EFFECTS OF *Cassia auriculata* AND *Cardospermum halicacabum* TEAS ON THE STEADY STATE BLOOD LEVELS OF THEOPHYLLINE IN RATS

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SUMMARY

The effects of concurrent administration of herbal tea prepared from dried flowers of *Cassia auriculata* or aerial parts of *Cardiospermum halicacabum* and steady state serum levels of theophylline was investigated in Wistar rats. Results obtained demonstrate that a significant increase in the steady state levels of theophylline occur when this drug is administered concurrently with herbal tea prepared from either of the above plants. *C. auriculata* and *C. halicacabum* enhanced the steady state levels of theophylline by 32.5% ($p < 0.02$) and 48.2% ($p < 0.02$), respectively, when compared with the levels in animals receiving theophylline alone for the same time period. Herbal teas prepared from *C. auriculata* or *C. halicacabum* should therefore be avoided by patients treated with theophylline as these herbal teas have the potential to influence the bioavailability of the prescription drug.

KEY WORDS

theophylline, *Cassia auriculata, Cardospermum halicacabum,* herbal tea, drug interaction

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INTRODUCTION

Herbal medicines and herbal teas are widely used in Sri Lanka and they are becoming increasingly popular in many other countries of the world /1/. On the assumption that herbal products are harmless, they are often consumed concurrently with conventional medications prescribed for specific disease conditions. Very often, people who consume herbal products do not inform their physicians.

Co-administration of herbal products with conventional drugs can result in drug interactions that may influence the efficacy as well as the toxicity of either medication; conventional drugs are affected most because they are generally more pharmacologically active /2-4/.

Theophylline is a drug that is widely used as a bronchodilator in obstructive airway diseases such as bronchial asthma /5,6/. Recent investigations have shown that concurrent use of some herbal preparations with theophylline can result in an alteration in the blood levels of the prescribed drug /4,7,8/. In Sri Lanka, herbal teas prepared from dried flowers of *Cassia auriculata* or aerial parts of *Cardospermum halicacabum* are widely used by the general population. *C. halicacabum* is considered to be beneficial for individuals with rheumatic ailments, nervous disease, and digestive and pulmonary disorders, while *C. auriculata* is reputed to be useful in the treatment of diabetes mellitus, constipation and diseases of the urinary tract /9/. These teas are also consumed by individuals being treated with theophylline. Recent investigations have shown that tea prepared from *C. auriculata* flowers can significantly alter blood levels of the anti-epileptic drug carbamazepine /10/. In view of these observations and drug interactions already reported between herbal products and theophylline /4,7,8/, an investigation to determine whether the therapeutic potential of theophylline could be altered by herbal teas prepared from *C. auriculata* or *C. halicacabum* due to drug-herb interactions was considered to be appropriate. The objective of the present study was to determine the impact of co-administration of teas prepared from *C. auriculata* or *C. halicacabum* with theophylline, on the steady state serum levels of the prescribed drug, using rats as the experimental model.

MATERIALS AND METHODS

Experimental model

Male Wistar rats (200-250 g body weight) purchased from the Medical Research Institute, Colombo, Sri Lanka, were used in all experiments. The animals were housed under standard animal house conditions of temperature and humidity, and maintained on a rat chow diet prepared by the Medical Research Institute, Sri Lanka, according to a formula recommended by the WHO /11/. Animals had free access to water at all times.

Plant collection and identification

Flowers of *C. auriculata* and plants of *C. halicacabum* were collected from Matara, Sri Lanka, dried in a low temperature oven (60°C), ground to a powder and stored at -20°C. Yields of the dried plant material was 28.2% and 30.5% of the fresh weights of *C. halicacabum* and *C. auriculata,* respectively. The fresh plant material was identified by the Botanist (Mr. Gunaratne Silva) of the Bandaranayake Memorial Ayurvedic Research Institute (BMARI), Nawinna, Sri Lanka. Sample specimens of *C. auriculata* and *C. halicacabum* have been placed in the Museum, BMARI, Sri Lanka (voucher specimen numbers BMARI 18367286 and BMARI 18367563, respectively).

Preparation and administration of the herbal tea and theophylline

Herbal tea

When required, weights of the *C. auriculata* and *C. halicocabum* powders corresponding to 200 g wet weight of the plant material were steeped in boiling water (200 ml) for 30 min. After filtration through gauze, a volume of the extract providing a weight equivalent to 20 g fresh plant material/kg body weight was orally administered by gavage to each rat in the test groups. This dose corresponded to approximately ten times the daily dose of herbal tea normally consumed by a human adult. The dosages of each drug (herbal and theophylline) administered to the rats corresponded to ten times the normal therapeutic dosage administered to humans because, in general, rodents have been shown to be approximately 10 times less sensitive than humans

(because of their greater surface area relative to weight) to the effects of drugs /12/.

Theophylline

From a suspension containing 6.7 mg drug (Deriphylline®)/ml distilled water, a volume providing the equivalent of 33 mg drug/kg was administered orally by gavage to each rat/day. In normal clinical practice, theophylline is administered with water. Therefore, to simulate natural conditions, an aqueous suspension of the drug, rather than a solution in an organic solvent such as methanol, was used in the present study.

Experimental protocol

When testing for the effects of each of the herbal teas, rats (n = 20) were randomly divided into two groups (Groups A and B) of 10 animals each. Initially both groups were orally administered the test drug (theophylline), the dose of which was calculated on a body weight basis. After a 1-week period of treatment with this drug only, to allow the serum levels to stabilize, Group A was fed concurrently with theophylline and the herbal preparation for a 1-week period. During this period, animals in Group B continued to receive only the test drug in the same dosage. At the end of this week, the herbal preparation was discontinued and both groups continued to receive theophylline only for a further week. At the end of this week, group B was administered the herbal preparation together with theophylline for 1 week while animals in group A continued to receive only the test drug.

This protocol was based on the assumption that a significant difference in the drug levels in the two groups at the end of week 2 and week 4 would validate the hypothesis that the herbal tea has an effect on the metabolism of the drug, while the lack of such a significant difference would negate the hypothesis.

Blood collection for preparation of serum

At the beginning of the experimental period and at the end of each subsequent week after treatment daily with theophylline only or theophylline plus herbal drug, blood (1 ml) was drawn from the tail

vein of each rat into heparinized containers. Blood was collected each week 2 h after the last dose of theophylline. Thirty minutes after collection, the blood samples were centrifuged in a bench centrifuge at 4,000 rpm for 15 min, to separate the serum.

Preparation of the serum for HPLC

Each of the specimens for analysis was prepared according to the following procedure: To serum sample (0.5 ml) containing the internal standard, β-hydroxyethyl theophylline (50 μg/ml; 200 μl) purchased from Aldrich Chemical Co., USA, ethyl acetate (3.0 ml) was added and mixed well for 5 min with the assistance of a Vortex mixer. The mixture was then centrifuged at 2,500 rpm for 10 min in a bench centrifuge. The resulting upper ethyl acetate layer was carefully transferred to a glass test tube and evaporated to dryness at 40°C for approximately 1 h on a Bucher Vortex Evaporator coupled to a Savant Universal Vacuum System (UVS 400 A). The residue was redissolved in acetonitrile (100 μl). After filtration through a 0.45 μm, 13 mm Millipore filter, 20 μl of the solution was injected onto the analytical column (Symmetry® C8, 5 μm, 100 A) of a high pressure liquid chromatography system (Waters Gesellschaft mbh, Austria), connected to a photodiode array detector (Model 996).

HPLC analysis

The HPLC conditions for detection of theophylline in serum samples were as follows: mobile phase, 5 mM potassium phosphate, pH 2/acetonitrile (95/5 v/v); flow rate, 1.0 ml/min; detection, 270 nm /13/. Linearity of the HPLC system was determined based on a standard curve prepared by using standard solutions of theophylline. System stability was verified by use of the internal standard β-hydroxyethyl theophylline. The peak areas and peak heights of theophylline were analyzed by the Oracle software package provided with the HPLC system. The peak area ratio of theophylline to that of the internal standard was found to be linear between concentrations of 1.0 and 30 μg/ml of the prescribed drug. Estimations of the steady state levels of theophylline in the animal experiments were based on the peak areas and not on peak heights.

Recovery of theophylline from serum samples was in the range 90-95%.

Statistical analysis

Student's t-test was used for determination of means and standard deviations within each group. For comparison of data between groups, Student's t-test and one way analysis of variance (ANOVA) were used. A difference of $p < 0.05$ was considered to be statistically significant.

RESULTS

Steady state blood levels of theophylline

Results presented in Tables 1 and 2 summarize the steady state serum concentrations of theophylline in animals of Groups A and B

TABLE 1

Changes in the concentrations of theophylline in the HPLC profile of rat serum (representing the steady state concentration of the drug) on concurrent administration of the drug with *C. auriculata* tea

	Week 1	Week 2	Week 3	Week 4
Group A	**(T only)**	**(T + H)**	**(T only)**	**(T only)**
T concentration at end of week (μg/ml)	13.2 ± 2.4	17.6 ± 2.4[a,b]	15.0 + 1.4	11.5 ± 3.0
Group B	**(T only)**	**(T only)**	**(T only)**	**(T + H)**
T concentration at end of week (μg/ml)	12.8 ± 2.1	12.6 ± 1.6	13.5 + 1.4	16.9± 1.4[c,d]

T = theophylline; H = *C. auriculata* tea.
Results are presented as means ± SEM.
In Group A, [a,b] = significantly different from animals in the same group at the end of week 1 and the corresponding animals in group B, respectively ($p = 0.014$ and 0.003, respectively).
In Group B, [c,d] = significantly different from animals in the same group at the end of week 1 and from corresponding animals in group A, respectively ($p = 0.044$ and 0.017 respectively).

TABLE 2

Changes in the concentration of theophylline in the HPLC profile of rat serum (representing the steady state concentration of the drug) on concurrent administration of the drug with *C. halicocabum* tea

	Week 1	Week 2	Week 3	Week 4
Group A	**(T only)**	**(T + H)**	**(T only)**	**(T only)**
T concentration at end of week (μg/ml)	12.3 + 2.4	18.5 ± 3.1[a b]	13.8 ± 1.9	11.9 + 2.1
Group B	**(T only)**	**(T only)**	**(T only)**	**(T + H)**
T concentration at end of week (μg/ml)	12.5 ± 1.8	13.2 ± 1.7	12.8 + 2.1	18.3 + 2.4[c d]

T = theophylline; H = *C. halicacabum* tea.
Results are presented as means ± SEM.
In Group A, [a b] = significantly different from animals in the same group at the end of week 1 and the corresponding animals in group B,respectively (p = 0.012 and 0.039, respectively).
In Group B, [c,d] = significantly different from animals in the same group at the end of week 1 and from corresponding animals in group A, respectively (p = 0.047 and 0.037, respectively).

receiving theophylline only or theophylline plus herbal tea. When the concentrations of theophylline at the end of each week between the two groups as well as within each of the individual groups (A and B) are compared, it is evident that concurrent administration of theophylline and the herbal tea prepared from *C. auriculata* (Table 1) or *C. halicacabum* (Table 2) results in a significantly altered serum level of theophylline when compared to that in animals receiving only theophylline.

In animals receiving either *C. auriculata* or *C. halicacabum,* a comparison of the concentrations of theophylline for Group A at the end of week 1 (theophylline only) and week 2 (theophylline + herbal tea) showed that the drug concentrations were significantly (p = 0.014 and 0.012, respectively) higher at the end of week 2. Similarly, in Group B, when the concentrations at the end of the week 1

(theophylline only) were compared with those at the end of week 4 (theophylline + herbal tea), the concentrations at the end of the fourth week were found to be significantly ($p = 0.044$ and 0.039, respectively) higher than those at the end of week 1.

A comparison of the serum concentrations between Groups A and B of animals receiving the *C. auriculata* or *C. halicacabum* teas shows that significant differences could be observed only at the end of weeks 2 and 4. At the end of week 2 (theophylline + herbal tea), the concentrations in Group A were significantly higher ($p = 0.003$ and $p = 0.039$ for *C. auriculata* and *C. halicacabum,* respectively) than in Group B (theophylline only). Similarly, at the end of the fourth week (theophylline + herbal tea), Group B exhibited significantly higher concentrations than group A (theophylline only) ($p = 0.017$ and $p = 0.037$ for *C. auriculata* and *C. halicacabum,* respectively). Thus, the serum concentrations of theophylline in each group receiving the *C. auriculata* or *C. halicacabum* preparation plus theophylline were significantly higher than in the corresponding animals of the second group receiving only theophylline during that time period.

DISCUSSION

Results of the present investigation demonstrate that a significant increase in the steady state serum level of theophylline in rats occurs when the drug is administered concurrently with herbal tea prepared from either *C. auriculata* or *C. halicacabum.* This observation is similar to that reported previously by Kuang *et al.* /14/. According to the above investigation, on concurrent administration of the drug enoxin® together with theophylline to humans, there is a dose-dependent enhancement of the plasma concentration of theophylline. In a previous investigation, the authors /10/ observed that *C. auriculata* tea could significantly enhance the steady state blood levels of the anti-epileptic drug carbamazepine. However, in contrast to the results of the present investigation, *C. halicacabum* tea did not produce any significant changes in blood levels of carbamazepine. From these observations, it may be inferred that the influence that *C. halicacabum* could have on steady state serum levels of a prescribed drug will depend on the class of conventional drug with which it is being concurrently administered, thus supporting the views expressed by Burstein *et al.* /15/.

Cytochrome P1A2 (CYP1A2) is the major cytochrome P450 isoform involved in the metabolism of theophylline /16/. Alterations of CYP1A2 expression can be mediated by extracts of some medicinal plants, such as St. John's wort /7/ and *Rhazya stricta* /8/. For example, induction of this cytochrome P450 isoform is believed to be responsible for the reduction in plasma concentration of theophylline that occurs upon co-administration of an extract of St. John's wort and theophylline /7/. In the present investigation, the herbal teas mediated an increased serum concentration of theophylline, perhaps suggesting inhibition of CYP1A2. Estimation of the serum concentration of a theophylline metabolite in rat serum is necessary to reach a more definite conclusion regarding the effects of the herbal teas on cytochrome P450 function. Whether the effects of *C. auriculata* or *C. halicacabum* on the bioavailability of theophylline are mediated via an alteration in cytochrome P450 isoform concentrations or another mechanism, such as changes in intestinal absorption or serum protein binding of the conventional drug /1/, or modulation of Phase I or Phase II drug metabolizing enzymes in rat liver, as has been demonstrated for a variety of other herbal teas /17/, needs further investigation. However, in view of the ability of *C. auriculata* and *C. halicacabum* teas to alter serum levels of theophylline, it would be best to avoid concomitant use of these herbal teas with theophylline or related drugs.

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