BIOLOGIA (LAHORE)
December, 2019, 65 (II), 243-248

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Caffeine concentrations in locally availableenergy drinks of Lahore, Pakistan

MUHAMMAD ASLAM[1*], MISBAH IRSHAD[1], AMINA ASGHAR[1], ASAD GULZAR[1], ZAHRA NOREEN[2], ABRAR HUSSAIN[2], SHAHID MASOOD[3], MUHAMMAD KAMRAN SHAHID[1], RASHAD MEHMOOD[4], KAYNAT SALEEM[5] & SAJID IQBAL[1]

[1]Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan
[2]Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan
[3]Food and Biotechnology Research Centre,Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Pakistan
[4]Department of Chemistry, University of Education, Vehari Campus, Vehari, Pakistan
[5]Department of Basic Sciences and Humanities, KhwajaFareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan
Institute of Industrial Biotechnology, Government College University, Lahore-54000, Pakistan

ARTICLE INFORMAION

Received: 19-12-2018
Received in revised form: 23-07-2019
Accepted: 28-10-2019

***Corresponding Author:**

Muhammad Aslam:
maslamchemist@hotmail.com

Original Research Article

ABSTRACT

Caffeine is a stimulant drug and beneficial to most human being when taken in at low-to-moderate amount, whereas at high amount it may have negative impact on health. Recently the caffeine is being added by companies in energy drinks and other beverages. The aim of this work was to determine the caffeine concentration, pH and acidity in a few energy drinks brands namely sting, booster six B, gourmet spark and powerful locally available in Lahore, Pakistan, and to compare the results with the labeled caffeine concentration and FDA recommended concentration, which is maximum 400 mg/day. The maximum amount of caffeine determined in Gourmet spark was 9.89 mg /100 ml, whereas powerful brand had minimum caffeine amount (9.34 mg/100 ml).pH were measured by pH meter at 20 ^{o}C and were found 3.47, 3.37, 3.20 and 3.57 for sting, booster six B, gourmet spark and powerful, respectively. The %age of acidity were found to be 1.267, 1.670, 1.868 and 1.785 for sting, booster six B, gourmet spark and powerful, respectively. The results showed that caffeine concentration would remain below moderate level if anybody took two serving size packs of anyone selected sample and consequently would have no negative impact on health.

Keywords: Caffeine, Energy drinks, Toxicity,Sting, Booster six B, Gourmet spark, Powerful

INTRODUCTION

Caffeine is a bitter, white crystalline methylxanthine alkaloid and acts as a stimulant drug and is a mild diuretic. Round about sixty plant species are known to have caffeine (Palatini *et al.,* 2009), and the major commonly known sources are coffee, cocoa beans, cola nuts and tea leaves (Runge & Friedlieb, 2014). Some other sources include yaupon holly leaves, South American holly yerba mate leaves and seeds of Amazonian maple guarana berries (Nathanson, 1984). In 1819, a German chemist isolated the caffeine in laboratory (Jarvis, 2002; Anna & Kurek, 2013). Many anthropologists believe that people have started using caffeine from Stone Age. Now a days, caffeine has become one of the most widely used drug and is used as a psychoactive substance. In recent years, use of caffeine in food and pharmaceutical manufacturing has increased because of its pharmacological characteristics like stimulation of central nervous system (CNS), myocardial stimulation, peripheral vasoconstriction and relaxation of the smooth muscle (Nehlig *et al.,* 1992) to boost human behaviour (Smith, 2002) and formation of kidney stone decelerate (Ferraro *et al.,* 2013). Caffeine is beneficial to human in weight loss, cognitive function and alertness. It is less harmful when it is taken at low-to-moderate amount (≤ 300 mg/day) (Smith,2005; Rogers and Dernoncourt, 1998), but sleep can be disrupted by caffeine with an amount of 200 mg (Frary *et al.,* 2005).100–200 mg caffeine

Author's Contribution: M. A., Wrote manuscript; **A. A., Z. N. & A. H.,** Wrote & submitted manuscript; **A. G. & K. S.,** Analyzed data; **M. K. S. & S. I.,** Did experimental work; **S. M., R. M. & M. I.,**Designed the study, experimental design and laboratory experiments

increases the alertness, wakefulness, decreases tiredness and improves performance (Stanton & Gray, 1995). It is also used in medication to manage drowsiness, headaches, migraines and restoring alertness (Crwaford*et al.,*2014). When it is consumed at high concentration it may have negative impact on pregnancy, fertility (Lyngs*øet al.,*2017), sugar control and other aspects of health. An acute dose of caffeine usually more than 250 mg can result in overstimulation of CNS called caffeine intoxication (Sfetcu, 2006). At the times of stress, anxiety or during pregnancy, less than 200 mg/day of caffeine is recommended by FDA (Food and Drug Administration). FDA recommended safe and moderate caffeine concentration is 250-300/day and the maximum permissible recommended concentrationis 400 mg/day. Above this concentration caffeine can cause serious side effects. Caffeine increases the amount of acid in stomach and may cause heartburn or upset stomach. Caffeine is highly addictive and increases stress level, and accelerates aging and wrinkles (Mrvos *et al.,* 1989). Taking 150–300 mg caffeine after 10 hours fast increases the urinary calcium excretion in adolescent men and women (Thelle, 1993). So, dehydration is also a major drawback of caffeine consumption.

Due to the most common effect and to make the drinks addictive, caffeine is a most common ingredient of beverages (Andrews *et al.,* 2007). The beverages containing caffeine coffee, tea, soft drinks, energy drinks, are getting great popularity (Torres & Francis, 2009). Adults take approximately three-quarters caffeine from coffee in their daily life. The caffeine content in an average cup of coffee is around 100 mg, tea 85 mg, green tea 60 mg and white tea 55 mg (Bonnie & Weinberg, 2004). Studies show that people who drink coffee regularly have a lower risk of developing Alzheimer's and dementia, and cut suicide risk by 45 percent. Some people consider it as a health drink, but over drinking of coffee can cause side effects. Most of youngsters obtain one half of caffeine from different soft drinks.

Energy drinks are fast-growing beverages that contain caffeine in different concentration ranging from 50 to 300 mg in a commercially available energy drink pack and its amount also depends upon the size of the pack (O'Brien *et al.,* 2008). Caffeine is used in the energy drinks due to its pleasant flavor and addictive behavior (Andrews *et al.,* 2007) and it also provides the main energizing boost.

Caffeine metabolizes in the liver by cytochrome P-450 oxidase enzyme system (specifically, the 1A2 isoenzyme) into three metabolic dimethyl xanthines: paraxanthine (84%), theobromine (12%) and theophylline (4%) (David *et al.,* 2015; Khalid *et al.,* 2016; David *et al.,* 2015) (Fig. 1) and each has its own effects on the body (Bolton&Null, 1981). Paraxanthine increases the free fatty acid levels in the blood plasma, theobromine increases urine volume and theophylline relaxes smooth muscles of the bronchi and is used to treat asthma (Newton *et al.,* 1981).

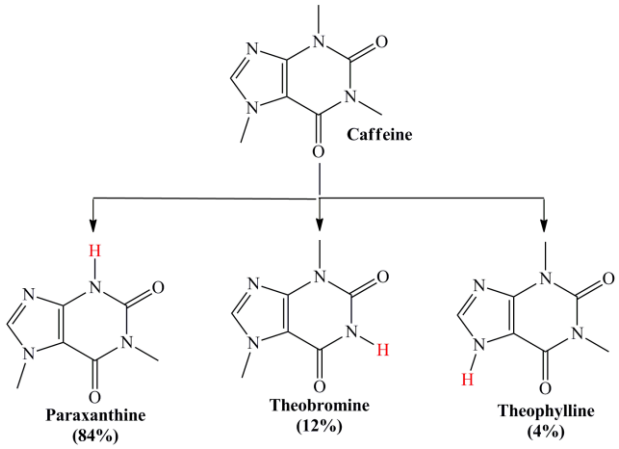

Fig. 1: Structure of caffeine and its metabolized products

A lot of work has been done to find out the caffeine amount in frequently used energy drinks. The main motive of this research work was to determine the amount of caffeine in locally available energy drinks and also to aquent people of its adverse effects on human health as many companies' sale their items only by mentioning a few benefits of caffeine without labeling the caffeine contents in drinks.

MATERIALS AND METHODS

Instruments

The samples of caffeine and activated charcoal powder were purchased from Sigma-Aldrich. Distilled water was used for washing all glassware and dried after washing in oven at 100 ºC. UV-Visible spectrophotometer (Thermo Electron Corporation, range 190-400nm)was used for quantitative determination of caffeine in energy drinks. Electronic balance (Electric Mettler Toledo balance, model AL 204) was usedmass measurement. pH was determined with WTW 1F10-220 Inolab Level 1 Multiparameter Meter without Probe, 110 V. High quality sweetness test refractometer sugar measuring reader meter (range 0-20% brix) was used to calculate the sugar contents in the energy drinks samples.

Determination of caffeine level

Caffeine amount/concentration in the energy drinks was measured by reported protocol (Uddin*et al.,* 2016).

Preparation stock solution

The standard stock solution (100 mL) of caffeine was prepared by dissolving 10 mg of caffeine in distilled water making it up to 100ml.

Preparation of standard solution

Working standards were prepared by taking 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL of aliquots of the standard stock solution into ten volumetric flask (100 mL) and making the volume up to the mark by adding distilled water (Table I).

Table I: Preparation of stock standard solution of caffeine.

Sr. No.	Stock standard solution (mL)	Distilled water (mL)
1	01	99
2	02	98
3	03	97
4	04	96
5	05	95
6	06	94
7	07	93
8	08	92
9	09	91
10	10	90

10 mm quartz cuvettes were used in the determination of absorbance of caffeine with the help of UV-Vis spectrophotometer at 272 nm wavelength. This is the wavelength at which spectrophotometer shows the maximum absorbance of caffeine (Table II and Fig., 2).

Table II: Absorbance values of standard stock solution of caffeine.

Sr. No.	Concentration (µg)	Absorbance (nm)
0	0	0
1	1	0.05
2	2	0.10
3	3	0.15
4	4	0.20
5	5	0.25
6	6	0.30
7	7	0.35
8	8	0.40
9	9	0.45
10	10	0.50

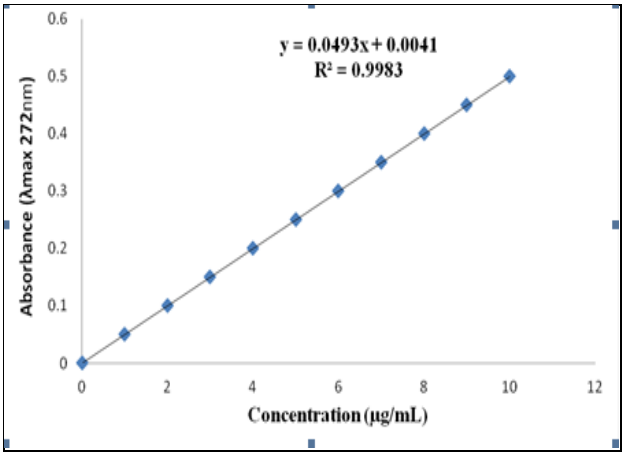

Fig., 2: Absorbance of standard solutions of caffeine

From the above calibration curve, the following equation was used for determining the strength of caffeine in all four samples of energy drinks.

$$y = 0.049x + 0.004$$
$$x = y - 0.004/0.049$$

Here,

x = concentration

y = absorbance

Collection of sample

Samples of four energy drinks having different brands namely sting, booster six B, gourmet spark and powerful were collected locally from Lahore, Pakistan.

Sample preparation

First of all, the de-carbonation of all energy drink samples was done as by the removal of the CO_2. Each bottle of energy drink sample was kept unsealed for 72 hours to remove whole carbon dioxide. Then decolorization of all the energy drink samples was carried out by adding 0.150 gm activated charcoal / $Na_2S_2O_4$ to 15 ml sample of each energy drink and kept for 5 minutes. After 5 minutes, sample was filtered with the help of filter paper in case of activated charcoal, but there was no need of filtration where sodium dithionite was used. Aliquots of the prepared energy drinks samples were kept into quartz cuvettes and

determined the absorbance of caffeine by using UV-Vis spectrophotometer at λ_{max} 272 nm.

Standard curve preparation

The contents of caffeine in all energy drinks samples were determined from the standard curve (Fig., 2).

Determination of pH

The pH of all energy drink samples was determined by using WTW 1F10-220 Inolab Level 1 Multiparameter Meter without Probe, 110 V.

Determination of Brix level

BRIX level (symbol °Bx) can be analyzed in the given samples by using the Digital Brix Refractometer. 1 - 2 drops of the given sample of energy drinks were added on the slide of the BRIX meter. Clean, the Digital Brix Refractometer with the cotton properly before and after use.

Determination of acidity

The acidity was determined on the basis of concentration of the citric acid. 10 mL of sample solution of the given energy drinks was added in 90 mL of distilled water and the 1-2 drops of phenolphthalein was added as indicator and titrated it against the standard solution of 0.1N NaOH. The value of acidity was calculated with the help of following formula:

$$\text{Acidity (\%age)} = \frac{\text{Standard solution used (mL) x 0.064 x 100}}{10}$$

RESULT AND DISCUSSION

Four energy drinks viz. sting, booster six B, gourmet spark and powerful, available locally in Lahore, were used to find their caffeine concentration through UV-Vis spectrophotometer, pH at 20 °C by pH meter, BRIX by Digital Brix refractometer and acidity through titration. Furthermore, the obtained results of caffeine concentration were compared with the labeled amount of caffeine with respective energy drink and with the recommended concentration of caffeine by US Food and Drug Administration (FDA) for a person per day, as high amount of caffeine per day cause side effects.

Caffeine content in each sample was determined by UV-Vis absorption method in distilled water. The results showed the caffeine contents ranged in energy drinks from 9.89 mg/100 mL to 9.34 mg/100 mL. The gourmet spark contains the highest caffeine content 9.89 mg/100 mL and powerful contains the lowest caffeine content 9.34 mg/100 mL while the caffeine contents in booster six B and sting are 9.45 mg/100 mL and 9.52 mg/100 mL, respectively (Table III). The determined caffeine contents when compared with the labeled caffeine contents on respective energy drink; these show that only sting brand had the comparable caffeine content (9.52 mg/100 mL) with the labeled caffeine content (10.0 mg/100 mL). In Booster six B, the calculated caffeine amount (9.45 mg/100 mL) is too low as compared to the labeled amount (45.0 mg/100 mL). On the other hand, the calculated amount of caffeine in Gourmet spark (9.89 mg/100 mL) and Powerful (9.834 mg/100 mL) are very high as compared to the labeled amount (0.01 mg/ 100 mL). On the basis of comparison, we can say that the last three companies betray the customer by labeling too much wrong contents of caffeine. The obtained results of caffeine suggest that if any person drinks daily two serving size packs from selected energy drinks, the total intake amount of caffeine will remain in the low-to-moderate range (250-300 mg/day), but not exceed the FDA recommended limit (400 mg/day). The maximum amount of caffeine recommended by FDA in a 355 mL (12 oz) bottle of energy drinks is almost 71 mg.

Table III: Obtained results of caffeine concentration, pH, brix and acidity of energy drinks.

Sample	Labeled conc. (mg/ 100mL)	Calculated conc.		Serving size (mL)	pH at 20 ºC	BRIX (units)	Acidity %
		(mg/ 100mL)	%age				
Sting	10.0	9.52	46.26	500	3. 47	12.5	1.267
Booster six B	45.0	9.45	23.64	250	3. 37	13.0	1.670
Gourmet spark	0.01	9.89	26.69	300	3. 20	15.0	1.868
Powerful	0.01	9.34	23.37	250	3. 57	12.0	1.785

The all four energy drinks having pH ranging from 3.20 to 3.57 at 20 ºC. The powerful brand has the highest pH which is 3.57 while the gourmet spark has lowest pH among all which is 3.20. The pH values indicate that powerful brand is less acidic while gourmet spark is more acidic among all energy drinks. These pH values are due to the presence of different acids that are utilized as preservatives by manufactures of these energy drinks (Table IV). These acids inhibit the growth of several microorganisms like bacteria and fungi which may contaminate energy drinks. The pH of mouth must remain in the range of 6.5 - 7.5 and pH 5.5 is considered as a threshold level for the development of dental decay. If pH of mouth drops from 5.5 for long time or frequently, the tooth enamel will be demineralized rapidly.

Table IV: Comparison of composition of energy drinks under study

<table>
<tr><th colspan="4">Composition of energy drinks</th></tr>
<tr><th>Sting</th><th>Booster six B</th><th>Gourmet spark</th><th>Powerful</th></tr>
<tr><td>PepsiCo</td><td>Six B Food Industries (pvt) Ltd.</td><td>Gourmet, Pakistan</td><td>King Beverages Industries, Sialkot Pakistan</td></tr>
<tr><td>Carbonate water</td><td>Carbonated water</td><td>Carbonated water</td><td>CO_2</td></tr>
<tr><td>Sugar</td><td>Sucrose</td><td>Sugar</td><td>Sugar, Glucose</td></tr>
<tr><td>Citric acid</td><td>Citric acid</td><td>Citric acid</td><td>Citric acid E330</td></tr>
<tr><td>Artificial flavor</td><td>Artificial flavor</td><td>Artificial flavor</td><td rowspan="2">Fructose</td></tr>
<tr><td>Maltodextrin</td><td>Dextrose</td><td rowspan="3">Trisodium citrate</td></tr>
<tr><td>Sodium citrate</td><td rowspan="2">Guarana (Energizer)</td><td rowspan="2">Acidity regulator sodium citrate E331</td></tr>
<tr><td>Sodium hexametophosphate</td></tr>
<tr><td>Taurine</td><td rowspan="2">Taurine</td><td rowspan="2">Taurine</td><td rowspan="2">Taurine</td></tr>
<tr><td>Potassium sorbate</td></tr>
<tr><td>Caffeine (200mg/L)</td><td>Caffeine (Brain relaxant)</td><td>Caffeine</td><td>Caffeine</td></tr>
<tr><td>Sodium benzoate</td><td>Sodium benzoate</td><td>Sodium benzoate</td><td>Ammonia</td></tr>
<tr><td>Allura red dye</td><td>Food color</td><td>Caramel red</td><td>Riboflavin elol, Caramel E150 C</td></tr>
<tr><td>Calcium disodium EDTA</td><td>Niacin</td><td>Inositol</td><td>Niacin, Inositol</td></tr>
<tr><td>Vitamins B_3, B_6, B_{12}</td><td>Vitamins B_2, B_6, B_{12}, C (antioxidant), pantothenic acid (vitamin B_5)</td><td>Vitamins B_2, B_6, B_{12}, Nicotinamide (vitamin B_3), Pantothenic acid (vitamin B_5)</td><td>Vitamins B_6, B_{12}, Pantothenic acid</td></tr>
<tr><td>Ginseng powder</td><td>Ginseng (Vitality regulator)</td><td>Glucuronolactone</td><td>Gingseng extract</td></tr>
<tr><td colspan="4">Three energy drinks have ginseng that cause low tension, relieve stress, stimulate metabolism (Khalid et al., 2016).</td></tr>
</table>

CONCLUSION

Due to psychoactive/stimulant property of caffeine, companies normally add the caffeine in beverages including energy drinks. In this study, caffeine amount, pH, BRIX and acidity were measured in four energy drinks locally available in Lahore. The results indicate that in all four brands caffeine amount is in the FDA recommended range, but companies labeled the different amount than the actualand this is direct falsity with the customers. Pakistan Government must ensure the contents in those beverage/energy drinks with the labeled amount that companies want to sale in local markets. If determined amounts of ingredients are different to the labeled amounts on any brand, this brand should be banned to sale in Pakistan and Government should take action against the company. There is also need to aware the public about these things, so that health of costumers remains good.

ACKNOWLEDGMENT

Dr. Muhammad Aslam expresses his gratitudes to Dr. Ijaz Ahamd, Principal Scientific Officer,Food and Biotechnology Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, and Department of Chemistry, Division of Science and Technology, University of Education, Township, Lahore for providing research facilities and financial support, respectively.

REFERENCES

Andrews, K., Schweitzer, A., Zhao, C., Holden, J. M., Roseland, J. M., Brandt, M., Dwyer, J., Picciano, M., Saldanha, L., Fisher, K., Yetley, E., Betz, J., and Douglass, L., 2007. The caffeine content of dietary supplements commonly purchased in the US: Analysis of 53 products having caffeine containing ingredients. *Anal.Bioanal. Chem.,* 389(1): 231-239.

Anna, K. and Kurek, J. P., 2013.*Natural products and pharmaceuticals.*UAM, Poznań 2nd ed. pp. 227-256.

Bolton, S. and Null, G., 1981. Caffeine: Psychological effects, use and abuse. *Orthomolecular Psychiatry,* 10(3): 202-211.

Bonnie, K. B. and Weinberg, B. A. 2004.*The worlds of caffeine.The science and culture of the world's most popular drug.*4th Ed.

Crwaford, P., Robert, C. and Atkins, V., 2014. California centre for public Health Advovacy. Hiding Under a health Halo.

David, Gabriela, I., Bizgan, Cristina, A. M., Buleandra, Mihaela, Popa, Elena, D., Moldovan, Zenovia, ndea and Adriana, I., 2015. Rapid determination of total polyphenolic content in tea samples based on caffeic acid voltammetric behaviour on a disposable graphite electrode.*Food Chem. J.,* 17(3): 1059-1065.

Ferraro, M. P., Taylor, E. N., Gambaro, G. and Curhan C. G., 2013.Soda and other beverages and the risk of kidney stones.*Clin. J. Am. Soc. Nephrol.,* 8(8): 1389-1395.

Frary, C. D., Johnson, R. K. and Wang, M. Q., 2005.Food sources and intakes of caffeine in the diets of persons in the United States.*Am. Diet. Assoc. J.,* 105(1): 110-113.

Jarvis, G., 2002. *The Rise and fall of cocaine cola.* 1st ed. pp. 302-311.

Khalid, A., Ahmad, S., Raza, H., Batool, M., Lodhi, R. K., Ain Q. T. and Naseer, F., 2016. Determination of caffeine in soft and energy drinks available in market by using UV/Visible spectrophotometer. *Fam. Med. Med. Sci. Res.,* 5(4): 206. Doi:10.4172/2327-4972.1000206.

Lyngsø, J., Hansen, C. H. R., Bay, B., Ingerslev, H. J., Hulman, A. and Kesmodel, U. S., 2017. Association between coffee or caffeine consumption and fecundity and fertility: a systematic review and dose–response meta-analysis. *Clin.Epidemiol.,* 9: 699-719.

Mrvos, R. M., Reilly, P. E., Dean, B. S. and Krenzelok, E. P., 1989.Massive caffeine ingestion resulting in death.*Vet. Hum. Toxicol.,* 31(6): 571-582.

Nathanson, J. A., 1984. Caffeine and related methylxanthines: Possible naturally occurring pesticides. *Sci. J.,* 226(4671): 184-187.

Nehlig, A., Daval J. L. and Debry, G., 1992. Caffeine and the central nervous system, mechanisms of action, biochemical, metabolic, and psycho stimulant effects.*Brain Res. Rev.,* 17(2): 139-170.

Newton, R., Broughton, L. J., Lind, M. J., Morrison, P. J., Rogers, H. J. & Brad, B. I. D. 1981. Plasma and salivary pharmacokinetics of caffeine in man.*European Clin. Pharm. J.,* 21(1): 45-52.

O'Brien, M. C., McCoy, T. P., Rhodes, S. D., Wagoner, A. and Wolfson, M., 2008. Caffeinated cocktails: Energy drink consumption, high-risk drinking, and alcohol-related consequences among college students. *Acad. Emerg. Med.,* 15(5): 453-460.

Palatini, P., Ceolotto, G., Ragazzo, F., Dorigatti, F., Saladini, F., Papparella, I., Mos, L., Zanata, G. and Santonastaso, M., 2009. CYP1A2 genotype modified the association between coffee intake and the risk of hypertension. *J. Hypertens.,* 27(8): 1594-601.

Rogers, P. J. and Dernoncourt, C., 1998. Regular caffeine consumption: A balance of adverse and beneficial effects for mood and psychomotor performance. *Pharmacol. Biochem. Behav.,* 59(4): 1039-1045.

Runge, F. and Friedlieb, F., 2014.*Latest phytochemical discoveries for the founding of a scientific phytochemistry*.Joseph publications, 20th ed. American. pp. 144-159.

Sfetcu, N., 2006. *Health and drugs: Diseases prescription and medication. Health and medicine*. 5thed.

Smith, A. P., 2005. Caffeine at work.*Hum. Psychopharmacol.Clin. Exp*., 20(6): 441-445.

Smith, A., 2002. Effects of caffeine on human behavior.*Food Chem. Toxicol.,* 40(9): 1243-1255.

Stanton, C. K. and Gray, R. H., 1995. Effects of caffeine consumption on delayed conception. *Agr. Eng. J.,* 14(2): 1322-1329.

Thelle, D. S. 1993. *Metabolic effects of coffee and caffeine intake on the cardiovascular system.Caffeine, Coffee and Health.* Edited by S. Garattini (New York: Raven), pp. 151-155.

Torres, A. and Francis, M., 2009.*Caffeine (health aspects), caffeine (physiological aspects) mental illness (risk factors)*. 11th ed. pp. 39-51.

Uddin, M. M. N., Dutta, S., Kabir, M., Bhuiya, M. A. M., Ajrin, M., Ibban, S. S. and Hossain, M., 2016. The quantitative determination of caffeine in energy drinks available in Bangladesh. DOI: 10.13140/ RG.2.1.2073.0005.