

Full Length Research Paper

Comparative evaluation of caffeine content in Arabian coffee with other caffeine beverages

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It is well documented that caffeine is the world's most widely consumed drug with its main source found in coffee. In the Kingdom of Saudi Arabia (KSA), diabetes and obesity are major health problems. Caffeine is attested as a potential drug for treating obesity, hepatic fibrosis, and preventing or delaying diabetes. The aim of this work is to evaluate the caffeine content of the Arabian coffee in comparison to Turkish coffee and instant coffee, in order to better adjust daily caffeine consumption. All types of coffee were prepared based on traditional ways in KSA. The average consumed coffee per normal person is assumed to be, 6 Arabian, 2 Nescafe or 1 Turkish cups per day. High performance liquid chromatography technique was used for caffeine measurement using paracetamol as an internal standard. Generally, coffee is prepared with other additives, liquid-liquid extraction was used for the extraction caffeine and paracetamol as an internal standard. HPLC method validated was over the range of 1 to 100 µg with good linearity ($r^2=0.991$). Validation data proved that the method is accurate with average of 102%. Caffeine contents of Arabian coffee, Nescafe®, and Turkish coffee were found to be 4.1, 43.4 and 82.8 mg/cup, respectively. One cup of Turkish coffee contains caffeine as much as 2 Nescafe® and 20 Arabian cups. Gold Nescafe® contains about 20% less caffeine than classic. The caffeine content of each type of marketed coffee was accurately measured. An individual Arabian coffee consumer, who is drinking an average of 6 cups/day, can safely increase the number of cups or cup size in order to obtain more caffeine. The other choice for increasing caffeine ingestion is to think of Turkish coffee and/or Nescafe® as a substitute, in order to maintain caffeine at a therapeutic range for better health.

Key words: Caffeine, Arabian coffee, Turkish coffee, Nescafe®, high performance liquid chromatography (HPLC).

INTRODUCTION

Caffeine (1,3,7-Trimethylpurine-2,6-dione) (Figure 1) is consumed naturally as a drug through normal drinking or eating habits. Coffee beans, tea leaves, and cocoa beans are the main sources of caffeine. Caffeine is an alkaloid

of the methylxanthine family occurring substance found in the leaves, seeds or fruits of over 63 plants species in different countries (Violetaa et al., 2008; Abdul et al., 2006; Violeta et al., 2010; Stovner et al., 2006). The most

commonly known source of caffeine is coffee beans. In its pure state, caffeine is white crystalline powder with strong bitter taste (Burge and Raches, 2003). Caffeine products have been in use for long time due to their pleasant flavor and the strong stimulant effects (John, 1992).

Caffeine is a pharmacologically active substance depending on the dose if taken as medicine or its concentration in food. It may produce mild effects on central nervous system. Caffeine is now used to addict drinkers to soda, although the major soft drink producers adhere to the claim that it is essential to the taste. Many coffee drinkers experience withdrawal symptoms, such as headaches, irritability, sleepiness, and lethargy, when they stop drinking coffee (Yu, 1995). There are several evidences suggesting that caffeine may contribute to the health benefits (Weinberg and Bealer (2001). About 200 mg of caffeine contains bang-up pharmacological effect. At this level, it stimulates the central nervous system, decreases fatigue leading to clearer flow of thoughts, sustained intellectual effort and a generation of perfect ideas with a better appreciation of sensory stimuli in human. At this level, it has a diuretic effect on the kidney hence affect fluid balance in the body. More than 1.0 g of caffeine leads to insomnia, nervousness, nausea, ear ringing, flashing of light delirium and tremulousness (Butt and Sultan, 2011).

Recently, numerous published research articles confirmed the high potency of caffeine in several extremely important health issues such as obesity, diabetes, hepatic cirrhosis and cancer. In an observational study done on a large population, high coffee intake proved to be associated with low risk of obesity and metabolic syndrome and type 2 diabetes (Nordestgaard et al., 2015). One study published in the journal of American society of clinical oncology confirmed that higher coffee intake could be significantly reducing cancer recurrence and even death in patients with the last stage of colon cancer (Guercio et al., 2015). Studies recently published by Guercio et al. (2015) and Lui (2015) have confirmed that caffeine consumption could significantly boost the risk for hepatic fibrosis and cirrhosis.

Obesity and diabetes are the most common health challenges in Saudi Arabia. Large portion of Saudi population are suffering from one or both illness. Young men are more heavily affected than older men and women. One fifth of Saudi children and adolescents counted obese. Caffeine is one proper choice as a potent drug agent for treating many medical issues. Arabian coffee is considered the number one drink in KSA, nationwide. Traditionally, the coffee is cooked by every

nation in certain idiomatic way with special flavors. To the best of our knowledge, there is no data available to suggest the optimal effective caffeine amount of Arabian coffee per day. This information is necessary in order to maintain caffeine therapeutic level of 5 to 20 mg/L (2.5 to 10.0 mmol/L) (Natarajan, 2007). It is documented that up to 400 mg caffeine per day is safe for adult and recommended 100 mg for children.

MATERIALS AND METHODS

Chemicals

Caffeine (purity 99%) and paracetamol (purity 99%) analytical grade were obtained from Sigma Aldrich (Seelze, Germany). Methanol HPLC grade and diethyl ether (absolute) were obtained from Sigma Aldrich (Seelze, Germany). Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA).

Equipment

An HPLC system, consisted of Shimadzu Prominence system equipped with LC-20AD quaternary gradient pump, Prominence SPD-M-20A Diode Array detector, CBM-20A communication bus module, CTO-20A column oven, SIL-20AP autosampler and Shimadzu LC solution software (ver. 1.21 SP1 from Shimadzu, Japan). All samples and standards were filtered through 0.2 μm (Millipore) filters. For analytical column, compounds were separated isocratically on Thermo BDS Hypersil C18 column (150 mm \times 4.6 mm, 5 μm i.d.). Separation was maintained at ambient temperature (25 \pm 2 $^{\circ}\text{C}$). Mobile phase was a mixture of methanol and water (30:70, v/v). The flow rate was 1.0 mL min $^{-1}$ and detection was adjusted at wavelength $\lambda = 270$ nm. The mobile phase was filtered and degassed by sonication before use. In addition, ultrasonic cleaner Ultrasons-HD from Selecta S.A. (Barcelona, Spain) was used.

Standard and internal standard preparations

Caffeine standard was prepared by dissolving 100 mg caffeine in 100 mL (methanol/water: 50/50). Caffeine then serially was diluted in the same solvent to obtain working standard solution for calibration curve. Calibration curve was constructed for 6 points (100, 50, 10, 5, 1, and 0.5 $\mu\text{g}/\text{mL}$). Similarly, quality control samples (50, 5 and 1 $\mu\text{g}/\text{mL}$) were prepared.

Paracetamol was the chosen internal standard for this method. Paracetamol 50 mg was weighted and dissolved in 50 mL methanol to achieve final concentration 1.0 mg/mL. To make working standard, this stock solution was diluted in methanol/water: 50/50 to achieve 100 $\mu\text{g}/\text{mL}$.

Beverages

Three types of coffee, Arabian coffee, Instant coffee (Nescafe $^{\circ}$) and Turkish coffee were purchased from local market. All types of

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Table 1. Amount of crude coffee and additives of Arabian, instant and Turkish.

Type of coffee	Coffee powder amount (g/cup)	Cup volume (mL)	Total volume (mL)	Additives	Amount of additives to each cup (g)
Arabian coffee	0.74	25	150	No additives	-
	0.74	25	150	Cardamom	0.28
	0.74	25	150	Saffron	0.1
	0.74	25	150	(Cardamom + saffron)	0.28 + 0.1
Nescafe	5.3	150	450	Classic	-
	5.3	150	450	Gold	-
Turkish coffee	7.1	60	180	Without additives	-
	7.1	60	180	With Ginger	0.31
	7.1	60	180	With Cloves	0.24
	7.1	60	180	With (Ginger + Cloves)	0.31 + 0.24

coffee were prepared according to the common preparation methods in Saudi Arabia.

Methods: Cocking coffee

Arabian coffee

The average coffee consumed by normal person per day is six cups; each cup contains about 25 mL. The Arabian coffee crude powder comes out from smashed coffee beans mixed with additives (types of additives and amounts are listed in Table 1) and boiled for 20 min to prepare six cups. In order to obtain accurate results, every six cups were prepared separately to obtain average of different preparations. Similarly, six cups of Arabian coffee were prepared (regular size) without additives. Each set of six cups were mixed in 500 mL conical flask, with volume adjusted to 500 mL using distilled water. Amount of weighted coffee powder and related additives are listed in Table 1.

Nescafe coffee

The average Nescafe® coffee consumed by a normal person per day is two cups. Each cup contains about 150 mL. Generally, preparing Nescafe is as simple as potting a small amount of coffee granules in a cup and adding boiling water. Every cup contents were mixed using regular tablespoon for 30 s. Each type was prepared in triplicates and then the contents of each three cups was poured into 500 mL volumetric flask. Using distilled water, the final volume was adjusted to 500 mL. Weight of powder of each type and dilution are listed in Table 1.

Turkish coffee

The average Turkish coffee consumed by normal person per day is one-two cups. Each cup contains about 60 mL. Turkish coffee was prepared by adding the coffee powder and additives onto the cooking containers. The mixture was dissolved in cold water. Containers were gently heated until just before boiling. Contents of three cups (every cup prepared separately) were transferred to 500 mL conical flask, and volume was adjusted to 500 mL using distilled water. Similarly, three cups without additive were prepared. Amount

of powder and types of additives are listed in Table 1. Turkish coffee additives are ginger only, cloves only, ginger + cloves.

Table representing the weight (g per each cup) of crude coffee powder was in column No. 2. Normal size of liquid in the corresponding coffee mugs 25, 150 and 60 mL. Each set of samples (6 cups of Arabian, 2 Nescafe®, and 1 Turkish coffee) was scaled up to one unified volume (500 mL). All types of coffee and additives were also represented in column No. 5. Amount of additive based on the normal preparation for each sample is represented in column No. 6.

Sample preparation

Each sample (1.0 mL) was pipetted onto a 10.0 mL volumetric flask, and the volume was brought up to 10.0 mL using methanol. It was mixed well and the samples were stored for the next step of extraction.

Sample extraction procedure

A volume of 200 µL was pipetted out of Blank, Standards and QC's (each tube from the aforementioned) to labeled tubes 1.8 mL of hexane in another glass tube. Then, vortex-mixed for 5 min and centrifuged for 3.0 min (low speed). All tubes were placed in -70/-80°C freezer for 7.0 min. The upper organic layer was transferred to another clean tube and evaporated until dried. The residue was reconstituted in 200 µL of method mobile phase. Then 50 µL was injected into HPLC column for analysis.

RESULTS

HPLC method data

The method of calibration curve was structured out of eight calibrators ranging from 1 to 100 µg/mL. The absorbance data was calculated as peak area ratio (caffeine/paracetamol). Method low limit of quantification (LLOQ) was chosen to be 1.0 µg/mL. Method a high limit of quantification (HLOQ) was 100 µg/mL and low limit of

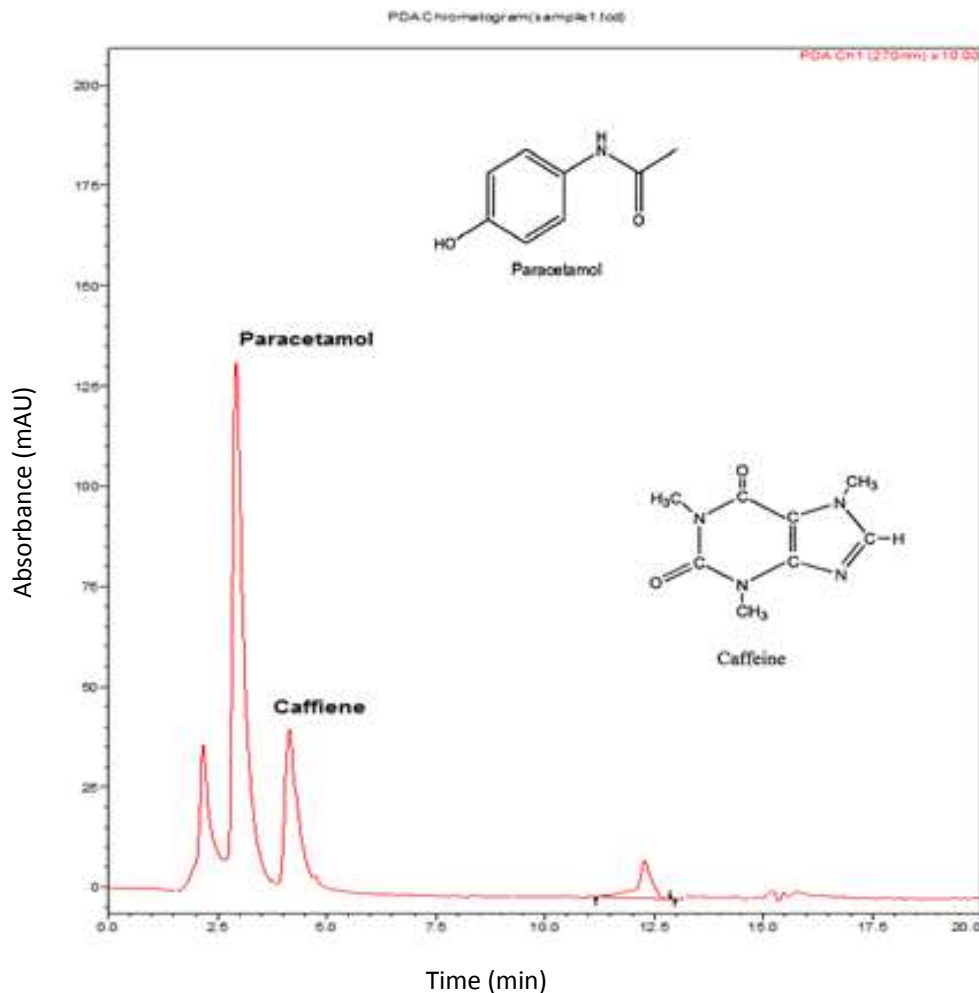


Figure 1. Chromatogram and chemical structure.

detection (LOD) has been identified to be 0.3 $\mu\text{g/ml}$.

Validation data was represented as method accuracy (Acc %). Accuracy percentages were calculated as $\text{Acc \%} = (\text{calculated concentration/nominal concentration}) \times 100$. Four different quality control (QC in triplicate) concentrations were prepared for validations. The method accuracy range is from 93 to 112%. Based on the guidelines of validation method (Guidance for Industry on Bioanalytical Method Validation, 2011) which is defined as 115% at high level and 120% at lower level; the method is accurate, trusted and meets the accuracy criteria. The precision of developed method was tested using %RSD of five readings for the three quality control samples. The RSD% values ranged from 0.87 to 2.5%, indicating good repeatability and precision. Figure 1 shows a chromatogram for an actual extracted sample. The plot indicates a complete separation between paracetamol and caffeine peaks. The elution order of paracetamol and caffeine peaks was 2.9 and 4.1 min, respectively. The chosen wavelength for this method was 270 nm.

Caffeine data

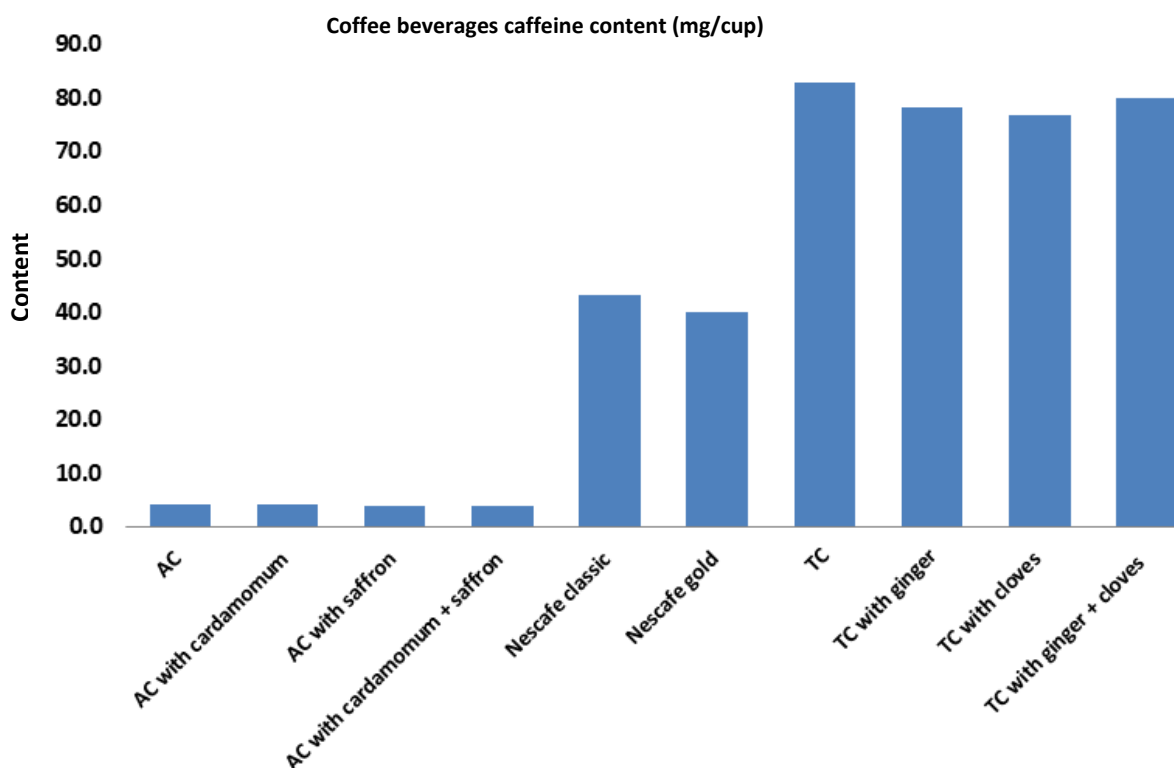
Table 2 shows the caffeine content in mg for each type. The amount of caffeine found per cup in the actual volume, when coffee beverages were traditionally cooked and served. Each set of samples (6 cups of Arabian, 2 Nescafe®, and 1 Turkish coffee) was calculated to represent daily consumed caffeine amount per normal person.

As shown in Figure 2, the lowest caffeine concentration was found in a traditional cup of Arabian coffee, 25 mL. Nescafe in both forms (classic and gold) contains around 40 mg of the normal volume of 150 mL coffee. Turkish coffee appeared as the highest caffeine concentration in small volume mug of 30 mL. Apparently, coffee additives weakened caffeine concentration within Arabian and Turkish coffee.

As shown in Figure 3, out of six cups of Arabian, two mugs of instant and one cup of Turkish coffee per day, normal person ingested 24.0, 83, and 80 mg/day, respectively.

Table 2. Calculated amount of caffeine of each cup represented as (mg/cup) and the total caffeine amount of daily consumption (mg/day).

Type of coffee	Number of cups/day	Additives	Caffeine/cup mg	Calculated caffeine content mg/day
Arabian coffee	6	No additives	4.1	24.6
	6	Cardamom	4.3	26.0
	6	Saffron	4.0	24.1
	6	Cardamom + saffron	3.9	23.6
Nescafe®	2	No additives (classic)	43.4	86.7
	2	No additives (gold)	40.0	79.9
Turkish coffee	1	No additives	82.8	82.8
	1	Ginger	78.2	78.2
	1	Cloves	76.9	76.9
	1	Ginger + cloves	79.8	79.8

**Figure 2.** Caffeine content of coffee beverages (mg/cup).

DISCUSSION

Caffeine has been used as a medicine for several centuries. Recently, caffeine was clinically proved to be a safe and effective drug based on many recent publications in 2015. A level of 5.6 mg/kg body weight

(BW) per day is the safe with no concern for adult. Children and adolescent can consume up to 3 mg/kg BW per day with no side effects (EFSA, 2015). Several clinical studies carefully reviewed with strong evidences that caffeine represents an excellent therapeutic tool in cases of Parkinson disease (Prediger, 2010).

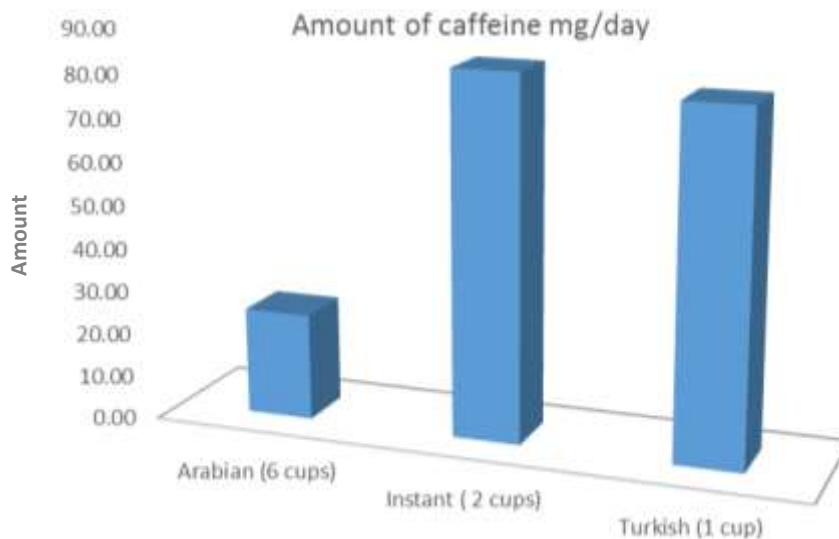


Figure 3. Amount of consumed caffeine mg/day out of 6 Arabian, 2 Nescafe®, and 1 Turkish coffee.

Vast number of studies validate the health benefits of caffeine in delaying diabetes, preventing liver cirrhosis/fibrosis, increasing the effect of chemotherapy in cancer treatment and very good source for obesity treatment. In this research, new methodology is been introduced for accurate evaluation of the amount of caffeine in cooked/served caffeine beverages. All coffee beverages were traditionally cooked exactly based on the averages of cups ingested per day. To the best of our knowledge, this is the first research study to accurately quantify the amount of caffeine in cooked Arabian coffee when compared with other common types of coffee.

The Kingdom of Saudi Arabia has one of the highest percentages of diabetes in the world. Some studies have presented that in every 100 persons, 25 are living with the disease. Obesity has also become a serious health problem. It has been documented that obesity has significantly increased in the Saudi population since the beginning of 21st century and is still a growing health concern. The prevalence of obesity among adolescents is increasing rapidly. Children and adolescents male (5 to 18 years) are considered to represent the highest rate of obesity. Health officials state that, obesity is one of the leading causes of preventable deaths in Saudi Arabia. According to Forbes, Saudi Arabia is ranked 29 on the 2007 list of the fattest countries with a percentage of 68.3% of its citizens being overweight (BMI>25).

Arabian coffee or Gahwa is a very important drink in every Saudi Arabian home and in the Arabian Gulf countries as well. HPLC was the chosen technique to measure caffeine concentration in coffee drinks. The technique was used to separate, identify and quantify different chemical components. HPLC has a number of

advantages and disadvantages as compared to other techniques. The advantages are quick, automated and highly accurate, but it can also be costly, complex and not highly sensitive to certain compounds as compared to mass spectrometry (MS). In this study, caffeine was measured using HPLC method.

Caffeine was extracted from the cooked beverages and then injected into HPLC. Caffeine and internal standard (paracetamol) have been extracted using a Liquid-Liquid extraction procedure. The extraction method has been adapted from Rezk et al. (2009) method with slight modifications. Several HPLC methods for measuring caffeine are found in the studies by Altun (2002), Franeta et al. (2002), Ramos-Martos et al. (2001) and Sawyer and Kumar (2003) was found in literature. These methods were published for quantification of caffeine in coffee or other food sources, some of them used RP-C18 column and water/methanol (60/40). When these conditions were applied in our HPLC system, the internal standard peak was not perfectly separated from the analyte (caffeine) peak. Therefore, we were able to have complete separation when our mobile phase was modified to be water/methanol (70/30). Caffeine was detected using DAD; the spectra was compared over four wavelengths. However, the highest absorbance was found at λ_{max} 190 nm, although the chosen absorbance measurement, 270 nm, was a better choice for the analyte and internal standard in terms of baseline and reproducibility.

The Arabian coffee has much less caffeine content than Nescafe® and Turkish coffee. Even though Saudi population believe they are heavy coffee drinkers, they consume very low amounts of caffeine. In fact, each Arabian cup of coffee contains only 4.0 mg of caffeine.

Table 3. Safe level of caffeine and related number of coffee cups.

The safe level of caffeine for adult and children	Safe level	
	Adult	Children and adolescent
mg/kg	5.6	3.0
mg/day	400	214
Arabian coffee cups	100	56
Nescafe® cups	10	5.6
Turkish cups	5	2.7

Interestingly, the traditionally prepared instant coffee and Turkish coffee contains 42, 80 mg/cup, respectively (Figure 2). It appears that, a person who drinks one cup of Turkish coffee ingested 20 folds of caffeine more than one who obtained it as one cup Arabian coffee. Also, caffeine in Turkish coffee equals 2-fold of one cup of instant coffee. Normalizing these data as per day, Figure 2 clearly represents the amount of caffeine of each type in mg/day.

There are some variations of the caffeine content in Arabian coffee with additives. However, these variations are not significant, but it appears some of the additives are lowering caffeine strength. The decrease of caffeine level could be interpreted as caffeine degradation by heat in the presence of such additives. Nescafe® coffee marketed in two forms (classic and gold); the market price value of gold is double the classic form. The regular customer, who drinks Nescafe® gold because of its desirable taste, might not be aware of how much caffeine he/she ingested on a daily basis. Our data presents the difference between the caffeine levels in both gold and classic varieties. In fact, the results indicated that gold Nescafe® contains less caffeine than classic. Similar to Arabian coffee, Turkish coffee is showing also some variation caused by additive such as ginger and cloves. The combination of both additives appears to cause slight change in caffeine concentration (Table 1). The safe level of caffeine set forth as a number of cups from every type of coffee (Table 3).

Conclusion

Conclusively, the results of this study indicated that caffeine measurement in three types of coffee was accurate. Arabian coffee users, who are drinking average of 6 cups/day, should increase the number of cups and/or the size to be more than just 25 mL. The other choice for increasing caffeine ingestion is to shift to Turkish coffee or Nescafe®, in order to maintain caffeine at a therapeutic level. Based on the results of this study, Arabian coffee was recommended for users to increase the size and the number of cups to reach a higher level of caffeine so they may benefit from its therapeutic effects. In the literature, caffeine consumption up to 400 mg/day

is considered safe. Since, the Saudi society is suffering from obesity and diabetes, increasing coffee drinking every day and maintaining a constant dosage of caffeine could benefit citizens in terms of preventing fat accumulation, reducing the possibility of diabetes, or controlling both illnesses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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