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Theobromine: A Comprehensive Review of Its Pharmaceutical Profile, Analytical Methods, and Clinical Insights

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ABSTRACT: Theobromine, which is a naturally occurring methylxanthine and mainly found in cacao plants, is now recognized for its various pharmacologic activities including bronchodilation, diuresis, and relaxing the smooth muscle. This review will discuss the pharmacologic implications of theobromine, reviews its absorption, distribution, metabolism, and excretion (ADME) aspects, as well as its use for therapeutic purposes. The review emphasizes analysis methods of theobromine through high-performance liquid chromatography (HPLC), LC-MS/MS methods which provide quantitative and quality control analysis of theobromine. In addition, the review discusses clinical implications, showing theobromine as a historical and modern pharmacologic agent.

KEYWORDS: Theobromine, Methylxanthine, HPLC Methods, ADME, LC-MS/MS.

I. INTRODUCTION

A naturally occurring methylxanthine alkaloid, theobromine is found predominantly in cacao plants and cacao-derived products like chocolate and cocoa powder (Alifiya & Guntarti, 2022). The Molecular formula for theobromine, which is $C_7H_8N_4O_2$, indicates that theobromine is a methylxanthine (a xanthine with three methyl groups) in which the xanthine has been methylated at positions 3 and 7, which contributes to its lipophilicity and its ability to penetrate biological membranes (Estelle et al., n.d.).

Adenosine receptor antagonism and phosphodiesterase inhibition, contributes to reduced drowsiness, relaxation of smooth muscle, and bronchodilation as necessary pharmacological effects of theobromine. Although overshadowed by its caffeine sibling, theobromine boasts several benefits, such as milder CNS stimulation and a longer half-life, providing a safer alternative in susceptible individuals to be overstimulated (Baggott et al., 2013).

Although it is consumed widely, thorough studies of the full therapeutic profile, safety, and efficacy of theobromine are still lacking (Mitchell et al., 2011). Furthermore, while theobromine is well defined in blood plasma and urine through clinical pharmacokinetics, specificity with analytical methods to determine this compound in biological matrices needs to be enhanced for sensitivity and validated for clinical interpretation (Ptolemy et al., 2010).

The aim of this review is to bridge those gaps with a systematic appraisal of the pharmacological profile of theobromine and evaluation of various analytical methods used, as well as clinical applications and safety profile. In doing so, this review attempts to spell out the wider implications for theobromine as a therapeutic agent and fill the gap of available knowledge for subsequent research forward.

II. Chemical and Pharmacological Profile

The molecular formula for theobromine, known as 3,7-dimethylxanthine, is C7H8N4O2, and its molecular weight is 180.16 g/mol. With two methyl groups attached to nitrogen atoms at positions 3 and 7 of the xanthine core, it has a planar bicyclic structure made up of an imidazole ring fused to a pyrimidinedione ring. Its biological action depends on this structure, which enables it to bind with adenosine receptors, albeit less strongly than caffeine, producing milder stimulant effects (Valada et al., 2022).

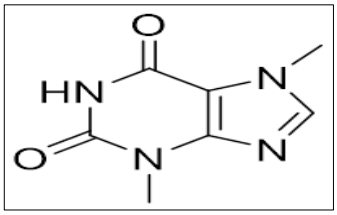

Fig. No.1: *Structure of Theobromine.*

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Table1: *Chemical and Pharmacological Profile of Theobromine*

Property	**Value**
Chemical Abstract Name	3,7-Dihydro-3,7-dimethyl-lH-purine-2,6-dione
Synonym	3,7-Dimethylxanthine
Molecular Formula	$C_7H_8N_4O_2$
Molecular Weight	180.16 g/mol
Melting Point	Around 357°C
Boiling Point	Sublimes at elevated temperatures
Solubility	Soluble in water (1.0 g/2l), boiling water (1.0 g/0.15l) and 95% ethanol (1.0 g/2.2l), slightly soluble in chloroform (1.0g/6l), almost insoluble in benzene, diethyl ether and carbon tetrachloride
Sublimation-point	290–295°C
Equilibrium constants	acidic (Ka) 0.9×10^{-10} and basic (Kb) 1.3×10^{-14} at 18°C
Log P (Partition Coefficient)	-0.8
pKa	10.2
Appearance	White or colourless crystalline powder
Stability	Stable under normal conditions but sensitive to light, heat, and extreme pH
UV Absorption	Maximum absorption at approximately 272 nm

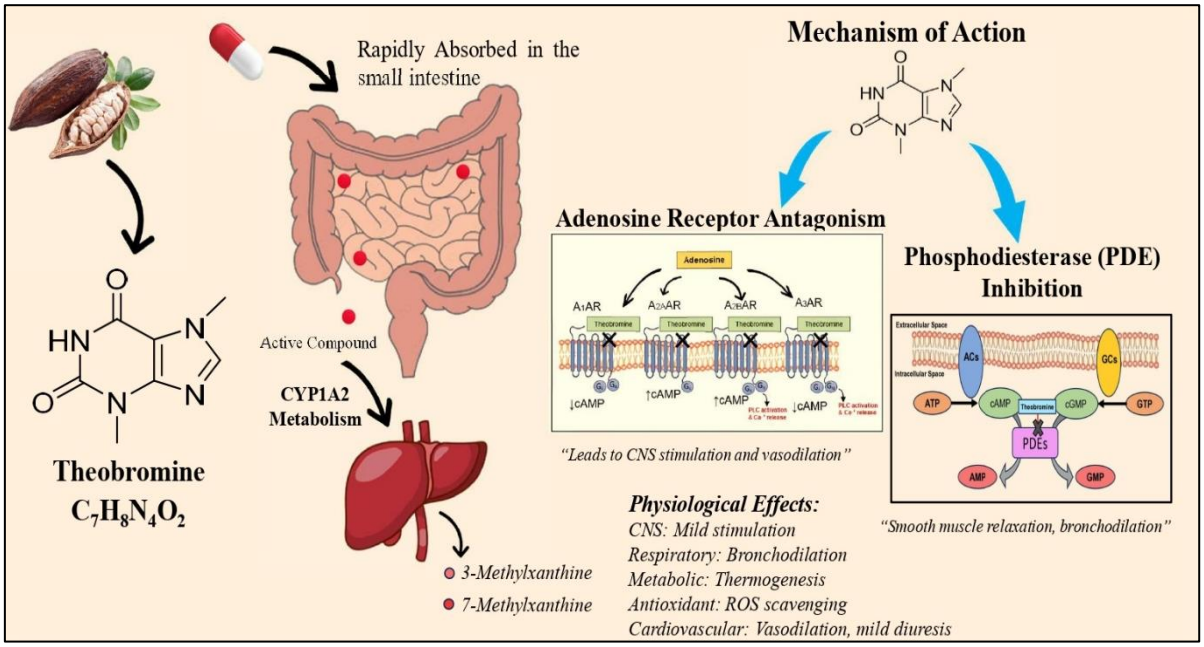

Fig. No.2: *Mechanisms of action and physiological effects of theobromine*

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III. Pharmacological Profile

A methylxanthine that is closely linked to caffeine, theobromine has a broad pharmacological profile with a range of mechanisms of action. Its effects on the central nervous system and its therapeutic uses are mostly due to its primary function as a weak antagonist of adenosine receptors, namely the A1 and A2 subtypes (Zhang et al., 2024).

IV. Mechanisms of Action

i. Adenosine Receptor Antagonism:

Theobromine's stimulatory effects, including increased alertness and decreased weariness, are facilitated by its capacity to oppose adenosine receptors. The A1 receptor, which is known to mediate sedative and anxiolytic effects, is the primary site of this antagonism. Moreover, it has been demonstrated that theobromine alters the levels of cyclic adenosine monophosphate (cAMP), which affects several physiological functions, such as respiration and lipolysis.

ii. Bronchodilation:

Theobromine also helps to treat respiratory disorders like asthma because of its bronchodilator qualities, which have also been reported (Dutra et al., 2024; Estelle et al., n.d.). Like theophylline, it works by blocking phosphodiesterase enzymes, which raises cAMP levels and causes the smooth muscle in the bronchi to relax. In individuals with impaired respiratory function, this action may help increase oxygenation in addition to improving airflow (Boushey, n.d.).

iii. Cardiovascular Effects:

Theobromine causes relatively low cardiovascular side effects, such as vasodilation and increased heart rate (Dutra et al., 2024). Its effect on adenosine receptors and inhibition of phosphodiesterase facilitate these effects, leading to increased blood flow and cardiac contractility. Although theobromine is not as effective as caffeine in lowering blood pressure, studies have shown that its cardiovascular advantages may also extend to this area (Edo et al., 2023).

iv. Neuroprotective Effects

Because of its antioxidant benefits and ability to modulate adenosine receptor activation, theobromine has neuroprotective activity (Bhat et al., 2021).

V. Pharmacokinetics (ADME)

i. Absorption:

Absorption of Theobromine is mainly in the small intestine, especially when consumed orally since it is lipophilic, which allows passive diffusion through biophysical membranes.

ii. Distribution:

After absorption, theobromine distributes evenly among the fluids of the body, detectable in plasma, brains, and many other tissues. With low protein plasma binding (15%–21%), it is believed to freely circulate and penetrate widely into many tissues, including the central nervous system, and has been discovered in saliva, indicating it could also pass into breast milk.

iii. Metabolism:

Primarily via N-demethylation and oxidation in the liver, CYP1A2 enzyme involved in metabolizing theobromine into various metabolites such as 7-methylxanthine, 3-methylxanthine, and 7-methyluric acid (Oduro-Mensah et al., 2018). The ethnicity, culture, compulsions, and social environment of the individuals carrying the aforementioned gene will determine how fast the metabolism occurs (Eteng et al., 1997).

iv. Excretion:

The excretion occurs through the kidneys mainly by glomerular filtration and tubular secretion. About 1% to 18% of theobromine is eliminated unchanged in urine. It's half-life elimination generally ranges from 6 to 10 hours, with variations according to age, liver competency, and possibly drugs being taken at the same time.

VI. Pharmacodynamics

Theobromine primarily acts via inhibiting phosphodiesterase and adenosine receptors. By inhibiting A1 and A2A adenosine receptors, the psychologically uplifting effect of less sedative action is achieved, improving alertness and function (Martínez-Pinilla et al., 2015). Higher phosphodiesterase inhibition results in higher adenyl cyclase levels, which in turn produce bronchodilation, relaxed parachute muscles, and an increase in cardiac output. Theobromine would also stimulate the central nervous system, cause vasodilation, and slightly dilute blood vessels, all of which would be helpful in syncapes from preterm deliveries with hyperemia. Its pharmacological profile is very similar to that of theophylline and caffeine in terms of its diuretic, respiratory, and cardiovascular actions (Cova et al., 2019).

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VII. Analytical Methods for Theobromine Detection and Quantification

i. High-Performance Liquid Chromatography (HPLC)

Sr. No.	Research work	Description		Ref. No
1.	HPLC method for urinary theobromine determination: effect of consumption of cocoa products on theobromine urinary excretion in children	***Mobile phase:***	A]. Ammonium acetate (20 mmol/L, pH 7.5) : ACN (98:2, vol/vol); B]. ACN	(Rodriguez et al., 2015)
		Stationary phase:	5-µm reversed-phase column (Gemini C18 110 A; 150 × 4.6 mm) protected with a Phenomenex security C18 guard cartridge (4 × 3.0 mm)	
		Retention time:	15 min	
		λ max:	273nm	
2.	Simultaneous HPLC determination of caffeine, Theobromine, and theophylline in food, Drinks, and herbal products	***Mobile phase:***	A]. Water–THF (0.1% THF in water, pH- 8); B]. ACN (90:10, v/v)	(Branislava Srdjenovic et al., 2008)
		Stationary phase:	Zorbax Eclipse XDB-C8 (4.6 × 150 mm, i.d., 5-µm particle size).	
		Retention time:	8 min	
		λ max:	273nm	
3.	Improved high-performance liquid chromatography method to Determine theobromine and caffeine in cocoa and cocoa products	***Mobile phase:***	A]. ACN B]. Water (20:80, v/v)	(Pura Naik, 2001)
		Stationary phase:	5-µm C18 reverse-phase column pore size A:80, 250 × 4.6 mm size, cartridge	
		Retention time:	1ml/min.	
		λ max:	5.091 min	
4.	Simple and sensitive method for the simultaneous determination of Enofylline, theobromine, paraxanthine, theophylline and caffeine using High-performance liquid chromatography	***Mobile phase:***	3% Tetrahydrofuran solution containing 10 mM disodium phosphate with the pH adjusted to 6.5 using dilute phosphoric acid.	(N. Grgurinovich, 1986)
		Stationary phase:	Regis octyl Hi-Chrom reversed-phase column, 5 um particle size, 25 cm x 4 mm	
		Flow rate:	2.0 ml/min	
		Retention time:	2.5 min	
		λ max:	280 nm	
5.	Simultaneous determination of caffeine, theobromine, and theophylline by high-performance liquid chromatography	**Mobile phase:**	Ethanol – Water– Acetic acid (20:75:5, v/v/v)	(Marcia S. Bispo et al., 2002)
		Stationary phase:	a Bondesil C18 5-µm column (4.0-mm × 15-cm)	
		Flow rate:	0.7 mL/min	
		λ max:	273 nm	
6.	Determination of theobromine, theophylline, and Caffeine in by-products of cupuacu and cacao seeds by High-performance liquid chromatography	***Stationary phase:***	Supelcosil LC-18 column (250 × 4.6 mm, 5 µm, Supelco, Sigma-Aldrich, Milan, Italy)	(F. Lo Coco et al., 2007)
		Flow rate:	1 mL/min	
		λ max:	275 nm	

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7.	The simultaneous determination of theophylline, Theobromine and caffeine in plasma by high Performance liquid chromatography	***Mobile phase:***	A]. Acetate buffer (10 mmol/1, pH 4); B]. ACN (88:12)	(Foenander et al., 1980)
		Stationary phase:	15 cm x 4.6 mm (i.d.) reverse phase S50DS column	
		Flow rate:	2 mL/min	
		Retention time:	2.1 min	
		λ max:	276 nm	
8.	High-performance liquid chromatographic determination Of dimethylxanthine metabolites of caffeine in human Plasma	***Mobile phase:***	A]. Acetate buffer (10 mmol/1, pH 4) B]. ACN (88:12)	(Wahllander et al., 1985)
		Stationary phase:	Hibar@ RT 250-4 columns filled with LiChrosorb Si 60, 5 pm particle size	
		λ max:	280 nm	
9.	HPLC analysis of methylxanthines in human breast milk	***Mobile phase:***	A]. Acetate buffer (10 mmol/1, pH 4); B]. ACN (88:12)	(James Blanchard et al., 1990)
		Stationary phase:	Ultrasphere ODS, 5-µm, 25-cm × 4.6-mm i.d.	
		Retention time:	13 min	
		λ max:	272 nm	

ii. High-Performance Thin Layer Chromatography (HPTLC)

Sr. No.	Research work	Description		Ref. No
1.	Extraction and estimation of Theobromine in marketed tea by HPTLC and UV method	***Mobile phase:***	A]. Ethyl acetate B]. Methanol (27:3 v/v)	(Kasabe & Badhe, n.d.)
		Stationary phase:	Silica gel 60 F254 HPTLC plates	
		LOD	30 ng/spot	
		LOQ	140 ng/spot	
		λ max:	274 nm	

iii. Mass Spectrometry and Spectroscopic Techniques

Sr. No.	Research work	Description		Ref. No
	Validation of an LC-MS/MS Method for the Quantification of Caffeine and Theobromine Using Non-Matched Matrix Calibration Curve	***Column:***	3 µm Gemini C18 column (50 × 2.0 mm, 110Å, Phenomenex)	
		Mobile Phase:	A] 0.1% formic acid in water B] 0.1% formic acid in ACN	
		Mass Conditions:	Ion Spray Voltage: 550 V; GS1: 35 psi; GS2: 20 psi; Curtain Gas: 30 psi; Temperature: 450 °C; EP: 10 eV; CXP: 15 eV; CAD: 8 psi	
		Mode:	Positive	
		RT:	3.8 min	

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2.	Degradation of exogenous caffeine by Populus alba and its effects on endogenous caffeine metabolism	***Column:***	50/4.6 Phenomenex Gemini 5-µm C18 column.	(Pierattini et al., 2016)
		Mobile Phase:	A] 0.1% formic acid in ACN B] 0.1% formic acid in water	
		Mass Conditions:	MS/MS experiments in APCI positive ion mode with nitrogen as collision gas; Source type: Heated nebulizer; Nebulizer gas: 6 (arbitrary units); Curtain gas: 6 (arbitrary units); Temperature: 450 °C; Needle current: 5 µA; Entrance potential: 10 V	
		Mode:	Positive	
3.	Quantification of theobromine and caffeine in saliva, plasma and urine via liquid chromatography–tandem mass spectrometry: A single analytical protocol applicable to cocoa intervention studies	***Column:***	1.7 um particles × 2.1 mm × 50 mm, Waters	(Ptolemy et al., 2010)
		Mobile Phase:	A] 0.1% formic acid in water B] ACN	
		Mass Conditions:	Capillary Voltage: 3 kV; Temperatures: Source: 120 °C, Desolvation: 350 °C; Gas Flow Rates: Cone Gas: 100 L/h, Desolvation Gas: 800 L/h; Multipliers: Set at 650 V, Argon used as the collision gas at a regulated flow rate of 0.35 mL/min; Multiple Reaction Monitoring (MRM): Cone voltage and collision energy optimized for each target compound's precursor and product ions	
		Mode:	Positive	
		RT:	0.9 min	
4.	Control of methylxanthines in the competition horse: pharmacokinetic/ pharmacodynamic studies on caffeine, theobromine and theophylline for the assessment of irrelevant concentrations	***Column:***	Nucleodur® C18 Pyramid (70 x 4 mm i.d., 5 µm particle size)	(Machnik et al., 2017)
		Mobile Phase:	A] Ammonium acetate buffer (5 mmol ammonium acetate, 0.1% acetic acid, pH 3.5) B] ACN.	
		Mass Conditions:	ESI Source Temperature: 550 °C; Multiple Reaction Monitoring (MRM): Protonated ions and specific product ions monitored after collision-induced dissociation with nitrogen at 3.3 × 10^{-3} Pa; Ion Transitions for Theobromine: m/z 181/138; Internal Standard Fragments (Theobromine-D6): m/z 187/144; Collision Energies: Optimized for each analyte and internal standard	
		Mode:	Positive	

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VIII. Clinical Insights and Therapeutic Potential

i. Clinical Pharmacology

When taken orally in different dose forms, theobromine has effects that start to show up within 30 minutes and remain for up to 6 hours. Although therapeutic indicators point to a reasonable level of safety, cautious dosage is necessary due to interindividual variability.

ii. Clinical Applications

Theobromine contains bronchodilator qualities, according to research, which can be very beneficial for treating respiratory conditions including asthma and chronic obstructive pulmonary disease (COPD). Additionally, it has two prominent effects on the cardiovascular system: it has a low diuretic impact and stimulates vasodilation.

These characteristics suggest that it could be a useful addition to hypertension therapy. Studies emphasize its importance in managing respiratory conditions, thanks to its bronchodilator effects, while its cardiovascular benefits, including vasodilation and slight diuretic activity, position it as a potential support in hypertension treatment.

iii. Adverse Effects and Safety Profile

Reported side effects include nausea, headache, and increased heart rate, particularly at high doses. Long-term safety data remain sparse, underscoring the need for further investigation (Edo et al., 2023).

IX. Conclusion

This review summarizes the pharmacological significance, ADME profile, and analytical techniques mainly HPTLC, HPLC and Mass Spectroscopy used for the quantification of theobromine in various matrices. The mentioned methods are simple, precise, and reproducible. Although centuries old, most of the therapeutic potential of theobromine remains to be explored. This review can prove to be a useful guide for researchers and students who want to explore further the analytical and pharmacokinetic features of theobromine.

REFERENCES

[1]. Alifiya, F., & Guntarti, A. (2022). Theobromine content in chocolate products: a review. Journal of Halal Science and Research, 2(1), 24–32. https://doi.org/10.12928/jhsr.v2i1.4434

[2]. Baggott, M. J., Childs, E., Hart, A. B., De Bruin, E., Palmer, A. A., Wilkinson, J. E., & De Wit, H. (2013). Psychopharmacology of theobromine in healthy volunteers. Psychopharmacology, 228(1), 109–118. https://doi.org/10.1007/s00213-013-3021-0

[3]. Bhat, J. A., Gupta, S., & Kumar, M. (2021). Neuroprotective effects of theobromine in transient global cerebral ischemia-reperfusion rat model. Biochemical and Biophysical Research Communications, 571, 74–80. https://doi.org/10.1016/j.bbrc.2021.07.051

[4]. Boushey, H. A. (n.d.). Drugs Used in Asthma.

[5]. Branislava Srdjenovic, Vukosava Djordjevic-Milic, Nevena Grujic, Rade Injac, & Zika Lepojevic. (2008). Simultaneous HPLC Determination of Caffeine, Theobromine, and Theophylline in Food, Drinks, and Herbal Products. Journal of Chromatographic Science, Volume 46. https://doi.org/10.1093/chromsci/46.2.144

[6]. Cova, I., Leta, V., Mariani, C., Pantoni, L., & Pomati, S. (2019). Exploring cocoa properties: is theobromine a cognitive modulator? In Psychopharmacology (Vol. 236, Issue 2, pp. 561–572). Springer Verlag. https://doi.org/10.1007/s00213-019-5172-0

[7]. Dutra, N. S., da Silva D'Ávila, C. M., da Silva, T. C., de Oliveira Mendes, T., Livinalli, I. C., Bertoncelli, A. C. Z., Saccol, F. K., & Cadoná, F. C. (2024). Biological properties of caffeine, (+)-catechin, and theobromine: an in silico study. 3 Biotech, 14(4). https://doi.org/10.1007/s13205-024-03934-7

[8]. Edo, G. I., Samuel, P. O., Oloni, G. O., Ezekiel, G. O., Onoharigho, F. O., Oghenegueke, O., Nwachukwu, S. C., Rapheal, O. A., Ajokpaoghene, M. O., Okolie, M. C., Ajakaye, R. S., Ndudi, W., & Igbodo, P. C. (2023). Review on the Biological and Bioactive components of Cocoa (Theobroma Cacao). Insight on Food, Health and Nutrition. In Natural Resources for Human Health (Vol. 3, Issue 4, pp. 426–448). Visagaa Publishing House. https://doi.org/10.53365/nrfhh/174302

[9]. Estelle, F., Simons, R., Becker, A. B., Simons, K. J., & Gillespie, C. A. (n.d.). The bronchodilator effect and pharmacokinetics

of theobromine in young patients with asthma.

[10]. Eteng, M. U., Eyong, E. U., Akpanyung, E. O., Agiang, M. A., & Aremu, C. Y. (1997). Recent advances in caffeine and theobromine toxicities: a review. In Plant Foods for Human Nutrition (Vol. 51). Kluwer Academic Publishers.

[11]. F. Lo Coco, F. Lanuzza, G. Micali, & G. Cappellano. (2007). Determination of Theobromine, Theophylline, and Caffeine in by-Products of Cupuacu and Cacao Seeds by High-Performance Liquid Chromatography. Journal of Chromatographic Science, Volume 45.

[12]. Foenander, T., Birkett, D. J., Miners, J. O., & Wing, L. M. H. (1980). The Simultaneous Determination of Theophylline, Theobromine and Caffeine in Plasma by High Performance Liquid Chromatography. In Clin. Biochem (Vol. 13, Issue 3).

[13]. James Blanchard, Charles W. Weber, & Luz-E . Shearer. (1990). HPLC Analysis of Methylxanthines in Human Breast Milk.

[14]. Kasabe, A. J., & Badhe, G. B. (n.d.). Extraction and estimation of theobromine in marketed tea by hptlc and uv method. www.ijabpt.com

[15]. Machnik, M., Kaiser, S., Koppe, S., Kietzmann, M., Schenk, I., Düe, M., Thevis, M., Schänzer, W., & Toutain, P. L. (2017). Control of methylxanthines in the competition horse: pharmacokinetic/pharmacodynamic studies on caffeine, theobromine and theophylline for the assessment of irrelevant concentrations. Drug Testing and Analysis, 9(9), 1372–1384. https://doi.org/10.1002/dta.2097

[16]. Marcia S. Bispo, Márcia Cristina C. Veloso, Heloísa Lúcia C. Pinheiro, Rodolfo F.S. De Oliveira, José Oscar N. Reis, & Jailson B. De Andrade. (2002). Simultaneous Determination of Caffeine, Theobromine, and Theophylline by High-Performance Liquid Chromatography. Journal of Chromatographic Science, Volume 40.

[17]. Martínez-Pinilla, E., Oñatibia-Astibia, A., & Franco, R. (2015). The relevance of theobromine for the beneficial effects of cocoa consumption. Frontiers in Pharmacology, 6(FEB). https://doi.org/10.3389/fphar.2015.00030

[18]. Mitchell, E. S., Slettenaar, M., vd Meer, N., Transler, C., Jans, L., Quadt, F., & Berry, M. (2011). Differential contributions of theobromine and caffeine on mood, psychomotor performance and blood pressure. Physiology and Behavior, 104(5), 816–822. https://doi.org/10.1016/j.physbeh.2011.07.027

[19]. N. Grgurinovich. (1986). Simple and sensitive method for the simultaneous determination of enprofylline, theobromine, paraxanthine, theophylline and caffeine using high-performance liquid chromatography. In Biomedical Applications Elsevier Science Publishers B.V (Vol. 380).

[20]. Oduro-Mensah, D., Ocloo, A., Lowor, S. T., Bonney, E. Y., Okine, L. K. N. A., & Adamafio, N. A. (2018). Isolation and characterisation of theobromine-degrading filamentous fungi. Microbiological Research, 206, 16–24. https://doi.org/10.1016/j.micres.2017.09.006

[21]. Pierattini, E. C., Francini, A., Raffaelli, A., & Sebastiani, L. (2016). Degradation of exogenous caffeine by Populus alba and its effects on endogenous caffeine metabolism. Environmental Science and Pollution Research, 23(8), 7298–7307. https://doi.org/10.1007/s11356-015-5935-z

[22]. Ptolemy, A. S., Tzioumis, E., Thomke, A., Rifai, S., & Kellogg, M. (2010). Quantification of theobromine and caffeine in saliva, plasma and urine via liquid chromatography-tandem mass spectrometry: A single analytical protocol applicable to cocoa intervention studies. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 878(3–4), 409–416. https://doi.org/10.1016/j.jchromb.2009.12.019

[23]. Pura Naik, J. (2001). Improved high-performance liquid chromatography method to determine theobromine and caffeine in cocoa and cocoa products. Journal of Agricultural and Food Chemistry, 49(8), 3579–3583. https://doi.org/10.1021/jf000728z

[24]. Rodriguez, A., Costa-Bauza, A., Saez-Torres, C., Rodrigo, D., & Grases, F. (2015). HPLC method for urinary theobromine determination: Effect of consumption of cocoa products on theobromine urinary excretion in children. Clinical Biochemistry, 48(16–17), 1138–1143. https://doi.org/10.1016/j.clinbiochem.2015.06.022

[25]. Valada, P., Alçada-Morais, S., Cunha, R. A., & Lopes, J. P. (2022). Thebromine Targets Adenosine Receptors to Control Hippocampal Neuronal Function and Damage. International Journal of Molecular Sciences, 23(18). https://doi.org/10.3390/ijms231810510

[26]. Wahllander, A., Renner, E., & Karlaganis, G. (1985). High-performance liquid chromatographic determination of dimethylxanthine metabolites of caffeine in human plasma. In Journal of Chromatography (Vol. 338).

[27]. Zhang, M., Zhang, H., Jia, L., Zhang, Y., Qin, R., Xu, S., & Mei, Y. (2024). Health benefits and mechanisms of theobromine. In Journal of Functional Foods (Vol. 115). Elsevier Ltd. https://doi.org/10.1016/j.jff.2024.106126