

# Validated analytical study of the effect of Lycopene on the pharmacokinetics of Paracetamol and Chlorzoxazone in rats

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Lycopene was reported to influence some cytochrome P450 enzymes activity. The present study investigates the effect of lycopene on the pharmacokinetics of paracetamol and chlorzoxazone. Lycopene (20 mg/kg) was intra-peritoneally administered to two groups of rats for eight consecutive days and two other groups were given vehicle. On the eighth day, chlorzoxazone and paracetamol were separately intravenously administered to a lycopene group and a control group. Blood samples were collected at different time intervals, treated and analyzed using HPLC. The HPLC method used for paracetamol analysis was based on isocratic elution using a mobile phase consisting of water: methanol, (77:23 v/v) at a flow rate 1 mL min<sup>-1</sup>, Kromasil C18 column, and UV detection at 254 nm using caffeine as internal standard. About chlorzoxazone, separation was carried out using water: acetonitrile (60: 40, v/v) as the mobile phase at a flow rate 1 mL min<sup>-1</sup>, Inertsil ODS-3 C18 column, UV detection at 283 nm and esomeprazole as internal standard. Statistical analysis of the pharmacokinetic data using student t test showed a significant increase in  $AUC_{0-p}$ ,  $AUC_{0-inf}$  and  $t_{1/2}$  of paracetamol ( $P < 0.05$ ) and of chlorzoxazone ( $P < 0.05$ ) in the groups pretreated with lycopene (20 mg/kg), significant increase in the volume of distribution of paracetamol ( $P < 0.05$ ), but no significant difference in that of chlorzoxazone. In other words, paracetamol and chlorzoxazone showed significant decrease ( $P < 0.05$ ), respectively. These results demonstrate that treatment of rats with Lycopene (20mg/kg, ip) has a significant effect on the metabolic clearance and the pharmacokinetics of both drugs.

**Keywords:** Lycopene/ chemistry. Paracetamol/pharmacokinetics. Chlorzoxazone/pharmacokinetics. Acetaminophen/pharmacokinetics. AUC. Area under curve. Volume of distribution. Validation studies. Metabolic clearance rate/drug effects. HPLC/methods. Chromatography. High Pressure Liquid/methods. Rats/metabolism.

## INTRODUCTION

Lycopene is a non provitamin A carotenoid, concentrated in tomatoes and other red fruits (Schulzová, Hajšlová, 2007). Great interest to lycopene was recently observed because most studies proved that those who consume more lycopene and accordingly have high concentrations in plasma have lower incidence for chronic illness such as cancer and coronary heart diseases (Gerster, 1997; Rao, Agarwal, 1999). More studies proved the effect of lycopene to decrease bone resorption and thus improve osteoporosis (MacKinnon *et al.*, 2011).

In a previous study, Tomato juice showed a potent inhibitory effect on nifedipine oxidation activity, which was comparable to that on testosterone 6 $\beta$ -hydroxylation

activity, and weak inhibitory effect on midazolam 1'-hydroxylation activity, by one or more mechanism-based and competitive inhibitor(s) of CYP3A4 (Sunaga *et al.*, 2012).

Hence, the carotenoid lycopene is increasingly gaining scientific attention because of its potential health effects. However, little is known about the metabolic interactions between lycopene and clinically used drugs like paracetamol and chlorzoxazone.

Paracetamol (acetaminophen, *N*-acetyl-*p*-aminophenol, PAR) is one of the most popular and widely used drugs for the treatment of pain and fever. Due to its good tolerability profile, PAR is often the analgesic or antipyretic of choice, especially in patients in whom salicylates or other non-steroidal anti-inflammatory drugs are contraindicated (Portolés *et al.*, 2003; Wang *et al.*, 2018).

Cytochrome P450 enzymes, CYP2E1 and CYP1A2, catalyze oxidation of PAR to the reactive metabolite

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N-acetyl-p-benzoquinone imine (NAPQI) (McGill, Jaeschke, 2013). Numerous drugs have been reported to interact with PAR leading to exacerbation of its toxicity, so PAR should be administered carefully to people on isoniazid treatment or consuming excessive amounts of alcohol to prevent hepatotoxicity due to the induction of CYP2E1. PAR toxicity can occur when CYP2E1 metabolism is impaired by drug-drug interactions (Zand *et al.*, 1993).

Chlorzoxazone (5-chloro-2(3*H*)-benzoxazolone; CZX), a skeletal muscle relaxant used for the treatment of painful muscle spasms is primarily metabolized to 6-hydroxychlorzoxazone (OH-CZX), which is subsequently glucuronidated and excreted in the urine (Kar, 2005; Kramer *et al.*, 2003). Hepatic microsomal cytochrome P450 enzyme (CYP2E1) plays an important role in the formation of OH-CZX from CZX in humans and rats (Rockich, Blouin, 1999; Peter *et al.*, 1990). Previous studies showed that lycopene has the ability to inhibit CYP2E1 in the rat microsomal model (Louisa *et al.*, 2009). So far, there has been no information about the effects of Lycopene on the pharmacokinetics of CZX and PAR. The aim of this study was to determine this influence in rats using bioanalytical HPLC method.

## MATERIAL AND METHODS

### Chemicals and solvents

Lycopene extract was kindly supplied from UG PHARMA company, Badr City, Egypt. Paracetamol, chlorzoxazone, esomeprazole and caffeine were kindly supplied from Memphis Company, Cairo, Egypt. Heparin (Calheparin 5000UI AMP) was purchased from Amoun Pharmaceutical Industries Company, Kaliobeya, Egypt). Methanol for HPLC and ethyl acetate were purchased from Sigma-Aldrich, Munich, Germany. Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK).

### Instrumentation

A chromatographic system (Younglin instrument Acme 9000, Korea) equipped with vacuum degasser, mixer, gradient pump and UV/VIS detector was used. Separation and quantitation were made on Kromasil® C18 column (150 x 4.6 mm, 5µm), Agilent, USA and Inertsil ODS-3:C18 column (250 x 4.6 mm, 5µm), Japan. Magnetic stirrer model Labinco L33, Fronteir™ Centrifuge FC5706, OHAUS, Germany and Ultrasonic processor; Soniclean 120T, Barton SA, Australia were used.

### Lycopene stock solution

Lycopene extract 10% equivalent to 200 mg was dissolved in 10 mL water to obtain a final concentration 20 mg/mL of pure lycopene.

### Animal experiment

24 Male Wister rats, 250–450 g, obtained from the Laboratory Animal Center, Faculty of Pharmacy, Cairo University, were housed, six rats per cage, and given free access to food and water in a temperature-controlled room (25°C) with a 12 h light/dark cycle. The study protocol was reviewed and approved (PC (1687) on 26<sup>th</sup> April 2016 by the Institutional Review Board (REC-FOPCU; Research Ethics Committee-Faculty of Pharmacy, Cairo University) in Egypt. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The rats were randomly divided into four groups: (1) Lycopene group of PAR (pretreated with lycopene (20 mg/kg, n=6) (2) Control group of PAR (pretreated with vehicle (water for injection), n=6) (3) Lycopene group of CZX (pretreated with lycopene (20 mg/kg), n=6) (4) Control group of CZX pretreated with vehicle (normal saline), n=6).

All the rats were given vehicle (control group) or lycopene (lycopene group) intraperitoneally on a daily basis for 8 consecutive days. On the eighth day, access to the diet was removed and only water was provided. After treatment with water or lycopene, PAR (25 mg/kg) was given to group (1) and group (2) and CZX (20 mg/kg) was given to group (3) and group (4) by intravenous administration in the tail.

After administration of PAR and CZX, 300 µL blood samples were collected from the vein of the eye at different time intervals (0, 0.25, 0.5, 1, 2, 4, 6 hr). The plasma samples were separated via Eppendorf containing 50µL of diluted heparin (1:10) by centrifugation at 4,000 rpm for 15 min and stored at -20°C until analysis.

### Extraction method of PAR

A liquid-liquid extraction method was used for extraction of PAR from plasma matrix. In an Eppendorf, an aliquot of 20 µL internal standard stock solution (Caffeine 400µg/mL) and 60 µL water were added to 120 µL plasma samples taken from groups (1) and (2) and then 1000 µL of a mixture of methanol and ethyl acetate (1:9) was added. The solution was vortexed for 30 s and the supernatant was separated by centrifugation at 4,000 rpm for 10 min. The organic layer was separated and evaporated to dryness at

40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 µL mixture of methanol and water (77: 23), then 20 µL was injected onto HPLC. The final concentration of IS is 80µg/mL.

### Extraction method of CZX

A protein precipitation method was used for extraction of CZX from plasma matrix. An aliquot of 20 µL internal standard stock solution (Esomeprazole, 880µg/mL) and 60 µL water were added to 120 µL plasma samples taken from groups (3) and (4) in Eppendorf and then 200 µL of a mixture of water and acetonitrile (4:6) was added. The solution was vortexed for 30 s and the supernatant was separated by centrifugation at 6,000 rpm for 15 min, then 20 µL was injected onto HPLC. The final concentration of IS is 44µg/mL.

### Assay of PAR in rat plasma

PAR plasma concentration was determined using HPLC. Chromatographic separation was achieved on a Kromasil C18 column (150 X 4.6 mm, 5µm) using a mobile phase consisting of water: methanol (77:23, v/v) at a flow rate 1 mL min<sup>-1</sup>. The column temperature was 25 °C. UV detector was operated at 254 nm. The injection volume was 20 µL.

### Assay of CZX in rat plasma

Chromatographic separation of CZX plasma concentration was achieved on an Inertsil C18 column (250 X 4.6 mm, 5µm) using a mobile phase consisting of water: acetonitrile (60:40, v/v) at a flow rate 1 mL min<sup>-1</sup>. The column temperature was 25 °C. UV detector was operated at 283 nm. The injection volume was 20 µL.

### Bioanalytical method validation (EMA, 2015)

#### *Preparation of Calibration standards and quality control samples*

Stock solutions of PAR (2.5-200 µg mL<sup>-1</sup>) were diluted in water to get concentrations of calibration curve (0.5-40 µg mL<sup>-1</sup>). Stock solutions of PAR (2.5, 7.5, 100, 150 µg mL<sup>-1</sup>) were diluted in water to get quality control samples (LLOQ = 0.5, Low = 1.5, Med =20, High = 30 µg mL<sup>-1</sup>). Stock solution of caffeine (400 µg mL<sup>-1</sup>) was diluted in distilled water to get concentration (80 µg mL<sup>-1</sup>) (IS). A 100 µL aliquot of rat plasma was spiked with 20 µL of stock solution, 20 µL of Caffeine stock solution (IS) and 60 µL water. To 200 µL

of the spiked calibration plasma standards or QC samples, 1000 µL mixture of ethyl acetate and methanol (9:1) was added and the extraction procedure was continued as mentioned in “Extraction method of PAR”.

Stock solutions of CZX (10-800 µg mL<sup>-1</sup>) were diluted in acetonitrile to get concentrations of calibration curve (0.5-40 µg mL<sup>-1</sup>). Stock solutions of CZX (10, 30, 400, 600 µg mL<sup>-1</sup>) were diluted in acetonitrile to get quality control samples (LLOQ = 0.5, Low = 1.5, Med = 20, High=30 µg mL<sup>-1</sup>). Stock solution of Esomeprazole (880 µg mL<sup>-1</sup>) was diluted in distilled water to get concentration (44 µg mL<sup>-1</sup>) (IS). A 100 µL aliquot of rat's plasma was spiked with 20 µL of stock solution, 20 µL of Esomeprazole stock solution (IS) and 60 µL water. To 200 µL of the spiked calibration plasma standards or QC samples, 200µL mixture of acetonitrile and water (6:4) was added and the extraction procedure was continued as mentioned in “Extraction method of CZX”.

Calibration curves were constructed by plotting peak area ratio of the drug to IS versus the corresponding drug concentration in both PAR and CZX. The constructed calibration curves were found to be linear and precise for PAR and CZX over the linearity range of 0.5-40 µg mL<sup>-1</sup>. The regression equations were also computed ( $Y = 0.0287x + 0.0013$ ,  $R^2 = 0.9998$ ) for CZX and ( $Y = 0.0448x + 0.0099$ ,  $R^2 = 0.998$ ) for PAR.

#### *Selectivity*

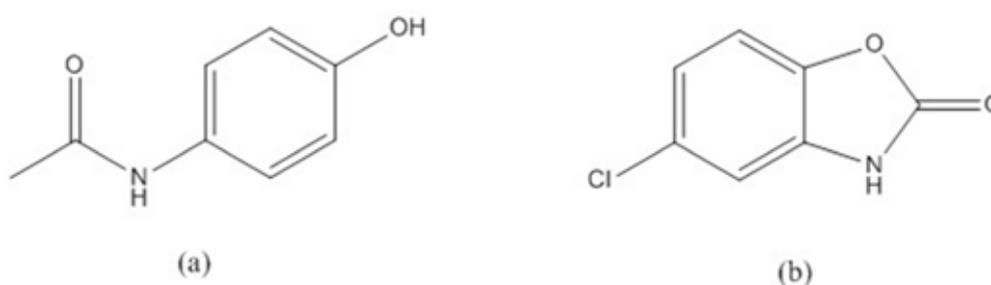
Selectivity was checked by using 6 randomly selected drug-free rats' plasma, (processed by the protein precipitation extraction procedure for CZX or liquid-liquid extraction for PAR) which was analyzed to determine the extent to which endogenous plasma components may contribute to the interference at the retention time of analytes and IS. There was no significant interference at the retention times of both CZX, PAR or IS from the six different batches of drug-free rat plasma used for analysis, as shown in Figures 2 and 3.

#### *Carry-over*

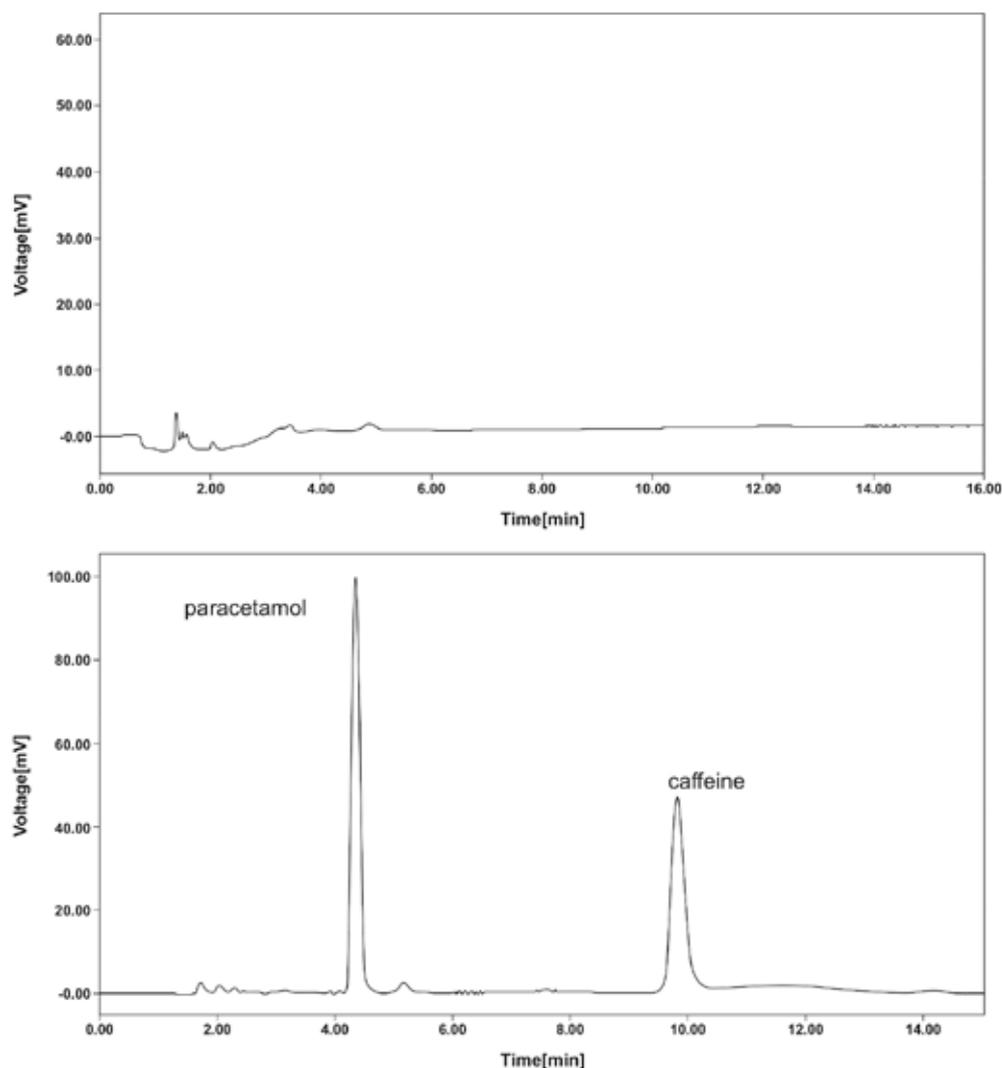
Carry-over was assessed by injecting blank samples after a high concentration sample (30 µg mL<sup>-1</sup>) of both PAR and CZX. Carry over in the blank sample did not exceed 20% of the lower limit of quantification and 5% for the internal standard.

#### *Accuracy and precision (inter and intra-day)*

For the evaluation of precision, the deviation of each concentration level from the nominal concentration should be within ±15.0%. Similarly, the mean accuracy should not deviate by ± 15.0% of the nominal



**FIGURE 1** - Chemical structure of (a) paracetamol, (b) chlorzoxazone.



**FIGURE 2** - Chromatogram of (a) blank rat's plasma (b) Rat plasma spiked with paracetamol (30  $\mu\text{g/mL}$ ) and caffeine (80  $\mu\text{g/mL}$ ).

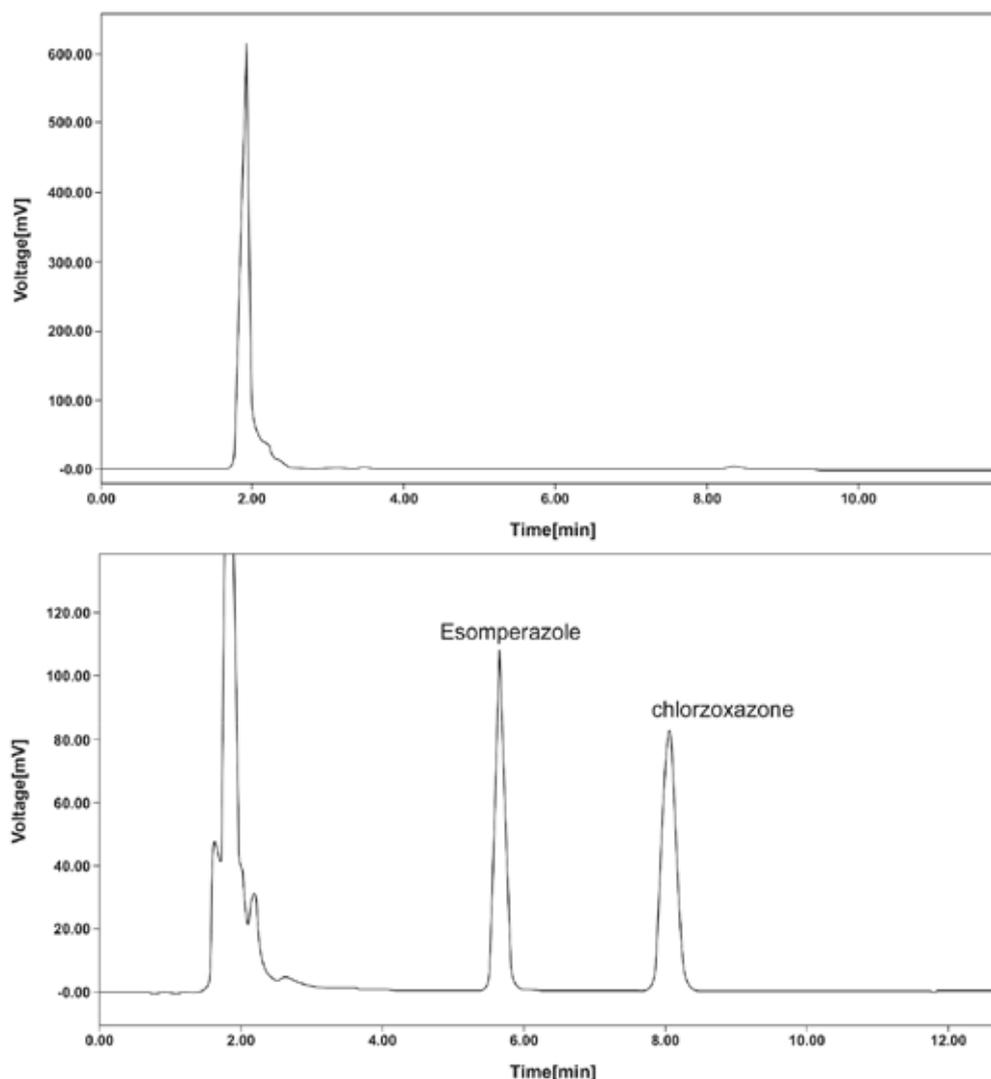
concentration except for the LLOQ which should be within 20% of the nominal value. The results are presented in Tables I and II.

#### Stability

To evaluate the stability of both PAR and CZX under different storage conditions, three aliquots of each low and high QC samples were stored in a deep freezer at  $-80 \pm$

$2^\circ\text{C}$  for two weeks. After two weeks, the samples were processed along with precision and accuracy batches. Concentrations obtained were compared with nominal concentrations to determine the long-term stability of both PAR and CZX in rat plasma.

The short-term stability was determined by keeping three aliquots of unprocessed QC samples at ambient temperature for 6.0 h. After 6.0 h, the samples



**FIGURE 3** - Chromatogram of (a) blank rat's plasma (b) Rat plasma spiked with chlorzoxazone (30 µg/mL) and esomeprazole (44 µg/mL).

**TABLE I** - The results of Intra- and Inter-day accuracy and precision for the determination of paracetamol in rat plasma

Accuracy and Precision	QC samples	Concentration of PAR (µg/mL) in rat plasma	Mean Found Concentration (µg/mL) of PAR	±SD	CV	Recovery %
Intraday	LLOQ	0.5	0.473	0.066	13.953	94.600
	Low	1.5	1.490	0.099	6.644	99.333
	Medium	20	19.950	0.426	2.135	99.750
	High	30	28.707	1.398	4.869	95.690
Interday	LLOQ	0.5	0.500	0.049	9.800	100.000
	Low	1.5	1.429	0.089	6.228	95.266
	Medium	20	18.840	0.722	3.832	94.200
	High	30	28.793	0.603	2.094	95.797

**TABLE II** - The results of intra- and inter-day accuracy and precision for the determination of chlorzoxazone in rat plasma

Accuracy and Precision	QC samples	Concentration of CZX ( $\mu\text{g/mL}$ ) in rat plasma	Mean Found Concentration ( $\mu\text{g/mL}$ ) of CZX	$\pm\text{SD}$	CV	Recovery %
Intraday	LLOQ	0.5	0.525	0.040	7.619	105.000
	Low	1.5	1.560	0.152	9.744	104.000
	Medium	20	20.981	1.634	7.869	104.905
	High	30	30.765	1.177	3.826	102.550
Interday	LLOQ	0.5	0.538	0.007	1.301	107.600
	Low	1.5	1.503	0.114	7.585	100.200
	Medium	20	20.620	1.447	7.017	103.100
	High	30	31.794	0.272	0.855	105.980

were processed, analyzed and compared with nominal concentrations. The stability of QC samples was also studied after three freeze and thaw cycles. Three aliquots of unprocessed QC samples were stored at  $-80 \pm 2^\circ\text{C}$  and subjected to three freeze and thaw cycles. After the completion of the third cycle, the samples were processed, analyzed and the results were compared with nominal values and the results represented in Tables III and IV.

### Pharmacokinetic analysis

Non-compartmental pharmacokinetic analyses were performed using Excel (Microsoft, Redmond, WA, USA) add-in program, PK solver. The estimated parameters included area under the plasma concentration–time curve ( $AUC_{0-t}$ ), area under the plasma time–concentration curve from time zero to infinity ( $AUC_{0-\infty}$ ), maximum plasma concentration ( $C_{max}$ ) and time to maximum plasma

**TABLE III** - The results of short, long and freeze and thaw Stability of paracetamol in rat plasma

Stability	QC Samples	Concentration of PAR ( $\mu\text{g/mL}$ ) in rat plasma	Mean Found Concentration ( $\mu\text{g/mL}$ ) of PAR	$\pm\text{SD}$	CV	Recovery%
Short Term Stability	Low	1.5	1.512	0.019	1.257	100.800
	High	30	28.793	0.738	2.563	95.977
Long Ter Stability	Low	1.5	1.437	0.085	5.915	95.800
	High	30	28.832	1.897	6.579	96.107
Freeze & Thaw stability	Low	1.5	1.533	0.080	5.219	102.200
	High	30	30.083	2.367	7.868	100.277

**TABLE IV** - The results of short, long and freeze and thaw Stability of chlorzoxazone in rat plasma

Stability	QC Samples	Concentration of CZX ( $\mu\text{g/mL}$ ) in plasma	Mean found Concentration of CZX ( $\mu\text{g/mL}$ ) in plasma	$\pm\text{SD}$	CV	Recovery%
Short term Stability	Low	1.5	1.553	0.139	8.950	103.533
	High	30	29.251	0.700	2.400	97.503
Long term stability	Low	1.5	1.610	0.034	2.112	107.533
	High	30	29.354	0.462	1.574	97.847
Freeze & Thaw Stability	Low	1.5	1.566	0.138	8.812	104.400
	High	30	30.091	0.364	1.209	100.303

concentration ( $T_{max}$ ), volume of distribution ( $V_d$ ), clearance ( $cL$ ) and the elimination half-life ( $t_{1/2}$ ) of both PAR and CZX, Figures 4 and 5 represent the chromatograms of the drugs in rat plasma after IV administration.

The plasma concentration–time curves after IV administration at PAR (25 mg/kg) and CZX (20 mg/kg) with either control or lycopene pretreatment (20mg/kg, ip) are shown in Figure 6.

The pharmacokinetic parameters of PAR and CZX indicated that pretreatment of rats with Lycopene at daily dosage of 20mg/kg for eight consecutive days resulted in a (20.374% & 45.997%,  $P < 0.05$ ) reduction in the total clearance ( $cL$ ), respectively, compared with the control group. Nevertheless, the difference in volume

of distribution ( $V_d$ ) was not significant between the two groups of CZX, where there was a significant increase (1.694 fold) in  $V_d$  of PAR compared to the control group.

The difference in  $C_{max}$  and  $T_{max}$  did not reach statistical significance in both PAR and CZX, but the  $AUC_{0-p}$ ,  $AUC_{0-inf}$ ,  $t_{1/2}$  were significantly increased by 1.245, 1.295 and 2.09 folds in PAR and 1.631, 1.914 and 2.216 folds in CZX ( $P < 0.05$ ), respectively, following pretreatment with Lycopene ( $P < 0.05$ ).

### Statistical analysis

The different parameters ( $AUC$ ,  $C_{max}$ ,  $T_{max}$ ,  $V_d$ ,  $cL$  and  $t_{1/2}$ ) of both PAR and CZX between control group

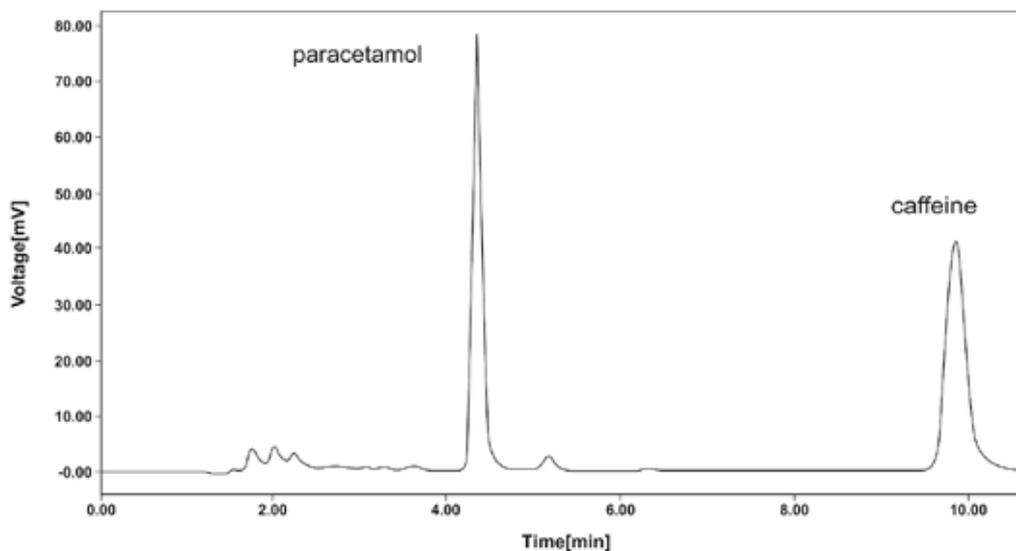


FIGURE 4 - Chromatogram of paracetamol in plasma taken from a rat after IV administration and spiked with caffeine (80  $\mu$ g/mL).

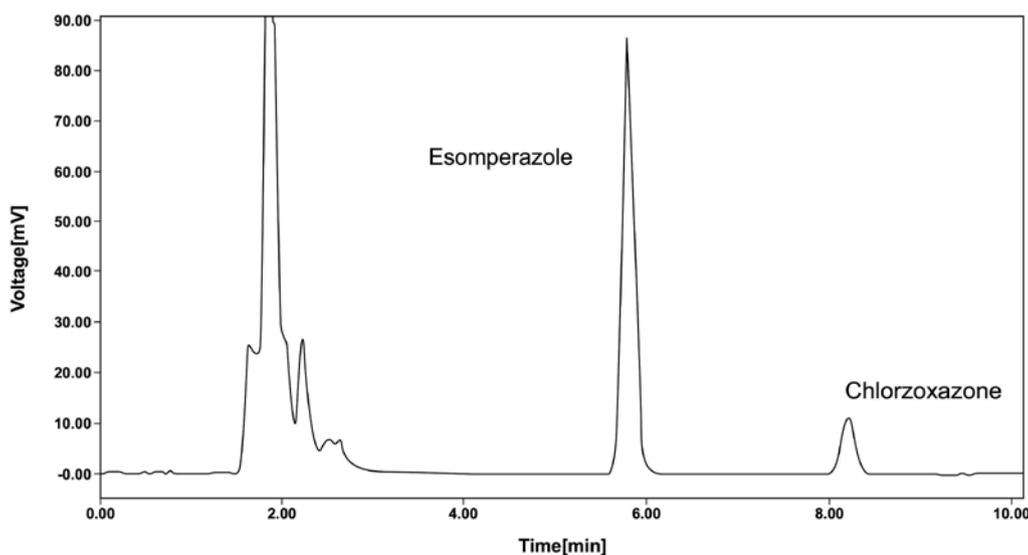
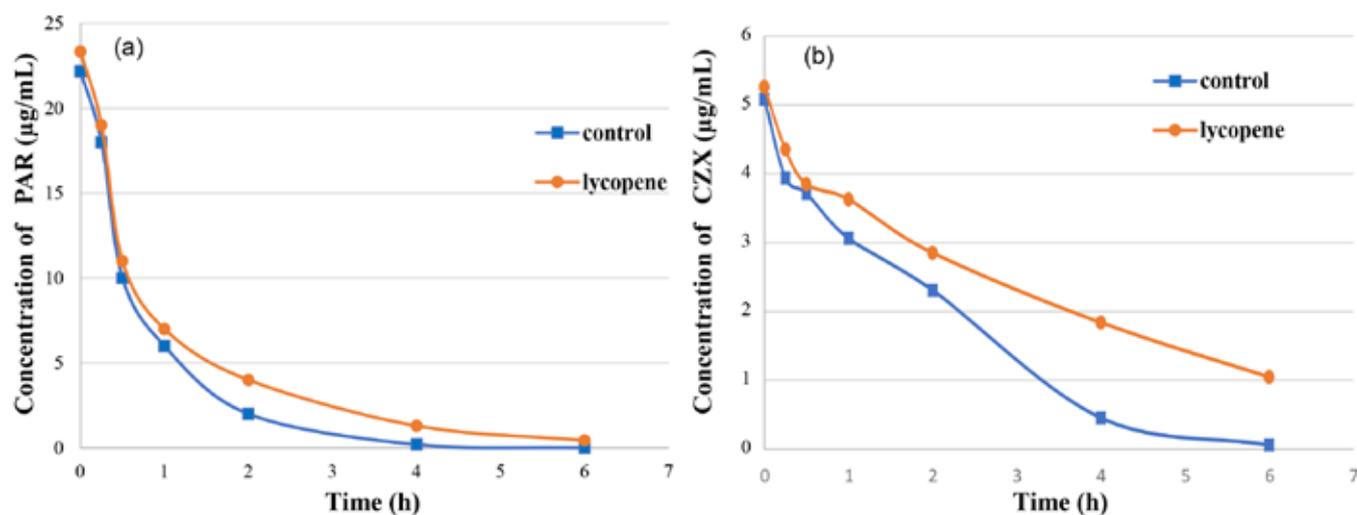


FIGURE 5 - Chromatogram of chlorzoxazone in plasma taken from a rat after IV administration and spiked with esomeprazole (44  $\mu$ g/mL).



**FIGURE 6 - (a)** The concentration–time profile of paracetamol (25 mg/kg, IV) after treatment with water (control) or lycopene (20 mg/kg, ip) in rats **(b)** The concentration–time profile of chlorzoxazone (20 mg/kg, IV) after treatment with saline (control) or lycopene (20 mg/kg, ip) in rats.

and lycopene group were compared. All statistics were calculated using IBM SPSS Statistics, Version 22, Copyright IBM Corporation and others 1989, 2013. The significance of difference between groups was analyzed

by student's t-test. Differences were considered significant when  $P < 0.05$ . The results are represented in Tables V and VI.

**TABLE V** - Statistical analysis of the results obtained by comparing the control group and lycopene group for the determination of pharmacokinetics parameters of paracetamol (25mg/kg) using student's t-test

Parameter	Lycopene	Control	$P_{cal}$
$C_{max}$ (µg/mL)	23.044±2.254	23.224± 3.032	0.905
$t_{1/2}$ (h)*	1.327 ±0.355	0.635±0.118	0.001
$V_d$ (mg/kg)/(µg/mL)*	1.936± 0.701	1.143± 0.242	0.026
cL (mg/kg)/(µg/mL)/h*	1.0001± 0.141	1.256± 0.178	0.020
$AUC_{0-t}$ (µg/mL*h)*	24.62± 3.032	19.773±3.227	0.038
$AUC_{0-inf}$ (µg/mL*h)*	26.102±3.720	20.150±3.023	0.015

\* Significant difference between two groups

**TABLE VI** - Statistical analysis of the results obtained by comparing the control group and lycopene group for the determination of pharmacokinetics parameters of chlorzoxazone (20mg/kg) using student's t-test

Parameter	Lycopene	Control	$P_{cal}$
$C_{max}$ (µg/mL)	5.264±0.725	5.298±0.424	.9230
$t_{1/2}$ (h)*	2.988±1.0498	1.348±0.112	.0030
$V_d$ (mg/kg)/(µg/mL)	4.260±0.725	3.834±0.926	.3460
cL (mg/kg)/(µg/mL)/h*	1.059±0.256	1.961±0.352	0.000
$AUC_{0-t}$ (µg/mL*h)*	14.915±1.519	9.143±2.360	0.001
$AUC_{0-inf}$ (µg/mL*h)*	20.020±5.758	10.460±1.749	.0030

\* Significant difference between two groups

## DISCUSSION

To determine the affecting dose of lycopene, many preliminary trials were carried out. Lycopene (10 mg/kg) was given IP to rats for six consecutive days followed by IV administration of PAR (25 mg/kg) or CZX (20mg/kg). Blood samples were collected from each group at different time intervals (0 - 6 h). The results of this experiment were compared to that carried out using lycopene (20 mg/kg). Control group was carried out for each drug by pretreating with the vehicle instead of lycopene. Both experiments showed an increase in  $AUC_{0-p}$   $AUC_{0-inf}$  and a decrease in cL of both PAR and CZX when pretreated with lycopene but this effect was more significant in the rats pretreated with (20 mg/kg) IP lycopene dose.

This experiment was repeated but with pretreating the rats with lycopene 20 mg/kg for eight consecutive days instead of six then PAR or CZX was IV administered. This showed that the effect of lycopene was more significant when the duration of lycopene administration was increased.

A herbal or food drug interaction occurs when a particular food alters an enzyme activity leading to changes in drug metabolism by this enzyme (Saxena *et al.*, 2008; Schmidt, Dalhoff, 2002).

Recent study showed the inhibitory effect of tomato juice on the metabolism of some substrates of CYP3A4 such as nifedipine, testosterone and midazolam due to the inhibitor effect of lycopene and other flavonoids on CYP3A4 (Sunaga *et al.*, 2012). But this work showed the inhibitory effect of lycopene on pharmacokinetic of paracetamol and chlorzoxazone, which is mainly metabolized by CYP2E, due to the inhibitory effect of lycopene on CYP2E1.

There are three main metabolic pathways for PAR leading to the formation of toxic or non-toxic products and eventually excreted in urine (Veronese, McLean, 1991). In the third pathway, however, the intermediate product *N*-acetyl-*p*-benzoquinone imine (NAPQI) is toxic. NAPQI is primarily responsible for the toxic effects of PAR. Production of NAPQI is due primarily to two cytochrome P450 enzymes: CYP2E1 and CYP3A4 (Foye, Lemke, Williams, 2008).

On the other hand, CZX is metabolized to OH-CZX by the hepatic microsomal cytochrome P450: CYP2E1 in humans (Lucas *et al.*, 1995) and rats (Rockich, Blouin, 1999).

According to experimental results, it can be deduced that pretreatment of rats with lycopene in a dose of 20 mg/kg lead to an increase in the AUC and a decrease in cL of both PAR and CZX and this could be a result of

inhibiting CYP2E1. Because both PAR and CZX have low to intermediate hepatic extraction ratio ((Coleman, 2006; Mehvar, Vuppugalla, 2006), therefore, their hepatic clearance depends mainly on the intrinsic clearance ( $cL_{int}$ ) ((Bryant, Knights, 2014; Davies, Morris, 1993; Lin, Lu, 1997). So, according to equation (1), AUC increases when clearance (or intrinsic clearance in case of low clearance drugs) decreases (Lin, Lu, 1997):

$$AUC_{total} = dose/cL = dose/f_p \cdot Cl_{int} \quad (1)$$

When the rats were pretreated with Lycopene, the intrinsic and hepatic clearances decreased and  $AUC_{0-p}$   $AUC_{0-inf}$  of both PAR and CZX increased, whereas Lycopene had no significant effect on  $C_{max}$  as both were administered intravenously.

The elimination half-life  $t_{1/2}$  increased when the volume of distribution increased, and the total clearance decreased (Lin, Lu, 1997) as shown in Equation (2), and this was consistent with the experimental results that showed an increase of the elimination half-life of both PAR and CZX more than 2 folds:

$$t_{1/2} = 0.693 \times Vd/Cl \quad (2)$$

Using FDA rodent-human dose conversion based on 60 kg average human weight (Nair, Jacob, 2016), should consume 193 mg of lycopene per day ( $20 \times 60 / 6.2 = 193$  mg) to show the difference of the pharmacokinetic parameters for the studied drugs. 6.2 is the ratio that compares the body surface area of rats with humans.

## CONCLUSION

In conclusion, the pharmacokinetic behavior of paracetamol and chlorzoxazone was remarkably changed in rats after intra-peritoneal administration of lycopene (20mg/kg), and thus a human being should consume 193 mg of lycopene to show the difference of pharmacokinetic parameters for the studied drugs. Hence, it could be presumed that the increase of AUC and the decrease of cL might be due to the inhibitory effect of lycopene on CYP2E1, the main enzyme in the metabolism of paracetamol and chlorzoxazone. The results of our research might contribute to guide future clinical studies.

## NOTES

The authors declare no competing conflict of interest and no financial contributions were received.

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