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Review Article

Discover How Ashwagandha May Impact Health: A Comprehensive Review

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ABSTRACT

Withania somnifera (Ashwagandha) is a medicinal herb that has been commonly utilized in traditional medicine for millennia, particularly in Ayurvedic practices. The root of the plant is pharmacologically active and has been used for its aphrodisiac, diuretic, anti-helminthic, narcotic, tonic, and stimulant properties. Additionally, other parts of ashwagandha, including the leaves, shoots, seeds, and berries, contribute to its health-promoting effects and the potential for improving longevity. This plant is composed of many bioactive compounds which exhibit a myriad of health-enhancing properties. Contemporary research has focused on the multifaceted bioactivities of ashwagandha, revealing promising impacts such as anticancer, antioxidant, and anti-inflammatory activities, among other therapeutic applications. This review was planned to find the most recent findings, providing an examination of the active constituents of ashwagandha, their biological activities, and a critical assessment of any associated safety concerns and potential toxicity.

Keywords: Ashwagandha, Withania somnifera, Anti-inflammatory, Antioxidant, Anticancer dosage, Toxicity.

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INTRODUCTION

Ashwagandha, where "ashwa" signifies horse and "gandha" denotes smell or Withania somnifera; the species is designated as 'somnifera', translating to "sleep-inducer" in Latin, because of its remarkable anti-stress properties. Ashwagandha refers to the roots' distinctive 'wet horse' aroma, and it is also known as Indian ginseng or Indian winter cherry because it resembles the pharmacological effects and traditional applications of Korean ginseng tea [1,2]. It is indigenous to India, but it is additionally planted in regions such as Africa, the Mediterranean countries, the Himalayas, the Cape of Good Hope, the Canary Islands, and Australia. Historically, ashwagandha has been utilized in Ayurvedic medicine as a tonic for augmenting the neurological system, which is demonstrated by its therapeutic applications and adaptogenic properties, referred to as "Rasayana." The utilization of it in traditional medicine traces back over 3000 years in India. The root of ashwagandha has been utilized as a narcotic, aphrodisiac, diuretic, anti-helminthic, tonic, and stimulant [1]. Traditionally, ayurvedic practitioners prepare this remedy by putting the fresh roots in milk and boiling them. On the other hand, they may crush the roots into a fine powder known as "churn" and mix them with several fluids, such as milk, honey, or water. In addition, for promoting longevity and enhancing health, other parts of the plant, such as leaves, shoots, seeds, and berries, are also utilized [3].

In Ayurvedic medicine, ashwagandha is designated as a "Rasayana", indicating its role as a regenerating agent that may foster health, augment physical energy, and possibly help prolong life. This medicinal plant has a various array of bioactive phytochemical compounds, including alkaloids, steroidal lactones (particularly withanolides and withaferins), saponins, and glycol-withanolides. The roots are commonly utilized for their therapeutic uses. However, all parts of the plant—leaves, flowers, seeds, and roots—demonstrate health-promoting characteristics. Among the phytochemical constituents, withanolides are considered important because of their well-reported pharmacological features. Withaferin A, a withanolide present in the plant, has attracted significant importance due to its various bioactivities, encompassing immunomodulatory, anti-inflammatory, anti-angiogenic, antioxidant, pro-apoptotic, and anti-adipogenic properties [4]. This review includes the biological activities of ashwagandha, especially its antioxidant properties, anticancer and anti-inflammatory properties, and bioactive substances, dosage, and toxicity profile.

Ashwagandha's active substances

Based on the raw material cultivation environment, the chemical constituents of ashwagandha are characterized by a variable complicated phytochemical composition [1]. The phytochemical analysis of Withania somnifera reveals a diverse distribution of bioactive compounds within its various morphological parts, including fruits, leaves, stem bark, and roots. The leaves are found to be rich in compounds, including twelve distinct withanolides, flavonoids, glycosides, condensed tannins, and free amino acids. However, the roots contain a variety of secondary metabolites, including alkaloids, volatile oils, reducing sugars, and steroids. These bioactive compounds have shown considerable scientific interest globally due to their multifaceted pharmacological properties [5].

The main biochemical ingredients of the plant are steroidal alkaloids and lactones; components that are collectively referred to as withanolides.

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However, more than twelve alkaloids, forty withanolides, and various sitoindosides (a withanolide including a glucose molecule at carbon-27) have been extracted and identified from roots, berries, and aerial parts of Withania species [6]. Withanolides have a core structure known as ergostane and feature a six-lactone ring located at either Carbon-8 or Carbon-9. *This category includes various compounds such as withanopherin A, withanolides A-Y, and withanone.* The composition of alkaloids includes substances such as witanin, somnin and tropin as well as other compounds such as choline, kuskohigrin and anaferin. Furthermore, the natural ingredients include flavonoids like 3-O rutinoside, 6-8 dihydroxycaffeoyl, quercetin, and its glycosidic variations such as 3-O-rutinoside-7-O glucoside. Withanolide glycosides can be known by the presence of a glucose component at position C-27, contain compounds like sitoinsides IX and sitoindoside X. In addition, steroidal saponins containing acyl groups—such as sitoinsides VII and VIII—are also present alongside coumarins, sterols, chlorogenic acid, resins, lipids, carbohydrates and fatty acids [1]. Additionally, it has been shown that sitoindosides and withanamides along with substances, like withanicil, reducing sugars, starch, glycosides, peroxidases, benzyl alcohol, 2-pheyl ethanol, dulciol, , benzoic acid, 3,4,5-trihydroxycinnamic acid, and phenylacetic acid are components found in the extract derived from both the roots and leaves[5,7].

Natural product chemists and medical practitioners are interested in the possible bioactive compounds that synthesized and accumulated in the roots and leaves such as withanolides, such as withanolide D, withaferin A, withanolide A, and withanone, The leaves are rich in withaferin A, an anticancer compound, whereas the roots have a high concentration of withanolide A, an immunomodulatory agent[8]. The pharmacological profile of ashwagandha is extremely documented, demonstrating several therapeutic properties, such as antioxidant, anti-inflammatory, immunostimulant, neurological aphrodisiac, anti-microbial, analgesic, adaptogenic, anti-arthritic, cardioprotective, anti-stress, and anticancer effects. A variety of functions have been attributed to its secondary metabolites, such as steroids, alkaloids, flavonoids, steroidal lactones, glycol-withanolides, and saponins [9].

Ashwagandha's biological activity

Anticancer Activity

Withania somnifera and its bioactive compounds have significantly enhanced various cancer types and cancer-related changes in cell lines. Due to its pleiotropic mode of action, it was shown that it has a significant effect as an antitumor agent, in which it targets multiple oncogenic pathways simultaneously [10,11]. There are several anticancer pathways through which Withania somnifera may be utilized. **First**, it could serve as an adjuvant therapy, potentially mitigating the adverse outcomes linked with radiotherapy and chemotherapy through its anti-inflammatory mechanisms. **Second**, it could be combined with conventional chemotherapeutic regimens to synergistically improve the therapeutic effects of both radiotherapy and chemotherapy via its capabilities for radio- and chemo-sensitization [12].

The leaf extract of ashwagandha and its constituents reveal cytotoxic effects on cancer cells through at least five variuos pathways: apoptosis signaling, the granulocyte-macrophage colony-stimulating factor signaling pathway, the death receptor signaling pathway, the p53 signaling pathway, and the G2-M DNA damage checkpoint pathway. Withaferin-A demonstrated anticancer activity by facilitating apoptosis in melanoma cells through the induction of reactive oxygen species (ROS). The withanolides are involved in the early generation of ROS and perturbations of mitochondrial membrane potential, which precede cytochrome-C release and nuclear translocation of apoptosis-inducing factor. Moreover, withaferin-A induces tumor necrosis factor receptor (TNFR)-1 overexpression, while down-regulating the expression of the pro-apoptotic factor Bid. Enhanced Poly-ADP-ribose-polymerase PARP cleavage, caspase-3 activation, B-cell lymphoma-2 (Bcl-2) down-regulation, ROS production, and the mitogen-activated protein kinase (MAPK) signaling cascade are fundamental constituents of the apoptotic processes induced by withaferin-A in human lymphoma cells[11].

Various in vitro and in vivo studies have indicated that ashwagandha may possess a significant role in the treatment of breast cancer, particularly in cases of estrogen receptor/progesterone receptors-positive (ER/PR positive) and triple-negative breast cancer. Preclinical evidence indicates that ashwagandha exhibits chemo-preventive properties in the context of breast cancer. In contrast, a few clinical trials have been conducted to date that explored its efficacy as an adjunct therapy, which suggested a possible improvement in the quality of life in breast cancer patients[13]. Recent clinical trials conducted randomized double-blind placebo-controlled methodologies have assessed the therapeutic effects of different dosages of Withania somnifera extracts, ranging from 200 mg/kg to 1000 mg/kg, demonstrating that Withania somnifera was effective at these dosages as well as it is safe and well-tolerated by participants. Moreover, numerous studies have established that both the plant and its essentil bioactive component, Withaferin-A, had an antitumor efficacy in human cancer cell lines and murine models[12]

Antioxidant Activity

Many studies have evidenced the effect of ashwagandha on the alterations found in antioxidant markers[14]. The plant is commonly indicated for use as an energy booster and adaptogen because of its free radical scavenging and antioxidant properties. Its antioxidant and free radical scavenging activities are primarily due to the presence of specific withanolides, including withaferin-A, withanone, withanolide-B, withanoside-V, and 1,2-deoxywithastramonolide. These bioactive compounds are demonstrated to play an essential role in mitigating oxidative stress and enhancing physiological resilience[5].

Ashwagandha has been shown to exhibit significant antioxidant properties and free radical scavenging capabilities, along with its role in modifying immune system function. A portion of the bioactive constituents within ashwagandha has demonstrated efficacy in scavenging free radicals linked with the onset and progression of Alzheimer's disease. In vitro and in vivo studies demonstrated that withanolide-A can effectively inhibit the degeneration of axons, dendrites, and synapses in the cerebral cortex and hippocampus. Furthermore, withanolide-A has been found to ameliorate memory deficits in murine models[15]A study revealed that Withania somnifera extract has antioxidant properties by reducing free radical generation in human embryonal neuroblastoma cells. Furthermore, it was found that withaferin- A extract from the plant markedly inhibits the expression of the neuro-inflammatory molecules gene and the production of amyloid[1].

Previous studies revealed that the different concentrations of Withania somnifera considerably raise the activity of antioxidants such as glutathione S-transferase, glutathione peroxidase, superoxide dismutase, catalase, and glutathione reductase. Another study demonstrated the antioxidant aspects of the plant in the aging spinal cords of laboratory mice. Additionally, it has been found that the treatment with the plant extract effectively inhibits lipid peroxidation, a process linked with the pathogenesis of heart disease in humans[16]. Previous research demonstrated that after testing the radical scavenging activities of the ashwagandha roots extract on different solvents, the methanol extract has the most potent radical scavenging activities, and a correlation was found between the total polyphenolic contents in the extract and the antioxidant activity. Overall, the methanolic extract of Withania somnifera has revealed strong antioxidants, hydrogen

peroxide scavenging, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, metal chelating, and superoxide-anion scavenging activities, which may be linked with high medicinal importance[17].

Anti-inflammatory activity

Many studies have evidenced the anti-inflammatory effects of ashwagandha, which can be due to its alkaloid and withanolide contents; this effect may occur by the synergistic effect rather than a single component[18]. Due to the plant's properties, it was explored for the treatment of disorders linked with the body's inflammation, such as diabetes mellitus, cardiovascular, autoimmune, cancers, neuro-degenerative, and pulmonary disorders. Preclinical trials have shown the ability of the plant to control mitochondrial function and apoptosis, as well as decrease inflammation by inhibiting nitric oxide, inflammatory markers such as cytokines (including Interleukin (IL)-6 and TNF-α), and reactive oxygen species. Additionally, ashwagandha has been investigated for its efficacy in proteinuria, nephritis, rheumatoid diseases, and skin inflammation by reducing the biomarkers of inflammation and pro-inflammatory cytokines expression and upregulating anti-inflammatory cytokines[1,19,20].

Withania somnifera has shown significant anti-inflammatory effects in several disease models. The extract of the root demonstrated anti-inflammatory and much-restorative activity by alleviating edema, neutrophil infiltration, and necrosis in trinitrobenzene sulfonic acid-induced inflammatory bowel disease. The root powder form demonstrated a strong inhibitory role on nephritis, proteinuria, and other inflammatory biomarkers in a mouse lupus model, including a reduction in cytokines such as IL-6 and TNF-α, as well as nitric oxide and reactive oxygen species reduction [11]. In addition, experimental animal studies have found that ashwagandha supplementation considerably reduces the blood levels of the pro-inflammatory cytokines and suppresses the synthesis and secretion of pro-inflammatory cytokines[21]. A study investigated that a water-soluble extract of ashwagandha exhibits anti-inflammatory properties by reducing the gene expression of C-C Motif Chemokine Ligand 2 (CCL2) and C-C Motif Chemokine Ligand 5 (CCL5) in response to TNF-α or Lipopolysaccharide (LPS) stimulation. This effect may be linked with a reduction in nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-Kb) activity. These findings indicate that ashwagandha may serve as a significant botanical therapeutic for the management of renal dysfunction [22].

It has been found that ashwagandha had dose-dependent inhibition properties in the production of inflammatory biomarkers[18,20,23] It exhibits dose-dependent inhibition of pro-inflammatory cytokines IL-1β and TNF-α in Lipopolysaccharide (LPS)-induced Tamm-Horsfall Protein-1 (THP-1) human monocytes, as well as superoxide generation in Phorbol 12-myristate 13-acetate (PMA)-induced HL-60 human monocytic cells. These findings indicate a reduction in neuroinflammation, potentially elucidating the anxiolytic properties of Withania somnifera. The in vitro study reveals significant inhibitory effects on IL-1β and TNF-α production and antioxidant role through superoxide inhibition, which may underline its beneficial roles on chronic unpredictable stress (CUS)-induced anxiety. Subsequent in vivo studies further support these results, demonstrating that Withania somnifera efficiently mitigates stress-induced anxiety and corrects CUS-induced physiological alterations in an animal model [20]. These findings promote the fact that ashwagandha possesses significant anti-inflammatory aspects against the denaturation of protein in vitro.

Miscellaneous activities

Treatment with the extract of ashwagandha root was shown to cause up-regulation of low-density lipoprotein receptor-related protein, which facilitates enhanced clearance of β-amyloid peptides and results in a reversal of Alzheimer's disease pathology in mice models. Additionally, oral administration of a semi-purified extract of ashwagandha possesses efficacy in mitigating behavioral deficits and inhibiting the accumulation of β-amyloid peptides in Alzheimer's disease model due to the presence of the active compound andrographolide. The observed therapeutic roles of Withania somnifera were mediated through the modulation of hepatic low-density lipoprotein receptor-related protein activity, indicating its role as a novel intervention for alzheimer-related pathology[15].

It was found that the phenolic compounds present in ashwagandha root are significant contributors to its metal chelation activity. The capacity for metal chelation is fundamental, as it mitigates the concentration of transition metals implicated in lipid peroxidation processes. Additionally, it has been shown that chelating agents, which involve sigma bonds with metal ions, function efficiently as secondary antioxidants. They lower the redox potential, thereby improving the stabilization of the oxidized states of these metal ions. Moreover, the extract has a dose-dependent response in chelating hydroxyl-free radicals, indicating that the methanolic extracts of the plant have a significant effect on scavenging superoxide radicals [17].

Ashwagandha's Dosage and Toxicity

Previous studies investigating the toxicity of various formulations, such as methanolic extracts, decoctions, root pastes, seed powders, and hydroalcoholic extracts, have shown that the active ingredients are present in different portions of the plant in varying concentrations. A recent study evaluated the acute and sub-acute oral toxicities of ashwagandha in animals; the results indicated that all the animals showed a gradual weight gain, and there were no signs of intoxication or significant alterations in blood biochemistry as well as the histo-pathological examinations of the organs remained within normal limits. The plant root powder extract shows no significant abnormalities, even with repeated doses of up to 800 mg/kg, which is five-fold higher than the recommended dose for humans[24]. Another study analyzing the toxicity profile of ashwagandha indicated that it is safe in mice at doses of 2000 mg/kg and 500 mg/kg in acute and repeated dose toxicity, respectively, with a low oral bioavailability[25]. Similarly, ashwagandha root and leaf extract at 1,000 mg/kg for 90 days showed no harmful effects on treated rats as well as it was found that the hematological and biochemical profiles were comparable to controls, and major organs appeared normal in histopathological examinations[26]. Furthermore, no adverse outcomes were reported for a methanolic extract standardized to 4.5% withaferin-A when administered to rats at doses of 500, 1,000, and 2,000 mg/kg per day for 28 days[27]

A human study demonstrated that the plant extract, when administered in capsule form as an aqueous solution, was found to be well-tolerated at gradually increasing dosages ranging from 750 to 1250 mg per day. The formulation was tested to assess hematological and biochemical organ function and found to be safe. Furthermore, in line with its historical use, this study showed improvements in sleep quality, reductions in lipid levels, and enhancements in muscle strength[28]. A recent clinical study conducted on eighteen healthy male subjects aimed to assess the tolerability and safety of standardized capsules of the ashwagandha root extract at 1000 mg/day dose upon oral administration found that following four weeks of administration, no appreciable changes or anomalies were seen in safety metrics such as kidney, liver, and thyroid functions, and the participant's hematological, biochemical, and physical features were all normal[29].

In recent years, its application as a dietary supplement has increased in Western countries, often used for unverified indications related to mental health disorders and as an ergogenic aid among fitness enthusiasts. This report involves eight cases of hepatotoxicity associated with ashwagandha supplementation. All patients had pre-existing hepatic conditions, and the observed mortality rates were

significantly elevated due to delays in liver transplantation[30]. In another study, five liver injury cases were associated with the ashwagandha dietary supplement. The liver injury pattern was consistent among all cases, showing a mixed and cholestatic profile, along with significant hyperbilirubinemia. Each patient was subjected to a thorough and detailed diagnostic evaluation, and a confirmation of all cases was conducted individually by a committee of experts in the field, utilising the Drug-Induced Liver Injury Network structured expert opinion causality assessment method[31]. Similarly, a study concluded that while glutathione detoxifies with withanone, which is a metabolite of ashwagandha, low levels of glutathione may contribute to DNA damage. This could explain the reported liver damage linked with ashwagandha use [32].

LIMITATIONS

This mini review provides a brief overview of the main biological activities, dosage, and toxicity of ashwagandha. However, the availability of clinical trials and the variability in extract standardization across studies limit the evidence. Additionally, the studies conducted were derived from small-scale studies, and large-scale studies needed to be conducted. Caution must be taken when conflicting reports regarding its toxicity and safety profile, particularly at high doses or with long-term use. Future, large-scale, randomized, controlled trials are needed to validate these findings.

CONCLUSION

In conclusion, this review showed that ashwagandha may have an essential role in the management of cancer and exhibits notable antioxidant and anti-inflammatory properties. Nevertheless, additional human-based studies are required to comprehensively evaluate the side effects and potential toxicity of ashwagandha and its active constituents. All those findings are substantiated through rigorous clinical trials prior to their application in clinical settings.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest. M.N. and Y.N. are family members who collaborated on this work. This relationship has been disclosed in the interest of transparency and does not compromise the integrity or objectivity of the review.

AUTHORS CONTRIBUTION

O.N. and **M.N.** contributed equally to conceptualizing the article, outlining its structure, and editing and revising the manuscript.

Y.N. and **D.M.** contributed to the literature review, wrote the first draft, and compiled references.

All others reviewed and approved the final manuscript.

ABBREVIATIONS

ROS	Reactive oxygen species
TNFR	tumor necrosis factor receptor
PARP	Poly (ADP-ribose) polymerase
MAPK	mitogen-activated protein kinase
Bcl-2	B-cell lymphoma 2
ER/PR	positive Estrogen receptor/progesterone receptors -positive
DPPH	2,2-diphenyl-1-picrylhydrazyl
IL-6	Interleukin-6
TNF-α	Tumor Necrosis Factor-Alpha
CCL2	C-C Motif Chemokine Ligand 2
CCL5	C-C Motif Chemokine Ligand 5
LPS	Lipopolysaccharide
NF-Kb	Nuclear Factor kappa-light-chain-enhancer of activated B cells.
LPS	Lipopolysaccharide
THP-1	Tamm-Horsfall Protein-1
PMA	Phorbol 12-myristate 13-acetate
HL-60	human promyelocytic leukemia cell line
CUS	chronic unpredictable stress

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