CHAPTER 1

INTRODUCTION

Enema is an alternative medical procedure. It involves the infusion of purified water or other liquid solutions into the rectum through the anus. The purpose of this procedure is to remove waste material from the colonic wall. In ancient Egyptian era, enema and other "cleansing rituals" were commonly used to get rid of body toxic waste products which were believed to cause various diseases and death (1, 2). Since the 19th century, large intestine has been described as a sewage system where toxins are formed and absorbed, leading to the theory of "autointoxication" (2, 3). Autointoxication has been an ancient theory based on the belief that intestinal waste products can poison the body and might be a major contributor to many, if not all, diseases (4-7). Laxatives and enemas therefore have been routinely recommended by some physicians to detoxify the body through the removal of accumulated waste from the colon (2).

Coffee enema has been one of the oldest medical procedures still in use today for “detoxification” since Dr. Max Gerson introduced them into cancer therapy in 1930s (8). Some experts in complementary and alternative medicine believe that caffeine from coffee enema causes dialysis of toxic products from blood across the colonic walls (1, 8) as well as causing dilation of the bile ducts, which facilitates excretion of toxic products by the liver. Thus, the coffee enema is claimed to have a very specific purpose in lowering serum toxins (9, 10). It has been also shown that substances found in coffee, kahweol and cafestol, are potent enhancers of glutathione

S-transferase (GST), a major antioxidant enzyme that catalyses the binding of a vast variety of electrophiles from the blood stream to the sulphhydryl group of glutathione (GSH) (7). In mice, for example, this system is enhanced by 600% in the liver and 700% in the bowel when coffee beans are added to the mice's diet (7, 11). Some researchers have shown that the average consumption of coffee in Italian drinkers (5 cups/day) significantly increases plasma concentration of GSH by 16% (5). Additionally, melanoidins formed during roasting of coffee beans, exhibit strong antioxidant activity (12), and significantly inhibit lipid oxidation (13-15). Phenolic compounds in coffee such as chlorogenic acid and caffeic acid have antioxidant activity *in vitro* (16), whereas caffeine and its metabolites, theobromine and other xanthines, appear to possess strong DNA-protective effects (4). Thus, some practitioners propose that coffee enema can eliminate toxins and exert antioxidant effects in the same manner or even better than coffee consumption (1, 9-10, 17). However, no scientific research regarding detoxifying or antioxidant effects after coffee enemas has been reported (17). Furthermore, some practitioners debate about dangers of the coffee enemas such as systemic adverse effects from caffeine (2, 9). Enemas can be dangerous because illness and even deaths could be the results from contaminated equipment, electrolyte (salt and mineral) imbalance, or perforation of intestinal walls. People with diverticulitis, ulcerative colitis, Crohn's disease, severe hemorrhoids, rectal or colon tumors, or recovering from bowel surgery may be at higher risk of bowel injury. People with kidney or heart failure may be more likely to experience fluid overload or electrolyte imbalances. In addition, many substances can be absorbed into the body from the colon walls, which can cause toxic or allergic reactions (4, 18).

So far, the usage of coffee enema for detoxification has been highly prevalent and increasing among patients in many countries including Thailand, especially in those with cancers, allergy, asthma, urticaria, migraine, dyslipidemia, obesity, chronic constipation, etc (10, 19), despite scientific support of its safety and benefits is still lacking. Thus, the purpose of this study is to compare the pharmacokinetic parameters of caffeine after single dose of coffee enema and coffee consumption, and to determine the antioxidant effects after single and multiple doses of both coffee procedures.

1.1 Caffeine

Physical and chemical properties of caffeine

Caffeine is a xanthine alkaloid compound that acts as a stimulant in humans. The chemical name of caffeine is 3,7-dihydro-1, 3, 7-trimethyl-1-purine-2, 6-dione or 1,3,7-trimethylxanthine and its chemical formula is $C_8H_{10}N_4O_2$ with a molecular weight of 194.19 g/mol. Its chemical structure is shown in Figure 1. The chemical is also known as caffeine, theine, mateine, guaranine, or methyltheobromine (20). Caffeine is white crystalline powder that and is odorless with bitter taste (21). The pKa value of caffeine is 13.9 (22).

Figure 1. Chemical structure of caffeine

Pharmacokinetics of caffeine

Caffeine is readily absorbed after oral or parenteral administration with 99-100% bioavailability (23-26). Following oral administration of 100-mg caffeine, peak plasma concentrations of about 1.5-1.8 μg/mL are reached after 50-75 min (27). After oral ingestion of 120-mg caffeine by 13 subjects, peak plasma concentration average 3 μg/mL (range 2.0-4.0 μg/mL) at 1 h and fall to 2.5 μg/mL by 2 h (28). Following a single oral 175-mg dose, peak plasma level average 5.0 μg/mL of caffeine at 90 min (23). Caffeine is rapidly distributed to all body compartments; readily crosses the placenta and blood brain barrier and passes into breast milk. Caffeine concentration in cerebrospinal fluid of preterm neonates approximates the plasma concentration (29). Volume of distribution (Vd) in adults ranges from 0.4-0.6 L/kg. Vd in neonates averages between 0.78 and 0.92 L/kg and approximately 25-36% of caffeine is bound to plasma protein (21).

Caffeine is extensively metabolized in the liver by the cytochome P450 isoenzyme 1A2 (21, 25-27). Such metabolism is complex, with at least 17 metabolites are formed. However, these metabolites arise from three primary pathways that contribute to over 95% of the drug's overall metabolic clearance. In adults, about 84% of a dose of caffeine is metabolized to paraxanthine (1,7-dimethylxanthine), 12% is metabolized to theobromine (3,7-dimethylxanthine), and about 4% is metabolized to theophylline (1,3-dimethylxanthine). The dimethylxanthines are pharmacologically active and may contribute to the effects of caffeine in humans (25, 27).

Caffeine has a plasma half-life ($t_{1/2}$) ranging from 4-6 h in healthy adults, and is increased by twofold in women during the later stage of pregnancy or with long term use of oral contraceptive steroids (27, 29). Smoking enhances caffeine clearance

such that the half-life may be shortened. Smoking and polycyclic aromatic hydrocarbons such as polychlorinated or polybrominated biphenyls or rifampin similarly increase the rate of demethylation of caffeine and its half-life is thus reduced (30-31). On average, the half-lives are 4-6 h in adults and 65-130 h in neonates (27).

Dimethylxanthine metabolites are further demethylated to monomethylxanthines and to methyl uric acids and then excreted mainly by kidneys, only about 1-5 % of the dose of caffeine is excreted unchanged in urine (26, 32-33). In adults, marked interindividual variability in the rate of caffeine elimination occurs, mean clearance is 1.6 ml/min (33). The biotransformation of caffeine is shown in Figure 2.

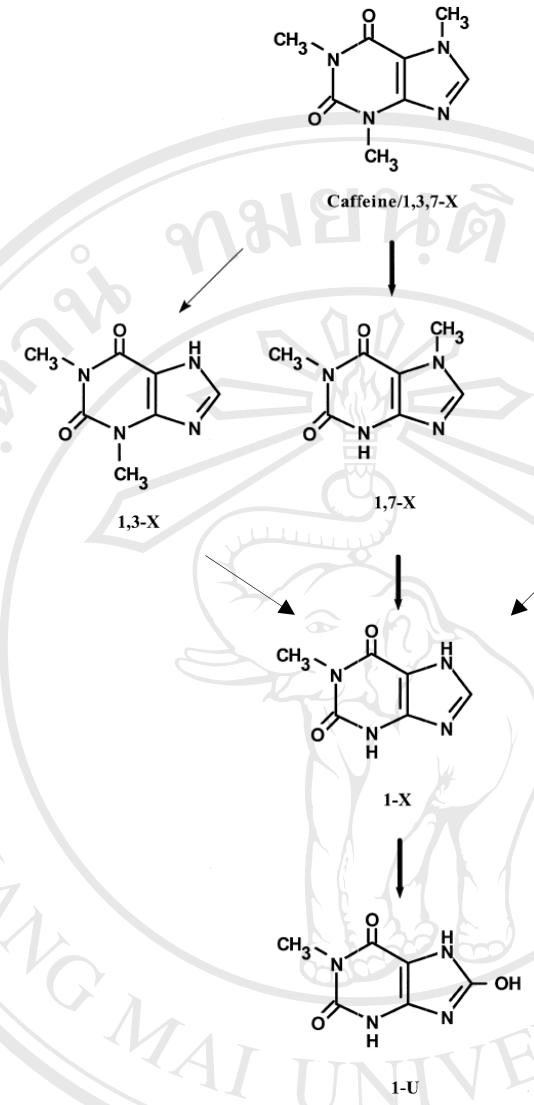

Figure 2. Biotransformation of caffeine in humans. It is demethylated by the hepatic cytochome P450 1A2 (CYP1A2) enzyme primarily to 1,7-dimethylxanthine (paraxanthine or 1,7-X) 3,7-dimethylxanthine (theobromine or 3,7-X) and 1,3-dimethylxanthine (theophylline or 1,3-X) in humans. Further demethylation of dimethylxanthine follows the same CYP1A2 enzyme and results in 1-methylxanthine (1-X) and 1-methyluric acid (1-U) which are the main metabolites of caffeine excreted in urine (34-35).

Mechanism of action

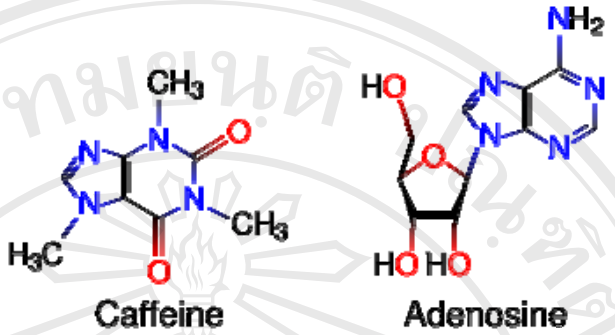

Figure 3. Caffeine and adenosine structure

Caffeine acts through multiple mechanisms involving both action on receptors and channels at the cell membrane, as well as intracellular action on calcium and cAMP pathways (36). Several mechanisms of caffeine have been proposed.

The most important mechanism of action of caffeine is the antagonism of adenosine receptors since this effect is exerted at dose or plasma concentrations achieved therapeutically (24-25, 30, 37). The caffeine molecule is structurally similar to adenosine (Figure 3) (36). Adenosine receptor located presynaptically is responsible for regulation of the release of several transmitters, and inhibition of this receptor by caffeine lead to the increased release of transmitters notably norepinephine which is responsible for the increased heart rate and force of contraction (24, 26). The reduction in central adenosine activity also results in increased activity of the neurotransmitter dopamine and norepinephrine, largely accounting for the stimulatory effects of caffeine. Acute usage of caffeine also increases levels of serotonin, causing positive changes in mood (22).

The inhibition of adenosine may be relevant in its diuretic properties. Because its inhibition may cause vasodilation, with an increase in renal blood flow (RBF) and glomerular filtration rate (GFR). This effect, called competitive inhibition, interrupts a pathway that normally serves to regulate nerve conduction by suppressing postsynaptic potentials.

Caffeine is also a known competitive inhibitor of the enzyme cyclic AMP-phosphodiesterase (cAMP-PDE), which converts cAMP in cells to its noncyclic form (AMP), allowing cAMP to build up in cells. cAMP participates in the messaging cascade produced by cells in response to stimulation by epinephrine, so by blocking its removal caffeine intensifies and prolongs the effects of epinephrine.

The metabolites of caffeine contribute to caffeine's effects. Theobromine is a vasodilator that increases the amount of oxygen and nutrient flow to the brain and muscles and also increases urine volume. Theophylline, the second of the three primary metabolites, acts as a smooth muscle relaxant that chiefly affects bronchioles and acts as a chronotrope and inotrope that increases heart rate and efficiency and is used to treat asthma. The third metabolic derivative, paraxanthine, is responsible for an increase in the lipolysis process, which releases glycerol and fatty acids into the blood to be used as a source of fuel by the muscles (27, 29, 35).

Caffeine effects

Central effects

Caffeine is primarily a stimulant, increasing arousal and vigilance, reducing fatigue, and decreasing motor reaction time for some tasks (38, 39). Caffeine is a central nervous system (CNS) stimulant, having the effect of temporarily warding off drowsiness and restoring alertness. In higher doses, caffeine may produce insomnia,

anxiety, tremors, and seizures. Caffeine may interfere with sleep by increasing sleep latency and decreasing total sleep time. Caffeine also decreases cerebral blood flow, presumably by antagonizing adenosine-mediated cerebral vasodilation (27, 40).

Cardiovascular effects

In doses typically found in 2 cups of coffee, caffeine increases blood pressure (5-10 mmHg), decreases heart rate slightly, and causes systemic release of epinephine, norepinephine, and renin. The primary mechanism of the pressor effect is peripheral vasoconstriction. Caffeine increases renal excretion of sodium and water, both by increasing glomerular filtration rate slightly and by inhibiting tubular reabsorption of sodium and water (27).

Respiratory effects

The primary effect of caffeine on the respiratory tract is to increase the respiratory rate, probably by sensitizing the medullary center to carbon dioxide (27).

Endocrine and metabolic effects

Caffeine increases circulating catecholamines, predominantly epinephine, plasma rennin activity, free fatty acids, cortisol, metabolic rate, and blood glucose (38).

Gastrointestinal effects

Caffeine stimulates gastric secretion of acid and pepsin; however, coffee per se, even in the absence of caffeine, may be as potent in this action (27).

Antioxidant effects

Caffeine has been reported as a scavenger of the hydroxyl radical at millimolar concentrations in the study of electron spin resonance (ESR) spin trapping (40). There is neither antioxidant activity nor protective ability present with caffeine, 1,7-

dimethylxanthine, 3,7- imethylxanthine, or 1,3-dimethylxanthine at micromolar concentration. The antioxidant activity is however significant with 1- methylxanthine (1-X) and 1-methyluric acid (1-U), the main metabolites of caffeine in humans. These compounds also significantly reduce the level of TBARS and conjugated dienes produced from the LDL peroxidation *in vitro*. However, it has not yet been shown if caffeine at physiologically relevant micromolar concentrations has antioxidant ability (34).

Dosage and administration

Caffeine is used orally or sometimes rectally in combination with ergotamine tartrate to abort vascular headache such as migraine and cluster headaches. Caffeine citrate is used intravenously or orally in short-term (10-12 days) treatment of apnea of premature neonate who are between 28 and 33 weeks of gestational age. Caffeine at the dose of 100-200 mg is widely used orally every 3-4 h for those who wish to stay awake or to restore mental alertness. Coffee and tea are among the most popular beverages used for this purpose (37).

The major current therapeutic use for caffeine is treatment of apnea in newborns. Caffeine is widely used in combination with analgesic drugs and caffeine has been shown in some studies to enhance the analgesic effect of acetaminophen or aspirin (41). Other recent therapeutic uses for caffeine include the treatment of postprandial hypotension in patients with chronic autonomic failure (42) and, given intravenously, as pretreatment to facilitate and prolong seizures during electroconvulsive therapy (43).

Acute toxicity and overdose

An acute overdose of caffeine, usually in excess of 400 mg (more than 3–4 cups of brewed coffee), can result in a state of central nervous system over stimulation called caffeine intoxication. The symptoms of caffeine intoxication may include restlessness, nervousness, excitement, insomnia, flushing of the face, increased urination, gastrointestinal disturbance, nausea, vomiting, diarrhea, hyperglycemia, hypokalemia, muscle twitching, a rambling flow of thought and speech, irritability, irregular or rapid heart beat, and psychomotor agitation. Adverse reactions to lower doses of caffeine, such as those that may be achieved through coffee consumption include tachycardia, palpitations, insomnia, restlessness, nervousness, tremor, headache, abdominal pain, nausea, vomiting, and diuresis (44-45).

In cases of extreme overdose, death can result. The median lethal dose (LD_{50}) of caffeine is 192 mg/kg in rats (46). The LD_{50} of caffeine in humans is dependent on weight and individual sensitivity and estimated to be about 150-200 mg/kg of body mass, roughly 80-100 cups of coffee (47). Oral doses of 5–50 g (mean 10 g) have resulted in fatalities in adults, and the lethal dose is estimated at 100–200 mg/kg of body weight. Ingestion of 15–30 mg/kg has resulted in significant toxicity. Symptoms of caffeine overdose may include agitation, delirium, seizures, dyspnea, cardiac arrhythmia, myoclonus, nausea, vomiting, hyperglycemia and hypokalemia (44).

Treatment of severe caffeine intoxication is generally supportive, providing treatment of the immediate symptoms, but if the patient has very high serum levels of caffeine then peritoneal dialysis, hemodialysis, or hemofiltration may be required.

Disease and age affecting caffeine pharmacokinetics

Liver disease can decrease the rate of caffeine metabolism thus resulting in accumulation of caffeine in the body. In preterm infants half-life of caffeine is reported to be 65-103 h (32). Neonates have a greatly reduced capacity to metabolize caffeine, and it is excreted unchanged in the urine until their hepatic metabolism becomes significantly developed, usually about 6 months (31, 44). Rate of caffeine clearance is similar in older and younger adults (32).

Drug interactions

Habitual caffeine consumption increases CYP1A2 activity, which has implications for the metabolism for a number of medications (29, 32, 44). Conversely, drugs that inhibit the activity of CYP1A2 interfere with the metabolism and elimination of caffeine, increasing the risk of toxic effects. Caffeine and other methylxanthines may enhance the effects and side effects of β-adrenergic stimulating agents, such as epinephrine and albuterol (49). Caffeine could theoretically increase the risk of hypertensive crisis in individuals taking monoamine oxidase inhibitors. Caffeine may inhibit the hepatic metabolism of the antipsychotic medication, clozapine, potentially elevating serum clozapine levels and increasing the risk of toxicity. Caffeine consumption can decrease the elimination of theophylline, potentially increasing serum theophylline levels. Caffeine has been found to decrease the systemic elimination of acetaminophen and to increase the bioavailability of aspirin, which may partially explain its efficacy in enhancing their analgesic effects. Caffeine may decrease serum concentrations of lithium by enhancing its elimination (32, 44). The following medications may impair the hepatic metabolism of caffeine, decreasing its elimination and potentially increasing the risk of caffeine-related side

effects: cimetidine, disulfiram, estrogens, fluconazole, fluvoxamine, mexiletine, quinolone class antibiotics and terbinafine. Phenytoin and cigarette smoking increase the hepatic metabolism of caffeine, resulting in increased elimination and decreased plasma caffeine concentrations (44).

Precaution and contraindications

Since it has been suggested that caffeine increase gastric secretion including gastric acid, caffeine should be used cautiously in patients with a history of peptic ulcer. Because of its suspected arrhythmogenic potential, it is generally recommended that caffeine be avoided in patients with symptomatic cardiac arrhythmias and/or palpitation and during the first several days to weeks after an acute attack of myocardial infraction. Although caffeine has not been proved to be teratogenic, its cautious use in pregnant women is prudent. Pregnant women should be cautioned in their consumption of food and beverages since they may contain significant amount of natural or added caffeine (30, 36).

Currently, available evidence suggests that it would be prudent for women who are pregnant, lactating, or planning to become pregnant to limit coffee consumption to 3 cups/day providing no more than 300 mg/day of caffeine. Health Canada's recommendation that children should not consume more than 2.5 mg/kg/day of caffeine (44).

1.2 Coffee and antioxidant effects in humans

The majority of studies on the health effects of coffee consumption in humans are observational (44). Coffee contains a variety of bioactive compounds including caffeine and other purine derivatives, Maillard reaction products, specific diterpenes

such as kahweol and cafestol and polyphenolics including chlorogenic acid derivatives and its degradation product caffeic acid. The consequences of coffee consumption on human health have been studied intensely during the last decades (49). Earlier studies have revealed that kahweol and cafestol, whose molecular structures are shown in Figure 4, are two rather abundant diterpenic components of coffee beans that also appear at high concentrations in unfiltered coffee (50). These diterpenes are extracted from ground coffee during brewing, but are mostly removed from coffee by paper filters. Unfiltered coffee such as Scandinavian boiled coffee, Turkish coffee, and French press (cafetiere) coffee contains relatively high levels of cafestol and kahweol (6–12 mg/cup), while filtered coffee, percolated coffee, and instant coffee contain low levels of cafestol and kahweol (0.2–0.6 mg/cup) (44).

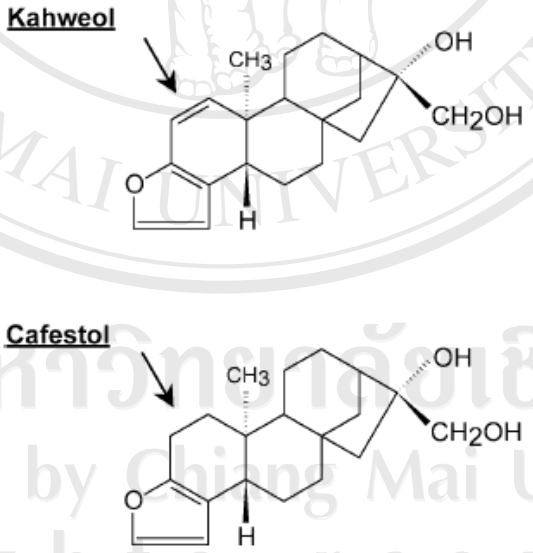

Figure 4. Molecular structures of kahweol and cafestol. Arrows indicate the chemical difference between the two compounds; a double bond in kahweol is lacking in cafestol (50).

Among the antioxidant or detoxifying mechanisms of xenobiotic metabolism, a key role is played by glutathione S-transferase (GST), a system of phase II enzymes that catalyzes the conjugation of a great variety of electrophilic compounds with the tripeptide glutathione (GSH) (51).

Cafestol and kahweol have been implicated in stimulation of glutathione S-transferase (GST) activity in rat. These diterpines also increase the glutathione (GSH) concentration in mice and they are found to increase the GSH and γ-glutamylcysteine synthetase (γ–GCS) level in a hepatoma cell line in a dose-dependent manner (7, 52-53). Molecular evidence has been provided of the ability of some phenolic compounds to activate γ–GCS, the rate-limiting enzyme in GSH synthesis. GSH is an important endogenous antioxidant and cofactor of detoxifying metabolism. These data suggest that diterpenes have chemoprotective effects through stimulation of the endogenous antioxidant system (5, 52-54). Several studies suggest that melanoidins and phenolic compounds such as chlorogenic acid and caffeic acid in coffee exhibit strong antioxidant activity and inhibit significantly lipid oxidation *in vitro* (12-13, 15, 55). Although chlorogenic acid and caffeic acid have antioxidant activity, it is unclear how much antioxidant activity they contribute *in vivo* because they are extensively metabolized, and the metabolites often have lower antioxidant activity than the parent compounds (44, 56-57).

Grubben *et al* have demonstrated that consumption of 1 L of unfiltered coffee (French press) daily in 64 healthy volunteers, with 2 intervention periods of 2 weeks separated by a washout period of 8 weeks, significantly increases the GSH content in the colorectal mucosa by 8% and in plasma by 15% (58). Additionally, Esposito *et al* have found that moderate coffee consumption increases plasma GSH but not

homocysteine in 22 healthy subjects who consume 5 cups of coffee/day for 1 week. The intervention trial was preceded and followed by 7 coffee-free days. Plasma GSH increases by 16% (p<0.05) on coffee consumption, and returns to the original concentration after the washout period. The increase in plasma homocysteine concentration (13% after 1 week of coffee intake) is not significant (5).

Yukawa *et al* conducted an *in vivo* study in 11 healthy men aged between 20 and 31 yr. The subjects began drinking coffee, 24 gm total per day, for 1 week. This was followed by a 1 week "washout period" during which mineral water was consumed. Fasting venous blood samples were taken at the end of each 1 week period. LDL oxidation lag time is approximately 8% greater (p<0.01) after the coffee drinking period than the other periods. Serum levels of total cholesterol and LDL cholesterol (LDL-C) and malondialdehyde (MDA) as thiobarbituric acid reactive substances (TBARS) are significantly decreased after the coffee drinking period. It therefore seems likely that effects of coffee are a reduction of serum lipid and MDA levels, and a decrease in the susceptibility of LDL to oxidation (59).

Steinkellner *et al* have shown that consumption of 1 L of unfiltered coffee/day over 5 days in 10 participants causes induction of GST in humans (60). This may lead to protection towards DNA-damage caused by (±)-anti-B[*a*]P-7,8-dihydrodiol-9,10-epoxide (BPDE), the DNA-reactive metabolite of benzo[*a*]pyrene (60). Several studies suggest that coffee extracts show inhibitory effects on the mutagenicity of benzo[*a*]pyrene (49, 61).

Caffeine inhibits carcinogenesis in mouse skin induced by chemical agents found in cigarette smoke (49) and glandular stomach carcinogenesis induced by lipid peroxidation in rats. Although the mechanism of anticarcinogenic effect of caffeine is

not clear, much of clinical interest underlies its potential role as an antioxidant in the control of oxidative damage. Uric acid that is structurally similar to caffeine is an important scavenger of both hydroxyl and peroxyl radicals (40). Caffeine and its metabolites, theobromine and paraxanthine, appear to possess strong DNA-protective effects (4).

Natalle *et al* have reported that coffee drinking influences plasma antioxidant capacity in humans. They have shown an increase in plasma antioxidant capacity after consumption of 200 ml brewed coffee in 10 healthy nonsmoking moderate coffee drinkers (2-4 cups per day) (56).

1.3 The scope and aims of this research

The objectives of the present study were to

1. Compare the pharmacokinetic parameters of caffeine after single dose of coffee enema and coffee consumption.
2. Determine the antioxidant effects using serum levels of glutathione (GSH), trolox equivalent antioxidant capacity (TEAC) malodialdehyde (MDA) as antioxidant parameters after single and multiple doses of coffee enema or coffee consumption,.
3. Evaluate the effect of single dose of coffee enema versus coffee consumption on blood pressure and heart rate.
4. Evaluate the effects of multiple doses of coffee enema on blood electrolytes.