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Withania somnifera: A Comprehensive Review of Its Pharmacological Properties, Therapeutic Applications, and Mechanisms of Action

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Abstract: *Withania somnifera* (WS), a medicinal plant used in traditional medicine for thousands of years, is attracting increasing attention in modern research. This review aims to collect and analyze the current scientific evidence regarding WS's pharmacological properties, therapeutic applications, and mechanisms of action. The methods used in this review included a comprehensive literature search of the PubMed and Scopus databases, with inclusion criteria that included relevant clinical and preclinical studies. Research shows that the bioactive compounds in WS, such as withanolides, alkaloids, and flavonoids, have significant antioxidant and anti-inflammatory activities, contributing to cellular protection and reducing inflammation. In the context of mental health, WS is effective in reducing anxiety and stress. Additionally, WS exhibits positive effects on the reproductive system, including increased fertility and sexual function, which may be attributed to the modulation of hormones such as testosterone and estrogen. Regarding neuroprotection, WS has been shown to improve cognition by reducing oxidative stress and increasing neurogenesis. Cardiovascular protective effects, demonstrating WS's ability to reduce damage from oxidative stress. This review shows that the bioactive compounds in WS have antioxidant and anti-inflammatory activities that contribute to cellular protection and reduction of inflammation.

Keywords: *Withania somnifera*; Ashwagandha; pharmacological properties; mechanisms of action.

1. Introduction

Withania somnifera, better known as Ashwagandha (Figure 1), is one of the most well-known medicinal plants in traditional medicine, especially in the Ayurvedic system. This plant has been used for many years for various therapeutic purposes, including treating stress, anxiety, and other health conditions. In the context of Ayurveda, WS is considered a "Rasayana," meaning a medicine that provides strength and vitality and prolongs life. The history of using this plant in traditional medicine shows that WS has therapeutic value and deep cultural significance in health practices in India and other Asian countries [1].

In recent years, scientific interest in WS has increased significantly, driven by studies demonstrating the broad therapeutic potential of its bioactive compounds, particularly withanolides and withaferin A. These compounds have been shown to have a variety of

therapeutic effects, including anti-inflammatory [2], antioxidant [3], and anticancer [4,5]. Studies have shown that withanolides can modulate cellular signaling pathways involved in cancer cell proliferation and apoptosis, making it a promising candidate in developing cancer therapies [5,6]. In addition, the adaptogenic effects of WS have also been studied, demonstrating its ability to increase the body's resistance to physical and mental stress [7,8].

Statistics show that herbal medicines, including WS, are increasing worldwide. According to a recent report, the global market for herbal supplements is expected to reach USD 104.78 billion by 2026 [9], with WS being one of the most sought-after herbs. This growing interest not only reflects people's desire for natural alternatives to medicine but also indicates a growing recognition of the scientific evidence supporting the health benefits of this medicinal plant.

This review aims to provide a comprehensive overview of current research related to WS, focusing on its therapeutic effects, mechanisms of action, and clinical applications. This review covers various studies, from basic research to clinical applications. Thus, this review will not only provide valuable information to academicians and researchers but will also help in understanding the potential of WS as a therapeutic agent in modern medical practice [10–12].

Figure 1. Botanical illustration of *Withania somnifera*. Original figure created by the authors.

2. Materials and Methods

To compile this review article, we conducted a comprehensive literature search through several internationally recognized databases, namely PubMed and Scopus. To maximize the search results, we used specific keywords and search terms, namely *Withania somnifera*, ashwagandha, pharmacological activity, pharmacokinetics, clinical study, preclinical study, mechanism of action, safety, and toxicology.

In the study selection process, we established clear inclusion criteria. These criteria included clinical trials and animal studies that directly addressed the therapeutic effects, mechanisms of action, phytochemical composition, pharmacokinetics, therapeutic applications, and safety and toxicology of WS. On the other hand, we also set exclusion criteria that include year range (2014-2024), document type (journal), subject area (Medicine, Pharmacology, Toxicology and Pharmaceutics, Biochemistry, Genetics and Molecular Biology, Chemistry, Neuroscience, Immunology and Microbiology).

After identifying studies that meet the inclusion criteria, the next step is data extraction. We used this method to extract important information from selected studies, including abstracts, methodology, and content data. Articles that passed the selection became the basis for the literature review. The selection of search results can be seen in Figure 2.

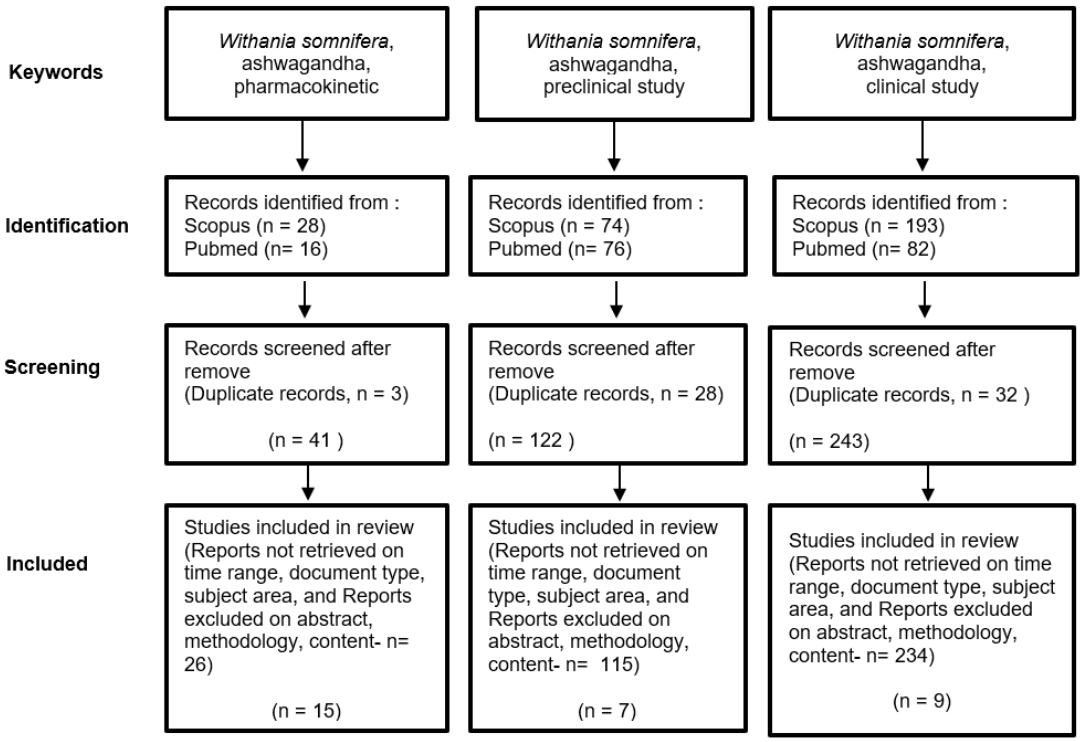

Figure 2. Literature search selection. Original figure created by the authors.

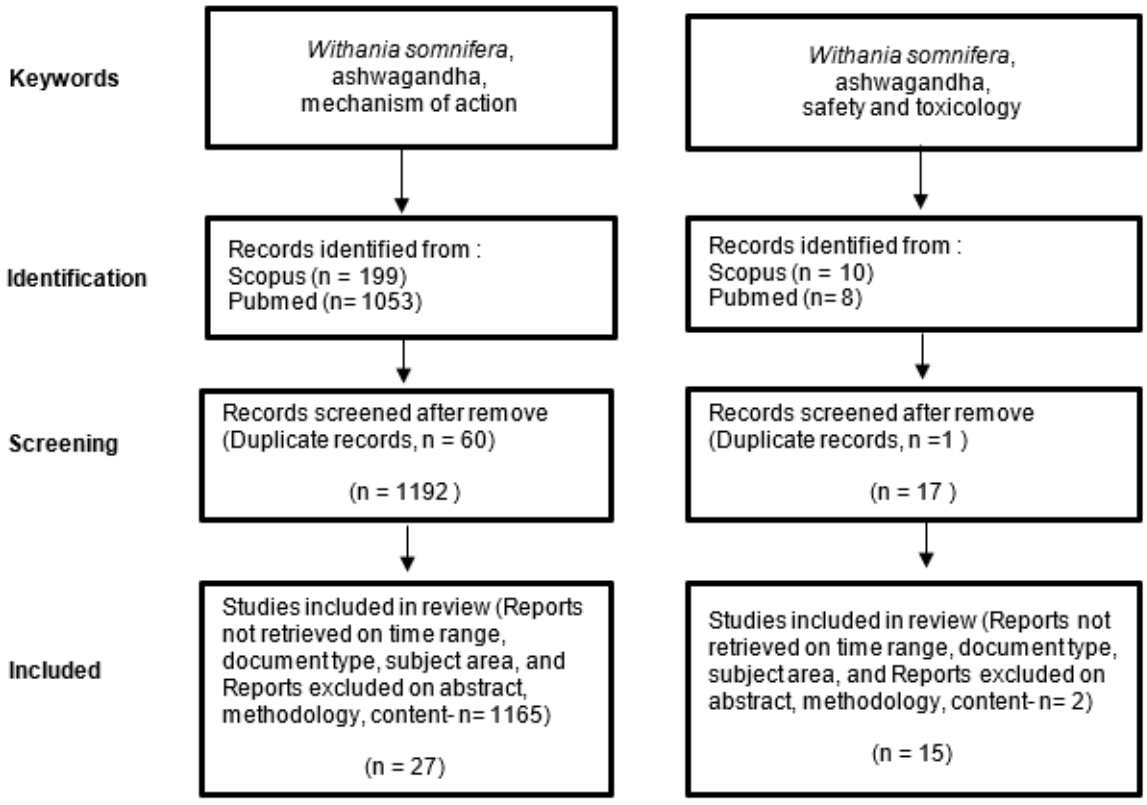

Figure 2 (cont.). Literature search selection (continued). Original figure created by the authors.

3. Results and Discussion

3.1. Phytochemical composition.

3.1.1. Major bioactive compounds.

Phytochemical analysis of WS has revealed the presence of diverse bioactive compounds, including alkaloids, steroidal lactones, saponins, tannins, flavonoids, and various other secondary metabolites [13–15]. The most studied and pharmacologically significant bioactive compounds in WS are withanolides, a group of naturally occurring C28 steroidal lactone triterpenoids formed from an intact or rearranged ergostane skeleton, in which C-22 and C-26 are appropriately oxidized to form a one-membered lactone ring [16–18]. Some major withanolides in WS include Withaferin A, Withanolide A, Withanolide D, Withanone, and Withanoside (Figure 3) [17,19]. Withaferin A is one of the most studied withanolides and has been shown to have a wide range of therapeutic properties, including anti-inflammatory, anticancer, and [20–22]. Withanolide A has also been reported to exhibit potent anticancer, anti-inflammatory, and immunomodulatory activities [6,18]. In addition to withanolides, WS also contains other bioactive compounds, such as alkaloids, saponins, and flavonoids. These bioactive compounds have been shown to contribute to the diverse pharmacological properties of WS, including its antioxidant, anti-inflammatory, neuroprotective, immunomodulatory, and anticancer activities [1,3,4,7,23–28,29–34]. The synergistic and complementary actions of these bioactive compounds are believed to be responsible for the therapeutic potential of the plant and its extensive use in Ayurvedic and traditional systems of medicine [16,35–38].

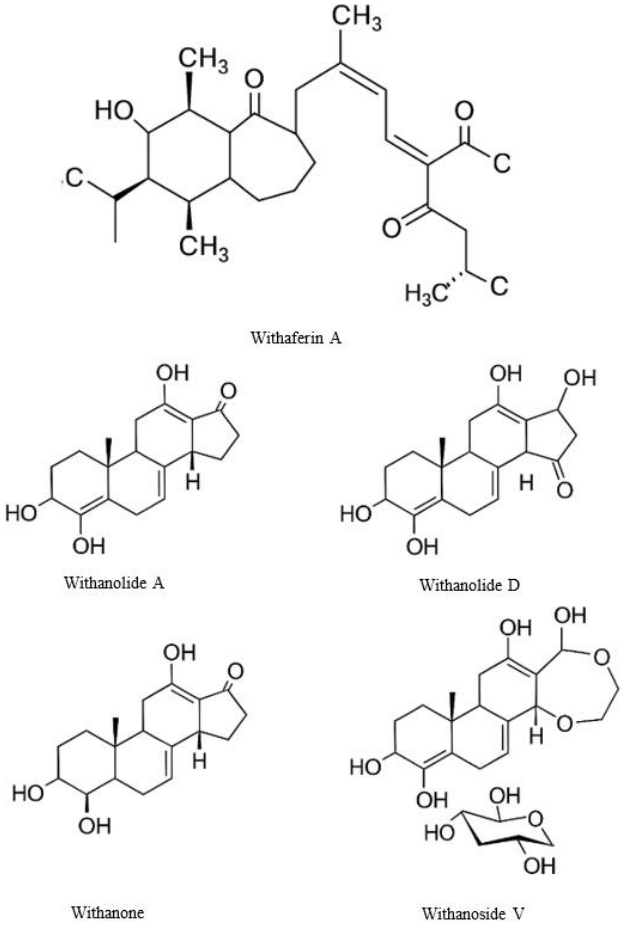

Figure 3. Chemical structure of major bioactive compounds from *Withania somnifera.*

3.2. Pharmacokinetics.

The pharmacokinetics of WS and its bioactive compounds have been extensively studied to understand their absorption, distribution, metabolism, and elimination in the body [39–42]. These studies have provided valuable insights into plant phytochemicals' bioavailability and pharmacokinetic properties. One of the key aspects of WS pharmacokinetics is the bioavailability of its active compounds, particularly withanolides. Studies have shown that the oral bioavailability of Withaferin A, the major withanolide, is about 32.4% in rats [43]. This relatively low bioavailability is due to the compound's poor solubility and extensive first-pass metabolism in the liver [43]. To improve the bioavailability of WS phytochemicals, various formulation strategies have been explored, such as the use of nanoparticle-based delivery systems [44,45]. This approach has enhanced plant bioactive compounds' solubility, dissolution, and absorption, improving pharmacokinetic profiles. In addition to the bioavailability of its phytochemicals, WS has also been found to interact with various drug-metabolizing enzymes and transporters, which may affect the pharmacokinetics of co-administered drugs [46–48]. These herb-drug interactions have been studied to understand the potential of WS in modulating the pharmacokinetics and pharmacodynamics of other therapeutic agents. The pharmacokinetic properties of WS phytochemicals have also been investigated in the context of their therapeutic applications. For example, the plant's neuroprotective and cognitive-enhancing effects have been attributed to the ability of its compounds, such as Withaferin A and Withanolide A, to cross the blood-brain barrier and exert their effects on the central nervous system [10]. Furthermore, the anticancer properties of WS have been extensively studied, and the pharmacokinetics of its bioactive compounds, especially Withaferin A, have been investigated in the context of their potential as anticancer agents [5,49,50].

3.3. Pharmacological properties

WS belongs to the Solanaceae family and is native to the dry regions of India [35,37,38,51]. This plant has been used for centuries to treat various diseases and to improve health [35–38]. Phytochemical analysis of WS has revealed the presence of different bioactive compounds [35–38,52]. The most studied and pharmacologically significant compounds are withanolides, such as Withaferin A and Withanolide A, which have been proven to have various therapeutic properties [5,35,53]. WS exhibits a remarkable series of pharmacological activities, which have been extensively studied and documented in the literature.

3.3.1. Antioxidant and anti-inflammatory.

Bioactive compounds in WS, such as withanolides, have been found to have the ability to scavenge free radicals and modulate the activity of various antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. These activities contribute to cellular protection and reduction of inflammation, which are particularly important in the context of various chronic diseases. Studies have shown that WS can reduce damage from oxidative stress, which is a major risk factor in the development of cardiovascular disease [1,3,7,23–25,27–30].

3.3.2. Effects on mental health.

In mental health, WS has been the focus of significant research. A meta-analysis conducted by Akhgarjand *et al*. [54] pooled data from 12 randomized controlled trials involving 800 participants. The analysis showed that WS supplementation significantly reduced anxiety scores by an average of 1.5 points on the Hamilton anxiety scale (HAM-A) compared to placebo, with a 95% confidence interval (CI) ranging from 1.2 to 1.8. This suggests the potential of WS as an alternative therapy for managing anxiety and stress [55,56].

3.3.3. Neuroprotective and cognitive enhancement effects.

Regarding neuroprotection, WS has been shown to improve cognition by reducing oxidative stress and increasing neurogenesis. Compounds in WS have been shown to modulate neurotransmitter systems, contributing to improved cognitive function. Further research is needed to explore the specific mechanisms involved in these neuroprotective effects [55,57,58]

3.3.4. Immunomodulatory and anticancer.

WS has been found to possess immunomodulatory and anticancer properties, with the ability to inhibit the growth and proliferation of various cancer cell lines, including breast, prostate, and colorectal cancers [4,31–34].

3.3.5. Cardioprotective.

WS has also been found to have protective effects on the cardiovascular system, helping to reduce damage caused by various toxins and stressors. These cardioprotective effects may be attributed to WS's ability to reduce oxidative stress and inflammation, which are major risk factors for heart disease [59–62].

3.3.6. Antimicrobial and antivirus.

WS has been reported to exhibit antimicrobial and antiviral activities, which may be useful in the management of infectious diseases, including COVID-19 [13,19,63].

3.3.7. Reproductive effects and endocrine modulation.

WS also shows positive effects on the reproductive system, including increased fertility and sexual function. Studies have shown that this compound can modulate the activity of hormones such as testosterone and estrogen, which are important for reproductive health. Thus, WS may be an effective therapeutic option for individuals experiencing fertility problems or hormonal disorders [23,64].

The various pharmacological properties of WS can be attributed to the synergistic and complementary actions of its various phytochemical constituents, particularly withanolides [65,66]. These compounds have modulated various cellular and molecular pathways, including those involved in oxidative stress, inflammation, apoptosis, and cell signaling [4,31,33].

3.4. Therapeutic applications.

3.4.1. Preclinical studies.

In the context of geriatric health, Bharani *et al*. [67] evaluated the effects of WS root extract in healthy geriatric dogs. In this randomized, double-anonymized, placebo-controlled study, 30 dogs were divided into two groups, one receiving WS extract and the other receiving a placebo. Results showed a significant increase in superoxide dismutase (SOD) levels by 20% and catalase activity by 25% in the group receiving WS, indicating the ability of WS to enhance the body's antioxidant defenses.

Khan *et al*. [68] evaluated the effects of WS root extract on rats with induced arthritis with collagen. This study showed that treatment with WS extract reduced autoantibody production and oxidative stress, with a 30% decrease in malondialdehyde (MDA) levels compared to the control group. In addition, there was a significant decrease in C-reactive protein (CRP) levels, indicating a strong anti-inflammatory effect of WS. RajaSankar *et al*. [69] examined the effects of WS root extract on a rat model of Parkinson's disease. The results showed that treatment with WS extract increased the brain's catecholamine levels (dopamine and norepinephrine) by up to 50% compared to the control group. This shows the potential of WS in improving neurochemical disorders associated with Parkinson's disease. Rasool & Varalakshmi [70] reported the protective effect of WS root powder on lipid peroxidation and antioxidant status in rats with adjuvant-induced arthritis.

The study showed a significant decrease in lipid peroxide (LP) levels and increased antioxidant status, indicating that WS may act as a protective agent against oxidative damage. Rehman *et al*. [71] reported that treatment with WS extract reduced stress-induced anxiety symptoms and airway inflammation in rats induced with allergic asthma, with a decrease in pro-inflammatory cytokines (IL-4 and IL-5) levels by 45% after 15 days of treatment. Tripathi *et al*. [72] studied the effect of the NMITLI 101R chemotype of WS on hamsters infected with Leishmania donovani. The results showed that treatment with WS extract enhanced the immune response mediated by IFN-γ and IL-12, contributing to antileishmanial drug efficacy. The increase in IFN-γ levels reached 60% compared to the control group. Yin *et al*. [73] reported that WS extract showed GABA-mimetic action on substantia gelatinosa neurons in the trigeminal subnucleus caudalis in rats. This study suggests that treatment with WS extract can increase GABAergic activity, which is potentially beneficial in managing neuropathic pain.

3.4.2. Clinical study.

Clinical Studies of WS, more commonly known as WS, have been the focus of significant research in the context of anxiety and stress management. A systematic review and meta-analysis by Akhgarjand *et al*. [54] pooled data from 12 randomized controlled trials involving 800 participants. The analysis showed that WS supplementation significantly reduced anxiety scores by an average of 1.5 points on the Hamilton Anxiety Scale (HAM-A) compared to placebo, with a 95% confidence interval (CI) of 1.2 to 1.8. The study also noted a decrease in cortisol levels, with an average reduction of 7.5 µg /dL, indicating WS's potential to normalize physiological stress responses. Lopresti *et al*. [74] conducted a more in-depth study on the pharmacological effects of WS, where they found that WS supplementation for 60 days resulted in a significant decrease in cortisol levels ($p < 0.01$) and an increase in DHEA (dehydroepiandrosterone) levels by 15% in the group receiving WS extract compared to the

placebo group. This study involved 64 participants who were divided into two groups, and the group receiving WS showed significant improvements in sleep quality and reduced anxiety symptoms. Chaudhari *et al*. [10] reported the use of WS as an adjunct treatment for restless legs syndrome in Parkinson's patients. In this case report, patients receiving WS showed significant improvement in symptoms, with a reduction in the frequency of restless legs syndrome episodes from 10 times per night to 2 times per night after 4 weeks of treatment. Although this is a single case report, these results suggest the potential of WS in managing neurological symptoms associated with sleep disorders. Majeed *et al*. [75] conducted a placebo-controlled study involving 120 participants with symptoms of anxiety and depression. The results showed that a standardized extract of WS combined with piperine can significantly increase serotonin levels, with an average increase of 30% compared to the placebo group ($p < 0.001$). This study emphasizes the importance of combining piperine to increase the bioavailability of active compounds in WS, contributing to its therapeutic effects. A systematic review and meta-analysis by Durg *et al*. [76] evaluated scientific evidence from experimental studies to clinical applications related to the effects of WS on diabetes. In this analysis, researchers collected data from 15 studies involving 1,200 participants, including both animal and human studies. The results showed that WS supplementation significantly reduced fasting blood glucose (FBG) levels with an average 15 mg/dL decrease. Several studies included in the meta-analysis showed that WS can improve insulin sensitivity. One of the studies reviewed involved 60 patients with type 2 diabetes who received 500 mg of WS extract daily for 8 weeks. The results showed a significant increase in the HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) index, indicating improvements in insulin sensitivity.

In a study by Biswal *et al*. [77], the effects of WS extract on breast cancer patients experiencing fatigue due to chemotherapy were evaluated. The study involved 60 patients who were divided into two groups, with one group receiving WS extract and the other group receiving a placebo. The results showed that the group receiving WS experienced a 30% decrease in fatigue scores ($p < 0.01$) and a significant improvement in quality of life, with quality of life scores increasing by an average of 20% compared to the placebo group. These findings suggest the potential of WS in improving the quality of life of cancer patients. Rasool & Varalakshmi [70] conducted a randomized, double-anonymized, placebo-controlled study to evaluate the use of standardized extract of WS as an adjunct treatment in patients with schizophrenia. In this study, 80 patients treated with antipsychotics were divided into two groups, one group receiving WS extract and the other group receiving a placebo. The results showed that the group receiving WS experienced a greater reduction in psychotic symptoms, with scores on the Positive and Negative Syndrome Scale (PANSS) decreasing by an average of 15 points ($p < 0.05$) compared to the placebo group. This study supports the use of WS as an adjunct therapy in the management of schizophrenia. In a study by Dongre *et al*. [78], the efficacy and safety of WS root extract in improving sexual function in women were evaluated. The study involved 50 women with sexual dysfunction who were divided into two groups, one group receiving WS extract and the other group receiving a placebo for 8 weeks. The results showed a significant improvement in sexual function, with Female Sexual Function Index (FSFI) scores increasing by an average of 25% ($p < 0.01$) in the group receiving WS. These findings suggest that WS may be an effective therapeutic option to improve sexual function in women. R. Kumar *et al*. [79] conducted a comparative study to evaluate the effect of WS as an adjuvant in treating newly diagnosed pulmonary tuberculosis patients. In this study, 100 patients were divided into two groups, where one group received DOTS (Directly Observed

Treatment, Short-course) therapy with the addition of WS extract. In contrast, the other group received DOTS therapy alone. The results showed that the group receiving WS had a higher cure rate, with 85% of patients cured after 6 months compared to 70% in the control group ($p < 0.05$). This study shows WS's potential to enhance the effectiveness of tuberculosis treatment.

3.5. Mechanisms of action.

One of WS's main mechanisms of action is its potent antioxidant and anti-inflammatory properties. Plant phytochemicals, such as withanolides, alkaloids, and flavonoids, have been found to possess potent free radical scavenging properties and the ability to modulate the activity of various antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase [1,3,30,7,23–29]. WS has also been shown to exhibit neuroprotective and cognitive-enhancing effects, which may be attributed to its ability to modulate neurotransmitter systems, reduce oxidative stress, and enhance neurogenesis and synaptic plasticity [10,55,57,58]. The adaptogenic and anti-stress properties of WS are believed to be mediated through its ability to regulate the hypothalamic-pituitary-adrenal (HPA) axis, as well as its ability to enhance the body's resistance to various forms of stress [55,56]. In addition, WS has been found to possess immunomodulatory and anticancer properties. Plant phytochemicals, especially withanolides, have been shown to modulate the immune system by enhancing the activity of immune cells, such as T cells and macrophages, and by inhibiting the growth and proliferation of various cancer cell lines [4,31–34]. WS has also been reported to exhibit cardioprotective and hepatoprotective effects, which are attributed to its ability to reduce oxidative stress, improve endothelial function, and modulate various signaling pathways involved in regulating cardiovascular and liver functions [24,80,81].

Furthermore, WS is known to have antimicrobial and antiviral properties, which may be useful in the management of infectious diseases, including COVID-19 [13,19,63]. WS plant phytochemicals, particularly withanolides, have inhibited the growth and proliferation of various pathogens, including bacteria, fungi, and viruses. Lastly, WS is known to have positive effects on the reproductive and endocrine systems, including the ability to improve fertility and function [23,64]. These plant phytochemicals have been shown to modulate the activity of various hormones, such as testosterone, estrogen, and progesterone, which are important for reproductive health. The overall mechanism of action of WS can be seen in Figure 4.

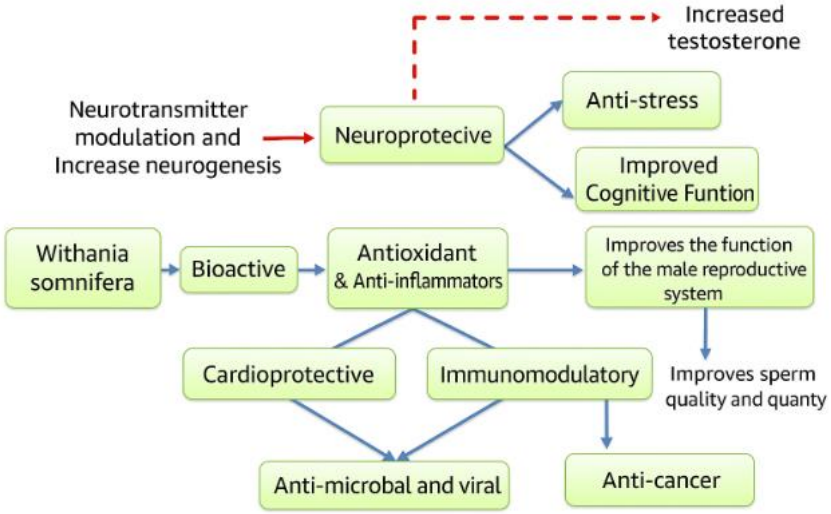

Figure 4. Mechanism of action of *Withania somnifera*. Original figure created by the authors.

3.6. Safety and toxicology.

3.6.1. Clinical studies.

In recent years, significant research has been conducted on the safety and efficacy of supplements containing WS. Numerous clinical studies and case reports have revealed potential side effects, particularly related to liver health. In a study conducted by [82], five cases of liver injury associated with using WS supplements were identified. Three cases occurred in Iceland between 2017 and 2018, and two others were reported by the Drug-Induced Liver Injury Network (DILIN) in 2016.

One interesting case report is that presented by [83], who reported a 20-year-old man with an anxiety disorder who developed severe intrahepatic cholestasis after taking multiple doses of WS. Another case was also reported by Bhushan [84] about a 28-year-old man who used WS for anxiety about two months before going to bed, and when physical examination showed jaundice. Tóth *et al*. [85] also reported that a patient developed jaundice 2 weeks after starting WS and recovered within 5 months after discontinuation without any specific treatment.

Furthermore, some cases have reported the potential for WS to cause liver toxicity, especially in individuals with pre-existing liver disease or when taken in combination with other medications. As reported by Gneco *et al*. [86], a case of a 36-year-old man with complaints of fatigue, jaundice, nausea, and subjective fever for 1 week was reported. Physical examination showed jaundice. Previously, the patient had used several medications, namely cetirizine, diphenhydramine, testosterone supplements, OTC apple cider vinegar gummies, and OTC WS gummies. In addition, Liu [87] also reported a 28-year-old man who presented with cholestatic liver injury after increasing the dose of WS. Biopsy showed canalicular cholestasis. The second case was a 57-year-old man who presented with elevated aminotransferases in the setting of WS use, with laboratory values consistent with hepatocellular injury. The third case was a 47-year-old woman with a history of immune inactive hepatitis B without advanced liver fibrosis who presented with new-onset mild hepatocellular liver injury without hyperbilirubinemia before WS use. Another case was reported by Philips [88], who retrospectively analyzed electronic medical records for WS-HILI using WS or as part of a multi-herbal formulation with supplements. Twenty-three patients had WS-associated liver injury (January 2019 to December 2022), and eight had HILI associated with the single-ingredient WS formulation. Wayman *et al*. [89] also reported a case of a 19-year-old man with severe pruritus and new-onset jaundice after starting WS. Additionally, some studies have reported the presence of heavy metals and pesticide residues in certain WS products, which may contribute to potential safety concerns, Kumar & Singh [90].

Furthermore, a study conducted by Sharma *et al*. [91] reported that administration of WS root extract (600 mg daily) to four subjects showed that treatment with WS can normalize thyroid index in hypothyroid patients. In addition, research conducted by Verma *et al*. [92] revealed that consuming WS root extract for 8 weeks is safe for male and female volunteers. In addition, a study was conducted as a randomized, double-blind, placebo-controlled, and parallel-group study, 80 healthy participants (40 men, 40 women) who received WS 300 mg or placebo at the same dose twice a day showed that consuming WS root extract for 8 weeks is safe for male and female volunteers. Furthermore, a study conducted by Langade *et al*. [93] with a randomized, parallel-group, stratified, and placebo-controlled design on 80 participants

showed that administering WS root extract for 8 weeks was well tolerated across various health conditions and age groups.

Despite these reports, the evidence suggests that WS is generally well tolerated and safe when used as directed. Reported cases of liver damage may be rare and may be related to the specific formulation or individual susceptibility. Nevertheless, healthcare providers should be aware of the potential for liver damage from WS, especially in patients with pre-existing liver disease or those taking other medications.

3.6.2. Preclinical studies.

Preclinical studies play a vital role in assessing the safety and efficacy of bioactive compounds, including Withaferin-A (WA), a major component of the medicinal plant WS. Gupta *et al*. [94] comprehensively assessed the safety, toxicity, and pharmacokinetics of orally administered WA in rats. This study revealed that WA can undergo significant first-pass metabolism, mainly through its interaction with P-glycoprotein and cytochrome P450 3A4 (CYP3A4) enzymes. To increase the bioavailability of WA, researchers investigated the effects of piperine, a known bioenhancer, when administered together with WA. The results showed that the combination of piperine with WA significantly increased the bioavailability of WA, which was likely due to piperine's ability to inhibit the activity of P-glycoprotein and CYP3A4, thereby reducing the first-pass metabolism of WA. In addition, physiological, serum chemistry, hematology, and histopathology examinations showed no toxicity at all tested doses, confirming the presence of a No-Observed Adverse Effect Level (NOAEL).

In a separate study, Wangikar *et al*. [95] evaluated WS root extract's acute and subchronic oral toxicity in Sprague Dawley rats. This study followed OECD and Good Laboratory Practice (GLP) guidelines. The results of the acute toxicity study showed that the LD50 of WS root extract was higher than 2,000 mg/kg, indicating that this extract has a relatively safe toxicity profile. In the subchronic toxicity study, rats were orally given repeated doses of WS root extract. Doses of 250, 500, and 1,000 mg/kg for 90 days, followed by an additional recovery period of 14 days. The results showed no significant toxicological changes, with hematological and serum chemistry parameters within the normal range, and terminal necropsy showed no gross or striking histopathology. These findings provide valuable insights into the toxicological profile of WS and its bioactive compounds and support the safe use of WS and its derivatives in various therapeutic applications.

Patel *et al*. [96] also conducted a toxicity study on Wistar rats by oral administration of WS root extract. In an acute toxicity study conducted at doses of 500, 1000, and 2000 mg/kg body weight/day for 28 days, the results showed that the oral LD50 of WS extract in Wistar rats was greater than 2000 mg/kg, and no side effects were observed at that dose. These findings align with previous studies showing that WS has a good safety profile, supporting its potential use in treating various health conditions.

Although generally considered safe, it is important to provide balanced information regarding the potential side effects or drug interactions that may be associated with WS use. More research is needed to evaluate the safety of long-term use and potential interactions with other medications.

3.8. Future directions.

3.8.1. Research gaps.

More robust and large-scale clinical trials are needed to further validate the efficacy and safety of WS in various health conditions, especially in comparison with standard treatments. Additionally, given the variability in the phytochemical composition of WS due to factors such as geographic origin, cultivation practices, and processing methods, further research is needed to establish robust quality control measures and standardization protocols.

3.8.2. Potential for drug development.

This review highlights that the pharmacokinetics of WS bioactive compounds, especially withanolides, have been studied, but there is still room for improvement in increasing their bioavailability. Further research may explore novel drug delivery systems or formulation strategies to enhance the absorption and distribution of these compounds. In addition, further investigation into the signaling pathways and cellular targets modulated by WS phytochemicals is needed, which may provide valuable insights for developing targeted Withania-derived drugs.

Next, this review discusses WS's neuroprotective and cognitive-enhancing effects. Further investigation into the mechanisms of action and potential therapeutic applications in neurological disorders may lead to the development of WS-based drugs for the management of conditions such as Alzheimer's disease, Parkinson's disease, and other neurodegenerative disorders.

4. Conclusions

WS contains a variety of pharmacologically significant bioactive compounds, especially withanolides such as Withaferin A and Withanolide A. WS exhibits a remarkable range of pharmacological activities, including antioxidant and anti-inflammatory properties, neuroprotective and cognitive enhancing effects, adaptogenic and anti-stress properties, immunomodulatory and anticancer properties, cardioprotective and hepatoprotective effects, and antimicrobial and antiviral activities. These diverse pharmacological properties can be attributed to the synergistic and complementary actions of its various phytochemical constituents, especially withanolides.

The main mechanisms of action of WS include potent antioxidant and anti-inflammatory properties, the ability to modulate neurotransmitter systems and enhance neurogenesis, regulation of the hypothalamic-pituitary-adrenal axis, modulation of the immune system, and cardioprotective and hepatoprotective effects. Further in-depth research is needed to understand the complex interactions between the bioactive compounds in WS and the various biological pathways involved in human health.

The pharmacokinetics of WS and its bioactive compounds have been extensively studied. The oral bioavailability of Withaferin A, the major withanolide, is only about 32.4% in rats due to the compound's poor solubility and extensive first-pass metabolism in the liver. Various formulation strategies have been explored to improve the bioavailability of WS phytochemicals, such as using nanoparticle-based delivery systems.

Although WS is generally well tolerated, there have been reports of potential liver toxicity associated with the use of WS products, particularly in individuals with pre-existing

liver disease or when taken in combination with other medications. Therefore, healthcare providers should be aware of this potential side effect. In the future, more robust and large-scale clinical trials are needed to validate the efficacy and safety of WS further, as well as further research to improve the bioavailability of its bioactive compounds and explore the mechanisms of action underlying its various therapeutic applications.

Author Contributions

Conceptualization, H.R. and R.T.I.; methodology, H.R.; validation, H.R. and R.T.I.; formal analysis, H.R. and R.T.I.; data curation, H.R.; writing—original draft preparation, H.R. and R.T.I.; writing—review and editing, H.R. and R.T.I.; visualization, H.R.; supervision, H.R. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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