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Original Article

Quantitative analysis of caffeine in energy drinks and their consumption patterns in undergraduate pharmacy students: Bangladesh perspective

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Abstract:

Caffeinated energy drinks (EDs) consumers are mostly unaware of the side effects due to long term use of excess amount of caffeine. Excess caffeine content in ED brands is not that much concerned public health issue in Bangladesh perspective. A two-part study was conducted to determine the quantitative profile of caffeine used in the most popular EDs available in Bangladesh, as well as to determine the frequency of usage and popularity of energy drinks among its young educated public health concerned customers' segment. UV-Visible spectroscopy (UV 1800 Spectrophotometer, Shimadz, Kyoto, Japan) for the quantification analysis of caffeine content of the most popular EDs available in Bangladesh. A descriptive, cross-sectional survey to determine ED consumers was also conducted to 300 undergraduate pharmacy students through face-to-face interviews using structured questionnaire. Returned questionnaires were double-checked for accuracy. All data documentation and graphs were prepared using Microsoft Excel 2019. From the quantitative analysis study of caffeine content within six different local ED brands, the determined concentration range was approximately within 22.6mg/250ml to 64.9mg/250ml. From the survey study, it was found that 45% of the total sampled population, were ED consumers, It was seen that 33% of the male and 12% of the female participants were used to energy drinks. Among energy drink users, roughly 22% reported feeling more wakefulness in works than usual, and 37% reported feeling more energized after consuming such drinks. A good amount of participants about 20% reported feeling excited, while 27% said they felt dizzy after taking EDs. Consumers may develop lifelong caffeine addiction if they are unaware of the hazards associated with ED consumption. To assess the risks that EDs bring to the general population and to get a complete understanding of their pharmacological activities and toxicity on the human body, extensive research is necessary.

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1. Introduction

The rate of consumption of energy drinks within the modern young population has been increasing drastically owing to the sophistication and multiplication of workload, improvement of living conditions, personal inclinations, and a multitude of other factors [1]. Next to multivitamins, energy drinks are the most popular dietary supplement consumed by American teens and young adults. Males between the ages of 18 and 34 years consume the most energy drinks, and almost one-third of teens between 12 and 17 years drink them regularly [2] .

Caffeine (1,3,7-trimethylxanthine) a white, crystalline alkaloid belonging to the methylxanthine group can interact with GABA receptors [3] cause better alertness, improve focus and concentration[4] . However, increasing caffeine consumption beyond the recommended level, negative effects such as mood sensitivity, anxiety, and restlessness can be observed[5].

Caffeine, in small doses, serves as a useful supplement in temporarily boosting concentration. However, modern society demands a consistent onslaught of productive task completion, thereby often leading to the formation of dependency upon caffeine as a drive-inducer within individuals. Unregulated usage of such compounds can lead to undesired physiological effects such as unsettling palpitations of the heart, amongst others[6]. However, a 'non-determinable dose' loophole created as a result of ignorance by the mass population is exploited by energy drink companies quite often, especially in third-world countries, leading to overconsumption of energy drinks by the general people. Caffeine is the psychoactive drug of choice worldwide[1].

Caffeinated energy drinks (EDs) contain caffeine and sugar, among other ingredients. They are the primary inducer of effects, with the exception of caffeinated alcoholic drinks, which include enhancing cognitive function and decreasing weariness. Food and Drug Administration, Health Canada, and European Food Safety Authority set the limit for caffeine consumption to less than 400 mg per day [1,7] whereas the UK Food Standards Agency and American Congress of Obstetricians and Gynecologists (ACOG) limit caffeine consumption to <200 mg daily for pregnant women[8]. However, commercial formulations of these brands are inept at considering the frequency of usage within a day and, therefore, manufacture products without regard to dose accumulation.

There are several categories of energy drinks found in the market in Bangladesh. Primarily, the advertisements cater to the spirit of youth, as the target segment for energy-inducing beverages are young adults[9]. Moreover, these advertisements often overshadow the standards set internationally in order to boost short-term energy spikes and productivity, despite the longstanding harmful effects of overdosed caffeine on the human body. The motto these companies hold surrounds 'recharging energy,' yet the chronic use of energy drinks can propel a chain of disabilities or ailments within the human body remains under-broadcasted, if at all.

Many studies focusing on consumption trends of caffeine from energy drinks and their effects in students and sports population have been published in 2023 in many parts of the world, including the United States [2], the United Kingdom [10], Australia [11], Canada [12], Japan [13], Europe [14-17], Saudia Arabia [18] and other countries [19]. From current literature review it was found there are very few studies conducted in Bangladesh highlighting the caffeine content and effects of high caffeine content presence in various energy drinks. As per our knowledge, there shows a lack of availability of public data in Bangladeshi population regarding the correlation between caffeinate content in caffeinated energy drinks and their consumption patterns among the young university going students. Therefore, this paper aims to provide adequate insight into the current energy drink issue through a bi-phased study consisting of quantitative analysis and questionnaire to understand the prevalence of energy drink consumption and their side effects on the enrolled population.

2. Materials and Methods

2.1 The Quantitative Analysis

2.1.1 Materials

Standard caffeine (powder) was collected from a local pharmaceutical company located in Tejgaon, Dhaka, Bangladesh. The six popular energy drinks were collected from local stores where they are available. For labeling, "ED" for energy drinks was used. For quantification analysis, UV-vis spectroscopy (UV 1800 Spectrophotometer, Shimadzu, Kyoto, Japan) was used. All data documentation and graphs were prepared using Microsoft Excel 2019.

2.1.2 Standard Preparation

A 100 ml stock standard of caffeine was prepared by dissolving 100 mg of caffeine in 100 ml of purified water. Working standards were prepared by pipetting 0.1, 0.2, 0.3, 0.4, and 0.5 ml of aliquots of the stock standard solution into separate 50 ml volumetric flasks, thereby acquiring solutions of 2, 4, 6, 8, and 10 µg/ml concentration. The absorbance of the prepared solutions was measured immediately after fabrication at 273 nm using a spectrophotometer (UV 1800 Spectrophotometer, Shimadzu, Kyoto, Japan). The absorbance was collected for each diluted concentration **(Table 1)** and a standard curve was prepared **(Figure 1).** The standard curve was used for the caffeine quantification purpose.

2.1.3 Sample Preparation

The initial step consisted of decarbonating the energy drinks by keeping the bottles open for one hour and decolorization via filtration through filter papers. Afterward, 1 ml of each drink was mixed with 24 ml of distilled water, hence obtaining diluted samples with a dilution factor of 25. Aliquots of the samples were placed into quartz cuvettes and analyzed using UV-Visible Spectrophotometer at 273 nm wavelength. Absorbance for each sample was documented in Microsoft Excel. The caffeine concentration was calculated using the standard curve

for each sample. To acquire the caffeine level in the corresponding 250 ml of purchased bottles, the acquired concentration was multiplied by 250. **Table 3** shows the absorbance of energy drinks and the corresponding caffeine concentration.

2.2 Survey Design and Questionnaire

A descriptive, cross-sectional study was conducted during June 2023- July 2023 based on a pre-validated, structured questionnaire of 10 questions. The respondents comprised 300 undergraduate pharmacy students from top 10 private and public universities located in Dhaka city, Bangladesh who consented to the use of their data for research. The study population was chosen irrespective of the gender, between age 18 and 26 years. The study was conducted according to the ethical guideline in the Declaration of Helsinki. The calculation of the sample size was performed using G*Power version 3.2. Based on a statistical power of 80% and an acceptable alpha error rate of 5%, the minimum sample size required for this study was calculated to be 118 participants. Eventually, a total of 300 patients who satisfied the specified inclusion criteria and provided their informed consent were included in this study.

2.2.1 Questionnaire

The respondents were required to respond to open-ended questions primarily regarding their background information, the timing, and frequency of their consumption, the reasoning behind their consumption, and the effects and the symptoms they incurred afterward. The questionnaire was formulated after reviewing a number of contemporary pieces of literature, which aided in mapping out a pragmatic schema for the survey. The surveyed were also required to answer questions on their reasoning behind energy drink consumption, the frequency of consumption, the feelings and aftereffects of consumption, preferred energy drink brands, and money spent on energy drink purchases.

Out of 10- item questionnaire. Questions 1 and 2 assessed demographic information (age and sex). Questions 3 was about participants' weight, Question 4 was used to identify energy drink users, Question 5 and 6 were about reasoning behind energy drink consumption and the feelings after consuming energy drinks. Question 7 asked about an average consuming quantity of energy drinks. Questions 8 and 9 were about the side effects of energy drinks and the time of consuming energy drinks. Last Question 10 was about the brand names of energy drinks. For content validity the questions were reviewed by experts before conducting the survey. Finally, the questions were reviewed by an expert to ensure content validity and relevance.

2.2.2 Data Collection and Analysis

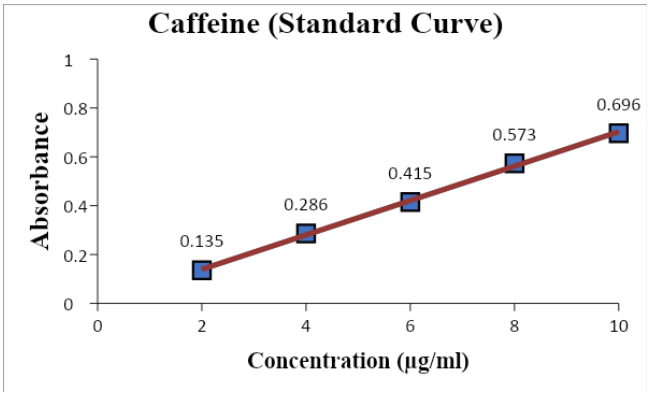
The participants who consented to the use of their response data for the study were provided with a questionnaire worded in English and were briefed on the purpose and significance of the study. Six brand names

were given in the questionnaire and those respondents who didn't mention these brand names were excluded. After completion of the survey, the questionnaire with responses was collected to be processed by statistical analysis. At first, the collected data were recorded in a Microsoft Excel 2019 spreadsheet and then the data was processed to produce graphs and diagrams for interpretation.

3. Results

3.1 This Quantitative analysis

Figure 1 demonstrates the standard curve of purchased pure caffeine. The R^2 value was found to be 0.9987, which elucidates that the plotted curve can be reliably used in calculating unknown concentrations of sample solutions. This plotted curve provides us with the given equation to determine the unknown concentration of sample solutions.



$$y = 0.70x - 0.0017$$

Figure 1: Standard curve of standard caffeine plotted from diluted solutions with concentration measurement equation

Table 1 shows the obtained concentrations and absorbance from serially diluted standard caffeine solutions. **Table 2** shows the concentration of caffeine quantifies from the energy drink sample solutions. The caffeine concentrations were measured from 22.6mg/250ml to 64.9mg/250ml. The mean value of caffeine concentrations found in energy drink samples was 44.76mg/250ml. After comparative quantification, the following sequence based on caffeine concentration can be made.

ED2 > ED6 > ED5 > ED1 > ED3 > ED4.

Test tube no	Concentration (µg/ml)	Absorbance
5	10	0.696
4	8	0.573
3	6	0.415
2	4	0.286
1	2	0.135

Table 1: Obtained concentrations and absorbance from serially diluted standard caffeine solutions.

Table 2: Absorbance of sample energy drinks (25 times diluted) and corresponding caffeine concentrations in 250 ml obtained from UV spectrophotometer

Sample Energy Drinks	**Absorbance**	**Concentration of 25 times diluted (µg/ml)**	**Concentration (mg/250ml)**
ED1	0.483	6.878	42.9
ED2	0.731	10.394	64.9
ED3	0.443	6.311	39.4
ED4	0.253	3.618	22.6
ED5	0.491	6.992	43.7
ED6	0.62	8.821	55.1

Figure 2 showcases that ED2 and ED6 have higher caffeine content than FDA provided limit [20]. Out of the investigated beverages, ED2 had the highest caffeine level of 64.9mg/250ml bottle. The standard deviation of 14.4 indicated that there is significant variation among the studied energy drinks. Furthermore, in energy drink samples, only ED1 and ED2 mentioned the quantity on the label which is ≤14.5 and 75 mg/250ml bottle respectively. Other sample energy drinks do not mention the quantity but rather only the presence of caffeine, as shown in **Table 3**.

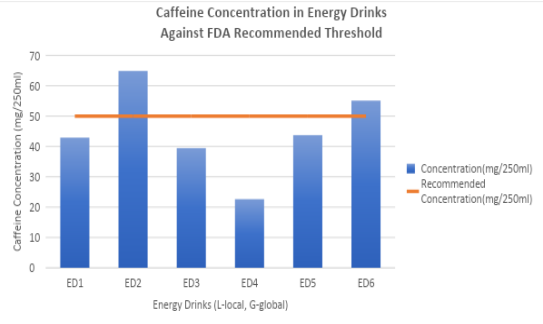


Figure 2: Bar chart showing caffeine concentration in energy drinks against US FDA given recommended level (71mg/12ounce). All Energy drink samples except ED2 and ED6 are under the 50mg/250ml level.

Table 3: Information on supposed caffeine concentration provided on bottle labels

Energy Drinks	Claimed Caffeine Concentration in Bottle (mg/250ml)
ED1	≤14.5
ED2	75
ED3	Quantity not mentioned
ED4	Quantity not mentioned
ED5	Quantity not mentioned
ED6	Quantity not mentioned

3.2 Survey data

In the survey study, the sample population was 300 undergraduate pharmacy students from 10 different private universities located in Bangladesh. The number of consumers among the participants (n = 300) was 135 (45%), and non-users were 165 (55%). **Figure 3[a]** shows the distribution of energy drink consumers within the two genders, male and female, within the sample population. Out of the 300 respondents, 135 people (45%) claimed to consume energy drinks, while the remaining 165 people (55%) claimed not to consume energy drinks.

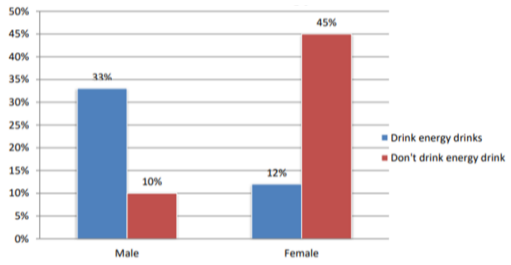


Figure 3a: Candidates who consume energy drinks (%)

Furthermore, according to **Figure 3b**, 33% of the male respondents have claimed to consume energy drinks, while 10% have not consumed these beverages. Among the female respondents, 12% replied affirmatively, while 45% denied consumption. The responses do not take into consideration the act of trying out energy drinks by respondents within their lifetime. As per **Table 4a** 96% of candidates reported having marital status as single. Only 3% of the candidate has reported being married and 1% of them are divorced. None of the candidates has reported being separated. In **Table 4b**, the age demography of the respondents has been showcased. Among the participants' majority was from the age group 21-23 (57%). The second-highest group of participants was 24-26 (25%), followed by 18-20 (18%).

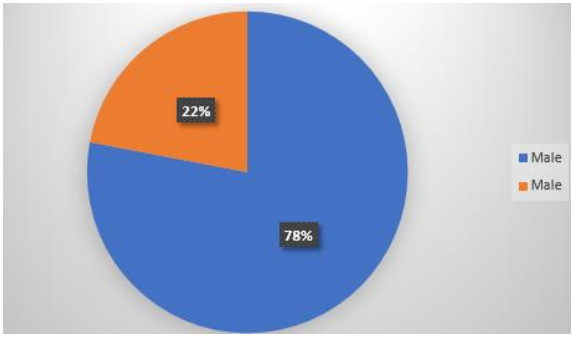


Figure 3b: Percentage of candidates according to gender, who consume energy drinks (%).

Table 4a: Martial status of the participants of the survey.

Marital status	Single	Divorced	Married
% of participants	96%	3%	1%

Table 4b: Age of the candidates who drink energy drinks (%).

Age Group	**% participants**
18-20	18%
21-23	57%
24-26	25%

One of the major reasons for consumption of caffeinated beverages [**Table 5**] is "To fell energetic" that comprises 77% of candidates. 11% of candidates have given a reason for "For its taste" and 6% claimed "To increase concentration while studying" and 7% claimed for refreshment, sexual and other reasons.

Table 5: Reasons for taking energy drinks.

Reasons of Consuming Energy Drinks	% of Consumers
To feel energetic	77%
For its taste	11%
To increase concentration during study	6%
Refreshment, sexual and other reasons	7%

In **Figure 4**, the weight distribution of the energy drink consumers has been laid out, with the dominant group being of the weight category 70-79 (34%). The second highest group was 60-69 (30%), followed by 50-59 (20%), >80 (12%), and <50 (4%).

Figure 5 shows a spectrum of positive stimuli felt by energy drink consumers. The highest option chosen by the respondents was "Energetic" (37%). The second most chosen option was "Dizziness" (27%), followed by Wakefulness (22%), "Excited" (20%), Confident (15%), and "Better" (4%). The figure shows that the utility of the act of consumption is primarily associated with uplifting energy levels.

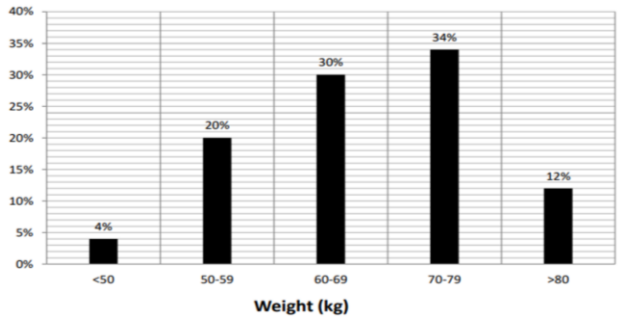


Figure 4: Weight of energy drink Consumers (%)

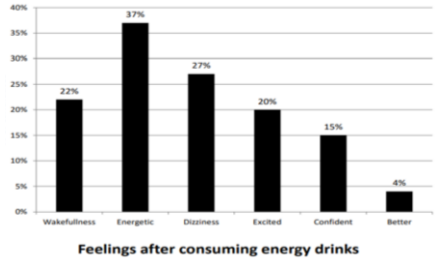


Figure 5: Positive stimuli felt after consuming energy drinks (%).

In **Figure 6**, the side effects gained after an hour of consumption were recorded from the responses of the Energy Drink consumers. The greatest response was "No symptoms" (41%), followed by "Sleep disturbance"

(24%) and "Headache" (16%), which can be correlated to the data from Table 7, which shows a prevalence of energy drink consumption at night. In addition, "Burning sensation" and "Vomiting" consisted of 13% and 5% of respondents, while 1% of respondents claimed to have suffered from anxiety.

In **Table 6**, the frequency of consumption of energy drinks within a week is displayed, while Table 7 shows the preferred timing of consumption of ED users within a single day. On an average scale, 61% of respondents claimed to have consumed energy drinks 1-3 times within a week, whereas 22% consumed energy drinks 4-6 times, while the remaining 17% consumed more than seven times.

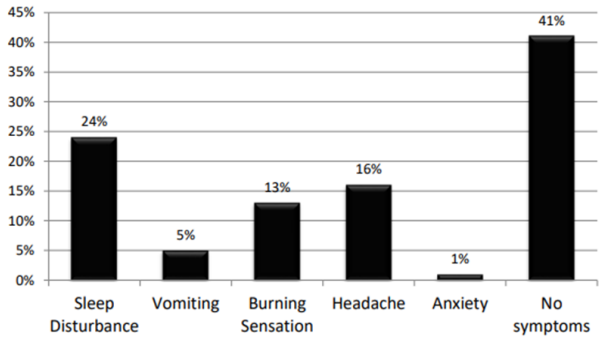


Figure 6: Negative stimuli felt after consuming energy drinks (%).

Table 6: The frequency of energy drink consumption per week (%).

Weekly ED consumes (Bottles/Can)	% of consumers
1-3	61%
4-6	22%
7+	17%

Likewise, in **Table 7**, the dominant group of consumers enjoy energy drinks late at night (47%), followed by "Anytime" (32%), "Evening" (14%), and "Morning" (7%).

Figure 7 shows the popularity of energy drinks among ED consumers, within local brands 1 - 6. It can be seen that Brand 1 [ED1] garnered the greatest response, with 58% of consumers claiming this to be their go-to brand. It was followed by Brand 4 [ED4] (24%), Brand 3 [ED3] (17%), Brand 6 [ED6] (16%), Brand 5 [ED5] (13%), and Brand 2 [ED2] (5%).

Table 7: Consumption period of energy drinks within a day (%).

Time of Consuming Energy Drinks	% of Consumers
Morning	7%
Evening	14%
Late Night	47%
Anytime	32%

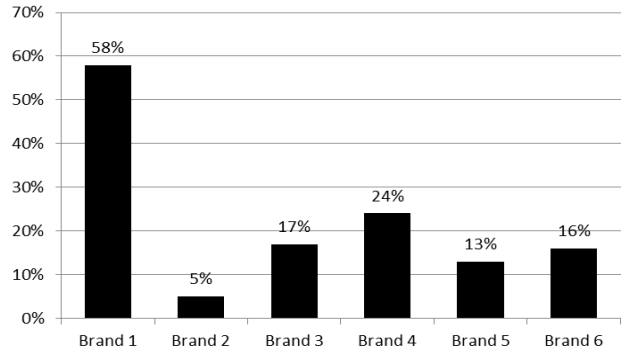


Figure 7: Most popular energy drink brands.

4. Discussion

The aim of this study is to elucidate the harmful effects of caffeinated energy drinks by analyzing the caffeine content of energy drinks as well as the quantifiable survey data procured from university going pharmacy students. Moreover, the study also aims to establish any significant correlation between the acquired data and educated guesswork.

Energy drinks have been seen to be more widely used among male consumers than females. These drinks are also more commonly consumed within the age group 21-23, which shows **Table 4b** that late adolescents and young adults are more prone to build energy drink consumption habits. This can be further upheld by previous research has estimated between 34% and 71% of college students use energy drinks [7] even though most of the youth are blissfully unaware of the constituents making up the beverage as well as their potential side effects [21,22]. Most of the ED containers do not mention the quantity but the presence of caffeine. The bottles must maintain labeling regarding the caffeine content to keep the consumers aware of the level of caffeine consumption they are partaking in.

Within the consumers, the ratio between male and female respondents is 39:11. According to **Figure 3b**; therefore, males are more than thrice as avid consumers as females, which can be attributed to an increased tendency to retain higher energy levels and increased exhaustion rates owing to greater average lean muscle and a higher rate of metabolism. Furthermore, the tropical climate can contribute further to the quicker decline of energy levels [23] and, therefore, create increased demand for these beverages.

The demography of the sample population showed a probable correlation between the consumption of energy drinks and the weight of the respondents. A previous study conducted over a one-year period provided apt reasoning as to how sugar-added beverages can contribute to weight increase. Furthermore, other studies also portray the visibility of the effects of sweet energy drinks on obesity[24]. For further insight into the data on weight distribution displayed in **Figure 4**, a correlation might be presented between consumption habits and body mass. In an anthropometric study by Khadem et al., the mean body weight was estimated to be 66.5 +- 9.59 kg for the Bangladeshi male population, with a mean stature of 167.7 +- 5.25 cm (~5 ft 6 inches) [25]. Therefore, the mean weight of the sample population exceeds weight recommendations as set by BMI metrics (>18.5-24.9), considering the average female height tends to be lower than males. Excluding socioeconomic factors, consumption of these beverages with high sugar content can be considered as both a causal factor for weight gain [24] , as well as a consequence of the weight gain itself as higher weight demand instigates greater energy consumption. Furthermore, sugar addiction can also be attributed to this phenomenon [26], a deduction that can be made from the 'overweight' frequency of consumers. An inverse correlation between social stratification and energy drink consumption frequency can also be detected, as reinforced by Benkert and Abel [26].

The unceasing usage of energy drinks, from the above results, shows a significant correlation with the fact that the sample population primarily comprises students who study late into the night and have less use of it in the morning, with little concern over the disruption of their circadian rhythm. In **Figure 6**, it is seen that sleep disturbance is the second most experienced negative stimulus, thereby reinforcing the claim. From this study, for adults, intake of more than 8 energy drinks per day may cause caffeine-associated adverse effects. Headache, vomiting, rapid heartbeat, and stupor are the associated adverse effects of excessive intake of caffeinated energy drinks as seen in **Figure 6**. However, it is seen that "No Symptoms" are felt by the majority of respondents (41%), which can be attributed to the majority of the respondents having a lower frequency of caffeine usage throughout the week, as well as brand preference tilting towards brands with comparatively less caffeine content as discussed below.

According to FDA, 400mg caffeine intake per day is considered to be the threshold point for potentially dangerous effects for adults [27]. From our study **Figure 2 & Table 6,** for adults, intake of more than 16 soft drinks and 8 energy drinks per day may cause caffeine associated adverse effects. Diarrhea, vomit, rapid heartbeat, stupor are the associated adverse effects of excessive intake of caffeinated soft and energy drinks [28]. One study

conducted in an university of Bangladesh, out of 323 students 43.7% of the participants take ED occasionally and 3.7% take regularly [29]. Although the numbers are not alarming, however, it is recommended to retrain the intake of caffeine by not more than 6 mg/kg/day, 100 mg/day, and 400 mg/day for children, adolescents and adults respectively [30]. A study found reduced cerebral blood flow by 27% with the consumption of caffeine in 405mg/day and 950 mg/day [31].

The preferences of brands **Figure 7** within the community can be attributed to the hype built by marketers of the brand, the prevalence of brand usage amongst peers, pricing, and availability. However, considering similar standings on all the other factors as all these brands are local and of similar stature, correlating brand preference with caffeine content seems to provide additional comprehension regarding consumer behavior. Brand 1[ED1] seems to hold the most significant ardor compared to other brands, constituting caffeine concentration approximately the median of the caffeine concentration (43.3 mg/ 250 ml) of all the discussed brands. This phenomenon could be accredited to the Brand's attribute of providing a caffeine concentration that provides a balance between efficacy to usage frequency. It was followed by Brand 4 [ED 4], containing the lowest concentration of caffeine (22.6 mg/ 250ml), and Brand 3 [ED3] containing the second lowest caffeine concentration of 39.4 mg/250 ml, both indicating an inclination towards lower caffeine dose which probably allows a higher frequency in consumption for the drinkers. Brand 6 [ED6], despite containing the second highest level of caffeine concentration (64.9 mg/ 250 ml), takes the immediate position, indicating that a group of high-dose caffeine dependents are present in the sample population. However, despite Brand 5 [ED 5] containing a similar level of caffeine concentration to Brand 1 [ED1], its popularity lags behind, therefore the inference of median caffeine concentration value being significant enough to influence brand preference has to be examined further, taking into consideration the marketing efforts, organoleptic properties, and other factors. Brand 2 [ED2], with the highest caffeine concentration of 64.9 mg/ 250 ml, stands at the lowest in terms of brand preference. However, a sample population consisting of varied demography might provide a different result overall.

5. Conclusions

Concerns regarding excessive caffeinated energy drinks usage and its potential side effects, particularly among Bangladeshi youth should be immediately addressed as it was found that the majority of Bangladeshi beverage firms do not adhere to the rules governing energy drinks, including correct content labeling and health warnings. According to our study, 45% of the 300 individuals regularly take caffeinated energy beverages, which contained higher-than-recommended caffeine concentration. 39% of these customers exceeded the US FDA's advised daily caffeine intake recommendation by consuming more than four daily drinks. High levels of caffeine use have been linked to negative side effects, including headaches, anxiety, nausea, and sleep disturbances and other difficulties found in this study. This study has been intended to increase awareness of the government's less attention of this rising problem and to encourage government authorities to take action. Failure to raise public

awareness regarding the usage of energy drinks today could result in consumers experiencing caffeine's long-term negative effects and developing a caffeine dependent.

Author Contributions: SSJ and AA drafted the primary manuscript. YH conducted the survey; NA did the UV spectroscopic study; WRK drafted the final manuscript, compiled and analyzed the data. FK, JAS, MHS and AAC edited the final draft. MBU conceptualized, supervised the entire study drafted and edited the final manuscript. All authors contributed to the manuscript, and read and approved the final manuscript.

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