REVIEW
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Update on the preclinical and clinical assessment of *Withania somnifera*: from ancient Rasayana to modern perspectives

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Author contributions

Alok Sharma conceived the original idea of the manuscript, whereas Akanksha has done extensive literature searches using various searching tools. Akanksha and Rashmi Singh wrote the manuscript and discussed it with Alok Sharma and Harjeet Singh. Atiqul Islam and Rimpi Arora prepared the theoretical framework of this manuscript and assisted in deriving figures and tables. All authors provided critical feedback and guided in the compilation and data analysis of the manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

W. Somnifera, *Withania somnifera*; WSREt, *Withania somnifera* root extract; WSLEt, *Withania somnifera* leaf extract.

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Abstract

The Rasayana plant *Withania somnifera* (*W. somnifera*) Dunal, also known as "Ashwagandha", has been mentioned in various classical Ayurvedic texts, such as Charaka Samhita, Sushruta Samhita, and Nighantus. This Ayurvedic drug has been referred to as a tonic that renews the body, provides physical and mental vigor in weakened states, and promotes endurance and longevity. *W. somnifera* possesses notable biological activity in many ailments, such as diabetes, conjunctivitis, insomnia, senile dementia, Parkinson's disease, nervous system disorders, rheumatism, and arthritis. These pharmacological activities are due to the presence of diverse active components and their derivatives. Some lead compounds are found to be effective against anxiety and other central nervous system disorders. *W. somnifera* has been proven to be effective and safe for a wide range of ailments from ancient to modern times. Its reported properties represent the traditional use of *W. somnifera* as indicated in the literature; furthermore, *W. somnifera* is one of the most important prescribed drugs in Ayurveda for its multimodal effects. This current review highlights the bioactive present and provides an overview of the toxicological and pharmacological studies on *W. somnifera*, including preclinical and clinical studies. From its earliest utilization to its current application, *W. somnifera* has been recognized to be effective at clinical levels for human health and welfare. Greater attention to the safety and efficacy of *W. somnifera* would provide more scientific evidence, promoting global acceptance of the Ayurvedic plant.

Keywords: *Withania somnifera*; Ayurveda; Rasayana; pharmacological profile; preclinical study; clinical study

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Highlights

This *Withania somnifera* (*W. somnifera*) is a Rasayana plant that has been referred to as a tonic and provides physical and mental vigor and also promotes endurance and longevity. The present work summarizes the role of *W. somnifera* in various disorders along with numerous pharmacological activities and preclinical, clinical, and toxicological studies. Besides, the safety aspects of *W. somnifera* have not been sufficiently addressed in modern research. This work focuses on bioactive and pharmacological studies, and mechanisms, as per published literature. *W. somnifera* extracts and bioactive could provide justification behind the traditional medicinal uses of *W. somnifera*.

Medical history of objective

W. somnifera is a much-praised Ayurvedic drug known for its beneficial effects on a multitude of disorders which can be dated back to the Vedic era (originating in ancient India). People of India believe that Ayurveda, a system of medicine and lifestyle developed in Ancient India, contains enumerate basic health and wellness information. The market of *W. somnifera* is gaining attention along with a boost in demand for nutritional supplements and herbal formulations. However, the acceptance is still growing higher and gaining momentum as customers become more aware of and believe in herbal products. The present review offers up-to-date information on the pharmacological, toxicological, studies and compiles them all together to get a concept of the safety aspect of the *W. somnifera* which is still so far having limited advances in modern research.

Background

Withania somnifera (*W. somnifera*) has been highly acclaimed for its beneficial effects on a variety of ailments since ancient times [1]. *W. somnifera* Dunal (family Solanaceae) is commonly known as Ashwagandha, Indian ginseng, or winter cherry (smell of its root, meaning “like a horse”). The roots of *W. somnifera* have often been used for medicinal purposes (Figure 1). It is one of the most important plants in South Asia, owing to its established therapeutic potential [2]. The plant is widely distributed in the drier parts of tropical and subtropical zones, including the areas of the Canary Islands, South and East Africa, and the region from Palestine to North India, covering Israel, Jordan, Egypt, Sudan, Iran, Afghanistan, Baluchistan, and Pakistan. In India, the plant grows wild in the northwestern regions, extending to the mountainous regions of Punjab, Himachal Pradesh, and Jammu, up to an altitude of 1,500 m [3].

W. somnifera is a much-praised Ayurvedic drug known for its beneficial effects on a multitude of disorders. This knowledge can be dated back to the Vedic era (originating in ancient India). Ashwagandha has been used in Ayurveda as a liver tonic, anti-inflammatory, astringent, aphrodisiac, and adaptogen for disorders such as insomnia, bronchitis, asthma, ulcers, emaciation, and dementia [4]. *W. somnifera* is referred to as “Asvabati” in the *Rigveda and Atharvaveda*, religious texts originating in ancient India (unknown publish date) [5]. Acharya Charaka, one of the principal contributors to Ayurveda, incorporated *W. somnifera* in Balya because of its effects in promoting longevity and anti-aging, enhancing vital strength, and improving sexual performance. *W. somnifera* is among the foremost medicinal herbs in Ayurveda material medic, which provide information on formulation, dosage, and traditional uses of *W. somnifera* [6].

Various investigations on active bioactive constituents have been conducted, which serve to provide a rationale for drug design with enhanced pharmacological properties [7]. Phytochemical studies have revealed the presence of many chemical ingredients in various sections of *W. somnifera*. More than 40 withanolides, 12 alkaloids, and many uncommon sitoindosides have been discovered in the plant thus far [8]. In a nutshell, withanolides (steroidal lactones), the major phytochemical in *W. somnifera*, play a key role in demonstrating synergistic multimodal effects. Furthermore, additional polyphenols are found in significant levels in *W. somnifera* extracts. The combination of such diverse phytochemicals enhances the potency of *W. somnifera* as a powerful therapeutic agent [9, 10]. Modern preclinical and clinical research has confirmed a wide range of pharmacological activities, including immunomodulatory, anti-inflammatory, antioxidant, anti-stress, antihypertensive, neuroprotective and antidiabetic activities, along with organ-protective properties [11, 12]. The graphical abstract illustrates the translational approach of *W. somnifera,* an Ayurvedic herb that is found to be effective against various ailments, with reported studies supporting their diverse pharmacological activities, including pre-clinical and clinical studies [13].

This review aims to assess and summarize the literature regarding the role of *W. somnifera* in various disorders. Here, up-to-date information on the numerous pharmacological activities and preclinical, clinical, and toxicological studies are presented and compiled to reveal the safety aspects of *W. somnifera*, which have not been sufficiently addressed in modern research [14]. This work mainly focuses on the reported active phytoconstituents, pharmacological studies, mechanisms, and toxicology in the published literature related to the potential activities of *W. somnifera* extracts and its isolated compounds, with the aim of providing a better understanding of the biological justification behind the traditional medicinal uses of *W. somnifera* [15].

Phytochemistry of W. somnifera

W. somnifera is an herbal drug with potential pharmacological and traditional properties. Since ancient times, this plant has been utilized, singly or in combination, in a variety of drug formulations to advance human vitality and longevity [16]. *W. somnifera* has extensive clinical and scientific validation and is promoted as a versatile therapeutic agent. *W. somnifera* exhibits a wide range of withanolide phytochemicals (triterpene lactones withanolides, withaferin A, alkaloids, steroidal lactones, and cuscohygrine) (Figure 2), resulting in a wide range of biological effects [8]. The presence of several active moieties with a steroidal lactone ring in their structure, known as withanolide (22-hydroxyergostan-26-oic acid-26,-22-lactone)3, which are structurally similar to the ginsenosides of *Panax* ginseng, gives *W. somnifera* the common moniker, “Indian ginseng” [17].

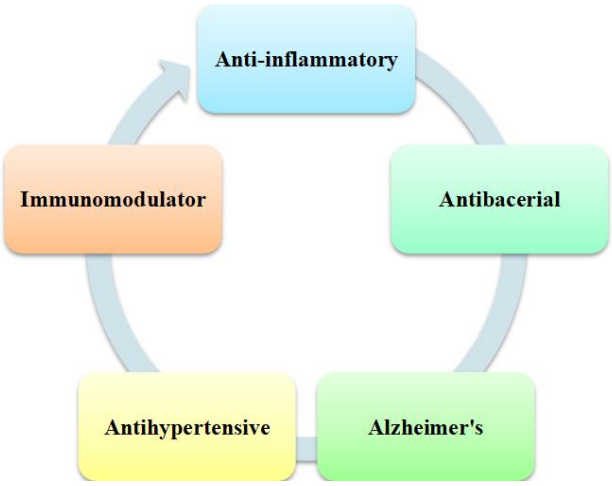


Figure 1 ***Withania somnifera*** **activity**

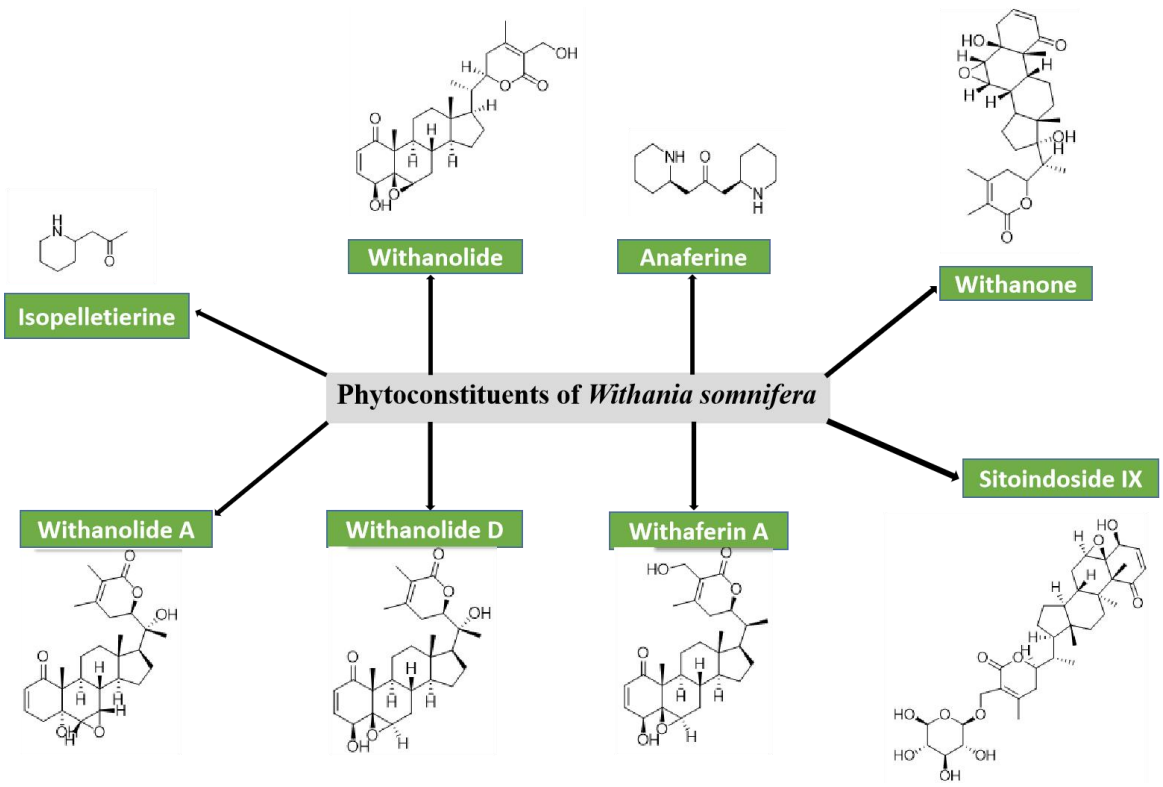


Figure 2 Constituent of *Withania somnifera*

Approximately 35 withanolides have been isolated from various portions of *W. somnifera*, including withanolide A, withanolide D, withanolide, withanone, and withaferin A. Most of the active phytochemicals needed for pharmacological activity are found in the roots of *W. somnifera* [18]. 12-Deoxywithastramonolide, withanolide A, and withanolide B are all derivatives of withaferin A. Alkaloids, steroidal lactones (withanolides and withaferins), and saponins are some of the other biologically active chemical elements in *W. somnifera* roots. Alkaloids, amino acids, steroids, volatile oils, starches, reducing sugars, glycosides, hentriacontane, dulcitol, and withaniol are all said to be present in the roots [19]. A proteolytic enzyme, amino acids, condensed tannins, and flavonoids are all found in the fruits of *W. somnifera* [20]. Fruits have also been shown to contain squalene and tocopherol. Two steroidal lactones of the withanolide-type have also been isolated from the fruits of *W. somnifera*. Withanolides are also present in the leaves of *W. somnifera*. In fact, the leaves of the plant are reported to contain 12 withanolides, 5 unidentified alkaloids, many free amino acids, chlorogenic acid, glycosides, glucose, condensed tannins, and flavonoids [21]. Recent preclinical pharmacological studies (Figure 3) have revealed that *W. somnifera* possesses innumerable chemotypes, including anti-inflammatory, antioxidant, antihypertensive, neuroprotective, immune modulation, and anticancer activities, as well as improves stress-related disorders. Several clinical studies have proven the safety and efficacy of *W. somnifera* [22].

Preclinical study

Preclinical pharmacodynamics identifies the clinical utility, risk factors, and conditions under which the drug/herbal formulation may be subjected to reflective modifications in the concentration-effect relationship. *W. somnifera* is gifted with diverse pharmacological activities, including pre-clinical activities as shown in Table 1.

Neuroprotective activity

Ashwagandha has gained interest during the last three decades owing to its potential in the treatment of brain-related disorders. *W. somnifera* bioactives have been documented for their utility in the effort to cure brain-related disorders. For example, Dar et al. investigated the effects of withanone, one of the active ingredients of *W. somnifera*, on N-methyl-D-aspartate induced excitotoxicity in retinoic acid-differentiated Neuro2a cells. The findings showed that N-methyl-D-aspartate causes significant cell death, as measured by malondialdehyde levels, and the cleavage of poly (ADP-ribose) polymerase-1, which is indicative of DNA damage [23, 24]. Apart from cholinergic inhibition, scopolamine-induced memory loss has been linked to oxidative stress; Ashwagandha i-extract and withanone

Figure 3 Clinical and pre-clinical activity of *Withania somnifera*

could be used to prevent and treat such neurodegenerative disorders [25]. Jain et al. investigated the antistress and neuroprotective effects of *W. somnifera* root extract (WSREt) on neuron cell bodies in the hippocampal subregions of the adult female rat brain [26]. A recent study showed that the oral administration of semi-purified WSREt, containing primarily withanolides and withanosides, corrected behavioral impairment, plaque pathology, and the buildup of β-amyloid peptides and oligomers in the brains of middle-aged and elderly APP/PS1 Alzheimer's disease transgenic mice [38]. Moreover, *W. somnifera* has demonstrated neuroprotective effects in 6-hydroxydopamine-induced Parkinsonism in rats, indicating its potential as a remedy for Parkinson's disease [27].

In chronically stressed mice, the oral administration of a chemically standardized and identified aqueous fraction of *W. somnifera* root at doses of 25, 50, 100, and 200 mg/kg orally enhanced the stress-induced depleted T-cell population and raised the expression of Th1 cytokines [28]. Treatment with withanolide A, withanoside IV, and withanoside VI resulted in the considerable restoration of pre- and post-synapses, as well as the regeneration of axons and dendrites in neurons [30]. Mukherjee et al. investigated the ability of withanolide A to penetrate the brain following intra-nasal injection and examined its neuroprotective properties in an adult mouse model of cerebral ischemia-reperfusion injury [31]. The chronic administration of WSREt (100 and 200 mg/kg) dose-dependently restored biochemical alterations induced by chronic 3-Nitropropionic acid treatment ($P < 0.05$). These findings suggest that the neuroprotective behavior of *W. somnifera* is mediated through its antioxidant activity [33].

Analgesic activity

Extensive efforts made during many decades have revealed the analgesic activity of the different types of *W. somnifera* and its bioactive constituents [39]. Recently, various reported studies have supported the pain-revealing potential of *W. somnifera*. According to Orru et al., the co-administration of its water-soluble extract with morphine altered its analgesic effectiveness, depending on the morphine dose and behavioral test employed [40]. In addition, receptor binding assays suggest the possible involvement of γ-aminobutyric acid type A&B, N-methyl-D-aspartate, and δ opioid receptors in the observed behavioral effects. This study reveals that *W. somnifera* extract can be used as an important adjuvant agent in opioid-sparing therapies [41].

Furthermore, another study conducted by Dey et al. showed that stress resistance is another benefit of its centrally acting analgesic activity, depending on factors such as dosage and number of days of treatment [42]. Rasool et al. revealed the analgesic potential of *W. somnifera* root powder and indomethacin for the acetic acid-derived writhing response in rats, showing a pronounced analgesic activity regardless of dose concentration, as compared with that of the control group [43]. Sabina et al. investigated the analgesic, antipyretic, and ulcerogenic activities of withaferin A (an active component of *W. somnifera*), a steroid lactone, using a variety of experimental models in mice. The study revealed that withaferin A (20/30 mg/kg body weight, intraperitoneal) produced significant analgesic and antipyretic effects at both doses in comparison to the standard drug [34].

Anti-arthritic activity

Rasool et al. showed the diminution in paw oedema and activity of lysosomal enzyme after the oral intake of *W. somnifera* root powder. The result showed that *W. somnifera* inhibits the release of lysosomal enzymes by stabilizing the lysosomes, which may be achieved through the fusion of lysosomal membranes with plasma membranes, preventing the release of lysosomal enzymes [44]. An experiment conducted by Giri found that the ethanolic WSREt and hydrocortisone (used as a standard compound) effectively decrease redness and swelling in both injected and non-injected paws [45]. Gupta et al. demonstrated that arthritic rats that have been given *W. somnifera* root powder demonstrate a significant reduction in their radiological score, illustrating the drug's ability to control joint inflammation and bone erosion. The study suggested that the inhibition of tumour necrosis factor can potentially prevent radiological progression and, thereby, prevent disability [46]. In another study by Khan et al., *W. somnifera* root powder was shown to exhibit anti-arthritic properties and inhibit inflammation in rats, promoting its use as a new treatment for arthritis [47].

Table 1 Diverse pharmacological activity of the *Withania somnifera* and its mechanism of action along with potential constituent

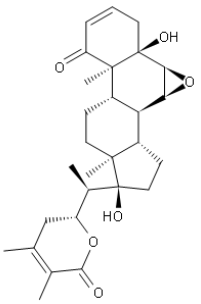
No.	Pharmacological activity	Mechanism of action	Potential constituent	References
1	Neuroprotective	Withanone attenuates the accumulation of intracellular Ca^{2+}, generation of reactive oxygen species, loss of mitochondrial membrane potential, crashing of Bax/Bcl-2 ratio, the release of cytochrome c, and increased caspase expression.	Withanone 	[23, 24]
		Withanone antagonizes muscarinic receptors, withanone attenuates scopolamine-mediated alterations of both brain-derived neurotrophic factor and glial fibrillary acidic protein expressions by alteration of cAMP-response element-binding protein signaling, normalizing the activity of Microtubule-associated protein 2, neurofilament subunit and growth-associated protein 43.	Withanone	[25]

Table 1 Diverse pharmacological activity of the *Withania somnifera* and its mechanism of action along with potential constituent (*Continued*)

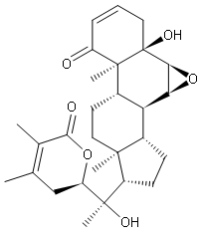
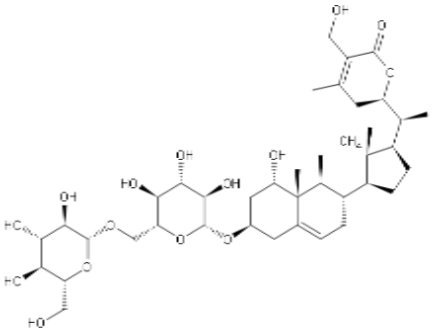
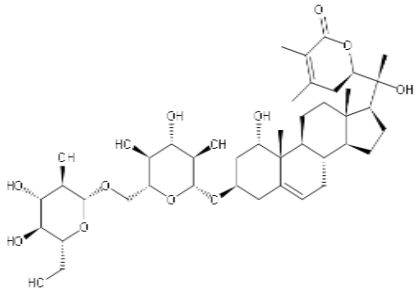
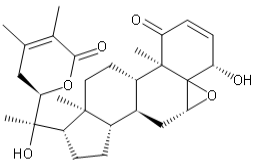
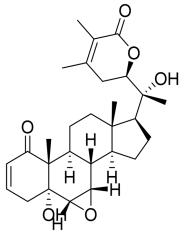
No.	Pharmacological activity	Mechanism of action	Potential constituent	References
		Withanolide A attenuates lipid peroxide formation.	Withanolide A 	[26]
	Parkinson's disease	Withanolide IV produces neurite regeneration, inhibition of acetylcholinesterase activity, and prevents amyloid fibril formation.	Withanolide IV 	[27]
	Alzheimer's disease and epilepsy	Withanoside VI modulation of GABAergic system.	Withanoside VI 	[27]
2	Anti-stress	Withanolide stimulate CD4+ and CD8+ T-cell surface markers resulting in increased secretion of Th1 cytokines i.e., interferon-γ and interleukin-2 which is the central regulator of immune response.	Withanolide 	[28]
		Glycowithanolide prevents the immobilization stress-induced increase in the dopamine receptor population in the corpus striatum as indicated by [3H]-spiroperidol ligand binding scavenger and inhibits lipid peroxidation in the central nervous system.	Glycowithanoide 	[29]

Table 1 Diverse pharmacological activity of the *Withania somnifera* and its mechanism of action along with potential constituent (*Continued*)

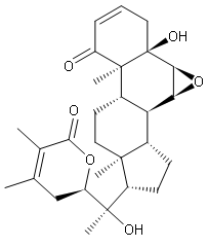
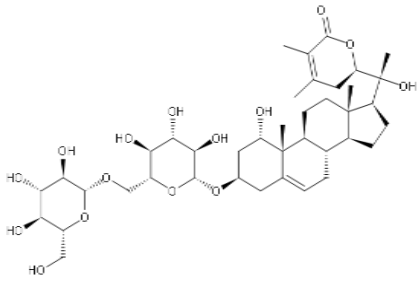
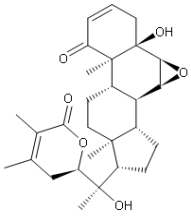
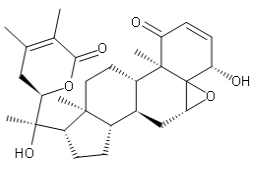
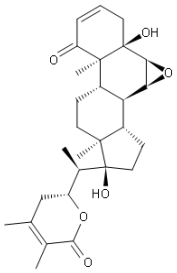
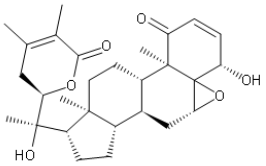
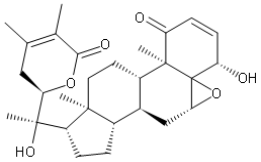
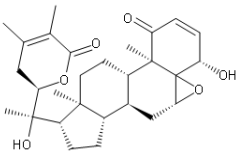
No.	Pharmacological activity	Mechanism of action	Potential constituent	References
3	Anti-dementia	Withanolide A and Withanolide VI increased the expression of phosphorylated neurofilament subunit (axon marker), microtubule-associated protein 2 (dendrite marker), and synaptophysin (dendrite marker). It facilitates the reconstruction of presynaptic and postsynaptic regions in neurons.	Withanolide A  Withanolide VI 	[30]
4	Cerebral ischemia reperfusion	Withanolide A have an inhibitory effect towards matrix metalloproteinases-2 and increases endogenous glutathione levels.	Withanolide A 	[31]
5	Anxiety chronic foot shock stress	Glycowithanolides reduce the activity of natural antioxidant enzyme superoxide dismutase with the increase in catalase and glutathione peroxides activities resulting in decreased production of hydrogen peroxide and hydroxyl ions, finally leading to decreased lipoperoxidation.	Glycowithanolides	[32]
6	Huntington's disease	Withaferin A has direct free radical scavenging activity, thereby decreasing lipid peroxidation and nitrite levels and sparing the antioxidant defense enzymes.	Withaferin A	[33]
7	Anti-diabetic	Withanolide decreases the elevated level of lipid peroxidation products and elevates cellular defense mechanisms such as superoxide dismutase and catalase.	Withanolide 	[29]

Table 1 Diverse pharmacological activity of the *Withania somnifera* and its mechanism of action along with potential constituent (*Continued*)

No.	Pharmacological activity	Mechanism of action	Potential constituent	References
8	N-methyl-D-aspartate-induced excitotoxicity in neuron-like cells	Withanone decreases the elevated level of lipid peroxidation products in reserpine-treated animals and elevates cellular defense mechanisms such as superoxide dismutase and catalase.	Withanone 	[34]
9	Antiproliferative, antimetastatic, antiangiogenic, anti-invasive	Withanolides suppress nuclear factor-kappa B and nuclear factor kappa B regulated gene products.	Withanolide 	[23]
10	Analgesic and antipyretic	Withaferin A blocks the release of the endogenous mediators of pain i.e. the prostaglandins. Withaferin A has an inhibitory action on the cyclooxygenase pathway.	Withaferin A	[34]
11	Ulcerogenic test	Withaferin A decreases the number of gastric lesions.	Withaferin A	[34]
12	Antipyretic test	Withaferin A has an inhibitory action on the cyclooxygenase pathway which is actually involved in the synthesis of prostaglandin biosynthesis.	Withaferin A	[34]
13	Antibacterial	Withanolide produce macrophage activation along with antibacterial property.	Withanolide 	[35]
14	Immunomodulator	Withanolide inhibit T-cell proliferation.	Withanolide 	[36]
15	Pancreatic cell cancer	Withaferin A deceases Hsp90 client proteins Akt, Cdk4, and glucocorticoid receptor.	Withaferin A	[37]
16	Memory enhancer	Sominone produces De-glycosylation by intestinal bacteria in this neuronal regeneration. Induce axonal regeneration and synaptic reconstruction in the brain and enhance object location memory.	Sominone	[23]

Anti-diabetic activity

Gorelick et al. studied the anti-diabetic properties of WSREt and *W. somnifera* leaf extract (WSLEt). The author concluded that withanolides and withaferin A, in particular, have anti-diabetic activity as observed in cellular models [48]. In another study by Anwer et al., aqueous WSREt reduced levels of blood glucose, improved hyperinsulinemic condition, and promoted glucose tolerance in non-insulin-dependent diabetes mellitus rats [49]. These results highlighted the fact that *W. somnifera* can improve insulin sensitivity. Thus, the insulin sensitivity index and homeostasis model assessment of insulin resistance levels were determined to check insulin sensitivity. The results obtained showed that the insulin sensitivity index was significantly improved upon *W. somnifera* treatment in non-insulin-dependent diabetes mellitus rats, and a rise in homeostasis model assessment of insulin resistance was prevented [50].

Furthermore, the existence of phenolic compounds, flavonoids, and antioxidant properties in WSREt and WSLEt has been demonstrated [10]. This study was carried out by Udayakumar et al., which indicates evidence of recovery from diabetic damage caused by alloxan-induced in rats. In another investigation by Udayakumar et al., the induction of WSREt, WSLEt, and glibenclamide in diabetic rats reestablished the alterations of the stated parameters to their usual levels after eight weeks of treatment, showing hypolipidemic and hypoglycemic effects in the rats with WSREt and WSLEt [51]. Verma et al. demonstrated a steady decline in blood glucose, comparable to that of an oral hypoglycemic drug. Results showed a significant increase in urine sodium, volume, serum triglycerides, cholesterol, low-density lipoprotein, and very low-density lipoprotein. This observation implies that the root of *W. somnifera* contains a possible metabolite, which can attack diabetes and induce diuresis without adverse effects [52].

Anti-cancer activity

Leyon et al. found that withanolide decreased biochemical levels significantly, demonstrating its ability to inhibit metastasis [53]. The authors also found that metastasis inhibition varied dramatically, depending on the modality of administration. Oza et al. first reported the medicinal significance of L-asparaginase in *W. somnifera* [54]. The investigations suggest the efficiency of L-asparaginase against leukemia cells, with a 24-hour lethal dose of 50% of 1.45 ± 0.05 IU. Using the assays, L-asparaginase from *W. somnifera* was found to exhibit a strong dose-response relationship. Szic et al. performed a pathway-derived transcriptase analysis in epithelial-like MCF-7 and triple-negative mesenchymal MDA-MB-231 breast cancer cells, which were exposed to different concentrations of withanolide A that could be estimated systemically in vivo settings [55]. A concentration- and time-dependent reduction in cell viability was observed in cultured breast cancer cells treated with *W. somnifera*. Wadhwa et al. conducted a study in vitro and in vivo on the anticancer potential of *W. somnifera* leaf water extract [56]. Nuclear magnetic resonance analysis and bioactivity-based size fractionation were carried out to recognize the active anti-cancer metabolite(s). Biochemical assays were used to assess the mechanism of the anticancer activity of the extract and its purified components. Yu et al. investigated the efficacy and mechanism of Hsp90 inhibition of withaferin A, a steroidal lactone occurring in *W. somnifera*, in pancreatic cancer in vitro and in vivo [37].

W. somnifera leaf water extract has been reported to be cytotoxic against cancerous cells and cause suppression of tumors in vivo [58]. Muralikrishnan et al. highlighted the reduced activities of key enzymes in the tricarboxylic acid cycle, such as succinate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase, in colon-cancer-induced animal models and reported that *W. somnifera* root powder normalized these enzyme concentrations in azoxymethane-stimulated experimental mice. The results from the study suggested that *W. somnifera* is a promising agent for treating colon cancer with chemotherapy [59].

Ichikawa et al. identified three components of withanolides that inhibited cell growth in human tumor cells: withaferin A, viscosalactone B, and physagulin D [60]. In all four cancer cell lines tested, these withanolides exhibited a similar pattern of antiproliferative activity. Using withaferin A as an anti-metastatic agent, Thaiparambi et al. demonstrated that the anti-metastatic activity of withaferin A is linked to vimentin and vimentin ser56 phosphorylation [61]. Padmavathi et al. examined the effects of dietary administration of Withania root on hepatic phase I and phase II and antioxidant enzymes, as well as the reduction of the carcinogen-induced face and stomach tumors in Swiss albino mice. The investigations concluded that the roots of *W. somnifera* inhibit phase I enzymes, while phase II and antioxidant enzymes are activated [62]. According to the body weight gain profile of mice, Withania does not appear to have any toxic effects. The findings suggest that *W. somnifera* root produces chemo-prophylactic effects against stomach and skin cancer development, which may provide insight into the synthesis of antitumor agents [63]. A study by Yu et al. highlighted the anti-proliferative effects of withaferin A against pancreatic cancer cell lines MiaPaCa2, BxPc3 and Panc-1 (with half-maximal inhibitory concentrations of 1.24, 2.93, and 2.78 mM, respectively). withaferin A was demonstrated to inhibit Hsp90 chaperone activity and bind to Hsp90 via a mechanism independent of adenosine triphosphate, causing protein degradation and inducing anticancer activity in vivo [64].

Prakash et al. conducted chemo-preventive studies of hydro-alcoholic-based WSREt in Swiss albino mice. In mice treated with the extract, liver biochemical factors showed decreased glutathione and glutathione-S-transferases, lipid peroxidation, and catalase, as compared with mice injected with 20-methylcholanthrene. The chemo-preventive activity of *W. somnifera* extract may be due to detoxifying and antioxidant properties [65].

Immunostimulant activity

Agarwal et al. conducted a study involving Coded Ashwagandha extracts, labeled WST and WS2 (provided by BIO-VED Pharmaceuticals Pvt, Pune, Maharadhtra, India). WS2 (a compound made by using carboxymethyl cellulose) was found to decrease ovalbumin-induced paw swelling [66]. *W. somnifera* preparations exhibit immunomodulatory properties regarding hyper-reactivity mediated by immunoglobulin E and cells. Animals treated with WST showed an increase in platelet count and white blood cells. There was a significant increase in white blood cell and platelet counts in animals treated with WST and *W. somnifera* 2A during cyclophosphamide-induced myelosuppression [67]. Glycowithanolides appear to reduce stress-induced alterations by restoring superoxide dismutase activity and reversing the stress-induced inhibition of catalase and glutathione peroxidase. As a result of these effects, lipid peroxidation decreases, resulting in a reduction in the negative effects of chronic stress [32].

Cyclophosphamide-induced immunosuppression was countered by WS2, producing an increase in hemolytic antibodies and hemagglutinating antibodies against sheep red blood cells [68]. The root powder of *W. somnifera* contains immunomodulator compounds, which specifically affect inflammation. It may have an immunosuppressive effect, owing to the steroidal lactones, withanovides, and flavonoids it contains. Further investigations into the underlying mechanisms are in progress with the active principle isolated from the *W. somnifera* root powder [36]. Verma et al. assessed the hematological parameters of tumor-induced mice using the methanolic extract of the plant, showing an increase in red blood cell count but an opposite decrease in white blood cell count, as compared with the control mice. Through Leishman staining, these data suggest a differential leucocyte count. The active metabolites isolated and characterized from a number of plants used in traditional medicine have either stimulated or inhibited immune responses [69]. The clinical value of the immunomodulatory potential of *W. somnifera* has been well recognized. Zahran et al. illustrated that *W. somnifera* root powder produced immunity 5% more effectively during the challenge

period, as well as afterward. *W. somnifera* root powder may have immunotherapeutic and protective functions in Nile tilapia against *Aeromonas hydrophila* infection, potentially helping to control important fish bacterial infections [70].

Anti-bacterial activity

Arora et al. estimated the synergistic antibacterial activity of *W. somnifera* by agar plate disc-diffusion assay against *Escherichia coli* and *Salmonella typhimurium* [71]. In another antibacterial study from Owais et al., both the alcoholic and aqueous extracts of the plant were found to be highly antibacterial, as demonstrated by in vitro diffusion experiments on agar wells. A variety of solvents were used to sub-fractionate the methanolic extract, and the butanolic fraction was found to be the most active against *Salmonella typhimurium* and other bacteria [71]. The antioxidant and antibacterial activity of aqueous WSREt against methicillin-resistant *Staphylococcus aureus* was also investigated. Mehrotra et al. found that aqueous WSREt had strong antibacterial activity against methicillin-resistant *Staphylococcus aureus* in an in vitro agar well diffusion assay [72]. The results of a study of Guinea pigs performed by El-Boshy et al. showed that the intake of the plant extract resulted in the improvement of certain biochemical and hematological parameters in the animals. It could be stated that *W. somnifera* extract possesses antibacterial activities corresponding to its immunological results [73]. In another study, the antibacterial activity of Ashwagandha (root and leaves) of both aqueous and alcoholic extracts of the plant against pathogenic bacteria was examined. The oral administration of the aqueous extracts was found to efficiently eliminate salmonella infection in Balb/c mice, as evidenced by a higher survival rate and lower bacterial load in numerous important organs of the treated mice [35].

Anti-inflammatory activity

Inflammatory diseases are still considered one of the largest threats to the modern world. Recently, various studies have demonstrated that *W. somnifera* holds promising anti-inflammatory properties [74]. A study performed by Giri found that *W. somnifera* can effectively decrease inflammation after only six hours of treatment. Sivamani et al. conducted a study on carrageenan-induced rat paw oedema, a well-accepted and popular model for evaluating anti-inflammatory activity. In this model, the early phase is related to the production of histamine, leukotrienes, platelet-activating factors, and possibly cyclooxygenase products. The anti-inflammatory activity of the thin layer chromatography-separated portion of the supernatant of *W. somnifera* might be due to the presence of rich phenolic acids and flavonoids [75]. In vitro data on withanolides demonstrate inhibitory effects on the cyclooxygenase enzyme and confirm the scientific support for the use of *W. somnifera* leaf in the treatment of inflammation, according to Ichikawa et al. This study represents the first time that this group of chemicals has been shown to suppress cyclooxygenase-2 enzyme activity [76].

Gupta et al. found that the oral administration of *W. somnifera* root powder effectively suppresses the symptoms of collagen-induced arthritis in rats. The sciatic functional index for the functional recovery of motor activity in arthritic rats treated with *W. somnifera* was also used to confirm the protective effects of *W. somnifera* [77]. The observations conducted in this study on human peripheral blood mononuclear cells demonstrated that *W. somnifera* is an inhibitor of T-cell proliferation and could be effective as an immunosuppressive or anti-inflammatory medication with special relevance to arthritis, according to Rasool et al. [78].

Heyninck et al. demonstrated that *W. somnifera* directly inhibits IkappaB kinase catalytic activity. The study states that withanolide A exerts its anti-inflammatory effects by targeting the critical Cys179 residue in the catalytic region of IkappaB kinase-b. It can create a hydrogen bond to stabilize its interaction with Cys179, when docked to an IkappaB kinase-b homology structure model [79]. The anti-inflammatory efficacy of *W. somnifera* was also investigated by Chandra et al., which found that *W. somnifera* has a significant anti-inflammatory impact against protein denaturation in vitro. Furthermore, Singh et al. found that the ethanol extract of *W. somnifera* inhibited the formation of pro-inflammatory chemicals in vitro. This effect is mediated in part by the component withanolide, which inhibits transcription factors nuclear factor-κB and activator protein [80].

Clinical study

The clinical study of herbal medicine provides scientific evidence of the safety and efficacy found in that herbal drug. The data could be used to assess quality, purity, and accuracy. Several clinical studies have proven the safety and efficacy of *W. somnifera*. Published studies suggest that *W. somnifera* has significant effectiveness and may cure various ailments. An overview of the clinical studies is summarized below (Table 2).

Adaptogenic activity

Plants that are adaptogens are non-toxic by nature and help to combat stress conditions of all types, including those that are chemical, physical, and biological. Traditional medicines have employed roots and herbs for centuries to treat various ailments. *Withania* has also been reported as having an adaptogenic effect [88]. A double-blind clinical trial was performed by Joshi et al. to determine the adaptogenic effect of root powder. It was found that daily consumption of *W. somnifera* (in 100 mL milk) did not result in toxic effects, and there was an improvement in hemoglobin and red blood cell, as well as an improvement in hair melanin levels [81, 82].

Another clinical study carried out by Bone et al. with healthy males aged 50–59 showed significant improvement in red blood cell count, hair melanin, hemoglobin, seated statues, serum cholesterol depletion, and nail calcium preserved with root powder [83, 84]. Sexual performance improved significantly when the erythrocyte sedimentation rate was reduced by 71.4 [89].

Analgesic and anti-arthritic activity

Analgesic and anti-arthritic activities of *W. somnifera* were studied in a randomized double-blind test [90]. In the study, a hot air pain model and a double-blind, placebo-controlled cross-over study model were used, employing a sole oral dose of 1,000 mg of an ethanolic extract to 39 subjects, out of which 20 obtained the formulation and 19 were given a placebo for 6 weeks. In addition, 42 patients suffering from osteoarthritis were randomized to receive a formula enriched with *W. somnifera* (Ashwagandha, Boswellia, turmeric, and zinc complex) or a placebo for three consecutive months. A significant reduction in pain severity ($P < 0.001$) and disability ($P < 0.05$) scores were observed after three hours of drug consumption, although no noteworthy modifications in radiological appearance or erythrocyte sedimentation rate were observed [91].

Anti-stress activity

The antistress effect was examined in two studies using 300 mg extract and root and leaf *W. somnifera* (130 patients) or placebo groups (130 patients) in a double-blind, randomized, placebo-controlled design. Stress levels were reviewed at 0, 30, and 60 days, using a modified Hamilton anxiety scale [92]. Clinical and biochemical parameters were deliberately calculated at 0 and 60 days, and stress levels were evaluated at 0, 30, and 60 days using a modified Hamilton anxiety scale. A noteworthy reduction ($P < 0.0001$) was found in the scores of all the stress-assessment scales, as compared with the placebo group. The levels of serum cortisol were substantially smaller in the *W. somnifera* group than that in the placebo group. A notably large number of patients met a priori response criteria in the drug group, as compared with the placebo group, in patients with the international classification of diseases-10 anxiety disorders [93]. Another clinical investigation looked at the safety and efficacy of a high-concentration full-spectrum extract of Ashwagandha roots to reduce stress and anxiety in 64 people over 60 days. The results showed that the treatment group exhibited a substantial drop ($P = 0.0001$) in scores on all stress scales, as compared with the placebo group [85]. Langade

Table 2 Reported clinical trials of *Withania somnifera*

No.	Name of the activity	Dose and methods	Participants	Duration	Therapeutic effects	References
1	Adaptogenic	1) Root powder (3g/day) (in 100 mL milk).	60 healthy children (8–12 years age)	2 months	Increases body weight, total protein and mean corpuscular hemoglobin.	[81, 82]
		2) Root powder (3 g /day).	50 healthy male volunteers (age 50–59 years)	1 year	Increases in hemoglobin, red blood cells, improvement in hair melanin, and seated stature in the treated group.	[82]
		3) Root powder in a group at a dosage of 3 grams daily.	101 healthy males, 50–59 years old	1 year	Significant improvement in hemoglobin, red blood cell count, hair melanin, and seated stature.	[83]
		4) Dried roots of *W. somnifer* were powdered and made as tablets of 0.5 g each and administered in the dose of 2 tabs 3 times a day with milk.	60 healthy volunteers	1 year	Serum cholesterol decreased in the treated group.	[84]
2	Anti-stress	Root and leaf extract (300 mg).	64 subjects	60 days	Reduces levels of serum cortisol and decrease adrenocorticotropic hormone secretion.	[85]
3	Hypothyroidism	Root extract 600 mg daily.	50 subjects	8 weeks	Lowers serum cortisol levels by down-regulation of the hypothalamic-pituitary-adrenal axis, which in turn up-regulates the hypothalamic-pituitary-thyroid axis to normalize the levels of the thyroid indices.	[86]
4	Transient insomnia	Root extract 300 mg.	60 subjects	10 weeks	Significant improve sleep onset latency, Pittsburgh sleep quality index, Hamilton anxiety rating scale, etc.	[87]

et al. conducted a randomized, double-blind, placebo-controlled study to determine the efficacy and safety of Ashwagandha root extract in patients with insomnia and anxiety. The results revealed a significant improvement in sleep efficiency ratings with Ashwagandha, with an initial value of 75.63 (2.70) and an increased value of 83.48 (2.83) after 10 weeks; meanwhile, the sleep efficiency scores for placebo changed from 75.14 (3.73) at the start to 79.68 (3.59) after 10 weeks [87].

Cardiovascular protections

Sandhu et al. conducted a double-blind placebo-controlled study of *W. somnifera* with various parameters, such as increased power, velocity, VO_2 max, and augmented VO_2 max, and observed a significant reduction in the resting systolic blood pressure [94] In this study, normal healthy subjects received standardized WSREt in the form of capsules with a proper dose for a particular period and were evaluated for cardiovascular protection and cardiorespiratory endurance. Results confirmed that *W. somnifera* showed a prominent effect as a cardioprotective [95].

Improving sexual function in women

In Ayurveda, *W. somnifera* is described as an aphrodisiac and is reported to treat male sexual dysfunction and infertility. Swasti et al. demonstrated the spermatogenic activity of WSREt (675 mg/d in three doses for 90 days, randomized) in oligospermic patients (n = 46) [96]. The results also show the enhancement of serum hormone levels with *W. somnifera* treatment (n = 21), as compared with the placebo (n = 25). Another study performed by Dongre Swasti suggests that high-concentration full-spectrum WSREt could effectively ameliorate the underlying pathologies and improve the variables of female sexual dysfunction [97]. Hence, this full-spectrum WSREt can be used safely as an adaptogen in women to improve sexual function [98].

Bipolar disorder

W. somnifera offers affordability, accessibility, and fewer side effects. In 2013, Roy performed a double-blind, placebo-controlled, randomized study with subjects having bipolar disorder [99]. A standardized dose of *W. somnifera* extract was given and was found to be beneficial to the health of the subject (n = 24), as compared with the placebo (n = 29). Mood and anxiety scale scores remained stable, and adverse events were minor. *W. sominfera* extract appears to improve auditory-verbal working memory, response time, and social cognition in bipolar disorder, at least at the preclinical level [100].

Hypothyroidism

A detailed analysis by Sharma et al. revealed that daily treatment with Ashwagandha for a period of eight weeks produced a significant decrease in serum thyroid-stimulating hormone levels ($P < 0.01$) and an increase in serum triiodothyronine ($P < 0.01$) and thyroxine ($P < 0.01$) levels, as compared with placebo. Ashwagandha treatment effectively normalized the thyroid indices during the eight-week treatment period in a significant manner (time effects: thyroid-stimulating hormone ($P < 0.001$), triiodothyronine ($P < 0.001$), thyroxine ($P < 0.001$)). Treatment with Ashwagandha was found to be safe and tolerable, with few mild and temporary adverse events [86].

Overview of toxicology

Since ancient times, several portions of *W. somnifera* have been utilized in the Ayurvedic system of medicine and have been found to be safe. There are several toxic studies that support the safety and efficacy of *W. somnifera*. The findings from acute and sub-acute toxicity studies (Figure 4) suggest that *W. somnifera* is safe and effective [101].

Acute toxicity study

Sharada et al. studied alcoholic WSREt for its acute (24 h) toxicity in conventional Swiss albino mice and sub-acute toxicity (30 days) in Wistar rats. In this study, mice demonstrated a relatively high tolerance for *W. somnifera*. No acute mortality was observed at 1,100 mg/kg; however, with a subsequent increase in the dose, there was a quick rise in the mortality rate. No animal survived after an injection of 1,500 mg/kg. Thus, the lethal dose of 50% of the extract was found to be 1,260 mg/kg [102].

"Acute Oral Toxicity Study" was conducted according to Organisation for Economic Co-operation and Development guidelines for the assessment of chemicals. The dose of 2,000 mg/kg did not cause any clinical signs of toxicity in any of the treated rats. At the end of the study, all animals were sacrificed, and no abnormalities were detected. In addition, an external examination of rats sacrificed at the end did not reveal any abnormality with pathological implications. No animal died in the study, either in the treated or the control groups. When all animals (treated and control groups) had surpassed the 90-day dosing period (120-day dosing period for recovery groups), they were sacrificed and inspected to determine the overall pathological results [103].

Sub-chronic toxicity study

A sub-chronic toxicity study was performed as per Organisation for Economic Co-operation and Development guidelines for the testing of chemicals (No. 408). In this study, three groups of 20 rats each (10 males and 10 females) were given purified ashwagandha extracts orally at the dosage levels of 100 mg/kg body weight (low dose), 500 mg/kg body weight (medium dose), and 1,000 mg/kg body weight (high dose), respectively, seven days a week for 90 days with the assistance of cannula joined with a syringe [104]. Furthermore, the fourth group of 20 rats (10 males and 10 females) were fed distilled water exclusively (vehicle) for 90 days and were designated as a "control group". Low dose group, medium dose group, and high dose group animals did not exhibit any treatment-related toxic signs or symptoms when compared with their respective control counterparts. There were no notable differences in body weight gain or feed consumption between the control and treated group animals [105].

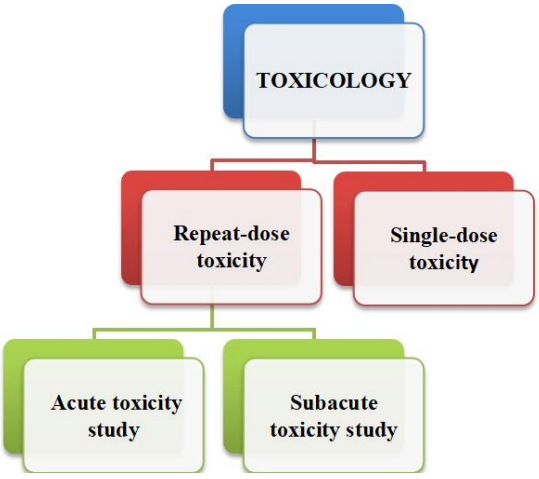


Figure 4 Overview of toxicology

Conclusion

W. somnifera is one of the most beneficial and fruitful medicinal plants used in Ayurveda, the ancient system of Indian medicine. *W. somnifera* is a Rasayana adaptogen as well as a nervine tonic. The plant has also been extensively researched for its numerous pharmacological attributes, including its antioxidant, anxiolytic, adaptogenic, memory-boosting, anti-parkinsonian, anti-inflammatory, and anti-tumor qualities. *W. somnifera* is chemically enriched with a variety of active compounds, including withanolides, sitoindosides, and other important alkaloids. These components have been shown to be beneficial in the treatment of a variety of ailments. Several publications on Ashwagandha have reported its effectiveness for a wide spectrum of diseases. The majority of published data is based on in vitro and in vivo investigations, and there are little clinical data to support its activity in multiple disorders. Although the effects of the herb are quite promising, *W. somnifera* is still a relatively new plant in modern research; as such, there have not yet been many advances in the study of its toxicology. In this review, we assessed all toxicological studies published to date and compiled the findings together for a composite understanding of the safety of *W. somnifera*. The findings presented above clearly show that the usage of and its ingredients have a reasonable and scientific basis. More robust scientific evidence regarding its pharmacological evaluation, including drug-like qualities, is required to promote Ashwagandha as a possible drug candidate for commercialized products. The key area that needs further exploration is the clinical trials of bioactive substances alone and in synergistic combinations. In addition, more studies and clinical examinations are required to discover the actual mechanisms involved and the optimal dosage range. This present work outlines the status of *W. somnifera* in terms of its safety and efficacy and endeavors to provide approaches to deal with safety issues that could offer scientific evidence and clinical assessment for the application of these drugs.

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