

REVIEW

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Ashwagandha (*Withania Somnifera*): a comprehensive narrative review of its role in enhancing physical performance and health

Hadi Nobari^{1,5*}, Nazila Parnian-Khajehdizaj², Kelly Johnson³ and Juan José Ramos-Álvarez⁴

Abstract

Ashwagandha (*Withania somnifera*), commonly known as Indian ginseng or winter cherry, is a prominent adaptogenic herb recognized for its profound impact on physical performance and health. As an adaptogen, Ashwagandha stands out for its unique ability to restore system functions to normal, enhance the body's resilience to stress, and elevate overall performance. This comprehensive narrative Review underscores the diverse health benefits of Ashwagandha, emphasizing its roles in immunomodulation, stress reduction, antioxidant effects, cardioprotection, anti-inflammatory, anti-tumor, and anti-cancer activities, neuroprotection, memory enhancement, cognitive function, physical performance, and sexual health improvement effects. With a rich phytochemical profile, including alkaloids and withanolides like Withaferin A, Ashwagandha showcases efficacy in mitigating stress-related markers, improving sleep quality, and regulating mitochondrial function. In addition to its anticancer potential against various types of cancer, Ashwagandha improves lipid profile and protects the heart from necrosis. Also, Ashwagandha has physical performance benefits, which indicate its potential to increase muscle strength, facilitate muscle regeneration, and improve cardiorespiratory endurance. This narrative review delves into the most recent findings and provides a comprehensive overview of the current understanding of Ashwagandha's potential uses and effects on physical performance and health.

Keywords Ashwagandha, *Withania somnifera*, Withaferin a, Anti-cancer activity, Anti-inflammatory activity, Physical performance, Anti-stress, Cognitive function

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Introduction

Ashwagandha, a well-regarded herb celebrated for its diverse health benefits, derives its name from the Sanskrit term “ashwa,” signifying equine characteristics. There exists a conjecture that the ingestion of ashwagandha root may engender attributes reminiscent of those observed in horses. The word “gandha,” which alludes to the distinctive scent of the plant’s fresh roots and is also known as Indian ginseng or winter cherry, has a second element that signifies “fragrance.” [1, 2]. The group of plants known as “adaptogens” includes ashwagandha. An herb with phytonutrients is referred to as an “adaptogen” if it helps the body adapt by (a) restoring system functions to normal, (b) building resistance to future stress, and (c) raising the body’s functioning to a higher level of performance [3]. Among the various members of the herb family known as adaptogens are ashwagandha, rhodiola, ginseng, schisandra, and maca [3].

As an adaptogen, ashwagandha can control metabolic functions, promote homeostasis, and restore the organism. Ashwagandha roots are known for their role in immunomodulation [4, 5] stress reduction [6], as an antioxidant, cardioprotective function [7] cardiorespiratory endurance [8], inflammation prevention, anti-tumor activity, neuroprotection, and anti-cancer activity [9] improves memory and cognition [10] augments stamina, strength, and muscle recovery [11] boosts sexual function in both men and women [12, 13] aids in body weight management [14] and acts as an antiaging agent [15] (Fig. 1). The roots increase defense against disease, slow down aging, revitalize the body in times of weakness, increase resistance to harmful environmental elements, and foster a sense of well-being [15–17].

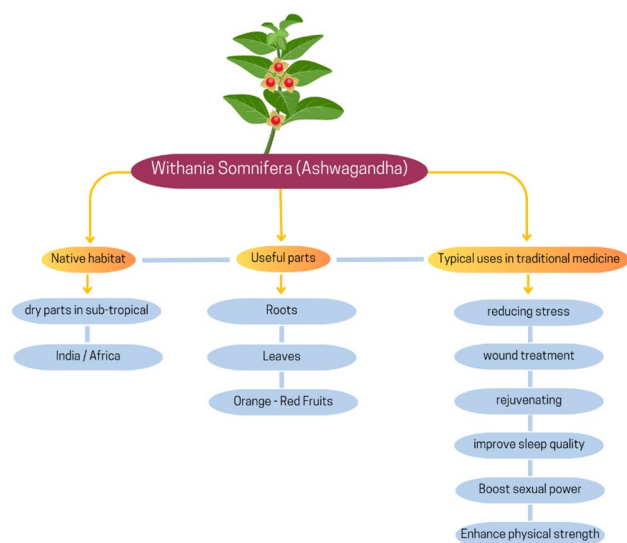


Fig. 1 *Withania somnifera*: native habitat, useful plant parts, traditional medicinal uses

Ashwagandha is distinguished by having a high phytochemical content. Depending on the location of the raw material, it exhibits a diverse composition of chemical compounds. Its active ingredients, witanolides and alkaloids, are essential to pharmacological action [18]. Alkaloids (isopelletierine, anferine), steroidal lactones (withanolides, withaferins), saponins with an extra acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitonidoside XI and X) are the biologically active chemical components. WS also contains a lot of iron [19]. The withanolides, the primary secondary metabolites in charge of this plant’s beneficial characteristics, are found within the steroid lactones. Withanolides, a class of steroidal lactones, are thought to be responsible for the pharmacological properties of WS’s roots [20]. The healing potential of *Withania somnifera* mainly arises from its wide range of biologically active compounds. Among these, withanolides a class of steroidal lactones such as withaferin A and withanone have been most thoroughly investigated for their anti-inflammatory, anticancer, and neuroprotective effects. Furthermore, sitoindosides a subclass of withanolide glycosides along with various alkaloids, play a crucial role in enhancing the plant’s adaptogenic and immunomodulatory properties [20]. Alkaloids and steroidal lactones are the primary components of ashwagandha, according to chemical analysis. Among the various alkaloids, withanine is the main constituent.

In the Ashwagandha extract, withaferin A dramatically reduces the expression of neuroinflammatory molecules linked to NF- κ B and amyloid formation [21]. according to studies, Ashwagandha is well tolerated in humans and linked to lower cortisol and testosterone levels [12]. Research suggests that ashwagandha may reduce increases in blood urea nitrogen, lactic acid, and corticosterone in response to stress [22] and, additionally, lessen the likelihood that dopamine receptors in the brain will activate during times of stress [23–25]. Ashwagandha contains several active components, which may account for the various mechanisms of action by which it exerts its effects (Fig. 2). These include saponins, alkaloids like isopelletierine and anaferine, steroidal lactones (withanolides, withaferins), and steroidal lactones [24, 26].

Numerous studies have revealed that patients who took ashwagandha extract for 8 + weeks at doses of 240–600 mg showed significantly decreased stress hormone levels. This included lower levels of Cortisol, the stress hormone that controls your body’s stress response [27–29].

Furthermore, empirical evidence has shown that the administration of 600 mg of Ashwagandha root on a daily basis over a duration of 12 weeks significantly enhances the quality of sleep, particularly exhibiting heightened efficacy in individuals suffering from insomnia [30].

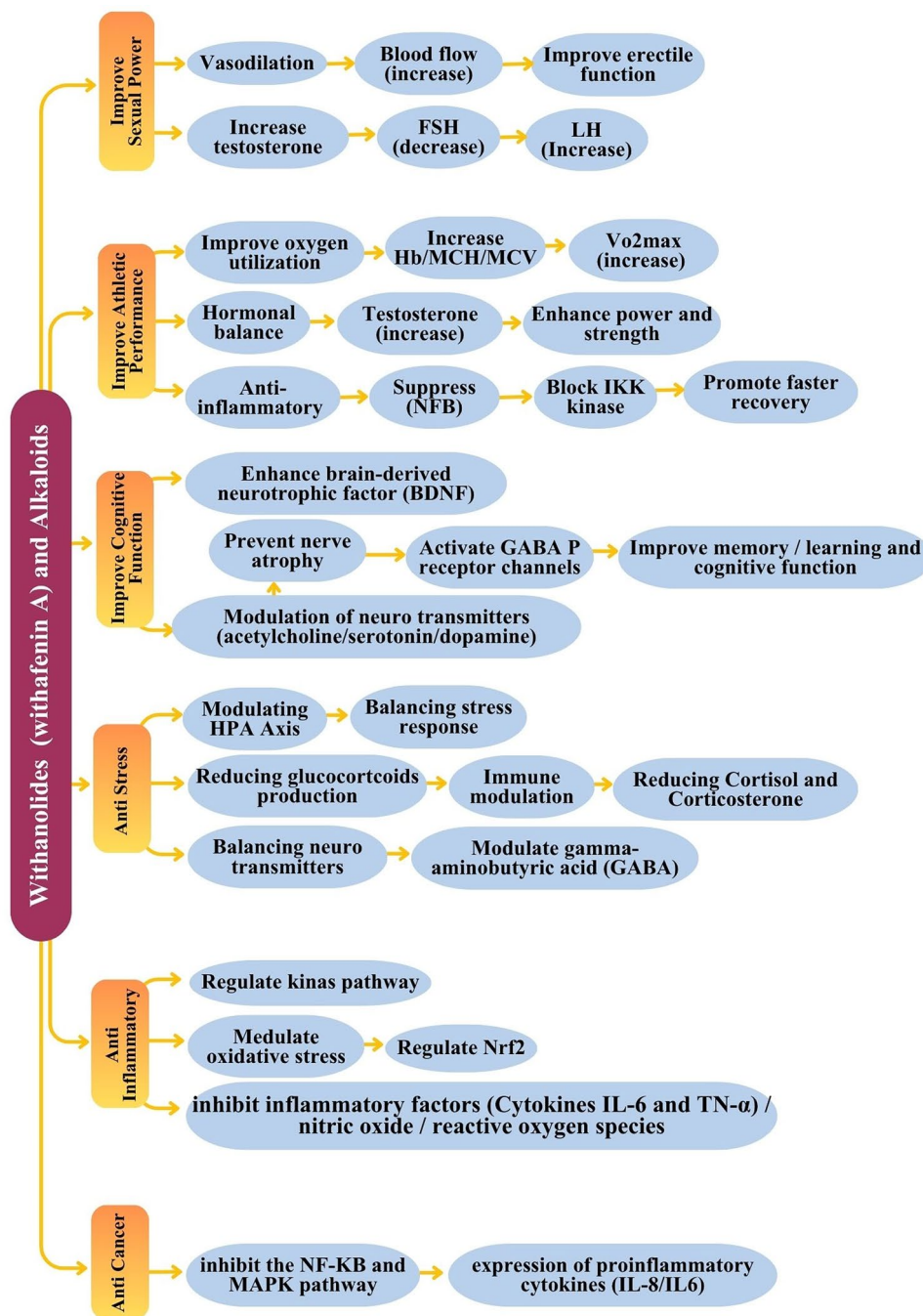


Fig. 2 Withanolides (withaferin A) pathways: sexual power, performance, cognitive function, anti-stress, anti-inflammatory, anti-cancer effects

Ashwagandha supplementation could potentially play a pivotal role in promoting overall health, as disruptions in sleep patterns or insufficient sleep have been correlated with detrimental effects on stress modulation and holistic well-being.

Preclinical investigations have showcased the plant's capacity to modulate mitochondrial function and apoptosis, as well as mitigate inflammation through the inhibition of inflammatory markers such as cytokines

(including IL-6 and TNF- α), nitric oxide, and reactive oxygen species. In a study using the HaCaT human keratinocyte cell line, an aqueous solution from Ashwagandha root was found to inhibit the NF- κ B and MAPK (mitogen-activated protein kinase) pathways by decreasing the expression of proinflammatory cytokines, including interleukin (IL)-8, IL-6, tumour necrosis factor (TNF- α), IL-1 β , and IL-12, and increasing the expression of anti-inflammatory cytokines. Based on these findings, it can

be said that Ashwagandha's anti-inflammatory properties may be used to avoid skin inflammation [31]. Additional findings suggest that the adaptogenic and anti-inflammatory effectiveness of *Withania somnifera* is strongly dependent on both the administered dose and the type of extract used. Alcohol-based extracts (methanolic and ethanolic), which contain higher concentrations of major withanolides such as withaferin A, generally demonstrate greater bioactivity than water-based extracts [32]. They exhibit a greater ability to inhibit the pro-inflammatory NF- κ B signaling pathway and to activate the antioxidant Nrf2 pathway more effectively. These effects are dose-dependent: low to moderate doses mainly enhance adaptive stress responses through mild Nrf2 activation, while higher doses of concentrated alcoholic extracts produce stronger pharmacological effects by directly suppressing NF- κ B and intensifying Nrf2 activation [33]. This dual mechanism provides a focused therapeutic approach for managing severe oxidative stress and inflammation.

A randomised, double-blind, placebo-controlled experiment with an open-label extension was carried out to determine the impact of Ashwagandha extract on the immune system of healthy individuals. The study's findings demonstrated that, compared to a placebo, Ashwagandha extract dramatically boosted natural killer cell activity and cytokine levels [34]. As a result, ashwagandha seems to be a useful supplement to pharmacotherapy in managing bacterial infections. Ashwagandha is a herb known for its safety, lacking toxicity and significant adverse effects. Research studies have demonstrated its efficacy in inhibiting the growth of methicillin-resistant *Staphylococcus aureus* and *Enterococcus* supplement [35]. Its features have been linked to its antimicrobial action, which has an immunomodulatory effect since it increases immunological reactivity (immunopotential), has cytotoxic effects, and silences genes [36]. Studies using animal models also demonstrate that *Withania somnifera* is a potent remedy for salmonellosis since it dramatically shortens the duration of the illness after contracting the pathogen [37].

Studies have revealed several substances extracted from Ashwagandha's root, stem, and leaves have anti-cancer potential. Consequently, they can treat cancer alone or with chemotherapy drugs [38]. Withanolides are alkaloids present in the plant that show great anti-cancer potential. They are also the most promising compounds showing this action, as they play a major role in the induction of apoptosis. Ashwagandha is effective against breast, colon, lung, prostate, and blood cancers [39]. It is a chemotherapeutic agent against numerous breast cancer subtypes, including ER/PR-positive and triple-negative breast cancer [40]. Research has shown that withaferin A from Ashwagandha is similarly successful in treating melanoma. This substance inhibits melanoma cell migration,

decreases cell proliferation, and promotes apoptosis [41]. Jawarneh et al. demonstrated that a combination of Ashwagandha extract and intermittent fasting has potential as an effective breast cancer treatment that may be used in conjunction with cisplatin [42]. By triggering apoptosis, the combination reduced the multiplication of cancer cells while lowering the liver and renal damage caused by cisplatin.

According to Tekula et al., Withaferin A has tremendous therapeutic potential because it can successfully manage generated type 1 diabetes in mice by modulating Nrf2/ NF- κ B signaling [43].

Multiple studies have indicated a beneficial influence on lipid profiles. In a study involving white albino rats exhibiting hypercholesterolemia, *Withania somnifera* demonstrated a reduction in cholesterol levels alongside antioxidant effects [44–46].

The broad therapeutic potential of Ashwagandha stems from its ability to simultaneously regulate multiple cellular signaling pathways, resulting in a synergistic interplay of biological effects. Its anti-inflammatory and immune-regulating functions are primarily achieved by downregulating the pro-inflammatory NF- κ B and MAPK pathways, which consequently decrease cytokine production [31, 47, 48]. Ashwagandha engages in a finely tuned regulatory balance by also stimulating the Nrf2 antioxidant pathway, which not only mitigates oxidative stress but also indirectly suppresses NF- κ B activity, thereby amplifying its anti-inflammatory potential. This coordinated modulation of NF- κ B/MAPK and Nrf2 pathways forms the mechanistic foundation for its therapeutic effects in a range of conditions, including cancer, metabolic dysfunctions, and cardiovascular protection. At the same time, the herb exhibits notable neuroactive properties through direct interaction with the GABAergic system, where it is thought to enhance GABA_A receptor signaling [49, 50]. This GABAergic mechanism forms the basis of Ashwagandha's anxiolytic and sedative effects, which function largely independently but synergistically support the overall physiological balance maintained through its modulation of inflammatory and antioxidant pathways. Consequently, the therapeutic efficacy of Ashwagandha arises from its intricate, multi-target regulation involving NF- κ B, MAPK, Nrf2, and GABAergic signaling networks. Despite the pharmacokinetic challenges posed by the low systemic bioavailability of key withanolides in human plasma, their substantial *in vivo* effectiveness seems to result from multiple interconnected mechanisms. These include preferential distribution and retention within specific tissues, the biological activity of secondary metabolites, and synergistic interactions across various pharmacological targets [51]. Through these mechanisms, even relatively low levels of active compounds can generate a fine-tuned

yet coordinated regulation of critical signaling pathways such as Nrf2, NF- κ B, and apoptosis ultimately producing a pronounced and clinically meaningful physiological effect [52].

Several studies have highlighted a beneficial influence on lipid profiles. In an investigation involving albino rats induced with cardiac necrosis via isoprenaline administration, the effects of Ashwagandha were examined. Among the rats administered *Withania Somnifera*, reductions were observed in glutathione levels, as well as in the activities of superoxide dismutase, catalase, creatinine phosphokinase, and lactate dehydrogenase. Additionally, notable decreases were recorded in lipid peroxidation levels. These findings suggest that *Withania somnifera* protects the heart in a rat model of isoprenaline-induced necrosis [53]. The cardioprotective effect of withaferin A, a component of Ashwagandha known for its anticancer properties, was also studied. In this study in rats, low doses of withaferin A were shown to have a cardioprotective effect by upregulating the mitochondrial anti-apoptotic pathway due to an increase in AMP-activated protein kinase (AMPK) phosphorylation and an increase in the Bcl-2/Bax ratio (AMPK) [54]. This enzyme works in some procedures that keep the body's overall and cellular energy balance in check.

ashwagandha promotes p38/MAPK and energy use in response to hormonal cues, controlling glucose, protein, and fat levels in the nervous system and peripheral tissues [55]. It is also known that AMPK is activated by calorie restriction and plays a role in several aging-related processes and disorders commonly affecting older persons. AMPK restores energy balance and is thought to improve quality and length of life [55, 56].

Supplementation with ashwagandha has been shown to notably enhance muscle strength and expedite muscle regeneration processes. In a controlled trial, young, healthy men were orally administered 300 mg of *Withania somnifera* root extract twice daily for a duration of eight weeks. Concurrently, these subjects engaged in a structured resistance training program adhering to guidelines outlined by the National Strength and Conditioning Association (NSCA). Following supplementation, significant increases in muscle mass within the arms and chest were observed among the treated individuals, along with notable improvements in muscle strength. Furthermore, stabilization of plasma creatine kinase levels indicated a reduced degree of exercise-induced muscle myocyte damage in the ashwagandha-supplemented cohort compared to the placebo group. Additionally, the treatment group showed a significant rise in testosterone levels and a considerable drop in body fat [11]. Shenoy et al.'s study corroborated that individuals supplemented with Ashwagandha exhibited significant enhancements in multiple indices of cardiorespiratory endurance compared

to those in the placebo cohort [57]. Significantly, the group administered Ashwagandha demonstrated notable improvements in ventilatory threshold, duration to exhaustion, and maximal aerobic capacity. Furthermore, serum cortisol levels, a biomarker associated with stress, were lower within the Ashwagandha-supplemented cohort. In another investigation involving adult athletes, individuals were provided with a specific dosage of Ashwagandha, while the control group received a placebo. Upon completion of the study, a considerable elevation in VO₂ max (maximum aerobic capacity) was discerned among the athletes receiving Ashwagandha compared to those in the placebo group [8].

The components in WS, mainly withanolide glycosides exert their immunomodulatory action by mobilizing and activating macrophages and inducing proliferation in murine splenocytes [58]. The involvement of immunoregulatory cells induced by WS extracts might have several functions, such as regulating antigen presentation and control of immunosuppressive microenvironment along with a physiological cytokine milieu for an effector T cell function [59].

Although extensive research has focused on Ashwagandha root, the plant's leaves are also abundant in withanolides, notably withaferin A, a compound recognized for its robust immunomodulatory and anti-inflammatory properties. Comprehensive extracts of *Withania somnifera* (WS), encompassing both roots and leaves, have demonstrated enhanced activity of immune cells, particularly macrophages, including heightened lysosomal enzyme activity responsible for detoxification and removal of waste products from immune cells. Alcoholic extracts derived from the entire WS plant have further been found to augment macrophage phagocytic activity, mitigate immunological hypersensitivity reactions, and stimulate the production of T lymphocyte immune cells [60].

In a 2020 review of five studies, it was found that the supplementation of higher doses (330-1000 mg) of Ashwagandha over a period of 24 weeks led to a significant increase in VO₂max in both trained and untrained individuals. A heightened VO₂max indicates improved oxygen utilization during physical exertion, thereby potentially reducing the risk of cardiovascular diseases [61]. It has also been demonstrated that daily doses of Ashwagandha between 750 mg and 1250 mg have favorable impacts on testosterone levels, muscular size, strength, and absolute power output [62].

Across various studies, Ashwagandha has consistently demonstrated enhancements across all cognitive domains, with particular emphasis on attention, concentration, and memory. In a double-blind, placebo-controlled clinical trial involving 30 healthy volunteers, the effects of *Withania somnifera*, Panax ginseng, and

placebo on psychomotor performance were examined. Comparing the group administered *Withania somnifera* to those receiving Panax ginseng and placebo, improvements were noted in sensory-motor function, auditory reaction speed, and mental arithmetic ability [12, 22]. The glycowithanolides mimic the body's stress-reducing relaxation hormones, which reduce cortisol. By improving mitochondrial function, overall energy levels can be increased.

Methodology

Approach to literature review

This narrative review employs a qualitative and exploratory methodology, amalgamating diverse literature sources to offer a comprehensive elucidation of Ashwagandha's efficacy in augmenting physical performance and overall health.

Literature search strategy

Sources of information: The primary sources for this review include academic databases such as PubMed, ScienceDirect, and Google Scholar. Additionally, reference lists of relevant studies were manually searched to identify further pertinent literature.

Search terms: Key phrases used in the search encompassed "Ashwagandha", "*Withania somnifera*", "health benefits", "physical performance", "adaptogenic properties", and "phytochemical composition".

Time frame and selection criteria: The focus was primarily on articles published in the last two decades, with particular attention to comprehensive reviews, landmark studies, and significant clinical trials. The selection was not limited strictly by study design or methodology to encompass a broad spectrum of research.

Data synthesis and analysis

Thematic organization: The collected literature was organized into themes based on the various health benefits and properties of Ashwagandha, such as stress reduction, anti-inflammatory effects, cognitive enhancement, and physical performance benefits.

Interpretative analysis: Instead of statistical analysis, this review emphasizes an interpretative approach, discussing and synthesizing findings from various studies to provide a holistic understanding of the subject.

Quality and relevance assessment

Study relevance: Each source was evaluated for its relevance to the key themes of the review. Priority was given to studies providing comprehensive and clear findings.

Diversity of sources: A variety of sources were included to present a well-rounded view, such as clinical trials, laboratory studies, and expert opinions.

Ethical and bias considerations

Ethical standards in sourced studies: The ethical standards of the studies referenced were considered, particularly for human and animal trials.

Acknowledgment of potential biases: The narrative nature of this review inherently includes interpretative elements. We acknowledge this potential bias and strive for a balanced and objective overview.

Limitations

The narrative nature of this review implies certain limitations, such as the potential for selection bias and the lack of quantitative analysis commonly found in systematic reviews. The review aims to provide an insightful, though not exhaustive, overview of the subject matter.

Anti-stress and adaptogenic effects of Ashwagandha

An external physical or mental strain can lead to a condition known as stress. It may cause someone to feel threatened, nervous, uncomfortable, or otherwise less able to respond fully and normally to environmental demands. Long-term stress exposure can throw a person's mental and physical state out of balance, which can lead to various diseases like depression, hypertension, heart conditions, and metabolic problems. Adaptogens are herbs that increase a person's capacity to handle stress [63]. An adaptogen should ideally: (a) lessen the harm caused by stress; (b) be safe and have a positive effect even if it is administered more frequently than is necessary; (c) be free of any side effects, such as withdrawal syndromes; and (d) not interfere with the body's normal functions more than is necessary [64]. There are several pharmacologically and medicinally significant compounds in ashwagandha, including withanolides, sitoindosides, and other alkaloids. The results of a series of experiments used to determine if sitoindoside VII and sitoindoside VIII had anti-stress properties suggested that both sitoindosides do [65]. Rats were used to assess the immunomodulatory and central nervous system effects of sitoindoside IX and X on stress, memory, and learning. A significant reduction was noticed in the incidence of stress-induced gastric ulcers [58].

Historically, WS formulations have been used to treat neurological problems, anxiety, and illnesses like arthritis, asthma, goiter, and ulcers. These applications are related to the plant's purported adaptogenic, anti-stress, and anti-inflammatory qualities [66, 67]. Stress has been linked to the development of most neuropsychiatric diseases, such as anxiety, depression, and insomnia, and can alter the structural and functional makeup of the brain [68, 69]. Hypothalamic-Pituitary-Adrenal (HPA) axis hyperactivity and immune system dysregulation are two mechanisms by which stress causes these illnesses [70,

71]. Given the known link between stress and neuropsychiatric diseases, it is most likely that WS's anti-stress action contributes significantly to any potential health advantages it may have for depression, anxiety, and sleeplessness.

Stress that is experienced suddenly raises heart rate and blood pressure as well as gluconeogenesis, glycolysis, lipolysis, and hepatic glucose release. These in turn increase the body's levels of catecholamines and cortisol [72]. Stress, whether it be physical or mental, increases ACTH secretion, which in turn raises cortisol levels, at times, the level may increase even 20-fold [73]. According to Chandrasekhar et al.'s study, full-spectrum Ashwagandha root extract in high concentrations lowers serum cortisol levels, which rise under stressful circumstances [6]. Similar results in patients with stress were seen in a prior study by Abedon et al. [74]. These two sets of results combined imply that high-concentration full-spectrum Ashwagandha root extract attenuates some of the stress' antecedents, effects, and symptoms in addition to its main benefits. This might be interpreted as Ashwagandha providing direct and indirect support. This implies that a high-concentration full-spectrum Ashwagandha root extract has the capacity to enhance a person's general well-being [6].

Ashwagandha supplementation potentially improves sleep quality, a critical aspect of stress mitigation. A study published in *Cureus* reported that Ashwagandha administration markedly reduced sleep onset latency, enhanced sleep efficiency, and prolonged overall sleep duration among individuals with insomnia [75]. These trials hint at possible advantages, but more analysis is required to determine Ashwagandha's effectiveness in reducing stress.

In a study by Kumar and Kalonia (2007), rats were given an intraperitoneal injection of WS prior to undergoing a 24-hour period of sleep deprivation. The researchers used a grid suspended over water to administer the WS injection [76]. WS dramatically reduced sleep latency, REM sleep, and overall waking time while increasing slow-wave sleep and total sleep time. WS dramatically reduced sleep latency, REM sleep, and overall waking time while increasing slow-wave sleep and total sleep time [77]. Extracts of WS have been demonstrated to lessen the negative consequences of sleep loss in addition to their sleep-promoting benefits. A well-defined water extract of WS (ASH-WEX) was given orally to rats for 15 days, after which they were sleep-deprived for 12 h [78]. In comparison to untreated sleep-deprived rats, rats given WS showed better learning and memory while maintaining their motor performance. The activation of GABAergic neurotransmission is one potential route through which WS may exert its beneficial effects on sleep. Kumar and Kalonia (2008) found that the sleep-promoting activity

of WS in rats was significantly reversed by picrotoxin (a GABA antagonist) and potentiated by muscimol (a GABA agonist), supporting a mechanistic role for GABA [77]. Additionally, there is proof that WS affects the brain's chronomodulation.

Anti-inflammatory and Immunomodulatory effects of Ashwagandha

In comparison to conventional adjuvants, natural botanical compounds used as immunomodulators have demonstrated fewer adverse effects. Many of these botanical agents have been utilized by humans for millennia. *Withania somnifera*, an herbal remedy with a rich historical background spanning thousands of years, has served multiple purposes including nutritional supplementation and therapeutic interventions for various ailments. Clinical trials incorporating *Withania somnifera* as an adjuvant or immunomodulator in both murine models and human subjects have yielded promising results [47, 79]. It has been established that the water-insoluble Withaferin-A is the primary organic solvent extract of Ashwagandha, and that it has anti-inflammatory, anti-angiogenesis, anti-metastasis, and anticancer properties [80].

Inhibiting inflammatory markers including cytokines (including IL-6 and TNF- α), nitric oxide, and reactive oxygen species is how Ashwagandha reduces inflammation, according to preclinical studies. It also could modulate mitochondrial function and apoptosis. These findings suggest that Ashwagandha may have therapeutic applications in managing inflammation-associated diseases [81–83]. It's crucial to remember that more investigation, including clinical trials, is required to properly comprehend the efficacy and security of Ashwagandha in the treatment of these ailments.

In Swiss mice (15–25 g, 5–6 months old) and Wistar breeds albino rats (120–150 g and 250–300 g), the immunomodulatory and central nervous system (antistress, memory, and learning) effects of glycowithanolides and a combination of sitoindosides IX and X isolated from withaferin A were examined [84]. In addition to phagocytosis and increased lysosomal enzyme activity, both medications dramatically increased peritoneal macrophage recruitment and activation. Both drugs (50–200 mg/kg orally) markedly reduced stress in albino mice and rats and greatly enhanced learning and memory retention in both young and old animals. Three animal myelosuppression models using cyclophosphamide, azathioprine, or prednisolone were used to assess the root extract of withaferin A for immunomodulatory activities [85]. Withaferin in comparison to untreated control mice, a drug caused statistically significant increases ($p < 0.05$) in hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight. Further research was done into how withaferin A affected

the actions of macrophages obtained from mice that had been exposed to the carcinogen ochratoxin A (OTA) [86]. The chemotactic activity of macrophages significantly decreased in mice receiving OTA therapy for weeks.

Additionally, there is a lot of hope for the therapeutic effects of WS on strong Th2 immune responses and airway inflammation. WS balances the Th2-Th1 immune responses in mice by upregulating Th1 immune responses along with an increase in CD4 and CD8 T cells [87]. Withaferin A (derived from WS) pre-administration in asthma mouse models proved successful in reducing airway inflammation by reducing the production of pro-inflammatory cytokines in the lungs [48]. Evidently, WS works as an immunomodulator that mitigates the airway overresponsiveness seen in mice asthmatic models suggesting that botanical drugs, like known adjuvants, can also modulate diseases in a favorable manner [48].

Antimicrobial and Immunomodulatory effects of Ashwagandha

natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action [88, 89]. Withanolides possess remarkable antibacterial, anti-arthritic and immunosuppressive. The antibacterial effectiveness of ashwagandha against different bacterial strains has been the subject of numerous investigations. For instance, a 2017 study reported in the *Journal of Clinical and Diagnostic Research* examined the antibacterial efficacy of Ashwagandha extract against widespread oral infections. The findings showed that Ashwagandha significantly inhibited the growth of microorganisms linked to periodontal and dental illnesses such *Streptococcus mutans* and *Porphyromonas gingivalis* [90]. Another study published in the *International Journal of Pharmaceutical Sciences and Drug Research* in 2011 assessed the antimicrobial potential of Ashwagandha root extracts against human pathogenic bacteria. The results showed that Ashwagandha was effective at killing microorganisms such *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [91].

According to the research done by Darshan et al., the methanol extract of WS stem has the most antibacterial activity, followed by the aqueous extract. In comparison to methanol and aqueous extract, hexane and ethyl acetate extracts show less antibacterial activity. Higher antibacterial activity was reported in WS leaf, root, and stem methanol extracts [92]. Different phytochemicals are present in different extracts of stem, root and leaf of WS. *Leishmania donovani* could undergo apoptosis-like cellular death in vitro when withanolides containing ethanolic extracts (60 and 15 mg/ml) were isolated from WS. These extracts caused DNA nicks, apoptosis, and cell cycle arrest in a dose- and time-dependent manner

by increasing the production of reactive oxygen species (ROS) and lowering mitochondrial potential [93] possibly by blocking the Protein Kinase C (PKC) [94]. According to Rizwana research, acetone extracts of stem, leaves, and roots had the strongest inhibitory impact on the test organisms. The strongest antibacterial activity against the test organisms was demonstrated by the inhibitory zones for the acetone extracts of leaves, followed by extracts of the stem and root. There have been reports of similar studies where acetone extracts significantly inhibited the growth of harmful microorganisms [95, 96]. The growth of *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. pyogenes* was suppressed by methanolic and ethanolic extracts of the stem and leaves of WS. These results demonstrate that enolides are used to extract steroid lactones from a range of polar solvents, including acetone, methanol, and ethanol, which are potent bacterial growth inhibitors. Furthermore, it may be inferred that the aforementioned WS extracts may be employed as herbal medicines to treat a variety of infectious disorders brought on by these harmful organisms.

A 28kDA nontoxic, acidic protease inhibitor glycoprotein extracted from WS tubers imparted a fungistatic effect to many phytopathogenic fungi like *Fusarium oxysporum*, *Aspergillus flavus*, and *Fusarium verticillioides* by inhibiting hyphal growth and spore germination [97]. Additionally, flavonoids from WS extracts were observed to have a Minimal Fungicidal Concentration (MFC) and Minimum Inhibitory Concentration (MIC) of 0.039 against *Candida albicans* [2]. Although the exact antibacterial processes of Ashwagandha are not entirely understood, several studies have shown that the presence of active molecules such withanolides and withanosides may be responsible for the antimicrobial action [90]. According to Mishra et al., the immunomodulatory actions of the drug may be responsible for its antibacterial activity by boosting the body's inbuilt defenses against microbial infections [9]. According to research by Jayaprakasam et al. on the growth suppression of tumor cell lines by withanolides generated from Ashwagandha, these substances may have their effects through a variety of methods, such as altering cellular signaling pathways and DNA synthesis [81].

Anti-cancer effects of Ashwagandha

Antigen recognition and the capacity of both B-cells and T-cells to proliferate are specifically impacted by withaferin A [98]. *Withania Somnifera*, an Ayurvedic medicinal herb, contains a steroidal lactone called withaferin A, which has been investigated for its biological properties, including anti-inflammatory, anti-angiogenic, and anti-tumor actions [99]. Additionally, WA, the plant extract's main ingredient, exhibits potential as a major anti-cancer therapeutic [100]. WA has been reported to inhibit

proliferation and induce cell death in a variety of tumor models such as pancreatic [101], breast [102], lung [103], cervical [104], and prostate [105].

A recent study found that withaferin contributes to suppressing Zap70 kinase activity, which is necessary for human T-cell function in both health and disease [106]. Withaferin A decreases the ability of cancer cells to invade and spread throughout the body by inducing Par-4 [107]. The capacity of cancer cells to infiltrate the extracellular matrix (ECM) is a crucial determinant in the progression of cancer, and withaferin prevents the spread of cancer cells by stopping them from penetrating. In a dose-dependent way, withaferin derivatives inhibit the ability of cancer cells to form colonies [107]. Actin is essential for three biological processes: directed growth, adhesion, and migration. By creating a covalent bond with the adaptor protein annexin II, which is flexible, and by increasing the activity of the basal F-actin cross-linking pathway, withaferin A can change how the cytoskeleton is organized [108]. NF- κ B is a major therapeutic target because it is constitutively active in many hematologic malignancies [109]. Inhibition of TNF- α -induced nuclear translocation of the NF- κ B p65 subunit by WA has been demonstrated in human lung epithelial cells [110, 111].

Peripheral blood mononuclear cells (PBMC) and leukemic THP-1 cell viability can be affected by aqueous root extracts of WS (0.05–0.4 mg/ml), which can also increase oxidant scavenging and caspase [99, 100] activities while decreasing levels of tumor necrosis factor- α (TNF- α), interleukin (IL)-10, and glutathione (GSH) [112]. In the Leukemic Murine mouse model, withanolide D is said to suppress the anti-apoptotic genes (TERT, Bcl-2, and Puma) [113]. Additionally, the crude water extract of WS (0.5%) changed the signaling pathway including pro-apoptotic and tumor-promoting proteins, which assisted in reducing tumor growth. Thus exhibiting a pleiotropic anti-glioma phenomenon in both in vitro and in vivo systems by inhibiting proteins involved in cell survival [(Nuclear factor-kappa B (NF κ B), Phospho-Akt (p-Akt), B-cell lymphoma-extra-large (Bcl-xl) and Heat shock protein 70 (HSP70)], proliferation (cyclin D1), angiogenesis [Vascular endothelial growth factor (VEGF)], invasion [Matrix metalloproteinases (MMPs), Neural cell adhesion molecule (NCAM) and polysialylated neuronal cell adhesion molecule (PSA-NCAM)] of glioma cells [114].

According to existing evidence, the anticancer effects of WA operate through multiple mechanisms, reflecting its broad and multifaceted influence on cancer cell survival. Research indicates that WA promotes apoptosis via several signaling routes. For example, in prostate cancer cells, it elevates the expression of the pro-apoptotic protein Par-4 and activates downstream caspases, while in cervical cancer cells, it reinstates p53 expression, thereby

stimulating Bax and other pro-apoptotic members of the Bcl-2 family to induce cell death [109]. In most cancer cell lines, WA prevents tumor cell growth by arresting the cell cycle in the G2/M phase and suppresses NF- κ B activation by interacting with the IKK γ subunit to prevent I κ B phosphorylation [115, 116]. Yu et al. showed that treatment with WA causes a range of Hsp90 chaperone client proteins to degrade in pancreatic cancer cells, indicating that WA inhibits heat shock proteins [101]. This mechanism of action has not yet been described in any other cancer cells. Although various WS formulations demonstrated highly encouraging anti-cancer activity in both in vitro and in vivo settings, there are currently no authorized therapeutic candidates available.

Cardioprotective effects of Ashwagandha through antioxidant and lipid-modulating

WS most known as 'Ashwagandha' is widely used in Ayurveda and Unani Systems of Medicine. Studies on Ashwagandha have shown that taking supplements of this herb can lessen lipid peroxidation, perhaps by scavenging free radicals, and can aid in the development of a strong antioxidant defense system. Ashwagandha is promoted as a preventative medicine for coronary heart disease, hypertension, and atherosclerosis [117]. It shields the heart from sympathetic outbursts by lowering the heart's sensitivity to adrenergic stimulation. According to Moharana, Ashwagandha roots and leaves are typically used as a powder, decoction, or oil. These have been employed in folk medicine to treat wounds, inflammation, hypertension, and general infirmity [118]. Ashwagandha was shown to have energy-boosting qualities by Thirunavukkarasu et al., who suggested using it as a dietary supplement for cardioprotection [119].

Lipid peroxidation in stress was assessed in relation to Ashwagandha root's action. The herb's extremely strong antioxidant activity may help to explain some of its anti-stress, congestion-easing, anti-inflammatory, and anti-aging properties [118]. Ashwagandha's flavonoids reduce the incidence of CDV via increasing coronary vasodilation, lowering blood platelet clotting, and inhibiting the oxidation of low-density lipoproteins. A group of albino rats were used in Mohanty and colleagues' study, and isoprenaline administration caused cardiac necrosis. In this study, rats given *Withania Somnifera* showed a significant decline in glutathione levels as well as superoxide dismutase, catalase, creatinine phosphokinase, and lactate dehydrogenase activity [53].

Cardiovascular disease has been linked to high amounts of circulating cholesterol and its buildup in heart tissue [120]. High levels of LDL-C and VLDL-C are adversely connected with HDL-C, but have been positively correlated with Myocardial Infarction (MI). HDL-C inhibits the uptake of LDL-C by the arterial walls and

facilitates the transport of cholesterol from peripheral tissues to the liver, where it is catabolized and excreted from the body [121]. However, pretreatment with WS leaf extract (WSLEt) greatly lessens these alterations, keeping the myocardial membrane's regular fluidity and functionality. There has been evidence that polyphenols, notably gallic acid and catechin, can block cholesterol esterase [122]. Generally, the hydrolysis of dietary cholesterol esters by pancreatic cholesterol esterase is crucial for the release of free cholesterol in the small intestine's lumen [123]. Therefore, it is anticipated that inhibiting cholesterol esterase will lower dietary cholesterol absorption by limiting its absorption. Additionally, it has been suggested that polyphenols' ability to bind with bile acids and promote their fecal excretion may be the mechanism by which they lower plasma cholesterol levels [122].

LPO is a recognized process of cellular damage and has been used as a sign of the oxidative stress that contributes to the development of MI [7]. In rats receiving ISO therapy, WSLEt pretreatment dramatically lowers the levels of lipid peroxides. It is therefore conceivable that some components in WSLEt with antioxidant properties scavenge the excess LPO products produced by ISO and provide protection to the heart tissue. The quinone metabolites of ISO that form superoxide anions and other reactive oxygen species (ROS) when they react with oxygen might cause oxidative stress by interfering with antioxidant enzymes [124]. For the prevention of oxygen-free radical-induced tissue damage, the presence of the endogenous antioxidant defense is crucial [125]. The principal free radical scavenging enzymes SOD,

catalase (CAT), and GPx play a role in the first-line cellular defense against oxidative harm by breaking down oxygen (O₂) and hydrogen peroxide (H₂O₂) before they combine to create the more dangerous hydroxyl radical [126].

According to histopathological investigations, giving WS to patients dramatically lessens ischemia-related cardiac damage. Because of its anti-apoptotic qualities and ability to restore the normal balance of oxidative substances, ashwagandha has a cardioprotective effect [127]. Additionally, withaferin A, a part of Ashwagandha renowned for its anticancer activities, was investigated for its cardioprotective effects. By upregulating the mitochondrial anti-apoptotic pathway through an increase in AMP-activated protein kinase (AMPK) phosphorylation and an increase in the Bcl-2/Bax ratio (AMPK), modest dosages of withaferin A were found to have a cardioprotective effect in this rat investigation [54]. Additionally, it is known that AMPK is triggered by caloric restriction and that it plays a role in a few aging-related processes and diseases that frequently affect older people. Since AMPK maintains energy balance, it is believed to increase life expectancy and quality of life [55, 56, 128].

Effects of Ashwagandha in enhancing physical performance

According to sources, WS has been utilized in the conventional Ayurvedic medical system to boost cognition, increase memory, and boost other aspects of physical performance [129–132] (Fig. 3). Ashwagandha acts as a gamma-aminobutyric acid (GABA) mimetic and shows

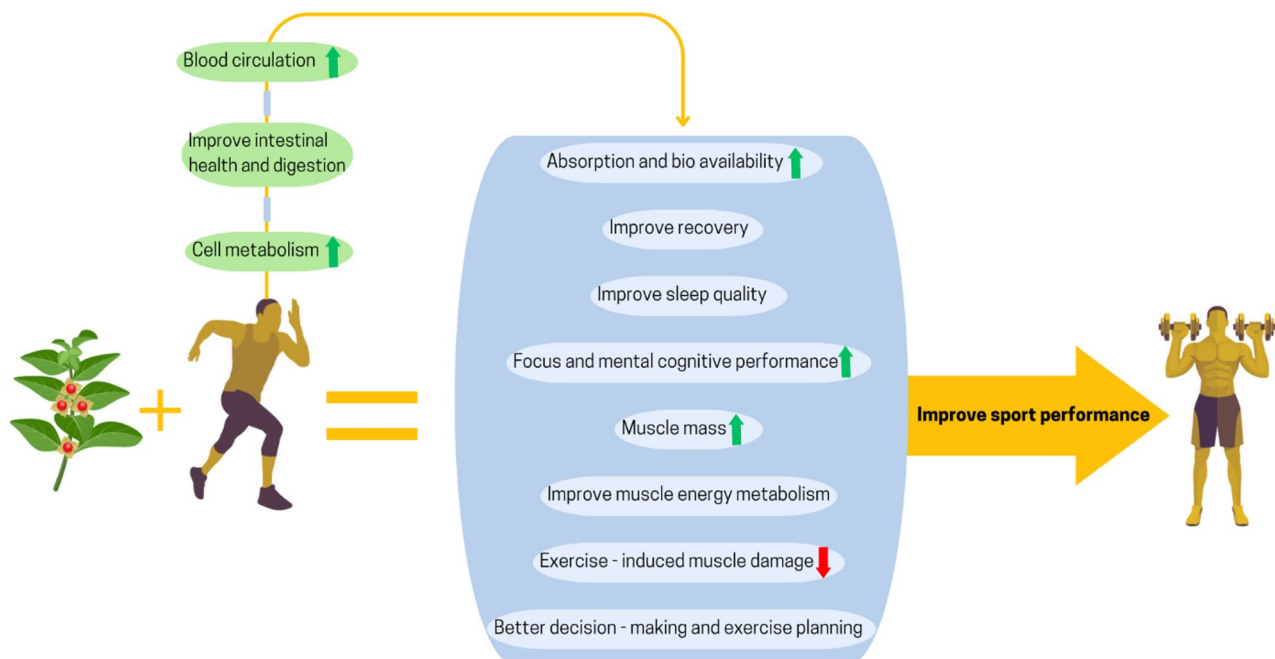


Fig. 3 Ashwagandha-mediated physiological and metabolic pathways enhancing recovery, muscle function, cognition, and overall sport performance

cholinomimetic activity [133]. Additionally, it appears that ashwagandha's secondary metabolites act as agonists for the nicotinic receptor α -7 [134]. *Withania somnifera* supplements have been found in animal trials to improve memory and cognitive function [133, 135]. *Withania somnifera* has been related in human intervention studies to improved cognition in people with early dementia or bipolar illness [10, 136]. However, there are few research on populations that are healthy [134, 137]. For eight weeks, Indian adults with early dementia who were taking 600 mg of Ashwagandha-root extract saw improvements in their memory, executive function, sustained attention, and processing speed [136]. In male and female bipolar patients with an average age of 47, supplementing for eight weeks with 500 mg of *Withania somnifera* appeared to improve a select few cognitive abilities, including auditory-verbal working memory, a measure of reaction time, and a measure of social cognition [136]. Gopukumar et al. examined the impact of Ashwagandha SR capsules (300 mg) on cognitive functioning, stress levels, sleep quality, and overall wellbeing in randomized placebo-controlled clinical research. They claimed that stress impairs cognitive function and can cause a number of illnesses, including mental and anxiety problems, coronary heart disease, high blood pressure, and diabetes [138]. This study demonstrated how Ashwagandha SR can enhance cognition, lower stress levels, and enhance sleep quality. Given the negative effects of conventional anti-anxiety drugs (such as benzodiazepines), which cause sleepiness and impair cognitive abilities, Ashwagandha SR's dual efficacy on cognitive capacities and sleep quality is advantageous [139]. They demonstrated that participants using Ashwagandha SR pills improved in three areas of cognition: visual memory, new learning, and persistent and prolonged attention.

WS could help with preventing physical tiredness and enhancing speed, lower limb muscle strength, and neuromuscular coordination. There are currently no studies outlining the molecular processes via which ashwagandha extracts enhance athletic performance and lessen muscular fatigue in humans. Although it is well known that sufficient physiological levels of reactive oxygen species (ROS) are necessary for the processes of physiological adaptation to exercise training, overtraining, insufficient energy supply, or poor sleep hygiene may increase the ROS production, which in turn may have a negative impact on the time course of exercise-induced adaptations [140–142]. For instance, it has been demonstrated that these chemicals cause structural changes in myofibrillar proteins, which have an impact on how well they function (e.g., a reduced sensitivity to intracellular Ca^{2+}). In this regard, numerous studies have shown that antioxidant supplementation (such as N-acetylcysteine) may prevent the onset of muscular exhaustion after

prolonged exercise in humans, but excessive doses have also been linked to diminished health benefits of exercise [143–145]. Several animal and cell studies have identified potential molecular targets of the secondary metabolites of this herbal extract that could be involved in the regulation of oxidative stress at the cellular level. metabolism in people of induced liver damage, Palliyaguru et al. (2016) found that withaferin A is a strong inducer of Nrf2, a transcription factor that controls the production of antioxidant enzymes in response to oxidative stress through activation of the PTEN/PI3K/Akt pathway [146]. These physiological consequences may serve as important explanations for the rise in VO_2max [147, 148].

Effects of Ashwagandha in enhancing immune system

There are several possible candidates in the exciting field of research on plant-derived immunomodulators. One of them, Ashwagandha, which is included in the Indian Ayurvedic tradition's Rasayana class of drugs and has been extensively studied as an immunomodulatory agent. The components in WS mainly withanolide glycosides exert their immunomodulatory action by mobilizing and activating macrophages and induce proliferation in murine splenocytes [58]. The activation of immune-regulatory cells by WS extracts may serve multiple purposes, including modulation of antigen presentation, regulation of the immunosuppressive tumor microenvironment, and maintenance of a balanced cytokine profile that supports effective T cell responses [59]. Tharakan et al. reported that extracts derived from the root and leaf of WS can enhance immunoglobulin levels key elements of innate immunity as well as increase IFN- γ production and the population of $\text{CD}3^+$ and $\text{CD}4^+$ T cells in humans. These results suggest that the plant plays an important role in strengthening both innate and adaptive immune responses, which are vital for the recognition and effective defense against common bacteria, viruses, and allergens [34]. The results of the present study align with previous in vivo research demonstrating an increase in $\text{CD}4^+$ and $\text{CD}8^+$ T cell populations, along with elevated expression of T-helper 1 (Th1) cytokines. Likewise, in immune-suppressed mice, administration of WS extracts led to a dose-dependent enhancement of natural killer (NK) cell activity and a faster restoration of $\text{CD}4^+$ T cells [149, 150]. After 30 days of administration, the WS extract used in this study led to a significant increase in $\text{CD}3^+$, $\text{CD}4^+$, $\text{CD}8^+$, $\text{CD}19^+$, and NK cell populations. Multiple in vivo studies on WS have highlighted its capacity to modulate both innate and adaptive immune responses across various levels of the immune system. However, while these immunostimulatory effects appear beneficial for individuals with weakened immune systems, they also introduce important safety concerns. Enhanced NK cell activity and a Th1-skewed cytokine

profile (marked by elevated IFN- γ levels) could theoretically heighten the risk of immune overactivation, which is particularly worrisome in individuals with pre-existing autoimmune disorders, where such stimulation may aggravate disease progression [151, 152]. Therefore, the clinical use of ashwagandha as an immune-supportive agent should be approached with caution, ensuring a careful balance between its immunostimulatory benefits and the potential risk of disturbing immune homeostasis, particularly in individuals predisposed to autoimmune or inflammatory disorders.

Alcoholic extracts of WS have been shown to increase the formation of white blood cells in a number of animal investigations [85, 153]. While Ashwagandha root has been the subject of numerous studies, WS leaves also contain a significant amount of withanolides, particularly withaferin A, a substance with substantial immunomodulating and anti-inflammatory properties. Whole-plant extracts of WS, including the roots and leaves, boosted the activity of immune cells (macrophages), particularly lysosomal enzymes that are responsible for detoxifying and getting rid of immune cells' waste products. Alcoholic preparations of the entire WS plant have also been discovered to increase macrophage phagocytic activity, lessen immunological hypersensitivity reactions, and promote T lymphocyte immune cell production [60]. After being exposed to pathogens like bacteria and viruses, the effect of WS on adaptive immunity may be especially helpful. An enhanced Th1 response was observed in mice given a standardized WS leaf extract for two weeks. This response was characterized by increased production of CD3+, CD4+, and CD8 + T cells, as well as co-stimulatory CD80 and integrins [79, 154]. The root extract of W.S., according to Malik et al.'s 2007 study, improves cell-mediated immunity mostly through boosting Th1 immunity [155].

An herb with phytonutrients is referred to as a "adaptogen" if it helps the body adapt by (a) restoring system functions to normal, (b) building resistance to future stress, and (c) raising the body's functioning to a higher level of performance [3]. An ergogenic aid is a method or practice that improves training quality, exercise capacity, work efficiency, ability to recover from exercise, and recovery from exercise, leading to larger training adaptations [149]. According to a 1996 study, Ashwagandha raised the RBC and Hb counts. A direct result of the increase in RBC mass is an increase in the blood's ability to deliver oxygen directly to working muscles, so directly increasing aerobic capacity [150]. Given that stress can be considered as a byproduct of exercise and that ashwagandha supplementation improves human physical performance, the adaptogenic qualities of ashwagandha raise the prospect that it could be a useful ergogenic aid. The study by Wankhede et al. shows that the group

receiving Ashwagandha supplementation achieved significant improvement in all parameters (muscle strength, muscle size and body fat percentage, testosterone and muscle recovery) compared to the placebo group [11]. The beneficial benefits of ashwagandha supplementation on resistance training and performance improvements in this study may have been mediated by several modes of action. From the perspectives of muscle development and muscular recovery, they can be examined.

A function of muscle growth is muscular size, which is influenced by two actions of ashwagandha: (i) an increase in testosterone, which promotes muscle growth, and (ii) a decrease in cortisol levels, which, as a catabolic agent, reduces muscle mass. Ashwagandha (i) can improve mitochondrial energy levels and functioning and decrease the activity of the Mg²⁺-dependent ATPase enzyme that breaks down ATP in terms of energy generation [156], and (ii) can raise creatine levels, which may then trigger the production of ATP [62]. Lastly, ashwagandha's effects on the nervous system as an anti-anxiety drug and in fostering focus and concentration [6]. The various extract components' synergistic effects, which include antioxidant effects to prevent free radical damage to the muscles and central nervous system, analgesic and anti-inflammatory effects, as well as a decrease in lactic acid and blood urea nitrogen, could all contribute to the faster recovery [9, 24, 157].

Mechanisms of action: ashwagandha's multifaceted pathways of effect

- Ashwagandha as an adaptogen.

Through a number of mechanisms of action connected to the homeostatic preservation of the hypothalamic-pituitary-adrenal (HPA) axis and the regulation of important mediators of the stress response, ashwagandha is regarded as an adaptogen that aids in the restoration of homeostasis by counteracting external stimuli as non-specific regulators [158]. The ideal adaptogen should minimize the negative effects of stress, perform safely and beneficially even when administered in doses greater than necessary, and be devoid of undesirable side effects, such as not impairing bodily functions beyond what is necessary [64]. Researchers have hypothesized that Ashwagandha may reduce cortisol levels, hence reducing the HPA axis activity. Particularly, the effect of WS on GABA and serotonergic pathways may reduce the activation of the HPA axis, diminishing the secretion of corticotropin-releasing hormone and adrenocorticotrophic hormone from the hypothalamus and pituitary, respectively, resulting in lower levels of cortisol released from the adrenal cortex [159]. These findings are uncertain in populations with low cortisol levels [160], but they appear to exist in

populations with high or moderate levels of cortisol [6, 28, 29, 138, 161]. However, although this offers a reasonable mechanistic explanation for how Ashwagandha may elevate testosterone levels through modulation of the HPA axis and reduction of cortisol current evidence does not definitively confirm that this is the predominant pathway for all individuals [160, 162]. The association tends to be stronger at higher dosages and is particularly evident in individuals who are experiencing substantial stress [160]. As an alternative, WS supplementation may also alter the activity of critical enzymes in physiological processes linked to the creation of testosterone, increasing levels in people with low cortisol levels [11, 12, 160], but not in people with high cortisol levels [29]. Because of their broad biological activities, the anti-inflammatory and Odant properties of aqueous, organic, and hydroalcoholic extracts of *Withania somnifera* may also contribute to their impact on testosterone levels [163–165]. These findings suggest the use of ashwagandha as an adaptogen to balance and rebalance biomarker levels and produce hormonal level balances.

- Ashwagandha's effects on the stress response.

WS has been shown to have an anti-stress effect through several pathways, including glucocorticoid decrease and immunological regulation. The anti-stress activity of WS is most commonly attributed to its effects on the glucocorticoids cortisol and corticosterone (the major stress hormones in humans and rodents), respectively [166]. Stress-related HPA axis dysfunction is largely caused by elevated levels of cortisol and corticosterone, which are also elevated during stressful times. Various WS root preparations reduced the rise in peripheral cortisol and corticosterone levels brought on by stress [167–169]. Additionally, it was discovered that WS might counteract the drop in adrenal cortisol caused by stress [170, 171]. WS might potentially reduce stress by influencing the immune system. In the reviewed studies, the effect of WS on immune markers depended on the animal model used. White blood cell count and lymphocyte percentage fell in a horse model of exercise, separation, and noise-induced stress, although the pro-inflammatory cytokine IL-6 rose [169]. These effects were reversed with WS root powder, which also showed immune-stimulating and anti-inflammatory properties. Similar results were observed in a rat model of restraint stress, where WS in the form of a root preparation reduced the effects of stress on peripheral T-lymphocyte counts (CD3+, CD4+, and CD8 + populations), as well as IL-2, INF-, and polymorphonuclear leukocyte counts [172].

- Ashwagandha's anti-inflammatory effects.

Most of the antiinflammatory activities of WS have been attributed by researchers to withanolides, particularly Withaferin A [173, 174]. Withanolides (WS) may work by interacting with mediators of the inflammatory cell signaling pathway such as NF- κ B, signaling kinases, HSP90, Nrf2, and the inflammasome complex, although the exact processes underlying their anti-inflammatory effects are still poorly understood. For people with raised NF- κ B levels, the nuclear factor NF-kappa-B (NF- κ B) transcription factor family is a therapeutic target since it plays a role in several inflammation-driven chronic disorders. WS can obstruct and mediate the inhibition of the NF- κ B pathway in this situation [173]. WS relies on the development of potent protein kinase inhibitor activity. WS has the ability to inhibit protein kinase signaling cascades, which are crucial to inflammatory processes [173, 175]. Additionally, it appears that the kinase inhibition takes place when nitric oxide generation is reduced, which benefits the inflammation process. Another method to explain the anti-inflammatory action of WS is the down-regulation and instability of the activity of the regulatory kinase HSP. As previously mentioned, Ashwagandha can reduce oxidative stress via controlling Nrf2 [146, 176, 177]. Last but not least, WS may inhibit some inflammasomes, cytokines, and other multiprotein pro inflammatory proteins to lessen inflammation [173]. The reduction in CK and CRP levels found in some studies may suggest anti-inflammatory effects of WS, even in individuals with low levels of inflammation (Table 1).

- Ashwagandha's effects on cognitive function.

Traditional and clinical uses of WS point to a probable GABAergic activity as a cause of the effects of WS [178–181]. Indeed, recent research has demonstrated that picrotoxin, a GABAA antagonist, inhibited the inward currents that methanolic WS root extracts produced in gonadotropin-releasing hormone neurons [49]. According to earlier studies, withanolides extracted from WS can be administered orally to mice to reverse oxidation, inhibit neuritic atrophy, and aid in the removal of amyloid-peptides from the brain [50, 181, 182]. The C28-steroidal lactones withanolide A (WLA) and withaferin A (WA) appear to be essential for a significant portion of this action. The traditional use of WS as an adaptogen is supported by data by Candelario et al., which indicate that it may have additional neuroprotective properties through activation of GABA receptor channels [183]. In the hippocampal region of the brain, there was evidence that rats fed ashwagandha inhibited acute alterations in the expression of proteins involved in synaptic plasticity, cell survival, and death. Ashwagandha, according to the

Table 1 Summary of ashwagandha's impact on inflammatory pathways

Inflammatory pathway	Specific effects of ashwagandha	References
NF- κ B Pathway	- Inhibits NF- κ B activation leading to reduced expression of pro-inflammatory genes. - Downregulates the nuclear translocation of NF- κ B p65 subunit.	[81, 110]
MAPK Pathway	- Modulates the MAPK pathway, leading to reduced expression of pro-inflammatory cytokines. - Affects key signaling proteins like ERK, JNK, and p38 in the pathway.	[31, 79]
Cytokine Production	- Reduces the levels of pro-inflammatory cytokines like TNF- α , IL-6, IL-1 β . - Increases the expression of anti-inflammatory cytokines.	[31, 47, 48]
Oxidative Stress	- Reduces oxidative stress markers, thereby mitigating inflammation. - Enhances the activity of antioxidant enzymes like SOD, catalase, and GPx.	[81, 124]
Immune Cell Modulation	- Promotes macrophage phagocytic activity and increases lysosomal enzyme activity. - Affects T cell proliferation and balances Th1/Th2 responses.	[60, 79, 85]
Apoptosis and Cell Signaling	- Induces apoptosis in inflammatory cells. - Modulates cell signaling pathways related to inflammation and cell survival.	[175, 176]

authors, may help to lessen cellular stress and apoptosis [184].

Ashwagandha has been demonstrated in research on human brain cells to counteract the harmful effects of β -amyloid, which may contribute to neurocognitive impairment during HIV infection [185]. A study was conducted on rats that were orally administered vitanon an ingredient isolated from the root of WS. The suppression of amyloid β -42 was associated with significant improvements in cognitive function, as well as a decrease in nitric oxide, lipid peroxidation, and the pro-inflammatory cytokines TNF- α , IL-1 β , IL-6, and MCP-1. Additionally, the activity of β enzymes and γ -secretase, which create insoluble neurotoxic clumps of β -amyloid, decreased [186].

Animals can also suffer from neurodegenerative illnesses, unlike humans, yet in both situations the disease's course and pathomechanism are extremely similar. Canine cognitive dysfunction (CCD) is an age-dependent condition marked by pathological alterations in the brain that impair motor coordination and cause memory loss. In both dogs and humans, a progression of oxidative damage in the brain is noted with age. In a study conducted on human embryonal neuroblastoma SK-N-SH cells, Ashwagandha extract was shown to have antioxidant properties (significantly reduces free radical generation). Additionally, it altered cholinergic transmission, possibly lowering acetylcholinesterase activity, which

may be helpful in the treatment of Alzheimer's disease and canine cognitive impairment [187]. Additionally, it has been observed that withaferin A in the form of Ashwagandha extract greatly reduces the expression of neuroinflammatory markers linked to NF- κ B as well as amyloid formation [21].

- Ashwagandha's effects on athletic performance.

The antioxidant qualities of Ashwagandha may play a role in the benefits on muscle strength/power, cardiorespiratory fitness, and fatigue/recovery that most studies have reported when using its aqueous root extract [132]. Ashwagandha has been demonstrated to have anti-inflammatory qualities, which may help reduce inflammation brought on by exercise and speed up recovery. However, nothing is known about the molecular mechanism underlying its immunomodulatory activity. Kaileh et al. show that pure WA or WS extract containing WA totally reduced (NFB) activation caused by inflammatory agents regardless of the type of cell, but not AP1 activity [116]. In fact, they demonstrated that pure WA and WS extract inhibit IKK kinase activity, which in turn prevents NFB/DNA binding, I κ B phosphorylation and degradation, p65 translocation, and subsequent NFB-driven gene expression. This ultimately leads to the downregulation of various NFB-regulated gene products induced by tumor necrosis factor TNF. It was clearly demonstrated that WS and WA inhibit NFB activation by directly suppressing IKK kinase activity, which prevents I κ B phosphorylation prior to its ubiquitination and degradation. This is contrary to Mohan et al.'s earlier report that suggested WA might inhibit NFB activity by impairing I κ B degradation and ubiquitination [98].

Withaferin A therapy enhanced cell survival in myoblast cells exposed to simulated ischemia/reperfusion or treated with H₂O₂, as shown by Yan et al. This was accomplished by promoting the production of proteins with antioxidant activity, such as HO-1, SOD2, SOD3, and Prdx-1, which is reliant on Akt activation [176]. It has been confirmed that Ashwagandha activates nuclear factor erythroid 2-related factor 2 (Nrf2), which possesses cytoprotective properties, in a variety of cellular settings and when combined with other herbal extracts [188–190]. On the other hand, after the administration of Ashwagandha, a few anabolic and catabolic signaling pathways controlling muscle protein synthesis and energy metabolism may be impacted. Of them, the NF- κ B signaling pathway has been connected to the control of myogenesis in both human and animal models of skeletal muscle. Although several authors have described a promyogenic function after the activation of this pathway, others have demonstrated the potent inhibitory effect of myogenesis and muscle regeneration, suggesting

a complex regulation of this signaling pathway during muscle formation [191]. Numerous investigations in various in vivo and in vitro experimental settings have demonstrated the impact of withanolides on the control of Nuclear factor kappa B (NF- κ B) transcriptional activity [116].

According to Ichikawa et al. (2006), withaferin A, viscosalactone, and their acetylated derivatives suppress the phosphorylation and degradation of I κ B, which prevents the production of NF- κ B-related genes [192]. In a MEK1/ERK1 dependent redox mechanism sensitive to thiol alkylation, Kaileh et al. showed that withaferin A directly suppresses IKK kinase activity, which phosphorylates I κ B prior to its ubiquitination and destruction [116]. Similar to this, withaferin A and withanolide D inhibited the breakdown of I κ B and promoted the production of the antioxidant enzyme HO-1 to prevent the angiogenic activity of endothelial cells [193]. Among other secondary metabolites, withaferin A has been recognized as a regulator of several cellular settings that may support myogenesis and oxidative metabolism in people.

Ashwagandha supplementation has been associated with possible enhancements in testosterone levels and overall hormonal regulation in both men and women an effect that holds particular relevance for athletic performance, strength development, and muscle growth [11]. It is often noted that elevated testosterone levels can improve physical capability, support muscle development, and enhance overall athletic performance.

However, it is important to recognize that research outcomes are inconsistent. While some studies have documented notable increases in testosterone levels [194], other clinical trials have reported no statistically significant differences when compared with placebo groups [162]. This tendency to highlight only favorable findings may lead to an overly optimistic and biased view of the herb's effectiveness. Apart from its possible hormonal influences, ashwagandha is well recognized for its traditional role as an adaptogen that helps reduce stress. Research shows that by lowering cortisol levels, it can enhance psychological well-being, which in turn may indirectly support athletic performance and recovery by improving stress regulation and promoting better sleep quality [29, 195].

In a mouse model of induced liver damage, Palliyaguru et al. (2016) found that withaferin A is a strong inducer of Nrf2, a transcription factor that controls the production of antioxidant enzymes in response to oxidative stress through activation of the PTEN/PI3K/Akt pathway [146]. A 2020 review analyzing five studies reported that supplementation with high doses of Ashwagandha (330–1000 mg) over 8 to 24 weeks led to a significant increase in VO₂max in both trained and untrained participants [61]. A higher VO₂max indicates more efficient oxygen

utilization during exercise, which is commonly associated with better cardiorespiratory fitness.

Supporting these results, several studies suggest that Ashwagandha supplementation can enhance hematological parameters such as mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV), potentially offering a physiological basis for the improvements observed in endurance performance and VO₂max [147, 148].

However, the evidence is not entirely uniform. While the previously mentioned review and studies report beneficial effects, other rigorously controlled trials have found no significant changes in VO₂max after Ashwagandha supplementation [57, 132]. Excluding studies with null results in certain discussions may create an overly favorable impression of Ashwagandha's effectiveness. Thus, although the herb shows potential to improve cardiorespiratory endurance, the overall evidence from the scientific literature is mixed and warrants further investigation.

- Ashwagandha's effects on sexual function.

Ashwagandha has historically been used to treat a variety of conditions, including weakness, erectile dysfunction, performance anxiety in males, and decreased desire for sex in both sexes [171, 196, 197]. Men who take the herb have been shown to have higher levels of blood testosterone, lower levels of the follicle-stimulating hormone (FSH), and higher levels of luteinizing hormone (LH) synthesis [12, 197]. According to some findings, greater psychological stress is linked to a rise in the synthesis of oxidants, and prolonged exposure to stressors may increase the formation of reactive oxygen species (ROS). Because spermatozoa have a high lipid content, they are more vulnerable to oxidative stress, which can lead to lipid peroxidation due to a lack of cytoplasmic enzymes that neutralize ROS [198]. According to a study by Chauhan et al., ashwagandha root extract powder effectively increases male libido in mature males with normal levels of prolactin and testosterone. Positive physiological changes were also documented. The research also suggested that people who took the ashwagandha supplement had their quality of life improve and remain stable [199].

Ashwagandha's action on GABA receptors, which helps the production of GnRH, is one likely explanation for how it improves sexual function [200]. The roots of this herb may include a variety of alkaloids, ergostane steroids, and amino acids, such as tryptophan, as well as inhibitors of the central nervous system, centrally acting hypotensive drugs, GABA agonists, and serotonin agonists [201]. There are claims that WS contains many

additional alkaloids and flavonoids in addition to being high in neurotransmitters [202]. The action of withanolides, steroidal lactone triterpenoids, which share chemical structural similarities with testosterone and may therefore mimic the effects of male steroidal hormones, is another way that ashwagandha affects the reproductive system and fecundity [203]. It has been previously established that WS flavonoids have strong antioxidant activity, and therapy with WS may prevent ROS from forming in infertile males [204]. Sitoindosides VII–X and withaferin A (glycowithanolides), the active components of WS, have been demonstrated to reactivate the main enzymes that scavenge free radicals in vivo [205]. The use of WS in the Agmo trial provided concrete proof of its ability to reduce stress. The percentage deviation of the parameter from the normal values determined how much of an effect the treatment had, suggesting the adaptogenic character of WS. Further investigation would be needed to confirm these findings, though. In Table 2, a summary of the health benefits of this supplement is presented, which were explained above.

- Ashwagandha's cardioprotection and anti-cancer effects through Mitochondrial Regulation.

The complex pharmacological profile of Ashwagandha, especially its principal bioactive constituent Withaferin A, stems from its context-dependent regulation of key cellular signaling nodes, with the mitochondria acting as the central control organelle. This dual action is reflected in its capacity to safeguard healthy cells while targeting malignant ones for elimination. In the context of cardioprotection, low doses of Withaferin A activate AMPK, which shifts the balance of the Bcl-2 protein family toward anti-apoptotic members. This increase in the

Bcl-2/Bax ratio stabilizes the mitochondrial membrane, prevents cytochrome c release, and enhances cell survival [54–56]. Beyond its cardioprotective effects, Ashwagandha's anti-inflammatory properties evidenced by the downregulation of pro-inflammatory genes such as IL-6, IL-1, IL-8, Hsp70, and STAT-2, along with activation of p38/MAPK in a prostate cancer model help create a less tumor-promoting microenvironment [206]. Conversely, in cancer settings, Ashwagandha mounts a multi-faceted antitumor response, with well-documented cytotoxic effects across a range of cell lines, including breast, colon, lung, central nervous system, and leukemia cells [81, 207, 208]. The primary mechanism centers on mitochondria-mediated apoptosis, which is tightly controlled by the Bcl-2 protein family. Bioactive compounds such as Withaferin A and Withanone trigger the production of reactive oxygen species (ROS) and activate the tumor suppressor p53 [207, 209–211]. Activated p53 promotes pro-apoptotic signaling by upregulating Bax, Bak, and Noxa while downregulating anti-apoptotic proteins such as Bcl-2, Bcl-xL, and Survivin [212–214]. This shift triggers mitochondrial outer membrane permeabilization (MOMP), resulting in cytochrome c release and the initiation of caspase-dependent apoptosis [215, 216]. Importantly, this apoptotic pathway operates independently of p53 status, as evidenced by Withaferin D, which triggers Bax/Bak-dependent apoptosis in both wild-type and p53-deficient cells [217]. Additional complexity arises from the extrinsic apoptosis pathway, in which Caspase-8 cleaves Bid into tBid, which then cooperates with Bax to permeabilize the mitochondrial membrane [217, 218]. Beyond its central mitochondrial-mediated effects, Ashwagandha's anticancer activity is further enhanced through multiple mechanisms, including inhibition of angiogenesis, suppression of metastasis, induction of cell cycle arrest, disruption of mitosis via Withanone's interaction with the TPX2-Aurora A complex, and impairment of cellular energy metabolism through inhibition of succinate dehydrogenase [94, 98, 219, 220]. Moreover, Ashwagandha exhibits notable therapeutic synergy by potentiating the effects of radiation therapy through lowering tumor glutathione (GSH) levels, mitigating paclitaxel-induced neutropenia, and potentially enhancing immune function by promoting the generation of cytotoxic T lymphocytes [60, 221–223]. Therefore, leveraging its diverse array of phytochemicals, Ashwagandha exerts anticancer effects through a coordinated network of pathways, with the direct and indirect regulation of mitochondrial apoptosis serving as the central mechanism (Fig. 4).

Table 2 Health benefits of Ashwagandha compounds

Health benefits	Supported by research
Stress Reduction	✓
Anti-inflammatory Effects	✓
Antioxidant Properties	✓
Cognitive Enhancement	✓
Improved Physical Performance	✓
Enhanced Recovery from Exercise	✓
Immune System Modulation	✓
Neuroprotective Effects	✓
Cardiovascular Health	✓
Antimicrobial Activity	✓
Anti-cancer Properties	✓
Sexual Health Improvement	✓

This table summarizes the diverse health benefits of Ashwagandha, particularly focusing on its impact in conjunction with physical activity. Improved Physical Performance and Enhanced Recovery from Exercise are key benefits supported by research, highlighting Ashwagandha's role in physical health and fitness

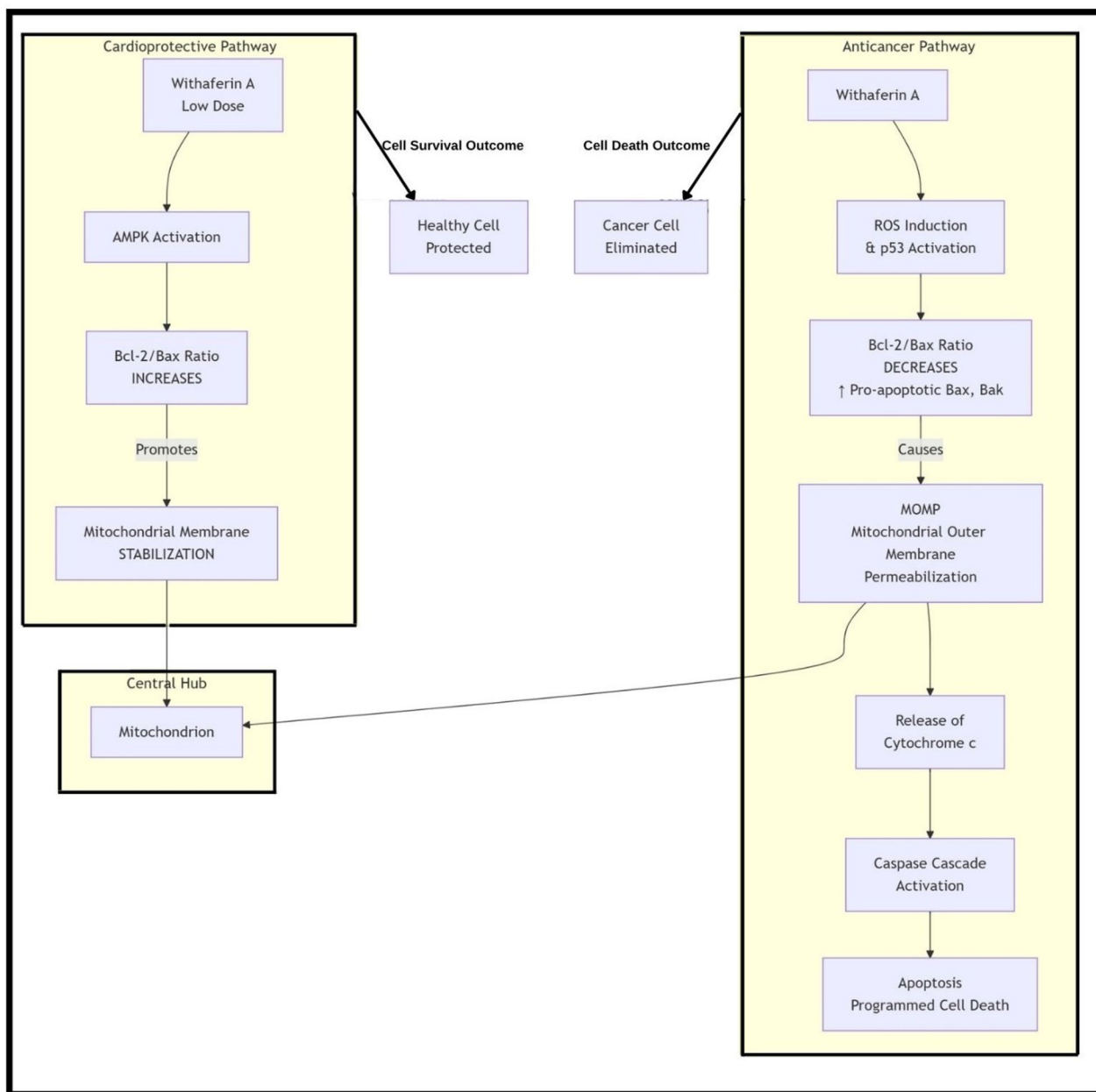


Fig. 4 Integrated cardioprotective and anticancer mechanisms of Withaferin A via Mitochondrial Modulation

Safety and side effects of taking Ashwagandha supplement

Ashwagandha has attracted considerable attention for its adaptogenic properties, which are largely attributed to key bioactive compounds such as withaferin A and withanolide D [224]. The clinical interpretation and application of these pharmacological effects are shaped by the herb’s variable regulatory status across different regions. In the United States, the Food and Drug Administration (FDA) classifies ashwagandha as a dietary supplement, meaning it is not approved for the treatment or prevention of specific diseases and is subject to less stringent

pre-market review compared with pharmaceuticals [225]. Ashwagandha is acknowledged in numerous pharmacopeias and authoritative references worldwide, including the Ayurvedic Pharmacopoeia of India (API), Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States Pharmacopoeia (USP), American Herbal Pharmacopoeia (AHP), the Pharmacopoeia of the People’s Republic of China, and the World Health Organization’s monographs [226, 227]. In Europe, the European Medicines Agency (EMA) takes a middle-ground approach, designating it as a traditional herbal medicinal product for alleviating stress-related symptoms based on its long

history of use [227]. This regulatory context highlights that current evidence supports the use of ashwagandha primarily within supplement or traditional medicine frameworks, rather than as an approved pharmaceutical, with direct implications for safety monitoring and risk assessment. While its traditional use and numerous clinical studies indicate relative safety at typical therapeutic doses [228], emerging data call for a more nuanced evaluation of potential risks. Contrary to earlier claims of complete harmlessness, recent clinical reports have linked ashwagandha to cases of clinically apparent liver injury, placing it among a growing group of herbal and dietary supplements (HDS) with recognized hepatotoxic potential [229]. Such HDS-induced liver injury can present severely, often as acute hepatocellular damage with jaundice, and, in rare instances, may progress to acute liver failure necessitating transplantation [230]. Causality assessment is often challenging due to the multi-component nature of HDS products, which can obscure the specific agent responsible for hepatotoxicity. In addition, ashwagandha exerts a dual effect on thyroid function. Clinical studies have shown that it can significantly increase thyroxine (T4) levels and decrease thyroid-stimulating hormone (TSH) in individuals with subclinical hypothyroidism, highlighting its therapeutic potential [231]. However, this thyroid-stimulating effect also carries a risk of iatrogenic thyrotoxicosis in individuals with normal thyroid function or pre-existing hyperthyroid conditions, such as Graves' disease. Therefore, the use of ashwagandha in patients with thyroid disorders requires careful medical supervision. The use of ashwagandha in individuals with endocrine disorders requires careful pharmacokinetic and pharmacodynamic consideration, as its adaptogenic and hormonal effects may be contraindicated. Pharmacodynamically, its thyroid-stimulating activity demonstrated by increased thyroxine (T4) levels and decreased thyroid-stimulating hormone (TSH) poses a notable risk of iatrogenic thyrotoxicosis in patients with hyperthyroidism or autonomous thyroid tissue [231]. Furthermore, while ashwagandha's ability to modulate the hypothalamic-pituitary-adrenal (HPA) axis may enhance stress resilience, it could theoretically disturb the delicate hormonal balance in patients with adrenal insufficiency, potentially worsening their condition [6]. From a pharmacokinetic perspective, the herb's bioactive withanolides are metabolized in the liver, and emerging evidence of its hepatotoxic potential adds a critical layer of complexity. Any drug-induced liver injury could alter the metabolism and clearance of both Ashwagandha's constituents and a patient's concomitant endocrine medications (antithyroid drugs, corticosteroid replacement therapy), leading to unpredictable plasma levels and effect. This risk is compounded by Ashwagandha's interaction with the GABAergic system, which may potentiate

sedative medications often used for symptom management in these populations [183]. Therefore, a thorough risk benefit assessment, careful medical supervision, and regular monitoring of thyroid function, adrenal status, and liver enzymes are essential in these vulnerable patient populations. Beyond hepatic and endocrine concerns, ashwagandha is associated with other adverse effects. Commonly reported reactions in human studies are generally mild to moderate gastrointestinal issues, including nausea, vomiting, and diarrhea. While most clinical evidence supports a favorable safety profile at typical therapeutic doses, caution is warranted as higher doses may cause sedation, cardiovascular effects such as hypotension and arrhythmias, and, in rare cases, respiratory depression. These more serious effects are generally linked to specific risk factors such as overdose, pre-existing respiratory conditions, or concomitant use of other central nervous system depressants rather than standard therapeutic use. A key pharmacological consideration is ashwagandha's interaction with the GABAergic system. Studies indicate that its methanolic root extract can influence dopaminergic neurons through GABA-A receptors [232]. This mechanism raises concerns about potential synergistic effects when co-administered with sedative-hypnotic medications, such as benzodiazepines or barbiturates, highlighting the need for caution.

In conclusion, while ashwagandha is a valuable botanical with well-documented therapeutic effects, a thorough safety assessment is essential, rather than assuming universal harmlessness. Its regulatory classification as a dietary supplement in many regions underscores the need for vigilant post-market surveillance. Future research should aim to elucidate its hepatotoxic potential, identify patient-specific risk factors, and develop clear, evidence-based usage guidelines. Clinicians must exercise caution, carefully balancing potential benefits against risks particularly in patients with pre-existing liver disease, thyroid disorders, or those taking sedative medications or other hepatotoxic agents.

Conclusion

Ashwagandha acts as a potent adaptogen, enhancing athletic performance by helping the body manage both physiological and psychological stress. Its primary mechanism involves modulating cortisol secretion, the body's principal stress hormone. By reducing elevated cortisol levels, ashwagandha mitigates the negative effects of stress on physical performance while fostering a more anabolic environment conducive to muscle growth. This is particularly important because cortisol and testosterone often have an inverse relationship; lowering cortisol can support optimal testosterone levels, which are essential for protein synthesis and muscle development. Additionally, ashwagandha's interaction with GABA receptors

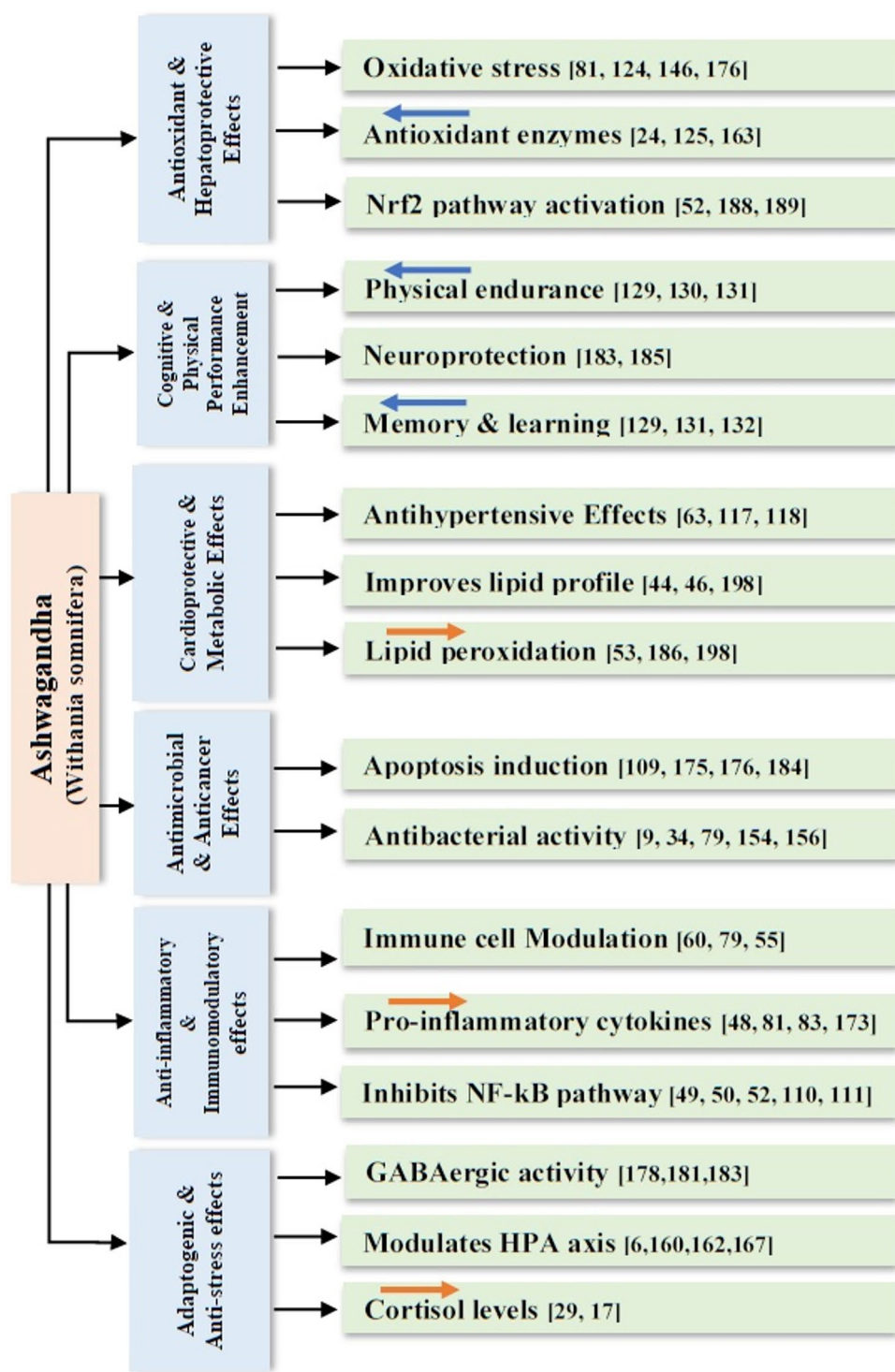


Fig. 5 Multidimensional Outcomes of Ashwagandha

in the nervous system provides a calming, sedative effect, improving sleep quality a critical factor for muscle repair, hormonal balance, and mental readiness. Together, these effects create a robust foundation for recovery and enhanced athletic performance.

The benefits of ashwagandha extend well beyond hormonal regulation and sleep improvement, encompassing direct restorative effects on the body. Its bioactive compounds, particularly withanolides, exhibit potent anti-inflammatory and antioxidant properties. Intense physical training induces substantial oxidative stress

and inflammation, which can lead to muscle damage, soreness, and delayed recovery. Ashwagandha helps neutralize harmful free radicals and reduces inflammatory markers, protecting muscle cells from exercise-induced damage. This effect translates into faster recovery, reduced muscle soreness, and decreased exercise-induced fatigue, allowing athletes to train more consistently, increase training volume, and achieve performance goals with fewer physical setbacks. Thus, ashwagandha serves as a valuable tool for enhancing resilience and accelerating athletic progress.

Moreover, the physical and psychological benefits of ashwagandha converge in a synergistic cycle that optimizes overall athletic output. By reducing stress, improving sleep, and accelerating physical recovery, it supports brain health and cognitive function. Additionally, ashwagandha provides direct neuroprotective effects by enhancing antioxidant defenses in the brain, promoting neurotransmitter balance, and stimulating neural connectivity. These cognitive enhancements manifest as improved memory, sharper focus, and greater mental clarity enabling athletes to make quicker decisions, plan exercise more effectively, and maintain motivation and psychological resilience during competition. Through this holistic approach, ashwagandha bridges enhanced physical capabilities with fortified mental strength, allowing athletes to maximize their overall performance potential (Fig. 5).

Abbreviations

IL-6	Interleukin 6
MMPs	Matrix metalloproteinases
TNF- α	Tumor Necrosis Factor Alpha
NCAM	Neural Cell Adhesion Molecule
MAPK	Mitogen-Activated Protein Kinase
PSA-NCAM	Polysialylated neuronal cell adhesion molecule
(IL)-8	Interleukin 8
IKKg	Inhibitor of nuclear factor kappa-B kinase subunit G
IL-1 β	Interleukin-1 beta
CDV	Cardiovascular diseases
IL-12	Interleukin 12
LDLC	Low-density lipoprotein cholesterol
ER/PR	Estrogen Receptor/Progesterone Receptor
VLDL-C	Very-low-density lipoprotein
Nrf2	Nuclear factor erythroid 2-related factor 2
HDL-C	High-density lipoprotein cholesterol
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
LDL-C	Low-density lipoprotein cholesterol
AMPK	AMP-activated protein kinase
WSLEt	W. somnifera leaf extract
NSCA	National Strength and Conditioning Association
LPO	Lipid peroxidation
VO ₂ max	Maximum aerobic capacity
ISO	Isoproterenol
WS	Withania somnifera
SOD	superoxide dismutase
HPA	HypothalamicPituitary-Adrenal
CAT	Catalase
ACTH	Adrenocorticotrophic hormone
GPx	Glutathione peroxidase
REM	Rapid eye movement
H ₂ O ₂	Hydrogen peroxide

ASH-WEX	Water extract of Ashwagandha leaves
PTEN	Phosphatase and Tensin Homolog
GABA	Gamma-aminobutyric acid
MCH	Mean Corpuscular Hemoglobin
OTA	Ochratoxin A
MCHC	Mean Cell Hemoglobin Concentration
ROS	Reactive Oxygen Species
MCV	Mean corpuscular volume
PKC	Protein Kinase C
Th1	T-helper 1
MFC	Minimal Fungicidal Concentration
ATP	Adenosine triphosphate
MIC	Minimum Inhibitory Concentration
HPA	Hypothalamicpituitary-adrenal
ECM	Extracellular matrix
MCP-1	Monocyte Chemoattractant Protein-1
PBMC	Peripheral Blood Mononuclear Cells
CCD	Canine Cognitive Dysfunction
GSH	Glutathione
NFB	Neurofeedback
TERT	Telomerase reverse transcriptase
LH	Luteinizing Hormone
Bcl-2	B-cell lymphoma-2
FSH	Follicle-Stimulating Hormone
p-Akt	Phospho-Akt
GnRH	Gonadotropin-releasing hormone
Bcl-xl	B-cell lymphoma-extra-large
STAT-2	Transducer and activator of transcription 2
HSP70	Heat shock protein 70
ER+	Estrogen receptor-positive
VEGF	Vascular Endothelial Growth Factor
ER-	Estrogen receptor-negative

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