SOBOTA, Weronika, PISKORZ, Przemysław, ZEMSTA, Katarzyna, ZWOLIŃSKI, Michał, TYNIEC, Mateusz, KAMAL, Morshed. Ashwagandha and stress Journal of Education, Health and Sport. 2024;70:55514. eISSN 2391-8306.
https://dx.doi.org/10.12775/JEHS.2024.70.55514
https://apcz.umk.pl/JEHS/article/view/55514

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences); Health Sciences (Field of medical and health sciences).
Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).© The Authors 2024;
This article is published with open access at Licensee Open Journal Systems of Nicolaus Copernicus University in Torun, Poland

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received:4.10.2024. Revised: 4.11.2024. Accepted: 7.11.2024. Published: 7.11.2024.

Ashwagandha and stress

Weronika Sobota (28%)
werasobota@gmail.com
ORCID: 0009-0000-7778-7030

Przemysław Piskorz (16%)
przemekpiskorz95@o2.pl
ORCID: 0009-0000-9812-2230

Katarzyna Zemsta (16%)
k.zemsta99@gmail.com
ORCID: 0009-0005-8336-2094

Michał Zwoliński (16%)
michzal714586@gmail.com
ORCID: 0009-0008-5093-5337

lek. Mateusz Tyniec (12%)
mateusztyniec@yahoo.com
ORCID: 0000-0002-0191-319X

prof. dr hab. n. med. Kamal Morshed (12%)
k.morshed@uthrad.pl
ORCID: 0000-0003-0061-0362

Abstract

Ashwagandha, also known as Withania somnifera, is an adaptogen gaining popularity as a natural stress reducer. Stress is a common phenomenon caused by factors such as lifestyle, work pressure or social conflict, and has a negative impact on both mental and physical health. In response to these challenges, more and more people are looking for natural solutions, and adaptogens such as ashwagandha offer support in dealing with stress without significant side effects. Scientific research shows that ashwagandha can lower levels of the stress hormone cortisol and improve results on tests assessing stress, anxiety and depression. Numerous studies conducted on both animals and humans confirm its beneficial effects on mental and physical health, including improvement of cognitive functions, sleep quality and emotional well-being. The mechanism of action of ashwagandha includes regulation of the HPA axis, reduction of glucocorticoids and modulation of the immune system. Despite promising results, further research is needed to fully understand its mechanism of action, assess long-term effects, and investigate possible interactions with other drugs. Ashwagandha seems to be a safe and effective agent in the fight against stress, but it is recommended to consult a doctor before starting supplementation.

Keywords: ashwagandha; stress

Introduction

Modern lifestyles, professional pressures, financial problems, illnesses, social conflicts, or traumatic events are just some of the factors contributing to stress. Recently, there has been an increase in this phenomenon, already affecting almost a third of the population [1]. Stress mainly affects mental health, affecting people of all ages, ethnic groups and social classes. It is characterized by feelings of nervousness, anxiety, anguish and difficulty responding normally to environmental demands. There is a subtle line between stress and anxiety, as both of these emotional reactions manifest with overlapping symptoms. Long-term exposure to stress can negatively affect an individual's mental and physiological state, leading to potentially irreversible health problems such as metabolic syndromes, cardiovascular problems, endocrine

complications, affective and anxiety disorders, and even infectious diseases [2, 3]. Numerous scientific reports confirm that stress levels are inversely related to quality of life [4].

In an attempt to cope with stress, people often seek the help of therapists [5] or turn to pharmacological agents, the effects of which can carry adverse health effects. In an effort to deal with stress in a more natural and less stressful way, substances of plant origin, often referred to as adaptogens, are gaining popularity. These are herbal extracts that enhance the body's ability to adapt to environmental stressors while minimizing damage from such factors. They are characterized by safety of use, are non-habit forming and harmonize metabolic systems, enhancing the body's response to external factors. One plant adaptogen that is attracting scientific attention is ashwagandha.

Withania somnifera Dunal, popularly known as ashwagandha, plays an important role in the Ayurvedic system of medicine. It has long been used as an adaptogen, promoting vitality by increasing muscle strength, endurance and overall health. Ashwagandha's main chemical constituents of pharmacological importance are steroidal lactones and their glycosides, known as vitanolides [2].

It is necessary to conduct research on natural substances of plant origin that will not only contribute to stress reduction, but also be fully legal and free of side effects. The purpose of this study is to evaluate the effects of ashwagandha on stress levels based on current scientific reports. The research material included publications available in PubMed and Google Scholar databases. The search process used a combination of keywords, such as "ashwagandha," "Withania somnifera," "stress," and "adaptogen." The first step was to select publications from the last five years, and the second step was a detailed analysis of the papers found.

Scientific reports

2.1

Alex B. Speers and colleagues published a meta-analysis in 2021 on the effects of ashwaganda on stress and neuropsychiatric disorders. They reviewed studies on both animal and human models [6].

Based on animal studies, the researchers proved that ashwaganda has anti-stress effects. They evaluated primarily the root extract, but also the leaves and defatted seeds.
Various methods were used to test stress levels before and after ashwaganda application. The most commonly used was the forced swim test (FST), originally developed as an animal model of depression. It consisted of placing an animal in a cylindrical container filled with water and observing whether it actively swam or adopted a passive posture [7]. Increased swimming time after taking ashwaganda was indicative of good stress management, as seen in many studies [8]. Other methods included exposure to low temperatures, sleep deprivation [9], hypoxia [10], and the use of electric shocks [11]. Ashwaganda had a positive effect on various symptoms with which the animal compensated for stress, such as: memory impairment [12], stomach ulcers, elevated glucocorticoids [13].
A number of studies have been conducted on a human model. Participants in the projects were healthy individuals between the ages of 18 and 75 who were stressed [14], experiencing chronic stress at work [15], overweight or diagnosed with anxiety.
Eligible subjects were required to take one capsule of an ashwaganda preparation with various supplemental ingredients daily for a period of 8-12 weeks [16]. Stress levels were assessed using serum cortisol levels and questionnaires. The Perceived Stress Scale (PSS) tested the respondent's level of stress experienced in the past month. The General Health Questionnaire-28 (GHQ-28) assessed anxiety, insomnia, social dysfunction, and depression. The Depression and Anxiety Stress Scale (DASS) measured symptoms of depression, anxiety, and stress.
As a result, ashwaganda supplementation led to significant reductions in serum cortisol levels, as well as improved scores on questionnaires assessing stress, depression and anxiety.

In conclusion, the studies used in the meta-analysis suggest that ashwaganda may be a promising option for treating chronic stress in both animals and humans.

2.2

Kumarpillai Gopukumar et al. in their randomized, prospective, double-blind, placebo-controlled clinical trial tested the efficacy of Ashwagandha root extract (Ashwagandha SR 300 mg capsule) in extended-release (SR) form on cognitive function, stress levels, sleep, and quality of life in people experiencing stress. They also studied the safety and tolerability of Ashwagandha SR capsules.

The study included healthy individuals aged 20-55, with a body mass index of 18-29 kg/m2, who are under stress and scored PSS 14-24 on the Perceived Stress Scale. Participants were instructed to take one capsule daily after breakfast for 90 days. The effectiveness of the product was evaluated by measuring changes in cognitive function using the CANTAB method from baseline to the end of the study period. The change in PSS-10 score, serum cortisol level (9-11 a.m.), OHQ score, PSQI score and serum BDNF level from baseline to the end of the study period were also considered. Safety was assessed as the percentage of patients who terminated participation in the study due to adverse events, i.e. changes in vital signs and laboratory tests (blood count, alanine aminotransferase [ALT], aspartate aminotransferase [AST] and serum creatinine levels).

125 subjects completed the study. The Cambridge Neuropsychological Test Automated Battery (CANTAB) showed a significant improvement in recall memory, and the total error rate in recall patterns decreased significantly in the group taking ashwaganda compared to the placebo group. The Perceived Stress Scale Index (PSS-10), serum cortisol levels and Pittsburgh Sleep Quality Index (PSQI) score significantly decreased, and scores on the Oxford Happiness Questionnaire (OHQ) were higher in the Ashwaganda SR group compared to the placebo group. Those taking the Ashwagandha SR capsule were 83.4% more likely to achieve stress control than those taking the placebo.

The study found that taking 1 Ashwagandha SR capsule daily in healthy adults provided improved memory and concentration, lowered stress levels, better sleep quality and improved mental well-being. No adverse effects were observed, and safety parameters were normal [17].

2.3

Muhammed Majeed et al. in 2023 published the results of their randomized, double-blind, placebo-controlled study that tested the effects of Ashwagandha root extract (ARE-500 mg) standardized to 2.5% vitanolides according to the USP protocol with piperine (5 mg 95% piperine) to relieve stress and anxiety in healthy individuals with mild/moderate symptoms.

The study invited healthy adults 21 to 54 years old with a PSS score = 14-25 and GAD-7 < 15 who experienced fatigue, insomnia, loss of concentration and appetite. Study participants took

ARE-500 mg with 95% piperine once a day before bed for 60 days. To determine how the formula worked, they used the PSS scale (to assess individual stress levels), the GAD-7 scale (to assess the severity of generalized anxiety disorder), the World Health Organization's QOL-BREF questionnaire (to assess quality of life), and the CANTAB test (to assess cognitive function). Changes in salivary cortisol, urinary serotonin, dopamine, nitric oxide, glutathione, serum malondialdehyde levels were also checked
from the initial value to the end of the study. The safety of the formulation was evaluated based on laboratory tests and the number of adverse events.

50 subjects completed the study. Significant improvements were observed in concentration, decision-making time and multitasking on the CANTAB test, and significantly better PSS, GAD-7 and QOL scores in the ARE group compared to the group taking placebo. An increase in urinary serotonin levels was also noted, as well as a greater reduction in morning salivary cortisol in the Ashwaganda group.

The study was considered safe, and the formulation was found to be well tolerated by the body.

The results suggest that ARE with 2.5% vitanolides can effectively reduce stress levels and reduce anxiety in healthy adults experiencing mild to moderate stress. This happens by reducing cortisol and increasing serotonin levels. The formulation also works to improve cognitive function and the subjects' quality of life. Further research is needed, particularly on a larger study group [2].

2.4

A study conducted by the team of S.J. Smith et al. in 2023 provides further confirmation of the link between ashwagandha use and stress reduction. The purpose of the study was to evaluate the effects of supplementation with 200 mg of ashwagandha root extract (Witholytin®) twice daily for 12 weeks on stress levels, fatigue and other health parameters in healthy men and women aged 40 to 75 years with overweight or moderate obesity.The study included 120 participants and was a two-arm, parallel, randomized, placebo-controlled, double-blind study. Participants were randomly divided into an ashwagandha group or a placebo group, taking the respective capsules for 12 weeks. Adherence was monitored using an app to track capsule intake and weekly questionnaires.

The results of the study showed that ashwagandha supplementation resulted in a significant reduction in stress levels, as assessed by the PSS scale, compared to the placebo group. However, this improvement did not reach statistical significance. In addition, a significant reduction in fatigue symptoms was observed in the ashwagandha-taking group, according to the CFS scale, compared to the placebo group. The ashwagandha group also showed an increase in heart rate variability (HRV), suggesting increased parasympathetic activity, although differences in this parameter between groups were not statistically significant. In the context of hormonal parameters, men taking ashwagandha showed an increase in free testosterone (FT) and luteinizing hormone (LH), and a tendency to increase MDA (lipid peroxidation products). On the other hand, an increase in estradiol concentrations was noted in women, especially in those in the peri-menopausal period.

The results of this study support the beneficial effects of ashwaganda on stress and fatigue levels, but there are some limitations, such as the small number of participants and lack of statistical significance in some parameters. Further research is needed to fully understand the potential health benefits of ashwaganda [18].

2.5

A study conducted by an Indian team led by KrishnaRaju AV is an important contribution to understanding the potential anti-anxiety and antidepressant properties of ashwagandha (Withania somnifera). In in vitro studies on lipopolysaccharide (LPS)-induced human THP-1 monocytes and PMA-stimulated HL-60 monocytic cells, AshwaSR demonstrated the ability to inhibit the production of interleukin IL-1β and tumor necrosis factor alpha (TNF-α) in a dose-dependent manner. In the context of PMA stimulation, AshwaSR also showed an inhibitory effect on superoxide production, suggesting potential antioxidant properties of the compound.

Based on in vitro studies, an in vivo method was designed where rats were given chronic stress (CUS), administration of ashwagandha (AshwaSR) showed beneficial effects on stress response. A reduction in anxiety symptoms was observed, measured as a reduction in the number of entries and time spent in open arms in the Elevated Plus maze test. In addition, ashwagandha had an effect on cognitive parameters, reducing goal latency and distance traveled in the Morris maze test.

The results of these studies suggest that ashwagandha may have effects on the body's response to stress, both at the cellular and behavioral levels. These include inhibition of the inflammatory

response, protection against oxidative stress, and improved cognitive function. Despite the promising results, further clinical studies are needed to confirm these observations and evaluate the efficacy of ashwagandha in humans [19].

2.6

Using a randomized, double-blind, placebo-controlled design, a team of researchers led by A. Remenapp set out to confirm the hypothesis that 30 days of Ashwagandha supplementation would result in improvements in cognitive ability, feelings of stress/anxiety, depressive disorders, food cravings and cortisol levels compared to the placebo group.

The study conducted included healthy adults (43 women and 17 men, mean age 34.41 years) who reported experiencing stress.Participants were randomly assigned to three groups receiving Ashwagandha (400 mg/d), Ashwagandha (225 mg/d) and placebo, participants took the supplements for a period of 30 days. The study was conducted on days 0, 15 and 30, assessing salivary cortisol levels, cognitive performance and self-reports related to anxiety, stress, depression and food cravings.

The results of the study showed significant improvements in study participants' self-assessments related to anxiety, depression, stress and food cravings in the groups receiving ashwagandha. Moreover, vital CNS parameters such as cognitive flexibility, visual memory, reaction time, psychomotor speed and executive functioning improved, often surpassing the results of the placebo group.

In terms of cortisol levels, a significant reduction was observed in the group receiving ashwagandha 225 mg/d compared to baseline, confirming the beneficial effect of ashwagandha in regulating levels of this stress hormone.

It is worth noting that ashwagandha supplementation appeared to be safe, with participants reporting no adverse events. The results of the study suggest that ashwagandha may be an effective agent in improving physiological, cognitive and psychological responses to stress within 30 days. These findings open up new perspectives for potential applications of ashwagandha in improving the body's mental health and adaptability. Despite the promising

results, further research is recommended, especially in the context of the longitudinal effects of ashwagandha supplementation in different population groups [20].

Mechanisms - how does ashwaganda act on stress?

3.1 Reduction of glucocorticosteroids

Ashwagandha can affect the activity of the hypothalamic-pituitary-adrenal (HPA) axis, leading to a weakening of its action. In response to stress, the HPA axis triggers a series of reactions that result in increased secretion of cortisol and DHEA(dehydroepiandrosterone) [21]. Although higher levels of DHEA, known as the hormone of youth, are often associated with increased health and longevity [22], when stressed, their elevation can be an indicator of increased stress reactivity (or HPA activity). For example, increased secretion of DHEA-S (dehydroepiandrosterone sulfate) has been observed in adults after exposure to stress [23], and higher levels of DHEA have been found in adults suffering from PTSD (post-traumatic stress disorder), according to recent reports [24]. Increased levels of DHEA have also been linked to cigarette smoking and alcohol consumption in middle-aged men [25].

The above information suggests that elevated DHEA levels (along with cortisol) could be a marker of increased stress. Accordingly, its acute decrease could signal a reduction in stress. The production of cortisol and DHEA occurs in different layers of the adrenal cortex, with cortisol produced in the bundle sheath and DHEA in the reticular sheath. Although there is a negative feedback regulatory system that restores cortisol levels after exposure to stress, ample evidence suggests that anxiety and depressive disorders are associated with abnormalities in HPA axis activity, often leading to excessive cortisol secretion [26]. Reductions in morning cortisol and DHEA-S levels in participants of Dr. Lopresti's team's study taking ashwagandha suggest that it may have a role in attenuating HPA axis activity, as evidenced by reductions in morning fasting cortisol (down 0.5% in the placebo group and 23% in the study group) and DHEA-S (up 2.5% and down 8.2%, respectively). Statistically significant changes in the levels of these hormones occurred in both sexes [27].

In order to fully understand the mechanism of ashwagandha's stress-reducing effects through its effects on glucocorticosteroid metabolism, further studies, including randomized controlled clinical trials, will be needed to confirm these observations and better understand the mechanism of action of this herbal preparation.

3.2 Immune modulation

In the context of immune modulation, ashwagandha may exert anti-stress effects by influencing the immune system. In various animal models, ashwagandha has been observed to have the ability to regulate immune markers. In an equine model of stress induced by exercise, separation and noise, ashwagandha increased the number of white blood cells and the percentage of lymphocytes, while decreasing the level of the pro-inflammatory cytokine IL-6 [14]. In a rat model of constrictive stress, ashwagandha attenuated the decrease in the number of T lymphocytes, IL-2, INF-γ and multinucleated leukocytes [28]. In contrast, in cold exposure and forced swimming stress models, ashwagandha reversed the increase in white blood cells, neutrophils, lymphocytes and eosinophils, suggesting an immunomodulatory effect [8].

Ashwaganda contains active substances such as withanolides and withaferins, which exhibit anti-inflammatory properties [29]. In the body, mental stress can lead to excessive secretion of pro-inflammatory cytokines, which can affect brain function and cause symptoms of depression and anxiety [30]. Ashwaganda can inhibit the production of these pro-inflammatory cytokines (tumor necrosis factor TNF-α, interleukin IL-1β and IL-6) [31], which helps balance inflammatory responses and reduce mental stress [19].

NK (natural killer) cells are key in fighting cancer cells and viral infections. Mental stress can weaken immune system function, making the body more susceptible to infections and other diseases. Ashwaganda can increase NK cell activity, which strengthens the body's immune system and reduces the risk of stress-related diseases. Ashwaganda can also affect the activity of T and B lymphocytes which are responsible for fighting pathogens and producing antibodies. Stimulating the production of T and B lymphocytes may help improve the body's immune function during exposure to mental stress [32].

4 Summary

The above chapters have discussed various aspects related to ashwagandha, and its potential anti-stress effects. Stress is a common problem that can have a negative impact on mental and physical health. More and more people are looking for natural ways to deal with stress, and

ashwagandha seems to be one promising solution. Research suggests that ashwagandha may have a beneficial effect on reducing stress levels. Its adaptogenic properties may help the body better cope with stress and regulate levels of cortisol, the stress hormone. In addition, ashwagandha has shown the potential to improve mental well-being and cognitive function, which may further help reduce perceived stress.

Despite the promising results, further research is needed to fully understand ashwagandha's mechanism of action and to evaluate its efficacy in different population groups. Studies on the long-term effects of ashwagandha and potential interactions with other drugs are also worthwhile.

These findings suggest that ashwagandha may be an effective and safe means of treating chronic stress and improving mental and physical health. Appropriate dosage recommendations also seem necessary. However, a doctor should always be consulted before starting ashwagandha supplementation, especially if a person is taking other medications or has existing health conditions.

References:

1. Salari N, Hosseinian-Far A, Jalali R, Vaisi-Raygani A, Rasoulpoor S, Mohammadi M, Rasoulpoor S, Khaledi-Paveh B. Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis. Global Health. 2020 Jul 6;16(1):57. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7338126/
2. Majeed M, Nagabhushanam K, Mundkur L. A standardized Ashwagandha root extract alleviates stress, anxiety, and improves quality of life in healthy adults by modulating stress hormones: Results from a randomized, double-blind, placebo-controlled study. Medicine (Baltimore). 2023 Oct 13;102(41):e35521. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10578737/
3. Wu J, Yan J. Editorial: Stress and Cognition. Front Psychol. 2017 Jun 9;8:970. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5465286/
4. Hohls JK, König HH, Quirke E, Hajek A. Anxiety, Depression and Quality of Life-A Systematic Review of Evidence from Longitudinal Observational Studies. Int J Environ

Res Public Health. 2021 Nov 16;18(22):12022. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8621394/

5. Aziraj-Smajić V, de la Fosse C. EMDR Techniques and Behavioral Therapy in Group Training Dealing with Stress. Psychiatr Danub. 2021 Feb;33(Suppl 1):24-28. https://pubmed.ncbi.nlm.nih.gov/33638953/
6. Speers AB, Cabey KA, Soumyanath A, Wright KM. Effects of Withania somnifera (Ashwagandha) on Stress and the Stress- Related Neuropsychiatric Disorders Anxiety, Depression, and Insomnia. Curr Neuropharmacol. 2021;19(9):1468-1495. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8762185/
7. Commons KG, Cholanians AB, Babb JA, Ehlinger DG. The Rodent Forced Swim Test Measures Stress-Coping Strategy, Not Depression-like Behavior. ACS Chem Neurosci. 2017 May 17;8(5):955-960. https://pubmed.ncbi.nlm.nih.gov/28287253/
8. Puri S, Kumar B, Debnath J, Tiwari P, Salhan M, Kaur M, & Mittal A. Comparative pharmacological evaluation of adaptogenic activity of Holoptelea integrifolia and Withania somnifera. International journal of drug development and research. 2011;3(1): 84-98. https://scholar.google.com/scholar_lookup?journal=International+Journal+of+Drug+Development+and+Research&title=Comparative+pharmacological+evaluation+of+adaptogenic+activity+of+Holoptelea+integrifolia+and+Withania+somnifera.&author=S.+Puri&author=B.+Kumar&author=J.+Debnath&volume=3&issue=1&publication_year=2011&pages=84-98&
9. Manchanda S, Mishra R, Singh R, Kaur T, Kaur G. Aqueous Leaf Extract of Withania somnifera as a Potential Neuroprotective Agent in Sleep-deprived Rats: a Mechanistic Study. Mol Neurobiol. 2017 May;54(4):3050-3061. https://pubmed.ncbi.nlm.nih.gov/27037574/
10. Baitharu I, Jain V, Deep SN, Hota KB, Hota SK, Prasad D, Ilavazhagan G. Withania somnifera root extract ameliorates hypobaric hypoxia induced memory impairment in rats. J Ethnopharmacol. 2013 Jan 30;145(2):431-41. https://pubmed.ncbi.nlm.nih.gov/23211660/
11. Dey A, Chatterjee SS, Kumar V. Triethylene glycol-like effects of *Ashwagandha* (*Withania somnifera* (L.) Dunal) root extract devoid of withanolides in stressed mice. Ayu. 2018 Oct-Dec;39(4):230-238. https://pubmed.ncbi.nlm.nih.gov/31367146/

12. Alzoubi KH, Al Hilo AS, Al-Balas QA, El-Salem K, El-Elimat T, Alali FQ. Withania somnifera root powder protects againist post-traumatic stress disorder-induced memory impairment. Mol Biol Rep. 2019 Oct;46(5):4709-4715. https://pubmed.ncbi.nlm.nih.gov/31218539/
13. Priyanka G, Anil Kumar B, Lakshman M, Manvitha V, Kala Kumar B. Adaptogenic and Immunomodulatory Activity of Ashwagandha Root Extract: An Experimental Study in an Equine Model. Front Vet Sci. 2020 Sep 29;7:541112. https://pubmed.ncbi.nlm.nih.gov/33134345/
14. Salve J, Pate S, Debnath K, Langade D. Adaptogenic and Anxiolytic Effects of Ashwagandha Root Extract in Healthy Adults: A Double-blind, Randomized, Placebo-controlled Clinical Study. Cureus. 2019 Dec 25;11(12):e6466. https://pubmed.ncbi.nlm.nih.gov/32021735/
15. Choudhary D, Bhattacharyya S, Joshi K. Body Weight Management in Adults Under Chronic Stress Through Treatment With Ashwagandha Root Extract: A Double-Blind, Randomized, Placebo-Controlled Trial. J Evid Based Complementary Altern Med. 2017 Jan;22(1):96-106. https://pubmed.ncbi.nlm.nih.gov/27055824/
16. Chengappa KNR, Brar JS, Gannon JM, Schlicht PJ. Adjunctive Use of a Standardized Extract of Withania somnifera (Ashwagandha) to Treat Symptom Exacerbation in Schizophrenia: A Randomized, Double-Blind, Placebo-Controlled Study. J Clin Psychiatry. 2018 Jul 10;79(5):17m11826. https://pubmed.ncbi.nlm.nih.gov/29995356/
17. Gopukumar K, Thanawala S, Somepalli V, Rao TSS, Thamatam VB, Chauhan S. Efficacy and Safety of Ashwagandha Root Extract on Cognitive Functions in Healthy, Stressed Adults: A Randomized, Double-Blind, Placebo-Controlled Study. Evid Based Complement Alternat Med. 2021 Nov 30;2021:8254344. https://pubmed.ncbi.nlm.nih.gov/34858513/
18. Smith SJ, Lopresti AL, Fairchild TJ. Exploring the efficacy and safety of a novel standardized ashwagandha (*Withania somnifera*) root extract (Witholytin®) in adults experiencing high stress and fatigue in a randomized, double-blind, placebo-controlled trial. J Psychopharmacol. 2023 Nov;37(11):1091-1104. https://pubmed.ncbi.nlm.nih.gov/37740662/
19. KrishnaRaju AV, Somepalli V, Thanawala S, Shah R. Efficacy and Anti-Inflammatory Activity of Ashwagandha Sustained-Release Formulation on Depression and Anxiety

Induced by Chronic Unpredictable Stress: in vivo and in vitro Studies. J Exp Pharmacol. 2023 Jul 25;15:291-305. https://pubmed.ncbi.nlm.nih.gov/37521489/

20. Remenapp A, Coyle K, Orange T, Lynch T, Hooper D, Hooper S, Conway K, Hausenblas HA. Efficacy of Withania somnifera supplementation on adult's cognition and mood. Journal of Ayurveda and Integrative Medicine. 2022;13(2):100510. https://www.sciencedirect.com/science/article/pii/S0975947621001558
21. Doom JR, Gunnar MR. Stress physiology and developmental psychopathology: past, present, and future. Dev Psychopathol. 2013 Nov;25(4 Pt 2):1359-73. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3869040/
22. Baylis D, Bartlett DB, Syddall HE, Ntani G, Gale CR, Cooper C, Lord JM, Sayer AA. Immune-endocrine biomarkers as predictors of frailty and mortality: a 10-year longitudinal study in community-dwelling older people. Age (Dordr). 2013 Jun;35(3):963-71. https://pubmed.ncbi.nlm.nih.gov/22388931/
23. Lennartsson AK, Kushnir MM, Bergquist J, Jonsdottir IH. DHEA and DHEA-S response to acute psychosocial stress in healthy men and women. Biol Psychol. 2012 May;90(2):143-9. https://pubmed.ncbi.nlm.nih.gov/22445967/
24. van Zuiden M, Haverkort SQ, Tan Z, Daams J, Lok A, Olff M. DHEA and DHEA-S levels in posttraumatic stress disorder: A meta-analytic review. Psychoneuroendocrinology. 2017 Oct;84:76-82. https://pubmed.ncbi.nlm.nih.gov/28668711/
25. Nagaya T, Kondo Y, Okinaka T. Serum dehydroepiandrosterone-sulfate reflects age better than health status, and may increase with cigarette smoking and alcohol drinking in middle-aged men. Aging Clin Exp Res. 2012 Apr;24(2):134-8. https://pubmed.ncbi.nlm.nih.gov/22842832/
26. Faravelli C, Lo Sauro C, Lelli L, Pietrini F, Lazzeretti L, Godini L, Benni L, Fioravanti G, Talamba GA, Castellini G, Ricca V. The role of life events and HPA axis in anxiety disorders: a review. Curr Pharm Des. 2012;18(35):5663-74. https://pubmed.ncbi.nlm.nih.gov/22632471/
27. Lopresti AL, Smith SJ, Malvi H, Kodgule R. An investigation into the stress-relieving and pharmacological actions of an ashwagandha (Withania somnifera) extract: A

randomized, double-blind, placebo-controlled study. Medicine (Baltimore). 2019 Sep;98(37):e17186.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6750292/

28. Khan B, Ahmad SF, Bani S, Kaul A, Suri KA, Satti NK, Athar M, Qazi GN. Augmentation and proliferation of T lymphocytes and Th-1 cytokines by Withania somnifera in stressed mice. Int Immunopharmacol. 2006 Sep;6(9):1394-403.
https://pubmed.ncbi.nlm.nih.gov/16846833/

29. Mikulska P, Malinowska M, Ignacyk M, Szustowski P, Nowak J, Pesta K, Szeląg M, Szklanny D, Judasz E, Kaczmarek G, Ejiohuo OP, Paczkowska-Walendowska M, Gościniak A, Cielecka-Piontek J. Ashwagandha (Withania somnifera)-Current Research on the Health-Promoting Activities: A Narrative Review. Pharmaceutics. 2023 Mar 24;15(4):1057.
https://pubmed.ncbi.nlm.nih.gov/37111543/

30. Calcia MA, Bonsall DR, Bloomfield PS, Selvaraj S, Barichello T, Howes OD. Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. Psychopharmacology (Berl). 2016 May;233(9):1637-50.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4828495/

31. Gupta M, Kaur G. Aqueous extract from the Withania somnifera leaves as a potential anti-neuroinflammatory agent: a mechanistic study. J Neuroinflammation. 2016 Aug 22;13(1):193.
https://pubmed.ncbi.nlm.nih.gov/27550017/

32. Tharakan A, Shukla H, Benny IR, Tharakan M, George L, Koshy S. Immunomodulatory Effect of Withania somnifera (Ashwagandha) Extract-A Randomized, Double-Blind, Placebo Controlled Trial with an Open Label Extension on Healthy Participants. J Clin Med. 2021 Aug 18;10(16):3644.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8397213/