Ashwagandha

A

Dissertation

On

(Ashwagandha)

For

Title Registration to the Degree of

BACHELOR OF PHARMACY

under the

School of Pharmacy

Submitted by

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Under the Supervision of

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DECLARATION

I, Ayush Tiwari Enrollment number 2021BPBP001 Student of B Pharmacy (Session 2024-25) hereby declare that the dissertation entitled "Ashwagandha" is my own compilation. I have strictly adhered to the guidelines provided by the department for the preparation of project report.

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CERTIFICATE

It is certified that outlines of the project which is prepared by Mr.Ayush Tiwari have been verified and submitted to School of Pharmacy, Sangam University, Bhilwara

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Date: **Signature & Seal of the Dean**

Ashwagandha

(Withania somnifera)

Safety Dossier

Contents

Scope of work

The Terms of References (TOR) for the committee to prepare a technical dossier

on the safety study of Ashwagandha are included below:

Description of Ashwagandha in authoritative literature for Ayurveda, Siddha, and Unani drugs as mentioned in the First Schedule of Drugs and Cosmetics Act, 1940.

- Description of Ashwagandha plant, part used, and its important formulations along with their indications as per authoritative literature for ASU drugs / Ayurvedic Pharmacopoeia of India / Ayurvedic Formulary of India.

- Description regarding Safety and pharmacologic properties of Ashwagandha with special reference to its root.

- Relevant references and outcomes of research studies conducted on Ashwagandha with special reference to its root.

- International regulatory provisions in respect of Ashwagandha.

- Any other information related to Ashwagandha which may be useful for its propagation/ creating awareness among the general public and for its international regulatory approvals from authorities like European Union and US-Food and Drug Administration (FDA) etc.

Committee

[Committee that prepared the technical dossier on Ashwagandha]

(F.No. T-1102/4/2022-DCC dated 24.08.2022, Ministry of Ayush, Govt. of India)

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Prologue

Ashwagandha (Withania somnifera), also known as “winter cherry”and “Indian ginseng”, is one of the most celebrated and widely used herbs, renowned globally for its health benefits. It was very popularly used during the COVID-19 pandemic as a herbal supplement, particularly for its immune-boosting and adaptogenic properties. It has been used extensively for its preventive, promotive, and therapeutic outcomes. The trust reposed in the beneficial properties of this herb is based on knowledge acquire dover thousands of years, through empirical uses and observational field trials. In recent years, the scientific community has further scrutinized this knowledge and validated the same by employing tools and methods of modern research.

The main objective of this technical dossier is to bring out the current evidence-based knowledge and understanding about this herb to the attention of academicians, researchers, practitioners of conventional and traditional medicine as well as the public at large.

The publication of this technical dossier was partly necessitated by the fact that the regulatory bodies of certain European countries have suspected some detrimental effects of Ashwagandha on human health, following its consumption. Herbal and natural products are often under the lens by practitioners of modern medicine. This is sometimes owing to well-founded principles of scientific inquiry but more often than not, borne out of human nature to be suspicious of the unknown.

In the case of herbal preparations, the biggest concern has been the standardization of preparation, batch and quality parameters, soil quality, environmental and geographical variations, etc. The use of fertilizers, insecticides, and pesticides have impact on the quality of cultivars and products thereof. All these affect the physical and phytochemical properties of the plant and product so derived. The process of preparation, storage, and shelf life are other areas of concern. Heavy metal and chemical contamination of preparation during the processing and packaging may add extraneous chemicals which may potentially harm human health, if good manufacturing practices are not followed. During the growing, harvesting

and storage of the herb, many infections and infestations may occur. Fungal, bacterial, viral and pest presence may take place which all may have an effect on the human organ system.

A recent and crucial fact of practice needs to be highlighted here. The use of herbal preparations found widespread use during the COVID-19 pandemic, including use of Ashwagandha as part of multi-pathy, multi-herbal management practices guided by physicians, or as a means of self-medication. During this period, many new and undesirable outcomes were encountered, which were alleged to be labeled due to the herbal supplement being used, as a matter of convenience, without undertaking a rigorous analysis of various possible causative factors from among the possible reasons, including infection itself, concurrently used modern medicines and other natural products, concomitantly used food items and supplements, and co-existing ailments or co-morbidities.

Another aspect, which has an important bearing on health outcomes,pertains to the part of the plant to be used. In the case of Ashwagandha, it is the root thatis the classically recommended herbal part for human use. It has been observed that many other parts of the plant, including the leaf, have been used with not-so-desirable outcomes. The current dossier focuses on root preparations only.

In this dossier, we have presented a comprehensive account of Ashwagandha with reference to molecular issues, preclinical and clinical studies using the root of this herb. Combinations using multi-herbal preparations as well as multi-part preparations of Ashwagandha containing components of the plant along with root were excluded. Many poly-herbal preparations, containing Ashwagandha as an ingredient,are in vogue. We have sparingly referred to publications based on such combinations, as they present the difficulty of discerning the efficacy and safety of individual herbs. Drug-drug interaction is a specialized area of enquiry, which requires full and separate scrutiny.

To prepare this dossier we have relied mostly on the original research published in indexed journals. We do not claim this dossier to be all inclusive, final or perfect document on this topic. Neither do we claim that all available evidence on the safety and efficacy of Ashwagandha, in all the languages of the world, are incorporated in this dossier. However, we have tried to include the maximum possible reliable and credible scientific information in an effort to make this document a comprehensive account on the topic. Our intention was not to make this dossier exhaustive or all-

inclusive. On the contrary, an attempt has been made to keep it sufficiently concise so as to enable easy readability.

As I conclude this prologue, I remind the readers that one must not overlook the utility of this herb, by millions, through millennia, following the original research of Rishis and Maharishis of Bharat. Historical accounts bear witness to the continuous use of this health supplement by the masses as recommended by conventional and traditional practitioners of the ancient wisdom of India. Controversies in medicine are not new but denying the benefits of this celebrated herb to many, based on insufficient proof or mere suspicion may not be in the best interest of human health. Many issues of safety and efficacy may remain unquenched, which shall be the responsibility of the present and future generations of researchers and academicians to answer. Answers, which will be found within the rigors of modern medical research and scientific enquiry. I hope this dossier is able to ignite that flame of inquiry.

All the committee members and their teams have contributed selflessly and diligently in the preparation of this document. I sincerely thank all the members, respective team members and other contributors for their most sincere hard work in this endeavor. There is no conflict of interest for any member of the committee.

I sincerely hope that this technical dossier on Ashwagandha will adequately serve the purpose for which it is being created.

I wish you all a good read.

Background

Ashwagandha is one of the most frequently used plants in Indian traditional systems of medicine including Ayurveda, Siddha and Unani with a variety of medicinal effects attributed to its multi-dimensional uses. The plant is botanically identified as Withania somnifera (L.) Dunal(Family-Solanaceae) which is a small perennial shrub with white flowers and orange-red berry commonly found in warmer regions of India. These days the herb is completely domesticated and is cultivated extensively in central and western India, viz. Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Punjab, Haryana and Rajasthan etc. The plant is also known as “Indian winter cherry” and “Indian ginseng”. The roots of the plant are, mainly used in Ayurved, Unani and Siddha (ASU) system for its various health purposes.

Several bio-actives, including withanolide glycosides (also known as sitoindosides) and withanolide aglycones, are considered responsible for the medicinal properties of Ashwagandha. More than 12 alkaloids, 40 withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27) have been isolated and reported from aerial parts, roots, and berries of Withania species. The major chemical constituents of these plants, withanolides, are mainly localized in leaves.1,2,3 Withaferin A (4β,27-dihydroxy-1-oxo-5β,6β-epoxywitha-2-24-dienolide) is another important steroidal lactone, isolated from the plant, which is largely valued for its anti-cancerous properties.4A quantitative analysis of Indian chemotypes of W. somnifera by thin layer chromatography (TLC) densitometryobserved that withaferin A is totally absent in roots, stems, seeds, and persistent calyx of fruits of intact plants but present in leaves (1.6%).5 Ashwagandha leaves are used traditionally for the management of various ailments. In Punjab, the paste of leaves is applied on the affected parts to relieve pain. In Rajasthan, the boiled leaves are applied to locally relieve pain; the paste of leaves is applied on boils. Bhil and Bhilala tribes of Madhya Pradesh use whole plant as a tonic; the leaves are used in asthma and dysentery in Dharmabad taluka of Nanded district, Maharashtra. In Eastern Rajasthan, mature leaves smeared with butter or oil are warmed and tied locally on boils, pimples and around the neck to relieve tonsillar enlargement.6

Ashwagandha- Classical contrive & Quality profiles

Introduction:

Ashwagandha [Withania somnifera (L.) Dunal; Family: Solanaceae], is one of the most important medicinal plants known for its 'rasāyana'property in Ayurveda- a traditional Indian medicine.The use of Ashwagandha in Ayurveda dates back to1000-1500 BC. Several texts and treatises on Ayush Medical literature especially Ayurveda cite a wide range of its health benefits. The review of Ayurvedic texts revealed that the root of Ashwagandha used as rejuvenating, strengthening tonic, weight promoting, aphrodisiac and to alleviate bleeding disorders, chest injury, consumption, cough, diseases of the ear, diseases of the eye, disorders of the abdomen, disorders of the skin, disorders of vāta, dyspnoea, dysuria, fracture, gout, hemorrhoids, vitiligo, lump in the abdomen, oligospermia, phthisis, poisonous disorders, pox, psychosis, scrotal swelling, sleeplessness, urticaria, wound etc.,

Apart from Ayurveda, Ashwagandha has been used in other Ayush Systems viz., Siddha, Unaniand Sowa Rigpa Medical Systems and even in Homoeopathy.

In Siddha, W. somnifera(Amukkarā) isclaimed to be anti-inflammatory, diuretic, rejuvenator, hypnotic, tonic, restorative, and to improve body strength, vitality and memory.It is also useful in pain, fever, eczema, tuberculosis, seizures, swelling, oligospermia etc.

In Unani Medical literature, the actions attributed toW. somnifera (Asgand) are anti-inflammatory, tonic, stomachic, bulk promoting, nervine tonic, and hypnotic; and it is used in the treatment of leucorrhoea, spermatorrhoea, sexual debility, rheumatism etc.

In Homoeopathy, it is used in debility, fatigue, spermatorrhoea etc., and in Sowa Rigpa

(Ba-dzi-gandha), it is used to pacify cold in the lower part of the body and diseases caused by the affliction of lymph.

Ashwagandhais administered either singly or in combination in therapeutics. It is commonly found as the main ingredient or co-ingredient in different dosage forms viz., juice (svarasa), powder (cūrṇa); decoction

(kaṣāyaor kvātha); electuary (avaleha/ lehya); medicated ghee (ghṛta); medicated oil (taila); medicated milk (kṣīrapāka); pills (guṭikā, vaṭi); alkali (kṣāra); distillate (arka); self-generating alcoholic preparation obtained through the fermentation process (āsava and ariṣṭa); paste (kalka) for internal use; paste (lepa) for external application; eye salve (añjana), herbo-mineral preparations (rasayoga) etc., and various dosage forms are commonly employed through oral, topical, nasal and ocular routes and also in the form of medicated enema (vasti) and massage etc.

A wide range of pharmacological activities, both in in-vivo and in-vitro models have been studied for the plant material, extracts, and isolated components of W. somniferawhich showed anti-oxidant, anti-cancer, anti-arthritic, anti-osteoporotic, anti-protozoal, adaptogenic, hepato-protective, nephron-protective, anti- diabetic, anti-cataract, anti-tussive, immune-modulatory, useful in the disorders of the nervous system and reproductive system, endocrinal disorders, systemic lupus erythematous (SLE) and portal hypertension. Several well-designed clinical studies on rheumatoid arthritis, osteoarthritis, obesity, stress, anxiety, sleep disorders (insomnia), for improving memory and cognitive function, muscle strength and recovery, menopausal disorders, COVID-19 etc. have demonstrated the safety and efficacy of W. somnifera.

Ashwagandha botanical Name

Withania somnifera (L.) Dunal

Family

Solanaceae

Habitat and Distribution

An under shrub found growing as a weed and cultivated in plains mainly in Madhya Pradesh and neighbouring districts of Rajasthan and throughout the arid, warmer zones of, as well as sub-Himalayan tracts ascending up to 1000m altitude.7

Official Part

Root8

Synonyms9 Alicabonsomniferum (L.) Raf. Hypnoticumsomniferum Boiss. Physalis flexuosa L.

Physalis flexuosa Wall.

Physalis scariosa Webb &Berthel.

Physalis somnifera L. Physalis tomentosa Thunb.

Physaloidessomnifera (L.) Moench

Withania chevalieri A.E.Gonç. Withaniakansuensis K.Z. Kuang & A.M. Lu Withaniamacrocalyx (Chiov.) Chiov.

Withaniamicrophysalis Suess. Withaniamucronata Chiov.

Withaniaobtusifolia Täckh. WithaniasiculaLojac.

Withania somnifera subsp. obtusifolia (Täckh.) Abedin, M.A. Al-Yahya, Chaudhary & J.S. Mossa.

Names of Ashwagandha in AYUSH Medical Systems

Ayurveda - Ashwagandha

Unani - Asgan

Siddha - Amukkarā Homoeopathy - Withania somnifera Sowa-Rigpa - Ba-dzi-gandha

ASHWA- GANDHA

अश्वगधा

اسگند

催眠睡茄

A SH WAG ANDH A S AFETY DOSSIER

Other Names 8,10,11,12

Arabic: Bahman, ubad, Kaknajehendi

Bengali: Ashvagandha, dhuppa

Chinese: Cui mian shui qie (催眠睡茄), nan feizuiqie (南非醉茄)

Danish: Withania, blærebæge

English: Indian ginseng, Winter cherry

French: Ashwagandha, cerise d'hiver, coqueretsomnifère, ginseng indien

German: Ashwagandha, Indischer Ginseng, Schlafbeere, Winterkirsche

Gujarati: Aasundha, Asana, Ghodakun

Hindi: Asgandh, Asagand, Asagandha

Italian: Ashwagandha, ciliegia d'inverno, ginseng indiano Kannada: Angarberu, Hiremaddina gadde, Hiremaddina gida Malayalam: Amukkuram, Ammukaram

Marathi: Asgund, Asvagandhi, Asagandha, Asandha

Nepalese: Aasoganda

Norwegian: Withania, indisk ginseng

Odiya: Asugandha

Persian: Meheman

Punjabi: Asgand

Pustu: Kutilad

Sanskrit: Ashvagandha, Hayagandha, Vajigandha, Turagagandha

Sinhalese: Amukkara

Spanish: Ashwagandha, cerezo de invierno, ginseng indiano, oroval

Swedish: Withania, indisk ginseng

Tamil: Amukira, Amukkara, Ammukkarakizangu

Telugu: Pennerugadda

Tibetan: Ba-dzi-gandha

Urdu: AsagandhaNagaori

A Chronological Review on Medico- Historical aspect

Ayurveda, is a comprehensive system of medicine, and its origin can be traced from the knowledge in Ṛgveda and Atharvaveda. Apart from Vedic literature, other ancient Sanskrit literature like Purāṇa, Upaniṣad, Rāmāyaṇa, Māhābhārata etc., discuss medicine-related information. Several authors have made an attempt to record health-related information and enumerated medicinal substances discussed in ancient Sanskrit literature.Indian Ancient Sanskrit Non-medical literature .

The direct reference of Ashwagandha is not found in Vedic literature. Acharya Sharma P.V., (1984) opined that the plant was mentioned by the name Aśvāvatī13in Ṛgvedaand Ashwagandha14 in Aśvalayānagṛhasūtra which probably denote present day Ashwagandha (W.somnifera).

Ayurvedic Medical Literature

The core strength of Ayurvseda lies in the systematically documented literature dating years on fundamental concepts, exhaustivemateria medica,diagnosis, and diagnostic methods, treatment through holistic approach etc. The major works, such as Carakasaṁhitā, Suśrutasaṁhitā (~400 BC–200 AD) and Aṣṭāṅgahṛdaya (500 AD) provided descriptions of over 700 herbs and 6,000 formulations.15Followed by this, several works during the medieval and modern period on therapeutics and materia medica enriched Ayurveda formulary by addition of new formulations and new drugs. An overview on Ashwagandha as mentioned in the texts and works

on Ayurveda,which were composed during the ancient, medieval and modern period provide a clear idea on Ashwagandha in Ayurveda practice during different time periods. In Carakasaṁhitā (~1500 BC), Ashwagandha is referred in the group of ten drugs (mahākaṣāya)for promoting weighta andstrength.b This drug used as single or in combination is indicated in itching, diseases of skin, swelling;c oligospermia;d for massage in consumption;efor internal use in disorders of abdomen;ffor fumigation in haemorrhoids;g ash of Ashwagandha mixed with honey and ghee for internal use in dyspnoea;h for medicated smoking in cough;I for external application in erysipelas;j as anti-toxic;k stiffness in thights;l disorders of vāta; mgoutn and as galactodepuranto etc. In Suśrutasaṁhitā (~2000 BC) there are several citations for Ashwagandha. Some of the therapeutic uses referred in the text are: Ashwagandha for internal and external use in emaciation and to provide nourishment to bodyp; Ashwagandha in combination with other drugs in the form of fomentation in earacheq; medicated enema in bleeding disorders and bloody diarrhoearetc.,and for external application in goutsetc.16

Nāvanitaka- the Bower Manuscript (400 AD), mentioned the administration of Ashwagandha in form of medicated enema ‘Ashwagandha vasti’ to promote strength, complexion and muscle tissuetetc.

Vāgbhaṭa, the author of Aṣṭāṅgahṛdaya (500- 600 AD),emphasizedintake of Ashwagandha with milk, ghee, oil or warm water for six months to nourish the emaciated body as rain do for the younger plantsu; mentionedit is one among drugs to be used to promote intellect, longevity of life, stability and strengthv; and best drug to promote healing of wounds.w The drug has been referred either single or in combination form in the treatment of coughx; dyspnoea;yphthisisz; for fumigation in haemorrhoidsaa; disorders of skinab; scrotal enlargementac; abdominal lumpad; feverae; to promote strength and nourishment in childrenaf; wound healingag; as aphrodisiacah etc.

Synonyms of Ashwagandha

Ayurveda adopts a polynomial system of nomenclature i.e., a single medicinal plant is denoted by different names, which provide comprehensive information on its form, habitat, action etc.

The word ‘Ashwagandha’ is derived from Sanskrit- ‘aśva’ meaning horse and ‘gandha’ meaning odour; describing‘ the smell (of root) is similar to the smell of a horse.’ It is commonly referred by the names with gandhā prefixed with Sanskrit names of a horse13 for example as vājigandhā, hayagandhā, turagagandhā, turaṅgagandhā, gandharvagandhā etc.

There are about 70 synonyms of Ashwagandha cited in different texts of Ayurveda (Table-2).

These synonyms help to enhance the perspective in understanding the drug in all aspects i.e., action, characteristics, habit, conventional meaning etc. Some of such synonyms are given here under:

Synonyms Describing the Action

Baladā, balyā (promotes strength), mārutaghnī (alleviatesvāta), puṣṭidā (provides nourishment/ bulk promoting), putradā (bestows progeny), vājīkarī (aphrodisiac), varadā (fulfill the requirements, means provide desired effects), vātaghnī (alleviates vāta), vraṇojjhita (heals wound) and vṛṣā (aphrodisiac).

SynonymsDescribing Characteristic/ Strong Smell of Root

Ashwagandha, aśvagandhakā, aśvagandhikā, aśvakanda, gandharvagandhā, hayagandhā, saptigandhā, turagagandhā, vājigandhā, vājīgandhā (smell of root resembling smell of horse); bahugandhā, gandhā, gandhātā, kuṣṭhagandhā, kuṣṭhagandhinī, ṛkṣagandhā, śiṣṭagandhā, gandhapatrī (having characteristic/ strong odour).

Synonyms Describing Leaves

Gokarṇa, gokarṇī (leaves resembling ear of a cow), palāśaparṇī (leaves resembling leaves of Palāśa-Butea monosperma); varāhakarṇī, vārāhakarṇī, varāhapatrī (leaves resembling ear of a boar).

Synonyms Describing Colour

Pītā (yellow in colour), śyāmālā (dark in colour).

Properties and Therapeutic uses described in other Ayush systems of Medicine

Siddha92

- Āṇmaiperukki (virility enhancer), Ciṟunīrperukki (diuretic), Kāyakaṟpamākki(~rejuvenator), Uṟakkamuṇṭākki (hypnotic), Uramākki (tonic), Uṭalveppakaṟṟi (febrifuge), Uṭaṟteṟṟi (~restorative), Vīrkkamurukki (~swelling resolver).

- Cūlai (~lancinating pain), Curam (~fever), Karappān (~eczema), Kayam (~tuberculosis), Toṭam (~disordered humour), Uṭalvaṇmaikkuṟaivu (~loss of body strength), Vaḷi noy (~convulsions), Veḷuppu noy (~anaemia), Vīkam (~swelling), Vintukkuṟaivu (~oligospermia).

Unani93

- Mohallil-e-waram (~anti-inflammatory), Muqawwi-e-aam (~general tonic), Muqawwi-e-meda (stomachic), Muwalli- d-e-mani (haematogenic), Musammin-e- badan (~adipogenous), Musakkin-e-Asab (~nerve sedative), Munawwim (~soporific).

- Sailan-ur-rahem (~leucorrhoea),

Jiryan (~spermatorrhoea),

Riqqat-e-mani (attenuated semen),

Waj-ul-qutn (~lumbago), Waj-ul-mafasil

(~rheumatism), Zof-e-bah

(~sexual debility).

Homoeopathy94

Debility, nervousness, brain fatigue, loss of memory, spermatorrhoea, speedy emission, nocturnal emission, backache, weak sight, flushes of heat, infertility, impotency, dull headache, cough, asthma, bronchitis, palpitation, nausea, vomiting, burning and frequent urge for urination, vertigo.

Sowa Rigpa95

Cold in the lower part of the body and diseases caused by the affliction of lymph.

Dose

Ayurveda

3-6 g drugin powder formalong with specified adjuvants.7

Siddha

3-6 g drug in powder form along with specified adjuvants.92

Unani

5-10 g drug in powder form along with specified adjuvants.93

Homoeopathy

Mother Tincture Ø Drug strength 1/10.96

Some of the Therapeutic Applications as mentioned in classical literature

1. As Rasayana (~rejuvenating)

Ashwagandhataken with milk, ghee,oil or warm water for a fortnight promotes nourishment of body (Aṣṭāṅgahṛdaya Uttarasthāna 39.157;39 Cakradatta 66.15) 97. Ashwagandhashould be used to promote intellect, life span, stability and strength40. One who takes powder of Ashwagandha root in late winter mixed with honey and ghee along with milk regains youthfulness even if old (Rājamārtāṇḍa33.11).98

2. Śoṣa (~consumption) The powder of Ashwagandha, sesame and black gram taken with goat's ghee and honey.34 Candied sugar, Ashwagandha and Pippalīmixed with ghee and honey.34,99 Milk cooked with Ashwagandha provides nourishment to the body or ghee obtained from that

milk should be taken after adding with sugar and followed by intake of milk (Suśrutasaṁhitā Uttaratantra 41.42).34

Ashwagandha, barley and punarnavā should be used for rubbing externally (Suśrutasaṁhitā Uttaratantra 41.42).34

Ghee extracted of the milk boiled with Ashwagandhais cooked with candied sugar, milk and meat along with the paste of jivaniya group of drugs. It is useful in consumption and allied disorders (Aṣṭāṅgahṛdaya Cikitsāsthāna Ci.5.25).45

3.Kārśya (~excessive emaciation) Ashwagandhashould be used in case of excessive emaciation (Suśrutasaṁhitā Sūtrasthāna.15.33).101

4.Śvāsā(~dysponea) The ash (alkali) of Ashwagandhataken with honey and ghee in dyspnoea (Carakasaṁhitā Cikitsāsthāna17.11722; Aṣṭāṅgahṛdaya Cikitsā- sthāna4.39).44

5.Granthi visarpa (one among seven types of ~erysipelas caused by kapha and vāta) Warm paste of Ashwagandha applied externally on the affected part (Carakasaṁhitā Cikitsāsthāna 21.123).24

6.Ūrustambha (~stiffness in thighs) The root of Ashwagandha mixed with honey, mustard and ant-hill earth anointed thickly and applied as paste in ūrustambha (Carakasaṁhitā Cikitsāsthāna 27.50-51).29

7.Nidrānāśa (~Insomnia) The powder of Ashwagandhamixed with sugar and taken with ghee alleviates insomnia and brings sleep quickly (VaṅgasenasaṁhitāJala- doṣādiyogādhikāra13).102

8.Accidental Wound One afflicted with accidental wound should lick the powder of Ashwagandhawith jaggery or ghee, or should take with milk. It also acts as rasāyana (Vaidyamanoramā 4.2).103

9. Mūtravibandha (~suppression of urine) Decoction of Ashwagandha alleviates suppression of urine and promotes urination (Siddhabheṣaja Maṇimālā 4 Mūtrāghāt- acikitsā 1).104

10.Vandhyā (for conception in sterility) Milk processed with Ashwagandhais added with milk and to be taken by the woman at proper time. It helps in conception (Vṛṁdamādhava64.10;105 BhāvaprakāśaCi.70.26).106

Arka(distillate)of Ashwagandhaprocessed with milk and taken with ghee helps in conception (Arkaprakāśa7.75).107

11.Bālaśoṣa(~emaciation in children) Ghee is cooked with one-fourth paste of Ashwagandhaand ten times milk is beneficial in development of body and is useful in emaciated children (Vṛṁdamādhava 67.10).108

12.Udararoga(~disorders of abdomen) Ashwagandha pounded with cow's urine and taken alleviates advanced udararoga, worms and oedema (Cakradatta 37.48).109

13.Sarpaviṣa(Snakebite) The root of Ashwagandhaground with milk and taken internally destroys snake bite effects. (Sahsrayogam 10.4).110

14.Vātaroga Ghee cooked with decoction and paste of Ashwagandhaalong with four times milk alleviates vāta, acts as aphrodisiac and promotes muscle tissue (Vṛṁdamādhava 22.115).111

15 Wholesome to Uterus Powder of Ashwagandhawith milk administered internally for seven days is wholesome to uterus (Ārogya Rakṣākalpadruma,Pg. 435).112

16. Masūrikā (Pox) Juice of Ashwagandhamixed with pāradaand applied overmasūrikā (Vaidyamano

Formulations in formularies of Ayurveda, Siddha and Unani

Ayurveda113, 114, 115

1. Ashwagandha dyariṣṭa [A.F.I., Part I, 1:6; p.8-9]
2. Mṛtasañjīvanīsurā [A.F.I., Part I, 1:28; p.18-19]
3. Sārasvatāriṣṭa [A.F.I., Part I, 1:36; p.22]
4. Ashwagandha di lehya[A.F.I., Part I, 3:2; p.33-34]
5. Guḍūcyādimodaka[A.F.I., Part I, 3:9; p.36-37]
6. Pūgakhaṇḍa[A.F.I., Part I, 3:17; p.41]
7. Madhusnuhīrasāyana [A.F.I., Part I, 3:19; p.42]
8. Rāsnādikvāthacūrṇa (mahā) [A.F.I., Part I, 4:28; p.60-61]
9. Trayodaśāṅgaguggulu [A.F.I., Part I, 5:4; p.68]
10. Lākṣāguggulu [A.F.I., Part I, 5:8; p.70]
11. Dadhikaghṛta [A.F.I., Part I, 6:20; p.87-88]
12. Phalaghṛta [A.F.I., Part I, 6:30; p.92]
13. Vastyāmayāntaka ghṛta [A.F.I., Part I, 6:40; p.97-98]
14. Sukumāraghṛta [A.F.I., Part I, 6:44; p.99-100]
15. Candanabalālākṣāditaila [A.F.I., Part I, 8:15; p.134]
16. Triphalāditaila [A.F.I., Part I, 8:21; p.136-137]
17. Dhanvantara taila [A.F.I., Part I, 8:22; p.137-138]
18. Nārāyaṇa taila [A.F.I., Part I, 8:23; p.138]
19. Prabhañjanavimardanataila [A.F.I., Part I, 8:30; p.140-141]
20. Pramehamihiritaila [A.F.I., Part 1, 8:31; p.141-142]
21. Balāguḍūcyāditaila [A.F.I., Part I, 8:34; p.143]
22. Balāśvagandhalākṣāditaila [A.F.I., Part I, 8:36; p.144-145]

Siddha116, 117

1. IracaKantiMezuku [S.F.I., Part I, 10.2; p. 68-69]
2. IṭivallāthiMezuku [S.F.I., Part I, 10.3; p. 69]
3. Nanti Mezuku [S.F.I., Part I, 10.8; p. 70-71]
4. Makā elāthi Kuḷikai [S.F.I., Part I, 13.21; p. 90-91]
5. Makā Vallāthi Iḷakam [S.F.I., Part I, 15.7; p. 120-121]
6. Nārathai Iḷakam [S.F.I., Part I, 15.8; p. 121]
7. Kanthaka Irācayaṉam [S.F.I., Part I, 16.2; p. 126]
8. Paṟaṅaki Paṭṭai Irācayaṉam [S.F.I., Part I, 16.3; p. 126-127]
9. Amkkura Cūranam [S.F.I., Part I, 21.1, p. 152]
10. CanthāṉaIḷakam [S.F.I., Part II, 6.6; p.72-74]
11. CowpākkiyacCuṇṭiIḷakam [S.F.I., Part II, 6.8; p. 76-79]
12. KarppaviyathikkuEṇṇai [S.F.I., Part II, 7.6; p. 107-108]

Purification

Water filled in an earthen vessel is covered with a cloth and Ashwagandha is kept on a cloth,and another earthen vessel or lid is placed on it. The edges are covered with a wet cloth to prevent steam escaping. The plate or the covering vessel with multiple holes is used to facilitate steaming. The drug is spread over a clean wet cotton cloth which is spread over this perforated vessel. The top of this perforated plate is covered with a suitable lid -just like baking idli or puttu. Boil the water over mild fire until three-fourth (25% remaining) of the liquid is reduced.121

Identification

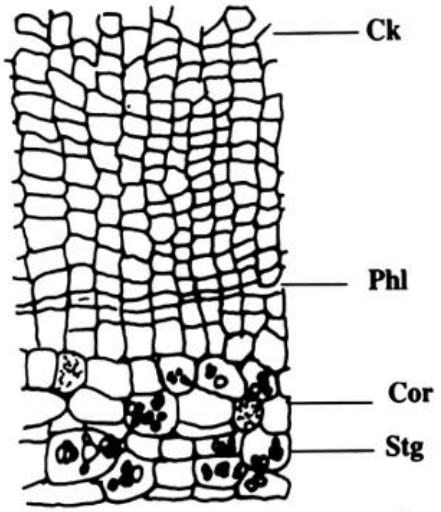
Plant Material of Interest: Root

Macroscopy:

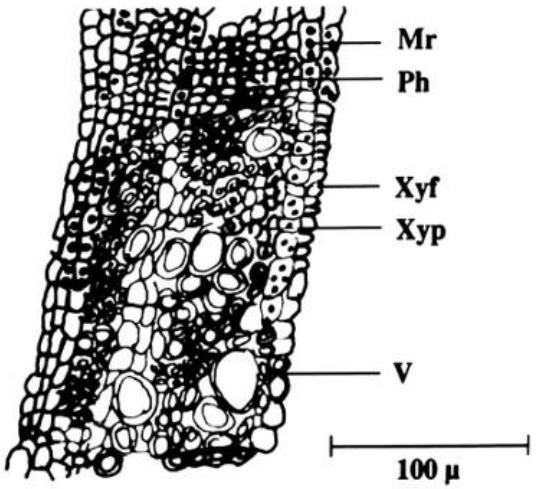
Roots straight, unbranched, thickness varying with age, roots bear fibre-like secondary roots, outer surface buff to grey-yellow with longitudinal wrinkles, crown consists of 2-6 remains of stem base, stem bases variously thickened, nodes prominent only on the side from where petiole arises, cylindrical, green with longitudinal wrinkles, fracture, short and uneven, odour- characteristic, taste- bitter and acrid (API, Part-1, Vol-1).

Microscopy:

Transverse section of root shows cork (Ck) exfoliated or crushed; when present isodiametric and non lignified; cork cambium of 2-4 diffused rows of cells; secondary cortex (Cor) about twenty layers of compact parenchymatous cells; phloem (Ph) consistof sieve tubes, companion cells, phloem parenchyma; cambium 4-5 rows of tangentially elongated cells; secondary xylem (Xy) hard, forming a closed vascular ring separated by multiseriate medullary (Mr) rays; and a few xylem parenchyma (Xyp).



T S root showing cork and cortex region



TS root showing phloem and xylem woody portion

Phyto-chemistry of Leaves:

Leaves of the plant extracted with methanol show the phyto-chemical presence of ashwagandhine, cuscohygrine, dl-isopelletierine, somniferine, tisopelletierine, 3α-tigloyloxtropine, 3-tropyltigloate, hygrine, hentriacontane, mesoanaferine, visamine, withanine, w it ha n a nine, w it ha somnine, a nd pseudowithanine144. Alcoholic extract of the plant leaves constitutes withanolide D, E145,146, withanolides F–M147,148, withanolides N, O149. Leaves of the plant extracted with ethanol also contain (5R, 6S, 7S, 8S, 9S, 10R, 13S, 14S, 17S,

20R, 22R)- 6, 7α- epoxy 5, 17-α, 27-trihydroxy-

1-oxo-22R-witha-2,24-dienolide.144

Phyto-chemistry of Whole Plant:150

The W. somnifera whole plant extract is rich in phyto-chemicals, such as alcoholic extract of the plant contains anaferine, anahygrine, choline, cuscohygrine, pseudotropine, dl-isopelletierine, and tropine. Besides, the methanolic extracts of the plant also contains starch, acylsteryl glucosides, iron, ducitol, hantreacotane, withaniol, and amino acids such as alanine, aspartic acid, cysteine, tyrosine, glutamic acid, glycine, proline, and tryptophan.151 The aqueous extract of the whole plant contains withanone and tubacapsenolide F150, while similar extracts with equimolar ratios of water and methanol constitute chlorinated withanolide and 6α-chloro-5β,17α- dihydroxywithaferin A, along with nine withanolides namely, 6α-chloro-5β- hydroxywithaferin A, (22R)- 5β-formyl- 6β,27- dihydroxy-1-oxo-4-norwith24-enolide, 2,3-dihydrowithaferin A, withanone, withanoside IV, withaferin A, 2,3-didehydrosomnifericin, 3-methoxy-2,3-dihydrowithaferin A, and withanoside X150. The ethanol extract of the whole plant containsisosominolide, sominone, withasomniferin A152.

Global Demand &

Developments and International Regulatory Provisions

Pharmacopeial and Regulatory Status:

Monograph of W.somniferaroot is available in the Ayurvedic Pharmacopeia of India (API, Part-I, Vol-I)153, Siddha Pharmacopeia of India154, Unani Pharmacopeia of India155. There are monographs published on the roots of Ashwagandha (Withania somnifera) in the World Health Organization (WHO) Monographs (Vol. 4, 2009)156, British Pharmacopoeia (BP 2012)157, Indian Pharmacopoeia (IP 2010)158, and United States Pharmacopeia (USP 36)159 also (Annexure 1a &1b).

As such, the monograph ofthe leaf is not available in any pharmacopeia160As per the information provided by the Industry representatives, Ashwagandha extract (including leaves) is offered as dietary supplement in the US, where non-weighted average daily exposure of Ashwagandha exceeds 1100 mg. These supplements are based on Ashwagandha extracts of roots and leaves and not roots alone.Country-wise regulatory status of Ashwagandha (Withania somnifera) is given as Annexure

Summary and Excerpts of Research Studies

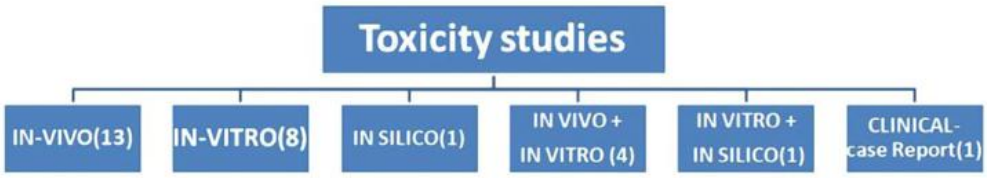
Summary of pre-clinical studies conducted for evaluation of toxicity of Ashwagandha

Using the keywords “Ashwagandha AND Toxicity”, a total of 208 studies were identified. The list of articles was then screened with keyword “toxicity” and the abstracts were screened to determine the actual articles that were dealing with toxicity study. It was found that only 39 studies were original articles related to actual toxicity study. Of the 39 studies, 2 were review articles, full text for one study was not available, two studies were conducted on different plant species namely, Withania frutescens and four studies were pertaining to environmental study and two studies were identified as in-silico studies. Of the 28 studies,one case study reported the toxic effect of the herb. The one study by Björnsson HK et al included case studies of five patients that suffered liver injury suspected by Ashwagandha consumption with similar patterns of liver injury and outcome. Of the five cases of DILI (Drug Induced Liver Injury), three were men and two women. The daily dose of Ashwagandha ranged from 450 to 1350mg in the study. However, the dose was recorded according to the label information and no direct dose measurement was performed(161). The rest of the 27 studies were observed closely and a summary of 9 studies is included (Fig.1).

Fig. 1: Summary of toxicity studies

It was observed that many of the toxicity- related studies were done on the in-vitro models particularly the cytotoxicity effect on different cell lines such as; CCRF-CEM leukemia cell line and MDA-MB-231-pcDNA3 breast cancer cells, HeLa (FR-α positive, FR+), and MCF7 (FR-α negative, FR-) cancer cells, ECV- 304 cell line (human bladder carcinoma cell line with endothelial properties) etc. The In- Vivo studies were conducted mostly on rats and one study was conducted on rotifers. Actual articles that studied the acute and sub- acute toxicity were six and they used animal models such as, wistar rats, sprague-dawley rats, Charles foster rats etc. Dose of Ashwagandha extract upto 2000mg/kg body weight/day have been studied in the acute toxicity studies.

Balkrishna A et al (2022), conducteda 28day subacute toxicity evaluation under Good Laboratory Practice (GLP)compliance in Sprague Dawley rats of either sex and were orally administered with Withania somnifera whole plant material [Methanol (1:1 v/v) as the solvent] (WSWPE) for 28-consecutive days at the doses of 100, 300 and 1000 mg/kg/day. The parameters that were assessed in the study included body weight, food consumption, ophthalmic examinations, clinical pathology observations, haematology, coagulation,



clinical chemistry, urinalysis and necropsy and macroscopic observation. In the present study, as compared to the vehicle-treated groups, WSWPE did not reveal any clinically significant alterations in the evaluated parameters. Accordingly, founded on the aforesaid premise, the NOAEL (No-Observed-Adverse-Effect- Level) for WSWPE was determined to be

1000 mg/kg/day in both male and female rats. The authors concluded that Withania somnifera Whole Plant Extract (WPE) was found to be safe up to the dose level of 1000 mg/kg/day as no toxicologically relevant findings could be detected.(162)

Patel SB et al (2016), conducted a study to investigate the potential adverse effects (if any) of a standardized Withania somnifera root extract (WSE), given orally in wistar rats following acute and sub-chronic administration. The acute toxicity study was done at the dose of 2000 mg/kg. In the sub- acute study, Wistar rats (10/sex/group) were administered via gavage 0 (control), 500, 1000, 2000 mg/kg body weight/day of WSE for 28 days. Among two additional satellite groups, one group did not receive any drug while the second group received 2000 mg/kg/day for 28 days. It was observed that in acute toxicity studies, oral LD50 of WSE in Wistar rats was greater than 2000 mg/kg body weight. Compared to the control group in sub-acute toxicity study, the administration of extract did not show any toxicologically significant treatment-related changes in clinical observations, ophthalmic examination, body weight gain, feed consumption, clinical pathology evaluation, and organ weight. The authors concluded that the no-observed-adverse-effect-level of WSE is 2000 mg/kg body weight, the highest level tested. (163)

Prabu PC et al (2015), evaluated the prenatal developmental toxicity of Withania somnifera root extract given orally to pregnant rats during the period of major organogenesis and histogenesis (days 5 to 19 of gestation) at the dose levels of 500, 1000 and 2000 mg/kg/day. The authors observed the clinical parameters including mortality, moribundity, behavioural changes, signs of overt toxicity, body weight, gross pathological changes of dams and foetal analyses including external malformations, skeletal and soft tissue malformations. The authors observed that there was no evidence of maternal or foetal toxicity. Withania somniferaroot extract caused no change in body weight of parental females, number of corpora lutea, implantations, viable foetuses, external, skeletal, and visceral malformations. It was concluded from the study that the no- observed-effect level (NOEL) of Withania somniferaroot extract for maternal and developmental toxicity was concluded to be at least 2000 mg/kg/day. (164)

Prabu PC et al (2013), conducted an acute and sub-acute oral toxicity assessment of a hydroalcoholic extract of Withania somnifera roots in wistar rats. For the acute toxicity study, Withania somnifera root extract was administered to five wistar rats at 2000 mg/kg, once orally, and were observed for 14 days. No toxic signs/mortality were observed in the experimental animals. In the sub-acute study, Withania somnifera root extract was administered once daily for 28 days to rats at 500, 1000, and 2000 mg/kg, orally. The authors observed that no toxic signs/mortality were observed in the animals. Also, there were no significant changes ($P < 0.05$) in the body weights, organ weights, and haemato- biochemical parameters in any of the dose levels. No treatment-related gross/ histopathological lesions were observed. The authors concluded that the no observed adverse effect level (NOAEL) was 2000 mg/kg body weight per day of hydroalcoholic extract of W. somnifera in rats and hence may be considered as non-toxic. (165)

Kalpana K et al (2020), conducted an in-vitro study for the toxicity/benefit analysis of lipopolysaccharides in plants for which two commercial supplements of Ashwagandha, namely, Daily Nutra™ KSM-66® Ashwagandha (AS1) and Organic India™ Ashwagandha (AS2) were used.In the present study, the authors established a simple cell-line based assay (THP-1 monocytic cells) that can be used to screen the toxicity and benefit of lipopolysaccharides (LPSs) in medicinal plants. The assay can distinguish endotoxic diphosphoryl lipid A (DPL) from beneficial monophosphoryl lipid A (MPL), which is a clinically used immunological adjuvant for vaccines.The authors concluded that the commercial Ashwagandha supplements are similarto MPL in terms of IL-6 and CCL5 induction. Although more comprehensive studies are needed to fully define the similarities and differences between the LPSs in Ashwagandha and MPL, howeverthe current finding opens a possibility that Ashwagandha and other immune-boosting plants may serve as an untapped source of new LPSs with therapeutic potential.

Anwar MF et al (2015), evaluated the acute toxicity of different shapes and sizes of silver nanoparticles (Ag NPs) and their modulations by using Withania somnifera and evaluated their effect on liver and kidneytissues of Wistar rats through biochemical and histopathological changes. The cytotoxicity in specific murine macrophages through confocal microscopy was also evaluated. Cytotoxicity analysis indicated that median lethal dose (LD50) for 20, 50, and 100-nm size spherical and 100-nm rod-shaped Ag NPs was 0.25, 0.35, 0.35, and

0.35 mg/ml, respectively. The authors observed that 20, 50, and 100-nm spherical Ag NPs (35 mg/kg, 23 days) increased the biochemically important enzymes and substrate levels glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT),

alkaline phosphatase (ALP), creatinine, and urea concentration in serum, showing liver and kidney tissue damage. After 23 days of treatment of Ag NPs (20, 50, and 100 nm spherical), along with W. somnifera, toxicity of Ag NPs significantly decreased and marginalized. The authors concluded that no significant changes were observed for 100-nm rod-shaped Ag NPs on normal liver and kidney architecture. Given their low toxic effects and high uptake efficiency, these have a promising potential as to lower the toxicity of Ag NPs. (167)

Singh B et al (2001), evaluated the antistress activity of an active fraction (BF) isolated from Withania somnifera. A withanolide-free aqueous fraction was isolated from the roots of the plant. The most active subfraction named as BF (186 g, yielding 20% of aqueous fraction, 1.43% of the starting material) was used for the study. The activity was assessed using a battery of tests (Swimming performance time, Swimming induced gastric ulceration¸ Immobilization induced gastric ulceration, Antifatigue effect, Immobilization induced auto-analgesia, Swimming induced hypothermia in rats, and Immobilization stress and biochemical parameters evaluation in adrenal glands) employing widely different stress situations in Charles Foster rats (3 months old, 100–150 g) and Swiss albino mice (3 months old, 24–30 g) of either sex, for 15 days period. The toxicity study conducted revealed that BF appears to possess a reasonable margin of safety. It does not produce any gross behavioral changes or mortality even at an oral dose of 3000 mg/kg in mice, thus it may be considered relatively safe. The authors concluded that oral administration of BF of W. somniferacan increase the capacity to tolerate non-specific stress in experimental animals as evident from the restoration of a large number of parameters studied during different types of stress and it does not interfere with the normal physiological functions of the body. There was no mortality or any gross behavioural changes for 72 h when BF was fed to mice upto 3000 mg/kg. There was no mortality of animals in any of the groups used in different models for determining antistress activity during the period of treatment. (168)

Pretorius E et al (2009), conducted a study on MRC-5 cells, a human embryonic lung- derived diploid fibroblast cell line, to compare the cytotoxicity of water and methanolic extracts of Withania somnifera. Five concentrations of extracts of both water and methanol extracts (using leaves, stem, and roots) were added to the cells in culture. The concentrations were: 0.007, 0.042, 0.250, 1.151, and 9.090 µg/ml. The cells were then exposed to these concentrations for 48 h. Cell number, cell viability, and lysosomal membrane integrity, were determined. The authors observed that the three lowest concentrations (0.007, 0.042, and 0.250 µg/ml) of the plant material extracted in water and methanol seem not to be significantly different in any of the assays. At these three concentrations, cell numbers, cell viability, and lysosomal membrane activity were preserved. The authors concluded that low concentrations of methanol extracts (up to 0.250 µg/ml plant material) do not cause cell damage to the human embryonic lung-derived diploid fibroblast cell line. However, higher levels should be avoided as particularly cell viability and cell numbers are negatively influenced. (169)

Devi PU et al (1996), conducted a study in Chinese hamster V79 cells by administering Withaferin A (WA) extracted and isolated from commercially available root powder and was added to the 24-h cultures at a dose of 2.1, 5.25, 10.5, 21, 31.5, 42 or 52.5 µM. Also, to study the radio-sensitizing effect of the drug, 24-h old cells were treated for 1 h with 2.1, 5.25 or 10.5 µM with Withaferin A and then exposed to 1-8 Gyγ radiation from a cobalt teletherapy source at a dose rate of 1 Gy/ min. From this study, the authors concluded that LD50 for survival was 16 µM. One-hour treatment with a nontoxic dose of 2 .1 µM before irradiation significantly enhanced cell killing, giving a sensitizer enhancement ratio (SER) of 1.5 for 37% survival and 1.4 for 10% survival. SER increased with drug dose, but at higher doses the increased lethality appears to be due to two effects - drug toxicity and radio-sensitization. The drug induced a G2/M block, with a maximum accumulation of cells in G2-M phase at 4 h after treatment with 10.5 µM withaferin A for 1 h. (170)

Summary of Studies Cconducted on

Withania somnifera for Assessment of Safety

Using the keywords “Ashwagandha”or “Withania somnifera” on Pubmed, articles were searched and a total of 1507 articles were available till 26-08-2022. Using the keywords “Ashwagandha AND Safety” a total of 88 articles were identified. The list of articles was then screened with the

keyword “safety” and the abstracts were screened to determine the actual articles that were dealing with safety studies. It was found that 19 studies were related to actual safety studies. Of these 19 studies, 16 were conducted on humans and the rest of 3 studies were on animal models.

Summary of clinical studies conducted for safety

A total of 16 clinical studies were identified of which 10 studies were conducted on diseased subjects and the rest 6 studies included healthy participants. The number of participants in these studies ranges from 13 subjects to 251 subjects. The duration of the studies ranges from 4 weeks to 18 months. The dose of Ashwagandha extract ranged from 600mg/day (KSM -66) to 1250mg/day. In one of the studies, Ashwagandha powder was used as an intervention with a daily dose of 10gm/day in two divided doses for 3 weeks. Few studies used polyherbal formulations such as Immu-25, Livwin, and Revivin. A detailedsummary of nine studies is given below.

Verma N. et al (2021), conducted a randomized, double-blind, placebo-controlled, and parallel-group study on 80 healthy individuals (40 male; 40 female) to evaluate the safety of Ashwagandha root extract. The participants were divided into 2 groups to receive either Ashwagandha 300 mg or a starch placebo of the same dosage, twice daily, orally for 8 weeks. The authors considered laboratory assessment of haematological parameters, serum biochemistry analysis along with hepatotoxicity evaluation, and thyroid function parameters as the primary outcome measures. The clinical adverse events and the vital parameters namely, body weight, pulse rate, body temperature, respiratory rate, Body Mass Index (BMI), systolic and diastolic blood pressure were assessed for secondary outcomes. The result of the study did not indicate any untoward effect in any of the treated volunteers. There was no statistically significant change or abnormality observed in the parameters considered including thyroid hormonal profile in both the groups. Also, no adverse events were reported by any of the participants during the study period. The authors concluded that the consumption of Ashwagandha root extract for eight weeks was found to be safe in both male and female volunteers.(171)

Gopukumar K et al (2021), in a randomized, double blind, placebo-controlled study, evaluated the effect of Ashwagandha root extract (sustained-release) capsule 300 mg (Prolanza™; one capsule daily) for 90 consecutive days, on cognitive function, stress level, sleep quality, overall well-being, and safety in stressed subjects [130 healthy cognitively sound adults (20–55 years of age, body mass index:18–29 kg/m2)]. A total of 125 subjects completed the study and were evaluated. The Cambridge Neuropsychological Test Automated Battery (CANTAB) reported significantly improved recall memory, and the total error rate in recalling patterns were significantly reduced at visit 4 in the Ashwagandha group as compared to the placebo group. At visit 4, lower PSS-10 score (p

< .0001), serum cortisol levels (p =0.0443), and

Pittsburgh Sleep Quality Index (PSQI) score (p

< .0001) but higher Oxford Happiness Questionnaire (OHQ) scores (p < .0001) were seen in the Ashwagandha group when compared to the placebo group, which suggests significantly lower stress level and significantly better psychological well-being and sleep quality in the former. No adverse events were reported during the study. The authors concluded that treatment with one Ashwagandha capsule (sustained release) once daily for 90 days improved memory and focus, psychological well-being, and sleep quality, reduced stress level, and was safe and well- tolerated.(172)

Langade D et al (2019), conducted adouble-blind, randomized, placebo-controlled study to determine the efficacy and safety of Ashwagandha root extract in 60 patients [test (n = 40) and placebo (n = 20)] with insomnia and anxiety. In the test group, one capsule containing high concentration, full spectrum Ashwagandha root extract (300mg) was administered twice daily with milk or water for 10 weeks. The parameters assessed were sleep actigraphy (Respironics Philips), for assessment of sleep onset latency (SOL), total sleep time (TST), sleep efficiency (SE) and wake after sleep onset (WASO). Other assessments were total time in bed (sleep log), mental alertness on rising, sleep quality, Pittsburgh Sleep Quality Index (PSQI), and Hamilton Anxiety Rating Scale (HAM-A). Two patients, one from each group, did not complete the study. The baseline parameters were similar in the two groups. The sleep onset latency was improved in both test and placebo groups at five and 10 weeks. However, the SOL was significantly shorter (p= 0.019) after 10 weeks with test group compared to placebo. Also, significant improvement in SE scores was observed with Ashwagandha which was for test at the baseline and increased after 10

weeks. Similarly, significant improvement in sleep quality was observed with test compared to placebo (p=0.002). Significant improvement was observed in all other sleep parameters, i.e., SOL, SE, PSQI and anxiety (HAM-A scores) with Ashwagandha root extract treatment for 10 weeks. The authors concluded that Ashwagandha root extract was well tolerated and it improved sleep quality and sleep onset latency in patients with insomnia at a dose of 300 mg extract twice daily.(173)

Chandrasekhar K et al (2012), in their prospective, randomized double-blind, placebo-controlledstudy demonstrated the safety and efficacy of a high concentration, full spectrum extract of W. somnifera root extract conducted on 64 adults with a history of chronic stress. Ashwagandha root extract in a dose of one capsule (300mg) was taken twice

a day for a period of 60 days. The subjects were assessed for measurement of serum cortisol, and assessing their scores on standard stress- assessment questionnaires. The authors found that subjects taking Ashwagandha root extract exhibited a significant reduction (P<0.0001) in scores on all the stress-assessment scales on Day 60, as compared to the placebo group. Also, the serum cortisol levels were substantially reduced (P=0.0006) in the Ashwagandha group, relative to the placebo group. The adverse effects were mild in nature and were comparable in both the intervention and the placebo arms and the difference was not statistically significant. There were six adverse effects reported in the Ashwagandha group and five in the placebo group. No serious adverse events were reported in the study. The authors concluded that a high-concentration, full- spectrum Ashwagandha root extract can be used safely as an adaptogen in adults who are under stress.(174)

Pires N et al (2020), conducted a phase I dose escalation study using the classical 3 + 3 design (C33D) in advanced stage, high-grade osteosarcoma. Dose escalation cohorts comprised of 72, 108, 144 and 216 mg of Withaferin A administered in two to four divided doses per day. A standardized root extract of W. somnifera containing 4.5% of WithaferinA w/w was used for this study (AshwaMAX 400, Pharmanza Herbal Pvt Ltd., Gujarat, India). Each 400 mg capsule of AshwaMAX contained 18 mg of Withaferin A. Three patients were enrolled in each cohort and the last patient was observed for at least 30 days for any dose-limiting toxicity before progressing to a higher cohort. Safety evaluation including clinical examination, detailed history of adverse events, liver function tests, renal

function tests and complete blood counts were performed at each visit. The formulation used for the study was generally well tolerated. Eleven adverse events of grade 1 or grade 2 severity were observed. No grade 3 or grade 4 adverse events were observed. Elevation of liver enzymes (5/11) and skin rash (2/11) were the most common adverse events. Other adverse effects included fatigue, fever, oedema, and diarrhoea (one each). None of the patients had detectable levels of Withaferin A in circulation. The authors concluded that the formulation was well tolerated and oral Withaferin-A in patients with advanced stage, high grade osteosarcoma has a good safety profile. However, Withaferin A appears to have low oral bioavailability.

Kumar G et al (2015), in a pilot prospective study conducted on 86 patients of rheumatoid arthritis,evaluated the efficacy and safety of ayurvedic treatment (Ashwagandha powder and SidhMakardhwaj). Of the 86 patients, 76 completed the study. The subjects were administered 5g of Ashwagandha powder twice a day for three weeks with lukewarm water or milk and then for the next four weeks, SiddhaMakaradhwaja (100 mg) with honey was administered daily. The authors found that a significant change in post-treatment scores of tender joint counts, swollen joint counts, physician global assessment score, patient global assessment score, pain assessment score, patient –self-assessed disability index score, and ESR level as compared to baseline scores. American College of Rheumatology (ACR) 20 response was observed in 56.4 percent (44/78) patients and moderate response in 39.74 percent (31/78) patients [European League Against Rheumatism (EULAR) criteria]. The treatment regimen showed normal kidney and liver function tests. However, increased urinary mercury levels were observed after treatment. The authors concluded that the ayurvedic treatment (Ashwagandha powder and SidhMakardhwaj) has a potential to be used for the treatment of rheumatoid arthritis. However, small sample size, short duration, non - randomization and lack of a control group were the study limitations, warranting further studies to confirm these findings.

Raut AA et al (2012), conducted a prospective, open-labelled study to evaluate the dose-related tolerability, safety, and activity of Withania somnifera formulation in eighteen apparently healthy volunteers (12 Males:6 Females, age:18-30 years, and BMI: 19-30 Kg/ m2). W. Somnifera was given in the form of capsules (aqueous extract) daily in two divided doses with increase in daily dosage every 10 days for 30 days (750 mg/day

x10 days, 1000 mg/day x 10 days, 1250 mg/day x 10 days). The authors assessed the subjects for vital functions, symptoms/signs, haematological and biochemical organ function tests. The volunteers were also assessed for muscle activity by hand grip strength, quadriceps strength, and back extensor force. Using the cycle ergometry, exercise tolerance was determined. Using the skinfold thickness measurements, lean body weight and fat percentage were computed. All but one volunteer tolerated W. Somnifera without any adverse event. One volunteer was withdrawn from the study because of the symptoms exhibited such as; increased appetite, libido, and hallucinogenic effects with vertigo at the lowest dose. The authors concluded that W. Somnifera, when given in escalating doses from 750 to 1250mg/day, was well tolerated. The formulation was found to be safe on haematological and biochemical organ function tests. This study has also demonstrated muscle strengthening, improved quality of sleep and lipid lowering potential in view of its traditional use as balya.

Keche Y et al (2010), conducted a randomized double-blind placebo-controlled clinical trial to assess the efficacy and safety of Livwin® (polyherbal formulation) in 58 patients (in two groups) with acute viral hepatitis. The formulation Livwin® comprised of Ashwagandha, Arjuna, Bhumyamalaki, Daruharidra, Guduchi, Kutki and Punarnava and was given orally, two capsules two times a day for eight weeks followed by treatment free period of four weeks. The assessment parameters included noting symptomatic recovery and by measuring levels of serum bilirubin, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase at baseline, 2, 4, 8 and 12 weeks. The authors found that significant earlier recovery of weakness was observed with Livwin® as compared to placebo at 2, 4 and 8 weeks. Serum bilirubin and ALT were observed in normal range in significantly higher number of patients with Livwin® treatment as compared to placebo at 2, 4 and 8 weeks. AST was observed in normal range in significantly higher number of patients with Livwin® treatment as compared to placebo at 2 and 4 weeks. The authors concluded that Livwin® is effective in uncomplicated

Usha PR et al (2003), in an open-label pilot study,evaluated the clinical efficacy and safety of a polyherbal preparation, named Immu-25® [100mg extract each of: Tinospora cordifolia, Withania somnifera, Emblia officinalis andOcimum sanctum] in 36 patients (10 female, 26 male) with a mean age of 35 ±10

years, with confirmed HIV infection with a CD4 count <500 cells/μL. The subjects received two capsules of the test drug twice daily for 18 months. Patients were evaluated at monthly intervals for general signs and symptoms, development of opportunistic infections, and changes in weight and performance index. Lymphocyte phenotyping and routine haematological, biochemical, hepatic and renal parameters were recorded after every six months of drug therapy. Viral load was evaluated before and after every six months of treatment. The authors found that the polyherbal test preparation produced good symptomatic improvement within six months. There was an increase in mean weight after 6, 12 and 18 months of treatment. The incidence and severity of symptoms such as diarrhoea, fatigue, anorexia, cough and fever decreased with drug treatment. There was a decrease in the mean viral load after 6 and 12 months of treatment. The decrease in viral load was associated with an increase in mean CD4 count from baseline after 6 months of therapy, and this continued to rise after 12 and 18 months of treatment. With the exception of mild gastrointestinal adverse effects, the drug was well tolerated. Both patients and investigators rated the treatment as good or very good. The authors concluded that the herbal drug may have a good immunomodulatory effect and has potential as a co-therapeutic agent in the management of HIV infection.

Clinical Reports on Ashwagandha

Methods

Literature search

An extensive literature search was done using a combination of various keywords and MeSH terms to identify relevant articles. Using the keywords “Ashwagandha OR Withania somnifera” on Pub Med, articles were searched and a total of 1507 articles were available. After screening the search result, 36 randomized controlled trials on Ashwagandha root as standalone intervention were included. Search results are summarized below in Fig.2 and Fig.3.We critically reviewed these studies regarding the efficacy and toxicity of Withania somnifera (WS) in various medical conditions which are summarised below. Details are available in Annexure 2.

Figure 2: Categorization of article on the basis of study type

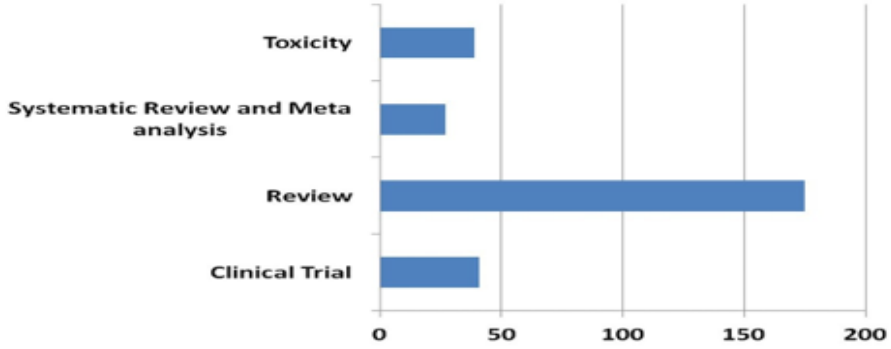
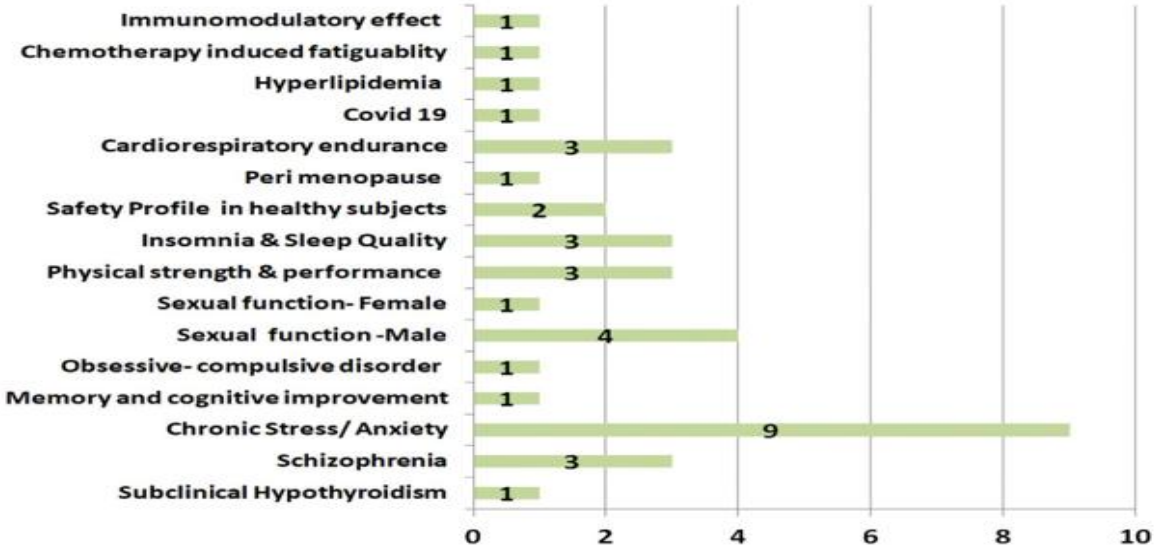


Figure 3: Categorization of article on the basis of conditions/effects studied



Subclinical Hypothyroidism

A study by Sharma et al., 2018180 on 50 adults with subclinical hypothyroidism using an Ashwagandha root extract at a daily dose of 600 mg for 8weeks concluded that root extract was found more effective in improvingthyroid function compared to the placebo. During the study,greater increase in thyroxine (T4) and triiodothyronine (T3) and a greater reduction in thyroid-stimulating hormone (TSH) were found in the Ashwagandha root extract group.

Schizophrenia

In a study conducted byGannon et al. 2019181, it was proved that in schizophrenic patients, symptoms of anxiety and depression were treated with benefits by W. somnifera extract. Significant reductions in the Positive and Negative Syndrome Scale were proved with adjunctive treatment with Withania somnifera in schizophrenic patients in the study by Chengappa et al., 2018182. Another study conducted by Agnihotri et al., 2013183, concludedthe positive effects of Ashwagandha extract on metabolic parameters.

ChronicStress, Anxiety and Sleep

Nine RCTs were found evaluating the effect of Ashwagandha on stress and anxiety symptoms and sleep quality. In three studies, Ashwagandha root extract was given ata dose of 600 mg (Salve et al., 2019184, Chandrasekhar et al., 2012185; Choudhary et al., 2017186;) and 250 mg (Salve et al., 2019184) daily for 8-weeks and significantly greater reductions in stress and serum cortisol were observed in Ashwagandha group compared to the placebo. Study by Salve et al. (2019)184also reported better improvement in sleep quality in both groups(250 mg and 600 mg dose), and a greater reduction in anxiety levels in 600 mg dose group. A study conducted by Choudhary et al. (2017b)187also reported significant reduction in body weight, food cravings, and

emotional eating in the Ashwagandha group. Khyatiet al., (2013)188in their study reported greater anxiolytic effects of Ashwagandha as compared to placebo. Study conducted by Andrade et al., 2000189, reported an 88% response rate in the Ashwagandha group and a 50% response rate in the placebo group(based on a HAM-A score < 12). However, changes were not significantly different between the two groups. The study conducted by Kelgane et al., 2020190 reported significantly better results in quality-of-life scores (WHOQOL- BREF), and global, physical, psychological, and environment domain scores, but not in the social relationship domain score compared to the placebo. Ashwagandha

intake was also associated with significantly greater improvements in mental alertness and sleep quality. One more study by Gopukumar K et al 2021191 reported that Ashwagandha had better clinical improvement in memory and focus, psychological well-being, and sleep quality, stress levels as compared to placebo, and was safe and well-tolerated.Also, in patients suffering from insomnia and anxiety, Ashwagandha root extract helped in improving sleep quality and had anxiolytic properties (Langade et al., 2019)173. Further, a study conducted in Iran by Narges Hosseini et al 2019192reported that the result of Revised Children's Manifest Anxiety (RCMA) showed a decrease in the scores of both groups(Ashwagandha and Placebo) during the third and sixth weeks, compared to the beginning of the study. Moreover, the difference between the groups was statistically significant and Withaniasomnifer a root extract was found more effective on the anxiety symptoms among children with ADHD.

Memory and Cognitive Improvement

A total of three randomized, double-blind, placebo-controlled studies comprising 128 participants were identified, examining the effects of Ashwagandha on memory and cognitive performance. In the study conducted by Choudhary et al., 2017187, Ashwagandha root extract was administered to 50 participants with mild, subjective symptoms of memory impairment, with a previous diagnosis of early dementia, mild cognitive impairment, or a Mini-Mental State Exam (MMSE) score ≥19. In this study, the Ashwagandha group had better results on immediate memory, general memory, executive function, and attention and information processing speed than the placebo group.

Obsessive-compulsive Disorder

A study by Jahanbakhshet al.,2016193 examined the effect of Ashwagandha in this disease condition, and they found a positive impact of Ashwagandhaon the Yale-Brown Obsessive- Compulsive scale.

Sexual Function -Male

Four RCTs comprising 291 participants were identified, examining the effects of Ashwagandha on sexual functions in males.In a 12-week, randomized, double-blind, placebo- controlled study conducted by Ambiye

et al., 2013194,on 46 infertile men, 675 mg daily of Ashwagandha root extract was associated with significant increase in sperm concentration, semen volume, sperm motility, serum testosterone, and luteinizing hormone levels. However, no statistically significant changes occurred in the placebo group.In a study by Nasimi Doost Azgomi et al., 2018195, Ashwagandha root extract was associated with significant increase in sperm count, motility, and morphology but not semen volume or round cells in infertile men. In another study by Mamidiet al., 2011196,95 men with a psychogenic type of erectile disorder, Ashwagandha root extract was associated with a statistically significant improvement in the International Index of Erectile Function (IIEF) total score. However, theseimprovements were

not significantly different from the placebo. No relief in erectile dysfunction occurred in 83% and 75% of participants in the Ashwagandha and placebo groups, respectively.

Sexual function -Female

In their study, Durg et al 2018197 showed that Ashwagandha supplementation improvesthe count and motility of sperm along with the volume of semen. This study was in concordance witha study by Dongre et al., 2015198 which concluded that the root extract of Ashwagandha increases sexual function in females.

Physical strength &Performance

In a study by Wankhede et al., 2015199, Ashwagandha intake was associated with greater increase in muscle strength (bench press and leg extension), muscle size (arms and chest), and serum testosterone; and greater reductions in creatine kinase and body fat percentage in healthy adult males participating in an 8-week resistance training program compared to participants on the placebo. In another placebo-controlled study by Shenoy et al., 2012200, Ashwagandha root extract was associated with greater increases in VO2max, metabolic equivalents, and time to exhaustion, but not respiratory exchange ratio during a graded exercise treadmill test. Standardized aqueous extract of Withania somnifera

significantly increased pain threshold force and time and pain tolerance force against mechanical pain (Sandhu et al., 2010)201.

Insomnia and Sleep quality

In a study conducted by Langade et al., 2019173,Ashwagandhahad significantly greater improvement in latency of sleep onset and sleep efficiency compared to the placebo group. Also, Ashwagandha was found more effectiveto improve sleep quality compared to the placebo group. Moreover, there was a significantly greater reduction in anxiety as measured by the HAM-A. Another study by Deshpande et al., 2020202 reported a 72% increase in self- reported sleep quality in the treatment group, compared with 29% in the placebo group. The treatment group also showed significant improvement in sleep efficiency, total sleep time, sleep latency and wake after sleep onset versus placebo after six weeks. It was reported in the study ofRaut AA et al., 2012203 thatW. Somnifera, when given in escalating doses from 750 to 1250mg/day, it was well tolerated and has also demonstrated muscle strengthening, improved quality of sleep and lipid lowering potential.

Peri-menopause

Study conducted by Sriram Gopal et al., 2021204 reported that, in comparison with the placebo, Ashwagandha supplementation was associated with a statistically significant reduction in total MRS score,total MENQoL scores, serum Follicle Stimulating Hormone levels and serum LeutinizingHormone levels; and was also associated with an statistically significant increase in serum estradiol levels.

Cardiorespiratory endurance

Study conducted by Tiwari S et al 2021205, Choudhary, B et al.2015206and Shenoy et al., 2012200, found that Ashwagandha enhances cardio-respiratory endurance and improves the quality of life in healthy athletic adults.

Hyperlipidemia

A study conducted by Andallu et al, 2000207, reported that Ashwagandha has a significant role in reductions of lipid profile parameters like total cholestrol, triglyceride, LDL-C, and VLDL-C.

Immunomodulatory effect In the study conducted by Tharakan, M et al208, Ashwagandha extract significantly improved the immune profile of healthy subjects by modulating the innate and adaptive immune systems.

COVID- 19

In the study conducted by Chopra, A et al 2021209, Ashwagandha was not found inferior to hydroxychloroquine (HCQ) and its efficacy was within the 15% non-inferiority margin set a priori. Ashwagandha as an immunomodulator had other clinical benefits including reducing mental stress.

Normal population studies

Eighty volunteers in a study in Lucknow by Verma N et.al.2020210provided results of normal thyroid and liver profile for use of Ashwagandha for8 weeks with a dose of 300mg but all were healthy adults. Fifty athletes took 300mg Ashwagandha twice daily for 8 weeks with enhanced cardiovascular endurance and increased anti-oxidant level.

Children

Two studies have investigated the use of Ashwagandha in children: one by Elgar K. Et.al. 2021211 was an uncontrolled open-label study in 8−12-year-olds with mild nutritional deficiencies; and one double-blind, placebo- controlled trial in healthy 8−12-year-olds. In both studies, children took 2 g per day of an Ashwagandha root powder for 60 days. Increase in body weight, haemoglobin and hand grip strength were seen in those children receiving the active herb, and no adverse effects were observed. Another study by Mishra, R K et.al. 2010212compared two different traditional Ashwagandha formulations, Ghrita (made with ghee and Ashwagandha) versus granules versus placebo in 111 children aged 3−12 years. Aerobic capacity, body composition and muscular strength improved the most in the Ghrita formulation group, and the least in the placebo group. Children aged 3−7 years old received 2.5−4 g, and 8−12-year-olds received 6−8 g daily for 6 weeks.

Conclusion

Ashwagandha (Withania somnifera (L.) Dunal), also known as “Indian ginseng” or “winter cherry,” is one of the most used and revered medicinal plants in Ayurveda and other traditional systems of medicines in India due to its myriad uses in human health and wellbeing. The use of Ashwagandha in Ayurveda medicine can be traced back to the time of Charaka Samhita, wherein it was lauded for its prominent use as a Rasayana (rejuvenating tonic), the most acclaimed of all types of medicine and vital to the philosophy of health and wellness in Ayurveda. Rasayanais a specialty in Ayurveda, aiming at rejuvenation, holistic care, wellness, etc., enabling an individual to have a long and healthy life. Ayurveda classifies therapeutics for the maintenance of health, focusing on preventive and promotive aspects and managing diseases, if required. Based on the Ayurvedic treatise, Ashwagandha is a potent herb with multitude of effects ranging from the promotion of health, maintenance of general well-being, rejuvenation, and as therapy for diseases that manifest with emaciation, neurological and neuropsychological symptoms.

Ashwagandha is well known among traditional medicine practitioners for its restorative and regenerative qualities. It is used, among others, for the treatment of nervous exhaustion, cognitive disorders, insomnia, fatigue, reproductive disorders in males, anxiety, and stress, and is also a well-known aphrodisiac and adaptogen. Ashwagandha stimulates the immune system, combats inflammation, improves learning ability and memory, and enhances energy, youthful vigor, endurance, strength, and health. Numerous pre-clinical and clinical studies on Ashwagandha are available in the public domain, establishing a solid evidence base for its potentially large array of therapeutic applications. Data from

pre-clinical safety and efficacy studies havereported its antioxidant, anti-tumor, antibacterial, spermatogenic, anti-ulcer, hypolipidemic, hepatoprotective, thyroid- stimulating, anti-inflammatory, anti-stress, nootropic, Anti-venom, immuno-modulatory, anti-osteoporotic, cytoprotective, cardio- protective, hypoglycemic activity, etc. The chemistry of Ashwagandha has been extensively studied, and over 35 chemical constituents have been identified, extracted, and isolated.

Available scientific evidence from numerous clinical studies reports the positive effects of Ashwagandha in stress and anxiety symptoms, sleep

quality, insomnia, schizophrenia, subclinical hypothyroidism, memory and cognitive impairment, obsessive-compulsive disorder, sexual function, physical strength &performance, peri-menopausal symptoms, cardio-respiratory endurance, hyper-lipidemia, immunomodulation and even in prevention and therapy of COVID-19.

While Ashwagandha has been in clinical practice of AYUSH systems of medicine, for generations and through time immemorial, its use has come under scrutiny in recent years, with some European regulatory bodies attributing detrimental effects, and subsequently scientific evidence surrounding its use has been debated in clinical arena andacademic communities. This Dossier on Ashwagandha presents an evidence-based knowledge synthesized from available information on Ashwagandha to cater to the interests of academicians, clinicians & researchers and for the benefit of public at large. A comprehensive search was performed to identify studies involving Ashwagandha root, which included animal models, pre-clinical and clinical studies. A total of 39 pre-clinical studies presented information on toxicity studies conducted on Ashwagandha. Among these, 2 were review articles, full text for one study was unavailable, and two studies were conducted on different plant species, namely, Withania frutescens; four studies pertained to environmental studies, and two were identified as in-silico studies. About 27 studies concluded that the use of Ashwagandha is safe for human consumption and safely administrable dose level of Withania somnifera is 2000 mg/kg body weight with no observed adverse effect level (NOAEL).Also, one case study reported the toxic effect of the herb. Among the clinical studies evaluated, 16 were safety studies, which includedten conducted on participants with disease, and 06 were conducted on healthy participants. These studies demonstrated that Ashwagandha was efficacious with benefits in a wide variety of clinical situations. It was well tolerated, and was found safe on hematological and biochemical organ function tests. The toxicity studies also demonstrated an acceptable toxicity profile; however, larger-scale studies may be required in the future. It is also important to note that the benefits observed from Ashwagandha were consistent across various medical conditions, including metabolic derangements, lifestyle disorders, and mood and personality disorders.

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Background

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