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Forensic Analysis of Caffeine Content in Tea Samples using UV-Visible Spectroscopy

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Abstract:

Tea is widely used as a refreshing beverage to reduce fatigue, tiredness and create alertness. It contains caffeine that enhances the capacity of a person to work if consumed in a limited amount. However, in some cases, it is also used by athletes or by bodybuilders as supplement to enhance their workout potential and burn fat. Caffeine when consumed in combination with different drugs, narcotic substances and alcohol produces toxic symptoms that can lead to fatality. The objective of the present study is to determine and compare the caffeine concentration in 25 different brands of commonly available tea found in India using UV-visible spectroscopy. Caffeine was extracted from samples by liquid-liquid extraction method using chloroform as solvent. The concentration of caffeine in different tea samples ranged between 3.273 mg/g - 37.380 mg/g. It has been concluded that higher caffeine concentration in tea samples may produce toxic effects or even become fatal if taken in excess or in combination with other drugs. The technique UV-visible spectroscopy offers the advantage of being sensitive, reliable, accurate and economical for the analysis of caffeine concentration in tea samples.

Keywords: Forensic science, Forensic toxicology, Caffeine, Drug of Abuse.

1. Introduction

Tea is widely used as a refreshment beverage that enhances the capacity of a person to work if consumed in a limited amount. It is extracted from the plant *Camellia sinensis* which is commonly found in China and India. It is an evergreen shrub with plant height about 0.6-1.5m [1]. Tea plant has shiny green coloured leaves with leathery surface and vibrant white colour flowers. The leaves, internodes or leaf buds of *Camellia sinensis* plant can be used for the preparation of tea [2]. Tea leaves are processed for preparing different types of tea available in the market, for example black tea is prepared from fermented leaves whereas green tea is prepared from unfermented leaves. Chemically tea contains caffeine, polyphenols, flavonoids, theaflavins, minerals and vitamins. Tea has anti-inflammatory and neuroprotective effects due to presence of flavonoids which possess detoxifying properties [2-4].

Caffeine is the most important constituent of tea which boost the performance of an individual. It belongs to the family of methylxanthines [5-6]. Xanthine is the oldest form of stimulants which is found naturally in tea plants [7]. Other than tea, caffeine can also be found naturally in leaves and seeds of other plants, like coffee beans, cocoa or cola seeds etc. Various properties of caffeine have been mentioned in table-1.

Caffeine is metabolized in the liver by cytochrome P450 oxidase into three active metabolites of xanthine- para xanthine (84%), theobromine (12%), and theophylline (4%). Paraxanthine (84%) increases the amount of free fatty acids in blood plasma, theobromine (12%) increases urine volume and theophylline (4%) releases smooth muscles of the bronchi [2,8-9]. Caffeine is a methylxanthine molecule that temporarily affects the central nervous system and works as a stimulant in contrast to adenosine's effects on the brain. The adenosine receptors control the release of neurotransmitters in the brain

and play a significant role in controlling arousal, cognition, sleep, memory, and learning. Due to its similar structure caffeine binds to adenosine receptors and blocks its ability to slow nerve activity. The release of neurotransmitters including norepinephrine, dopamine, acetylcholine, serotonin, glutamate, and gamma-aminobutyric acid (GABA) are impacted by the blocking of adenosine receptors. These neurotransmitters affect mood, memory, awareness, and cognitive function when they are released in excess [4,10].

Caffeine can cause toxic symptoms after roughly 1g of oral consumption like insomnia, tremors, tachycardia, arrhythmia, nervousness, nausea, altered perception, flashes, vomiting, seizures, abdominal pain, headache, fever, weakness, anxiety, restlessness, vomiting, dizziness, tinnitus, cerebral oedema, anxiety and irritability, while more than 3g of it can be fatal [6,10-14]. Caffeine overdose results in gastrointestinal problems (diarrhoea), high blood pressure, restlessness, insomnia, auditory hallucination, accelerated pulse, nausea, increased urination, cardiac palpitations, and dizziness [3][15].

Drug abuse is frequent and intentional use of a substance for creating pleasure effects. Repetitive use of some drugs may lead to drug addiction. The individual may also have withdrawal and tolerance issues [16-17]. Drugs like marijuana, narcotics, and alcohol enhance the dependence of caffeine in an individual which increases concentration of caffeine in the body [5,13,19-20]. A few case studies about caffeine intoxication are mentioned below in table-2. Therefore, the present work aims to study the concentration of caffeine in tea samples using UV-Visible spectroscopy which can contribute to determine the threshold value for caffeine that could be abused as a drug.

2. Materials and Methods

Sample collection

25 samples of tea (6 green tea and 19 black tea) were collected from different Indian states as mentioned in table-3.

Sample preparation

2 g of each dried tea sample was weighed using a weighing machine and 20 ml of distilled water was added to it and boiled. The solution was then filtered. 2g of sodium carbonate (NA_2CO_3) was then added to each solution then stirred and filtered. The filtrate was heated and concentrated to 5 ml and kept aside for cooling. Caffeine was extracted by adding 5 ml chloroform to the above volume by liquid-liquid extraction using a separating funnel [2]. The organic layer that contains caffeine was separated and 0.1 ml of this tea extract was taken in the test tube and 10 ml of chloroform was added [11]. Absorbance was measured at 276 nm. All the samples were examined under UV-Visible to check the caffeine content.

Standards preparation

Caffeine standard (spectroscopic grade) was purchased from Molychem Ltd (Mumbai, India). A caffeine stock solution of 1000 ppm was prepared by dissolving 0.1g of standard caffeine in 100 mL of chloroform (CCL_3) in an airtight container [2]. Among all other solvents, chloroform was used in this study as it gives better solubility of caffeine. From the caffeine stock solution following dilutions were prepared – 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 35 ppm, 40 ppm, 45 ppm, 50 ppm, and 55 ppm.

Sample analysis

The UV-Visible spectrophotometer Hitachi- UH3500 (Hitachi High- Tech Science Corporation, Tokyo, Japan) was used for the analysis of caffeine in different tea samples. A quartz cuvette with a path length of 1cm was used. A full range scan from 200 nm to 800 nm with the scanning speed of 100nm/minute was done to determine the wavelength at which caffeine absorbs maximum when dissolved in chloroform.

3. Results

Caffeine concentration is variable in different types of tea such as green tea and black tea. Some tea samples have high concentration of caffeine which may lead to accumulation of caffeine in human body. Higher concentration of caffeine in body may affect psychological and physiological functioning of the body.

In the present study, UV-Visible spectrophotometer Hitachi 3500 has been used to check the caffeine concentration present in 25 different tea samples chosen for the study in triplicates and the average results are shown in Table-4. The calibration curve of standard caffeine was prepared in MS Excel given in figure-1. The correlation coefficient was 0.9934 which suggests that the developed calibration is robust and can be used to determine the concentration of caffeine in unknown samples with great accuracy.

It is observed that some of the tea samples contain higher amounts of caffeine and others contain a very less amount. The concentration of caffeine in different tea samples ranges from 3.273- 37.380 mg/g. Caffeine content of green valley tea (T2), ACB special tea (T11), red label brooke bond (T23) ranges from 37.380-12.095 mg/g respectively. Green tea samples have caffeine concentration ranging from 3.425 mg/g to 37.380 mg/g. The highest amount of caffeine was found in green valley tea (T2) at 37.830 mg/g followed by black tea ACB special (T11) at 13.311 mg/g. Concentration of caffeine found in local tea sample (L2) is 11.410 ppm. and the lowest caffeine concentration was found in doko tea (T19) at 3.273 mg/g. It is evident that many samples contained high amount of caffeine and when consumed in high quantity can result in severe ailments, and possibly death.

4. Discussion

In most of the reported cases of caffeine intoxication, the form of caffeine is either tablets or 100% pure anhydrous caffeine powder which produced signs and symptoms such as organ congestion, vomiting, seizures, cardiovascular collapse, chronic hepatitis, chronic pulmonary emphysema, coronary artery sclerosis, cardiac arrhythmia, pulmonary edema, congestion, etc [2,10-11,13,21]. As discussed in table-2, a 28-year-old person after consumption of 640 mg of caffeine in the form of energy drinks has suffered from cardiac arrest due to caffeine intoxication [23-24]. Followed by another case study, in which a 25-year-old having a medical history of mitral valve prolapse after consumption of 550 mg of caffeine in the form of energy drinks has suffered from ventricular arrhythmias which eventually lead to the death of the individual [25]. Caffeine not necessarily causes toxic effects only when taken in higher doses as its metabolism is affected by a number of factors in human body. Diseases related to liver may lead to slower metabolism of caffeine. Disease like hepatitis and cirrhosis may cause hindrance in the metabolism of caffeine by reducing the level of CYP1A2 [26]. For example, there is a death reported from the consumption of mint having caffeine by a person having cirrhosis even the dose of caffeine is not considered lethal in normal case [27].

Another factor which can influence the caffeine metabolism in the human body is the presence of some drugs which can increase the toxic effect of caffeine or in some cases it proves to be fatal [26]. Cardiovascular drugs like propafenone, verapamil and mexiletine reduce the metabolism of caffeine [28].

In a case study it was reported that a lower amount of caffeine when interacted with other drugs like 60 mg ephedrine, 240 mg caffeine, and 60 mg aspirin may increase the toxic effects of other drugs, which was seen when a runner has taken combinations of drugs mentioned above which led to severe rhabdomyolysis [29]. A study conducted by George j et al. shows that alcohol consumption with caffeine may interact and reduce 36% clearance of caffeine from the body [30].

It has been reported that caffeine concentration may contribute to the threshold value for being used as a drug of abuse or for leading to accidental intoxication. By comparing the results from Vuletic et al. (2021) it is observed that the caffeine concentration in tea ranges from 588.138-14171.021 ppm. The highest amount of caffeine is found in black tea (14171.021 ppm) and the lowest concentration is found in green tea (588.138 ppm) [3]. In a comparison of Rehman et al. (2017), the caffeine concentration ranges from 0.251 mg/g to 16.111 mg/g. The highest amount of caffeine was found in tetley tea (16.111 mg/g) and the lowest concentration in ghatnctr tea (0.251 mg/g) [8]. When the results of the present study were compared to the previous studies, it was observed that there was no similarity between the previous findings and this research. It is found that green tea basically contains less amount of caffeine as compared to black tea which contains more caffeine. Green valley tea contains 37.380 mg/g which is a greater concentration of caffeine as compared to the ACB special tea, which contains 13.311mg/g. A single cup of tea contains 85 mg of caffeine; when 300mg or more is consumed, it may cause caffeine intoxication [3-4]. However, it has been concluded that green valley tea contains a high concentration of caffeine that may not cause caffeine intoxication. But when taken in combination with other drugs like marijuana, methamphetamine, MDMA, or alcohol at nearly 32-200 mg, it can increase the blood plasma level up to the concentration of 80 mg/L which can cause toxic symptoms that can be fatal for an individual [13,15,22].

5. Conclusion

Forensic toxicological significance of caffeine is often neglected due to its wide applications in medicine and recreational purposes. However, deadly overdoses may occur when caffeine is taken in combination with other drugs of abuse and accidental cases involving the misuse of dietary supplements. In this study, UV-visible spectroscopy is used for the analysis of tea samples, and it has been observed that the various brands of tea samples contain higher amounts of caffeine. The excessive intake of caffeine concentration in tea can lead to toxic effects in an individual. This technique offers various advantages such as sensitivity, reliability, accuracy and consumes less chemicals. Additionally, in future further studies investigating forensic perspective of toxic effects of caffeine can be explored.

Conflict of Interest

Declared none

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Table 1- Properties of caffeine [8]

Sl. No	Characteristics	Caffeine
1	IUPAC name	1,3,7-trimethyl xanthine
2	Physical properties	White, crystalline and bitter taste
3	Chemical Formula	$C_8H_{10}N_4O_2$
4	Family	Methyl xanthines
5	Molecular mass	194.19 g/mol
6	Density	1.2g/ cm^3
7	Boiling point	178^{o}c
8	Melting point	237^{o}c
9	Solubility	Chloroform, dichloromethane, petroleum ether, and benzene

Table 2- Case Studies of caffeine intoxication

Sl. No	Source of caffeine	Age of individual	Drug interaction/s (if any)	Signs and symptoms	Amount of caffeine found in analyse	Manner of death	Ref
1	Plastic bag label 100 g caffeine	48	-	Organ congestion, cyanosis of both arms and legs	401 mg/L	Suicide	[6]
2	2 boxes of 100 pills of caffeine	21	Venlafaxine, O-desmethylvenlafaxine	-	210 mg/L	Suicidal	[21]
3	Bottle of caffeine pills	29	-	Vomiting, seizures, intubation, aspiration, cardiovascular collapse	567 mg/L	Accidental	[12]
4	100% pure caffeine anhydrous powder	39	-	Pulmonary edema, congestion	350 mg/L	Accidental	[22]
5	Caffeine tablets	44	25 different medications	Cardiac arrhythmia	80 mg/L	Unknown	[10]
6	250 caffeine tablets	54	Orphenadrine, thioridazine	Chronic hepatitis, chronic pulmonary emphysema, coronary	173 mg/L	Unknown	[21]

				artery sclerosis			
7	7-8 cans of caffeinated energy drink	28	-	Cardiac arrest, chest pain, dizziness	640 mg	-	[23]
8	100 tablets of letigen	47	-	Cardiac arrest	200mg	uncertain	[21]
9	Energy drinks	25	-	Ventricular arrhythmias	550 mg	-	[24]

Table 3- Sample details

Sl. No	**Brand**	**Manufacturer**	**Locality**	**Sample Code**
1	Supriya green tea	Kolkata	Jaipur	T1
2	Green valley tea	Tamil Nadu	Jaipur	T2
3	Organic green tea	Uttar Pradesh	Uttarakhand	T3
4	T-chai green tea	Kolkata	Uttarakhand	T4
5	Tata tulsi green tea	Kolkata	Mumbai	T5
6	Organic India green tea	Uttar Pradesh	Mumbai	T6
7	Minakshi tea	Jaipur	Jaipur	T7
8	Taaza brooke bond tea	Mumbai	Punjab	T8
9	CTC leaf tea	Delhi	Uttarakhand	T9
10	Jadu gold elaichi tea	Delhi	Punjab	T10
11	ACB Special tea	-	Punjab	T11
12	Mohan tea	Jodhpur	Jaipur	T12
13	Mahak tea	Delhi	Jaipur	T13
14	Lal ghoda tea	Jaipur	Jaipur	T14
15	Tata agni tea	Assam	Haryana	T15
16	Madhushree tea	Delhi	Jaipur	T16
17	Utah tea	New Delhi	Jaipur	T17
18	Girnar royal tea	Mumbai	Mumbai	T18
19	Doko tea	Delhi	Mumbai	T19
20	Hill tree tea	Navi Mumbai	Mumbai	T20
21	Wagh bakri tea	Gujarat	Mumbai	T21
22	Society’s tea	Gujarat	Mumbai	T22
23	Red label brooke bond	Kolkata	Mumbai	T23
24	Local tea sample 1	Rajasthan	Jaipur	L1
25	Local tea sample 2	Rajasthan	Jaipur	L2

Table 4- Caffeine concentration obtained from tea samples

Sample code	Absorbance	Caffeine concentration (in mg/g)
T1	0.068	5.707
T2	0.901	37.380
T3	0.008	3.425
T4	0.178	9.889
T5	0.054	5.174
T6	0.066	5.631
T7	0.058	5.327
T8	0.040	4.642
T9	0.083	6.277
T10	0.148	8.749
T11	0.268	13.311
T12	0.067	5.669
T13	0.03	4.262
T14	0.029	4.224
T15	0.013	3.615
T16	0.011	3.539
T17	0.024	4.034
T18	0.041	4.680
T19	0.004	3.273
T20	0.197	10.616
T21	0.084	6.315
T22	0.179	9.927
T23	0.236	12.095
L1	0.087	6.429
L2	0.218	11.410

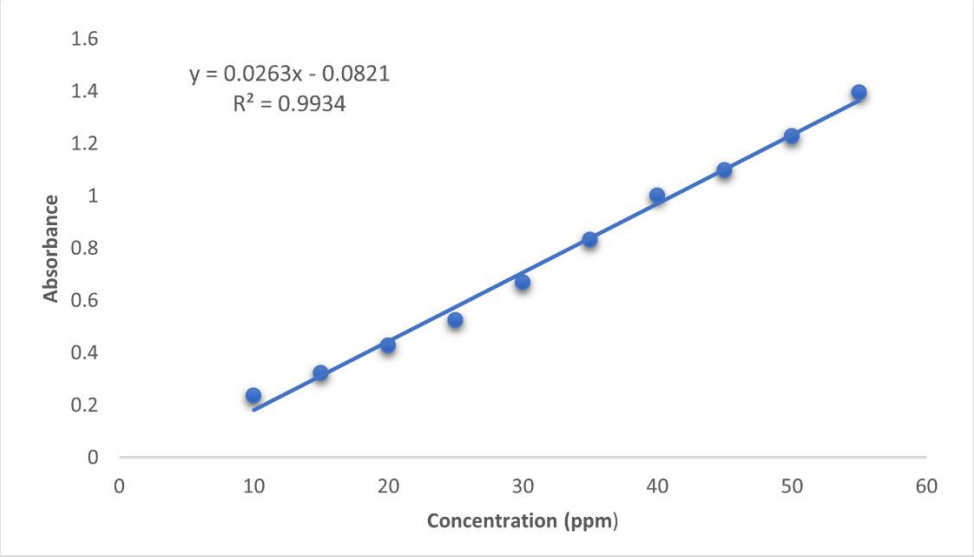

Figure 1- Standardization of caffeine