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Review Article

A Comprehensive Scientific Claims on Ethnobotany, Phytochemical Pharmacological and Toxicology of *Withania somnifera* (L.) Dunal

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Abstract

For more than 3,000 years, *Withania somnifera*, commonly known as Ashwagandha, has been utilised in traditional and Ayurvedic medicine. It belongs to the ginger family. Dry powder, crude extract, and refined metabolites of the plant have all demonstrated potential therapeutic advantages in animal trials because it is a medicinal plant. Withanolides are the primary metabolites of the plant, and they are responsible for the therapeutic effects of the herb. Despite the fact that other review papers on this plant have previously been published, this review article is being provided in order to gather all of based on the most recent data on its pharmacological and phytochemical effects, which have been investigated using a variety of approaches. According to research, Ashwagandha has anti-inflammatory, adaptogen, anti-parkinsonian, anti-oxidant, and memory-boosting properties, and anticancer effects in addition to other benefits. Other effects, such as immunomodulation, hypolipidemia, antimicrobial activity, Investigations have also been done on cardiovascular defence, sexual behaviour, tolerance, and reliance. These outcomes are extremely encouraging, and they recommend more research into this herb to confirm these results and find further potential medicinal characteristics. Clinical studies including the use of ashwagandha for a number of illnesses should be carried out as well. The new evidence on its pharmacological and phytochemical activities is described in the current publication. In order to give thorough information on the ethnobotany, traditional applications, phytochemistry, and pharmacological efficacy of the medicinal plant, W. somnifera, from reliable sources, the present review has primary objectives.

Keywords: *Withania somnifera,* Solanaceae, traditional claim, ethnobotany, phytochemistry, phytopharmacology

Introduction

For thousands of years, both developing and developed countries have made substantial use of traditional herbal therapy and its preparations, due to their natural origins and the fact that they have less adverse effects than modern pharmaceuticals. For a long time, these remedies were sold as unprocessed medications, including tinctures and teas, poultices and powders, and other herbal preparations. The use of plants for medicinal reasons predates recorded history and is credited with laying the foundations for most of contemporary medicine. These ancient remedies, which were mostly produced from plants, were the subject of clinical, pharmacological, and chemical investigations [1]. For over 75–80 percent of the world's population, particularly in less developed countries, herbal medicine continues to be the main source of basic healthcare. This is largely because it's a common misperception that herbal remedies, apart from being inexpensive and readily accessible, have no negative side effects and are thus safe to take. The World Health Organization (WHO) reports that the usage of herbal remedies globally outpaces the use of standard medicines by a factor of two to three [2, 3]. Indigenous herbal remedies are ones that have traditionally been used in a particular community or area and have become extremely well known to the local people as a result of their long-standing use in terms of composition, treatment, and dose. It is available for free usage by members of the confined public or by anybody in the surrounding area. But if the medications in this category are offered for sale, or distributed outside of the neighboured or area where they were developed, they must fulfil the safety and effectiveness standards set out in the national laws for herbal medicines. Modified herbal medications have had their shape or form changed, and this pertains to the dosage, dosage form, mode of administration, and ingredients in herbal medicines, techniques of manufacture, and medical indications. Modified herbal medicines are also available in generic form. They must ensure that herbal medications are safe and effective in accordance with national regulatory regulations. Imported items having a herbal medicine foundation includes all herbal medicines, including raw ingredients and finished products, that are imported [2]. Because of this, traditional and plant-derived medicine is relied upon by more than 80 percent of the world's population at this time. Plants are key suppliers of pharmaceutical ingredients, and approximately 25% of American prescriptions for pharmaceuticals contain at least one plant-derived ingredient substance at any given point in time. Approximately 121 pharmaceutical items were developed during the course of the twentieth century, all of which were based on traditional knowledge gathered from a

variety of sources [3]. According to current estimates, nature provides up to 90% of the new therapeutic molecule. The World Health Organization (WHO) has demonstrated a keen interest in documenting how tribes from around the world, particularly in Africa and Asia, use medicinal herbs. Many developing nations have stepped up their efforts to record ethno-medicinal data on medicinal plants, particularly in the last few years. The hunt for scientific proof to support assertions made by tribal healers about the efficacy of Indian herbs has been stepped up. Once these ethno-medicinal concoctions from the local area have been scientifically analysed and adequately distributed, people will be better educated about the effectiveness of pharmacological treatment and the improvement of their health state [4-5].

As a result of its significant pharmacological and nutraceutical characteristics, *Withania somnifera (L.)* In the Indian Ayurvedic medical system, is regarded as one of the most significant medicinal herbs [6]. Only two of the twenty-three known species of Withania (*Withania somnifera* and *Withania coagulans*) are economically significant, making them the most valuable [7]. *W. somnifera,* commonly referred to as "Ashwagandha, Asgandh, Winter Cherry, and "Indian ginseng" is a medicinal herb that has been utilised in Ayurvedic and traditional medicine for over 3,000 years. It belongs to the ginger family. The dried powder, unprocessed extract, and purified metabolites of the plant are used as medicines have all demonstrated promise therapeutic benefits in animal studies [8-9].

Distribution

From the southern Mediterranean to the Canary Islands, from South to East Africa, from Palestine to North India, *W. somnifera* is extensively dispersed throughout the world., where it can be found in Israel, Jordan, Egypt, Sri Lanka, Sudan, Iran, Afghanistan, Baluchistan, Pakistan; and from the Middle East to the Far East. It can also be found at great altitudes in the Himalayas, where it can reach 5,500 feet in elevation. In Bombay and Western India, there are plenty of these shrubs, and it is also occasionally encountered in Bengal. It spreads like wildfire throughout India, particularly in the hotter regions, on waste land, and along roadside embankments. Fields and open fields all across India are devoted to the cultivation of this plant for therapeutic uses [10-13].

Morphological Characters *W. somnifera*

A strong foul odour, similar to that of horse's urine, emanates from the erect, green, branched or unbranched small or medium undershrub reaching up to 1.25m in height, and a branching perennial with height up to 1.25m. Almost the entire plant is covered and encircled by very short, tiny, fine, branching, silver-grey coloured hairs and very short in length [14].

- **Stem:** The tall, brownish-dark stems of *W. somnifera* have a rounded appearance; occasionally, leaves are absent or very sparsely distributed on the bottom section of the stem [15, 16].
- **Leaves:** The simple, somewhat wavy margined leaves are placed intermittently (opposite on blooming stems) and narrow into the 5-20mm long petioles, and are generally broadly oval or oblong in shape, 29–80mm long and 21–50mm broad, depending on the variety. It is sometimes referred to as stellate tomentose, and it is a greyish, under shrub that grows to 30-150cm in height and has long woody tuberous roots that are stellate in shape [17-19].
- **Flowers:** Generally, Flowers are small and greenish, axillary, monoceous or bisexual, and can be found alone or in little flower clusters called cymes. Numerous, discoid, reniform, and yellow seeds are frequently seen. The number of chromosomes in the human genome is 2n = 48. The 5-lobed, constrictly campanulate, 5-8mm-long, pale yellow to yellow-green corolla has five petals [19-21].
- **Fruits:** The fruit of *W. somnifera* is typically a spherical, hairless berry that ranges in size from 5-8 mm, turns orange-red to scarlet when ripe, and is encircled by an enlarged calyx. Most of the seeds have a very light brown colour, are 2.5 mm wide, occasionally kidney-shaped, compressed, have a rough, netted surface on one side, and a smooth surface on the other. The flowering season in *W. somnifera* typically lasts from October to June, and from October to July is the fruiting season. The red fruit of *W. somnifera* is distinguished by its brownish, papery, turgid calyx, which is brownish and papery. It has been noted by observers to be an offensive-smelling bush with generally pungent roots and leaves that smell strongly like green tomatoes[22].
- **Roots**

When dry, the roots of W. somnifera are fleshy and traight, cylindrical, tapering downward, gradually becoming unbranched, and measuring roughly 10–17.5 cm in length and 6–12 mm in thickness. The major roots are brownish on the outside and creamy on the inside, and they produce subsidiary roots that are fiber-like, taste bad, and are bitier (Anonymous 1982). The roots are robust and meaty, and they are a white brown colour. The parts of plant are shown in Figure 1 (A-D).

Figure 1(A – D): Exomorphic features of *W. somnifera.*

Traditional uses

According to historical records, The Indian traditional medical method known as Ayurveda has been practised since 6000 BC (Charak Samhita, 1949). Ashwagandha has been utilised as a Rasayana throughout the majority of the last 6000 years. Ashwagandha root is a tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic, and stimulant, among other things. It has historically been used to treat a wide range of conditions, including sex-related problems, heart disease, pain, liver disorders, fever, and respiratory infections [23]. Alkaloids, steroids, phenolics, flavonoids, and other phytochemicals are examples of bioactives that are thought to contribute to the medicinal properties of plants [31-32]. Among plants Due to its abundance in the aforementioned bioactives, *W. somnifera* is the herb of choice for traditional healers [24-26].

Therapeutic Uses

W. somnifera roots are mostly used for medicinally in the Unani and Ayurvedic schools of medicine, respectively. Due to the fact that the plant loses its pharmacologic effectiveness after two years, newly dried roots are preferable for optimal effects [27, 28]. Bitter in taste, the plant's leaves have some medical applications in the treatment of fever and uncomfortable swell. In contrast to their other qualities, the blossoms have astringent, depurative, diuretic, and aphrodisiac characteristics. In addition to having anti-helminthic properties, the seeds can erase silver acnes from the cornea and boost sperm count and testicular development both. Tradition has claimed that the fruits are used topically to cure tumours and tubercular glands, carbuncles, and skin ulcers, among other conditions [29]. In Asgand is a traditional Chinese medicine herb that has a variety of therapeutic applications. A small justification for designating *W. somnifera* Indian Ginseng is that its pharmacologic effects and folklore usage are similar to those of Korean Ginseng tea [30].

Table 1: Various portions of *W. somnifera* have been used medicinally. [31-33]

Plant part used	Therapeutic uses
Roots	Treatment of arthritis, aphrodisiac, anticancer, antioxidant, bronchitis, leucoderma, TB, liver issues, and cardiac conditions.
Leaves	Treatment of edoema, haemorrhoids, boils, eyesores, syphilis, uncomfortable swelling, external aches, and ulcers
Seeds	possess diuretic, narcotic, and hypnotic properties
Fruits	Treatment for TB and ulcers. antihelmintic action
Stem	function as a herbicide, antibacterial, and anticancer
Whole plant	Act as a countermeasure, insecticide, larvicide, antioxidant, immunomodulator, neurotic regenerator, adaptogenic hepatoprotective, and cardioprotective.

Phytochemistry of *W. somnifera*

There are several compounds that have been identified from *W. somnifera.* Various chemotypes. Alcoholic, alkaloid, and withanolides substances are examples of such chemicals. In contrast to the alkaloids, the plant also contains 40 withanolides and several sitoindosides, which have been identified and documented [34] (Table 2).

Table 2: The Important chemical constituents of *W. somnifera* [35]

Class	Phytoconstituents	References
Alkaloids	Nicotine, tropeltigloate, somniferinine, somnine, withanine, withananine, Nicotine, tropeltigloate,	[35]
Steroidal lactones	Withaferin A, withanone, Withaferin A, withanone, withanolide E, F, Withanolide A, Withanolide G, Withanolide H, Withanolide I, Withanolide J, Withanolide K, Withanolide L, and Withanolide M	[35, 36]
Steroids	cholesterol, stigmasterol, diosgenin, stigmastadien, β-sitosesterol, and sitoinosides: VII, VIII, IX, and X.	[37, 39]
Salts	Cuscohygrine, sanahygrine, stropine, pseudotropine, anaferine	[37]
Flavonoids	quercetin Kaempferol	[38]
Glycosides	Sitoindosides VII and VIII	[39]

Alcoholic compounds

The first study on the phytochemistry of the plant now accessible was based on research into the chemical principles of *W. somnifera*, which showed the existence of many chemicals in the plant's roots and leaves. Withaniol, $C_{25}H_{33}O_4OH$, and somnirol, $C_{32}H_{43}O_6OH$, two novel monohydric alcohols, somnitol, $C_{33}H_{44}O_5(OH)_2$, a brand-new dihydric alcohol called somnitol, withanic acid, $C_{29}H_{45}O_6$ COOH, phytosterol, $C_{27}H_{46}O$, and ipuranol, $C_{25}H_{38}O_2$ $(OH)_2$, as well as a nitrogen-containing component. The researchers also

discovered a combination of sugar, an essential oil, and fatty acids and an essential oil [40].

Alkaloids

Nicotine and the drugs withanine, withananine, withananinine, pseudo-withanine, somniferine, somniferinine, and witherine, which was the eighth component of their investigation, were among the seven amorphous bases that Majumdar and colleagues 1952 and 1955 partially characterised in their [41] study of Bengali and South African variations served as the ancestors of Indian types. During their examination, they found many nitrogenous bases as well. All six of the chemicals were discovered to be alkaloids, while the seventh component was discovered to be a breakdown result of withanine. Withanine was discovered to be the most abundant alkaloid among these, accounting for 38 percent of the complete alkaloid content. Eight bases were found in the extract, according to Schwarting et al. 1963 [42], with the last two being the unique ones.

Withanamides A-I were isolated and purified from the methanolic extract of *W. somnifera* fruits by Jayaprakasam et al., (2004) [43].

Withanolides

Withanolides are a novel family of steroidal lactones from Withania somnifera that Lavie and colleagues described in a number of studies. They were distinguished by a C28 basic skeleton with a side chain of 9 C atoms and a lactone ring of 6 membered." During the time from August 1996 to March 2010, 360 new naturally occurring withanolides were isolated and identified.

The withanolides contain a side chain of the cholestane type that is highly oxygenated and contains an additional methyl group at the position of C-24. Specifically, withaferin A. In recent years, chemical investigations and X-ray crystallography have both helped to uncover the 2,3-dihydro derivative of withaferin A structures [44, 45]. It was revealed that withaferin A had a basic skeleton by a series of selenium dehydrogenations that resulted in the separation of a cyclopentenophenantharene derivative and a trimethylnaphthalene derivative. Most people are familiar with it because of its anti-cancerous properties. It has three potentially active sites that could engage in an biomedical nucleophiles are used in an in vivo alkylation process to provide metabolic activity. Position 3 in ring A, the epoxide at position 5 (or 6) in ring A, and position 24 in the unsaturated lactone ring E are a few examples of these locations. It was shown that the analogues with cholesterol side chains had considerably less anti-tumor action than withanolides, proving that the action requires the side chain to include an unsaturated lactone to take place [46]. Several withanolides that resemble withaferin A in structure include those that have an epoxy group at positions 5 and 6 and unsaturation at positions 2 and 24 (Fig. 1). The identical structure as withaferin A was discovered, However, it was found 71 that a hydroxyl group exists at position 20 as opposed to position 27. In 1971, the roots of *Withania somnifera* were used to produce withanolide A, which had previously been extracted from *W. coagulans* [46]. Withanolide C, a chlorinated withanolide, was discovered in *W. somnifera* chemotype III (Lavie et al., 1992) [47]. The number 5 chloro group and the opening of the 5b, 6b epoxide ring were disclosed by the structure of withanolide C.

- Nine withanolides were isolated by Glotter et al., (withanolides E-M) in 1973 [48] and discovered that five of them (withanolides G–K) have a special D8–14 double bond. Currently, there are only a few reports pertaining to the presence of the D8 (14) double bond in natural steroids. When compared to withanolide E, withanolide F has a double bond where withanolide E has epoxy groups at positions 5b and 6b. Withanolide E and F were subjected to X-ray analysis, which revealed that the side-chain exhibited a peculiar 17-α oriented conformation [49]. During research on the biogenesis of withanolides in *W. somnifera*, withanolide S was found, which involved the cross pollination of various types of withanolides to produce the compound. On the basis of nuclear magnetic resonance, it has been found to be very similar to withanolide E, with the only difference being a secondary, axial hydroxyl group at D4.10 instead of an epioxide ring signal at D3.20

- According to Glotter et al. (1972) [48], withanolide F, G, G2, H, J, L, M, N, O, P, Q, and U were among the compounds that included 2,5-dien-1-one in the AB ring system. Given that withanolide Q and withanolide G exhibit the identical NMR signal for three vinylic protons in the low field region, the 2,5-dien-1-one structure may be categorised as belonging to the AB ring system. The sole difference between the side chains of withanolide Q and R, according to a comparison of the NMR signals, is the hydroxyl group's presence or absence at position 27 in the former. By using X-ray single structure analysis, It has been determined that the chemical formula of withanolide Y is 5a, 6a-epoxy-7a, 17a, 20R-trihydroxy-1-oxo-22Rwitha- 2, 24-dienolide. It is the sole example of a withanolide with a hydroxyl group in the seventh position [50].

- Withanone and tubacapsenolide F were two of the eight novel steroidal lactones that Kirson et al., 1971 [50], who researched six compounds of *W. somnifera*, which is growing in North-Western India, have been documented.

- It was reported in 1982 by A.Velde [51] and colleagues that a steroidal lactone of withanolide G that occurs naturally. It is sometimes referred to as dunawithagenin and D16-withanolide, was extracted from the plant *W. somnifera*. Chemotype III is the most common.

- In 1992, Rahman et al., [52] isolated sominolide and sominone from *W. somnifera* in addition to withasomniferin-A.

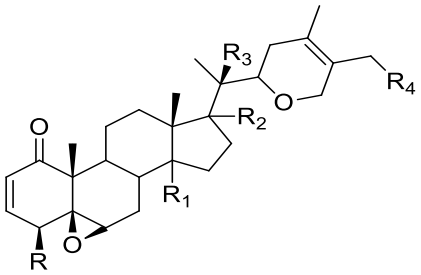
- Withasomidienone has also been isolated by [53, 54] Instead of position 2 and 24, as in the majority of withanolides, spectroscopic tests revealed the existence of three double bonds at positions 1, 4, and 24.

- From the stem bark of W. sativa, five novel withanolides were found by Ali et al. in 1997 [55] *W. somnifera*, specifically withasomnilide, withasomniferanolide, somniferanolide, somniferawithanolide, and somniwithanolide.

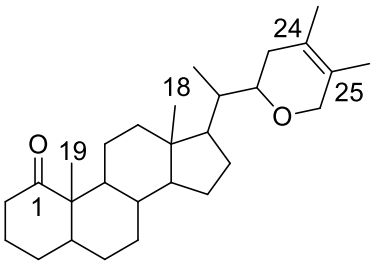
- Withasomniferol A, withasomniferol B, and withasomniferol C were obtained by Anjaneyulu and Rao in 1997 [56] from the non-basic part of the roots of W. somnifera extracted using benzene and ethyl acetate. In 2003, Jayaprakasam and Nair found the plant compound *W. somnifera's* viscosalactone B. Due to the presence of a 6,7 epoxy group, viscosalactone B is structurally similar to withaferin A; however, it only contains one double bond at position 24 as opposed to two double bonds at position 24 as in withaferin A, and it also has three hydroxyl groups at positions 3, 4 and 27. Viscosalactone B is a cyclic peptide that is structurally analogous to withaferin A.

Miscellaneous compounds

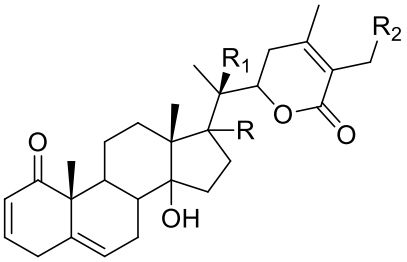
Two substances were identified from *W. somnifera* by Misra et al., in 2012 [57].



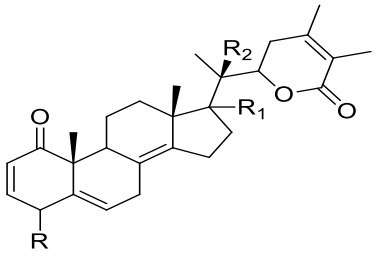
Withaferine A



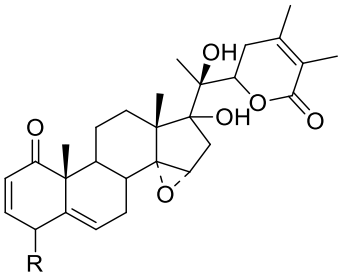
Skeleton of Withanolides



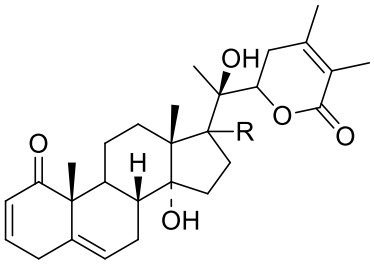
Withanolide C



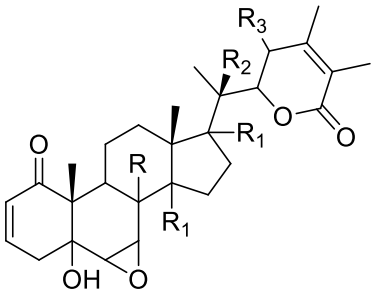
Withanolide A



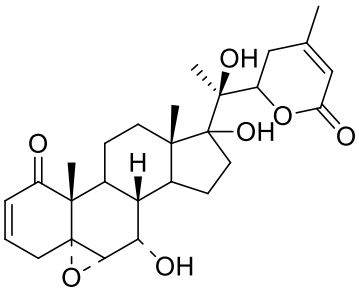
Withanolide G



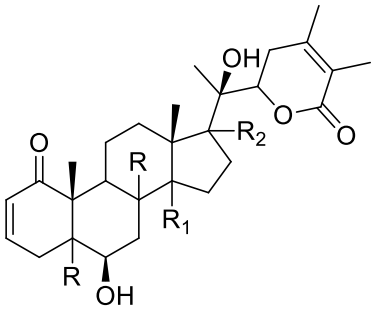
Withanolide F



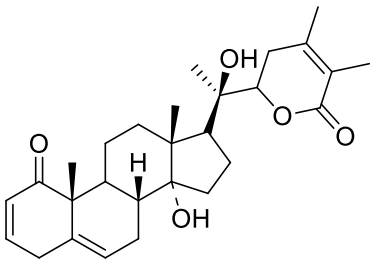
Withanolide I



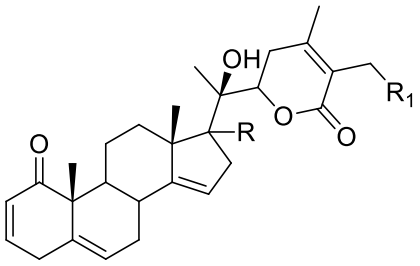
Withanolide G2



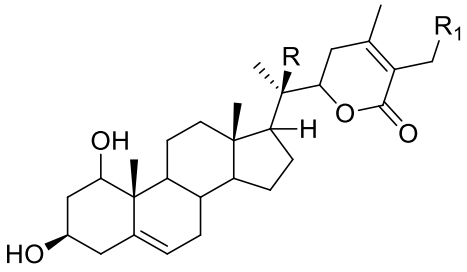
Withanolide Q



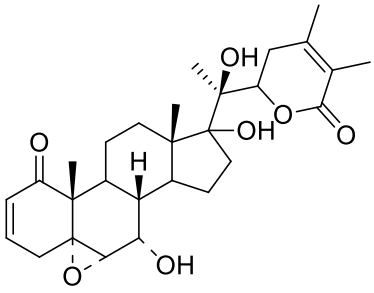
Withanolide M



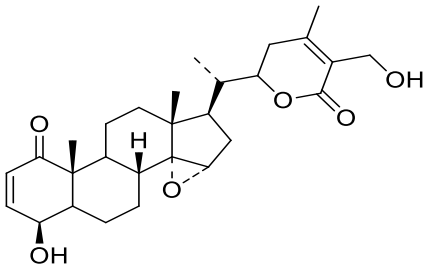
Sominone



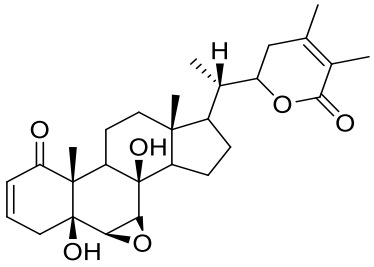
Withanolide Y



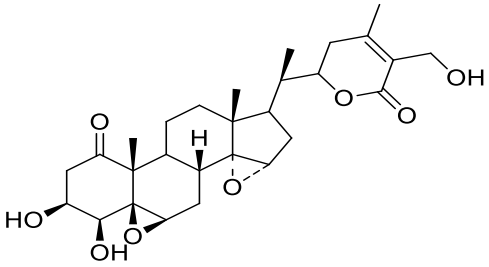
Withasomniferin A



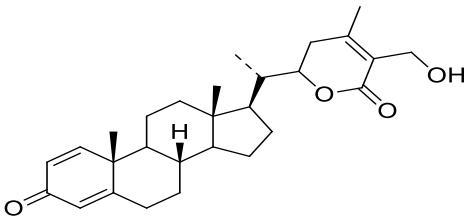
D16 Withanolide



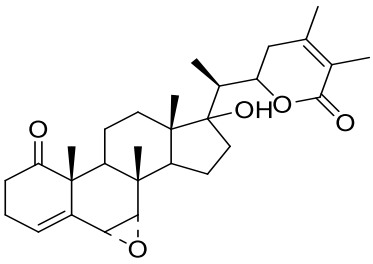
Withasominodienone



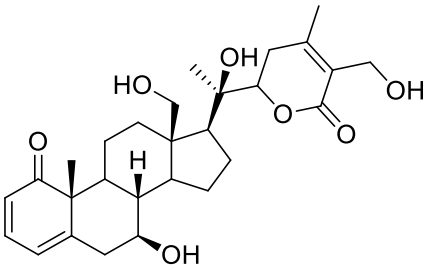
Seminosides



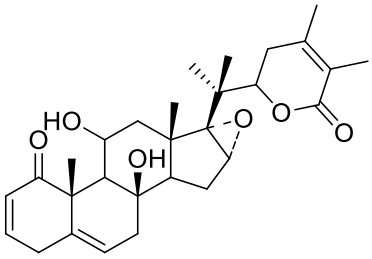
Somnifericin



Withoxylactone



Somniferanolide



Withasomniferinolide

Previously reported bioactivities

The herb has been professionally studied in animal models to prove its ability to treat a number of illnesses. The applications listed below are folk and traditional usage.

Toxicologic studies

Since antiquity, *W. somnifera* has been used for a range of pharmacological purposes in both sexes and all age groups, as well as during pregnancy, without creating any unfavourable side effects [58].

In Wistar rats, a hydro-alcoholic root extract of *W. somnifera* was evaluated for acute and sub-acute oral toxicity by Prabu *et al.,* 2013 [59] found to be non-toxic even at doses as high as 2000 mg/kg body weight (very high). Moreover, there was no noticeable variation in body weight, organ weight, or hemato-biochemical parameters when any amount of the extract was administered. The extract was supplied at a dosage of 2000 mg/kg for 14 days to assess acute toxicity, and at doses of 500, 1000, and 2000 mg/kg for 28 days to assess subacute toxicity. Secondly, the toxicity profile of W. somnifera on developing rat foetuses was investigated with mortality, structural abnormalities, and growth variations all being observed, although no obvious modifications were observed in either the mother or the foetus. There were no variations in the skeletal and visceral forms, the number of corpora lutea, and the percentage of implantations, pregnant female body weight, or the number of viable offspring [60, 61]. Even though 1100 mg/kg intraperitoneal doses were given to Swiss albino mice and Wistar rats within 24 hours without experiencing any fatalities in acute and subacute toxicity tests, little increases in body weight resulted in death, with an LD_{50} of 1260 mg/kg body weight. There were no alterations identified in the composition of peripheral blood. The weights of the spleen, thymus, and adrenal glands, on the other hand, were significantly reduced [62, 63]. *W. somnifera* has the potential to be utilised as a medicinal plant a secure medication for a range of clinical disorders in this way.

Anti-microbial activity

- *W. somnifera* methanolic leaf extract has exhibited significant antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Enterococcus species*, which is consistent with the folklore usage of *W. somnifera* for the treatment of infections [64].
- The effectiveness of *W. somnifera* has been shown to vary depending on the organism being researched in various studies. Cytotoxicity, gene silencing, and immunopotentiation were cited as the mechanisms of action for the anti-microbial effectiveness [65].
- In vitro, *W. somnifera* has significant anti-Salmonella typhimurium activity [66]. Additionally, It has been noted that the administration of W. somnifera increased the survival rate of mice infected with salmonellosis and reduced the amount of germs in several vital organs [67]. Tibrim (rifampicin and isoniazid) and Tibrim's antibacterial activity was synergistically increased by *W. somnifera* extracts *Salmonella typhimurium* and *E. coli* when used together [68]. Oral bacteria, *Streptococcus mutans* and *Streptococcus sobrinus*, were shown to be inhibited in acid production, acid tolerance, and biofilm formation when exposed to *W. somnifera* at concentrations below the minimum inhibitory concentration (MIC).

Anti-inflammatory activity

- *W. somnifera* has been demonstrated to have potent anti-inflammatory effects in a range of medical disorders. In cases of IBD brought on by TNBS, its root extract is anti-inflammatory and restores mucous membranes resolving necrosis, edoema, and neutrophil infiltration, as well as reversing the progression of the disease [69]. In a mouse model of lupus, researchers found that a powder prepared from the roots of this plant significantly reduced inflammation as measured by proteinuria, nephritis, interleukin (IL)-6, tumour Nitric oxide (NO), reactive oxygen species (ROS), and necrosis factor-alpha [70].
- Withaferin-A blocks nuclear factor-kappa B activation, interleukin-6 and TNF-α production, leukocyte adherence and migration, hyperpermeability, and the expression of cell adhesion molecules (CAM) to protect the integrity of the vascular barrier [71]. Additionally, it prevents the production of cell adhesion molecules that TNF-α induces in human pulmonary epithelial cells by inactivating AKT and NFkβ [72]. Withaferin-A also inhibits NFkβv activation by concentrating on the cysteine 179 in the IKK-β catalytic site. NFk and IL-8 are inhibited by cystic fibrosis cell models with withaferin-A. [73].

Anti-arthritic activity

- There is a lot of data to suggest that W. somnifera is effective in treating arthritis. In vitro damage to human osteoarthritic cartilage was shown to be temporarily protected by aqueous extracts of W. somnifera root powder in 2007, as demonstrated by a significant and repeatable suppression of collagenase type-2 activity and a considerable reduction in NO release [74, 75].
- *W. somnifera* root powder was tested on a rat model of adjuvant-induced arthritis, and it was shown to lessen cartilage disintegration as indicated by the quantity of bone collagen in the joints [76].
    - In rats with arthritis brought on by collagen, an aqueous extract of the root of *Withania somnifera* reduced a rise in the arthritic index [77].
    - In a investigation on arthritis-prone rats, *W. somnifera* root powder administration significantly enhanced both the radiological score and the functional recovery of motor activity, which led to a striking decrease in the arthritis' severity [78]. However, by inhibiting collagenase, *W. somnifera* is able to aid in the stability of collagen fibres [79].

Anti-Cancer activity

- Many malignancies and changes associated with cancer have been demonstrated to be inhibited by *W. somnifera* or its chemical constituents in cell lines. A molecular docking research proved the value of withaferin-A and withanone for the development of cancer medication [80].
- At least five distinct processes are used by *W. somnifera* and its constituent parts to eliminate cancer cells. These include the G2-M DNA damage regulation system, the death receptor signalling pathway, the p53 tumour suppressor gene pathway, granulocyte macrophage colony stimulating factor (GM-CSF), and apoptosis signalling [81].
- When withaferin-A was administered to melanoma cells, it was shown to have anti-cancer effect by decreasing the Bcl-2/Bax and Bcl-2/Bim ratios, which were both stimulated by ROS. In this particular apoptotic cascade, the mitochondrial route was used, and it was connected with Bcl-2 downregulation, Caspases-9 and 3 are activated, Cytochrome C is released into the cytosol, the transmembrane potential is eliminated, and Bax is translocated to the mitochondrial membrane [82].
- Withaferin-A also resulted in a reduction in the expression of the Bid gene and an increase in the expression of the

tumour necrosis factor receptor (TNFR)-1. More importantly, withaferin-A prevented NFk- from binding to caspase-3 was activated by DNA and triggered nuclear cleavage of p65/Rel., which led to cell death. According to these findings, withaferin-A kills malignant cells by apoptosis, which might be reliant on mitochondrial processes or independent of mitochondrial mechanisms [83].

- Through the reduction how many breast cancer stem cells there are as well as the size and area of tumours in a transgenic mice model, withaferin-A was shown to significantly slow the progression of mammary cancer. Similarly, withaferin-A treatment of human breast cancer cells resulted in a dose-dependent inhibition of mammosphere development, this was related to complex-III activity decrease and apoptosis activation [84-85]. Autophagy has no bearing on any of these consequences [86]. It does, however, activate Notch-2 and Notch-4, which causes their migration to be stopped [87]. Furthermore, withaferin-A stops the G2 and M phases of the cell cycle in human breast cancer cells [88].
- Withaferin-A therapy suppresses the proliferation of experimental mammary cancer cells by the inhibition of vimentin protein production [89]. This is accomplished by interfering with the cytoskeletal architecture of the cell by interfering with the -tubulin protein [90].
- Hep2 cells from human laryngeal cancer were killed by W. *somnifera* and caused them to enter a cell cycle arrest, which resulted in a blockage of angiogenesis as a result [91]. Human umbilical vein endothelial cells treated with withaferin-A exhibit decreased cell proliferation as a result of anomalies in the ubiquitin-mediated proteasome pathway, cyclin-D1 expression, and protein ubiquitination [92]. Withaferin-A also inhibits the proteasome and causes tumour cells to die, which prevents the growth of patient-derived mesothelioma [93].
- Withaferin-A led to dose-dependent apoptotic cell death and PARP cleavage in a kidney cancer cell line through down-regulating the STAT-3 pathway [94, 95].
- Using doses of 10, 12, and 15 mg/kg body weight, withaferin A, withanolide D, and E demonstrated significant anti-tumor activity in vitro against cells derived from human epidermoid carcinoma of the nose (KB) and in vivo against Ehrlich ascites carcinoma, Sarcoma 180, Sarcoma Black (SBL), and E 0771 mammary adenocarcinoma in mice. During the study, more than half of the mice lived for more than 100 days without showing any signs of tumour development, indicating that the Ehrlich ascites carcinoma was entirely suppressed. As a mitotic toxin, they stopped the division of cultivated human laryngeal carcinoma cells at metaphase and in HeLa cultures, which looked similar to star metaphase. Mitotic arrest was triggered in embryonic chicken fibroblast cells by the antibiotic withaferin A. The action of Withaferin A was enhanced by the addition of methylthiodeacetyl colchicine [96, 97]. Withaferin A has been proven to have growth inhibitory and radio-sensitizing effects on experimental mouse tumours, according to the researchers [98]. The administration of Withaferin A to mice that had been injected with Ehrlich ascites carcinoma cells was shown to suppress tumour development and enhance tumor-free animal survival in a dose-dependent manner, according to the findings [99-100]. Cell death was considerably increased by exposing cells to Withaferin A at a nontoxic dosage of 2.1 micrograms per millilitre for one hour prior to irradiation. Using withaferin A at a safe dosage of roughly 2 μM, we were able to achieve a sensitizer enhancement ratio (SER) of 1.5 for the in vitro cell death of V79 Chinese hamster cells. The serum SER rose as the medication dosage increased [101].

Cardio protective activity

This study investigated the cardioprotective effects of *W. somnifera* in relation to ischemia and reperfusion injury Wistar rats [102]. Post-ischemic reperfusion damage caused significant myocardial necrosis and apoptosisWhen compared to the IR control group, there was a decrease in antioxidant grade and an increase in lipid peroxidation in the heart and the sham group. Prior treatment with *W. somnifera* resulted in strong anti-apoptotic effects [upregulation of Bcl-2 protein and reduction of TUNEL positive, as well as an improvement in the myocardial oxidant-antioxidant balance and histopathologic evaluation of the heart revealed less myocardial injury.

The cardioprotective effects of polyherbal formulations containing *W. somnifera* were demonstrated in animal models [103, 104] through activation of phase-II detoxification enzymes are stimulated by the apoptosis is prevented in a way that is reliant on Nrf-2 [105-106]. Moreover, it aided in the production of hemopoiesis [107]. In a rat model of coronary artery blockage, prophylactic therapy with *W. somnifera* considerably improved the myocardial antioxidant/oxidant balance, consequences of anti-/pro-apoptosis, decreased TUNEL positivity, and compact histopathologic degeneration of the myocardium. In addition to maintaining the oxidant/antioxidant equilibrium, several benefits were observed. Comparable to this, a standardised Cardiotoxicity was treated and biological abnormalities were reversed by *W. somnifera* extract brought on by doxorubicin [108].

Anti-diabetic activity

Indian Systems of Medicine polyherbal formulations (Dianix, Trasina) were shown to have significant anti-diabetic efficacy in human subjects [109]. When administered orally for 30 days, In patients, *W. somnifera* root powder was proven to have blood glucose stability comparable to the oral hypoglycemic drug daonil. *W. somnifera* therapy also substantially improved insulin sensitivity index and prevented the increase in insulin resistance measured using the homeostasis model in rats with non-insulin-dependent diabetic mellitus. *W. somnifera* leaf and root extracts were shown to increase glucose absorption in skeletal myotubes and adipocytes with the leaf extract in a daily dosage manner having more dramatic benefits than the root extract In rats with alloxan-induced diabetes mellitus, root and leaf extracts considerably decreased the amounts of tissue glycogen, glucose-6-phosphatase, blood glucose, and urine sugar and significantly increased the levels of insulin. Additionally, there was a reduction in the ability of the non-enzymatic and enzymatic anti-oxidant defences to be strengthened. SoRelle *et al.,* 2013 [110]. Withaferin-A suppresses the inflammatory response following cytokine-induced islet destruction in culture and after transplantation, and it also has a substantial anti-glycating activity.

Antihepatotoxic activity

Following ten days of oral doses of 10, 20, and 50 mg/kg glycowithanolides isolated from the roots of *W. somnifera* ameliorate silymarin at a dosage of 20 mg/kg did not produce hepatotoxicity in rats while iron overload (FeSo4, 30 mg/kg, i.p.) did [111]. Based on the levels of lipid peroxidation products like TBARS (thiobarbituric acid and reactive substances), HP (hydroperoxides), and liver marker enzymes like AST (alanine transaminase), ALT (alanine transaminase), and ALP, it was determined whether *W. somnifera* root powder had a hepatoprotective effect (alkaline phosphatase). *W. somnifera* provides hepatoprotection when taken in experimental hyperammonemia by reducing the levels of lipid peroxidation

products and liver markers. This might be because *W. somnifera* alkaloids, withanolids, and flavonoids, can maintain normal amounts of urea and urea-related substances, has antioxidant properties, and may scavenge free radicals [112].

The Sabina et al. 2013 [113] study investigated the protective effect of *W. somnifera* against paracetamol-induced hepatotoxicity and found that treatment with *W. somnifera* considerably decreased elevated levels of liver marker enzymes and bilirubin in the blood. Additionally, it helped to restore the histological results, antioxidant status, and total protein content, all of which had been damaged by paracetamol treatment [114].

Anti-oxidant activity

That oxidative damage and reactive oxygen species (ROS) are crucial elements in the processes under study frequently demonstrates the therapeutic value of antioxidants. In an experiment using a model system based on large unilamellar vesicles, we looked at the capacity of withanamides A through I (alkaloids produced from *W. somnifera*) and 3 withanolides to suppress lipid peroxidation. We found that withanamides A through I (alkaloids derived from *W. somnifera*) were effective at inhibiting lipid peroxidation. At concentrations of 1 and 0.5 lg/mL, all nine withanamides decreased lipid peroxidation, and 1 withanolide prevented lipid peroxidation by 82 percent at a concentration of 10 lg/mL. This experiment was also performed using commercial antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) all of which demonstrated 80, 81, and 85 percent inhibition, respectively [115]. Consequently, the findings imply that the hydroxylated long-chain acyl group present in new withanamides may be responsible for the high antioxidant action shown by these compounds. Levels of glutathione peroxidase (GPX), catalase, and superoxide dismutase (SOD) were shown to be increased in the frontal cortex and striatum of the rat brain by other drugs, such as sitoindosides VII-X and withaferin A [116]. Antioxidant activity that is increased and a protective effect on brain tissue would be represented by an increase in the levels of these enzymes. Immunomodulatory activity and hematopoiesis are two important aspects of the immune system.

Antigenotoxic activity

When 7, 12-dimethylbenz (a) anthracene was administered to Syrian golden hamsters, pretreatment with Micronucleated polychromatic erythrocytes (MnPCEs) and chromosomal abnormalities such chromosomal break, gap, minute, and fragment were greatly decreased by withaferin A as well as the number of MnPCEs (DMBA). Accordingly, the findings demonstrated that withaferin-A has an antigenotoxic effect on the DMBA-induced genotoxicity in the bone marrow of golden Syrian hamsters [117].

Neuroprotective activities

A large number of researches have shown that *W. somnifera* has a neuroprotective property. Both neuronal and glial cells are protected against scopolamine-induced harmful alterations when the leaf extract and its component withanone are used. *W. somnifera* significantly decreased the inactivation of glial cell marker glial fibrillary acidic protein and other neuronal cell indicators (GFAP), as well as the inactivation of DNA destruction and oxidative stress markers in a scopolamine-induced inactivation model [118]. In glial cells, by reestablishing the equilibrium between the expression of GFAP, heat shock protein (HSP70), mortalin, and neural cell linkage molecule (NCAM), *W. somnifera* extract reduced lead-induced toxicity [119]. *W. somnifera* glycowithanolides were shown to have considerable antioxidant action in the cortex and striatum of the rat brain, as evidenced by an increase in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity in the cortex and striatum of the rat brain [120]. The extract of *W. somnifera* also protected mice against streptozotocin-induced oxidative damage by reducing the production of reactive oxygen species (ROS) [121]. In human neuroblastoma cell lines, *W. somnifera* root extract or its derivatives induced neurite outgrowth extensions and enhanced neurite outgrowth extensions. Withanolide-A is primarily responsible for axonal extension, while withanolides-IV and VI are responsible for dendritic extension. Contrarily, in rat cortical neurons that had been harmed by 4450, withanolide-IV caused both axonal and dendritic regeneration as well as synaptic repair. N. J. Dar and colleagues 123 amyloid-β (Ab) [122].

Anti-Parkinson activity

There is evidence in the literature that *W. somnifera* contributed significantly to Parkinson's disease development. In a 6-hydroxydopamine (6-OHDA) rat model, *W. somnifera* has been shown to lessen the pathology and symptoms of Parkinson's disease. The study's findings showed that LPO significantly reduced the amount of glutathione (GSH) in the body and increased the activity of the antioxidant enzymes glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPX), SOD, and CAT. This suggests that LPO has a pronounced anti-oxidant effect. It is possible that the increase in striatal catecholamine content caused by *W. somnifera* restored functional deficiencies like as locomotor activity and muscle coordination, as well as drug-induced rotating behaviour. Furthermore, this study revealed an increase in the number of dopaminergic D2 receptors in the striatum, which may act as a protective mechanism to ensure that every dopamine molecule is collected when Parkinsonism is produced. W. somnifera has also been demonstrated to enhance the amount of dopaminergic neurons that have survived in the brain, as shown by tyrosine hydroxylase labelling [123]. The root extract of *W. somnifera* restored anti-oxidant status and decreased oxidant stress in the midbrain of MPTP-intoxicated parkinsonian mice, resulting in a return to normal catecholamine content in the midbrain of parkinsonian mice with normal catecholamine content. The improvement in functional activity of the model was matched by biochemical alterations in the model [124].

Anti-Alzheimer activity

According to earlier research, *W. somnifera* may be crucial in the creation of medications to treat Alzheimer's disease. Healthy research participants who received standardised water extract of *W. somnifera* experienced enhanced cognitive and psychomotor function. *W. somnifera* root extract restored behavioural deficiencies and pathological signs in Alzheimer's disease mice, as well as Ab clearance, via up-regulating lipoprotein receptor-related protein in the liver, according to the findings. This shows that withanamides have the capability to suppress fibril materialization and so protect cells from the toxicity of Ab. Additionally, withanolide-A may inhibit human acetyl cholinesterase, which is crucial for the treatment of Alzheimer's disease, according to docking simulation studies. By reversing memory deficits and preventing the loss of axons, dendrites, and synapses, withanoside-IV and its active metabolite, sominone, greatly reduced the severity of Ab(25-35)-induced neurodegeneration in mice. In rats, subchronic administration to propoxur results in a protective response that totally reverses the inhibition of acetylcholine esterase (AChE) activity and cognitive impairment [125]. W. somnifera has been demonstrated to be beneficial in a model of cognitive impairment by reducing the oxidative damage brought on by streptozotocin, which has been shown to be beneficial. After being treated with Ab, *W. somnifera* increased the peroxisome proliferator-activated receptor-c (PPAR-c) levels in the SK-N-

MC cell line as well as its viability. As a side effect, it also led to the inhibition of acetylcholinesterase activity [126].

Anti-stress activity

Animals that were given *W. somnifera* had better stress tolerance. Researchers were able to reverse chronic stress-related declines in T cell population in mice while also raising Th1 cytokines by extracting the aqueous fraction from *W. somnifera* roots. A clinical experiment including humans was conducted to evaluate the efficacy and safety of *W. somnifera* root extract, broad spectrum, high concentration.The findings demonstrated that blood cortisol levels were decreased without causing any appreciable adverse effects. Additionally, over an extended period of time, the polyherbal formulation EuMil dramatically decreased the levels of brain monoamines chronic electroshock stress results in the release of (nor-adrenaline, dopamine, and 5-hydroxytryptamine). Further research revealed that EuMil normalised male sexual behaviour, corrected chronic stress-induced glucose intolerance, and reduced behavioural despondency in male participants. Additionally, it decreased corticosterone levels in the circulation, immune suppression, stomach ulcers, and cognitive impairment. Another poly-herbal formulation's depressive and anxiolytic effects were demonstrated in rats, and they were partly attributable to the activation of the adrenergic and serotonergic systems. When combined with pentylenetetrazole, W. somnifera's glycowithanolides were shown to have an anxiety-relieving impact on rats that was comparable to that of recognized anti-depressant drugs. Additionally, it abridged tribulin heights, an endocoid marker of clinical anxiety, in the rat brain. In addition, it reduced the levels of lipid peroxidation (LPO) and oxidative free radical scavenging enzymes in the striatum and frontal cortex of chronically footshock stressed rats, respectively [127].

Anxiety and depression

It has been demonstrated that *W. somnifera* aids people with behavioural problems in mood regulation and anxiety reduction. W. somnifera roots were used to extract the bioactive glycowithanolides (WSG), which were tested for their anxiolytic and depressive effects in rats at dosages of twenty and fifty mg/kg administered orally once daily for five days. It was demonstrated that WSG generates superior results in the anxiolytic tests when compared to standard tricyclic anti-depressant imipramine in the dose of 10 mg/kg, ip for anti-depressant studies and standard bendodiazepine lorazepam in the dosage of 0.5 mg/kg, ip for anxiolytic research. In a different study, oral administration of *W. somnifera* at dosages of 100, 200, or 500 mg/kg significantly increased the amount of time spent and the number of entries into the open arms on the EPM test, indicating anxiolytic activity. Additionally, when combined with other anxiolytic medicines, it helped to increase the anxiolytic effect of diazepam (0.5, 1 or 2 mg/kg, ip) at subeffective levels, such as 50 mg/kg, oral. Kaurav et al., (2012) obtained similar results [128], They found that the activity of parachlorophenylalanine, ritanserin, and aqueous and methanolic extracts of W. somnifera (50 mg/kg) decreased marble burying behaviour activity without changing motor activity [129].

Nootropic effect

W. somnifera has been utilised for hundreds of years as a memory-enhancing drug in traditional Ayurvedic therapy. Its neuropharmacological effects have been thoroughly studied in a variety of laboratories, and various studies have been published demonstrating its nootropic activity. The neuropharmacological effects of the plant, plant extract, and isolated withanolides—the main active principles—have all been well studied. Oral W. somnifera root extract reversed the scopolamine (0.3 mg/kg)-induced disruption of acquisition and retention and attenuated the amnesia produced by acute treatment with electroconvulsive shock (ECS) given right after training; oral W. somnifera root extract improved retention in a step-down paradigm in mice administered orally at doses of 50, 100, and 200 mg/kg; -induced interference with recruitment and retention [130]. Alzheimer's disease developed as a result of an ibotenic acid (IA) lesioning of the nucleus basalis magnocellularis and showed up as a substantial cognitive decline. Equimolar dosages of sitoindosides VII-X and withaferin A administered within two weeks after the commencement of treatment successfully reversed the cognitive deterioration brought on by IA as well as the drop in cholinergic markers in the brain [131- 134]

Conclusions

The herb has been utilised in several medicinal practises dating back to ancient times, including Ayurveda, Unani, Siddha, and homoeopathy. It is believed to work well. To support its potential medical uses, further thorough clinical investigations are still needed. This paper provides a comprehensive overview of *W. somnifera* and its active components' geographic distribution, traditional applications, phytochemistry, and pharmacological effects. It also provides a full evaluation of the number of commercial medications that contain it as an active component and have therapeutic benefits and favourable health consequences. The clinical studies and toxicological features of its extracts and components are also discussed. *W. somnifera* extracts and phytochemicals have significant anticancer, anti-inflammatory, apoptotic, immunomodulatory, antimicrobial, anti-diabetic, hepatoprotective, hypoglycemic, hypolipidemic, cardio-protective, and spermatogenic activity, according to a review of pertinent in-vitro, in-vivo, and clinical studies. Numerous neurological and psychological disorders have been discovered to be particularly active against *W. somnifera*, including Parkinson's disease, Alzheimer's disease, Huntington's disease, ischemic stroke, sleep deprivation, amyotrophic lateral sclerosis, attention deficit hyperactivity disorder, bipolar disorder, anxiety, depression, schizophrenia, and obsessive-compulsive disorder. Modern conventional medications include drawbacks such rising resistance, inevitable side effects, effectiveness loss with repeated use, and high cost. Researchers have been motivated by this to produce bioactive therapeutic compounds and drugs from natural sources, such herbal plants. Clinically available neuroprotective and psychoactive drugs are rare, and the majority of those that have been shown to be effective in animal models but fall short in human studies. In this regard, more study is required to determine if this species may be harmful after sub-chronic and chronic administration. As a consequence, this study points to prospective directions for further research on the W. somnifera species in the area of human health. Therefore, it is anticipated that further study of this species will lead to the identification of model molecules that might be used in the creation of innovative herbal medicines, and that new species-related patent applications will be made soon.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported.

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