International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 3; Issue 6; November 2018; Page No. 14-21

Caffeine content of products in Egyptian markets and its daily intake among Alexandria University employees

Dalia I Tayel[1], Samar M Abo-Rhyem[2], Doaa Tawfik[3]

[1] Assistant Professor of Nutrition, Department of Nutrition, High Institute of Public Health, Alexandria University, Egypt
[2] Lecturer of Food Analysis, Department of Nutrition, High Institute of Public Health, Alexandria University, Egypt
[3] Lecturer of Nutrition, Department of Nutrition, High Institute of Public Health, Alexandria University, Egypt

Abstract
Caffeine is a widely consumed stimulating agent. Health authorities recommends daily intake below 3 mg/kg body weight. The study assessed caffeine content in different Egyptian caffeinated products and estimated the mean daily intake among Alexandria University Employees. Different caffeine containing products were analyzed using HPLC also a cross sectional study was conducted on randomly selected 500 Alexandria University Employees of both sexes to collect data about their frequency consumption using FFQ. Instant coffee black had the highest content of caffeine (0.891±0.094 mg/g). Daily caffeine Intake was more than the recommended level among 36.6% of studied sample while 61.4% consumed at least one caffeinated beverage per day. Mean daily caffeine intake was 254.58±230.78 mg. Caffeine intake among higher consumers was double the safe level which recommended by EFSA (190.86±100.35%). Tea followed by instant coffee black and cola beverages were the key contributors to daily caffeine intake.

Keywords: caffeine, intake, consumption, caffeinated beverages, analysis, HPLC

1. Introduction
Caffeine (1, 3, 7-trimethylxanthine) is a natural alkaloid found in coffee, chocolate, tea, some soft drinks, and energy drinks. Caffeine has a mild stimulating effect on the central nervous system, resulting in feeling of strength reinforcement. (Heckman, *et al*., 2010) [17] In 1978, Food and Drug Administration (FDA) described caffeine as Generally Recognized as Safe (GRAS). (FDA, 2015) [38] In 2015 the European Food Safety Authority (EFSA) suggested that caffeine intake of 3 mg/kg body weight per day are safe for adults. (EFSA, 2015) [10].

Caffeine intake varies across different types of beverages and in different population groups. (Frary, *et al*., 2005) [13] A study held in 2013 showed that 85% of the U.S. population consumes at least one caffeinated beverage per day. More than 78% consumed above the recommended 200 mg of caffeine per day (e.g. more than 2-3 cups of coffee). (Mitchell, *et al*., 2014) [27].

The average and median potential daily intake of caffeine in the studied population of survey on the potential intake of caffeine in Brazil were 2.74 and 1.85 mg/kg of body weight, respectively. (Camargo, *et al*., 1999) [5 6] In Portugal survey concerning caffeine levels in retail beverages, daily caffeine intake was estimated to range from 4.7 to 200 mg/kg of body weight. (Pena, *et al*., 2005) [34] While in another survey concerning caffeine levels in Turkish city, daily caffeine intake was estimated to range from 0 to 400 mg/kg of body weight. (Kucer, 2010) [21] A study held in Marshal University in 2011 to determine mean caffeine consumption in college freshmen found out that it was 12.08 mg/kg/day, which is 5 times more than the recommended amount and only 5.33%, reported none consuming any caffeine containing product during the last 2 weeks. (McIlvain, *et al*., 2011) [26].

It has been shown that moderate caffeine consumers can experience symptoms of withdrawal when daily consumption of caffeine is terminated. Common caffeine withdrawal symptoms include headache, fatigue, irritability, sleepiness/drowsiness, increased work difficulty, lack of concentration/decreased alertness, decreased energy and activeness, decreased feelings of well-being/contentment, decreased sociability/friendliness/talkativeness, flu-like symptoms, and blurred vision. Some studies have demonstrated that caffeine, especially from coffee, tea, and soft drinks, disrupts sleep. (Brice and Smith, 2002) [4].

Number of reports is available for the determination of caffeine in both official and published research papers. But there is a lack of simple inexpressive method for direct estimation of caffeine from different caffeinated beverages. (Venkatesh *et al*., 1994; Barone and Roberts, 1996; Verster and Koenig, 2018) [19, 1, 40] The reported caffeine content in the main dietary sources varies significantly. The differences have been attributed to the variety of coffee bean or tea leaf, method of preparation (e.g. the brewing of coffee and tea), volume of a cup and analytical methods utilized for caffeine determination. (Camargo and Toledo, 1999) [5, 6] Although the level of caffeine in chocolate is less variable, it still depends on the origin of the beans. In the case of carbonated beverages the variability occurs among brands, since most of the caffeine content in these products is added from other natural sources, i.e. less than 5% of the total present caffeine is from cola nuts. (Barone and Roberts, 1996; Camargo and Toledo, 1999) [1, 5, 6] All over the world, the caffeine contents in soft drinks varies

according to the type of the brand. (Nour, *et al*., 2010; Somogyi, 2010) [32, 36].

In the Arab world, consumers are interested in knowing the exact amounts of caffeine existing in beverages. However, studies on caffeine content in beverages and caffeine consumption are limited in all age groups. So it was valuable to conduct this study that aimed to determine mean content of caffeine in various brands of Egyptian caffeinated beverages and products, and to estimate the amount of daily caffeine intake comes from coffee, instant coffee, tea, cola drinks, energy drinks, cocoa, and chocolate products consumed by staff members and employees of Alexandria University.

2. Materials and methods

2.1 Determination of caffeine content

2.1.1 Preparation of samples

Different kinds of beverages brands including coffee, instant coffee, tea, regular and diet cola drinks, energy drinks, cocoa, and chocolate products were purchased from different Egyptian markets, 70 samples were analyzed using the High Performance of Liquid Chromatography (HPLC) method. All measurements were performed in triplicate.

Five grams of tea and coffee samples were weighed and put into 250 ml beakers. One hundred ml of boiling distilled water was added and let to stand for five minutes with stirring; the solution was cooled and filtered into conical flasks. 5 ml of the filtrate were pipetted into clean 50 ml volumetric flasks and 25 ml of dichloromethane was added. The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion. The dichloromethane layer was removed to a clean flask and the extraction procedure was repeated twice more. The extracted layer was evaporated, finally the crystals was dissolved in 1 ml distill water. While soft drinks were degassed by sonication. Each sample was filtered then a filtered sample of 2 ml was 5 times diluted in distilled water. Twenty micro-milliliters (µl) of each diluted sample were injected into the HPLC column. The relative peak areas were determined for three replicates of each dilute sample. Then the concentration of caffeine in each sample was calculated from the peak area in mg/g of the studied samples of beverages. (Horžić, *et al*., 2009) [18].

2.1.2 Conditions of HPLC

Filtered samples were injected for HPLC analysis according to the method. (Nour, *et al*., 2008) [32] Equipment used consisted of a Surveyor Thermo Electron system including vacuum degasser, Surveyor Plus LCPMPP pump, Surveyor Plus ASP auto sampler, "diode array" detector with 5 cm flow cell and Chrom-Quest 4.2 software. The determinations were made under isocratic conditions, at 20 °C, by using a mobile phase made of 88% phosphate solution (KH2PO4 0.02 mol/L), adjusted to pH 4.3 with 5% phosphoric acid, filtered through a polyamide membrane (0.2 µm) and 12% acetonitrile. The volume injected was 5 µl and the flow rate of the mobile phase was 1 ml/min. Caffeine in the degassed and filtered sample is separated by reversed phase chromatography on a 250 × 4.6 mm, 5 µm particle DS HYPERSIL C18 column, detected by absorbance at the wavelength of 217 nm and quantified with a calibration graph. Caffeine was identified by comparing the retention times and spectral data with those of authentic standards. All analyses were repeated three times. The relative peak areas were determined for three replicates of each dilute sample. The reagents used in caffeine analysis were; standard caffeine, dichloromethane, sodium thiosulphate and purified water.

2.2 Study design and sampling

A cross-sectional study was conducted on 500 apparently healthy staff members, clerks and workers of Alexandria University of both sexes. The sample size was calculated using Epi Info version 7 and statistically assuming a 50% prevalence of caffeine consumption among adults; using precision of 5 and α of 5%, the estimated required sample size was 384 which was rounded to 500 subjects. Two faculties (Arts and Commerce) and two institutes (High Institute of Public Health and Medical Researches Institute) were selected at random from a list containing all faculties and institutes of Alexandria University. One hundred and twenty five subjects from staff members, clerks and workers from both sexes were selected at random from each selected faculty and institute. Subjects were informed about the purpose of the study and their verbal consent was obtained.

2.3 Data collection and study questionnaire

A structured questionnaire was used for interviewing each enrolled university staff members, clerks and workers; and to collect data about socio-demographic characteristics (age and sex, education level, marital status, and occupation), medical history (presence of any chronic disease and presence of obesity), and physical activity if sedentary or active was considered as life style. Education was categorized into low education (primary, and preparatory), middle (secondary education), and high education (university education or above).

Daily dietary intake of caffeine data were collected from every member of the selected sample using food frequency questionnaire method (FFQ) at the time of interview. FFQ used to estimate the dietary intake and frequency of consumption of beverages and products containing caffeine commonly consumed by the participants. (Hammod, 2012) [16] These beverages and products were tea (high and low extracted tea, tea with milk, and green tea), coffee (regular coffee, instant coffee black, instant coffee with creamer, instant coffee with creamer and sugar, and decaffeinated instant coffee), cola drinks (regular and diet), cocoa (regular and with milk), chocolates, and energy drinks. The frequency of consumption was considered high if it was followed daily or more than once daily while it was considered low if the frequency of consumption was less than 7 times weekly.

Daily dietary intake of caffeine consumed by the studied sample was estimated as follows: (1) the mean daily intake from each item of consumed beverages was calculated in g/day for each subject of the study; (2) the mean content of caffeine which was estimated by HPLC method in each beverage in mg/g; (3) Item No.1 was multiplied by item No.2 to get the mean daily caffeine intake from each food item in mg/day; (4) the daily caffeine intake was divided by the body weight for each subject to get this intake in mg/kg body weight/day; and (5) the values obtained in item No.4 were compared with high dietary exposure estimates by the

European Food Safety Authority (EFSA) (3 mg/kg body weight per day) (EFSA, 2015) [10]. Percent contribution of caffeine in consumed products by the studied sample was calculated as follows: daily intake of this products (g/day) x its content of caffeine (mg/g) / total daily intake of caffeine from all consumed products (mg/day) x 100. High consumers of caffeine were considered if their daily intake of caffeine more than 3 mg/kg body weight and normal ones were considered among those who had daily intake of caffeine equal and less 3 mg/kg body weight.

Anthropometric measurements and blood pressure were assessed for each participant at the time of the interview. Weight and height were measured according to the criteria of Gibson. (Gibson, 2005) [15] Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of height in meters. Obese subject was defined if BMI ≥ 30 kg/m2. (WHO, 1995) [42] A mercury sphygmomanometer with a suitable cuff size was used to measure blood pressure. After 5 minutes rest, the right arm blood pressure of a seated participant was assessed twice, 5 minutes apart, and the average was reported as the final blood pressure measurement. (Perloff, *et al*., 1993) [35].

3. Statistical analysis

Data management was conducted using the Statistical Package for Social Science (SPSS) version "21" software (Chicago, Illinois, US). (Levesque, 2007)[22] Data was presented tabular, graphically and mathematically using the mean and standard deviation (SD). For all analyses P value < 0.05 was used to detect statistically significant difference. Data were analyzed using Chi squared test for analysis of categorical data and Student's t test was used to evaluate the significance of the difference between the 2 means.

4. Ethical considerations

This study was conducted according to the guidelines laid down for medical research involving human subjects and was approved by Ethics Committee of High Institute of Public Health, Alexandria University, Egypt. Weight measurement was taken following all privacy procedures and all collected data were kept confidential. Verbal Consent was obtained from all subjects after informing them about the study purpose and they had the right to participate or not in the study. There was no conflict of interest.

5. Results & Discussion

5.1 Chemical composition

According to the theory, black tea has the highest caffeine content among teas due to some factors such as strength of brew, growing condition, processing techniques, soil chemistry, position and altitude of the leaf on leaf bush and cultivation practices. (Wanyika, *et al*., 2010) [41] Data presented in Table (1) showed that caffeine content in brew tea ranged from 0.029-0.078 mg/g, the largest content was for high extracted tea while the lowest was for tea with milk, our results agreed with the study (Chin, *et al*., 2008) [8] which stated regular Lipton with low extraction tea was 0.058 mg/g and this result within our range. Data concerning tea products were agreed with the study (Belščak, *et al*., 2009) [3] which stated that caffeine content decreased from black to green tea due to fermentation process, but their level were higher than the obtained results that may be due to different extraction methods and brands.

Table 1: Caffeine content in some Egyptian products and its intake from each one

Product	Number of samples (n=70)	Caffeine Content (mg/g) Mean± SD	Caffeine Intake (mg/day) Mean± SD
High extracted tea	5	0.078±0.091	37.16±3.91
Low extracted tea	5	0.046±0.080	21.5±3.83
Tea with milk	5	0.029±0.005	1.05±0.11
Green tea	5	0.049±0.006	0.20±0.02
Coffee	5	0.335±0.022	19.74±6.96
Instant coffee black	5	0.891±0.094	57.08±7.57
Instant coffee with creamer	5	0.282±0.087	22.48±2.25
Instant coffee with creamer and sugar	5	0.146±0.032	8.09±0.74
Decaffeinated instant coffee	3	0.008±0.001	0.06±0.02
Cola beverages	5	0.135±0.063	31.63±2.44
Energy drinks	2	0.295±0.091	1.14±0.52
Chocolates	10	0.073±0.052	0.24±0.02
Cocoa	5	0.077±0.034	0.26±0.07
Cocoa with milk	5	0.018±0.011	0.33±0.04

SD: standard deviation

High extracted tea contained more caffeine than low extracted tea that due to time and temperature factors. There were shortage in the researches concerning high extraction tea due to it's a newly produced product but it may compared with steep time as (Chin, *et al*., 2008) [8] which stated that longer steep time increase caffeine content of brewed tea and that agreed with the present results. Caffeine content in brewed teas tends to be lower than specialty coffees and energy drinks but similar or even higher than decaffeinated coffee and carbonated soda, (McCusker, *et al*., 2006) [24, 25] that agreed with current findings as tea products was higher than decaffeinated coffee but lower than coffee, energy drinks, and cola by 0.822%, 6.3%, 5.4%, and 1.95% respectively. The obtained results concerning caffeine content in green tea were less than those results by 1.9% that may be due to difference in brands or species used in the analysis. (Juliano, *et al*., 2005) [20] Data concerning energy drinks were agreed with the study (McCusker, *et al*., 2006) [24, 25] which obtained 0.267 mg/g

caffeine and this nearly the same of our findings 0.295 mg/g, but in case of cola beverages our finding was much higher than the study (Igelige, *et al*., 2014) [19] and the others (Barone and Roberts; Igelige, *et al*., 2014) [19] which were 0.0819 and 0.018 mg/g, respectively that may be due to difference in the extraction method and type of brands which were purchased in the study.

The concentration of caffeine in energy drinks were noticeably higher than of soft drinks and that agreed with (Elithabith and Barbara, 1999) [9] that stated caffeine content in energy drinks were ranged 0.168 ±0.12 to 0.39 ±0.08 mg/g, while soft drinks were ranged from 0.097 to 0.143m g/g which were approximately the same of the present results. Nour (2010) [30, 31] stated that caffeine content in soft drinks ranged from 0.085- 0.38 mg/g and in energy drinks was 0.32 mg/g which agreed with the results of the current study; 0.335 and 0.29 mg/g respectively, this wide range due to different cola brands were analyzed.

Caffeine content in Starbucks hot chocolate was 0.0502 mg/g which lower than our finding 0.077 mg/g this may be different brands were used in the analysis. (Juliano, *et al*., 2005) [20] Data concerning chocolates are widely varied because majority of them are imported and small numbers of local brands. The obtained results were much lower than the range suggested by sport dietitian Australia this may be explained due to difference in brands or/and method of analysis. (Belščak, *et al*., 2009) [3] It stated that caffeine content of dark and milk chocolates ranged from 0.67 - 0.25 mg/g, while milk and hot chocolates were 0.02- 0.032 mg/g and these results were higher than the obtained results 0.073- 0.018 mg/g that may due to different brands were analyzed. (Belščak, *et al*., 2009) [3].

Caffeine content in coffee is varied and widely dependent on type of coffee bean and method of preparation. Results of the present analysis showed caffeine based products ranged from 0.008±0.001 to 0.891±0.094 mg/g. For coffee based products; coffee had the highest content of caffeine then instant black coffee 0.981±0.094 and 0.335 mg/g respectively which may be due to roasting reduced the caffeine content and that agreed with the study(36); while the study (Wanyika, *et al*., 2010) [41] stated that Nescafe coffee contained 0.624 mg/g caffeine which lower than the mean value 0.891mg/g in the present study, which may be explained by the difference in method and instrument used in the analysis and also may be different in coffee bean its self. The study (Belščak, *et al*., 2009) [3] also agreed with the current results as stated that coffee and instant black coffee were ranged from 0.54-0.71 mg/g and 0.304-0.424 mg/g, respectively. Decaffeinated instant coffee had the lowest caffeine content among all sample due to decaffeination process. Amount of caffeine in food products varies according to product type and method of preparation. Coffee bean is higher in caffeine than cocoa bean. (Ogah and obebe, 2012) [33].

5.2 Caffeine intake

Daily caffeine intake among the entire studied sample was 254.58±230.78 mg (corresponding to 2.99±1.83 mg/kg of body weight) and that was in agreement with the national representative studies from across the globe where the average daily caffeine intake is below the recommendations of Health Canada and European Food Safety Authority (EFSA) (400 mg per day for adults). (EFSA, 2015; Verster and Koenig, 2018; Wanyika, *et al*., 2010) [10, 40, 41].

5.3 Frequency of caffeine consumption among the studied samples

Table (3) and figure (3) showed that 63.4% of the studied sample was considered normal consumers of caffeine (a mean of 131.76±80.76 mg/day and 1.42±0.79 mg/kg/day) that was less than what recently estimated in adults in the U.S. where 85% of adults regularly consume caffeine, with an average daily intake of about 180 mg/day (2 mg/kg/day). (Fulgoni, *et al*., 2015) As well more than 95% of caffeine consumers in UK (a national representative sample) ingest caffeine in amounts below the limits. (Fitt, *et al*., 2013) Figure (3) revealed that nearly one third of the present study (36.6%) were high consumers (a mean of 467.34±250.83 mg/ day and 5.73±3.01 mg/kg/day) compared to the safe and recommended amounts. (EFSA, 2015; Wanyika, *et al*., 2010) [10, 41] This figure is also in accordance with the data presented by the EFSA Panel (scientific opinion on the safety of caffeine); where in 7 out of 13 countries, the highest daily caffeine intake exceeded 400 mg/day in a proportion ranged from 6% to almost one-third of the adult population (EFSA, 2015) [10].

While Figure (4, 5) showed the presence of large differences between countries in caffeine sources. According to EFSA (2015) [10]; the major sources of caffeine for adults are coffee and tea, followed by carbonated soft drinks while the main source of caffeine in the UK and Irish adult population is tea, compared to coffee in all other investigated Europe countries. (EFSA, 2015; Verster and Koenig, 2018) [10, 40] The later was the same as found in the present study where tea (high and low extracted) was the main source of caffeine by about 40% in both normal and high consumers followed by cola beverages in 20% of the normal consumers while the second highly consumed in higher consumers was instant coffee black by about 24.38% followed by cola beverages by 11.86%. These were with higher frequency of consumption for low extracted tea, instant coffee black, high extracted tea, coffee, cola beverages, instant coffee with creamer, and green tea among high consumers than normal consumers.

Table (4) showed that near two thirds (64.6%) of the studied sample had no chronic diseases that may be owed to that patients with chronic diseases specifically cardiac disease are often warned about the potential harmful effects of caffeine. (Temple, *et al*., 2017) [37] Also, many drugs are known to interact with dietary caffeine even may lead to major interactions. (Carrillo and Benitez, 2000) [7] Despite the prospective cohort studies on the relationship between habitual caffeine intake and long-term changes in blood pressure and on the risk of incident hypertension are conflicting; (Lovallo, *et al*., 2004; Noordzij, *et al*., 2005; Farag, *et al*., 2010) [23, 29, 11] the present study found a significant statistical difference between high and normal consumers as regards blood pressure readings. However, habitual caffeine consumption from all sources up to 400 mg per day consumed throughout the day doesn't lead to safety concerns for healthy adults. (EFSA, 2015; Nawrot. *et al*., 2003) [10, 28].

Table 2: Socio-demographic characteristic, medical history and life style of the studied sample

Item	High consumers (n=183) No. (%)	Normal consumers (n=317) No. (%)	*P* value
Sex			
Male	77 (42.1)	90 (28.4)	0.002*
Female	106 (57.9)	227 (71.6)	
Age			
<25	13 (7.1)	24 (7.6)	0.904
25-	120 (65.6)	212 (66.9)	
45+	50 (27.3)	81 (25.6)	
Mean±SD	40.42±12.21	38.58±12.72	0.115
Education level			
Low	12 (6.6)	17 (5.4)	0.001*
Middle	28 (15.3)	17 (5.4)	
High	143 (78.1)	283 (89.3)	
Marital status			
Single	29 (15.8)	95 (30.0)	0.000*
Married	126 (68.9)	186 (58.7)	
Widowed	16 (8.7)	30 (9.5)	
Divorced	12 (6.6)	6 (1.9)	
Occupation			
Staff member	54 (29.5)	105 (33.1)	0.120
Clerk	123 (67.2)	190 (59.9)	
Worker	6 (3.3)	22 (6.9)	
Presence of chronic diseases			
Diseased	67 (36.4)	110 (34.7)	0.667
Not diseased	116 (63.4)	207 (65.3)	
Presence of Obesity			
Obese	221 (69.7)	78 (42.6)	0.000*
Non-obese	96 (30.3)	105 (57.4)	
Daily physical activity			
Sedentary	109 (59.6)	187 (59.0)	0.900
Active	74 (40.4)	130 (41.0)	

SD: standard deviation, *P* value: based on Chi Squared test,*****$P<0.05$: significant

Table 3: Frequency of caffeine containing products consumption among the studied sample

Item	High consumers (n=183) No. (%)	Normal consumers (n=317) No. (%)	*P* value
High extracted tea			
High	47 (25.7)	42 (13.2)	0.000*
Low	136 (74.3)	275 (86.8)	
Low extracted tea			
High	84 (45.9)	89 (28.1)	0.000*
Low	99 (54.1)	228 (71.9)	
Tea with milk			
High	58 (31.7)	180 (56.8)	0.000*
Low	125 (68.3)	137 (43.2)	
Green tea			
High	43 (23.5)	30 (9.5)	0.000*
Low	140 (76.2)	287 (90.5)	
Coffee			
High	46 (25.1)	42 (13.2)	0.001*
Low	137 (74.1)	275 (86.8)	
Instant coffee black			
High	60 (32.8)	7 (2.2)	0.000*
Low	123 (67.2)	310 (97.8)	
Instant coffee with creamer			
High	44 (24.0)	22 (6.9)	0.000*
Low	139 (76.0)	295 (93.1)	
Instant coffee with creamer and sugar			
High	30 (16.4)	48 (15.1)	0.710
Low	153 (83.6)	269 (84.9)	
Decaffeinated instant coffee			
High	1 (0.5)	7 (2.2)	0.051
Low	182 (99.5)	310 (97.8)	

Cola beverages			0.000*
High	45 (24.6)	15 (4.7)	
Low	138 (75.4)	302 (95.3)	
Energy drinks			0.020*
High	36 (19.7)	38(12.0)	
Low	147 (80.3)	279(88.0)	
Chocolates			0.906
High	1 (0.5)	2 (0.6)	
Low	182 (99.5)	315 (99.4)	
Cocoa			0.049*
High	3 (1.6)	1 (0.3)	
Low	180 (98.4)	316 (99.7)	
Cocoa with milk			0.142
High	6 (3.3)	20 (6.3)	
Low	177 (96.7)	297 (93.7)	

SD: standard deviation, *P* value: based on Chi Squared test, ***P*<0.05: significant

Table 4: Anthropometric measurements, blood pressure and caffeine daily intake (mg) among the studied sample

Item	High consumers (n=183) Mean ± SD	Normal consumers (n=317) Mean ± SD	*P* value
Wt (kg)	94.47±21.57	83.29±19.41	0.000*
Ht (cm)	163.94±10.42	166.27±10.45	0.017*
BMI (kg/m^2)	35.82±10.18	30.72±9.07	0.000*
Systolic BP (mmHg)	124.69±13.65	120.22±12.62	0.000*
Diastolic BP (mmHg)	91.40±11.97	88.03±10.96	0.002*
Caffeine intake (mg/day)	467.34±250.83	131.76±80.76	0.000*
Caffeine intake (mg/kg BW/day)	5.73±3.01	1.42±0.79	0.000*
% Caffeine intake relative to EFSA safe amount for adults estimates (3 mg/kg body weight/day)	190.86±100.35	47.29±26.49	0.000*

Wt: weight, Ht: height, BMI: body mass Index, BP: blood pressure, EFSA: European Food Safety Authority, BW: body weight, SD: standard deviation, *P* value: based on Student's t test, **P*<0.05: significant

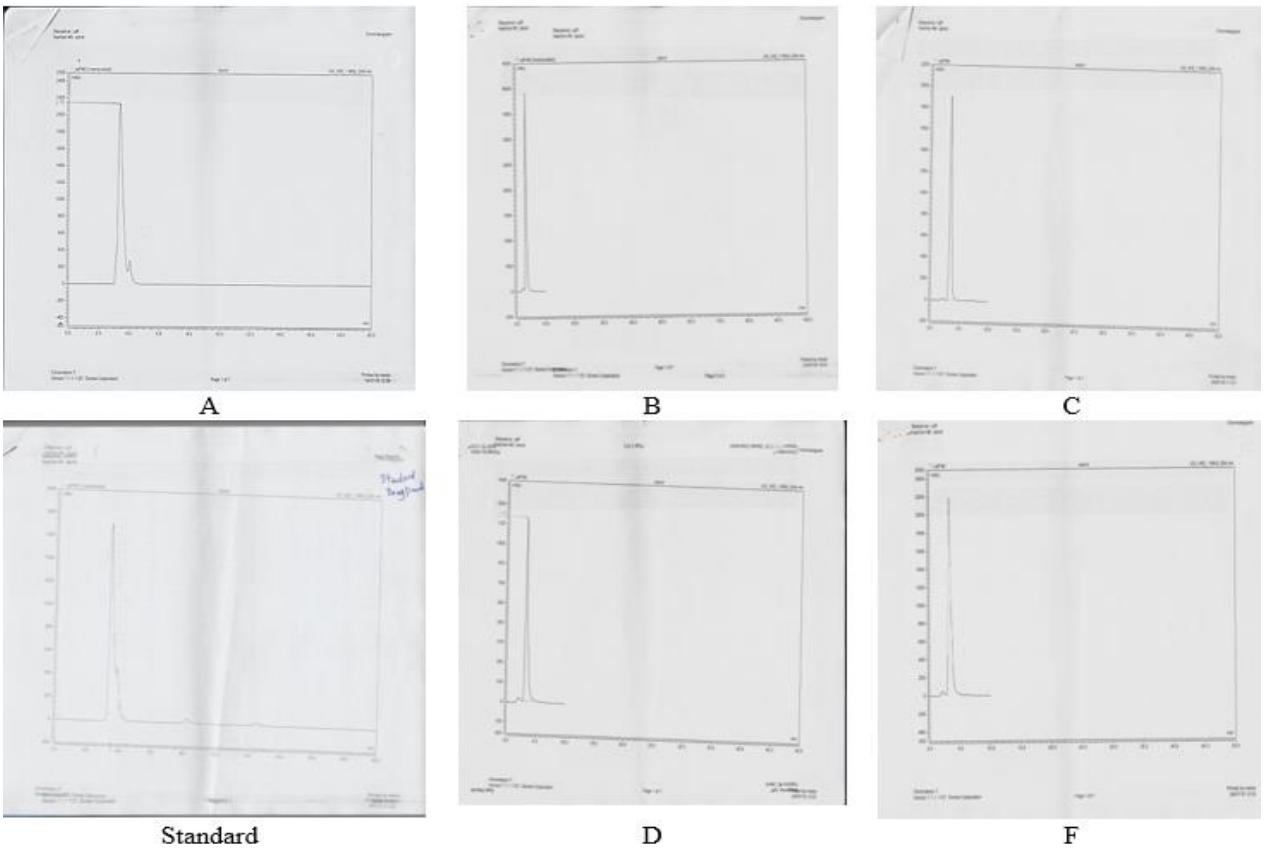

Fig 1: Examples of some HPLC sheets for caffeine content of the studied sample

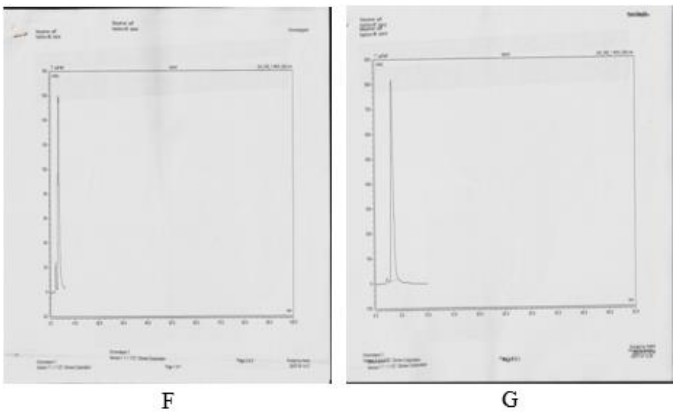

Fig 2: Examples of some HPLC sheets for caffeine content of the studied sample

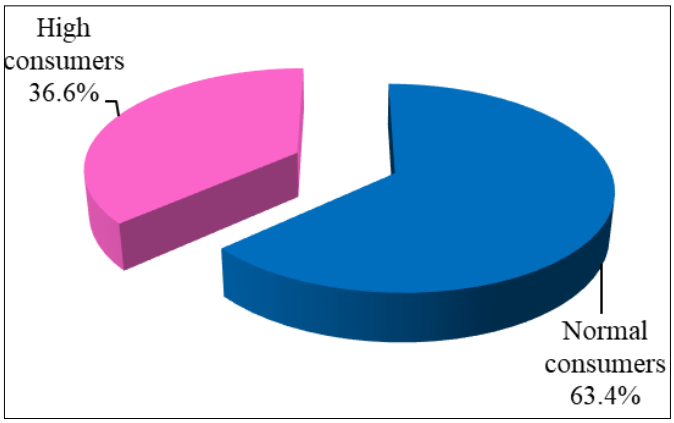

Fig 3: Level of caffeine consumption among the studied sample

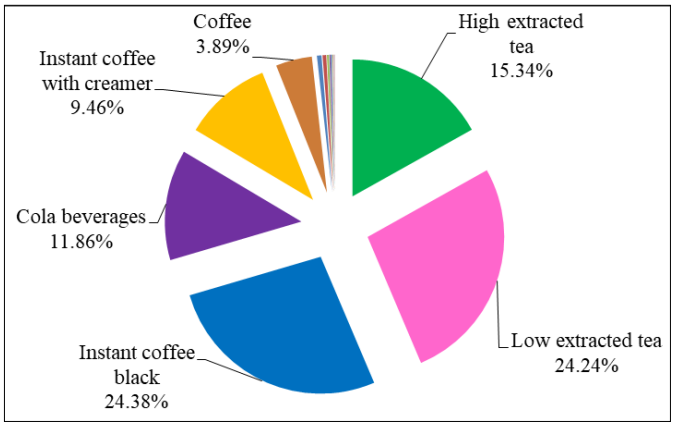

Fig 4: Percent contribution of caffeine containing products in caffeine intake among high consumers

6. Conclusions

Instant coffee black had the highest content of caffeine followed by coffee, energy drinks, and instant coffee with creamer. Daily caffeine intake was more than the recommended level among 36.6% of Alexandria University Employees (higher consumers) and 61.4% of the studied sample consume at least one caffeinated beverage per day. Caffeine intake among higher consumers was double the safe level. Tea (high and low extracted) was the key contributor of daily caffeine intake followed by instant coffee black and cola beverages. The most of Alexandria University Employees consume caffeine within the safe level and further wide studies should be carried out on different age groups of Egyptian.

7. Acknowledgments

Authors would like to express the deepest appreciation to data collectors, who do great efforts in collecting data, also to participants who accepted to be a part of the research.

8. References

1. Barone JJ, Roberts HR. Caffeine consumption (Review). Food and Chemical Toxicology. 1996; 34 (1):119-29.
2. Barone JJ, Roberts L. The health effects of caffeine. 2nd ed. New York. Docket No. 80N-0418, pp59-73.
3. Belščak A, Komes D, Horzic D, Ganic KK, Karlovic D. Comparative study of commercially available cocoa products in terms of their bioactive composition. Food Research International. 2009; 42:707-16.
4. Brice CF, Smith AP. Effects of caffeine on mood and performance: a study of realistic consumption. Psychopharmacology. 2002; 164:188-92.
5. Camargo MC, Toledo MC, Farah HG. Caffeine daily intake from dietary sources in Brazil. Food Additives and Contaminants. 1999; 16:79-87.
6. Camargo MCR, Toledo MCF. HPLC determination of caffeine in tea, chocolate products and carbonated beverages. Journal of the Science of Food and Agriculture. 1999; 79(13):1861-64.
7. Carrillo JA, Benitez J. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. Clin Pharmacokinet. 2000; 39(2):127-53.
8. Chin JM, Merves ML, Goldberger BA, Sampson-Cone A, Cone EJ. Caffeine Content of Brewed Teas. Analytical Toxico J. 2008; 32:702-4.
9. Elithabith EE, Barbara SM. A comprehensive guide book. Caffeine in nonalcoholic carbonated beverages. Docket No.82N-0318. 1999, 52(97).
10. European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the safety of caffeine. EFSA Journal. 2015; 13(5):4102-222.
11. Farag NH, Whitsett TL, Mckey BS, Wilson MF, Vincent AS, Susan AE, *et al*. Caffeine and blood pressure response: sex, age and hormonal status. 2010; 19:1171-6.
12. Fitt E, Pell D, Cole D. Assessing caffeine intake in the United Kingdom diet. Food Chem. 2013; 140(3):421-6.
13. Frary CD, Johnson RK, Wang MQ. Food sources and intakes of caffeine in the diets of persons in the United States. J Am Diet Assoc. 2005; 105:110-3.
14. Fulgoni VL, Keast DR, Lieberman HR. Trends in intake and sources of caffeine in the diets of US adults: 2001-2010. Am J ClinNutr. 2015; 101(5):1081-7.
15. Gibson RS. Principles of Nutritional Assessment. 2nd ed. Oxford: Oxford University Press, 2005.
16. Hammod KA. Intake: Analysis of the diet. In: Mahan LK, Escott-stumps S, Raymond JL, editors. Krause's food and nutrition care process. Missouri: Elsevier Saunders; 2012.p.129-143.

17. Heckman MA, Weil J, De Mejia EG. Caffeine (1, 3, 7-trimethylxanthine) in foods: A comprehensive review on consumption, functionality, safety and regulatory matters. J Food Science. 2010; 75:R77-R87.
18. Horžić D, Komes D, Belščak A, Kovačević Ganić K, Iveković D, Karlović D. The composition of polyphenols and methylxanthines in teas and herbal infusions. Food Chemistry. 2009; 115:441-8.
19. Igelige G1, David EA, Adebiyi A. Determination of caffeine in beverages: A review. American Journal of Engineering Research (AJER). 2014; 3(8):124-37.
20. Juliano LM, Griffiths RR, Lowinson JH, Ruiz P, Millman RB, Langrod JG. Substance Abuse: A Comprehensive Textbook, 4thEdition. Baltimore: Lippincott, Williams and Wilkins. 2005; pp403-421.
21. Kucer N. The relationship between daily caffeine consumption and withdrawal symptoms: a questionnaire-based study. Turk J Med Sci. 2010; 40(1):105-8.
22. Levesque R. SPSS Programming and data management, 4th edition: a guide for SPSS and SAS users. Chicago, IL: SPSS Inc.
23. Lovallo WR, Wilson MF, Vincent AS, Sung BH, Mckey BS, Whitsett TL. Blood pressure response to caffeine shows incomplete tolerance after short-term regular consumption. Hypertension. 2004; 43:760-5.
24. McCusker RR, Fuehrlein B, Goldberger BA, Gold MS, Cone EJ. Caffeine content of decaffeinated coffee. J Anal Toxicol. 2006; 30:611-3.
25. McCusker RR, Goldberger BA, Edward J. Caffeine content of energy drinks, carbonated sodas and other beverages. Analyti Toxico J. 2006; 30:112-4.
26. McIlvain GE, Noland MP, Bickel R. Caffeine consumption patterns and beliefs of college freshmen. Am J Health Educ. 2011; 42(4):235-44.
27. Mitchell DC, Knight CA, Hockenberry J, TeplanskyR, Hartman TJ. Beverage caffeine intakes in the U S. Food and Chemical Toxicology. 2014; 63:136-42.
28. Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M. Effects of caffeine on human health. Food Addit Contam. 2003; 20(1):1-30.
29. Noordzij M, Uiterwaal CS, Arends LR, Kok FJ, Grobbee DE, Geleijnse JM. Blood pressure response to chronic intake of coffee and caffeine: a meta-analysis of randomized controlled trials. J Hypertens. 2005; 23:921-8.
30. Nour V, Trandafir I, Ionică ME. Chromatographic determination of caffeine contents in soft and energy drinks available on the Romanian market. Sci. Study Res J. 2010; 11(3):351-8.
31. Nour V, Trandafir I, Ionică ME. Chromatographic determination of caffeine contents in soft and energy drinks available on the Romanian market. Scientific Study & Research: Chemistry & Chemical Engineering, Biotechnology, Food Industry. 2010; 11(3):351-8.
32. Nour V, Trandafir I, Ionica ME. Quantitative determination of caffeine in carbonated beverages by an HPLC method. Journal of Agroalimentary Processes and Technologies. 2008; 14(1):123-7.
33. Ogah CO, obebe OT. Caffeine Content of Cocoa and Coffee Beverages in Lagos, Nigeria. Innovative Research in Engineering and Sciences J. 2012; 3(1):404-11.
34. Pena A, Lino C, Silveira MIN. Survey of caffeine levels in retail beverages in Portugal. Food Additives and Contaminants. 2005; 22:91-6.
35. Perloff D, Grim C, Flack J, Frohlivh ED, Hill M, McDonald M. Human blood pressure: Determination by sphygmomanometer. Circulation. 1993; 88:2460-70.
36. Somogyi LP. Caffeine Intake in the U.S, 2010. Population.http://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofFoods/CFSAN/CFSANFOIAElectronicReadingRoom/UCM333191.pdf (accessed 15 Julay 17).
37. Temple JL, Bernard C, Lipshultz SE, Czachor JD, Westphal JA, Mestre MA. The safety of ingested caffeine: a comprehensive review. Front Psychiatry. 2017; 8:80.
38. US. Food and Drug Administration [Internet]. Maryland: the Administration; 2015 [cited 2016 Jan 15]. U. S. Food and Drug Administration; [about 5 screens]. Available from:http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/ucm256650.htm
39. Venkatesh S, Swamy MM, Reddy YSR, Suresh B, Sethuraman M. A simple method for determination of caffeine content in tea samples. Ancient Science of Life. 1994; 14(1/2):35-38.
40. Verster JC, Koenig J. Caffeine intake and its sources: A review of national representative studies. Critical Reviews in Food Science and Nutrition. 2018; 58(8):1250-9. DOI: 10.1080/10408398.2016.1247252.
41. Wanyika HN, Gatebe EG, Gitu LM, Ngumba EK, Maritim CW. Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. African Food Sci. J. 2010; 4(6):353-8.
42. World Health Organization. Physical status. The use and interpretation of anthropometry. Report of a WHO Expert Committee, WHO Technical Report Series. 1995; 854:263-311.