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REVIEW

Clinical applications of Ashwagandha plant in depression and anxiety

Depresyon ve anksiyetede Ashwagandha bitkisinin klinik uygulamaları

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Abstract

Ashwagandha is an adaptogenic herb that has been long used in traditional Indian medicine and has garnered attention in modern medicine in recent years. Known for its ability to restore balance in the body due to its adaptogenic properties, Ashwagandha is believed to offer potential benefits in addressing anxiety and depression, which are growing public health concerns in modern society. The bioactive components found in Ashwagandha, particularly withanolides, may contribute to reducing symptoms of anxiety and depression through various biological mechanisms that affect the nervous system. This can involve the regulation of neurotransmitters, anti-inflammatory effects, and support for stress coping mechanisms. The fact that Ashwagandha tends to cause fewer side effects compared to conventional antidepressants and anxiolytic drugs, along with its broad mechanism of action as a natural adaptogen, presents a significant advantage in terms of potential future therapeutic options. Many studies in the literature suggest that Ashwagandha could serve as a natural adjunct in these areas. However, more clinical trials and long-term effect analyses are necessary to fully evaluate this potential. This review aims to lay the groundwork for future research by assessing current literature on the effectiveness of Ashwagandha in addressing anxiety and depression.

Keywords:. Anxiety, depression, Ashwagandha.

Öz

Ashwagandha, geleneksel Hint tıbbında uzun süredir kullanılan adaptogenik bir bitkidir ve son yıllarda modern tıpta dikkat çekmektedir. Ashwagandha, adaptogen özellikleri sayesinde vücutta dengeyi sağlama yeteneği ile bilinmektedir. Anksiyete ve depresyon, modern toplumda giderek artan bir halk sağlığı sorunudur. Ashwagandha'nın içerdiği biyoaktif bileşenler, özellikle withanolid, sinir sistemini etkileyen bir dizi biyolojik mekanizma üzerinden anksiyete ve depresyonun hafifletilmesine katkıda bulunabilir. Bu durum, nörotransmitterlerin düzenlenmesi, anti-inflamatuar etkiler ve stresle başa çıkma mekanizmalarını destekleme yoluyla gerçekleşebilir. Ashwagandha'nın, geleneksel antidepresan ve anksiyolitik ilaçlara kıyasla daha az yan etkiye neden olması ve doğal bir adaptogen olarak geniş bir etki mekanizmasına sahip olması, gelecekteki potansiyel terapötik seçenekleri açısından önemli bir avantaj sunmaktadır. Literatürdeki birçok araştırma, Ashwagandha'nın bu alanlarda potansiyel bir doğal yardımcı olarak rol oynayabileceğini öne sürmektedir. Ancak, bu potansiyeli tam anlamıyla değerlendirebilmek için daha fazla klinik çalışma ve uzun vadeli etki analizlerine ihtiyaç duyulmaktadır. Bu derleme, Ashwagandha'nın anksiyete ve depresyonla mücadeledeki etkinliği konusunda mevcut literatürü değerlendirerek, gelecekteki araştırmalara yönelik bir temel oluşturmayı amaçlamaktadır.

Anahtar kelimeler: Anksiyete, depresyon, Ashwagandha.

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INTRODUCTION

Mental health problems such as depression and anxiety are an important global public health problem. These conditions affect the emotional, cognitive, and physical health of millions of individuals, negatively impacting their activities of daily their daily life and reducing their overall quality of life. These problems are prevalent in all segments of society, across different ages, genders, and socioeconomic groups, and can seriously affect individuals' social relationships, work performance, and general life functioning[1,2].

Major depressive disorder is characterized by depressed mood, anhedonia, sleep disturbances, marked changes in weight, psychomotor agitation, retardation, fatigue, loss of energy, and difficulty concentrating, and recurrent thoughts of death or suicide. According to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), these symptoms should persist for at least two weeks. According to the International Statistical Classification of Diseases and Related Health Problems, 10th Edition (ICD-10), depression is characterized by persistent sadness, anhedonia, loss of energy, difficulty concentrating, feelings of worthlessness or guilt, and suicidal thoughts. These symptoms must last at least two weeks and cause significant impairment in normal functioning. The overall prevalence of major depression is 3-5.8%. One-year prevalence is 2.6-6.2%. The lifetime risk is 3-12% for men and 10-26% for women. Epidemiological data show that 13% of women and 8% of men are depressed at any given time [1,3].

DSM-5 defines anxiety disorders as a mental change in which physical symptoms such as excessive anxiety, restlessness, muscle tension, fatigue, lack of mental discharge, sleep problems, lack of attention and concentration are observed. Daily life is negatively affected as a result of these findings. ICD-10 defines anxiety as a mental change that usually lasts for at least six months and is characterized by worry, anxiety, tension, muscle tension, insomnia, impaired concentration, and physical symptoms that adversely affect daily life[2,3]. An estimated 4.05 percent of the global population has an anxiety disorder, which corresponds to approximately 301 million people. The number of people affected increased by more than 55 per cent from 1990 to 2019[4].

Modern medicine adopts a holistic approach to depression and anxiety disorders, usually involving a treatment plan that includes pharmacotherapy and psychotherapy. Antidepressant medications aim to regulate neurotransmission by modulating the levels of neurotransmitters like serotonin and norepinephrine. Cognitive behavioral therapy, psychoanalysis, and emotionally oriented therapies are included in psychotherapy practices and aim to increase individuals' ability to cope with emotional difficulties[5].

The evolution of the therapeutic paradigm in the management of mental health problems has increased the use of herbal therapies in recent years. This phenomenon reflects a demand for solutions of natural origin, beyond pharmacotherapy and psychotherapy. Herbal therapies include plant extracts and supplements, often containing biologically active components. Herbal therapies encompass plant extracts and supplements, typically containing biologically active compounds. Notable herbs, such as Ashwagandha, St. John's Wort, and Ginkgo biloba, are recognized for their efficacy in alleviating mental health issues like depression and anxiety. This increased interest is primarily a result of individuals' desire for active involvement in their own health and the confidence associated with the perceived lower potential side effects of natural products[6,7].

The interest in alternative therapies is increasing day by day among patients who do not respond to existing treatments for mental problems such as depression and anxiety, or who cannot tolerate the side effects of these treatments. The potential of the Ashwagandha plant to reduce the effects of depression and anxiety, due to its neuroprotective and adaptogenic properties, has attracted attention for its use in treatment[8].

Ashwagandha, also known as Withania somnifera, is a plant with a long history in Ayurveda, the traditional Indian system of medicine. Its traditional uses encompass a wide range of diseases. For example, hypertension, asthma, cancer, schizophrenia, chronic stress, insomnia, anxiety, depression, memory/cognitive enhancement, obsessive-compulsive disorder, rheumatoid arthritis, type-2 diabetes, male infertility, supporting fertility in women, growth-promoting activity in children and reducing fatigue and improving quality of life in cancer patients undergoing chemotherapy. In addition, this plant attracts attention as a plant believed to have adaptogenic properties. Adaptogens are plants or plant extracts that enhance the body's

ability to cope with stress. Research indicates that Ashwagandha may be effective in combating stress and supporting overall mental health due to its adaptogenic properties[9,10].

Ashwagandha has been widely practiced in Ayurvedic medicine, especially for the purpose of coping with stress, increasing energy, and mental clarity. The roots of the plant are usually powdered and consumed in capsule or tea form. This traditional use is also being studied by modern science. It is thought that Ashwagandha may contribute to the alleviation of mental health problems such as depression and anxiety by increasing the ability to cope with the mental and physical effects caused by stress[10].

The main aim of this study is to scientifically evaluate the therapeutic potential of Ashwagandha by examining its clinical applications in the treatment of depression and anxiety. The goal is to uncover the biochemical and pharmacological mechanisms underpinning the antidepressant and anxiolytic effects of Ashwagandha, thereby increasing the knowledge in this field. Within this framework, the review seeks to offer comprehensive, evidence-based insights into the efficacy and safety of Ashwagandha. The contribution of the study to the literature is to evaluate the pharmacological profile of Ashwagandha in the treatment of depression and anxiety in a more comprehensive manner and to provide new perspectives for future research in this field. Firstly, compiling the results of existing clinical trials allows for a better understanding of the potential therapeutic effects and mechanisms of Ashwagandha.

ASHWAGANDHA (WITHANIA SOMNIFERA)

Withania somnifera, belonging to the Solanaceae family, is a plant species popularly known as "Ashwagandha". This plant usually grows in regions such as India, the Middle East, and North Africa. The roots and leaves of the Ashwagandha plant are known to have various medicinal and adaptogenic properties[11].

Ashwagandha, particularly its roots, contains numerous biologically active compounds. Withania somnifera is especially rich in triterpenoid compounds known as withanolides, which are believed to be primarily responsible for the plant's adaptogenic properties. Additionally, Ashwagandha contains other bioactive constituents such as alkaloids, flavonoids, tannins, and ferulic acid[12].

ACTIVE COMPOUNDS AND CLINICAL EFFECTS OF ASHWAGANDHA

The major bioactive components of Withania somnifera are withanolides, which are triterpene lactones. There are more than 40 withanolides, about 12 alkaloids, and various sitoindosides[12,13].

Withanolides are ergostane-type steroid compounds characterized by a δ-lactone functionality between the C-22 and C-26 atoms and an oxidized C-1 position[11,12]. Two prominent compounds among withanolides are withanolide D and withaferin A. Withanolides have antitumor, anti-inflammatory, and immunosuppressant properties. Furthermore, the plant extract contains potent antioxidants that contribute to the neuroprotective effect. In vitro studies have shown that withanolide A, withanosides IV, and VI are effective on axons and dendrites. The ability of withanolide A to regenerate neuronal networks has also been determined. It was also observed that withanolides have the capacity to inhibit acetylcholinesterase and increase dendrite formation in human neuroblastoma cells. Research indicates that these components have neuroprotective properties and may protect nerve cells from damage caused by oxidative stress. The neuroprotective properties of withanolides may prevent protein damage in nerve cells and stabilize neurotransmitter levels. Ashwagandha's effects on the nervous system are often linked to stress management and mental health promotion. It has been suggested that withanolides may regulate cortisol levels, the stress hormone, and slow neurodegenerative processes by protecting nerve cells[14-17].

Other components found in Ashwagandha may also influence the nervous system. For example, the potential of alkaloids to regulate communication between nerve cells and the antioxidant properties of flavonoids may contribute to mechanisms to protect nerve cells from the effects of oxidants. In addition, ferulic acid and tannins possess antioxidant and anti-inflammatory properties, which may benefit the nervous system by combating oxidative stress and reducing inflammation[18]. Other groups of compounds in Withania somnifera that are not withanolides have also been shown to play an important role in anxiolytic and antidepressant

effects. For example, cytoindosides VII and VIII have been found to reduce stress and depressive symptoms in rodents. Additionally, trimethylene glycol from Withania somnifera leaves has been observed to induce sleep in mice and reduce anxiety and depression-like behaviour[8,19].

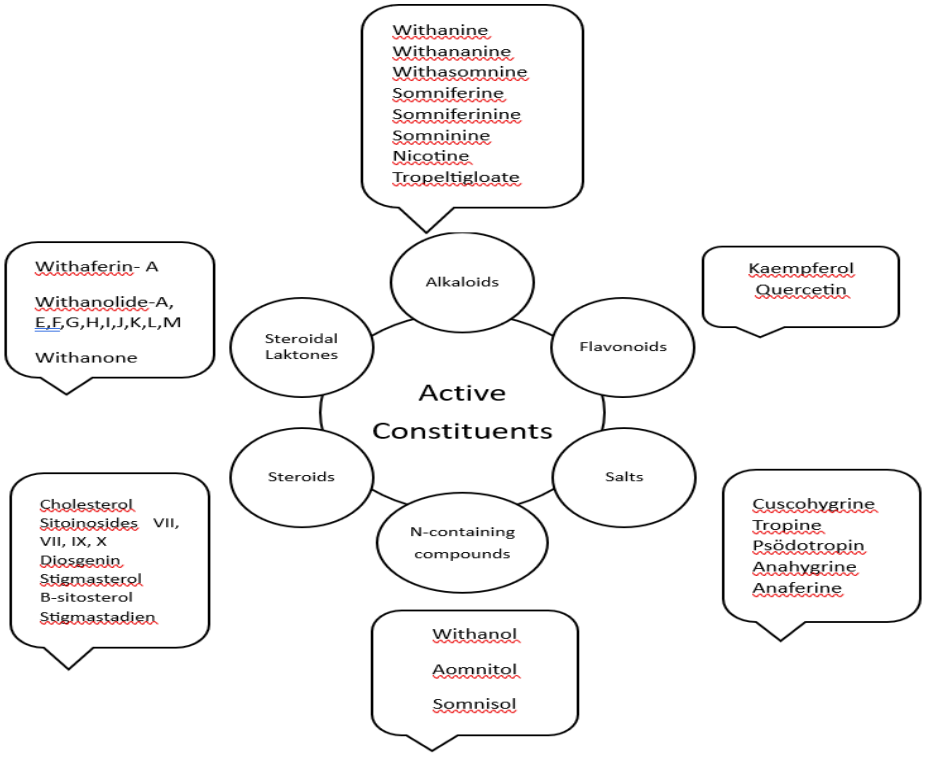

Figure 1. Different phytochemical compounds found in Withania somnifera.

EFFECTS OF ASHWAGANDHA ON ANXIETY

Animal studies

The therapeutic effects of Withania somnifera (WS) on anxiety were investigated using root extracts, leaf extracts, and mixtures of compounds such as withaferin-A and cytoindosides VII-X isolated from this plant. While investigating the effects of Withania somnifera on anxiety, extracts obtained from both root and leaf were applied. While obtaining these extracts, different extraction methods and different solvents such as water, ethanol, methanol, and hydroalcoholic were used (Table 1). This indicates the possibility of the interaction of multiple bioactive compounds. Furthermore, Withania somnifera extracts have been shown to enhance the effects of known anti-anxiety drugs. For example, in a rat social isolation model, a subtherapeutic dose of Withania somnifera root extract (50 mg/kg, oral) was found to increase the anxiolytic effect of diazepam (0.5 mg/kg, ip) when administered at a subtherapeutic dose. This observation suggests that Withania somnifera may have a synergistic effect with drugs used in the treatment of anxiety. As a result, WS may be included in current treatment options for anxiety[20]. Similarly, in the rat alcohol withdrawal model, WS was observed to potentiate the anxiolytic effect of ethyl alcohol at subtherapeutic doses (0.5 or 1 g/kg, i.p.) [21].

Bhattacharya et al. investigated the anxiolytic and antidepressant effects of bioactive glycowitanolides isolated from Withania somnifera roots in rats. In the studies, glycowithanolides were compared with lorazepam (0.5 mg/kg, i.p.), a benzodiazepine derivative, and imipramine (10 mg/kg, i.p.), a tricyclic antidepressant, in terms of their anxiolytic and antidepressant effects. The results showed similar anxiolytic and antidepressant effects with the compared drugs. These results support the use of Withania somnifera as a mood stabilizer in anxiety and depression[22].

In an in vivo study conducted by Krishna Raju et al., the efficacy of AshwaSR (sustained release formula of Ashwagandha) on comorbid anxiety and depression was tested by creating a chronic unpredictable stress model in rats. The AshwaSR-treated group and the group treated with escitalopram, a selective serotonin reuptake inhibitor, showed significantly more favorable outcomes in coping with stress and managing stress-related anxiety and depression compared to the group exposed to chronic unpredictable stress[23].

Table 1. Anti-anxiety and Anti-depressant effects of Ashwagandha (Withania somnifera, WS) in animal studies

Nature of Extract	Dosage	Model	Behavioral Effects	Biological Effects	Advers effects	Refs.
Root extract	50, 100, 200, or 500 mg/kg p.o. on days 38-42 of social isolation and 1 hr prior to assessment	Rat (social isolation)	▼anxiety-like behavior (EPM) ▲anti-anxiety action of diazepam at subtherapeutic dose (50 mg/kg) of WS (EPM) ▼depression-like behavior (FST)	Not assessed	No Advers effects	19
Ethanolic root extract	50, 100, 200, or 500 mg/kg p.o., one hr prior to assessment	Rat (acute ethanol induced anxiolysis and withdrawal from chronic ethanol consumption)	▼anxiety-like behavior (EPM) ▲anti-anxiety action of a subtherapeutic dose of ethanol at subtherapeutic dose (50 mg/kg) of WS (EPM)	Not reported	No Advers effects	20
Glycowithanolide rich fraction (WS,containing sitoindosides VII-X and withaferin) isolated from aqueous root extract, standardized to 1.13% total steroid content	WS 20 and 50 mg/kg p.o. for 5 days	Rat (anxiety and depression)	▼depression-like behavior (LHT, FST) ▼anxiety-like behavior (EPM, SIT, NSFLT)	▼PTZ-induced increase in rat brain tribulin activity (PTZ)	No Advers effects	21
Ashwagandha sustained-release formulation (AshwaSR)	AshwaSR 50mg/kg/day Orally for 35 days escitalopram orally at a dose	Rat (chronic unpredictable stress-CUS)	▼depression-like behavior (OFT, EPM, FST, MWM) ▼anxiety-like behavior (OFT, EPM, MWM)	▼ pro-inflammatory cytokines (TNFα, IL-1β, superoxide generation)	No Advers effects	22

	of 20mg/kg for 35days					
Ashwagandha Root extract	25, 37.5, 50, 100 and 200 mg/kg i.p., 30mins before assessment	Mouse (untreated, clonidine, reserpine)	▼depression-like behavior (FST; 50 to 200 mg/kg WS) ▲imipramine and fluoxetine antidepressant activity at subtherapeutic dose (37.5 mg/kg) of WS (FST) ▼reserpine- and clonidine-induced depression-like behavior (FST; 100 mg/kg WS)	Not assessed	No Advers effects	37
Withanolide-free root extract	3.3, 10, 33.3, and 100 mg/kg p.o. for 12 days	Rat (stress)	▼depression-like or anxiety-like behavior (MBT)	▼stress induced weight loss ▼stress-induced increase in rectal temperature ▼transient hyperthermic response ▼stress-induced increase in adrenal weight ▼stress-induced increase in plasma cortisol and blood glucose	No Advers effects	38
Mamsyadi Kwatha are Jatamamsi (Nardostachys jatamansi DC.), Ashwagandha (Withania somnifera Linn.) and Parasika Yavani (Hyocymus niger Linn.) in an 8:4:1 ratio,	Mamsyadi Kwatha 8 ml/kg for 7 days reserpine (2.5 mg/kg) An hour after the drug administration	Swiss Albino Mice	▼immobility time in behavioural despair test ▼reserpine-induced – ptosis, catatonia, sedation ▼immobility time in Chronic Fatigue Syndrome (CFS) test	Not assessed	No Advers effects	39
Asvagandha root extract	100mg/kg orally) for 4 and 8 weeks	Albino rats	▼depression-like behavior (OFT)	▲sensitivity of postsynaptic 5HT2 receptors in brain	No Advers effects	41

				▼5HT1A receptor responsiveness in brain		

▲ – Increased; ▼ – Decreased; WS=Withania Somnifera; EPM = Elevated plus maze test; FST = Forced swim test; LHT = Learned helplessness test; NSFLT = Novelty-suppressed feeding latency test; SIT = Social interaction test;5-HT= 5-hidroksitriptamin; OFT = Open field test; EPM = Elevated plus maze test; FST = Forced swim test; MWM = Morris water maze test; MBT = Marble burying test; LHT = Learned helplessness test; CFS= Chronic Fatigue Syndrome Test.

Phase studies

In most of the studies in which Withania somnifera was applied, significant improvements were observed in clinical findings of anxiety (Table 2). For example, in the study conducted by Muhammed Majeed et al. Ashwagandha root extract containing withanoliden was administered according to the United States Pharmacopoeia (USP) protocol. The effect of Withanoliden, administered once daily and standardized at 12.5 mg/day, on individuals with mild to moderate depression and anxiety was evaluated. Randomly selected 70 participants were divided into a treatment group and a placebo group for 90 days and evaluated double-blind. The degree of improvement was higher in the group receiving Ashwagandha root extract. Serotonin levels increased in the Ashwagandha root extract group and decreased in the placebo group. These results suggest that Ashwagandha root extract may be useful in the treatment of depression and anxiety by increasing serum serotonin levels[24].

In a study conducted by Remenapp and colleagues, 43 women experiencing stress and 17 healthy male adults were followed for 30 days. Ashwagandha extract was administered in two different doses of 225 mg/day and 400 mg/day in these groups. In the study, it was found that Ashwagandha extract had a positive effect on cognitive ability, stress, anxiety, depression, and food cravings control and was safe in terms of side effects. It was also observed that the Ashwagandha extract used reduced the cortisol levels of the participants. These results show that Ashwagandha supplements can alleviate the physiological, cognitive and psychological effects of stress[25]. In another double-blind, placebo-controlled study, it was determined that the ethanol extract of Withania somnifera may be a potential anxiolytic for anxiety disorders and may not show any adverse effects compared to the placebo group[26].

Table 2. Effects of Ashwagandha (Withania somnifera, WS) on anxiety and depression in human trials.

Nature of Extract	Dosage	Model	Behavioral Effects	Biological Effects	Advers effects	Refs.
Ashwagandha root extract (ARE) standardized for 2.5% full-spectrum withanolides	12.5 mg withanolide/day once Daily for 90 days piperine (500 mg with 5 mg of 95% piperine) for 90 days	70 adults with mild to moderate depression and anxiety Randomised, double-blind, placebo-controlled study	▼HARS, ▼HDRS, ▲GSQS, ▲QOL	▲serum serotonin levels	headache, nausea, diarrhea, drowsiness, fever, back pain, and stomach pain	23
Ashwagandha (Withania somnifera) root and leaf extract (NooGandha® Specnova LLC, USA)	Ashwagandha (400 mg/d), Ashwagandha (225 mg/d), and placebo for 30 days.	Healthy adults (43 women and17 men; mean age 34.41 years) who reported experiencing perceived stress Randomised,	▼PSS score ▼DASS-21 ▼FCQ- T ▲CNS vital signs:complex attention, cognitive flexibility,	▼saliva corti sol level	No Advers effects reported	24

		double-blind, placebo-controlled study	processing speed, executive functioning, and visual memory,			
Tablets of ethanolic Ashwagandha plant extract	250 mg twice daily 6 weeks	39 adults (41.3 + 13.8 yrs; 61.5% male) with GAD, mixed anxiety and depression, panic disorder and adjustment disorder with anxiety Randomised, double-blind, placebo-controlled study	▼Global Rating Scale score ▼HAS score	Not assessed	Increased appetite, Decreased sleep, Gastritis, Heaviness in stomach drowsiness, heaviness of head	25
KSM-66 Ashwagandha extract (Ixoreal Biomed) full spectrum	300 mg twice daily after food with water 8.5 weeks (60 days)	64 stressed adults (18-54 yrs; 41 males, 23 females; WHO-5 well being score <5; PSS score of at least 14) Randomised, double-blind, placebo-controlled study	▼PSS score, ▼GHQ score, ▼DASS score	▼serum cortisol	No Advers effects reported	42
Sensoril®(Natreon Inc.) Capsules of aqueous extract of Ashwagandha	250 mg twice daily for 1 week, then 500 mg twice daily for 11 weeks 12 weeks	66 adults (18-75 yrs, 21:13 male to female for WS group, 14:20 for placebo group) with schizophrenia or schizoaffective disorder (PANSS score ≥60) and recent symptom exacerbation. On stable antipsychotic dose Randomised, double-blind, placebo-controlled study	▼PANSS single item depression and anxious/depression scores	Not assessed	No Advers effects reported	43

Shoden; (Arjuna Natural Ltd.) Capsules of etha nol:water (70:30) extract	240 mg once daily after dinner with 250 mL of water 8.5 weeks (60 days)	60 healthy adults (18-65 yrs; 37 males, 23 females) with HAM-A scores 6-17 Randomised, double-blind, placebo-controlled study	▼HAM-A score, ▼DASS-21 (near significant)	▼serum cortisol, ▼serum DHEA S, ▲serum testosterone (males only)	No Advers effects reported	44

▼= significant decrease compared to placebo; ▲= significant increase compared to placebo; WS= Withania Somnifera; DASS = Depression Anxiety Stress Scale; DASS-21 = Depression Anxiety Stress Scale-21; FCQ-T = Food Cravings Questionnaire – Trait; HAM-A/HARS = Hamilton Anxiety Rating Scale; IL-6 = Interleukin 6; PANSS = Positive and Negative Syndrome Scale; PSS = Perceived Stress Scale; QoL = Quality of Life; HAS=Hamilton Anxiety Scale; GHQ= General Health Questionnaire; GSQS= Groningen Sleep Quality Questionnaire; HDRS=Hamilton Depression Rating Scale.

Possible mechanisms for effects on anxiety

Studies investigating the mechanisms behind the anti-anxiety effects of Withania somnifera (WS) have identified several key factors. Notably, its effects on the GABA (Gamma-Aminobutyric Acid) receptor system, along with its antioxidant and anti-inflammatory activities, may play significant roles.

GABA is recognized as the primary inhibitory neurotransmitter in the central nervous system. GABAergic neurotransmission is believed to play a crucial role in regulating anxiety. GABA type A (GABAA) receptors are the main targets of GABA agonist drugs, which enhance GABAergic activity and are widely used to treat anxiety disorders[27]. There is substantial clinical evidence that molecules in Withania somnifera modulate these receptors, particularly by interacting with GABA-A receptors. This interaction may account for some of the anxiolytic effects of Withania somnifera[28].

Mehta et al. demonstrated the direct GABA-mimetic effect of Withania somnifera. Their research showed that the methanol extract of Withania somnifera increased chloride ion entry in mammalian spinal cord neurons in the absence of GABA, exhibiting GABA-mimetic activity. Additionally, studies have indicated that molecules in the methanol root extracts of Withania somnifera have a high affinity for GABA-A receptors while exhibiting a lower affinity for GABA-B, glutamatergic, and opioid receptors[29,30].

The effect of Withania somnifera on the GABA-A receptor has been demonstrated in various animal studies. For example, the methanol root extract of Withania somnifera was shown to suppress the stimulant effects of morphine and ethanol on dopaminergic neurons in rats via the GABA-A receptor. In this study, the spontaneous firing rate and dopamine transmission of Ventral Tegmental Area neurons were significantly stimulated by morphine and ethanol. However, the extract of Withania somnifera, used at concentrations of 200-400 μg/ml, was found to significantly reduce both morphine and ethanol-mediated spontaneous neuronal firing of dopaminergic neurons in the Ventral Tegmental Area through a GABA-A-mediated mechanism, but not the GABA-B receptor-mediated mechanism[30].

Withania somnifera is also a potent agonist of GABAϱ1 receptors, demonstrating greater sensitivity to these receptors than to GABA-A receptors. GABAϱ1 receptors are a subclass of GABA-A receptors, and the effect of Withania somnifera on these receptors is more pronounced compared to other GABA-A receptor subtypes. For example, a study investigated the pharmacological effects of withaferin A and withanolide A, compounds found in Withania somnifera root extract, on natural rat brain GABA-A channels microtransplanted into Xenopus oocytes and GABAϱ1 receptors heterologously expressed in oocytes. The results indicated that Withania somnifera has a strong agonist effect on GABAϱ1 receptors, with these receptors being 27 times more sensitive to Withania somnifera than GABA-A receptors[31].

The brain is an organ sensitive to oxidative stress and studies show that anxiety disorders are associated with increased oxidative damage with decreased antioxidant defence[32]. In animal studies investigating the anxiolytic effects of Withania somnifera, a link between anxiety-like behaviors and oxidative stress

and inflammatory markers has been shown. It was determined that these inflammatory markers returned to normal levels as a result of Withania somnifera administration. For example, both root and leaf extracts of Withania somnifera were found to increase catalase activity in the brains of mice with acute sleep deprivation and zebrafish with neurotoxicity induced by benzopyrene administration. Consequently, reduced glutathione (GSH) levels increased, and lipid peroxidation decreased. In another study, Withania somnifera administered in an ischemic stroke rat model was shown to enhance antioxidant activity by reducing lipid peroxidation in the brain[33].

In animal models, the aqueous leaf extract of Withania somnifera decreased pro-inflammatory cytokines, reactive gliosis, neuroinflammation markers, and modulated inflammatory pathways[34]. Moreover, in a rat obesity model induced by a high-fat diet, Withania somnifera dry leaf powder regulated the NFκB pathway and decreased pro-inflammatory cytokine levels, reactive gliosis, neuroinflammation markers, and apoptosis[35].

These findings show that the anti-anxiety effects Withania somnifera might be based on its ability to regulate oxidative stress, neuroinflammation, and the GABA A receptor.

EFFECTS OF ASHWAGANDHA AGAINST DEPRESSION

Animal studies

The antidepressant effects of Ashwagandha have been demonstrated in various studies using root extracts, leaf extracts, and components isolated from Withania somnifera (Table 1). In particular, the anti-depressant activities of components such as withanoside X and cytoindosides VII-X were determined. Water, methanol, and hydroalcohol-based extracts of Withania somnifera and traditional root extract have all been shown to produce anti-depressant effects[36]. In addition, Withania somnifera has been found to increase the effects of antidepressant drugs used in the treatment of depression. Withania somnifera administered in depression models developed in mice and rats has been found to enhance the effects of antidepressant drugs such as imipramine, a tricyclic antidepressant, and fluoxetine, a selective serotonin reuptake inhibitör [37,38]. Additionally, it was determined that Withania somnifera regulates blood glucose and insulin levels altered by stress, alongside its antidepressant effects, when administered at doses higher than 33.3 and 100 mg/kg/day[39].

The antidepressant effects of Mamsyadi Kwatha, an Ayurvedic compound (composed of Nardostachys jatamansi, Withania somnifera, and Hyoscyamus niger in an 8:4:1 ratio), were investigated in albino mice subjected to an experimental depression model. To measure the antidepressant effect of Mamsyadi Kwatha, the Behavioral Despair Test, Antireserpine Test, and Chronic Fatigue Syndrome (CFS) test were conducted on the albino mice. The results from these tests indicated that Mamsyadi Kwatha significantly inhibited behavioral despair in the mice, exhibited mild to moderate anti reserpine effects (such as ptosis, catatonia, and sedation), and demonstrated a moderate effect in the CFS test. These findings clearly show that Mamsyadi Kwatha possesses antidepressant properties[40].

In another study, C. elegans nematodes with a serotonin deficiency mutation were evaluated for their resistance to oxidative, osmotic, or heat stress and their expected lifespan after treatment with withanolide A. It was found that withanolide A increased resistance to stress, extended lifespan, and reduced ROS (reactive oxygen species) activity. These results were confirmed by molecular docking analyses, which indicated strong binding between the ligand and the target protein. Given the high similarity of neuronal genes between C. elegans and vertebrates, these findings are promising for developing new therapeutic strategies to combat depression[41].

In a study by Tripathi et al, rats were orally administered 100 mg/kg Ashwagandha (Withania somnifera) root extract for 4 and 8 weeks. Treated rats showed an increase in open-field behavior and emotional stability. In addition, a moderate increase in the functional sensitivity of 5-HT2 receptors in the brain was observed. Chronic Ashwagandha treatment successfully prevented behavioral impairments in open field activity in animal models of depression, which are associated with adaptive hypersensitisation of postsynaptic 5-HT2 receptors in the brain. The effects of Ashwagandha on 5-HT receptor subtypes are similar to those of chronic Electroconvulsive Therapy (ECT), tricyclic antidepressants, and MAO inhibitors[42].

Phase studies

Chandrasekhar et al. (2012) investigated the antidepressant effects of a high-concentration full-spectrum extract derived from the roots of the Ashwagandha plant. In this study, sixty-four individuals aged 18-57 with a history of chronic stress were included for 60 days (Table 2). Participants were randomly divided into two groups: a placebo group and a treatment group. The treatment group received 300 mg of full-spectrum Ashwagandha root extract twice daily. By the end of the study, the treatment group that received the high-concentration full-spectrum Ashwagandha root extract demonstrated a significant reduction in all stress rating scales compared to the placebo group. Additionally, serum cortisol levels in this group were found to be significantly lower than those in the placebo group[43].

In a study conducted by Gannon et al. (2019), the effects of Withania somnifera extract on depression and anxiety symptoms in individuals diagnosed with schizophrenia were examined in a randomized, placebo-controlled clinical trial. The study lasted 12 weeks and included 66 participants who were randomly assigned to either a placebo group or a treatment group. The treatment group received 1000 mg of Withania somnifera extract daily. Participants were assessed using the Positive and Negative Syndrome Scale (PANSS), a widely used measure for evaluating symptoms in schizophrenia and other psychotic disorders. This scale is designed to assess the patient's overall psychiatric condition and monitor changes in specific symptomatic areas. At the beginning of the trial, there were no significant differences between the two groups in terms of single-item depression scores and anxiety-depression cluster scores according to PANSS ratings. By the end of the trial, the group treated with Withania somnifera showed significantly higher single-item depression scores and anxiety-depression cluster scores compared to the placebo group. Withania somnifera extract was generally well tolerated, and there were no significant differences in side effects between the treatment and placebo groups. The findings suggest that Withania somnifera extract may be a promising treatment for depression and anxiety symptoms in patients with schizophrenia[44].

Lopresti et al. (2019) investigated the stress-reducing and other pharmacological effects of Ashwagandha extract in healthy adults under stress. Sixty adults participated in the study, and the participants were randomly assigned to either a placebo group or a treatment group. The treatment group received 240 mg of Ashwagandha extract once daily for 60 days. The results were evaluated using the Hamilton Anxiety Rating Scale (HAMA) and the Depression Anxiety Stress Scale-21 (DASS-21). Additionally, the cortisol, dehydroepiandrosterone sulfate (DHEA-S), and testosterone hormone levels of the participants were measured. The Hamilton Anxiety Rating Scale is a questionnaire used to assess anxiety levels, containing specific symptoms, each corresponding to a particular score, providing an objective reflection of anxiety levels based on the total score. The Depression Anxiety Stress Scale-21 is designed to measure levels of depression, anxiety, and stress. It consists of 21 items, with 7 questions in each subscale. Participants respond by their emotional states. Depression, anxiety, and stress levels are determined by calculating total scores on the subscales. There was a statistically significant decrease in HAMA scores and a partial decrease in DASS-21 in the Ashwagandha group compared to the placebo group. Large decreases in morning cortisol and DHEA-S were observed in the group receiving Ashwagandha compared to placebo. Testosterone levels in men increased over time. However, this change was not considered statistically significant compared to placebo. These results suggest that the stress-reducing effects of Ashwagandha are due to its regulatory effect on the hypothalamus-pituitary-adrenal axis[45].

Possible mechanisms for effects on depression

The bioactive components in Withania somnifera are known to enhance antioxidant capacity and regulate inflammatory responses. Therefore, the effectiveness of Withania somnifera in combating depression may be attributed to fundamental mechanisms such as reducing oxidative stress and modulating inflammation. These potential mechanisms may provide the scientific basis for the antidepressant activity of Withania somnifera and suggest its role as a potential natural supplement in the treatment of depression[46].

Experimental studies suggest that Withania somnifera may have potential serotonergic activity and this feature may explain its antidepressant effects. For example, in a study conducted by Priyanka and colleagues (2020) by creating a stress model in horses, it was found that Withania somnifera root powder showed a protective effect against the decrease in

serotonin levels associated with stress caused by exercise, separation, and noise[47]. In another study with mice, similarly, Withania somnifera root extract was found to protect against stress-induced decrease in hippocampal serotonin levels[48]. Furthermore, withanolide A compound was found to increase the mRNA expression of serotonin receptors and transporters in Caenorhabditis elegans. Molecular studies have shown that withanolide A binds to human and C. elegans serotonin receptors and serotonin transporters with a higher affinity than serotonin and fluoxetine[41]. The possible mechanism of action of Ashwagandha in mental disorders such as stress and stress-related neuropsychiatric disorders depression and anxiety is presented in Figure 2.

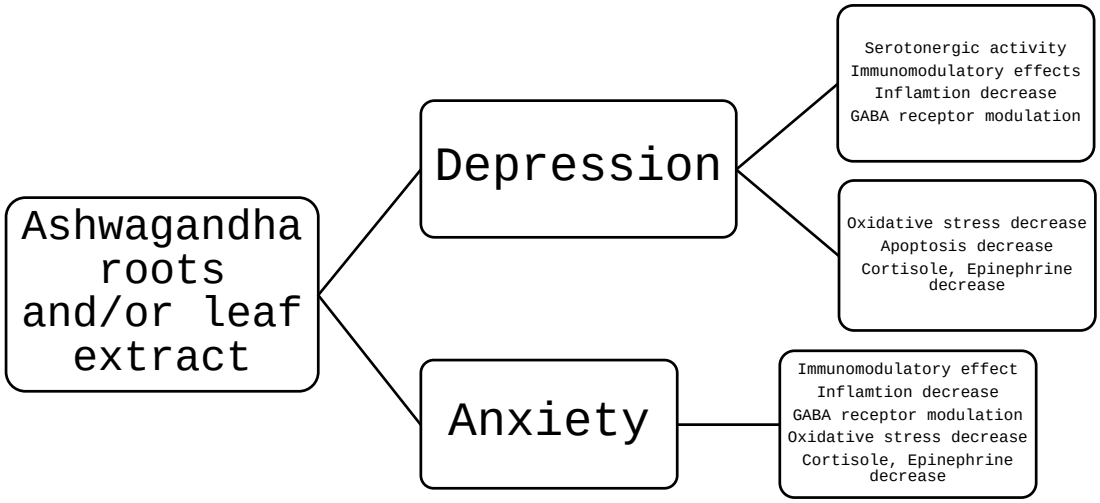

Figure 2. Potential mechanisms by which Ashwagandha exerts positive benefits on depression and anxiety.

SIDE EFFECTS AND HERB-DRUG INTERACTIONS IN CLINICAL TRIALS

Before starting the therapeutic use of any new medicinal plant, the evaluation of its possible side effects and toxicity in various body systems is the first procedure that should be performed. Many studies have reported that Withania somnifera extract is safe for all age groups, male and female individuals[49]. For example, 40 children aged 8-12 years with mild nutritional deficiencies were administered 2.0 g/day of dried Ashwagandha root powder for 60 days and no adverse effects were observed[50]. In another randomized double-blind placebo-controlled study, 58 healthy children aged 8-12 years were administered 2.0 g/day dried Ashwagandha root powder and no side effects were observed[51]. The same situation has been observed in healthy adults in many studies. The same situation has been observed in healthy adults in many studies. For example, in a randomized double-blind placebo-controlled study, 101 healthy men were administered 3.0 g/day of dried Ashwagandha root powder for 60 days, and no side effects were observed[52]. In another randomised double-blind placebo-controlled study, 50 healthy women were given 300 mg Ashwagandha root extract capsules twice daily for 8 weeks and no side effects were reported[53]. In a study evaluating the effects of Withania somnifera extract on pregnant rats, the focus was on the period between the 5th and 19th days of gestation. This is a particularly sensitive period due to increased organogenesis and histogenesis in the fetus. In Ashwagandha, doses as high as 2000 mg/kg/day were administered orally. No toxic effects were observed and no changes in body weight, corpus luteum count, or embryo implantation were observed in pregnant rats. Furthermore, no external appearance, skeletal or visceral deformities were detected in the foetuses[54]. However, there are no studies in the literature regarding the gestation period of women. Nevertheless, based on this study, Ashwagandha can

be considered pregnancy category B. In a study in elderly people, a 12-week, prospective, randomized, double-blind, placebo-controlled, double-blind, placebo-controlled study was conducted on individuals of both sexes aged 65-80 years. Participants were randomized to receive Ashwagandha root extract orally at a dose of 600 mg/day and no adverse effects were reported[55].

Withania somnifera supplementation is typically administered in doses ranging from 240 mg to 1250 mg, with the most commonly used dose being 600 mg. The duration of use varies from 2 weeks to 6 months, with most studies utilizing an 8-week period[56].

In clinical studies, no serious side effects or changes in hematological, biochemical, and vital parameters have been reported. Mild and generally transient side effects have been observed. The most common side effects reported in clinical studies are epigastric pain/discomfort and loose stools, while less common side effects include dizziness, drowsiness, hallucinations, vertigo, nasal congestion, cough, cold, loss of appetite, nausea, constipation, dry mouth, hyperactivity, night cramps, blurred vision, hyperacidity, skin rash, and weight gain[49,57]. However, there is no information in the literature on possible withdrawal symptoms.

It is known that most xenobiotics and herbal medicines are metabolized by phase I enzymes. In vitro experiments have shown that extracts of Withania somnifera prepared with water, methanol, ethanol, and ethyl acetate solvents affect liver microsomal enzymes. For example, it has been demonstrated that methanolic and ethyl acetate extracts inhibit CYP2B6 enzyme activity but moderately induce CYP3A4 mRNA expression in HepG2 cells. According to these findings, it is important to consider potential herb-drug interactions when using Withania somnifera root extracts or supplements in patients treated with drugs metabolized by CYP2B6 or 3A4 enzymes (such as artemisinin, bupropion, cyclophosphamide, efavirenz, ketamine, atorvastatin, cyclosporine, diazepam, estradiol, simvastatin, sildenafil, and verapamil). Therefore, caution should be exercised when administering such drugs concurrently with Withania somnifera [58].

In a study examining hydroethanolic extracts from 30 of the best-selling and widely used herbal dietary supplements in the USA, the ability of these plants to activate receptors such as the human pregnane X receptor (hPXR) and the human aryl hydrocarbon receptor (hAhR), as well as their potential to enhance the activities of drug-metabolizing cytochrome P450 enzymes (CYP3A4 and CYP1A2, respectively) regulated by hPXR and hAhR, were investigated. Withania somnifera was found to increase the activity of both CYP3A4 and CYP1A2 enzymes by more than 50% at various concentrations[59].

Side effects seen during the simultaneous use of Withania somnifera with other drugs are another important point. The most important group of drugs that draws attention in this regard are antidepressants. Generally, the most common interaction is seen with the use of selective serotonin reuptake inhibitors (SSRIs). It is suggested that the most important mechanism underlying this interaction is the cytochrome 450 isoenzymes (CYP3A4 and CYP2B6) responsible for Withania somnifera and antidepressant metabolism. For example, an increase in side effects was observed when Withania somnifera was used in combination with escitalopram (SSRI), paroxetine (SSRI), reboxetine (Noradrenaline selective reuptake inhibitors -NARI), and sertraline (SSRI). Another mechanism by which Withania somnifera interacts with other drugs may be its effect on p-glycoprotein. This protein complex is widely present in the intestinal epithelium and blood-brain barrier and is responsible for the removal of xenobiotics from cells. Active compounds found in Withania somnifera have been shown to inhibit the activity of the p-glycoprotein complex. As a result, it has been shown that Withania somnifera may affect the distribution of antidepressant drugs, which are p-glycoprotein substrates and may cause an increase in antidepressant drug concentrations in the central nervous system. Antidepressants metabolized by this mechanism include sertraline (SSRI), agomelatine (melatonin agonist and selective serotonin antagonist-MASS), citalopram (SSRI), escitalopram (SSRI), trazodone (serotonin antagonist and reuptake inhibitor-SARI) and paroxetine (SSRI)[60].

CONCLUSION

Many studies in the literature reveal that the Ashwagandha plant has positive effects on mental health issues such as anxiety and depression. Thanks to its adaptogenic properties, Ashwagandha can enhance the ability to cope with anxiety and depression, offering an effective coping strategy. The fact that Ashwagandha has fewer side effects

compared to chemical drugs and possesses a broad mechanism of action as a natural adaptogen presents the potential for further research and application in the treatment of mental health problems in the future. Ashwagandha may offer an alternative approach to traditional antidepressant and anxiolytic drugs, which are often not preferred or not effective enough due to side effects.

However, further clinical studies and evaluation of long-term effects are required. Firstly, more clinical studies should be carried out to understand the effects of Ashwagandha on certain types of depression or anxiety disorders in more detail. It is also important to expand research on the effects of different formulations and dosages of the herb. Elucidation of the mechanisms of action at the molecular level is also critical. Ashwagandha's effects on the nervous system and neurotransmitter regulation should be more deeply understood and its effects on biochemical changes, especially those associated with depression and anxiety, should be determined.

In conclusion, Ashwagandha should be considered a promising natural shield in the fight against anxiety and depression, but more scientific studies and research are needed.

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