Int J Ayurvedic Med,Vol 17 (3), 2026;

Ayurveda Sahiti Prabha's

International Journal of Ayurvedic Medicine

ISSN No: 0976-5921

Review Article

Ayurvedic Neuroprotective Phytochemicals and Their Evaluation in Zebrafish Models: Pharmacological Insights and Translational Challenges

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Received: 01-12-2025 Accepted: 21-06-2026 Published: 30-09-2026

Abstract

Ayurvedic medicine has been traditionally employed for the management of numerous neurological and psychological disorders through the use of medicinal plants enriched with biologically active phytochemicals. Several Ayurvedic herbs possess flavonoids, alkaloids, tannins, sterols, glycosides, and polyphenolic compounds that exhibit antioxidant, anti-inflammatory, neuroprotective, anxiolytic, and cognition-enhancing activities. Recent advances in experimental neuroscience have identified zebrafish (Danio rerio) as an efficient vertebrate model for investigating central nervous system (CNS) disorders and evaluating neuroactive compounds because of its genetic homology with humans, conserved neurotransmitter pathways, rapid embryonic development, and suitability for high-throughput screening. This review summarizes the CNS related pharmacological effects of important Ayurvedic medicinal plants, including *Withania somnifera* (L.) Dunal, *Bacopa monnieri* (L.) Wettst., *Curcuma longa* L., *Centella asiatica* (L.) Urb, *Ocimum tenuiflorum* L., and *Clitoria ternatea* L., with particular emphasis on their assessment in zebrafish models. Evidence from experimental studies indicates that these herbal interventions demonstrate neuroprotective, anti-stress, anxiolytic, anti-amnesic, and locomotor-regulating effects in zebrafish models of neurological dysfunction. Nevertheless, limitations associated with extract standardization, mechanistic validation, and interspecies translational variability continue to restrict clinical extrapolation. Furthermore, the review discusses the translational relevance of zebrafish in understanding molecular, behavioral, and neurochemical mechanisms associated with Ayurvedic therapies. Important challenges, including variability in phytochemical composition, dose standardization, pharmacokinetic limitations, species-specific metabolic differences, and safety concerns, are also critically evaluated. In addition, the potential integration of nanotechnology-based delivery systems with Ayurvedic medicines is highlighted as a promising strategy for improving bioavailability and therapeutic efficacy. Collectively, zebrafish models represent a valuable experimental platform for advancing Ayurvedic neuropharmacology and translational CNS drug discovery.

Keywords: Neuroprotective phytochemicals, Zebrafish (Danio rerio), Neuropharmacology, Central nervous system disorders, Neurodegenerative diseases, Ayurvedic medicinal plants, Translational research

Access this article online

Website: https://ijam.co.in

DOI: https://doi.org/10.47552/ijam.v17i3.6772

Introduction

For over five millennia, the traditional Indian healing practice known as Ayurveda has been utilized to manage a broad spectrum of diseases, including cancer, infectious, metabolic, cardiovascular, and neurological condition(1)(2). The word *Ayurveda*, derived from Sanskrit meaning "Knowledge of Life," emphasizes the therapeutic application of native herbs abundant in phytoconstituents such as flavonoids, lignans, sterols, tannins, and alkaloids, which possess notable anti-inflammatory, antioxidant, anti-arthritic, and other restorative properties (3). Considered one of the world's most ancient and enduring healthcare traditions, its global acceptance continues to expand. At present, nearly 10% of the adult population in the United States and about 90% of individuals in India make use of Ayurvedic formulations, valued for their historical relevance and minimal adverse reactions.

Medicinal plants like curcumin, *Ashwagandha (Withania somnifera* (L.) Dunal), and holy basil (*Ocimum tenuiflorum*) have long been prescribed for managing neurological disorders (4). In Sanskrit, such ailments are termed "vata-vyadhi," where "vata" refers to the vital force governing neural and mental activities, and "vyadhi" denotes disease (2). According to Ayurvedic principles, physiological balance is regulated by three *doshas—vata, pitta,*

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and kapha which are believed to influence neurological and systemic health. Imbalances in these functional energies are traditionally associated with various CNS related disorders (5)(6). Generally, each individual has one predominant *dosha*, forming their unique constitutional type termed *prakriti*. This *prakriti* determines a person's tendency toward certain health conditions based on their physical, emotional, physiological, and behavioral characteristics, regardless of cultural, ethnic, or environmental background (7). Ayurveda has traditionally been used for the management of several CNS disorders, including anxiety, depression, Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD), many of which are increasingly investigated using zebrafish neuropharmacological models. (Tables 1 and 1–Supplementary material).

In addition to clinical research, animal models particularly rodents are increasingly utilized in translational studies to evaluate the CNS effects of Ayurvedic therapies (discussed later) (8)(9). Growing evidence also indicates that alternative models, such as the zebrafish (*Danio rerio* Hamilton), offer valuable platforms for investigating the neurological actions of Ayurvedic interventions. In this review, we examine how zebrafish can be used to study the CNS effects of various Ayurvedic plants, based on a thorough analysis of literature from major biomedical databases including Web of Science, PubMed, ScienceDirect, Scopus, and Google Scholar. Moreover, we discuss the current challenges and limitations of using fish models in Ayurvedic pharmacological research and suggest future directions for advancing translational studies in this field.

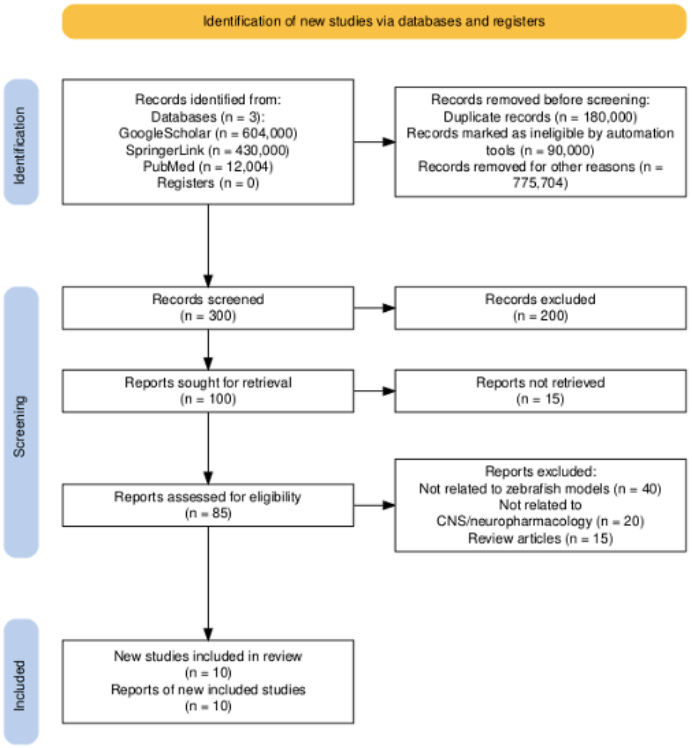
Methods — Search strategy and selection criteria

A systematic literature search was conducted to identify studies relevant to the neuropharmacological evaluation of Ayurvedic medicinal plants in zebrafish models. The search was performed using three major scientific databases: PubMed (n = 12,004), Google Scholar (n = 604,000), and SpringerLink (n = 430,000), yielding a total of 1,046,004 records. No additional records were identified through registers. A comprehensive search strategy was employed using combinations of keywords related to Ayurveda, medicinal plants, zebrafish (Danio rerio), neuroprotection, neuropharmacology, central nervous system disorders, Alzheimer's disease, Parkinson's disease, anxiety, and depression. Boolean operators (AND, OR) were used to optimize retrieval of relevant studies.

During the identification stage, 180,000 duplicate records, 90,000 records identified as ineligible through automated screening tools, and 775,704 records removed for other reasons, including irrelevant article types and lack of relevance to the review objectives, were excluded prior to screening. Consequently, 300 records remained for title and abstract screening. Following screening, 200 records were excluded because they did not involve zebrafish models, lacked CNS related outcomes, or were unrelated to Ayurvedic medicinal interventions.

A total of 100 reports were sought for full-text retrieval, of which 15 could not be obtained because of access limitations or unavailability of complete articles. The remaining 85 reports underwent full-text eligibility assessment. Subsequently, 40 studies were excluded because they were not related to zebrafish models, 20 studies were excluded because they did not evaluate CNS or neuropharmacological outcomes, and 15 studies were excluded because they were review articles, editorials, conference abstracts, or book chapters. Ultimately, 10 studies satisfied all predefined inclusion criteria and were included in the final review. These studies provided relevant evidence regarding the neuropharmacological effects of Ayurvedic medicinal plants in zebrafish models. The complete study selection process is illustrated in the PRISMA flow diagram.

Figure 1: PRISMA flow cart



Illustrative examples of experimental models used to investigate the central nervous system (CNS) effects of Ayurvedic medicines;

Robust clinical data are supported by various experimental animal models employed to assess the therapeutic potential of Ayurvedic medicines (6). For instance, Indian ginseng (*Withania somnifera* (L.) Dunal), recognized for its effectiveness in treating stress, epilepsy, and Parkinson's disease (PD) in humans (10), has also shown promising outcomes in experimental models of Alzheimer's disease (AD). It helps reduce neurofibrillary tangles and neutralizes free radicals, thereby protecting dopaminergic neurons in rodent studies (11). Commonly known as *Ashwagandha*, this plant has been part of Ayurvedic treatment for thousands of years to alleviate pain, inflammation, insomnia, and stress.Extracts from *Ashwagandha* decrease lipid peroxidation, enhance tyrosine hydroxylase (TH) activity in the substantia nigra, and exhibit antioxidant effects in mice (12).

In transgenic AD mouse models, root extracts demonstrate neuroprotection against amyloid beta (Aβ)-induced toxicity and oxidative stress, while also reducing Aβ accumulation in the brain (13). *Ashwagandha* supports neurite regeneration and increases central gamma-aminobutyric acid (GABA) activity, making it useful in epilepsy treatment (14). Furthermore, it improves cognitive functions, restores motor abilities, and regulates acetylcholine esterase (AChE) activity in Huntington's disease (HD) models (15). Both methanolic and aqueous extracts of Indian ginseng (*Withania somnifera* (L.) Dunal) have been shown to boost serotonergic activity via 5HT 2A/2C receptors in mouse models of obsessive-compulsive disorder (16).

Another notable Ayurvedic herb is water hyssop (*Bacopa monnieri* (L.) Wettst), referred to as brahmi ("the plant of Lord

Brahma"), which is frequently used to support cognitive function, reduce anxiety, and manage epilepsy (17). The therapeutic effects of this plant are largely due to its bioactive saponins, including bacoside A3, bacopaside II, bacopasaponin C, and bacopaside I. Water hyssop is recommended for individuals with neurological diseases (18) and has been shown in animal studies to enhance cognition while alleviating anxiety and stress (5). It facilitates neuroplasticity by upregulating brain-derived neurotrophic factor (BDNF) expression in mice (19). At lower doses, the extract reduces pro-inflammatory interleukins in microglial cells (20), mitigates oxidative stress, protects neuronal and glial populations, and decreases neuroinflammation in rat models (21)(22). Moreover, its cognitive-enhancing effects have been well documented in humans.

Curcumin, the primary bioactive substance in turmeric (*Curcuma longa* L.), offers neuroprotective effects in Alzheimer's disease (AD) and displays anti-inflammatory, antioxidant, and anxiolytic properties in both in vitro and in vivo studies (3). In Ayurveda, turmeric is esteemed for its anti-amyloid and anti-inflammatory effects, providing therapeutic advantages in neurological disorders including tardive dyskinesia, Alzheimer's disease (AD), depression, epilepsy, and other CNS-related conditions (23). The key active ingredient, curcumin, boosts brain monoamine (24) levels in mice by suppressing monoamine oxidase (MAO)-A and demonstrates complementary effects when used alongside serotonergic antidepressants (25). Currently marketed as Longvida and undergoing Phase II clinical trials for AD with FDA approval, curcumin also diminishes microglial and astrocyte activation, lowers cytokine release, and interferes with the NFκB signaling pathway actions that support its therapeutic potential against AD. Furthermore, it is applied clinically as an adjunctive therapy for the management of neuropathic pain(26) (27).

Indian pennywort (*Centella asiatica* (L.) Urb.), traditionally called *gotu kola or mandukaparni* in Ayurveda, is another widely utilized medicinal plant (28). It possesses strong antimicrobial, antioxidant, antiulcer, antidiabetic, anticancer, antidepressant, and cognitive-enhancing effects (29). Rich in flavonoids, antioxidants, and vitamins, Indian pennywort traditionally supports cognition and mindfulness, as well as epilepsy, depression, wound healing, and infection control. It is classified as a key 'Medhya Rasayana' (a term meaning 'mind-enhancing' or nootropic) herb, which improves learning and memory in rodent studies (30)(31). The plant inhibits enzymes like phospholipase A2 (PL-A2) and acetylcholine esterase (AChE), protects cerebellar cells against amyloid-beta toxicity (32), exhibits anti-inflammatory effects in neurons treated with lipopolysaccharides (LPS), and shows anxiolytic, anti-stress, and antidepressant actions in live models (33).

Morning glory (*Convolvulus pluricaulis* Choisy), known as S*hankhapushpi* and classified as another important *Medhya Rasayana* nootropic herb, along with spikenard (*Nardostachys jatamansi (D.Don) DC.*), which has promnestic, antidepressant, and antiepileptic properties, both demonstrate cognitive-enhancing effects in clinical and rodent studies (34). Likewise, root extracts from Asian pigeon wing (*Clitoria ternatea* L.), a traditional Ayurvedic nootropic called aparajita or 'butterfly pea,' improve learning and memory retention in rats. The medicinal effects of this plant are primarily due to its bioactive constituents, including triterpenoids, glycosides, anthocyanins, and steroids, which contribute to its calming, mood-enhancing, anticonvulsive, and sedative properties (35).

Regarded as a key herb in Ayurveda, *Tulsi* (*Ocimum tenuiflorum* L.) is known to enhance overall mental and physical rejuvenation, boost cognitive function and metabolism, and alleviate stress, anxiety, and depression (36)(37). It also shows anticonvulsant effects in clinical settings (38). Similar to its effects in humans, ethanol extracts of tulsi produce anti-stress effects in rodent models by lowering Plasma corticosterone concentrations in response to both short-term (acute) and long-term (chronic) noise-induced stress, while also enhancing memory performance (39). In an experimental rat model of Neurodegenerative diseases, tulsi extract facilitates to reverse lipid peroxidation, restores deficits in active avoidance learning and memory retention, and normalizes superoxide dismutase enzyme activity (40).

Table 1: Representative Ayurvedic medicinal herbs, their key active ingredients, and the diverse effects on the central nervous system (CNS)

Herb	Botanical Name	Primary Bioactive Constituents	Neurological / Cognitive Disorders Addressed	Ref
Ashwagandha	*Withania somnifera* (L.) Dunal	Somnine, somniferine, sominone, and withanolides (A, B, Q, IV, and V)	Neurodegenerative disorders such as AD, PD, HD; also beneficial in stress and epilepsy	(11)(14)
Brahmi	Bacopa monnieri	Bacopasaponins, Bacoside A, Bacoside B, and D-mannitol	Cognitive impairment in AD and PD; stress and anxiety	(21)(22)
Jatamansi	*Nardostachys jatamansi* (D.Don) DC	Sesquiterpenoid derivatives and valerianan oid compounds	Memory impairment in AD and insomnia	(99)(100)
Curcumin	Curcuma longa	Curcumin	Symptomatic management in AD and PD, and anxiety-related conditions	(2)(3)
Mandukaparni	Centella asiatica	Asiaticoside A & B, asiatic acid	Cognitive impairment in AD and PD	(101)
Shankhpushpi	Convolvulus prostratus	flavonols, Glycosides, steroidal components	cognitive fatigue and sleep disturbances	(97)(98)
Shatavari	Asparagus racemosus	Shatavarin IV	AD- and PD-related dysfunction and neuroinflammatory states	(2)
Guggul	Commiphora wightii	Guggulsterols (I–V), E/Z-guggulsterone	Dementia and age-related cognitive impairment	(102)

The brain–gut axis has emerged as a critical factor in numerous central nervous system (CNS) disorders, including depression, anxiety, and neurodegenerative diseases (41). For instance, inflammatory bowel disease (IBD), which involves persistent

inflammation of the gastrointestinal tract and includes Crohn's disease and ulcerative colitis, is linked to elevated CNS inflammation, worsening mood and affective disturbances (42). Individuals with Crohn's disease frequently show increased anxiety compared to healthy subjects (43) and have a higher risk of depression (44). Traditionally, Ayurvedic treatments have been employed to help restore homeostasis within the brain–gut axis in clinical settings (45). For instance, an 8-week course of curcumin extract (Curcugen) at 500 mg daily has been linked to notable improvements in digestive symptoms and reductions in anxiety among adults reporting digestive issues (46).Likewise, an 8-week course of ashwagandha (*Withania somnifera* L. Dunal) significantly enhanced cognitive performance, including both short-term and overall memory, compared to placebo (47). In addition to clinical observations, research in rodent models has demonstrated that Ayurvedic treatments such as *Withania somnifera* , aqueous extracts of *Azadirachta indica* A. Juss (48), and combined probiotics with curcumin alleviate anxiety- and depression-like behaviors (49)(50). Overall, these results highlight the essential role of animal models in deepening our understanding of the central nervous system effects of Ayurvedic therapies.

Evaluating specific Ayurvedic medications in zebrafish central nervous system models

Alongside clinical and rodent studies, zebrafish have recently been acknowledged as a valuable vertebrate in vivo model for exploring central nervous system (CNS) disorders and supporting drug discovery (51). Both adult and larval stages of zebrafish serve as effective vertebrate models for investigating CNS functions (52), owing to their significant genetic and physiological resemblance to humans, rapid developmental processes, and conserved neurotransmitter systems (53).Approximately 70% of human genes and a substantial proportion of disease-associated genes have functional homologs in zebrafish, supporting their translational utility in neurological research. In addition, zebrafish possess a functional blood-brain barrier (BBB) with structural and physiological similarities to mammals, making them useful for evaluating CNS drug permeability and neuroactive compounds. Zebrafish possess well-characterized neuroanatomical structures and conserved neurotransmitter systems comparable to mammals, including dopaminergic, serotonergic, cholinergic, glutamatergic, and GABAergic pathways. These conserved neurochemical systems enable effective investigation of neurobehavioral responses and pharmacological modulation relevant to human CNS disorders. Further benefits of using zebrafish include their neuroanatomical similarity to humans (54), ease of care, high reproductive capacity, and access to well-established behavioral testing methods. Commonly employed zebrafish behavioral paradigms include novel tank exploration, scototaxis, thigmotaxis, social interaction assays, predator avoidance tests, and locomotor activity assessments, which collectively facilitate evaluation of anxiety-like behavior, cognitive impairment, stress responses, and neurotoxicity. Zebrafish respond to all main categories of CNS drugs, providing a cost-efficient in vivo platform for drug screening with the capability for high-throughput screening (HTS) (55)(56). Zebrafish models are increasingly utilized in neurodegenerative disease research because they enable rapid visualization of neuronal damage, oxidative stress responses, neuroinflammatory changes, and dopaminergic dysfunction associated with disorders such as Alzheimer's disease and Parkinson's disease.Given their promising application in screening a broad spectrum of medicines, zebrafish have recently been employed as a preclinical model to explore molecular mechanisms and develop new neurological therapies derived from Ayurvedic herbal treatments (57). Growing evidence, outlined in Table 2 and illustrated with select detailed examples, strongly reinforces the zebrafish's role in investigating how Ayurvedic botanicals affect the central nervous system. Examples of these CNS effects are shown in Figure 1. Nevertheless, most available studies predominantly rely on behavioral observations without extensive molecular or mechanistic validation. Additional investigations integrating neurochemical, genomic, and proteomic analyses are necessary to better clarify the pathways underlying the observed CNS effects of Ayurvedic interventions in zebrafish models.

Table 2: A few instances of screening the central nervous system effects of Ayurvedic medications utilizing zebrafish models.

Herbal/Traditional Medicine	Scientific Name	Test Sex	Central Nervous System Activity	Ref
Curcumin	Curcuma longa L	Both	Improved motor performance and reduced neurotoxicity in rotenone-induced Parkinsonian models	(61)
Indian pennywort	*Centella asiatica* (L.) Urb.	Both	Protected dopaminergic neurons by increasing BDNF levels and minimizing α-synuclein build-up under rotenone stress	(28)(103)
Carob	Ceratonia siliqua	Both	Demonstrated antioxidant and anti-AChE properties, improving cognition in 6-OHDA Parkinsonism	(62)
Indian ginseng	*Withania somnifera* (L.) Duna	Both	Enhanced CNS antioxidant capacity and protected neural tissue from benzo[a]pyrene-related damage	(60)
Tulsi	Ocimum tenuiflorum L.	Male	Prevented scopolamine-induced memory impairment by preserving cognitive function	(63)
Asian pigeonwings	Clitoria ternatea L	Both	Reduced stress-related responses caused by reserpine	(65)
Brahmi	*Bacopa monnieri* (L.) Wettst.	Not specified	Decreased cortisol levels and alleviated anxiety-like behavior following stress exposure	(66)
Suvarna Bhasma[a]	–	Not specified	Ameliorated motor and behavioral impairments in rotenone-based Parkinson's disease models	(70)
–	–	Both	Reduced anxiety-like behavior	(71)
Neem	Azadirachta indica	Not specified	Reduced locomotor activity coupled with heightened anxiety-like responses	(104)

[a] Gold nanoparticles.

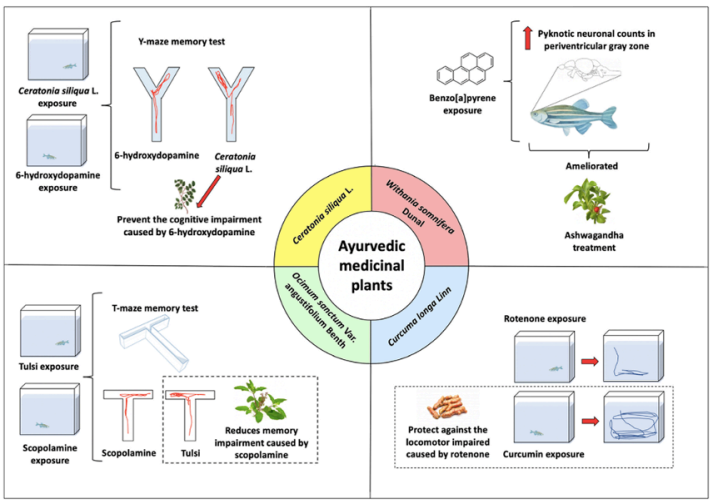


Figure 2: Several Ayurvedic medicinal plants have demonstrated neuroprotective effects in zebrafish models of central nervous system disorders. Ceratonia siliqua L. (carob), a plant native to the Mediterranean region, was found to prevent cognitive deterioration in the 6-hydroxydopamine (6-OHDA) model of Parkinson's disease (PD). Leaf extracts of *Ashwagandha* decreased the number of pyknotic neurons in the periventricular gray zone induced by benzo[a]pyrene-mediated neurotoxicity. In Zebra fish, Tulsi leaf extract showed efficacy in reducing memory deficits triggered by scopolamine. Moreover, curcumin, the principal bioactive constituent of turmeric, conferred protection against rotenone-induced locomotor impairments, a commonly used model for PD-related symptoms (105).

Indian pennywort

Extensive studies have been conducted on Indian pennywort using zebrafish models. In experiments involving rotenone-induced neurotoxicity, adult zebrafish treated with Indian pennywort extract demonstrated an increase in midbrain brain-derived neurotrophic factor (BDNF) levels, protection of dopaminergic neurons from damage, and exhibited fewer Parkinson's disease-like symptoms such as reduced alpha-synuclein accumulation, enhanced movement, and elevated dopamine levels in the brain (28). These findings suggest that *Centella asiatica* may exert neuroprotective effects through dopaminergic pathway regulation, antioxidant activity, and suppression of α-synuclein aggregation. The observed behavioral improvements in zebrafish locomotor assays further support its translational relevance in Parkinsonian neurodegeneration models.The herb's key compounds, including brahmoside and brahminoside, are traditionally used to promote CNS relaxation in humans and produce comparable effects in animal models (58). Another key bioactive molecule, asiaticoside, protects against cell damage and diminishes reactive oxygen species generated by hydrogen peroxide in vitro, and additionally lowers amyloid beta (Aβ) levels in the hippocampal region of rats (59).

Indian ginseng

Exposure to Indian ginseng extract has been shown to reduce benzo[a]pyrene-induced neurotoxicity in adult zebrafish by enhancing brain antioxidant defenses and restoring typical dark preference behavior (scototaxic response) (60). Interestingly, in Ayurvedic practice, this plant is frequently used as an anxiolytic, cognitive enhancer, and agent against CNS pathology, hyperactivity, and epilepsy (10). The effects observed in zebrafish models closely mirror these clinical applications, underscoring its therapeutic potential. The therapeutic actions of *Withania somnifera* are believed to involve enhancement of antioxidant defense systems, regulation of GABAergic and serotonergic neurotransmission, and attenuation of neuronal oxidative damage. Nevertheless, further mechanistic investigations are required to establish conserved molecular pathways between zebrafish and mammalian models.

Carob and curcumin

Curcumin has been reported to boost locomotor abilities in adult zebrafish models of Parkinson's disease induced by rotenone (61). Curcumin mediated neuroprotection in zebrafish may involve suppression of oxidative stress, regulation of dopaminergic signaling, and reduction of neuroinflammatory responses. In a similar manner, tannin-enriched leaf extract from carob (St. John's bread, *Ceratonia siliqua* L.) enhances cognitive functions and diminishes anxiety in adult zebrafish exposed to the neurotoxin 6-hydroxydopamine (6-OHDA), a standard model for Parkinson's disease. These benefits are likely due to the restoration of brain antioxidant levels and the modulation of acetylcholinesterase (AChE) activity (62). The neuroprotective effects of curcumin may involve suppression of oxidative stress, modulation of dopaminergic signaling pathways, inhibition of neuroinflammatory cytokines, and regulation of mitochondrial dysfunction. However, most zebrafish studies primarily rely on behavioral endpoints, and detailed molecular validation remains limited.

Areca palm and Tulsi

Reflecting its traditional nootropic use in humans, tulsi leaf extract has been shown to alleviate scopolamine-induced memory deficits in adult zebrafish, where scopolamine acts as an anticholinergic agent commonly used to create memory impairment . In Ayurveda, the areca palm (Areca catechu Linnaeus), known as puga, is widely used clinically to treat ulcers, pain, and digestive issues through extracts derived from its leaves and nuts. The main psychoactive substance in betel nuts, arecoline, along with water extracts of these nuts, has been investigated in zebrafish, revealing central nervous system effects similar to those observed in clinical and rodent studies, including notable anxiolytic-like behaviors and increased levels of norepinephrine and serotonin(63). These findings not only highlight the evolutionary preservation of the psychoactive and neurochemical actions of Ayurvedic medicinal compounds across vertebrate species, but also suggest opportunities for repurposing Ayurvedic medicines. Although anxiolytic action is not traditionally emphasized for puga, the consistent CNS effects seen in zebrafish, rodents, and humans following arecoline treatment indicate that zebrafish-based CNS screening could uncover new therapeutic uses of Ayurvedic drugs for CNS effects(64).

Asian pigeonwing

Asian pigeonwing, known for its strong stress-reducing and cognitive-enhancing effects in humans and rodent studies, has also been evaluated using zebrafish. In a zebrafish model of drug-induced affective disorder, an extract from the plant's roots, rich in clitorienolactone and isoflavonoids, alleviates motor impairments such as freezing and reduced movement caused by the dopamine-depleting agent reserpine (65). These results closely mirror the stimulant effects on the brain seen with this well-known Ayurvedic treatment for people. These behavioral effects may be associated with modulation of monoaminergic neurotransmission and stress-response pathways. The zebrafish reserpine model also provides translational relevance for evaluating compounds targeting affective and neuropsychiatric disorders

Water hyssop

Water hyssop, a conventional neurotonic in Ayurveda, effectively mitigates anxiety-like responses, including thigmotaxis and scototaxis, in larvae of zebra fish exposed to osmotic stress, relative to untreated stressed larvae. In addition, it lowers the cortisol release triggered by stress in these larvae, indicating its potential use in alleviating stress and anxiety (66). The neurocognitive benefits of *Bacopa monnieri* may be associated with modulation of cholinergic transmission, reduction of neuroinflammation, and enhancement of synaptic plasticity through BDNF-related pathways. The anxiolytic effects observed in zebrafish may be associated with modulation of cholinergic neurotransmission, regulation of cortisol-mediated stress pathways, and attenuation of neuroinflammatory responses. Moreover, behavioral paradigms such as thigmotaxis and scototaxis provide translationally relevant tools for evaluating stress- and anxiety-related neuropharmacological effects of Ayurvedic compounds.

Black Myrobalan with Satawari

Building on the effectiveness of Ayurvedic medicines in modulating the brain–gut axis and the emerging role of zebrafish as models for this axis, several compounds have been evaluated in zebrafish to enhance understanding of the gut-brain connection and its impact on central nervous system disorders. Increasing evidence links gut inflammation with CNS dysfunction and affective disorders. Zebrafish models are increasingly utilized to investigate gut-brain interactions and evaluate Ayurvedic interventions targeting neuroinflammatory pathways(68) . Overall, these results highlight the utility of zebrafish as an effective species for exploring the impact of Ayurvedic medicinal plants on inflammatory processes and central nervous system disorders as well as for developing new treatments targeting the gut-brain axis (67). Despite these advantages, zebrafish models cannot fully replicate the anatomical complexity, higher cognitive functions, and pharmacokinetic responses observed in mammalian central nervous system models.

Suvarna Bhasma

Beyond herbal remedies, other Ayurvedic therapies can be evaluated using zebrafish models. Nano-sized gold particles known as Suvarna Bhasma (SB), traditionally considered rejuvenating, cognitive-enhancing, and stress-relieving in Ayurveda, show strong potential for treating neurological conditions such as Parkinson's disease (PD) in clinical settings (69). In a zebrafish model of PD induced by rotenone, treatment with SB alleviated behavioral impairments and prevented dopamine depletion in the brain, reflecting its neuroprotective effects observed in PD (70). Additionally, when administered at non-toxic levels, SB demonstrated anxiolytic-like effects in zebrafish during the novel tank test, closely mirroring its recognized calming and anti-stress properties in humans (71).

Zebrafish as a Promising Model for Neuroscience Studies and Ayurvedic Drug Evaluation

Studies on the central nervous system (CNS) effects of Ayurvedic plants in animal models (see Table 1–Supplementary material) mainly highlights their in vivo promnestic (cognitive-enhancing) and anti-stress activities, which generally correspond to the clinical conditions for which these herbal remedies are traditionally employed (Table 1).Nevertheless, discovering new clinical applications for traditional Ayurvedic plants is important, particularly for disorders currently lacking targeted drug therapies, such as neurodevelopmental conditions like autism spectrum disorder (72). Ayurvedic medicines have also proven effective in managing pain due to their strong analgesic and anti-inflammatory properties (73) and may have potential in treating complex brain disorders such as schizophrenia (74). For example, chronic administration of water hyssop extracts has been shown to improve both positive and negative symptoms of psychosis without major side effects (75).

With zebrafish emerging as a useful model for schizophrenia (76) and related CNS disorders, testing Ayurvedic treatments in this system is now feasible.Despite encouraging experimental findings, the translational applicability of zebrafish data to complex human CNS disorders remains limited because of interspecies differences in physiology, metabolism, and neuropharmacological responses.A critical factor influencing the efficacy of Ayurvedic plants is the sustained bioavailability of their active compounds. To address this, advanced nanotechnologies have been developed to improve the absorption and activity of these medicines.Nanoparticle-based delivery systems may improve CNS bioavailability of Ayurvedic compounds and enhance their evaluation in zebrafish neuropharmacological models (77). Utilizing water immersion for drug administration in zebrafish allows for similar protracted treatment strategies, which could further optimize zebrafish CNS models and Ayurvedic drug screening (78). Zebrafish models offer considerable advantages for translational neuropharmacology because of their conserved neurotransmitter systems, rapid behavioral responses, optical transparency during early development, and compatibility with high-throughput screening approaches. Behavioral paradigms such as scototaxis, thigmotaxis, novel tank exploration, and locomotor assays enable efficient assessment of anxiety-like behavior, stress responses, cognitive dysfunction, and neurotoxicity following exposure to Ayurvedic compounds. Furthermore, zebrafish provide opportunities for investigating oxidative stress pathways, neuroinflammatory signaling, mitochondrial dysfunction, and neurotransmitter modulation in vivo. Nevertheless, species-specific physiological and metabolic differences may limit direct extrapolation of zebrafish findings to mammalian and human CNS disorders.In addition, supercritical fluid micronization technology is another effective method to boost drug bioavailability and therapeutic efficiency (79). As an example, micronized resveratrol has demonstrated promising anticonvulsant effects in adult zebrafish by preventing tonic-clonic seizures in an epilepsy model (80).

These techniques can be applied to improve the delivery and efficacy of Ayurvedic medicines in zebrafish models of various CNS disorders. It is crucial to acknowledge that intrinsic factors, including individual variability, sex, anatomical distinctions , physiological characteristics and behavioral affect pharmacological responses in both clinical and preclinical studies. Body temperature is a key determinant of drug effects in humans and rodents, and this parameter may differently impact Ayurvedic treatments in poikilothermic zebrafish (81), which are typically maintained at 25–28°C in laboratory settings, compared to mammals that maintain body temperatures around 35–37°C (82) (83). In addition, extrinsic factors may affect behavioral and CNS drug responses differently across species; for example, the sex of the experimenter influences these responses in rodents , but this effect is not seen in zebrafish (84).

For large-scale studies involving Ayurvedic medicines, zebrafish offer potential for high-throughput imaging assays to assess drug neurotoxicity and enhance the understanding of drug pharmacokinetics and pharmacodynamics (85). An example includes *rasasindura*, a mercury-based Ayurvedic formulation

which exhibited no signs of toxicity or morphological alterations in zebrafish larvae, even at elevated concentrations of 1000 ppm indicating its safety at these levels in vivo (86)(87). While many Ayurvedic drug studies utilize adult zebrafish (Table 2), larvae models are promising due to their small size enabling use in multi-well plates with just 200 µL of medium and necessitating only small quantities of the compound for screening (88). While the extraction of bioactive constituents from Ayurvedic plants generally requires extensive chromatographic methods and substantial sample quantities challenging for larval assays new methods combining micro-fractionation with larval behavioral testing have been developed to identify active substances from traditional herbal medicines (89).

A noteworthy research direction is rasa shastra, an Ayurvedic branch that integrates medicinal plants with inorganic elements, primarily metals. For example, combining plant extracts with metals has shown potential therapeutic effects in zebrafish models, warranting further investigation. Lastly, understanding interactions between Ayurvedic medicines and conventional pharmaceuticals is crucial for clinical and translational applications. Indian ginseng, for instance, enhances the antiepileptic effects of diazepam; gugul works synergistically with lipid-lowering drugs in humans, while tulsi has exhibited hepatotoxicity in rats when combined with paracetamol (90)(91). Considering the inherent responsiveness of zebrafish in drug evaluation, particularly for high-throughput screening (HTS) purposes, these models are invaluable for examining CNS interactions among Ayurvedic medicines and their interplay with other drug classes such as allopathic treatments. Although zebrafish studies demonstrate behavioral and neuroprotective effects comparable to those observed in rodent models, important interspecies differences remain. Rodent studies often provide more extensive molecular, histopathological, and pharmacokinetic characterization, whereas zebrafish models are primarily advantageous for rapid behavioral screening and high-throughput analysis. Clinical evidence supporting Ayurvedic interventions in humans remains comparatively limited for several compounds, thereby restricting direct translational interpretation of preclinical zebrafish findings.

Zebrafish models provide valuable opportunities for translational neuroscience research because of their conserved neurotransmitter systems, genetic similarity to humans, and suitability for high-throughput neurobehavioral screening. These features facilitate early-stage evaluation of Ayurvedic neuroactive compounds and support investigation of molecular pathways associated with neurodegeneration, neuroinflammation, and neurotransmitter dysfunction. However, translational extrapolation to human CNS disorders requires careful validation using mammalian and clinical studies(92).

Future directions

While growing evidence supports the evaluation of Ayurvedic medicines in zebrafish central nervous system (CNS) models, several important scientific and translational challenges remain unresolved. The precise molecular pathways underlying the neuroprotective, anxiolytic, cognitive-enhancing, and anti-inflammatory effects of many Ayurvedic compounds are still insufficiently characterized. Future studies should consequently focus on elucidating signaling mechanisms associated with oxidative stress regulation, neuroinflammation, neurotransmitter modulation, synaptic plasticity, and neuronal survival pathways. In addition, advanced omics-based approaches, including genomics, transcriptomics, proteomics, metabolomics, and epigenomics, may provide deeper insight into the complex biological responses induced by Ayurvedic interventions in zebrafish CNS models. The identification of reliable translational biomarkers linking zebrafish findings with mammalian and human neurological responses is also essential for improving clinical relevance and predictive validity. Moreover, many currently available studies lack integrated mechanistic validation combining behavioral, molecular, and translational endpoints, thereby limiting comprehensive interpretation of the neuropharmacological effects of Ayurvedic compounds. Moreover, although zebrafish offer several advantages for high-throughput neuropharmacological screening, limitations associated with species-specific metabolism, physiological differences, drug absorption patterns, and variations in blood-brain barrier permeability may restrict direct extrapolation of findings to humans. Standardization of plant extracts, dose optimization, and validation of long-term safety profiles remain additional challenges requiring further investigation. Integrating nanotechnology-based delivery systems with Ayurvedic formulations may improve bioavailability, CNS targeting, and therapeutic efficacy. Overall, combining mechanistic studies with advanced molecular approaches and translational validation strategies will strengthen the application of zebrafish models in Ayurvedic CNS drug discovery and neuropharmacological research. “Fig. 2. Overview of frequently observed behavioral and physiological central nervous system (CNS) effects. Nevertheless, mechanistic evidence explaining the precise molecular pathways underlying these neuroprotective and behavioral effects remains insufficient. Future studies should focus on elucidating molecular pathways associated with oxidative stress regulation, neuroinflammatory signaling, neurotransmitter modulation, mitochondrial dysfunction, apoptosis, and synaptic plasticity in zebrafish CNS models exposed to Ayurvedic compounds.

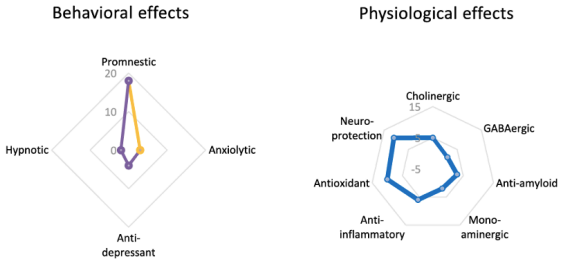


Figure 3: Overview of frequently observed behavioral and physiological central nervous system (CNS) effects associated with the preclinical use of traditional system of medicines. The data illustrate the occurrence of specific effects reported in the plants discussed here and listed in Table 1-Supplementary material. Note: GABA denotes gamma-aminobutyric acid (105).

Potential challenges and limitations

Treating brain disorders is generally challenging due to issues such as low drug bioavailability, ineffective delivery methods, unclear genetic risk factors, the use of model organisms that may not be well-suited for drug discovery and the absence of individualized therapeutic strategies. Although a range of animal models is being increasingly utilized to evaluate the medicines of ayurveda (Tables 1 and 2), the field faces several obstacles. These include difficulties in obtaining standardized raw materials, identifying active compounds and conducting pharmacognostic analyses as well as concerns regarding low bioequivalence and safety. Standardizing the dosage of bioactive constituents from Ayurvedic plants is crucial but often overlooked, particularly since

animal doses frequently differ from those traditionally recommended for human use.

Potential side effects of Ayurvedic herbs also raise safety concerns, as limited human and animal studies have investigated them. For instance, water hyssop has been shown to reduce fertility in rats, although this effect is not seen in humans, while Indian pennywort can cause serious neurological symptoms like headaches, nausea, dizziness and intense drowsiness at high doses (58). Consequently, clinical trials are necessary to validate the safety profiles of Ayurvedic plants and to compare them with conventional allopathic medicines for their efficacy and safety in humans.

Additionally, the biotransformation of herbal constituents in vivo may reduce their effectiveness and can vary among species such as fish, rodents and humans (94). Assessing active compounds during pharmacokinetic studies is also complicated, partly because the raw herbal materials can vary depending on geographic origin, climate, harvesting techniques and collection protocols, making drug standardization difficult.Moreover, standardization of extract composition remains a major challenge in Ayurvedic research, as variations in cultivation conditions, harvesting methods, extraction procedures, and phytochemical content can significantly influence pharmacological outcomes and reproducibility across studies. Zebrafish models offer several advantages, including genetic conservation, rapid development, low maintenance cost, and suitability for high-throughput neurobehavioral screening. Neverthless, limitations such as species specific metabolism, differences in thermoregulation, variations in blood-brain barrier permeability, and incomplete replication of complex human CNS pathology may reduce direct translational applicability to mammalian systems.

Despite their numerous experimental advantages, zebrafish models possess several important limitations in translational neuropharmacology. Species-specific metabolic pathways, differences in thermoregulation, and temperature-dependent pharmacokinetic responses may influence drug absorption, distribution, metabolism, and elimination differently compared to mammals. Furthermore, interpretation of zebrafish behavioral endpoints can sometimes be challenging because locomotor alterations may reflect multiple overlapping neurological or physiological processes. Although zebrafish exhibit conserved neurochemical pathways, they cannot completely replicate the structural complexity and higher-order cognitive functions of the human brain.

Conclusion

Ayurvedic medicines represent promising traditional therapeutic candidates that may contribute to the identification of novel neuropharmacological targets and future CNS drug development strategies.The use of zebrafish models in CNS drug discovery, including studies of Ayurvedic medicines (Table 2), is expanding rapidly, though there are translational and practical challenges as noted earlier. Several important questions remain unanswered in this area (Table 3), highlighting the need for additional research. These include how closely zebrafish physiology mimics human clinical responses, whether there are dependable, evolutionarily conserved biomarkers that indicate the therapeutic potential of Ayurvedic treatments shared between fish and humans, and whether Ayurvedic therapies produce long-lasting genomic, epigenetic, or epigenomic changes in the zebrafish brain. In addition, the potential of modern nanotechnology to improve the therapeutic effects of Ayurvedic interventions in zebrafish CNS models warrants investigation. Nanotechnology-based medicine is progressively adopted in therapeutics to minimize toxicity to healthy cells and maximize dosing effectiveness (95). Integrating nanotechnology with traditional Ayurveda may help overcome major challenges such as metal toxicity by transforming treatments into biologically compatible nanoparticles, as seen with Suvarna Bhasma which are preparations involving incinerated metals(96).However, additional mechanistic investigations, standardized preclinical studies, and well-designed clinical trials are required before definitive therapeutic conclusions can be established for human CNS disorders. Assessing these and other Ayurvedic interventions in zebrafish will further promote the use of this model organism to explore Ayurvedic drug therapies and uncover potential molecular mechanisms underlying their CNS effects.

Table 3: Key unresolved questions regarding the application of Ayurvedic plants in zebrafish models

Questions
• Which molecular and cellular mechanisms drive the central nervous system (CNS) responses to different Ayurvedic compounds in zebrafish?
• How do factors such as sex, genetic background, or age modulate the CNS effects of Ayurvedic plant interventions in zebrafish?
• What are the pharmacologically active and toxic concentration ranges of Ayurvedic plant extracts in zebrafish models?
• In what ways do different routes of administration of Ayurvedic medicines influence screening results in zebrafish assays?
• To what extent does the unique metabolic system of zebrafish, compared to humans, alter the efficacy of Ayurvedic drugs?
• How consistent are the behavioral effects of Ayurvedic plant extracts in zebrafish with those observed in rodent and human studies?
• Which genomic and epigenomic modifications occur in the CNS of zebrafish following treatment with Ayurvedic medicines?
• Are there heritable or transgenerational epigenetic consequences of Ayurvedic interventions in zebrafish?
• What behavioral and cognitive changes occur in zebrafish following short-, medium-, and long-term exposure to Ayurvedic plant extracts, including parameters such as aggression, anxiety, and motor activity?
• Can zebrafish CNS models be employed to investigate interactions between Ayurvedic medicines and other drugs (e.g., antidepressants, anesthetics)?
• What strategies can be employed to overcome issues of blood-brain barrier (BBB) permeability for Ayurvedic compounds?
• How do various extraction methods of Ayurvedic plants influence their efficacy in zebrafish CNS research?
• How do neuroglial cells in zebrafish and mammals respond to active components of Ayurvedic drugs?
• How can Ayurvedic remedies be applied to neurodevelopmental research using zebrafish?
• Are there any delayed CNS effects from early exposure to these medicines?
• How do factors like body or environmental temperature influence the efficacy of Ayurvedic treatments in zebrafish compared to clinical conditions?
• What toxicological and other adverse effects, both neurological and systemic, are induced by Ayurvedic medicines in zebrafish?
• How do Ayurvedic treatments impact glial cells in zebrafish?
• Which proteomic and metabolomic alterations occur in zebrafish following acute or chronic exposure to Ayurvedic medicines?
• Do Ayurvedic interventions elicit placebo- or nocebo-like effects on the central nervous system?
• If such effects exist, can they be replicated in zebrafish, and what underlying physiological mechanisms might contribute?
• How could modern nanotechnology enhance the efficacy of Ayurvedic medicines in zebrafish CNS models?

Funding sources: Nil

Conflict of interest: Nil

Acknowledgements: Nil

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process

During the preparation of this work, the author(s) used **ChatGPT (OpenAI)** in order to **assist with language refinement, grammar correction, and improving clarity of expression**. After using this tool/service, the author(s) carefully reviewed, revised, and edited the content as necessary, and take full responsibility for the final content of the manuscript.

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