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Review Article

Evaluating Ashwagandha: A Comprehensive Scientific Review of Safety Data and Clinical Implications

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Abstract

Ashwagandha (*Withania somnifera*), commonly known as Indian ginseng or winter cherry, has been used in Ayurvedic medicine for over 3,000 years. Recognized for its therapeutic properties, Ashwagandha contains bioactive compounds, such as withanolides, alkaloids, and steroidal lactones. Ethnomedically, it has been utilized to address various ailments such as anti-inflammatory, anti-anxiety, and neuroprotective effects. This review complies information from databases such as Google Scholar, PubMed, ScienceDirect, and ResearchGate, highlighting Ashwagandha's favorable safety profile across various dosages and formulations. Toxicity studies reveal no significant adverse effects. Recent studies have demonstrated its efficacy in reducing stress, anxiety, and inflammation while also enhancing cognitive health and athletic performance. Clinical trials further validate its safety and effectiveness, demonstrating improvements in cognitive function and physical performance. This comprehensive review underscores the importance of ongoing research to bridge traditional knowledge with contemporary scientific validation, potentially integrating Ashwagandha into healthcare practices. By confirming its safety and therapeutic benefits, further exploration can enhance its role in modern medicine.

Keywords: Ashwagandha; Ayurveda; Traditional Medicine; Safety Studies; Withanolides; Phytochemistry

Abbreviations

WHO: World Health Organization; GC-MS: Gas Chromatography-Mass Spectroscopy; LC-MS: Liquid Chromatography-Mass Spectroscopy; NMR: Nuclear Magnetic Resonance; HPA: Hypothalamic-Pituitary-Adrenal; DHEA-S: Dehydroepiandrosterone Sulfate; HMG-CoA: Hydroxymethylglutaryl-CoA; LD_{50}: Lethal Dose; PCV: Packed Cell Volume; DMBA: Dimethylbenz(a)anthracene; MnPCEs: Micronucleated Polychromatic Erythrocytes; P/N: Polychromatic to Normochromatic; GSH: Glutathione; SOD: Superoxide Dismutase; MMSE: Mini-Mental State Examination; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders

Introduction

Traditional Indian medical systems, including Ayurveda, Siddha, and Unani, have a rich history of utilizing therapeutic herbs to promote health and longevity. Ayurveda, often referred to as the "science of life," integrates holistic practices to maintain well-being and address various health conditions, reflecting its deep roots in ancient Vedic texts [1,2]. A key component of this tradition is the study and application of medicinal plants, which is explored through the interdisciplinary field of ethnopharmacology [3]. This field aims to isolate and characterize bioactive compounds from plants used in Ayurvedic medicine. Ashwagandha is a popular Indian medicinal plant that has been used for over 3000 years in Ayurvedic medicine to treat diverse range of diseases [4]. Ashwagandha (*Withania somnifera* Dunal) commonly referred to as winter cherry or Indian ginseng, is an evergreen branched shrub of the family Solanaceae [5]. This plant is native to Asia, particularly India, and has also been found in the Middle East and Africa [6].

The term 'Ashwagandha' is commonly used and is derived from two Sanskrit words: 'ashwa,' meaning horse, and 'gandha,' meaning fragrance, which refers to the aroma of the plant's fresh roots [7]. In Ayurvedic practice, the herb is classified as a rasayana, meaning it functions as a tonic for life and longevity [8].

Ashwagandha is referred to as Balya (physical and mental power) for the first time in Charaka Samhita and Sushruta Samhita due to its unique qualities [9]. Widely cultivated, Ashwagandha is used as an ingredient in more than 100 formulations in Ayurveda, Unani, and Siddha systems of medicine [10]. WHO recognizes Ashwagandha, as a medicinal herb. Various parts of the plants, particularly roots, have been utilized for thousands of years to treat a range of diseases [11].

The major biochemical constituents of Ashwagandha are steroidal alkaloids and lactones, commonly known as withanolides. The Withania species contain approximately 12 alkaloids, 40 withanolides, and several sitoindosides, which have been isolated from aerial parts, roots, and berries [12]. Ashwagandha provides numerous health benefits, including anti-inflammatory, antioxidant, antimicrobial, anti-anxiety, aphrodisiac, immunomodulatory, antihyperglycemic, anticancer, central nervous system depressant, hepatoprotective, hypolipidemic, cardiovascular protection, diuretic, adaptogenic, anti-stress, antiepileptic, anti-arthritic and impotence treatment properties. It is also beneficial in managing neurological disorders such as Parkinson's and Alzheimer's diseases [13].

Taxonomical classification of Ashwagandha [14-16]

Kingdom: Plantae
Division: Angiospermae
Class: Dicotyledons
Subclass: Asteriidae
Order: Tubiflorae (Solanales)
Family: Solanaceae
Genus: *Withania*
Species: *Somnifera* (L.)

Vernacular Names [17,18]

Sanskrit	Ashvagandha, Ashvakandika, Gandhapatri
Hindi	Asgandh, Punir
English	Winter cherry
Marathi	Askandha, Kanchuki, Tilli
Telugu	Asvagandhi, Penneru, Dommadolu
Tamil	Amukkira, Asubam, Asuvagandi, Asvagandhi, mukkura, amkulang, amukkuram-kilangu,
Bengali	Ashvaganda, Asvagandha
Malayalam	Amukkiram, pevetti
Persian	Kaknaj-e-Hindi, Asgand Nagaori
Gujarati	Asan, Asana, Asado, Asundha, Ghadaasoda
Arabic	Kaknaj-e-Hindi
Kannada	Viremaddlinagadde, Pannaeru, ashwagandha, Kiremallinagida
Urdu	Asgand, Asgand Nagori

Table 1: Vernacular names of Ashwagandha**.**

Botanical description

The ashwagandha plant is a small, upright shrub that typically grows to a height of 30 to 75 cm, distinguished by its woolly pubescence. It features simple, oval leaves with a smooth surface, measuring 4 to 10 cm in length, that are arranged alternately on the vegetative stems. The plant produces small, bisexual, pale green flowers with five petals and five sepals, grouped in axillary cymes, along with a bicarpellary ovary. As the flowers mature, they develop into small berries, about 5 mm in diameter, which start off green and turn orange-red upon ripening, containing numerous tiny, yellow, reniform seeds [Figure 1]. Ashwagandha thrives in the arid, dry soils of India and other subtropical regions of Asia and Africa. Primarily tetraploid with 2n = 48 chromosomes, the plant also exhibits variations in ploidy and has tuberous roots that contribute to its medicinal properties [16,17,19,20] [Figure 2].

Figure 1: Aerial parts of Ashwagandha.

Figure 2: Roots of Ashwagandha.

Ethnomedical use

Ashwagandha is a revered herb in ethnomedicine, valued for its wide-ranging therapeutic benefits. Different parts of the plant; roots, leaves, and flowers are utilized to address various ailments, including heart disorders, pain, liver issues, fever, and respiratory infections. In traditional Asian practices, particularly Ayurveda, the roots serve as a key ingredient in around 200 formulations targeting conditions such as asthma, anxiety, and fatigue. Known as a Rasayana, Ashwagandha has been used since 6000 BC, with its roots recognized for their tonic, aphrodisiac, and diuretic properties. The crushed root paste is often applied to reduce joint inflammation,

while the leaves and flowers help manage fever and painful swellings. The root is also believed to enhance cognitive function and stimulate sperm production, with the Nagori variety particularly sought after for its superior potency. In Africa, communities recognize Ashwagandha for its blood-purifying and diuretic effects, employing it to alleviate coughs, asthma, and epilepsy. Specific ethnic groups, such as the Zay people of Ethiopia, use it for chest pain, while in Somalia, its smoke is wafted over individuals to improve circulation. Healers in South Africa apply the leaves for wound healing. The extensive applications of Ashwagandha highlight its significance in both traditional and folk healing practices worldwide, reflecting its deep-rooted cultural importance and versatility as a medicinal herb [19,21-23].

Phytochemistry

In order to create novel treatments for a range of medical disorders, secondary plant metabolites must be isolated and characterized [24]. Various studies have used different analytical techniques, including Column Chromatography, Gas Chromatography-Mass Spectroscopy (GC-MS), Liquid Chromatography-Mass Spectroscopy (LC-MS), Nuclear Magnetic Resonance (NMR), and X-ray diffraction, to isolate and identify phytochemicals in Ashwagandha [5]. The root contains the highest concentration of physiologically active compounds, with fewer of them found in the stems and leaves [13]. The active constituents include steroidal lactones, alkaloids, saponins, flavonoids, tannins, starch, phenolic compounds, and carbohydrates, as well as withanolides, sitoindosides, anaferine, anahygrine, β-sitosterol, chlorogenic acid, cysteine, cuscohygrine, pseudotropine, withanine, scopoletin, withananine, somniferinine, somniferiene, tropanol, 14-α-hydroxywithanone, and 6,7β-epoxywithanon. Among these, withaferin A, withanoside IV withanoside V, withanoside VI, 12-deoxywithastramonolide, withanolide A, withanone, and withanolide B and withanolide D are primarily responsible for the plant's therapeutic effects, demonstrating significant antioxidant, anti-inflammatory, and anticancer activities. Recent studies have also identified novel compounds like ergosterol and various fatty acids, further enhancing the pharmacological potential of Ashwagandha. Collectively, these phytochemicals contribute to the efficacy of Ashwagandha in both traditional and modern medicinal applications [5,22,25,26]. The chemical structures of some of the components present in Ashwagandha are shown in [Figure 3].

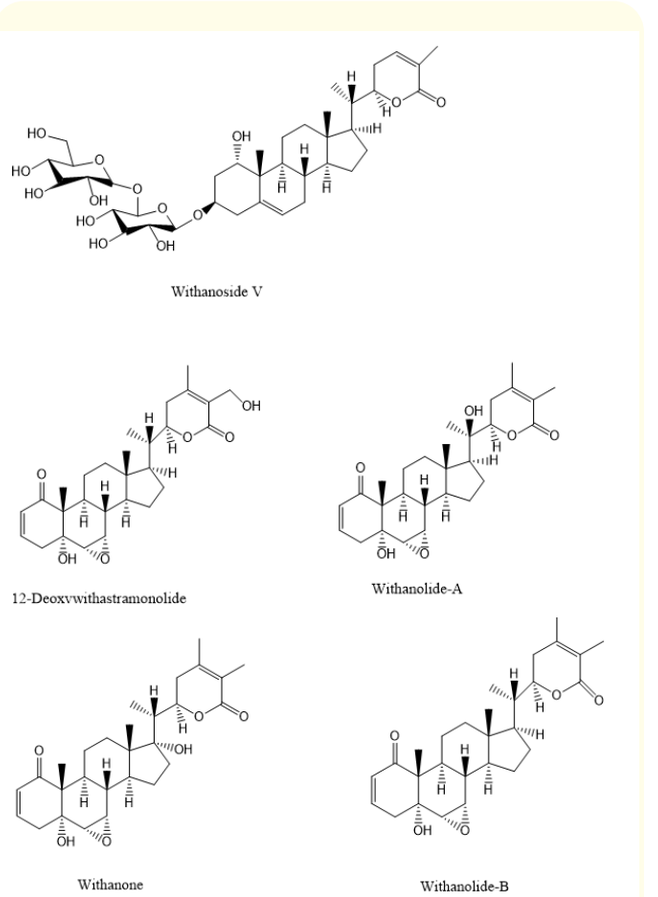

Figure 3: Structures of some of the components present in Ashwagandha.

Pharmacological activities

Anti-inflammatory activity

Ashwagandha demonstrates significant anti-inflammatory properties across various models. Studies show that Ashwagandha's withaferin-A reduces inflammation by inhibiting TNF-α and IL-6, blocking NF-κB activation, and decreasing phosphorylation of p38, ERK-1/2, and JNK, which protects endothelial integrity and reduces leukocyte adhesion [27]. Rasool., *et al.* demonstrated that Ashwagandha root powder significantly reduces paw volume and serum lysosomal enzymes in monosodium urate crystal-induced rats, indicating its safe anti-inflammatory potential [28]. In a study conducted by Giri, the ethanolic extract of Ashwagandha was shown to exhibit significant anti-inflammatory effects, comparable to hydrocortisone, in both carrageenan and Freund's adjuvant arthritis models [29].

Anti-stress activity

Ashwagandha alleviates stress by modulating the hypothalamic-pituitary-adrenal (HPA) axis, lowering cortisol and DHEA-S levels. Its adaptogenic properties enhance mood, cognitive function, and heart rate variability, alleviate anxiety, and improve sleep quality, thereby contributing to effective stress relief [30]. Studies have shown that animals given Ashwagandha prior to chronic foot

shock stress exhibited normalized levels of elevated superoxide dismutase and lipid peroxidation, while enhancing catalase and glutathione peroxidase activities, indicating its potential as an anti-stress adaptogen [31]. A high-concentration, full-spectrum extract of Ashwagandha root significantly reduced stress levels in adults, as data by decreased scores on stress-assessment scales and lower serum cortisol levels. This evidence supports its efficacy in enhancing stress resilience and overall well-being [32].

Antiparkinson activity

Parkinson's disease is a neurological condition characterized by the loss of midbrain substantia nigra dopamine neurons, leading to impaired motor control [33]. In an experimental study with mice, Ashwagandha effectively reduced catalepsy induced by haloperidol or reserpine, suggesting potential neuroprotective effects and promising therapeutic applications for Parkinson's disease [34]. Orally administered Ashwagandha extract significantly alleviated catalepsy and toxic effects in a 6-hydroxydopamine rat model of Parkinson's disease, indicating its potential as a neuroprotective agent [35].

Antioxidant effect

The brain and nervous system are highly susceptible to free radical damage due to their high concentrations of lipids and iron, which promote the generation of reactive oxygen species and increase vulnerability to oxidative stress [36]. *In vitro,* antioxidant activity was observed in the leaves, fresh tubers, and dry tubers of Ashwagandha using DPPH and lipid peroxidation models, with the leaves displaying the highest antioxidant potential, followed by the fresh and dry tubers [37]. The study demonstrated that Ashwagandha root powder significantly reduced plasma cholesterol, lipids, and triglycerides while increasing HDL-cholesterol and HMG-CoA reductase activity in male albino rats. Additionally, the root extract inhibited stress-induced lipid peroxidation in mice and rabbits caused by lipopolysaccharides and peptidoglycans [38].

Safety studies

The acute toxicity of a withanolide free hydrosoluble fraction from Ashwagandha was evaluated in mice by administering graded doses ranging from 100 to 3000 mg/kg to groups of ten, including a control group receiving only the vehicle. Researchers continuously monitored the animals for one hour, then at half-hour intervals for the next four hours, followed by a 72-hour observation period to assess mortality and severe adverse effects. Results demonstrated a favorable safety profile, with no fatalities reported, and calculated acute LD_{50} values indicating a high therapeutic index. Throughout the observation period, there were no significant behavioural changes noted at any dose [39].

In the acute toxicity study, the approximate LD_{50}, of Ashwagandha was found to be 1750 ± 41 mg in albino mice [40]. Orally administered graded doses (100–1000 mg/kg) of two test compounds, sitoindoside IX and sitoindoside X, were given to groups of ten albino mice to assess their LD_{50} values. No mortality was observed within 24 hours after the administration of either compound at doses up to 1000 mg/kg. The LD_{50} values determined via the intraperitoneal route were 518 ± 34 mg/kg for sitoindoside IX and 808 ± 68 mg/kg for sitoindoside X. These results indicate that both compounds exhibit low acute toxicity, with a wide margin between effective and toxic doses [41].

This study investigates the acute and subacute toxicity of Ashwagandha root extract in healthy young male albino mice. The acute toxicity assessment involved administering a single oral dose of 2000 mg/kg, with observations conducted over 14 days, revealing no toxic effects. For the subacute toxicity evaluation, mice received daily doses of 0 (control), 500, 1000, and 2000 mg/kg for 28 days. Post-treatment analysis of blood parameters, including PCV, indicated no significant differences among groups, suggesting that Ashwagandha extract is relatively safe at doses up to 2000 mg/kg/day. However, a slight decrease in PCV was noted, potentially attributable to saponins in the extract, which may influence plasma membrane permeability. Overall, these findings support the safety profile of Ashwagandha in the assessed dosage range [42].

In the study conducted by Sahni., *et al.* investigated the phytochemistry and toxicity of Ashwagandha root extract. The hydroalcoholic extraction process yielded an extractability of 17.4%, and subsequent chemical analysis identified various active principles, including alkaloids, glycosides, and saponins. The LD_{50} was found to exceed 1000 mg/kg in female albino rats. Acute and chronic toxicity assessments revealed initial excitement, followed by mild depression and decreased motor activity at the 1000 mg/kg dose, with no mortality reported. These results indicate a favorable safety profile for Ashwagandha while underscoring its pharmacological potential [43].

The antigenotoxic effects of a steroidal lactone from Ashwagandha were examined against DMBA-induced genotoxicity in golden Syrian hamsters. DMBA injection significantly increased micronucleated polychromatic erythrocytes (MnPCEs) and chromosomal aberrations. However, pretreatment with steroidal lactone markedly reduced these genotoxic effects, demonstrating its protective role in the bone marrow genotoxicity [44].

In vitro cytotoxicity studies of Ashwagandha root extract on human malignant melanoma A375 cells demonstrated safety up to 200 µg/ml, maintaining acceptable cell viability. The calculated IC_{50} values were 350 µg/ml, 250 µg/ml, and 200 µg/ml for 24, 48, and 72 hours, respectively. Notably, the treated cells exhibited significant morphological changes and DNA fragmentation, suggesting

the induction of apoptotic effects. These findings highlight the potential of Ashwagandha as a candidate for further investigation in cancer therapeutics [45].

The methanolic extract of Ashwagandha was investigated for its protective effects against Mitomycin C-induced damage in mice. Administering 250 mg/kg of Ashwagandha for 7 days prior to a 4 mg/kg Mitomycin C injection significantly reduced the micronucleated cells and improved the Polychromatic to Normochromatic (P/N) ratio. The extract also increased liver levels of SOD and GSH, which were depressed by Mitomycin C. Importantly, no toxic effects were observed, indicating the extract's safety. These findings suggest that Ashwagandha protects against oxidative stress and DNA damage by scavenging reactive oxygen species [46].

Clinical studies

A randomized, double-blind, placebo-controlled clinical study evaluated the efficacy and safety of BHC9612CP Ashwagandha extract in 30 adults aged 40 to 75 with mild cognitive impairment over 56 days. Cognitive function was assessed using the MMSE, focusing on parameters such as orientation, memory, attention, and verbal comprehension. The results indicated significant cognitive enhancements in the Ashwagandha group compared to the placebo. Safety analysis revealed no serious adverse effects, with participants generally tolerating the treatment well; mild side effects, such as nausea and headaches, were reported but not linked to the medication. Overall, the study demonstrated the safety and cognitive benefits of BHC9612CP Ashwagandha extract in addressing cognitive decline [47].

In a study, 32 young male hockey players were enrolled to investigate the effects of Ashwagandha supplementation on VO_2max and haemoglobin levels. Participants, with a mean age of 17.4 years, were randomly assigned to two groups: the experimental group received 500 mg capsules of Ashwagandha twice daily for eight weeks, while the placebo group received starch capsules. The results showed significant improvement in VO_2max ($t = 2.98$, $p < 0.01$) and haemoglobin levels ($t = 2.78$, $p < 0.01$) in the experimental group, with no significant changes noted in the placebo group. The treatment was deemed safe, with no adverse effects reported, indicating that Ashwagandha effectively enhances aerobic capacity and haemoglobin concentration in young athletes [48].

This study aimed to assess the cognitive effects of Ashwagandha in euthymic adults with Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) bipolar disorder. Sixty volunteers completed an 8-week, double-blind, placebo-controlled experiment in which they received 500 mg/d of Ashwagandha. Results showed substantial gains in auditory-verbal working memory (digit span backward, P =.035) and reaction time (Flanker test, P =.033) when compared to placebo. Safety assessments found minor, temporary adverse effects in both groups, with no serious incidents and steady vital signs throughout the trial. These data indicate that Ashwagandha may improve cognitive function in individuals with bipolar disorder while maintaining a positive safety profile, necessitating additional exploration [49].

In this study, 180 infertile male patients were evaluated for the efficacy of Ashwagandha on seminal plasma using high-resolution NMR spectroscopy. Participants received 5 g of Ashwagandha root powder daily for three months, with a control group of 50 healthy men. Results showed that Ashwagandha therapy significantly restored concentrations of key metabolites and improved semen quality in post-treatment samples compared to pre-treatment levels. Serum biochemistry also demonstrated significant enhancements. These findings suggest that Ashwagandha may effectively support metabolic pathways and serve as a potential therapeutic approach for male infertility [50].

The study evaluated the safety of Ashwagandha root extract in a randomized, double-blind, placebo-controlled trial involving 80 healthy adults. Participants were assigned to receive either 300 mg of Ashwagandha or a placebo twice daily for eight weeks. The primary safety outcomes included assessments of vital signs, hematological parameters, and biochemical markers, including thyroid function. Results indicated no significant changes or adverse events in either group, suggesting that Ashwagandha is safe for short-term consumption. The study emphasizes the need for further research on long-term effects and varying dosage ranges [51].

Raut., *et al.* conducted a study to evaluate the tolerability, safety, and activity of Ashwagandha in healthy individuals. Eighteen volunteers (12 males and 6 females; ages 18-30 years) received escalating doses of Ashwagandha capsules over a 30-day period, starting at 750 mg/day and increasing to 1,250 mg/day. Participants were assessed for adverse events, vital signs, and muscle strength using grip and quadriceps tests. Most volunteers tolerated the treatment well, with only one reporting increased appetite and hallucinogenic effects at the lowest dose. Improvements in sleep quality, muscle strength, and reductions in cholesterol were noted, indicating that Ashwagandha is safe and effective for enhancing muscle activity. Further research is planned to explore its potential in treating sarcopenia [52].

Conclusion

In conclusion, this review emphasizes the therapeutic potential of Ashwagandha, a medicinal herb known for its numerous health benefits. Numerous research studies investigate the botanical characteristics, historical applications, phytochemical composition, and efficacy in treating various health conditions by combining traditional knowledge with modern scientific results. Safety studies demonstrate that Ashwagandha exhibits a favourable tolerance profile at recommended dosages, indicating its potential as a safe

natural remedy for various ailments. This review lays the groundwork for future research, with a special emphasis on finding the bioactive chemicals that contribute to the pharmacological effects. Further research is essential for improving our understanding of Ashwagandha's medicinal potential.

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