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# ZEBRAFISH AS A MODEL FOR HERBAL TOXICOLOGY: A SCIENTIFIC REVIEW ON ASHWAGANDHA (*WITHANIA SOMNIFERA*) AND ITS SAFETY PROFILE

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## ABSTRACT

Herbal medicines remain integral to traditional and modern healthcare, with Ashwagandha (*Withania somnifera*) being one of the most widely utilised for its adaptogenic and neuroprotective properties. However, concerns regarding safety and herb–drug interactions necessitate systematic toxicological evaluation. Zebrafish (*Danio rerio*), owing to their genetic similarity to humans and high-throughput potential, have emerged as powerful models in preclinical toxicology. This review synthesises recent evidence from zebrafish-based assays, rodent studies, and clinical trials to evaluate Ashwagandha's safety profile. Literature was critically analysed to highlight experimental approaches, including embryotoxicity, neurotoxicity, and cardiotoxicity assays, alongside molecular insights into oxidative stress, mitochondrial dysfunction, and cytochrome P450 interactions. Findings indicate that Ashwagandha demonstrates a broad safety margin, with zebrafish models revealing dose-dependent therapeutic and toxic effects. At optimal concentrations, extracts confer neuroprotection, immunomodulation, and stress resilience, consistent with mammalian and clinical outcomes. Conversely, excessive dosing may induce developmental and metabolic disturbances, underscoring the need for standardised protocols. Zebrafish data also align closely with rodent and human evidence, reinforcing translational validity. Zebrafish provide an efficient, predictive, and ethically sustainable platform for assessing Ashwagandha's toxicological profile. Their integration into drug discovery and regulatory pipelines enhances the reliability of safety assessments, supporting the advancement of Ashwagandha as a standardised therapeutic.

**KEYWORDS:** Zebrafish toxicology, Ashwagandha, *Withania somnifera*, herbal safety, neuroprotection.

## 1. INTRODUCTION

Herbal medicine has been a cornerstone of health care for hundreds of years, producing therapeutic effects by complex combinations of bioactive substances. Unlike synthetic drugs, which act by affecting a specific pathway, herbal preparations are polyvalent and can enhance efficacy but raise issues about safety. New studies accentuate the growing usage of herbal extracts in human and veterinary care, backed by evidence of their capacity to enhance the immune system and reduce stress, particularly in aquaculture management (Alam et al., 2024). However, the growing use of herbal products necessitates rigorous examination of their toxicological profiles. Conventional toxicology experiments have highly considered the rodent and mammal models that are typically construed under the shadow of ethical, economical as well as translational constraints. The multidimensionality of the herbal preparations together with the possibility of interactions with synthetic drugs would require creating models that are both reliable and effective in practice. In this respect, zebrafish (Danio rerio) has been an appropriate species of fish to monitor the toxicological capability of therapeutic herbs. They are suitable in assessing systemic and developmental toxicity since it is to their great degree of homology in humans and their capability to screen a high number of chemicals within a short span of time. The issue of retaining the knowledge of the efficacy and safety of the herbal medicines has remained a subject of interest by pharmacological sciences globally since their increased popularity in the world (Aktary et al., 2025).

Their genetic, physiological and developmental properties which are comparable to those of human beings, zebrafish are a versatile tool to test the safety as well as efficacy of experimental drugs. Their clear embryos allow unequivocal observations on developmental toxicity as they allow observing the organization of the organs and toxicological manifestations of the animal without complications.

In a most important case, zebrafish are inexpensive and can be high throughput screened in natural products, therefore, useful with preclinical safety testing (Balkrishna et al., 2021). Second, zebrafish have behavioural and neurological characteristics associated with mammalian systems and, consequently, zebrafish are the preferred organisms in neuropharmacology and neurotoxicology research. It is also predetermined by the fact that they are used in biomedical research as they can replicate human pathophysiologic processes, e.g. viral or inflammatory diseases. In pharmacy The zebrafish models have assisted in the pharmacological safety assays, particularly in examining the application of natural substances, including the question of their protective properties in virus damage and has translational connections (Balkrishna et al., 2021).it is linking genetic technologies to high-resolution imaging due to the fact that zebrafish provide an interface between in vitro screens and mammalian systems thereby circumventing the constraints that the classic toxicological model poses. The adoption of the latter in studying how herb medicines works is therefore a critical step to facilitating development of standard safety measures of the versatile phytotherapeutics ashwagandha (Withania somnifera) (Aktary et al., 2025).The adaptogenic herb ashwagandha (Withania somnifera), is known to have been widely used in Ayurveda to enhance the overall health, stress tolerance, and energy provisions (Aktary et al., 2025). Most of these heritary statements can be validated with the help of recent pharma research works that indicate that ashwagandha is beneficial in prevention of inflammation, oxidative stress, and immunological control (Basudkar et al., 2024). Because of its neuroprotective and cytoprotective attributes of its bioactive compounds, including withanolides, it has become a recognized medicine and nutraceutical substance at the worldwide level. According to the toxicological study, Ashwagandha may be able to lead to the repair of the radiation-induced organ damage, and therefore, may be used as a treatment procedure based on severe physiological stress (Azab et al., 2022). Besides the medical use in human health, researchers describe the value of Ashwagandha in viral infections including SARS-CoV-2

combined with studies that predict its antiviral effect of prevents viral contacts in zebrafishes models (Balkrishna et al., 2021).Because of these results, it can be stated that Ashwagandha is of significance in terms of both therapeutic and safety evaluation. Although extensive use is an assurance of an excellent safety history, variation in the preparation, dose, and bioactive constituents requires proper toxicological testing. Consequently, its zebrafish models will be very critical in assessing its safety on the system, thus securing its presence in evidenced-based medicine and the appropriate scientific supporting data of its safety claims to development of its therapeutic effects (Basudkar et al., 2024).

**OBJECTIVES OF THE STUDY**

1. To critically evaluate the employment of zebrafish as a model system for testing the toxicological safety of Ashwagandha (*Withania somnifera*).
2. To synthesise evidence from preclinical and translational research explaining the drug-like action and toxicological consequences of Ashwagandha.

## 2. Zebrafish as a Model for Toxicology

### 2.1 Advantages of Zebrafish in Toxicology

Zebrafish possess unique advantages for toxicology as including their rapid development, optically clear tissue, and small size, which enable direct observation of the toxic effect. Their behavioural repertoire provides quantifiable endpoints for assessing the effect of herbal and synthetic compounds (Bhattacharya et al., 2024). The cost-effectiveness of keeping zebrafish in large numbers also provides statistically valid studies. Their fecundity is high such that parallel testing of several compounds becomes particularly feasible, minimising variability and maximising reproducibility. Notably, zebrafish have been effectively used in nutraceutical and herbal toxicology to serve as a good model for detecting adverse neurological and systemic effects (Bian & Pei, 2016).

### 2.2 Genetic Similarity and Developmental Biology

Zebrafish are ideal for toxicological analyses since they share a significant portion of their genome with humans. According to Bian and Pei (2016), around 70% of human genes have orthologs in zebrafish, including genes that contribute to disease pathways. They quickly finish their embryonic phases and go through exterior development, which allows scientists to track organogenesis and distinguish between teratogenic effects. When employed in herbal medicine research, conservation is observed in neurodevelopmental and metabolic pathways that support its translational relevance (Bhattacharya et al., 2024). this similarity, zebrafish can forecast toxicological results that are applicable to humans and helpful for moral and scientific reasons.

### 2.3 Applications of High-Throughput Screening

Due to their vulnerability to automated imaging and microplate assays, zebrafish are becoming more and more involved in high-throughput toxicology.They may be the plated embryos under the multiwell plates, and the exposure to a compound can be investigated systematically (Bian & Pei, 2016). The process is scalable to speed up the process of screening herbal extracts and natural products on efficacy and safety. Indian medicinal herbs, on the other hand, have been tested as the neurological modulatives of zebrafish paradigms of behaviour presenting both therapeutic and toxic outcomes (Bhattacharya et al., 2024). They have the ability to assess multifaceted safety endpoints of these basic molecules, behaviours, and physiology with multifaceted degrees of toxicological usefulness.

### 2.4 Limitations and Limitations of the Zebrafish Model

Some toxicological effects cannot be fully extrapolated to humans due to interspecific differences in drug metabolism and organ physiology (Bian & Pei, 2016). Those herbal components with complex pharmacokinetics can produce incomplete toxicity profiles in zebrafish and must be validated using mammalian models as shown in Figure 1. Secondly, some behaviour endpoints may be influenced by environmental factors and introduce variability (Bhattacharya et al., 2024). Regulatory tolerance of zebrafish-based data is still ongoing, and therefore, there is a need for standardised protocols. Thus, while zebrafish are powerful tools, they are best used when combined with complementary systems.

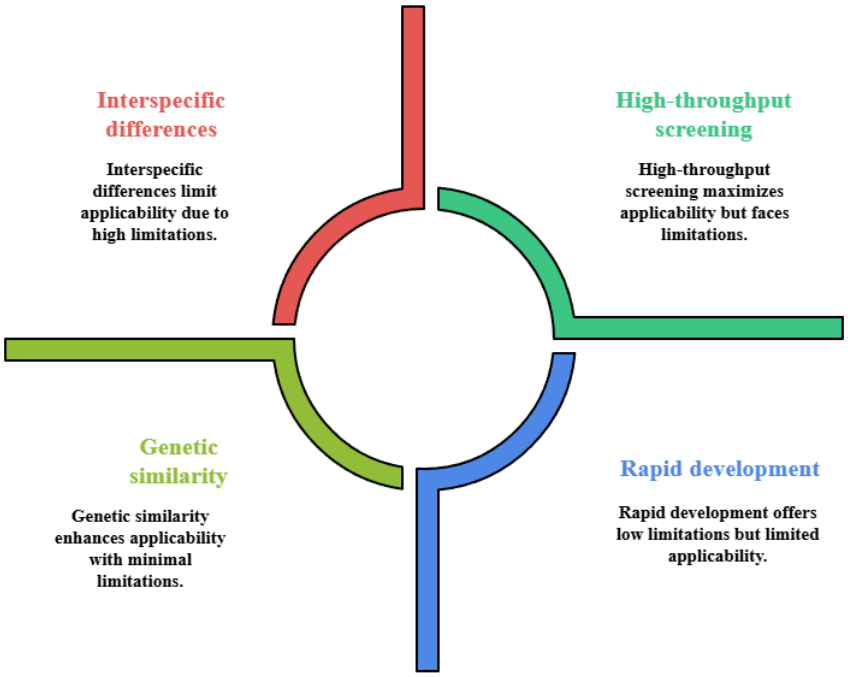


**Figure 1: Zebrafish in Toxicology: Advantages and Limitations.**

## 3. Overview of Ashwagandha

### 3.1 Botanical Classification and Phytochemistry

*Withania somnifera* or Ashwagandha belongs to the family Solanaceae and has a wide distribution in South Asia and the Middle East. Its leaves, berries, and roots possess a rich phytochemical makeup that is responsible for its medicinal value (Husain, 2025). This continues to comprise steroidal lactones, alkaloids, flavonoids, sitoindosides that are active, thanks to their adaptogenic as well as pharmacological activity. The heterogeneity of the structural withanolides as the dominant bioactive together with the complexity of its contents are justified by current phytochemical studies (Dipankar et al., 2025). Ashwagandha has lately been subject to global pharmacological interest due to its thick composition that underlies its pharmacological use across a wide range.

### 3.2 Bioactive Constituents (Withanolides, Alkaloids, Flavonoids, etc.)

Particularly, the pharmacological performance of Ashwagandha can be attributed to its bioactive phytoconstituents composed of neuroprotective, anti-inflammatory, and adaptogenic potential of especially withanolides steroidal lactones (Dipankar et al., 2025). All of them enhance its cytoprotective and antioxidant activity: alkaloids, flavonoids,

and withanolides, whereas its immunomodulatory effect is assisted by sitoindosides (Husain, 2025). The combination of these phytoconstituents changes neurotransmissions and cell stress pathways. An experimental approach that depicts the clinical worth of withanolides is also that some of them are either neurodevelopmental toxin and are protective in vitro (Dwivedi et al., 2025). The biochemical variety of this herb is to be ascribed to the phenomenal ability of Ashwagandha to exert systemic physiological toys to a vast majority of organ systems.

### 3.3 Uses that are traditional and those that are modern therapeutic ones

Ashwagandha in Ayurveda has traditionally been utilized to help to extend lifespan, decrease stress levels, and achieve energy. The primary reason is that current clinical and preclinical research supports these roles, particularly with regard to psychological illnesses as anxiety and depression (Gullu & Kiroglu, 2024). It also has adapting genic capabilities that leverage its efficacy with issues concerning stress and exhaustion. The modern will be through the use of anti-inflammatory and anxiolytic properties in neurological disorders and inflammatory disorders (Gupta and Kaur, 2018). Moreover, its recommended expanded therapeutic usage in integrative medicine evidences by its importance in dermatology, including hypopigmentary conditions (Husain, 2025).

### 3.4 Pharmacological Relevance in Stress, Neuroprotection and Immune Modulation

Ashwagandha has great pharmacological measures of stress reliever, neuro protection, and immune immunomodulation. It alters the hypothalamic-pituitary-adrenal axis, which decreases cortisol which blocks the pathology caused by stresses (Gullu & Kiroglu, 2024). Neuroprotection is also observed in the capacity to reverse neuroinflammation and guarantee neuron survival, and withanolides can be used with enormous therapeutic potential against degenerative diseases (Gupta & Kaur, 2018; Dipankar et al., 2025). It also has immunomodulatory effects, which boost host defence, and therefore it is applicable in treating chronic and autoimmune conditions as indicated in Table 1. All these pharmacological activities render Ashwagandha an all on therapeutic plant which coordinativates the traditional style of medicinal treatment with the contemporary therapeutic treatments.

**Table 1: Botanical classification, phytochemistry, uses, and pharmacological relevance of *Withania somnifera.***

| Botanical Classification | Phytochemicals | Bioactive Constituents | Traditional Uses | Modern Therapeutic Applications | Pharmacological Relevance | References |
|---|---|---|---|---|---|---|
| Family: Solanaceae; distributed in South Asia & Middle East | Steroidal lactones, alkaloids, flavonoids, sitoindosides | Withanolides (neuroprotective, anti-inflammatory), alkaloids, flavonoids, sitoindosides | Vitality, stress relief, longevity (Ayurveda) | Anxiety, depression, inflammation, fatigue | Adaptogenic, neuroprotective, immunomodulatory | Husain (2025); Dipankar et al. (2025) |
| Root | Withanolides | Withaferin A, withanolide D | Rejuvenation, stress management | Neurodegenerative disorders | Anti-apoptotic, anti-inflammatory | Dipankar et al. (2025) |
| Leaf | Alkaloids, flavonoids | Somniferine, anaferine | Local poultice in Ayurveda | Dermatology (hypopigmentary disorders) | Antioxidant, cytoprotective | Husain (2025) |
| Berry | Sitoindosides | Immunomodulatory glycosides | Fertility and energy booster | Integrative medicine | Enhances immune response | Husain (2025) |
| Whole plant extract | Mixed phytochemicals | Complex phytochemical synergy | General health tonic | Psychological disorders (anxiety, depression) | Modulates HPA axis, reduces cortisol | Güllü & Kıroğlu (2024) |
| Seed/derivatives | Secondary metabolites | Secondary alkaloids | Minor Ayurvedic use | Experimental pharmacology | Potential neuroactive properties | Dwivedi et al. (2025) |
| General summary | Multi-constituent | Structural heterogeneity of withanolides | Ayurveda's Rasayana (longevity practice) | Neurological & autoimmune diseases | | |

## 4. Preclinical and Clinical Evidence on Ashwagandha Safety

### 4.1 Animal testing in rodents and mammals

Removing the rodent preclinical studies, all of them indicated the relative safety of Ashwagandha at therapeutic concentrations. Animal studies have demonstrated to use their bioactive phytochemicals to induce immunomodulatory, antiviral, and anticancer effects without causing significant side effects on the system (Jadaun et al., 2023; Jahagirdar et al., 2024). Nevertheless, herbal constituents may interfere and disrupt metabolic and enzymatic functions, which can be reported to cause dose-dependent toxicity thus necessitating special attention when exposed to a high dose (Kanungo et al., 2024). In silico models can also be used to assure safety, by predicting binding affinities that act as constraints to off-target effects (Kandagalla et al., 2022). All in all, the offered toxicological profile of rodent and mammalian models is rather positive, which proves the therapeutic benefit of ashwagandha again.

### 4.2 Clinical Trials of Humans and Safety Observations.

Clinical outcomes support preclinical studies by describing the positive safety profile of Ashwagandha in different groups of patients in the clinical setting. It is highly tolerated and minimally toxic with according to the results of the research on its immunomodulatory, anxiolytic, and adaptogenic properties (Jayasinghe & Jayawardena, 2019). It is reported to have typical safety outcome with no remarkable organ-specific damage in its therapeutic effects on a number of ailments including viral infections, immunological defects and chronic stress (Jadaun et al., 2023). Importantly, as with its long history in traditional medicine, drug dosages over extended periods of time administered in controlled dosages have been found to be very tolerated. These findings support the idea that it is now integrated in modern healthcare with the focus on the importance of normalized regimens of dosing (Kanungo et al., 2024).

### 4.3 Adverse effects and Contraindications.

Although the ashwagandha in most cases is safe, some risks are involved. The adverse effects are generally drowsiness, upset stomach, and, in the rare cases, hepatotoxicity, which are related to the excessive or uncontrolled intake of the herb (Kanungo et al., 2024).The interactions with drugs may result in contraindications in patients who received hyperthyroidism, pregnancy, or some immunosuppressive treatment (Jayasinghe et al., 2019). The discrepancies in extract preparation and content of withanolides might also have an effect on safety outcomes, which highlights the need to use standard preparation (Jahagirdar et al., 2024) as demonstrated in Figure 2. Nevertheless, Ashwagandha is comparatively a safe alternative medicine in herbs that require clinical caution and regulatory supervision to keep the risks away.

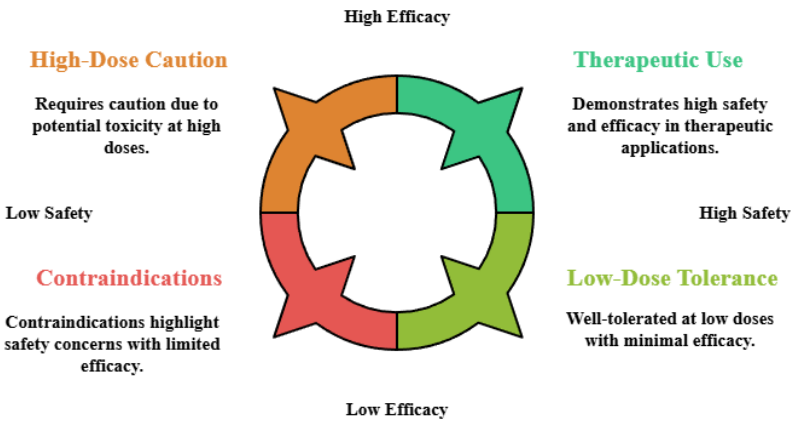


**Figure 2: Ashwangandha Safety and Efficacy Profile.**

## 5. Zebrafish-Based Toxicological Studies of Herbal Compounds

### 5.1 Standardised Protocols for Zebrafish Herbal Toxicity Assays

Zebrafish tests of herbal toxicity employ standardised protocols such as embryotoxicity, cardiotoxicity, and neurobehavioral tests, which produce measurable endpoints in precise developmental windows. Transparent embryos of zebrafish enable malformations and organ-specific toxicities to be examined visually, where survival rates and hatching are good indicators of toxicity (Mandlik & Namdeo, 2021). These assays are complemented with sophisticated imaging and molecular techniques, yielding mechanistic insights into the herbal compound's mechanism of action at the cellular level. These techniques have been particularly useful in the evaluation of *Withania somnifera* and other botanicals' systemic effects, wherein doses cause dose-dependent effects with therapeutic as well as potentially toxic effects (Kayesth et al., 2024).

### 5.2 Case Examples of Herbal Toxicology Studies Using Zebrafish

Some studies identify zebrafish as a potential model to assess the safety of herbal medicines. *Withania somnifera* extracts in experiments display neuroprotective and anticancer effects and allow researchers to determine developmental toxicity at elevated doses (Kulavi et al., 2024). Similarly, Withaferin A, a key bioactive compound, has been assessed for systemic safety, reporting its therapeutic value with dose-limiting toxicity (Kumar et al., 2024). Apart from Ashwagandha, certain nootropic herbs have been examined in zebrafish models for their cognitive-enhancing and toxicological activities (Malík & Tlustoš, 2023). The case studies emphasise the utility of zebrafish in the evaluation of different phytoconstituents.

### 5.3 Benefits of Zebrafish in Early Detection of Herb-Drug Interaction

Zebrafish models are particularly beneficial for the prediction of herb–drug interaction since herbal drugs more often than not coexist with traditional drugs. Their conserved metabolic pathways facilitate the detection of cytochrome P450-mediated interactions, offering information on pharmacokinetic alterations (Mandlik & Namdeo, 2021). For instance, *Withania somnifera* zebrafish tests have provided preliminary hints at potential adverse synergy effects when co-administered with other bioactive compounds (Kayesth et al., 2024). Also, neurochemical and behavioural endpoints can sense subtle interaction-induced alterations that might escape detection in in vitro systems as shown in Table 2. These capabilities render zebrafish a predominant model for research in integrative toxicology.

**Table 2: Zebrafish-based toxicological studies of herbal compounds: protocols, case examples, and interaction insights.**

| Assay Type / Focus | Key Features | Endpoints | Case Example | Toxicological Insights | Benefits in Herb–Drug Interaction | References |
|---|---|---|---|---|---|---|
| Embryotoxicity | Transparent embryos, rapid development | Survival, hatching, malformations | *W. somnifera* embryonic exposure | Dose-dependent malformations observed | Enables early-stage developmental toxicity detection | Mandlik & Namdeo (2021) |
| Cardiotoxicity | Zebrafish heart development observable in real time | Heart rate, rhythm, structural anomalies | *W. somnifera* crude extracts | Identified arrhythmic effects at high doses | Predicts cardiac liabilities with herb–drug co-administration | Kayesth et al. (2024) |
| Neurotoxicity / Neurobehavior | Locomotion and anxiety paradigms | Swimming patterns, startle response, anxiety markers | *W. somnifera* extracts | Neuroprotective at therapeutic doses; neurotoxic at extremes | Detects CNS-related herb–drug interaction changes | Kulavi et al. (2024) |
| Anticancer activity | Tumor xenograft zebrafish models | Tumor regression, apoptosis markers | Withaferin A in zebrafish | Anticancer efficacy with dose-limiting toxicity | Assesses synergistic/antagonistic cancer therapy outcomes | Kumar et al. (2024) |
| Cognitive / Nootropic evaluation | Behavioral cognition assays | Memory, learning endpoints | Nootropic herbs in zebrafish | Showed cognitive enhancement with toxicity at high dose | Evaluates interactions with nootropic drugs | Malík & Tlustoš (2023) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Systemic safety profiling | Combined organ-level readouts | Liver, kidney, survival indices | Whole plant extracts of *W. somnifera* | Demonstrated systemic safety at therapeutic doses | Predicts multi-organ herb–drug interactions | Kayesth et al. (2024) |
| Herb–drug interaction prediction | Conserved CYP450 pathways in zebrafish | Pharmacokinetics, metabolic alterations | *W. somnifera* + co-treatments | Highlighted potential adverse synergy at high dose | Robust for early screening of herb–drug interactions | |

## 6. Zebrafish Studies on Ashwagandha

### 6.1 Experimental Methods used (Embryotoxicity, Neurotoxicity, Cardiotoxicity)

The experimental techniques used in the study of Ashwagandha in zebrafish are different in order to investigate therapeutic effectiveness and systemic safety. Embryoshow toxicity assays use the developmental malformations and survival as parameters of exposure to plant extracts, whereas neurotoxicity tests measure behavioural and cellular outputs of importance to neurologic disease (Moise et al., 2024). The cardiotoxicity tests are based on the study of the heart rhythm and rate of the zebrafish larvae to identify the slight disruption. Each of the models offers a comprehensive view of the pharmacological and toxicological effect of Ashwagandha in terms of whole-profile. Most importantly, they provide translational evidence of how bioactive compounds, such as withanolides, influence organ systems in a diverse range of somatic changes distributed across many different stages of embryonic development (Mikulska et al., 2023).

### 6.2 Results of Dose-Response Relationships and Lethal Concentration (LC50)

The dose-response curves of ashwagandha can accurately be determined through the series of experiments involving zebrafish and this allows applications in distinguishing between doses that are deleterious and therapeutic. It has been found that at moderately high doses, the appropriate amount leads to a more favorable outcome as it boosts neuroprotection and organ homeostasis (Mazurkiewicz et al., 2024). The acute toxicity of Ashwagandha is low as compared to botanicals in general as evidenced by the LC50 of the embryotoxicity test (Naseem et al., 2023). The following findings underscore the importance of dosage accuracy in herbal medicine as excessive exposure compromises the form of safety despite according to the preset pharmacological effects. Thus zebrafish is a good model in testing the toxicological window of ashwagandha.

### 6.3 Developmental, Behavioural and Physiological Endpoints

Physiological indicators and behavioral response to developmental growth patterns have been found to be used in studies that utilize ashwagandha in zebrafish and have very broad objectives. Whereas behavioral endpoints can be movement, anxiety-like behavior, and cognitive functioning, developmental milestones can be morphological integrity and hatching successful (Murthy and Shyamala, 2024). The endocrine pathways have proven to be affected by ashwagandha, so one of the physiological outcomes is the alteration of thyroid activity (Naseem et al., 2023). A combination of this information sustains an integrative perspective of both toxic and curative properties of ashwagandha as a superb model organism to use when considering toxicological and therapeutic outcomes on a system-level (Moise et al., 2024).

### 6.4 Comparison with Mammalian Data

Zebrafish and mammalian research are compared, the pharmacology and toxicity profiles of ashwagandha are strongly in agreement. Zebrafish neuroprotective activity reflects findings in invertebrate and mammalian illness models, specifically oxidative stress pathways and motor dysfunction (Murthy & Shyamala, 2024). Similar to the species-independent significance of ashwagandha's bioactivity, changes in thyroid function in zebrafish mirror endocrine

consequences in mammals (Naseem et al., 2023). Interspecific variations in bioavailability and metabolism, however, caution against unqualified extrapolation (Mikulska et al., 2023). Overall, as Figure 3 illustrates, zebrafish provide a useful predictive model to enhance mammalian research and expand the translational platform for ashwagandha studies.

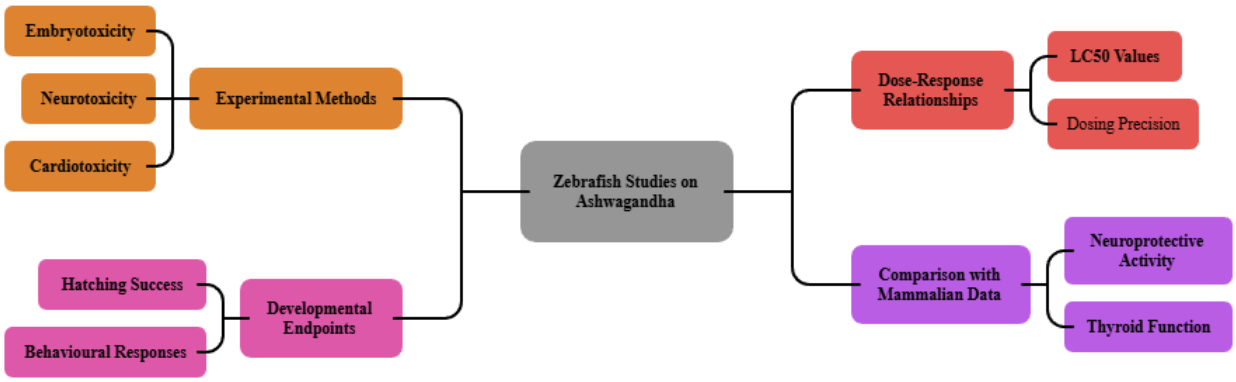


**Figure 3: Zebrafish Studies on Ashwangandha: Methods and Findings.**

## 7. Mechanistic Insights into Ashwagandha Toxicity

### 7.1 Molecular Pathways and Gene Expression Changes in Zebrafish

Zebrafish has demonstrated that the bioactive chemicals in ashwagandha, especially withanolides, alter several molecular pathways related to stress adaption and brain health. According to Pullaiah et al. (2025), transcriptome study demonstrates a bivalent effect of neuroprotection and potential toxicity depending on dosage by confirming regulation of genes linked to synaptic plasticity, apoptosis, and inflammatory signaling. Additional evidence suggests that withanolides alter the expression of genes linked to tau and amyloid in Alzheimer's disease pathways (Olaniyi et al., 2025). The therapeutic potential of ashwagandha is highlighted by this redirection, which also raises concerns about dose-dependent genomic reprogramming. These findings highlight how useful zebrafish models are for defining safety margins using gene-level toxicological insights.

### 7.2 Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress is one of the key functions of the toxicological and therapeutic activity of Ashwagandha. Extreme concentrations of it inhibit mitochondrial functions leading to increased production of reactive oxygen species (ROS) and inhibited cell respiration, whilst intermediate levels enhance antioxidant defenses in zebrafish models (Prajapati and Singh, 2025). There are potential risks in case one abuses or over uses ashwagandha since neurodegenerative processes are susceptible to mitochondrial mal functioning. Phytochemical screening also authenticates these activities and reveals that part of withanolides could cause oxidative imbalances at toxic doses (Ramli et al., 2023). Zebrafish is hence a convenient system of assessing the mobility of mitochondria and oxidative biomarkers in toxicity.

### 7.3 Pathways and CNS Action of Neurobehavior

The effects of ashwagandha in central nervous system (CNS) functionality have been explained using behavioral models of zebrafish. In accordance with their neuroprotective characteristics, withanolides enhance memory under therapeutic doses, reduce anxiety-like behavior, and modulate locomotor response in zebrafish, indicating that withanolides have neurotoxicity potential (Pullaiah et al., 2025).Nonetheless, withanolides enhance memory in high concentrations, induce hyperactivity, desynchronized circadian patterns, and decreased cognition growth in zebrafish

(Olaniyi et al., 2025). These effects are definition of the modulation of neurotransmitter system and synaptic transmission, as in the mammals. Of particular concern is the fact that such a CNS dose-dependency is indicative of the razor-edge between therapeutic activity and neurotoxicity of the razor. Zebrafish therefore is a delicate bioassay in detecting subtle changes in behaviour that may be as a result of exposure to Ashwagandha.

### 7.4 Potential Interaction with Cytochrome P450 Pathways

Components of ashwagandha may have an impact on cytochrome P450 (CYP450) enzymes, which could lead to herb-drug interactions. Studies on zebrafish show that ashwagandha administration changes the expression of CYP450 isoforms, which can change the metabolism of endogenous and xenobiotic substrates (Ramli et al., 2023). As Table 3 illustrates, these kinds of interactions will change the pharmacokinetics of medications taken together, either increasing toxicity or decreasing efficacy. Additionally, they raise the possibility that altering metabolic processes may either enhance or diminish therapy approaches for viral diseases (Rao et al., 2024). Determining safety standards will require knowledge of CYP450-mediated effects, and zebrafish provide an inexpensive system for predictive testing of such herb–drug interaction concerns.

**Table 3: Mechanistic insights into *Withania somnifera* toxicity in zebrafish models: pathways, effects, and safety implications.**

| Mechanistic Domain | Molecular Target/ Pathway | Dose Effect | Key Outcomes | Toxicological Implications | Experimental Utility | References |
|---|---|---|---|---|---|---|
| Gene expression | Synaptic plasticity, apoptosis, and inflammation | Dose-dependent modulation | Neuroprotection at low dose; apoptosis at high dose | Safety margins narrow; dual effects possible | Transcriptome analysis in zebrafish | Pullaiah et al. (2025) |
| Alzheimer's pathways | Amyloid and tau-related genes | Altered transcription | Potential therapeutic modulation | Dose-linked risk of neurotoxicity | Zebrafish CNS disease models | Olaniyi et al. (2025) |
| Oxidative stress | ROS balance, antioxidant enzymes | Moderate vs. high dose | Antioxidant defence vs. ROS surge | Mitochondrial dysfunction at excess doses | Biomarker screening in zebrafish | Prajapati & Singh (2025) |
| Mitochondria | Cellular respiration, ATP synthesis | Inhibition at higher doses | Reduced energy metabolism | Neurodegenerative risk | Imaging of mitochondrial mobility | Ramli et al. (2023) |
| Neurobehavior | Locomotion, memory, anxiety | Dose-dependent | Memory enhancement, anxiolysis vs. hyperactivity & impairment | CNS toxicity at excess doses | Behavioural assays | Pullaiah et al. (2025); Olaniyi et al. (2025) |
| CYP450 pathways | Xenobiotic metabolism enzymes | Altered isoform expression | Changed pharmacokinetics | Herb–drug interaction risk | Predictive metabolic screening | Ramli et al. (2023) |
| Herb–drug interactions | CYP450-mediated | Variable | Efficacy alteration, toxicity synergy | Hazard with co-medications | Zebrafish predictive tool | |

## 8. Translational Relevance and Regulatory Perspectives

### 8.1 Implications of Zebrafish Discoveries for Human Safety Evaluation

Studies in zebrafish offer important translational information about the safety profile of herbal bioactives like ashwagandha for humans. Early prediction of human toxicological repercussions is made possible by their conservation of human genes and quantifiable behavioral endpoints (Saleem et al., 2020). For example, similar to the therapeutic effects observed in human systems, zebrafish models of neurodegeneration have demonstrated protective mechanisms of herbal bioactives (Sen et al., 2024). Furthermore, zebrafish support high-throughput screening of herbal extracts for potential to induce adverse effects, thereby bridging the gap between in vitro tests and clinical data in humans. Such findings position zebrafish as the focal point of preclinical models for predicting safety outcomes in humans.

### 8.2 Regulatory Guidelines for Herbal Toxicology Evaluation

Regulatory toxicology evaluation of herbal medicines continues to be burdened by variability in phytochemical content, extraction method, and dosing unit. While rodent models are the basis for traditional toxicology, zebrafish are increasingly being set up as a valid model in early safety profiling (Salman et al., 2025). Global regulatory bodies emphasise the need for standard methodologies and validated endpoints when it comes to reproducibility and safety compliance. Regulatory thought in aquaculture and human medicine emphasises herbal testing as essential in mitigating the risk of toxicity and in product uniformity (Semwal et al., 2023). Integration of zebrafish data in official guidelines would significantly accelerate evidence-based regulatory approval of herbal drugs.

### 8.3 Integration of Zebrafish Models into Drug Discovery and Risk Assessment

Integration of zebrafish in drug discovery platforms enhances efficacy screening as well as risk assessment of herbal constituents. Their use for neurodegenerative and oncological disease modelling is promising for the identification of bioactives of therapeutic as well as safety potential (Sharma et al., 2025). For Ashwagandha, zebrafish assays allow the measurement of adaptogenic and anticancer activities with a simultaneous check for systemic toxicity (Saleem et al., 2020). This two-fold utility allows for a translational range from early discovery to regulatory approval, as shown in Figure 4. Finally, by adding zebrafish models to mammalian systems, herbal medicines can be transported to clinical and integrative medicine pipelines with greater safety and effectiveness.

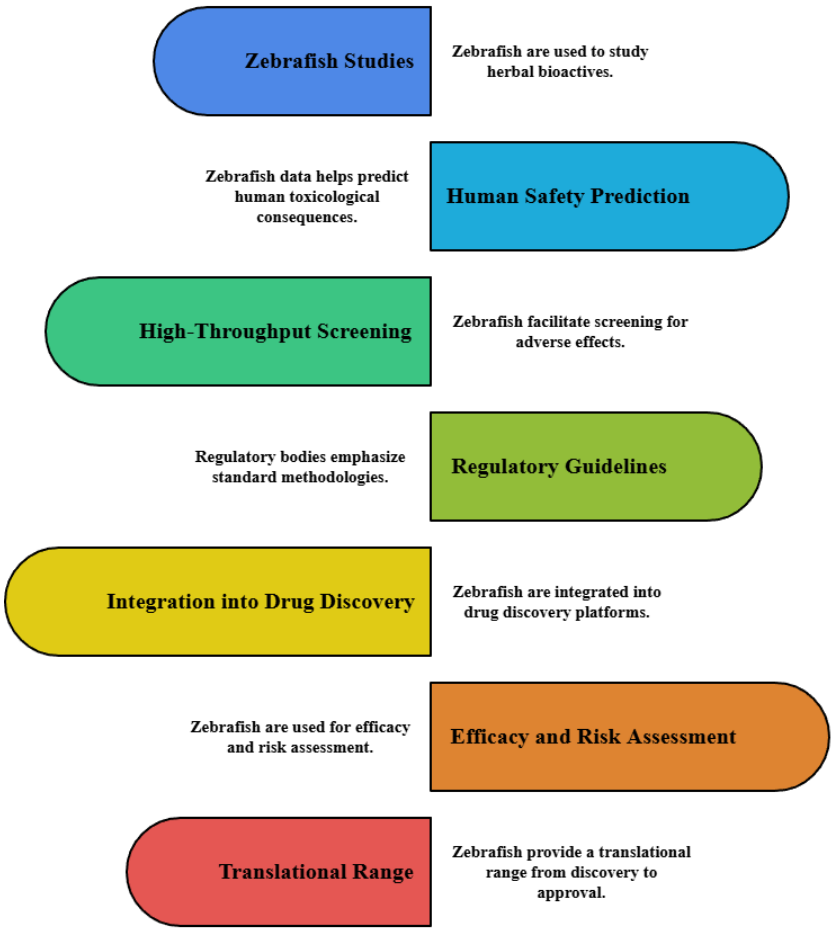


**Figure 4: Zebrafish in Herbal Safety Evaluation.**

## 9. Future Directions

### 9.1 Emerging Technologies: Omics Approaches, Imaging, and CRISPR Models

In the studies that evaluate the safety of ashwagandha in zebrafish performed in the future, there will come the use of cutting-edge technologies such as transcriptomics, proteomics and genomics. Greater mechanistic understanding is possible with the aid of these technologies to determine molecular signs of within-toxicity and within-therapeutic effect (Siddiqui et al., 2021). The high-resolution imaging technologies will also allow the monitoring of organ-specific activity in real time, and CRISPR models will be capable of generating targeted knockouts to study the interactions between the genes and the herbs (Singha et al., 2024). This inclusion of new methodology within the greater context of Ashwagandha will improve the neurological, immunological and metabolic disease standardization intent by increasing the value of the others in toxicity evaluation and balancing the ancient pharmacological and modern precision assessment methodologies.

### 9.2 Standard Process Zebrafish Requirement of Standardised Protocols in Herbal Toxicity Studies

One of the most significant issues about herbal toxicology is the lack of proper established procedures. Depending on extract preparation, dose-time series and endpoint selection, reproducibility, and good interpretation of zebrafish toxicology can be inacquired across labs (Sumran & Aggarwal, 2019). The case of Ashwagandha requires the compatibility of sustaining guidelines on reproducibility and good interpretation of zebrafish toxicology information. It will improve scientific cross-study comparability because they will have the same measures of developmental, behavioural and molecular endpoints. Second, there will be the validation of mammalian and human information through standardised protocols and thus be accepted by the regulatory authorities (Speers et al., 2021). The development of consensus-driven systems will eventually be used as a base in the use of herbal toxicology and integrative safety testing.

### 9.3 Bridging Gaps Between Zebrafish, Rodent, and Human Data

To maximise translational worth, zebrafish results must be mechanistically correlated with rodent and human information. Zebrafish models have successfully replicated Ashwagandha's action on neuroprotection, memory, and stress pathways (Valavan et al., 2022; Speers et al., 2021). However, metabolic and pharmacokinetic variations necessitate rigorous cross-validation to avoid misinterpretation, as shown in Table 4. A combination of zebrafish endpoints with rodent behavioural assays and clinical biomarkers in humans will lead to an integrated pipeline giving greater predictive accuracy (Siddiqui et al., 2021). Such triangulation not only verifies safety profiles but also accelerates Ashwagandha's ascension as a validated therapy in precision medicine.

**Table 4: Future research directions for *Withania somnifera* safety evaluation using zebrafish models: technologies, protocols, and translational integration.**

| Research Focus | Technology/Approach | Application | Expected Outcomes | Challenges | Translational Value | References |
|---|---|---|---|---|---|---|
| Omics tools | Genomics, transcriptomics, proteomics | Detect molecular toxicity & therapeutic markers | Deep mechanistic mapping | Data integration complexity | Precision toxicology | Siddiqui et al. (2021) |
| Imaging | High-resolution real-time imaging | Monitor organ-specific activities | Visualise organogenesis & pathology | Requires advanced equipment | Refined phenotypic endpoints | Singha et al. (2024) |

| | | | | | | |
|---|---|---|---|---|---|---|
| CRISPR models | Targeted knockout zebrafish lines | Study gene–herb interactions | Functional validation of toxicity pathways | Ethical & technical hurdles | Predictive mechanistic assays | Singha et al. (2024) |
| Standardisation | Harmonised zebrafish toxicity protocols | Dose/extract reproducibility | Reliable, comparable data across labs | Lack of consensus globally | Regulatory acceptance | Sumran & Aggarwal (2019) |
| Protocol validation | Cross-check with mammalian data | Confirm zebrafish endpoints | Improves reliability | Differences in metabolism | Integrative toxicology frameworks | Speers et al. (2021) |
| Bridging models | Zebrafish ↔ Rodent ↔ Human | Multispecies triangulation | Enhanced predictive accuracy | PK/PD variability | Translational pipeline | Valavan et al. (2022) |
| Integrated medicine | Precision safety evaluation | Link traditional use with modern methods | Safer therapeutic positioning | Balancing efficacy vs safety | Validation for clinical guidelines | Siddiqui et al. (2021); Speers et al. (2021) |

## 10. CONCLUSION

This review highlights the multifaceted application of zebrafish as a proven and translatable model for herbal toxicology, with a specific focus on Ashwagandha (*Withania somnifera*). Results from embryotoxicity, neurotoxicity, and cardiotoxicity tests demonstrate the ability of zebrafish to provide early and comprehensive data on systemic safety. Homology to humans in genetics and physiology, combined with economic and high-throughput feasibility, makes zebrafish pivotal in bridging the gap between in vitro screening and mammalian testing. Ashwagandha, the foundation of Ayurvedic medicine, continues to be of significant international scientific interest today due to its adaptogenic, neuroprotective, and immunomodulatory activities. Its favourable safety profile is well established across preclinical and clinical data, with sporadic reports of side effects as a reminder to standardise doses and be careful in patient selection. Zebrafish models have proven informative in defining therapeutic windows and delineating dose-dependent toxicological limits, thereby cementing the herb's position in evidence-based practice. In conclusion, zebrafish research complements mammalian research through the provision of mechanistic understanding, high-throughput toxicology screening, and predictive information on human safety outcomes. Their use in regulatory and drug development pipelines can accelerate the process of developing standardised, safe, and effective herbal drugs. Hence, zebrafish are an innovative tool towards advancing Ashwagandha's toxicological evaluation and clinical translation.

## REFERENCES

1. Aktary, N., Jeong, Y., Oh, S., Shin, Y., Sung, Y., Rahman, M., ... & Kim, B., Unveiling the therapeutic potential of natural products in Alzheimer’s disease: insights from in vitro, in vivo, and clinical studies. *Frontiers in Pharmacology*, 2025; *16*: 1601712.
2. Alam, S., Urva-til-wusqa, Z. I., Qamar, M. R., Moeen Afzal, G. M., Ali, H. M., & Hussain, R., Immunostimulation and Stress Reduction in Farmed Fish Fed Herbal Extracts. *Complementary and Alternative Medicine: Botanicals/Homoeopathy/Herbal Medicine; Unique Scientific Publishers: Faisalabad, Pakistan*, 2024; 422-430.
3. Azab, K. S., Maarouf, R. E., Abdel-Rafei, M. K., El Bakary, N. M., & Thabet, N. M., *Withania somnifera* (Ashwagandha) root extract counteract the acute and chronic impact of γ-radiation on liver and spleen of rats. *Human & Experimental Toxicology*, 2022; *41*: 09603271221106344.

4. Balkrishna, A., Pokhrel, S., Singh, H., Joshi, M., Mulay, V. P., Haldar, S., & Varshney, A., Withanone from *Withania somnifera* attenuates SARS-CoV-2 RBD and host ACE2 interactions to rescue spike protein induced pathologies in humanized zebrafish model. *Drug design, development and therapy*, 2021; 1111-1133.
5. Basudkar, V., Gujrati, G., Ajgaonkar, S., Gandhi, M., Mehta, D., & Nair, S., Emerging vistas for the nutraceutical *Withania somnifera* in inflammaging. *Pharmaceuticals*, 2024; *17*(5): 597.
6. Bhattacharya, S., Chatterjee, D., Goswami, R., Pal, B., Paul, S., Sarkar, N., & Chakraborty, M., Reconnoitring antiepileptic and anxiolytic effects of Indian herbs: Findings from zebrafish behavioral research. *Pharmacological Research-Natural Products*, 2024; *5*: 100125.
7. Bian, W. P., & Pei, D. S., Zebrafish model for safety and toxicity testing of nutraceuticals. In *Nutraceuticals* (pp. 333-339). Academic Press, 2016.
8. Brunetti, P., Lo Faro, A. F., Tini, A., Busardò, F. P., & Carlier, J., Pharmacology of herbal sexual enhancers: a review of psychiatric and neurological adverse effects. *Pharmaceuticals*, 2020; *13*(10): 309.
9. da Silva, T. M. H. R., Herbal Solutions for Longevity: Anti-Ageing Compounds From Traditional Medicine. In *Drug Discovery and Antiaging Approaches for Human Longevity* (pp. 325-350). IGI Global Scientific Publishing, 2025.
10. de Oliveira, M. C., Attia, Y. A., Khafaga, A. F., Alqurashi, A. D., Asiry, K. A., Taha, A. E., & Abd El-Hack, M. E., *Withania somnifera* as a Phytoherbal Growth Promoter for Broiler Farming–A Review. *Annals of Animal Science*, 2024; *24*(1): 53-64.
11. Dipankar, S. P., Dani, M. M., Anirudhan, R., Tripathi, D., Mishra, C., Devi, S. H., & Dani Sr, M. M., Pharmacological Insights Into Ashwagandha (*Withania somnifera*): A Review of Its Immunomodulatory and Neuroprotective Properties. *Cureus*, 2025; *17*(8).
12. Dwivedi, S., Dusane, A., Goli, S., Medishetti, R., Kulkarni, P., & Perumal, Y., Characterization and optimization of neurosphere culture as in vitro screening tool against valproic acid-induced neurodevelopmental toxicity, 2025.
13. Güllü, H., & Kıroğlu, O., Clinical applications of Ashwagandha plant in depression and anxiety. *Cukurova Medical Journal*, 2024; *49*(3): 818-833.
14. Gupta, M., & Kaur, G., *Withania somnifera* as a potential anxiolytic and anti-inflammatory candidate against systemic lipopolysaccharide-induced neuroinflammation. *Neuromolecular medicine*, 2018; *20*(3): 343-362.
15. Husain, T., Phytochemical and Therapeutic Evaluation of Psoralea corylifolia and *Withania somnifera*: A Comprehensive Review of Their Use in the Treatment of Hypopigmentary Disorders, 2025.
16. Jadaun, P., Harshithkumar, R., Gaikwad, S. Y., Seniya, C., Borse, S., Gawai, A. A., ... & Mukherjee, A., *Withania somnifera* extracts induced attenuation of HIV-1: a mechanistic approach to restrict viral infection. *Virology Journal*, 2023; *20*(1): 173.
17. Jahagirdar, S., Praveen Kumar, H., Bhat, S. S., Poddar, A., Chattaraj, P. K., Ahmad, S. F., & Prasad, S. K., In silico evaluations of phytochemicals from *Withania somnifera* exhibiting anticancer activity against NAD [P] H-quinone oxidoreductase. *Human & Experimental Toxicology*, 2024; *43*: 09603271241291399.
18. Jayasinghe, C. D., & Jayawardena, U. A., Toxicity assessment of herbal medicine using zebrafish embryos: A systematic review. *Evidence-Based Complementary and Alternative Medicine*, 2019; *2019*(1): 7272808.
19. Kandagalla, S., Rimac, H., Gurushankar, K., Novak, J., Grishina, M., & Potemkin, V., Withasomniferol C, a new potential SARS-CoV-2 main protease inhibitor from the *Withania somnifera* plant proposed by in silico approaches. *PeerJ*, 2022; *10*: e13374.

20. Kanungo, J., Sorkin, B. C., Krzykwa, J., Mitchell, C. A., Embry, M., Spencer, P., ... & Westerink, R. H., Screening tools to evaluate the neurotoxic potential of botanicals: building a strategy to assess safety. *Expert Opinion on Drug Metabolism & Toxicology*, 2024; *20*(7): 629-646.
21. Kayesth, S., Gupta, K. K., Tyagi, K., Mohan, R. R., Arora, J., & Nissapatorn, V., Natural products and human health—A special focus on Indian Ginseng *Withania somnifera* (L.) Dunal. *Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)]*, 2024; *15*(2): 244-259.
22. Kulavi, S., Dhar, D., Iyer, K., Ghosh, A. K., & Bandyopadhyay, J., Unveiling the Potentials of *Withania somnifera* (L.) Dunal as a Precise Therapeutic Intervention Against Glioblastoma Multiforme, 2024.
23. Kumar, P., Banik, S. P., Goel, A., Chakraborty, S., Bagchi, M., & Bagchi, D., Revisiting the multifaceted therapeutic potential of Withaferin A (WA), a novel steroidal lactone, W-ferinAmax ashwagandha, from *Withania somnifera* (L) Dunal. *Journal of the american nutrition association*, 2024; *43*(2): 115-130.
24. Malík, M., & Tlustoš, P., Nootropic herbs, shrubs, and trees as potential cognitive enhancers. *Plants*, 2023; *12*(6): 1364.
25. Mandlik, D. S., & Namdeo, A. G., Pharmacological evaluation of Ashwagandha highlighting its healthcare claims, safety, and toxicity aspects. *Journal of dietary supplements*, 2021; *18*(2): 183-226.
26. Mazurkiewicz, A., Mądry, W., Kołodziej, M., Męczyńska, J., Saiuk, N., Kozicz, M. A., ... & Salasa, W., Ashwagandha (*Withania somnifera*)-Its Antibacterial and Anticancer Activity. *Journal of Education, Health and Sport*, 2024; *66*: 50081-50081.
27. Mikulska, P., Malinowska, M., Ignacyk, M., Szustowski, P., Nowak, J., Pesta, K., ... & Cielecka-Piontek, J., Ashwagandha (*Withania somnifera*)—current research on the health-promoting activities: a narrative review. *Pharmaceutics*, 2023; *15*(4): 1057.
28. Moise, G., Jîjie, A. R., Moacă, E. A., Predescu, I. A., Dehelean, C. A., Hegheș, A., ... & Vlad, C. S., Plants' Impact on the Human Brain—Exploring the Neuroprotective and Neurotoxic Potential of Plants. *Pharmaceuticals*, 2024; *17*(10): 1339.
29. Murthy, M. N., & Shyamala, B. V., Ashwagandha-*Withania somnifera* (L.) Dunal as a multipotent neuroprotective remedy for genetically induced motor dysfunction and cellular toxicity in human neurodegenerative disease models of Drosophila. *Journal of Ethnopharmacology*, 2024; *318*: 116897.
30. Naseem, A., Karande, V., Srivathsan, M., Signapurkar, S., & Langade, D., Effect of ashwagandha on thyroid functions in a zebra fish (daniorerio) model. *IJMPR*, 2023; *4*(5): 341-346.
31. Olaniyi, O. M., Gbayisomore, T. J., Farinde, R. A., Oke, J. O., Adedotun, O. A., Oluwagbamila, O. B., & Iteire, K. A., A Review on Some Medicinal Plants with Therapeutic Potential for Alzheimer Disease and Other Related Dementia (ADRD). *Journal of Experimental and Clinical Anatomy*, 2025; *22*(2): 464-472.
32. Prajapati, D., & Singh, P., Exploring nature's pharmacy: a comprehensive review of herbal plants with neuroprotective properties. *Current Green Chemistry*, 2025.
33. Pullaiah, C. P., SP, P. P., SK, P., Nemalapalli, Y., Birudala, G., Lahari, S., ... & Mitta, R., Exploring Withanolides from *Withania somnifera*: A Promising Avenue for Alzheimer's Disease Treatment. *Current Pharmacology Reports*, 2025; *11*(1): 1-15.
34. Ramli, S., Wu, Y. S., Batumalaie, K., Guad, R. M., Choy, K. W., Kumar, A., ... & Ramachawolran, G., Phytochemicals of *Withania somnifera* as a future promising drug against SARS-CoV-2: pharmacological role, molecular mechanism, molecular docking evaluation, and efficient delivery. *Microorganisms*, 2023; *11*(4): 1000.

35. Rao, D. M. S., Mishra, J., Damodar, S. V., Bagal, J. G., Joseph, R. A., Karra, T., ... & KARRA, T., Uncovering the Role of Indian Medicinal Botanicals in COVID-19 Prevention and Management: A Review. *Cureus*, 2024; *16*(12).
36. Saleem, S., Muhammad, G., Hussain, M. A., Altaf, M., & Bukhari, S. N. A., *Withania somnifera* L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iranian journal of basic medical sciences*, 2020; *23*(12): 1501.
37. Salman, A., Eid, A. H., Khalaf, S. S., El-Dessouki, A. M., El-Shiekh, R. A., & Aly, S. H., Natural products proposed for the management of Huntington's disease (HD): a comprehensive review. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 2025; 1-31.
38. Semwal, A., Kumar, A., & Kumar, N., A review on pathogenicity of Aeromonas hydrophila and their mitigation through medicinal herbs in aquaculture. *Heliyon*, 2023; *9*(3).
39. Sen, D., Rathee, S., Pandey, V., Jain, S. K., & Patil, U. K., Comprehensive Insights into Pathophysiology of Alzheimer's Disease: Herbal Approaches for Mitigating Neurodegeneration. *Current Alzheimer Research*, 2024; *21*(9): 625-648.
40. Sharma, P., Sudan, R., Mishra, A., Choudhury, A., Sharma, N., Kumar, K., & Vyas, M. K., Ayurvedic medicinal plants for cancer treatment: Current insights and future perspectives. *Ayush Journal of Integrative Oncology*, 2025; *2*(2): 68-76.
41. Siddiqui, M. A., Farshori, N. N., Al-Oqail, M. M., Pant, A. B., & Al-Khedhairy, A. A., Neuroprotective effects of *Withania somnifera* on 4-hydroxynonenal induced cell death in human neuroblastoma SH-SY5Y cells through ROS Inhibition and apoptotic mitochondrial pathway. *Neurochemical Research*, 2021; *46*(2): 171-182.
42. Singha, P. S., Ghosh, R., & Ghosh, D., Immunomodulatory Phytocompounds from *Withania somnifera* (L.) Dunal (Ashwagandha) Against COVID-19. *Trends in Immunotherapy*, 2024; *8*(1).
43. Speers, A. B., Cabey, K. A., Soumyanath, A., & Wright, K. M., Effects of *Withania somnifera* (Ashwagandha) on stress and the stress-related neuropsychiatric disorders anxiety, depression, and insomnia. *Current neuropharmacology*, 2021; *19*(9): 1468-1495.
44. Sumran, G., & Aggarwal, A., Prospect of Indian herbs as sources of antioxidants in combating oxidative stress. *Chemistry & Biology Interface*, 2019; *9*(1).
45. Valavan, R., Kharkwal, H., Rajput, S. K., Hoerr, R., & Chander, S., Aqueous-ethanolic extracts of Curcuma longa and *Withania somnifera* improve memory in the dementia model. *Journal of Research in Pharmacy*, 2022; *26*(6): 1842-1856.