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Quantitative analysis of caffeine content in some of energy drinks that are commercially available on the Egyptian market

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Abstract

This study was carried out to determine the levels of caffeine in the samples of the selected brands of energy drinks that are available in local market in Egypt. Quantitative analysis of caffeine content in the samples of energy drinks was performed using UV/Vis spectrophotometric method. $CHCl_3$ was used as the extracting solvent, and the absorbance of caffeine extract was measured at λ_{max} = 274 nm. The caffeine concentration in the samples (ppm) was subsequently determined using the standard calibration curve. This study gave preliminary information about caffeine levels in often consumed energy drinks in Egypt. The results indicated that the concentrations of caffeine in energy drink samples were in the range of 226.12 to 388.69 ppm with an average value of 310.37 ppm. Among the energy drink samples analyzed, the highest caffeine concentration (388.69 ppm) was measured in the energy drink sample of Red Bull, while the lowest caffeine concentration (226.12 ppm) was measured in the energy drink sample of Twist. Concentrations of caffeine in energy drinks measured in this study were lower than the one specified on their labels for all the analyzed drinks except Sting, Red Bull and Power horse which showed slightly higher concentrations of caffeine than those labels specified. The levels of caffeine in the energy drink samples analyzed in this study were below the maximum allowable limits set by the food regulatory bodies.

Key Words:

Energy drinks, caffeine content, quantitative analysis, adverse effects, awareness

1. Introduction: Caffeine is one of the most frequently used psychoactive substances in the world. It is present in the leaves and seeds of various plants. It is typically extracted from coffee beans, tea leaves, and cola seeds. Caffeine is commonly found in energy beverages. It is incorporated into these beverages not only for taste but also to enhance their addictive qualities (Khalid et al., 2016, 1–5).

Caffeine is a naturally occurring compound present in humans and acts as a stimulant for the central nervous system (CNS). It effectively delays drowsiness and enhances alertness temporarily. The effects of caffeine can be felt as soon as 15 minutes after consumption, lasting for several hours. Consuming between 100–200 mg of caffeine improves alertness, boosts vigilance, accelerates thought processes, increases concentration, and enhances overall body coordination. However, ingesting more than 250 mg may lead to acute caffeine overdose, which causes CNS overstimulation known as caffeine intoxication (Nowaczewska et al., 2020, 1–16).

Beverages that include caffeine, such as coffee, tea, soft drinks, and energy drinks, are extremely popular. Over the last 10 to 15 years, there has been a notable rise in the consumption of energy drinks. Products containing caffeine are commonly consumed by various age groups for multiple reasons ranging from social interactions to enhancing mental and physical alertness (Samoggia and Rezzaghi, 2021, 1–17).

Energy drinks are classified as non–alcoholic beverages that include caffeine along with herbal extracts such as guarana, ginseng or Ginkgo biloba; they may also incorporate B vitamins, amino acid derivatives like carnitine, and sugar derivatives including ribose and glucuronolactone (Boyle and Castillo, 2006, 116–122).

The consumption of energy drinks has become a regular habit not just among adults, but also increasingly among youngsters, adolescents, and even children today. After intake, individuals can experience a variety of reactions, producing effects that may be positive, neutral, or negative to performance. These responses are influenced by factors such as genetic makeup, training level, regular caffeine usage, gender, source of caffeine, and age (Temple, 2018, 36–45).

Adults typically consume caffeine through beverages like tea and coffee. In contrast, youngsters, adolescents, and Children often obtain caffeine from soft and energy drinks. They believe that these drinks improve performance and boost energy. The primary motivations for energy drinks consumption were taste, to feel energized, or to remain alert, with some users admitting they try them out of curiosity. Usually, a cup of coffee has about 100 mg of caffeine, while a regular cup of tea contains approximately 85 mg. In soft drinks, the caffeine content can vary between 30 and 72 mg per 335 mL. For commercially available energy drinks, one can may have anywhere from 80 to 280 mg of caffeine depending on its size (Amos–Tautua et al., 2014, 155–158).

There are no benefits associated with energy drinks in terms of enhancing brain development, psychomotor skills, or social growth. Conversely, there are numerous adverse side effects linked to regular or excessive consumption of energy drinks, particularly notable in younger individuals (Watson et al., 2017, 533–543). So, it is important to describe the effects of caffeine and energy drinks, just like their effects in physical activity and performance, to know their health benefits and consequences.

The purpose of this study was to determine the amount of caffeine in samples from various energy drink brands that are sold in Egypt's local market, alongside increasing awareness about the adverse effects of caffeine on health. The quantitative analysis of caffeine concentrations in these energy drinks was carried out using a UV/Vis spectrophotometric technique with chloroform utilized as the extraction solvent at a wavelength of 274 nm.

2. The Theoretical Framework

Caffeine is a bitter white crystalline alkaloid from the methylxanthine group. It is a heterocyclic organic compound with a purine base. The systematic name of caffeine is 1,3,7–trimethylxanthine. The structure of caffeine can be seen in Figure 1. Caffeine ($C_8H_{10}N_4O_2$) has a molar mass of 194.19 g/mol and a density of 1.2 g/mL. It exhibits limited solubility in cold water but shows improved solubility when dissolved in hot water, acetone, pyrrole, ethyl acetate, and pyrimidine. Additionally, it dissolves very effectively in solvents such as chloroform, benzene, petroleum ether, and ether (Sharangi, 2009, 529–535).

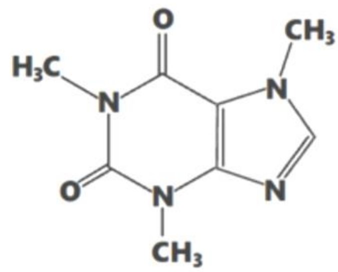

Figure (1): Chemical structure of caffeine.

Numerous products and beverages that contain caffeine are available, such as tea, coffee, energy drinks, chocolate, cocoa, cola nuts, mate, guarana, as well as some medications, dietary supplements, and soft drinks. Energy drinks are soft drinks containing caffeine, taurine, glucuronolactone, carbohydrates, different types of vitamins, niacin, pyridoxin, riboflavin (B2), ginseng substrate, inositol (B8), guarana (caffeine, theobromine, and theophylline), Ginkgo biloba extracts, herbs, and l–carnitine (Willson, 2018, 1140–1152).

Caffeine is the primary active ingredient in energy drinks (Arenas–Jal et al., 2021, 1–17), it is used to improve alertness and reduce fatigue. Sugar provides a quick energy boost, however, the high sugar content (up to 54–62 grams per can) can lead to weight gain, insulin spikes, and dental problems. Taurine is an amino acid thought to enhance cognitive and athletic performance, excessive intake may pose neurological or cardiovascular risks.

B Vitamins are essential for energy metabolism, including niacin B3, B6, and B12. They are often included in quantities exceeding daily requirements. Guarana is a natural source of caffeine, adding to the overall caffeine load in energy drinks. Glucuronolactone is believed to combat fatigue, but more research is needed to confirm its effectiveness. The caffeine levels in these drinks are influenced by the quality and type of processing applied to the raw ingredients (Cappelletti et al., 2015, 71–88).

The beneficial impacts of energy drinks on mental function and physical performance depend on the combination of the various components. These beverages frequently include a variety of plant extracts that work together to enhance energy metabolism and boost mood. When caffeinated alongside other botanical elements, they have been found to improve alertness, concentration, enhance mood, and reduce fatigue (Hoffman et al., 2009, 1–8).

Upon consumption, caffeine is quickly taken up from the gastrointestinal tract into the circulatory system. Peak concentrations of caffeine in the blood are observed approximately one hour after ingestion. Following absorption, caffeine promptly gets into all the body tissues and crosses the blood–brain. In humans, the half–life of caffeine varies between 2 to 5 hours (Jodra et al., 2020, 1–11).

Caffeine intake offers numerous benefits. It is known to reduce physical tiredness and possesses various medicinal properties. The ability of caffeine to enhance cognitive performance is widely accepted. Studies have demonstrated that moderate amounts of caffeine can lead to improvements in attention, reaction times, memory retention, alertness, and verbal reasoning skills. Additionally, caffeine consumption is associated with a reduced formation of kidney stones. Caffeine may promote hair growth, shield against eyelid spasms and cataracts, and it is linked with a likely reduction in the risk of various cancers, including skin, liver, breast, colorectal, colon, endometrial, and prostate, as well as decreased likelihoods of cardiovascular diseases and mortality rates. Moreover, it contributes to a decreased likelihood of developing Type 2 diabetes (Einöther and Giesbrecht, 2013, 251–274).

Numerous studies have supported the association between higher levels of caffeine intake and enhanced performance on cognitive assessments among older adults. Moreover, an inverse relationship exists between caffeine consumption and the chances of developing Alzheimer's disease, while also showing a reduced risk for stroke events. Evidence suggests that caffeine might reduce pain sensations by acting on adenosine receptors. Furthermore, caffeine can be combined with specific pain relievers to effectively treat migraines. Caffeinated medications are frequently utilized by patients suffering from headaches either alone or alongside other treatments. Research shows that combining caffeine with analgesics like acetaminophen, acetylsalicylic acid, or ibuprofen results in significantly better outcomes compared to using pain relievers

without added caffeine for treating tension–type headaches (TTH) or migraines (Wolk et al., 2012, 243–251).

On the other hand, there are notable drawbacks to caffeine intake. The actual caffeine levels in the energy beverages often differ from what is indicated on their labels due to the presence of taurine and glucuronolactone, which increase caffeine's effects; additionally, guarana also contains caffeine. Sleep can be significantly affected by as little as 200 mg of caffeine. Another major issue associated with caffeine intake is dehydration due to its diuretic properties. It also fosters strong addiction, heightens stress levels, and may contribute to premature aging and the development of wrinkles. The excessive consumption of caffeine (300 mg or more) can lead to feelings of anxiety and irritability, and it has the potential to be toxic, resulting in side effects such as vomiting, heart rate issues, shock, or even death. The overdose of caffeine, particularly at doses around 1000 mg per day can result in high blood pressure, restlessness, hyperactivity, headaches, a rapid heartbeat, nausea, increased urination, heart palpitations, hypoglycemia, muscle spasm, gastrointestinal disturbances such as diarrhea, and dizziness. After caffeine consumption of around 2000 mg/day, hospitalization, toxic symptoms and cardiovascular, gastrointestinal, psychological/neurological, and metabolic symptoms might appear (Winkelmayer et al., 2005, 2330–2335).

Children and teenagers might be especially vulnerable to the adverse effects of caffeine, which can include symptoms like anxiety, diarrhea, and dehydration. Even at recommended doses, caffeine intake during the afternoon and evening may negatively affect both sleep quality and duration. The group most at risk among all consumers is children, teens, and young adults because they tend to ingest a higher amount of energy drinks, resulting in a greater prevalence of negative side effects and symptoms. For healthy adult individuals, the safe consumption levels of caffeine are around 400 mg per day. In contrast, it is advised that children and teenagers should limit their caffeine intake to no more than 100 mg daily (Higgins and Babu, 2013, 730.e1–8).

Caffeine is metabolized in the liver via the cytochrome P450 oxidase enzyme system, producing three key metabolites, as shown in Figure 2. Each of these metabolites has specific effects on the body. Two of the cP450 isoenzymes, CYP1A2 and CYP2C9, play an important role in caffeine demethylation. Among these enzymes, CYP1A2 is particularly significant for caffeine metabolism, responsible for about 95% of its breakdown. The primary metabolites of caffeine are paraxanthine (84%), theobromine (12%), and theophylline (4%). Paraxanthine (84%) breaks down fats and boosts levels of free fatty acids in blood plasma. Theobromine (12%) leads to an increase in urine output. Theophylline (4%) has a relaxing effect on the smooth muscles of the bronchi and is utilized for asthma treatment (Reddy et al., 2024, 100138.).

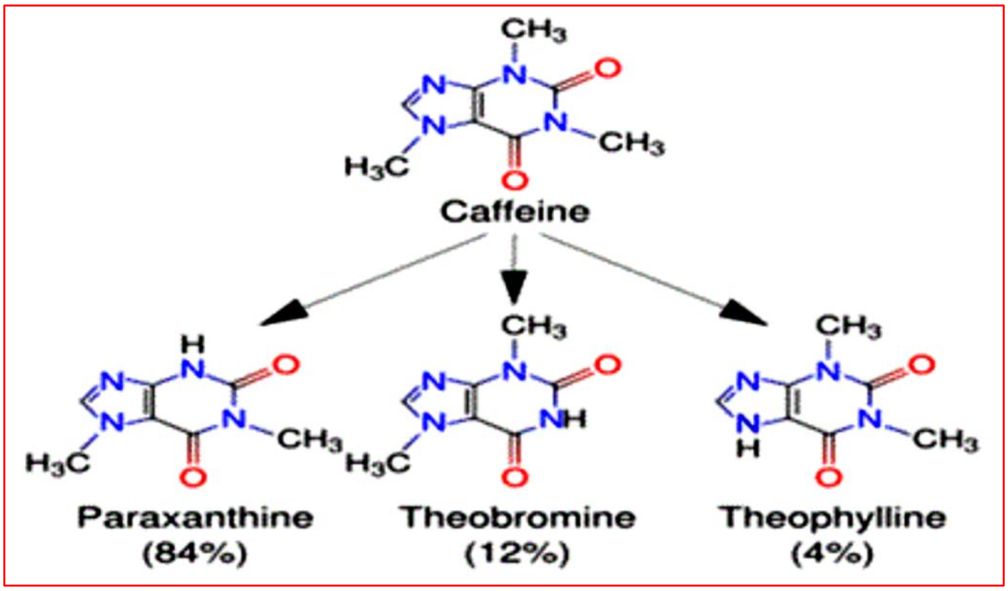

Figure (2): Caffeine and its metabolites.

Caffeine and its metabolites act as weak nonselective competitive inhibitors of phosphodiesterase, which is an enzyme that hydrolyzes phosphodiester linkages in molecules such as cyclic adenosine monophosphate (cAMP), thereby preventing their degradation. At high doses, caffeine inhibits phosphodiesterase, preventing cAMP breakdown and leading to its accumulation (Cappelletti et al., 2015, 71–88).

Elevated cAMP levels extend its action and amplify its effects within the cell. cAMP acts as a key second messenger that participates in various signal transduction pathways associated with numerous biological processes. The heightened levels of cAMP activate hormone–sensitive lipase in adipose tissue, promoting lipolysis. This process releases fatty acids and glycerol, contributing to caffeine's ability to stimulate fat breakdown. (Chen and Parrish, 2009, 647–652).

The most well–known mechanism for caffeine's cellular effects is that caffeine acts in CNS as a competitor of adenosine in its receptors. Caffeine is structurally like adenosine as shown in Figure 3. It can bind non–selectively to the adenosine receptors and competitively inhibit them. Consequently, caffeine acts as an antagonist by inhibiting the stimulation of these receptors by adenosine, where it inhibits the negative impacts that adenosine induces on neurotransmission, excitation, and pain perception. For example, binding adenosine to the receptors in the CNS will promote drowsiness. Nerve cells are unable to differentiate between adenosine and caffeine. Consequently, upon ingestion of caffeine, it prevents adenosine from binding to and stimulating the receptors. This will result in temporary relief of drowsiness, which explains why we feel more alert after consuming caffeine. (McLellan et al., 2016, 294–312).

By blocking adenosine receptors, caffeine appears to competitively antagonize their action and promote enhanced release of dopamine, noradrenaline, and glutamate. Resulting in lower pain perception, more sustainable and forceful muscle contraction, and maintaining or increasing the firing rates of motor units, it consequently allows for greater strength production. Caffeine can affect the mobilization of energy substrates during exercise. It has been proposed that caffeine enhances the mobilization of free fatty acids through adrenaline stimulation, which helps preserve glycogen levels (Mielgo–Ayuso et al., 2019, 1–17).

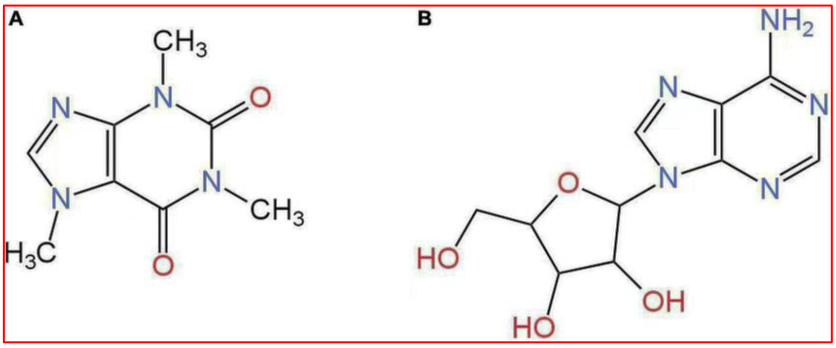

Figure (3): Similarity in the chemical structures of caffeine (A) and adenosine (B).

3. Materials and Methods used

3.1. Materials

The chemicals used in this study include caffeine standard ($C_8H_{10}N_4O_2$), sodium carbonate (Na_2CO_3), and analytical grade chloroform ($CHCl_3$) of 99.9% purity. 20% (w/v) aqueous Na_2CO_3 solution was prepared by using distilled water. Different samples of commonly consumed energy drinks including Monster, Red Bull, Power horse, Fury, String, Twist, and Volt were purchased from the local markets in Cairo, Egypt.

3.2. Methods

3.2.1. Instruments and measurements

The UV/Vis spectrophotometer (Jasco model V–550) was used for the quantitative analysis of caffeine in different samples of energy drinks. The maximum absorption wavelength of caffeine was identified by scanning a diluted caffeine standard solution across a range of 200–400 nm. The resulting absorption spectrum revealed a single intensive absorption band in the UV range at λ_{max} = 274 nm (Rehman and Ashraf, 2017, 823–828).

3.2.2 preparation of caffeine standard solutions

A 200 ppm caffeine stock standard solution was prepared by dissolving 40 mg of caffeine in 200 mL of $CHCl_3$ in a volumetric flask. From this stock solution, standard dilutions were prepared at concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm and 100 ppm. The absorbance of each solution was measured at the wavelength of 274 nm using quartz cuvettes. The absorbance values were subsequently plotted against their respective concentrations to generate a standard calibration curve.

3.2.3. procedure of caffeine extraction from energy drinks

Energy drink samples were heated to remove CO_2. The samples were left to cool to room temperature. Then 5 mL of each sample was taken and placed in the separatory funnel. 10 mL of distilled water, 1 mL of 20% (w/v) aqueous Na_2CO_3 solution and 20 mL of $CHCl_3$ were added to each sample. The caffeine was extracted by shaking the funnel for a few minutes, and then the non–aqueous chloroform layer was separated, as shown in Figure 4. Another 20 ml portion of chloroform was added to the aqueous solution in separatory funnel and extraction procedure was repeated twice more and chloroform layers combined. The volume was made up to 50 mL with the chloroform in a volumetric flask (Vuletić et al, 2021, 325–330). The absorbance of each extract was measured on UV/Vis Spectrophotometer at 274 nm using quartz cuvette. The caffeine concentration in the samples (ppm) was subsequently determined using the standard calibration curve.

Figure (4): procedure of caffeine extraction from energy drinks.

4. Results and discussion

The absorbances of caffeine standard solutions are given in Table 1. The standard calibration curve obtained from the standard solutions of caffeine is presented in Figure 5. It showed a good linear relation between the absorbance and concentrations of standard solutions. The standard calibration curve was linear over the range 10–50 ppm caffeine with an accepted correlation coefficient (R^2) value (0.9999), and a regression line equation: $y = 0.0529x + 0.0138$

Table (1): Absorbance of standard caffeine solutions.

No	Concentration (ppm)	Absorbance
1	10	0.548
2	20	1.082
3	30	1.615
4	40	2.123
5	50	2.653

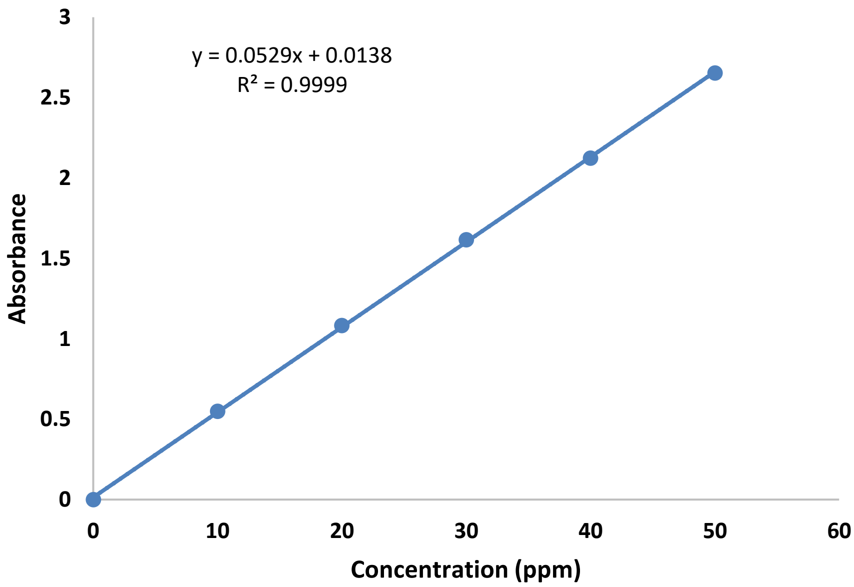

Figure (5): Standard calibration curve of caffeine.

Caffeine content levels in investigated energy drink samples are illustrated in Table 2 and presented and Figure 6. The concentrations of caffeine in energy drink samples were in the range of 226.12 to 388.69 ppm with an average value of 310.37 ppm. The highest caffeine concentration (388.69 ppm) was measured in the energy drink sample of Red Bull, while the lowest caffeine concentration (226.12 ppm) was measured in the energy drink sample of Twist.

Table (2): Caffeine contents of energy beverage samples.

Sample name	Serving size (ml)	Caffeine conc. (mg/L)	Caffeine conc. (mg/100 mL)	Labelled caffeine conc. (mg/100 mL)
Sting	275	320.64	32.06	29
Red Bull	250	388.69	38.86	32
Twist	250	226.12	22.61	28
Monster	500	318.75	31.87	32
fury	400	273.38	27.33	32
Power horse	250	352.77	35.27	32
Volt	200	292.28	29.22	32

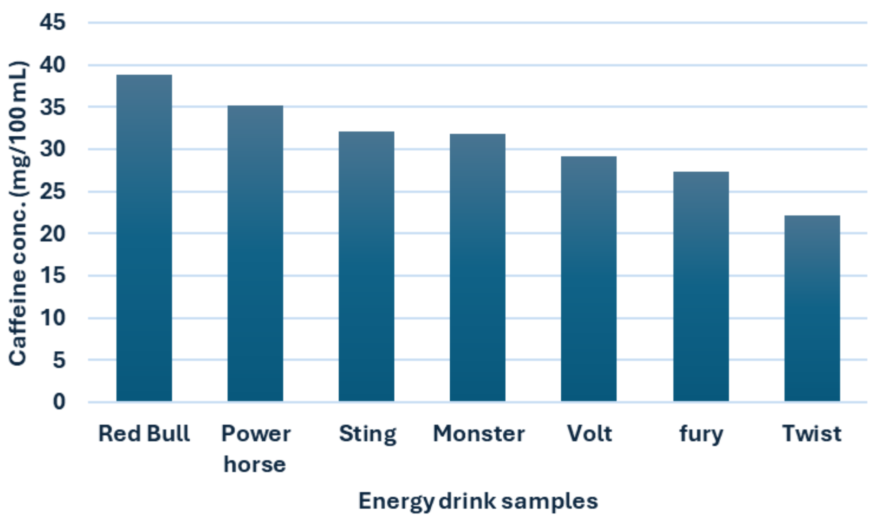

Figure (6): Caffeine concentrations (mg/100 mL) of energy drink samples.

The caffeine content in energy drinks around the world varies depending on the type of brand. Since caffeine is an addictive substance and when consumed can cause different health concerns, it would be appropriate that the amount of caffeine was specified on the labels of all energy caffeine–containing drinks.

Food regulatory bodies, US Food and Drug Administration (FDA) and European Food Safety Authority (EFSA), have limited the maximum allowed amount of caffeine in energy drinks to 320 mg/L. All energy drinks under investigation were labeled with a maximum allowed concentration of caffeine (32 mg/100 mL) except the energy drinks Sting and Twist which were labeled with 29 and 28 mg/100 mL of caffeine, respectively. Concentrations of caffeine in energy drinks measured in this study were lower than the one specified on their labels for all the analyzed drinks except Sting, Red Bull and Power horse which showed slightly higher concentrations of caffeine than those labels specified.

Moderate caffeine consumption of 300 mg/day, is considered generally safe for healthy adults (Smith, 2005, 441–445). According to the European Food Safety Authority (EFSA, 2015, 4102), single doses of caffeine that do not raise safety concerns recommended for healthy adults are up to 200 mg. Caffeine intakes up to 400 mg per day do not give rise to safety concerns for healthy adults. Single doses of 100 mg of caffeine, especially if consumed before bedtime in some adults may affect sleep. A safety level of 3 mg per kilogram of body weight (3 mg/kg bw) of caffeine is also recommended for adolescents. While caffeine intake is generally not recommended for children.

The obtained results showed that the levels of caffeine in the energy drink samples that analyzed in this study are below the permitted limits (200 mg caffeine per dose) that are set by the food regulatory bodies.

The World Health Organization has warned against excessive consumption of energy drinks and their risks to public health, especially among children and adolescents. The risks of consuming energy drinks lie in their high caffeine content. Unlike other caffeinated beverages, energy drinks can be consumed in a single dose, which may exceed the safe limit of caffeine, leading to many adverse side effects on health such as insomnia, anxiety, increased heart rate, high blood pressure, and may increase the probability of caffeine intoxication. Therefore, it is recommended to reduce consumption of energy drinks, and the number of energy drink cans consumed daily does not exceed the recommended limits of caffeine (300 mg/day on average).

5. Conclusion

Determination of caffeine content in energy drinks is a very important analytical process to maintain the health of people who are unaware of adverse effects of caffeine. Determination of caffeine can be done by many analytical methods, UV/Vis spectrophotometric method was employed in this study for the quantitative analysis of caffeine because it is simple, rapid, inexpensive, highly sensitive and accurate in determination of the concentration. The current study gave a preliminary information about the caffeine in energy drinks content often consumed in Egypt. The results of this study revealed that the concentrations of caffeine in the energy drink samples that obtained from the local market in Egypt were lower than the maximum authorized level. Since caffeine can be a cause for potential health concerns, the study indicated that the caffeine content should be stated precisely on the labels of caffeinated drinks, especially due to the great popularity and easy accessibility of caffeine–containing drinks. Moreover, awareness should be raised about excessive energy drinks consumption and their risks to public health, especially among children and adolescents.

Recommendations

According to the results obtained from this study, we can state the following recommendations:

- Since caffeine is an addictive substance and when consumed can cause different health concerns, it seems appropriate that warning labels, indication of the presence and amounts of caffeine should accompany all caffeinated energy drinks.
- It is recommended to reduce consumption of energy drinks, and the number of energy drink cans consumed daily does not exceed the recommended limits of caffeine.
- It is necessary to work on raising awareness among those who drink caffeinated beverages, especially children and adolescents, about the amounts of caffeine they consume and the risks that can result from excessive consumption of energy drinks that contain high caffeine content.

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