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Home Dna Testing For Neuropsychiatric Disorders Like Stress, Anxiety, Depression And Personalised Ashwagandha Dosage For Prevention And Cure: A Review Of Recent Development

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ABSTRACT

Worldwide 284+ million people suffer from stress, anxiety and depression disorders and modern lifestyle, environments and unhealthy diets are causing an epidemic of stress, anxiety & depression (SAD) disorders. Other risk factors which contribute to SAD disorder are compromised genetics, weak immune system, low response to stress & brain neuroplasticity. Several studies had investigated genes, single nucleotide variation, Indels, large deletions, inversions & triple repeat polymorphism involved in various molecular and biological mechanisms underlying the disease initiation & progression. Recent genome-wide association studies (GWAS) have identified 150+ genes and genetic association of 44 risk variants [1, 2] for detection of SAD disorders. Nowadays At-home DNA tests (direct-to-consumer DNA tests) are gaining popularity for wellness and healthy longevity. These tests predict risk for micronutrient & vitamin deficiency, antioxidant activity, neuro-muscular functionality, risk for SAD disorders, efficacy of pharmacologically active herbal formulations for prevention & cure. On the basis of genetic outcome, medicinal plants/ herbs have been greatly employed to combat and reduce stress, anxiety & depression and thereby enhance general wellbeing in recent years. Ashwagandha (*Withania somnifera*, fam. Solanaceae) is a most versatile and potential herb of the Indian Ayurvedic system of medicine as a Rasayana. It is also accredited as a 'royal herb' having potential therapeutic applications. There are more than 33,787 publications for genetic markers for SAD & due to health awareness Ashwagandha research has gained scientific importance as evidenced by 77 publications documented since 2010 in NCBI PubMed [3]. The present review concludes with a discussion of prospects for clinical translation of genetic findings based on DNA testing, mode of action & personalized dosage of Ashwagandha as potent anti-SAD medicinal supplement

Keywords: Home DNA testing, stress, anxiety, depression, Ashwagandha

1. Introduction

Mental health is an integral component of health and well-being that determines individual abilities to make decisions, learn well and work well, to cope with the stresses of life, build relationships and contribute to community. A person's mental health is affected by multiple

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risk factors including genetic composition, diet intake, fitness exercise, yoga & vulnerabilities to stress at different stages of life leading to stress, anxiety & depression (SAD). As per WHO 280+ million people suffered from stress, anxiety & depression disorders worldwide [4]. In India 44·9 million people are suffering from anxiety & 45·7 million are suffering from depression [5]. Core symptoms of *stress* includes chest pain, rapid heartbeat, eating too much or not enough, using alcohol to relax; *anxiety* includes feeling nervous, restless or tense, an increased heart rate, breathing rapidly (hyperventilation), experiencing gastrointestinal (GI) problems, trouble concentrating; depression includes feelings of sadness, angry outbursts, sleep disturbances, including insomnia, tiredness and lack of energy & frequent or recurrent suicidal thoughts. In addition SAD patients are more likely to develop hypertension, coronary artery disease and type 2 diabetes. Therefore, the growing number of reports on anxiety and stress compelled us to seek herbal remedies & dietary supplements as an effective solution to prevent & manage SAD [6]. Since 1978 [7], many genetics studies have been conducted to identify the genes responsible for the progression of SAD disorder & 150+ candidate gene variants have been associated with the genetic risk & disease onset. Development of DNA microchip technology has made it possible to carry out GWAS studies for predisposition of disease. This leads to the development of home DNA testing kit technologies combined with Artificial Intelligence & Machine learning based predictive methods from large genome databases for easy & efficient risk assessment for personalized treatment with herbal formulations & supplements. Use of active ingredients/herbal/Ayurvedic formulation of medicinal plants Ashwagandha is one of the important herbs of Ayurveda (the traditional system of medicine in India) also used for millennia as a Rasayana for its wide ranging health benefits and commonly known as “Indian Winter cherry” or “Indian Ginseng”. Numerous active compounds of Ashwagandha such as steroids, alkaloids, flavonoids, phenolics, saponins and glycosides etc. have been identified, tested & validated their efficacy in neuroprotective application. Therefore the objective of this review is to provide an insight of the research regarding genetic markers to identify predisposition for SAD disorder and antistress, ant-depressent & anxiolytic activity of Ashwagandha along with active ingredients, and underlying molecular mechanism(s) for brain pathologies.

2. Ashwagandha and Medicinal Usage

Ashwagandha & usage of its roots, leaves and fruits for medicinal purposes is described in various Indian ancient texts such as Charaka Samhitas, Sushruta Samhitas, Ashtanga Hridayam and Bhava Prakasha. Ashwagandha (Dunal.) belongs to the *Withania somnifera* family, solanaceae is an important medicinal plant, which has been cultivated for centuries in Indian states such as Gujarat, Haryana, Madhya Pradesh, Maharashtra, Punjab, Rajasthan and Uttar Pradesh,, and Ashwagandha is also grown in countries like Afghanistan, Bangladesh, Congo, Egypt, Jordan, Morocco, Nepal, Pakistan, South Africa and Sri Lanka. In India annual production of Ashwagandha ~1500 tonnes and current demand is ~7000 tonnes per annum due to its application in Ayurvedic medicine & pharmaceutical industry as a nutrition & health supplement [8].

Pharmacological studies have concluded that Ashwagandha health benefits as anti-inflammatory, relieve stress, reduce anxiety, improve sleep quality, memory-enhancing and hematopoietic. It is also used to treat various diseases associated with nerve damage, heart problems, atherosclerosis and cancer. It normalizes the body functions by targeting the hypothalamic-pituitary-adrenal gland axis and decreases cortisol levels and acts as antioxidant and vasorelaxant by enhancing endothelial NO generation [9, 10] under chronic stress. In traditional Ayurveda and Unani systems of medicine, the roots/leaves/extract of Ashwagandha have a long history of use as an adaptogen and its molecular mechanism has been explored as well. Since ancient times, this herb has been claimed to be safe when used

within the recommended dosages and formulations.

3. Chemical constituents of Ashwagandha

The bioactive compound of Ashwagandha has been isolated, identified & studied for its medicinal usage which includes steroidal lactones (withanolides, withaferins), alkaloids (isopelletierine, anaferine), saponins containing an additional acyl group (sitoindoside VII and VIII), phenolics, flavonoids, phytophenols, glycosides and withanolides with a glucose at carbon 27 (sitoindoside IX and X) [11-23] Figure 1. At present, more than 12+ alkaloids, 40+ withanolides, and several sitoindosides have been isolated and reported from aerial parts, roots and berries of Ashwagandha [24-30]. Therefore, the compounds of Ashwagandha have multifarious medicinal applications such as anti-inflammatory, antistress, anti-depressant, antiangiogenic and anticancer.

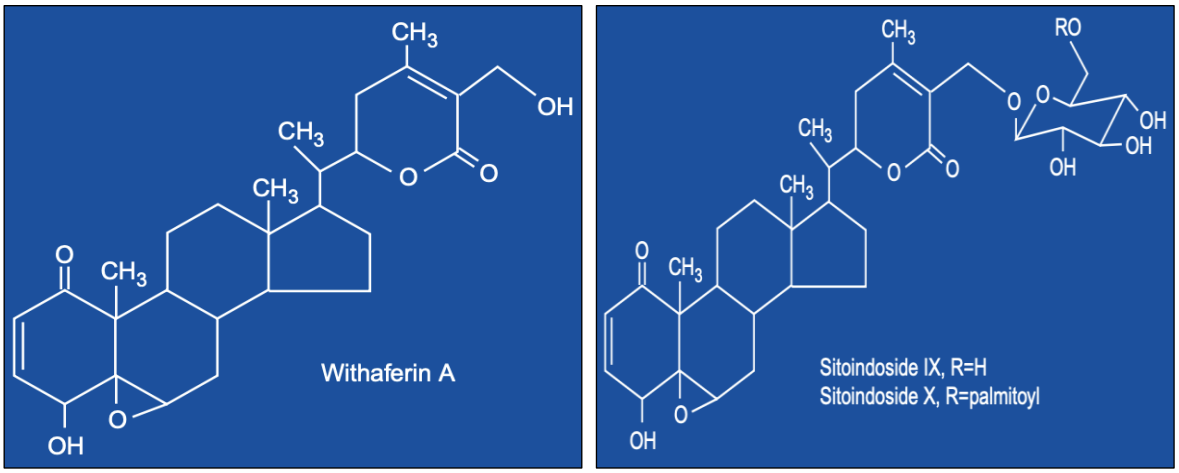

Fig 1: Chemical Structure of (a) Withaferin A (b) Sitoindosides IX and X.
https://chiro.org/Graphics_Box_NUTRITION/Scientific_Basis.pdf

4. Proposed Mechanism of Ashwagandha in SAD Disorder

The anti-SAD disorder effect of Ashwagandha has been attributed by several mechanisms mentioned below:

4.1 Glucocorticoid reduction

Glucocorticoids (Cortisol, Cortisone, Prednisone & Prednisolone) are a class of steroid hormones regulated by hypothalamic-pituitary-adrenal axis, which are hypersecreted by adrenal glands in response to stress & anxiety allows the body to stay on high alert. These hormones play a pivotal role for daily functioning such as protein: carb: lipid metabolism, homeostasis of water and electrolyte, immune response, cardiovascular activity and cognitive psychological behaviour. The human body encounters many types of external and internal stress based on the degree of threats. The root of Ashwagandha is widely used as an adaptogen to relieve stress, reduce anxiety, improve cognitive function & prevent other diseases like type 2 diabetes, cardiovascular & cancer by reducing glucocorticoids [32].

4.2 Immune modulation

Immune modulation is the change in the functioning of the immune cells by the action of an immunomodulator. It is one of the effective therapies against immune-related diseases & various infections. To reduce stress & anxiety Ashwagandha has been used as an

immunomodulator due to its diverse & bioactive chemical constituents which regulate immune pathways via protein-protein interactions, immune cell activation (macrophages) and lysosomal enzymes production [34], increase in IFN-γ, IL4 cytokines, increase in the production of antibodies by activating T & B cells [35].

4.3 Gamma-Aminobutyric Acid (GABA) Related Activity

Gamma-Aminobutyric Acid **(**GABA), the main inhibitory neurotransmitter produced in the central nervous system. It showed its inhibitory role through two types of specific receptors called GABA-A & B. GABA-A receptors are the primary site of action for GABA agonist drugs, which stimulate GABAergic activity and are commonly used in the treatment of SAD disorders [36]. Many studies revealed that bioactive compounds found in Ashwagandha enhance GABA receptors due to its GABA-mimicking effects which regulate serotonin in the brain. *Ashwagandha* can also help prevent and repair damage caused by Alzheimer's, Parkinson's and Huntington's disease.

4.4 Antioxidant and Anti-inflammatory activity

Antioxidants are substances that protect the body from damage caused by free radicals and lower the risk for oxidative stress, anxiety, infections, cardiovascular disease, and cancer. Many studies revealed that the roots, leaves and fruits of Ashwagandha plant have high antioxidant potential. Extract of Ashwagandha increases the levels of various natural antioxidant enzymes like superoxide dismutase, glutathione S-transferase, catalase, glutathione reductase and glutathione peroxidase. Alcoholic extract of Ashwagandha improves gut dysbiosis by reducing LPS producing pathogenic bacteria like *E. coli*, Staphylococcus & Streptococcus species to reduce inflammation.

5. Modulation of genes governed by Ashwagandha

SAD disorder is strongly associated with differential expression of genes due to ROS signalling pathways, neurotrophic factors which are produced by the brain serves as a neurotransmitter modulator; very crucial for brain plasticity, impairment of the action of cytokines in response to neurotransmitters which is responsible to combat different types of stresses. Various neurodegenerative diseases such as depression, multiple sclerosis, Alzheimer's disease, and Huntington's disease occur due to a decrease in the level of neurotrophic factors. Chemokines (chemotactic cytokines) are a superfamily of secreted protein by various immune cells (macrophages, lymphocytes) & play a central role in homeostasis of the immune system & many disease processes. These *CCL5* & *CCL2* genes are one of several chemokine genes clustered on the q-arm of chromosome 17. Accumulating data have suggested functional impairment and modifications of these molecules during stress, anxiety & depression. Here, Ashwagandha has been proven by various studies to enhance neurotic regeneration and synaptic reconstruction by differential expression of genes which is mainly responsible for increase of anti-inflammatory cytokines & decrease the pro-inflammatory cytokine such as that alcoholic extract of Ashwagandha treated cells showed high levels of γH2AX, p53, p21 and ROS that protected against the DNA damage and oxidative stress [41]. Similar study was conducted by Elizabeth Grunz-Borgmann in 2015 that concluded that Ashwaganda completely prevented TNF-α-induced increases in *CCL5*, while attenuating the increase in *CCL2* expression and NF-κB activation [42] also similar result was observed about Ashwagandha protection against the LPS as the pro-inflammatory stimuli. Abudubari Sikandan (2018) also revealed that Ashwagandha treatment inhibited the MAPK and NF-κB pathways to decrease the expression of pro-inflammatory cytokines and increase

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the expression of anti-inflammatory cytokines [43]. *Therefore* Ashwagandha has been shown to prevent the expression of the genes *CCL2* and *CCL5*. The proteins produced by *CCL2* and *CLL5* are critical chemokines, which are small signalling molecules that are activated in response to inflammation; specifically, *CCL2* and *CCL5* are linked to various inflammatory diseases. By limiting their activity, Ashwagandha can *tranquilize* the progression of inflammation, a known factor in stress, anxiety & depression.

6. Home DNA Test and Dietary intake of Ashwangandha to manage SAD disorders

Home DNA Test is an essential step in diagnosis of various genetic disorders based on changes that occur in a person's DNA. Human health is the result of constant interaction between genes and environmental factors mainly diet & daily lifestyle has been proved by research studies. Of the over 3 billion base pairs of the human genome, 97 to 99% are identical among any two given individuals. However, the 1 to 3% genome difference among us makes us respond differently to different types of food. This is why personalized diets are necessary based on DNA. DNA contains all the genetic information of a living being, including hair, eye and skin color, as well as the risk of predisposition to stress, anxiety & depression. These hereditary traits are inherited through genes, which are an average of 25,000 in humans. The exome of the human genome consists of 288 654 unique exons from 46 275 transcripts of 20 921 Ensembl-protein-coding genes, which constitute about 1% of the total genome, or about 35.1 megabases of DNA. Whole exome & whole genome sequencing are carried out using NovaSeq6000 NGS platform to generate 100x and 30x paired end sequencing data. The high quality data will be aligned to the human reference genome (GRCh38 build P.13) using BWA-MEM. Coverage across Exome targeted regions will be analyzed using a kit specific Bed file. Variant Analysis Pipeline included the GATK4 best practices framework for identification of variants in the sample(s). Gene annotation of the variants is performed using VEP program [44] against the Ensemble release 91 human gene model [45]. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases-BioneVaInGDbv1, ClinVar, OMIM (updated on 21st November 2018), GWAS, HGMD (v2018.3) and SwissVar [46-50]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD(v2.1), EVS, dbSNP(v155), 1000 Japanese Genomes and our internal Indian population database [51-53]. Non-synonymous variant effects are calculated using multiple algorithms such as PolyPhen-2, SIFT, Mutation Taster 2 and LRT. Only non-synonymous and splice site variants found in the whole exome panel genes (19000+) were used for clinical interpretation. The Infinium Global Screening Array-24 Bead Chip containing 654,027markers obtained from multi-ethnic genome-wide content, curated clinical research variants include from disease associations data, important pharmacogenomics markers, and curated exonic content based on ClinVar, ExAC, NHGRI and Pharm GKB databases. The genome-wide content was selected for high imputation accuracy at minor allele frequencies of >1% across all 26 populations of 1000 Genomes Project. (https://www.illumina.com/products/by-type/microarray-kits/infinium-global-screening.html)

Ultimately, Predisposition genetics aim is to provide diagnoses and forecasts of future disease risk. For complex disease prediction of polygenic risk prediction allows for personally and clinically useful stratification of risk. In the context of clinical diagnostics the possibilities offered by AI are important for analyzing the relationship between heredity and the appearance of genetic diseases. Machine learning (ML) is a subset of artificial intelligence in which ML models acquire and integrate knowledge through large-scale observations and improve and extend themselves by learning new knowledge rather than being programmed with that knowledge. AI-assisted DNA decoding has grown increasingly popular in the fitness industry for its customized approach for achieving fitness goals in a unique and wiser

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way. A DNA test that can be done at home can identify gene changes that can put you at risk for stress, anxiety & depression. Genetic markers for SAD disorders along with vitamin B12 & vitamin D genetic markers which play a vital role in progression of these disorders. Depression is the most common mental disorder in India with 45.7 + million people suffering from it. Genome-wide studies of genetic variation in several important genes, has identified the specific risk variants, which is evidence for genetic contributions to stress, anxiety and the depression disorders. Genetic markers on chromosome 3:8762685 (GRCh38) allele A>G,T in the oxytocin receptor gene (*OXTR*), on chromosome 5:63962738 (GRCh38), allele C>A,G in the 5-hydroxytryptamine/serotonin receptor gene (*HTR1A*), and on chromosome 5:143399010 (GRCh38), allele G>C in the glucocorticoid receptor gene (*NR3C1*), marker on chromosome 4:71742666 (GRCh38), allele T>G in *GC* gene for vitamin D & marker on chromosome 19:48703728 (GRCh38), allele G>A in *FUT* gene for vitamin B12 associated with brain health which play a crucial role in behavior & leads to stress, anxiety & depression [55-60]. One promising avenue for preventing SAD disorders and informing its clinical management lies in uncovering both the genetic and dietary determinants of the disorder as well as their interaction (i.e. gene-diet intervention; GxD).

Ashwagandha can address many of the health and psychological disorders that affect 284+ million individuals worldwide as per WHO. Various studies based on human trials revealed that the oral administration of Ashwagandha varied between 2 and 12 weeks with intakes between 300 to 1000 mg/day effective against the negative effects of stress by reducing the cortisol level upto 26% [66]. The clinical studies in human beings [Table: 1] has proved the potential application of Ashwagandha to manage SAD disorders effectively. Simultaneously Ashwagandha intake increases energy, reduces fatigue, better sleep, enhanced sense of well-being. Genomics based advanced studies, meta-analysis and further research is needed to establish the Ashwagandha usage for management of several lifestyle disorders.

Table 1: Summary of Ashwagandha product human clinical studies

Product details	# of Subjects	Dosage & Duration	Biological Outcomes	Conclusions
Sensoril®, (Natreon Inc.) Capsules of aqueous extract [61] **Active Ingredient:** ≥8% Withanolide glycosides (with- anosides and sitoindosides)	66 subjects	500 mg daily for the first week, which was titrated up to 1000 mg daily for the second week, then maintained for 10 additional weeks.	↓ hsCRP (C-reactive protein)	↓stress, depression and anxiety
Sensoril® (Natreon Inc.) or Essentra®; (NutraGenesis) Capsules of aqueous root and leaf extract of a withaferin [62] **Active Ingredient:** 11.90% withanolide glycosides, 1.05% withaferin A	130 subjects	125 mg daily OR 250 mg twice daily for 8.5 weeks	↓serum cortisol	↓stress, anxiety
KSM-66 Capsules of Ashwagandharoot extract (Ixoreal Biomed) [63] **Active Ingredient:**≥5% withanolide content	64 subjects	300 mg twice daily for 8.5 weeks	↓serum cortisol	↓stress
(Prolanza™ [Inventia Healthcare Ltd. and Laila Nutraceuticals]) is a sustained-release (SR) capsule containing *Withania somnifera* root extract [64] **Active Ingredient:** 15 mg withanolides	125 subjects	300 mg capsule daily for 12 weeks	↓serum cortisol	↑ memory ↑sleep quality ↓stress
Shoden; (Arjuna Natural Ltd.) Capsules Ashwgandha extract [65] **Active Ingredient:** 35% withanolide glycosides	60 subjects	240 mg once daily after dinner for 8.5 weeks	↓serum cortisol	↓anxiety, stress

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7. Conclusion

Genomics technologies like whole genome sequence, whole exome sequence, microarray are revolutionizing and discovering new genes & genetic markers associated with SAD disorders as well as genes responsible for efficacy of ayurvedic/nutraceutical/herbal formulations for the prevention & cure of various human lifestyle disorders & other diseases. Preventive DNA testing based on comprehensive genetic markers is expanding personalized health & wellness. Therefore, the burgeoning & alluring area of gene-by-environment (G × E) interactions has resulted in new biological insights, particularly in the realm of stress, anxiety, and depression-related disorders. To date, the association between an individual's SAD disorders and their genetic predisposition based on 100+ candidate gene variants has been explored by various studies. Efficacy of Ashwagandha derived group of alkaloids called withanolides, has been well established in the treatment of stress, anxiety & depression, other active ingredients, and underlying molecular mechanism(s). In the omics era, home-DNA testing has created a new breakthrough for clinical management & treatment of SAD disorders by using a personalized dosage form of Ashwagandha.

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