Consumption Rate of Energy Drinks among Kabul University Students: Caffeine Contents and Microbiological Examinations of their Available Varieties at Kabul City

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ABSTRACT

Energy drinks (EDs) have gained popularity as a beverage all around the world, but as their usage has grown, questions have been raised concerning their high caffeine concentration and potential for microbial contamination. The objective of this study was to evaluate EDs consumption rate among Kabul University students, the determination of caffeine contents, microbiological quality, and pH of EDs available in Kabul City. 184 Kabul University students were selected using simple random selection to assess the types, amounts of consumption, and awareness about the side effects of EDs. The caffeine levels in EDs were quantified using a UV/Vis spectrophotometer. The EDs were cultured in various culture media for microbiological studies. 23 of the 184 students were regular users of EDs. The mean concentration of caffeine was 277.43 ppm with an SD of 137.49 to 143.10. The EDs had no microbiological contamination, and their pH ranged from 2.64 to 3.64. 12.5% of the students regularly used EDs, Caffeine levels in all of the evaluated EDs were below recommended levels, and none of them contained any bacteria contamination.

Keywords: Caffeine content, Consumption rate, Energy drinks, Microbiological quality.

1. INTRODUCTION

Energy drinks (EDs) are frequently used alongside nutritious foods and regular meals [1] and are now a common beverage [2]. In 2010, the global EDs market was worth $6.7 billion, with consumers under the age of 35 making up more than 50% of the market [3]; this amount increased to a value of 45.8 billion dollars in 2020 and is expected to rise by 8.2% yearly to 108.4 billion dollars by 2031 [4]. There are more than 400 different trade names for EDs as of 2019, and they all have quite different ingredients [5]. However, there have recently been concerns raised about the safety of EDs. According to the United States Food and Drug Administration, in November 2012 detailing 18 deaths were linked to the intake of two different EDs [6]. Between 2000 and 2012, the US Poison Control Center received 5,105 reports of adverse effects of EDs on the body, including 554 cases of general health deterioration, 1 death, 25 cases of serious cardiovascular diseases, and 528 cases of mild cardiovascular disorders [5].

EDs include 30 to 60 mg of caffeine (1, 3, and 7-trimethyl xanthine) per can [7], in addition to calories, along with other substances that are thought to increase energy, such as taurine, herbal extracts, and B vitamins [3], [6], [8]. The amount of guarana, taurine, and ginseng in most EDs is low. Therefore, they are neither therapeutic nor cause side effects. However, the caffeine and sugar content of most EDs causes greater negative side effects [9]. According to...
one prior study, a total EDs with caffeine, glucose, ginseng, and ginkgo biloba improved memory and attention, but each component by itself had no impact on mood or cognition because no cross-over design was utilized to assess the substances of interest, it is difficult to say if the effects were caused by an interaction between two or more factors [2]. It has been suggested that caffeine is the active component of EDs and is therefore responsible for their physiological and behavioral effects [3], which is a common ingredient in EDs because of its ability to stimulate the central nervous system [1, 7, 8, 10–13] and is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs [10]. Caffeine is diuretic that can cause dehydration and water loss [1], [8], [10], [12]. It increases the heart rate and blood pressure and stimulates gastric secretion [10]. Reduced food intake [8] and reduced glucose tolerance have also been connected to caffeine consumption [14]. It encourages lipolysis in both animals and people [8]. Average caffeine doses of between 85 and 250 mg may produce feelings of alertness, reduced weariness, and improvement in concentration [1], [8], while higher doses, such as 250–500 mg, could cause tremors, hyperactivity, nervousness, anxiety, and insomnia. Undesirable effects can occur after ingestion of as little as 50 mg. These side effects could include gastrointestinal upset, anxiety, and insomnia. More significant toxicity occurs after the ingestion of 15–30 mg/kg and is marked by symptoms of muscle spasms, myocardial irritability, myocardial arrhythmias, seizures, and vomiting [1].

As there are numerous varieties of energy drinks, each with a different level of caffeine, there are no established standards for the preservation of EDs [15]. The three main variables that affect the type of microbial development and deterioration are the temperature, oxygen content, and moisture content of the storage environment. This has led to evidence that even imported products like energy beverages may pose a danger for the emergence of microbial risks other than caffeine intoxication [16]. Therefore, in this study, we aimed to find the frequency and pattern of EDs intake among Kabul University students, the caffeine contents, and the microbiological qualities of EDs variety available in Kabul City.

2. Materials and Methods

2.1. Survey

184 students from Kabul University, both male and female, were chosen using a simple random sampling method. A questionnaire was designed to know the gender, age, the most known EDs to the student, the EDs that he/she usually consumes, frequency of consumption, quantity of consumption, stickiness to a brand, the occasions on which he/she consumes EDs, and their knowledge about the side effects EDs. The study duration was from 10/12/2022 to 08/06/2023.

2.2. Sample Collection

13 different EDs brands were purchased according to their popularity and accessibility at supermarkets and neighborhood shops and brought into the lab in a cold box. These beverages were often consumed by students at Kabul University students.

2.3. Determination of Caffeine Content

1. A sodium carbonate solution was prepared by dissolving 20 g of sodium carbonate in 100 mL of deionized water in a 100 mL volumetric flask.

2. The caffeine stock standard was prepared by dissolving 20 mg of caffeine in 250 mL of chloroform in a 250 mL volumetric flask. Working standards were prepared by pipetting 0.1, 0.2, 0.3, 0.4, and 0.5 mL, respectively, aliquots of stock standard solution into separate 100 mL volumetric flasks and diluting it with chloroform, which formed 10, 20, 30, 40, and 50 mg/L standard solutions.

The absorbance of each solution was measured at a wavelength of 274 nm three times for each dilution in quartz cuvettes by using the Shimadzu UV/Visible Spectrophotometer UVmini-1240 (Table I).

2.4. Caffeine Extraction

A total of 10 mL of each EDs sample was taken and placed in a separatory funnel. 1 mL of 20% sodium carbonate (Merck, Germany) solution that was prepared with 5 mL of chloroform (Merck, Germany) was added. Caffeine was extracted by stirring the funnel a few times, and then the lower layer was separated. An aliquot of 0.1 mL of each extract was mixed with 5 mL of chloroform and placed in a quartz cuvette. The absorbance of each EDs sample was measured at 274 nm using a Shimadzu UV/visible spectrophotometer (UVmini-1240, Japan). The amount of caffeine in each of the two cans of the same EDs brand was measured (Table II).

2.5. Microbiological Tests

First of all, to avoid external contamination, the tops of the cans of each EDs sample were cleaned with an alcohol pad. After a few inversions, 1 mL of each EDs sample was added to the 10 mL of BHI broth in the Pyrex tube to evaluate for the presence of bacterial contamination. A spread plate technique was used to cultivate 0.5 mL of sample on each Mueller-Hinton agar for total plate count, mannitol salt agar for Staphylococcus aureus, and MacConkey agar for gram-negative bacteria. All the plates were incubated at 37 °C for 48 hours.

2.6. pH of Energy Drinks

The EDs pH was determined using the pH meter (HM25G-E0201, Germany).
3. Results

Of 184 students, 86 (46.7%) were male (M), and 98 (53.3%) were females (F). 56 (30.4%) were aged 18–20 years, 119 (64.7%) were 21–25, 8 (4.3%) were 26–30, and 1 (0.5%) was above 30. Fig. 1 shows that regular users of EDs were 23 (12.5%) out of 184. 80 (43.5%) used to drink once a week, 20 (10.9%) twice a week, 26 (14.1%) three or more times a week, and 28 (15.2%) did not use EDs.

44 out of 184 (23.9%) students did not know the side effects of EDs, while 70 (38%) students had partial knowledge about the side effects associated with EDs (Fig. 2).

50 out of 184 (27%) of these students used to drink EDs during parties, 27 (14.7%) during their studies and exams, 37 (20.1%) at work, and 54 (29.3%) needed no special occasions to drink (Table III).

4. Discussion

Based on our results, out of 184 people, 23 (12.5%) consumed EDs regularly, 80 (43.5%) drank once per week, 20 (10.9%) twice per week, 26 (14.1%) three times per week, 28 (15.2%) never did. Based on the amount of caffeine in it, it has no harmful effects on our health, although each individual’s sensitivity to the adverse effects of caffeine is different [17]. Caffeine intake is widespread; thus, it’s crucial to gather accurate data on how much is in EDs. The majority of research has concentrated on chromatographic techniques, yet spectrophotometric analysis is preferred due to its speed, high precision, and reproducibility. In addition, UV-visible spectrophotometers are inexpensive and are common in labs [18].

Since caffeine is the primary component of EDs, caffeine overdoses are the main reason for negative health effects. The findings of this study demonstrate that the caffeine content of EDs purchased from the local market ranged from 38.30 ppm to 514.44 ppm, with a mean of 292.76 ppm (Fig. 4).

Seven of the 13 brands of EDs had labels indicating the amount of caffeine in each can, as opposed to the other five brands, which lacked labeling (Table IV).

The microbial quality results indicated that none of the evaluated EDs contained bacteria and did not grow in the above-mentioned culture media. The pH of EDs was shown to be acidic, and ranged from 2.64 to 3.60 with a mean of 3.25.
Consumption Rate of Energy Drinks among Kabul University Students

### TABLE III: Occasions in Which Students Drink Energy Drinks

<table>
<thead>
<tr>
<th>Occasions</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percentage</th>
<th>Cumulative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid Parties</td>
<td>50</td>
<td>27.2</td>
<td>27.2</td>
<td>27.2</td>
</tr>
<tr>
<td>Before deadlines and exams</td>
<td>27</td>
<td>14.7</td>
<td>14.7</td>
<td>41.8</td>
</tr>
<tr>
<td>At work</td>
<td>37</td>
<td>20.1</td>
<td>20.1</td>
<td>62.0</td>
</tr>
<tr>
<td>No need for a special occasion</td>
<td>54</td>
<td>29.3</td>
<td>29.3</td>
<td>91.3</td>
</tr>
<tr>
<td>Other</td>
<td>16</td>
<td>8.7</td>
<td>8.7</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>184</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

- **Fig. 3.** Caffeine standard calibration curve.
- **Fig. 4.** Caffeine content of energy drinks in ppm.

### TABLE IV: Caffeine Content Measured Versus Labeled in a Can

<table>
<thead>
<tr>
<th>No</th>
<th>Caffeine amount measured mg/l</th>
<th>Caffeine amount labeled in can mg/250 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED2</td>
<td>348.04</td>
<td>32 mg</td>
</tr>
<tr>
<td>ED4</td>
<td>289.74</td>
<td>32 mg</td>
</tr>
<tr>
<td>ED7</td>
<td>83.6</td>
<td>80 mg</td>
</tr>
<tr>
<td>ED8</td>
<td>134.42</td>
<td>32 mg</td>
</tr>
<tr>
<td>ED10</td>
<td>38.28</td>
<td>200 mg</td>
</tr>
<tr>
<td>ED11</td>
<td>221.32</td>
<td>31 mg</td>
</tr>
<tr>
<td>ED12</td>
<td>514.47</td>
<td>32 mg</td>
</tr>
<tr>
<td>ED13</td>
<td>492.25</td>
<td>77 mg</td>
</tr>
</tbody>
</table>

- **Note.** ED = Energy Drink, ppm = parts per million.

Because using it has health hazards [12]. Not all EDs were labeled, and the labeled caffeine content for certain EDs did not match the amount that was measured. The caffeine content of EDs (ED2) was measured to be 348.04 ppm (87 mg/250 ml), which was labeled 32 mg/250 ml; ED4 was measured to be 289.74 ppm (72.43 mg/250 ml), labeled 32 mg/250 ml. ED7 was measured to be 83.6 ppm (20.9 mg/250 ml) labeled 80 mg/250 ml. ED8 was measured to be 134.42 ppm (33.60 mg/250 ml), labeled 32 mg/250 ml. ED10 was measured to be 38.28 ppm (9.57 mg/250 ml), labeled 200 mg/250 ml. ED11 was measured to be 221.32 ppm (55.33 mg/250 ml) labeled 80 mg/250 ml. The same inequalities were seen when Said Kudema et al. [17] compared the caffeine content of EDs to the caffeine content labeled.

Toxicity of caffeine has been linked with a dose greater than 3 g. Although the amount of caffeine required to cause negative side effects varies from person to person, the daily suggested limit for the majority of healthy people is under 500 mg. During pregnancy, the European Food Safety Authority (EFSA) advises that daily caffeine intake from all sources should not exceed 200 mg [6]. Numerous studies have examined the benefits and drawbacks of consuming caffeineated beverages, and the majority of them have concluded that their consumption must be moderated and that their marketing and advertising must be controlled [15].

The microbiological quality evaluation findings indicate that none of the evaluated EDs contained bacteria. This might be due to the caffeine’s antimicrobial property and low pH value (2.64 to 3.60) due to the presence of CO2 gas, or other acids employed as preservatives by manufacturers, such as phosphoric acid, malic acid, ascorbic acid, citric acid, and tartaric acid. These acids hinder the growth of bacteria, mold, and other microorganisms that could contaminate EDs [12].

Moreover, a similar study on microbial tests of EDs carried out in Saudi Arabia in 2016 on 20 samples of various brands found no dangerous bacteria, although two of the 20 tasting samples contained bacterial populations of 2.3 and 2.5 log CFU/mL, respectively [16]. In comparison to other samples, these samples contained lower caffeine concentrations and higher pH values (160 mg/L, pH 3.95) (50 mg/L, pH 4.2), which may have contributed to the bacterial population’s survival. Furthermore, Saudi Arabia’s cities are quite hot; the highest recorded temperature in Riyadh is 47.2 °C, and the lowest recorded temperature is −1.1 °C. Kabul, in contrast, has temperate weather; the lowest temperature there is −20 °C, and the maximum is +38 °C. This temperature variation may also contribute to microbial growth in Saudi Arabia’s EDs. The optimal pH of the mouth is between 6.5 and 7.5 (neutral). The pH level at which dental decay begins to develop is thought to be 5.5 [19].

The most crucial variables that affect the type of microbial development and deterioration are the temperature, oxygen content, and moisture content of the storage environment [19]. Effective education and proactive measures are crucial for improving health awareness and reducing EDs consumption among young people to promote public health and the prevention of diseases in the population [18].

Studies have shown that these EDs destroy the natural flora found in the human gastrointestinal tract, including Lactobacillus and Bifidobacterium species. These bacteria contribute to several aspects of human health and are crucial to it [13]. Consumers should be informed about the potential health effects of EDs intake to maintain a healthy lifestyle. Specifying the maximum can size, lowering the
cafeine content of EDs, prohibiting the sale of EDs to those under the age of 18, regulating the advertising of products with high caffeine contents, monitoring cafeine consumption (especially by children and adolescents), and enhancing the advisory role of healthcare professionals are some measures that could reduce the consumption of cafeine by the vulnerable category of the population [15].

This is the first study that evaluated the rate of consumption, the cafeine concentration, and the microbiological quality of EDs available in Kabul City, despite the fact that several surveys and research on microbiological contamination and cafeine levels in EDs globally have been undertaken [16]. It is impossible to estimate the number of EDs consumed nationally from this survey, which only covers Kabul University students. Furthermore, components other than cafeine in EDs should also be quantified.

5. CONCLUSIONS

12.5% of Kabul University students were regular users of EDs. The mean cafeine concentration in EDs was much lower than the permitted maximum level. There were no bacteria contaminations in any of the assessed EDs.

AUTHOR CONTRIBUTIONS

Mohammad Homayoun Hashimi conceived and designed the experiments; performed the experiments; analysed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper. Ziauddin Azimi analysed and interpreted the data and wrote the paper. Hashmatullah Yusufi and Sahar Mohammad contributed reagents, materials, analysis tools or data.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES