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Withania somnifera (L.) Dunal (Ashwagandha): A Narrative Review of Phytochemistry, Standardization, Pharmacology, Bioavailability and Safety

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ABSTRACT

Background: *Withania somnifera* (L.) Dunal (Ashwagandha) is a major Rasayana herb of Indian medicine and a widely marketed dietary supplement, yet inconsistent extract quality and emerging safety signals limit its translation. This review integrates current evidence on its phytochemistry, standardization, pharmacology, bioavailability and safety.

Methods: A narrative review of PubMed literature searched in October 2026, prioritizing systematic reviews, randomized trials, validated analytical studies, pharmacokinetic studies and safety reports; 27 records were included.

Results: The bioactive profile is dominated by withanolides (withaferin A, withanolide A, withanoside IV) and alkaloids. High-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods now quantify marker withanolides in extracts, Ayurvedic formulations and plasma. Meta-analyses of randomized trials report modest reductions in perceived stress, anxiety and serum cortisol and improved sleep, but heterogeneity is high and trials are small. Human pharmacokinetic studies show rapid withanolide absorption, and formulation influenced systemic exposure. Case reports and a scoping review of 25 patients describe idiosyncratic, mostly cholestatic or mixed liver injury, rarely requiring transplantation.

Conclusions: Ashwagandha is promising, but standardized and well-characterized extracts, longer trials with liver monitoring, and stronger pharmacovigilance are required before evidence-based use can be recommended.

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1. Introduction

Withania somnifera (L.) Dunal (family Solanaceae), commonly called Ashwagandha, Indian ginseng or winter cherry, is a key Rasayana (rejuvenative) herb of Ayurveda. It has been used for several thousand years, and its roots in particular have been prescribed for stress, hypertension, diabetes, asthma and cancer, among other conditions [1,2].

In recent years the herb has moved from classical formulations into the global dietary-supplement market, largely because of its reputation as an adaptogen [3]. Published studies attribute to it anti-stress, anti-inflammatory, antimicrobial, anticancer, antidiabetic, anti-obesity, cardioprotective and hypolipidemic properties, as well as neuropsychiatric effects in depression, insomnia and anxiety disorders [4].

Three issues now shape the translational story of Ashwagandha. First, extract composition differs widely between studies, which has prevented consensus on an optimal preparation or dose [3]. Second, analytical methods for marker withanolides have matured, and their routine application to finished products is essential for reproducible results. Third, a growing series of case reports has linked Ashwagandha supplements to liver injury [5,6]. This review therefore brings phytochemistry, standardization, pharmacology, pharmacokinetics and safety together in one pharmacy-oriented account. Its objectives are to:

- summarize the principal phytoconstituents of *W. somnifera* and their analytical relevance;
- compare validated chromatographic approaches used for standardization and bioanalysis;
- appraise clinical evidence for stress, anxiety and sleep, alongside neuropharmacological and anticancer findings;
- review pharmacokinetics, bioavailability and delivery strategies; and
- evaluate safety, with emphasis on hepatotoxicity, and identify research gaps.

2. Materials and Methods

This is a narrative review. PubMed was searched on 18 October 2026 using combinations of the terms “Withania somnifera”, “ashwagandha”, “withanolides”, “withaferin A”, “standardization”, “HPLC”, “pharmacokinetics”, “bioavailability”, “nanoformulation”, “randomized controlled trial”, “anxiety”, “sleep” and “hepatotoxicity”. Records were limited mainly to publications from 2015 onwards. Priority was given to systematic reviews and meta-analyses, randomized trials, validated analytical studies, pharmacokinetic studies and safety reports. Titles and abstracts were screened for relevance, and 27 records were retained. Quantitative findings were extracted from the published abstracts, and full texts were not systematically verified. This is not a PRISMA-registered systematic review, and publication and selection bias cannot be excluded.

3. Results and Discussion

3.1 Botany and Traditional Use

W. somnifera is a perennial shrub of the family Solanaceae; the root is the principal drug, although leaf extracts have also been studied [3]. Ayurvedic classics such as the Charaka Samhita and Sushruta Samhita describe its use for improving weight and strength and for nourishing the body, and it is listed as a medicinal plant in the Ayurvedic Pharmacopoeia of India. In the Unani tradition the root is regarded as a tonic, aphrodisiac and emmenagogue [7].

3.2 Phytochemistry

The pharmacologically important constituents are ergostane-type steroidal lactones, collectively called withanolides, together with alkaloids [8]. Withaferin A is the best-known withanolide and has been used in traditional medicine for many disorders [2]. Five withanosides and withanolides (withanoside IV, withanoside V, withanolide A, withaferin A and 12-deoxywithastramonolide) have been quantified together in human plasma, which makes them the most practical marker set for clinical pharmacokinetics [7]. Importantly, reviewers note that some neuropsychiatric benefits cannot yet be attributed to specific withanolides and that additional, still unidentified, active compounds may be present [3]. Table 1 lists the main markers and their analytical relevance.

Table 1. Principal marker constituents of *W. somnifera* and their analytical relevance

Constituent	Chemical class	Relevance in the reviewed literature
Withaferin A	Withanolide (steroidal lactone)	Marker for HPLC standardization of Ayurvedic formulations [9]; subject of rat pharmacokinetic and tissue-distribution work [10]; C_{max} 2.833 ± 0.981 ng/mL after a 500 mg root extract in humans [7].
Withanolide A	Withanolide	Co-marker in HPTLC fingerprinting of a lehyam formulation (R_f 0.45) [9]; used as internal standard in a rat LC–MS/MS assay [10]; human C_{max} 2.926 ± 1.317 ng/mL [7].
Withanoside IV	Glycosylated withanolide	Quantified in human plasma after oral dosing; C_{max} 0.639 ± 0.211 ng/mL [7].
Withanoside V	Glycosylated withanolide	Detected in plasma but below the lower limit of quantification [7].
12-Deoxywithastramonolide	Withanolide	Highest plasma C_{max} among the quantified markers (5.498 ± 1.986 ng/mL) [7].
Alkaloids	Nitrogenous compounds	Contribute, with withanolides, to the antioxidant and neuroprotective effects described in preclinical reviews [8].

Abbreviations: C_{max}, peak plasma concentration; R_f, retardation factor; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry.

3.3 Standardization and Quality Control

3.3.1 Why standardization matters

Variability in extract composition is a recurring obstacle to comparing clinical studies [3]. Quality assurance is also a safety issue, because adulteration, contamination and inadequate regulatory oversight of herbal and dietary supplements complicate the identification of the product responsible when adverse events occur [11].

3.3.2 Pharmacopoeial and classical quality parameters

Standard quality control of the raw drug and finished product normally includes authentication of the plant part (macroscopy and microscopy), foreign matter, moisture content, total ash and acid-insoluble ash, extractive values, phytochemical screening, thin-layer chromatography (TLC)/HPTLC fingerprinting, assay of markers, and limits for heavy metals, pesticide residues, aflatoxins and microbial load. Stability data are added for finished dosage forms. These are standard pharmacopoeial practices and should be reported in every experimental study using Ashwagandha material.

3.3.3 Chromatographic methods

HPTLC fingerprinting combined with HPLC assay has been used to standardize the Ayurvedic formulation Ashwagandhadi lehyam, where withaferin A and withanolide A showed R_f values of 0.35 and 0.45 respectively and withaferin A content was 0.092% [9]. A rapid gradient HPLC method validated to International Council for Harmonisation (ICH) guidelines now allows simultaneous estimation of withaferin A together with markers of *Convolvulus pluricaulis* and *Bacopa monnieri* in a polyherbal mixture, with a 20-minute run time that the authors describe as eco-friendly [12]. For bioanalysis, LC–MS/MS methods have been developed for withaferin A in rat plasma and tissues [10], and an ultra-high-performance liquid chromatography (UHPLC)–MS/MS method has been validated for five withanolides in human plasma [7]. A UHPLC–MS/MS assay for six withanolides in *Withania coagulans* illustrates how the approach can be extended to other species and polyherbal products [13]. Table 2 compares these methods.

Table 2. Representative analytical methods for withanolides

Technique	Matrix	Analytes	Key features
HPTLC and HPLC	Ashwagandhadi lehyam (formulation)	Withaferin A, withanolide A	R_f 0.35 (withaferin A) and 0.45 (withanolide A); HPLC retention time about 5.0 min; withaferin A 0.092% [9].
Gradient HPLC	Mixture of three herbal extracts	Withaferin A, scopoletin, bacosides and others	ICH-validated; 20 min run; $r > 0.999$; precision < 2%; withaferin A LOD 48.3 and LOQ 146.5 ng/10 µL [12].
LC–MS/MS	Rat plasma and tissues	Withaferin A	MRM m/z 471.1 → 281; withanolide A as internal standard; liquid–liquid extraction with ethyl acetate containing 5% ascorbic acid [10].
UHPLC–MS/MS	*W. coagulans* extract	Six withanolides	9 min separation; LOD 0.040–4.80 ng/mL; LOQ 0.13–16 ng/mL; RSD mostly < 5% [13].
UHPLC–MS/MS	Human plasma	Five withanosides and withanolides	Selective and linear; no carry-over or matrix effect; applied to a pharmacokinetic study [7].

Abbreviations: ICH, International Council for Harmonisation; LOD, limit of detection; LOQ, limit of quantification; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MRM, multiple reaction monitoring; R_f, retardation factor; RSD, relative standard deviation; UHPLC, ultra-high-performance liquid chromatography.

3.3.4 Practical recommendations

- State the plant part, extraction solvent, drug–extract ratio and marker content (for example, percentage total withanolides) for every study material.
- Report a batch-specific HPTLC/HPLC fingerprint and a certificate of analysis, including contaminant limits.
- Declare the withanolide grade of the product. Commercial extracts range widely, for example 1.5%, 5% and 10% withanolide preparations in one bioavailability trial [14].
- Archive voucher specimens and retained samples so that adverse events can be traced to a product batch.

3.4 Pharmacological Activities

Reviews describe mechanisms involving reduction of reactive oxygen species and inflammation, modulation of mitochondrial function, regulation of apoptosis and improved endothelial function [2]. The anti-stress and anti-anxiety actions are thought to occur mainly through the hypothalamic–pituitary–adrenal (HPA) and sympathetic–adrenal–medullary axes, with GABAergic and serotonergic pathways also implicated [3].

3.4.1 Stress, anxiety and sleep

A meta-analysis of nine randomized controlled trials (RCTs; 558 patients) found significantly lower scores on the Perceived Stress Scale and Hamilton Anxiety Scale and lower serum cortisol with Ashwagandha than with placebo, and four of the trials reported mild to moderate adverse events [15]. A separate meta-analysis of five RCTs (n = 254) reported a mean difference of −5.96 on the Hamilton Anxiety Scale together with better sleep-onset latency, total sleep time, sleep efficiency and Pittsburgh Sleep Quality Index scores, but heterogeneity was extreme (I^2 = 98%) [16]. For sleep alone, a meta-analysis of five RCTs (400 participants) found a small but significant benefit, stronger in adults with insomnia, at doses of 600 mg/day or more, and with treatment of eight weeks or longer; no serious side effects were reported, although long-term safety data were limited [17]. In a network meta-analysis of herbal anxiolytics, *W. somnifera* ranked among the efficacious herbs, but the trials were small [18]. Table 3 summarizes these syntheses.

Table 3. Summary of meta-analytic evidence for *W. somnifera* in stress, anxiety and sleep

Study	Design	Evidence base	Principal findings
Arumugam et al., 2024	Systematic review and meta-analysis	9 RCTs, 558 patients	PSS MD −4.72 (95% CI −8.45 to −0.99); HAM-A MD −2.19 (−3.83 to −0.55); cortisol MD −2.58 (−4.99 to −0.16) [15].
Fatima et al., 2024	Systematic review and meta-analysis	5 RCTs, n = 254	HAM-A MD −5.96 (−10.34 to −1.59), I^2 = 98%; improved SOL, TST, PSQI and SE; no change in WASO or TIB [16].
Cheah et al., 2021	Systematic review and meta-analysis	5 RCTs, 400 participants	Sleep SMD −0.59 (−0.75 to −0.42), I^2 = 62%; larger effect in insomnia, ≥ 600 mg/day and ≥ 8 weeks [17].
Zhang et al., 2022	Network meta-analysis (12 herbs)	29 RCTs	*W. somnifera* HAM-A MD −4.90 (credible interval −9.70 to −0.17); small samples limit confidence [18].

Abbreviations: CI, confidence interval; HAM-A, Hamilton Anxiety Scale; MD, mean difference; PSQI, Pittsburgh Sleep Quality Index; PSS, Perceived Stress Scale; RCT, randomized controlled trial; SE, sleep efficiency; SMD, standardized mean difference; SOL, sleep-onset latency; TIB, total time in bed; TST, total sleep time; WASO, wake after sleep onset.

3.4.2 Endocrine effects

A review of Ashwagandha root extract and the endocrine system reported improved thyroid secretory function (higher T3 and T4 with lower TSH), normalization of adrenal activity and effects on the reproductive axis, including lower cortisol and higher LH and FSH in men [4]. These findings are promising but derive from heterogeneous studies, and safety and toxicity in specific patient groups remain open questions [4].

3.4.3 Neuroprotection and cognition

A systematic review of plant-derived nootropics concluded that Ashwagandha mainly modulates anxiety and social-related cognitions [19]. Preclinical work supports neuroprotective, antioxidant and anti-inflammatory effects in Alzheimer's disease models [8]. In 5XFAD transgenic mice, a combination of a bioavailability-enhanced coenzyme Q10 formulation and ethanolic Ashwagandha extract preserved brain volumes, reduced amyloid pathology and inflammation, and showed sex-specific responses [20]. Clinical evidence in Alzheimer's disease remains limited, heterogeneous and inconclusive [21]. In schizophrenia, randomized evidence exists for a limited number of plant-derived candidates including *W. somnifera*, and such agents should be positioned as adjuncts rather than alternatives to antipsychotics [22].

3.4.4 Anticancer research

Withaferin A is among the phytochemicals reviewed for suppressing cancer stem-cell self-renewal and stemness pathways such as Wnt/β-catenin, STAT3 and NF-κB [23], and it is also listed among natural compounds examined in ovarian cancer models [24]. This evidence is preclinical, and both reviews highlight poor bioavailability as a major barrier to clinical translation [23,24]. A recent green-synthesis study built a cellulose-nanocrystal/MgO nanoconjugate from *W. somnifera* and reported selective *in vitro* cytotoxicity against HepG2 and PANC-1 cells, but the authors state that *in vivo* pharmacokinetic and safety studies are still required [25].

3.5 Pharmacokinetics, Bioavailability and Delivery

In 18 healthy male volunteers given a single 500 mg standardized root extract (containing not less than 7.5 mg total withanolides), withanolides were absorbed rapidly, with T_{max} of roughly 0.9–1.6 h for the four quantified markers, and the product was well tolerated [7]. A randomized, double-blind, crossover study in 20 healthy adults compared a 125 mg extract containing 1.5% withanolides with 600 mg of a 5% extract and 500 mg of a 10% extract and found 2.1-fold and 1.3-fold higher total-withanolide bioavailability respectively, indicating that formulation can change systemic exposure independently of nominal dose [14]. Both human studies had authors affiliated with the extract manufacturers, and independent replication would strengthen the conclusions. In rats given withaferin A orally, tissue distribution followed the order stomach > heart > lung > kidney > small intestine > spleen [10]. Table 4 summarizes these studies.

Table 4. Pharmacokinetic and bioavailability studies of *W. somnifera* constituents

Study	Design	Dose and analytes	Principal findings
Vaidya et al., 2024	Non-randomized, open-label, single-dose; 18 healthy males, fasting	500 mg root extract (≥ 7.5 mg total withanolides); five markers by UHPLC–MS/MS	T_{max} about 0.9–1.6 h; C_{max} 0.639–5.498 ng/mL; well tolerated [7].
Ramapalaniappan et al., 2025	Randomized, double-blind, crossover; 20 healthy adults, fasting	125 mg (1.5%) vs 600 mg (5%) vs 500 mg (10%) extract; total withanolides by LC–MS/MS	2.1-fold and 1.3-fold higher bioavailability for the 125 mg product; well tolerated [14].
Wang et al., 2019	Rat study, i.v. and oral	Withaferin A, 0.5–4.5 mg/kg orally; 4.5 mg/kg i.v.	Tissue order after oral dosing: stomach > heart > lung > kidney > small intestine > spleen [10].

Abbreviations: C_{max}, peak plasma concentration; i.v., intravenous; LC–MS/MS, liquid chromatography–tandem mass spectrometry; T_{max}, time to peak plasma concentration; UHPLC, ultra-high-performance liquid chromatography.

3.5.1 Nanotechnology and advanced delivery

Poor solubility, rapid metabolism and limited bioavailability restrict the clinical performance of many free phytochemicals, and polymeric nanoparticles, lipid carriers, hybrid nanocapsules and ligand-targeted systems have been reported to improve stability, uptake and tumour accumulation in preclinical models [23]. For neurological indications, poor brain delivery and variability in herbal preparations are named as translational barriers, with nanotechnology-based delivery listed as an emerging approach [21]. In this search, no clinical study of a nano-formulated *W. somnifera* product was retrieved, so the benefit of such systems for Ashwagandha remains largely unproven. Lipid-based self-emulsifying systems, liposomes, phytosomes and polymeric nanoparticles are logical candidates for future work, and any such formulation should be judged by validated LC–MS/MS measurement of plasma withanolide exposure, as in the studies above [7,14].

3.6 Safety, Toxicity and Herb–Drug Interactions

Ashwagandha is generally described as well tolerated, and the RCT meta-analyses report mostly mild to moderate adverse events [15]. However, safety evidence is limited for long-term use [17], and hepatotoxicity has emerged as the main concern. Two new cases of Ashwagandha hepatotoxicity were reported together with a review of earlier cases: a 36-year-old man (450 mg three times daily for six months) with hepatocellular injury, and a 30-year-old woman (45 days of use) with a mixed pattern; the updated Roussel Uclaf Causality Assessment Method (RUCAM) score was 7 (“probable”) in both, and both improved after discontinuation [5]. A 2026 scoping review of 13 publications covering 25 patients found that injury typically appeared after weeks of use, that cholestatic or mixed patterns predominated with jaundice and pruritus, and that most patients recovered over weeks to months; however, one case required liver transplantation and three deaths occurred in patients with pre-existing cirrhosis who decompensated [6]. Herbal and dietary supplements now account for about 20% of cases in the US Drug-Induced Liver Injury Network (DILIN),

with Ashwagandha among the implicated agents [11]. Reviews of Ayush herbs likewise place *W. somnifera* among commonly used herbs with hepatotoxic potential; most injuries are self-limiting but some progress to liver failure [26]. Table 5 summarizes the key evidence.

Table 5. Key safety evidence for hepatotoxicity

Source	Type	Principal findings
Bokan et al., 2023	Case reports and review	Two new cases with RUCAM score 7 (probable); recovery after stopping the supplement [5].
McIntyre et al., 2026	Scoping review	13 publications, 25 patients; cholestatic or mixed injury predominant; one transplant and three deaths in cirrhotic patients [6].
Halegoua-DeMarzio and Navarro, 2025	Narrative review	Herbal and dietary supplements about 20% of DILIN cases; Ashwagandha among implicated agents [11].
Philips and Theruvath, 2024	Narrative review	Hepatotoxic potential of commonly used Ayush herbs, including *W. somnifera* [26].

Abbreviations: DILIN, Drug-Induced Liver Injury Network; RUCAM, Roussel Uclaf Causality Assessment Method.

Herb–drug interactions deserve equal attention. Authors of a neuropsychiatric review stress the need to investigate interactions when Ashwagandha is used with pharmaceutical interventions [3], and a Society of Toxicology symposium report highlights the need for better tools to predict botanical-induced liver injury and botanical–drug interactions [27]. Practical points for pharmacists and clinicians include:

- ask every patient with unexplained hepatitis or cholestasis about herbal and supplement use;
- advise patients with advanced chronic liver disease to avoid Ashwagandha [6];
- advise prompt medical review if jaundice, itching, dark urine or nausea develops, with liver function tests and discontinuation of the product; and
- review concurrent prescription drugs for possible interactions, and record suspected adverse reactions through the national pharmacovigilance system (Pharmacovigilance Programme of India, PvPI).

3.7 Research Gaps and Future Perspectives

- Develop and use standardized, chemically characterized extracts across trials, with reporting of marker content, so that results can be pooled meaningfully [3].
- Conduct larger, longer RCTs with pre-specified liver safety monitoring, since existing meta-analyses rest on small trials with high heterogeneity.
- Identify the constituents responsible for specific effects and for idiosyncratic hepatotoxicity.
- Evaluate nano-formulations and other delivery systems in head-to-head pharmacokinetic and clinical studies using validated assays.
- Include both sexes in preclinical designs, because sex-specific responses have been observed [20].
- Strengthen product traceability and pharmacovigilance so that adverse events can be linked to specific batches.

4. CONCLUSION

Withania somnifera is a chemically rich, pharmacologically versatile medicinal plant with a long traditional record and an expanding evidence base. Meta-analyses suggest modest benefits for stress, anxiety and sleep, and robust chromatographic methods now exist to standardize withanolide markers in extracts, formulations and plasma. Yet inconsistent extract composition, small trials and a clear signal of rare but serious liver injury temper enthusiasm. For pharmacy practice and research, the priority is standardized, traceable products used with appropriate counselling and monitoring. If these conditions are met, Ashwagandha can be evaluated rigorously as a candidate for evidence-based phytopharmaceutical development.

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