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Health benefits of Ashwagandha (*Withania somnifera*): A comprehensive review of its therapeutic potential

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Abstract
Ashwagandha (*Withania somnifera*) is a widely recognized medicinal herb with a long history of use in traditional systems of medicine, particularly Ayurveda. Ashwagandha has gained increasing attention for its potential therapeutic properties. Studies have revealed that Ashwagandha exhibits adaptogenic qualities, aiding the body in managing stress and promoting overall well-being. It possesses anti-inflammatory, antioxidant, immunomodulatory, and anti-cancer properties, which contribute to its diverse therapeutic effects. Ashwagandha has shown promise in reducing anxiety, improving cognitive function, enhancing physical performance, and stamina. It has demonstrated potential in managing metabolic disorders, such as diabetes and obesity, by regulating blood glucose levels, improving insulin sensitivity, and modulating lipid profiles. The herb also exhibits hepatoprotective effects and has been investigated for its potential in preventing and treating liver diseases. Studies have indicated cardio-protective effects of Ashwagandha, including lowering blood pressure, reducing cholesterol levels, and protecting against oxidative stress. Furthermore, it has positive effects on reproductive health, including fertility enhancement and improving sexual function. While most studies have been conducted on animal models and *in vitro* systems, clinical trials have provided evidence supporting the efficacy and safety of Ashwagandha supplementation in humans. The present study mainly focusing on Immunomodulatory, anti-cancer, anti-diabetic, anti-microbial and cardio protective effect of Ashwagandha.

Keywords: Anti-inflammatory, antioxidant, immunomodulatory

Introduction
Withania somnifera (L.) Dunal, a member of the Solanaceae family, is widely recognized as "Ashwagandha," a term originating from its traditional medicinal use in India. Within Ayurvedic medicine, Ashwagandha holds a prominent position as a Rasayana herb, renowned for its potential to revitalize the body and enhance the health of various tissues. This classification as an adaptogen further characterizes Ashwagandha indicating its ability to promote overall body balance and homeostasis through a diverse range of pharmacological mechanisms and complex responses. Notable attributes attributed Ashwagandha encompass improved concentration, memory, and mood, along with bolstering resilience against pathogens and diseases (Speers *et al*., 2021) [1].

Table 1: Scientific classification of Ashwagandha (*Withania somnifera*).

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	Withania
Species	somnifera

Ashwagandha, commonly referred to as Indian Ginseng, is a shrub plant from the Solanaceae family. Traditional Indian Ayurvedic medicine has relied on Ashwagandha for numerous centuries to address various health concerns. Its historical uses encompass aiding in sleep, reducing inflammation, addressing sexual issues, repairing nerve tissue damage, managing stress and anxiety, alleviating insomnia, and treating other ailments (Deshpande *et al*., 2020) [2]. The roots of Ashwagandha contain a total of 35 chemical constituents. Among these, the biologically active compounds include alkaloids such as iso pelletierine and anaferine, steroidal lactones such as withanolides and withaferins, saponins with an additional acyl group like sitoindoside VII and VIII, as well as withanoloides with glucose at carbon 27, including sitonidoside XI and X. Furthermore, Ashwagandha is abundant in iron. The primary components found in the roots of Ashwagandha are withanolides, which are believed to contribute to its remarkable medicinal properties (Bharti *et al*., 2016) [4].

Plant part	Bioactive compound	reference
Roots 	Withanolides, Withaferins	(Kaur *et al*., 2013) [6]
Leaves 	Alkaloids, Flavonoids	(Rault *et al*., 2012) [7]
Seeds 	Fatty Acids, Sterols	(Rani *et al*., 2016) [8]
Berries 	Withanosides, Polyphenols	(Jahan *et al*., 2017) [9]
Stem 	Triterpenoids, Sitoindosides	(Parveen *et al*., 2012) [10]

The plant is a vertically growing, grayish evergreen shrub with elongated tuberous roots, short stems, oblong leaves with petioles, and greenish bisexual flowers that emerge in the leaf axils. While it thrives in arid regions of India, it is also capable of growing at high altitudes reaching up to 1700 meters in the Himalayan region, particularly in Himachal Pradesh, Uttarakhand, and Jammu and Kashmir. Among the 23 identified species of Withania, only *Withania somnifera* and Withania Coagulans (S) Dunal, commonly known as W. coagulans or Rishyagandha, are recognized for their potential therapeutic properties (Bhat *et al*., 2022) [3].

The utilization of plants for therapeutic purposes dates to 4000-5000 BC, with the Chinese being the pioneers in using herbal remedies. However, the ancient Indian text Rigveda, believed to have been written between 3500 and 1600 BC, contains the earliest references to the medicinal use of plants in India. Subsequently, ancient physicians in Ayurveda, the oldest medical profession in India, extensively explored and documented the qualities and therapeutic benefits of medicinal plants through empirical investigations. (Bharti *et al*., 2016) [4]. In India, the cultivation Ashwagandha, holds great significance in meeting the increasing demand for medicinal plants used in pharmaceutical applications. However, conventional propagation methods have proven inadequate in satisfying the global requirement for Ashwagandha due to various challenges. The low viability of seeds and the limited growth period of one year pose significant obstacles to meeting the demand and maintaining storage supplies. Additionally, the plant faces additional constraints such as poor germination, seed rot, and blight diseases that further reduce its population. Therefore, the cultivation and propagation of Ashwagandha in India, as an essential medicinal plant, face considerable difficulties, hindering its ability to fulfil the high global demand for this valuable herb (Namdeo *et al*., 2021) [5].

Immunomodulatory effect

According to research findings, Ashwagandha has been identified as a substance with the potential to impact different facets of immune function, which include both innate and adaptive immunity (Tharakan *et al*., 2021) [11]. This plant, known as Ashwagandha, holds significant value in Indian traditional medicinal practices such as Ayurveda and Unani. It is highly regarded for its root and herb, which are utilized as tonics, hypotonic, sedatives, and diuretics (Srivastava *et al*., 2018) [12].

Ashwagandha is recognized as a significant herbal Rasayana and is commonly referred to as "Sattvic Kapha Rasayana." Rasayana refers to a herbal or metallic preparation that possesses various pharmacological properties, including aphrodisiac, adaptogenic, diuretic, anti-helminthic, astringent, tonic, narcotic, immune-stimulating, anti-inflammatory, anti-stress, rejuvenative, rheumatism-relieving, goiter-alleviating, boils and pimples treating, piles relieving, flatulent colic soothing, oligospermia addressing, health-promoting, leucoderma managing, constipation alleviating, insomnia combating, nervous breakdown addressing, snake venom neutralizing, and scorpion sting mitigating effects (Deepa *et al*., 2020) [35]. Ashwagandha holds significant importance as an herbal rasayana and is referred to as "Sattvic Kapha Rasayana." Rasayana refers to herbal or metallic preparations with diverse pharmacological properties, including aphrodisiac, adaptogenic, diuretic, anti-helminthic, astringent, tonic, narcotic, immune-stimulating, anti-inflammatory, anti-stress, rejuvenative, rheumatism, goiter, boils, pimple, piles, flatulent colic, oligospermia, health-promoting, leucoderma, constipation, insomnia, nervous breakdown, snake venom, and scorpion sting remedies (John 2014) [13].

The suppression of cyclophosphamide-induced potentiation of delayed type hypersensitivity reaction was observed in animals treated with three immunosuppressive drugs: azathioprine, cyclophosphamide, and prednisolone (Deepa *et al*., 2020) [35]. Cyclophosphamide is well known anticancer drug used for the treatment of several types of cancers. In combination with other drugs cyclophosphamide is prescribed to treat breast cancer, leukaemia, and ovarian cancer. But cyclophosphamide reduces the production of blood cells from the bone marrow (Kim *et al*., 2017) [14]. cyclophosphamide itself a immunosuppressant drug and cancer patients also suffer from immunity hence it reduces the production of platelets, number of red blood cells and number of white blood cells. Cancer chemotherapy drugs are immunosuppressant's, cytotoxic, and exert variety of side effects. Hepatoprotective protective activity of Ashwagandha. In experiments conducted on Balb/c mice, the administration of Ashwagandha extract resulted in a significant increase in white blood cell (WBC) counts and platelet counts. The treatment involved the use of Ashwagandha root extract at a dosage of 20 mg/kg, administered intraperitoneally (i.p.). These experiments demonstrated that the Ashwagandha root extract had the ability to stimulate immunological activity in the mice. Furthermore, when the mice were treated with five doses of Ashwagandha root extract, it led to enhancements in WBC counts, bone marrow cellularity, the number of alpha-esterase positive cells, circulating antibody titers, and the number of plaque-forming cells in the spleen. Additionally, the extract exhibited inhibitory effects on delayed type hypersensitivity reactions and improved the phagocytic activity of peritoneal macrophages in mice (Deepa *et al*., 2020) [35]. steroidal lactone, known as Withaferin A. along with the whole extract of Ashwagandha, possesses antioxidant and anti-inflammatory properties that help counteract the oxidative stress caused by ZnO (Zinc oxide) nanoparticles (NPs). Moreover, the administration of WA and WS demonstrates potential in restoring macrophage activity, thereby mitigating the cytotoxic effects induced by ZnO NPs in male Balb/c mice. The immuno-protective properties of WS provide defense against oxidant-induced cellular damage by ZnO NPs. As evidenced in our study, Ashwagandha can be considered as a phytomedicine to counter the toxicity associated with ZnO NPs. Furthermore, it is worth noting that our research indicates that ZnO NPs exhibit toxicity only at a dosage of 50 mg/kg/b.w. in the mouse model (Kumar *et al*., 2019) [15].

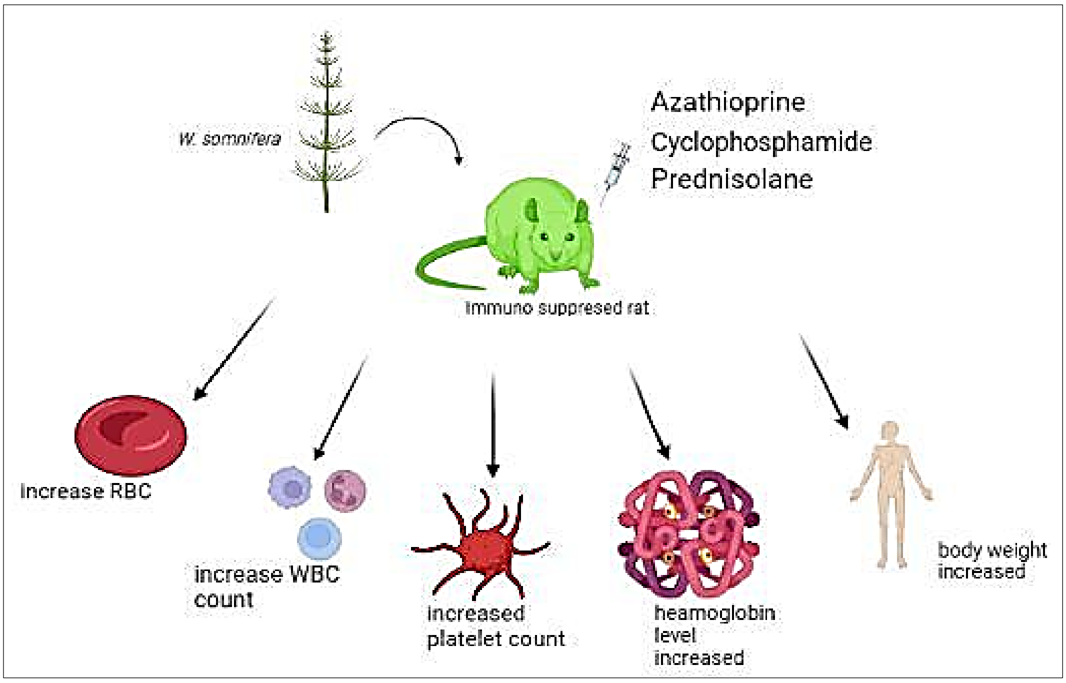

Fig 1: immunomodulatory effect of ashwagandha on immunosuppressed rat treatment with W. somnifera extract exhibited beneficial effects on blood parameters, weight, and leukopenia induced by cyclophosphamide or gamma radiation. Furthermore, ashwagandha extract showed potential in mitigating cyclophosphamide-induced immunosuppression, as evidenced by its effects on delayed-type hypersensitivity reactions. (Deepa *et al*., 2020) [35].

Anti-cancer effect

The major bioactive compounds found in W. somnifera, namely withaferin A (WA) and withanolide D, play a significant role in its biological effects, including its anti-cancer properties. Research has shown that these withanolides, derived from W. somnifera, have demonstrated effectiveness in treating various types of cancers, such as colon, mammary, lung, prostate, skin, blood, liver, and kidney cancers (Sherawat *et al*., 2017) [16]. The anticarcinogenic potential of these compounds, particularly WA and withanolide D, has been highlighted. Notably, WA has been identified as a novel inhibitor of nuclear factor kappa β (NFκ), which is crucial for cancer progression. The suppression of NFκ activity by WA suggests its potential as an anti-cancer agent in the treatment of different types of cancers (Deepa *et al*., 2020) [35].

withaferin A is an active steroidal lactone produced from the herb Ashwagandha (demonstrates anticancer action through regulating reactive oxygen species (ROS) In several cancer types, including breast cancer, lung cancer, and pancreatic cancer (Xia *et al*., 2018) [17]. Withaferin A, along with its analogues, has the ability to act as inhibitors of p97 chaperones, thus affecting proteostasis and demonstrating cytostatic activity *in vitro* (Dutta *et al*., 2019) [18]. The proliferation of various cancer cells can be inhibited by Withaferin A, primarily through the induction of G2/M-phase cell cycle arrest. This compound has been observed to induce G2/M phase arrest in human prostate cancer cells (PC-3 and DU-145) by increasing the phosphorylation of Wee-1, histone H3, p21, and Aurora kinase-B, as well as downregulating cyclins and reducing Cdc2 phosphorylation (Lee *et al*., 2016) [19]. Activation of Cdc2 triggers cell cycle arrest in the M phase and initiates mitotic catastrophe (Roy *et al*., 2013) [21].

Treatment with Withaferin-A reduces the development of breast tumours by preventing the production of the vimentin protein and disrupting the cytoskeletal architecture of β-tubulin (Deepa *et al*., 2020) [35]. The G2 and mitotic arrest brought on by Withaferin-A exposure in MCF-7, SUM159, and SK-BR-3 cells was correlated to a pronounced drop in β-tubulin protein levels in both MCF-7 cells and a cell-free system, WA covalently binds to Cys^{303} of β-tubulin. The ability of WA to covalently modify the cysteine residues in the b-tubulin protein is yet unknown. Molecular docking simulations, however, verified the stability of the Cys^{303}-WA adduct in the pocket (Antony *et al*., 2013) [22].

Withanolide D (C4b-C5b, C6b-epoxy-1-oxo-,20b, dihydroxy20S,22R-witha-2,24-dienolide; WithaD) is a steroidal lactone isolated from the leaves of Ashwagandha. Withanolide D increases ceramide formation by activating N-SMase 2, modifies JNK and p38MAPK in phosphorylation, and induces death in myeloid and lymphoid cells as well as primary cells obtained from patients with leukaemia (Mondal *et al*., 2012) [23]. Unrestrained proliferation of immature myeloid cells and bone marrow failure are two features of the heterogeneous clonal illness known as leukaemia (Saultz *et al*., 2015) [24]. The extract of Ashwagandha, withanaloid D, induced apoptosis in both myeloid (K562) and lymphoid (MOLT-4) cells and inhibited tumour cell growth in K562 xenografts through ceramide accumulation by activating neutral sphingomyelinase (N-SMase), which in turn modulated the phosphorylation of stress kinases, c-Jun N-terminal and p^{38} MAPK guiding to apoptosis in these cells. (Guerreiro *et al*., 2019) [25]. Withanaloid-D function on the pancreatic carcinoma cells leads to the destruction in β-catenin, which is a useful in organogenesis and oncogenesis (Rai *et al*., 2016) [26].

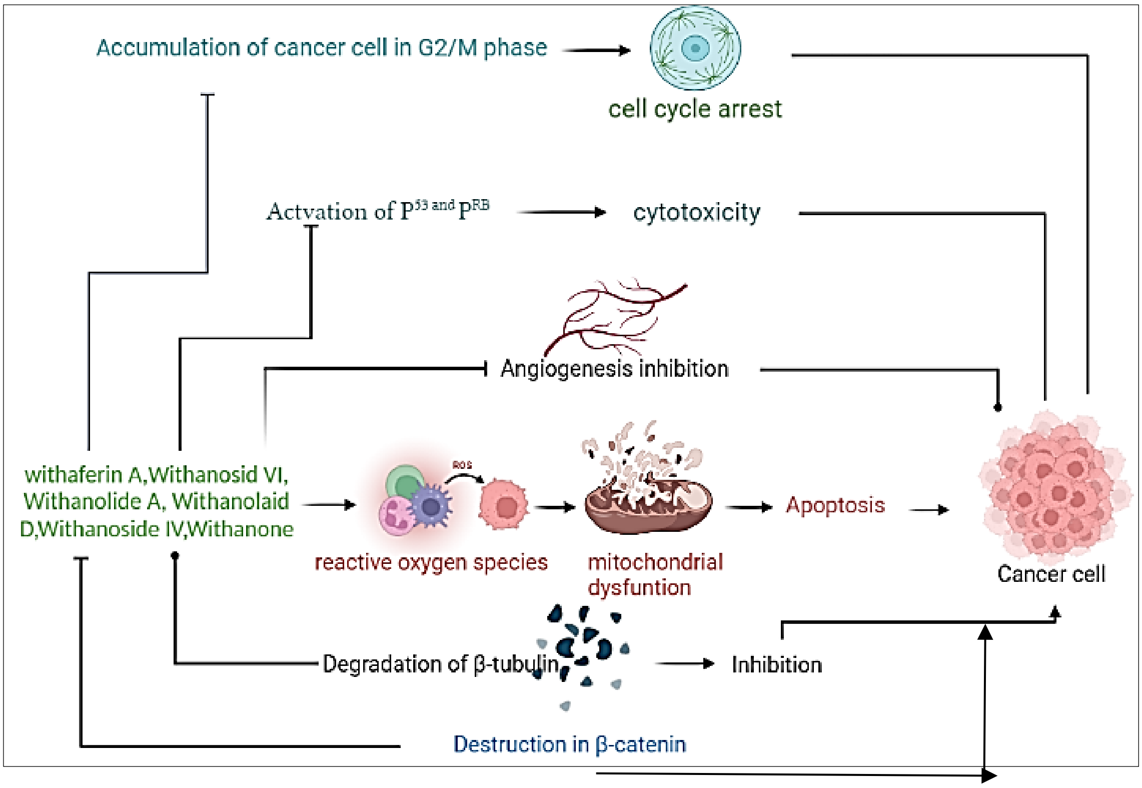

Fig 2: Potential mechanisms of action for withanolides

Anti-diabetic effect

Withania somnifera is a key plant in Indian medical systems that has anti-diabetic properties. Many Ayurvedic polyherbal formulas, including Dianix and Trasina, showed blood sugar-lowering effectiveness in human test participants (Deepa *et al*., 2020) [35] Based on glucose absorption in skeletal myotubes, six withanolides isolated from W. somnifera were examined for anti-diabetic action. The fact that withaferin A increases glucose uptake—10 M produced a 54% increase over control—indicates that withaferin A is at least largely responsible for Ashwagandha anti-diabetic effect (Gorelick *et al*., 2015) [27]. Through enzymatic and non-enzymatic antioxidant processes, W. somnifera root and leaf extracts significantly stabilized blood glucose, urine glucose, glucose-6-phosphatase, and tissue glycogen levels in rats with diabetes mellitus caused by alloxan (Deepa *et al*., 2020) [35].

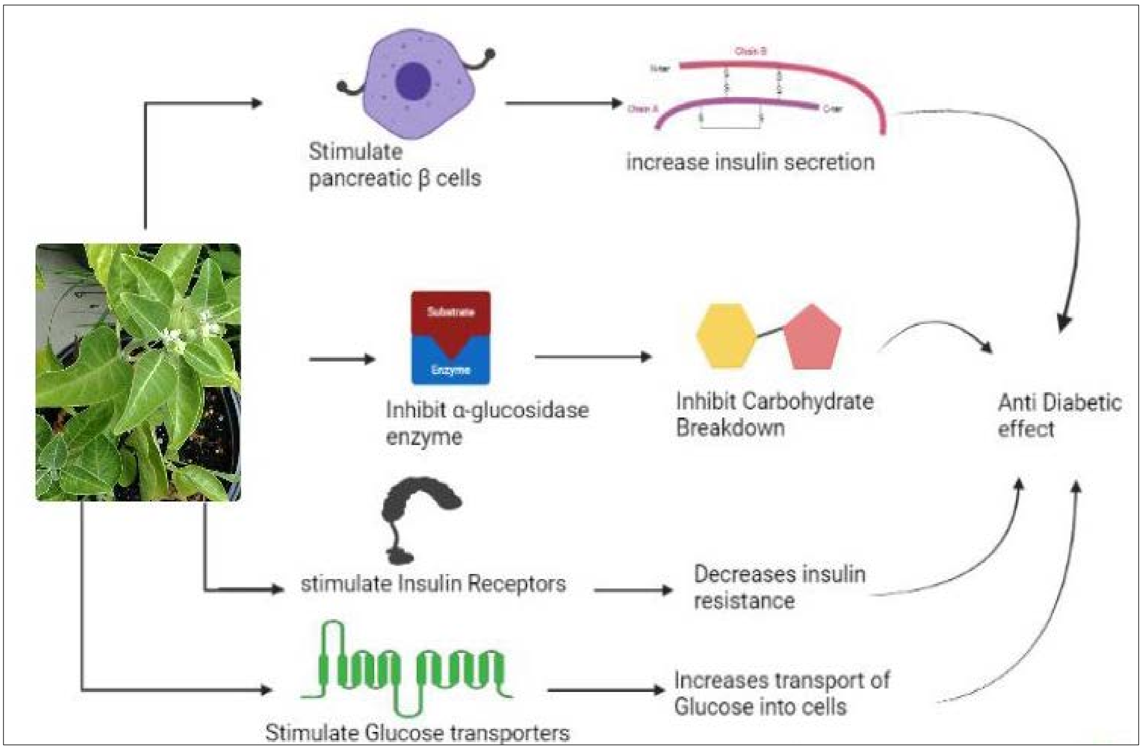

Fig 3: Anti-diabetic effect of Ashwagandha

Anti-microbial Activity

The requirement to test medicinal plants for potential antibacterial activity has arisen from the growing failure of chemotherapeutics and antibiotic resistance showed by microbial infections. Ashwagandha has been found useful in treatment of microbial diseases (Santhi *et al*., 2011) [28]. Ashwagandha extract was subjected to testing against various bacterial strains and fungi. The bacterial strains included Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The fungi tested were Aspergillus Niger, Aspergillus flavus, Candida albicans, Candida tropicalis, Cryptococcus neoformans, and Candida kefyr. The results indicated that different extracts, such as ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane, and aqueous extract, exhibited anti-bacterial and anti-fungal properties. Specifically, the ethanol extract of Ashwagandha demonstrated higher efficacy against Staphylococcus aureus (Velu *et al*., 2012) [29].

Ashwagandha shows inhibitory activity on fungal growth by supressing spore germination and hyphal growth, A glycoprotein present ashwagandha is responsible for this fungistatic property in different species like Aspergillus flavus, *Fusarium oxysporum*, and *Fusarium verticillioides* (Deepa *et al*. 2020, Dar *et al*., 2015) [35, 20]. With a minimum fungicidal dosage (MFC) of 0.039 and MIC of 0.039, flavonoids derived from Ashwagandha have been observed to be useful over Candida albicans (Dar *et al*., 2015) [20].

Ashwagandha exhibits potent antibacterial activity, which can be attributed to the presence of several bioactive constituents (Jeyanthi *et al*., 2013) [30]. Notably, Withanolide A and withaferin A are two secondary metabolites that play a significant role in the bioactive properties of Ashwagandha. Additionally, Ashwagandha contains various therapeutic compounds, including 12-deoxywithastramonolide, 12-deoxy withanstramonolide, withanoside-IV, withanoside-V, ashwagandhanolide, withanone, withanolide-B, withanolide-D, withanolide-E, pseudo-withanine-27-hydroxywithanone, somnine, 20-deoxywithanolide A, somniferinine, withastramonolide, somniferine, and tropine (Ajay *et al*., 2020). n vitro studies have shown that Withanolides induce a death mechanism similar to apoptosis in Leishmania donovani. This effect is achieved by inducing DNA nicks, causing cell cycle arrest at the sub G0/G2 phase, and leading to outward manifestations of phosphatidylserine. These effects occur in a dose- and time-dependent manner and are accompanied by an increase in reactive oxygen species (ROS) and a decrease in mitochondrial potential. Additionally, the protein kinase-C signalling pathway is blocked, contributing to the observed effects (original study citation is needed for verification) (Dar *et al*., 2015) [20]. Dental caries is an oral condition related to dental biofilms and excess dietary sugar consumption. Bacteria are able to convert dietary carbohydrates into organic acids and can resist abrupt and significant changes in the pH These bacterial traits cooperate to enable *Streptococcus mutans* and *Streptococcus sobrinus* to successfully colonise the surface layer of tooth and it cause dental diseases. Methanol extract of Ashwagandha inhibited acid production, acid tolerance, and biofilm formation of *Streptococcus mutans* and *Streptococcus sobrinus* (Pandit *et al*., 2013) [31].

Cardio protective effect

Ashwagandha, a medicinal herb, has been demonstrated to possess cardio-tropic and cardio-protective properties in both preclinical and clinical studies. Notably, when Ashwagandha is included as a constituent in polyherbal formulations, it has shown promising cardio-protective effects in animal models. These effects are attributed to the activation of nuclear factor-erythroid-2-relatetranscription factor (Nrf)2, a transcription factor involved in cellular defence mechanisms. Activation of Nrf2 by Ashwagandha leads to the stimulation of phase-II detoxification enzymes, which play a crucial role in eliminating harmful substances from the body. Moreover, Ashwagandha has been found to abrogate the apoptosis process, a form of programmed cell death that can contribute to cardiovascular diseases. By interfering with apoptosis, Ashwagandha helps in preserving the integrity and function of cardiac cells, further supporting its cardio-protective activity. (Mandlik *et al*., 2021) [32]. The use of Ashwagandha extracts in prophylactic treatment has been found to effectively preserve the equilibrium between oxidant and antioxidant levels. (Ashour *et al*. 2012) [33], In a rat model of coronary artery occlusion, the administration of Ashwagandha extracts resulted in a reduction in histopathological damage to the myocardium. This protective effect was observed through the measurement of anti-apoptotic activity using the terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) assay. Additionally, the standardized extract of Ashwagandha demonstrated inhibition of doxorubicin-induced cardio-toxicity by mitigating the biochemical changes occurring in the rat myocardium. (Deepa *et al*., 2020) [35]. When rats were orally pre-treated with a 100 mg/kg dose of Ashwagandha extract for a period of 4 weeks, a notable cardio-protective effect was observed. This was evident by a significant decrease in cardiac troponin-I level, lipid peroxidation (LPO) level, and lipid profiles, as well as a decrease in cardiac marker enzymes. Additionally, there was an increase in the activity of antioxidant enzymes in the rat myocardium. These findings suggest that the cardio-protective activity of Ashwagandha extract in this model may be attributed to two potential mechanisms. Firstly, it may enhance the endogenous antioxidant system, leading to improved defence against oxidative damage. Secondly, it may inhibit LPO in the myocardial membrane, further contributing to the preservation of cardiac health. (Khalil *et al*.,. 2015) [34].

Future perspective of Ashwagandha

Ashwagandha (*Withania somnifera*), a revered herb in traditional medicine, holds great importance in the medicinal field due to its diverse health benefits. Looking ahead, there is a promising future for Ashwagandha in both the food industry and pharmaceutical field. In the food industry, Ashwagandha has the potential to be utilized as a functional ingredient due to its adaptogenic properties and rich nutritional profile. It can be incorporated into various food and beverage products to promote overall well-being and stress management. With its antioxidant and anti-inflammatory properties, Ashwagandha can enhance the nutritional value of functional foods, offering consumers a natural and holistic approach to improving their health. Furthermore, its adaptogenic qualities may contribute to increased energy levels, improved cognitive function, and reduced anxiety, making it an appealing ingredient for products targeting mental wellness and stress reduction. In the pharmaceutical field, the therapeutic potential of Ashwagandha continues to be explored. The herb has already shown promising results in preclinical and clinical studies for various conditions such as diabetes, cancer, cardiovascular diseases, and neurodegenerative disorders. As research advances, more targeted and precise formulations can be developed to harness the full potential of Ashwagandha's bioactive compounds. These formulations may include standardized extracts, isolated compounds, or nano-formulations to enhance their efficacy and bioavailability. Additionally, with further investigation into the mechanisms of action and identification of specific molecular targets, Ashwagandha-based drugs or therapeutic interventions may be developed to address specific diseases or conditions. Moreover, the utilization of Ashwagandha in combination therapies or as an adjuvant to conventional treatments holds promise. Its synergistic effects with other natural compounds or drugs may enhance treatment outcomes and reduce potential side effects. By leveraging Ashwagandha's multi-faceted properties, novel therapeutic approaches can be developed to tackle complex diseases and improve patient outcomes.

The future of Ashwagandha holds significant potential in both the food industry and pharmaceutical field. With its proven health benefits, the herb can be incorporated into functional foods and beverages to promote wellness and stress management. Additionally, further research and development in the pharmaceutical field may lead to the creation of targeted formulations, combination therapies, and novel interventions to address a wide range of health conditions. As scientific understanding deepens, Ashwagandha is poised to play a pivotal role in the advancement of natural therapeutics and holistic health solutions.

Conclusion

The review of on Ashwagandha's health benefits reveals its potential as a valuable therapeutic agent in the realms of diabetes management, cancer treatment, cardio protection, and immunomodulation. Ashwagandha exhibits anti-diabetic properties by regulating glucose levels and improving insulin sensitivity. Its anti-cancer effects are attributed to its ability to inhibit tumour growth, induce apoptosis, and mitigate metastasis. Additionally, Ashwagandha shows promise in protecting the heart from damage, reducing blood pressure, and modulating lipid profiles, thus demonstrating cardioprotective potential. Moreover, its immunomodulatory properties contribute to enhanced immune response and protection against various diseases. These findings highlight Ashwagandha as a multifaceted medicinal herb with significant potential for therapeutic application, calling for further research and exploration to fully understand its mechanisms of action and optimize its clinical utility in these areas.

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