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# Study of Caffeine Consumption Rate and Concentration in Different Food and Beverages Consumed by Libyan Children

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**Abstract:** ***Caffeine is the most commonly used psychoactive substance throughout the world. It is found in coffee, black tea, and chocolate, as it is produced naturally in the beans and leaves of the plants used to manufacture these products. This study aimed to make a survey study among children 2-11 years in some Tripoli, Libya schools to determine caffeine concentration in number of beverages and foods consumed by studied children. About 313 copies of the questionnaire were distributed to students to be filled by their parents. Caffeine was extracted from samples using dichloromethane. Purity of isolated caffeine was estimated by using TLC method.Quantitative analysis of caffeine was performed by using a UV-Visible spectrophotometer. Chocolate biscuits, chocolate cake, cocoa milk and chocolate ice cream were the highest consumed products among the studied children (81%, 79%, 67% and 63% respectively). The highest side effects of caffeine consumption was hyperactivity was the highest side effect in children (76%). Some children also suffered from severe side effects such as panic attack (14%). Naseem ice cream sample showed the highest caffeine content (28 mg/serving) among solid samples.Whereas in the beverages, energy drink samples Red pull, XIR and Shark were 268, 262 and 250 mg/serving respectively. In conclusion, there was excessive consumption of caffeine among the studied group of children and some of them showed side effects and even severe side effects. Variety of tested foods and beverages under the study contained uncontrolled concentrations of caffeine. In addition, chocolate milk (Al Rayhan), contained no caffeine.***

**Keywords:** Caffeine, concentration, consumption rate, dichloromethane, side effects

## 1. Introduction

Caffeine is a bitter white crystalline xanthine' alkaloid. It is metabolized in the liver into three primary metabolites, paraxanthine (84%), theobromine (12%) and theophylline (4%) (Figure 1)(Aurnaud 1987). Caffeine is the most commonly used psychoactive substance throughout the world (Nehlig 1999). It is classified as a stimulant drug that is typically used for its ability to arouse the central nervous system. Although generally recognized as safe by the Food and Drug Administration, caffeine use in excess can result in serious health hazards and, in rare cases, death(Broderick and Benjamin 2004, Kerrigan and Lindsey 2005). The safety of caffeine use among children is understudied and poorly understood. Given that some caffeine containing beverages are marketed directly to children (Bramstedt 2007) and that caffeine use is on the rise among children (Frary*et al*. 2005), it is important to understand the potential effects of caffeine usewithin this population.

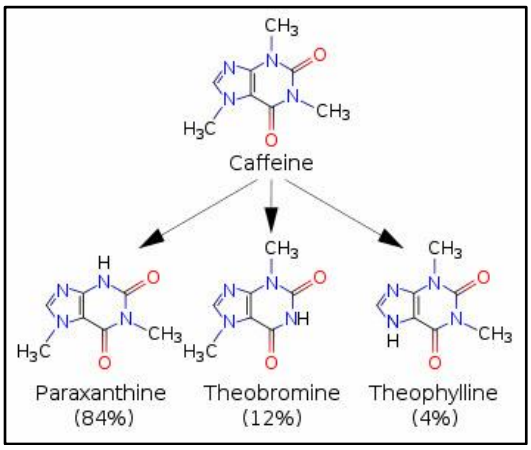


**Figure 1:** Caffeine and its main metabolic products

## 2. Materials and Methods

Questionnaires were prepared including questions like age and gender in addition to other some important information regarding caffeine consumption. About 313 copies of the questionnaire were distributed to students to be filled by their parents, the students included public and private schools in Tripoli to cover children cases from 2-11 years old. Questionnaires were collected and statistically analyzed.Samples were collected randomly depending on kind of food and beverages consumed by children under the study. Samples were listed in details in (table 1).

**Table 1:** Samples containing caffeine that were continuously consumed by students under the study

| Sample name | Brand 1 | Brand 2 | Brand 3 | Brand 4 |
|---|---|---|---|---|
| Cola | Pepsi cola | Coca cola | Stars cola | |
| Instant coffee | Nescafe | | | |
| Chocolate | Kit Kat | Kinder | | |
| Frozen yoghurt | Danette | | | |
| Biscuits | Gardena | | | |
| Chocolate cake | Tiyo | Luppo | | |
| Red tea | Al Baraka | | | |
| Green tea | Lipton | | | |
| Energy drinks | Xir | Shark | Red bull | |
| Chocolate milk | Al rayhan | | | |
| Cocoa | Nesquik(Nestle) | | | |
| Corn flakes | Nesquik(Nestle) | | | |
| Ice cream | Gilice | Oreo (local)* | Nutella (local)* | Chocolate (local)* |

* Local ice cream were prepared in local cafeteria

Caffeine standard was prepared by dissolving 1 mg of caffeine in 100 mL dichloromethane (Merck, Germany)in a volumetric flask (100 mL). The working standard solutions (100, 80, 60, 40, 20, 10, 5, 2.5 and 1 ppm) were used in this study (Amos-Tautua*et al*. 2014). The absorbance of each solution was measured at 271 nm wavelength using UV-Visible spectrophotometer 6506 UV/VIS. (JENWAY). The absorbance values were then plotted against concentrations to generate a standard calibration curve. The results must show a good linear relationship between the absorbance and concentrations of the standard solutions.Aliquots of boiling purified water (200ml) wasadded to each of two 250ml beakers containing2g of instant coffee, 2g of cocoa and a single PG pyramid teabag (3.2g of dried tea leaves) respectively. Thecoffee and tea preparations were stirred for 30seconds using a magnetic stirrer (500rpm) andallowed to cool to room temperature. The softdrink samples were used as supplied by themanufacturer. Finally Cakes, biscuit, chocolatesand frozen yoghurt were weighed and prepared as one serve then dissolved in 200ml hot water and treated like coffee and tea samples. A 50 ml aliquot was taken from each working standard or sample solution. This aliquot was placed into a separating funnel 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 and the solvent layers combined (Vishnoi 2003). Purity of isolated caffeine was estimated by using TLC method and was compared with standards. This method can be applied for the determination of caffeine in different types of commercially available samples. The test samples and standards were dissolved in ethanol-water (8:2 v/v) and applied to precoated TLC. The chromatographic separations were done on the silica gel F254. TLC plates were developed with chloroform-acetone-methanol (1:1:1 v/v/v). The detection was performed under UV lamp at 254 nm and the evaluation of the chromatographic plate was based on processing of chromatographic images and Rf value was calculated (Harborne 2005, Kumar *et al*. 2010). Quantitative analysis of caffeine was performed by using a UV-Visible spectrophotometer 6506 UV/VIS. (JENWAY). The λmax was determined by scanning the standard solution from 200-400 nm and the obtained results gave an absorption spectrum, which was characterized by a single intensive absorption band located in the UV range at λmax = 271 nm. Standard linear calibration curve was run to obtain the linear range of sample analysis, correlation factor was with accepted value (0.9983). The quantitative amount of caffeine in samples (ppm) was then determined using the standard curve.

The final caffeine content of the solid food and beverage under test is then calculated from the extracted sample solution's concentration using equation 1 dividing this value by the volume of the drink gives the caffeine content per ml.

Caffeine content (mg)

$$= conc(ppm)\ x\ \left(\frac{(total\ sample\ vol\ (ml))}{(measured\ sample\ vol\ (ml) x\ 1000}\right)$$

(Equasion1)

## 3. Results and Discussion

Questionnaires were collected and analyzed statistically to produce some logical information regarding studied student samples who consumes food and beverages containing caffeine in regular manner.Among the studied children, the average weight of the vast majority (181 out of 300) was ranging from 16 to 30 kg, where the least weight average was 61 to 75 kg. (Figure 2).The effects of caffeine are affected by size, weight. Because the largest group of children were relatively of small weight, this indicates that they may be affected by caffeine in bad way.The highest age of the studied sample was 10 years (69 children), while the least age was 14 and 15 years (Figure 3). This also might indicate that these children are susceptible to caffeine side effects because of their relatively small age. Chocolate biscuits, chocolate cake, cocoa milk and chocolate ice cream were the highest consumed products among the studied children (81%, 79%, 67% and 63% respectively). The least consumed product was instant coffee (7%) (Figure 4). It was noticed that children prefer consumption of chocolate and its product the most among other products. Chocolate contain moderate amount of caffeine but if the consumption was in high amount with repetition, it might exceed the limited amount and cause side effects. This was proved by time of consumption in the studied sample. 64% of children consumed caffeine containing products 4 times a day and 32% used it 2 times a day (Figure 5) which also considered high consumption rate according to the age and weight of the studied sample. According to the information filled by children parents, they suffered from some side effects that might were due to caffeine consumption. Among these side effects, hyperactivity was the highest side effect in children (76%). This effect is a typical side effect of caffeine. This was followed by loss of appetite (29%) and sleep disturbance (19%). The least side effect was bed wetting and diabetes (Figure 6). Some children also suffered from severe side effects such as panic attack (14%), and rapid heart rate (9%). These effects might because of caffeine over consumption. The least effect was tremor (3%) (Figure 7).

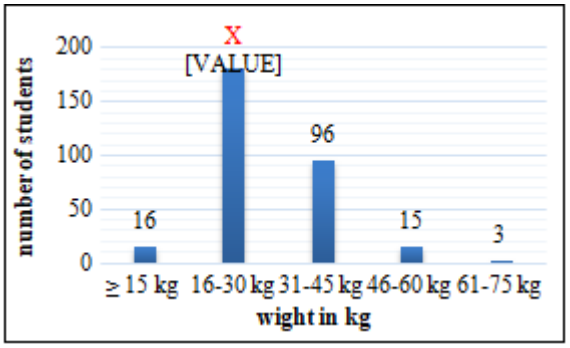


**Figure 2:** Average weight of studied sample of children.

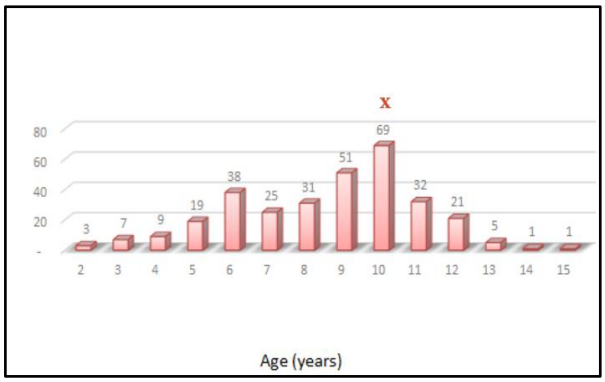


**Figure 3:** Average age of studied sample of children.

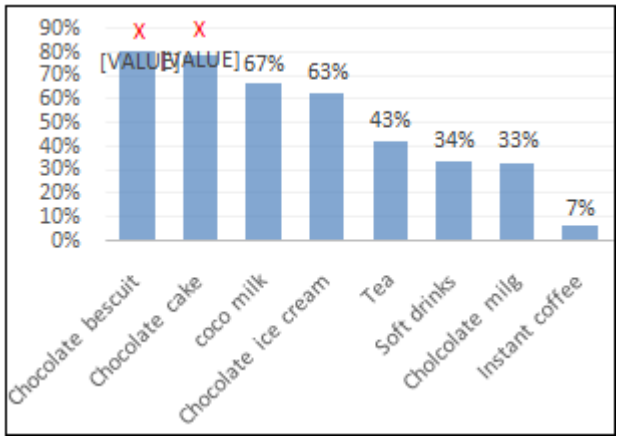


**Figure 4:** Percentage of consumption of caffeine containing product among studied sample of children.

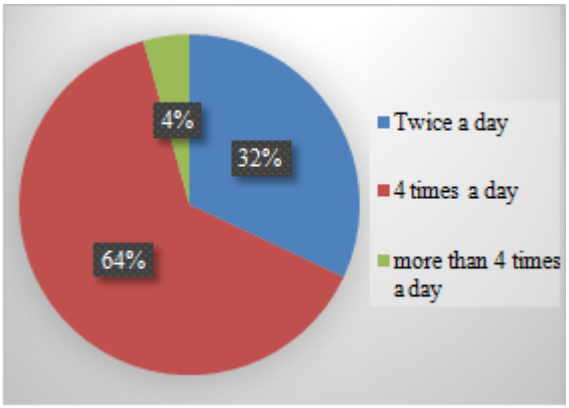


**Figure 5:** Times of consumption of caffeine containing product among studied sample of children.

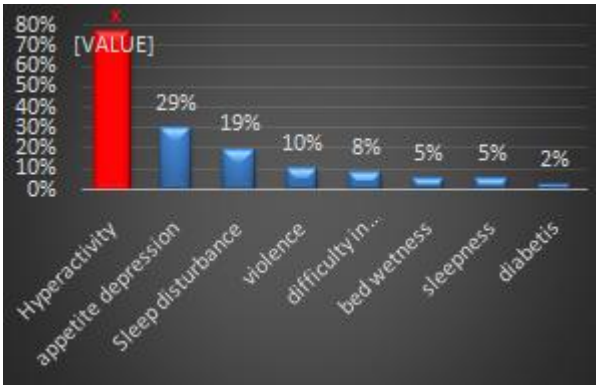


**Figure 6:** Side effects of caffeine consumption among studied sample of children.

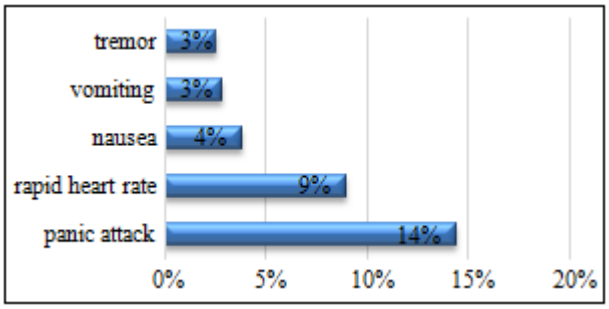


**Figure 7:** Sever Side effects of caffeine consumption among studied sample of children.

The standard linear calibration curve obtained of spectrophotometric UV/Vis method for the determination of caffeine is presented in Figure 8. The results showed a good linear relationship between the absorbance and concentrations of the standard solutions.Correlation factor was with accepted value = (0.9983) and the standard calibration curve was linear over the range (10-60) ppm caffeine.

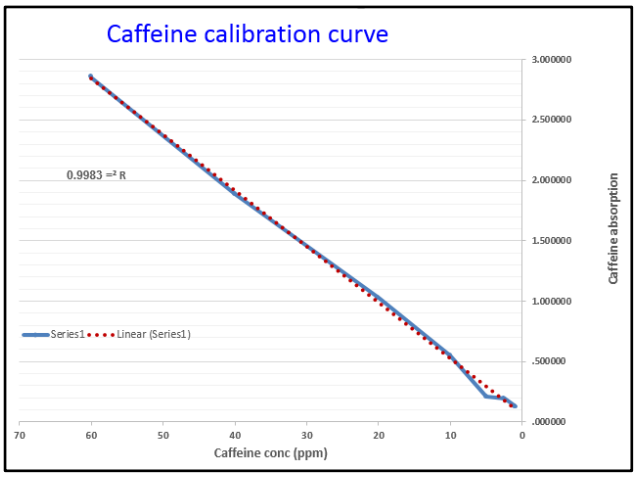


**Figure 8:** Calibration curves of spectrophotometric UV/Vis method for the determination of caffeine.

Qualitative analysis of caffeine was performed by TLC method (Figure 9) for the determination of the caffeine in studies food and beverage samples. UV/Vis spectroscopic study and TLC of the isolated compound were found almost similar to that of the standard caffeine Table 2. (Harborne 2005, Stahl 2007).

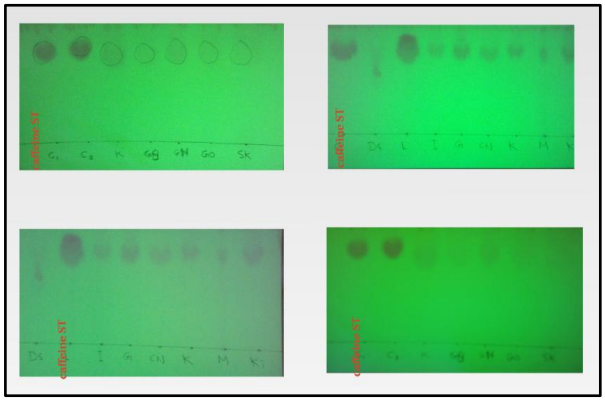

**Figure 9:** TLC analysis of investigated samples and standard solution of caffeine.

**Table 2:** Results of TLC and UV Spectrophotometric study of caffeine

| Sample | UV-Spectra (nm) | TLC Studies (Rf) |
|---|---|---|
| Tested samples | 271 | 0.78 - 0.81 |
| Standard | 271 | 0.80 |

Regarding solid food samples, the minimum caffeine level was observed in kinder chocolate (1 mg/serving), while the Naseem ice cream gilice sample showed the highest caffeine content (28 mg/serving) (Figure 10). The caffeine content in the other solid sample group ranged between 2 and 25 mg/serving; while in the beverages, energy drink samples Red pull, XIR and Shark were 268, 262 and 250 mg/serving respectively. While the least caffeine content was in cocoa (21 mg/serving). The average caffeine quantity in the other drinks was found to be ranging from 87 to 152 mg/serving (Figure 11). This is in agreement with previous work reported by (Mei*et al*. 2012). Moderate caffeine consumption of 300 mg/day, is considered generally safe for adults (Rogers and Dernoncourt 1998). This amount typically corresponds to about 1 and 1/2 cans of energy drink. However the US Food and Drug Administration (FDA 2006)limits the maximum amount of caffeine in children of 4 to 6 years old to 45 mg/day, children age from 7 to 9 is 62.5 mg/day and age of 10-12 is 85 mg/day. Therefore caffeine content allowed in soft drinks may be in the range between 30 and 72 mg/355 mL (12 oz) or 8.45-20.28 mg/100 mL (NSDA 1999). Obviously, the levels of caffeine in the solid food and beverage samples analysed in this study are above the maximum allowable limits set by the above food regulatory bodies.It was noticed that the chocolate milk (Al Rayhan$^{TM}$), contained no caffeine. This was indicated by lack of caffeine on TLC. Although the presence of cocoa as ingredient was mentioned on the package.

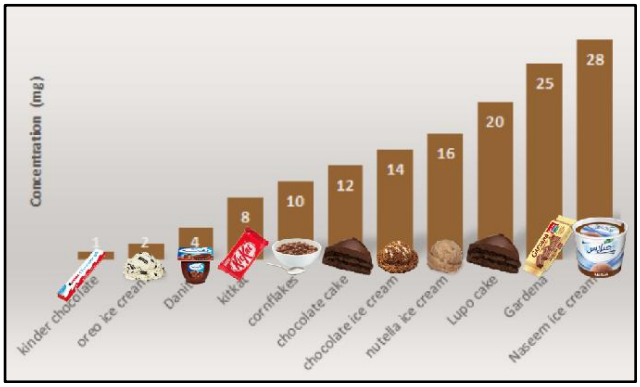


**Figure 10:** Caffeine content (mg) in serving of tested solid samples.

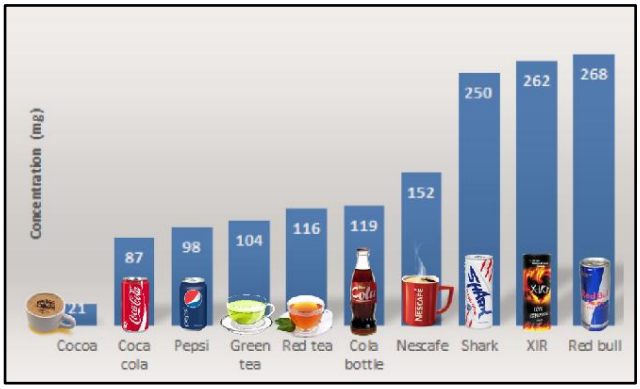


**Figure 11:** Caffeine content (mg) in serving of tested beverage samples.

## 4. Conclusion

It can be concluded from this study that there was excessive consumption of caffeine among the studied group of children and some of them showed side effects and even severe side effects. Variety of tested foods and beverages under the study contained uncontrolled concentrations of caffeine. The levels of caffeine in the solid food and beverage samples analysed in this study are above the maximum allowable limits set by the food regulatory bodies. In addition, chocolate milk (Al Rayhan), contained no caffeine. This emphasise the importance of supporting quality control system in the country to control the local and exported food and beverage items.

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