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Methylxanthine content in hot drinks consumed in the State of Kuwait

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Abstract

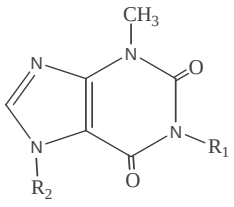
Apart from four major catechins present in *Camellia sinensis* (tea), four major methylxanthines are also present in tea aqueous extract. Caffeine with its structural similarity to uric acid is the predominant methylxanthine in tea and it is known to contribute to hypertension and obesity leading to diabetes. Caffeine intake from tea, soft drinks and candies may be contributing to high level of obesity and diabetes prevalent in Kuwait. There are a variety of hot drinks that are traditional to the culture of Kuwait and their caffeine content has not been established. In this study we have undertaken to establish caffeine content in traditional hot drinks used in Kuwait and have found that Arabic Gahwa, a very popular hot drink in Kuwait, has one of the lowest caffeine content among coffee drinks but its high frequency of consumption may have an additive effect on caffeine intake. Caffeine content of commercial brands and herbal teas used in Kuwait has also been established and found that Rabea black tea had the highest caffeine and theobromine content, while Red label black tea has the lowest caffeine. Reverse phase high performance liquid chromatography (HPLC) was used to establish methylxanthine content in different tea aqueous extracts. A comparison of caffeine content in various hot drinks is presented.

Key words: Tea, coffee, methylxanthine, caffeine, theophylline, theobromine, HPLC.

Introduction

Camellia sinensis commonly known as tea is one of the primordial hot drink that came into human use as early as 4th century BC, introduced from China into Indian subcontinent. Currently, worldwide tea consumption is second only to that of water. Green, black and oolong tea are all derived from leaves of the same shrub *C. sinensis*. Green tea is made from unfermented leaves which are slightly steamed to inactivate enzymes and then dried. The leaves of oolong tea are partially fermented and black tea is fully fermented. All these varieties are endowed with high level of polyphenols with pharmacological activities. The greater the fermentation, the lower the polyphenol content and higher is the caffeine content. Thus bioavailability of caffeine in black tea is 2-3 times more than in green tea [1]. Tea is known to possess medicinal properties such as hepatoprotective [2-5], antimicrobial [6], antiatherosclerotic [7], antioxidant [5], anticarcinogenic [8], and supportive of human immune system [9]. Several studies have also shown that tea extract is beneficial to control viral hepatitis [10, 11], diabetes [12, 13] and inhibits calcium oxalate precipitation forming renal calculi [14-16]. In lipidology, green tea consumption has been associated with decreased cholesterol and triglyceride and an increased level of HDL [17]. Most of these activities are associated with antioxidants like polyphenolic compounds present in tea leaves and coffee [2, 11]. Largely prevalent catechins in tea leaves are (-)-epicatechin, (-)-epigallocatechin; (-)-epicatechingallate and (-)-epigallocatechingallate (EGCG) [1]. An average cup of tea is known to contain 50-150 mg of polyphenols. Black tea, with an equivalent amount of polyphenol antioxidants to green tea, contains EGCG as the most physiologically active component [18].

In addition to polyphenols, tea leaves are also rich in alkaloids. Four most prevalent alkaloids in tea leaves are paraxanthine, caffeine (caf, 1), theophylline (tph, 2) and theobromine (tbr, 3)(Fig. 1). All these compounds are xanthine alkaloids and caffeine, (1,3,7-trimethylxanthine, 1) is derived from theobromine [19]. Caffeine is structurally related to uric acid and is most widely used as psychoactive stimulant drug of abuse [20]. The mechanism of action of caffeine has been postulated through inhibition of phosphodiesterase activity and production of its stimulant effects by blocking adenosine receptors [21-23]. Theophylline and theobromine are both bronchodilators, tbr being a weaker bronchodialator than tph. However, tbr content in tea and coffee is higher than that of tph [24]. Tbr is the main component of coca and can cause prostate cancer [25, 26]. Coffee contains caf and tph but no tbr [27]. So far, caffeine has been identified from over 60 plants but its content depends upon the type of the plant, age of plant and the method of preparation for the hot drink. Tea



(1): $R_1 = R_2 = CH_3$
(2): $R_1 = CH_3$; $R_2 = H$
(3): $R_2 = CH_3$; $R_1 = H$

Figure 1. Chemical formula of methylxanthines caffeine (caf, 1), theophylline (tph, 2) and theobromine (tbr, 3).

methylxanthines have been separated using many different techniques [27-29] but high performance liquid chromatographic (HPLC) separation has been a method of choice.

Caffeine is commonly used for flavor in food and drinks but it has been linked with hypertension [30] and obesity [31, 32]. In the Arab world, there is a wide spread use of fermented tea and on the average twelve 5-oz cups of tea are consumed per person per day making an average intake of 100-220 mg of caffeine/day. This is in addition to caffeine that is consumed in soft drinks and candies, popularly used in daily life. Many types of traditional hot drinks such as Arabic Gahwa, Turkish coffee, coffee Arabica and Robusta etc. are served and for many of these drinks, caffeine content has not been established. A high intake of caffeine in the Arab world may be the cause of increased obesity leading to cardiovascular diseases in the society.

Thus, Kuwait with a population of 2.5 million has one of the highest levels of obesity in the world. Of the population 75% is obese and an estimated cost of obesity to Kuwait government is a minimum of $2.8 billion/annum in direct and indirect costs. Obesity related diabetes in Kuwait has reached an epidemic level. Of Kuwait population 26% is suffering from diabetes. In 1-29 years age group, diabetes is prevalent in 22% [33-35]. This study was undertaken to establish caffeine, theobromine and theophylline content in different brands of tea and coffee consumed in Kuwait, including traditional hot drinks like Gahwa and Turkish coffee.

Materials and Methods

Reagents and standards: Chemicals and solvents were obtained from Fluka (Switzerland). Solvents were redistilled before use. All solvents used in HPLC were of chromatographic grade. Other chemicals were of analytical grade. Caffeine, theophylline and theobromine were purchased from Sigma-Aldrich St. Luis, USA.

Samples: All tea/coffee/herbal tea samples were purchased from the local market and were kept in sealed plastic bags until use. In total fourteen tea and coffee samples were used in this study. Samples were divided into three categories as follows. Four types of coffee consisted of a) green beans; b) Turkish coffee; c) Arabic roasted coffee (Gahwa) and d) Nescafe. Six types of tea samples included a) Alwazah black tea; b) Alwazah green tea; c) Lipton black tea; d) Lipton red label; e) Al-Rabea green tea and f) Al-Rabea black tea. Five types of herbal teas included a) Marmeya; b) Babonage; c) Zatar; d) Mint and e) Habit Halwa.

Ground leaves sample (250 mg) was boiled in 20 ml of HPLC grade water on a hot plate for 5 minutes. The extract was centrifuged at 1000 rpm for 20 minutes. Clear supernatant was taken and the volume was adjusted to 20 ml with HPLC grade water. A part of the supernatant was membrane filtered (0.45 µm) and made to a specific dilution, and 20 µl extract was injected into HPLC without any further pretreatment. Standard solutions of caffeine, theophylline and theobromine were prepared, and four serial dilutions were made for HPLC analyses.

HPLC analysis of methylxanthines: HPLC analyses were carried out on a Shimadzu prominence equipment consisting of LC-20AT dual pump, SPD-M20A photodiode array detector, DGU-20A3 degasser, CBM-20A communications bus module, SIL-10AE autosampler and CTO-20A column oven at 35°C. A reverse phase Waters symmetry C_{18}, 5 µm column (250 mm × 4.6 mm) protected with 20 mm × 4.6 mm C_{18} guard column was used for separation. Sample injection was 20 µl and flow rate was maintained at 1 ml/minute. Mobile phase acetonitrile:0.1% trichloroacetic acid in water (1:9 v/v) was used in isocratic mode. Identification of methylxanthines and catechins was made from retention time of the standards run under exactly similar conditions. Calculation of the methylxanthine content was made from the peak area and using it in the linear equation generated from the standard curves generated from caffeine, theophylline and theobromine.

Results and Discussion

Four coffee samples were first assessed for their methylxanthine content, and it was found that Nescafe coffee had the highest caffeine content (21.96 mg/g) with lowest theophylline content (1.22 mg/g), while Arabic Gahwa had the lowest caffeine (5.12 mg/g) and the highest theophylline (2.34 mg/g) content (Table 1). In the Arab world, Arabic Gahwa and Turkish coffee are popular drinks consumed much more frequently than Nescafe. Although caffeine content of Arabic Gahwa and Turkish coffee is lower than Nescafe, their frequency of consumption makes them a rich source of caffeine intake. In addition, Arabic Gahwa had twice the theophylline content as compared to Nescafe. This might have a protective role against frequent dust storms in the desert that cause frequent asthmatic problems among Kuwaiti population. In green bean coffee and Nescafe, theophylline content is low while Turkish coffee did not show any presence of theophylline (Table 1). Presence of theophylline, which is the least of all methylxanthines in tea extract [24], was not confirmed in any type of the tea or coffee samples used in this study. A typical HPLC separation of caffeine is shown in Fig 2.

Among six tea samples included in this study, Rabea black tea had the highest amount of caffeine (15.27 mg/g) with highest amount of theobromine (1.55 mg/g), while Red label tea had the lowest amount of caffeine (8.19 mg/g) with its theobromine (0.38 mg/g) content. All other tea samples included in this study had lower caffeine content than Rabea black tea (Table 1). Theophylline could not be detected in any of the tea samples used in this study. In five types of herbal tea samples, which are commonly used in the Arab world, no methylxanthines could be detected indicating that these types of tea may be nonaddictive and safer to use. This study concludes that although Kuwaiti population consumes low caffeine containing Arabic Gahwa, the frequency of use may be the cause of higher caffeine intake leading to obesity and diabetes in general public.

Table 1. Methylxanthine content of tea and coffee samples used in Kuwait Value are given in mg/g of dried sample.

No.	Sample	Caffeine	Theophylline	Theobromine
1	Green bean coffee	6.6	1.27	-
2	Turkish coffee	6.23	-	-
3	Arabic Gahwa	5.12	2.34	-
4	Nescafe coffee	21.96	1.22	-
5	Alwazah black tea	14.7	-	1.26
6	Red Label black tea	8.19	-	0.38
7	Lipton black tea	8.79	-	0.73
8	Rabea black tea	15.27	-	1.55
9	Alwazah green tea	13.63	-	0.67
10	Rabea green tea	11.53	-	0.39

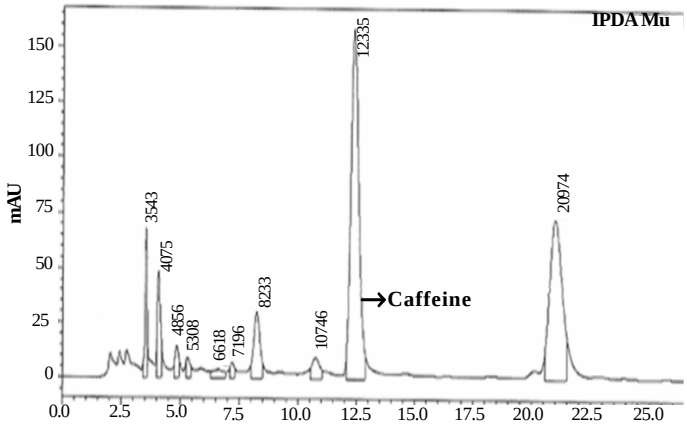


Figure 2. HPLC separation of caffeine in Rabea green tea.

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