

Antiviral, Anticancer and Immunoboosting Efficacy of Ashwagandha (*Withania somnifera* L.) with Special Reference to COVID-19

Narendra Kumar¹, Awad Y Shala² and SM Paul Khurana^{3*}

^{1, *3} Amity Institute of Biotechnology, Amity University Haryana, Manesar, Gurgaon, Haryana, India

² Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

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ABSTRACT

Currently novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2/2019- nCoV) pandemic has resulted in millions of deaths around the globe. *Withania somnifera* (L.) Dunal is an effective popular medicinal plant. This is locally called Ashwagandha or Indian Ginseng. The People of India call it 'Queen of Indian herbs'. This is the storehouse of antiviral activity which calls for discovering new strategies for fighting serious virus infections. This article summarizes the antiviral and Immuno boosting efficacy of the plant for exploiting its potential as a great (natural) antiviral agent in modern medicine for combating various virus diseases. The study records it as a “*Rasayana*” (=drug, which reduces the blood sugar levels). This is rich in biochemicals and found highly useful for thousands of years for human health. This promotes the death of tumor cells. It lowers cortisol, reduces stress, reduces depression, increases testosterone levels, boosts sperm quality, and thereby fertility in men. This increases muscle mass, reduces body fat, and increases virility/ strength in men. It increases natural killer cell activity and decreases markers of inflammation. It helps reduce the risk of heart disease by decreasing cholesterol and triglyceride levels, improves brain function, memory, reaction time, and the human ability to perform tasks. It is an elixir and truly a great Immuno- booster. *W. somnifera* has a distinct effect on the viral receptor-binding domain (RBD) and host angiotensin-converting enzyme 2 (ACE2) receptor complex. *W. somnifera* may now be the first choice of herbs in this direction and to curb the COVID-19 infection. Due to many medicinal values, it can serve as a miraculous drug and/or a great health supplement.

Keywords: *W. somnifera*, Antiviral, Anticancer, Withanolides, Withaferin A, Immuno booster

INTRODUCTION

Medicinal plants are rich sources of secondary metabolites that can cope with the disease problems of human beings. One of the most potent plants is Ashwagandha (*Withania somnifera* (L.) that has been in use for over 5000 years. It has been concluded from studies undertaken that this plant possesses various medicinal properties contributing to many health benefits [1]. *Withania somnifera* (L.) Dunal (Solanaceae), commonly known as Ashwagandha, is one of the most valued medicinal plants of the traditional Indian systems of medicines, used in more than 100 formulations of Ayurveda, and is thought to be therapeutically equivalent to Ginseng [2]. *W. somnifera* has been used as an antiviral herb for the treatment of genital disease caused by Herpes Simplex Virus among African tribes [3], and shown to have anti-influenza properties [4]. The present study aims to review the antiviral potential of *W. somnifera* ingredients against COVID-19.

The current Coronaviruses pandemic has great importance. The infectious strains of the virus, originally named based on their 'Crown like appearance', due to the glycoprotein projections/spikes on its envelope, as seen under the electron microscope and grouped into the family Coronaviridae; order Nidovirales. They invade the respiratory tract via the nose. After an incubation period of about 3-7 days, they cause the symptoms of a mild common cold/bronchitis (nasal obstruction, sneezing, runny nose, cough, headache,

Corresponding author: SM Paul Khurana, Amity Institute of Biotechnology, Amity University Haryana, Manesar, Gurugram, India, Tel: +919650484759; E-mail: smpaulkhurana@gmail.com

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fever, pneumonia, asthenia, and inflammation in the airway) in avian and mammalian species.

In contrast to animals, wherein they have been shown to infect several tissues causing a large variety of diseases, mainly human respiratory infections with mild common cold-like symptoms, with occasional gastrointestinal problems have been reported. The infected individuals shed virus in nasal secretions and mucosa resulting in its transmission that can often be controlled, at least partially, by following hygienic measures. Vaccines for coronaviruses are not yet available and treatment remains only symptomatic. Designing and development of antiviral medicines from herbal sources is the need of the time. Among the several strains of coronaviruses are known so far, including HCoV229E, HCoV-OC43, HCoV-NL63, SARS-CoV, MERS-CoV, and 2019-nCoV/SARS-CoV-2 [5-7] the latter was designated as a novel strain of coronavirus that caused pneumonia outbreak in Wuhan city of China in December 2019 (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020).[8,9] As of May 14, 2020, it has infected over 4,258,666 individuals globally with 294,190 deaths, as reported to WHO (WHO, 2020). It has been declared as an international public health emergency and advocated for rapid research efforts.

Indian Ayurvedic herb, Ashwagandha (*W. somnifera*) has been heavily used in traditional home medicine systems. known to boost the immune function, possess a variety of prophylactic and therapeutic activities [10]. Besides, Withaferin-A, one of the withanolides from Ashwagandha, was shown to possess inhibitory activity for HPV and influenza viruses [4].

W. somnifera, commonly known as "Indian ginseng" or "ashwagandha", is popular as a functional food because of its diverse purported therapeutic efficacies including invigorating, improvement of cognitive ability, and relieving stress [11]. The roots are the most commonly used part and find enormous medicinal uses. The powder of the roots is the key ingredient of almost any antioxidant, antistress, and antiaging formulation for human or veterinary uses in India. The root powder and its preparations are consumed extensively as a functional food for promoting vitality and virility. The plant is cultivated in the states of Madhya Pradesh, Uttar Pradesh, Punjab, Gujarat, and Rajasthan. India produces more than 1500 only tonnes of *Withania* roots which is much less than meeting the industry requirements. Thus, a need arises for the enhancement of its cultivation and productivity [12].

Studies indicate that *Withania* has immuno-modulatory, and more vital properties besides positively influencing the other metabolic systems, and also acts on anti-serotogenic and arthritis [13]. *Charaka Samhitā* describes Ashwagandhā in the treatment of liver diseases. *W. somnifera* being a multidrug constituent needs to be extensively studied. The

plant extract contains many bioactive compounds: Alkaloids, withanolides, and several sitoindosides which have been reported to be present in the roots. It also contains withanolides (withaferin A and withanolide D) [14]. Seeing its need and popularity in the present pandemic of COVID - 19 for getting informed about its power and health-boosting capacity, the present study reviews its antiviral and also health-boosting powers. It records how it is useful as a health tonic, power booster, and in reducing blood sugar levels, promoting the death of tumor cells, lowering cortisol, reducing human stress, depression, increase testosterone levels, etc. This also deals about how it helps lower risk of heart diseases (decreasing cholesterol and triglyceride levels), improves brain function, memory, reaction time, etc.

MATERIALS AND METHODS

The comprehensive literature searches were conducted for Antiviral, anticancer, and Immuno boosting efficacy of Ashwagandha through searching PubMed, SCOPUS, and CINAHL Google Scholar, Science Direct, Medline, Embase, Cochrane Library, and Indian Medical databases. The Search terms covered "ashwagandha," "*Withania somnifera*," related to antiviral, phytochemical, anticancer, anxiety, and stress, increases virility/ strength natural killer cell activity inflammation. decreasing cholesterol and triglyceride levels, improves brain function, memory, reaction time, Immuno booster for COVID-19 infection. More efforts were done through Google Scholar searches of Ashwagandha relation in terms of anxiety disorders and searched for its various attributes on AYUSH Research Portal.

BOTANY AND SIGNIFICANT CHEMICAL OF WITHANIA SOMNIFERA

W. somnifera is a small and woody shrub belongs to the Solanaceae family. This grows about 35-75 cm in height. It has observed growing in Africa, the Mediterranean, and India. An erect, evergreen, tomentose shrub, have been found throughout the drier parts of India in waste places and on bunds. Roots look stout fleshy, whitish brown; leaves simple ovate, glabrous, those in the floral region smaller and opposite; flowers inconspicuous, greenish/ lubrid-yellow, in axillary or umbellate cymes; berries small, globose, orange-red when mature. This is enclosed in the persistent calyx; seeds yellow, reniform. The roots being the main portions of the plant taken therapeutically. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring. Parts used: Whole plant, roots, leaves, stem, green berries, fruits, seeds, and bark [15].

The major chemical constituents of the *Withania* genus, the withanolides, are a group of naturally occurring C28-steroidal lactone triterpenoids built on an intact or rearranged ergostane framework. Where C-22 and C-26 are appropriately oxidized to form a six-membered lactone ring. In recent years, numerous pharmacological investigations

have been carried out into the components of *W. somnifera* extracts. (Figure 1).

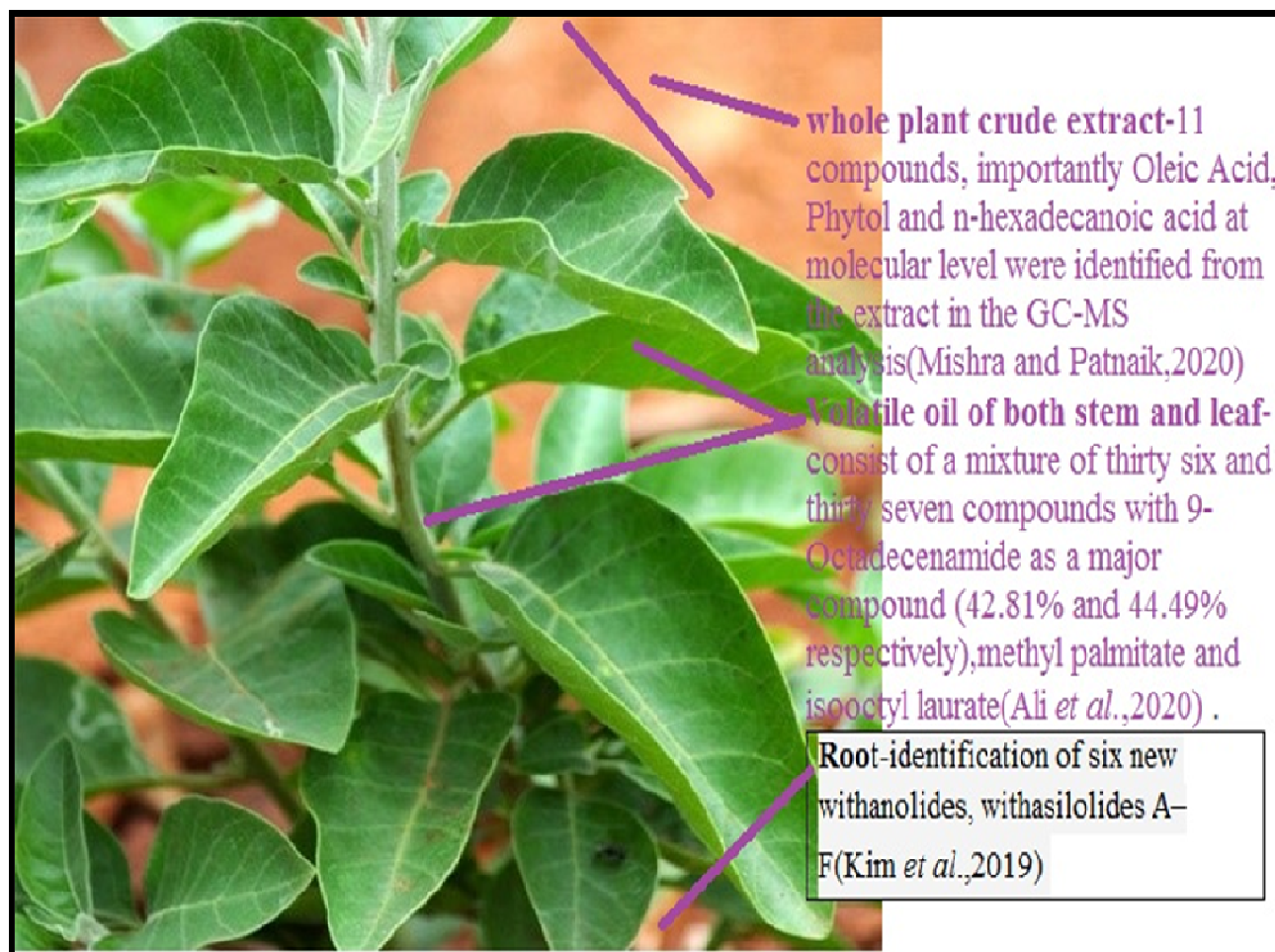


Figure 1. Extracts of *W. somnifera*.

ANTIVIRAL ACTIVITY

A lot of viruses are known which afflict humankind. The antiviral activity of this plant is recorded in **Table 1**. This is the time of COVID-19. Coronaviruses, discovered in 1960, are infectious strains of viruses originally named based on their 'Crown- like appearance, due to the glycoprotein projections on its envelope, under the electron microscope and grouped into the family Coronaviridae; order Nidovirales. They invade the respiratory tract via the nose. After an incubation period of about 3-7 days, they cause the symptoms of a mild common cold/bronchitis (nasal obstruction, sneezing, runny nose, cough, headache, fever, pneumonia, asthenia, and inflammation in the airway) in avian and mammalian species. In contrast to animals, wherein they have been shown to infect several tissues causing a large variety of diseases, mainly respiratory infections with mild common cold-like symptoms,

occasional gastrointestinal problems (like diarrhea) have been reported for humans. The infected individuals shed virus in nasal secretions and mucosa resulting in disease transmission that can often be controlled, at least partially, by following hygienic measures. Vaccines for coronaviruses are not yet available (except the reported effect of MMR vaccine) and treatment remains symptomatic. Newly emerged COVID-19 has been shown to engage the host cell ACE2 through its spike protein receptor-binding domain (RBD). A natural phytochemical from a medicinal herb, *Withania somnifera*, has distinct effects on the viral receptor-binding domain (RBD) and host ACE2 receptor complex. Natural phytochemicals could well be the viable options for controlling COVID-19 entry into host cells. The antiviral power of *W. somnifera* may project these herbs as a number one in fighting to remove COVID-19 infections.

Table 1. Antiviral activity of *W. somnifera*.

Tested against virus	Results	References
COVID-19, molecular docking to screen thousands of phytochemicals against the ACE2-RBD complex,	<i>W. somnifera</i> compound, Withanone, docked very well in the binding interface of AEC2-RBD complex and was found to move slightly towards the interface center on simulation, incorporation of Withanone destabilized salt bridges and decreased their occupancies. interruption of electrostatic interactions between the RBD and ACE2 would block or weaken COVID-19 entry and its subsequent infectivity.	Balkrishna [16]
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2/2019- nCoV)	Natural compounds may possess the potential to inhibit the functional activity of SARS-CoV-2 protease (an essential protein for virus survival),	Kumar [17]
Chikungunya Virus	Chikungunya-infected mice got relieved of morbidity and joint swellings. Analysis of virus clearance in the brain and joint tissues on formulation treatment revealed a direct correlation of viral load in the brain to morbidity during the infection.	Jaspreet [18]
Infectious Bursal Disease (severe viral disease of chicken)	The root extract of the <i>W. somnifera</i> inhibits the Infectious Bursal Disease virus in vitro.	Ganguly [19]
Newcastle disease virus (NDV) belongs to the Paramyxoviridae family	Potential to improve the immunity of infected poultry birds	Raza [20]
H1N1 Influenza	<i>Withaferin A</i> (WA), has the potential to attenuate the neuraminidase of H1N1 influenza	Cai [4]
Against Infectious Bursal Disease Virus Replication	Hydro-alcoholic extract of <i>W. somnifera</i> roots showed the inhibition of virus at maximum 99.9 % in its highest nontoxic concentration, 25µg/ml in Cytopathic Effect Reduction Assay	Pant [21]
Herpes simplex virus	Antiviral activity of <i>Aloe ferox</i> and <i>Withania somnifera</i> aqueous extracts seen on HSV-1 in vitro. The extracts showed detectable activity at 1000µg/ml concentration against the virus in monolayers of Vero cell cultures	[3]
Herpes simplex virus	A potential ligand to target/inhibit DNA polymerase of the Herpes simplex virus	Grover [22]

IMMUNO BOOSTING ACTIVITY AND VARIOUS HEALTH BENEFITS

This is an ancient herbal medicine that may be projected as an adaptogen which means this may be helpful in metabolism and also managing body stress. It may provide numerous other benefits both for the body and brain like boosting brain function, lowers blood sugar and

cortisol levels. This is much useful in fighting symptoms of anxiety and depression.

Ashwagandha finds a place as the most important herb in Ayurveda. This places as an alternative medicine based on Indian principles of natural healing. Ashwagandha is Sanskrit stands for the smell of the horse. That refers to both its unique smell and ability to increase strength.

Because of its varied therapeutic potential, this has been the subject of considerable modern scientific attention. This project's tumor inhibition and antiangiogenic properties of withaferin A. The withanolide A have effects on Alzheimer's disease. So, it needs biotechnological production (of withanolides) [23,1].

Reduces Blood Sugar Levels

The studies support that ashwagandha lowers blood sugar levels. A study was done on the anti-diabetic activity of *W. somnifera* extract and purified withanolides, as well as the effect of various elicitors, evidenced that *W. somnifera* leaf and root extracts increased glucose uptake in myotubes and adipocytes in a dose-dependent manner. This was observed that leaf extract is more active in comparison to root extract [24]. Leaf but not root extracts increased insulin secretion in basal pancreatic beta cells. Six withanolides isolated from *W. somnifera* were tested for anti-diabetic activity based on glucose uptake in skeletal myotubes. Withaferin A increased glucose uptake, with 10 μ M producing a 54% increase in comparison to the control. This projects that withaferin A is at least partially responsible for anti-diabetic activity. Elicitors applied to the root growing solutions affected the physiological state of the plants. This is by altering membrane leakage or osmotic potential. Methyl salicylate and chitosan have increased withaferin A content by 75% and 69%, respectively. The extracts from elicited plants increased glucose uptake to a higher extent in comparison to non-elicited plants. This demonstrates a correlation between the increased content of withaferin A and the anti-diabetic activity. So, it increased insulin secretion and improved insulin sensitivity in muscle cells [24]. Andallu [25] investigated hypoglycemic, diuretic, and hypocholesterolemic effects of roots of *W. somnifera* (ashwagandha). Where six mild NIDDM subjects and six mild hypercholesterolemic subjects were treated with the powder of roots of *W. somnifera* for 30 days. The decrease in blood glucose levels was comparable to an oral hypoglycemic drug. There was a significant increase in urine sodium, urine volume. This showed a significant decrease in serum cholesterol, triglycerides, LDL (low-density lipoproteins), and VLDL (very low-density lipoproteins) cholesterol. This indicates that the root is a potential source of hypoglycemic, diuretic, and hypocholesterolemic agents. Clinical observations revealed no adverse effects.

Various human studies suggested that it may reduce blood sugar levels in both healthy and diabetic people [26,27]. The formulation appeared safe. This strengthened muscle activity. Because of its traditional Rasayana value, studies were planned for evaluating the potential of this drug in patients [26].

A randomized double-blind, placebo-controlled, clinical trial was conducted in the psychiatry OPD of a tertiary

care teaching hospital after approval of the Institutional Ethics Committee [27]. Schizophrenia patients receiving second-generation antipsychotics for 6 months or more, having serum triglycerides more than 150 mg/dl, high-density lipoprotein (HDL) cholesterol less than 40 mg/dl in men and less than 50 mg/dl in women, fasting blood glucose (FBG) level more than 100 mg/dl, aged above 18 years were included. Patients suffering from other psychiatric/systemic illnesses, receiving concurrent medicines, pregnant/lactating women were excluded. Patients were briefed about the study and written informed consent was obtained from those willing to participate. Baseline investigations (routine hematological, liver and kidney function tests, FBG, serum triglycerides, HDL cholesterol) were performed. Blood pressure and weight were recorded. Out of 30 patients, 3 from WS and 2 from the placebo group were lost to follow-up. There was however no change in the body weight and blood pressure after 1 month of treatment in both groups. Hypoglycemic and hypolipidaemic effects of WS observed in this trial are significant and deserve to be explored further [27].

Anticancer Properties

Animal and test-tube studies have found that withaferin helps induce apoptosis. That is the programmed death of cancer cells [28]. This impedes the growth of new cancer cells in several ways. This has been used in India for thousands of years in treating a variety of disorders. The WS which is rich in biochemicals is potentially useful in the prevention and treatment of cancer. The root and leaf extracts are shown to confer protection against chemically-induced cancers in experimental rodents. This retards tumor xenograft growth in athymic mice. The anticancer effect of *W. somnifera* is generally attributable to steroidal lactones. This is collectively referred to as withanolides. Withaferin A (WA) appears most active against cancer among structurally divergent withanolides. Which is isolated from the root or leaf of *W. somnifera*. The cancer-protective role for WA has now been established. This is by using chemically-induced and oncogene-driven rodent cancer models [28].

Firstly, withaferin is believed to promote the formation of reactive oxygen species (ROS) within cancer cells. Thus, disrupting their function. Secondly, it causes cancer cells to become less resistant to apoptosis [29]. Withaferin A (WA) which is a major bioactive component of the Indian herb *W. somnifera*, induces cell death (apoptosis/necrosis) in multiple types of tumor cells. They report here that 2 μ M WA induced cell death selectively in androgen-insensitive PC-3 and DU-145 prostate adenocarcinoma cells. Their toxicity was less severe in androgen-sensitive LNCaP prostate adenocarcinoma cells and normal human fibroblasts (TIG-1 and KD). WA also killed PC-3 cells in the spheroid-forming medium. DNA microarray analysis

suggested WA significantly increased mRNA levels of c-Fos and 11 HSPs (heat-shock proteins) in PC-3 and DU-145. But not in LNCaP and TIG-1. Western blot analysis revealed increased expression of c-Fos and reduced expression of the anti-apoptotic protein c-FLIP (L). Expression of HSPs such as HSPA6 and Hsp70 was conspicuously elevated. But siRNA-mediated depletion of HSF-1, an HSP-inducing transcription factor does a reduction in PC-3 cell viability. The heat-shock genes were involved in protecting against cell death. WA induced generation of reactive oxygen species (ROS) in PC-3 and DU-145 though not in normal fibroblasts. Immunocytochemistry and immuno-electron microscopy depicted WA disrupted the vimentin cytoskeleton. This is possibly by inducing ROS generation, c-Fos expression, and c-FLIP (L) suppression. These depict those multiple events followed by disruption of the vimentin cytoskeleton play pivotal roles in WA-mediated cell death [29]. The animal studies depict it can help in several types of cancers such as breast, lung, colon, brain, and ovarian [30]. The cancer-preventive activity of an extract of WS roots was examined in female transgenic (MMTV/Neu) mice which received a diet having WS extract (750 mg/kg of diet) for 10 months. Mice in the treated group (n=35) had an average of 1.66 mammary carcinomas while mice in the control group (n=33) had 2.48. This showed a reduction of 33%. The average weights of the carcinomas were 2.36 g for mice in the treated group and 2.63 g for the controls, a difference of 10%. Labeling indices for Ki67 and proliferating cell nuclear antigen marker in mammary carcinomas of the treated group were 35% and 30% lower, respectively than those of the corresponding control group. The expression of the chemokine was reduced by 50%. These results indicate that the root extract reduced the number of mammary carcinomas that developed and also reduced the rate of cell division in the carcinomas [30]. The therapeutic effects of WS along with paclitaxel on lung tumors induced through benzo (a) pyrene in male Swiss albino mice were studied. The study recorded that treatment displays a protective role through inhibiting free radical-mediated cellular damages. It depicts that WS along with paclitaxel provides stabilization of membrane-bound enzyme profiles and decreased lipid peroxidation against benzo (a) pyrene-induced lung cancer in mice [31].

GBM (Glioblastoma multiforme) is a gressive cancer. Neurosphere cultures (U87-MG, GBM2, and GBM39) were inhibited by AshwaMAX at IC₅₀ of 1.4, 0.19, and 0.22 μ M equivalent respectively and by Withaferin A with IC₅₀ of 0.31, 0.28, and 0.25 μ M respectively. Oral dosage, every other day, of AshwaMAX (40 mg/kg per day) significantly reduced bioluminescence signal (n = 3 mice, p < 0.02, four parameters non-linear regression analysis) in preclinical models. (After 30 days of

treatment), the bioluminescent signal increased which suggests an onset of resistance. BLI signal for control, vehicle-treated mice increased and then plateaued. Bioluminescent imaging revealed diffuse growth of GBM2 xenografts. With Ashwa MAX, GBM neurospheres collapsed at nanomolar concentrations. Oral treatment studies on murine models also revealed that AshwaMAX is effective against orthotopic GBM [32].

Mice with ovarian tumors treated with withaferin alone or in combination with an anti-cancer drug showed a 70-80% reduction in tumor growth. And complete inhibition of metastasis to other organs. Histochemical and Western blot analysis of the tumors revealed that WFA (2 mg/kg) resulted in a significant elimination of cells expressing CSC markers - CD44, CD24, CD34, CD117, and Oct4 and down regulation of Notch1, Hes1, and Hey1 genes and prevented the spread of cancer to other organs [33].

Reduces cortisol levels and stress/anxiety/depression

The Cortisol is known as a stress hormone given that adrenal glands release it in response to stress and as well as when blood sugar level gets too low. But unfortunately, in some cases, the cortisol levels might be chronically elevated. Studies have shown that ashwagandha helps reduce cortisol levels [34]. The adverse effects were mild and were comparable. There was no adverse effect reported which suggests that a high-concentration full-spectrum Ashwagandha root extract may safely and effectively improve an individual's resistance towards stress. So, it helps to improve the self-assessed quality of life [34].

Stress has also been reported to be a cause for male infertility. So selected normozoospermic but infertile individuals (N = 60) were given root powder of *W. somnifera* at a rate of 5 g/day for 3 months. Studying the biochemical and stress parameters before and after treatment depicted a definite role of stress in male infertility and the power of *W. somnifera* in treating stress-related infertility (decrease in stress) by improving the level of anti-oxidants. Upon treatment, the sperm concentration in normozoospermic men, cigarette smokers, and those having psychological stress was increased by 17, 20, and 36%, respectively while motility of spermatozoa increased by 9, 10, and 13% along with a decrease in their semen liquefaction time by 19, 20, and 34% [35].

Ashwagandha as a drug is well known for its ability in reducing stress. This blocks the stress pathway in the brains of rats. Thereby controlling of chemical signaling in the nervous system [36]. Various other controlled human studies projected that it may reduce symptoms in people with stress and anxiety disorders [37,38,34]. The studies have projected that ashwagandha can help in alleviating depression [37,34].

Andrade [37] instructed to take a daily WS dose of 1000 mg and were assessed via the Hamilton Anxiety Scale (HAM) and the Global Rating Scale (GRS) at baseline, week 2, and week 6; a systematic assessment for treatment-emergent effects (SAFETY) at weeks 2 and 6. Clinical response was coded as a reduction of HAM score to 12 or below. At week 6, 15 of 17 intervention participants were classified as meeting criteria for response, with only 8 of 16 control participants showing response ($p=0.026$).

Cooley and colleagues' study [38] was composed of 81 participants distributed into the naturopathic care (NC) group or the psychotherapy (PT) group. Participants in the NC group received daily WS dosages of 600 mg, while the PT group received cognitive-behavioral therapy (CBT) sessions and placebo. Beck Anxiety Inventory (BAI) scores decreased significantly in the NC group compared with the PT group; mean changes in the BAI were -13.31 points in the NC group and -7.15 points in the PT group ($p=0.004$) [38].

In one controlled 60-day study in 64 stressed adults, those who took 600 mg of high-concentration ashwagandha extract per day, there was a 79% reduction in severe depression. But in the placebo group, a 10% increase was noticed. Significant differences were found for all outcome measures, including scores on the Perceived Stress Scale ($p<0.0001$), the General Health Questionnaire ($p<0.0001$), and levels of cortisol in the bloodstream ($p=0.0006$). The authors describe WS as a safe and well-tolerated medicinal herb [34]. The treatment group in Khyati and Anup's study received the highest WS dose of all evaluated studies (12,000 mg/d) but resulted in mostly nonsignificant differences in HAM scores compared with the placebo group. The only item that WS participants scored significantly higher on was for "anxious mood" ($p<0.001$) [39].

Boosts testosterone and (increases) male fertility

Ashwagandha supplements show a powerful effect on testosterone levels and even on reproductive health. A study demonstrated that men who received ashwagandha for stress experienced higher antioxidant levels and better sperm quality. After 3 months of treatment, 14% of the men's partners had become pregnant [35,40-42]. A study in 75 infertile men, group treated by ashwagandha revealed an increased sperm count and motility. The researchers also reported that the group who took the herb had increased antioxidant levels in their blood [35].

Increases muscle mass and strength

The study projected that ashwagandha can improve body composition and increase muscle mass and [43,42].

In an experiment for determining a safe and effective dosage of ashwagandha the healthy men who took 750-

1,250 mg of ashwagandha root powder per day had gained muscle strength after 30 days [26]. In another investigation ashwagandha also projected a significant gain in muscle strength and size [42]. The group treated with ashwagandha had significantly greater increases in muscle strength on the bench-press exercise (Placebo: 26.4 kg, 95% CI, 19.5, 33.3 vs. Ashwagandha: 46.0 kg, 95% CI 36.6, 55.5; $p = 0.001$) and the leg-extension exercise (Placebo: 9.8 kg, 95% CI, 7.2, 12.3 vs. Ashwagandha: 14.5 kg, 95% CI, 10.8, 18.2; $p = 0.04$), and significantly greater muscle size increase at the arms (Placebo: 5.3 cm(2), 95% CI, 3.3, 7.2 vs. Ashwagandha: 8.6 cm(2), 95% CI, 6.9, 10.8; $p = 0.01$) and chest (Placebo: 1.4 cm, 95% CI, 0.8, 2.0 vs. Ashwagandha: 3.3 cm, 95% CI, 2.6, 4.1; $p < 0.001$). Compared to the placebo subjects, the subjects receiving ashwagandha also had a significantly greater reduction of exercise-induced muscle damage as indicated by the stabilization of serum creatine kinase (Placebo: 1307.5 U/L, 95% CI, 1202.8, 1412.1, vs. Ashwagandha: 1462.6 U/L, 95% CI, 1366.2, 1559.1; $p = 0.03$) [42].

Reduces inflammation

Various animal studies have projected ashwagandha helping decrease inflammation [44,45]. In humans, this may increase the activity of natural killer cells, which fight infections and are helpful for people to stay healthy [46]. Ashwagandha was administered with *anupana* (whole milk) to participants. Peripheral blood samples were collected at 0, 24, and 96 h and compared for differences in cell surface expression of CD4, CD8, CD19, CD56, and CD69 receptors by flow cytometry. Significant increases were observed in the expression of CD4 on CD3+ T cells after 96 h. At 96 h of use, mean values of receptor expression for all measured receptor types were increased indicating that a major change in immune cell activation occurred [46].

The effect of a tea fortified with five herbs selected from Indian traditional medicine (Ayurveda) for their putative immune-boosting effect (*W. somnifera*, *Glycyrrhiza glabra*, *Zingiber officinale*, *Ocimum sanctum*, and *Elettaria cardamomum*) on innate immunity a study was done. The study demonstrated that NK cell activity increased that is an important aspect of the early innate immune response to infections. *Ex vivo* natural killer (NK) cell activity was assessed after consumption of fortified tea compared with regular tea in two independent double-blind intervention studies on healthy volunteers (age ≥ 55 years) selected for a relatively low baseline NK cell activity and a history of recurrent coughs and colds. In a pilot study conducted with 32 volunteers, the consumption of Natural Care tea significantly improved the NK cell activity of the volunteers in comparison with a population consuming regular tea [47].

Lowers Cholesterol and Triglycerides

Despite its anti-inflammatory effects, the ashwagandha can help in improving heart health by reducing cholesterol and triglyceride levels. The Animal studies depicted that it significantly decreases levels of these blood fats. One study in rats reported that this lowered total cholesterol and triglyceride levels by 53% and nearly 45%, respectively [48]. The Hypocholesteremic and antioxidant effects of *W. somnifera* (WS) were investigated in hypercholesteremic male albino rats. When the root powder of WS was added to the diet at 0.75 and 1.5 gm/rat/day in hypercholesteremic animals registered a significant decrease in total lipids (-40.54%; -50.69%), cholesterol (-41.58%; -53.01%) and triglycerides (-31.25%; -44.85%) in plasma. The study projected that WS root powder is also effective in normal subjects in decreasing lipid profiles [48].

Improves Brain Function/Memory

The Test-tube and animal studies demonstrated that ashwagandha can mitigate memory loss and brain function problems resulted from injury or disease [49,50]. The rats having epilepsy which were treated with ashwagandha projected nearly a complete reversal of spatial memory impairment. That was due to a reduction in oxidative stress [49]. Although ashwagandha has traditionally been used to boost memory in Ayurvedic medicine, little human research has been conducted in this area. In a controlled study, the healthy men that had 500 mg of standardized extract daily showed significant improvements in their reaction time and task performance in comparison to the men that received a placebo [51]. Another 8-week study in 50 adults demonstrated that taking 300 mg of ashwagandha root extract twice daily significantly improves general memory, task performance, and even attention. The ashwagandha treatment group demonstrated significant improvements compared with the placebo group in both immediate and general memory, as evidenced by Wechsler Memory Scale III subtest scores for logical memory I ($p = 0.007$), verbal paired associates I ($p = 0.042$), faces I ($p = 0.020$), family pictures I ($p = 0.006$), logical memory II ($p = 0.006$), verbal paired associates II ($p = 0.031$), faces II ($p = 0.014$), and family pictures II ($p = 0.006$). Ashwagandha may be effective in enhancing both immediate and general memory in people with MCI as well as improving executive function, attention, and information processing speed [52].

CONCLUSION

WS is a plant used in medicine from the time of Ayurveda which is an ancient system of Indian medicine. The plant is highly antiviral. This has a high potential for inhibiting pathogenic viruses. So clinical trials need to be conducted to support and make use of its possible

therapeutic use for fighting against viral diseases. The outcome may be an alternative medicine as a natural drug that may be useful in treating several infectious diseases. *W. somnifera* contains several groups of phytochemicals such as Alkaloids, withanolides, and several sitoindosides which have been reported to be present in the roots [53-55].

Ashwagandha is a “*Rasayana*” (= drug) that is recommended for *balavardhan* (strength) and being *smamsavardhan* (muscles). The evidence records ashwagandha reduces blood sugar levels. This is through its effects on insulin secretion and sensitivity. The animal and test-tube investigations have projected that withaferin, a bioactive compound in ashwagandha may promote the death of tumor cells. This may be effective against several types of cancers. Ashwagandha supplements can help in lowering cortisol levels in chronically stressed individuals. It can help in reducing stress and anxiety in both animals and human beings. The limited research available project that ashwagandha helps reduce depression in increasing the testosterone levels. This significantly boosts sperm quality and fertility in men. (Ashwagandha may help increase muscle mass. This reduces body fat and even increase strength in men). This increases natural killer cell activity decreases the markers of inflammation. Ashwagandha can help reduce the risk of heart disease through lowering of cholesterol and triglyceride levels. The Ashwagandha supplements can improve the functioning of the brain, memory, reaction time. This may improve the ability to perform tasks. The ashwagandha is safe for most people but certain individuals shouldn't use it due to lack of knowledge unless prescribed. The standardized root extract is commonly taken @ 450–500-mg capsules once or twice per day. This plant has enough immuno-boosting power, which can be exploited for fighting COVID-19 after successful clinical trials.

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REFERENCES

1. Nigam PSN, Apenten RO (2019) Current Aspects of Medicinal Properties and Health Benefits of Plant *Withania somnifera*. In: Khurana S., Gaur R. (eds) Plant Biotechnology: Progress in Genomic Era 1: 311-325.
2. Sangwan RS, Chaurasiya ND, Misra LN, Lal P, Uniyal GC, et al. (2004) Phytochemical variability in commercial herbal products and preparations of

- Withania somnifera* (Ashwagandha). Curr Sci 10: 461-465.
3. Kambizi LG, Goosen BM, Taylor MB, Afolayan AJ (2007) Anti-viral effects of aqueous extracts of *Aloe ferox* and *Withania somnifera* on herpes simplex virus type 1 in cell culture. S Afr J Sci 103: 359-360.
 4. Cai Z, Zhang G, Tang B, Liu Y, Fu X, et al. (2015) Promising Anti-influenza Properties of Active Constituent of *Withania somnifera* Ayurvedic Herb in Targeting Neuraminidase of H1N1 Influenza: Computational Study. Cell Biochem Biophys 72: 727-739.
 5. Graham RL, Donaldson EF, Baric RS (2013) A decade after SARS: Strategies for controlling emerging coronaviruses. Nat Rev Microbiol 11: 836-848.
 6. Hoek LVD, Pyrc K, Jebbink MF, Oost WV, Berkhout RJ, et al. (2004) Identification of a new human coronavirus. Nat Med 10: 368-373.
 7. Woo PC, Huang Y, Lau SK, Yuen KY (2010) Coronavirus genomics and bioinformatics analysis. Viruses 2: 1804-1820.
 8. Wu F, Zhao S, Yu B, Chen YM, Wang W, et al. (2020) A new coronavirus associated with human respiratory disease in China. Nature 579: 265-269.
 9. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, et al. (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579: 270-273.
 10. Kaul SC, Wadhwa R (Eds) (2017) Science of Ashwagandha: Preventive and therapeutic potentials. Springer International Publishing.
 11. Kim S, Sik J, Yu Ji, Lee YJ, Choi SU, et al. (2019) Cytotoxic Withanolides from the Roots of Indian Ginseng (*Withania somnifera*) J Nat Prod 82: 765-773.
 12. Bara KJ, Soni R, Jaiswal S, Saksena P (2016) Phytochemical study of plant *Withania somnifera* against various diseases. J Agr Vet Sci 9: 109-112.
 13. Saidulu C, Venkateshwar C, Gangadhar RS, Ashok VT (2014) In-vitro Antimicrobial Activity of *Withania somnifera* Leaf and Root Extracts grown in Heavy Metal toxic soils. Int J Adv Pharm Biol Chem 3: 872-879.
 14. Umadevi M, Rajeswari R, Sharmila RC, Selvavenkadesh S, Pushpa R, et al. (2012) Traditional and medicinal uses of *Withania somnifera*. Pharm Innov 1: 102-110.
 15. Narinderpal K, Junaid N, Raman B (2013) A review on pharmacological profile of *Withania somnifera* (Ashwagandha). Res Rev: J Bot Sci 2: 6-14.
 16. Balkrishna A, Pokhrel S, Singh J, Varshney A (2020) Withanone from *Withania somnifera* may inhibit Novel Coronavirus (COVID-19) entry by disrupting interactions between Viral S-Protein Receptor Binding Domain and Host ACE2 Receptor.
 17. Kumar V, Dhanjal JK, Bhargava P, Kaul A, Wang J, et al. (2020) Withanone and Withaferin-A are predicted to interact with transmembrane protease serine 2 (TMPRSS2) and block entry of SARS-CoV-2 into cells. J Biomol Struct Dyn 0: 1-13.
 18. Jaspreet J, Vimal N, Shivam C, Somnath P (2018) In Vivo Evaluation of *Withania somnifera*- Based Indian Traditional Formulation (Amukkara Choornam), Against Chikungunya Virus-Induced Morbidity and Arthralgia. J Evid Based Integr Med 23: 1-7.
 19. Ganguly B, Umapathi V, Rastogi SK (2018) Nitric oxide-induced by Indian ginseng root extract inhibits Infectious Bursal Disease virus in chicken embryo fibroblasts in vitro. J Anim Sci Technol 60: 2.
 20. Raza A, Muhammad F, Bashir S, Anwar M, waiss AM, et al. (2015) Antiviral and immune-boosting activities of different medicinal plants against Newcastle disease virus in poultry. World Poultry Sci J 71: 523-532.
 21. Pant M, Ambwani T, Umapathi V (2012) Antiviral activity of Ashwagandha extract on Infectious Bursal Disease Virus Replication. Indian J Sci Technol 5: 2750-2751.
 22. Grover A, Agrawal V, Shandilya A, Bisaria VS, Sundar D (2011) Non-nucleoside inhibition of Herpes simplex virus DNA polymerase: mechanistic insights into the anti-herpetic mode of action of herbal drug withaferin A. BMC Bioinformatics 12: S22.
 23. Mirjalili MH, Moyano E, Mercedes B, Rosa M Cusido, Javier P (2009) Steroidal Lactones from *Withania Somnifera*, an Ancient Plant for Novel Medicine. Molecules 14: 2373-23793.
 24. Gorelick J, Rosenberg R, Smotrich A, Hanuš L, Bernstein N (2015) Hypoglycemic activity of withanolides and elicited *Withania somnifera*. Phytochemistry 116: 283-289.
 25. Andallu B, Radhika B (2000) Hypoglycemic, diuretic and hypocholesterolemic effect of winter

- cherry (*Withania somnifera*, Dunal) root. *Indian J Exp Biol* 38: 607-609.
26. Raut AA, Rege NN, Tadv FM (2012) Exploratory study to evaluate tolerability, safety, and activity of Ashwagandha (*Withania somnifera*) in healthy volunteers. *J Ayurveda Integr Med* 3:111-114.
 27. Akshay P, Smita DA, Vijay RS, Saoji TA, Shishir SV, et al. (2013) Effects of *Withania somnifera* in patients of schizophrenia: A randomized, double-blind, placebo-controlled pilot trial study. *Indian J Pharmacol* 45: 417-418.
 28. Vyas AR, Singh SV (2014) Molecular targets and mechanisms of cancer prevention and treatment by withaferin a, a naturally occurring steroidal lactone. *AAPS J* 16: 1-10.
 29. Nishikawa Y, Okuzaki D, Fukushima K (2015) Withaferin A Induces Cell Death Selectively in Androgen-Independent Prostate Cancer Cells but Not in Normal Fibroblast Cells. *PLoS One* 10: e0134137.
 30. Khazal KF, Hill DL, Grubbs CJ (2014) Effect of *Withania somnifera* root extract on spontaneous estrogen receptor-negative mammary cancer in MMTV/Neu mice. *Anticancer Res* 34: 6327-6332.
 31. Senthilnathan P, Padmavathi R, Magesh V, Sakthisekaran D (2006) Stabilization of membrane-bound enzyme profiles and lipid peroxidation by *Withania somnifera* along with paclitaxel on benzo(a) pyrene-induced experimental lung cancer. *Mol Cell Biochem* 292: 13-17.
 32. Chang E, Pohling C, Natarajan A, Witney TH, Kaur J, et al. (2016) AshwaMAX and withaferin A inhibits gliomas in cellular and murine orthotopic models. *J Neurooncol* 126: 253-264.
 33. Kakar SS, Ratajczak MZ, Powell KS (2014) Withaferin an alone and in combination with cisplatin suppresses growth and metastasis of ovarian cancer by targeting putative cancer stem cells. *PLoS One* 9: e107596.
 34. Chandrasekhar K, Kapoor J, Anishetty S (2012) A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults. *Indian J Psychol Med* 34: 255-262.
 35. Mahdi AA, Shukla KK, Ahmad MK (2009) *Withania somnifera* Improves Semen Quality in Stress-Related Male Fertility [published online ahead of print, 2009 Sep 29]. *Evid Based Complement Alternat Med* 2011: 576962.
 36. Candelario M, Cuellar E, -Ruiz JMR (2015) Direct evidence for GABAergic activity of *Withania somnifera* on mammalian ionotropic GABAA and GABAp receptors. *J Ethnopharmacol* 171: 264-272.
 37. Andrade C, Aswath A, Chaturvedi SK, Srinivasa M, Raguram R (2000) A double-blind, placebo-controlled evaluation of the anxiolytic efficacy of an ethanolic extract of *Withania somnifera*. *Indian J Psychiatry* 42: 295-301.
 38. Cooley K, Szczurko O, Perri D, Mills EJ, Bernhardt B, et al. (2009) Naturopathic care for anxiety: A randomized controlled trial *ISRCTN78958974*. *PLoS One*. 4: e6628.
 39. Khyati S, Anup T (2013) A randomized double-blind placebo-controlled study of ashwagandha on generalized anxiety disorder. *Int Ayurvedic Med J* 1:1-7.
 40. Ahmad MK, Mahdi AA, Shukla KK (2010) *Withania somnifera* improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. *Fertil Steril* 94: 989-996.
 41. Gupta A, Mahdi AA, Shukla KK (2013) Efficacy of *Withania somnifera* on seminal plasma metabolites of infertile males: a proton NMR study at 800 MHz. *J Ethnopharmacol* 149: 208-214.
 42. Wankhede S, Langade D, Joshi K, Sinha SR, Bhattacharyya S (2015) Examining the effect of *Withania somnifera* supplementation on muscle strength and recovery: A randomized controlled trial. *J Int Soc Sports Nutr* 1243.
 43. Sandhu JS, Shah B, Shenoy S, Chauhan S, Lavekar GS, et al. (2010) Effects of *Withania somnifera* (Ashwagandha) and *Terminalia arjuna* (Arjuna) on physical performance and cardiorespiratory endurance in healthy young adults. *Int J Ayurveda Res* 1:144-149.
 44. Noshahr ZS, Shahraki MR, Ahmadvand H, Nourabadi D, Nakhaei A (2015) Protective effects of *Withania somnifera* root on inflammatory markers and insulin resistance in fructose-fed rats. *Rep Biochem Mol Biol* 3: 62-67.
 45. Orrù A, Casu MA, Tambaro S, Marchese G, Casu G, et al. (2016) *Withania somnifera* (L.) Dunal root extract alleviates formalin-induced nociception in mice: involvement of the opioidergic system. *Behav Pharmacol* 27: 57-68.
 46. Mikolai J, Erlandsen A, Murison A (2009) In vivo effects of Ashwagandha (*Withania somnifera*) extract on the activation of lymphocytes. *J Altern Complement Med* 15: 423-430.

47. Bhat J, Damle A, Vaishnav PP, Albers R, Joshi M, et al. (2010) In vivo enhancement of natural killer cell activity through tea fortified with Ayurvedic herbs. *Phytother Res* 24: 129-135.
48. Visavadiya NP, Narasimhacharya AV (2007) Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. *Phytomedicine* 14: 136-142.
49. Soman S, Korah PK, Jayanarayanan S, Mathew J, Paulose CS (2012) Oxidative stress-induced NMDA receptor alteration leads to spatial memory deficits in temporal lobe epilepsy: Ameliorative effects of *Withania somnifera* and Withanolide A. *Neurochem Res* 37: 1915-1927.
50. Baitharu I, Jain V, Deep SN (2013) *Withania somnifera* root extract ameliorates hypobaric hypoxia induced memory impairment in rats. *J Ethnopharmacol* 145: 431-441.
51. Pingali U, Pilli R, Fatima N (2014) Effect of standardized aqueous extract of *Withania somnifera* on tests of cognitive and psychomotor performance in healthy human participants. *Pharmacognosy Res* 6:12-18.
52. Choudhary D, Bhattacharyya S, Bose S (2017) Efficacy and Safety of Ashwagandha (*Withania somnifera* (L) Dunal) Root Extract in Improving Memory and Cognitive Functions. *J Diet Suppl* 14: 599-612.
53. Ali AS, Bashir SH, Abdelshafeek KA (2020) Isolation, Identification of Some Chemical Constituents and Antimicrobial Activity of Different Extracts from *Withania somnifera* Growing at Albaha Region, KSA. *Biomed Pharmacol J* 13.
54. Andallu B, Radhika B (2000) Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera*, Dunal) root. *Indian J Exp Biol* 38: 607-609.
55. Mishra D, Patnaik S (2020) GC-MS Analysed Phyto-Chemicals and Antibacterial Activity of *Withania Somnifera* (L.) Dunal Extract in the Context of Treatment to Liver Cirrhosis. *Biomed Pharmacol J* 13.