

A Comparative Analysis of Caffeine Extraction Efficiency from Different Tea Varieties and Its Effect on Human Physiology: A Spectrophotometric Investigation

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Abstract

The current work imitates the trivial method which intricates the abstraction along with classification of caffeine accessible in variability of decoction shrubberies which are expended in two different states of India (Telengana & Uttar Pradesh). As per observation individuals of both the states are identical tender of consumption of tea. Abstraction progression tangled variability of stages which are discoursed in the work. In addition, the outcome of drinking of caffeine or the permitted ingestion of caffeine over tea has also been conferred through the work and linked the data with the literature data. In this study, we used a UV-visible spectrophotometer as well as liquid-liquid extraction method to determine the caffeine content in various tea samples. The significance of this study lies in the fact that accurate determination of caffeine content is essential for quality control and labeling of tea products. The UV-visible spectrophotometer method was found to be simple, reliable, and sensitive for the detection of caffeine in tea samples.

Keywords

Caffeine, Tea Shrubberies, Separation, Health Impacts, Spectrophotometry

1. Introduction

Tea, the beloved beverage of many cultures across the globe, has been consumed for centuries for its rich flavor, soothing aroma, and therapeutic properties. From the British afternoon tea tradition to the Japanese tea ceremonies, tea has been an integral part of social rituals and daily routines. One of the reasons for its

widespread popularity is its natural source of caffeine, a natural stimulant that helps to increase alertness and focus. Caffeine is also known to have potential health benefits, such as reducing the risk of chronic diseases like Parkinson's and Alzheimer's. Recent research has highlighted the potential of tea and caffeine for improving brain function, boosting metabolism, and reducing the risk of heart disease. Studies have also shown that tea consumption may have a positive effect on mental health, reducing symptoms of depression and anxiety [1]. In addition, the tea industry has been thriving, with new and innovative blends and flavors being introduced regularly. For example, matcha, a powdered form of green tea, has gained popularity in recent years due to its high levels of antioxidants and potential health benefits. Innovative approaches to tea consumption have also emerged, such as tea-infused cocktails, which combine the health benefits of tea with the enjoyment of a refreshing drink [2] [3]. The various products of caffeine have been illustrated in **Figure 1**.

Tea is consumed in various forms around the world, including hot tea, iced tea, and bubble tea. The most commonly consumed types of tea include black tea, green tea, white tea, oolong tea, and herbal tea [5]. There are various techniques used to extract the flavors and nutrients from tea leaves, including steeping, infusion, and decoction. Steeping is the most common method used to prepare tea, where tea leaves are steeped in hot water for a certain amount of time. The optimal steeping time varies depending on the type of tea and personal preference. Infusion involves adding tea leaves to a liquid, such as milk or hot water, and allowing the mixture to sit for a certain amount of time. This technique is

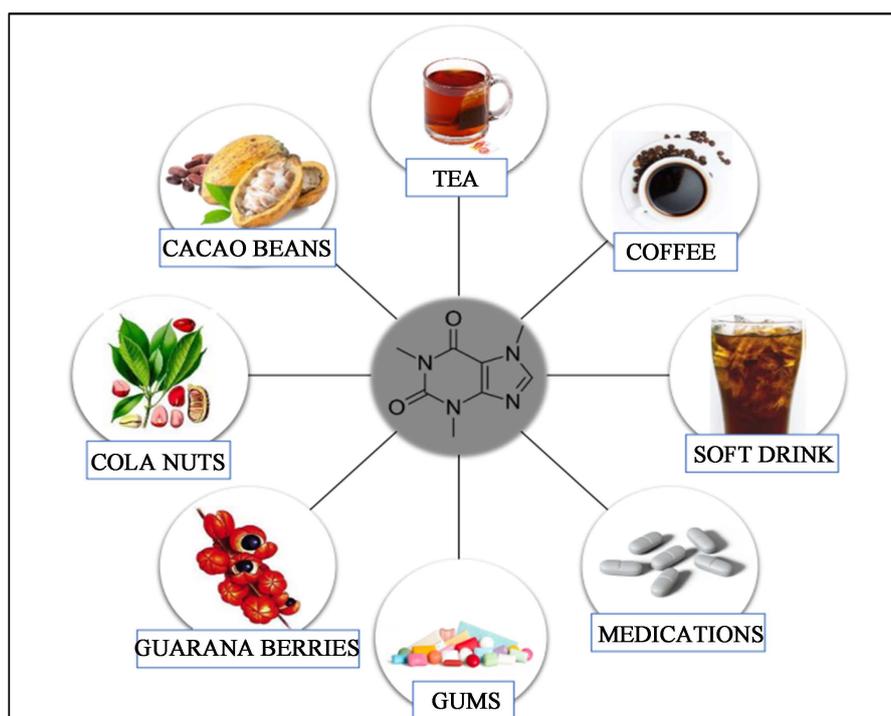


Figure 1. The most popular plants and products containing caffeine. Based on de Mejia *et al.* [4].

commonly used to prepare chai tea, which is made by infusing tea leaves with spices, milk, and sweetener. Decoction involves boiling tea leaves and other ingredients, such as herbs or spices, for an extended period of time to extract their flavors and nutrients. This method is commonly used to prepare traditional Chinese herbal teas, such as chrysanthemum tea [6]. Caffeine is a central nervous system stimulant that can improve cognitive function, increase alertness, and reduce fatigue. It has been shown to have positive effects on memory, mood, and physical performance. However, caffeine can also have negative effects, such as anxiety, sleep disturbances, and increased heart rate. In addition to its stimulating effects, caffeine in tea has also been studied for its antioxidant properties. Tea contains a variety of antioxidants, including catechins and flavonoids, which have been shown to have anti-inflammatory and anti-cancer properties. Some studies suggest that caffeine may enhance the antioxidant activity of these compounds [7] [8]. However, Caffeine consumption is growing globally. The essential inspirations are generally absorption as well as remembrance improvement also physical act development. Numerous studies projected that, ingesting caffeine recurrently while in acceptable capacities, can affect in protracted migraines and nuisances [9]. The purpose of this study is to fill a significant gap in the literature regarding the detection of caffeine content in tea from two states in India where tea is highly popular. Despite the widespread consumption of tea in these regions, there is currently a lack of research on the caffeine content of tea produced in these areas. Therefore, this study aims to advance our understanding of the caffeine levels in tea from these two states by employing advanced methods of analysis. By doing so, this study can contribute valuable insights into the composition of tea from these regions and provide important information for consumers, researchers, and tea industry professionals alike.

Chemical Structure and Properties of Caffeine

Caffeine is a trimethylxanthine in which the three methyl groups are placed at positions 1, 3, also 7. A purine alkaloid that takes place certainly in coffee as well as tea. Caffeine is well-defined as an alkaloid, which is a group of nitrogenous biological complexes of herbal source that have marked physiological actions on humans. Numerous of this nitrogen formed biological centers are recognized for esurient a malicious then savor additional to alkaloids entail of morphine, cocaine, toxins and nicotine. A sum of effects of caffeine are: untainted anhydrous caffeine savor malicious, white in color and odorless powder with melting point $235^{\circ}\text{C} - 237^{\circ}\text{C}$. Caffeine is somberly resolvable in water at ambient high temperature (2 g/100ml), yet real resolvable in hot water (66 g/100ml) with a biochemical formulation $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$ also biochemical arrangement as shown in **Figure 2**.

It is somewhat solvable in Benzene and petroleum ether but then soberly soluble in acetone as well as alcohols solvents. Pure caffeine sublimates at 178°C and is feebly basic with a pH range of 6.5 to 10.4 needing sturdy acid to protonate it [10] [11]. Caffeine is equally fat plus water-solvable, which permits it to clearance over closely every tissues simply. Likewise, caffeine may be supported over the blockade by conversing to a transporter that is regularly connected by adenosine,

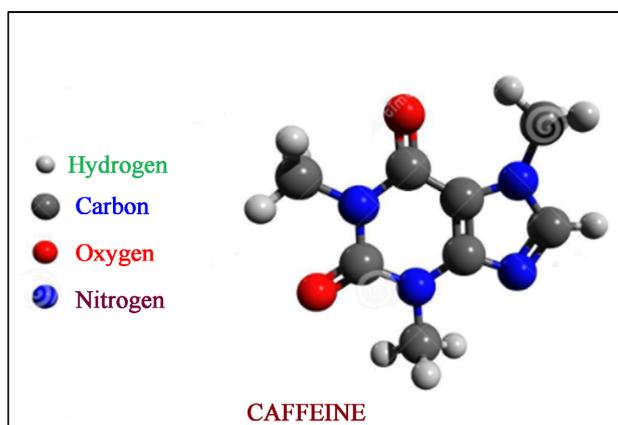


Figure 2. Structure of caffeine.

a constituent part formed in entirely social slots [12].

2. Materials and Methods

Weight Analysis Technique-Liquid-liquid extraction method has been used for caffeine from each tea sample as reported by many authors [13] [14]. Tea samples collected from two states of India namely Uttar Pradesh (town Shikohabad) also Telengana's capital Hyderabad homegrown marketplace is: Tata tea, Wagh bakri, Taj Mahal, Red label, Hyderabad local and Assam tea. Each sample of tea kept in dry clean separate beakers for further treatment. For clear identification mark all six beakers by A [Tata], B[Wagh], C[Taj], D[Red], E[Hyd], F[Assam]. Tea samples, lead acetate, chloroform, beakers, glass rod, filter paper, funnel, water. First of all, 50 g of tea sample were taken and 150 ml of distill water was further added to it in a beaker. Formerly the beaker was fiery equal to electrifying hot. The solution was purified then lead acetate was further added to the deposit, foremost to the creation of curdy brown colored precipitate.

Continuous adding lead acetate till no more precipitate has been shaped. Over, solution was sifted. Here and now the filtrate was boiled till it had developed 50 ml. Then the solution was permissible to cooled up to 15°C - 20°C. Afterward, 20 ml of chloroform was added. Soon afterward, two coatings looked in the unravelling funnel in **Figure 3**, and then unglued the lower coating. The solution formerly uncovered to air in direction to permit chloroform to get disappeared. The filtrate leftward late was caffeine. The weight of caffeine powder was considered as the final weight. On subtracting the initial weight from the final weight, the quantity of caffeine extracted was found. Then balanced it in addition to verifying the explanations. Alike process was accomplished with diverse illustrations of tea shrubberies and extent of caffeine was detected in them.

Instrumentation Analysis Technique

The solvent used for the experiment was chloroform, and the concentration of caffeine in each selected tea sample was measured at a wavelength of 274 nm. Both chloroform (CHCl₃) and sodium carbonate (Na₂CO₃) were obtained from Merck (Merck INDIA), and all reagents used in the study were of analytical



Figure 3. Sample of two layers.

grade. Additionally, all solutions were prepared using distilled water. The UV/V is spectrophotometer used for the analysis of caffeine in the tea samples was the Secomam UVi Light-XTD5, manufactured in Ales, France.

Wavelength selection—The extreme wavelength at which caffeine absorbs light was resolute via scanning the range of 190 - 400 nm. The wavelength at which caffeine captivates light the most was found to be 274 nm and was chosen for further analysis.

Standardization resolutions preparation—A solution of caffeine stock (100 parts per million) was created by dissolving 0.01 grams of purified caffeine in 100 milliliters of chloroform in a volumetric flask. Subsequently, dilutions of the caffeine stock solution were made to create solutions of 1 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. The absorbance of these solutions was measured three times at a wavelength of 274 nanometers using quartz cuvettes.

3. Result and Discussion

The fine quality powdered has been extracted from the different tea samples. The extraction method cast-off for defining the quantity of caffeine has been verified to be extremely effective. The quantity of caffeine existing in diverse tea examples has been displayed in **Table 1**. Fraction of caffeine from six diverse tea examples has been evaluated by the given formula

$$\frac{\text{Mass of caffeine recovered (gm)} \times 100}{\text{Mass of tea sample (gm)}}$$

The caffeine content found maximum in Taj Mahal tea (1.9%) and minimum has been reported in Assam tea (1.2%) sample. The Tata tea sample is also showing second highest quantity of caffeine present in the available sample. It has been suggested that the variation of caffeine content in tea shrubberies over the heap-fermentation be calculated over not merely on the growth and reproduction of microorganisms, however on the tea conformation [15]. The out-

comes projected that the Taj Mahal and Tata tea sample shrubberies displayed a trivial exhilarating significance linked to other discrepancies of tea samples. The graphical representation of each tea sample has been mentioned in **Figure 4**.

The quantity of caffeine too differs dependent on the change of tea, product of tea as well as unswervingly ascribed to the treating then leaf ripeness [16]. The special effects of environs for example limited weather (sunlight/humidity) agricultural as well as soil features may be a smaller amount significant than general deviation in the controller of caffeine substances in both coffee beans plus tea leaves [11]. Amount of caffeine has been reported in **Table 2** from literature.

The absorbance of calibration solutions of caffeine changes with different concentrations of caffeine in the solution. As the concentration of caffeine increases, the absorbance of the solution also increases. This relationship between absorbance and concentration is described by the Beer-Lambert law, which states that the absorbance of a solution is directly proportional to the concentration of the absorbing substance in the solution. To create a calibration curve for caffeine, solutions of known concentrations of caffeine are prepared and their absorbance values are measured using a spectrophotometer. The absorbance values are then plotted against the corresponding caffeine concentrations, resulting in a

Table 1. Quantity of caffeine recovered from different tea samples from extraction process.

S. No.	Tea Samples	Amount of Caffeine Extracted (gms)	Percentage of caffeine (%)
1.	Assam tea	0.60	1.20
2.	Hyderabad local tea	0.65	1.30
3.	Red label tea	0.70	1.40
4.	Wagh bakri	0.85	1.70
5.	Tata tea	0.92	1.84
6.	Taj Mahal	0.95	1.90

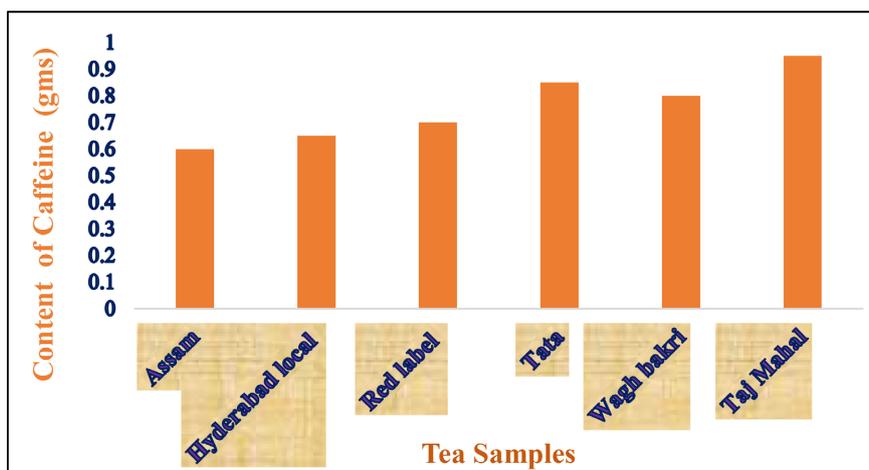


Figure 4. Graphical representation of extracted caffeine from different tea samples (gms).

linear relationship. This calibration curve can then be used to determine the concentration of caffeine in an unknown sample by measuring its absorbance and comparing it to the calibration curve. It is important to use a consistent method and instrument for measuring absorbance to ensure accuracy and reproducibility of results. Additionally, the choice of solvent and wavelength of light used for measurement can also affect the accuracy of the results. The results showed that the caffeine content in the tea samples varied widely, with the highest levels found in Taj Mahal tea and the lowest levels in Assam tea. The caffeine content in Red label and Wagh Bakri teas was found to be intermediate respectively. The results of this study are consistent with previous studies that have reported the caffeine content in different tea types [17] [18]. However, the use of a UV-visible spectrophotometer provides a simple and reliable method for the detection of caffeine in tea samples. This method is more sensitive than other methods such as HPLC and GC-MS, which require more complex and time-consuming sample preparation. The usual absorbance values for every dilution are delivered in **Table 3**. These absorbance values were used to generate the calibration curve for examining caffeine content, and wavelength value at 274 nm as shown in **Figure 5** and **Figure 6**.

Caffeine is rapidly in addition to generally engrossed in the liver compartments

Table 2. Reported quantity of caffeine in different tea and food samples.

Tea Samples/Food Samples	Caffeine Level (%)
Soft Drink	0.02 [13, 18]
Chocolate	0.074 [13, 18]
Green tea	3.11 [13, 18]
Black tea	3.57 [13, 18]
Earl grey tea	4.27 [13, 18]
Mambila beverage tea	0.040 [1]
Chinese green tea	0.023 [1]
White tea	88 [5]
Red tea	60 [5]

Table 3. Absorbance of standardization solutions of caffeine.

S. No.	Tea Samples	Concentration (ppm)	Absorbance
1.	Assam tea	1	0.010
2.	Hyderabad local tea	5	0.370
3.	Red label tea	10	0.700
4.	Wagh bakri	15	0.985
5.	Tata tea	20	1.195
6.	Taj Mahal	25	1.537

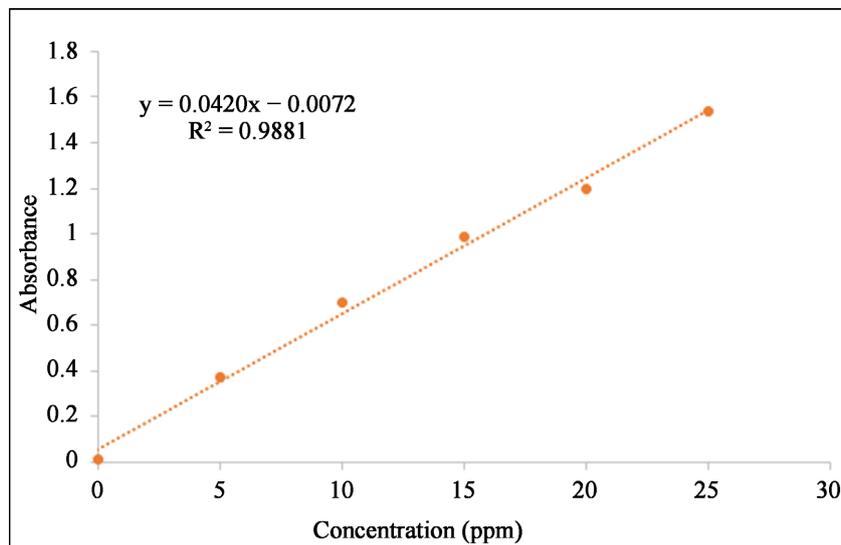


Figure 5. Calibration curve for examining caffeine content.

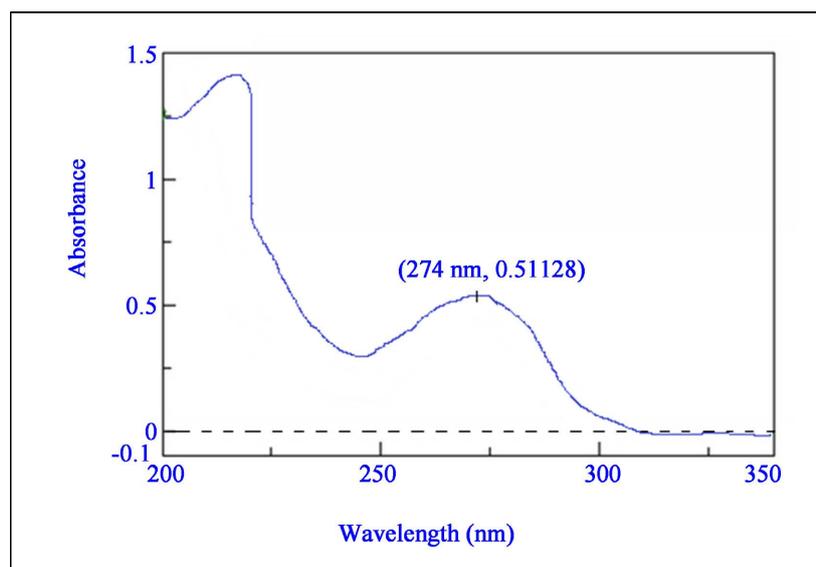


Figure 6. Graphical representation of absorbance versus wavelength of Caffeine.

to model dimethyl surplus monomethylxanthines, dimethyl also uracil derivatives and monomethyluric acids [19]. Caffeine consumption fluctuates through diverse forms of drinks then in diverse populace clusters [2] [20] [21]. The utmost renowned severe properties of caffeine ingestion are motivation of brain purpose also development in attitude, as well as bodily presentation [22]. However, alongside the earlier eternities, several epidemiologic studies have correlated diffident coffee ingestion through process of decreasing in the comparative opportunity of progress of prolonged deteriorating ailments and decease [23] [24] [25] [26], besides that caffeine is one of the developments answerable for numerous other benefits. They contain reduction in the plausibility of Parkinson's also Alzheimer's illnesses along with hepatoprotective symptoms. The devices include anti-inflammatory and antioxidant actions, amongst other. Caffe-

ine pays an expectant outcome on resolve furthermore of trial capability allocated to the overhead-revealed neuronal devices that stimulate a system of biotic responses, which marks it an ergogenic reserve [27]. The biosynthesis of Caffeine from tea has been displayed in **Figure 7**. Caffeine also upsurges synchronization [28] plus diminutions the estimation of tiredness and ache [29]. Caffeine ingesting has also remained related with the incidence of arrhythmias in persons. It profits a traditional impetus of myocardial nerve, leading to distended emotion amount in addition to strength of decrease [18]. Several studies support caffeine's ability to supplement defiance and alertness [30] [31], training repetitive [32], the fleetness through which information is managed, consciousness, kindness, and response period [33]. Furthermore, investigation has endorsed that caffeine can sustenance in dipping symbols associated with Parkinson's disease (PD) for instance the weakening of distant and minor motor services, and trembles [34] [35].

Since PD is a neurodegenerative illness which marks in advanced damage of dopaminergic neurons of the substantia nigra, caffeine a nonselective adenosine antagonist is supposed to relief in refining the enactment of the dopaminergic scheme by obstructive the AA2 receptors, therefore, motivating dopamine discharge [37].

Impression of caffeine on Several Parts of Human Physique

There's a high-minded bargain of wrangle about almost the wellbeing impacts of caffeine, and whether these impacts are basically positive or negative. Caffeine utilization has a few shocking benefits on the indications of individuals enduring from certain unremitting conditions, as well as really bringing down the chance of some maladies within the to begin with place.

Sensitivity Acuity

Circulating adenosine heighten the sweetened tasting signals within the taste buds [38]. Caffeine squares adenosine receptors [39] and adversely influences the discernment of sweet tastes [40]. The severe taste of caffeine may have an impact on how the human body responds. There comes about of a few ponders recommend that sharp products can upgrade execution additionally allow the

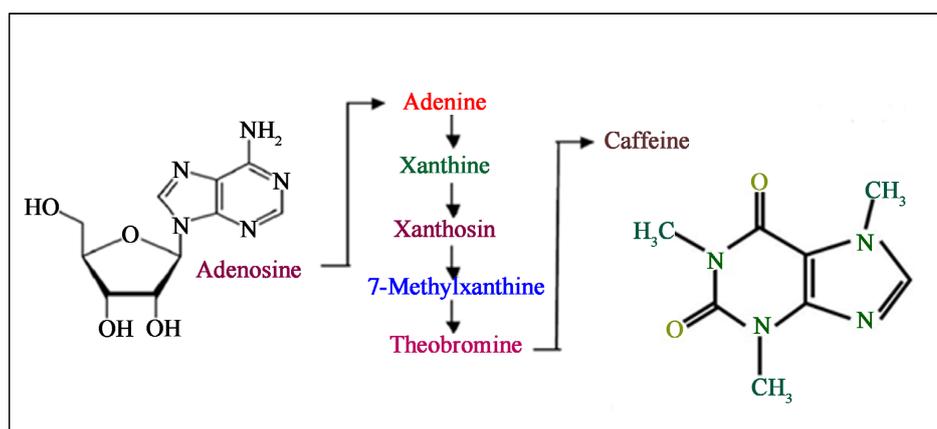


Figure 7. Biosynthesis of Caffeine from tea [36].

signal to our brain that the life form is prepared for activity [41]. These viewpoints are still to be inspected, and appear to be possibly vital for investigate within the field of sports medication.

Parkinson's Ailment

Parkinson's contagion (PD) may be a neurodegenerative malady with engine and non-motor indications [42]. A few thinks about detailed that caffeine admissions are useful for PD patients, additionally to Alzheimer's infection. To begin with of all, caffeine admissions are related with lower chance of Parkinson's ailment. Caffeine moreover appears to secure humanoid mind cubicles. In a meta-investigation that united 13 considers, consumers of customary coffee—but not decaffeinated coffee, had a 30% lesser hazard of creating Parkinson's infection than non-drinkers. There are at slightest six considers demonstrating that individuals who sip coffee on a normal premise are up to 80% a lesser amount of possible to create Parkinson's, with three appearing that the further coffee they sip, the lesser the menace.

Understanding of Ache

Caffeine may be a component of numerous over-the-counter torment relievers and medicine drugs since the vasoconstricting and anti-provocative impacts of caffeine performance as an accolade to anesthetics, in a few cases, expanding the viability of torment reliefs via equal to 40% [43]. The component by means of which caffeine can diminish torment sensation shows up to be thoroughly correlated to its coordinate impacts on adenosine receptors, particularly through the vital obstructive of those receptors that impact torment signaling or piece fringe adenosine receptors on tangible afferents.

Psychological Vigor

Discouragement, uneasiness, and suicide are getting to be a progressively common issue among children and grown-ups. This prompts not as it were the hunt for unused treatments, but too for the reasons of these afflictions more closely. Over a long time, caffeine has been examined as a potential defensive or hazard calculate for psychiatric disarranges [44]. It has as of now been recorded that caffeine admissions is related with depressive symptoms.

The Invulnerable System

The safe framework is composite and is complete of different cubicles and proteins that are dependable for the physique's confrontation, especially through the safe reaction, which may be a weapon against remote antigens [45]. Increasingly consideration is remunerated to the two-mode connection amongst the resistant framework and diet [46]. The part of caffeine in the working of the safe framework comes to the cells themselves.

Bladder Malignance

The defensive part of caffeinated chocolate in bladder tumor is still being well-thought-out. A few meta-analyses consider appeared no relationship between coffee utilization and chance of bladder cancer [47], but then other meta-investigates of event-controller thinks about give restricting information, proposing a straight increment within the risk of bladder cancer together with

the sum of coffee admissions (15% - 29% increment for 2 - 4 glasses of coffee a daytime, relatively) [48]. In outline, the influence of caffeine on the urinary framework may be coordinate or backhanded, also caffeine can perform over the items of its digestion system, which are defaecated through the kidneys, and this handle hinge on stage of development and gender. Ladies are further probable to involvement urinary incontinence issues. There are too positive impacts such as a decrease in kidney stone arrangement much appreciated to diminish within the quartz-obligatory measurements of renal tube-shaped epithelial cells, and lesser renal fibrosis since of its capacity to dispense with ROS. The part of caffeine in bladder malignance is still.

Oxidative Strain

Oxidative Strain is the outcome of the adverse impact of receptive oxygen in addition to nitrogen types, which, beneath advantageous situations, are a source of the trouble of oxidative-antioxidant adjust. Receptive oxygen and receptive nitrogen are delivered persistently within the humanoid physique over oxidative digestion system, mitochondrial bioenergetics, and safe work. In a roundabout way, supplements that actuate inflammation are too included within the advancement and upkeep of oxidative push. There are diverse sorts of bases of nutriment-intermediated oxidative stretch that show a vital part within the improvement of numerous human maladies [49]. Caffeine can demonstrate oxidative stretch of shifting seriousness liable on the individual's age, sex, welfare, body mass, BMI, and way of life, additionally reliant on kind, dosage, also approach of arrangement of coffee [50].

4. Conclusion

Overall, while caffeine in tea can have both positive and negative effects, it is generally considered safe and can be enjoyed as part of a healthy diet. However, it is important to be mindful of caffeine intake and to limit consumption if experiencing negative effects. Caffeine is an operative approach to stand both cognitive as well as physical deprivation accompanying with sleep deficiency. It could be established this caffeine remains a prospective regular, antimicrobial mediator in contradiction of different microorganisms, and so, could be recycled in nutrients as a natural protective, to regulate their development. It's important to note that caffeine sensitivity can vary from person to person, and some people may experience negative side effects from consuming too much caffeine, such as anxiety, insomnia, or digestive issues. It's also important to be aware of other sources of caffeine in your diet, such as coffee, soda, or energy drinks. In terms of applications, knowing the caffeine content in tea can help you make informed decisions about your caffeine intake and choose the right type of tea based on your individual needs and preferences. For example, if you're sensitive to caffeine or trying to cut back, you may opt for a white or green tea over a black tea. On the other hand, if you need a boost of energy, a cup of black tea may be a better choice. Additionally, adjusting brewing time and water temperature can also help control the amount of caffeine in your tea. This study shows various

volume of caffeine extant in different tea leaves available and used by the people of two different states of India. In conclusion, the UV-visible spectrophotometer method is a simple, reliable, and sensitive method for the detection of caffeine in various tea samples. Accurate determination of caffeine content is essential for quality control and labeling of tea products, and this method can be easily implemented by tea manufacturers and researchers.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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