CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Discussion

It has been demonstrated that caffeine absorption from gastrointestinal tract, especially in small intestine, is rapid and complete with the bioavailability of 99-100% after oral administration (29-30, 32, 38, 69-71). T_{max} is observed approximately 30 to 60 min after oral consumption, although the range can be as wide as 15 to120 min because of variation in gastric emptying (32, 35, 72). Our results, as in previous reports (32, 73), demonstrated that single dose of coffee consumption resulted in rapid absorption of caffeine with average T_{max} of 0.44 h (26.4 min). When the C_{max} of oral caffeine absorption in our study is compared to those reported in other studies, it is found that single administration of coffee consumption, containing 96.34±1.39 mg caffeine, resulted in average C_{max} of 2465.45 ng/mL comparable to C_{max} of 1500-1800 ng/mL following single oral administration of coffee containing 100 mg caffeine and 3000 ng/mL following single oral administration of coffee containing 120 mg caffeine.

Caffeine can be also used in combination with some medications in suppository dosage form, for example ergotamine/caffeine rectal suppository. Rectal administration of this combination is indicated for the treatment or prevention of migraine headache. Caffeine is added in order to further enhance the vasoconstrictive effect without the necessity of increasing ergotamine dosage (74). The data lend

support to caffeine absorption into systemic circulation when administered via rectum or colon.

So far, the usage of coffee enema for detoxification and as antioxidant has been highly prevalent and increasing among patients in many countries including Thailand, despite scientific support of its safety and benefits is still lacking. The present study is the first to determine pharmacokinetics of caffeine after coffee enema in comparison to coffee consumption in healthy Thai male volunteers. The ready to drink coffee beverage was chosen for coffee consumption because it is the beverage commonly consumed by population worldwide including Thailand and the amount of caffeine in each serving is consistent and standardized by the manufacturing company. Whereas, unfiltered ground coffee was chosen for preparation of enema solution according to the instruction recommended by Dr. Max Gerson (75). The choice of different preparations of coffee (ready to drink coffee versus ground coffee) is based on the way commonly practice in real-life situation.

When the comparable doses of caffeine were administered, coffee enema resulted in significantly lower extent of absorbed caffeine comparing to coffee consumption. Lower extent of absorption from coffee enema might be explained by at least 2 possibilities: Firstly, the coffee solution administered by enema was retained only 10 minutes, limiting the time for caffeine absorption into systemic circulation via large intestine. The unabsorbable caffeine was then emptied from large intestine by defecation afterwards. In contrast, caffeine from coffee consumption can be rapidly and readily absorbed while its moves along gastrointestinal tract, especially small intestine, without time limitation for absorption. Secondly, absorption surface area in large intestine is enormously less than that of small intestine. Physiologically, the

inner walls of the small intestine have thousands of finger-like outgrowths called villi. These villi not only increase the surface area but also increase the capacity for absorption of the digested food and chemical sustances including caffeine, whereas the inner wall of the large intestine posses no villi for absorption of the digested food. Its function is to absorb water and electrolyte from the remaining indigestible food matter, and then to pass this useless waste material out of the body (76).

Nonetheless, the mean plasma $t_{1/2}$ of caffeine derived form coffee enema or coffee consumption (4.68±1.36 versus 4.87±1.39 h) did not significantly difference. This indifference is resulted from the fact that caffeine is eliminated by first-order kinetics. With first order elimination, the elimination rate constant is independent of the plasma concentrations. The plasma $t_{1/2}$ of caffeine demonstrated in the present study is comparable to those of 2.5-5.7 h reported in other studies (33, 69, 77-78).

This study showed that single dose of coffee consumption containing 96.34±1.39 mg/serving of caffeine exerted no statistical and clinical changes in systolic blood pressure, diastolic blood pressure and heart rate when compared to their own baseline values. These data are consistent with the results reported in previous studies. Astrup *et al* have shown that oral administration of 100 mg and 200 mg doses of caffeine do not affect systolic blood pressure, diastolic blood pressure and heart rate in healthy volunteers (79). In addition, Castiglia *et al* have reported no change in systolic blood pressure, diastolic blood pressure and heart rate after 100 mg caffeine either in the form of coffee or purified caffeine in normal volunteers (80-81). Newcombe *et al* have demonstrated that subjects drinking 4-12 cups of coffee equivalent to 280-840 mg/day of caffeine show no significant change in heart rate (82).

Although comparable doses of caffeine were administered but coffee enema resulted in significantly lower extent of caffeine absorption comparing to coffee consumption. It is therefore not surprising that coffee enema did not produce statistical and clinical changes in systolic blood pressure, diastolic blood pressure and heart rate when compared to their own baseline values. The findings from this study confirm that neither coffee consumption nor coffee enema could produce deleterious effects on hemodynamics in healthy subjects. Nevertheless, since most of caffeine in systemic circulation is normally eliminated within 4-5 half-lives (approximately 24 h) (83), it could be postulated that even multiple doses of coffee enema (e.g., once a day or once every other day) would not result in accumulation of caffeine in the body, and hence should not adversely affect hemodynamic parameters.

Coffee is the most frequently ingested beverage worldwide (84). More recently, coffee consumption has been associated with reduced risk of several chronic diseases (45, 85-87) by its antioxidant effect (88). Coffee is a rich source of caffeine and it also contains numerous substances, many of which are antioxidants, for example, melanoidins, polyphenolic compounds and diterpenoid alcohols. Two diterpenoid alcohols, cafestol and kahweol, are abundant in coffee. They are natural constituents of coffee beans (89), and are released from roast and ground coffee beans by hot water, but are largely trapped by paper filter in coffee drink preparation (84, 90-92). Several lines of evidence suggest that coffee substances exert beneficial effects via enhancement of endogenous antioxidant activities (see more detail in "Chapter 1 and 2") (84).

GSH is a tripeptide (γ-glutamylcysteinylglycine) widely distributed in both human and animals (93). GSH serves as a nucleophilic co-substrate to GST in the

detoxification of xenobiotics and is an essential electron donor to glutathione peroxidases in the reduction of hydroperoxides (93-94). Concentration of GSH ranges from a few micromolar in plasma to several millimolar in tissues such as liver (95-96).

In this study, 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). These baseline GSH concentrations are comparable with the values of 5.5±1.8 μmol/L (58), 4.1±1.4 μmol/L (5) and 3.5±2.1 μmol/L (97) in healthy volunteers reported in other studies. However, since single dose of coffee enema or coffee consumption did not significantly alter serum concentrations of GSH at any time points (10 min - 12 h), these data suggest that both acute coffee procedures might not produce any beneficial effects in term of enhancement of serum GSH levels. Even though tissue concentrations of GSH were not measured in this study, there is evidence to support that the concentration of plasma GSH reflects the intrahepatic concentration (90). Nonetheless, so far, acute effect on serum concentrations of GSH following coffee procedures has not been reported elsewhere.

Although multiple doses of coffee consumption in the present study showed a trend of increasing serum GSH concentrations by about 25% at day 6 and day 12, but these effects did not reach statistical significant level. In contrast, consumption of unfiltered coffee 1 L/day for 14 days or 5 cups/day for 7 days has been found to significantly increase plasma GSH by approximately 15%-16% and by 8% in colorectal mucosa (5, 58). These discrepancies may reflect the different type and dose

of coffee used, the smaller sample size and large variation of serum GSH responses in the present study albeit greater increase in serum GSH.

Similar to multiple doses of coffee consumption, multiple doses coffee enema also increased serum GSH by about 22% at day 6 and 16% at day 12, but again these changes did not attain significant level. Although these magnitude of changes were comparable to those resulted from coffee consumption, we do believe that absorption of antioxidant substances from enema solution into systemic circulation, if any, should not be equivalent to that of oral administration, similar to the case of caffeine absorption described above. However, the trend towards enhancement of serum GSH levels from coffee enema might be the result from mechanical cleansing of large intestine from enema procedure, thus preventing absorption of toxic waste products including free radicals into the body (4-5). The reduction in bioavailability of waste products secondarily to evacuation of the colon causes preservation of endogenous antioxidants and probably increases the pool of GSH in the serum. To prove this possibility, the effect of tap water enema on serum GSH levels warrants further investigation.

MDA is a naturally occurring product of lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in both human and animals and is used as an indicator of oxidative stress in cells and tissues (98-99). Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds, such as MDA. Assay of total antioxidant status utilizing TEAC can be used to determine antioxidant levels in serum or plasma samples, solubilized food and drug samples.

The assay relies on the ability of antioxidants in the sample to inhibit oxidation of ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^{\bullet+}$ (100).

At baseline, serum MDA concentrations of coffee enema and coffee consumption groups were not significantly different and were in close approximate to the results of previous studies (101-102). Although serum TEAC concentrations of both treatment groups at baseline were statistically different but these may not be of clinical significance. The values of baseline serum TEAC concentrations in the present study were comparable to those reported previously in healthy volunteers (103-104)

Single dose of coffee enema or coffee consumption did not significantly affect serum concentrations of MDA or TEAC at any time points (10 min-12 h), suggesting that single dose of both treatments might not produce any antioxidant advantage in term of lowering serum MDA or enhancing TEAC levels. Similar to the case of GSH, acute effect on serum concentrations of MDA and TEAC following both coffee treatments has not yet been reported elsewhere. Nevertheless, Natella *et al* have shown significantly increases in plasma total radical-trapping antioxidant parameter (TRAP) at 2 h after single consumption of 200 ml brewed coffee in 10 healthy nonsmoking moderate coffee drinkers (2-4 cups/day) (56). This discrepancy in total antioxidant status following coffee consumption between the present study and other might be due to different antioxidant parameters measured and type of coffee consumption used in each study (ready-to-drink coffee versus unfiltered coffee). It has been postulated that unfiltered coffee contains abundant antioxidant substances, for example; melanoidins, polyphenolic compounds and diterpenoid alcohols, than ready-to-drink coffee used in our study (84, 105), possibly causing larger extent of

antioxidant substances absorbed into systemic circulation following oral administration, and hence higher total antioxidant status in plasma.

In this study, multiple doses of coffee consumption insignificantly changed serum concentrations of MDA from the baseline value at day 6 and 12, respectively. In contrast, Yugawa *et al* have demonstrated that drinking unfiltered coffee, 24 g total per day, for 1 week in 11 healthy volunteers significantly decreases serum level of MDA (59). The possibilities of this discrepancy might be in part due to different type of coffee consumed as mentioned above and lower amount of coffee consumption used in the present study. On the other hand, although multiple doses of coffee enema showed a trend towards insignificant increase in serum concentrations of MDA from the baseline value at day 6 and 12. These changes seemed to be of no clinical significance because previous reports have demonstrated that serum MDA levels in pathologic conditions are markedly higher than the values reported here, such as 0.130±0.02 mmol/L in patients with diabetes (101), and 47.9±7.1 mmol/L in patients with stroke (106).

Despite statistically significant reduction in serum concentrations of TEAC at day 12 following multiple doses of either coffee consumption and coffee enema, these reductions were and might not correlate to clinical significance because previous reports have shown that the serum TEAC levels in pathologic conditions should be much lower than the values reported here, such as 0.79±0.68 mmol/L in patients with hypothyroidism (104) and 0.004±0.00007 mmol/L in patients with cardiovascular diseases (68). However, the mechanisms underlying significant reductions in serum TEAC following multiple doses of both coffee treatments, despite a trend towards enhancement of serum GSH, are still unknown. It could be postulated that either

procedure may adversely increase the oxidative stress as documented by a decrease in serum TEAC, a trend towards increase in serum GSH could be the adaptive response to an increase in oxidative stress. In summary, these data suggest that multiple doses of coffee enema or coffee consumption seemed not to produce any beneficial antioxidant effect regarding decrease in serum MDA levels, or enhancement of serum GSH and TEAC levels.

In order to verify whether coffee consumption is beneficial to consumers, study in larger sample size using higher dose of unfiltered ground coffee should be further determined. On the other hand, effect on caffeine pharmacokinetics and antioxidant effect from coffee enema using higher concentration and/or larger volume as well as prolongation of time to retain enema fluid should be of interest and further studied. In addition, antioxidant effect of coffee consumption and coffee enema also warrants further investigation in Thai patients who manifest abnormal baseline antioxidants, such as heavy smokers or patients with diabetics, AIDS, cancers, etc.

Although beneficial effects from coffee enema are not clearly demonstrated, the procedure in routine practice is unlikely to produce any adverse effects on cardiovascular system or electrolyte balance, coffee enema could therefore be employed according to personal preference unless contraindications (i.e., hemorrhoids, gut obstruction, diverticulitis, ulcerative colitis, Crohn's disease, irritable bowel syndrome (IBS), colostomy, recent bowel surgery and colorectal cancer) are documented.

4.2 Conclusion

Single dose of coffee enema containing comparable amount of caffeine yields 28% bioavailability of single orally consumed coffee despite slightly but significantly faster T_{max}. Single dose of coffee enema and coffee consumption did not adversely affect systolic and diastolic blood pressure, heart rate, or electrolyte balance. Acute or multiple dose of coffee enema and coffee consumption up to 12 days seemed not be of any beneficial effects with respect to enhancement of serum GSH and TEAC levels or decrease in serum MDA concentrations in the present experimental setting.