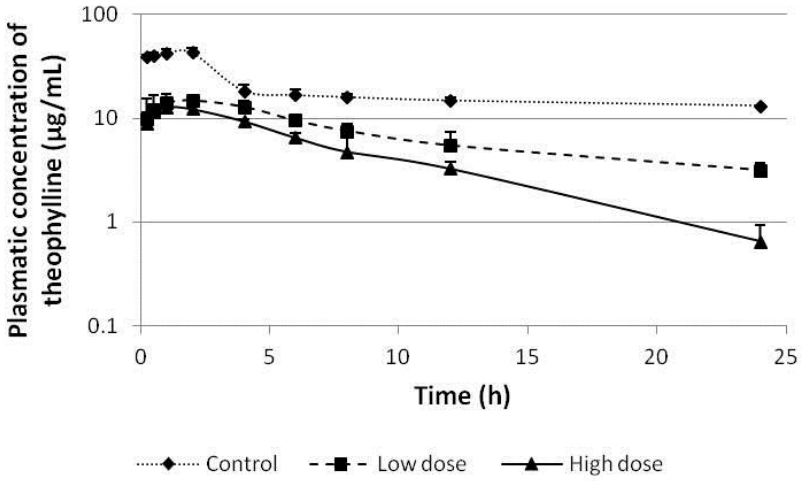

Fig. 1. Kinetic disposition of theophylline obtained in the plasma samples of rats submitted to subchronic treatment with aqueous solution of *P. cupana* (82.1 or 821 mg/kg/day) or vehicle (n = 6 per sampling time)

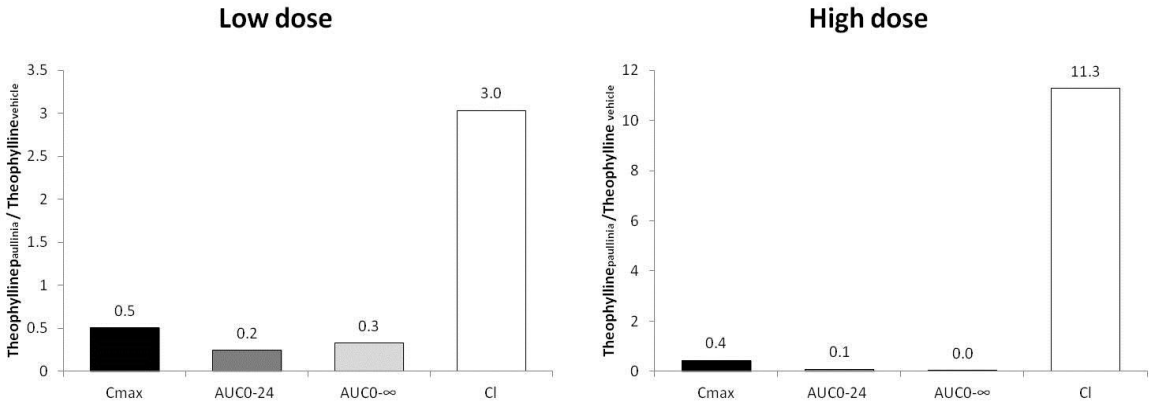

Fig. 2. Ratios of the main pharmacokinetic parameters (C_{max}, AUC_{0-24}, $AUC_{0-\infty}$ and Cl) estimated by the concentration of theophylline in rats treated simultaneously with a single dose of *P. cupana* extract (low dose - 82.1 or high dose - 812 mg/kg) or vehicle

4. CONCLUSION

The pharmacokinetics of TPH was altered by the oral pre-administration of multiple doses of *Paullinia cupana* aqueous extracts, since the C_{max} and AUC of this drug were reduced in the plasma samples assayed, while the clearance was enhanced. Further studies should be done to clarify the results obtained.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The manuscript was approved by the local Institutional Review Board (IRB) as reported in the Methodology section and all authors hereby

declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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