BIOANALYTICAL STUDY OF THE EFFECT OF LYCOPENE ON THE PHARMACOKINETICS OF THEOPHYLLINE IN RATS

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Lycopene, a natural pigment, was reported to have a modulatory effect on cytochrome P450, hence, metabolic clearance of some drugs. This research is concerned with studying the influence of lycopene on the pharmacokinetics of theophylline, a drug with a narrow therapeutic index. Lycopene (10 mg/kg) was intraperitoneally given to rats for eight consecutive days and on the eighth day of treatment, theophylline (5 mg/kg) was intravenously administered. Blood samples were withdrawn from rats at various time periods, treated and analyzed using HPLC. The HPLC method was based on isocratic elution of theophylline and caffeine as internal standard on ZORBAX Eclipse plus C_8 column using a mobile phase consisting of water and methanol (80:20, v/v) at a flow rate of 1 mL min^{-1}. Statistical analysis of the pharmacokinetic data using Paired t-test showed a significant decrease in AUC_{0-t} and $AUC_{0-\infty}$ (about 22.140 and 23.824%, respectively, $P < 0.05$) and a significant increase in V_d and CL (1.28- and 1.40-fold, respectively, $P < 0.05$) of theophylline administered to rats pretreated with lycopene. Treatment with lycopene has a significant effect on the metabolic clearance and pharmacokinetics of theophylline in rats.

Keywords: lycopene; theophylline; rat plasma; pharmacokinetics; HPLC-UV.

1. INTRODUCTION

Lycopene (LYC) is a carotenoid, an acyclic isomer of β-carotene, which is found in fruits and vegetables mainly in tomatoes, watermelon, and guava and has no vitamin A activity [1]. LYC was reported to possess strong antioxidant potency [2, 3]. Many studies demonstrated the importance of LYC in the prevention of various types of cancer such as prostate cancer [4 – 6], gastric cancer, breast cancer, and lung cancer [7 – 9]. More recent studies proved the ability of LYC to decrease bone resorption and thus improve osteoporosis [10]. Hence, the carotenoid LYC is increasingly gaining scientific attention because of its potential health effects. However, there is no reported information about the pharmacokinetic interactions between Lyc and commonly used drugs such as theophylline.

Theophylline (THEO), 1,3-dimethylxanthine (Fig. 1) [11], is a drug commonly used in the treatment of bronchial constriction and obstruction caused by asthma and various lung diseases such as acute asthma, bronchitis, and chronic obstructive pulmonary disease [12, 13]. Because of its narrow therapeutic range of 5–20 mg/L THEO levels should be carefully adjusted in people who use many drugs at the same time [14, 15].

THEO is a typical substrate of cytochrome P450 family 1 subfamily A member 1 (CYP1A2) enzyme *in vivo* [16 – 18]. CYP1A2 is one of the cytochrome (CYP) enzyme
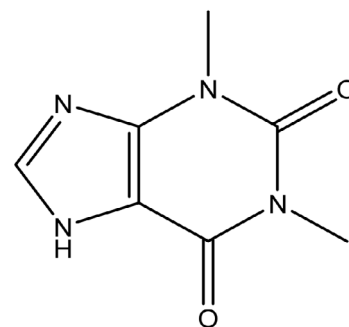

Fig. 1. Chemical structure of theophylline.

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subfamily which accounts for about 13% of human CYP [19, 20]. Many medicines could affect the activity of CYP enzymes, which made them one of the reasons of drug – drug interactions [21]. In addition, drug – drug interactions may alter CYP1A2 metabolism, thus causing the theophylline toxicity [22].

Previous studies showed the induction effect of LYC on many CYP enzymes such as 1A1/2, 2B1/2, and 3A when given to rats at doses within 0.001 – 0.1g [23, 24]. To date, there is no reported information about the effect of LYC on the pharmacokinetics of THEO. Therefore, this work was carried out to study this effect in rats using HPLC as an analytical method.

2. MATERIALS AND METHODS

2.1. Chemicals and Solvents

LYC was kindly supplied from UG PHARMA Company, Egypt. THEO and caffeine were kindly supplied from Memphis Company, Egypt. Methanol for HPLC and diethyl ether were purchased from Sigma-Aldrich, Germany. Membrane 0.45 μm filters were purchased from Sartorius Stedium Biotech Company. Heparin (Calheparin 5000UI AMP) was purchased from Amoun Pharmaceutical Industries Company, Egypt.

LYC stock solution. LYC (200 mg) was dissolved in 10 mL of water to obtain a final concentration (20 mg/mL) of pure LYC.

2.2. Instrumentation

A chromatographic system consisted of Younglin instrument Acme 9000 (Korea) equipped with vacuum degasser, mixer, gradient pump, and UV/VIS detector; magnetic stirrer model Labinco L33. Separation and quantitation were made on ZORBAX Eclipse plus C8 column (150 mm × 4.6 mm, 5 μm), Agilent, USA; Frontier Centrifuge FC5706 OHAUS, Germany; ultrasonic processor, Soniclean 120T, Barton SA, Australia.

2.3. Animal Experiment

Twelve male Wistar rats, 250 – 450 g, taken from the Laboratory Animal House, Faculty of Pharmacy, Cairo University, were placed in cages, six per cage, with free access to food and water; the temperature was set at 25°C. The study protocol was reviewed and approved (PC 1687, 26 April 2016) by the Institutional Review Board (REC-FOPCU; Research Ethics Committee-Faculty of Pharmacy, Cairo University, Egypt). The rats were randomly divided into two groups: (1) LYC group (pretreated with LYC extract, 10 mg/kg); (2) control group (pretreated with vehicle (normal saline)). All rats were given vehicle (control group) or LYC (LYC group) daily by (IP) for 8 consecutive days. On the eighth day food was stopped but only water was allowed.

After treatment with normal saline or LYC, THEO (5 mg /kg, i.v.) was administered to all test rats via the tail. After the administration of THEO, 300 μL blood samples were taken from the eye vein at various preset time intervals (0, 0.25, 0.5, 1, 2, 4, 6, 24 h). The plasma samples were separated in Eppendorf vials containing 50 μL of diluted heparin (1:10) by centrifugation at 4,000g for 15 min and stored at –20°C until analysis. The experiment was performed after a 7-day washout period with switching between the two groups.

Extraction method. Aliquots (20 μL) of internal standard (IS) stock solution (caffeine, 220 μg/mL) and 60 μL water were added to 120 μL plasma samples in Eppendorf vials and then 200 μL methanol was added. The solution was vortexed for 30 sec and the supernatant was separated by centrifugation at 6,000 rpm for 15 min, and 20 μL was injected into HPLC.

Assay of theophylline in rat plasma. The plasma THEO concentration was determined using HPLC method. ZORBAX Eclipse plus C8 column was used as the stationary phase using a water – methanol (80:20 v/v) mixture as the mobile phase at a flow rate of 1 mL/min. The column temperature was 25°C. The UV detector was set at 272 nm wavelength. The injection volume was 20 μL.

2.4. Analytical Method Validation [25, 26]

Preparation of calibration standards and quality control samples. Six non-zero calibration standards of THEO (0.5 – 15 μg /mL) and the quality control (QC) samples were prepared similarly at three levels, (Low = 1.5, Medium = 7.5, High = 11.5 μg/mL) by adding 20 μL of the corresponding working solutions of THEO, 20 μL of caffeine stock solution (220 μg/mL), and 60 μL distilled water to 100 μL rat plasma. To 200 μL of spiked calibration plasma or QC samples, 200 μL methanol was added and the mixture was vortexed for 30 sec; the supernatant was separated by centrifugation at 6,000 rpm for 15 min, and 20 μL was injected into HPLC. The final IS concentration is 11 μg/mL.

Selectivity. Selectivity was checked using 6 drug-free rat plasma samples processed for protein precipitation and then analyzed to exclude any interference due to plasma components at the retention times of drug and IS. This was confirmed by HPLC patterns shown in Fig 2.

Carry-over. In order to assess carry-over, blank samples were injected after a high concentration sample (11.5 μg/mL). The carry-over in the blank sample was within the limits for LLOQ and IS.

Calibration curve. Calibration curve, with linearity range of 0.5 – 15 μg/mL THEO, was constructed by plotting peak area ratio of THEO to IS versus the corresponding concentration. The regression equation was also computed as $Y = 0.1103x - 0.003$ ($r^2 = 0.9995$).

Accuracy and precision (inter- and intraday). The accuracy recoveries for intraday precision were found to be be-
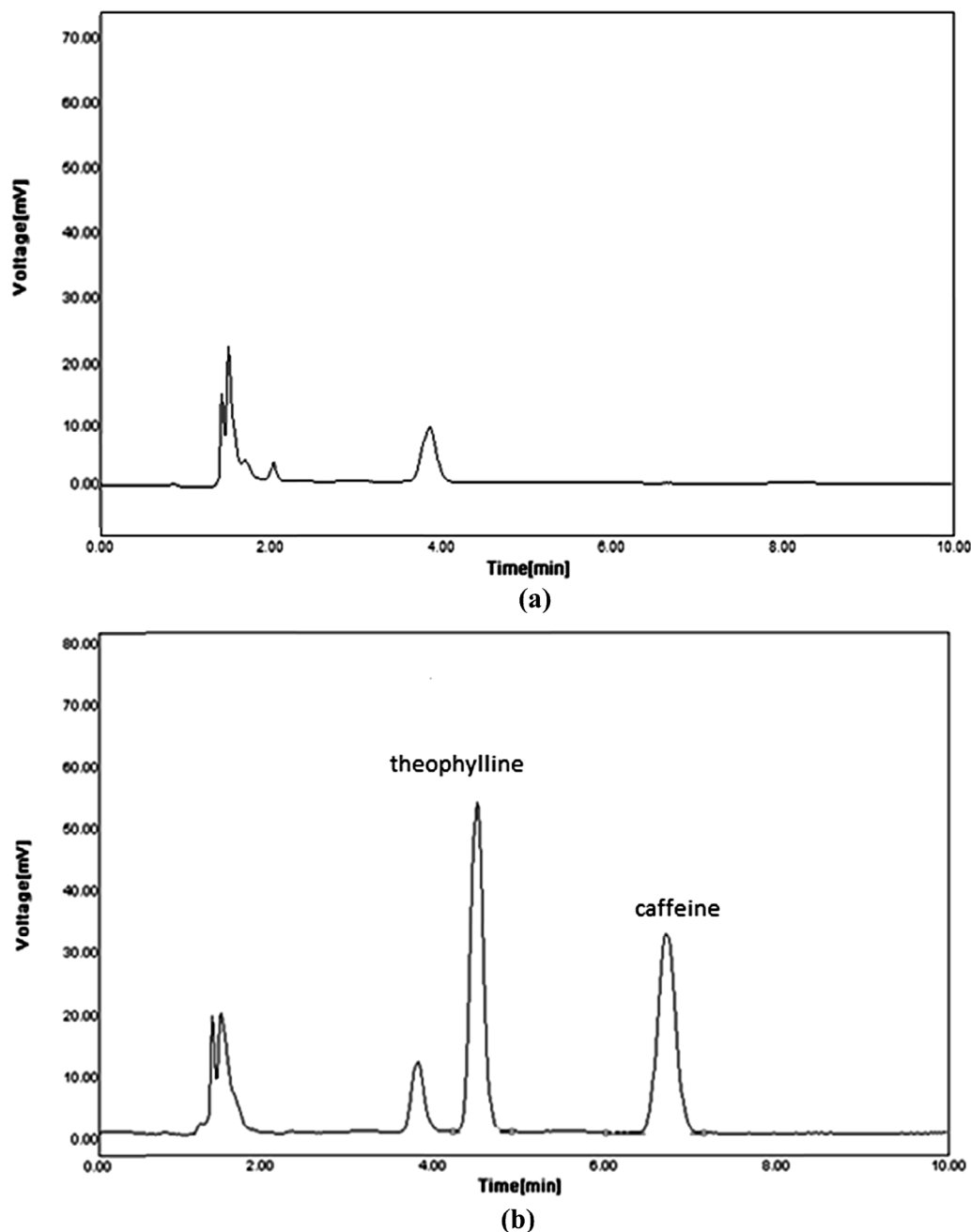
(a)

(b)

Fig. 2. Chromatograms of (a) blank rat plasma and (b) rat plasma spiked with theophylline (11.5 µg/mL) and caffeine IS (11 µg/mL).

tween 102.000 and 111.133% and the CV% was between 1.765 and 12.897%. The interday accuracy was between 100.800 and 108.400% with a coefficient of variation within 1.439 – 2.637%. The results are presented in Table I.

Stability. To evaluate long-term stability, low (1.5 µg/mL) and high (11.5 µg /mL) QC samples were stored in deep freezer (-80 ± 2°C) for two weeks and then analyzed. The results of long-term stability were test within the acceptance criteria. Two QC samples were kept at room temperature for 6.0 h and then analyzed. The results of short-term

stability test were within acceptance criteria. To study freeze/thaw stability, QC samples were exposed to three freeze and thaw cycles. Td then the samples were processed, analyzed, and compared with nominal values (Table 2).

2.5. Pharmacokinetic Analysis

Non-compartment pharmacokinetic analyses were carried out using WinNonlin software. The calculated parameters included area under the plasma concentration–time curve (AUC_{0-t}), area under the plasma time–concentration
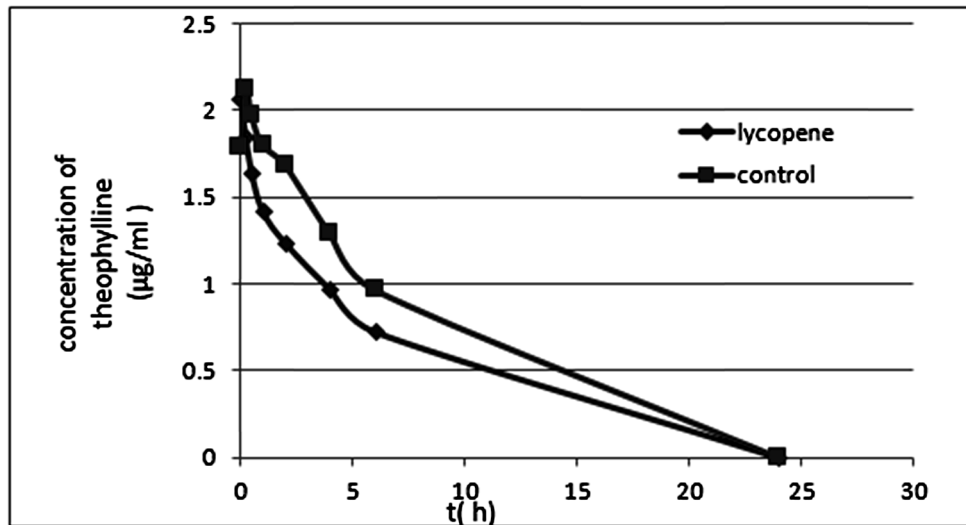
Fig. 3. The concentration–time profiles of theophylline in rats (5 mg/kg, i.v.) after treatment with water (control) and lycopene (10 mg/kg, i.p.).

curve from time zero to infinity ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), volume of distribution V_d, clearance (CL) and the elimination half-life ($t_{1/2}$).

The plasma concentration–time curves of THEO after i.v. administration at a dose of 5mg/kg with control and LYC pretreatment (10 mg/kg, i.p.) are shown in Fig. 3.

TABLE 1. Intraday and Interday Accuracy and Precision of Theophylline Determination in Rat Plasma

Accuracy and precision	QC samples	Concentration (µg/mL)		±SD	CV	Recovery%
		Added	Found			
Intraday	LLOQ	0.5	0.510	0.009	1.765	102.000
	Low	1.5	1.667	0.215	12.897	111.133
	Medium	7.5	7.980	0.178	2.231	106.400
	High	11.5	12.061	0.300	2.487	104.878
Interday	LLOQ	0.5	0.524	0.013	2.513	104.800
	Low	1.5	1.512	0.0377	2.493	100.800
	Medium	7.5	8.130	0.117	1.439	108.400
	High	11.5	12.057	0.318	2.637	104.843

TABLE 2. Short-Term, Long-Term, and Freeze/Thaw Stability of Theophylline in Rat Plasma

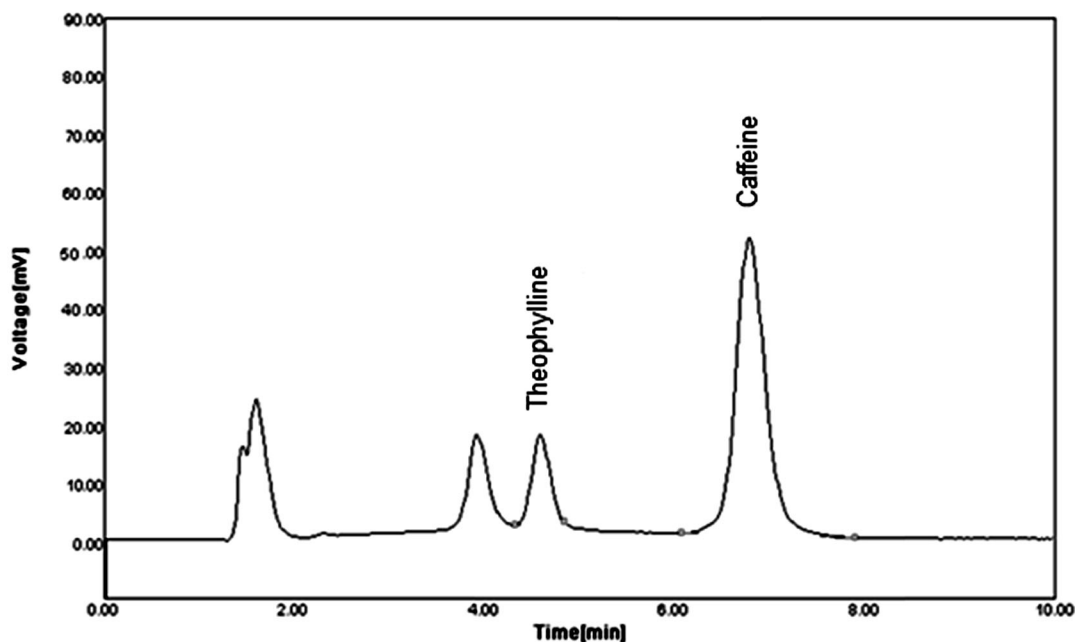| Stability	QC samples	Concentration (µg/mL)		±SD	CV	Recovery%
		Added	Found			
Short-term	Low	1.5	1.574	0.032	2.033	104.93
	High	11.5	10.837	0.146	1.347	94.235
Long-term	Low	1.5	1.293	0.021	1.618	86.533
	High	11.5	11.635	0.367	3.154	101.174
Freeze/thaw	Low	1.5	1.523	0.118	7.748	101.533
	High	11.5	10.422	0.143	1.369	90.626

Fig. 4. Chromatogram of theophylline in plasma taken from rat immediately (0 h) after i.v. administration and spiked with caffeine (11 μg/mL).

Pharmacokinetic analyses of THEO showed that the pretreatment of rats with LYC at a daily dose of 10 mg/kg for eight consecutive days resulted in 22.140%, 23.824% ($P < 0.05$) reduction in AUC_{0-t} and $AUC_{0-\infty}$ of THEO, respectively, compared to the control group. The difference in C_{max}, and $t_{1/2}$ did not reach statistical significance but the total clearance (CL) and the volume of distribution (V_d) were significantly (1.28-fold and 1.40-fold, respectively $P < 0.05$) increased following the pretreatment with LYC. The HPLC chromatogram of THEO in plasma taken from rats i.v. pretreated with LYC immediately (0 h) after THEO administration and spiked with caffeine is shown in Fig 4.

2.6. Statistical Analysis

The pharmacokinetic parameters of THEO were compared between the control and LYC groups using IBM SPSS Statistics, Version 22 (Copyright IBM Corporation and others 1989, 2013). The significance of differences between groups was analyzed by *t*-test; differences were considered significant for $P < 0.05$ (Table 3).

3. RESULTS AND DISCUSSION

Many mobile phases with different ratios between methanol and water or phosphate buffer with various pH and different columns (C_8, C_{18} and Cyano columns) were tried but found unsuccessful to separate the drug and the internal standard from the peaks of plasma components. Finally, C_8 column was chosen to be the stationary phase with a mobile phase of methanol – water (20:80, v/v) mixture. Also, many internal standards were tried (diclofenac, terbutaline,

ofloxacin and paracetamol) but caffeine was the best choice as it was completely separated from THEO and rat plasma components.

Moreover, many extraction methods were tried, including the protein precipitation using perchloric acid 20%, acetonitrile, liquid-liquid extraction, and solid phase extraction, but all showed interference between the peaks of plasma and drug or IS (caffeine). In protein prescription, the use of acetonitrile led to bad peak of caffeine, but when it was replaced by methanol, this method was the best choice to separate the drug and IS from plasma peaks.

On the other hand, to determine the effective dose of LYC, many preliminary trials were carried out. Finally, LYC (100 mg/kg) was suspended in sunflower oil and given orally to rat (LYC group) for six consecutive days followed by i.v. administration of THEO (5 mg/kg). Blood samples were withdrawn from rats at time periods within 0 – 24 h. The re-

TABLE 3. Pharmacokinetics of Theophylline (5 mg/kg, i.v.) in Rats With and Without Lycopene Pretreatment

Parameter		Lycopene	Control
C_{max}	μg/mL	2.060 ± 0.240	2.125 ± 0.175
$t_{1/2}$	h	4.963 ± 1.420	4.730 ± 1.330
V_d	mL/kg	2.878 ± 0.563	2.248 ± 0.367
CL	mL/h · kg	0.470 ± 0.191	0.336 ± 0.097
AUC_{0-t}	mg h/mL	6.882 ± 1.262	8.839 ± 0.757
$AUC_{0-\infty}$	mg h/mL	11.955 ± 3.679	15.694 ± 3.247

sults of this experiment were compared to that carried out using pretreatment of rats with i.p. dose of LYC (10 mg/kg). Control was carried out in each experiment by pretreating animals with the vehicle. Both experiments showed a decrease in AUC_{0-t}, $AUC_{0-\infty}$ and an increase in CL and V_d of THEO when pretreated with LYC, but this effect was more significant in the rats pretreated with i.p. LYC dose. This test was repeated in rats pretreated with LYC for eight consecutive days instead of six and this experiment showed that the effect of LYC was more significant when the duration of LYC administration was increased.

Foreign chemicals such as in food, herbal medicines or drugs can affect drug metabolism and this interaction can have clinical significant effect (27). This interaction can commonly be a result of inhibition or induction of the CYP enzyme isoform which is responsible for metabolizing a certain administered drug, consequently increasing or decreasing the clearance of that drug (28,29).THEO is mainly metabolized by CYP1A2 (around 60 – 80% of the drug) [30]. Administration of LYC in a dose of 10 mg/kg (i.p.) may induce CYP1A2 thus causing a decrease in the AUC and an increase in CL and V_d. If the drug is only eliminated through the liver, the total CL is nearly equal to the hepatic clearance (CL_H) [31]. Since THEO is a low-clearance drug, its CL_H is mainly affected by any factor that affects its intrinsic clearance (CL_{int}) (31). Accordingly, pretreatment of rats with LYC increased the CL_{int} and subsequently the CL_H, and decreased AUC_{0-t} and $AUC_{0-\infty}$ of THEO. The V_d of THEO also increased, whereas LYC produced no significant effect on C_{max} as THEO (i.v.) was administered. Since THEO is a low clearance drug, when both CL and V_d increase, the $t_{1/2}$ will be slightly affected as compared to that of high clearance drugs. The results of our present investigation might be a guide for further clinical studies.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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The three authors contribute equally to this work.

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