

Effects of *Withania somnifera* (Ashwagandha) on Stress and the Stress-Related Neuropsychiatric Disorders Anxiety, Depression, and Insomnia

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Abstract: Background: *Withania somnifera* (WS), also known as Ashwagandha, is commonly used in Ayurveda and other traditional medicine systems. WS has seen an increase in worldwide usage due to its reputation as an adaptogen. This popularity has elicited increased scientific study of its biological effects, including a potential application for neuropsychiatric and neurodegenerative disorders.

Objective: This review aims to provide a comprehensive summary of preclinical and clinical studies examining the neuropsychiatric effects of WS, specifically its application in stress, anxiety, depression, and insomnia.

Methods: Reports of human trials and animal studies of WS were collected primarily from the PubMed, Scopus, and Google Scholar databases.

Results: WS root and leaf extracts exhibited noteworthy anti-stress and anti-anxiety activity in animal and human studies. WS also improved symptoms of depression and insomnia, though fewer studies investigated these applications. WS may alleviate these conditions predominantly through modulation of the hypothalamic-pituitary-adrenal and sympathetic-adrenal medullary axes, as well as through GABAergic and serotonergic pathways. While some studies link specific withanolide components to its neuropsychiatric benefits, there is evidence for the presence of additional yet unidentified active compounds in WS.

Conclusion: While benefits were seen in the reviewed studies, significant variability in the WS extracts examined prevents a consensus on the optimum WS preparation or dosage for treating neuropsychiatric conditions. WS generally appears safe for human use; however, it will be important to investigate potential herb-drug interactions involving WS if used alongside pharmaceutical interventions. Further elucidation of active compounds of WS is also needed.

Keywords: Anxiety, depression, insomnia, stress, Ashwagandha, *Withania somnifera*.

1. INTRODUCTION

The botanical *Withania somnifera* (L.) Dunal, family Solanaceae, is widely known as “Ashwagandha”, a Sanskrit name deriving from its use in traditional medicine in India. *Withania somnifera* (WS) enjoys a formidable reputation in Ayurvedic medicine as a Rasayana herb, *i.e.*, one that can rejuvenate the body and promote the health of all the tissues [1]. WS is, therefore, also classified as an adaptogen: an agent that promotes homeostasis of the whole body not only by one specific pharmacological mechanism, but by eliciting

complex responses as well [2]. The reputed properties of WS include the ability to improve concentration, memory, and mood, as well as providing resilience against pathogens and disease [3].

The WS plant is a 0.5-2m high woody shrub found in the drier parts of tropical and subtropical zones, including the Canary Islands, the Mediterranean, Africa, China, South Asian countries such as India and Sri Lanka, and the Middle East [4]. It is known by several names, including Ashwagandha (Sanskrit), Asgand (Urdu), and Indian Winter Cherry or Poison Gooseberry (English), as well as the term “Indian Ginseng” owing to its adaptogenic effects [4, 5]. In India, WS is extensively cultivated for medicinal purposes, being used in more than 100 formulations in Ayurvedic, Unani, and Siddha medicine [6, 7]. Freshly dried roots are the predominant medicinal component; however, the leaves, flow-

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ers, seeds, and fruits of the plant are also used for therapeutic purposes [6].

Traditionally, WS preparations are used for conditions such as arthritis, asthma, goiter, and ulcers, as well as anxiety, insomnia, and neurological disorders. These uses are linked to the botanical's reputed adaptogenic, anti-stress, and anti-inflammatory properties [6, 7]. Numerous WS commercial products are now readily available to the public in India and other parts of the world. In the United States (US), WS products are classified as "botanical dietary supplements" by the Food and Drug Administration. According to the National Institutes of Health Office of Dietary Supplements, there are currently more than 1,300 products in the US market that contain WS, and its use is becoming increasingly popular [8]. WS was listed as the 6th top-selling herb in US in 2017 [9], rising from the 8th position in 2016 [10]. By 2019, WS was the 5th most popular dietary supplement, with sales exceeding \$10 million through mainstream channels (e.g., grocery stores, drug stores) and more than \$13 million in terms of natural channels (e.g., supplement and specialty retail outlets) [11].

The widespread and growing use of WS by the public highlights the need for a greater understanding of this botanical's biological properties and active phytochemicals to validate and optimize its use. Fortunately, WS is one of the most widely studied medicinal herbs. A recent review by Tetali *et al.* (2021) summarizes about 140 specialized compounds reported in WS [12]. Among these, the best known are a complex group of steroidal lactones known as withanolides, which also occur as glycosides (withanosides) [12]. Over 70 individual withanolide derivatives have been reported in WS leaf and root [12-14], with higher levels in the leaves than in the roots [15]. WS also contains four sitoindosides, of which sitoindosides IX and X are glycosylated derivatives of the withanolide, withaferin A [16], while sitoindosides VII and VIII are long chain acyl steryl glucosides [17]. Distinct WS chemotypes are recognized based on the withanolide substitution patterns [18, 19]. Multiple alkaloids, phenolic compounds, and organic acids have also been reported in WS [12]. The withanolide derivatives are the most common group to have been examined individually for biological activities [12]. Indeed, some withanolides contain electrophilic sites conferring thiol reactivity, which may play important roles in mediating biological activity associated with antioxidant activity and/or targeting other electrophile sensors that modulate transcriptional or post-transcriptional responses [20]. The structures of specific WS compounds mentioned in this review are shown in Fig. 1.

The varied biological effects of WS have been widely studied and reviewed, including its potential application in brain disorders [21-23]. Neuropharmacological effects of WS root and WS leaf have been studied in preclinical and clinical models [24-26], and two recent reviews have summarized the evidence for the efficacy of WS in neurodegenerative disorders, including Alzheimer's disease, Huntington's disease, and Parkinson's disease [4, 27]. However, one of the most common uses of Ashwagandha products is for stress relief. It is now well accepted that stress can cause

functional and structural changes within the brain and has been implicated in the development of most neuropsychiatric disorders, including anxiety, depression, and insomnia [28-30]. The mechanisms by which stress contributes to these disorders include hyperactivity of the Hypothalamic-Pituitary-Adrenal (HPA) axis and dysregulation of the immune system [31, 32]. Given the well-established relationship between stress and neuropsychiatric disorders, it is likely that WS's anti-stress activity plays a key role in its potential health benefits for depression, anxiety, and insomnia, and *vice versa*.

Numerous studies on WS have examined its effects on stress, mood, and insomnia in humans (Table 1) and animal models (Tables 2-5). The present article provides a detailed and comprehensive review focusing on these neuropsychiatric effects of WS. Specifically, it presents pre-clinical and clinical evidence of the effects of WS on stress and three of the most common stress-related neuropsychiatric disorders: anxiety, depression, and insomnia. The pathophysiology of these disorders will be discussed as they relate to the effects of WS; however, the reader is referred to previous reviews for more comprehensive discussions of these topics [32-35]. As stress is implicated in all three of the other common disorders, we first review the evidence for the anti-stress effect of WS, and then discuss the evidence for the use of WS in anxiety, depression, and insomnia.

1.1. Research Methods

Literature was gathered using the PubMed, Scopus, and Google Scholar databases, as well as from papers cited within the initial articles retrieved. Search terms included combinations of the following words: ashwagandha, ashwaganda, *Withania somnifera*, Indian ginseng, poison gooseberry, winter cherry, stress, adaptogen, adaptogenic, insomnia, sleep, anxiety, anti-anxiety, depression, anti-depressant, pharmacokinetics, safety, cytochrome P-450, interaction, animal, mouse, rat, rodent, preclinical, clinical, withaferin A, withanolide, cortisol, cytokines, inflammation, inflammatory, serotonin, oxidative stress, antioxidant, GABA, and immune. Only studies where WS and its derivatives were the sole agents being studied were included. Any studies that included WS as part of a multi-herb or multidomain intervention were excluded due to an inability to differentiate the effects of WS from other interventions used.

2. ANTI-STRESS EFFECTS

2.1. Animal Studies

As summarized in Table 2, WS has demonstrated anti-stress activity in several animal models of stress, the most common of which was the Forced Swim Test (FST) [17, 36-42]. The FST was originally developed as a model of depression-like behavior and involved placing a rodent in an inescapable tank filled with water [43]. When used as a model of stress, a drug's ability to increase the time duration an animal spends actively swimming suggests the ability to cope with stress [44]. In the studies reviewed here, WS consistently increased swim duration in the FST [38-42] (Table 2). Other methods of inducing stress included sleep deprivation

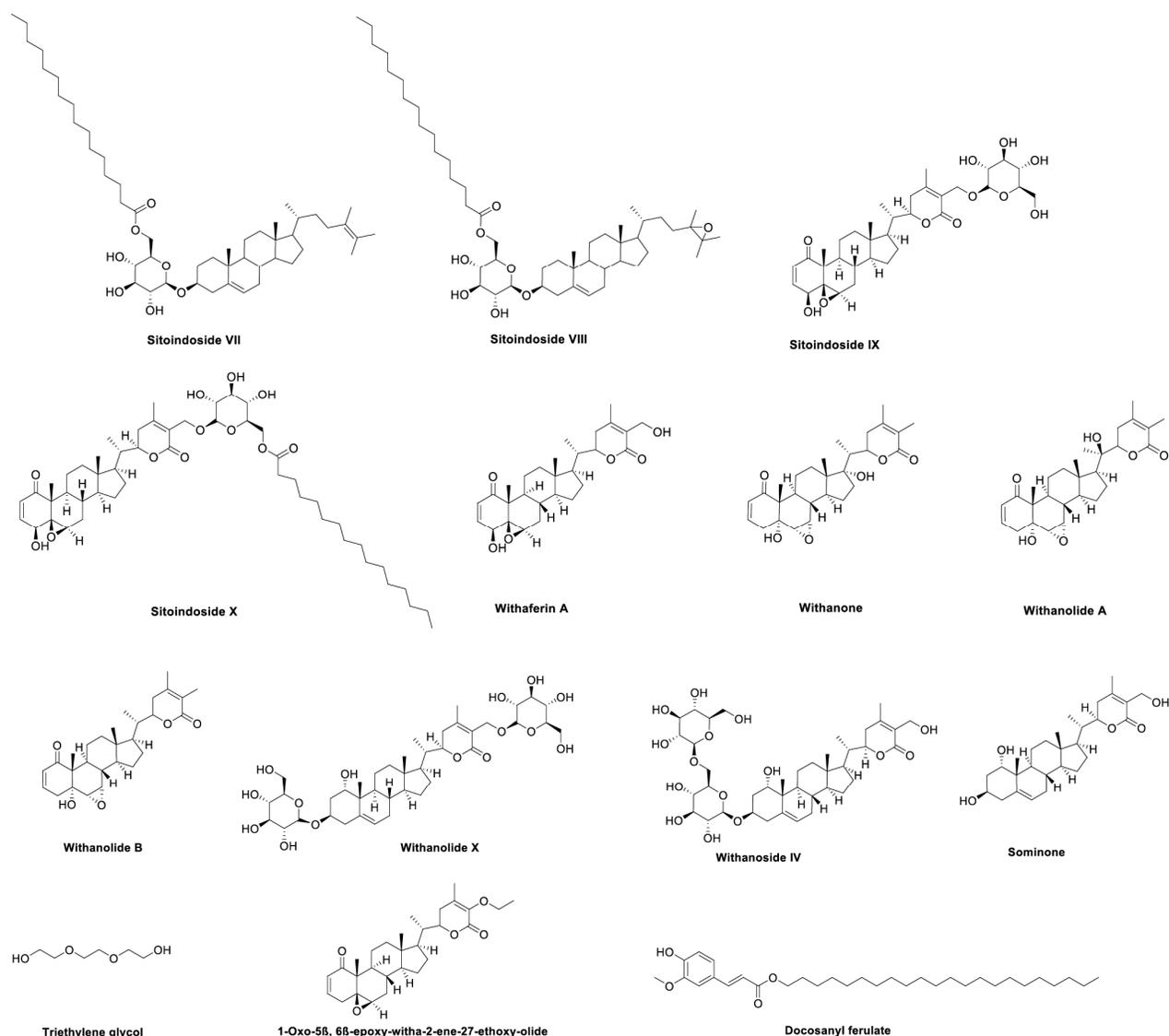


Fig. (1). Selected WS compounds tested, or found in WS extracts tested, for effects related to neuropsychiatric disorders.

[45], exposing animals to cold [40], hypoxia [46], and/or prolonged restraint [17, 38, 40, 47, 48], either alone or in combination [41, 42, 49, 50], or applying electrical shocks to the feet of animals [51-54]. In these studies, WS attenuated a variety of stress-induced changes, including behavioral changes (*e.g.*, memory impairment [45, 46, 52, 53, 55]), biochemical changes (*e.g.*, increased glucocorticoids [38, 39, 41, 42, 46-48, 50, 54, 56]) and physical changes (*e.g.*, gastric ulcers [17, 38, 40, 52]).

Most of the extracts studied were derived from the root of WS [37-39, 41, 42, 46-49, 51, 53-56]. Anti-stress effects were also seen for a leaf extract [45], a leaf and root extract [36] and an extract made from defatted seeds [40]. Extraction methods and test preparations varied, and included alcoholic extracts [40-42, 51], aqueous extracts [45, 49, 50], hydroalcoholic extracts [36-38, 46, 52, 53, 55], a traditional extract made with water, ghee, and honey [53], a withanolide-free fraction [37, 38], a glycowithanolide-rich [52]

fraction, and several isolated compounds, including sitoindosides VII and VIII [17], 1-oxo-5 β , 6 β -epoxy-witha-2-ene-27-ethoxy-olide [64], and a substance named Compound X [49].

2.2. Human Studies

Six studies evaluated the use of WS for anti-stress activity in adults aged 18-75 years [57-62] (Table 1). Study populations included participants who were healthy [61], stressed [57, 60], overweight, or obese, experiencing chronic work stress [58], diagnosed with anxiety [62], and diagnosed with schizophrenia or schizoaffective disorder [59]. The number of participants in each study ranged from 52 to 130 participants.

Each study administered WS as capsules of commercially available preparations (Table 1). Three studies evaluated

Table 1. Effects of Ashwagandha (*Withania somnifera*, WS) on stress, insomnia, anxiety, and depression in human trials.

WS Material Given	Standardization/ Chemical Com- position	Dosage	Control	Subjects	Behavioral Out- comes	Biological Outcomes	Authors’ Conclusions	Refs.
KSM-66; Batch#: KSM/19/S013; Ixoreal Biomed Inc) Capsules of aqueous root extract	>5% withanolide content	300 mg twice daily with milk or water 8 weeks	Starch	80 adults (18-50 yrs) (two-armed trial: 40 healthy and 40 insomnia, male to female ratio NR)	▼SOL, ▼WASO, ▲TST, ▲TIB, ▲SE, ▼PSQI, ▼HAM-A (insomnia only), ▼MARS rating, ▲sleep quality	Safety: physical exam and lab tests (blood pressure, body weight, routine blood test)	Improved sleep and anxiety	[63]
KSM-66 Ashwagandha Root extract (Ixoreal Biomed Inc.) Capsules of aqueous root extract	NR	300 mg twice daily with water 12 weeks	Starch	50 healthy older adults (60-85 yrs, male to female ratio NR)	▲WHOQOL-Bref score, ▼sleepiness scale score, ▼MARS rating, ▼sleep quality rating High PGAET rating, high PGATT rating	Not assessed	▲QoL, ▲sleep quality, ▲mental alertness	[64]
KSM-66 Ashwagandha root extract, (Ixoreal Biomed Inc.) Capsules of aqueous root extract, Batch#: KSM/VG/18/1085	NR	125 mg twice daily OR 300 mg twice daily 8 weeks	125 mg starch	60 adults (18-55 yrs, male to female ratio NR) with PSS score ≥20 and no other psychiatric conditions	▼PSS score, ▼HAM-A score (600 mg dose only), ▼sleep quality rating	▼serum cortisol	▼stress, ▼anxiety	[60]
KSM-66, (Ixoreal Biomed Inc.) Capsules of aqueous root extract, Batch#: KSM/14/270	>5% withanolides	300 mg twice daily with milk or water 10 weeks	Starch powder	60 adults (18-60 yrs; 31:9 male to female for WS group, 16:4 for placebo group) with insomnia	▼SOL, ▼WASO, ▲TST, ▲TIB, ▲SE, ▼MARS rating, ▲sleep quality rating, ▼PSQI score, ▼HAM-A score	Not assessed	▼insomnia, ▼anxiety	[65]
KSM-66 Ashwagandha; (Ixoreal Biomed) Capsules of root extract	5% withanolides	300 mg twice daily with water 8 weeks	“Inert filler”	52 overweight to obese adults (18-60 yrs; 38 males, 14 females) with chronic, routine work stress (PSS score ≥20, BMI 25 - 39.9 kg/m ²)	▼PSS score, ▲OHQ score, ▼TFEQ score ▼FCQ-T score	▼serum cortisol, ▼body weight, ▼BMI	▼stress, ▲well-being, ▼food cravings	[58]
KSM-66 Ashwagandha extract (Ixoreal Biomed) Capsules of root extract prepared “without using alcohol or synthetic solvents”	≥5% withanolide content	300 mg twice daily after food with water 8.5 weeks (60 days)	“Neutral substance”	64 stressed adults (18-54 yrs; 41 males, 23 females; WHO-5 well-being score <5; PSS score of at least 14)	▼PSS score, ▼GHQ score, ▼DASS score	▼serum cortisol	▼stress	[57]
Sensoril®, (Natreon Inc.) Capsules of aqueous extract	≥8% Withanolide glycosides (withanosides and sitoindosides), ≥32% carrier oligosaccharides, ≤2%Withaferin A	250 mg twice daily for 1 week, then 500 mg twice daily for 11 weeks 12 weeks	“Inactive ingredients”	66 adults (18-75 yrs, 21:13 male to female for WS group, 14:20 for placebo group) with schizophrenia or schizoaffective disorder (MINI and PANSS score ≥60) and recent symptom exacerbation. On stable antipsychotic dose.	▼PANSS score, ▼PSS score, ▼PANSS single item depression and anxious/depression scores	▼hsCRP, S100B (nsd), no significant difference in serum IL-6	▼stress, depression, and anxiety symptoms in schizophrenia	[66] [59]

(Table 1) contd....

WS Material Given	Standardization/ Chemical Composition	Dosage	Control	Subjects	Behavioral Outcomes	Biological Outcomes	Authors' Conclusions	Refs.
Sensoril®; (Natreon Inc., New Jersey) Capsules of aqueous extract	“Minimum concentration of the critical bioactive withanolide glycosides and carrier oligosaccharides, but only traces of Withaferin A”	250 mg twice daily for 1 week, then 500 mg twice daily for 11 weeks 12 weeks	“Inactive ingredients”	66 adults (18-75 yrs, 21:13 male to female for WS group, 14:20 for placebo group) with schizophrenia or schizoaffective disorder (MINI and PANSS score ≥ 60)	▼PANSS score, ▼PSS score	▼hsCRP, S100B (nsd), no significant difference in serum IL-6	▼stress in schizophrenia	[59]
Sensoril® (Natreon Inc.) or Essentra®; (NutraGenesis) Capsules of aqueous root and leaf extract of a withaferin A and corresponding withanolide glycoside-predominant genetically uniform chemotype	11.90% withanolide glycosides, 1.05% withaferin A, 40.25% oligosaccharides, 0.05% alkaloids, 3.44% polysaccharides	125 mg daily OR 125 mg twice daily OR 250 mg twice daily taken before lunch and dinner 8.5 weeks (60 days)	Excipient placebo	130 adults (18-60 yrs; 95 males, 38 females) with moderate to severe anxiety (mHAM-A score of 24-42)	▼mHAM-A score	▼serum cortisol, ▼CRP, ▼FBG, ▼TC, ▼TG, ▼LDL-C, ▼VLDL-C, ▲HDL-C, ▲Hemoglobin, ▼pulse rate, ▼systolic and diastolic blood pressure	▼stress, ▼anxiety	[62]
Shoden; (Arjuna Natural private Ltd.) Capsules of leaf and root extract	21 mg withanolide glycosides	120 mg daily with water in the evening 2 hrs before meal 6 weeks	Rice powder	150 adults (18-65 yrs; 72 males, 78 females) with non-restorative sleep (RSQ-W score ≤ 50)	▲RSQ-W total score, ▲WHOQOL-Bref score, ▼SOL, ▲SE, ▲TST, ▼WASO, ▼average awakening time	Hematologic safety markers (nsd)	improved sleep quality	[67]
Shoden; (Arjuna Natural Ltd.) Capsules of ethanol:water (70:30) extract	35% withanolide glycosides	240 mg once daily after dinner with 250 mL of water 8.5 weeks (60 days)	Roasted rice powder	60 healthy adults (18-65 yrs; 37 males, 23 females) with HAM-A scores 6-17	▼HAM-A score, ▼DASS-21 (near significant)	▼serum cortisol, ▼serum DHEA-S, ▲serum testosterone (males only)	▼anxiety, ▼stress	[61]
Tablets of ethanolic plant extract	NR / NR	250 mg twice daily 6 weeks	Placebo	39 adults (41.3 ± 13.8 yrs; 61.5% male) with GAD, mixed anxiety and depression, panic disorder and adjustment disorder with anxiety	Global Rating Scale score (nsd), ▼HAS score	Not assessed	“Anxiolytic potential”; Safe and well tolerated	[68]
Granules of dried root	NR	4 g thrice daily with milk 8.5 weeks (60 days)	Granules of wheat flour	86 participants (16-60 yrs, male to female ratio NR) with GAD	HARS score (▼anxious mood; other anxiety scores nsd between groups)	Not assessed	▼anxiety; potential placebo effect	[69]
Iranian WS (70% ethanol) Capsules of ethanolic root extract with lactose as excipient	Not assessed	1 g daily 6 weeks	Lactose	40 adults (39.29 ± 10.10 yrs; 11:7 male:female for WS group; 40.50 ± 7.76 ; 11:11 male:female for placebo group) with GAD on SSRIs	▼HAM-A score	Not assessed	▼anxiety in GAD in combination with SSRIs	[70]

▼ = significant decrease compared to placebo; ▲ = significant increase compared to placebo; BAI = Beck Anxiety Inventory; BDI = Beck Depression Inventory; BD-NOS = Bipolar disorder, not otherwise specified; BMI = Body Mass Index; CGI = Clinical Global Impressions scale; CRP = C-reactive protein; DASS = Depression Anxiety Stress Scale; DASS-21 = Depression Anxiety Stress Scale-21; DHEA-S = Dehydroepiandrosterone sulfate; FBG = Fasting Blood Glucose; FCQ-T = Food Cravings Questionnaire – Trait; FQ = Fatigue Questionnaire; GAD = General Anxiety Disorder; GHQ = General Health Questionnaire; HAM-A/HARS = Hamilton Anxiety Rating Scale; HDL-C = High density lipoprotein cholesterol; IL-6 = Interleukin 6; LDL-C = Low density lipoprotein cholesterol; MADRS = Montgomery-Asberg Depression Rating Scale; MARS = Mental Alertness on Rising; mHAM-A = modified Hamilton Anxiety Rating Scale; MINI = Mini-International Neuropsychiatric Interview; MYMOP = Measure Yourself Medical Outcomes Profile; NR = Not reported; NOS = Not otherwise specified; OHQ = Oxford Happiness Questionnaire; nsd = Non-significant decrease; PANSS = Positive and Negative Syndrome Scale; PGAET = Physician’s Global Assessment of Efficacy to Therapy; PGATT = Patient’s Global Assessment of Tolerability to Treatment; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index; RSQ-W = Restorative Sleep Questionnaire-Weekly; S100B = S100 Calcium-binding protein B; SE = Sleep efficiency; SF-36 = Short Form-36; SOL = Sleep Onset Latency; SSRI = Selective serotonin reuptake inhibitor; SST = Set Shifting Test; STDT = Strategic Target Detection Test; TBT = Total Bed Time; TC = Total Cholesterol; TG = Triglycerides; TIB = Total Time in Bed; TFEQ = Three-Factor Eating Questionnaire; TST = Total Sleep Time; VLDL-C = Very low density lipoprotein cholesterol; WASO = Wake After Sleep Onset; WHOQOL-Bref = WHO Quality of Life-BREF; YMRS = Young Mania Rating Scale; QoL = Quality of Life

Table 2. Anti-stress effects of Ashwagandha (*Withania somnifera*, WS) in animal studies.

Nature of Extract, Standardization	Dosage	Model	Outcomes	Refs.
Root powder (KSM-66), standardized to ≥5% withanolides	2.5, 5, or 10 g/day p.o. for 21 days	Horse (exercise-, separation-, and noise-induced stress)	<p>▲ stress-induced decrease in peripheral total erythrocyte count, total leukocyte count, hemoglobin, lymphocyte percentage, serotonin, GSH and SOD activity, HDL and LDL cholesterol</p> <p>▼ stress-induced increase in peripheral cortisol, epinephrine, TBARs, blood glucose, ALT, AST, creatinine, triglycerides, IL-6</p>	[56]
Water-ethanol (7:3) root extract, containing 0.23% (w/w) isowithanone	500 mg/kg p.o., 6 days per week for 6 weeks	Rat (SPS)	<p>▲ short- and long-term memory (RAWM)</p> <p>▲ hippocampal oxidative stress biomarkers (▼ GSSG, ▲ GSH/GSSG, ▲ GPx activity, catalase, and SOD)</p>	[55]
Withanolide-free root extract	3.3, 10, 33.3, and 100 mg/kg p.o. for 12 days	Rat (FSS)	<p>▼ stress-induced weight loss</p> <p>▼ stress-induced increase in rectal temperature</p> <p>▼ transient hyperthermic response (FSS)</p> <p>▼ anxiety-like or depression-like behavior (MBT)</p> <p>▼ stress-induced increase in adrenal weight</p> <p>▼ stress-induced increase in plasma cortisol and blood glucose</p>	[54]
Methanolic root extract, standardized to 2.7% (w/w) total withanolides	10, 20, and 40 mg/kg/day p.o. for 11 days, or as a single dose	Rat (FSS, HPT, TST)	<p>▼ stress-induced weight loss</p> <p>▼ transient hyperthermic response (FSS)</p> <p>▲ reaction time (HPT)</p> <p>▼ depression-like behavior (TST)</p>	[51]
Aqueous methanolic (75:25) root extract, NR	200 mg/kg p.o. for 21 days before hypoxia and 7 days during hypoxia	Rat (hypobaric hypoxia)	<p>▼ stress-induced increase in corticosterone[*], NO[^], AChE activity[^], ROS[^], lipid peroxidation[^], synaptophysin[^], nNOS[^], L-type calcium channels[^], pyknotic cells in CA3 region of hippocampus[^], Bax[^]</p> <p>▲ stress-induced decrease in ACh[^], GSH[^], SOD activity[^], BDNF[^], NCAM[^], Bcl-2[^]</p> <p>▼ stress-induced memory impairment (MWM)</p>	[46]
Aqueous leaf extract (ASH-WEX), NR	140 mg/kg/bwt, p.o. for 15 days prior to acute sleep deprivation	Rat (acute sleep deprivation)	<p>▼ stress-induced learning and memory impairments</p> <p>▼ stress-induced motor dysfunction</p> <p>▼ anxiety-like behavior (▼ grooming activity)</p> <p>Modulated markers of synaptic plasticity (▼ hippocampal PSA-NCAM and NCAM, ▲ PSA-NCAM in the pyriform cortex)[^]</p> <p>▼ mortalin[^], ▲ Akt-1 phosphorylation[^]</p>	[45]
Ethanollic root extract, NR	23 mg/kg p.o. for 7 days prior to assessment	Mouse (FST, CRS)	<p>▲ swim duration (FST)</p> <p>▼ stress-induced increase in WBC count, blood glucose, plasma cortisol, and serum triglyceride levels (CRS)</p>	[41]
Alcoholic root extract, NR	FST: 100 mg/kg p.o. for 7 days CRS: 100 mg/kg p.o. for 10 days	Rat (FST, CRS)	<p>▲ swim duration (FST)</p> <p>▼ stress-induced increase in blood glucose, total cholesterol, triglycerides, serum corticosterone, RBC count, WBC count, neutrophils, lymphocytes, and eosinophils (FST, CRS)</p> <p>▼ stress-induced increase in monocytes, liver weight, and adrenal weight (CRS)</p> <p>▲ stress-induced decrease in spleen weight (CRS)</p>	[42]
Root extract, NR	40 mg/kg/bwt p.o. for 30 days	Mouse (RST)	<p>▼ stress-induced increase in corticosterone* and hippocampal nNOS-positive neurons</p> <p>▲ stress-induced decrease in ChAT reactivity[^] and serotonin[^]</p>	[47]

(Table 2) contd....

Nature of Extract, Standardization	Dosage	Model	Outcomes	Refs.
Water:methanol 40:60 leaf and root extract ("Sensoril®"), standardized to 10% total withanolides	50 and 100 mg/kg p.o. for 7 or 30 days	Mouse (FST)	▲stress-induced decrease in ATP, AEC, TAN, and ATP/ADP ratio (7- and 30-day FST)	[36]
Root (preparation unclear), NR	25, 50, 100, and 200 mg/kg p.o. for 14 days	Mouse (RST)	▲stress-induced decrease in T lymphocyte count (CD3+, CD4+, and CD8+ populations)*, IL-2*, IFN- γ *, polymorphonuclear leucocyte count*, and organ weights (thymus, spleen, axillary lymph nodes) ▼stress-induced increase in serum cortisol	[48]
Glycowithanolide-rich fraction (containing sitoindosides VII-X and withaferin) isolated from aqueous ethanolic (1:1) root extract, standardized to 28-30% withanolide glycosides	25 and 50 mg/kg p.o. for 21 days, administered 1 hr before assessment	Rat (FSS)	▼stress-induced hyperglycemia, perturbed glucose tolerance test, and gastric ulcers (FSS) ▼depression-like behavior (FST, LHT) ▼stress-induced memory impairment (EPM, PAT), sexual behavior inhibition (FSS), and immunosuppression (FSS)	[52]
Isolated fraction and pure compound (1-oxo-5 β , 6 β -epoxy-witha-2-ene-27-ethoxy-olide), extracted from aqueous root extract	Fraction: 20 mg/kg/bwt p.o., administered 30 mins prior to assessment Compound: 2.5 mg/kg/bwt p.o., administered 30 mins prior to assessment	Rat (CHR)	▼stress-induced increase in serum corticosterone, CPK, LDH (fraction, compound) ▼stress-induced increase in LPO (compound) ▲time taken to achieve 23 °C rectal temperature (fraction, compound) ▼recovery time taken to achieve 37 °C rectal temperature (fraction, compound)	[50]
Traditional root extract consisting of water:ghee:honey (60:7.5:32.5) and 50% methanolic root extract, NR	Traditional extract: 250 mg/kg Methanolic extract: 250 mg/kg	Rat (FSS)	▼depression-like behavior (FST, methanolic extract) ▼stress-induced memory impairment (PAT, both extracts)	[53]
Withanolide-free aqueous fraction (BF), isolated from hydroalcoholic (30:70) root extract and a root extract, NR	Withanolide-free fraction: 12.5, 25, 50, and 100 mg/kg p.o., administered 2 hrs before assessment Root extract: 125 mg/kg p.o., administered 2 hrs before assessment	Rat (FST)	▼stress-induced increase in serum GPT, GOT, ALP, triglycerides, and hepatic LPO (fraction, extract) ▲stress-induced decrease in hepatic glycogen (fraction, extract)	[37]
Aqueous root extract and isolated compound (Compound X) from aqueous root extract, NR	Root extract: 360 mg/kg/bwt, administered 30 mins before assessment Compound: 20 mg/kg/bwt, administered 30 mins before assessment	Rat (CHR)	▲time taken to achieve 23 °C rectal temperature (extract, compound) ▼recovery time taken to achieve 37 °C rectal temperature (extract, compound)	[49]
Withanolide-free aqueous fraction (BF), isolated from hydroalcoholic (30:70) root extract and a commercial root extract, NR	Fraction: 12.5, 25, 50, 100 mg/kg p.o. for 15 days Root extract: 125 mg/kg p.o. for 15 days	Rat (HST, FST, RST, RTT)	▲hypoxia-induced time to convulsion (HST, fraction, extract) ▲swimming duration (FST, fraction, extract) ▼stress-induced gastric ulcers (FST, RST, fraction, extract) ▲antifatigue activity (RTT, fraction, extract) ▼stress-induced hypothermia (FST, fraction, extract) ▼stress-induced autoanalgesia, increase in adrenal weight (RST, fraction, extract) ▲stress-induced decrease in adrenal content of ascorbic acid, cortisol, and cholesterol (RST, fraction, extract)	[38]

(Table 2) contd....

Nature of Extract, Standardization	Dosage	Model	Outcomes	Refs.
Powdered root, NR	100 mg/kg p.o. for 7 days	Rat (FST)	▲swim duration ▼stress-induced increase in plasma corticosterone level, phagocytic index, and avidity index	[39]
Sitoinosides VII and VIII	PTZ: 20 mg/kg i.p., 30 mins prior to assessment FST: 20 and 50 mg/kg i.p., 30 mins prior to assessment OST, TCT: 50 mg/kg p.o. for 4 days RST: 50 and 100 mg/kg p.o. for 4 days; 100 mg/kg i.p., 30 mins before morphine administration Morphine toxicity: 50 mg/kg p.o. for 4 days	Mouse (FST, OST, TCT) Rat (PTZ, RST)	▼depression-like behavior (FST) ▼stress-induced autoanalgesia, gastric ulcers (RST) ▼morphine-induced hypothermia (RST) ▼morphine toxicity (OST, TCT) ▼anxiety-like behavior (PTZ-induced) ▼tribulin activity in urine (RST) ▲stress-induced decrease in adrenal content of ascorbic acid, corticosterone	[17]
Alcoholic extract from defatted seeds, NR	100 mg/kg i.p., administered 30 mins or 1 hr before assessment	Mouse (FST, MIL) Rat (CST, RST, ASA)	▲swim duration (FST) ▲stress-induced decrease in ascorbic acid and cortisol content in adrenals (FST) ▼stress-induced gastric ulcers (CST, RST, ASA) ▼milk-induced leukocytosis (MIL)	[40]

*Peripheral; ^CNS; ▲ – Increased; ▼ – Decreased; ACh = Acetylcholine; AChE = Acetylcholinesterase; ADP = Adenosine diphosphate; AEC = Adenylate energy charge; ALP = Alkaline phosphatase; ALT/GPT = Alanine aminotransaminase; ASA = Aspirin administration; AST/GOT = Aspartate aminotransferase; ATP = Adenosine triphosphate; Bax = Bcl-2-associated X protein; BDNF = Brain-derived neurotrophic factor; ChAT = Choline acetyl transferase; CHR = Cold-Hypoxia-Restraint test; CRS = Cold restraint stress test; CST = Cold stress test; EPM = Elevated plus maze test; FSS = Foot shock stress test; FST = Forced swim test; GPx = Glutathione peroxidase; GSH = Reduced glutathione; GSSG = Oxidized glutathione; HDL = High density lipoprotein; HPT = Hot plate test; HST = Hypoxia stress test; IFN- γ = Interferon gamma; IL-2 = Interleukin 2; IL-6 = Interleukin 6; LDL = Low density lipoprotein; LHT = Learned helplessness test; LPO = Lipid peroxidation; MIL = Milk-induced leukocytosis; MWM = Morris Water Maze test; NCAM = Neural cell adhesion molecule; nNOS = Nitric oxide synthase; NO = Nitric oxide; NR = Not reported; OST = Overcrowding stress test; PAT = Passive avoidance test; PTZ = Pentylentetrazol administration; RAWM = Radial arm water maze; RBC = Red blood cell; ROS = Reactive oxygen species; RST = Restraint stress test; RTT = Rotarod test; ROS = Reactive oxygen species; SOD = superoxide dismutase; SPS = Single prolonged stress; TAN = Total adenine nucleotide; TBARS = Thiobarbituric acid reactive substances; TCT = Tactile stress test; TST = Tail suspension test; WBC = White blood cell.

effects of KSM-66 Ashwagandha®, described as an aqueous WS root extract made without alcohol or synthetic solvents [57, 58, 60]. It is worth noting that the literature description of KSM-66 as an “aqueous” extract differs from the product website [71], describing the use of a traditional process involving milk (except for a vegan version), which may extract more lipophilic molecules due to its fat content. Two other studies used Sensoril®, an aqueous extract of WS leaf and root standardized to $\geq 10\%$ withanolide glycosides [59, 62]; and one study used Shoden®, a hydroalcoholic (70:30) root and leaf extract of WS standardized to 35% withanolide glycosides [61]. In addition, Auddy *et al.* (2008) included Es-sentra®, a version of Sensoril® created for the food and beverages market [62]. The daily dose of WS extract ranged from 240 mg to 1000 mg, with two of the studies using more than one dose in the intervention group [60, 62]. The treatment period was variable, with three studies lasting 8.5 weeks [57, 61, 62], two lasting 8 weeks [58, 60], and one lasting 12 weeks [59].

Stress was assessed *via* serum cortisol levels and three questionnaires: the Depression Anxiety Stress Scale (DASS), the Perceived Stress Scale (PSS), and the General Health Questionnaire-28 (GHQ-28). The DASS is a self-report questionnaire that measures symptoms of depression, anxiety, and stress and is available in 42- (DASS) and 21-item (DASS-21) versions. The PSS is a self-report questionnaire

used to evaluate the level of stress perceived by a respondent in the previous month [58]. The GHQ-28 is a 28-item questionnaire with four item-subsets (somatic, anxiety and insomnia, social dysfunction, and severe depression) that correspond to different categories of stress [57]. Four studies used versions of the PSS [57-60], two studies used versions of the DASS [57, 61], and one study used the GHQ-28 [57]. Five of the six studies measured serum cortisol levels [57, 58, 60-62].

WS supplementation improved stress markers and symptoms in the majority of the human trials as evidenced by statistically significant declines in PSS, DASS, and GHQ-28 scores, as well as decreased serum cortisol levels compared to placebo (Table 1). Lopresti *et al.* (2019) saw a non-significant decrease in DASS-21 scores [61].

2.3. Proposed Mechanisms for Anti-Stress Effects

The anti-stress effect of WS has been attributed to several mechanisms, including reduction of glucocorticoids and immune modulation (described below). A more comprehensive overview of mechanisms that may be related to the anti-stress effect of WS is illustrated in Fig. 2.

2.3.1. Glucocorticoid Reduction

The anti-stress activity of WS is most commonly attributed to its effects on the glucocorticoids cortisol and cor-

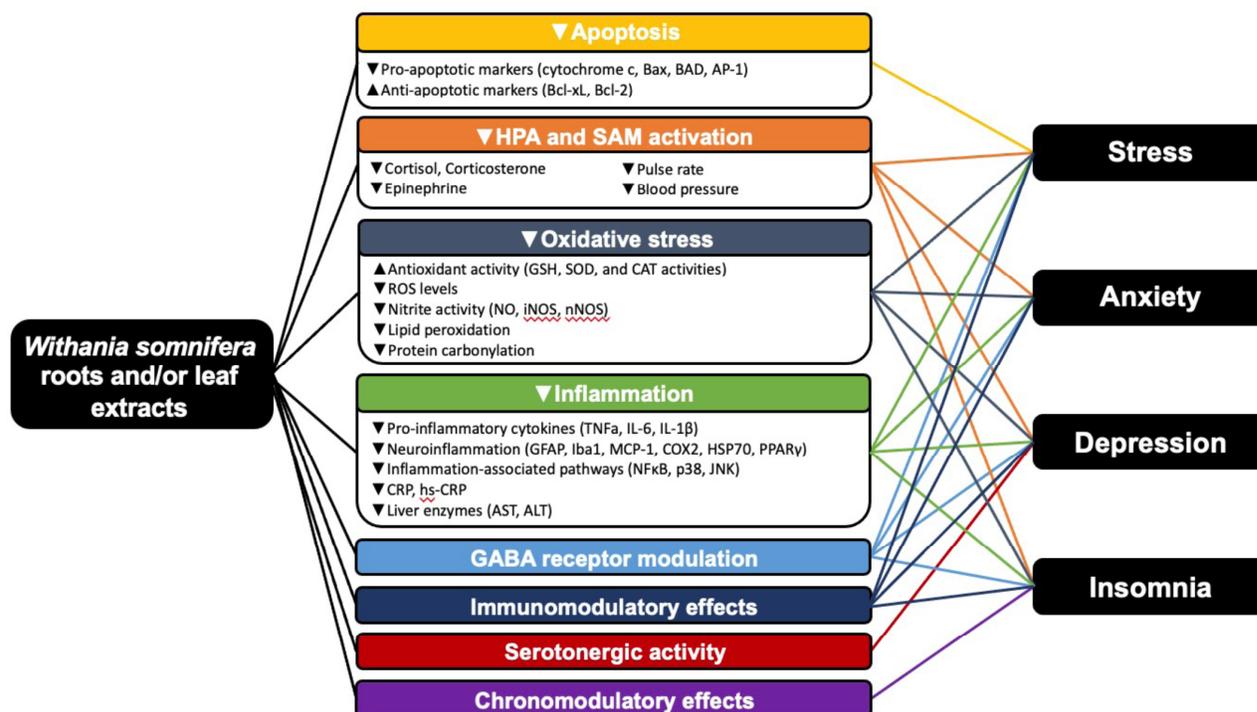


Fig. (2). Potential mechanisms by which WS exerts positive benefits on stress, anxiety, depression, and insomnia. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

ticosterone (the major stress hormones in humans and rodents), respectively [72]. Cortisol and corticosterone are elevated during periods of stress and play a central role in stress-induced HPA axis dysfunction. As described in Table 2, various preparations of WS root attenuated the stress-induced increase in peripheral cortisol and corticosterone levels [38, 39, 41, 42, 46–48, 50, 54, 56]. WS was also found to reverse the stress-induced decrease in adrenal cortisol content [38, 40]. In addition to its effects on peripheral cortisol and corticosterone, Baitharu *et al.* (2013) found that a hydroalcoholic root extract of WS decreased hippocampal levels of corticosterone in a rat model of hypobaric-hypoxia-induced stress [46].

2.3.2. Immune Modulation

WS may also exert its anti-stress effects *via* modulating the immune system. In the reviewed studies, the effect of WS on immune markers depended on the animal model used. In a horse model of exercise-, separation-, and noise-induced stress, white blood cell count and lymphocyte percentage decreased while the pro-inflammatory cytokine IL-6 increased [56]. A root powder of WS reversed these effects, demonstrating immune-stimulating and anti-inflammatory effects. Similar effects were seen in a rat model of restraint stress, where a root preparation of WS attenuated stress-induced declines in peripheral T-lymphocytes counts (CD3⁺, CD4⁺, and CD8⁺ populations), IL-2, INF- γ , and polymorphonuclear leukocyte counts [48]. However, in rodent models of cold restraint stress and forced swimming-induced stress, stress increased white blood cell counts, neutrophils, lymphocytes, and eosinophils [41, 42]. These effects were reversed by WS, suggesting immune-modulating activity

[41, 42]. Fig. 2 describes all demonstrated mechanisms of WS that may be involved with its anti-stress effects.

3. ANTI-ANXIETY EFFECTS

3.1. Animal Studies

To date, animal studies have demonstrated the ability of WS to decrease anxiety-like behavior in several animal models of disease, including stress [17, 54, 73–75], sleep deprivation [45, 76, 77], social isolation [78], and neuroinflammation [79] (Table 3). Anxiety-like behavior was most commonly evaluated using the elevated plus maze [74, 75, 77, 80, 81]: a test in which rodents are placed in an elevated plus-shaped maze with open and closed arms, where increased entries and/or time spent in the open arms reflect anti-anxiety behavior [82]. The elevated plus maze test has been validated to assess pharmaceutical agents for anti-anxiety activity in rodents [82]. The marble burying test, in which increased marble burying is said to indicate anxiety-like behavior, was another commonly used test [54, 73, 83]. However, the validity of this test as a measure of anxiety is questionable [84, 85]. Other measures used to evaluate anxiety-like behavior in rodent studies included the open field test [75], social interaction test [81], novelty-suppressed feeding latency test [81], and pentylenetetrazol-induced defecation and urination [17]. A zebrafish model of benzo[a]pyrene-induced neurotoxicity used the light/dark preference test and the novel tank diving test to assess anxiety-like behavior [86].

Anti-anxiety effects were observed for root extracts [48, 54, 73, 74, 77, 78, 80, 81, 83, 87], leaf extracts [76, 79, 86, 88],

Table 3. Anti-anxiety effects of Ashwagandha (*Withania somnifera*, WS) in animal studies.

Nature of Extract, Standardization	Dosage	Model	Behavioral Effects	Biological Effects	Refs.
Aqueous leaf extract (ASH-WEX), NR	140 mg/kg/day p.o. for 8 weeks	Rat (LPS-induced neuroinflammation)	▼ anxiety-like behavior (EPM)	▼ pro-inflammatory cytokines (TNF α , IL-1 β , IL-6)* [^] ▼ reactive gliosis and neuroinflammation (Iba-1, GFAP, NOX2, iNOS, COX2, MCP-1, HSP70) [^] ▼ inflammatory pathways (NF κ B, p38, JNK) [^]	[79]
Aqueous leaf extract (ASH-WEX), NR	140 mg/kg/bwt, p.o. for 15 days prior to acute sleep deprivation	Rat (acute sleep deprivation)	▼ anxiety-like behavior (EPM)	▼ reactive gliosis (GFAP), ▼ OX-18 ▼ pro-inflammatory cytokines (TNF α , IL-6) [^] ▼ cell survival proteins (NF κ B, AP-1) ▼ apoptosis (▲ Bcl-xL, ▼ Cytochrome c)	[76]
Aqueous leaf extract (ASH-WEX), NR	140 mg/kg/bwt, p.o. for 15 days prior to acute sleep deprivation	Rat (acute sleep deprivation)	▼ anxiety-like behavior (▼ grooming activity) ▼ stress-induced learning and memory impairments ▼ stress-induced motor dysfunction	Modulated markers of synaptic plasticity (▼ hippocampal PSA-NCAM and NCAM, ▲ PSA-NCAM in the pyriform cortex) [^] ▼ mortalin [^] , ▲ Akt-1 phosphorylation [^]	[45]
Dry leaf powder, NR	1 mg/g/bwt p.o. for 12 weeks	Rat (high fat diet-induced obesity)	▼ anxiety-like behavior (EPM)	▼ pro-inflammatory cytokines (TNF α , IL-1 β , IL-6)* [^] ▼ reactive gliosis and neuroinflammation (GFAP, Iba1, PPAR γ , iNOS, MCP-1, COX2) [^] ▼ NF κ B pathway [^] ▼ apoptosis (▼ AP-1, ▲ Bcl-xL, ▼ BAD) [^]	[88]
Aqueous leaf extract, NR	0.3% concentration (2.8 mg/L water), waterborne administration for 72 hrs	Zebrafish (benzo[a]pyrene-induced neurotoxicity)	▼ anxiety-like behavior (LDPT, NTDT)	▼ pyknotic cells in periventricular gray zone ▼ oxidative stress (▼ lipid peroxidation) ▼ protein carbonylation, ▲ catalase activity, ▲ GSH) [^]	[86]
Withanolide-free root extract	3.3, 10, 33.3, and 100 mg/kg p.o. for 12 days	Rat (stress)	▼ anxiety-like or depression-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	[54]
Root extract, 2.7% (w/w) withanolides	10, 20, and 40 mg/kg/day p.o. for 12 days, or as a single dose	Mouse (acute stress)	▼ anxiety-like behavior (MBT)	▼ stress-induced changes in weight, basal core temperature, and hyperthermic response	[73]
Hydro-alcoholic root extract, standardized to 2% (w/w) withanolides	300 mg/kg/bwt p.o. for 30 days	Rat (ischemic stroke)	▼ anxiety-like behavior (EPM) ▲ cognitive function (MWM) ▲ locomotor function (NDS, NBW, RTT)	▲ acetylcholinesterase activity [^] ▼ lipid peroxidation [^] ▲ antioxidant activity [^] ▼ infarct volume ▼ stroke-induced histopathological changes	[87]

(Table 3) contd....

Nature of Extract, Standardization	Dosage	Model	Behavioral Effects	Biological Effects	Refs.
Methanolic (MEWS) and aqueous root extracts (AEWS), NR	10, 25, 50, and 100 mg/kg i.p., 30 mins prior to assessment	Mouse (obsessive-compulsive behavior)	▼obsessive compulsive behavior (MBT) ▲anti-OCD action of subtherapeutic dose of fluoxetine at subtherapeutic dose (50 mg/kg) of WS (MBT)	Not reported	[83]
Several root extracts, using various solvents (alcohol, water, hydro-alcohol (50:50)), ratios (1:6, 1:8, 1:10), and methods (hot continuous percolation (10 hrs) and maceration (10 hrs)), NR	100 or 200 mg/kg p.o., 30 mins prior to assessment	Mouse (acute stress)	▼anxiety-like behavior (EPM) for water and hydro-alcoholic extracts prepared by maceration and for hydro-alcoholic extract prepared by hot continuous percolation	Not reported	[74]
Withaferin A and Hydroalcoholic (4:1) root extract, NR	Withaferin A: 10-50 mg/kg i.p., 1-2 hrs prior to assessment Root extract: 100-500 mg/kg p.o., 1-2 hrs prior to assessment	Rat (acute stress)	▼anxiety-like behavior (EPM, OFT)	Not reported	[75]
Ethanol root extract, NR	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	[80]
Root extract, NR	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	[78]
Root extract, NR	100 and 200 mg/kg p.o. for 5 days, beginning 3 days prior to sleep deprivation	Mouse (sleep deprivation)	▼anxiety-like behavior (EPM) ▲locomotor activity	▼sleep deprivation-induced weight loss ▼oxidative stress (▼lipid peroxidation, ▼nitrite activity, ▲catalase activity, ▲GSH) [^]	[77]
Glycowithanolide-rich fraction (WSG, containing sitoindosides VII-X and withaferin) isolated from aqueous root extract, standardized to 1.13% total steroid content	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)	[81]

(Table 3) contd....

Nature of Extract, Standardization	Dosage	Model	Behavioral Effects	Biological Effects	Refs.
Sitoindosides VII and VIII	PTZ: 20 mg/kg i.p., 30 mins prior to assessment FST: 20 and 50 mg/kg i.p., 30 mins prior to assessment OST, TCT: 50 mg/kg p.o. for 4 days prior to assessment RST: 50 and 100 mg/kg p.o. for 4 days prior to restraint; 100 mg/kg i.p., 30 mins before morphine administration Morphine toxicity: 50 mg/kg p.o. for 4 days	Mouse, rat (stress)	▼ anxiety-like behavior (PTZ-induced) ▼ depression-like behavior (FST)	▼ stress-induced autoanalgesia, gastric ulcers (RST) ▼ morphine-induced hypothermia (RST) ▼ morphine toxicity (OST, TCT) ▼ tribulin activity in urine (RST) ▲ stress-induced decrease in adrenal content of ascorbic acid, corticosterone	[17]

*Peripheral; ^CNS; ▲ – Increased; ▼ – Decreased; AP-1 = Activator protein 1; Bcl-xL = B-cell lymphoma extra-large; BAD = Bcl-2 associated agonist of cell death; COX-2 = Cyclooxygenase 2; EPM = Elevated plus maze test; FST = Forced swim test; GFAP = Glial fibrillary acidic protein; GSH = Reduced glutathione; HSP70 = Heat shock protein 70; Iba1 = Ionized calcium binding adaptor molecule 1; IL-1 β = Interleukin 1 beta; IL-6 = Interleukin 6; iNOS = Inducible nitric oxide synthase; JNK = c-Jun N-terminal kinase; LDPT = Light/dark preference test; LHT = Learned helplessness test; LPS = Lipopolysaccharide; MBT = Marble burying test; MCP-1 = Monocyte chemoattractant protein; MWM = Morris water maze test; NBW = Narrow beam walking test; NDS = Neurological deficit score; NF κ B = Nuclear factor kappa B; NOX2 = NADPH oxidase 2; NSFLT = Novelty-suppressed feeding latency test; NTDT = Novel tank diving test; OFT = Open field test; OST = Overcrowding stress test; PPAR γ = Peroxisome proliferator-activated receptor gamma; PTZ = pentylenetetrazol administration; RST = Restraint stress test; RTT = Rotarod test; SIT = Social interaction test; TCT = Tactile stress test; TNF α = Tumor necrosis factor alpha.

and isolated compounds from WS, including withaferin A [75] and a mixture of sitoindosides VII-X [17, 81]. WS extracts produced from leaf or root and with various solvents (water, ethanol, methanol, hydroalcoholic), solvent ratios, and extraction methods all produced anti-anxiety effects (Table 3), suggesting the possibility of multiple bioactive compounds. In addition, WS extracts potentiated the effects of well-known anti-anxiety drugs. A subtherapeutic dose of a WS root extract potentiated the anti-anxiety effect of a subtherapeutic dose of diazepam in a rat model of social isolation [78]. In a rat model of alcohol withdrawal, a subtherapeutic dose of an ethanolic root extract of WS potentiated the anti-anxiety action of a subtherapeutic dose of ethanol [80]. Of note, in a mouse model of Obsessive-Compulsive Disorder (OCD), subtherapeutic doses of a methanolic and aqueous root extract potentiated the anti-OCD action of fluoxetine at a subtherapeutic dose, which differentiates WS from an anti-anxiety to an anti-OCD agent, the authors concluded [83].

3.2. Human Studies

The anti-anxiety effect of WS was assessed in ten human trials of adults aged 18-75 years (Table 1). Study populations included participants described as healthy [61], stressed [57, 60], diagnosed with general anxiety disorder or a related condition [62, 68-70], with insomnia [63, 65], or with schizophrenia or schizoaffective disorder [66]. Sample sizes ranged from 39 participants [68] to 130 participants [62], with the majority of studies including between 60 and 80 participants [57, 60, 61, 63, 65, 66].

Most of the trials used commercially available preparations of WS (KSM-66®, Sensoril®, Essentra®, or Shoden®) for a duration of 6-12 weeks (Table 1). These formulations are described in Section 2.2. Three studies used various preparations of WS without reporting any standardization, including a 70% ethanolic root extract [70], an ethanolic extract [68], and the dried root [69]. All studies administered WS as capsules or tablets except for Khyati and Anup

(2013), in which dried root granules were used. Daily doses of WS were highly variable, ranging from 125-1000 mg in studies using capsules or tablets up to 12 g of the dried root granules (Table 1). Only two studies compared more than one dose in the intervention group [60, 62].

A majority of studies evaluated anxiety using some variation of the Hamilton Anxiety Rating Scale (HAM-A or HARS), the most commonly used instrument for measuring anxiety in clinical trials [89]. The HAM-A is a self-reported questionnaire used to assess the severity of anxiety as perceived by the respondent. Other instruments included the Depression Anxiety Stress Scale (DASS) described previously [57] and the Positive and Negative Syndrome Scale (PANSS) [66]. The PANSS is a validated tool used to evaluate persons with schizophrenia [90, 91]. Lopresti *et al.* (2019) used both the HAM-A and the DASS [61].

An improvement in anxiety after supplementation with WS was observed in a majority of studies as measured by changes in anxiety scores (Table 1). In a study of adults who self-reported high stress, Lopresti *et al.* (2019) found that daily administration of 240 mg Shoden® for 60 days significantly decreased HAM-A (but not DASS) scores compared to placebo [61]. In those with general anxiety disorder, Khyati and Anup (2013) found that administration of either 12 g of a dried WS root preparation or placebo decreased all measured anxiety scores from baseline values [69]. Although larger decreases were noted for all anxiety scores in the intervention group compared to the placebo group, the effect size only differed significantly between groups for anxious mood, as identified by the HARS. Changes in other anxiety score subgroups did not differ significantly between groups, suggestive of a potential placebo effect [69]. Lastly, Salve *et al.* (2019) found that KSM-66® aqueous root extract produced a significant decrease in HAM-A score with a daily dose of 600 mg but not with 250 mg [60].

Two papers, Cooley *et al.* (2009) and Chengappa *et al.* (2013), found during our literature review re excluded from

Table 1 [92, 93]. Cooley *et al.* (2009) evaluated the use of naturopathic care, which included the use of WS for treating anxiety using psychotherapy as the control [92]. This study was excluded because the intervention included many components in addition to WS supplementation, and not all of these components were included in the control, making it difficult to ascertain the effect of WS on anxiety. Chengappa *et al.* (2013) conducted a randomized, double-blind, placebo-controlled study to evaluate the cognitive effects of a standardized WS extract in bipolar disorder [93]. The investigators noted that symptoms were well-controlled at baseline (*i.e.*, euthymic), and there were no significant differences in anxiety between the treatment groups, as measured by the HAM-A. As a result, no comment can be made regarding the effect of WS on anxiety in this study.

3.3. Proposed Mechanisms for Anti-Anxiety Effects

Research investigating the mechanisms underlying WS's anti-anxiety effects has primarily focused on the GABA receptor system (described below) and antioxidant and anti-inflammatory activities. Fig. 2 illustrates additional mechanisms of WS that may play a role in its anti-anxiety effects.

3.3.1. Gamma-Aminobutyric Acid (GABA) Related Activity

GABA is the most important inhibitory neurotransmitter in the central nervous system, and GABAergic neurotransmission is thought to play a key role in the regulation of anxiety [94]. GABA type A (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 anxiety disorders [95, 96]. There is substantial pre-clinical evidence suggesting that compounds found in WS interact with and modulate GABA_A receptors, which may in part explain the anti-anxiety effect of WS.

Evidence of WS's direct GABA-mimetic activity was first demonstrated by Mehta *et al.* (1991), who found that a methanolic root extract of WS increased chloride ion influx in mammalian spinal cord neurons in the absence of GABA and also inhibited GABA binding in a manner similar to GABA_A receptor agonists [97]. Receptor-binding assays have shown that components in WS methanolic root extracts display high affinity for GABA_A receptors, with significantly less affinity for GABA_B, glutamatergic, and opioid receptors [98, 99]. WS's GABA_A receptor-specific activity is supported by several animal studies. The stimulatory actions of morphine and ethanol on ventral tegmental area dopaminergic neurons in rats were suppressed by a methanolic root extract of WS *via* a GABA_A- but not a GABA_B-mediated mechanism [100]. In a mouse model of pentylenetetrazol (PTZ)-induced seizures, a sub-effective dose of an undefined WS root extract increased the seizure threshold in mice in combination with sub-therapeutic doses of GABA, a GABA receptor agonist, and diazepam, a GABA_A receptor modulator [101]. In an earlier mouse study, Kulkarni *et al.* (1993) found that a methanolic extract of WS (plant part not specified) in combination with pentobarbital (a GABA_A receptor agonist) produced greater protection from PTZ-induced toxicities compared to either agent alone [102]. In addition, the combination of a sub-protective dose of WS with GABA

potentiated the protective effects of the extract. In a rat model of sleep disturbance, a selective GABA_A agonist (muscimol) potentiated the hypnotic effect of an undefined WS root extract, whereas a GABA_A receptor antagonist (picrotoxin) reversed the effect [103]. GABA_A receptor antagonists (picrotoxin, bicuculline) blocked the abilities of methanolic and aqueous root extracts of WS to induce depolarization in mice gonadotropin releasing hormone neurons or to increase inward ion currents in mouse substantia gelatinosa neurons and rat brain GABA_A channels [96, 104, 105].

Attempts to identify which compound or compounds found in WS are responsible for its GABA_A receptor activity have been difficult. Candelario *et al.* (2015) found that while an aqueous root extract of WS demonstrated GABA_A receptor agonist activity in rat brain GABA_A channels, neither withaferin A nor withanolide A, two of the major compounds found in WS, had any direct effect on GABA_A receptors [104]. Similarly, Schliebs *et al.* (1997) found that intraperitoneal injection of an equimolar mixture of withaferin A and sitoindosides VII-X had no effect on GABA_A receptors in a rat model [106]. Sonar *et al.* (2019) isolated nine compounds from a methanolic extract of WS, including withanolides and ferulic acid esters. They found no direct GABA-mimetic activity for any of these compounds (at 10 μM) in an *ex vivo* model [95]. However, two compounds (withanolide B and docosanyl ferulate) did modulate GABA_A receptor function by enhancing inhibitory postsynaptic currents, with a similar pharmacological profile to the methanolic extract from which they were isolated and to other known GABA_A allosteric modulators [95]. Interestingly, several of the compounds, including withanolide A, had an opposite effect on GABA_A receptor function, decreasing its activity [95]. Further research is needed to identify structure-activity relationships of withanolide interactions with GABA_A receptors, possible sites of interaction at the GABA_A receptor complex, and whether or not specific subpopulations of GABA_A receptors are more sensitive to WS compounds. Of note, Candelario *et al.* (2015) found that WS was also a strong GABA_{ρ1} receptor agonist and that these receptors were 27 times more sensitive to WS than GABA_A receptors [104]. GABA_{ρ1} receptors are a subclass of GABA_A receptors with unique pharmacological properties, including greater sensitivity to GABA, a lower rate of desensitization, and insensitivity to bicuculline: a GABA_A antagonist [104].

3.3.2. Antioxidant and Anti-Inflammatory Activity

The brain is uniquely susceptible to oxidative stress [107], and research suggests that anxiety disorders are marked by decreased antioxidant defenses combined with increased oxidative damage [108]. There are several proposed mechanisms by which oxidative stress contributes to anxiety, including both as a cause and a consequence of neuroinflammation, which similarly has been associated with anxiety disorders [109]. Peripheral inflammation is also thought to directly contribute to neuroinflammation and oxidative stress in the brain [109]. While both oxidative stress and inflammation have been implicated in the pathogenesis of anxiety, a definitive cause-effect relationship has yet to be established for either [108-110].

Animal studies investigating the anti-anxiety effects of WS (Table 3) have demonstrated an association between anxiety-like behavior and the dysregulation of oxidative stress and inflammatory markers, which are ameliorated after administration of WS. An undefined root extract [77, 86] and an aqueous leaf extract [77, 86] of WS both showed an ability to increase catalase activity and levels of reduced glutathione (GSH) in the brain, while reducing lipid peroxidation in a mouse model of acute sleep deprivation and a zebrafish model of benzo[a]pyrene-induced neurotoxicity, respectively. In addition, WS reduced nitrite activity in the mouse model, while reducing protein carbonylation in the zebrafish model [77, 86]. In a rat model of ischemic stroke, a standardized hydroalcoholic root extract of WS also reduced lipid peroxidation and increased antioxidant activity in the brain [87].

In animal models of neuroinflammation and sleep deprivation, an aqueous leaf extract of WS (ASH-WEX) reduced pro-inflammatory cytokines including TNF α and IL-6, both peripherally and centrally [76, 79]. ASH-WEX also reduced markers of reactive gliosis (e.g., GFAP) and neuroinflammation (e.g., NOX2, iNOS, COX2), while modulating several inflammatory pathways and reducing apoptosis [76, 79]. Similarly, in a rat model of high fat diet-induced obesity, a dry leaf powder of WS reduced levels of pro-inflammatory cytokines peripherally and centrally, reduced markers of reactive gliosis and neuroinflammation, modulated the NF κ B pathway, and reduced apoptosis [88].

4. ANTI-DEPRESSANT EFFECTS

4.1. Animal Studies

WS has been evaluated for anti-depressant activity in various animal models (Table 4). The most frequently used method of evaluating depression was the Forced Swim Test (FST), a common screening test for agents with potential antidepressant activity; however, the validity of the FST for this use has recently been called into question [111-113]. As described earlier, the FST involves placing a rodent in an inescapable tank filled with water; a drug's ability to reduce the amount of time an animal is immobile is considered indicative of anti-depressant properties [43, 114]. The FST is also used to assess non-depression endpoints, including coping ability, capacity to learn, endurance, or as a method to induce stress [111]. Another common depression test model was the Learned Helplessness Test (LHT). The LHT involves dividing study animals into three groups with differing exposure to electrical shocks [115, 116]. The first group is not exposed to shocks. A second group is exposed to shocks that the animal can control by a physical mechanism, such as by pressing a lever. A third group is exposed to shocks that are triggered by the activities of the animals in the first group. These shocks are uncontrollable and unavoidable, so the animals develop learned helplessness, which is considered a sign of depression-like behavior. A third method used to model depression was the Tail Suspension Test. Here, mice are suspended from their tail, and researchers measure the time until the mice become immobile, which is interpreted as depression-like behavior [51]. Effects of WS were tested in rodents exposed to these behavioral despair tests alone [81, 117-119], or in animals exposed to stress [17,

51, 52, 54], social isolation [78], or drugs such as clonidine or reserpine [120] prior to testing.

Anti-depressant effects were observed for WS root extracts [51, 52, 54, 78, 81, 117, 119], leaf and root extracts [120], and isolated compounds from WS, including 27-O-beta-D-glucopyranosylpubesanolide-3-O-beta-D-glucopyranoside (withanoside X) [120] and sitoindosides VII-X [17, 52, 81] (Table 4). Aqueous [81], methanolic [51], and hydroalcoholic extracts [52, 120], along with a traditional root extract made with water and ghee [117] of WS, all demonstrated anti-depressant activity. In addition, WS potentiated the effects of well-known anti-depressant drugs. In mouse and rat models of depression, WS potentiated the antidepressant activity of imipramine (a tricyclic antidepressant) [117-119] and fluoxetine: a selective serotonin reuptake inhibitor [119].

4.2. Human Studies

WS was evaluated for anti-depressant effects in three studies of adults aged 18-75 years who were either stressed [57], healthy [61], or diagnosed with schizophrenia or schizoaffective disorder [66] (Table 1). All studies included between 60 and 66 participants. Each study administered capsules of commercially available preparations of WS: KSM-66 Ashwagandha® [57], Sensoril® [62, 66], and Shoden® [61] (described previously). Daily doses of WS ranged from 240-1000 mg for a period of 8.5 weeks [57, 61] or 12 weeks [66]. Two studies assessed depression using the Depression Anxiety Stress Scale (DASS) [57, 61] (described previously). Chandrasekhar *et al.* (2012) also used the General Health Questionnaire-28 (GHQ-28), which includes a subset of items corresponding to stress that is categorized as "severe depression" [57]. Gannon *et al.* (2019) assessed depression using the Positive and Negative Syndrome Scale (PANSS) (described previously) [66].

Chandrasekhar *et al.* (2012) saw improvement in depression after supplementation with WS, as evidenced by a significant decrease in scores of the DASS and the "severe depression" subset of the GHQ-28 [57]. In Gannon *et al.* (2019), participants saw significantly increased PANSS scores, indicating an improvement in depression [66]. Lopresti *et al.* (2019) observed a decrease in DASS-21 score among participants, but the change was not statistically significant [61]. The results of these studies suggest that supplementation with WS may be beneficial for depressive symptoms (Table 1), but there are significant limitations. None of the studies evaluated patients diagnosed with major depressive disorder or seasonal affective disorder, representing a significant gap in the existing human research on WS for depression.

4.3. Proposed Mechanisms for Anti-Depressant Effects

The anti-depressant effect of WS demonstrated in the reviewed studies has been attributed to both its antioxidant and serotonergic activities (detailed below), though other mechanisms are likely involved (Fig. 2).

4.3.1. Antioxidant Activity

The antioxidant activity of WS is one mechanism by which WS may exert its anti-depressant activity. While

Table 4. Anti-depressant effects of Ashwagandha (*Withania somnifera*, WS) in animal studies.

Nature of Extract, Standardization	Dosage	Model	Behavioral Effects	Biological Effects	Refs.
Aqueous methanolic (40:60) root and leaf extract (AME), withanolide-rich fraction from AME (WF), flavonoid-rich fraction from AME (FF), compound isolated from WF (Withanolide X), Enteric-coated versions (EC-AME, EC-WF, EC-FF, EC-WX)	AME, WF, FF: 60mg/kg p.o. Withanolide X: 10mg/kg p.o. EC-AME: 20mg/kg p.o. EC-WF: 15mg/kg p.o. EC-Withanolide X: 2.5mg/kg p.o. All WS treatments given 4 hrs prior to assessment	Rat (reserpine)	▼depression-like behavior (FST)	Not assessed	[120]
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	[54]
Methanolic root extract, standardized to 2.7% (w/w) total withanolides	10, 20, and 40 mg/kg/day p.o. for 11 days, or as a single dose	Rat (stress)	▼depression-like behavior (TST) ▲reaction time (HPT)	▼stress-induced weight loss ▼transient hyperthermic response (FSS)	[51]
Traditional root extract (Ashwagandha ghrutha) consisting of root:ghree:water (1:4:16)	20 and 40 mg/kg, given 1 hr prior to assessment (acute) or for 7 days (chronic); Route of administration NR	Mouse	▼ depression-like behavior (TST, 40 mg/kg acute or 20 and 40 mg/kg chronic WS dosing; FST, 20 and 40 mg/kg chronic WS dosing) ▲ antidepressant activity of low-dose imipramine (FST, TST, 10 mg/kg each, acute or chronic dosing) ▼reserpine-induced catatonia and sedation (ART) alone (20 and 40 mg/kg WS) and in combination with low-dose imipramine 10 mg/kg each) all at chronic dosing	▼reserpine-induced ptosis (ART), alone (20 and 40 mg/kg) and in combination with low-dose imipramine (10 mg/kg each) all at chronic dosing	[117]
Powder, NR	50, 100 and 150 mg/kg p.o. for 2 weeks	Rat	▼depression-like behavior (LHT, FST) ▲antidepressant activity of low-dose imipramine (LHT, FST, 16 mg/kg imipramine, 50 mg/kg WS)	Not assessed	[118]
Root extract, NR	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)	▼depression-like behavior (FST) ▼anxiety-like behavior (EPM) ▲anti-anxiety action of diazepam at subtherapeutic dose (50 mg/kg) of WS (EPM)	Not assessed	[78]
Root extract, NR	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	[119]

(Table 4) contd....

Nature of Extract, Standardization	Dosage	Model	Behavioral Effects	Biological Effects	Refs.
Glycowithanolide-rich fraction (containing sitoindosides VII-X and withaferin) isolated from aqueous ethanolic (1:1) root extract, standardized to 28-30% withanolide glycosides	25 and 50 mg/kg p.o. for 21 days, administered 1 hr before assessment	Rat (chronic stress)	<ul style="list-style-type: none"> ▼depression-like behavior (FST, LHT) ▼stress-induced memory impairment (EPM, PAT), sexual behavior inhibition (FSS) 	<ul style="list-style-type: none"> ▼stress-induced hyperglycemia, perturbed glucose tolerance test, and gastric ulcers (FSS) ▼stress-induced immunosuppression (FSS) 	[52]
Glycowithanolide-rich fraction (WSG, containing sitoindosides VII-X and withaferin) isolated from aqueous root extract, standardized to 1.13% total steroid content	20 and 50 mg/kg p.o. for 5 days	Rat	<ul style="list-style-type: none"> ▼depression-like behavior (LHT, FST) ▼anxiety-like behavior (EPM, SIT, NSFLT) 	<ul style="list-style-type: none"> ▼PTZ-induced increase in rat brain tribulin activity (PTZ) 	[81]
Sitoindosides VII and VIII	PTZ, FST: 20 mg/kg i.p., 30 mins prior to assessment FST: 20 and 50 mg/kg i.p., 30 mins prior to assessment OST, TCT: 50 mg/kg p.o. for 4 days RST: 50 and 100 mg/kg p.o. for 4 days; 100 mg/kg i.p., 30 mins before morphine administration Morphine toxicity: 50 mg/kg p.o. for 4 days	Mouse, rat (stress)	<ul style="list-style-type: none"> ▼depression-like behavior (FST) ▼anxiety-like behavior (PTZ-induced) 	<ul style="list-style-type: none"> ▼stress-induced autoanalgesia, gastric ulcers (RST) ▼morphine-induced hypothermia (RST) ▼morphine toxicity (OST, TCT) ▼tribulin activity in urine (RST) ▲stress-induced decrease in adrenal content of ascorbic acid, corticosterone 	[17]

▲ – Increased; ▼ – Decreased; ART = Anti-reserpine test; EPM = Elevated plus maze test; FSS = Foot shock stress test; FST = Forced swim test; HPT = Hot plate test; LHT = Learned helplessness test; NR = Not reported; NSFLT = Novelty-suppressed feeding latency test; OST = Overcrowding stress test; PAT = Passive avoidance test; PTZ = pentylenetetrazol administration; RST = Restraint stress test; SIT = Social interaction test; TCT = Tactile stress test; TST = Tail suspension test.

the complex pathophysiology of depression is not yet fully understood, oxidative stress has been linked to many psychiatric disorders, including depression [108, 121]. The brain is prone to oxidative stress due to its high oxygen consumption, which can lead to increased production of reactive oxygen species (ROS) and the increased availability of lipids, which are susceptible to ROS-related peroxidation [121]. The oxidative stress hypothesis of depressive disorders proposes that oxidative stress, along with activation of pro-inflammatory and pro-apoptotic mediators, is a critical step in a cascading series of events leading to depressive symptoms [121]. As previously discussed, WS has demonstrated both antioxidant and anti-inflammatory activities in various animal models, making it a good candidate for investigation for antidepressant activity.

4.3.2. Serotonergic Activity

There is evidence from animal studies that WS may possess serotonergic activity, which could play a role in its antidepressant effects. In a horse model of stress, in which horses were exposed to exercise-, separation-, and noise-induced stress, Priyanka *et al.* (2020) found that administration of a root powder of WS for 21 days protected against a stress-induced decrease in serum serotonin levels [56]. Similarly, in a mouse model of restraint stress, an undefined root extract of WS protected against a stress-induced reduction in hippocampal serotonin levels [47]. Withanolide A promoted

mRNA expression of serotonin receptors and transporters in wild-type and mutant strains of *Caenorhabditis elegans* [122]. Furthermore, molecular docking studies showed that withanolide A bound to human and *C. elegans* serotonin receptors and serotonin transporters with greater affinity than serotonin, and the Selective Serotonin Reuptake Inhibitor (SSRI) fluoxetine [122]. Unfortunately, the limited number of available human studies have not measured neurotransmitter levels in response to WS administration as a possible mechanism, warranting further investigation.

5. SLEEP-PROMOTING EFFECTS

5.1. Animal Studies

Animal studies have demonstrated sleep-promoting effects of WS, as described in Table 5. Wang *et al.* (2020) used a *Drosophila* model of sleep deprivation to screen seven herbal extracts for their sleep-promoting properties, including a hydroalcoholic root extract of WS [123]. WS increased total sleep time and decreased sleep latency while not affecting fly activity during wake time [123]. Kaushik *et al.* (2017) found that an alcoholic leaf extract of WS high in withanolides, including withaferin A and withanone, was ineffective at inducing sleep in mice [124]. However, aqueous leaf extracts containing triethylene glycol significantly induced non-rapid eye movement sleep in mice at 30 mg/mouse [124]. Furthermore, mice administered isolated

Table 5. Sleep-promoting effects of Ashwagandha (*Withania somnifera*, WS) in animal studies.

Nature of Extract, Standardization	Dosage	Model	Behavioral Effects	Refs.
Hydroalcoholic root extract, ≥2.5% with-anolides	1 mg/ml added to fly food, given from two days after eclosion until the end of the experiment	<i>Drosophila</i> (sleep deprivation)	▲total sleep ▼sleep latency	[123]
Alcoholic leaf extract and aqueous leaf extracts	Alcoholic extract: 200 mg/kg p.o. prior to sleep Aqueous extract and cyclodextrin-assisted aqueous extract: 30 mg/mouse p.o. prior to sleep Triethylene glycol: 10, 20, and 30 mg/mouse p.o. prior to sleep	Mouse (sleep induction)	Water extracts and triethylene glycol ▲NREM sleep	[124]
Root extract, NR	100 mg/kg i.p., 30 mins before assessment	Rat (sleep deprivation)	▼sleep latency ▲duration of slow wave sleep ▲total sleep time ▼total waking	[103]

▲ – Increased; ▼ – Decreased; NR = Not reported; NREM = Non-rapid eye movement.

triethylene glycol (also 30 mg/mouse) experienced similar effects on non-rapid eye movement sleep, suggesting that this molecule may play a role in the hypnotic effects of WS. Kumar and Kalonia (2007) investigated the sleep-promoting activities of an undefined root extract of WS in sleep-deprived rats, in which rats were given an intraperitoneal injection of WS before 24 hours of sleep deprivation using a grid suspended over water method [77]. WS significantly increased total sleep time and slow-wave sleep, while reducing sleep latency, REM sleep, and total waking time [103].

In addition to their sleep-promoting effects, extracts of WS have also been shown to attenuate the adverse effects associated with sleep deprivation. A well-defined water extract of WS (ASH-WEX) was given orally to rats for 15 days, after which they were sleep-deprived for 12 hours [45]. Rats that were administered WS demonstrated improved learning and memory and maintained their motor function compared to untreated sleep-deprived rats [45]. In a mouse model of sleep deprivation, pretreatment with an undefined root extract of WS protected against sleep deprivation-induced weight loss, improved locomotor deficits, and reduced anxiety-like behavior compared to untreated sleep-deprived mice [77].

5.2. Human Studies

Five human studies have investigated WS for its effects on insomnia in adults aged 18-85 years (Table 1). Study populations included participants who were healthy [63, 64], stressed [60], diagnosed with insomnia [65], or had been experiencing non-restorative sleep [67]. Four of those studies included between 50 and 80 participants, while Deshpande *et al.* (2020) evaluated 150 participants [67].

All the studies used a commercially available preparation of WS, administered in capsule form. Four of the studies used KSM-66 Ashwagandha® [60, 63-65], while Deshpande *et al.* (2020) used Shoden® [67]. WS was administered at a daily dose of 120-600 mg for 6 weeks [67], 8 weeks [60, 63],

10 weeks [65], or 12 weeks [64]. Salve *et al.* (2019) evaluated multiple dosages [60].

Several outcome measures were used to assess changes in sleep, and the majority of studies evaluated more than one sleep measure (Table 1). The most commonly used instrument was the sleep quality rating, a seven-point Likert scale with higher ratings indicating worse sleep quality [60, 63-65]. Langade *et al.* (2019) and Langade *et al.* (2021) also used the Pittsburgh Sleep Quality Index (PSQI) to assess sleep quality [63, 65]. The mental alertness on rising rating, a three-point scale to assess morning alertness, was used in three studies [63-65]. Three studies used objective sleep actigraphy: a non-invasive, wearable sensor was used to assess various sleep parameters, such as sleep latency, sleep efficiency, total sleep time, and wake after sleep onset [63, 65, 67]. Kelgane *et al.* (2020) included the Sleepiness Scale, a 4-item questionnaire, to assess the likelihood of falling asleep during daytime activities [64]. Deshpande *et al.* (2020) included the Restorative Sleep Questionnaire – Weekly (RSQ-W), a 9-item questionnaire, to evaluate whether restful sleep occurred [67].

Overall, supplementation with WS improved various measures of insomnia across a variety of study populations and age groups (Table 1). Due to the small number of human trials involving persons experiencing sleep disturbances and the variability in doses and outcome measures, a minimum dose needed to improve sleep cannot be determined based on these studies alone. Additional studies are needed in the target population to determine the effectiveness of WS on improving insomnia.

5.3. Proposed Mechanisms for Sleep-Promoting Effects

One possible mechanism by which WS may exert its positive effects on sleep is by activating GABAergic neurotransmission. Kumar and Kalonia (2008) found that the sleep-promoting activity of WS in rats was significantly reversed by picrotoxin (a GABA antagonist) and potentiated by muscimol (a GABA agonist), supporting a mechanistic

role for GABA [103]. It is also evidenced that WS has chronomodulatory effects on the brain. Jagota and Kowshik (2017) demonstrated that a hydroalcoholic leaf extract of WS restored age-induced changes in several clock genes (*e.g.*, *rBmall*, *rPer1*, *rCry1*, *rPer2*) in the suprachiasmatic nucleus of middle- and old-aged rats [125]. In a subsequent study, Kukkemane and Jagota (2020) found that in old-aged rats, a hydroalcoholic leaf extract of WS restored the daily rhythms and phases of SIRT1 (a clock modulator) and NRF2, which is a transcription factor controlled by a clock that regulates several endogenous antioxidant enzymes [126]. It is likely that other mechanisms also contribute to the sleep-promoting effects of WS, as shown in Fig. 2.

6. DISCUSSION

WS has been extensively researched due to its prominence as a rasayana or rejuvenating herb in Ayurvedic medicine [1, 19] and its popularity as an adaptogenic botanical supplement in Western countries [11]. The classification of WS as a rasayana or adaptogen is related to its reputed effects on stress. The term “stress” is generally understood as a challenge to the organism that requires a response. Stressors can be physiological (*e.g.*, pathogens or an unfavorable physical environment) or psychological (*e.g.*, fear, anxiety, or social discomfort) [127]. Stress can be “good” (*e.g.*, a demanding situation leading to a beneficial outcome), “tolerable” (*e.g.*, where the individual has sufficient resilience to overcome or adapt to the stressor), or “toxic” (*e.g.*, where the stress response is insufficient, leading to disease) [128]. Rasayanas or adaptogens, such as Ashwagandha, are used to increase resilience to potentially toxic stressors, allowing the stress to become tolerable.

The effects of stress on the body are wide-ranging. While stress may be initiated centrally, the response to psychological stress can include both physiological changes and neuropsychiatric symptoms. Markers related to the sympathetic and parasympathetic nervous systems (neurotransmitters), the HPA axis (stress hormones), inflammation (cytokines), cardiac function, and glucose and lipid metabolism have all been used as objective biomarkers to study the physiological effects of stress [129]. Furthermore, stress has been implicated in the development of many neuropsychiatric disorders, including anxiety [130], depression [131], and insomnia [132]. The mechanisms by which stress may contribute to these disorders include hyperactivity of the HPA and sympathetic-adrenal-medullary (SAM) axes and dysregulation of the immune system [31, 32]. This review aimed to summarize the preclinical and clinical studies investigating the effects of Ashwagandha on stress and its effects on three common stress-related neuropsychiatric disorders: anxiety, depression, and insomnia.

6.1. Evidence for the Ability of WS to Ameliorate Stress and Stress-Related Neuropsychiatric Disorders

6.1.1. Preclinical Evidence

As summarized in Tables 2-5, WS has been widely evaluated in animal models of neuropsychiatric disorders, with most studies focusing on the effects of WS in anxiety and

stress. Various WS preparations and compounds have been shown to attenuate stress-related biological and behavioral abnormalities, reduce anxiety- and depression-like behavior, and improve the onset and duration of sleep in these models.

An advantage of animal models is that biological changes in the brain can be measured, providing insights into mechanisms of action not readily measurable in humans. Evidence from pre-clinical studies suggests multiple potential mechanisms for the effects of WS on stress and stress-related neuropsychiatric disorders, including anti-apoptotic activity, reduced activation of HPA and SAM axes, antioxidant and anti-inflammatory effects, GABAergic activity, immune modulation, serotonergic activity, and chronomodulatory effects on sleep (Fig. 2). The effect of WS on GABA and serotonergic pathways, in particular, is noteworthy given that GABA activation has been shown to decrease HPA axis activation [130], while associated serotonin pathways have appeared to reduce in stress [131], contributing to depression. Thus, the modulation of GABAergic and serotonergic pathways by WS may underlie its ability to reduce anxiety, stress, and depression simultaneously. In addition, the ability of WS to reduce NF κ B activation and levels of IL-1 β is noteworthy since these markers are related to NLRP3 inflammasome activation, which has been associated with stress-induced anxiety and depression in rodents [133]. A study by Xia *et al.* (2021) found that withaferin A inhibited NLRP3 inflammasome activation in a mouse model of fulminant hepatitis [134]. These potential mechanisms of WS require further exploration in future studies.

6.1.2. Clinical Evidence

The present review focused on 14 clinical studies, examining the neuropsychiatric effects of WS (Table 1). As with the animal studies, anxiety and stress were the most frequently studied conditions, with fewer studies specifically directed toward sleep and depression, though several studies evaluated more than one of these conditions. Effects of WS were, in most cases, evaluated using standardized, validated behavioral questionnaires and objective biological measures, *e.g.*, HPA activation (serum cortisol), SAM activation (pulse rate, blood pressure), or inflammation (CRP, inflammatory cytokines). The results of these clinical studies provide broad support for WS's ability to reduce stress and anxiety and to improve sleep quality when administered for two or more months. There is limited clinical evidence for the effect of WS on depression. Further studies are needed to determine the possible benefits of WS in individuals diagnosed with specific depressive conditions, such as major depressive disorder or seasonal affective disorder. In addition to the limited number of blood biomarkers evaluated in these clinical studies, a more comprehensive assessment of inflammatory cytokines and neurotransmitter levels would add to our understanding of the mechanisms through which WS achieves the clinical endpoints observed.

Strengths of these clinical studies include that all were reported as double-blind and placebo-controlled. However, the exact nature of the placebo and blinding and randomization methods were not always reported (Table 1). Also, most studies examined WS in participants already experiencing

symptoms of stress, anxiety, insomnia, or depression, thereby increasing the clinical relevance of the research. The age and sex of participants were not well controlled in these studies, however. Only three studies examined WS within a relatively narrow age range (25 years) and in middle-aged or older adults [64, 68, 70]. The other studies recruited participants covering a wide age range (e.g., 18 to 75). Given the known effects of age on cognition [135], mood [136], and sleep [137], it would be informative to evaluate WS in more closely defined age groups. In addition, there have been no studies assessing the effects of WS for neuropsychiatric conditions in pediatric populations. Only three studies [59, 66, 67] included similar numbers of male and female participants. Most of the studies had a preponderance of male participants, and four studies [60, 63, 64, 69] did not provide information on participant sex. Since sex is known to influence neuroendocrine stress-responses [138] and that stress-related neuropsychiatric disorders have a higher prevalence in women than men [139], it would be more appropriate to evaluate WS separately in each sex or to ensure even representation of men and women in the placebo and test groups.

While the evidence from pre-clinical and clinical studies generally supports a potential therapeutic role of WS for stress and stress-related neuropsychiatric disorders, significant questions remain unanswered, especially regarding the nature of active compounds found in WS, the variability of WS preparations tested, and the safety of WS products.

6.2. Active Compounds of WS

6.2.1. Identification of Active Compounds in WS

Withanolides are reported to be the main neurologically active compounds of WS [7, 12, 140]; however, only a small number of the withanolides in WS have been evaluated for their neurological effects, and the results are mixed. For example, withanone [141], withanolide A [142], sominone [142], and withanoside IV [142] all improved memory in cognitively impaired rodents. Conversely, withaferin A, withanolide A, three other withanolides, and sitoindosides VII-X did not appear to possess the GABAergic activity seen with extracts of WS [95, 104]. In contrast, a long-chain fatty acid of ferulic acid was active [95].

Only a few of the studies found in the literature tested pure WS compounds for their neuropsychiatric effects. A withanolide derivative (1-oxo-5 β , 6 β -epoxy-witha-2-ene-27-ethoxy-olide) showed anti-stress effects [50], while withaferin A reduced anxiety-like behavior in a rat stress model [75], and an anti-depressant compound (withanoside X) was identified through bioassay-guided fractionation [120]. An enriched WS extract containing 28-30% withanolide glycosides, sitoindosides VII-X, and withaferin reduced stress related end points, and depression- and anxiety-related behavior in rats [52, 81]. Interestingly, withanolide-free fractions improved endurance and reversed several stress-induced physiological changes [37], and showed anti-anxiety and anti-depressant effects in stressed mice [54], suggesting a role for other groups of compounds. For example, the sitoindosides VII and VIII, which are not withanolides, reduced stress and depressive symptoms in rodents [17]; and a

flavonoid-rich fraction was found to reduce depression-like behavior in rats [120]. Triethylene glycol, a compound from WS leaves, was found to induce sleep in mice [124] and reduce anxiety- and depression-like behavior in mice [54], although the concentrations used were high compared to the parent WS extract. Oligosaccharides of WS, despite being abundant in aqueous root extracts [59, 62], have not been evaluated independently for biological effects. Interestingly, the neurotransmitter GABA was produced by *in vitro* cultures of WS leaves [143], suggesting it may also be present in cultivated WS plants and possibly contributing to the reported GABAergic effects of extracts.

In summary, there have been limited studies linking the withanolides to the neuropsychiatric effects of WS, and other compounds may play a role in these effects. Evidence for specific mechanisms of action of WS compounds relevant to neuropsychiatric disorders is even more limited. Future studies comparing pure compounds, or compound groups, at doses equivalent to their content in active parent extracts would be informative to assess their contribution to WS's functional effects and mechanisms of action. The possibility of synergistic or additive effects [144] between components must also be considered [144] since these may be lost when single compounds are tested, giving a false impression of lack of biological activity.

6.2.2. Test Product Variability

An important feature of the clinical studies and preclinical studies reviewed here is the chemical variation in the type of WS preparation tested. Most of the clinical studies (Table 1) used commercial Ashwagandha products that included an aqueous extract of the root (KSM-66®), an aqueous extract of the root and leaf (Sensoril®, Essentra®), and a hydroethanolic extract of the leaf and root (Shoden®). Two studies used non-commercial ethanolic extracts of WS, and one used granules of dried WS root. For studies that did not report the plant part used, this information was inferred from other studies using the same product where those details were provided. Based on this assumption, all products tested included derivatives of WS root, although some commercial products also had WS leaf extract. The products tested varied in the compound groups measured or used for standardization (withanolides, withanosides, sitoindosides, or oligosaccharides), as well as in the content of these compounds (5% to 35%). Some studies did not report any standardization [60, 64, 68-70]. The products, in some cases, appeared to include excipients that were not fully elucidated, nor was it confirmed in all cases that the placebo used consisted of the inactive components of the test WS product. While the reviewed studies generally found similar benefits of WS on markers of anxiety, depression, and sleep, the variation in test products makes it difficult to compare the studies directly or draw any conclusions regarding the best extraction method, dosage or number of active constituents needed for clinical benefit.

Similarly, a wide variety of WS preparations were tested in preclinical models (Tables 2-5). While most tested extracts were derived from WS root, three studies reported anti-anxiety effects of WS leaf extracts (Table 3). The prepara-

tion of the extracts tested in the studies also varied. Extraction solvents included water, methanol, ethanol, or mixtures of these solvents, whereas in two cases, traditional extractions using a mixture of ghee and water [117] or water, ghee and honey were used [53]. Two studies [36, 56] were performed on commercial products (KSM-66®; Sensoril®) used in human trials; however, the description of the products differed. In a horse study, KSM-66® is described as a “root powder” [56], whereas the clinical studies refer to it as capsules of an aqueous root extract (Table 1). Interestingly, the literature description of KSM-66® as an “aqueous” extract differs from the product website describing the use of a traditional process involving milk [71]. Sensoril® is described in mouse study as a methanol:water (40:60) extract [36], whereas the clinical studies (Table 1) all refer to this product as containing an aqueous extract. Communication by the authors with representatives at Natreon Inc. confirmed that the Sensoril® product used in the clinical studies is an aqueous extract. The content of specific compounds or compound groups in the extracts tested was reported in less than half of the preclinical studies found. A few of these studies describe the content of individual compounds (*e.g.* isowithanone, withaferin and sitoindosides) [55, 81]. In contrast, other studies reported the percentage content of compound groups, such as withanolides, from 0.23% to > 10%, withanolide glycosides 28-30%, and total steroids 1.13% in a glycowithanolide-rich fraction [52, 81]. Further highlighting the variability in reporting for product composition, Chengappa *et al.* (2018) and Gannon *et al.* (2019) (two studies that used the same study population to evaluate different outcomes) described the composition of Sensoril® using varying levels of detail [59, 66].

A noteworthy point is that despite the wide variation in the preparation and chemical profiles of the extracts used in the clinical and preclinical studies, most showed biological activity, suggesting that the active compounds are broadly soluble in aqueous and polar organic solvents or that a subset of active compounds are extracted in any given solvent. One exception is that WS root extracts made with 100% ethanol did not show anti-anxiety activity [74], and a 100% alcoholic leaf extract did not induce sleep [124], whereas aqueous extracts from the same plant materials were active. Interestingly, a separate study did show anti-anxiety effects of an ethanolic WS root extract [80], which may have been due to differences in dose or starting WS plant material.

These examples illustrate a challenge commonly encountered in comparing studies performed on a particular botanical, where natural chemical variation in botanical materials is further amplified by the use of multiple extraction methods. The problem can be compounded by the lack of information, or even misinformation, on the test material and the absence of chemical information on the materials tested. As a result, data can at best be regarded as specific to the product tested with extrapolations to other products only possible where there is demonstrated chemical similarity. These issues are being addressed by many natural product journals, which now require chemical analysis information in conjunction with biological data. The application of both targeted and untargeted chemical profiling of botanicals can be used to

document the composition of botanical extracts more comprehensively [12, 145].

6.3. Pharmacological Properties

6.3.1. Bioavailability of WS Compounds

It is desirable to evaluate the *in vivo* oral bioavailability, Plasma Pharmacokinetics (PK) and tissue distribution (particularly to target organs) of putative active compounds, which are often identified through *in vitro* bioassays or parenteral administration *in vivo*. There have been relatively few studies on the PK and brain bioavailability of WS compounds. Plasma bioavailability and PK of withaferin A and withanolide A were recorded after intragastric administration of a WS root aqueous extract in mice [146]. Plasma bioavailability of withaferin A was also shown in rats after oral administration of a polyherbal formulation containing WS root [147]. Plasma PK of withanolide A was established in rats following oral administration of the compound, which also showed wide tissue distribution; notably, brain levels were considerably lower than in lung, kidney, liver, heart, and spleen [148]. Four major withanamides were detected in the mouse brain following the intraperitoneal administration of an extract of WS [149]. Oral bioavailability of a root extract of WS standardized to 4.5% withaferin A (AshwaMAX®) was assessed in 13 patients with advanced-stage high-grade osteosarcoma [150]. Patients consumed 1600 mg AshwaMAX®, corresponding to 72 mg of withaferin A. Withaferin A was not detected in plasma samples collected over the following 24 hours [150]. No other bioavailability studies of WS compounds in humans were found.

6.3.2. Safety

The widespread use of WS as a traditional medicine and dietary supplement, as well as current scientific literature, supports its general safety. In a review of clinical trials that used root preparations of WS for a wide variety of conditions, Tandon and Yadav (2020) noted that reasonable safety outcomes were seen with no serious adverse events or changes in vital signs, hematological and biochemical parameters [5]. Mild to moderate transient adverse events, including somnolence, giddiness, vertigo, and drowsiness, were reported in some studies, while other studies involving adults and children did not report any adverse events [5]. Similarly, an earlier review by Alam *et al.* (2012) reported that WS has been used in all age groups and both sexes, even during pregnancy, without any reported side effects [7]. The safety of KSM-66® Ashwagandha root extract (Ixoreal Biomed, Inc.) was assessed in a randomized, placebo-controlled study [151]. Eighty healthy participants (40 of each sex), aged 18-45 years, were randomized 1:1 to receive 300 mg KSM-66® or a matching placebo twice per day for 8 weeks. To assess safety, researchers assessed vital signs and various hematological and biochemical markers (hemoglobin, neutrophil percentage, platelet count, alkaline phosphatase, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), thyroid hormone panel). No significant changes were observed in any of the safety parameters compared to placebo, and no adverse events were reported. No serious adverse effects were reported in any of the clinical studies reviewed

here. One study [64] formally measured the tolerability of a WS root extract using the Patient's Global Assessment of Tolerability to Therapy and found it to have a high tolerability score.

WS extracts have also been shown to be safe in animal toxicity studies. Acute and sub-acute toxicity of a hydroalcoholic root extract of WS was investigated in female Wistar rats [152]. No behavioral signs of toxicity or gross pathological changes were observed with acute doses up to 2000 mg/kg. Similarly, no signs of subacute toxicity were observed in rats given 500, 1000, or 2000 mg/kg of the extract daily for 28 days. In a separate study, a hydroalcoholic root extract of WS was used to assess prenatal developmental toxicity in rats at doses of 500, 1000, and 2000 mg/kg daily for 28 days [153]. No signs of toxicity, gross pathology changes, or mortality were observed in the pregnant rats or fetuses. Further studies are required to examine the safety of WS extracts prepared using other extraction methods and plant parts.

While these studies suggest that WS does not cause significant toxicity, the possibility of adverse events arising from herb-drug interactions must be considered [154]. Pharmacodynamic interactions between WS and some groups of drugs may exist, as evidenced by additive effects seen in rodent studies between WS and the drugs imipramine [117-119], diazepam [78], and fluoxetine [119]. The GABA-mimetic and serotonergic activities seen with some WS extracts in rodents would suggest caution when co-administering WS with drugs that work by similar mechanisms. Pharmacokinetic interactions may also occur when botanicals alter the activity of drug transporters or drug-metabolizing enzymes [155]. It has been suggested that an IC_{50} of less than 100 $\mu\text{g/mL}$ for extracts or 100 μM for active constituents should be classified as potent inhibition that could lead to undesirable herb-drug interactions [156, 157]. Various root extracts of WS had IC_{50} values greater than 100 $\mu\text{g/mL}$ for the cytochrome P450 isoenzymes CYP3A4, CYP2D6, CYP1A2, and CYP2C9 in human liver microsomes (HLM). At the same time, withaferin A and withanolide A did not inhibit these enzymes at doses up to 50 μM [158, 159]. An aqueous extract of WS did not inhibit human recombinant CYP3A4 at doses as high as 1000 $\mu\text{g/mL}$ [160]. Methanol and ethylacetate extracts of WS root had IC_{50} values of 79 and 58 $\mu\text{g/mL}$, respectively, for CYP2B6 in HLM, whereas aqueous and ethanolic extracts were not inhibitory at up to 200 $\mu\text{g/mL}$ [161]. None of the extracts inhibited β -esterase-dependent rifampicin metabolism in HLM or induced mRNA of CYP2B6 or CYP3A4 in HepG2 cells. In a detailed biopharmaceutical study of withanone, the compound was found to have IC_{50} values $>100 \mu\text{M}$ for CYP2C9/11 in rat and human liver microsomes and IC_{50} values between 28.5 and 80 μM for other CYP isoenzymes (the lowest being 28.5 μM for CYP3A4 in human liver microsomes) [162].

The clinical relevance of these findings will depend on the systemic concentrations of these compounds achieved during regular use of WS. Further studies are required to evaluate this important aspect of the use of WS fully.

6.4. Clinical Considerations

While WS is generally regarded as safe and appears to have anti-anxiety, anti-depressant, and sleep-promoting properties in animal and limited human trials, there was significant heterogeneity of the products used, the level of product standardization, and the doses selected (Table 1). Additionally, it is unknown what herb-herb and herb-drug interactions may exist for WS beyond those previously described for diazepam [78, 101], imipramine [117-119], and fluoxetine [83, 119], and more specifically for the products that have been used in the available human trials. Due to the significant variability in the products used and lack of interaction studies, we are currently unable to make definitive clinical recommendations for the use of WS, both as an independent intervention or as an adjunctive therapy to those already in use for stress-related neuropsychiatric conditions. Additional rigorous studies using standardized products and doses in populations diagnosed with anxiety, depression, and insomnia rather than healthy participants are needed to provide enough definitive evidence to safely integrate WS into clinical management of stress-related neuropsychiatric conditions.

CONCLUSION

Extracts made from Ashwagandha root and/or leaf have shown remarkable anti-stress and anti-anxiety activity in numerous animal models and clinical studies. Fewer studies have examined the effects of WS on depression and sleep, but as before, positive activity was observed here as well. The ability of WS to potentially ameliorate all of these conditions, which often occur as co-morbidities, is of significance. Where a polypharmacy approach may be used in allopathic medicine, WS may offer a single treatment to address each of these conditions simultaneously. WS appears to exert this pan-condition effect by modulating the HPA and SAM axes, as well as GABAergic and serotonergic pathways, all of which are interconnected. Critical gaps in knowledge remain, however. The optimum extraction method and dosage to address any or all of these disorders has yet to be determined, as is the severity of the conditions that are responsive to WS. The association of biological activity to chemical content is a priority area of research for Ashwagandha. While withanolide derivatives are widely stated to be the active compounds of WS, only a few of the many compounds of this group have been specifically tested for their neuropsychiatric effects. Testing of additional withanolides, examination of their additive or synergistic effects, and structure-activity relationships are all warranted. The fact that many of the standardized, active extracts contain 70-95% of uncharacterized materials highlights the possibility of additional, unidentified active compounds. The oligosaccharides found in the roots, for example, have been largely ignored and may modulate the activity of WS, perhaps at the level of the gut. There is also a dearth of studies examining the pharmacokinetic characteristics and biological distribution of WS compounds in animal models and humans. Finally, with the increasing use of WS by the general public, its potential for herb-drug interactions requires attention. Nevertheless, the studies conducted so far suggest that WS by it-

self has a good safety profile, adding to the potential of this traditional herb to provide resilience against stress and stress-related neuropsychiatric ailments.

LIST OF ABBREVIATIONS

ACh	=	Acetylcholine	GAD	=	General Anxiety Disorder
AChE	=	Acetylcholinesterase	GFAP	=	Glial fibrillary acidic protein
ADP	=	Adenosine diphosphate	GHQ	=	General Health Questionnaire
AEC	=	Adenylate energy charge	GPx	=	Glutathione peroxidase
ALP	=	Alkaline phosphatase	GSH	=	Reduced glutathione
ALT/GPT	=	Alanine aminotransaminase	GSSG	=	Oxidized glutathione
AP-1	=	Activator protein 1	HAM-A/HARS	=	Hamilton Anxiety Rating Scale
ART	=	Anti-reserpine test	HDL	=	High density lipoprotein
ASA	=	Aspirin administration	HDL-C	=	High density lipoprotein cholesterol
AST/GOT	=	Aspartate aminotransferase	HPA	=	Hypothalamic-pituitary-adrenal
ATP	=	Adenosine triphosphate	HPT	=	Hot plate test
BAD	=	Bcl-2 associated agonist of cell death	HSP70	=	Heat shock protein 70
BAI	=	Beck Anxiety Inventory	HST	=	Hypoxia stress test
Bax	=	Bcl-2-associated X protein	Iba1	=	Ionized calcium binding adaptor molecule 1
Bcl-xL	=	B-cell lymphoma extra-large	IFN- γ	=	Interferon gamma
BDI	=	Beck Depression Inventory	IL-1 β	=	Interleukin 1 beta
BDNF	=	Brain-derived neurotrophic factor	IL-2	=	Interleukin 2
BD-NOS	=	Bipolar disorder, not otherwise specified	IL-6	=	Interleukin 6
BMI	=	Body Mass Index	iNOS	=	Inducible nitric oxide synthase
CGI	=	Clinical Global Impressions scale	JNK	=	c-Jun N-terminal kinase
ChAT	=	Choline acetyl transferase	LDL	=	Low density lipoprotein
CHR	=	Cold-Hypoxia-Restraint test	LDL-C	=	Low density lipoprotein cholesterol
CRP	=	C-reactive protein	LDPT	=	Light/dark preference test
CRS	=	Cold restraint stress test	LHT	=	Learned helplessness test
CST	=	Cold stress test	LPO	=	Lipid peroxidation
COX-2	=	Cyclooxygenase 2	LPS	=	Lipopolysaccharide
DASS	=	Depression Anxiety Stress Scale	MADRS	=	Montgomery-Asberg Depression Rating Scale
DASS-21	=	Depression Anxiety Stress Scale-21	MARS	=	Mental Alertness on Rising
DHEA-S	=	Dehydroepiandrosterone sulfate	MBT	=	Marble burying test
EPM	=	Elevated plus maze test	MCP-1	=	Monocyte chemoattractant protein
FBG	=	Fasting Blood Glucose	mHAM-A	=	modified Hamilton Anxiety Rating Scale
FCQ-T	=	Food Cravings Questionnaire – Trait	MIL	=	Milk-induced leukocytosis
FSS	=	Foot shock stress test	MINI	=	Mini-International Neuropsychiatric Interview
FST	=	Forced swim test	MWM	=	Morris Water Maze test
FQ=	=	Fatigue Questionnaire	MYMOP	=	Measure Yourself Medical Outcomes Profile
			NBW	=	Narrow beam walking test

NCAM	=	Neural cell adhesion molecule	SOD	=	superoxide dismutase
NDS	=	Neurological deficit score	SOL	=	Sleep Onset Latency
NFκB	=	Nuclear factor kappa B	SPS	=	Single prolonged stress
nNOS	=	Nitric oxide synthase	SSRI	=	Selective serotonin reuptake inhibitor
NO	=	Nitric oxide	SST	=	Set Shifting Test
NOS	=	Not otherwise specified	STDT	=	Strategic Target Detection Test
NOX2	=	NADPH oxidase 2	TAN	=	Total adenine nucleotide
NR=	=	Not reported	TBARs	=	Thiobarbituric acid reactive substances
NREM	=	Non-rapid eye movement	TBT	=	Total Bed Time
nsd=	=	Non-significant decrease	TC	=	Total Cholesterol
NSFLT	=	Novelty-suppressed feeding latency test	TCT	=	Tactile stress test
NTDT	=	Novel tank diving test	TG	=	Triglycerides
OFT	=	Open field test	TIB	=	Total Time in Bed
OHQ	=	Oxford Happiness Questionnaire	TFEQ	=	Three-Factor Eating Questionnaire
OST	=	Overcrowding stress test	TST	=	Total Sleep Time
PANSS	=	Positive and Negative Syndrome Scale	TNFα	=	Tumor necrosis factor alpha
PAT	=	Passive avoidance test	WASO	=	Wake After Sleep Onset
PGAET	=	Physician's Global Assessment of Efficacy to Therapy	WBC	=	White blood cell
PGATT	=	Patient's Global Assessment of Tolerability to Treatment	WHOQOL-Bref	=	WHO Quality of Life-BREF
PPARγ	=	Peroxisome proliferator-activated receptor gamma	YMRS	=	Young Mania Rating Scale
PSS	=	Perceived Stress Scale	QoL	=	Quality of Life
PSQI	=	Pittsburgh Sleep Quality Index			
PTZ	=	Pentylentetrazol administration			
RAWM	=	Radial arm water maze			
RBC	=	Red blood cell			
ROS	=	Reactive oxygen species			
RST	=	Restraint stress test			
RSQ-W	=	Restorative Sleep Questionnaire-Weekly			
RTT	=	Rotarod test			
ROS	=	Reactive oxygen species			
S100B	=	S100 Calcium-binding protein B			
SAM	=	Sympathetic-adrenal-medullary			
SE	=	Sleep efficiency			
SF-36	=	Short Form-36			
SIT	=	Social interaction test			

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CONFLICT OF INTEREST

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