CHAPTER 3

RESULTS

3.1 Subjects

Twelve healthy Thai male volunteers participated in the study. All completed study protocol, however, data from one subject was excluded from analysis since caffeine was detected in his serum at baseline. The demographic characteristics of the remaining 11 subjects completed the study without protocol deviations are shown in Table 2-4. Every subject was considered healthy on the basis of medical history and physical examination.

Table 2 The demographic characteristics of subjects who completed study without protocol deviation (n=11)

Subject No.#	Age (y)	Weight (kg)	Height (m)	BMI (kg/m^2)
1	20.00	60.00	1.69	21.01
2	25.00	52.00	1.69	18.21
4	19.00	84.50	1.84	24.96
5	19.00	59.20	1.68	20.98
6	19.00	53.80	1.68	19.06
7	22.00	53.50	1.60	20.90
8	22.00	51.00	1.61	19.68
9	20.00	54.00	1.60	21.23
10	19.00	55.20	1.67	19.79
11	21.00	66.50	1.64	24.72
12	19.00	57.80	1.78	18.24
Mean	21.09	58.86	1.68	20.80
SD	1.97	9.58	0.07	2.27

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 3 Blood pressure and heart rate of subjects who completed study without protocol deviation (n=11)

Subject No.#	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Heart rate (beats/min)
1	117	71	80
2	112	74	89
4	110	76	69
5	114	76	75
6	110	58	85
7	114	73	80
8	113	77	89
9	106	63	66
10	114	73	89
11	122	91	68
12	108	65	61
Mean	112.73	72.45	77.36
SD	4.38	8.65	10.19

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 4 The demographic characteristics and serum antioxidant levels at baseline of the study subjects at the initiation of coffee enema (CE) phase and coffee consumption (CC) phase

	Study phase		
	CE	**CC**	***p* value**
Age (y)	21.09±7.97		
Weight (kg)	58.86±9.58		
Height (m)	1.68±0.07		
BMI (kg/m^2)	20.80±2.27		
SBP (mmHg)	112.40±6.87	114.82±8.11	0.485
DBP (mmHg)	73.00±8.52	74.36±7.60	0.709
HR (beat/min)	69.20±12.62	66.82±9.90	0.632
Baseline GSH (μmol/L)	5.230±1.390	4.961±1.307	0.645
Baseline MDA (mmol/L)	0.011±0.005	0.017±0.009	0.056
Baseline TEAC (mmol/L)	1.584±0.095	1.509±1.963	0.020

Data represents mean ± SD

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; GSH, glutathione; MDA, malondialdehyde; TEAC, trolox equivalent antioxidant capacity

3.2 Caffeine content in coffee solutions

Six servings of each coffee solution were measured for caffeine contents. The mean caffeine contents were 107.24±2.22 mg/500 ml for coffee enema solution and 96.34±1.39 mg/180 ml for coffee consumption solution (Table 5). These mean values of caffeine contents were not statistically different between caffeine solution prepared for coffee enema or coffee consumption (p=0.972).

Table 5 Mean value of caffeine contents in 6 servings of coffee solution used in this study (n=6)

	Lot No.	Caffeine content (mg/serving)
Coffee enema (500 mL)	50851	107.24±2.22
Coffee consumption (180 mL)	363A41	96.34±1.39

Data represents mean ± SD

3.3 Validation of HPLC method

3.3.1 Specificity

Chromatogram of caffeine-free plasma is shown in Figure 8A, whereas chromatogram of plasma containing 4,000 ng/mL of acetaminophen (internal standard) is presented in Figure 8B. The chromatogram of plasma containing 4,000 ng/mL of caffeine and 4,000 ng/mL of acetaminophen (internal standard, IS) are presented in Figure 8C. The retention time of acetaminophen and caffeine were about 4.204 min and 7.082 min, respectively. All peaks were clearly separated and no interference from endogeneous substances was observed.

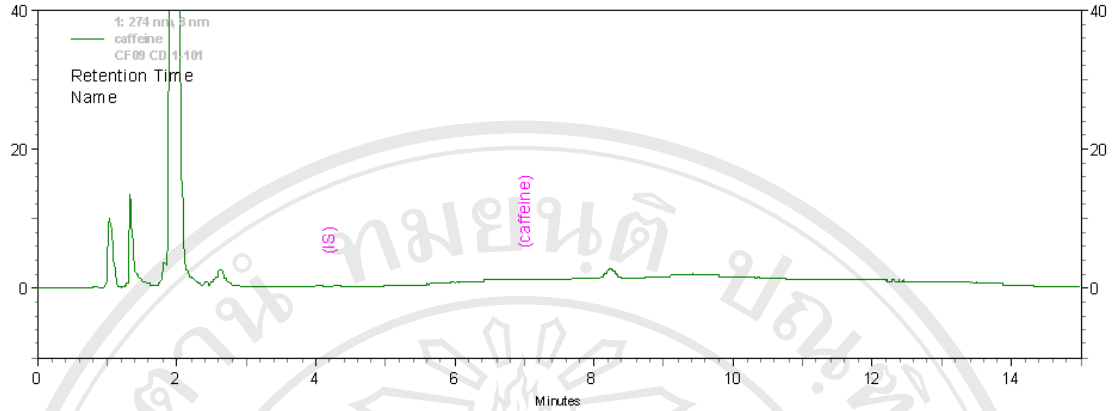


Figure 8A. Chromatogram of caffeine-free plasma

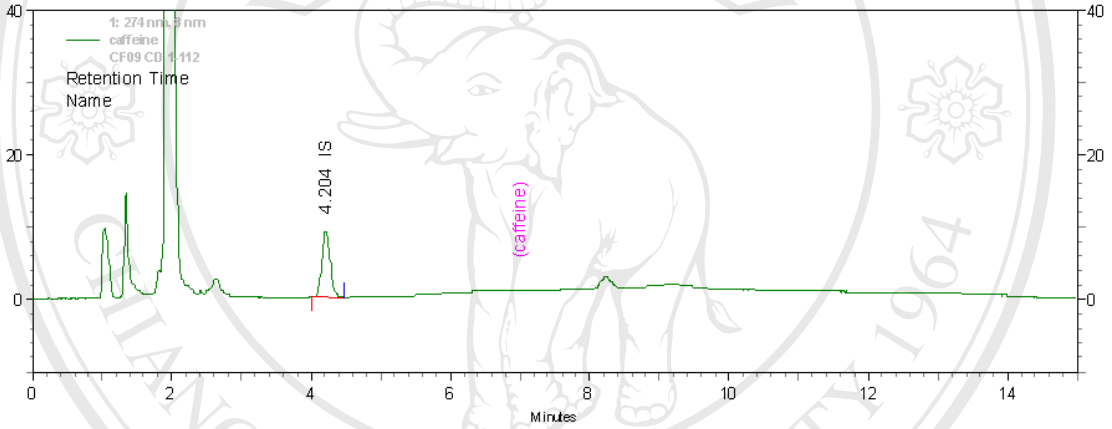


Figure 8B. Chromatogram of plasma sample containing 4,000 ng/mL of IS (retention Time= 4.204 min)

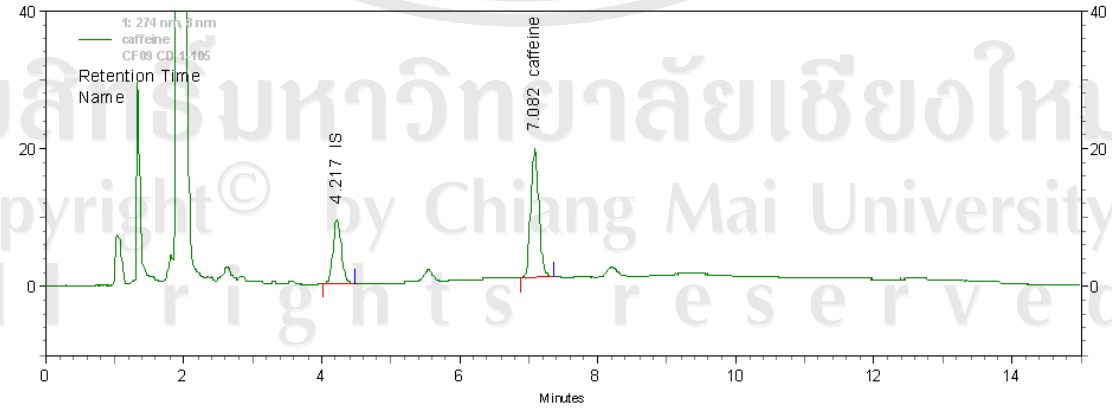


Figure 8C. Chromatogram of plasma sample containing 4,000 ng/mL of internal standard (IS) (retention time= 4.217 min) and 4,000 ng/mL caffeine (retention time = 7.082 min)

3.3.2 Linearity of calibration curve

Calibration curve of standard plasma containing 100-4,000 ng/mL of caffeine was constructed from the measured peak heights ratio of caffeine and acetaminophen chromatograms. Linearity of the calibration curve was determined by regression and correlation coefficient (r^2) analyses. The data of plasma caffeine used for constructing calibration curve are shown in Table 6. The regression equation for testing the linearity of standard calibration curve is shown below:

$y = 0.8094x + 0.113, \quad r^2 = 0.9999$

The linearity with good correlation coefficient (r^2) of calibration curves was also demonstrated (Figure9A). Pooled calibration causes from 5 replicated calibration data of caffeine are shown in Figure 9B.

Table 6. Calibration curve data of caffeine in plasma

No.	Caffeine concentration (ng/mL)	Peak height of caffeine	Peak height of IS	Caffeine/IS peak height ratio x 1000
1	100	691	7812	88.45
2	250	1340	6599	203.06
3	500	2330	6082	383.10
4	1,000	6197	7473	829.25
5	2,000	11697	7258	1,611.60
6	4,000	24647	7610	3,238.76

IS, Internal standard

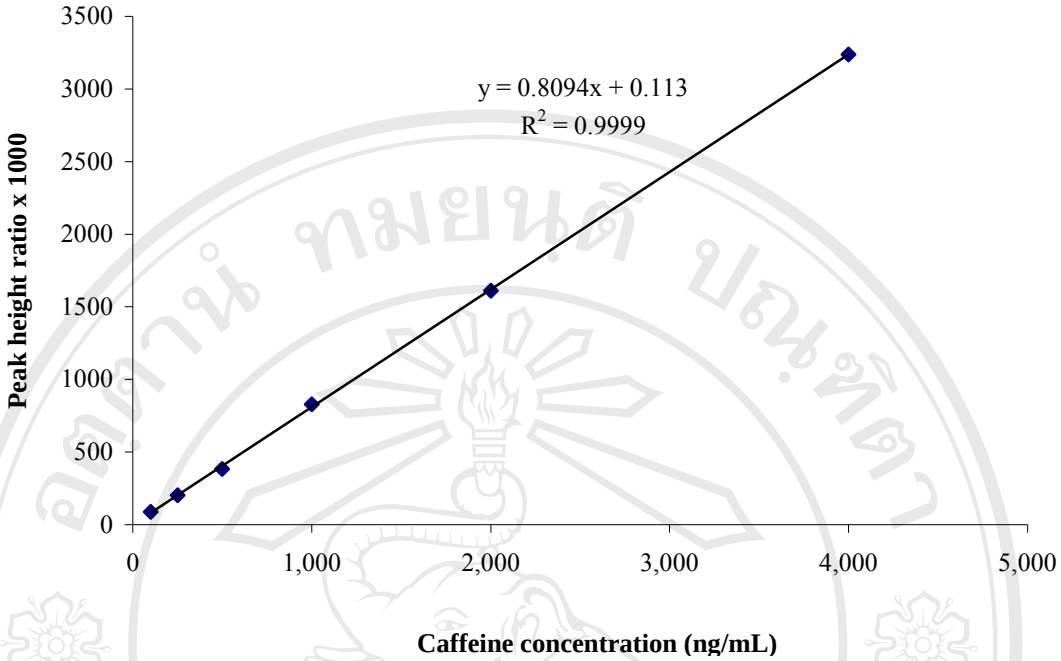


Figure 9A. A standard calibration curve of caffeine/internal standard peak height ratio versus plasma caffeine concentrations

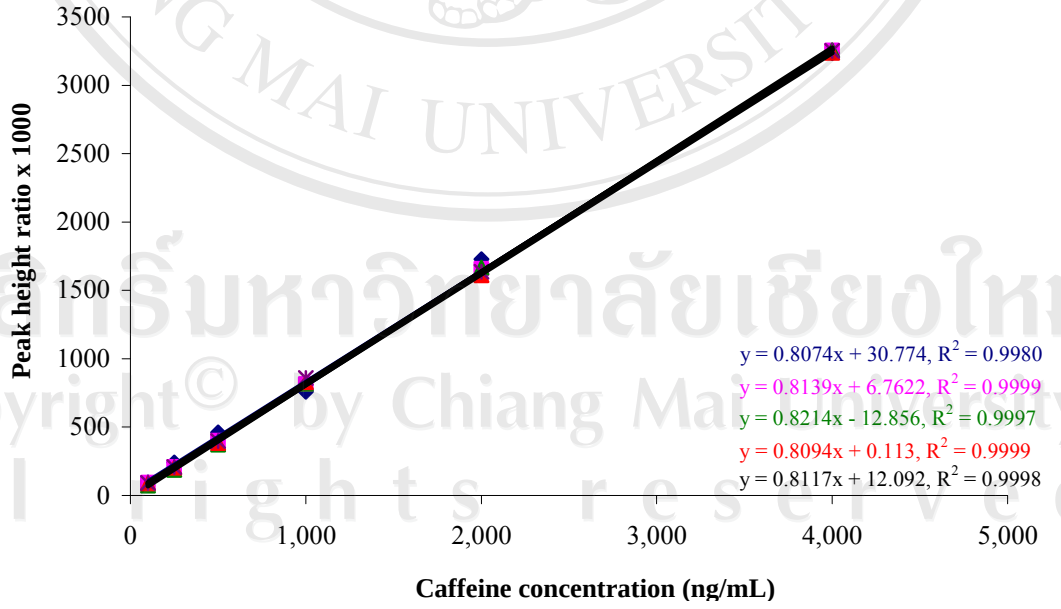


Figure 9B. Pooled calibration curve from 5 replicated calibration data

3.3.3 Precision and accuracy

Intra-day and inter-day precision were determined using 3 quality control (QC) samples of caffeine in plasma (300, 1900, 3800 ng/mL). Intra-day precision was determined in one day by assaying 5 replicates of each concentration whereas inter-day precision was determined daily with one sample of each concentration for 5 days. The precision was reported as the percentage of coefficient of variation (% CV) which was calculated as follow:

$$\% \text{ CV} = \left(\frac{SD}{\overline{X}}\right) \times 100$$

Where SD = standard deviation

$\overline{X}$ = mean value of caffeine concentration in plasma

The deviation was expressed as the percentage of inaccuracy calculated by the following equation:

$$\% \text{ Deviation} = \frac{(\text{Measured concentration} - \text{Spiked concentration})}{\text{Spiked concentration}} \times 100$$

The % CV of intra-day precision for low (100 ng/mL), medium (1900 ng/mL), and high (3800 ng/mL) plasma caffeine concentrations ranged from 1.69-3.91 (Table 7), whereas the % CV of inter-day ranged from 4.47-5.78% (Table 8). Likewise, the % deviation in intra-day and inter-day assay from low, medium, and high plasma caffeine concentrations ranged from -8.45 to 2.00% (Table 7) and -3.43 to 5.78%, respectively (Table 8). All these values were within rang of ±15% recommended by U.S. Food and Drug Administration guidance for bio-analytical method validation.

Table 7. Intra-day assay validation of caffeine in plasma

Low concentration

No.	Spiked concentration (ng/mL)	Peak height of caffeine	Peak height of IS	Caffeine /IS peak height ratio x 1000	Calculated concentration of caffeine (ng/mL)	Deviation (%)
1	300	2518	9677	260.20	306.51	2.17
2	300	2690	9717	278.83	324.92	8.31
3	300	2160	8737	247.22	292.14	-2.62
4	300	2495	9639	258.84	305.00	1.67
5	300	2661	10409	255.64	301.46	0.49
	Mean	2504.80	9635.80	259.75	301.01	2.00
	SD	210.86	564.55	10.80	11.96	
	% CV	8.42	6.17	4.16	3.91	

IS, Internal standard

Medium concentration

No.	Spiked concentration (ng/mL)	Peak height of caffeine	Peak height of IS	Caffeine /IS peak height ratio x 1000	Calculated concentration of caffeine (ng/mL)	Deviation (%)
1	1,900	14073	8608	1634.87	1828.68	-3.75
2	1,900	13491	8424	1601.50	1791.72	-5.70
3	1,900	14402	8757	1644.63	1839.48	-3.19
4	1,900	14336	8809	1627.43	1820.43	-4.19
5	1,900	13469	8541	1576.98	1764.57	-7.13
	Mean	13954.20	8627.80	1617.08	1808.97	-4.79
	SD	450.11	157.31	27.54	30.49	
	% CV	3.23	1.82	1.70	1.69	

IS, Internal standard

High concentration

No.	Spiked concentration (ng/mL)	Peak height of caffeine	Peak height of IS	Caffeine /IS peak height ratio x 1000	Calculated concentration of caffeine (ng/mL)	Deviation (%)
1	3,800	28031	8780	3192.60	3553.54	-6.49
2	3,800	27783	8872	3131.54	3485.93	-8.27
3	3,800	27323	8588	3181.53	3541.29	-6.81
4	3,800	29465	9512	3097.67	3448.42	-9.25
5	3,800	28312	9368	3022.20	3364.86	-11.45
	Mean	28182.80	9024	3125.11	3478.81	-8.45
	SD	803.57	396.62	69.14	76.56	
	% CV	2.85	4.40	2.21	2.20	

IS, Internal standard

Table 8. Inter-day assay validation of caffeine in plasma

Spiked concentration (ng/mL)	Calculated concentration of caffeine (ng/mL)							Precision (% CV)	Deviation (%)
	day 1	day 2	day 3	day 4	day 5	Mean	SD		
300 (n=5)	306.01±11.96	328.80±8.10	340.20±2.44	313.82±23.58	293.95±7.92	316.56	18.29	5.78	5.52
1,900 (n=5)	1808.97±30.49	1944.62±16.38	2002.25±36.02	1788.10±35.35	1901.42±36.31	1889.07	90.36	4.78	-0.58
3,800 (n=5)	3478.81±76.56	3759.32±70.54	3712.78±62.86	3525.94±52.94	3871.85±72.30	3669.74	164.21	4.47	-3.43

3.3.4 Lower limit of quantification (LLQ)

The LLQ was defined as the lowest concentration on the calibration curve (100 ng/mL) that could be measured with acceptable precision (% CV of less than ± 20%) and accuracy (% deviation of less than ± 20%). The LLQ was determined by analyzing a series of 5 replicated samples of gradually lowering concentrations until the lowest concentration with acceptable precision and accuracy was obtained. The % CV and % deviation of LLQ were 2.34% and 10.52%, respectively (Table 9).

Table 9. The LLQ of caffeine in plasma

No.	Spiked concentration (ng/mL)	Peak height of caffeine	Peak height of IS	Caffeine /IS peak height ratio x 1000	Calculated concentration of caffeine (ng/mL)	Deviation (%)
1	100	714	7833	91.51	112.48	12.48
2	100	699	7743	90.28	111.39	11.39
3	100	719	7933	90.63	111.84	11.84
4	100	701	7801	89.86	110.88	10.88
5	100	657	7646	85.93	106.02	6.02
	Mean			89.57	110.52	10.52
	SD			2.09	2.58	
	% CV			2.33	2.34	

IS, Internal standard

3.3.5 Recovery

Recovery was determined by comparing the peak height ratios of caffeine/acetaminophen extracted from plasma with that of caffeine/acetaminophen standard sample in mobile phase from 5 sets of 3 different concentrations of QC samples. The mean recoveries (%) of caffeine in plasma from the extraction procedure are presented in Table10. The mean recoveries (%) at the concentrations of 300, 1900 and 3800 ng/mL were 104.51, 97.65 and 88.75, respectively and their mean recoveries was 96.97%.

Table 10. Recovery of caffeine in plasma

	Concentration (ng/mL)	Peak height of caffeine		Peak height of IS	
		in mobile phase	in plasma	in mobile phase	in plasma
Low	300	2299	2320	9066	9645
	300	2364	2352	8939	9465
	300	2278	2418	8735	8833
	300	2323	2547	9107	8793
	300	2260	2407	8695	8548
	Mean	2305	2409	8908	9057
	SD	40.60	87.03	187.63	472.01
	% Recovery		104.51		101.67
Medium	1,900	13136	13432	8189	8368
	1,900	13146	13126	8197	8057
	1,900	12701	12792	7903	7872
	1,900	14542	13103	9086	8430
	1,900	13336	12838	8316	8138
	Mean	13372	13058	8338	8173
	SD	694.12	257.72	444.77	228.78
	% Recovery		97.65		98.02
High	3,800	28148	25205	8781	7931
	3,800	28587	25336	8929	8117
	3,800	27436	24788	8597	7914
	3,800	28111	24795	8692	8006
	3,800	29540	25742	9043	7931
	Mean	28364	25173	8808	7980
	SD	775.33	400.77	179.28	84.56
	% Recovery		88.75		90.59

3.3.6 Freeze/thaw (F/T) stability

The freeze/thaw stability of caffeine in plasma was obtained by 3 repeating analyses of plasma caffeine concentrations in QC samples (300 and 3,800 ng/mL) kept frozen at -20 ºC after 3 freeze-thaw cycles compared to caffeine concentrations in freshly prepared QC samples without freeze-thaw processing. The stability of caffeine in plasma after three freeze and thaw cycles is presented in Table 11. The percentages of caffeine remaining were 105.46 and 104.92, respectively, with overall percentage remaining of 105.19

Table 11. Concentrations of caffeine in plasma before and after 3 cycles of freeze and thaw (F/T) stability test

		Before F/T	After F/T
		Caffeine (ng/mL)	Caffeine (ng/mL)
Low concentration (ng/mL)	300	301.51	313.72
	300	293.94	307.27
	300	301.37	324.81
	Mean	298.94	315.27
	SD	4.33	8.87
	% Remaining[1]		105.46
High concentration (ng/mL)	3,800	3418.35	3567.55
	3,800	3482.85	3654.07
	3,800	3395.05	3581.19
	Mean	3432.08	3600.94
	SD	45.48	46.51
	% Remaining[1]		104.92
Average F/T stability (%)[2]			105.19

[1] (mean value of concentration after F/T) x100/(mean value of concentration before F/T)

[2] (% remaining of low concentration + % remaining of high concentration)/2

3.4 Pharmacokinetics of caffeine after single dose of coffee enema or coffee consumption

The individual as well as mean plasma caffeine concentration-time profiles from 11 subjects after single dose of coffee enema or coffee consumption are presented in Table 12A and 12B, respectively. The plasma concentration-time curves of caffeine of each subject are shown in Figure 10.

Table 12A. Plasma concentrations of caffeine (ng/mL) after single dose of coffee enema in study subjects

Subject No.#	Plasma concentration of caffeine (ng/mL) at various sampling time (h)										
	0.00	0.17	0.33	0.50	0.67	1.00	1.50	2.00	4.00	8.00	12.00
1	<LLQ	538.93	473.77	460.58	440.71	432.06	380.95	363.53	278.91	179.91	108.67
2	<LLQ	782.77	727.91	673.06	714.53	653.53	603.86	614.94	407.91	191.21	129.55
4	<LLQ	372.11	723.67	592.99	607.22	550.54	482.46	463.65	346.63	206.58	126.96
5	<LLQ	403.39	724.76	782.07	614.94	570.17	573.36	504.54	356.21	148.61	<LLQ
6	<LLQ	884.75	701.97	644.65	586.07	528.03	465.79	457.60	399.99	126.26	<LLQ
7	<LLQ	750.27	865.05	817.68	714.83	750.94	675.24	645.72	484.25	285.94	158.97
8	<LLQ	399.82	865.06	924.51	901.06	798.97	695.83	629.69	344.79	157.04	<LLQ
9	<LLQ	879.54	828.98	785.26	703.55	635.77	602.45	585.33	431.83	284.13	155.50
10	<LLQ	373.50	633.63	630.18	618.85	532.14	491.58	467.98	298.43	153.48	<LLQ
11	<LLQ	742.03	857.41	748.17	694.90	706.23	705.32	663.02	570.61	402.64	271.36
12	<LLQ	424.85	564.91	388.30	472.10	464.70	423.32	398.81	279.99	109.52	<LLQ
Mean	<LLQ	595.63†	724.28†	677.04†	642.61†	602.10†	554.56†	526.80†	381.78†	204.12†	158.50††
SD	<LLQ	212.50	128.53	157.92	126.19	117.21	112.58	105.00	89.90	87.26	58.42
% CV	<LLQ	35.68	17.75	23.32	19.64	19.47	20.30	19.93	23.55	42.75	36.86

† Mean values calculated from 11 subjects (n=11)

†† Mean values calculated from 6 subjects (n=6), samples with plasma concentration below LLQ were not taken into account

Data from subject No. 3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 12B. Plasma concentrations of caffeine (ng/mL) after single dose of coffee consumption in the study subjects

Subject No.#	Plasma concentration of caffeine (ng/mL) at various sampling time (h)										
	0.00	0.17	0.33	0.50	0.67	1.00	1.50	2.00	4.00	8.00	12.00
1	<LLQ	306.24	1516.36	2431.07	2429.69	2119.89	1910.24	1823.78	1567.39	986.16	609.20
2	<LLQ	965.14	2478.51	3124.12	2887.36	2438.70	2215.78	2041.81	1680.52	862.31	479.78
4	<LLQ	1074.97	1791.47	1639.45	1891.85	1735.70	1598.57	1474.80	1139.87	732.69	524.93
5	<LLQ	1836.43	2215.21	2875.06	2747.64	2308.58	1759.79	1662.63	981.12	490.02	185.29
6	<LLQ	1753.52	2126.82	2058.51	1924.13	1829.54	1693.74	1625.75	1057.09	540.91	221.35
7	<LLQ	422.95	2318.70	1782.97	1825.73	2129.41	1970.79	1889.20	1455.96	962.17	601.97
8	<LLQ	600.14	1204.58	1729.58	2315.19	2250.31	1843.64	1720.63	1138.96	516.24	213.38
9	<LLQ	1093.37	2425.24	2517.56	2446.57	2201.71	2041.28	1968.02	1372.63	909.33	545.56
10	<LLQ	1541.02	2954.58	2458.37	2437.45	2133.45	1969.77	1746.54	1268.16	696.17	390.70
11	<LLQ	1600.76	1864.52	2242.86	2134.96	2022.99	1597.73	1680.27	1282.46	924.96	650.68
12	<LLQ	1747.07	2415.40	2016.66	1830.75	1734.81	1714.91	1514.77	1055.70	438.33	204.39
Mean	<LLQ	1176.51	2119.22	2261.47	2261.03	2082.28	1846.93	1740.75	1272.71	732.66	420.66
SD	<LLQ	559.12	492.91	474.77	369.64	231.35	195.08	177.89	226.05	207.94	183.59
% CV	<LLQ	47.52	23.26	20.99	16.35	11.11	10.56	10.22	17.76	28.38	43.64

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

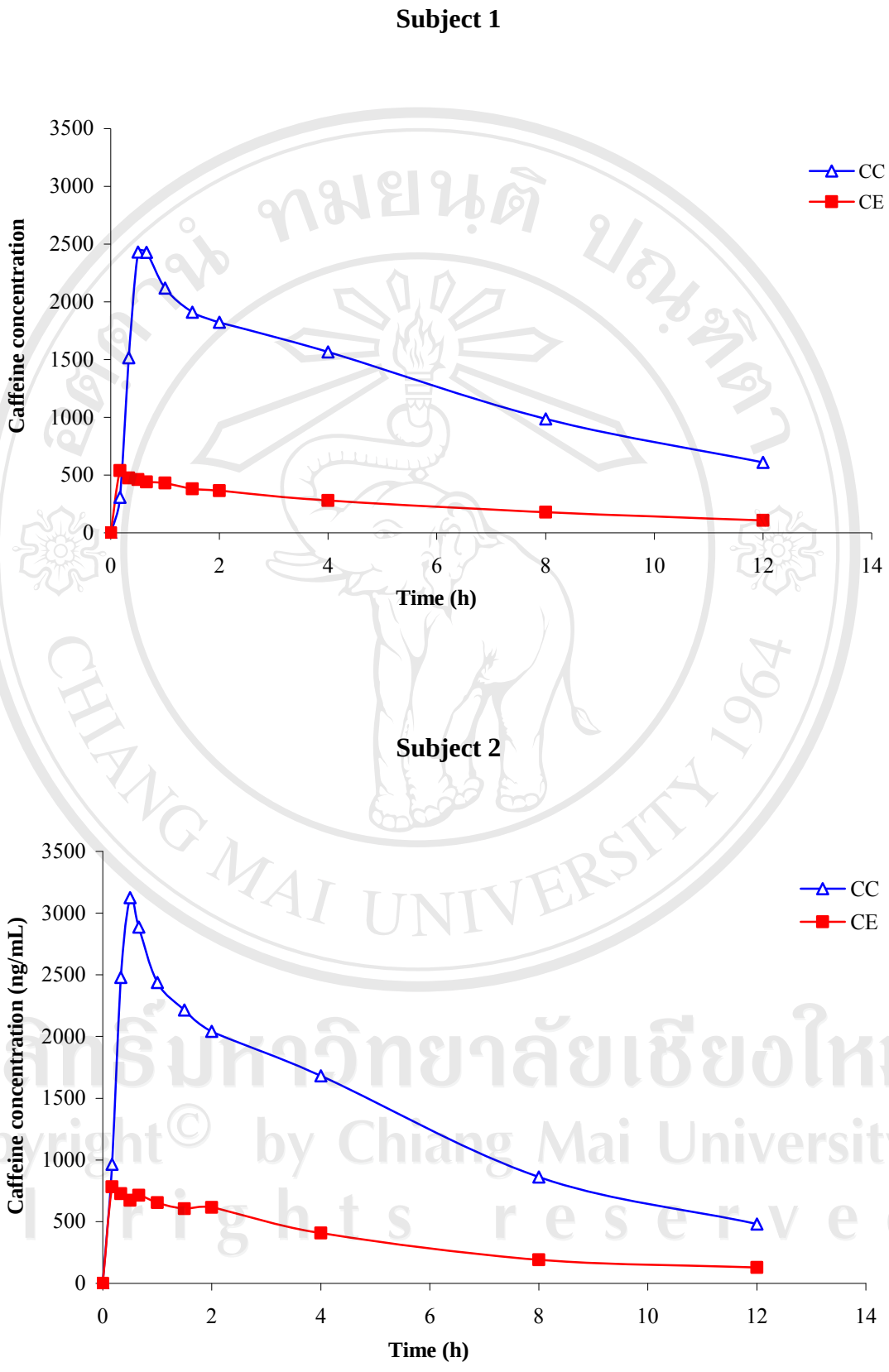
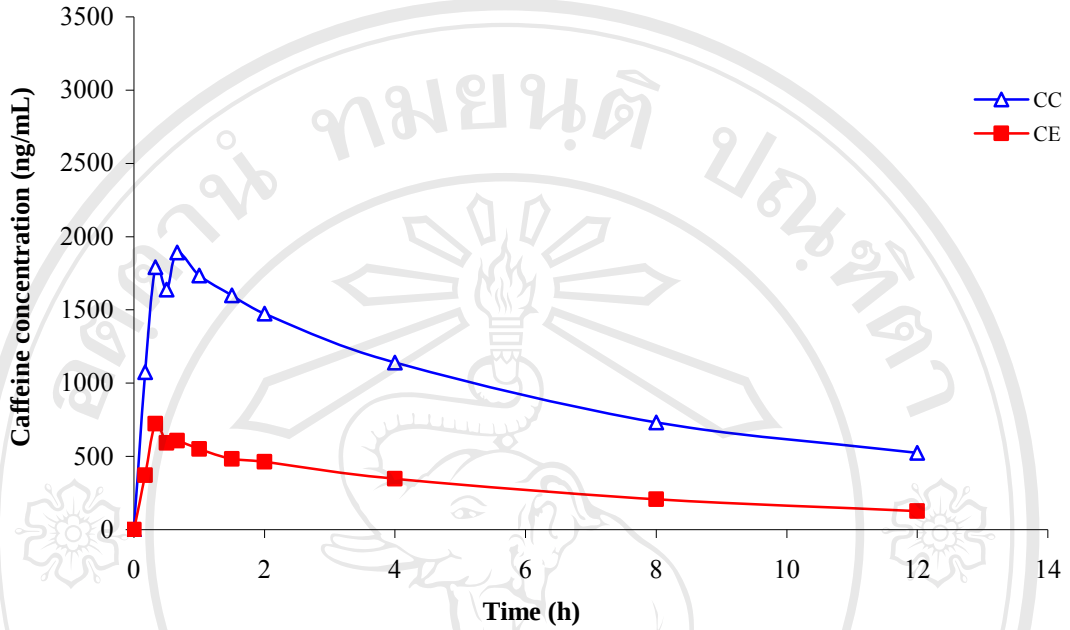


Figure 10. Plasma concentration-time curves of caffeine after single dose of coffee enema (CE) or coffee consumption (CC)

Subject 4



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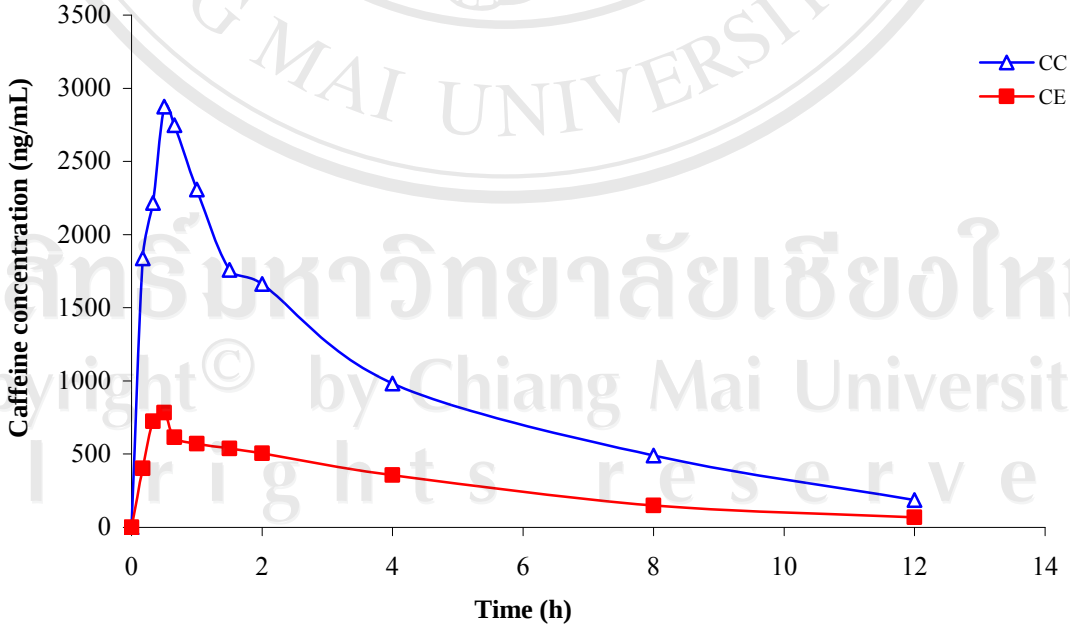

Figure 10. (Continued)

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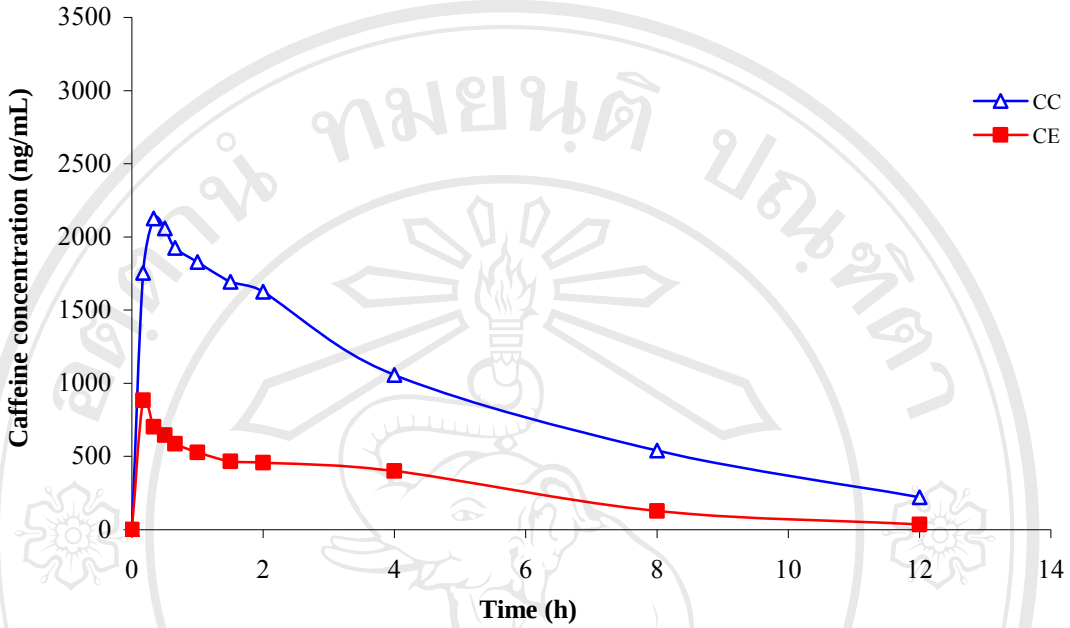

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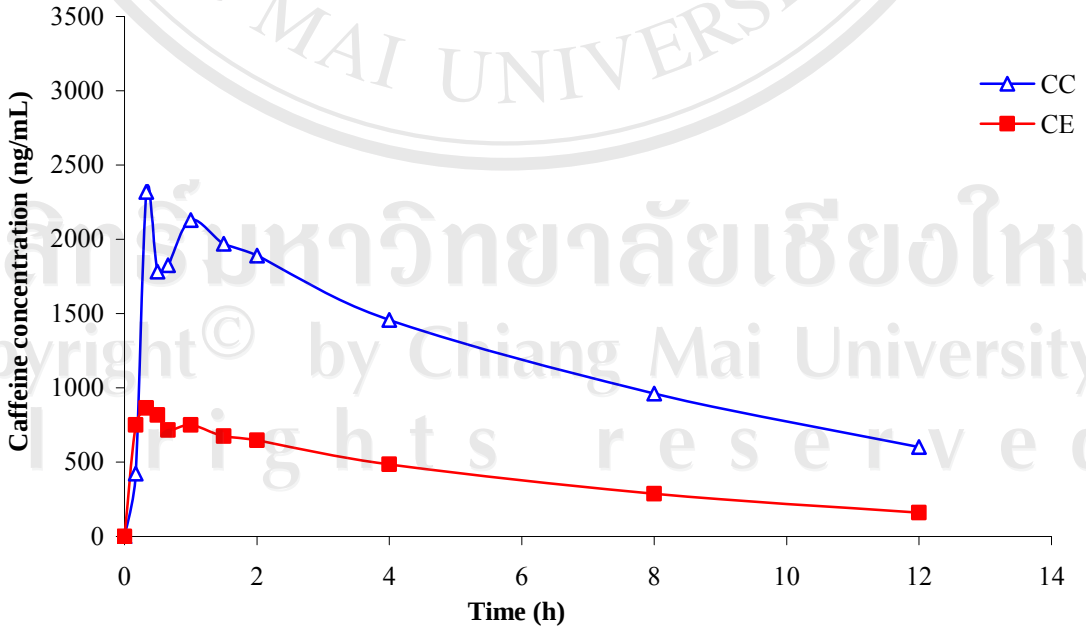

Figure 10. (Continued)

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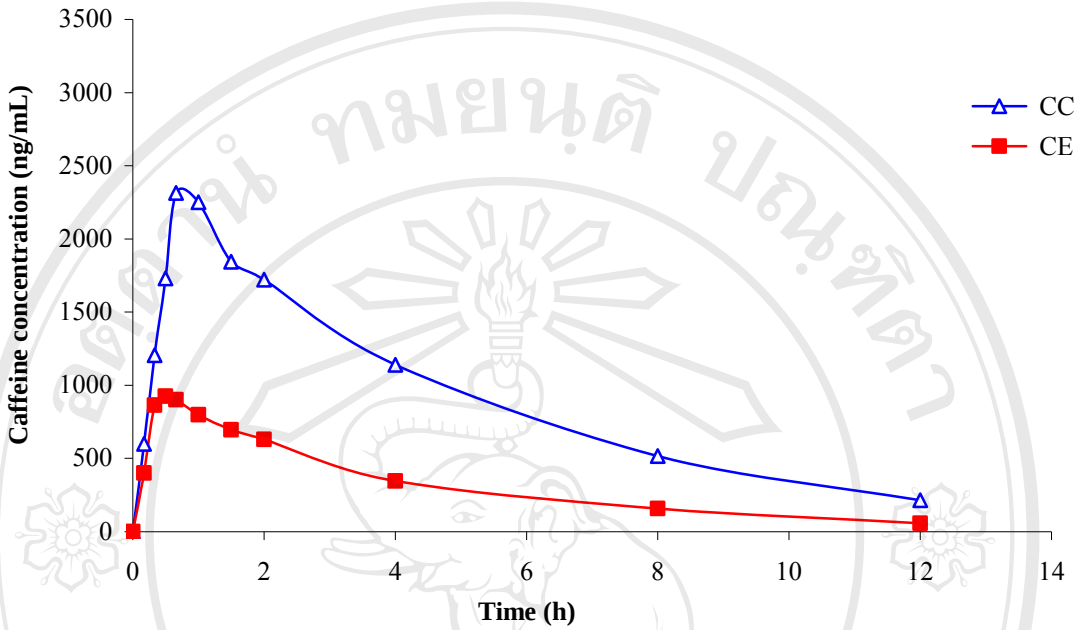

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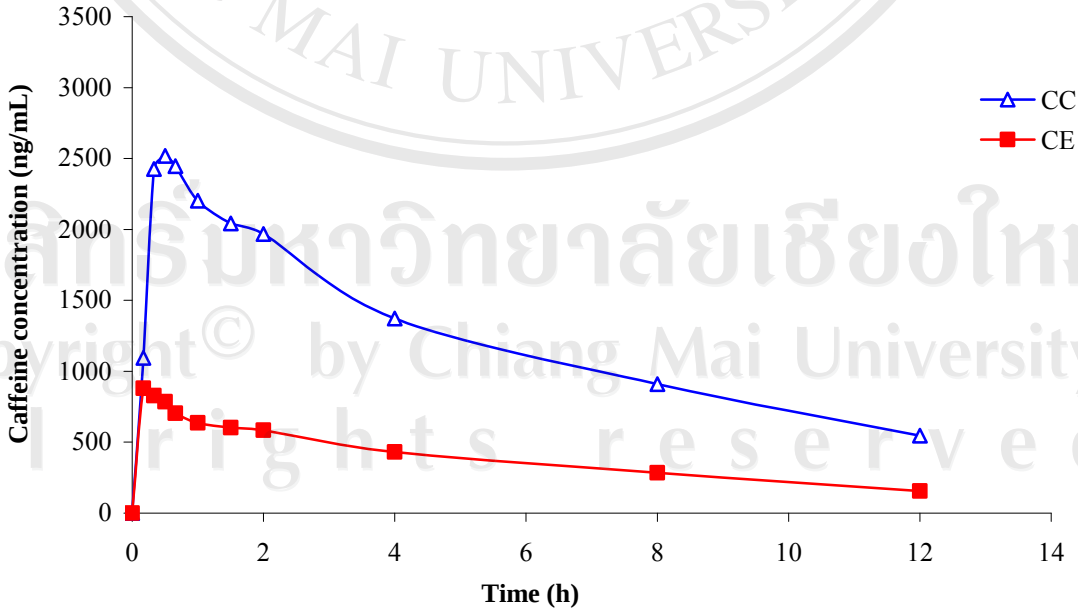
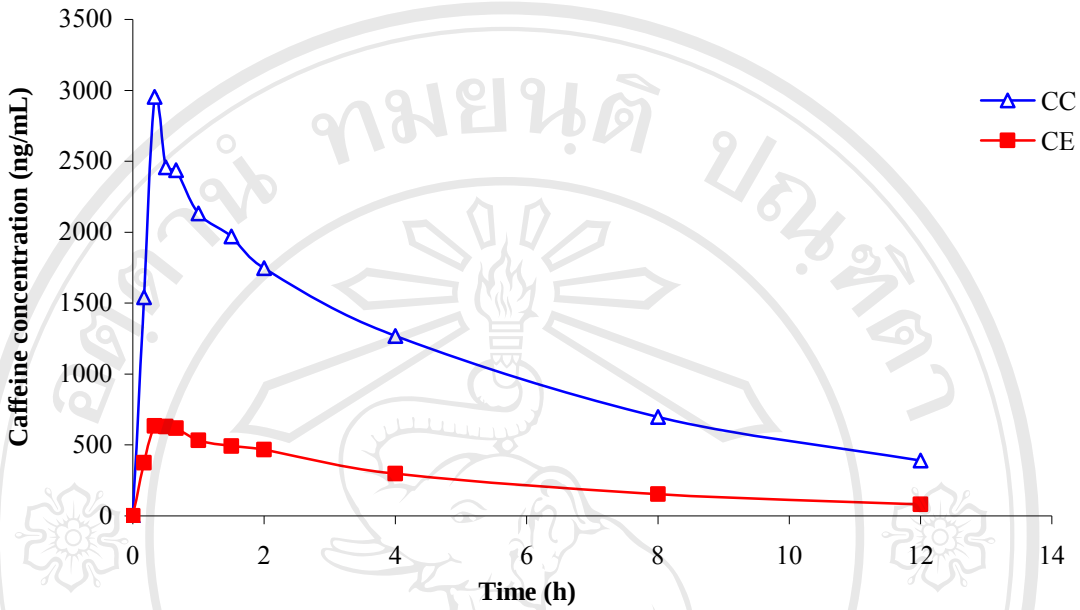


Figure 10. (Continued)

Subject 10



Subject 11

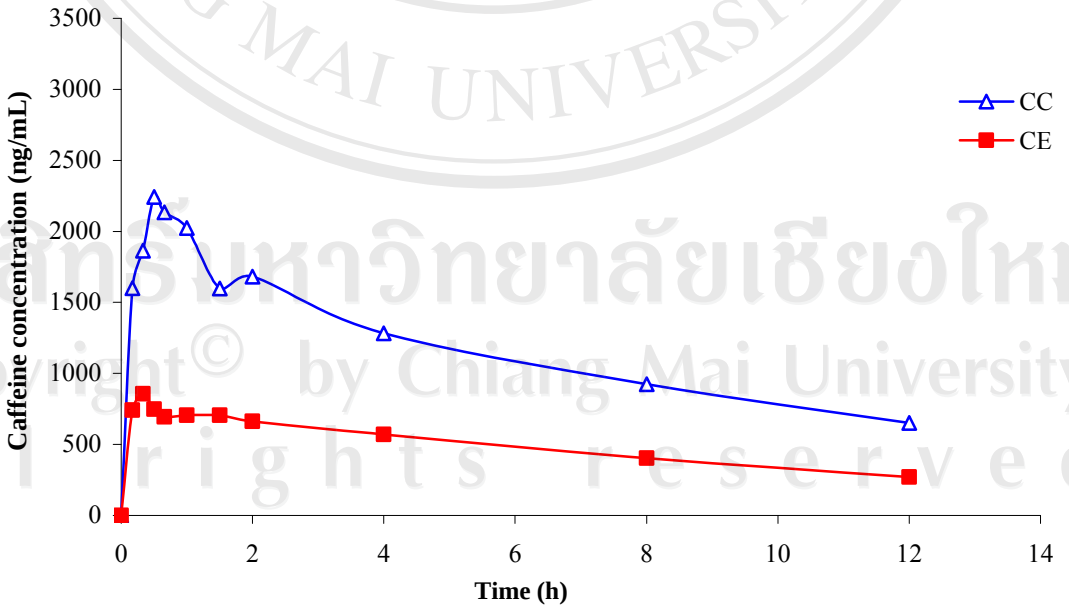


Figure 10. (Continued)

Subject 12

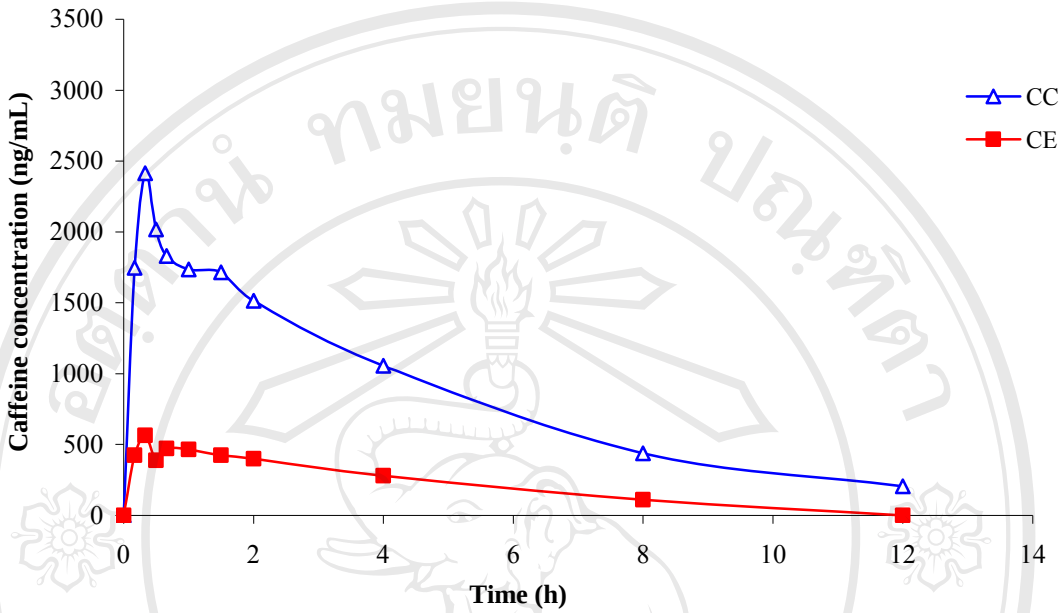


Figure 10. (Continued)

Individual plasma caffeine concentration-time curves of 11 subjects after single dose of coffee enema or coffee consumption are respectively depicted in Figures 11A and 11B. Their mean concentration-time profiles are also presented in Figure 11C. The pharmacokinetic parameters of caffeine (C_{max}, AUC_{0-12}, $AUC_{0-\infty}$, T_{max}, $t_{1/2}$) after single dose of coffee enema or coffee consumption were determined and are shown in Table 13.

When pharmacokinetic parameters of caffeine from coffee enema were determined in comparison to those of coffee consumption (Table 13), the mean maximum plasma caffeine concentrations (C_{max}, ng/mL) were 757.18 versus 2465.45. The times of their occurrences (Tmax, h) were 0.30 versus 0.44. The elimination $t_{1/2}$ (h) was 4.68 versus 4.87. The AUC_{0-12} (ng.h/ml) was 3685.58 versus 13046.26. The $AUC_{0-\infty}$ (ng.h/ml) was 4731.85 versus 16323.12. The pharmacokinetic parameters of caffeine from coffee enema and coffee consumptions (C_{max}, T_{max} , AUC_{0-12} and $AUC_{0-\infty}$), except $t_{1/2}$, were reach statistically significant differences (Table 13).

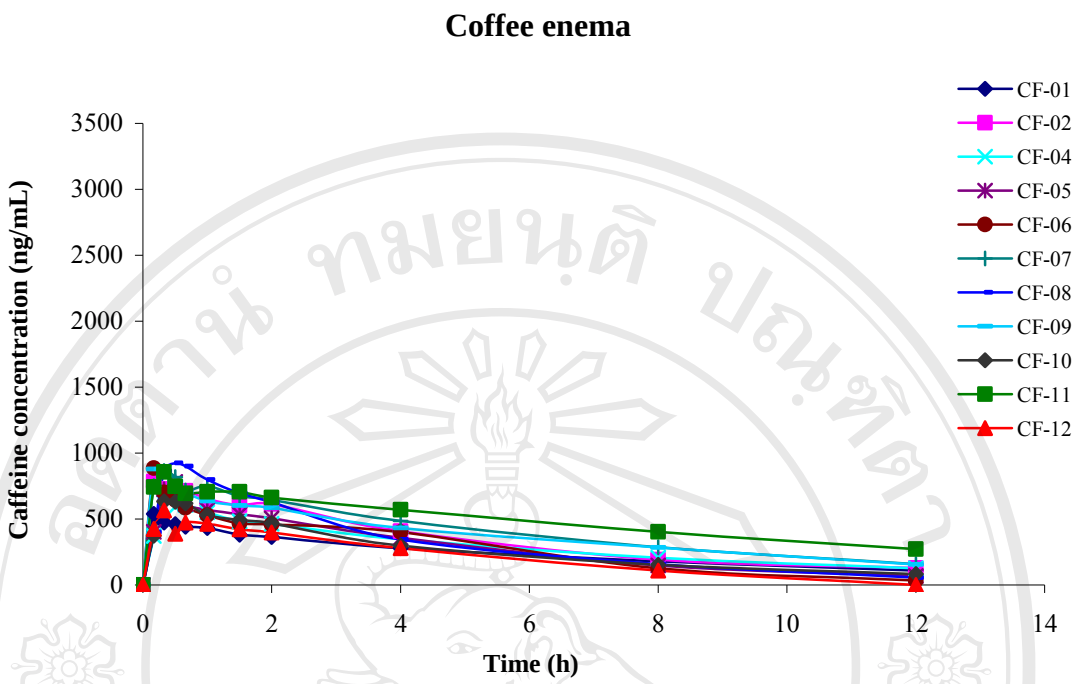


Figure 11A. Plasma caffeine concentration-time curves of individual subject (n=11) after single dose of coffee enema (CE)

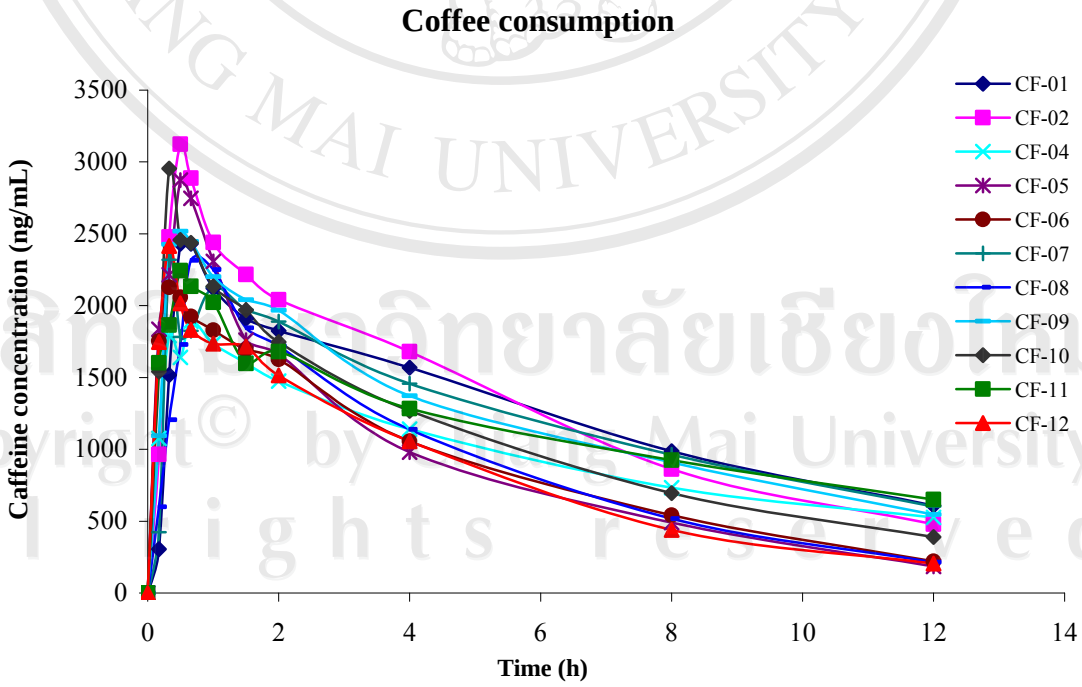


Figure 11B. Plasma caffeine concentration-time curves of individual subject (n=11) after single dose of coffee consumption (CC)

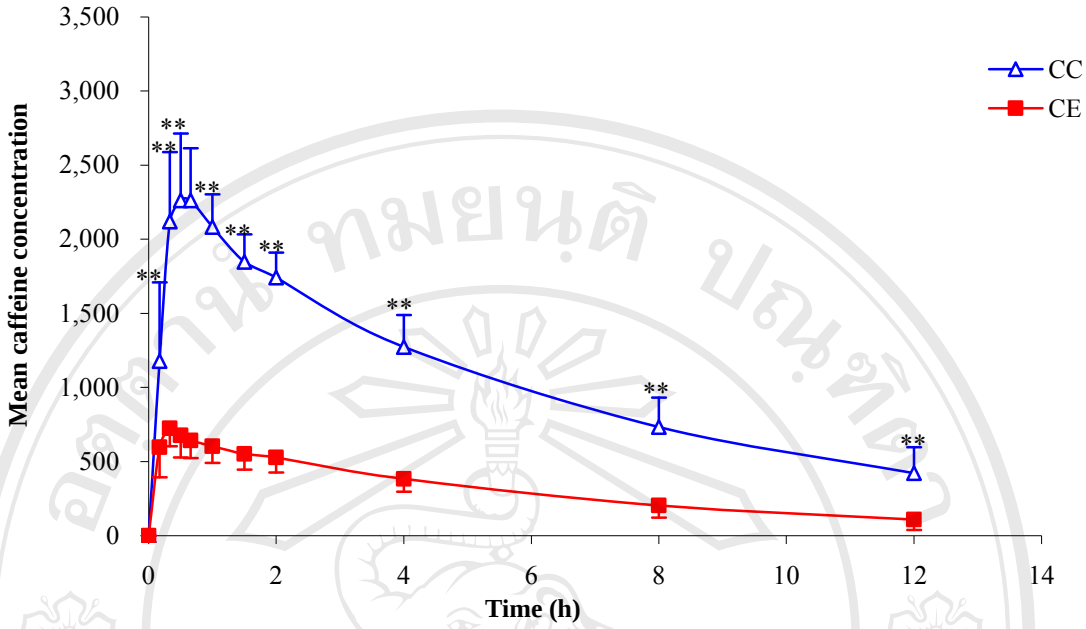


Figure 11C. Mean plasma caffeine concentration-time curves after single dose of coffee enema (CE) or coffee consumption (CC)

** Statistically significant between group (p<0.05, paired t-test)

Table 13. Pharmacokinetic parameters of caffeine after single dose of coffee enema (CE) or coffee consumption (CC) in 11 subjects completed the study without protocol deviation

Subject	C_{max} (ng/mL)		AUC_{0-12} (ng.h/ml)		$AUC_{0-\infty}$ (ng.h/ml)		T_{max} (h)		$t_{1/2}$ (h)	
No.#	CE	CC	CE	CC	CE	CC	CE	CC	CE	CC
1	539.00	2430.00	2953.33	15299.69	3828.98	20836.36	0.17	0.50	5.59	6.30
2	783.00	3120.00	4132.04	15940.29	4966.65	19123.05	0.17	0.50	4.47	4.60
4	724.00	1790.00	3602.60	11988.44	4543.37	16678.30	0.33	0.33	5.14	6.19
5	782.00	2880.00	2973.60	11031.62	3746.01	11857.10	0.50	0.50	3.60	3.09
6	885.00	2130.00	2993.85	10886.02	3616.72	12056.45	0.17	0.33	3.42	3.67
7	865.00	2320.00	4954.77	14864.74	6122.02	20208.53	0.33	0.33	5.09	6.15
8	925.00	2320.00	3405.53	11088.29	4065.17	12112.85	0.50	0.66	2.91	3.33
9	880.00	2520.00	4630.28	14859.32	5792.21	19103.40	0.17	0.50	5.18	5.39
10	634.00	2950.00	2681.38	13191.44	3508.53	15650.49	0.33	0.33	3.74	4.36
11	857.00	2240.00	5910.18	14073.02	9037.99	20610.62	0.33	0.50	7.99	6.96
12	565.00	2420.00	2303.76	10285.98	2822.66	11317.21	0.33	0.33	3.38	3.50
Mean	767.18	2465.45**	3685.58	13046.26**	4731.85	16323.12**	0.30	0.44**	4.68	4.87
SD	134.97	387.72	949.84	2059.61	1740.89	3885.00	0.12	0.11	1.36	1.39
Median	783.00	2420.00	3405.65	13191.44	4065.17	16678.30	0.33	0.50	4.47	4.60

** Statistically significant between group ($p<0.05$, paired t-test)

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

C_{max}, Mean maximum plasma caffeine concentrations; AUC_{0-12}, area under the concentration-time curve from administration to 12 hours; $AUC_{0-\infty}$, area under the concentration-time curve from administration and extrapolation to infinity; T_{max}, time to reach maximum concentration; $t_{1/2,}$ half life

3.5 Cardiovascular effects after single dose of coffee enema and coffee consumption

Blood pressure and heart rate

The mean values of systolic blood pressure, diastolic blood pressure and heart rate before and after single doses of coffee enema or coffee consumptions are shown in Table 14 and depicted in Figure 12, respectively. Single administration of either coffee enema or coffee consumption produced no statistical change in systolic blood pressure, diastolic blood pressure and heart rate when compared to their own baseline values. In addition, the mean changes from baseline of these hemodynamic parameters were not statistically different between the two coffee procedures (data not shown).

Table 14. Blood pressure and heart rate before and after single dose of coffee enema (CE) or coffee consumption (CC) in 11 subjects competed the study without protocol deviation

Parameter	Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)		Heart rate (beat/min)	
Time (h)	CE	CC	CE	CC	CE	CC
0	112.40±6.87	114.82±8.11	73.00±8.52	74.36±7.60	69.20±12.62	66.82±9.90
0.17	118.73±6.68	115.55±9.34	78.64±7.34	77.55±5.96	70.00±8.88	68.18±8.09
0.33	116.82±8.73	119.27±5.45	78.09±12.11	80.09±5.88	71.45±9.63	66.82±8.68
0.50	115.91±10.06	115.64±5.01	73.91±10.25	78.27±4.79	67.73±7.40	63.82±7.52
0.66	111.00±12.32	117.73±4.57	68.64±10.80	80.36±5.03	66.45±8.08	66.45±8.05
1.00	112.09±8.66	116.91±4.93	71.91±9.31	77.73±5.56	65.36±10.95	69.18±8.79
1.50	112.91±8.23	113.91±8.63	71.27±6.78	77.18±4.97	62.27±8.33	68.09±8.47
2.00	109.36±6.69	115.64±5.09	70.73±14.37	75.73±7.29	63.64±9.80	69.91±7.53
4.00	112.09±7.15	113.91±6.24	63.91±8.18	71.55±7.19	76.27±14.25	65.91±8.08
8.00	108.91±11.63	114.45±6.11	68.64±9.91	72.36±6.68	72.82±9.06	68.64±9.18
12.00	115.91±9.59	119.36±9.18	71.18±9.76	75.09±8.51	75.36±10.28	71.36±8.90

Data represents mean ± SD

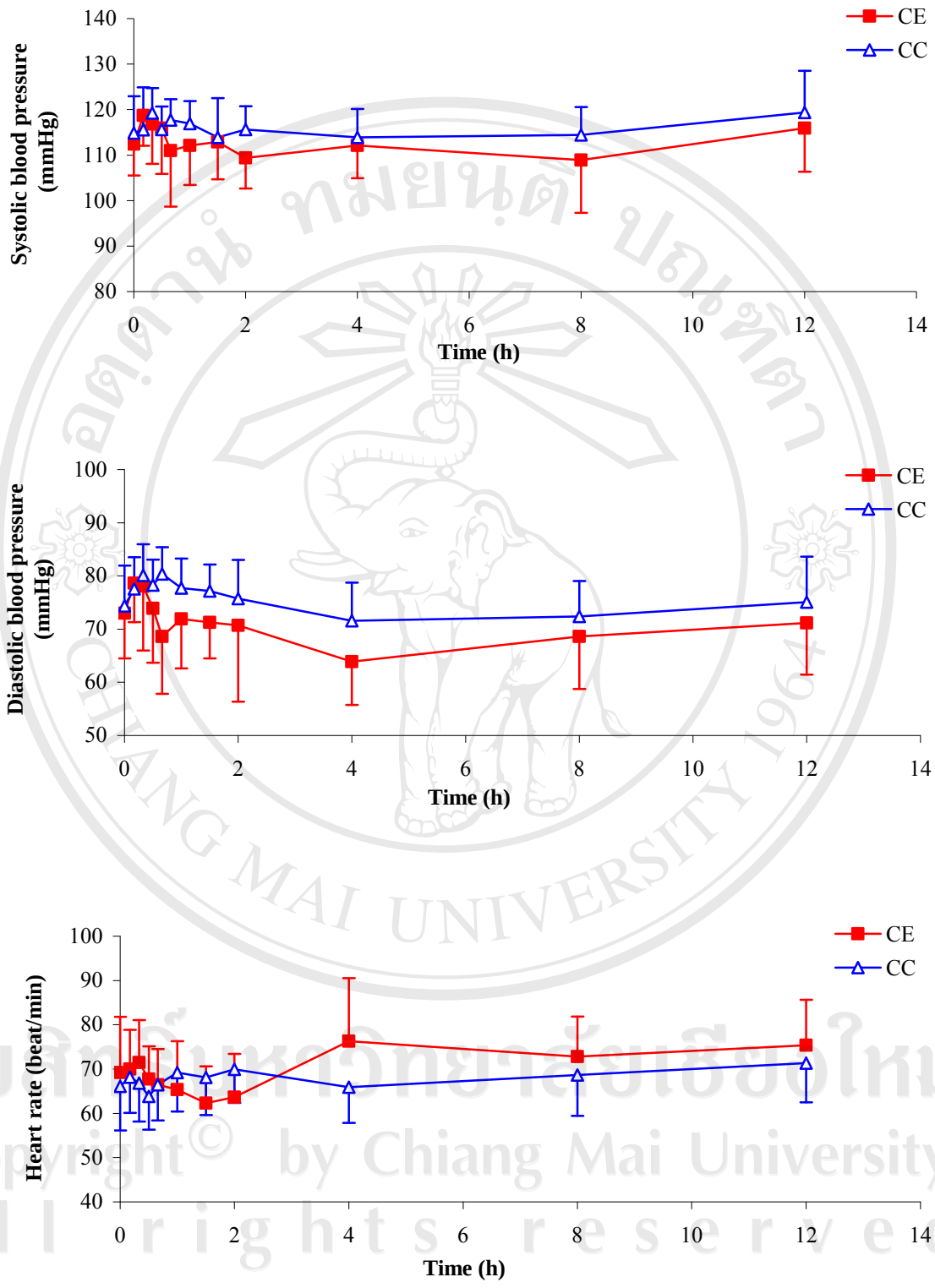


Figure 12. Effects of single dose of coffee enema or (CE) coffee consumption (CC) on systolic blood pressure, diastolic blood pressure and heart rate at various time points. Data represents mean±SD

3.6 Effects of coffee enema or coffee consumption on serum GSH concentrations

The individual as well as mean serum GSH concentration-time profiles from 11 subjects before and after single dose of coffee enema and coffee consumption are shown in Tables 15A-B and depicted in Figures 13A-B, respectively. Their mean concentration-time profiles are also presented in Figure 13C. Individual serum GSH concentrations of 11 subjects before and after multiple doses of coffee enema and coffee consumption are shown in Tables 16A-B and depicted in Figures 14A-B, respectively. Their mean concentration-time profiles are also presented in Figure 14C and percentages of mean change of serum GSH concentrations from baseline at day 6 and day 12 after multiple doses of coffee enema or coffee consumption are shown in Table 17.

The average serum concentrations of GSH at baseline were not statistically significant different between subjects assigned to coffee enema and coffee consumption (5.230±1.390 versus 4.961±1.307 μmol/L, p=0.645) (Tables 15A-B). Single dose of coffee enema or coffee consumption did not significantly alter serum concentrations of GSH at any time points (Tables 15A-B) comparing to their own baseline values. After multiple doses of coffee enema, serum concentrations of GSH insignificantly changed from the baseline value of 5.230±1.390 μmol/L to 6.160±1.152 μmol/L (22.13±25.339% increase) and 5.767±1.456 μmol/L (16.16±38.781% increase) at day 6 and 12, respectively (Tables 16A, 17 and Figure 14C). Likewise, multiple doses of coffee consumption insignificantly changed the serum concentrations of GSH from the baseline value of 4.961±1.307 μmol/L to 5.942±1.275 μmol/L and 5.861±1.213 μmol/L at day 6 and 12, accounting to mean

changes of +25.59±40.390% and +23.54±37.358%, respectively (Tables 16B, 17 and Figure 14C).

The percentages of mean changes from baseline of serum GSH concentrations at day 6 and day 12 after multiple doses of coffee consumption were not significantly different from treatment with coffee enema (Table 17).

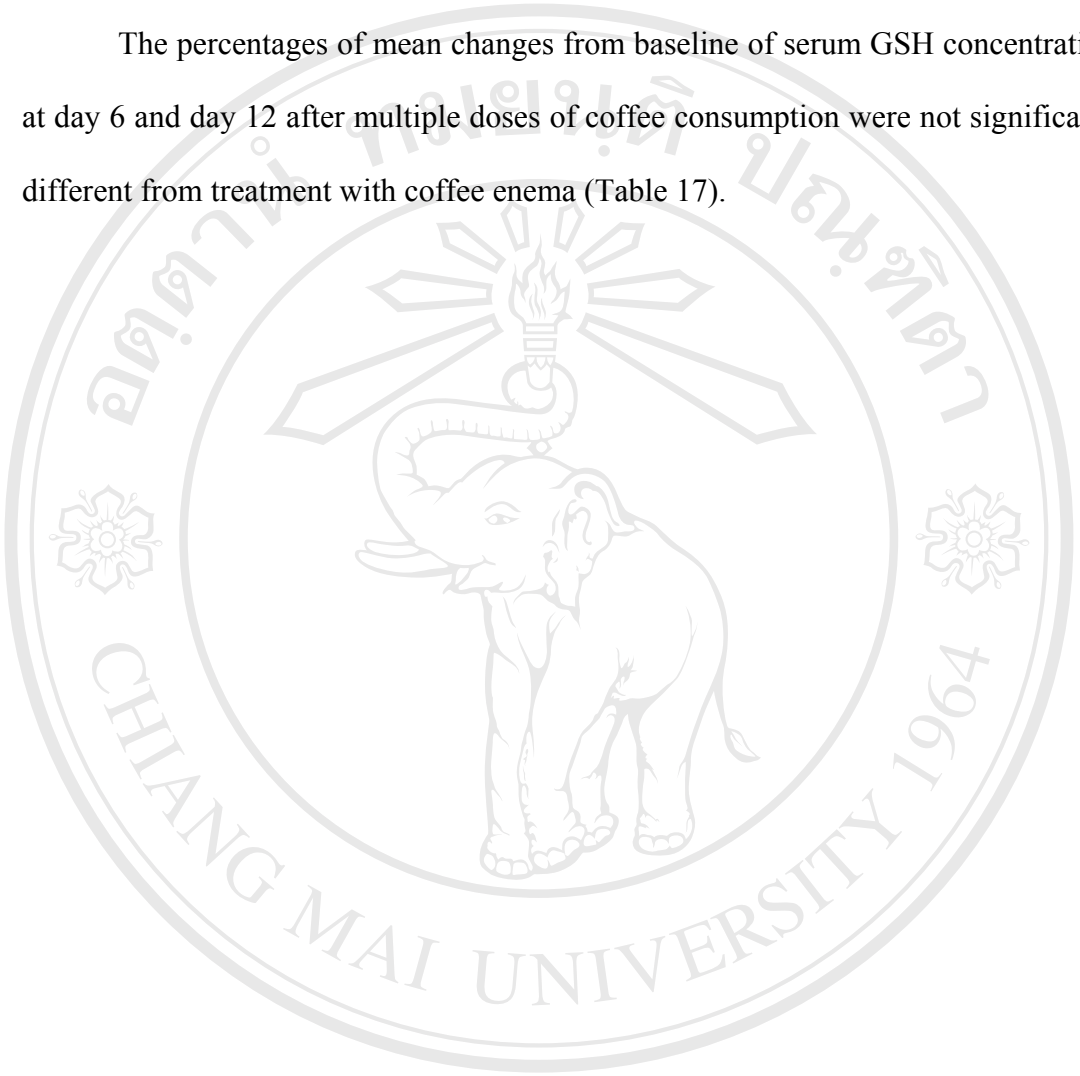


Table 15A. Serum GSH concentrations after single dose of coffee enema in 11 subjects completed the study without protocol deviation

Subject No. #	Serum GSH concentration (μmol/L) at various time (h)										
	0.00	0.17	0.33	0.50	0.67	1	1.5	2	4	8	12
1	3.180	3.752	4.165	3.530	3.353	3.410	4.143	3.406	3.226	3.410	2.564
2	3.808	4.090	3.947	3.846	3.812	4.414	3.459	3.703	5.320	4.241	4.609
4	5.586	6.173	6.301	6.459	6.015	6.932	6.579	4.846	4.883	5.985	6.447
5	6.139	5.132	5.233	5.429	5.368	5.684	5.492	6.451	2.880	5.398	5.778
6	5.951	4.774	5.481	4.808	5.154	5.699	5.199	6.707	9.120	6.263	6.489
7	5.650	5.489	5.771	5.669	5.180	6.643	5.857	5.256	5.944	5.722	6.312
8	3.940	3.744	4.071	4.353	4.060	4.568	3.447	4.207	4.218	4.413	3.259
9	3.805	3.868	4.056	3.383	3.504	5.492	3.602	4.301	5.244	5.519	3.771
10	7.109	6.662	6.680	6.211	6.015	5.560	5.692	6.583	5.188	4.462	6.556
11	5.075	4.192	8.492	8.248	4.883	4.154	5.417	3.511	8.071	4.974	5.086
12	7.282	6.045	7.278	7.184	5.823	6.323	7.203	6.530	6.038	5.056	6.233
Mean	5.230	4.902	5.589	5.375	4.834	5.353	5.099	5.045	5.467	5.016	5.191
SD	1.390	1.064	1.501	1.567	0.994	1.100	1.280	1.322	1.856	0.874	1.443

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 15B. Serum GSH concentrations after single dose of coffee consumption in 11 subjects completed the study without protocol deviation

Subject No. #	Serum GSH concentration (μmol/L) at various time (h)										
	0.00	0.17	0.33	0.50	0.67	1	1.5	2	4	8	12
1	3.429	3.041	3.368	3.252	4.117	2.951	3.184	3.380	3.391	3.353	2.929
2	3.699	3.470	3.331	4.113	3.703	5.030	4.654	4.398	5.086	5.102	4.154
4	6.782	4.812	7.263	7.692	5.639	6.011	5.974	7.086	5.613	5.380	6.109
5	4.955	5.609	5.455	4.248	4.989	3.936	5.128	5.628	5.846	5.455	4.951
6	3.545	3.583	3.756	4.571	4.132	6.214	3.880	6.545	5.320	5.447	3.436
7	5.391	5.620	5.203	5.966	5.162	5.474	5.511	5.850	5.102	6.353	4.613
8	4.207	4.079	3.549	4.049	3.568	3.620	3.955	3.850	3.778	3.857	4.368
9	4.015	5.034	4.895	3.936	4.508	4.624	3.846	3.985	5.519	4.590	6.026
10	6.278	5.752	3.688	7.034	7.308	5.613	7.195	5.917	6.383	5.376	5.658
11	5.214	3.763	4.910	3.034	2.970	3.481	2.556	4.425	4.361	7.244	5.192
12	7.053	6.654	6.756	6.293	6.598	7.500	5.398	8.064	6.158	6.917	6.635
Mean	4.961	4.674	4.734	4.926	4.790	4.950	4.662	5.317	5.141	5.370	4.916
SD	1.307	1.162	1.363	1.563	1.321	1.378	1.342	1.496	0.949	1.180	1.156

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

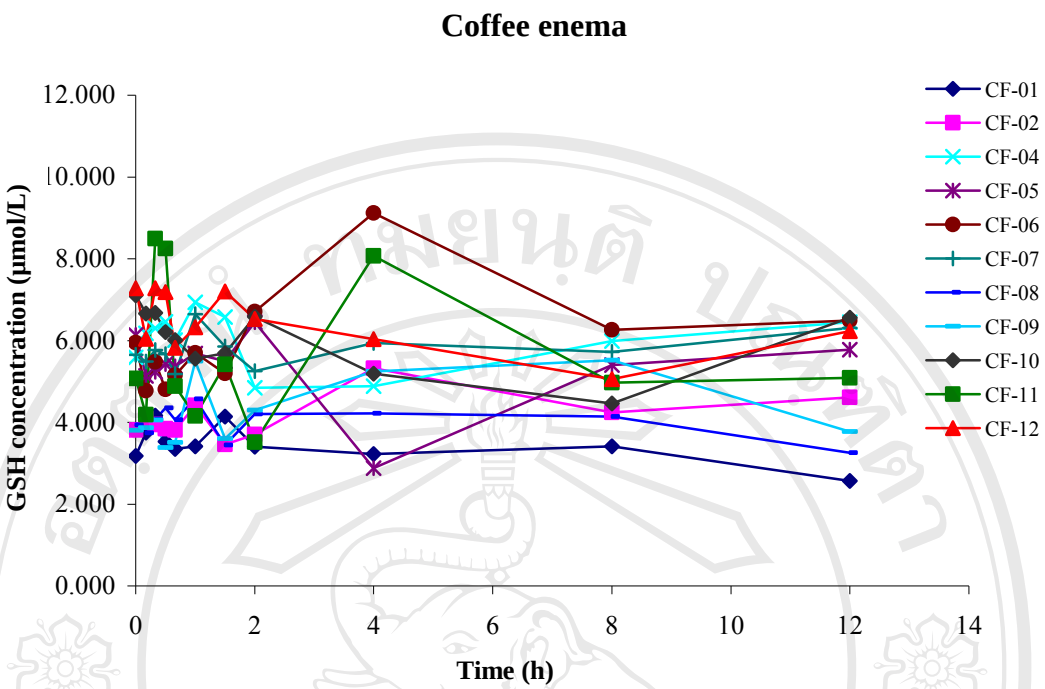


Figure 13A. Effects of single dose of coffee enema on serum GSH of individual subject (n=11)

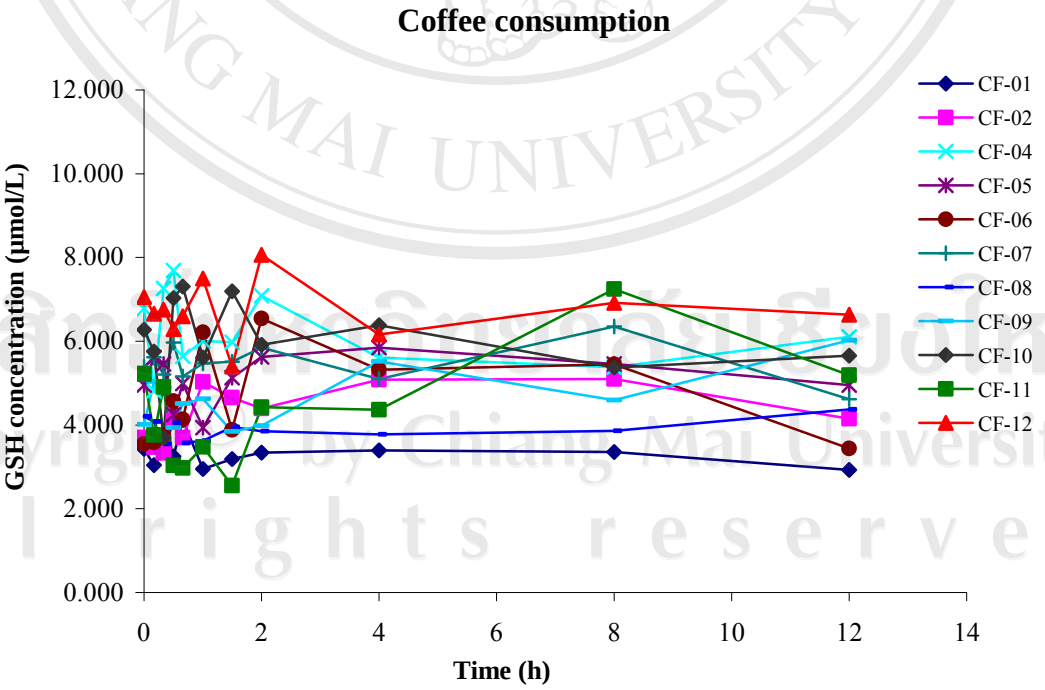


Figure 13B. Effects of single dose of coffee consumption on serum GSH concentrations of individual subject (n=11)

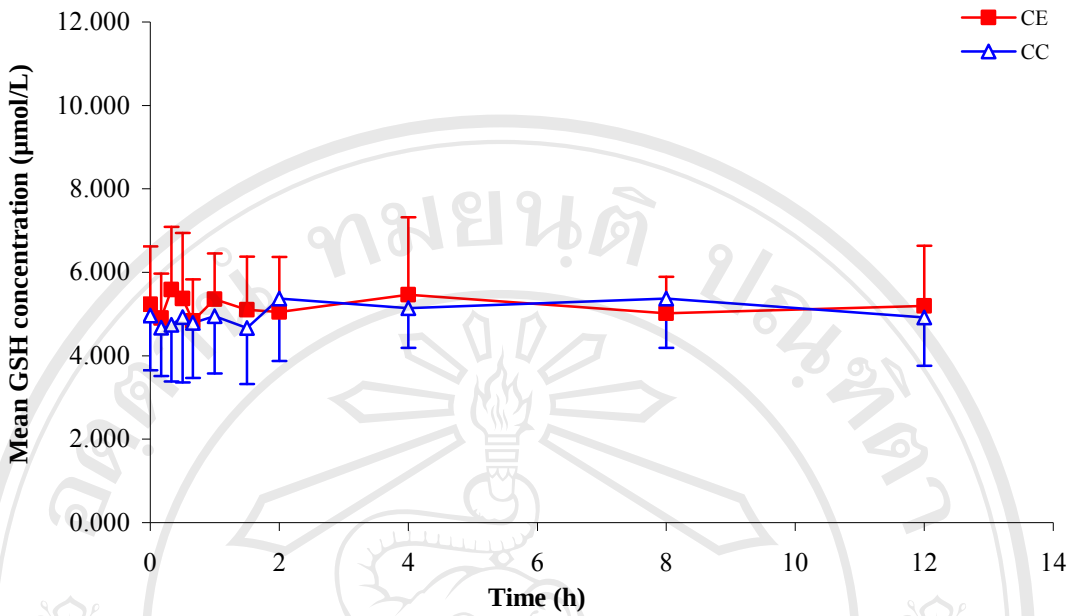


Figure 13C. Effects of single dose of coffee enema (CE) or coffee consumption (CC) on serum GSH concentrations

Table 16A. Effects of multiple doses of coffee enema (6 administrations within 12 days) on serum GSH concentrations

Subject No.#	Serum GSH concentration (µmol/L)		
	day 0	day 6	day 12
1	3.180	5.019	3.970
2	3.808	4.571	4.850
4	5.586	6.628	6.579
5	6.139	7.383	7.011
6	5.951	5.771	5.086
7	5.650	6.650	7.771
8	3.940	4.150	4.647
9	3.805	6.338	8.041
10	7.109	6.684	6.553
11	5.075	7.598	4.447
12	7.282	7.015	4.481
Mean	5.230	6.160	5.767
SD	1.390	1.152	1.456

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 16B. Effects of multiple doses of coffee consumption (24 consumptions within 12 days) on serum GSH concentrations

Subject No.#	Serum GSH concentration (µmol/L)		
	day 0	day 6	day 12
1	3.429	4.842	5.128
2	3.699	4.876	4.891
4	6.782	7.320	6.711
5	4.955	7.195	6.132
6	3.545	8.312	7.853
7	5.391	6.038	6.914
8	4.207	4.571	4.959
9	4.015	4.248	3.895
10	6.278	5.872	6.380
11	5.214	5.898	4.711
12	7.053	6.188	6.902
Mean	4.961	5.942	5.861
SD	1.307	1.275	1.213

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

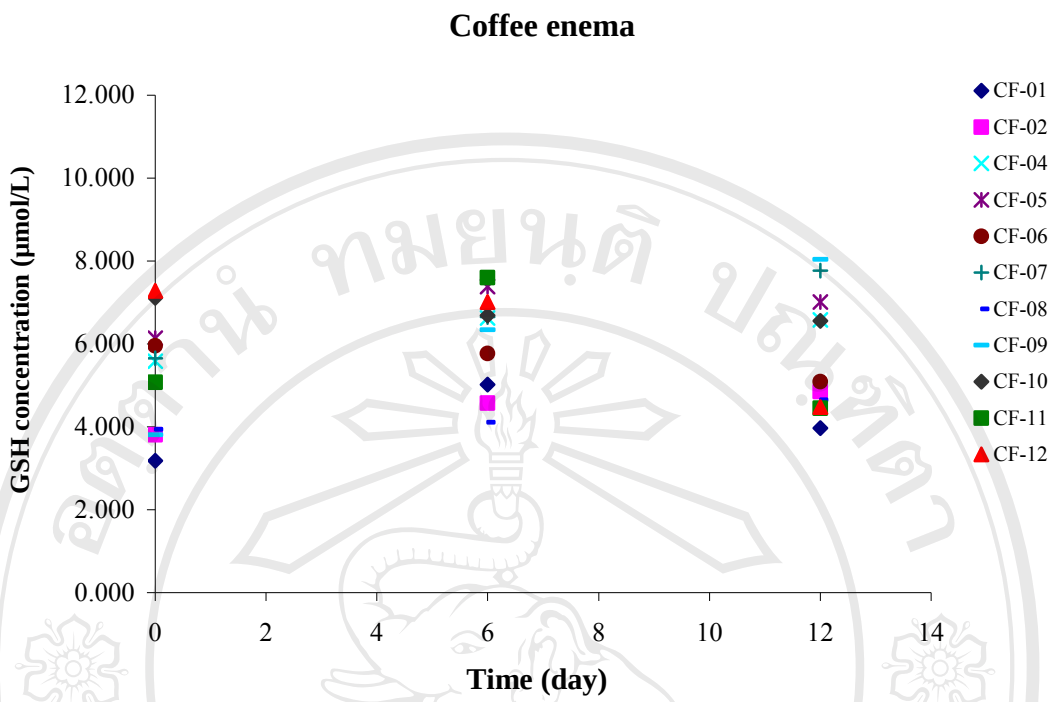


Figure 14A. Effects of multiple doses of coffee enema on serum GSH concentrations of individual subject (n=11)

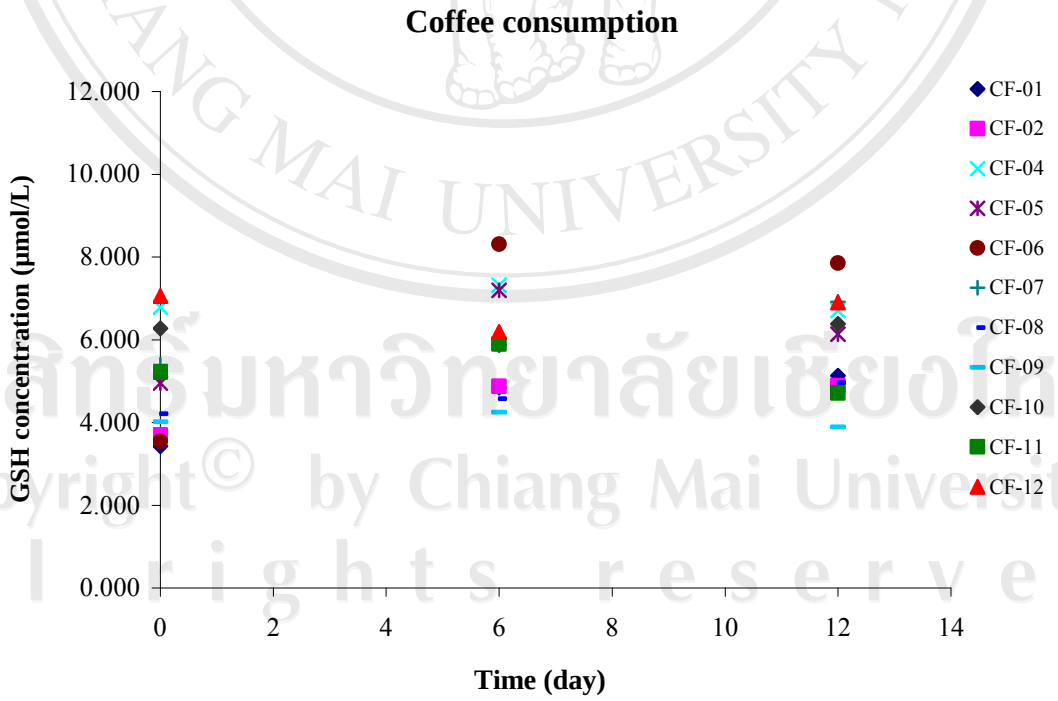


Figure 14B. Effects of multiple doses of coffee consumption on serum GSH concentrations of individual subject (n=11)

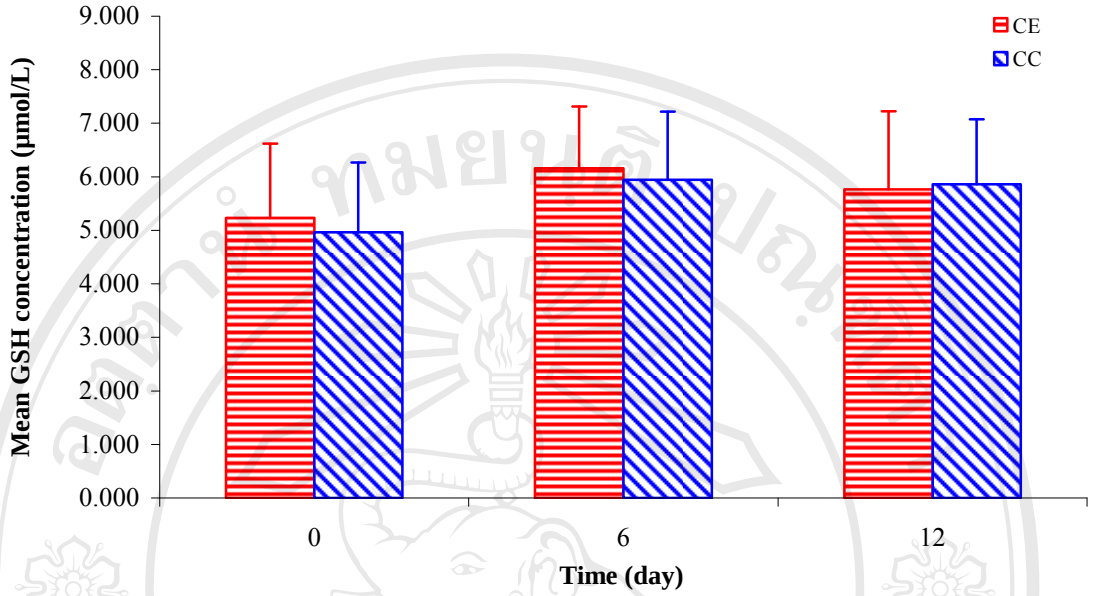


Figure 14C. Effects of multiple doses of coffee enema (CE) or coffee consumption (CC) on serum concentrations of GSH

Table 17. Percentages of mean change from baseline of serum GSH concentrations at day 6 and day 12 after multiple doses of coffee enema (CC) or coffee consumption (CC)

Subject No.#	% mean change of serum GSH concentration from baseline			
	day 6		day 12	
	CE	CC	CE	CC
1	57.830	41.207	24.843	49.548
2	20.037	31.819	27.363	32.225
4	18.654	7.933	17.777	-1.047
5	20.264	45.207	14.204	23.754
6	-3.025	134.471	-14.535	121.523
7	17.699	12.001	37.540	28.251
8	5.330	8.652	17.944	17.875
9	66.570	5.803	111.327	-2.989
10	-5.978	-6.467	-7.821	1.625
11	49.714	13.119	-12.374	-9.647
12	-3.667	-12.264	-38.465	-2.141
Mean	22.130	25.589	16.164	23.543
SD	25.339	40.390	38.781	37.358
p value	0.812		0.654	

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

3.7 Effects of coffee enema or coffee consumption on serum MDA concentrations

The individual as well as mean serum MDA concentration-time profiles from 11 subjects before and after single dose of coffee enema and coffee consumptions are shown in Tables 18A-B and depicted in Figures 15A-B, respectively. Their mean concentration-time profiles are also presented in Figure 15C. Individual serum MDA concentration of 11 subjects before and after multiple doses of coffee enema and coffee consumption are shown in Tables 19A-B and depicted in Figures 16A-B, respectively. Their mean concentration-time profiles are also presented in Figure 16C and percentages of mean change from baseline of serum MDA concentrations at day 6 and day 12 after multiple doses of coffee enema or coffee consumption are shown in Table 20.

The average serum concentrations of MDA at baseline were not statistically significant different between subjects assigned to coffee enema and coffee consumption (0.011±0.005 mmol/L versus 0.017±0.009 mmol/L, p=0.056) (Tables 18A-B and Figures 15A-C). Single administration of neither coffee enema nor coffee consumption did not significantly affect serum concentrations of MDA at any time points (Tables 18A-B) when compared to their own baseline values**.** Multiple doses of coffee enema showed a trend towards insignificant increase in serum concentrations of MDA from the baseline value of 0.011±0.005 mmol/L to 0.013±0.009 mmol/L and 0.017±0.010 mmol/L at day 6 and 12, respectively (Table 19A and Figure 16C). While multiple doses of coffee consumption insignificantly changed serum concentrations of MDA from the baseline value of 0.017±0.009 mmol/L to 0.014±0.005 mmol/L and 0.017±0.009 mmol/L at day 6 and 12, respectively (Table 19B and Figure 16C).

The percentages of mean change from baseline of serum MDA concentrations at day 6 and day 12 after multiple doses of coffee consumption were not significantly from coffee enema (Table 20).

Table 18A. Serum MDA concentrations after single dose of coffee enema in 11 subjects completed the study without protocol deviation

Subject No. #	Serum MDA concentration (mmol/L) at various time (h)										
	0.00	0.17	0.33	0.50	0.67	1	1.5	2	4	8	12
1	0.009	0.006	0.007	0.006	0.004	0.007	0.007	0.008	0.004	0.007	0.002
2	0.004	0.006	0.005	0.005	0.005	0.005	0.005	0.008	0.005	0.004	0.006
4	0.010	0.014	0.011	0.010	0.013	0.012	0.009	0.007	0.011	0.013	0.013
5	0.007	0.005	0.008	0.009	0.008	0.005	0.010	0.005	0.003	0.008	0.000
6	0.011	0.012	0.010	0.009	0.010	0.010	0.010	0.012	0.015	0.013	0.012
7	0.013	0.005	0.006	0.003	0.005	0.006	0.004	0.008	0.007	0.001	0.006
8	0.009	0.008	0.013	0.014	0.014	0.014	0.011	0.013	0.004	0.005	0.000
9	0.012	0.011	0.013	0.012	0.013	0.013	0.012	0.013	0.013	0.014	0.014
10	0.010	0.008	0.011	0.007	0.012	0.014	0.016	0.016	0.019	0.007	0.008
11	0.016	0.012	0.013	0.010	0.014	0.013	0.015	0.011	0.014	0.011	0.010
12	0.021	0.016	0.016	0.014	0.013	0.014	0.013	0.018	0.017	0.011	0.013
Mean	0.011	0.009	0.010	0.009	0.010	0.010	0.010	0.011	0.010	0.008	0.008
SD	0.005	0.004	0.003	0.003	0.004	0.004	0.004	0.004	0.006	0.004	0.005

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 18B. Serum MDA concentrations after single dose of coffee consumption in 11 subjects completed the study without protocol deviation

Subject	Serum MDA concentration (mmol/L) at various time (h)										
No. #	0.00	0.17	0.33	0.50	0.67	1	1.5	2	4	8	12
1	0.010	0.008	0.007	0.007	0.006	0.008	0.007	0.007	0.004	0.009	0.012
2	0.011	0.011	0.021	0.020	0.019	0.011	0.013	0.019	0.021	0.038	0.020
4	0.027	0.041	0.041	0.044	0.041	0.041	0.034	0.034	0.020	0.014	0.016
5	0.013	0.011	0.012	0.010	0.011	0.012	0.012	0.010	0.010	0.005	0.008
6	0.009	0.014	0.007	0.019	0.025	0.018	0.018	0.014	0.008	0.011	0.006
7	0.016	0.019	0.019	0.017	0.019	0.016	0.020	0.021	0.017	0.009	0.016
8	0.010	0.008	0.007	0.007	0.008	0.011	0.008	0.009	0.006	0.010	0.004
9	0.011	0.013	0.014	0.013	0.011	0.013	0.013	0.012	0.008	0.010	0.005
10	0.031	0.031	0.031	0.034	0.034	0.054	0.024	0.031	0.017	0.014	0.016
11	0.016	0.019	0.018	0.016	0.014	0.017	0.018	0.019	0.007	0.014	0.009
12	0.034	0.047	0.041	0.037	0.027	0.034	0.054	0.034	0.022	0.019	0.022
Mean	0.017	0.020	0.020	0.020	0.020	0.021	0.020	0.019	0.013	0.014	0.012
SD	0.009	0.014	0.013	0.013	0.011	0.015	0.014	0.010	0.007	0.009	0.006

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

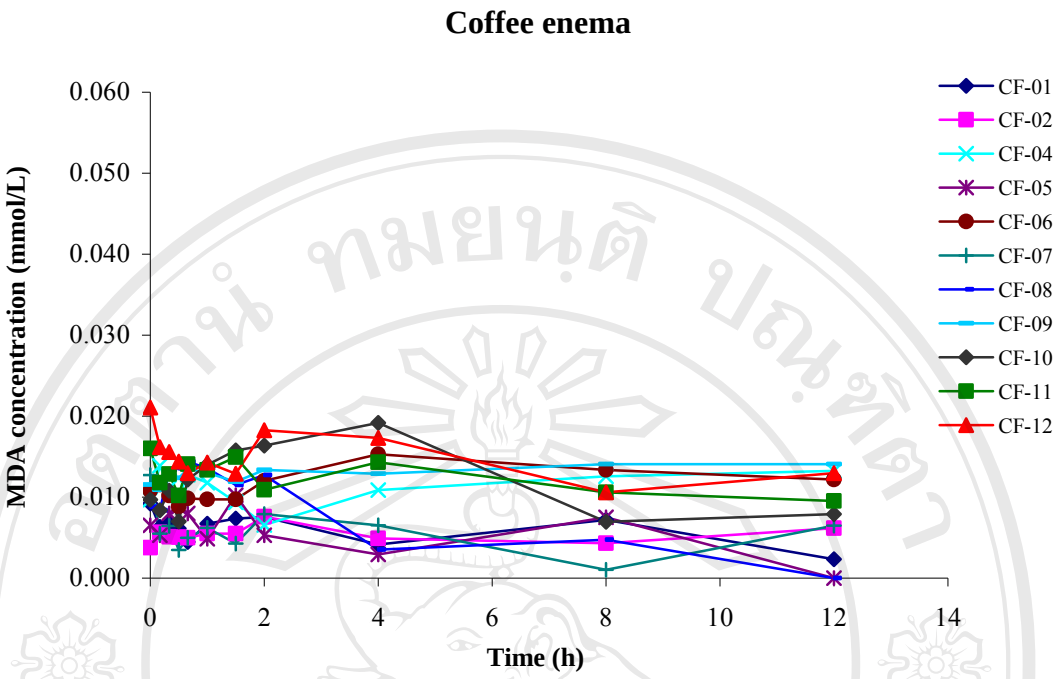


Figure 15A. Effects of single dose of coffee enema on serum MDA concentrations of individual subject (n=11)

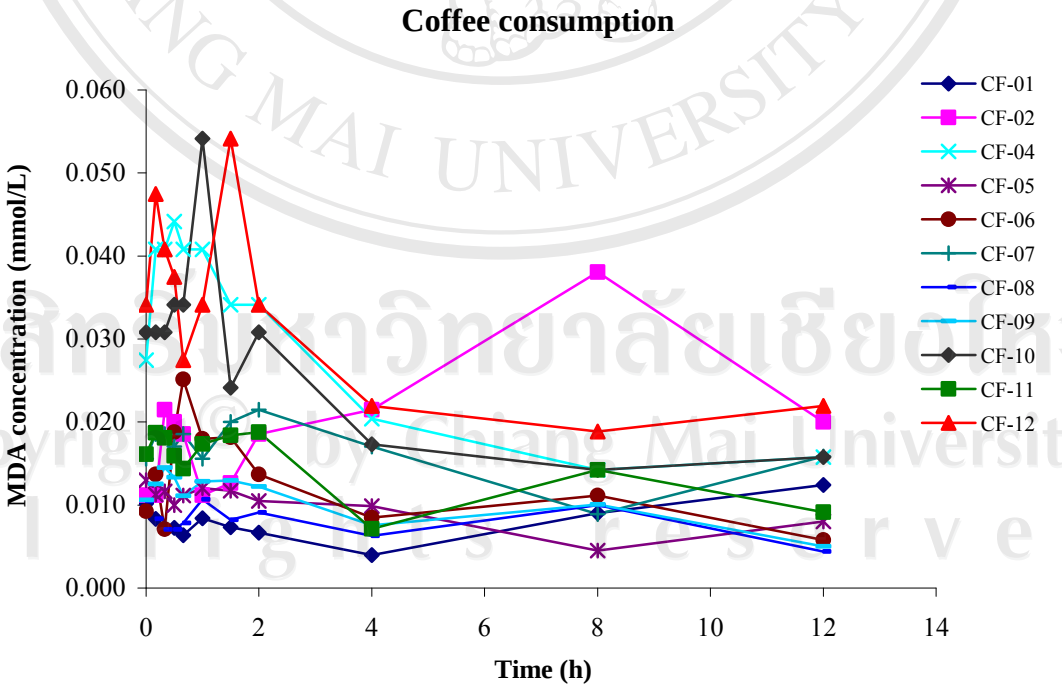


Figure 15B. Effects of single dose of coffee consumption on serum MDA concentrations of individual subject (n=11)

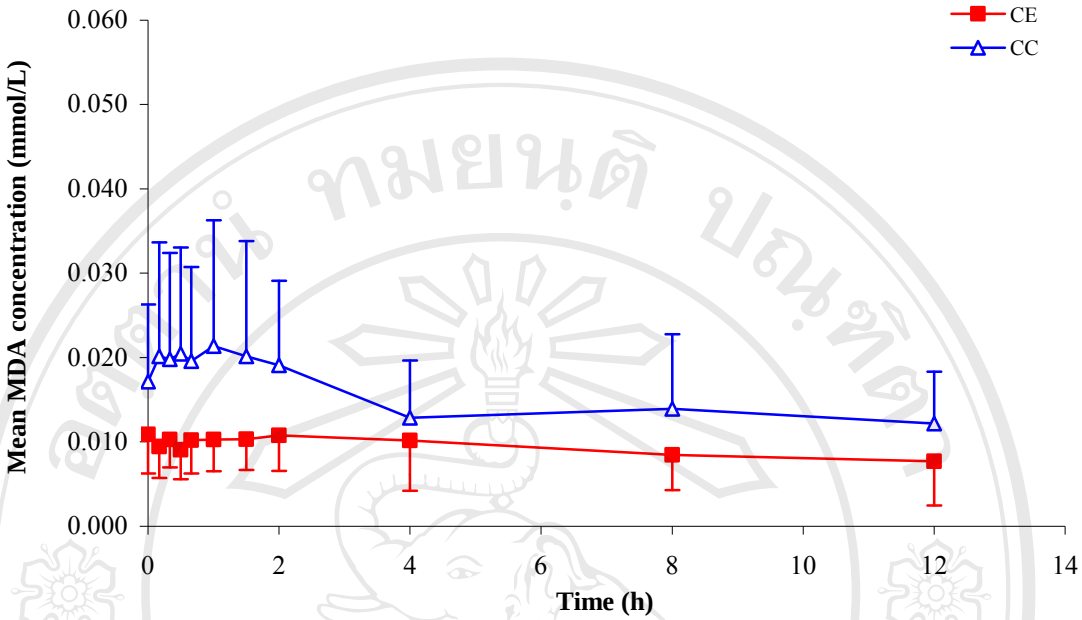


Figure 15C. Effects of single dose of coffee enema (CE) or coffee consumption (CC) on serum MDA concentrations

Table 19A. Effects of multiple doses of coffee enema (6 administrations within 12 days) on serum MDA concentrations

Subject No.#	Serum MDA concentration (mmol/L)		
	day 0	day 6	day 12
1	0.009	0.011	0.040
2	0.004	0.011	0.014
4	0.010	0.029	0.013
5	0.007	0.011	0.031
6	0.011	0.004	0.010
7	0.013	0.027	0.009
8	0.009	0.011	0.024
9	0.012	0.003	0.010
10	0.010	0.018	0.016
11	0.016	0.004	0.009
12	0.021	0.011	0.014
Mean	0.011	0.013	0.017
SD	0.005	0.009	0.010

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 19B. Effects of multiple doses of coffee consumption (24 consumptions within 12 days) on serum MDA concentrations

Subject No.#	Serum MDA concentration (mmol/L)		
	day 0	day 6	day 12
1	0.010	0.024	0.010
2	0.011	0.009	0.010
4	0.027	0.012	0.032
5	0.013	0.016	0.010
6	0.009	0.015	0.016
7	0.016	0.013	0.011
8	0.010	0.021	0.007
9	0.011	0.011	0.018
10	0.031	0.013	0.015
11	0.016	0.013	0.034
12	0.034	0.009	0.023
Mean	0.017	0.014	0.017
SD	0.009	0.005	0.009

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

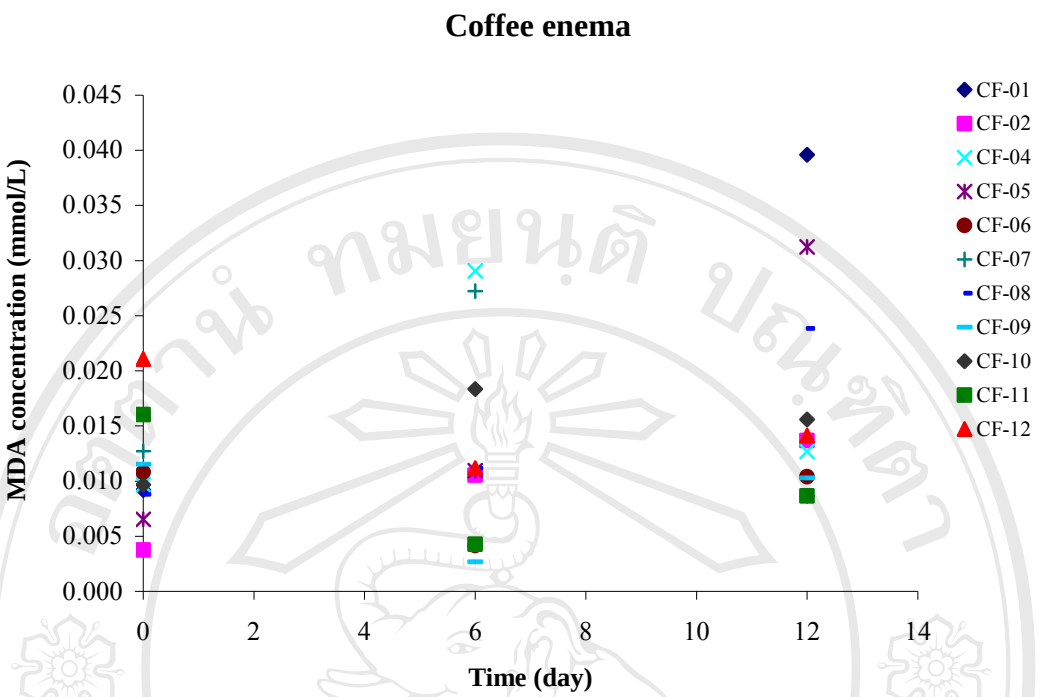


Figure 16A. Effects of multiple doses of coffee enema on serum MDA concentrations of individual subject (n=11)

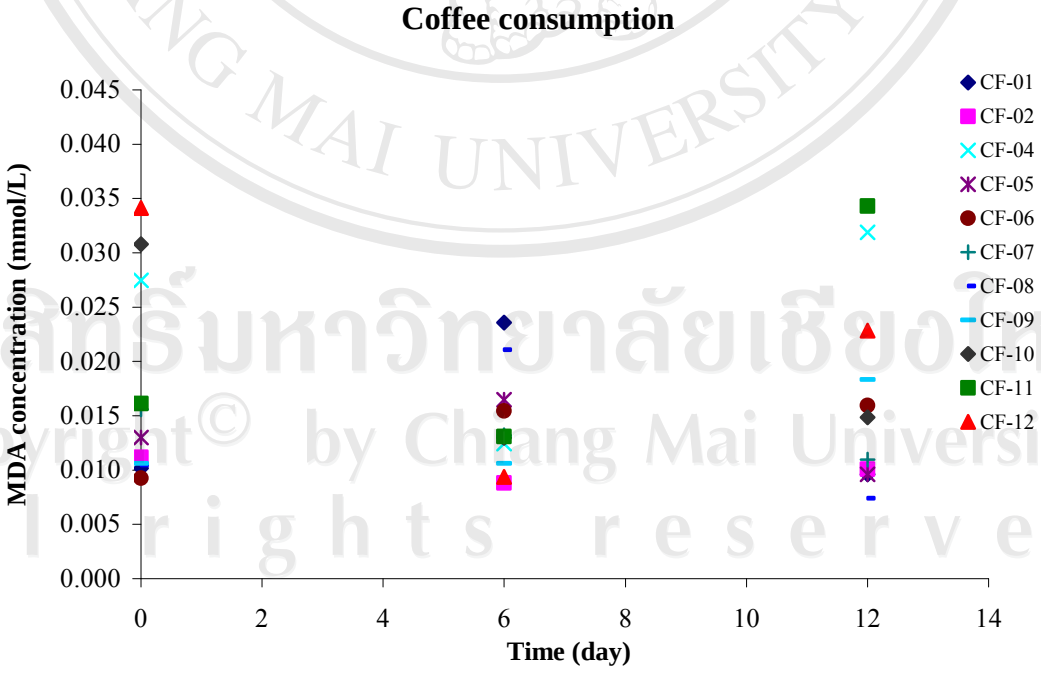


Figure 16B. Effects of multiple doses of coffee consumption on serum MDA concentrations of individual subject (n=11)

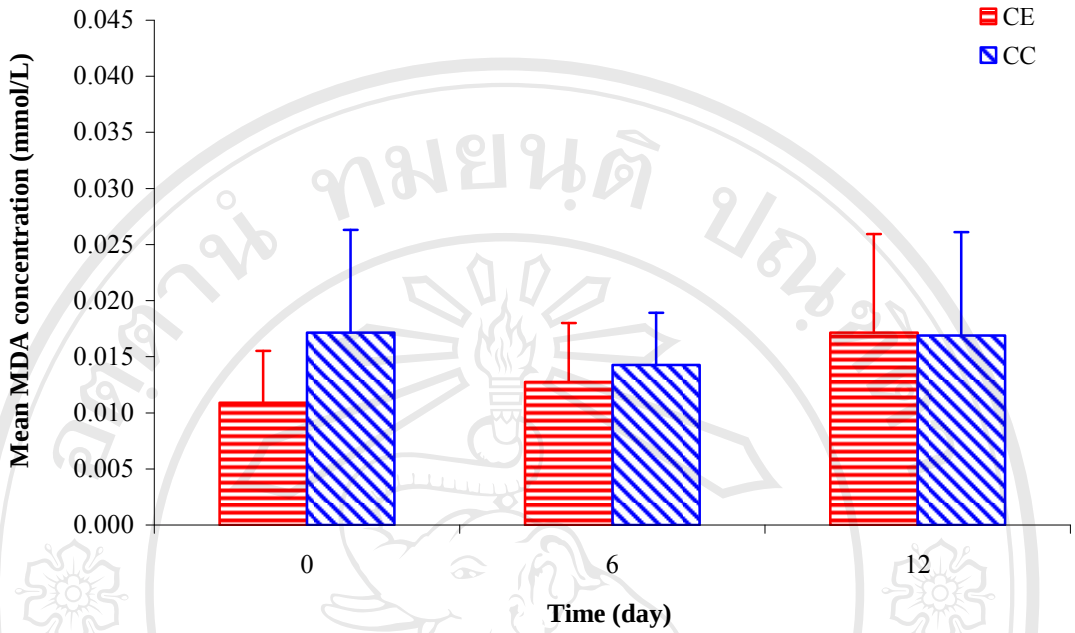


Figure 16C. Effects of multiple doses of coffee enema (CE) or coffee consumption (CC) on serum MDA concentrations

Table 20. Percentages of mean change from baseline of serum MDA concentrations at day 6 and day 12 after multiple doses of coffee enema (CE) or coffee consumption (CC)

Subject No.#	% mean change of serum MDA concentration from baseline			
	day 6		day 12	
	CE	CC	CE	CC
1	17.960	131.744	329.734	-5.651
2	180.559	-21.253	263.915	-9.888
4	200.103	-54.670	31.095	16.130
5	67.764	26.595	378.484	-26.211
6	-61.620	66.901	-3.796	72.246
7	114.157	-15.234	-31.852	-29.666
8	26.534	101.385	170.795	-29.417
9	-76.800	0.000	-10.755	73.053
10	89.411	-57.906	60.950	-51.753
11	-73.283	-18.915	-46.067	112.713
12	-46.917	-72.579	-32.946	-33.133
Mean	39.806	7.824	100.869	8.038
SD	94.928	63.815	150.25	51.477
p value	0.387		0.079	

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

3.8 Effects of coffee enema or coffee consumption on serum TEAC

The individual as well as mean serum TEAC concentration-time profiles from 11 subjects before and after single administration of coffee enema and coffee consumptions are shown in Tables 21A-B and depicted in Figures 17A-B, respectively. Their mean concentration-time profiles are also presented in Figure 17C. Individual serum TEAC concentrations of 11 subjects before and after multiple doses of coffee enema and coffee consumption are shown in Tables 22A-B and depicted in Figures 18A-B, respectively. Their mean concentration-time profiles are also presented in Figure 18C and percentages of mean change from baseline of serum TEAC concentrations at day 6 and day 12 after multiple doses of coffee enema or coffee consumption are shown in Table 23.

The average serum concentrations of TEAC at baseline were statistically significant different between subjects assigned to coffee enema and coffee consumption (1.584±0.095 mmol/L versus 1.509±0.030 mmol/L, p=0.02) (Tables 21A-B and Figures 17A-C). Single dose of coffee enema or coffee consumption did not significantly alter serum concentrations of TEAC at any time points (Tables 21A-B), comparing to their own baseline values. After multiple doses of coffee, serum concentrations of TEAC significantly changed from the baseline value of 1.584±0.095 mmol/L to 1.480±0.079 mmol/L (p=0.004) at day 12 (Table 22A and Figure 18C). Similarly, the average serum concentrations of TEAC after multiple doses of coffee consumption significantly changed from the baseline value of 1.509±0.030 mmol/L to 1.375±0.166 mmol/L (p=0.008) and 1.388±0.094 mmol/L (p=0.017) at day 6 and 12, respectively (Table 22B and Figure 18C).

The percentages of mean change from baseline of serum TEAC concentrations at day 6 and day 12 after multiple doses of coffee consumption were not significantly different from coffee enema (Table 23).

Table 21A. Serum TEAC after single dose of coffee enema in 11 subjects completed the study without protocol deviation

Subject No. #	Serum TEAC (mmol/L) at various time (h)										
	0.00	0.17	0.33	0.50	0.67	1	1.5	2	4	8	12
1	1.602	1.609	1.564	1.570	1.592	1.588	1.640	1.560	1.617	1.550	1.607
2	1.750	1.720	1.620	1.724	1.672	1.669	1.669	1.688	1.742	1.695	1.632
4	1.556	1.550	1.555	1.524	1.554	1.554	1.554	1.570	1.537	1.550	1.572
5	1.592	1.601	1.623	1.634	1.576	1.617	1.617	1.564	1.582	1.546	1.556
6	1.436	1.468	1.359	1.421	1.445	1.415	1.415	1.473	1.443	1.377	1.299
7	1.541	1.481	1.502	1.496	1.521	1.541	1.541	1.550	1.517	1.486	1.471
8	1.502	1.538	1.518	1.432	1.471	1.486	1.486	1.503	1.434	1.388	1.436
9	1.706	1.695	1.594	1.652	1.621	1.599	1.599	1.591	1.570	1.591	1.586
10	1.485	1.492	1.500	1.500	1.535	1.546	1.546	1.572	1.519	1.539	1.598
11	1.657	1.688	1.640	1.690	1.690	1.713	1.713	1.714	1.662	1.701	1.736
12	1.598	1.596	1.547	1.593	1.515	1.520	1.520	1.535	1.525	1.527	1.546
Mean	1.584	1.580	1.547	1.567	1.563	1.602	1.571	1.575	1.559	1.541	1.549
SD	0.095	0.098	0.079	0.102	0.077	0.116	0.075	0.071	0.091	0.103	0.114

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

Table 21B. Serum TEAC after single dose of coffee consumption in 11 subjects completed the study without protocol deviation

Subject No. #	Serum TEAC (mmol/L) at various time (h)										
	0.00	0.17	0.33	0.50	0.67	1	1.5	2	4	8	12
1	1.524	1.504	1.517	1.528	1.496	1.498	1.542	1.519	1.479	1.506	1.527
2	1.485	1.455	1.468	1.462	1.417	1.448	1.473	1.434	1.488	1.475	1.463
4	1.465	1.535	1.527	1.517	1.536	1.552	1.530	1.540	1.493	1.481	1.535
5	1.493	1.479	1.519	1.499	1.481	1.480	1.463	1.479	1.465	1.450	1.470
6	1.474	1.536	1.550	1.532	1.486	1.491	1.486	1.509	1.506	1.486	1.496
7	1.551	1.543	1.557	1.505	1.489	1.524	1.520	1.541	1.525	1.553	1.504
8	1.554	1.558	1.568	1.559	1.572	1.566	1.564	1.569	1.545	1.546	1.537
9	1.502	1.499	1.498	1.496	1.486	1.479	1.499	1.512	1.484	1.494	1.470
10	1.498	1.494	1.472	1.473	1.486	1.491	1.479	1.509	1.307	1.490	1.500
11	1.532	1.588	1.578	1.592	1.567	1.585	1.593	1.554	1.575	1.498	1.512
12	1.517	1.562	1.548	1.542	1.537	1.552	1.536	1.540	1.517	1.538	1.522
Mean	1.509	1.523	1.527	1.519	1.505	1.515	1.517	1.519	1.489	1.502	1.503
SD	0.030	0.040	0.037	0.038	0.045	0.043	0.041	0.038	0.068	0.032	0.027

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

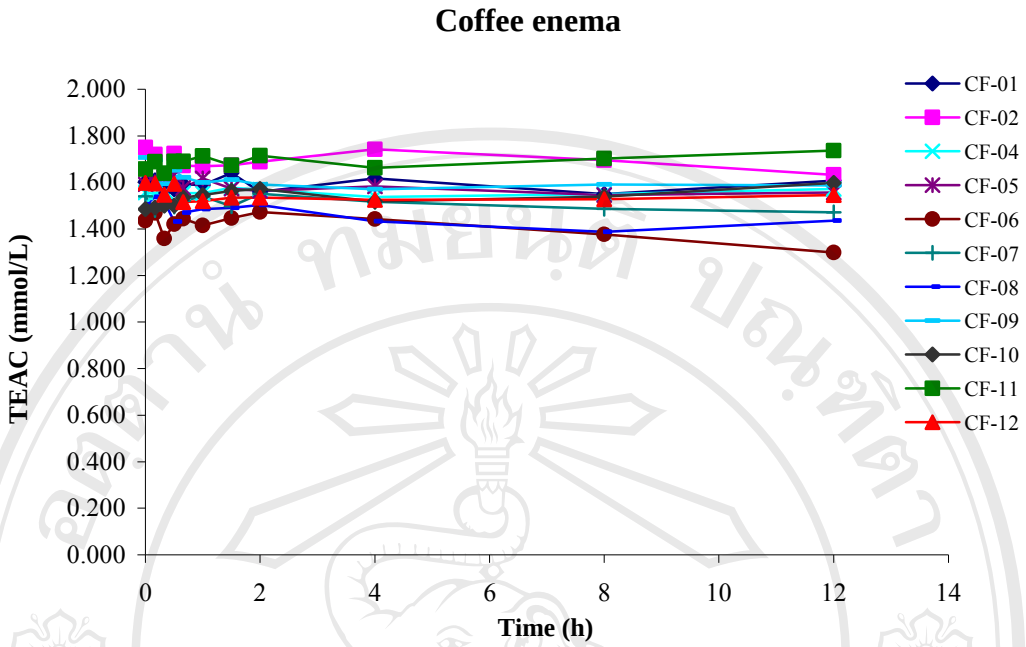


Figure 17A. Effects of single dose of coffee enema on serum TEAC of individual subject (n=11)

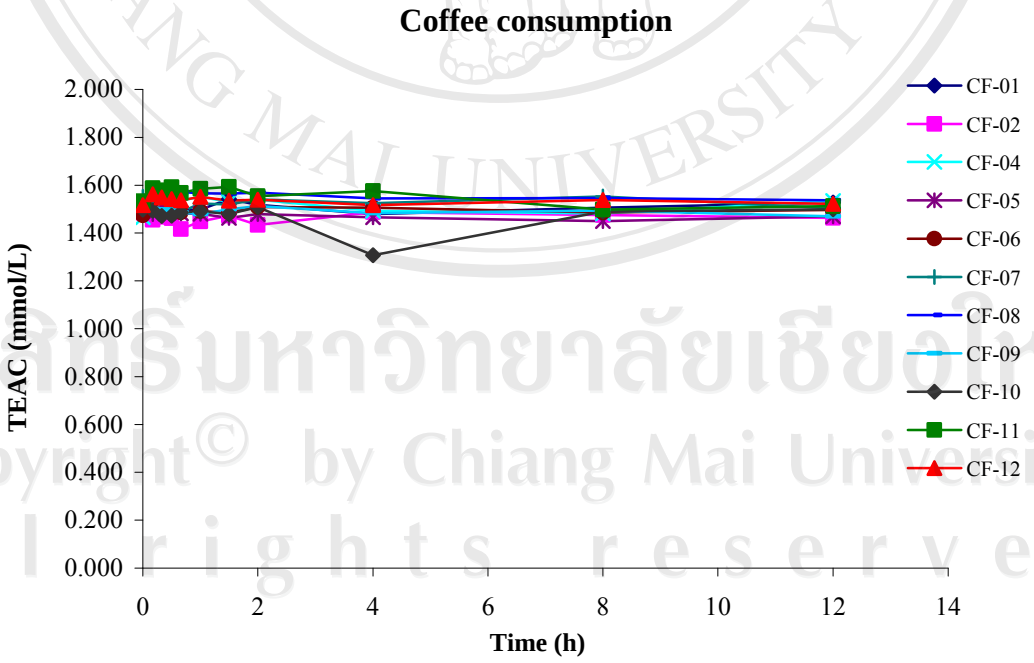


Figure 17B. Effects of single dose of coffee consumption on serum TEAC of individual subject (n=11)

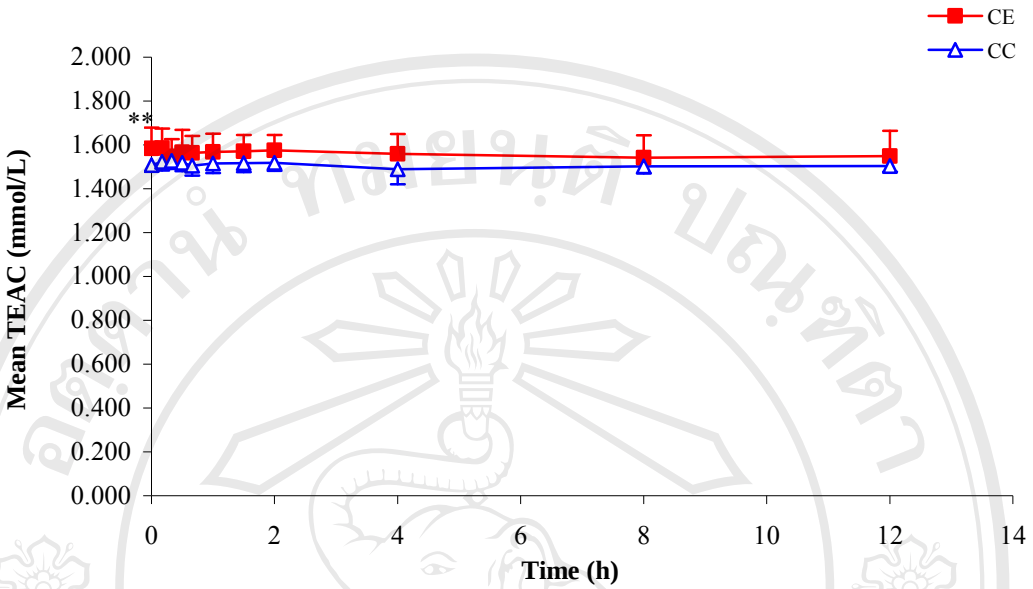


Figure 17C. Effects of single dose of coffee enema (CE) or coffee consumption (CC) on serum TEAC

** Statistically significant between group ($p<0.05$, paired t-test)

Table 22A. Effects of multiple doses of coffee enema (6 administrations within 12 days) on serum TEAC

Subject No.#	Serum TEAC (mmol/L)		
	day 0	day 6	day 12
1	1.602	1.564	1.554
2	1.750	1.581	1.497
4	1.556	1.552	1.401
5	1.592	1.540	1.548
6	1.436	1.540	1.398
7	1.541	1.495	1.464
8	1.502	1.542	1.604
9	1.706	1.629	1.498
10	1.485	1.495	1.369
11	1.657	1.672	1.545
12	1.598	1.498	1.399
Mean	1.584	1.555	1.480*
SD	0.095	0.056	0.079

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

* Statistically significant difference from baseline (day 0) ($p < 0.05$, one-way ANOVA with repeated measurement)

Table 22B. Effects of multiple doses of coffee consumption (24 consumptions within 12 days) on serum TEAC

Subject No.#	Serum TEAC (mmol/L)		
	day 0	day 6	day 12
1	1.524	1.498	1.338
2	1.485	1.203	1.494
4	1.465	1.524	1.342
5	1.493	1.516	1.325
6	1.474	1.200	1.517
7	1.551	1.209	1.469
8	1.554	1.587	1.355
9	1.502	1.167	1.476
10	1.498	1.517	1.233
11	1.532	1.250	1.433
12	1.517	1.453	1.288
Mean	1.509	1.375*	1.388*
SD	0.030	0.166	0.094

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

* Statistically significant difference from baseline (day 0) ($p < 0.05$, one-way ANOVA with repeated measuremen

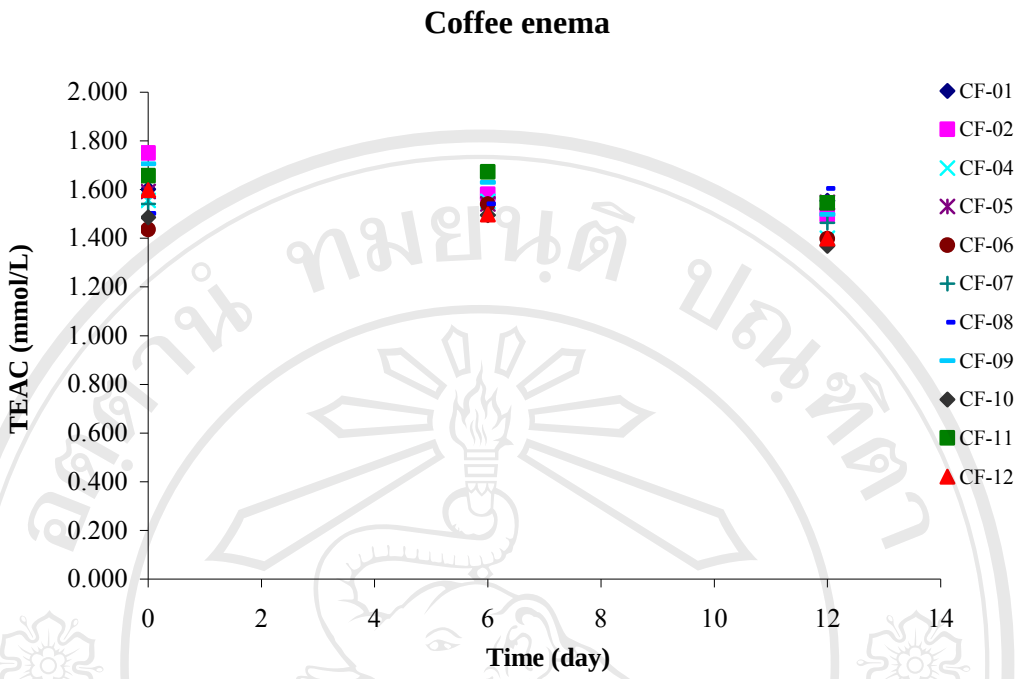


Figure 18A. Effects of multiple doses of coffee enema on serum TEAC of individual subject (n=11)

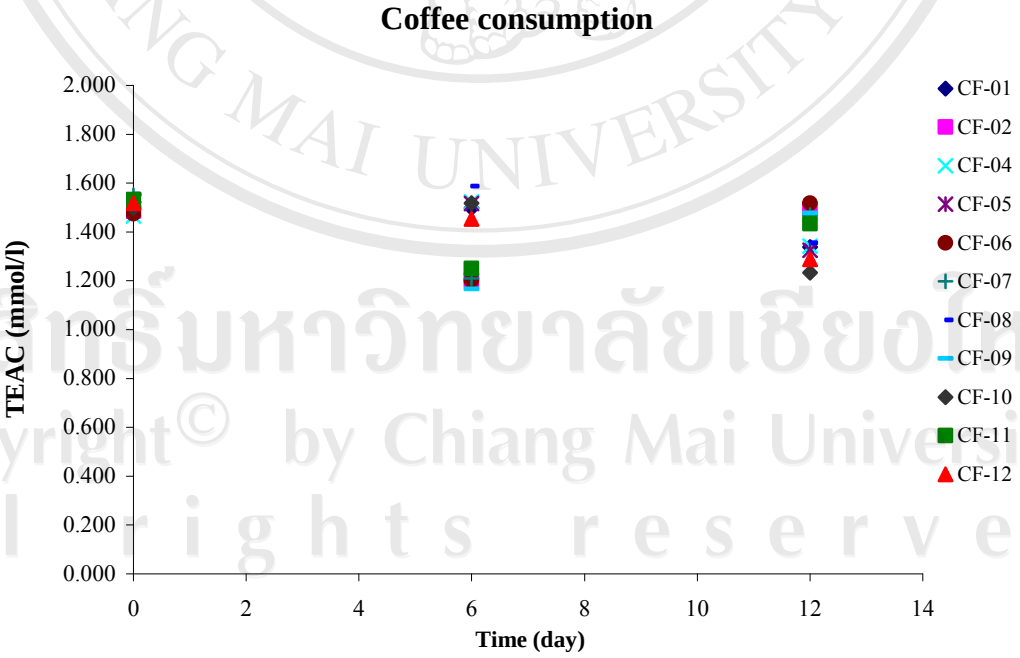


Figure 18B. Effects of multiple doses of coffee consumption on serum TEAC of individual subject (n=11)

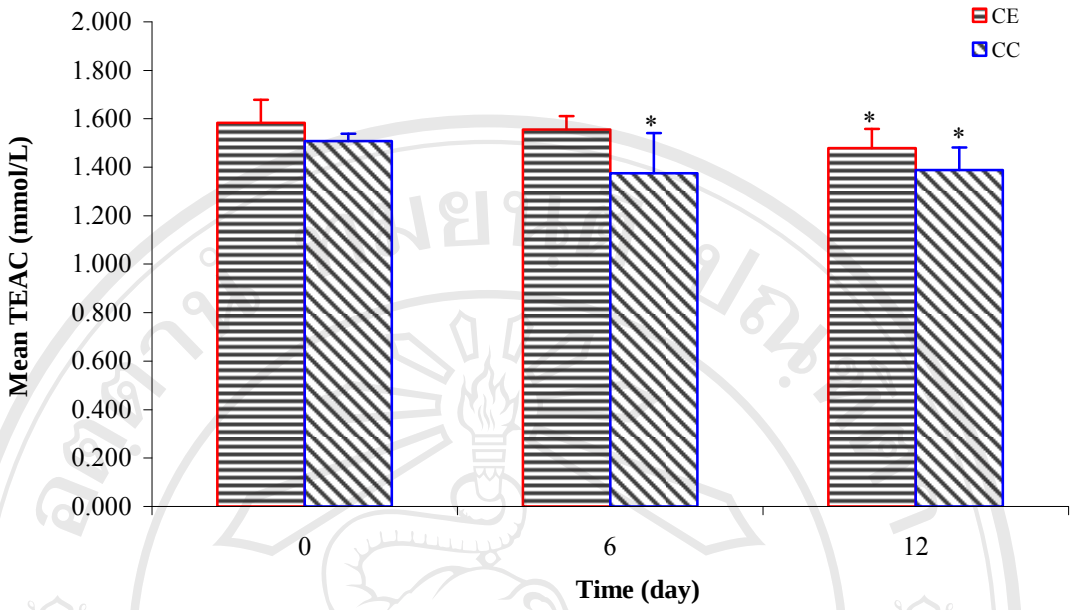


Figure 18C. Effects of multiple doses of coffee enema (CE) or coffee consumption (CC) on serum TEAC

* Statistically significant difference from baseline (day 0) ($p < 0.05$, one-way ANOVA with repeated measurement

Table 23. Percentage of mean changes from baseline of serum TEAC at day 6 and day 12 after multiple doses of coffee enema

Subject No.#	% mean change of serum TEAC from baseline			
	day 6		day 12	
	CE	CC	CE	CC
1	-2.367	-1.706	-2.991	-12.185
2	-9.665	-19.000	-14.444	0.583
4	-0.290	3.970	-9.964	-8.446
5	-3.237	1.575	-2.765	-11.194
6	7.236	-18.556	-2.638	2.939
7	-2.944	-22.027	-4.998	-5.267
8	2.667	2.070	6.814	-12.818
9	-4.515	-22.323	-12.174	-1.730
10	0.613	1.322	-7.849	-17.686
11	0.923	-18.417	-6.738	-6.462
12	-6.245	-4.241	-12.485	-15.090
Mean	-1.620	-8.848	-6.385	-7.942
SD	4.577	11.009	6.046	6.616
p value	0.058		0.571	

Data from subject No.3 was not taken into account because caffeine concentration was detected in plasma sample at baseline

3.9 Adverse events after coffee enema or coffee consumption

All subjects completed the study without any adverse events. The mean values of hemodynamic parameters (systolic blood pressure, diastolic blood pressure and heart rate) following multiple doses of each coffee procedure did not significantly alter from their own baseline values (data not shown). Additionally, the mean values of blood electrolytes following multiple doses of coffee enema were 138.91±1.70 mmol/L for sodium, 3.99±0.23 mmol/L for potassium, 104.64±2.38 mmol/L for chloride, and 26.45±1.75 mmol/L for bicarbonate, in comparison to the baseline values of 140.00±2.10 mmol/L for sodium, 4.25±1.72 mmol/L for potassium, 102.82±1.72 mmol/L for chloride, and 22.09±1.30 mmol/L for bicarbonate. These differences in the mean values of blood electrolytes before and after coffee enema were not clinically significant.