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Type of the paper: Review

Physiological effects of caffeine and its congeners present in tea and coffee beverages

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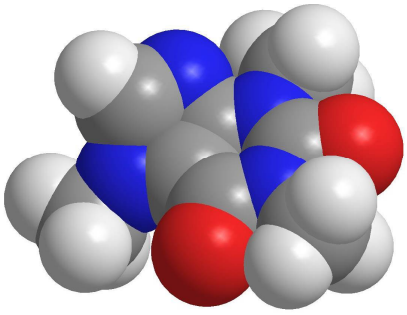
Running title: Caffeine



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Abstract: Tea and coffee are the most commonly used beverages throughout the world. Both decoctions are rich in small organic molecules such as phenolics/polyphenolics, purine alkaloids, many methylxanthines, substituted benzoic and cinnamic acids. Many of these molecules are physiologically chemopreventive and chemoprotective agents against many severe conditions such as cancer, Alzheimer, Parkinsonism, inflammation, sleep apnea, cardiovascular disorders, bradycardia, fatigue, muscular relaxation, and oxidative stress. Caffeine, a purine alkaloid, is a common metabolite of both tea and coffee aqueous decoctions and its concentration in tea/coffee depends on the fermentation process, preparation of the water extract and quality of tea leaves/coffee beans. A 250 ml of a coffee cup contains 100-150 mg caffeine while the same volume of strong tea contains 25-40 mg caffeine. The present paper presents the potential of caffeine as a potent chemopreventive agent that can be used for numerous physiological disorders.

Keywords: Caffeine, methylxanthine, chlorogenic acid, caffeic acid, inflammation, antimutagen, anticancer, antioxidant.

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1. Introduction

Caffeine from cocoa beans was first discovered in Ethiopia, and since then it has been a part of the global history for thousands of years. German scientist Friedrich Ferdinand Runge was the first to isolate pure caffeine in 1820 and the word caffeine is derived from the German word '*Kaffee'* and the French word 'Café.' Since then, the psychoactive substance caffeine is one of the most researched medications with more than 30,200 research publications. During 2016 more than 2000, while in 2017 in access of 1200, and in 2018 till March, more 300 publications have appeared on different aspects of caffeine. This data shows the global research interest in caffeine and related methylxanthines. On a daily basis, around the world, more than two billion cups of tea and coffee are consumed making it the most frenzied beverage after water and the second most traded commodity after oil when both are deliberated as black gold. Due to its diverse pharmacological activities, caffeine merits a focus for further scientific research.

The black decoction of coffee contains hundreds of compounds, the composition, and concentration of which depends upon many factors including cultivar, the origin of coffee and the method of its preparation. Roasting of coffee beans results in about 1000 volatile compounds, and 35 aroma compounds and caffeine is a common metabolite in both tea leaves and coffee bean. On the average, tea leaves contain 3% caffeine depending upon the tea quality and method of its preparation. An 8 fl oz. cup of coffee contains 100-150 mg caffeine while black and green tea provides 40-60 mg and 25-40 mg of caffeine, respectively. The total phenolic content of varies 19.2-108.6 mg mL^{-1} with an abundance of quinic acid and gallic acids (1). The seasonal variation of theanine, methylxanthines, and catechins such as Afzelechin (1a), in 21 cultivars of *Camellia Sinensis,* has been recently published [1, 1a].

Caffeine, a 1,3,7-trimethylxanthine ($C_8H_{10}N_4O_2$), purine alkaloid (2) and structurally related to adenosine (5), has an astringent taste. It is produced in more than 60 distantly related plant species such as coffee beans, tea leaves, kola nuts (*Cola acuminate*, Beauv and *Cola nitida*, Vent), cocoa beans (*Theobroma caca*o) and even in some citrus species [2]. In some cases, methylxanthines are recognized as remarkable markers for facilitating chemotaxonomy of plants while caffeine (2), theophylline (17), saxitoxin, and theobromine (4) are the representative examples of this class of methylxanthines.

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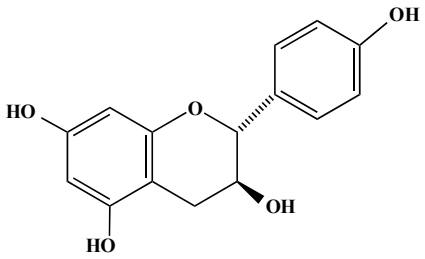
The bitter taste of black tea (*Camellia Sinensis*) and roasted coffee (two species of *Coffee*; *C. arabica and C. robusta*), is recognized due to the variable amounts of caffeine and 5-caffeoylquinic acid (6). A link between the mouth cavity, nasal cavity, and the brain is known, and caffeine can cross the blood-brain barrier [3].



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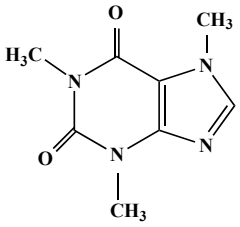

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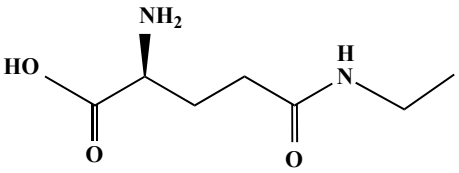

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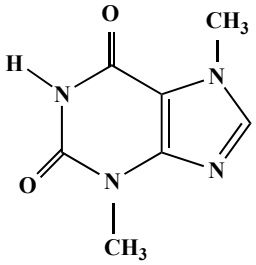

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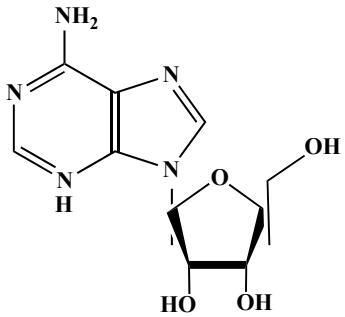

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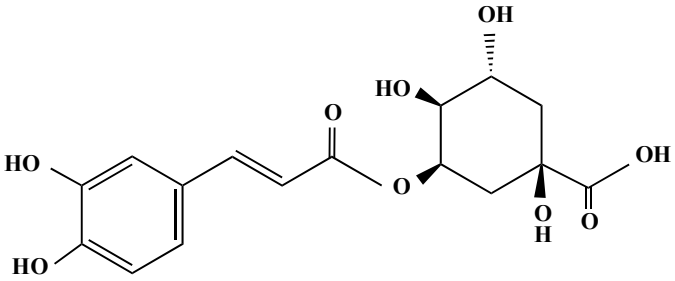

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Caffeine is frequently used as a CNS stimulant with numerous physiological consequences on cardiovascular, renal, respiratory, immuno-modulation, smooth muscle, mood forming, memory, alertness, and cognitive performance [4]. Due to their ergogenic effect, caffeine and creatine (10) are the two most widely used compounds in sports with a sizeable interindividual variation of caffeine absorption within and between groups [5]. As complex forming agents, methylxanthines, like caffeine can interact with lysozyme, bringing about a conformational change in the cationic proteins, thus lowering the antimicrobial activity. Synergistic cytotoxicity and mechanism of caffeine and lysozyme on hepatoma cells have been recently reported [6].

Tea and coffee extract, apart from methylxanthines, contain many other phytochemicals such as L-theanine (N-ethyl-L-glutamine) (3) that also affect mood, cognition, sustained attention and suppression of distraction [7]. Theanine enhances the toxicity when combined with strychnine or phenobarbital sodium [8]. The other tea congener, theobromine (4), is an inhibitor of physiologically essential phosphodiesterases and it increases intracellular second messenger molecules cAMP and cGMP in mice [9]. In green coffee beans, along with caffeine, chlorogenic acid, the most popular weight loss ingredient and its derivatives have also been identified [10].

While hot tea and coffee extracts contain variable amounts of caffeine, heating, and processing of coffee beans convert 5-caffeoylquinic acid (neochlorogenic acid, 6) into various phenylindanes (7) and lactones such as 3-caffeoylquinic-1,5-lactone (8) and 4-caffeoylquinic-1,5-lactone (9) [11]. Melanoidins, present in coffee, are the byproducts produced by Millard reaction between amino groups of proteins and carbonyl groups of reducing sugars and are poorly understood [12]. This convolution of the tea and coffee extracts shows a highly complicated mixture of hundreds of small molecules that have not even been investigated.

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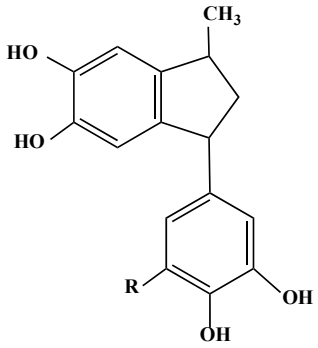

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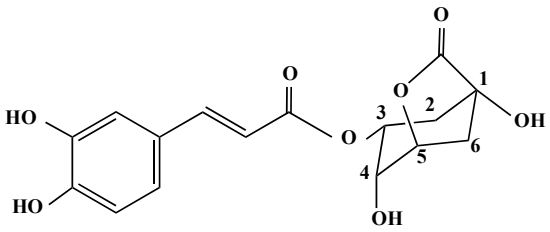

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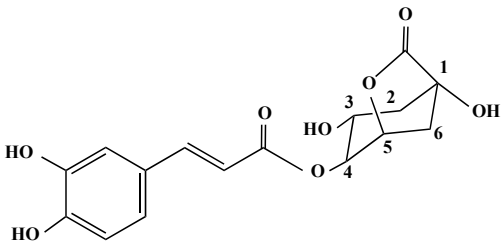

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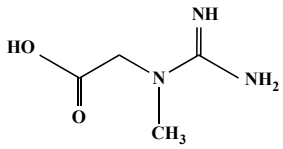

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2. Caffeine: Bioavailability and dose response

For healthy adults, the recommended amount of caffeine is around 400 mg/day, and an overdose may occur if one absorbs more than this amount. A moderate dose of caffeine may cause anxiety in consumers, and it can lead to central nervous system consequences in heavy consumers. Liver diseases, pregnancy, oral use of contraceptives, and interaction with therapeutic drugs may lower the metabolism and its excretion consequently increasing its concentration in the body. The safe amount of caffeine differs for everyone, and it depends on age, weight, gender and the state of general health. These variables make it difficult to know the exact amount of caffeine that can lead to an

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overdose or an inevitable consequence. Therefore, at a young age, a dose of 100 mg/day is safer.

The half-life of caffeine (HLC) is species dependent and is recorded as 3-5 hr. Thus, in an adult human, HLC is 0.7-1.0 hr., rats, and mice, 1-1.6 hr., rabbits 3-5 hr., monkeys, and baboons, 4-4.3 hr., while in dogs it is 11-12 hr [13]. After consumption, the absorption rate of caffeine is high while its excretion rate is low. Due to its rapid absorption and low excretion rate, the peak plasma concentration is achieved in 30-90 minutes after ingestion with a mean plasma half-life of 5 hr. It is excreted through urine that carries around 1-3% caffeine, and its clearance rate is documented as 0.078 L/h/kg. Conversely, the clearance rate may also depend on many other factors such as the state of health, pregnancy, and smoking, and gender [14].

Caffeine overdose, 150-200 mg/kg BW, can be lethal and causes overstimulation, sleeplessness, apnea, restlessness, convulsions, irritability, delirium, ventricular tachycardia, arrhythmia, hyperventilation, emesis and even tremors [15, 16]. After a prolonged consumption, its sudden withdrawal can also be unsafe and may cause anxiety, depression and mood disorders [17]. These kinds of maladies have been examined in a neotropical freshwater teleost, *Prochilodus lineatus* [18].

Caffeine can cross the placenta, and its higher concentration may cause abortion or miscarriage. A relationship between caffeine dose response, with a decreased depression and loss of pregnancy, low birth weight, preterm birth, hyperuricemia and endometrial cancer is known [19].

Being a potent medication, pharmacological effects of caffeine can occur even at low doses, but their severity is influenced by a wide individual variation and the development of tolerance. At the doses consumed by humans, there is little evidence, at present, to suggest the effects of caffeine on reproduction, teratogenesis, tumor formation or the incidence of myocardial infarction. Monterio and his co-workers [20] have reported its structure-activity relationship (SAR).

Table (1) shows the amount of caffeine in some of the conventional sources of caffeine, according to the Center for Science in the Public Interest.

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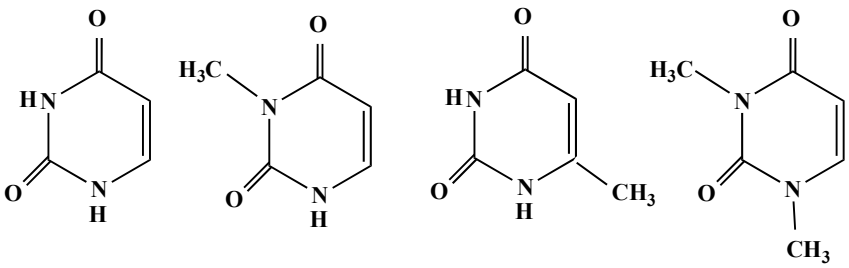
Table 1. Amount of Caffeine in different beverages

Serving	Size	Caffeine (mg)
Black coffee	12 oz.	260
Black tea	8 oz.	30-80
Soda	12 oz.	30-70
Red Bull	8.3 oz.	80
Chocolate bar (dark)	1.45 oz.	20

3. Caffeine: Metabolism and Biosynthesis

Caffeine (14) is one of the most studied methylxanthines in human and animals. Metabolism of caffeine is species dependent and produce demethylated and hydroxylated metabolites, resulting in the synthesis of uracil (10), 3-methyluracil (11), 6-methyluracil (12) and 1,3-dimetyluracil (13). Caffeine is also catabolized into a variety of diverse phenolic acid metabolites including chlorogenic acid (ester of caffeic acid and quinic acid), neochlorogenic acid (6), caffeic acid, and ferulic acid, that are eliminated by the kidney as sulfates, glucuronic acid, quinic acid or lactone conjugates (8, 9) [8].



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The metabolism of caffeine takes place in liver producing at least 17 metabolites excreted in urine, feces, saliva, and breast milk. The 3-demethylation of caffeine giving rise to 1,7-dimethylxanthine (paraxanthine, 18), is the dominant route of caffeine

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metabolism [22]. The first two demethylated metabolites of caffeine are theophylline (1,3-dimethylxanthine) (17) and theobromine (3,7-dimethylxanthine) (4). However, paraxanthine (18) is the primary product of catabolism of caffeine [22]. Although caffeine, theophylline (17) and theobromine (4) are structurally related, their physiological properties are reasonably distinctive. For example, the diuretic activity of these compounds is in the following order, caffeine<theophylline<theobromine but caffeine has fewer side effects compared with theophylline (14) [23]. The biosynthesis and metabolism of caffeine are shown in Fig. 1, 2.

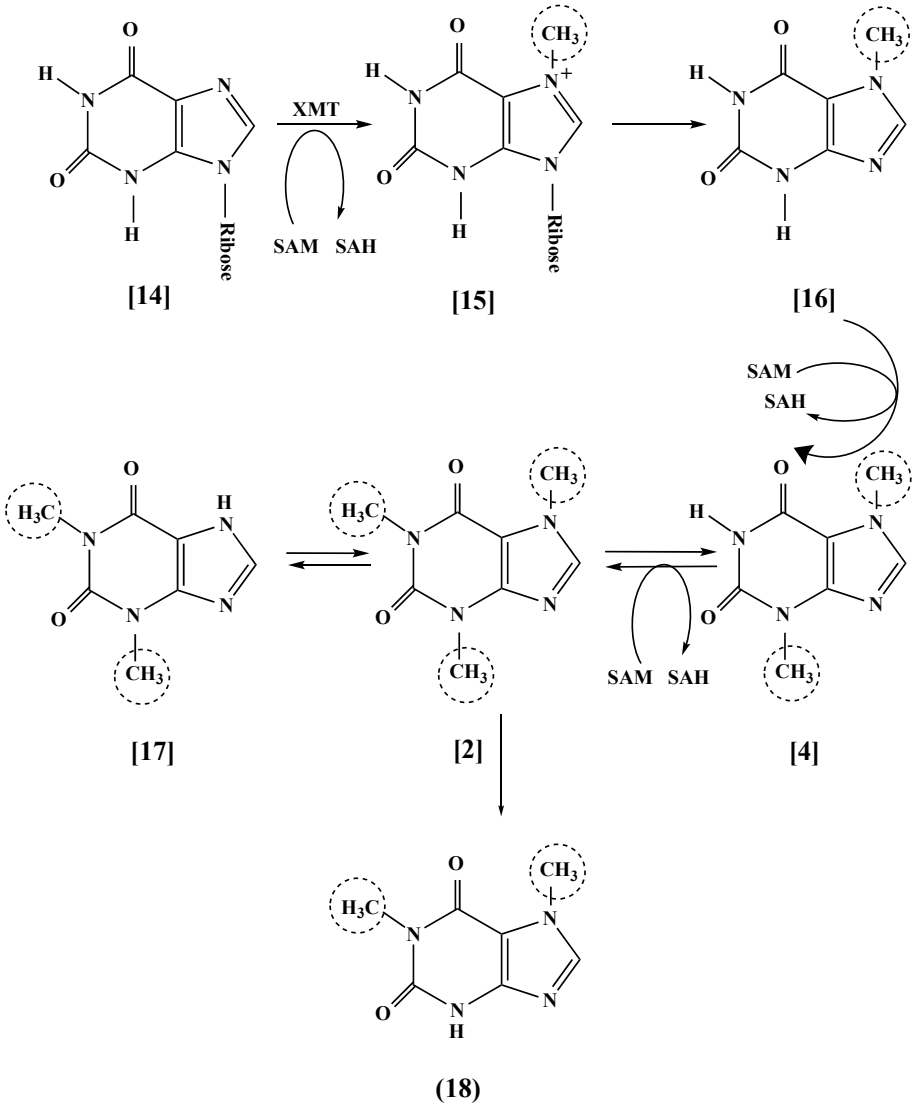

Fig. 1 Biosynthesis and Catabolism of Caffeine
SAM = S-adenosylmethionine

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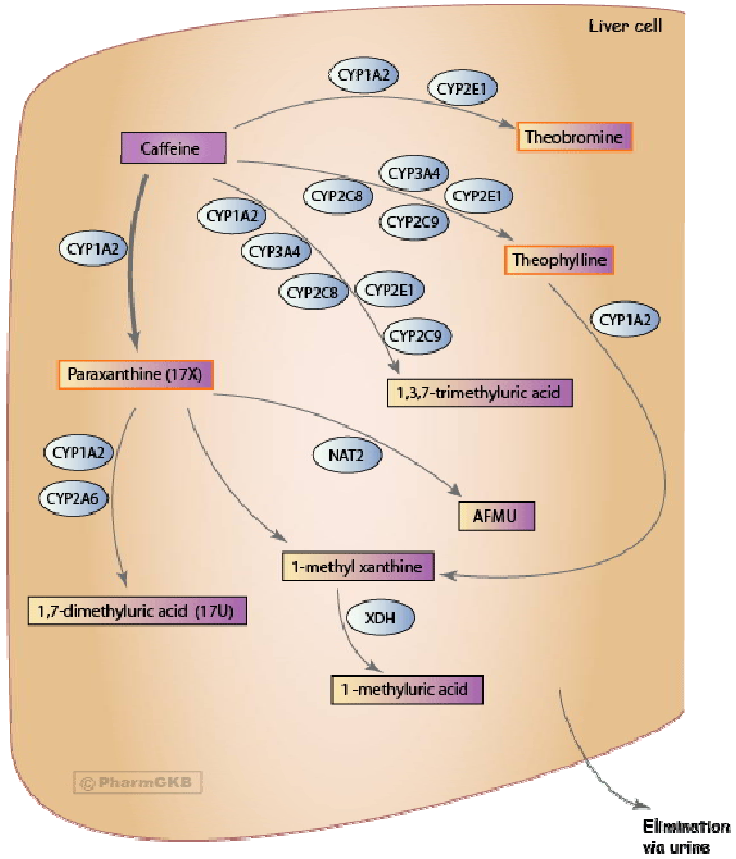

Fig. 2 Caffeine metabolism, with permission from Caroline F. Thorn, Eleni Aklillu, Ellen M. McDonagh, Teri E. Klein, Russ B. Altman. PharmGKB summary: caffeine pathway. Thorn Caroline F, Aklillu Eleni, McDonagh Ellen M, Klein Teri E, Altman Russ B in Pharmacogenetics and genomics (2012). PMID: 22293536. PMCID: PMC3381939. DOI: 10.1097/FPC.0b013e3283505d5e. https://www.pharmgkb.org/pathway/PA165884757

In rats and mice, caffeine is metabolized to theophylline (17), theobromine (4), paraxanthine (18), and 1,3,7-trimethyluric acid (22). In human, caffeine is metabolized through 3-demethylation to give paraxanthine (18) and other products that appear as 1-methylxanthine (19), 1-methyluric acid (20), 1,7-dimethyluric acid (21) and 5-acetylamino-6-formylamino-3-methyluracil (23). Its metabolism is induced by hepatic cytochrome oxidase 1A2 where it can be converted into caffeine citrate making it a more water-soluble salt. This facilitates caffeine interaction with specific medications that are

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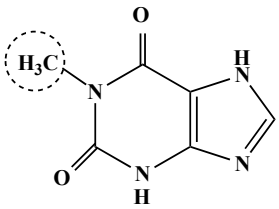
metabolized by CYP1A2 regulating drug-caffeine interaction. Metabolism of caffeine resulting in numerous xanthines is given in Fig. 1, 2. The coffee genome provides an insight into its biosynthesis, Fig. 3 [24].



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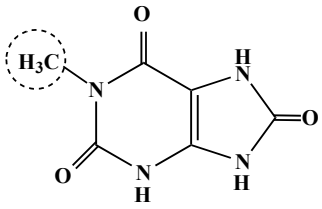

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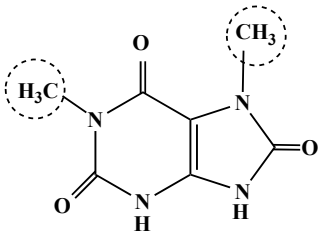

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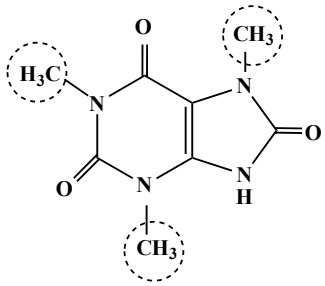

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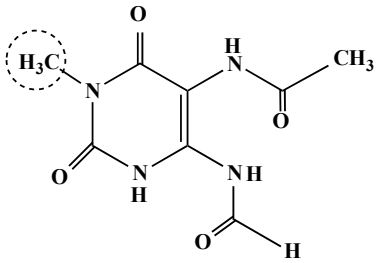

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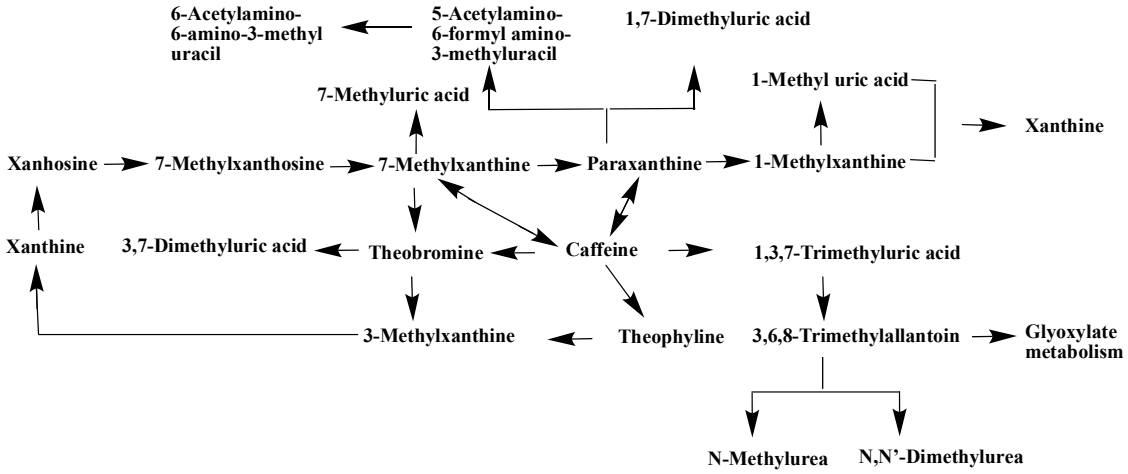

Fig. 3 Caffeine metabolism (From KEGG source record:ce 100232

4. Caffeine: pregnancy and other health effects

Caffeine and theophylline are interconvertible molecules. Asthmatic pregnant women consuming theophylline as a bronchodilator can convert theophylline into caffeine causing transient toxicity in the fetus [25]. The placenta and blood-brain barriers are readily crossed by caffeine causing severe toxicity to neonates when caffeine level in serum, crosses 50 µg/ml [25]. Many smokers including women take tea/coffee while smoking and it can be deleterious particularly during pregnancy since caffeine interacts with cigarette smoke resulting in a reduce placental and growth weights while there is evidence of the adverse effect of methylxanthines on the fetus during the first third parts of pregnancy compared with later parts of pregnancy [26]. In clinical trials, neonates receiving caffeine have been reported to suffer either hypoglycemia or hyperglycemia [27].

During pregnancy, caffeine metabolism declines but it elevates the serum level of the methylxanthines in the mother, and it can lead to a situation where the fetus could be at risk with caffeine/methylxanthines toxicity.

In healthy adults, no accumulation of caffeine or its metabolites, in any body organ, is reported, even at higher doses, therefore, the risk of caffeine is insignificant in male adults. Nonetheless, an interaction of caffeine and other methylxanthines with reproductive hormones like estradiol and interference with progesterone metabolism via aromatase inhibition, in women has been recorded [28]. Therefore, caffeinated beverages

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should be taken with caution. Although no association between gastric ulceration and caffeine/theophylline has been established, dental caries in rat model has been reported [29]. However, dental caries may also be related to organic alterations of salivary composition induced by caffeine.

5. Caffeine and Melatonin

The mechanism of induction of alertness by caffeine is through blocking neurotransmitter adenosine in brain cells, and this is done by the interaction of adenosine receptors with caffeine. Melatonin (N-acetyl-5-methoxytryptamine, 26), a hormone produced by the pineal gland in the brain, induces its effect opposite to caffeine, the feeling of drowsiness, and its inhibition leads to a wakeful phase. Melatonin and caffeine are thus antagonistic, and consumption of caffeine can decrease the production of melatonin in the body. In the pineal gland, two enzymes namely serotonin-N-acetyltransferase and hydroxyindole-O-methyltransferase, catalyze the conversion of brain serotonin (5-hydroxytryptamine, 24) to melatonin. Serotonin is primarily produced in the enteric nervous system of the gastrointestinal tract. The intermediates, N-acetylserotonin (25) and 6-hydroxy-melatonin (27) are both proxy radical scavengers better than melatonin (26) or Trolox and protect against oxidative stress.

The biosynthetic pathway of melatonin (5-hydroxytryptamine) is shown in (Fig. 4). Many drugs such as alcohol, nicotine, and marijuana can increase the level of serotonin in the human brain and cause a burst of serotonin triggering the feeling of elation. Contrarily, caffeine lowers serotonin level in the brain. The binding of caffeine to adenosine receptors may also affect conversion of tryptophan-serotonin (24)-melatonin (26) pathway (Fig. 4) in the pineal gland of the brain. The antioxidant, phyto-melatonin has also been reported in coffee and many other plants [30].

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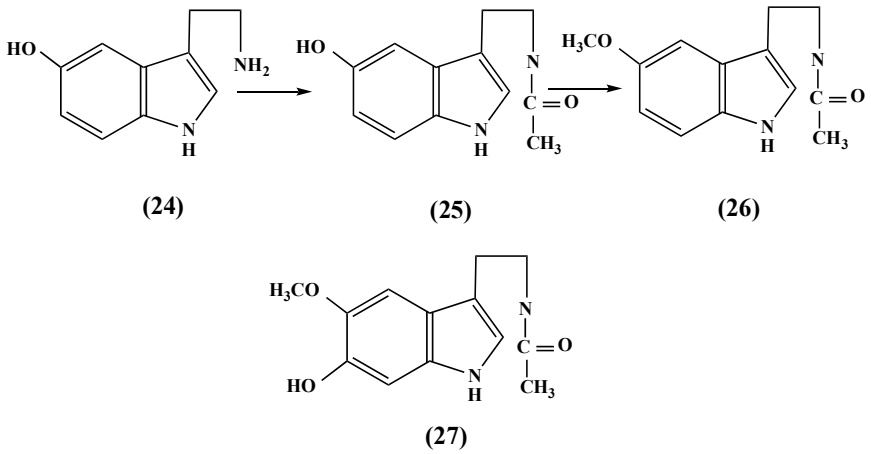

Fig. 4 Biosynthesis of melatonine

6. Caffeine: Physiological Activities

Adenosine receptors are present in many tissues including brain and human skeletal muscles, and caffeine/theophylline are both adenosine receptor antagonists inhuman. Caffeine binds with the adenosine receptors increasing nerve cell activity, producing adrenaline and inducing wakefulness [31]. A common mechanism, for the brain stimulation with addictive substances such as amphetamines, cocaine, and heroin is shared with caffeine. However, in some cases, at high blood concentrations (KI =9.8 mM), methylxanthines may be restricted to the brain causing an injury to the nervous system.

Purine based methylxanthines, including caffeine and theophylline, are known for their stimulatory action and have been successfully used in the control of preterm infants apnea/bradycardia and reduce the incidence of bronchopulmonary dysplasia [32]. The stimulant caffeine is also used to control nervousness, exhaustion, fluid retention related to menstruation. In three months old offspring rats, exposure to caffeine induces changes in neuroendocrine metabolism [33]. Interestingly, a caffeine-induced body weight loss through lipolysis and a decrease in stroke prevalence has also been reported [34].

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In the literature, there are contradictory reports about the relationship between caffeine and apnea. Tian and co-workers [35] have reported that there is no correlation between episodes of apnea and caffeine concentration in serum in neonates treated with caffeine citrate, in the post-intubation period. On the contrary, Pinheiro et al., [36] have reported a positive correlation between caffeine consumption and obstructive sleep apnea. A positive relationship also has been established in tachycardia and plasma caffeine concentration [37], and this may be due to an overproduction of adrenaline after binding of caffeine with adenosine receptors. It may be of advantage, as caffeine is the safest and most commonly used drug for respiratory stimulation and it even reverses the action of opioid-induced respiratory depression [38]. In other studies, caffeine is known to inhibit depression and memory dysfunction in rats [39].

Addition of caffeine (0.5-2.0 mM) to the cytosolic side of the membrane increases the probability of the calcium-activated calcium-release channel which increases the frequency of the channel opening without a significant alteration in the duration of open events causing a skeletal muscle contraction and reduced bone mass and increased fracture risk. This effect has been observed at both 0.1 and ten μM-activating cytosolic calcium [40].

In adipose tissue, for energy metabolism, caffeine catalyzes the process of lipolysis that releases free fatty acids in plasma, and their oxidation generates energy pool through β-oxidation and citric acid cycle. After caffeine consumption, the liver releases sugar into the bloodstream offering extra energy and alertness. However, caffeine is also known to inhibit glucose transport by binding at the GLUT1 nucleotide binding site thus lowering blood glucose [41]. In addition to tea and coffee, Guarani seeds (*Paullinia cupana*) are known for their numerous pharmacological properties including energy stimulant, antigen toxic, hyperlipidemia, and antidepressant, etc., and caffeine is present in its leaves, nuts, seeds, and other parts, with its highest content 2.5-5% in the seeds [42]. In plants, methylxanthines are known to introduce a chemical defense mechanism against fungal, insect and pest invasion [43].

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7. Caffeine: Analgesic activity

Caffeine itself has no analgesic activity. However, many over the counter analgesics, antipyretics and anti-inflammatory medications mixed with caffeine, are available that have proved to be better analgesics. These medications include aspirin, phenacetin, acetaminophen, and codeine. Caffeine potentiates the analgesic effect of these medications by increasing the absorption of salicylates resulting in pain reduction. Caffeine administration with ibuprofen kills pain in postoperative adults while, when admixed with naproxen, it enhances the permeation of the same with improved amelioration property [44]. Better pain management and inhibition of the inflammatory mediators with fibers of the spent coffee grounds containing short-chain fatty acids has been reported [45]. Millions of people have fibromyalgia, a chronic pain condition and current pharmacotherapies are not very useful and poorly tolerated. Caffeine in combination with other painkillers such as carisoprodol or acetaminophen has produced better results in the management of fibromyalgia in adults. Caffeine, when mixed with (S)-ketoprofen for treatment of arthritic pain, its pharmacokinetics and pharmacodynamics are altered. Caffeine is a powerful antioxidant, and when co-administered, it improves antioxidant activity and anti-choline esterase effect of donepezil, a drug used to strengthen dementia [46]. Similarly, caffeine augments the antidepressant action of mianserin and agomelatine drugs [47]. However, using combination drugs, caution needs to be taken since drug-drug interaction may cause adverse health effects or even they may be fatal.

8. Caffeine: Psychotropic activity

Caffeine is the world's most widely consumed psychoactive substance. After entering the bloodstream through the stomach and small intestine caffeine quickly passes through the blood-brain barrier and causes effects within 15 minutes. Caffeine when co-administered with N-methyl-D-aspartate (NMDA) receptor ligands in mice, an antidepressant-like synergistic impact is noticed [48]. NMDA is a specific agonist for glutamate, a neurotransmitter that binds with its receptor.

The mechanism of caffeine psychotropic activity is through its binding with adenosine receptors, down-regulating the activity of CNS and stimulating the brain

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366 respiratory centers [49]. Since it is a competitive antagonist of neurotransmitter,
367 adenosine and its receptors, it helps in the release of exciter neurotransmitters thus
368 stimulating CNS and increasing vigilance and generating anxiety. A beneficial effect of
369 caffeine admixed with aspirin concerning mood and performance is known [50].
370
371 **9. Caffeine: Interaction with hormones**
372 Caffeine relaxes smooth muscle, stimulates the cardiac tissue, plays a role in diuresis
373 and modulates intracellular theophylline (17), which is known to induce osteopenia by
374 altering calciotropic hormones [51]. Protection of neurological hypoxic tissue by caffeine
375 is well reported indicating a dilation of the blocked blood vessel in hypoxic-ischemic
376 brain injury [52]. Therefore, the influence of caffeine on the brain vasculature and
377 cerebral blood flow increase anxiety and modifies neuroendocrine signaling [53].
378 Caffeine combined with progesterone has been found to be useful to treat neonate apnea
379 [54]. At the same time, a reduction in thyroid hormone T3 caused by tea consumption
380 and endocrine hormone disruption with caffeine has been reported [54, 55]. An
381 interaction of caffeine with insulin, blood sugar level, and interleukin-10 are also known
382 [56]. α-glycosidase inhibit the hydrolysis of oligosaccharides/disaccharides in the small
383 intestine, thus lowering blood glucose and an inhibitory effect of Yixing black tea
384 extracts on α-glycosidase resulting in a decreased blood sugar level [57].
385
386 **10. Caffeine: Anticancer activity**
387 Numerous purine alkaloids are known for their antitumor and anti-inflammatory
388 characteristics [58]. Thus, caffeine, along with many other positive health effects, is
389 recommended for its ovarian anticancer activity and the risk of postmenopausal breast
390 cancer [59].
391 As mentioned above, caffeine alone or in combination with other drugs exhibit/potentiate
392 anticancer activity, and the mechanism of xanthine action involve targeting tubulin
393 polymerization [60, 61]. Thus, natural purine alkaloids and many xanthine derivatives of
394 caffeine with anticancer activities have been synthesized [61, 62]. Exploring new
395 xanthine derivatives for their anticancer activity can be a rewarding area of synthetic
396 organic chemistry. Tumor cells need a constant supply of nutrients and oxygen for their

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growth, and angiogenesis facilitates this. Caffeine and theophylline cause a decrease in mRNA of fibroblast growth factor 19, which is known to modulate the uptake of glucose and also regulate glucose transporter 1 inhibitors that are overexpressed in cancer cells [63].

Xanthine derivatives are also known to sensitize standard-anticancer drugs, by many folds, increasing their anticancer activities and overcoming natural resistance to anticancer drugs. Interaction of caffeine with anticancer medications enhancing their effectiveness is well known. Thus, caffeine synergistically enhances the effect of cyclophosphamide (44.8%), mitomycin C (44.8%), Adriamycin [doxorubicin] (27.8%), and cisplatin (77.8%). Interaction of caffeine with an antioestrogen drug, tamoxifen for breast cancer, enhances its anticancer activity. The encouraging factor is that the enhancement of antitumoricidal effects is without increasing the side effects of the anticancer drugs [62]. In radiation therapy, caffeine helps to repair damage caused by ionizing radiation or chemically induced damage to DNA lesions [64]. However, its synergistic action with vincristine and methotrexate is not known to enhance the antitumoricidal action of these drugs.

Caffeine has a diverse and dose-dependent action on different organs. Thus caffeine, in a dose-dependent fashion, inhibits skin cancer in mouse skin [65] but it is known to attenuate liver fibrosis in a cirrhotic model [66]. It should be appreciated that tea and coffee are complete pharmacopeias with tens of compounds in these decoctions and caffeine is only one of them that shows anticancer activity. Consumption of caffeinated beverages can offer protection and prevention from cancer propagation. However, their high sugar content and reduction in bone mass is prohibitive causing severe health concerns [67]. Daily consumption of 4-6 cups of coffee by adults may be enough for caffeine to exert its defensive and beneficial role in regular health maintenance.

In the western world, breast cancer is a significant problem where 7-10% of the population suffers from this endemic condition. However, in economically less developed countries, breast cancer accounts for less than 1% which directly reflects on the lifestyle, diet, and environment in the western world that may be responsible for this malady originating from alterations in the endocrine system and loss of the estrogen receptor-α. Many phytoestrogens with anticancer activity have been reported, and trigonelline (28) is

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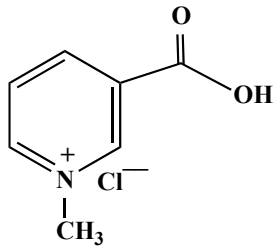
a novel phytoestrogen present in coffee beans [68]. Inhibition of breast cancer cell lines (MCF-7 & MDA-MB-231), by modifying estrogen receptors, with caffeine and caffeic acid is also known to sensitize cancer cells to tamoxifen and diminish breast cancer growth [69].



(28)

Many factors assist tumor progression, and insulin-like growth factors (IGFs) are the most important contributing factors [70]. Insulin-like growth factor and insulin resistance may induce many types of cancers including human breast cancer progression [71]. The estrogen and related hormones increase insulin-like growth factor-1 receptor (IGFIR), and antiestrogens protect from breast cancer [72]. Regulation of several genes, by both estrogen and IGFs, is known and a reduction in mitogenic insulin-like growth factor (IGF-IR) influenced by caffeine has been reported [69, 73]. In colorectal adenocarcinoma, caffeine decreases human fibroblasts growth factor (FGF 19) mRNA and down-regulates the expression of VEGF and EGFR in HeLa cells attesting to the cytotoxic effects of caffeine on these cells [74]. A down-regulation of mRNA level of p53α compared with p53β and consequently deregulation of SRSF3, is responsible for regulating VEGF, EGFR, COX-2 and Glut1 [74]. In addition, tea extract contains polyphenols i.e. EGCG, that are potent antioxidant/anticancer agents [75].

More than 50% of the anticancer drugs are natural products or derived from natural products and caffeine is no exception. According to a WHO report more than 80% of the world population relies on alternative medicine and believe in beneficial effects of caffeine, perhaps through the synergistic effect of many other compounds present in the extract [76]. Besides, many minor products co-occur with caffeine and have never been isolated, purified or identified. These secondary products may be physiologically more

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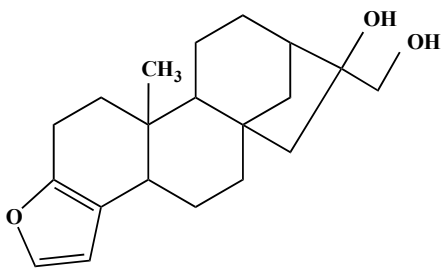
active than their primary counterparts. The modern purified advanced pharmaceutical products may have more healing potency, but they lack synergistic effects, not to name the minor natural products that are entirely overlooked resulting in harmful side effects. Therefore, in a quest for new natural products, it is indispensable to look into the transparent micro-components and harvest the whispers of nature.

Apart from methylxanthines, the other coffee components with anticancer activity are ROS scavenger diterpenoid molecules such as cafestol (29) and kahweol (30), which significantly upturn the level of DNA detoxification, DNA repair, P450 enzymes and potent anticancer activity against aggressive malignant pleural mesothelioma [77, 78]. Besides, many substituted benzoic and cinnamic acids like gallic acid, acid, chlorogenic acid (5-caffeoylquinic acid), ferulic acid, syringic acid, components of tea and coffee, are potent antioxidant and anticarcinogenic molecules [79, 80].



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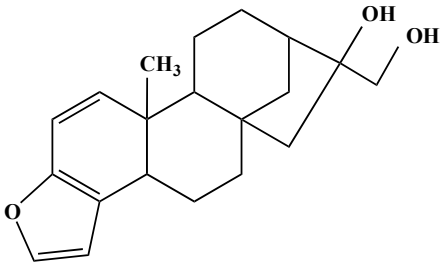

(30)

11. Caffeine: Anti-inflammatory activity

Caffeine facilitates the up-regulation of myeloperoxidase (MPO) activity indicating its anti-inflammatory activity [81]. At the same time, it reduces acetylcholine esterase (AChE) activity, increasing acetylcholine level, suppresses proinflammatory cytokines and protects against inflammation [82]. Caffeine exerts its anti-inflammatory activity through its nonselective competitive inhibition of phosphodiesterases that promote the intracellular concentration of cAMP and inositol phosphate receptors [83]. Accumulation of cAMP activates protein kinase-A, and disengage leukotriene synthesis, consequently

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leading to reduced inflammation. An interaction of caffeine with other secondary messengers such as acetylcholine esterase, monoamine oxidase, ryanodine and other receptors for controlling neurodegenerative and neuroplasticity diseases and immunity alterations are also known [84].

In immune cells, cytokines are the major signaling molecules, and they move cells towards inflammation or infection. Caffeine deregulates the proinflammatory cytokines like IL-Ira and IL-10 stimulated by cancer cells [85]. Caffeine also shields the two chronic inflammatory diseases, hepatic cirrhosis, and fibrosis that can lead to hepatic cancer [86, 87].

NFkB is a proinflammatory-signaling pathway activated by proinflammatory cytokines such as interleukin IL-1. In inflammation, NFkB is involved in the expression of other proinflammatory cytokines, chemokines and adhesion molecules. Increased caffeine consumption deregulates the production of NFkB and hepatic fibrosis [88]. The anti-inflammatory caffeine mixed with melanoidins reduces NFkB pathway activity [89].

12. Caffeine: Free radical scavenger

In biosystems, many free radicals create oxidative stress, and it alters many biomolecules including DNA and proteins. Free radicals accelerate the formation of end-glycation products resulting in aging and several age-related chronic diseases. These reactive molecules work at various molecular and cellular levels in a living system and in addition to cancer, cause age-related and neurodegenerative chronic disorders such as Parkinsonism, Alzheimer's, cardiovascular, and immune system [90]. In a biosystem, free radicals are naturally produced through an untested mechanism of electron leakage from the mitochondrial double membrane and during electron transport chain in oxidative phosphorylation. Natural defenses like vitamin E, ascorbate, glutathione peroxidase, NADPH oxidase cannot combat the overproduction of free radicals, and therefore, there is a need for antioxidants in the human diet.

Phytochemicals, especially phenols are considered to be the most potent antioxidants to combat free radicals [79]. A positive effect of green tea and its phytochemicals like EGCG have antioxidant activity on post-stroke depression [91]. Wheat bread enriched

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with green coffee is known to improve the phenolic content and its bioaccessibility through enhanced antioxidant activity [92].

Caffeine is a powerful antioxidant, and it can scavenge one of the most dangerous hydroxyl free radicals $^{\bullet}OH$ and also a modest scavenger for $^{\bullet}OCH_3$ [93]. The antioxidant activity of methylxanthines in tea and coffee make it a beverage of choice that is readily available [94]. Recently, caffeine is reported to induce conserved longevity pathways, including insulin-like signaling pathway and the oxidative stress response. The mechanism of this involves the regulation of heat shock response in HSF-1 dependent manner in *Caenorhabditis elegans* {95] and the arrangement includes the radical-adduct formation with caffeine [96].

Caffeine co-occurs with caffeic acid, and both are involved in the mechanism of cancer suppression [69]. Caffeic acid, an anti-inflammatory, antimutagenic, and anti-carcinogenic phenolic acid is a powerful antioxidant that quenches hydroxyl radical through ion chelating mechanism [97]. Besides, chlorogenic acid and caffeic acids are natural free radical scavengers, and they inhibit mutagenic and carcinogenic N-nitroso compounds and DNA damage [98, 99]. The phenolic acids and their esters including cinnamic acid, ferulic acid, chlorogenic acid and caffeic acid, components of tea and coffee, all show a good premise in cancer treatment [100-102]. Caffeic acid and caffeine have been shown to arrest cells in the G1/S phase of the cell cycle in breast cancer cell lines [100].

13. Caffeine: Mechanism of action

Central nervous system (CNS) and cardiovascular systems are principally affected by most methylxanthines [103]. Caffeine works through four different mechanisms. The first mechanism involves the translocation of intracellular calcium, the second mechanism includes cyclic nucleotides accumulation (3’5’ cAMP) and inhibition of phosphodiesterases, in the third mechanism, adenosine receptor blockage takes place, and the fourth mechanism of action of caffeine is as a free radical scavenger [104]. The predominant mechanism of action of caffeine depends on many factors including age, gender, and species. In the first mechanism of action, an upturn in the intracellular calcium facilitates the release of the inhibitory neurotransmitter GABA affecting

serotonin synthesis [105]. This release of calcium leads to muscle stimulation protecting the body from exhaustion and anxiety, encouraging consumption of caffeine and creatine, in sports [106]. However, reduction of bone mass by translocation of calcium by caffeine cannot be overlooked. A combination of amino acids like GABA and others with caffeine is used to enhance sprint-running capacity [107]. In the second and third mechanism of action of caffeine, inhibition of phosphodiesterases and an accumulation of cAMP play a profound role in stimulating vagal, vasomotor and respiratory centers leading to bradycardia, vasoconstriction and an elevated degree of respiration. The blockage of adenosine receptors by caffeine acts psychosomatically and further, helps in the stimulation of CNS [108]. The fourth mechanism of action of caffeine works through its antioxidant activity that prevents cellular damage and nutrients.

The above activities of caffeine are only a few to mention and the research area to explore xanthene type of molecules is wide open and additional research is needed to explore the full potential of these compounds, for use in a clinical setup. Caffeine is readily available at low cost, and its natural analogs must be investigated for the benefits of humanity. We hope this review will stimulate researchers to multiply their efforts in this rewarding area of research and come up with new methylxanthines, natural and synthetic, that may have good therapeutic potential.

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