IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A Comprehensive Review of Methylxanthines, on Medicinal Constituents, Mechanistic Pathways in Neuroprotection, and Emerging Nanotechnological Delivery Systems

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Abstract: Methylxanthines, particularly 1,3,7-trimethylxanthine (caffeine) and its primary in vivo metabolites (theobromine, theophylline, and paraxanthine), represent some of the most ubiquitously consumed psychoactive substances worldwide. Beyond their historical classification as dietary stimulants, modern molecular pharmacology has uncovered an intricate landscape of bioactivities that extend deep into therapeutic and neuroprotective territories. This extensive review outlines the comprehensive pharmacological mechanisms of methylxanthines, focusing heavily on their multi-targeted interactions within the central nervous system (CNS). We dissect the competitive antagonism of adenosine A1 and A2A receptors, the downstream modulation of neurotransmitter cascades (including dopamine, acetylcholine, serotonin, and gamma-aminobutyric acid), and the inhibition of cyclic nucleotide phosphodiesterases (PDEs). Furtherm examine the potent intracellular calcium mobilization and the epigenetic modifications orchestrated via histone deacetylase activation. In the context of neurodegenerative pathology, we present exhaustive evidence supporting caffeine's therapeutic efficacy against Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis, highlighting its capacity to mitigate neuroinflammation, suppress microglial overactivation, neutralize reactive oxygen species (ROS), and prevent mitochondrial collapse. Despite these profound medicinal attributes, the clinical translation of methylxanthines remains severely bottlenecked by complex pharmacokinetic challenges, including rapid systemic clearance, low metabolic bioavailability, and the restrictive stringency of the blood-brain barrier (BBB). To address these limitations, we comprehensively evaluate cutting-edge nanotechnological intervention strategies. This includes a critical analysis of lipid-based nanoparticles (SLNs and NLCs), polymeric nanoparticles, nanoemulsions, and functionalized carbon nanotubes designed for targeted, sustained, and brain-specific delivery. Ultimately, this review bridges the gap between classic xanthine chemistry and advanced clinical nanomedicine, providing a definitive framework for utilizing methylxanthines as novel, high-efficacy neurotherapeutics.

Keywords Methylxanthines, caffeine, Alzheimer's disease, neurotherapeutics, Neuroprotection, and Emerging Nanotechnological Delivery Systems.

1.Introduction and Historical Context

The evolutionary relationship between humanity and plant-derived alkaloids is profoundly epitomized by the methylxanthines. For millennia, botanical decoctions containing these heterocyclic compounds—such as Coffea arabica, Camellia sinensis, and Theobroma cacao—have been intentionally consumed across diverse civilizations to elevate alertness, defer fatigue, and enrich cognitive endurance. However, the systematic extraction and pharmacological characterization of these compounds did not materialize until the early 19th century, when the German chemist Friedlieb Ferdinand Runge isolated caffeine in its pure crystalline form in 1820. This milestone laid the foundational bedrock for modern central nervous system (CNS) pharmacology. In the subsequent decades, structural elucidation revealed that these molecules belong to the purine alkaloid family, consisting of a xanthine core adorned with varying degrees of methylation. While early medical applications restricted methylxanthines to primitive bronchodilation and cardiac stimulation, late 20th-century receptor pharmacology fundamentally revolutionized our comprehension of their structural purpose. The discovery of endogenous adenosine receptors in the mammalian brain established that caffeine acts as a structural analogue to adenosine, functioning as a non-selective competitive antagonist. This insight transformed caffeine from a simple metabolic stimulant into a sophisticated pharmacological tool capable of modulating intricate neurochemical circuits.

In contemporary molecular medicine, the research trajectory surrounding methylxanthines has expanded exponentially. No longer confined to the boundaries of lifestyle optimization or basic alertness, these compounds are at the forefront of neuroprotective therapeutic development. As global demographics shift toward an aging population, the incidence of debilitating neurodegenerative disorders—such as Alzheimer's disease (AD) and Parkinson's disease (PD)—has risen to critical, epidemic proportions. Current pharmaceutical regimens offer merely palliative relief, completely failing to halt or reverse the underlying progressive cascade of neuronal death. Methylxanthines present a highly compelling, multi-targeted therapeutic paradigm. Their ability to simultaneously suppress neuroinflammatory signaling, attenuate oxidative stress, preserve mitochondrial structural integrity, and optimize neurotransmitter homeostasis positions them as powerful candidates for disease-modifying interventions.

However, translating these robust in vitro and in vivo pre-clinical successes into predictable, reproducible human clinical outcomes faces severe obstacles. The systemic delivery of methylxanthines is heavily plagued by physiological hurdles. Upon oral ingestion, caffeine undergo rapid and extensive hepatic biotransformation via the cytochrome P450 enzyme system, specifically the CYP1A2 isoform. This rapid metabolism creates wild fluctuations in plasma concentrations and shortens the therapeutic window. More critically, reaching effective therapeutic concentrations within the brain requires crossing the highly restrictive endothelial network of the blood-brain barrier (BBB). Although lipophilic in nature, achieving sustained, targeted cerebral bio-distribution without causing systemic side effects—such as tachyarrhythmias, gastrointestinal distress, and profound psychomotor agitation—presents a major clinical challenge.

This review represents an exhaustive, state-of-the-art treatise designed to navigate this complex pharmacological landscape. We look deeply into the structural chemistry and metabolic pathways of primary methylxanthines, map their molecular mechanisms of action within the CNS, evaluate their neuroprotective performance across diverse neuropathological models, and provide a definitive analysis of the advanced nanotechnological delivery vehicles engineered to unlock their full therapeutic potential.

2.The Historical Odyssey of Methylxanthines

2.1Ethnobotanical Roots and Pre-Industrial Consumption Architecture

The historical narrative intertwining human civilization and plant-derived methylxanthines is an ancient, multi-millennial evolutionary saga. Long before the extraction apparatuses of modern chemical sciences could isolate a single molecule, ancestral human societies discovered, cultivated, and deeply integrated botanical decoctions containing purine alkaloids into their cultural, spiritual, and medical frameworks. These ancient groups identified a unique capacity within specific plants to alter human consciousness, defer somatic fatigue, enhance endurance, and sharpen psychological focus.

In Africa, the historical focus centers primarily on the wild shrubs of Coffea arabica within the highland forests of Ethiopia. Legendary lore often attributes the discovery to an Ethiopian goatherder named Kaldi, who allegedly noticed his herd displaying hyperactive, dance-like behaviors after eating the bright red berries of a native bush. Beyond folklore, historical records reveal that early Oromo communities actively crushed these coffee cherries, mixing them with animal fats to create dense, high-energy rations designed to sustain warriors during grueling long-distance migrations. This primitive practice reflects an early, practical understanding of sustained systemic energy liberation, long before the metabolic pathways of fatty acid oxidation or central nervous system stimulation were defined.

Simultaneously, in East Asia, the systematic cultivation of Camellia sinensis (tea) formed the cornerstone

of early Chinese medicinal and philosophical practices. Attributed to the mythical Emperor Shennong in

2737 BCE, tea emerged not merely as a pleasant beverage but as a profound medicinal potion used to

cleanse toxins, settle the digestive tract, and support prolonged, uninterrupted states of mental clarity

during meditation. This specific property was highly prized by Buddhist monks, who relied on the natural

synergy of caffeine and L-theanine within Camellia sinensis to sustain deep meditation without falling into

physical sleep or entering states of over-stimulated anxiety.

In Mesoamerica, the indigenous Mayan and Aztec civilizations pioneered the cultivation of Theobroma cacao (the cocoa tree). They transformed the bitter, methylxanthine-rich seeds into a specialized ceremonial drink known as 'xocolatl'. This beverage, heavily laced with natural spices and hot peppers, was strictly reserved for the ruling elites, high-ranking military commanders, and spiritual shamans. The Aztecs viewed cocoa as a divine gift from Quetzalcoatl, recognizing its capacity to act as a powerful cardiotonic stimulant and aphrodisiac—a physiological consequence we now know is driven by the subtle synergy between theobromine and various trace phenylethylamines.

2.2The Isolation Era: Moving from Botanical Mixtures to Pure Compounds

The scientific transformation of methylxanthines from raw, botanical mixtures into pure, isolated chemical compounds began during the early decades of the 19th century. This shift was fueled by the rapid expansion of analytical organic chemistry across Europe. The foundational milestone occurred in Germany in the year 1820. The legendary poet and statesman Johann Wolfgang von Goethe, who possessed an intense fascination with natural science, handed a small packet of rare Greek coffee beans to the brilliant young chemist Friedlieb Ferdinand Runge. Goethe urged Runge to use his analytical

techniques to uncover the exact, mysterious chemical agent responsible for the powerful psychoactive properties of coffee.

Using crude, sequential chemical extraction techniques involving acid-base washes and crystallization, Runge succeeded in isolating a pure, white, crystalline powder that he initially named 'Kaffeebase'. This historical isolate was later officially named caffeine. This successful experiment marked a major turning point, establishing that the psychological and physical alterations caused by coffee were driven by a distinct, quantifiable, and stable chemical molecule rather than a mystical, unmeasurable vital force.

Following Runge's discovery, a wave of chemical isolation swept across Europe. In 1827, the French chemist M. Oudry isolated an identical crystalline substance from tea leaves, naming it 'theine', which was later structurally confirmed to be identical to caffeine. In 1842, the Russian chemist Aleksandr Voskresensky successfully isolated the major dimethylated counterpart, theobromine, from the seeds of Theobroma cacao. Shortly thereafter, in 1888, the German pharmacologist Albrecht Kossel discovered and isolated another dimethylxanthine variant from Camellia sinensis, naming it theophylline. These historical discoveries proved that nature utilized a single, shared structural blueprint—the xanthine core—modifying it across different plant species through subtle changes in methylation to achieve unique botanical properties.

2.3Structural Clarification and Synthetic Organic Chemistry

With the primary methylxanthine molecules successfully isolated, the global chemical community shifted its focus toward unlocking their exact structural chemistry and atomic configurations. This proved to be a major intellectual challenge, requiring several decades of meticulous research. The ultimate breakthrough came through the systematic, relentless work of the legendary German chemist Hermann Emil Fischer during the late 19th century.

Fischer embarked on a comprehensive research project to map the structural relationships between uric acid, xanthine, adenine, guanine, and the isolated plant alkaloids. Through an extensive series of chemical degradation and reconstruction experiments, Fischer successfully determined that these compounds shared a fused, bicyclic heterocyclic ring structure. He introduced the overarching umbrella term 'purine' to define this chemical family in 1884.

In 1895, Fischer successfully synthesized caffeine from basic uric acid building blocks, conclusively demonstrating its exact atomic identity as 1,3,7-trimethylxanthine. He followed this with the total synthesis of theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine). This monumental achievement earned Fischer the Nobel Prize in Chemistry in 1902. His work effectively brought methylxanthines out of the domain of basic botanical extracts, positioning them as well-defined molecular targets ready for advanced pharmaceutical development and exploration.

2.4The Evolution of Clinical and Receptor Pharmacology

During the early to mid-20th century, the clinical application of methylxanthines was largely restricted to their peripheral physiological effects. Theophylline, because of its powerful capacity to relax bronchial smooth muscle, became a cornerstone therapy for treating severe acute asthma attacks and chronic obstructive pulmonary disease (COPD). Simultaneously, theobromine and caffeine were widely used as clinical diuretics and cardiovascular stimulants to treat congestive heart failure and optimize renal clearance.

However, the exact molecular mechanisms explaining why caffeine stimulated the central nervous system remained unknown. Early pharmacologists assumed that its psychostimulant actions were driven by its capacity to inhibit phosphodiesterase enzymes or mobilize intracellular calcium stores. This assumption was challenged when researchers realized that the doses required to activate these pathways

in vitro far exceeded the safe, non-toxic concentrations found in human plasma after standard consumption.

The ultimate breakthrough occurred in the 1970s and 1980s through pioneering work led by receptor pharmacologists like Solomon Snyder and Bertil Fredholm. Their research identified specific G-protein coupled receptors on mammalian cell walls that responded to endogenous adenosine. They discovered that at low, non-toxic, and physiologically relevant concentrations, caffeine acts as a competitive antagonist against both adenosine A1 and A2A receptors. This discovery transformed our understanding of the drug, revealing that caffeine does not directly flood the brain with artificial energy. Instead, it acts as an elegant structural analogue that blocks endogenous adenosine brakes, lifting the brain's natural inhibitory pathways. This crucial insight laid the groundwork for modern research into the powerful neuroprotective capabilities of methylxanthines.

3.Chemical Profiles and Medicinal Constituents

The pharmacodynamic identity of methylxanthines is fundamentally dictated by their precise molecular architecture. Chemically classified as purine-2,6-diones, these compounds consist of a fused, bicyclic ring system containing a six-membered pyrimidine ring coupled to a five-membered imidazole ring. The core xanthine skeleton is systematically methylated at specific nitrogen positions (N1, N3, and N7), generating distinct biochemical entities with radically divergent pharmacokinetic profiles, receptor binding affinities, and tissue-specific selectivities.

3.1Caffeine (1,3,7-trimethylxanthine)

Caffeine represents the fully methylated archetype of this chemical class. Its structure features three methyl groups located at the N1, N3, and N7 positions of the xanthine core. This precise configuration imparts a relatively high degree of lipophilicity, enabling the molecule to partition seamlessly through biological membranes and rapidly diffuse across the blood-brain barrier via simple passive diffusion. From a structural standpoint, the three methyl groups create a hydrophobic topography that enables close stacking interactions with the aromatic amino acid residues situated within the binding pockets of adenosine receptors. Caffeine serves as the master prodrug and central reference compound from which all subsequent systemic metabolites derive their pharmacological relevance.

3.2Theobromine (3,7-dimethylxanthine)

Theobromine is a dimethylated xanthine lacking the methyl substituent at the N1 position. Primarily sourced from Theobroma cacao, this structural modification severely reduces its affinity for central adenosine receptors compared to caffeine. However, the elimination of the N1 methyl group significantly alters its tissue tropism, shifting its primary pharmacological influence from the CNS to the cardiovascular, renal, and smooth muscle systems. Theobromine exhibits prolonged plasma half-life characteristics and acts as a potent smooth muscle relaxant and mild diuretic, operating primarily through mechanisms that bypass standard central adenosine antagonism.

3.3Theophylline (1,3-dimethylxanthine)

Theophylline possesses two methyl groups at the N1 and N3 positions, leaving the N7 nitrogen unmethylated. This structural layout generates a highly potent compound with an exceptional affinity for peripheral airway smooth muscle and unique intracellular enzymatic pathways. For decades, theophylline has been utilized as a classical bronchodilator in clinical medicine for treating asthma and chronic obstructive pulmonary disease (COPD). At the molecular level, its lack of an N7 methyl group enhances its capacity to inhibit phosphodiesterase enzymes and stimulate histone deacetylases, highlighting the profound impact that a single methyl deletion can have on clinical utility.

3.4Paraxanthine (1,7-dimethylxanthine)

Paraxanthine, resulting from demethylation at the N3 position, is the dominant metabolic consequence of caffeine ingestion in humans, accounting for over 80% of systemic caffeine clearance. Despite being historically categorized as an inactive breakdown product, modern molecular profiling has revealed that paraxanthine possesses exceptional pharmacological potency. It displays a significantly higher. binding affinity for both A1 and A2A adenosine receptors than its parent molecule, caffeine. Furthermore, paraxanthine exhibits unique properties in terms of enzymatic inhibition and synaptic plasticity modulation, making its comprehensive characterization essential to fully understanding the total in vivo impact of caffeine.

Methylxanthine	Chemical Structure	Primary	Log P	Primary Target Profile
Source				
Caffeine Coffea arabica	1,3,7-trimethylxanthine		-0.07	A1 / A2A Receptor Antagonist;
CNS Stimulant				
Theobromine	3,7-dimethylxanthine	Theobroma	-0.78	Cardiovascular modulator;
cacao Relaxant	Smooth Muscle			
Theophylline	1,3-dimethylxanthine	Camellia	-0.02	PDE Inhibitor; Powerful
sinensis HDAC Activator	Bronchodilator;			
Paraxanthine	1,7-dimethylxanthine	Caffeine	-0.62	Potent Adenosine Antagonist;
Metabolite	Lipolysis Stimulator			

3.5 Hepatic Metabolic Cascades

The systemic lifespan of caffeine is tightly regulated by a complex network of hepatic metabolic pathways. Upon absorption via the gastrointestinal tract, caffeine enters the portal circulation and reaches the liver, where it encounters the microsomal cytochrome P450 monooxygenase enzyme system. The primary catalyst driving this metabolic processing is the CYP1A2 enzyme, which is responsible for over 95% of initial caffeine clearance. Through highly coordinated phase-I biotransformation reactions consisting of parallel N-demethylation pathways, CYP1A2 strips specific methyl groups from the xanthine ring.

Demethylation at the N3 position yields paraxanthine (84%), demethylation at the N1 position produces theobromine (12%), and demethylation at the N7 position generates theophylline (4%).These secondary dimethylxanthines undergo subsequent, secondary demethylations driven by CYP2E1 and CYP1A2, ultimately breaking down into monomethylxanthines, urides, and complex uric acid derivatives (such as 1-methyluric acid and 1,3-dimethyluric acid) which are easily excreted by the kidneys. This metabolic cascade exhibits extreme inter-individual variability, heavily influenced by genetic polymorphisms within the CYP1A2 gene locus (such as the CYP1A2*1F allele), lifestyle factors like tobacco consumption (which strongly induces CYP1A2 expression), and the concurrent use of clinical pharmaceuticals that inhibit or accelerate these specific enzymatic pathways.

4.Molecular Mechanisms of Action in the Central Nervous System

The diverse neurological profiles exhibited by methylxanthines are not driven by a singular molecular trigger, but are the direct result of multi-layered, dose-dependent interactions across several critical neurochemical targets. At physiological concentrations, these compounds elegantly modulate synaptic transmission by blocking endogenous brake systems. At higher concentrations, they access deeper intracellular enzymatic and structural domains.

4.1 Competitive Antagonism of Adenosine A1 and A2A Receptors

The core mechanism underlying the neuropharmacological profile of caffeine is its competitive antagonism of G-protein coupled adenosine receptors, specifically the A1 and A2A subtypes. Under normal physiological conditions, extracellular adenosine concentrations accumulate progressively during waking hours, acting as a homeostatic indicator of metabolic strain and sleep debt. When adenosine binds to the A1 receptor, which is coupled to inhibitory G-proteins (Gi/o), it suppresses adenlyl cyclase activity, restricts intracellular cyclic adenosine monophosphate (cAMP) accumulation, blocks voltage-gated calcium channels, and opens inwardly rectifying potassium channels. This cascade decreases presynaptic neurotransmitter release, inducing sedation and slowing neural networks.

Simultaneously, adenosine activates the A2A receptor, which is coupled to stimulatory G-proteins (Gs or Golf). Primarily concentrated in the dopamine-rich regions of the striatum, A2A activation triggers adenylyl cyclase, elevates cAMP levels, activates protein kinase A (PKA), and suppresses psychomotor drive. Because caffeine possesses a structural core that perfectly mimics the purine ring of adenosine, it inserts itself cleanly into the orthosteric binding pockets of both receptor subtypes, blocking endogenous adenosine from binding. By preventing the inhibitory actions of A1 activation and reversing the sedative cascades of A2A stimulation, caffeine effectively removes the neural brakes, triggering a sustained increase in baseline neuroplasticity and alertness.

4.2 Downstream Neuromodulatory Cascades

The disruption of adenosine signaling by methylxanthines triggers a cascade of effects across multiple neurotransmitter networks. Within the striatum, A2A receptors form functional, physical heteromeric complexes with dopamine D2 receptors. When adenosine stimulates an A2A receptor, it induces a conformational change that reduces the binding affinity of the adjacent D2 receptor for dopamine, dampening locomotor drive. By blocking A2A receptors, caffeine removes this negative regulation, restoring dopamine D2 receptor affinity and augmenting dopaminergic transmission without directly stimulating dopamine release. This cross-talk enhances locomotor activity, sharpens focus, and drives the reinforcing behaviors linked to methylxanthines.

Furthermore, caffeine’s blockade of presynaptic A1 receptors on glutamatergic, cholinergic, and serotonergic terminals removes the inhibitory tone normally maintained by adenosine. This results in a synchronized, exocytotic surge of glutamate, acetylcholine, and serotonin into the synaptic cleft. The influx of glutamate drives post-synaptic NMDA and AMPA receptor signaling, enhancing long-term potentiation (LTP) within the hippocampus and strengthening memory consolidation. Simultaneously, the elevation of synaptic acetylcholine reinforces cortical arousal, sharpens sustained attention, and optimizes signal-to-noise processing across sensory networks.

4.3 Phosphodiesterase (PDE) Inhibition

At higher therapeutic concentrations, methylxanthines expand their pharmacological scope by directly inhibiting phosphodiesterase (PDE) enzymes, specifically the PDE1, PDE4, and PDE5 families. PDEs are responsible for breaking down the critical intracellular second messengers cAMP and cyclic guanosine monophosphate (cGMP) into their inactive monophosphate forms. By competitively blocking the catalytic core of PDEs, methylxanthines prevent this degradation, causing a sharp, sustained accumulation of intracellular cAMP and cGMP.

This elevation triggers downstream protein kinase cascades (PKA and PKG), which phosphorylate transcription factors like cAMP-response element-binding protein (CREB). The activation of CREB drives the transcription of essential neuroplasticity genes, including brain-derived neurotrophic factor (BDNF). Although achieving the high plasma concentrations required for robust PDE inhibition is

difficult through standard dietary consumption without risking cardiovascular side effects, this pathway serves as a vital target for synthetic methylxanthine derivatives engineered for targeted clinical applications.

4.4 Intracellular Calcium Mobilization via Ryanodine Receptors

At high concentrations, methylxanthines interact directly with intracellular calcium stores. Caffeine acts as a potent agonist for ryanodine receptors (RyR1 and RyR2) located on the membrane of the endoplasmic and sarcoplasmic reticulum. It lowers the threshold required for luminal calcium to trigger channel opening, causing an efflux of stored calcium ions into the cytosol.

This sudden surge in intracellular calcium activates various calcium-dependent enzymes, including calmodulin-dependent protein kinases (CaMK) and protein kinase C (PKC). These kinases modulate ion channel conductance, alter cytoskeletal dynamics, and regulate vesicle exocytosis. While this mechanism is highly pronounced in cardiac and skeletal muscle tissue—contributing to increased myocardial contractility and physical performance—it also influences central neurons during high-dose exposures, playing a key role in modulating synaptic transmission and, in extreme cases, driving xanthine-induced excitotoxicity.

4.5 Epigenetic Modifications via Histone Deacetylase (HDAC) Activation

An emerging frontier in methylxanthine pharmacology involves their capacity to induce precise epigenetic modifications, a mechanism heavily spearheaded by theophylline. At low micromolar concentrations, theophylline acts as a potent activator of histone deacetylase-2 (HDAC2). In chronic inflammatory states, the recruitment of inflammatory transcription factors (such as NF-κB) to gene promoters triggers the hyper-acetylation of core histones, unwinding chromatin and driving the expression of pro-inflammatory cytokines.

By actively stimulating HDAC2, theophylline promotes the deacetylation of these histones, tightly condensing the chromatin structure and physically blocking transcription factor access. This effectively silences the expression of toxic, pro-inflammatory genes. This unique epigenetic propertyallows theophylline to restore corticosteroid sensitivity in severe inflammatory diseases and offers a compelling new therapeutic angle for reversing the persistent, epigenetically driven microglial inflammation seen in chronic neurodegenerative disorders.

5.Mechanistic Pathways in Neuroprotection

The progression of neurodegenerative diseases is driven by a complex web of pathological mechanisms: the accumulation of misfolded proteins, chronic neuroinflammation, unchecked oxidative stress, and mitochondrial dysfunction. Rather than targeting a single endpoint, methylxanthines engage multiple pathways simultaneously, offering a comprehensive, multi-layered defense system that protects vulnerable neuronal populations from progressive degeneration.

5.1Attenuation of Neuroinflammation and Microglial Deactivation

Chronic, uncontrolled neuroinflammation is a primary driver of neurodegeneration. Microglia, the resident immune cells of the CNS, transition into a highly destructive, hyper-activated state (the M1 phenotype) when exposed to pathogenic aggregates like amyloid-beta or alpha-synuclein. In this state, they continuously secrete toxic pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and inducible nitric oxide synthase (iNOS). This creates a chronic inflammatory environment that damages neighboring neurons.

Methylxanthines, primarily through blocking adenosine A2A receptors on microglial membranes, halt this destructive cycle. A2A receptor activation normally amplifies the intracellular cascades that drive pro-inflammatory gene transcription. By blocking these receptors, caffeine suppresses the activation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. This shifts microglia away from the destructive M1 phenotype and toward the neuroprotective, resolving M2 phenotype. Consequently, the production of neurotoxic cytokines drops sharply, protecting nearby neurons and halting the self-perpetuating cycle of neuroinflammation.

5.2Mitigation of Oxidative Stress and Reactive Oxygen Species (ROS) Clearance

The metabolic demands of the human brain make it exceptionally vulnerable to oxidative stress. Left unchecked, the excessive accumulation of reactive oxygen species (ROS) causes widespread lipid peroxidation of neuronal membranes, denatures essential structural proteins, and induces catastrophic DNA strand breaks. Methylxanthines protect against this damage through a combination of direct chemical scavenging and the activation of endogenous antioxidant defenses. The molecular structure of caffeine contains electron-rich double bonds that allow it to directly neutralize highly reactive hydroxyl (.OH) and peroxyl radicals, converting them into stable, non-toxic chemical species.

Simultaneously, methylxanthines upregulate the expression of Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2), a master transcriptional regulator of antioxidant defenses. Under baseline conditions, Nrf2 is kept inactive in the cytoplasm by Keap1. Methylxanthines disrupt this binding, allowing Nrf2 to translocate into the nucleus and bind to Antioxidant Response Elements (ARE) on the genome. This triggers a robust upregulation of essential endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1). This two-pronged approach cleanses the cellular environment of ROS and strengthens the neuron's internal defenses against future oxidative insults.

5.3 Preservation of Mitochondrial Integrity and Inhibition of Apoptotic Cascades

Mitochondrial dysfunction is an early, critical checkpoint in the commitment of a neuron to apoptotic death. Exposure to neurotoxic stresses typically triggers an abnormal opening of the mitochondrial permeability transition pore (mPTP), causing a loss of mitochondrial membrane potential, the arrest of ATP synthesis, and the leakage of cytochrome c into the cytoplasm. Once in the cytosol, cytochrome c complexes with Apaf-1 to form the apoptosome, which activates the executioner caspase-3 cascade, leading to systematic cellular destruction.

Methylxanthines intercept this apoptotic cascade by regulating the Bcl-2 family of proteins. They actively upregulate the expression of anti-apoptotic Bcl-2 and Bcl-xl, while simultaneously suppressing the transcription of pro-apoptotic Bax and Bad. This preserves the structural integrity of the outer mitochondrial membrane, preventing the release of cytochrome c and halting caspase-3 activation. Furthermore, by optimizing complex I and complex IV activity within the electron transport chain, methylxanthines ensure stable, efficient ATP production even under pathological stress, providing neurons with the energy required to sustain vital cellular repair mechanisms.

6.Comprehensive Mechanistic Pathways in Neuroprotection

6.1The Adenosine Receptor Antagonism Architecture

The foundation of methylxanthine-mediated neuroprotection is rooted in its competitive antagonism of G-protein coupled adenosine receptors within the mammalian brain. Under baseline physiological conditions, extracellular adenosine concentrations follow a homeostatic sleep-wake cycle. As neurons consume ATP during hours of high cognitive activity, adenosine accumulates in the extracellular space, acting as a real-time molecular metric of metabolic stress and neural fatigue.

Adenosine A1 receptors are widely distributed throughout the brain, showing exceptionally dense expression in the cortex, hippocampus, and cerebellum. These receptors are coupled to inhibitory G-proteins (Gi/o). When adenosine binds to the A1 receptor, it triggers the alpha subunit to inhibit adenylyl cyclase, causing a sharp reduction in intracellular cyclic adenosine monophosphate (cAMP). This cascade closes voltage-gated N-type calcium channels and opens inwardly rectifying potassium channels, stabilizing the presynaptic membrane and suppressing the release of excitatory neurotransmitters like glutamate.

Conversely, adenosine A2A receptors are highly concentrated within the striatum and basal ganglia, where they link to stimulatory G-proteins (Gs/Golf). When activated, they stimulate adenylyl cyclase, increase cAMP, activate Protein Kinase A (PKA), and reduce the binding affinity of adjacent Dopamine D2 receptors, suppressing locomotor drive.

Because the xanthine core of caffeine closely mimics the purine ring of adenosine, it fits precisely into the orthosteric binding pockets of both A1 and A2A receptors without activating them. By physically blocking endogenous adenosine from binding, caffeine maintains presynaptic calcium channel open states and keeps adenylyl cyclase active. This action prevents the depressive, sedative cascades of adenosine and preserves normal synaptic plasticity and transmission even when the brain is under pathological stress.

6.2 Downstream Neurochemical Modulatory Cascades

The competitive blockade of adenosine receptors by methylxanthines triggers a highly coordinated cascade of downstream neurochemical adjustments across several vital neurotransmitter networks. Within the striatum, the removal of the adenosine A2A brake plays a critical role in restoring balance to dopamine transmission. Under normal conditions, A2A activation structurally alters adjacent Dopamine D2 receptors, dampening their affinity for dopamine. By blocking the A2A receptor, caffeine keeps the D2 receptor in its high-affinity state, significantly enhancing dopaminergic signaling. This optimization improves locomotor control, heightens attention, and supports goal-directed behaviors without forcing an artificial, addictive flood of dopamine into the synaptic cleft.

Simultaneously, blocking presynaptic A1 receptors on glutamatergic, cholinergic, and serotonergic terminals removes the natural inhibition maintained by adenosine. This causes a synchronized, controlled surge of glutamate and acetylcholine into the synaptic cleft. The controlled influx of glutamate stimulates post-synaptic NMDA and AMPA receptors, triggering the phosphorylation of Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and driving Long-Term Potentiation (LTP) in the hippocampus. This process strengthens synaptic networks and forms the physiological foundation for learning and memory consolidation.

Concurrently, the increased availability of synaptic acetylcholine reinforces cortical arousal, sharpens sustained attention, and optimizes signal-to-noise processing across sensory networks. This extensive, multi-system modulation acts as a strong protective shield for neurons, preserving functional synaptic communication and protecting neural networks from progressive cognitive decline.

6.3 Neutralizing Oxidative Stress and Activating the Nrf2 Shield

Due to its high consumption of systemic oxygen, high concentration of polyunsaturated fatty acids, and relatively low baseline levels of endogenous antioxidant enzymes, the human brain is exceptionally vulnerable to oxidative stress. In neurodegenerative states, defective cellular metabolism generates a massive excess of reactive oxygen species (ROS), including superoxide anions (O2.-) and highly destructive hydroxyl radicals (.OH). Left unchecked, these radicals attack cell membranes via lipid peroxidation, alter essential structural proteins, and cause catastrophic double-stranded DNA breaks, triggering apoptosis.

Methylxanthines counter this oxidative damage through a powerful two-pronged mechanism. First, they act as direct chemical scavengers. The core structure of methylxanthines features electron-rich double bonds that can directly interact with unstable free radicals, donating electrons to neutralize them into stable, harmless molecules like water and non-reactive xanthine derivatives.

Second, and more importantly, methylxanthines activate the **Nrf2** (Nuclear Factor Erythroid 2-Related Factor 2) genetic defense pathway. Under normal resting conditions, Nrf2 is kept inactive in the cytoplasm, bound to its inhibitor protein Keap1, which targets it for continuous degradation.

Methylxanthines modify specific cysteine residues on the Keap1 protein, changing its shape and freeing Nrf2 from its grip. The liberated Nrf2 translocates into the nucleus, where it binds to Antioxidant Response Elements (ARE) across the genome.

This genetic binding triggers a massive, coordinated upregulation of essential endogenous antioxidant enzymes, including Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Heme Oxygenase-1 (HO-1). This robust cellular defense rapidly clears accumulated ROS, minimizes lipid peroxidation across neuronal membranes, and significantly enhances the neuron's internal resilience against future oxidative stress.

6.4 Epigenetic Control via Histone Deacetylase (HDAC) Overactivation

An emerging frontier in methylxanthine research focuses on their capacity to induce precise epigenetic modifications within damaged neurons and surrounding glial cells. This specialized pathway is primarily driven by the dimethylxanthine variant theophylline. In chronic neurodegenerative states, persistent inflammatory signaling triggers the activation of histone acetyltransferases (HATs). HATs add acetyl groups to core histone proteins, opening up the chromatin structure and allowing transcription factors like NF-kB easy access to drive the continuous expression of pro-inflammatory cytokines.

At low, non-toxic micromolar concentrations, theophylline serves as a potent activator of **Histone Deacetylase-2 (HDAC2)**. HDAC2 reverses this process by removing acetyl groups from the lysine residues on histones H3 and H4. This deacetylation causes the chromatin structure to coil tightly back into a dense, closed configuration known as heterochromatin. This physical condensation blocks transcription factors from reaching the gene promoters, silencing the expression of toxic pro-inflammatory cytokines and inflammatory proteins.

Additionally, this HDAC2 activation helps restore normal gene expression for neuroprotective factors like Brain-Derived Neurotrophic Factor (BDNF). By dynamically shifting the epigenetic balance away from continuous inflammatory transcription toward tightly controlled chromatin structure, methylxanthines help reset glial cells, providing a valuable mechanism for halting long-term, self-perpetuating neuroinflammation in the brain.

7.Therapeutic Applications in Neurodegenerative Diseases

The clinical relevance of methylxanthines is vividly demonstrated across various models of debilitating neurodegenerative conditions. By targeting the fundamental drivers of cell death, these compounds show clear potential to alter the course of diseases that have long resisted traditional single-target therapies.

7.1Alzheimer's Disease (AD)

Alzheimer's disease is characterized by the accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein. In advanced transgenic models of AD, long-term administration of caffeine significantly reduces brain Aβ levels and slows cognitive decline. This therapeutic effect is driven by several complementary mechanisms.

First, caffeine modulates the processing of amyloid precursor protein (APP). It suppresses the expression of beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), the rate-limiting enzyme responsible for producing toxic Aβ peptides, while shifting APP processing toward the non-amyloidogenic alpha-secretase pathway. Second, caffeine blocks adenosine A2A receptors, which directly dampens the activity of glycogen synthase kinase-3 beta (GSK-3β)—the primary kinase responsible for abnormal tau hyperphosphorylation. By keeping GSK-3β inactive, caffeine prevents tau detachment from microtubules, preserving axonal transport and preventing the formation of neurofibrillary tangles. Finally, caffeine enhances the expression of neurotrophic factors like BDNF, which supports hippocampal synaptic plasticity and protects spatial memory networks from amyloid-induced decay.

7.2 Parkinson's Disease (PD)

Parkinson's disease involves the selective, progressive loss of dopaminergic neurons within the substantia nigra pars compacta, leading to severe motor deficits. Epidemiological studies consistently show a strong, dose-dependent inverse correlation between caffeine consumption and the risk of developing PD. The neuroprotective effects of caffeine in PD are highly localized and mechanistically precise.

Within the striatum, adenosine A2A receptors are highly co-expressed with dopamine D2 receptors. In PD, the loss of dopamine leads to an overactivation of the indirect pathway, causing severe movement suppression. By acting as a selective antagonist at these striatal A2A receptors, caffeine removes this inhibition, restoring balance to basal ganglia circuitry and improving motor coordination without inducing the dyskinesias often triggered by direct dopamine agonists. Furthermore, in animal models using toxins like MPTP or 6-OHDA, caffeine administration directly protects dopaminergic neurons by

suppressing microglial activation and preventing the down-regulation of dopamine transporters (DAT), demonstrating a clear capability to preserve vulnerable nigrostriatal pathways.

7.3Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive loss of upper and lower motor neurons, leading to muscle atrophy, respiratory failure, and death. The pathology of ALS is deeply rooted in excitotoxicity, driven by an accumulation of extracellular glutamate, and severe neuroinflammation in the spinal cord.

Methylxanthines, particularly theophylline and specialized caffeine derivatives, have shown encouraging protective effects in preclinical ALS models (such as SOD1-G93A transgenic mice). This protection is mediated primarily through the upregulation of glutamate transporter-1 (GLT-1/EAAT2) on astrocytes, which accelerates the clearance of excess glutamate from the synaptic cleft and prevents excitotoxic calcium influx in motor neurons. Additionally, by suppressing microglial-mediated inflammation around spinal motor neurons, methylxanthines slow the progressive loss of motor units, preserve diaphragmatic function, and extend survival windows, offering a valuable multi-targeted strategy for ALS drug development.

8.Pharmacokinetic Hurdles and Delivery Limitations

Despite the exceptional therapeutic potential demonstrated by methylxanthines in laboratory settings, their successful clinical application remains severely restricted by significant pharmacokinetic and physiological barriers. When administered orally in standard free forms, these molecules encounter a range of systemic obstacles that dramatically lower their therapeutic index.

8.1Rapid Clearance and Hepatic First-Pass Metabolism

Free caffeine is rapidly and completely absorbed in the upper gastrointestinal tract, leading to a sharp spike in plasma concentration. However, this peak is short-lived due to aggressive hepatic metabolism driven by the cytochrome P450 (CYP1A2) enzyme system. This extensive first-pass metabolism breaks down the parent molecule into its dimethylated derivatives, causing erratic fluctuations in plasma half-life and requiring frequent, large doses to maintain therapeutic concentrations.

These high doses often lead to systemic accumulation, which can trigger severe adverse side effects. These include cardiovascular complications like sinus tachycardia and atrial fibrillation, gastrointestinal irritation from increased gastric acid secretion, and profound psychomotor agitation. This narrow therapeutic window makes it exceedingly difficult to maintain safe, steady, and effective systemic levels over extended periods using conventional oral delivery methods.

8.2The Blood-Brain Barrier (BBB) Restriction

The ultimate obstacle to treating central neurodegenerative disorders is the blood-brain barrier (BBB). This highly specialized endothelial interface features continuous tight junctions (such as claudin-5, occludin, and ZO-1) and a high concentration of efflux transporters, including P-glycoprotein (P-gp) and multidrug resistance-associated proteins (MRPs). While free caffeine is lipophilic enough to cross the BBB via passive diffusion, achieving and maintaining effective therapeutic concentrations within specific deep-brain regions remains highly challenging.

To overcome efflux mechanisms and compensate for rapid systemic clearance, clinicians are often forced to escalate oral or intravenous doses. This approach floods peripheral tissues with the active drug, increasing the risk of adverse systemic side effects before adequate concentrations can be reached in the brain. Consequently, there is an urgent need for advanced delivery systems that can shield the drug from rapid hepatic breakdown, bypass peripheral targets, and safely guide methylxanthines directly across the BBB to vulnerable neural tissues.

9.Emerging Nanotechnological Delivery Systems

To overcome the pharmacokinetic limitations of traditional formulations, modern nanomedicine is developing sophisticated nanotechnological delivery platforms. By encapsulating methylxanthines within engineered, sub-micron scale vehicles, researchers can protect the active compounds from

premature breakdown, extend their circulation time, and direct them precisely to target sites within the central nervous system.

9.1Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs)

Lipid-based nanocarriers represent a major breakthrough in brain-targeted drug delivery. Solid Lipid Nanoparticles (SLNs) are constructed from biocompatible, solid-phase lipids (such as purified triglycerides or steroids) stabilized by non-toxic surfactants. Nanostructured Lipid Carriers (NLCs) represent a second-generation advancement, incorporating a precisely blended mixture of solid and liquid lipids. This liquid lipid component creates an imperfect, disrupted crystalline matrix that prevents drug expulsion during storage and significantly increases drug loading capacity.

The naturally lipophilic surface of SLNs and NLCs allows them to interact seamlessly with the endothelial membranes of the blood-brain barrier. Furthermore, these nanoparticles can be modified by adsorbing specific surfactants, such as Polysorbate 80. Once in circulation, Polysorbate 80 triggers the selective adsorption of endogenous Apolipoprotein E (ApoE) from the blood onto the nanoparticle surface. This biomolecular coating mimics natural lipoproteins, enabling the nanoparticles to bind to low-density lipoprotein (LDL) receptors on the BBB and cross into the brain via receptor-mediated endocytosis, bypassing traditional efflux pumps.

9.2Polymeric Nanoparticles

Polymeric nanoparticles offer exceptional structural stability, highly predictable release kinetics, and extensive options for surface modification. Biocompatible and biodegradable polymers, such as Poly(lactic-co-glycolic acid) (PLGA) and Chitosan, are widely used to encapsulate methylxanthines. PLGA nanoparticles release their therapeutic payload over extended periods through a controllable combination of matrix erosion and ester bond hydrolysis.

Chitosan, a natural cationic polymer derived from chitin, possesses unique mucoadhesive properties due to its positive charge, which interacts strongly with negatively charged sialic acid residues in mucus layers. This makes chitosan nanoparticles highly effective for intranasal delivery, allowing drugs to bypass the systemic circulation entirely and travel directly to the brain along the olfactory and trigeminal nerve pathways. Additionally, the surfaces of these polymeric spheres can be easily functionalized with hydrophilic Polyethylene Glycol (PEG) chains. This 'PEGylation' creates a protective water barrier that prevents detection by the mononuclear phagocyte system, extending circulation times and ensuring a steady, prolonged release of the drug.

9.3Nanoemulsions and Liposomes

Nanoemulsions are thermodynamically stable, transparent dispersions of oil and water with droplet sizes typically under 200 nm, stabilized by an optimized mixture of surfactants and co-surfactants. These systems can dissolve large quantities of hydrophobic molecules, shield them from rapid hepatic breakdown, and significantly enhance oral bioavailability. Liposomes, by contrast, are spherical vesicles composed of a lipid bilayer surrounding an aqueous core.

This dual-nature architecture allows liposomes to simultaneously encapsulate hydrophilic molecules within their core and lipophilic compounds within the lipid bilayer. Structurally optimized liposomes prevent premature chemical degradation, exhibit excellent stability in circulation, and fuse directly with cellular membranes. This fusion enhances the intracellular delivery of methylxanthines, making liposomes an effective platform for maximizing therapeutic impact at the cellular level.

9.4 Functionalized Carbon Nanotubes (CNTs)

At the cutting edge of nanomedicine, functionalized carbon nanotubes (CNTs)—including single-walled (SWCNTs) and multi-walled (MWCNTs) variants—are attracting significant interest as high-capacity drug delivery vehicles. Due to their unique needle-like shape, CNTs can penetrate cellular membranes via an energy-independent pathway known as 'nanopenetration', delivering therapeutics directly into the cytoplasm without disrupting cell structure.

Raw carbon nanotubes are inherently toxic and insoluble. However, precise surface functionalization through the covalent attachment of PEG chains or target-specific peptides renders them highly biocompatible and dispersible in biological fluids. By conjugating methylxanthines to the exterior walls or encapsulating them within the hollow inner core of functionalized CNTs, researchers can achieve highly targeted, localized delivery into deep brain tissues, opening new possibilities for ultra-high-efficiency neurotherapeutics.

Nanocarrier Type	Composition Matrix	Encapsulation Efficiency	Primary Delivery Route	Mechanistic Advantage
Solid Lipid Nanoparticles (SLNs)	Solid Lipids + Polysorbate 80	High (70-85%)	Intravenous / Oral	ApoE adsorption triggers receptor endocytosis across BBB
Nanostructured Lipid Carriers (NLCs)	Solid + Liquid Lipid Blend	Very High (85-95%)	Intravenous	Disrupted matrix prevents drug expulsion; high stability
PLGA Polymeric NPs	Poly(lactic-co-glycolic acid)	Moderate (60-75%)	Intravenous / Subcutaneous	Long-term sustained release via polymer erosion
Chitosan Nanoparticles	Cationic Chitosan Polymer	Moderate (65-80%)	Intranasal	Mucoadhesive properties enable direct olfactory brain delivery
Nanoemulsions	Oil / Water / Surfactant	High (75-90%)	Oral / Intranasal	Dramatically improves oral absorption and systemic bioavailability
Functionalized CNTs	Surface-modified Carbon Cylinders	Variable (50-70%)	Targeted Injection	Direct cellular penetration bypassing traditional transport limitations

10.Safety Profiles, Toxicological Thresholds, and Future Perspectives

While low to moderate consumption of methylxanthines is generally safe and well-tolerated, their transition into high-dose clinical therapies requires a thorough understanding of their toxicological boundaries. In adults, mild side effects like insomnia, anxiety, and mild muscle tremors can occur at daily doses above 400 mg. If plasma concentrations rise significantly higher due to accidental overdose or rapid, unregulated delivery, a severe condition known as acute xanthine toxicity can emerge.

This toxic state is characterized by profound electrolyte imbalances (such as severe hypokalemia), cardiac arrhythmias (including ventricular fibrillation), persistent generalized seizures, and metabolic acidosis. At the cellular level, these severe symptoms are driven by unchecked ryanodine receptor activation and widespread intracellular calcium overload, coupled with the systemic blockade of adenosine receptors throughout peripheral organs. Accordingly, the clinical development of methylxanthine therapies must prioritize precise, controlled delivery methods to avoid triggering these dangerous systemic responses.

The future of methylxanthine pharmacology lies at the intersection of precise molecular design and advanced nanotechnology. Researchers are actively synthesizing novel xanthine derivatives designed with a higher selectivity for specific receptor subtypes, such as targeting microglial A2A receptors while minimizing interactions with cardiac tissue. When combined with smart, responsive nanocarriers—such

as nanoparticles designed to release their therapeutic payload only in response to localized markers of neuroinflammation, like high ROS concentrations or acidic pH levels—these next-generation therapies will offer unprecedented precision. By pairing refined molecular targeting with intelligent delivery vehicles, methylxanthine-based nanomedicine is well-positioned to transition from a conceptual breakthrough into a powerful class of clinically viable neuroprotective treatments.

11.Conclusion

In summary, methylxanthines have evolved far beyond their traditional roles as simple dietary stimulants to become sophisticated multi-targeted agents with significant therapeutic potential. Their ability to simultaneously modulate adenosine receptors, inhibit phosphodiesterases, regulate intracellular calcium, and induce targeted epigenetic modifications allows them to effectively address the complex, interconnected pathological features of neurodegenerative diseases. By simultaneously suppressing neuroinflammation, neutralizing oxidative stress, and preserving mitochondrial function, these compounds offer a robust defense against progressive neuronal loss in disorders like Alzheimer's, Parkinson's, and ALS.

The long-standing challenges of rapid clearance and limited brain targeting are being systematically resolved through innovative nanotechnological approaches. Modern delivery systems like solid lipid nanoparticles, polymeric matrices, and functionalized carbon nanotubes provide a reliable means of transporting these compounds across the blood-brain barrier directly to affected tissues. As structural engineering and targeted nanomedicine continue to advance, methylxanthine-based therapies are uniquely positioned to overcome historical limitations, offering promising new options for disease-modifying interventions in clinical neurology.

12.References

1. Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A., & Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that influence its widespread use. Pharmacological Reviews, 51(1), 83-133.
2. Chen, J. F., Eltzschig, H. K., & Fredholm, B. B. (2013). Adenosine receptors as drug targets — what are the prospects? Nature Reviews Drug Discovery, 12(4), 265-286. DOI: 10.1038/nrd3930
3. Ribeiro, J. A., & Sebastião, A. M. (2010). Caffeine and adenosine. Journal of Alzheimer's Disease, 20(s1), S3-S15. DOI: 10.3233/JAD-2010-1379
4. Nehlig, A., Daval, J. L., & Debry, G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Research Reviews, 17(2), 139-170. DOI: 10.1016/0165-0173(92)90012-B
5. Xu, K., Xu, Y. H., Chen, J. F., & Schwarzschild, M. A. (2010). Neuroprotection by caffeine: time course and role of adenosine A2A receptors in mice. Journal of Neuroscience, 30(24), 8135-8142. DOI: 10.1523/JNEUROSCI.0345-10.2010
6. Wissing, S. A., Kayser, O., & Müller, R. H. (2004). Solid lipid nanoparticles for topical, cosmetic and pharmaceutical applications. Advanced Drug Delivery Reviews, 56(5), 665-672. DOI: 10.1016/j.addr.2003.10.026
7. Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Solid lipid nanoparticles (SLN) and
8. nanostructured lipid carriers (NLC) for dermal delivery. Advanced Drug Delivery Reviews, 54, S131-S155. DOI: 10.1016/S0169-409X(02)00118-7
9. Jokerst, J. V., Lobovkina, T., Zare, R. N., & Gambhir, S. S. (2011). Nanoparticle PEGylation for imaging and therapy. Nanomedicine, 6(4), 715-728. DOI: 10.2217/nnm.11.19
10. Garcia-Benefiel, C. M., & Cortès-López, M. (2022). Epigenetic modulations of methylxanthines in human microglia. Frontiers in Cellular Neuroscience, 16, 894502. DOI:
11. 10.3389/fncel.2022.894502

12. Prediger, R. D. (2010). Effects of caffeine in Parkinson's disease: from epidemiology to molecular mechanisms. Journal of Alzheimer's Disease, 20(s1), S117-S140. DOI: 10.3233/JAD-2010-1386

13. Daly, J. W., Padgett, W., & Shamim, M. T. (1986). Analogs of caffeine: antagonism at adenosine receptors and inhibition of phosphodiesterases. Journal of Medicinal Chemistry, 29(7), 1305-1308. DOI: 10.1021/jm00157a034

14. Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). Alpha-synuclein in Lewy bodies. Nature, 388(6645), 839-840. DOI: 10.1038/42166

15. Hardy, J., & Higgins, G. (1992). Alzheimer's disease: the amyloid cascade hypothesis. Science, 256(5054), 184-185. DOI: 10.1126/science.1566067

16. Yan, R., & Vassar, R. (2014). BACE1: the beta-secretase enzyme in Alzheimer's disease. Molecular Neurodegeneration, 9(1), 1-14. DOI: 10.1186/1750-1326-9-30