IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Understanding Regulation For The Traditional Medicine USFDA & CDSO Regulatory Countries

Miss.Shradhda Hemraj Badole, Mr. Chetan T. Choudhary

Student, Professor

Maharashtra Institute of Pharmacy Betala

➢ INTRODUCTION

Traditional medicine (TM) represents a comprehensive body of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, and treatment of physical and mental illnesses. According to the World Health Organization (WHO), traditional medicine remains a primary source of healthcare for a large proportion of the global population, particularly in developing countries, and its use is steadily increasing in developed nations as complementary and alternative medicine (CAM).[1]

The global resurgence of interest in traditional medicine has been driven by factors such as cultural acceptability, perceived safety, cost-effectiveness, and limitations associated with conventional pharmaceuticals, including adverse drug reactions and antimicrobial resistance [2]. Herbal medicines, Ayurveda, Unani, Siddha, Homeopathy, and other traditional systems are now widely marketed across international borders, increasing the need for robust regulatory frameworks to ensure product quality, safety, and efficacy.[3]

Regulation of traditional medicine varies significantly among countries due to differences in historical development, healthcare systems, and legal frameworks. In the United States, traditional medicine products are primarily regulated by the US Food and Drug Administration (USFDA) under categories such as dietary supplements, foods, or cosmetics, rather than as drugs.[4] The Dietary Supplement Health and Education Act (DSHEA) of 1994 defines dietary supplements and places responsibility for safety and labeling largely on manufacturers, with the FDA exercising post-market regulatory control. As a result, traditional medicine products in the US are subject to limited pre-market evaluation, particularly with respect to efficacy claims.[5]

In contrast, India has a long-standing and formally recognized system of traditional medicine regulated under a structured legal framework. The Central Drugs Standard Control Organization (CDSCO), along with the Ministry of AYUSH, governs the regulation of Ayurveda, Siddha, Unani, Sowa-Rigpa, and Homeopathy under the Drugs and Cosmetics Act, 1940 and its associated rules. Unlike the US system, traditional medicines in India are legally classified as drugs, requiring manufacturing licenses, adherence to Good Manufacturing Practices (GMP), and compliance with pharmacopoeial standards[6].

The divergent regulatory approaches adopted by USFDA and CDSCO present both challenges and opportunities for manufacturers, regulators, and researchers involved in the global traditional medicine market. Differences in product classification, approval pathways, possible claims, and quality validation[7]. Therefore, a comparative understanding of traditional medicine regulation in USFDA and CDSCO regulatory jurisdictions is essential to promote harmonization, ensure consumer safety, and support evidence-based integration of traditional medicine into modern healthcare systems.[8].

Natural medications alluded as plants materials or herbalism, includes the utilization of entire plants or parts of plants, to treat wounds or diseases. Herbal medications are utilization of restorative spices to forestall and treat illnesses and infirmities or to help wellbeing and recuperating. These are medications or arrangements produced using a plant or plants and utilized for any of such purposes. Natural medications are the most established type of medical services in the world. There are numerous Hm's items offered that state to treat the manifestations of an expansive scope of issues, from melancholy to cold and influenza. World Health Organization (WHO) has particular Hm's medications as complete, named restorative items that have lively fixings, ethereal or cryptic pieces of the plant or other plant material or mixes. World Health Organization has set exact rules for the assessment of the safety, adequacy, and quality of Hm's drugs. WHO gauges that 80% of the world populaces as of now utilize Hm's medications for significant medical care. Astoundingly, in certain nations Hm's medications may likewise encase by custom, common natural or inorganic dynamic constituents which are not of plant source.[9] Hm's medication is a central constituent in conventional medication and a typical constituent in ayurvedic, homeopathic, naturopathic and other medication frameworks. Seeds, leaves, stems, bark, roots,

blossoms, and concentrates of these have been utilized in natural medications over the course of the centuries of their utilization. Natural items have arrived at broad ampleness as valuable specialists like antimicrobial, antidiabetic, antifertility, antiaging, antiarthritic, narcotic, upper, antianxiety, antispasmodic, pain relieving, calming, hostile to HIV, vasodilatory, hepatoprotective, therapy of cirrhosis, asthma, skin break out, feebleness, menopause, headache, nerve stones, constant exhaustion, Alzheimer's illness and memory upgrading activities. Hm's medications have been perceived for roughly 4000 years. These medications have endure certifiable testing and millennia of human testing. A few medications have been ended because of their poisonousness, while others have been adjusted or joined with extra spices to offset results[10].

Advantages of Herbal Drugs

- Low/Minimum cost
- potency and efficiency
- enhanced tolerance
- More protection
- fewer side-effects
- complete accessibility
- recyclable

Disadvantages of Herbal Drugs

- Not able to cure rapid sickness and accidents
- Risk with self-dosing
- Complexity in standardizations

Herbal medications (HMs) have been acquiring expanded ubiquity among purchasers in both created and non-industrial nations. As indicated by the World Health Organization (WHO), 60% of the total populace, and 80% of the populace in non-industrial nations relies upon HMs for their medical care needs. Global consumption of HMs has grown significantly from $20 billion in 1997 to $83 billion in 2008.While a scope of definitions exist for HMs, in this examination, HMs are characterized as "herbal medications arrangements that are fabricated modernly in which the dynamic ingredient(s) is/are simply and normally unique plant substance(s), which is/are not synthetically adjusted and is/are liable for the general remedial impact of the item". The public commonly perceive HMs as safe, yet there are concerns about their safety too. A few antagonistic impacts, some of them perilous, can emerge from dynamic fixings themselves, just as contaminated of HMs with ordinary meds, herbal drug cooperation's improper HMs plans. Be that as it may, critical HM security issues additionally emerge fundamentally from the improper administrative order of HMs.

For instance, in the United Stated (US), HMs are delegated dietary enhancements, with prerequisites for assessing quality and wellbeing less rigid than those for restorative items. Implying that these items don't need evaluation by the public medication administrative power (DRA) before their advertising. This has specific ramifications for some nations in the Eastern Mediterranean Region (EMR), which import most of their HMs from different nations including the US.[11][12] Phytomedicines or natural prescriptions normally referred to in the market as Hm's drugs. Which is the utilization of plants box diverse route for remedial or restorative reason. This arising Hm's market is managed by AYUSH which is Ministry of Ayurveda, Yoga and naturopathy, Unani, Siddha and Homeopathy under the Drug and Cosmetic Act (D and C) 1940 and Rules 1945 in India. Makers are told to submit to AYUSH rule for showcasing of natural item. All the more vitally they should go in like manner Drug and corrective demonstration, segment C and D for definition structure, authorizing, naming, producing, pressing, quality and fare. Moreover great assembling practice (GMP) has additionally been actualized through schedule "T" in 2016. Hm's medications are controlled under the Drug and Cosmetic Act (D and C) 1940 and Rules 1945 in India, where administrative arrangements for Ayurveda, Unani, Siddha medication are obviously set down in Chapter IV-A. There are 18 diverse area are available from form 33C to 33 O.[13] Its goal to guarantee that the examinations are deductively and morally stable and that the clinical properties of the ASU medication under scrutiny are appropriately reported. The rules try to set up two cardinal standards: insurance of the privileges of human subjects and credibility of ASU medication clinical preliminary information generated [14].

Key Differences

Aspect	**USFDA**	**CDSCO**
Classification	Case-by case [drug,supplement, etc.]	Specific ASU category
Approval Pathway	NDA with trials for therapeutic claims.	Classical formula +GMP , minimal trials
Disease Claims	Prohibited without approval.	Allowed per traditional use
Enforcement Focus	Import alerts on unapproval/adulterated	Domestic GMP compliance

The Central Drugs Standard Control Organization (CDSCO) controls herbal drugs in India through the Drugs and Cosmetics Act of 1940 and the Rules of 1945. The rules consider Ayurvedic, Siddha, and Unani (ASU) drugs separately. CDSCO controls the licensing, formulation, manufacturing, packing, and quality control of herbal drugs. Schedule T of the Act sets Good Manufacturing Practices (GMP) of ASU drugs in order to render them safe and effective. Herbal drugs must be of defined standards in the First and Second Schedules of the Act, which set books to be maintained and import and domestic manufacturing standards of drugs.

But the CDSCO is significantly hampered in the regulation of herbal products. These are a lack of tight guidelines for herbal drugs, causing heterogeneity of the quality as well as the safety of such drugs. The allopathy-based regulatory system cannot avail itself to its optimal potential in herbal drugs of compound composition. There is also extremely limited scientific knowledge on the safety and efficacy of the majority of herbal drugs, which also leads to the problem. Inadequate pharmacovigilance systems and rudimentary infrastructure for testing and standardization also add to these problems[15,16]

The Central Drugs Standard Control Organisation (CDSCO) beneath Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India is the National Regulatory Authority (NRA) of India. Its common headquarters is situated at FDA Bhawan, Kotla Road, New Delhi 110002 and also has six zonal offices, four sub zonal offices, thirteen harbour offices and seven laboratories spread all over the country. The Drugs & Cosmetics Act,1940 and rules 1945 have provide different responsibilities to central & state regulators for regulation of drugs & cosmetics[17].

Major functions of CDSCO:

1. Control of imported drugs, new drug approvals and clinical trials.
2. Drug Control Committee (DCC) and Drug Treatment Advisory Board (DTAB) meetings
3. Central License Approving Authority.

Under the Drugs and Cosmetics Act, CDSCO is accountable for approval of New Drugs, Conduct of Clinical Trials, laying down the guidelines for Drugs, control over the quality of imported drugs in the nation and coordination of the activities of State Drug Control Organizations by giving expert advice with a view of bring about the uniformity in the enforcement of the Drugs and Cosmetics Act.

Functions of CDSCO

- Central licensing authorities are responsible for
  - o New drugs approval
  - o Performing clinical trials
  - o Building up for drugs
  - o Quality Control of imported drugs, import registration and licensing
  - o Coordination of the activities of state drug control authorities by giving expert opinion to consistently enforce the D&C Act.
- State licensing authorities are responsible for
  - o Regulation of production, sale and marketing of drugs.
- Other Functions
  - o Legalization of the Commercialization of Blood, Vaccines, Recombinant DNA Products, and Selected Medicinal Devices.
  - o Adjustments to the Rules Implementing the D&C Act
  - o Issuing Export Test License, Personal License, and No Objection Certificate are.
  - o Older pharmaceuticals and cosmetics should be outlawed.
  - o Preclinical testing and assessment of cosmetics and medicines Functions of Drug Testing Canters Centrally[18].

The FDA is one of the most well-known and respected regulatory agencies in the world. It is responsible for protecting public health in the United States by ensuring that drugs, vaccines, biologics, medical devices, and even food products are safe and effective.[19]

In pharmaceuticals, the FDA reviews and approves:

- Investigational New Drug Applications (NDA) for brand-new drugs,
- New Drug (IND) applications before clinical trials begin,
- Abbreviated New Drug Applications (ANDA) for generics,
- And Biologics License Applications (BLA) for biologics like vaccines.

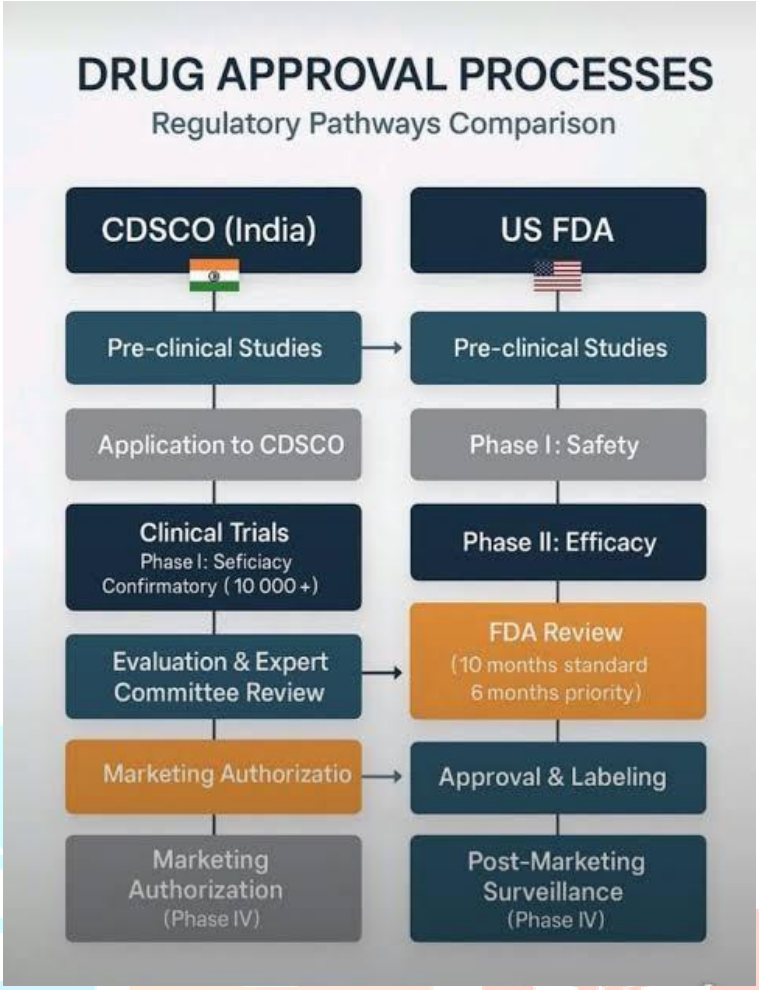
In United States, the term complementary/alternative medicines (CAM) are most commonly used for traditional medicine systems. "Complementary medicine" refers to use of CAM together with conventional medicine[20] In addition to usual care to help lessen pain FDA in its draft guidance "Guidance for industry on complementary and alternative medicine products and regulation by the food and their regulation by the food and drug administration" clarified different categories of Complimentary Alternative Medicines (CAM) products into cosmetic; device; dietary supplement; drug, as well as "new drug" and "new animal drug;" food; and food additive. These statutory definitions cover some CAM products. It was also clarified neither the FDA Act nor the Public Health Service Act exempts CAM products from regulation. The draft guideline was later withdrawn after recommendations of several agencies including The American Herbal Products Association (AHPA)[21].

The Food and Drug Administration (FDA or Agency) is the administrative, scientific, public health and consumer protection agency responsible for guaranteeing all human and animal drugs, medical devices, cosmetics, foods, food additives, drugs and medicated feeds for food producing animal, tobacco, and radiation emitting devices are safe, and that all such products marketed in the United States are satisfactorily, truthfully, and informatively labelled and safely and appropriately stored, transported, manufactured, packaged, and controlled. FDA's programs are national in scope and impact, and the agency's exercises have a direct and significant affect on multibillion dollar business, in expansion to securing the wellbeing and safety of American Consumers[22]. The work of the Agency is carried out by a staff of more than 18,000 researcher, physicians, 2 regulatory and other personnel stationed throughout the United States.FDA's Office of Regulatory Affairs (ORA) is the lead office for all organisation regulatory exercises. Over 5,000 ORA representative strategically located in district offices, resident posts, and research facilities throughout the United States perform assessment and examination (including criminal investigations), wharf exams, sample collections andanalyses, and carry out enforcement exercises, education, and outreach directly to consumers, industry agents, importers, and shippers, as well as other partners across the country. ORA also works with its government, state, local, tribal, territorial, and foreign counterparts to further the agency's mission. ORA is driven by the Associate Commissioner for Regulatory Affairs (ACRA)[23].

Key Functions of the FDA:

1. Regulation of Food Products:
   - o Oversees most food products, including dietary supplements, bottled water, food additives, and infant formulas.
   - o Note: The U.S. Department of Agriculture regulates certain aspects of meat, poultry, and egg products.

2. Regulation of Drugs:
   - o Evaluates and approves both prescription and over-thecounter drugs for human and veterinary use.
   - o Ensures that drugs are safe and effective before they reach the market
3. Regulation of Medical Devices:
   - o Assesses and monitors medical devices ranging from simple items like depressors to complex technologies such as pacemakers.
   - o Oversees the safety and effectiveness of devices that emit radiation, including X-ray machines and microwave ovens.
4. Regulation of Biological Products:
   - o Ensures the safety and efficacy of biological products, including vaccines, blood and blood products, and gene therapies.
   - o Monitors the production and distribution of these products to protect public health.
5. Regulation of Cosmetics:
   - o Oversees the safety of cosmetics, ensuring they do not contain harmful ingredients and are properly labelled.
   - o Takes action against products that are adulterated or misbranded.
6. Regulation of Tobacco Products:
   - o Regulates the manufacturing, distribution, and marketing of tobacco products to protect public health.
   - o Implements measures to reduce tobacco use, especially among minors.
7. Advancement of Public Health:
   - o Facilitates innovations that make medical products safer and more effective.
   - o Provides the public with accurate, science-based information to improve health.
8. Ensuring Security of Products:
   - o Protects the nation's food supply and medical products from intentional and unintentional contamination.
   - o Collaborates with other agencies to safeguard against bioterrorism and other public health threats[24].



➢ LITERATURE REVIEW

1] Traditional medicine has gained increasing global recognition due to its long history of use, cultural acceptance, and growing demand for natural and complementary therapies. The World Health Organization (WHO) has consistently emphasized the importance of integrating traditional medicine into national healthcare systems through appropriate regulatory frameworks to ensure safety, quality, and efficacy (WHO, 2014).

2]Tilburt and Kaptchuk et.al (2008) reported that while the USFDA framework supports innovation and market accessibility, it poses challenges related to inconsistent quality, misleading health claims, and limited scientific validation of traditional medicine products.

3] Ernst at.al (2002) raised concerns regarding adulteration, contamination with heavy metals, and undeclared pharmaceutical ingredients in herbal medicines, emphasizing the need for stronger regulatory controls.

4] Patwardhan and Mashelkar et.al (2009) noted that traditional medicine systems such as Ayurveda, Siddha, and Unani are legally recognized and regulated as drugs under the Drugs and Cosmetics Act, 1940, with oversight from the Central Drugs Standard Control Organization (CDSCO) and the Ministry of AYUSH [6].

5]Sharma and Kumar et.al (2016) highlighted that although India has a well-defined regulatory structure for traditional medicine, challenges remain in terms of standardization, clinical evidence generation, and global regulatory acceptance .

6] Jadhav Das Rajyaguru, et al. (2025)Regulations, Current Development, &Future Prospects of Phytopharmaceuticals in India. Discover Pharmaceutical Sciences.

➢ AIM AND OBJECTIVE

Aim-

study and compare the regulatory frameworks governing traditional medicines in India and the United States with special reference to the Central Drugs Standard Control Organization and the United States Food and Drug Administration, focusing on quality, safety, efficacy, approval procedures, and regulatory challenges**.**

Objective –

1. To understand the concept and importance of traditional medicine in global healthcare systems.
2. To study the regulatory framework of traditional medicines under the Central Drugs Standard Control Organization in India.
3. To analyze the regulatory approach of the United States Food and Drug Administration for herbal and traditional medicines in the USA.
4. To compare the approval processes, licensing requirements, and quality control standards followed by CDSCO and USFDA.
5. To evaluate the role of Good Manufacturing Practices (GMP), labelling regulations, and pharmacovigilance systems in traditional medicine regulation.
6. To identify major challenges associated with the regulation of herbal and traditional medicinal products such as standardization, contamination, and misleading claims.
7. To study the safety, efficacy, and quality assessment methods used for traditional medicines in both countries.
8. To examine international guidelines and recommendations related to herbal medicine regulation, including those of the World Health Organization (WHO).
9. To explore the need for harmonization of traditional medicine regulations at the global level.
10. To provide recommendations for improving regulatory systems and ensuring safe use of traditional medicines worldwide.

➢ PLAN OF WORK

Phase 1: Selection and Understanding of Research Topic

- Select the research topic related to regulation of traditional medicine.
- Understand the importance of herbal and traditional medicine in healthcare systems.
- Define the scope of study focusing on India and the United States.

Phase 2: Collection of Literature Review

- Collect information from:
  - Research articles
  - Review papers
  - WHO guidelines
  - CDSCO guidelines
  - USFDA regulations
  - AYUSH policies
  - Books and journals
  - Review previous studies related to traditional medicine regulation.

Phase 3: Study of Regulatory Authorities

➢ **India**

- Study the role of:
  - Central Drugs Standard Control Organization
  - Ministry of AYUSH
- Understand:
  - Drugs and Cosmetics Act, 1940
  - GMP requirements
  - Licensing procedures
  - Pharmacovigilance systems

➢ **USA**

- Study the role of:
  - United States Food and Drug Administration
- Understand:
  - Dietary Supplement Health and Education Act (DSHEA), 1994
  - Botanical drug regulations
  - Labeling and safety requirements
  - Clinical trial requirements

➢ **Phase 4**: Comparative Analysis

- Compare CDSCO and USFDA regulations based on:
  - Approval procedures
  - Quality control
  - Safety monitoring
  - Labelling requirements
  - Clinical evidence
  - Manufacturing standards
- Prepare comparative tables and regulatory charts.

➢ **Phase 5**: Case Study Evaluation

- Select herbal/traditional medicine examples such as:
  - Ashwagandha
  - Herbal supplements
  - Ayurvedic products
- Analyze:
  - Product approval process
  - Regulatory classification
  - Safety concerns
  - Market authorization

➢ **Phase 6**: Identification of Challenges

- Study major regulatory challenges:
  - Standardization issues
  - Heavy metal contamination
  - Adulteration
  - Misleading advertisements
  - Lack of scientific evidence
  - International trade barriers

➢ **Phase 7**: Data Interpretation and Discussion

- Interpret collected data and findings.
- Discuss differences and similarities between USFDA and CDSCO systems.
- Evaluate strengths and limitations of each regulatory framework.

➢ **Phase 8**: Preparation of Conclusion and Recommendations

- Summarize major findings of the study.
- Provide recommendations for:
  - Better regulation
  - International harmonization
  - Improved pharmacovigilance
  - Evidence-based traditional medicine

➢ **Phase 9**: Final Report Preparation

- Compile:
  - Introduction
  - Literature review
  - Aim and objectives
  - Methodology
  - Comparative analysis
  - Case studies
  - Conclusion
  - References
- Prepare the final review paper/project report according to academic guidelines.

- **RATIONALE**

Traditional medicine plays a significant role in public healthcare systems worldwide; however, its regulation differs substantially between countries due to variations in legal frameworks, healthcare priorities, and historical acceptance of traditional medical systems. India and the United States represent two major and contrasting regulatory environments for traditional medicine, governed by the Central Drugs Standard Control Organization (CDSCO) and the U.S. Food and Drug Administration (USFDA) respectively. Understanding these regulatory differences is essential for ensuring product safety, quality, efficacy, and global market accessibility.

In India, traditional medicine systems such as Ayurveda, Siddha, Unani, Sowa-Rigpa, and Homeopathy are formally recognized and regulated as drugs under the Drugs and Cosmetics Act, 1940, with oversight from the Ministry of AYUSH and CDSCO. This system-based regulatory approach allows therapeutic claims, mandates licensing, and enforces Good Manufacturing Practices and pharmacopoeial standards. Despite this structured framework, challenges remain in areas such as standardization, scientific validation, pharmacovigilance, and alignment with international regulatory expectations.

Conversely, in the United States, traditional medicine products are generally regulated by the USFDA as dietary supplements, foods, or cosmetics rather than as drugs, under the Dietary Supplement Health and Education Act (DSHEA), 1994. This product-based regulatory model emphasizes manufacturer responsibility, limited pre-market review, and post-market safety monitoring. While this approach facilitates market access and innovation, it also raises concerns related to inconsistent quality control, misleading health claims, and consumer safety, particularly for imported traditional medicine products[25].

The contrasting regulatory philosophies of CDSCO and USFDA create significant challenges for global manufacturers, exporters, and researchers engaged in traditional medicine. Products legally approved as medicines in India may not qualify for therapeutic claims or drug status in the US, leading to regulatory uncertainty and trade barriers. Furthermore, the absence of harmonized regulatory standards complicates efforts toward scientific validation, global acceptance, and integration of traditional medicine into modern healthcare systems. Therefore, the rationale of this study lies in the need to critically analyze and compare the regulatory frameworks of CDSCO and USFDA with respect to traditional medicine. Such an analysis is essential to identify regulatory gaps, assess compliance challenges. The findings of this study may contribute to improved policymaking, enhanced consumer protection, and the development of evidence-based regulatory strategies that support the safe, effective, and globally acceptable use of traditional medicine[26].

- **Case Study on Regulation of Ashwagandha Herbal Product in USFDA and CDSCO Regulatory Systems**

A .introduction to traditional medicine regulations

1. Introduction

Traditional medicine refers to the knowledge, skills, and practices based on indigenous beliefs, cultural traditions, and experiences used for the maintenance of health and treatment of diseases. It includes herbal medicines, spiritual therapies, manual techniques, and traditional exercises practiced in different countries for centuries.

The increasing global demand for herbal and traditional medicinal products has created the need for effective regulation to ensure their safety, quality, and efficacy. Governments and international organizations have established regulatory frameworks to monitor the manufacturing, marketing, import, export, and clinical use of traditional medicines.

The World Health Organization defines traditional medicine as "the sum total of knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to different cultures used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness." Traditional medicine plays an important role in healthcare systems worldwide, especially in countries such as India, China, Japan, Korea, and African nations. Herbal medicines are also gaining popularity in Western countries because of their natural origin and perceived lower side effects[27,28].

2. Historical Background of Traditional Medicine

Traditional medicine has existed since ancient civilizations. Early humans relied on plants, minerals, and animal products for healing purposes.

Ancient Systems of Medicine

- Ayurveda – Originated in India over 5000 years ago.
- Traditional Chinese Medicine (TCM) – Developed in China using herbal therapy and acupuncture.
- Unani System – Introduced by Greek and Arab physicians.
- Siddha Medicine – Practiced mainly in South India.
- Homeopathy – Developed by Samuel Hahnemann.
- African Traditional Medicine – Based on spiritual and herbal healing practices.

These systems contributed significantly to the development of modern pharmaceuticals [29].

3. Need for Regulation of Traditional Medicine

The rapid growth of herbal product markets has increased concerns regarding product quality, contamination, adulteration, false claims, and safety issues. Regulation is essential to protect public health.

✓ Objectives of Regulation

- Ensure safety of traditional medicines
- Maintain product quality and purity
- Establish efficacy through scientific evidence
- Prevent adulteration and contamination
- Standardize manufacturing practices
- Control misleading advertisements
- Promote rational use of herbal medicines
- Facilitate international trade

Without proper regulation, consumers may face risks such as toxicity, microbial contamination, heavy metal poisoning, and drug interactions.

4. Global Importance of Traditional Medicine

According to the World Health Organization, nearly 80% of the population in developing countries relies on traditional medicine for primary healthcare.

- ✓ Global Market Growth
- ✓ The global herbal medicine market has expanded rapidly due to:
- ✓ Increasing preference for natural therapies
- ✓ Rising healthcare costs
- ✓ Growing awareness about preventive healthcare
- ✓ Expansion of nutraceutical and wellness industries
- ✓ Countries like India and China are major exporters of herbal products.

5. Role of WHO in Traditional Medicine Regulation

The World Health Organization plays a major role in developing international guidelines for traditional medicine.

- ✓ WHO Traditional Medicine Strategy WHO developed strategies to:
- Integrate traditional medicine into healthcare systems
- Ensure safety and effectiveness
- Promote quality control
- Encourage research and development
- Strengthen regulatory policies
- ✓ WHO Guidelines

WHO has issued guidelines regarding:

- Good Agricultural and Collection Practices (GACP)
- Good Manufacturing Practices (GMP)
- Herbal medicine quality control
- Safety monitoring and pharmacovigilance
- Clinical evaluation of herbal medicines

These guidelines help member countries establish national regulatory systems.

6. Classification of Traditional Medicines

Traditional medicines are classified into different categories depending on the country's regulations.

- ✓ **Common Categories**

1. Herbal Medicines

Products containing plant materials such as leaves, roots, seeds, and extracts.

2. Finished Herbal Products

Prepared dosage forms like tablets, capsules, syrups, and ointments.

3. Nutraceuticals

Products providing health benefits beyond nutrition.

4. Dietary Supplements

Products intended to supplement diet with herbs, vitamins, or minerals.

5. Traditional Formulations

Classical preparations based on ancient texts.

7. Key Components of Traditional Medicine Regulation

A. Quality Control

Quality control ensures identity, purity, strength, and consistency of herbal products.

- ✓ Quality Parameters
- Authentication of raw materials
- Standardization of extracts
- Microbial testing
- Heavy metal testing
- Pesticide residue analysis
- Stability studies

B. Safety Evaluation

Safety evaluation includes:

- Toxicity studies
- Adverse effect monitoring
- Drug interaction studies
- Contamination assessment

C. Efficacy Evaluation

Efficacy is assessed through:

- Traditional evidence
- Clinical studies
- Scientific literature
- Pharmacological studies

D. Labelling Requirements

Labels should contain:

- Product name
- Ingredients
- Dosage instructions
- Warnings
- Manufacturer details
- Batch number
- Expiry date

8. Good Manufacturing Practices (GMP)

Good Manufacturing Practices are guidelines ensuring consistent product quality.

Importance of GMP

- Prevent contamination
- Maintain product consistency
- Ensure proper documentation
- Improve consumer confidence GMP covers:
- Personnel hygiene
- Equipment maintenance
- Quality assurance
- Packaging and storage
- Record keeping

WHO GMP guidelines are widely followed globally.

9. Pharmacovigilance in Traditional Medicine

Pharmacovigilance refers to monitoring adverse effects of medicines after marketing.

Importance

Traditional medicines are often assumed safe because they are natural. However, some herbal products may cause:

- Liver toxicity
- Kidney damage
- Allergic reactions
- Drug interactions

Pharmacovigilance programs help detect and prevent adverse drug reactions.

10. Challenges in Regulation of Traditional Medicine

1. Lack of Standardization

Variation in plant species, cultivation, and processing affects product quality.

2. Adulteration

Some products contain synthetic drugs or harmful substances.

3. Insufficient Scientific Evidence

Many traditional medicines lack clinical studies.

4. Weak Regulatory Systems

Developing countries may lack proper infrastructure.

5. Misleading Advertisements

False therapeutic claims may misguide consumers.

6. International Regulatory Differences

Different countries have different regulatory requirements.

11. Regulation of Traditional Medicine in India

India has one of the oldest traditional medicine systems in the world. Regulatory Authorities

Central Drugs Standard Control Organization Responsible for regulation of drugs and clinical trials.

- ✓ Ministry of AYUSH

Supervises Ayurveda, Yoga, Unani, Siddha, and Homeopathy systems. Important Acts

Drugs and Cosmetics Act, 1940

Provides legal framework for manufacturing and sale of Ayurvedic, Siddha, and Unani medicines.

- ✓ **Key Features**
- Licensing of manufacturing units
- GMP requirements
- Quality testing laboratories
- Pharmacopoeial standards

12. Regulation of Traditional Medicine in the United States

In the United States, herbal products are mainly regulated as dietary supplements. Regulatory Authority

United States Food and Drug Administration

USFDA regulates dietary supplements under the Dietary Supplement Health and Education Act (DSHEA), 1994.

Features of USFDA Regulation

Manufacturers are responsible for product safety Premarket approval usually not required

Good Manufacturing Practices mandatory Claims must not be misleading

USFDA can take action against unsafe or adulterated products.

13. Comparison Between CDSCO and USFDA Regulation

Parameter	CDSCO (India)	USFDA (USA)
Regulatory Category	Traditional medicine	Dietary supplements
Premarket Approval	Required in some cases	Usually not required
Governing Law	Drugs and Cosmetics Act	DSHEA
Focus	Traditional systems	Consumer safety
Evidence Required	Traditional + scientific	Safety evidence
GMP	Mandatory	Mandatory

14. Intellectual Property Rights and Traditional Medicine Traditional knowledge is valuable intellectual property. **Issues**

- ✓ Biopiracy
- Patent disputes
- Unauthorized commercial use

India established the Traditional Knowledge Digital Library (TKDL) to protect traditional medicinal knowledge from illegal patents.

15. Research and Development in Traditional Medicine

Scientific research improves acceptance of traditional medicines.

- ✓ Areas of Research
- Phytochemical analysis
- Clinical trials
- Standardization techniques
- Novel drug delivery systems
- Toxicological studies

Research helps integrate traditional medicine with modern healthcare.

16. Future Prospects of Traditional Medicine Regulation

Future regulatory developments may include:

- International harmonization
- Advanced quality testing
- Strong pharmacovigilance systems
- Digital traceability systems

- Increased clinical evidence generation

Global cooperation is important for ensuring safe and effective use of traditional medicines.

❖ USFDA FRAMEWORK DSHEA

DSHEA was enacted in 1994 to establish a regulatory framework for dietary supplements in the United States.

Under DSHEA:

- Dietary supplements are regulated as a category of food
- Manufacturers are responsible for product safety
- FDA monitors products after marketing Examples of supplements:
- Ashwagandha
- Turmeric
- Vitamin C
- Fish oil
- Probiotics

Definition of Dietary Supplement

According to DSHEA, a dietary supplement is a product intended to supplement the diet and contains:

- Vitamins
- Minerals
- Herbs or botanicals
- Amino acids
- Enzymes
- Dietary substances

These products are available in forms such as:

- Tablets
- Capsules
- Powders
- Soft gels
- Liquids

Main Objectives of DSHEA The objectives include:

- Ensuring consumer access to supplements
- Promoting public health
- Supporting safe use of herbal products
- Establishing labelling standards
- Defining FDA authority.

✓ **Botanical Drug Development Guidance 2016**

1. **Purpose & Scope**

Released December 2016, this guidance replaces the 2004 version. It describes FDA's Center for Drug Evaluation and Research (CDER) approach to botanical drug products submitted in NDAs.

Key principle: FDA recognizes botanicals are complex mixtures, not single molecules. The guidance provides flexibility vs. synthetic drug requirements, but still demands safety + efficacy for disease claims.

Definition: A _botanical drug product_ consists of vegetable materials, which may include plant materials, algae, macroscopic fungi, or combinations. It can be a powder, extract, or purified fraction. Does _not_ include highly purified, chemically modified substances [30][31][32].

Critical point: Marketing as a dietary supplement does _not_ prevent IND/NDA development. But "drug preclusion" clause 201(ff)(3)(B) means if FDA authorizes substantial clinical investigations _and_ public notice, that article may be barred from future supplement use unless marketed as food/supplement first.

Chemistry, Manufacturing, Controls (CMC) – Biggest Challenge

✓ **Description of Product**

- Botanical raw material: Scientific name _Withania somnifera_, plant part used = root, geographical source, growing conditions
- Voucher specimen: Deposited in herbarium for future reference
- Drug substance: Extraction solvent, ratio, manufacturing process

✓ Quality Controls

- Identity: At least 2 orthogonal methods. Example: HPTLC + HPLC fingerprint vs reference standard. DNA barcoding encouraged for Ashwagandha due to adulteration risk
- Active constituents Identify/quantify when known. For Ashwagandha: withanolides, withaferin A. If unknown, use "active markers" or chemical fingerprint
- Batch-to-batch consistency: Chromatographic fingerprint comparison, must demonstrate "sameness" between clinical, nonclinical, and commercial batches
- Contaminants: Heavy metals USP <2232>, pesticides USP <561>, residual solvents, microbial limits USP <2021>/<2022>, aflatoxins

Stability: Must show active constituents/markers are stable. Shelf-life based on real-time data.

Flexibility: Unlike synthetic drugs, you don't need 99% purity or structure elucidation of every constituent. "Totality of evidence" approach allowed.

Nonclinical Safety: Leveraging Prior Human Use

Standard drug: Requires full battery: safety pharm, genotox, 2-species acute/chronic tox, repro tox, carcinogenicity.

Botanical drug FDA may _reduce or waive_ some studies if there is adequate documentation of prior human experience.

For Ashwagandha

- 3,000+ years use in Ayurveda helps, but FDA wants details: formulation, dose,

duration, route, population, AEs

- If prior use differs significantly from proposed drug product in plant part, extraction, or dose, new tox studies required

- Genotox assays usually still required: Ames + in vitro chromosomal aberration[33].

✓ **Clinical Requirements: No Shortcut on Efficacy**

Must still conduct adequate and well-controlled trials per 21 CFR 314.126. No exemption for traditional use.

Phase 3: 2 trials typically needed. Endpoints must be clinically meaningful for the disease claim. Example: If seeking "treatment of generalized anxiety disorder", use HAM-A scale.

Challenge for Ashwagandha: Most U.S. clinical data is on stress/sleep = structure/function. To get a _drug claim_ for "anxiety disorder", new trials to FDA standard needed. Cost: $50M-$200M+.

✓ **NDI VS GRAS VS FOOD PATHWAY**

NDI may be the preferable regulatory strategy route. Under this scheme, use is based on recommended intake, and you can exclude higher-risk populations (young children, pregnant mothers, etc.). Under this scheme, notification to the FDA is mandatory, with the FDA responding within 75 days.

Both routes have advantages, but which route you should take will depend on several factors. Choosing the right path for your ingredient is difficult without expert support and, if you choose the wrong route, you might experience unnecessary delays.

SGS solutions

Partner with SGS for unrivaled expertise in filing GRAS and NDIN. We work with your team, guiding you towards the optimal regulatory route for your product. Our experts can conduct comprehensive gap analyses, oversee necessary testing, assemble safety and manufacturing data, and meticulously prepare and review your final dossier for submission [34].

✓ **Regulations Governing BE Studies for USFDA**

• 21 CFR 314.94(a)(7)-requires BE documentation in support of an ANDA.

• 21 CFR 320.21(b)-requires submission of evidence that the proposed drug product is bioequivalent to the reference listed drug or information supporting waiver of evidence demonstrating in vivo bioequivalence (i.e., 21 CFR 320.22).

• 21 CFR 320.22-contains requirements regarding the criteria for a waiver of the in vivo bioequivalence study requirement.

• 21 CFR 320.23(b)-contains requirements stating that: Rate and extent of absorption do not show a significant difference when administered at the same molar dose of the active moiety under similar experimental conditions – Bioequivalence may be demonstrated by scientifically valid methods for drug products not intended to be absorbed into the bloodstream.

• 21 CFR 320.24(a)– FDA may require in vivo or in vitro testing, or both, to measure the bioavailability of a drug product or establish the bioequivalence of specific drug products. – Applicants shall conduct bioavailability and bioequivalence testing using the most accurate, sensitive, and reproducible approach available [35].

• 21 CFR 320.24(b)-Approaches for demonstrating BE

- In vivo pharmacokinetic (PK) study in whole blood, plasma, serum or other appropriate biological fluid or a correlated in vitro study.
- In vivo urine excretion study.
- In vivo pharmacodynamic (PD) study.
- In vivo comparative clinical endpoint BE study.
- In vitro test acceptable to FDA

types of new drug applications:-

1. An application that contains full reports of investigations of safety and effectiveness section 505(b)(1) NDA;

2. An application that contains full reports of investigations of safety and effectiveness but where at least some of the information required for approval comes from studies not conducted by or for the applicant and for which the applicant has not obtained a right of reference section 505(b)(2) NDA; and

3. An application that contains information to show that the proposed product is identical in active ingredient, dosage form, strength, route of administration, labelling, quality, performance characteristics, and intended use, among other things, to a previously approved product section 505(j)ANDA.

❖ CDSCO FRAMEWORK

The Drug and Cosmetics Act, 1940 was enacted by the Department of Health under the Ministry of Health and Family Welfare after receiving the assent of the Governor General on April 10, 1940 and came into force on April 1, 1947.It regulates the import, manufacture, distribution and sale of drugs, biosimilars and medical devices. The primary objective of the act is to ensure that the drugs, biosimilars and medical devices sold in India are safe, effective and conform to state quality standards. The original version of Act has 5 Chapters, 38 Sections and 2 schedules [36].

The Rules are established by the government of India through the Drugs and Cosmetics Act, 1940. The Rules have also been amended time to time to meet the needs of the time and to rectify any deficiencies noticed during the implementation:

- The Drugs and Cosmetics Rules, 1945
- The New Drugs and Clinical Trial Rules, 2019

✓ **Objectives of Drug and Cosmetic Act 1940**

- To regulate the import, manufacture, distribution and sale of drugs & cosmetics through licensing.
- Manufacture, distribution and sale of drugs and cosmetics by qualified persons only.
- To prevent substandard in drugs, presumably for maintaining high standards of medical treatment.
- To regulate the manufacture and sale of Ayurvedic, Siddha and Unani drugs.
- To establish Drugs Technical Advisory Board (DTAB) and Drugs Consultative Committees (DCC) for Allopathic and allied drugs and cosmetics.

✓ **Schedules of Drug and Cosmetics Act 1940**

First Schedule- Names of books under Ayurvedic and Siddha systems.

Second Schedule– Standard to be compiled with by imported drugs and by drugs manufactured for sale, sold, stocked or exhibited for sale or distribution.

1. **Drugs and Cosmetics Act 1940 with respect to cosmetics**

Drugs and Cosmetic Act defines cosmetic as "cosmetic" means any article intended to be rubbed, poured sprinkled or sprayed on, or introduced into, or otherwise applied to, the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetic. It Regulate the import, manufacture and sale or distribution of drugs and cosmetics through Licenses and permits etc.

2. **Drug & Cosmetic Act 1940 with respect to Biologicals**

Drugs and Cosmetics Act and Rules made there under has stated rules for import, manufacture and sale of biological products in India:

Manufacturing for the purpose of Examination, test or analysis of vaccines, r-DNA products, Stem cells and Cell based products, Blood & Blood Products, Veterinary vaccines. Requirement of Clinical Trials in human and Field Trials in animals before marketing authorization. Issuance of Registration Certificate, Import License and Test Licence. Manufacturing license under CLAA Scheme for human and veterinary vaccines, rDNA products and blood products. Export NOC and Re-packaging Permission under Rule 37 for human and veterinary vaccines and rDNA products. Requirements of Shelf life and storage conditions for biological products. Reduced requirements for preclinical and clinical data based on demonstration of similarity in the comparative assessment Conditions for waiving local clinical trials to provide patients with earlier access to biological products. The time-bound review of applications.

3. **Drugs and Cosmetics Act 1940 with respect to Drugs**:

Drugs and Cosmetics Act and Rules made there under has stated rules for import, manufacture and sale of biological products in India:

Grant of NOC for issuance of Form 29 (manufacture of drugs for the purpose of Examination, test or analysis).

- Approval of Clinical Trials in human and Field Trials in animals.
- Grant of Marketing Authorization of drugs.
- Grant of Registration Certificate, Import License and Test Licence.
- Issuance of Form 28-D Licensing under CLAA Scheme for drugs.

- Issuance of Export NOC and Permission under Rule 37 for.
- Shelf life and storage conditions requirements
- Conditions for waiving local clinical trials.
- The time-bound review of applications.

4. Drug & Cosmetic Act 1940 with respect to Medical Device:

In India, the manufacturing, import, sale, and distribution of medical devices are regulated under India's Drugs & Cosmetic Act and Rules (DCA). Every single medical device in India pursues a regulatory framework that depends on the drug guidelines under the Drug and Cosmetics Act (1940) and Drugs and Cosmetics runs under 1945.

Drug & Cosmetic Rules, 1945

The Drugs and Cosmetics Rules, 1945 are the rules which the government of India established through the Drugs and Cosmetics Act, 1940. These rules classify drugs under given schedules and present guidelines for the storage, sale, display and prescription of each schedule. The drug rules were promulgated in December 1945 and enforcement of these rules started in 1947. The rules comprise of 19 Parts and 20 schedules. The part IV, part VII, part VIII and part IX states rules and regulations for Import, manufacturing and sale of drugs, biological and medical devices. The part X explains provisions solely applicable to biological products. The Part X describes naming, container, labelling, standards related to quality and testing of biological products [37].

✓ **AYUSH**

Herbal medications (HMs) have been acquiring expanded ubiquity among purchasers in both created and non-industrial nations. As indicated by the World Health Organization (WHO), 60% of the total populace, and 80% of the populace in non-industrial nations relies upon HMs for their medical care needs. Global consumption of HMs has grown significantly from $20 billion in 1997 to $83 billion in 2008. While a scope of definitions exist for HMs, in this examination, HMs are characterized as "herbal medications arrangements that are fabricated modernly in which the dynamic ingredient(s) is/are simply and normally unique plant substance(s), which is/are not synthetically adjusted and is/are liable for the general remedial impact of the item". The public commonly perceive HMs as safe, yet there are concerns about their safety too. A few antagonistic impacts, some of them perilous, can emerge from dynamic fixings themselves, just as contaminated of HMs with ordinary meds, herbal drug cooperation's and improper HMs plans. Be that as it may, critical HM security issues additionally emerge fundamentally from the improper administrative order of HMs. For instance, in the United Stated (US), HMs are delegated dietary enhancements, with prerequisites for assessing quality and wellbeing less rigid than those for restorative items. Implying that these items don't need evaluation by the public medication administrative power (DRA) before their advertising. This has specific ramifications for some nations in the Eastern Mediterranean Region (EMR), which import most of their HMs from different nations including the US. For a drug fabricating organization to import and disseminate HMs in these nations, it should choose nearby specialists, who follow up for the drug organization in correspondence with the mindful DRA toencourage the accommodation of all documentation and materials for showcasing the item. Any way in present day current medication is a lot of created in by far most of the world, yet at the same time world is searching for elective normal pathways where spices are assuming a vital part for public interest. Through the commitment of numerous analysts on decency of numerous spices are distinguished. Ayurveda has just referenced use of numerous spices for their remedial use. Contingent upon Ayurveda huge no of organization creating numerous items for restorative and valuable use. Prior it was accepted that Hm's items has no result except for now it is realized that spices are not generally protected. On the off chance that we investigate natural items advertised in India we effortlessly found the don't bears item handout; they don't have signs for use [38]. Nonetheless on the off chance that we contrast the equivalent and allopathic meds we will locate a colossal distinction. To be accessible in the market a drug organization goes through a lot of cash to build up its adequacy which goes through an alternate degree of models like bioavailabilityharmfulness, wellbeing, clinical information and so forth However, natural items don't nothing and results dis appointment of greatest Hm's item. On the off chance simply investigate the guideline status on Hm'item around the planet we will come to know how severeis different nations in wellbeing of human.

India is pioneer of Hm's medications the antiquated writing of spices is has placewith India. Yet, the cautiousness over natural meds in India is poor. Nations around the globe are promoted their Hm's item in India. Due to globalization and internet advertising low quality items are sold in India. Connection of natural items are not tried, clinical preliminary not performed, pharmacovigilance not successful. Any way AYUSH has acquainted guideline with control creation of natural items however in India in the time of 1945. Yet, it is as yet being worked on. Worldwide Trade of Herbal Drugs is dependent upon consistence with the International deals like Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Phytomedicines or natural prescriptions normally referred to in the market as Hm's drugs. Which is the utilization of plants box diverse route for remedial or restorative reason. This arising Hm's market is managed by AYUSH which is Ministry of Ayurveda, Yoga and naturopathy, Unani, Siddha and Homeopathy under the Drug and Cosmetic Act (D and C) 1940 and Rules 1945 in India. Makers are told to submit to AYUSH rule for showcasing of natural item. All the more vitally they should go in like manner Drug and corrective demonstration, segment C and D for definition structure, authorizing, naming, producing, pressing, quality and fare. Moreover great assembling practice (GMP) has additionally been actualized through schedule "T" in 2016. Hm's medications are controlled under the Drug and Cosmetic Act (D and C) 1940 and Rules 1945 in India, where administrative arrangements for Ayurveda, Unani, Siddha medication are obviously set down in Chapter IV-A. There are 18 diverse area are available from form 33C to 33
O. Its goal to guarantee that the examinations are deductively and morally stable and that the clinical properties of the ASU medication under scrutiny are appropriately reported.[39] The rules try to set up two cardinal standards: insurance of the privileges of human subjects and credibility of ASU medication clinical preliminary information generated. According to World Health Organization (WHO) Hm's prescriptions are of three kinds: Raw plant materials, prepared plant materials and Medicinal natural items. In India, Hm's drugs are controlled by the Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy (AYUSH). As indicated by meanings of natural therapeutic item, Hm's medication substances and Hm's arrangements are as per the following:

- Herbal therapeutic item: It is characterized as any restorative item, only containing as dynamic fixings Sadhika et.al International Journal of Drug Regulatory Affairs. 2021; 9(1): 78-86 e-ISSN: 2321-6794 at least one natural substance or at least one Hm's arrangements, or at least one such Hm's substances in blend with at least one such Hm's arrangements.
- Herbal substances: These are essentially entire, divided or cut plants, plant parts, green growth, parasites, and lichen in a natural, typically dried, structure, however at times new.
- Herbal arrangements: These are the arrangements acquired by exposing natural substances to medicines like extraction, refining, articulation, fractionation, refinement, fixation or aging. These incorporate comminuted or powdered Hm's substances, colors, removes, fundamental oils, communicated squeezes and prepared exudates [40].

❖ CASE STUDY ON ASHWAGANDHA

- **ABSTRACT**

Ashwagandha (Withania somnifera L.) root powder and extracts have long been used in Ayurvedic medicine to improve sleep and anxiety. Recent scientific investigations into its efficacy have shown promise for relief from anxiety, insomnia and stress and for improving the immune system. It has also been suggested that oxygen uptake in the cardiovascular system, muscle strength, cognitive function, the reproductive system and the aging process significantly benefit from ashwagandha treatment. Since the herbal remedy is taken daily by millions of people in India, China and parts of the West, it is interesting that there are very few case reports of side effects directly attributed to the treatment, suggesting that the administration of ashwagandha preparations may be safe. Currently, neither the European Medicines Agency nor the FDA considers ashwagandha as a drug or general health supplement. Therefore, ashwagandha products are marketed in the West as dietary supplements so that users may be exposed to unscrupulous vendors. In this narrative/literature review, scientific findings from basic research and

human clinical trials on herbal remedies, spanning the period from 1994 to date, were critically evaluated for the purpose of highlighting knowledge gaps to provide context for new research. Such investigations will provide evidence for the efficacy and safety of ashwagandha treatment, thus making the herbal preparations more accessible to a wider audience [41].

- **INTRODUCTION**

Ashwagandha (botanical identification: Whitania somnifera (L.)), also known as Dunal or winter cherry (in English), is of the family Solanaceae, like potato and tomato. The leaves and stems from members of the Solanaceae family, which also include auberges, can be harmful and even toxic due to high concentrations of glycoalkaloids and should not be eaten. However, the potato and the fruits from tomato and aubergine plants are known and used worldwide for their nutritional advantages. Ashwagandha has been known in the Ayurvedic tradition for almost 4000 years, with the leaves and stems being avoided due to their high content of cytotoxic and liver-damaging phytochemicals. By contrast, ashwagandha root, in the form of a powder or as a water extract, is very useful as a food and herbal remedy and does not have side effects.

Ashwagandha is often referred to as “Indian ginseng”, though this is very misleading, as the plant has genetically nothing in common with ginseng. Ashwagandha is a large family consisting of many different morpho- and chemo-subspecies. So, when speaking about ashwagandha, one should be aware of the huge variety in appearance and in the phytochemical content of the ashwagandha subspecies.

Ashwagandha is a perennial plant that grows well in India and in most Asian and African countries, as well as in Europe, mostly in Spain. The plant is an erect subshrub with a green stem and leaves and yellow or greenish flowers. It flowers all year, and the flowers are followed by small orange-reddish berries containing seeds (see Figure 1). Ashwagandha normally grows about 75 cm high but can be up to two meters high. It grows mainly on wasteland up to an altitude of 1800 m, but can also be cultivated in the lowlands, where the root is used as a nutritional supplement [43].

Figure 1) Ashwagandha plant, including the root. As is the case for other plants of the Solanacea family, the roots of the ashwagandha plant are not poisonous.

The fresh roots are pale to yellowish-brown with a creamy interior. A main root often bears much smaller secondary roots, which are normally removed before the main root is dried and powdered (Figure 1). The resulting powder is light grey to yellowish-brown. Commercial ashwagandha can be sourced from wild or cultivated plants. There are at least five different cultivated species in India, and other species of ashwagandha are also cultivated in the United States of America [42].

1. The Evolution and History of Ashwagandha

The species name “Somnifera” refers to the fact that in old times, the plant was used to promote sleep. Later in history, the plant was also known for its ability to generate energy and modify stress. The root has a strong aroma that may direct our thoughts towards horses, for “Ashwa” literally means horse in Sanskrit, while “ghanda”, in the same language, means smell. This can project the idea that the plant will make you “strong like a horse”. As the name suggests, ashwagandha has been used in ancient Ayurvedic

traditional medicine for centuries for treating different ailments, including tiredness, sleep disturbances, lack of energy, stress, hormonal disruptions and lack of sexual drive.

2. Utilization in Modern Times

During the last 50 years, interest in ashwagandha and especially the root of the plant has drawn increasing attention from scientists. Applications of modern technology in examining the constituents and effects of the plant parts have shown that extracts, especially of the root, can improve sleep, as well as mediate analgesic, antiarthritic, anti-inflammatory and muscle-strengthening effects. Results from these studies and the accompanying clinical studies, which also show promising results on stress, including a lowering of cortisol and a normalization of serum TSH and thyroid hormones in hypothyroid patients, are reviewed below in.

3. Content of Different Metabolites in Ashwagandha Root and Leaves

As stated earlier, only the main tap root, which is cylindrical and about 1 to 1.25 cm in diameter, is traditionally used for medicinal purposes. Smaller and more peripheral root fragments are discarded as they contain less of the active ingredients. Traditionally, the leaves were only used in topical applications or for treating specific conditions under the supervision of Ayurvedic doctors.

An example of the wide variety of metabolites detectible in ashwagandha roots, leaves and fruits is presented in Table 1. There is a distinct quantitative difference in the contents of secondary metabolites extractable from the leaf, as compared to those from root parts. For example, withanone (class—steroidal lactones; a subclass of triterpenoids) is about six times higher in the leaves than in the root, while withaferin A (a more toxic withanolide) is more than twenty times higher in the leaves than in

the root. Unfortunately, withanolides are often declared as one group on the labels of commercial ashwagandha preparations, whereas, as shown in Table 1, there are several types of steroidal lactones, each with differing biological effects. This practice makes it impossible to determine if leaves, which are rich in withaferin A and withanone, have been included in the so-called ashwagandha root product[44].

As the type of solvent used in the preparation of the plant extracts is also very important. This means that it matters if an extract was made with alcohol (e.g., methanol) or water or a combination of both, since there are water-soluble, as well as fat-soluble, phytochemicals from the same ashwagandha plant. The same paper also analyzed the phytochemical classes and went on to investigate the primary and secondary metabolites. For example, they identified porphyrin metabolites like chlorophylls and their degraded secondary metabolites, pheophytins (not included in the tables). These secondary metabolites are formed during the extraction process, where chlorophylls and pheophytins are observed in leaves and not in roots.

Total metabolic content in leaves and roots.

Extract Partition	Total Metabolite Content (mg/g of Dry Weight)	
	Leaf	Root
Hexane	34.29 ± 2.0	4.44 ± 0.8
Chloroform	35.71 ± 1.5	10.00 ± 1.0
n-butanol	28.57 ± 1.6	11.11 ± 1.2
Methanolic water	228.57 ± 5.2	15.00 ± 1.6

Although most products containing ashwagandha are declared as root-only products, it is not uncommon to find unscrupulous vendors partially replacing or augmenting the expensive root material with the significantly cheaper and easier-to-obtain aerial parts. As previously stated, ashwagandha leaves are rich in ingredients like withaferin A and whitanone, which can exert liver injury. Therefore, extracts from leaves should never be a part of an ashwagandha product[45].

4. The Need for Standardization for Ashwagandha Root Preparations

The main active ingredients in ashwagandha are the steroidal lactones or withanolides, and not surprisingly, six different types are the basis for the standardization of some leading products, like KSM66, which is by far the most sold and researched product worldwide. However, some other phytochemicals that may be contributing to the activity can be polyphenolic compounds and flavonoids. So, when speaking about standardization, the above-mentioned ingredients are candidates that should be used. In addition, the toxic withanone and withaferin A, which occur in high concentration in leaves, may be used for standardization. Ideally, these ingredients should be at very low concentrations in prospective ashwagandha

products. Better still, they should be below detection levels to ensure that ashwagandha leaves or other unsafe ingredients are not included in the formulation under consideration.

5. The Importance of Standardization

Root content of alkaloids: Data from 15 very promising genotypes of ashwagandha showed that the total root content of alkaloids differed from 0.24% to 0.90% dry weight. This study was followed by a similar study, where 25 different genotypes of ashwagandha root showed a variation in alkaloids from 0.16 to 0.96% (dry weight), indicating that when generating herbal medicine, the species chosen is also of great importance. This pattern with alkaloids was also observed when the total amount of withanolides was investigated. Here, the variance was from 0.23 to 1.11% when comparing levels in different cultivated ashwagandha and some wild varieties. The amount of starch and fibers also showed a wide variation when 25 different genotypes were tested. These data clearly indicate the importance of the subspecies chosen, in addition to the extraction methodology used when making the preparation.

As with all plant-sourced materials, the quality of the ashwagandha product is dependent on the soil properties, the use of fertilizers or manure, the amount of rain that falls during the growth process, the number of sunny hours, the altitude where the plants grew and the extent to which the plant matured before being harvested. For all these reasons, when determining what ashwagandha product one should choose, key factors to be considered, especially as regards the criteria used for the standardization of the product, should include natural variability in plant composition (due to genetics), environment and processing.

6. Are Raw Materials and Formulations of Commercial Ashwagandha Preparations the Same?

It is often puzzling when one goes to a store or a marketplace to buy a potato or an apple, where there are so many different species that taste different and perhaps do not contain the same ingredients. For just as with grapes used for making wine, the quality of the vegetables can differ from year to year and from location to location.

Similarly, when one buys an ashwagandha root product, one ought to be watchful of the subspecies of the source plant, where it was grown, when it was harvested and how the finished herbal remedy was prepared because of the factors outlined above in Section 2.2. There are many wild ashwagandha species around the world. In India, where the plant is cultivated, there are at least five different species grown, and the same is true for the USA. The tables presented in this review include the results of analyzing metabolites of subspecies that have been cultivated for two decades by an Indian family-owned company and marketed under the name “KSM66”. The KSM66 is extracted using a very standardized extraction procedure, involving the use of milk and water. Other established products in the market may have used ethanol, methanol or combined extractions, resulting in totally different results, as shown in. So, when considering whether a particular ashwagandha preparation is relevant or not, or when discussing if ashwagandha should be avoided due to possible side effects or not, two pertinent questions to be considered are as follows:

- Is the product a good-quality ashwagandha root extract?
- Are there chemically testable assurances that the product does not contain contaminations by elements from other plant parts or materials that are not from the ashwagandha root?

Given the wide range of dietary supplements and the varying quality of products in the market, it is preferable to choose a clinically well-documented and characterized product [46].

7. The Impact of Ashwagandha Root Preparations on Health and Disease

✓ Ashwagandha's Influence on the Immune System

The immunomodulatory effects of ashwagandha, defined as an increase in IgA, IgM, IgG, IgG2, IgG3 and IgG4, were seen in a placebo-controlled study in which humans were administered the herbal medicine for 30 days. Aside from the effect on immunoglobulin content, there was also a significant improvement in cytokines like IFN-gamma and IL4 and in CD45+, CD3+, CD4+, CD8+ and CD19+, as well as in natural killer (NK) cells. No such changes were observed in the placebo group. It is important to note that all cellular and biochemical markers of the immune system, which were improved, were still within the normal physiological range of the respective markers, indicating that the treatment did not give rise to uncontrolled immune stimulation. In a study with human monocyte cells, ashwagandha inhibited TNF alpha, IL-1 and superoxide production in a dose-dependent manner. Such findings were reinforced in an animal study with rats, where a dose-dependent reduction in CRP and oxidative stress was evidenced upon ashwagandha administration, as indicated by a decrease in lipid peroxidation and in glutathione-S-transferase activity. In another in vivo rat model, where gout was induced by monosodium urate crystals, edema was reduced to normal levels, indicating a potent anti-inflammatory effect with ashwagandha treatment. In contrast to the control group treated with NSAID, there were no gastrointestinal side effects, indicating that ashwagandha can retard amplification and propagation of the inflammatory response without causing any gastric injury [47].

✓ Ashwagandha's Influence on the Cardio-Respiratory System, Oxygen Uptake, Muscle Strength and Recovery

In a rat model with isoprenaline (isoproterenol)-induced myocardial necrosis, Withania somnifera exerted cardio-protective effects. An augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered hemodynamic parameters, initiated earlier by the artificially induced myonecrosis, were suggested as sources of the observed cardio protection observed with the treatment. In addition, there was improved cardiorespiratory endurance in healthy younger athletes in a placebo-controlled study of ashwagandha water extract (KSM66) administered for three months. The study, which assessed the maximal oxygen uptake and self-reported quality of life, showed a great and statistically significant improvement in VO2 max (maximal oxygen uptake/consumption) after a 20 m shuttle run test and in quality of life (Qoli), which was associated with KSM66 treatment. These data were also supported in a double-blinded, randomized, placebo-controlled study with 50 athletes, in which VO2 max, recovery, energy and the ability to manage stress were improved as a result of ashwagandha treatment (KSM66). The same was observed in an earlier study (2010), in which a group of young adults in a placebo-controlled clinical trial used ashwagandha for 8 weeks. In a double-blinded study, where younger adults were treated with ashwagandha for two months, muscle strength and recovery after strenuous work were tested. In accordance with the above-reported studies, the ashwagandha (KSM66)-treated group had a highly significant improvement in muscle strength when tested with the bench press exercise and leg extension exercise. Furthermore, the volunteers also developed a significantly greater muscle size and improved their endurance and VO2 max. Compared to placebo, ashwagandha-treated healthy volunteers showed a significant decline in exercise-induced muscle damage as indicated by serum creatinine kinase measurements, combined with an increase in serum testosterone and a decline in body fat percentage. Muscle force and serum contents of total cholesterol were likewise improved when healthy volunteers were treated with up to 1250 mg of ashwagandha in one month. An improvement in muscle recovery, as well as in levels of free and total testosterone, was also documented with daily administration of 600 mg daily to healthy younger volunteers for two months. The above studies are summarized by a meta-analysis on physical performance and ashwagandha, concluding that ashwagandha supplementation was more efficacious than the placebo for improving variables related to physical performance in healthy men and females. Recently, a 12-month study comprising 191 healthy volunteers with an age range of 18–65 years old, treated with 600 mg of ashwagandha KSM66 daily, likewise showed an improvement in serum testosterone levels, possibly due to a stress-reducing effect. It is important to state that although the levels are modified, they remained within normal range.

✓ The Impact of Ashwagandha on Stress, Anxiety, Insomnia and Sleep

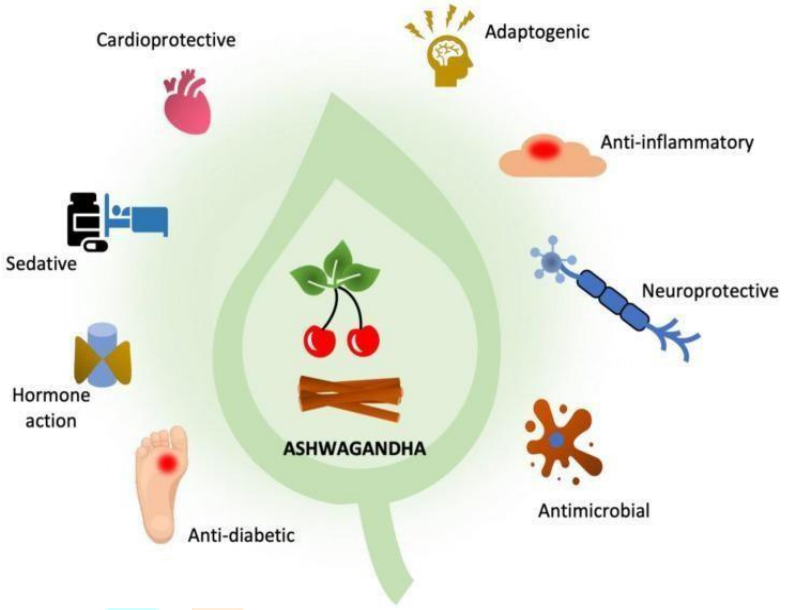
Ashwagandha (full-spectrum) root extract was tested in volunteers under chronic stress in a randomized, double-blind, placebo-controlled set-up. The study included measurement of stress using standard stress and anxiety questionnaires. Serum cortisol was measured throughout the study, which lasted for 8 weeks. The ashwagandha root extract group showed a highly statistically significant reduction in stress after 8 weeks of treatment when compared to the placebo. In accordance with the stress reduction, serum cortisol dramatically declined. It should also be noted that there was a significant reduction in anxiety and insomnia because of the treatment, in addition to a decrease in social dysfunction and depression, as revealed with participants' responses in GHQ-28 questionnaires. A normalization of serum cortisol and significant decline in stress levels (when evaluated through questionnaires) were also documented in another placebo-controlled, randomized, double-blind study. In a similar study, using the Hamilton anxiety rating scale, ashwagandha root extract treatment also resulted in a statistically significant lowering of anxiety and in a normalization of serum cortisol. A statistically significant reduction in serum levels of cortisol, a reduction in the level of stress and improvement of sleep were also observed when 60 and 50 middle-aged volunteers, represented by both sexes, were tested for 8 and 12 weeks, respectively.[48] An alleviation of stress, anxiety and improvement of quality of life, as well as a (lowering) normalization of serum cortisol, in addition to an increase in blood levels of serotonin, was reported. The above summary of the impact of ashwagandha on stress, anxiety and sleep is further supported by two systematic reviews and meta-analyses of randomized controlled trials. As an anecdote, it should finally be mentioned that even in randomized, placebo-controlled studies in cats and dogs, ashwagandha treatment for one month resulted in a (lowering) normalization of serum cortisol and in a decline in fear and anxiety [49].

✓ Ashwagandha and Cognitive Function

The efficacy and safety of ashwagandha root extract on affecting cognitive function in healthy stressed adults was tested in a randomized, double-blinded and placebo-controlled clinical trial, involving 130 men and women. The results showed that 300 mg of the root extract taken daily significantly improved memory and focus, psychological well-being and sleep quality, as well as reduced the level of stress. Aside from these positive effects on general well-being, the treatment, which lasted for three months, was safe, without reports of side effects. In another efficacy and safety study, ashwagandha root extraction was tested in volunteers with moderately impaired cognition. After 8 weeks of study, the ashwagandha-treated group (300 mg daily) showed statistically significant improvements in immediate and general memory. The treated group also showed enhancements in executive function, sustained attention and information processing speed. Ashwagandha extraction was later tested in Wistar rats of both sexes. Treatment resulted in increased brain function, as shown from electrophysiological changes in the delta and gamma bands, and the changes were statistically significant. It is generally agreed that herbal remedies need to be used for some time before any impact of relevance can be measured, as herbal remedies generally do not work within hours. Very interestingly, it turned out that acute supplementation with ashwagandha (400 mg) improved selected measures of executive function, helped to sustain attention and increased short-term memory in test subjects. There were also indications that administration of 225 mg of ashwagandha (nearly half the test dose) can improve measures of memory, attention and executive function while decreasing the perception of tension and fatigue.

- **BIOLOGICAL ACTIVITY**

Neuroprotective and Anti-Neurodegenerative Effects



✓ **. Ashwagandha Use in Alzheimer's Disease**

For many years, the phenomenon of aging populations has been observed, which also implies a significant increase in the percentage of people suffering from dementia syndromes. Dementia is a syndrome with a multifactorial aetiology characterized by a range of symptoms caused by a brain disease, typically with a chronic and progressive course. This condition affects higher cortical functions, including memory, thinking abilities, orientation, comprehension, learning abilities, and emotional control.

Neurodegenerative diseases cause the destruction of the central nervous system, resulting in irreversible damage. Over the course of Alzheimer's disease, an abnormal deposition of β-amyloid protein in the brain is observed. In its fibrillar form, it has a neurotoxic effect because it induces the formation of free radicals and impairs glucose transport in neurons, which leads to cell damage and death. Hyperphosphorylated τ proteins in Alzheimer's disease form clusters surrounding the core of the senile plaque, consisting of β-amyloid. Physiologically, τ proteins stabilize microtubules, along with other proteins. Accumulating senile plaques are accompanied by microglia (inflammatory response cells), attempting to break down and remove damaged and dead neurons as well as senile plaques. Microglia cells produce toxins, destroying both diseased and healthy cells and enhancing the brain's inflammatory response.

In studies conducted on human nerve cells, Ashwagandha has been shown to neutralize the toxic effects of β-amyloid, an implication in neurocognitive impairment during HIV infection [50]. A study was conducted on rats that were orally administered vitanonan ingredient isolated from the root of Whitania somnifera. Significant improvements in cognitive function were observed as a result of the inhibition of amyloid β-42, and a reduction in pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and MCP-1, nitric oxide, and lipid peroxidation was also observed. There was also a decrease in the activity of β and γ-secretase, enzymes responsible for the formation of insoluble neurotoxic aggregates of β-amyloid. In addition, withaferin A extracted from Ashwagandha appears to be a promising ingredient in terms of Alzheimer's disease treatment. It works by reducing β-amyloid aggregation and inhibiting τ protein accumulation. In addition, withaferin A is responsible for inhibiting oxidative and pro-inflammatory chemicals and regulating heat shock proteins (HSPs), the expression of which increases when cells are exposed to stressors. However, more medical studies are needed to assess the safety of withaferin A and to confirm its neuroprotective effects in the treatment of Alzheimer's disease. In addition, it has been noted that withaferin A from Ashwagandha extract significantly inhibits not only the production of amyloid β, but also the gene expression of neuroinflammatory molecules related to NF-κB . A study was also conducted in transgenic mice that were given a half-purified extract of Whitania somnifera root containing mostly withanolides for 30 days. After this time, it was noted that Ashwagandha offset the negative effects of Alzheimer's disease by increasing the number of the LDL receptor-related protein

LRP1 (low density lipoprotein related protein 1) in the liver. Increasing LRP1 levels reduced amyloid β and reversed the behavioural deficits in Alzheimer's disease. Studies suggest that LRP1 functionally modulates the steps required to form the β-amyloid precursor protein APP, which is critical for amyloid β production and APP processing. The study also shows that LRP1 is a key regulator of protein proliferation τ . The effect of Withania somnifera derivatives on the formation of β-amyloid 42 deposits in Alzheimer's disease was studied. It was observed that withanolide A, withanolide B, witanoside IV and witanoside V interact with the hydrophobic core of β-amyloid 1–42 in the form of an oligomer, which prevents further interaction with monomers and reduces aggregation. In another study, Withania somnifera extract was bioconverted by the fungus Beauveria bassiana. The resulting cysteine and glutathione derivatives of withaferin A were purified and fully characterized. It was shown that CR-777, a glutathione derivative of withaferin A, has neuroprotective effects and can be a protective agent against many neuronal stressors. It is known that one of the most important components of Ashwagandha–withanolide A combats neurodegenerative processes in Alzheimer's disease and Parkinson's disease. However, it was only relatively recent that the ability of withanolide A to penetrate the blood-brain barrier (BBB) was demonstrated. In another study, adult mice were administered three different doses of vitanolide A intranasally: 1 mg/kg, 5 mg/kg, 10 mg/kg. The intranasal administration allowed for the penetration of the test substance into the cortex and cerebellum. After treatment with withanolide A, a significant reduction in cerebral infarction, improvement in blood-brain barrier function, and reduction in cerebral oedema were noted. An improvement in biochemical parameters and a reduction in excessively high levels of neurotransmitters, which had been caused by previous ischemia, were also noted. The highest dose administered (10 mg/kg) significantly reduced morphological damage, apoptosis and necrosis in brain tissues[51].

- ✓ **Ashwagandha Use in Parkinson's Disease**

In Parkinson's disease, the degeneration of the dopaminergic neurons of the nigrostriatal system is observed. This leads to an imbalance between dopamine's inhibitory action and acetylcholine and glutamic acid's excitatory action. Factors that induce the degeneration of nigrostriatal cells include:

Genetic conditions;

Endo- and exogenous toxic factors; Neuroinfections;

Oxidative stress; Reduced growth factors;

The sum of the action of several of the above factors.

The disease is slightly more common in men than in women, and although the cause is not known, it is thought that this may be due to the protective role of estrogen.

A study was conducted on rats with 6-hydroxydopamine-induced Parkinson's disease. Prior to an injection of 6-hydroxydopamine into the striatum, the rats were orally administered a Withania somnifera extract at doses of 100, 200, and 300 mg/kg body weight for 3 weeks. It was observed that administration of Ashwagandha significantly reduced lipoperoxidation, increased glutathione concentration, increased glutathione S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase activities, catecholamines, and dopamine D2 receptor binding and enhanced tyrosine hydroxylase expression.

Although Withania somnifera significantly improves biochemical parameters in Parkinson's disease, its effects depend on the dose administered. In addition, in a study conducted on fruit flies, it was shown that administration of a standardized methanol extract of Ashwagandha root counteracts deficits associated with Parkinson's disease. In mice with Parkinson's disease, administration of Ashwagandha extract not only improved biochemical parameters, but also reduced motor impairment compared to the control group. It has been observed that oral administration of Withania somnifera extract (100 mg/kg, i.p.) to mice increases the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and also normalizes the levels of lipoperoxidation markers in the striatum of the mice.

✓ **Use of Ashwagandha in the Treatment of Huntington's Disease** Huntington's Disease is an incurable disease. Current medications only work on the symptoms and slow down the progression of the disease. The disease is inherited in an autosomal dominant manner, which means that statistically, half of the offspring will receive the disease-causing allele. A mutation in the IT15 gene encoding the huntingtin (htt) protein on chromosome 4 leads to a conformational change in huntingtin, into its insoluble form. The N-terminal part of the mutant huntingtin protein, containing expanded polyglutamine repeats, accumulates leading to accelerated neuronal apoptosis. This leads to an imbalance of dopamine, GABA, serotonin, and acetylcholine.

The compound 3-Nitropropionic acid (3-NP) is a potent neurotoxin. It induces oxidative and nitrosative stress, inhibits complex II of the mitochondrial electron transport chain (resulting in a deficit of moma energy), and is responsible for biochemical and neurobehavioural changes very similar to those observed in Huntington's disease. In an animal model, symptoms of Huntington's Disease were artificially induced by applying 3-NP intraperitoneally. It was observed that the chronic administration of Ashwagandha extract had a beneficial effect on biochemical parameters and motor function due to the antioxidant properties of the plant studied.

There was a decrease in lipoperoxidation, a decrease in the levels of lactate and nitrate dehydrogenase, an increase in the levels of superoxide dismutase and catalase, and an unblocking of the mitochondrial complex and thus a restoration of ATP synthesis. The effect was dose-dependent—100 mg/kg and 200 mg/kg. In another study in mice, the beneficial effect of withaferin A, isolated from Ashwagandha, was demonstrated. The inability of cells to maintain proteostasis is a sign of aging and a hallmark of many neurodegenerative diseases, including Huntington's disease. In this mouse model, withaferin A ameliorates the impaired proteostasis by activating the heat shock response and delaying disease progression. The mice with Huntington's Disease treated with withaferin A lived significantly longer, and restoration of behavioural and motor deficits was also observed, including a reduction in body weight. Biochemical studies confirmed the activation of heat shock, the reduction of mutant huntingtin aggregates, and the improvement of striatal function in the brain in mice. In addition, withaferin A significantly reduced inflammatory processes, as noted by reduced microglia activity [52].

✓ **Treatment of Obsessive-Compulsive Disorder, Alcohol Withdrawal Syndrome**

Obsessive-compulsive disorder (OCD) is a chronic psychiatric disorder that involves patients experiencing symptoms in the form of intrusive thoughts and imagery. Patients perceive them as undesirable, unwanted, compulsive, and irrational. Although the severity of the cognitive disturbance can vary considerably from patient to patient, OCD makes daily life significantly more difficult—especially in its severe form, where it can significantly impair psychosocial functioning. Genetic and psychological factors play a significant role in the aetiology of OCD, but structural and functional abnormalities within the central nervous system are equally important. Obsessive-compulsive disorder is thought to be associated with the dysregulation of the serotonergic system. Ashwagandha root extract may be a helpful adjunct to SSRIs in the treatment of patients with obsessive-compulsive disorder[53]. A study was conducted on mice that exhibited behavioural symptoms similar to those observed in OCD. In this animal model, mice were administered a methanolic extract of Withania somnifera (doses: 10, 25, 50, 100 mg/kg) and an aqueous extract of Withania somnifera. It was observed that the administration of the aqueous and methanolic extract of Ashwagandha significantly improved behavioural deficits in the mice, without affecting motor activity. The results obtained were similar to those of the standard treatments: fluoxetine, ritanserin, and para-chlorophenylalanine.

✓ **Anti-Inflammatory/Immunomodulatory Effects**

Due to its properties, Withania somnifera is being studied for the treatment of many diseases associated with inflammation in the body, such as cardiovascular, pulmonary, and autoimmune diseases and diabetes, cancers, and neurodegenerative diseases. Preclinical studies have demonstrated the ability of this plant to regulate mitochondrial function and apoptosis and reduce inflammation by inhibiting inflammatory markers such as cytokines (including IL-6 and TNF-a), nitric oxide, and reactive oxygen species. Meanwhile, in a mouse model with lupus, a potential inhibitory effect of Ashwagandha root

powder was demonstrated in conditions such as proteinuria and nephritis. Ashwagandha is also being investigated for its efficacy in rheumatoid diseases. In a study conducted in an animal model, Withania somnifera root powder was administered orally to rats for three days, one hour before inflammation was induced by an injection of CFA (complete Freund's adjuvant). In the control group (positive control), rats were administered phenylbutazone. Changes in the concentrations of a number of serum proteins, such as α2 glycoprotein, acute phase protein α1 and prealbumin, were demonstrated, along with a significant reduction in inflammation. In a study using the HaCaT human keratinocyte cell line, an aqueous solution from Ashwagandha root was found to inhibit the NF-κB and MAPK (mitogen-activated protein kinase) pathways by decreasing the expression of pro-inflammatory cytokines, including interleukin (IL)-8, IL-6, tumour necrosis factor (TNF-α), IL-1β, and IL-12, and increasing the expression of anti-inflammatory cytokines. Based on these results, it can be concluded that the anti-inflammatory effects of Ashwagandha could potentially be used in the prevention of skin inflammation. In a preclinical study of the anti-neuroinflammatory effects of Ashwagandha water extract (ASH-WEX) against lipopolysaccharide-induced systemic neuroinflammation, animals treated with ASH-WEX showed an inhibition of reactive gliosis; production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6; and expression of nitro-oxidative stress enzymes.[54][55] The underlying molecular mechanisms for the anti-inflammatory potential of ASH-WEX appear to involve inhibition of lipopolysaccharide (LPS)-activated NFκB, P38 and JNK/SAPK MAPK pathways. The results of this study suggest the potential use of Withania somnifera in suppressing nervous system inflammation associated with various neurological disorders.

- ✓ **Antibacterial Properties**

Drug resistance in micro-organisms is a major and growing threat, although now widely recognized. In recent years, a significant increase in infections caused by drug-resistant strains has become a major problem. It is known that the reckless and often unwarranted use of antibiotics has resulted in the development of drug-resistant strains, and in some situations, these drugs have become completely ineffective. Ashwagandha, therefore, appears to be a valuable addition to pharmacotherapy in the treatment of bacterial infections. Many of the drugs currently used to treat bacterial infections, despite their efficacy, have many dangerous side effects related to their toxicity. Ashwagandha is a safe, non-toxic plant with almost no side effects. In the studies that have been conducted, it has been proven to effectively inhibit the growth of methicillin-resistant Staphylococcus aureus and Enterococcus spp. Withania somnifera root extract has also been shown to effectively inhibit the growth of the Gram-negative bacteria Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Citrobacter freundi, and Klebsiella pneumoniae. The mechanism of its antimicrobial action is attributed to several of its properties; it has an immunomodulatory effect because it enhances immune reactivity (immunopotentiation), cytotoxic effects, and gene silencing.

Animal model studies also show that Withania somnifera is an effective treatment for salmonellosis, as it significantly alleviates the course of infection following infection with this pathogen. Withania somnifera can also be an effective anti-caries agent, depending on the concentrations used. It significantly slows the growth of bacteria present in the oral cavity, such as Streptococcus mutant and Streptococcus sobrinus. It also inhibits bacterial acid production, acid tolerance, and biofilm proliferation. It shows particular efficacy against Salmonella typhi. The witanolides isolated from Ashwagandha induce cell death (acts on promastigotes) in Leishamania donovani by activating the process of apoptosis (ROS release from mitochondria occurs) and disrupting the mitochondrial membrane potential.

- ✓ **Anticancer Effects**

Cancer is a group of diseases in which cell division proceeds in an uncontrolled manner. This is due to mutations in gene-encoding proteins that are involved in the cell cycle, such as proto-oncogenes and anti-oncogenes. Statistics show that cancer is a serious and growing health and social problem. Despite worldwide research efforts in the fight against cancer, it remains a major cause of death.

Studies have shown that various compounds isolated from parts of Ashwagandha, such as the root, stem, and leaves, exhibit anti-cancer properties. Therefore, they can be used to treat cancer alone or in combination with other chemotherapeutic agents. Witanolides are alkaloids present in the plant that show great anti-cancer potential. They are also the most promising compounds showing this action, as they play a major role in the induction of apoptosis. Ashwagandha is effective against cancers such as breast, colon, lung, prostate, and blood cancers. It acts as a chemotherapeutic agent against many different types of breast cancer, especially ER/PR-positive breast cancer and triple-negative breast cancer. In addition to its treatment, it also shows properties that prevent it. Research also suggests the potential of Ashwagandha in improving the quality of life of breast cancer patients. According to research, withaferin A derived from Ashwagandha is also effective in the treatment of melanoma. This compound induces apoptosis and also reduces cell proliferation and inhibits melanoma cell migration. The antitumor mechanisms of withaferin A in glioblastoma multiforme (GBM) were investigated. RNA-seq analysis, Western blot, immunofluorescence staining, qRT-PCR, and siRNA gene silencing were performed to determine the signaling pathways affected by withaferin A. It significantly inhibited GBM growth in vitro and in vivo and triggered intrinsic apoptosis of GBM cells. It arrested GBM cells in the G2/M phase of the cell cycle by defosphorylating Thr161 CDK1. This finding is important for the optimization of withaferin A-based regimens for the prevention and/or treatment of glioblastoma multiforme.

- ✓ **Antidiabetic Activity**

The use of Ashwagandha is also considered in the context of anti-diabetic effects. However, there are relatively few reports on this issue. The antidiabetic properties of the raw material were interestingly described in a review paper by Durg et al. The results of the preclinical studies were promising. Animal studies have shown its ability to lower blood glucose levels. In addition, Tekula et al.confirmed that Withaferin A can effectively control induced type 1 diabetes in rats through modulation of Nrf2/NFκB signaling and therefore has significant potential for therapy. In silico studies have also confirmed withaferin A's potential using molecular docking. However, only one clinical study from the year 2000 showed a direct blood-glucose-lowering effect.

On the other hand, many studies have shown a beneficial effect on the lipidemic profile. In a study conducted on white albino rats with hypercholesterolemia, a reduction in cholesterol levels and also in the antioxidant effects of Withania somnifera were observed. In the case of clinical trials on diabetes, despite not showing an effect on blood sugar levels, interesting results were achieved in improving the lipidemic profile, body weight, and blood pressure in a study by Agnihotri et al. Nayak et al. showed an improvement in the lipidemic profile and patient assessment with the DDS17 scale assessing patients' distress scale. Usharani et al. noted that the administration of a standardised Ashwagandha extract under the name SENSORIL improved antioxidant parameters and the lipidemic profile and demonstrated the tolerability and safety of the raw material. Usharani, et al., in spite of its tolerability and safety, demonstrated an effect on lipidemic profile and a change in the reflection index [RI].

- ✓ **Cardioprotective Properties**

The effect of Ashwagandha was studied in a group of albino rats in which myocardial necrosis was induced by isoprenaline treatment. A decrease in glutathione levels and a decrease in the activity of superoxide dismutase, catalase, creatinine phosphokinase, and lactate dehydrogenase were observed in the group of rats treated with Withania Somnifera. Lipid peroxidation levels also decreased significantly. These results indicate that Withania somnifera has a cardioprotective effect in an experimental model of isoprenaline-induced necrosis in rats. Studies were also conducted on rats in which cardiac ischemia was induced. This caused significant myocardial necrosis, an oxidation–antioxidation imbalance, and an increase in lipoperoxidation. Histopathological studies have noted that the administration of Withania somnifera significantly reduces damage to the heart caused by ischemia. Ashwagandha has a cardioprotective effect due to its anti-apoptotic properties and due to its restoring of the oxidative balance. The cardioprotective effect of withaferin A, a component of Ashwagandha known for its anticancer properties, was also studied. In this study in rats, low doses of withaferin A were shown to have a cardioprotective effect by upregulating the mitochondrial anti-apoptotic pathway due to an increase in AMP-activated protein kinase (AMPK) phosphorylation and an increase in the Bcl-2/Bax

ratio (AMPK) [87]. This enzyme is involved in a number of processes responsible for maintaining energy homeostasis at both the cellular and whole-body level[56]. AMPK regulates glucose, protein and fat levels in the nervous system and peripheral tissues and responds to hormonal signals by modulating food intake and energy consumption. It is also known that AMPK is activated by caloric restriction and is involved in a number of processes correlated with ageing and diseases that often occur in older people. AMPK restores energy balance and is therefore thought to improve quality and length of life [88,89,90]. Surprisingly, however, only low doses of administered withaferin A (1 mg/kg) showed a cardioprotective effect. Administration of higher doses (5 mg/kg) was not effective.

- ✓ **Treatment of Sleep Disorders**

Insomnia is defined as a situation in which the patient sleeps too little in relation to his or her needs, and this further leads to a deterioration in daytime functioning. Contrary to popular belief, insomnia is not just a condition in which nocturnal sleep is shortened, as the problem is much more complex and involves numerous complications. It is believed that insomnia can be related to difficulty initiating sleep, difficulty maintaining sleep or waking up too early. All of these symptoms can occur even when maintaining good sleep hygiene. The occurrence of these disorders leads to a poor sense of well-being, problems concentrating, emotional disturbances,cognitive disorders, and lack of motivation, which affects professional as well as social life. Researchers around the world agree that insomnia affects women more often than men. Old age is also a factor in insomnia. Unfortunately, there has been a significant increase in the proportion of people taking sleep medication, which indicates the scale of the insomnia problem. Nevertheless, it is believed that the condition is underdiagnosed. Epidemiological data differ by countries, which may be the reason for different methods of diagnosing this disorder. It is also worth noting that sleep constitutes approximately 30% of human life, hence the conclusion that the occurrence of any disorders in this area significantly disrupts the homeostasis of the organism.

- ✓ **Anxiolytic and Anti-Stress Effects**

Stress is defined as the body's biological resonse to external or internal stimuli. The compensatory response resulting from stress is called stress response and depends on the type of stress, its frequency, and its duration. It has been observed that stress as a factor disturbing homeostasis can not only exacerbate the symptoms of many diseases, but can also be their root cause. People who are under constant pressure at work or at home, and who feel overwhelming stress as a result, are much more likely to suffer from many diseases.

In the general population, anxiety disorders are the most common psychiatric problems. Unfortunately, due to their chronic nature and the compulsion to take multiple medications on a regular basis, some patients discontinue pharmacotherapy, which leads to relapses. The occurrence of numerous side effects, often unavoidable with conventional therapy, is cited as one of the reasons for discontinuing medication. Among other reasons, this is why it is so important to search for new therapeutics with fewer unwanted side effects. One study was conducted on a group of patients diagnosed with Generalized Anxiety Disorder (GAD). Participants were treated with SSRIs—Selective Serotonin Reuptake Inhibitors—and also took one capsule of Ashwagandha extract daily for six weeks. After the experiment, it was concluded that Withania somnifera extract could potentially support SSRI therapy in patients diagnosed with GAD syndrome.

It was also noted that Ashwagandha supplementation statistically and significantly reduced HAM-A (Hamilton Anxiety Rating Scale) and to a slightly lesser extent, reduced DASS-21 (Depression, Anxiety and Stress Scale). A reduction in morning cortisol and DHEA-S levels was also observed. In men, there was an increase in testosterone levels. In women, testosterone levels did not change. A significant reduction in PSS (perceived stress scale) scores was observed.

✓ Adaptogenic Effects

Adaptogens are herbs that improve an individual's ability to cope with stress and adapt to change. The most recent definition of an adaptogen is "a class of metabolic regulators that enhances the body's ability to adapt to environmental factors and avoid the damage they could imply."

The ideal adaptogen should reduce negative changes caused by stress, be safe and act beneficially even when the dose given is higher than required, and be free of adverse side effects, such as not affecting the functioning of the body more than needed. Based on the above-mentioned characteristics, Ashwagandha can be considered as an adaptogen.

A study was conducted on a group of horses given Ashwagandha root extract. The animals were subjected to various stressors, such as heavy exercise, separation, and noise. Haematological, biochemical, hormonal, and immunological parameters were studied during the experiment. After 21 days, a statistically significant decrease in cortisol, epinephrine, glucose, triglycerides, creatinine, IL-6, alanine aminotransferase, and aspartate aminotransferase was observed in the treated group. This indicates the adaptogenic, antioxidant, and immunostimulating effects of Ashwagandha. The adaptogenic effects of the standardised extract of Withania somnifera root and Panax ginseng were also studied in rats subjected to chronic stress (CS) using the Footshock method. Chronic stress induced the induction of hyperglycaemia, glucose intolerance, elevated plasma corticosterone levels, increased gastric ulcers, sexual dysfunction, cognitive deficits, immunosuppression, and mental depression. The entire range of the aforementioned disorders was significantly alleviated by the administration of Withania somnifera extract and Panax ginseng prior to the stressor. The effect of an aqueous fraction devoid of witanolides, which was isolated from the root of Ashwagandha, was also studied. The study investigated the adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of Withania somnifera in rats and found that it exhibited significant anti-stress effects, including improved swimming endurance and reduced adrenal gland weight, without causing any adverse effects.

✓ Treatment of Hypothyroidism

Thyroid diseases are among the many conditions that pose significant problems in the 21st century. Hypothyroidism is the most common in clinical practice. Ashwagandha extract lowers thyroid hormone levels in the blood and regulates glucose metabolism, which is impaired in thyroid disease.

Ashwagandha is one of the few medicinal plants that is free of iodine. Iodine is what stimulates the thyroid gland to produce the hormones T3, T4, and TSH. However, Ashwagandha has been shown to be more effective in the treatment of subclinical hypothyroidism than in advanced hypothyroidism. Despite this, it can be used as an adjunct in conventional therapy. The substance that mainly stimulates thyroid activity is withaferin A, which also has anticancer effects. The antioxidant effect of withaferin A normalises the function of the thyroid gland. A study designed to evaluate the efficacy and safety of Ashwagandha root extract in patients with subclinical hypothyroidism was conducted. A total of 50 subjects with elevated serum thyrotropic hormone (TSH) levels (4.5–10 μIU/L) aged between 18 and 50 years were randomly assigned to treatment (n = 25) or placebo (n = 25) groups for an 8-week treatment period.

Eight weeks of Ashwagandha treatment significantly improved serum TSH, T3, and T4 levels compared to placebo. Thus, it has been shown that treatment with Ashwagandha may be beneficial for normalising thyroid markers in patients with subclinical hypothyroidism.

✓ Increase Muscle Strength

Ashwagandha supplementation has been shown to significantly increase muscle strength and to stimulate muscle renewal processes. In one study conducted, young healthy men were orally administered 300 mg of Withania somnifera root extract twice daily for eight weeks. These men also performed physical exercise—subjects participated in a structured resistance training program based on the publications of the National Strength and Conditioning Association (NSCA). A significant increase

in muscle strength was observed in the treated patients, as well as an increase in muscle mass in the arms and chest. It was noted that in the Ashwagandha-supplemented patients, the level of exercise-induced muscle myocyte damage was significantly lower than in the placebo group, as indicated by the stabilisation of plasma creatine kinase levels. In addition, a significant increase in testosterone levels and a significant decrease in body fat were noted in the treated group. Shenoy et al, in their study, confirmed that the group receiving Ashwagandha supplementation had significant improvements in several measures of cardiorespiratory endurance compared to the placebo group. Specifically, the Ashwagandha group showed a significant increase in maximal aerobic capacity, time to exhaustion, and ventilatory threshold. Additionally, the Ashwagandha group had lower levels of serum cortisol, a hormone associated with stress. A study was also conducted on a group of adult athletes who were given a strictly defined dose of Ashwagandha, while the other group received a placebo. At the end of the study, a significant increase in VO2 max (maximum aerobic capacity) was observed in the treated group of athletes, compared to the placebo group. The athletes treated with Ashwagandha had significantly higher

Total Quality Recovery Scores (TQR)[57]. An improvement in quality of life was observed in Ashwagandha-treated athletes (this was investigated based on results obtained from the DALDA—Daily Analysis of Life Demands for Athletes—questionnaire). It was estimated from the Recovery Stress Questionnaire (RESTQ) scores that treated athletes recovered more easily from exercise—they were less tired and had more energy—compared to the placebo group. A significant increase in antioxidant levels was also noted in the treated group. No adverse effects were observed throughout the study, indicating that this plant can be used safely. In addition, an aqueous extract of Withania somnifera effectively increased muscle strength and induced fat growth. The study found that after 8 weeks of supplementation with Ashwagandha, the participants had significantly greater increases in muscle strength and power, compared to those who received a placebo. Additionally, the participants who took Ashwagandha had faster muscle strength recovery following a muscle-damaging exercise compared to the placebo group.

- **Ashwagandha and the Reproductive System**

a) Ashwagandha in Women

Ashwagandha has been credited with improving women's sexual function. In a double-blinded, randomized, placebo-controlled clinical pilot study, 50 women were either treated with ashwagandha extract (600 mg daily) or a placebo for eight weeks. Sexual function was evaluated using psychometric scales such as the Female Sexual Function Index (FSFI) and Female Sexual Distress Scale (FSDS). When compared to the placebo, the ashwagandha extract led to improvements in the registered arousal, lubrication, orgasm and satisfaction. This study was later confirmed in 80 women who were treated with a similar dosage and for the same period. Assessments were made using the same questionnaires. The mechanisms behind the significant changes observed in the blinded studies and evaluated using questionnaires gained some biochemical support, as a normalization of estrogen levels was observed in peri-menopausal women. This contrasts with a study on younger women still having their menstrual period, in which ashwagandha did not cause any changes in estrogen levels. However, the normalization of symptoms reported for peri-menopausal women can have many possible reasons. Ashwagandha has been reported to improve sleep, lower stress and impact several brain functions detectible from electrophysiological changes in electroencephalograms. This may impact sexual function. Moreover, ashwagandha contains isoflavones and flavonoids that may be estrogen analogs. Ashwagandha extract was also shown to exert GABA mimetic properties and increase the secretion of gonadotropin hormones.

Indeed, ashwagandha treatment alleviated menopausal symptoms such as hot flushes, somato-vegetative and urological domain problems in peri-menopausal women. In the same study, there was a significant increase in serum estradiol and no significant changes in serum testosterone when compared with the placebo.

Traditionally, ashwagandha root has been used to prevent miscarriages and to stabilize the fetus. Despite this, during the last 20 years, there has been a growing suspicion that ashwagandha can cause miscarriage—the primary reference has most often been the World Health Organization (WHO), which,

in turn, cited the American Herbal Pharmacopoeia (AHP) Ashwagandha Root Monograph and Therapeutic Compendium. However, according to the American Herbal Pharmacopeia, the WHO monograph is an example of what is known in the medical literature as "citation distortion" since it did not fully articulate the AHP review, which stated the following: "There are conflicting reports regarding the use of ashwagandha in pregnancy. Large but undefined doses have been reported to possess abortifacient activity". The statement can be considered controversial since ashwagandha is traditionally used in Ayurvedic practice to prevent miscarriage and stabilize the fetus. The latter notion is further supported by a recently published animal study, where pregnant Wistar rats received up to 2000 mg of ashwagandha extract per kg of body weight per day during the gestation period without showing any negative impacts on the reproduction process or on fetal development. Finally, the Ministry of Ayush (Government of India) released a safety dossier in 2024, noting the lack of abortifacient activity in ashwagandha root, citing all clinical and preclinical data that have investigated the use of ashwagandha and its root preparations in pregnancy. The conclusion was that no clinical or preclinical investigations revealed an abortifacient activity of ashwagandha root extracts, a claim which is supported by the American Herbal Pharmacopoeia (AHP) press release, 25 June 2024.

b) Ashwagandha in Men

From 2009 to 2024, there are several published scientific articles claiming that sexual activity, sperm count and motility, serum LH, FSH, testosterone levels and even erection and sexual arousal increased in sluggish male rats given ashwagandha for up to three months.

In conformity with animal studies, administration of 600 mg of ashwagandha extract daily for 2 months was also reported to elevate serum testosterone in healthy young male human volunteers, as well as in middle-aged overweight men. Similarly, serum testosterone, sperm count and motility were significantly improved as a result of ashwagandha treatment—675 mg extract taken daily for 3 months—in a group of men under infertility screening, as well as in another group of men who were infertile. Serum testosterone also significantly increased, as did sexual function, in a study on male volunteers with lowered sexual desire, who took 5 g of ashwagandha root

powder for 3 months. In three other studies on infertile men, the quality of sperm improved, and the harmonic balance of seminal plasma metabolites and reproductive hormones increased. By contrast, neither ashwagandha nor placebos provided any improvements when tested in volunteers with psychogenic erectile dysfunction. Five studies performed in male volunteers with low sexual desire or infertility indicate an improvement in sex hormones, including serum testosterone, as a result of ashwagandha treatment. There are, however, papers on healthy younger men, and one on middle-aged overweight men, which, irrespective of the age, show a modest but significant increase in serum testosterone levels as a result of 2–3 months of ashwagandha treatment. Interestingly, the initial level of testosterone was within normal range when the study started and remained so throughout the duration of treatment. As will be discussed below in Section 3.8, this increase in testosterone with ashwagandha treatment is a slightly different pattern than what was observed for thyroid hormones, in which individuals with hypothyroidism before ashwagandha treatment were normalized, while subjects starting with normal thyroid hormone levels remained unchanged at the end of the experiment.

Therefore, the mechanism or biochemical basis for the modest rise (up to 15% increase in serum testosterone) in the above studies and in another study, where the males were treated with ashwagandha, 600 mg daily for one year, is not clear. It should, however, be noted that increased physical activity can improve serum testosterone levels. Thus, considering that ashwagandha treatment improves energy and physical activity in the recipient, the improvement of serum testosterone might indirectly result from volunteers becoming more physically active. Furthermore, ashwagandha lowers cortisol levels in stressed subjects, which can, in turn, improve their testosterone levels [58].

- ✓ **Ashwagandha and Liver Protection**

Like hepatoprotective green tea, garlic and red grapes, the root extract of Withania somnifera has been shown to improve superoxide dismutase and catalase levels, as well as enhance antioxidation in liver tissue. In addition, ashwagandha was also shown to protect mouse livers from paracetamol-induced damage and protect rats from gentamicin- and thioacetamide-induced liver damage (fibrosis and

cirrhosis), as indicated by a significant reduction in the liver enzymes ALT and AST and a decline in bilirubin. In the latter experiment, Withania somnifera administration exerted liver protection in rats by influencing the levels of lipid peroxidation products. Histopathological alterations after carbendazim-induced liver and kidney injury were also amended by 48 days of ashwagandha treatment in rats. The root extracts of the herb were also shown to attenuate hepatic and cognitive defects in a rat model of hepatic encephalopathy induced by thioacetamide treatment. Specifically, the administration of withaferin A (WA, a major constituent of ashwagandha roots; refer to Table 1) significantly improved the pathology of non-alcoholic steatohepatitis (NASH) in mice. Here, WA prevented and therapeutically improved liver injury in two NASH mouse models, as revealed by lower serum aminotransaminases, hepatic steatosis, liver inflammation and fibrosis.

In old dogs, 28 days of ashwagandha treatment significantly improved ALT, AST and superoxide dismutase (SOD) and normalized glutathione (GSH) levels. These changes in GSH levels can indicate how ashwagandha exerts its hepatoprotection, as it is one of the most important enzymes protecting the liver from elements that can be poisonous or destructive. For example, oral administration of 300 mg of glutathione daily for 4 months to non-alcoholic fatty liver disease patients demonstrated a potential therapeutic effect of GSH, as indicated by a decline in the liver enzyme ALT. In another study with healthy elderly dogs, ashwagandha root extract was also shown to significantly lower AST and ALT, in addition to supporting kidney function by improving creatinine and blood urea. In a two-month study on healthy humans treated with 600 mg of ashwagandha (KSM66) daily, there were no changes in ALT and AST levels, indicating that the herb is neither hepatotoxic nor nephrotoxic under the tested conditions. Similarly, in a long-term study that lasted 12 months, where 191 participants took 600 mg of ashwagandha (KSM66) daily, there were no clinically relevant changes in AST and ALT values.

However, there are a few case reports of liver damage, mostly from undefined ashwagandha extracts and often during co-administration with other unknown herbal remedies. This anomaly will be discussed below in Section 3.10, together with results from a recently published 12-month study, which focused on the side effects of ashwagandha treatment.

✓ **Other Ashwagandha Effects, Including Possible Impacts on Longevity** There have been some indications that ashwagandha may have antidepressant effects, although the mechanisms are not fully understood. Various compounds isolated from the root, stem and leaves have also been claimed to exhibit anti-cancer properties. In randomized, double-blind, placebo-controlled studies, which involved testing a topical preparation of ashwagandha root, it was possible to demonstrate improved hair health and enhanced facial skin in photoaged healthy adults. These aging-related changes in skin have been reported to affect the anti-oxidative defense systems, like superoxide dismutase (SOD), catalase, glutathione and malondialdehyde in humans, as well as in animal models. In the study on elderly dogs, it is interesting to note that in addition to improvements in liver enzyme (ALT and AST) levels, anti-inflammatory markers and indicators of the anti-oxidative defense system, such as SOD, catalase, glutathione and malondialdehyde, also improved with ashwagandha root extract treatment. Some of these findings suggest a lifespan extension, which was also seen in another study where Caenorhabditis elegans were treated with ashwagandha root extract.

✓ **Ashwagandha and Thyroid Function, Including Thyrotoxicosis**

Herbs like Commiphora mukul, Humulus lupulus, Bauhinia purpura and Withania somnifera (ashwagandha) are thought to interfere with thyroid function when consumed in their raw form or as extracts. In a randomized, double-blind, placebo-controlled study, administration of 600 mg of KSM66 ashwagandha daily for eight weeks was found to normalize thyroid hormones in elderly volunteers with mild hypothyroidism. The positive effect was accompanied by an improvement in the levels of T3 and T4 hormones, as well as a reduction in TSH. This observation is particularly interesting because many of the elderly with cognitive impairment and a tendency for chronic depression often have a modest thyroid hypofunction as the underlying cause of their symptoms.

Since ashwagandha can improve thyroid levels in hypothyroidism, a very pertinent question is whether the treatment will continue to raise the hormone levels in volunteers with normal thyroid function. The result of such an undesired effect would be hyperthyroidism or an overactive thyroid gland, which is not

desirable, as symptoms include tachycardia, nervousness or irritability, hand tremors, muscle weakness and excessive sweating [59]. The frequency of hyperthyroidism in the general population, depending on where we are on our planet, is 1–2%. As mentioned earlier in this review, many people in Asia, Europe and the USA are taking ashwagandha daily and have been doing this for centuries, with only very rare reports of hyperthyroidism. Hence, it is doubtful whether the few incidences of hyperthyroidism are due to ashwagandha alone or whether they have been provoked by diet or other factors.

As the possibility that ashwagandha might induce hyperfunction of the thyroid gland in some volunteers cannot be excluded, the safety of ashwagandha root extract (KSM66) (600 mg administered daily for 8 weeks) was tested in a randomized, placebo-controlled trial in 80 healthy volunteers, representing both sexes. There were no indications that ashwagandha induced changes in T3, T4 or TSH in the actively treated group. Nevertheless, the study was only run for 8 weeks, and higher doses of ashwagandha could have been used. In 2022, another safety study was carried out, in which 2000 mg of KGM66 was administered daily to volunteers with normal thyroid hormone levels for 3 months. Results showed that the high doses of ashwagandha taken for three months did not lead to thyroid values outside the normal range or changes in TSH, T3 and T4 hormones. In accordance with what has been reported so far in humans, results from experiments with several animal models support the notion that ashwagandha treatment improves thyroid function where hypothyroidism is induced experimentally but has no effect in normo-thyroid animals.

However, a literature search showed four rare cases claiming hyperthyroid function as a result of ashwagandha treatment. One case is a 47-year-old man from Japan, who two months after he started taking ashwagandha (dose not provided), suffered from minor weight loss (4 kg) and fatigue. There was no thyroid enlargement, but TSH was low, and T3 and T4 were raised. Fifteen days after withdrawal of ashwagandha, symptoms and thyroid parameters that had been blamed on stimulation of the thyroid gland were normalized. In another instance, a 62-year-old female from the USA, who suffered stress, was diagnosed with thyrotoxicosis after self-administration of ashwagandha (1950 mg daily for two months). In addition to the ashwagandha treatment, the lady was also receiving estradiol (menopause) and self-administering collagen, astaxanthin, vitamin B complex and a “green powder”. She experienced physical fatigue, weight loss and palpitations two months after adding ashwagandha to the plethora of medicines she was taking. Her TSH was low, and T3 and T4 were raised. After discontinuation (time not given) of ashwagandha, the negative symptoms stopped, and thyroid parameters normalized. The suggested mechanism by the authors for the anomaly was direct stimulation of the thyroid tissue. The third report involved a 73-year-old woman from the USA with hypothyroidism. Two years before presenting with the symptoms, she had stopped her prescribed levothyroxine treatment. Instead, she was self-medicating with ashwagandha treatment (dose unknown). At the time of the report, she came to the hospital with symptoms like palpitations, tachycardia, fatigue and hair thinning. Her TSH level was markedly reduced, but T3 and T4 were within normal range. Two weeks after withdrawal of ashwagandha treatment, the hypothyroid symptoms vanished, and the TSH level normalized. Nevertheless, three weeks following ashwagandha stoppage, the TSH level was elevated again, while T3 and T4 became low due to her baseline hypothyroid status.The final case is a 32-year-old woman from Holland, who started out with ashwagandha (Holisan, Lelystad, The Netherlands; Lelystad capsules) at 250 mg daily for six weeks, followed by an increase to 500 mg daily. After a short while, she experienced weight loss and tachycardia. Upon admission into the hospital, thyroid hormone levels indicated thyroid dysfunction. Four weeks after discontinuation of ashwagandha treatment, hyperthyroid symptoms and thyroid values were normalized [60].

In conclusion, adverse side effects on the thyroid glands from ashwagandha treatment are rare. To the best of my knowledge, only four cases have been recorded. Although it is generally agreed that ashwagandha treatment improves thyroid function in volunteers with low thyroid function, while not affecting normo-thyroid patients, more detailed safety studies need to be carried out for clarification of this very important aspect.

- **Safety of Use**

The long history of the medicinal use of Ashwagandha is primarily a confirmation of its efficacy and of its good tolerance by the body. Nevertheless, nowadays, many researchers are trying to eliminate concerns regarding its use. Recent reports of liver damage are worrying. Herbal supplements are a large and growing component of pharmacological markets, both nationally and internationally. Therefore, the monitoring of its safety is ever more important.

In 2004, the first case linking Ashwagandha to liver disease was discovered in Japan. It concerned a 20-year-old man who had congestive liver damage and recovered without complications after withdrawal from Ashwagandha and 2 months of symptomatic treatment with ursodeoxycholic acid and phenobarbitone. Björnsson et al. reported that Ashwagandha was the cause of five cases of liver damage. These cases illustrate the hepatotoxic potential of Ashwagandha. Liver damage is usually cholestatic or mixed with severe jaundice and pruritus, but is self-limiting, with normalisation of liver test results within 1–5 months. In addition, in the UK, a case was reported where a 39-year-old woman was diagnosed with jaundice and nausea after taking an over-the-counter herbal supplement containing Ashwagandha root extract. There was also a report of a 41-year-old woman who, while taking Ashwagandha extract and progesterone, qualified for a liver transplant due to her deteriorating condition. Reports of hepatotoxic effects are so far scarce and inconclusive. However, further reports should be monitored.

A study conducted in India on a group of 80 fully healthy individuals confirmed the lack of toxicity of this raw material. The participants were each administered 300 mg of Ashwagandha root extract orally, twice daily for 8 weeks. This was assessed by monitoring parameters such as body weight, systolic and diastolic blood pressure, haemoglobin, alkaline phosphatase, alanine transaminase, aspartate transaminase and plasma neutrophil and platelet counts. The values of the above indicators at the end of the study showed no significant differences between the group using the extract (40 subjects) and the group taking placebo (40 subjects). Thyroid function was also monitored by measuring blood levels of triiodothyronine, thyroxine and TSH; however, there were also no significant differences in the levels of these hormones [61].

Another study on a smaller group of volunteers (18 people) confirms, among other things, the lack of significant effects on red blood cell count, white blood cell percentage, ESR value, bilirubin, and plasma protein levels. However, an increase in serum creatinine and a decrease in blood urea nitrogen levels were observed. The researchers attributed this phenomenon to the concomitantly observed increase in muscle mass during the study. Volunteers took aqueous extracts over a 10-day period in doses that increased over time, starting with the equivalent of 6 g and ending with the equivalent of 10 g of vitania sluggard root. Despite its many benefits, the plant should not be used during breastfeeding and pregnancy. Evidence is still lacking to unequivocally confirm the safety of taking Ashwagandha-based preparations during such sensitive periods of life. Some insight into this aspect of safety is provided by studies evaluating the effects of vitania sluggard extract on pregnant rats. The focus was primarily on the period between days 5 and 19 of pregnancy. Importantly, this is a particularly sensitive time due to increased organogenesis and histogenesis in the fetus. Doses were administered orally, the highest of which was 2000 mg/kg/day. No toxic effects were observed as a result of the study, and no changes were observed in the body weight of the pregnant females, the number of corpus luteum or embryo implantation. Furthermore, no external, skeletal or visceral deformities of the fetuses were detected [62].

- **Side Effects in General**

It is well known that herbal remedies used in the Ayurvedic tradition can cause side effects, including liver toxicity. Suspected hepatotoxic herbal remedies include the root of ginseng kianpi, a green tea, which can cause oxidative stress and apoptosis in liver cells; several lichen species; and the root of kava; and Withania somnifera (ashwagandha). However, as will be discussed below, the basis for the claim that ashwagandha can be hepatotoxic is conflicting.

In the clinical trials mentioned above in Section 3.6, where a total of 191 volunteers (male and female) took ashwagandha (KSM66) for up to 12 months, there were no indications that active treatment was different from the placebo, regarding side effects like itching skin, gastrointestinal complaints, drowsiness and headache [30]. In the same studies, there were no side effects related to the liver, as seen in changes in biochemical parameters suggestive of liver damage jaundice or pruritus, or to the thyroid gland, as seen in changes in biochemical markers, weight loss, tachycardia and hair loss. This trend is furthermore supported by all the review papers and meta-analyses cited in this review.[63][64] It should, however, be noted that most of the clinical studies, on average, lasted from 1 to 3 months, which is not comparable to taking the remedy for years. In some of the cases, the side effects occurred after 3 months of the treatment. Indeed, in a single case, the negative effects were reported after more than one and a half years of treatment.

When the reported cases of side effects were evaluated by Professor Nilsson, a specialist in medicine and gastroenterology at the University of Lund in Sweden, he could not find a clear relation between ashwagandha preparations and liver ailment [105]. His conclusion was that ashwagandha, in rare cases, may cause an idiosyncratic reaction (DILD), usually with a mixed cholestatic/hepatocellular profile. He further concluded that although there may be some underreporting of severe liver reactions worldwide, it is very unlikely that there should be a significant underreporting in Western Europe. The few reported severe side effects, despite the widespread use of ashwagandha, indicate that such reactions must be very rare, probably much rarer than Although ashwagandha root, at first glance, seems to be very safe, one must be watchful of the few case reports indicating ashwagandha root toxicity, especially when used in combination with other herbal remedies. In some of these reports, the root extract combined with extracts from the leaves may be responsible for liver toxicity. Thus, adulterations of the product, as well as the administration of high doses, have been suggested as possible explanations for the toxicity reported in some of the cases. However, in rare instances where ashwagandha root extract was taken as a monotherapy, liver injury has been reported, and possible biochemical pathways for the rare phenomenon have been discussed. It should, however, be mentioned that for all the cases reported above, except for one, the liver injury was self-limiting. A summary of different case reports on liver injury that could have been induced by ashwagandha treatment includes symptoms like jaundice and pruritus and elevated levels of key liver enzymes and markers (ALT, AST and bilirubin), which can occur after a few weeks of treatment but also after daily administrations lasting up to several months. As indicated earlier, values for the liver function test reverted to normal levels within 1 to 8 months of cessation of ashwagandha treatment in all the reported cases reviewed in this study, apart from one case. Nevertheless, there are no clear indications as to what could be the metabolic mechanisms or nature of the substances that cause disturbances of the liver in the very few reports of side effects associated with ashwagandha treatment. While discussing possible threats from ashwagandha treatment and how we should respond to them, Simon Mills points out that idiosyncratic reactions known from consuming general food, herbs or medicine could play a role in some of these rare cases [65][66]. Underlying enzyme deficiencies from genetic or hormonal variations have been known to cause reactions, and some can form a DNA adduct from unknown sources. In addition, low levels of glutathione in the individual, as mentioned earlier, may contribute to the “idiosyncratic reactions”. As the liver is the main site for drug metabolism, many drugs are bio-transformed there, and some of the toxic phytochemicals may harm the liver cells if the glutathione level is low. The combination of drugs (paracetamol) and alcohol can also cause severe liver injury. To the best of my knowledge, we do not know the pattern of possible (if any) interactions between ashwagandha and over-the-counter and prescription medicines, especially in patients who are prone to marginal or more severe liver injury. It might also make life easier (and safer) if it were mandatory to declare the content of withaferin A and withanone (mainly present in the leaves) on each package entering the shelves in a store.

From these figures, one would expect a far higher number of reported liver injuries than what has been described if the intake of ashwagandha was truly associated with serious liver side effects and should be avoided. Interestingly, some of the reported side effects mentioned above were from patients who already had severe liver injury prior to taking ashwagandha. These findings should also be seen in light of ~~daily reported liver damage from high intake of alcohol or from paracetamol and NSAIDs, which c~~an be bought without a prescription in most parts of the world. It is, ashwagandha has never been observed to confer liver toxicity. When animals were treated with 5 to more than 200 times the dose of what is relevant for humans, there were no histopathological changes in the different tissues and organs tested

and no signs of deleterious or disruptive changes in blood biochemistry. By contrast, there was an improvement in the level of antioxidants like superoxide dismutase and catalase in hepatic tissue, indicating some liver protection with ashwagandha treatment, including protection from paracetamol-induced liver damage and non-alcoholic steatohepatitis, possibly by improving glutathione levels. As glutathione was shown to protect the liver in humans suffering from alcohol-induced liver injury, improvement of glutathione levels may be important. Therefore, the thorough evaluation of this issue and deep diving into the literature regarding side effects and ashwagandha treatment are blurred and lack any cohesive conclusion. Ashwagandha seems to exert some liver protection, and at the same time, there are rare case reports in a few individuals where the changes in liver enzymes tend towards liver injury. Still, other more likely reasons for liver injury could be multiple, like adulteration of the product (possibly contamination with ashwagandha leaves or administration of very high doses, as well as combination with other herbs or medicines, which are dangerous to compromised livers). Finally, there is the possibility of idiosyncratic reactions in sensitive or predisposed individuals. Therefore, there is a need for more studies to elucidate both the safety and efficacy of ashwagandha products [67].

India: Eight cases were reported. Three of these cases were known to have pre-existing chronic liver disease, while two cases were diagnosed to have underlying chronic liver failure during the evaluation of herb-induced liver injury (HILI). Among the latter two, one was diagnosed with HILI on the background of non-alcoholic steatohepatitis (NASH) on liver biopsy, while the other had imaging features of cirrhosis and portal hypertension. One patient had a history of non-Hodgkin's lymphoma in remission. The various types of ashwagandha formulations consumed by patients included homemade powdered roots, jam preparations, manufactured branded ashwagandha root formulations as powdered root and syrups and nonbranded Ayurveda practitioner-prepared tablets.

Dosing in the different cases was not clear. Time to onset of symptoms and lab abnormalities varied from 14 to 540 days. The major symptom was jaundice, and biochemical disturbances were elevated liver enzymes and elevated bilirubin

[73][75]. Four had liver injury on a cholestatic basis, three on a hepatocellular basis, and one was mixed. Of the four patients with chronic pre-existing liver disease, three died with the development of acute-on-chronic liver failure (ACLF), while the one without ACLF survived. The mortality rate in ACLF is up to 89%. In all the patients, ashwagandha liver injury was self-limited, except in the ones who died from their pre-existing or underlying chronic liver failure, and where it was unlikely that ashwagandha was the cause of death [68][69].

USA: Six cases have been reported up to 2021, of which five were reversible and one ended up with liver transplantation. The one patient with liver transplantation was treated years earlier with thyroid hormones due to thyroid cancer. She went to an herbal practitioner three months before being admitted to the hospital, as she felt tired and was not in good shape. She was treated with ashwagandha (unknown dose) at the same time as she was taking corticoids. After two months, she discontinued treatment with ashwagandha as she felt the treatment was not working. One month into the ashwagandha treatment, she had gone to the hospital because, in her opinion, things were not getting better. It turned out that she had elevated liver enzymes and bilirubin, which continued to increase when measured a month later. The situation slowly got worse and ended with her getting a liver transplant. It is not easy to understand why liver enzymes and bilirubin were elevated one month after withdrawal of ashwagandha—instead, the enzymes continued to increase during the ashwagandha withdrawal period. Her case was different from other reviewed cases, where normalization of liver enzymes and bilirubin followed withdrawal of Ashwagandha treatment [70].

- **Effect Size**

There is clear evidence from the reviewed literature, 40 placebo controlled clinical trials, that ashwagandha positively impacts mood, sleep, the cardiovascular system, the immune system and endocrine functions. However, the effect size (ES) has not been estimated in any of the studies. When looking broadly at the data, the ES of ashwagandha on anxiety and sleep is not far from what can be expected when anxiety and sleep patients are treated with benzodiazepines. As benzodiazepines are often associated with the development of addiction and other undesirable side effects, it would be of great interest to investigate and compare the ES of ashwagandha to other prescription medicines used for

the areas where ashwagandha treatment shows promise. If herbal remedies (green therapy) can reduce the consumption of prescription medicine, including those like benzodiazepines, this would be of great interest to our society in general and to governmental organizations that determine which health alternatives should be promoted.

- . **Limitations of This Study**

As a narrative review, a major limitation of this study is the heterogeneity in the reviewed literature. Although a structured literature search was carried out for publications in Google Scholar using the search word "ashwagandha", the material collected included publications spanning nearly three decades (1998 to the present). The literature considered included published basic scientific studies of ashwagandha effects in cells and different animal models, as well as in human intervention studies and clinical trials with varying designs, protocols and outcome measures. Consequently, this study only provides a contextual basis for more systematic studies [71][72].

➢ CONCLUSIONS :-

All of the literature accessed for this review indicates that ashwagandha root powder, or extracts thereof, can improve the immune system, sex hormones and libido and the uptake of oxygen, as well as improve muscle strength. Ashwagandha can also positively impact stress, anxiety, insomnia and sleep and cognitive function. There are indications that ashwagandha can boost thyroid function in modest hypothyroid volunteers, while up until now, there are no indications that ashwagandha will improve thyroid hormones above normal levels in volunteers with normal thyroid function who are treated for up to twelve months. However, more long-term studies are still welcome to shed more light on this question. Ashwagandha is also reported to improve the reproductive system, especially in men, and to protect the liver.

In general, it should be noted that side effects with ashwagandha are rare. Indeed, the levels reported so far in clinical trials are comparable to what would be seen with a placebo. A few cases indicating liver injury have been reported during ashwagandha treatment, even when the dose is <600 mg daily. Symptoms like jaundice, pruritus, elevated liver enzymes and the elevation of bilirubin normalized after withdrawal of treatment in all the cases except one, who had been treated earlier for a cancer and had received glucocorticoids [74]. Conversely, ashwagandha is also reported to protect the liver, making it difficult to decipher the basis for liver injury reported in the rare cases mentioned above. However, idiosyncratic reactions that have also been reported for other herbs and medicines may be at play here. Other likely explanations include the possibility that a few volunteers who present with the rare side effects of liver injury may have enzyme deficiencies from genetic or hormonal variation, as exemplified in volunteers low in glutathione. In the few cases where hyperthyroid function was reported, symptoms and hormone levels normalized shortly after withdrawal of ashwagandha treatment [76].

➢ REFERENCES:-

1) World Health Organization. WHO Traditional Medicine Strategy 2014-2023. WHO Press, Geneva.

2) U.S. Food and Drug Administration. Dietary Supplement Health and Education Act of 1994 (DSHEA). FDA, USA.

3) U.S. Food and Drug Administration. Dietary Supplements: Regulatory Framework. FDA Guidance Documents.

4) Government of India. Drugs and Cosmetics Act, 1940 and Rules, 1945. Ministry of Health and Family Welfare, New Delhi.

5) Ministry of AYUSH. Good Manufacturing Practices (GMP) for AYUSH Drugs. Government of India.

6) Sharma V, Kumar R. Regulatory challenges of herbal medicines for global commercialization. Journal of Ethnopharmacology. 2016;194:768-774.

7) Ernst E. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends in Pharmacological Sciences. 2002;23(3):136-139.

8) Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: ethical and regulatory issues. Bulletin of the World Health Organization. 2008;86(8): 594-599.

9) Patwardhan B, Mashelkar RA. Traditional medicine-inspired approaches to drug discovery. Drug Discovery Today. 2009;14(15-16):804-811.

10) World Health Organization. Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. WHO Press, Geneva.

11) Chakraborty, A., Singh, H., & Panda, S. K. (2019). Herbal medicines in India: Regulation and development. Indian Journal of Drugs and Rare Applications, 33–39.

12) Singh, M., Kumar, N., & Shukla, R. (2024). Innovations in herbal drug delivery systems: Overcoming formulation challenges. Frontiers in Medical Technology, 6, 1358956.

13) Wang, Y., Zhang, X., & Li, J. (2018). Advances in herbal medicine for cardiovascular diseases: A review. Journal of Ethnopharmacology, 210, 123–135.

14) Gatt, Anthony Raphael, Patricia Vella Bonanno, and Raymond Zammit. "Ethical Considerations in the Regulation and Use of Herbal Medicines in the European Union." Frontiers in Medical Technology, vol. 6, 2024, Article 1358956

15) Anupama S, Vikas AS, Vandana K, Anil B. Current status of regulations for herbal medicine in Europe, USA and India. Journal of Natura Conscientia. 2011; 2(3):406-422.

16) Anupama S, Vikas AS, Vandana K, Anil B. Current status of regulations for herbal medicine in Europe, USA and India. Journal of Natura Conscientia. 2011; 2(3):406-422.

17) Ganesh G, Ramachandran A, Suresh KR, Senthil V, Baviya PR. Nutraceuticals - A regulatory review. International Journal of Drug Regulatory Affairs. 2015; 3(2):22.

18) Manish R, Lovedeep N, Aayushi G, Harvinder P. Cosmetics: Regulatory and market scenario for us and India. The Pharma Innovation Journal. 2018;7(6):164-169.

19) Omprakash G, Madhuri C, Vijayendra S, Sachin B, Giram P, Mahesh JB. Phytopharmaceuticals: An emerging platform for innovation and development of new drugs from botanicals. Journal of Drug Delivery & Therapeutics 2019; 9(3-s):1046.

20) Singh S, Shukla VK. Current regulations for herbal medicines in India.International Journal Of Drug Regulatory Affairs. 2021;9(2):30-

21) Qu L, Zou W, Wang Y, Wang M. European regulation model for herbal medicine: The assessment of the EU monograph and the safety and efficacy evaluation in marketing authorization or registration in Mem.

22) Liang Z, Hu H, Li J, Yao D, Wang Y, Ung CO. Advancing the regulation of traditional and complementary medicine products: A comparison of five regulatory systems on traditional medicines with a long history of use. Evidence-Based Complementary and Alternative Medicine. 2021;2021(1):5833945

23) Ostreikova T.O., Kalinkina O.V., Bogomolov N.G., Chernykh I.V. Glycoalkaloids of plants in the family Solanaceae (Nightshade) as potential drugs. Pharm. Chem. J. 2022;56:948–957. doi: 10.1007/s11094-022-02731-x.

24) Upton R., editor. American Herbal Pharmacopoeia and Therapeutic Compendium, Monograph, Ashwagandha Root (Withania somnifera) American Herbal Pharmacopoeia; Santa Cruz, CA, USA: 2000.

25) Mikulska P., Malinowska M., Ignacyk M., Szustowski P., Nowak J., Pesta K., Szelag M., Szklanny D., Judasz E., Kaczmarek G., et al. Ashwagandha (Withania somnifera)—Current Research on the Health-Promoting Activities: A Narrative Review. Pharmaceutics. 2023;15:1057. doi: 10.3390/pharmaceutics1504105

26) M.ishra L.-C., Singh B.B., Dagenais S. Scientific Basis for the Therapeutic Use of Withania somnifera (Ashwagandha): A review. Altern. Med. Rev. 2000;5:334–346.

27) Elgar K. Ashwagandha: A review of clinical use and efficacy. Nutr. Med. J. 2021;1:68–78.

28) Musamil A., Nawab J.D. Ethnobotany, Pharmacology and Therapeutic Functions. Elsevier Inc.; Amsterdam, The Netherlands: 2017. Withania somnifera: Ethnobotany, Pharmacology, and Therapeutic Functions; pp. 137–154.

29) Panossian A., Wikman G. Effects of Adaptogens on the Central Nervous System and the Muscular Mechanisms Associating with Their Stress—Protective Activity. Pharmaceuticals. 2010;3:188–224. doi: 10.3390/ph3010188.

30) Sharna K.S., Basu I., Sing S. Efficacy and Safety of Ashwagandha Root extract in Subclinical Hypothyroid Patients: A Double-Blind, Randomized Placebo-Controlled Trial. J. Altern. Complement. Med. 2018;24:243–248. doi: 10.1089/acm.2017.0183.

31) Chatterjee S., Srivastava S., Khalid A., Singh N., Sangwan R.S., Sidhu O.P., Roy R., Khetrapal C.L., Tuli R. Comprehensive metabolic fingerprinting of Withania somnifera leaf and root extracts. Phytochemistry. 2010;71:1085–1094. doi: 1016/j.phytochem.2010.04.001.

32) Namdeo A.G., Sharma A., Yadav K.N., Gawande R., Mahadik K.R., Lopez-Gresa M.P., Kim H.K., Choi Y.H., Verpoorte R. Metabolic characterization of Withania somnifera from different regions of India using NMR spectroscopy. Planta Med. 2011;77:1958–1964. doi: 10.1055/s-0031-1279997

33) Johri S., Jamwal U., Rasool S., Kumar A., Verma V., Qazi G.N. Purification and characterization of peroxidases from Withania somnifera (AGB 002) and their ability to oxidize IAA. Plant Sci. 2005;169:1014–1021.

34) Zhao J., Nakamura N., Hattori M., Kuboyama T., Tohda C., Komatsu K. Withanolide derivatives from the roots of Withania somnifera and their neuritis outgrowth activities. Chem. Pharm. Bull. (Tokyo) 2002;50:760–765. doi: 10.1248/cpb.50.760.

35).Alam N., Hossain M., Khalil M.I., Moniruzzaman M., Suleiman S.A., Gan S.H. High catechin concentrations detected in Withania somnifera (ashwagandha) by high performance liquid chromatography analysis. BMC Complement Altern. Med. 2011;11:65. doi: 10.1186/1472-6882-11-65.

36) Srivastava M.P., Gupta S., Dixit S., Yadav N., Yadav V., Singh H., Kanaujia P., Sharma Y.K. Withania somnifera (Ashwagandha): A wonder herb with multiple medicinal properties. Asian J. Pharm. Pharmacol. 2018;4:123–130. doi: 10.31024/ajpp.2018.4.2.5.

37) Chauhan S., Joshi A., Jain R., Devendra J. Estimation of withanolide A in diverse genotypes of Ashwagandha Withania somnifera (L.) Dunal. Indian J. Exp. Biol. 2019;57:212–217.

38) Sreerekha M.V., Patel K.V., Bhatnagar R., Sriram S. Distribution of total withanolides in various plant parts of ashwagandha (Withania somnifera) accessions as influenced by light and dark reaction cycle. J. Med. Aromat. Plant Sci. 2004;26:681–683.

39) Tharakan A., Shukla H., Benny I.R., Tharakan M., George L., Koshy S. Immunomodulatory Effect of Withania somnifera (Ashwagandha) Extract—A Randomized, Double-blind, Placebo Controlled Trial with an Open Label Extension on Healthy Participants. J. Clin. Med. 2021;10:3644. doi: 10.3390/jcm10163644.

40) Krishna Raju A.V., Somepalli V., Thanawala S., Shah R. Efficacy and Anti-Inflammatory Activity of Ashwagandha Sustained-Release Formulation on Depression and Anxiety Induced by Chronic Unpredictable Stress: In vivo and in vitro Studies. J. Exp. Pharmacol. 2023;15:291–305. doi: 10.2147/JEP.S407906.

41) Khan M.A., Subramaneyaan M., Arora V.K., Banerjee B.D., Ahmed R.S. Effect of Withania swomnifera (Ashwaganda) root extract on amelioration of oxidative stress and autoantibodies production in collagen-induced arthritic rats. J. Complement. Integr. Med. 2015; 12:117–125. doi: 10.1515/jcim-2014-0075.

42) Rasool M., Varalakshmi P. Suppressive effects of Withanin somnifera root powder on experimental gouty arthritis: An in vivo and in vitro study. Chem. Biol. Interact. 2006;164 :174–180. doi: 10.1016/j.cbi.2006.09.011.

43) Mohanty I., Arya D.S., Dinda A., Talwar K.K., Joshi S., Gupta S.K. Mechanisms of Cardioprotective Effect of Withania somnifera in Experimental Induced Myocardial Infarction. Basic Clin. Pharmacol. Toxicol. 2004 ;94:184–190. doi: 10.1111/j.1742-7843.2004 .pto940405.x.

44) Choudhary B., Shetty A., Langade D.G. Efficacy of Ashwagandha (Withania somnifera [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. Int. Q. J. Res. Ayurveda. 2015; 36:63–68. doi: 10.4103/0974-8520.169002.

45) Tiwari S., Gupta S.K., Pathak A.K. A double-blind, randomized, placebo-controlled trial on the effect of Ashwagandha (Withania somnifera dunai.) root extract in improving cardiorespiratory endurance and recovery in healthy athletic adults. J. Ethnopharm. 2021;272: 113929. doi: 10.1016/j.jep.2021.113929.

46) Sandhu J.S., Shah B., Shenoy S., Chauhan S., Lavekar G.S., Padhi M.M. Effects of Withania somnifera (Ashwagandha and Terminalia Arjuna) on physical performance and cardiorespiratory endurance in healthy young adults. Int. J. Ayurveda Res. 2010;1 :144–149. doi: 10.4103/0974-7788.72485.

47) Verma N., Gupta S.K., Patil S., Tiwari S., Mishra A.K. Effects of Ashwagandha (Withania somnifera) standardized root extract on physical endurance and VO2max in healthy adults performing resistance training: An eight-week, prospective, randomized, double-blind, placebo-controlled study. F1000Research. 2023; 12:335. doi: 10.12688/f1000research.130932.

48) Wankhede S., Langade D., Joshi K., Sinha S.R., Bhattacharyya S. Examining the effect of Withania somnifera supplementation on muscle strength and recovery: A randomized controlled trial. J. Int. Soc. Sports Nutr. 2015; 12:43. doi: 10.1186/s12970-015-0104-9.

49) Raut A.A., Rege N.N., Tadvi F.M., Solanki P.V., Kene K.R., Shirolkar S.G., Pandey S.N., Vaidya R.A., Vaidya A.B. Exploratory study to evaluate tolerability, safety and activity of Ashwagandha (Withania somnifera) in healthy volunteers. J. Ayurveda Integr. Med. 2012; 3:111–114. doi: 10.4103/0975-9476.100168.

50) Verma N., Gupta S.K., Tiwari S., Kumar R., Gupta S. Effect of Ashwagandha Root Extract on serum Testosterone and Muscle Recovery in Strength Training. Int.J. Med. Pharm. Res. 2023;4: 371–381.

51) Bonilla D.A., Moreno Y., Gho C., Petro J.L., Odriozola-Martínez A., Kreider

R.B. Effects of Ashwagandha (Withania somnifera) on Physical Performance:

Systematic Review and Bayesian Meta-Analysis. J. Funct. Morphol. Kinesiol. 2021; 6:20. doi: 10.3390/jfmk6010020.

52) Salve J., Kale S., Prajapati B.L., Sparavigna A., Savant M., Ademola J., Langade

D. Safety of 12-months Administration of Ashwagandha (Withania somnifera) Standardized Root Extract in Healthy Adults: A Prospective, Observational Study. Phytother. Res. 2025:1–24. doi: 10.1002/ptr.70096.

53) Chandrasekhar K., Kapoor J., Anishetty S.A. Prospective, Randomized, Double-Blind, Placebo-Controlled Study of Safety and Efficacy of High-Concentration Full-Spectrum Extract of Ashwagandha Root in Reducing Stress and Anxiety in Adults. Indian J. Psychol. Med. 2012; 34:255–262. doi: 10.4103/0253-7176.106022.

54) Choudhary D., Bhattacharyya S., Joshi K. Body Weight Management in Adults Under Chronic Stress Through Treatment with Ashwagandha Root Extract: A Double-Blind, Randomized, Placebo-Controlled Trial. J. Evi.-Based Complement. Altern. Med. 2016;1:11. doi: 10.1177/2156587216641830.

55) Lopresti A.L., Smith S.J., Malvi H., Kodgule R. An investigation into the stress-relieving and pharmacological actions of an ashwagandha (Withania somnifera) extract: A randomized, double-blind, placebo-controlled study. Medicine. 2019;

56) Salve J., Pae S., Debnah K., Langade D. Adaptogenic and Anxiolytic Effects of Ashwagandha Root Extract in Healthy Adults: A Double-blind, Randomized, Placebo controlled Clinical Study. Cureus. 2019;11:e6466. doi: 10.7759/cureus.

57) Kelgane S.B., Salve J., Sampara P., Debnath K. Efficacy and Tolerability of Ashwagandha Root Extract in the Elderly for Improvement of General Well-being and Sleep: A Prospective, Randomized, Double-blind, Placebo-controlled Study. Cureus. 2020;12:e7083. doi: 10.7759/cureus.7083.

58) Langade D., Kanchi S., Jaising Salve J., Debnath K., Ambegaokar D. Efficacy and Safety of Ashwagandha (Withania somnifera) Root Extract in Insomnia and Anxiety: A double-blind, Randomized, Placebo-controlled Study. Cureus. 2019;11:e5797. doi: 10.7759/cureus.5797.

59) Langade D., Vaishali T., Kanchi S., Kelgane S. Clinical evaluation of the pharmacological impact of ashwagandha root extract on sleep in healthy volunteers and insomnia patients: A double-blind, randomized, parallel-group, placebo-controlled study.

60) Majeed M., Nagabhushanam K., Mundkur L.A. Standardized Ashwagandha root extract alleviates stress, anxiety and improves quality of life in healthy adults by modulating stress hormones: Results from a randomized, double-blind, placebo-controlled study.

61) Akhgarjand C., Asoudeh F., Bagheri A., Kalantar Z., Vahabi Z., Shab-bidar S., Rezvani H., Djafarian K. Does Ashwagandha supplementation have a beneficial effect on the management of anxiety and stress? A systematic review and meta-analysis of randomized controlled trials. Phytother. Res. 2022;36:4115–4124. doi: 10.1002/ptr.7598.

62) Cheah K.L., Norhayati M.N., Yaacob L.H., Rahman R.A. Effect of Ashwagandha (Withania somnifera) extract on sleep: A systematic review and meta-analysis. PLoS ONE. 2021;16: e0257843. doi: 10.1371/journal.pone.0257843.

63) Singh N., Bhalla M., de Jager P., Gilca M. An overview on Ashwagandha: A Rasayana (rejuvenator) of Ayurveda. Afr. J. Tradit. Complement. Altern. Med. 2011; 8:208–213. doi: 10.4314/ ajtcam.v8i5S.9.

64) Langade D., Kanchi S., Salve J., Debnath K., Ambegaokar D. Efficacy and Safety of Ashwagandha (Withania somnifera) Root Extract in Insomnia and Anxiety: A Double-blind, Randomized, Placebo-controlled Study. Cureus. 2019;11: e5797. doi: 10.7759/cureus.5797.

65) Połumackanycz M., Forencewicz A., Wesołowski M., Viapiana A. Ashwagandha (Withania somnifera L.)—The plant with proven health-promoting properties. Farm. Pol. 2020; 76:442–447. doi: 10.32383/farmpol/127824.

66) Mirjalili M.H., Moyano E., Bonfill M., Cusido R.M., Palazón J. Steroidal lactones from Withania somnifera, an ancient plant for novel medicine. Molecules. 2009; 14:2373–2393. doi: 10.3390/molecules14072373.

67) John J. Therapeutic potential of Withania somnifera: A report on phyto-pharmacological properties. Int. J. Pharm. Sci. Res.

68) Dutta R., Khalil R., Green R., Mohapatra S.S., Mohapatra S. Withania Somnifera (Ashwagandha) and Withaferin A: Potential in Integrative Oncology. Int. J. Mol. Sci. 2019; 20:5310. doi: 10.3390/ijms20215310.

69) Tiwari S., Atluri V., Kaushik A., Yndart A., Nair M. Alzheimer's disease: Pathogenesis, diagnostics, and therapeutics. Int. J. Nanomed. 2019; 14:5541–5554. doi: 10.2147/IJN.S200490.

70) Kurapati K.R.V., Atluri V.S.R., Samikkannu T., Nair M.P.N. Ashwagandha (Withania somnifera) reverses β-amyloid1-42 induced toxicity in human neuronal cells: Implications in HIV-associated neurocognitive disorders (HAND) PLoS ONE. 2013;8:e77624. doi: 10.1371/journal.pone.0077624.

71) Pandey A., Bani S., Dutt P., Satti N.K., Suri K.A., Qazi G.N. Multifunctional neuroprotective effect of Withanone, a compound from Withania somnifera roots in alleviating cognitive dysfunction. Cytokine. 2018; 102:211–221. doi: 10.1016/j.cyto.2017.10.019.

72) Das R., Rauf A., Akhter S., Islam M.N., Emran T.B., Mitra S., Khan I.N., Mubarak

M.S. Role of Withaferin A and Its Derivatives in the Management of Alzheimer's Disease: Recent Trends and Future Perspectives. Molecules. 2021; 26:3696. doi: 10.3390/molecules26123696.

73) Atluri V.S.R., Tiwari S., Rodriguez M., Kaushik A., Yndart A., Kolishetti N., Yatham M., Nair M. Inhibition of Amyloid-Beta production, associated neuroinflammation, and Histone Deacetylase 2-mediated epigenetic modifications prevent neuropathology in Alzheimer's disease in vitro Model. Front. Aging Neurosci. 2020; 11:342. doi: 10.3389/fnagi.2019.00342.

74) Sehgal N., Gupta A., Valli R.K., Joshi S.D., Mills J.T., Hamel E., Khanna P., Jain S.C., Thakur S.S., Ravindranath V. Withania somnifera reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. Proc. Natl. Acad. Sci. USA. 2012; 109:3510–3515. doi: 10.1073/pnas.1112209109.

75) Zlokovic B.V., Deane R., Sagare A.P., Bell R.D., Winkler E.A. Low-density lipoprotein receptor-related protein-1: A serial clearance homeostatic mechanism controlling Alzheimer's amyloid β-peptide elimination from the brain. J. Neurochem. 2010; 115:1077–1089. doi: 10.1111/j.1471-4159.2010.07002.x

76) Lillis A.P., Van Duyn L.B., Murphy-Ullrich J.E., Strickland D.K. LDL receptor-related protein 1: Unique tissue-specific functions revealed by selective gene knockout studies. Physiol. Rev. 2008;88:887–918. doi: 10.1152/physrev.00033.2007.