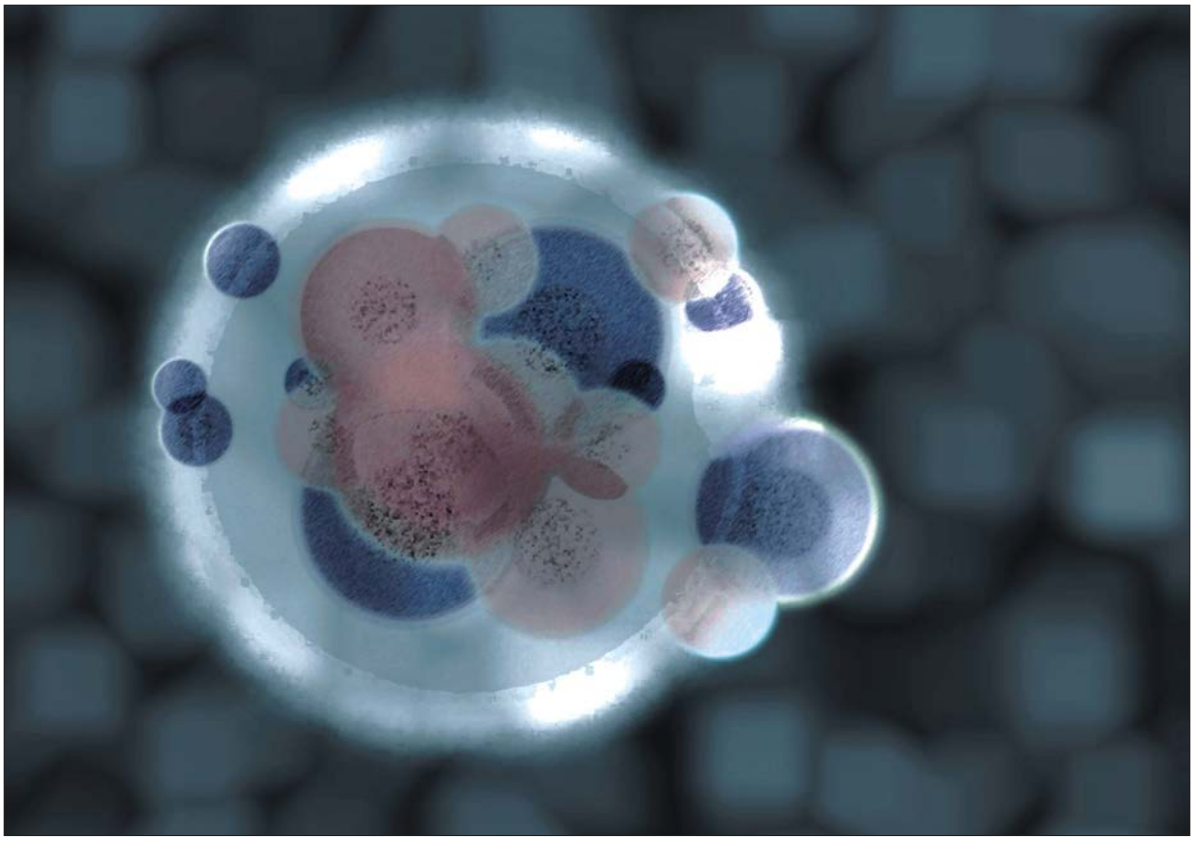

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Ashwagandha (*Withania somnifera*): Role in Safeguarding Health, Immunomodulatory Effects, Combating Infections and Therapeutic Applications: A Review

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Abstract: Ashwagandha (*Withania somnifera*) is a well known herb possessing several health benefits. The steroidal lactones (withanolides) obtained from its roots have been implicated in a wide range of therapeutic activities and maintaining general health: Immunomodulation, combating infectious agents, anti-cancer and anti-epileptic, memory enhancer, to promote good physical and mental health, mood elevator, diuretic, general tonic and rejuvenator, stress reliever, cardiorespiratory endurance enhancer, anti-ageing, anti-oxidant, hypoglycemic, hypocholesterolemic and in common an effective adaptogen. Steroidal alkaloids and lactones are the active constituents of the plant. Withanolides as per theory occupies the receptor sites in the cell membrane thereby preventing the attachment and subsequent exertion of the effect of actual hormone. Withanolides have got analgesic and anti-inflammatory activity due to cyclooxygenase-2 inhibition property. Ashwagandha enhances nitric oxide synthatase activity of the macrophages, which in turn increases the microbial killing power of these immune cells thereby enhancing the Cell Mediated Immune (CMI) response. A glycoprotein Glycowithanolides (WSG) commonly known as *W. somnifera* glycoprotein is responsible for antimicrobial activity. Milk supplemented with Ashwagandha has been reported to increase total proteins and body weight and the plant alone helps in inducing tolerance and dependence. Its anti-stress and radiosensitization action; beneficial effects on cardiovascular system and sexual behavior; curative properties against neurodegenerative diseases and poisoning due to toxins and chemicals (including snake venom) has made this plant a treasure of nature. Thus the plant is an important component of many polyherbal preparations. Important for researchers and scientists is that biotechnologically advanced techniques; novel disciplines of bioinformatics and genomics can help in identifying and generating bioactive principles of the plant. All these salient health applications of this herb in biomedicine and veterinary sciences are discussed in this review focusing its potent role in maintaining sound health, immunomodulatory effects, combating infections, therapeutic usages and other beneficial applications.

Key words: Ashwagandha, indian ginseng, ayurveda, health, beneficial application, immunomodulation, treatment, therapy, memory enhancer, anticancer

INTRODUCTION

Ashwagandha (*Withania somnifera*) is a well known herb possessing several health benefits and is an important 'Rasayana' as "Sattvic Kapha Rasayana" in Indian Ayurveda, used since centuries for its miraculous advantages (Mahima *et al*., 2012). Ashwagandha (*Withania somnifera*) is a traditional medicine with growing needs due to its remedial potentials. Chinese, Unani, Ayurveda and Siddha literatures admire the therapeutic merits of plant-derived medicines against almost all ailments. Herbal medicines strongly involve mass appeal being safer and inexpensive. An esteemed Rishi (sage) Punarvasu Atriya was the first person who gave the teaching regarding the use of Ashwagandha that extends back over 3000 to 4000 years ago wherein its use is widely extolled as a tonic particularly for emaciation in all age group of people.

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This causes enhancement of the reproductive function of both men as well as women (Mathur and Velpandian, 2009; Verma and Kumar, 2011; Mahima *et al*., 2012; Dhama *et al*., 2013a).

Ashwagandha is generally available in form of fine powder that can be used with water, ghee or honey (Gupta *et al*., 2006). The Nagori variety is the best among all Ashwagandha varieties. The health products made up of Ashwagandha (*W. somnifera,* Apocynaceae) are becoming popular as commonly used medicinal plants. The steroidal lactones (withanolides) obtained from its roots have been implicated in a wide range of therapeutic activities and maintaining general health like immunomodulation, combating infectious agents, anti-cancer, anti-epileptic, memory enhancer, to promote good physical and mental health, mood elevator, diuretic, rejuvenator, stress reliever, cardio-respiratory endurance enhancer, anti-ageing, anti-oxidant, hypoglycemic, hypocholesterolemic and in common an effective adaptogen (Scarfiotti *et al*., 1997; Adallu and Radhika, 2000; Hemalatha *et al*., 2006; Naidu *et al*., 2006; Mahima *et al*., 2012; Dhama *et al*., 2013a). Such alkaloids (withanolides) also work as "marker compounds/agents" for chemical standardization of Ashwagandha-based products (Dhuley, 2000; Shenoy *et al*., 2012).

Ashwagandha improves the memory by enhancing the brain and nervous function; promotes vigour and vitality along with cheerful sexual life and reproductive equilibrium; augments the body's pliability to stress being a powerful adaptogen; shows anxiolytic effect, has hepato-protective property, raises hemoglobin level and red blood cell count, improves energy levels, maintains mitochondrial health; has potent antioxidant properties so as to protect cellular damage caused by free radicals and improves the body's resistance against various ailments by improving the cell-mediated immunity (Scarfiotti *et al*., 1997; Bhattacharya and Muruganandam, 2003; Arora *et al*., 2004; Kuboyama *et al*., 2005; Harikrishnan *et al*., 2008; Sandhu *et al*., 2010). Ashwagandha is a potent adaptogen or vitalizer and has powerful antioxidant and detoxifying properties. Multiple actions of this miracle herb include anti-inflammatory, analgesic, anti-stress, immunomodulatory, ant-microbial, cytoprotective, bettering anabolic activities, active against air-pollution and anti-cancer effects (Mishra *et al*., 2000; Kushwaha *et al*., 2012; Mahima *et al*., 2012, 2013a, b; Dhama *et al*., 2013a).

The present review describes Ashwagandha (*W. somnifera*) and its active compounds, mechanism of action and biological chemistry and classical beneficial applications of Ashwagandha in biomedicine and veterinary sciences viz., immunomodulatory effects, activity against microbes and infection and usefulness as an alternative, chemotherapeutic agent, general health benefits, promoting vigour and vitality, stress reliever antidepressant, anti-inflammatory and adaptogenic property, effects on cardiovascular system, role in treating sexual disability, diseases and disorders, potent anti-cancer effects, reducing poisoning due to toxins/chemicals/drugs, anti-aging activities, memory enhancer, treating neurodegenerative disorders, role in development of drug tolerance and dependence.

CLASSIFICATION

Ashwagandha (*Withania somnifera*)
Family-solanaceae/apocynaceae
Popular/common name: Indian ginseng/winter cherry:
Ashwagandha is an exceedingly valuable medicinal plant with valuable and wide therapeutic benefits in the conventional system of medicine. The plant grows in form of shrub with branching, height reaches to around 150 cm, leaves are up to 10 cm long; flowers present greenish or lurid yellow color, fruits/berries when mature are orange colored and its seeds are sown mostly during month of June or July (Khanna *et al*., 2006a; Dasgupta *et al*., 2008). Yield as well as quality of plant and its metabolites are affected by seasonal temperature, method of sowing, duration of light and dark period, depth of tillage, time of harvesting, concentration of fertilizers i.e., nitrogen, phosphorus, potassium application, effect of manure and field space present in between crops or density of plant population etc. (Kothari *et al*., 2003; Agarwal *et al*., 2004; Patel *et al*., 2004; Sreerekha *et al*., 2004; Ajay *et al*., 2005; Panchbhai *et al*., 2006).

Active constituents/compound/principle: The root of *Withania somnifera* has more than 35 chemical constituents (Rastogi and Mehrotra, 1998). Steroidal alkaloids and lactones (Withanolides, Withaferins): Anaferine alkaloid, anahygrine, isopelletierine, cuseohygrine, Ashwagandhanolide (dimeric thiowithanolide), chlorogenic acid, beta-Sisterol, fruit cysteine, iron, scopoletin, somniferinine, somniferiene, tropanol, withananine, withanoside IV, withanolides A-Y (Steroidal lactones) and saponins sitoindosides and acylsterylglucosides. The sitoindosides VII-X and withaferin-A are anti-stress agents which support immunomodulatory actions and have antifungal properties also (Abraham *et al*., 1975; Choudhary *et al*., 1995; Singh *et al*., 2006). Most of the pharmacological activities of Ashwaganda have been attributed to two main withanolides, withaferin A and withanolide D (Singh *et al*., 2010). Five-dehydroxy withanolide-R and withasomniferin-A are obtained from the aerial parts of

W. somnifera and effect of withaferin-A has been also seen on human blood lymphocytes. *Withania somnifera* is a rich source of iron (Davis and Kuttan, 2000a; Kuboyama *et al*., 2006; Subbaraju *et al*., 2006; Mirjalili *et al*., 2009).

Ethnopharmacological aspects: The pharmacological as well as metabolic effects of ashwagandha reveals that it has both herbal tonic as well as health food. In rats the swimming time is increased by Ashwagandha as determined by physical working capacity test (swimming endurance test). By employing such test it has been found that the weight of the heart increases relatively and the content of glycogen in myocardium increased significantly (Dhuley, 2000).

Two major classes of compounds viz., steroidal alkaloids and steroidal lactones are responsible for the wide range of beneficial effects of Ashwagandha. Withanolides are a class of compound included in the group of steroidal lactones and are responsible for antioxidant properties as well as free radical scavenging activities. Till date at least 12 alkaloids and 35 withanolides have been studied. Several studies have also revealed the antimicrobial properties of ashwagandha along with antibacterial activity against potentially dangerous like *Salmonella* (food poisoning causing organism). The ability of macrophage and immune cells to eat pathogens is enhanced by the root extract of Ashwagandha in comparison to macrophages (in control group) that have not received ashwagandha (Davis and Kuttan, 2000b; Anonymous, 2004; Govindarajan *et al*., 2005; Owais *et al*., 2005).

Treatment with Ashwagandha affords resistance of heart muscle of frog towards the toxic action of strophanthin-K and the duration of contractility is increased. The coagulation time is significantly increased by ashwagandha treatment resulting in attainment of normalcy after 7 days of cessation of treatment. In the blood serum of rats there is no any significant change in biochemical parameters. On the basis of such observations adaptogenic, cardioprotective as well as anticoagulant properties of ashwagandha are well understood (Dhuley, 2000).

Mechanism of action: Due to the property of helping in regulation of important physiologic processes Ashwagandha is assumed to be amphoteric. As per requirement withanolides act as important hormone precursors that has got the capability to convert into human physiologic hormones. The plant-based hormone precursor as per theory occupies the receptor sites in the cell membrane thereby preventing the attachment and subsequent exertion of the effect of actual hormone. Small effect is exerted by the plant-based hormone if the level of original hormone is low (Misra, 2004). The anti-stress effect of ashwagandha was due to stimulation of respiratory function causing relaxation of smooth muscle along with stimulation of thyroid synthesis and secretion. Increase in dopamine receptors in the corpus callosum of brain induced by stress is suppressed by ashwagandha. Stress-induced increase in corticosterone in plasma along with blood urea nitrogen as well as blood nitric acid is also reduced. Anxiolytic effect of ashwagandha is exerted by acting as a gamma-aminobutyric acid (GABA) mimetic agent. The anticonvulsant activity by virtue of attachment to the GABA receptor is also a special feature of Ashwagandha (www.amazondiscovery.com).

Toxicological properties: Acute toxicity studies of ashwagandholine (total alkaloids from the roots of *Withania somnifera*) in 10% propylene glycol on the central nervous system. The acute LD_{50} has been found to be a bit higher in rats (465 mg kg^{-1}) than in mice (432 mg kg^{-1}) (Mishra *et al*., 2000). Sharada *et al.* (1993) tested acute (24 h) toxicity of alcohol extract from the roots of ashwaganda in swiss albino mice and subacute toxicity (30 days) in wistar rats. Single intraperitoneal injection of 1100 mg kg^{-1} of the extract in mice did not produce any deaths within 24 h, but small increases in dose led to mortality. LD_{50} value was calculated as 1260 mg kg^{-1} b.wt. Repeated injections of ashwagandha extract at a dose of 100 mg kg^{-1} b.wt. for 30 days in either sex of wistar rats for subacute toxicity studies did not produce any mortality and no change in peripheral blood constituents. But, significant reductions in the weights of spleen, thymus and adrenals were observed in male rats at the end of the experiment. The acid phosphatase content of peripheral blood in both sexes showed a significant increase from control whereas other biochemical parameters were in the normal range.

Acute toxicity studies of *Withania somnifera* (L.) Dunal, WSF did not reveal any mortality and clinical signs of toxicity up to 2000 mg kg^{-1} b.wt. Chronic administration of WSF did not cause any clinical signs of toxicity up to 1000 mg kg^{-1} b.wt. Genotoxic study of WSF did not showed increase in percentage abnormal metaphases up to 1000 mg kg^{-1} b.wt. Moreover, WSF was found to increase immunological response against antigenic stimuli (Sharma, 2011).

Dose-related tolerability, safety and activity of *Withania somnifera* formulation in normal individuals were evaluated in eighteen apparently healthy volunteers (12 male and 6 female) ageing about 18-30 years. The volunteers were treated with WS capsules (aqueous

extract, 8:1) daily in two divided doses with increase in daily dosage every 10 days for 30 days (750, 1000 and 1250 mg $day^{-1} \times 10$ days). Except one volunteer, all tolerated WS without any adverse effects. One volunteer showed increased appetite, libido and hallucinogenic effects with vertigo at the lowest dose and was withdrawn from study. In six volunteers, improvement in quality of sleep was noticed. Reduction in total and LDL cholesterol, normal values in organ function tests, reduction in total body fat percentage and increase of strength of muscle activity was significant (Raut *et al*., 2012).

Biological chemistry of *Withania sominifera*: Withanolide obtained from the plant possess analgesic and anti-inflammatory activity due to its cyclooxygenase-2 inhibition property (Nair and Jayaprakasam, 2007). Peroxidases enzyme have been purified from this herb. Withanolides I-III and IV-V isolated from *W. somnifera* inhibited cholinesterase, acetylcholinesterase and butyrylcholinesterase, toxic phospholipase enzymes and therefore, are under consideration to be among the potent therapeutic candidate for treatment of Alzheimer's disease (Choudhary *et al*., 2004, 2005; Johri *et al*., 2005; Kambizi *et al*., 2006). Important constituent withanolides can be isolated and purified by various techniques mainly by High Performance Liquid Chromatography (HPLC) and spectrometry (Khajuria *et al*., 2004; Sharada *et al*., 2007).

Classical applications of ashwagandha: Ashwagandha attains the special name as the root smells like horse ("*Ashwa*") and it is believed that on guzzling it provides power of a horse. Different parts of Ashwagandha have significant therapeutic potency either as a whole plant extract or as separate constituents (Bhatt *et al*., 2006; Gupta and Rana, 2007). Utmost benefits comes out when fresh powder from the plant is used.

The root of Ashwagandha is very useful due to properties of being effective narcotic, diuretic, tonic, aphrodisiac, anthelmintic, astringent, antiangiogenic in case of tumor, antimutagenic, thermogenic and stimulant (Mohan *et al*., 2004; Khanam and Devi, 2005a; Khanna *et al*., 2006b; Mahima *et al*., 2012).

Roots are equally effective against emaciation, constipation, debility, goiter, rheumatism, vitiated conditions of leucoderma, insomnia and nervine disorders, lead-induced DNA damage etc (Sharma *et al*., 1985; Khanam and Devi, 2005b).

The paste of roots prepared with water effectively reduces the incidences of asthma, arthritis, rheumatoid, osteoarthritis, carbuncles, ulcers, leucorrhoea, boils, pimples, flatulent colic, piles and painful swellings as anti-arthritic agent when applied locally (Rasool and Varalakshmi, 2006b; Salve *et al*., 2006).

The root in conjunction with other drugs is prescribed for snake venom as well as in scorpion-sting. Glycoprotein obtained from *W. somnifera* hampers activity of hyaluronidase and neurotoxic phospholipase A(2) and counteract the toxicity (Machiah and Gowda, 2006; Machiah *et al*., 2006).

Studies demonstrated that root extract upregulates Th1-dominant polarization due to the presence of withanolide-A and hence supports the humoral immunity (HI) and Cell-mediated Immune (CMI) responses in BALB/c mice (Bani *et al*., 2006; Malik *et al*., 2007).

The leaves are bitter and recommended in fever and tender swellings (Kaur *et al*., 2004).

It also shows phenomenon of cytomixis (Datta *et al*., 2005; Kaul *et al*., 2005).

The flowers are useful as astringent, depurative, diuretic and aphrodisiac (Singh *et al*., 2011).

Fruits of Withania have potent inhibitory effect on peroxidation of lipid (Jayaprakasam *et al*., 2004).

The seeds on one side have anthelmintic property to expel the worms from the body and on another hand they are capable of increasing the sperm count and testicular growth (Abdel-Magied *et al*., 2001).

Aqueous extract of plant can modulate the immune response of vaccine, such as DPT vaccine (Guatam *et al*., 2004).

Ashwagandharishta prepared from seeds is used against conditions of hysteria, anxiety, memory loss etc. It also acts as a potent stimulant (Dadkar *et al*., 1987).

Immunomodulatory effects: Extract of *W. somnifera* has been shown to significantly increase the Cell Mediated Immunity (CMI) in normal mice. Root extract enhances the level of interferon gamma (IFN-γ), interlukin-2 (IL-2) and granulocyte macrophage colony stimulating factor (GM-CSF) in mice, suggestive of an immunopotentiating and myeloprotective effect. Ashwagandha enhances nitric oxide synthatase activity of the macrophages, which in turn increases the microbial killing power of these immune cells (Iuvone *et al*., 2003). It activates and mobilizes macrophages for rendering increased phagocytic activity, potentiates activity of lysosomal enzymes and acts as an anti-stress molecule and anti-inflammatory agent in mice and rat (Rasool and Varalakshmi, 2006a). Immune enhancement with Ahswagandha has also been observed in mice with myelosuppression induced by cyclophosphamide, azathioprin and prednisolone. Root extract of *W. somnifera* has been reported to induce helper T-lymphocyte (Th1) polarised cell mediated immune

response in BALB/c mice (Davis and Kuttan, 1998; Iqbal and Datta, 2006; Malik *et al*., 2007). Both immunostimulatory and immunosuppressive properties are present in Ashwagandha. It induces inhibition of delayed hypersensitivities (Auddy *et al*., 2008; Verma *et al*., 2012). Powdered root extract from Ashwagandha has profound effect on production of healthy white blood cells thereby it is an effective immunoregulator as well as chemoprotective agent in mice. The delayed type of hypersensitivity is also inhibited by this extract along with enhancement of phagocytic activities of macrophages while comparing with a control group. It has been found that the nitric oxide activities of the macrophages are enhanced by *W. somnifera* via induction of nitric oxide synthase enzyme activity. The plant is also responsible to cause down regulation of the senescence-specific beta-galactosidase activity (Choudhary *et al*., 2004; Kiefer, 2006; Singh *et al*., 2010; Widodo *et al*., 2009).

Active against microbes and infection: Due to rapid emergence of antibiotic resistant strains of bacteria, treatment of infectious diseases is becoming challenging day by day and at the same time rapidly developing bacterial resistance is growing as a matter of global concern (Tiwari *et al*., 2013a). This alarming health concern particularly due to the continuous increase of immunocompromised patients demands various alternative therapeutic modalities such as bacteriophage, panchgavya, cytokine, herbal therapy and others (Tiwari and Hirpurkar, 2011; Mahima *et al*., 2012; Dhama *et al*., 2013b, c, d; Tiwari *et al*., 2013b, c, d).

Herbal therapy is an ancient revered therapy which is again gaining the momentum in lieu of need of alternative novel therapies and with least or no side effects this therapy is rapidly speeding the steps (Mahima *et al*., 2012; Dhama *et al*., 2013b). Though morphological, biochemical, functional and genetic variation exist but as a whole variety of herbs form a bouquet of safe, sound and easily available medicine (Dhar *et al*., 2006; Bandyopadhyay *et al*., 2007; Kumar *et al*., 2007; Mahima *et al*., 2012). In regards to Ashwagandha besides other biological health promoting effects, the herb has also been found to illustrate antibacterial, antifungal and anti-viral effects.

A glycoprotein Glycowithanolides (WSG), commonly known as *Withania somnifera* glycoprotein, 28 kDa isolated from the *W. somnifera* root has demonstrated potent antimicrobial activity against the pathogenic fungi and bacteria. WSG protein put forth fungistatic effect in terms of inhibiting fungal spore germination and reduction of hyphal growth of *Fusarium oxysporum*, *F. verticilloides* and *Aspergillus flavus*. Antibacterial effect has also been seen against *Clvibacter michiganensis* subsp. *Michiganensis bacteria*. *In vitro* antibacterial property of *Withania* plant in laboratory plant cell culture is also on hand. These findings persuade further studies to explore wide horizons of WSG as a budding therapeutic agent against various fungi and bacteria (Girish *et al*., 2006; Jamil *et al*., 2007; Kulkarni *et al*., 2007).

W. somnifera plant has potent antibacterial property even against multidrug resistant (MDR) strains of microbes as withaferin and withanolides are the chief compounds. *Withania* has been found to be effective in inhibiting the growth of several bacteria viz., *Neisseria gonorrhoea*, *Escherichia coli*, *Salmonella*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* or Oxacillin resistant *S. aureus* (MRSA or ORSA) (Akinyemi *et al*., 2004; Owais *et al*., 2005; Kambizi and Afolayan, 2008; Mehrotra *et al*., 2011; Sundaram *et al*., 2011; El-Boshy *et al*., 2013). Ashwagandha has been shown to provide immunoprotection against *Escherichia coli* infection in Guinea pigs, *Listeria monocytogenes* infection in mice and *Bordetella pertussis* infection in animals (Teixeira *et al*., 2006). It is a potent inducer of inhibiting Delayed Type of Hypersensitivities (DTH). Clinical health benefits of Ashwagandha have been reported in groups of Human Immunodeficiency Virus (HIV) infected patients and to treat the cases of genital herpes occurring due to herpes simplex virus type 1 and 2 (HSV1 and-2) (Kambizi *et al*., 2007). Ashwagandha is a potent antidepressant with the property to strengthen immunity against cold, flu and other common infections. Recently, ashwagandha has been found to ameliorate the effects of chicken infectious anaemia virus induced clinical parameters (haematological changes), pathology and pathogenesis in virus infected chicks, indicating protective potential of this herb in immunosuppressive viral disease of poultry (Latheef *et al*., 2013a, b).

General health benefits: Milk supplemented with Ashwagandha has been reported to increase total proteins and body weight (Venkataraghavan *et al*., 1980). It has a rejuvenative effect on the body mainly on the reproductive and nervous systems and is used to improve vitality and aid in recovery after chronic illness (Bhattacharya *et al*., 1987). Aphale *et al*. (1998) reported that the combination of Asgand (*Withania somnifera*) and Ginseng (Panax ginseng) was significantly increased the body weight, food consumption, liver weight and improved haematopoiesis when administered orally for 90 days using three doses in rats.

Anti-stress agent: Aswagandha is a potent anti-stress agent. It checks stress induced changes in adrenal function and augments protein synthesis. Ashawagandha with anti-stress activity is effective in increasing the physical endurance, plasma corticosterone level, sexual vigour, more sperm count, phagocytic index, cardiac activity, augmenting level of Th-1 cytokines, rising T lymphocytes proliferation and preventing stress induced ulcer, carbon tetrachloride (CCl_4) induced hepatotoxicity and mortality (Ilayperuma *et al*., 2002; Tomi *et al*., 2005; Khan *et al*., 2006; Al-Qirim *et al*., 2007). Experimental studies in rats and mice showed same aforesaid effects when pre-treated with the crude form of Ashawagandha. Several studies have indicate the potent clinical and beneficial use of Ashwagandha (*W. somnifera*) in various health related issues viz., in the prevention and treatment of cyclophosphamide induced urotoxicity, protection of gonads in case of carbendazim toxicity, many stress induced diseases like arteriosclerosis, early ageing, arthritis, diabetes, hypertension and malignancy due to its potent anti-stress, vitalizing and rejuvenating properties (Scarfiotti *et al*., 1997; Davis and Kuttan, 2000b; Bhattacharya and Muruganandam, 2003; Singh *et al*., 2011).

Rodents received a mild electric shock to their feet for a period of 21 days, resulting in stress induced effects like hyperglycemia, increase in plasma corticosterone levels, glucose intolerance, gastric ulcerations, male sexual dysfunction, immunosuppression and mental depression. Ashwagandha was given to the animals one hour before the electric shock and it is effectively reduces chronic stress in rodents (Bhattacharya and Muruganandam, 2003). The anti-anxiety effect of Ashwagandha was due to GABA-like activity, inhibitory neurotransmitter in the brain. It decreases the neuron activity and inhibits nerve cells from over firing, results in calming effect (Mehta *et al*., 1991).

Ashwagandha has been used to stabilize mood in patients with behavioural disturbances. It has an anti-depressant and anti-anxiety effect in rodents when compared to the anti-depressant drug imipramine and the anti-anxiety drug lorazepam (Ativan) (Archana and Namasivayam, 1999). Ashwagandha is one of the most widespread tranquillizers used in India (Singh *et al*., 2010). Bhattacharya and Muruganandam (2003) compared the ability of *Withania somnifera* and *Panax ginseng* to relieve chronic stress syndrome in a rat model. They showed that both Ashwagandha and *Panax ginseng* decreased the frequency and severity of stress-induced ulcers, reversed stress-induced immunosuppression, reversed stress-induced inhibition of male sexual behaviour and inhibited the effects of chronic stress on retention of learned tasks, but only the *Withania* extract increased peritoneal macrophage activity.

Anti-oxidant activity: Root powder of *W. somnifera* has been reported to prevent Cadmium-induced oxidative stress in chicken and lead-induced oxidative damage in mouse (Chaurasia *et al*., 2000; Mahadik *et al*., 2008; Bharavi *et al*., 2010). Plant extract protects myocardial cells from adverse effect of infarction or cardiac necrosis and stroke in rats which has molecular basis and high regeneration power as well (Arya *et al*., 2004; Gupta *et al*., 2004; Mohanty *et al*., 2004; Sivanesan, 2007). *Withania* has showed antiulcer property and antioxidant activity also in rats along with improved calcification of bone in calcium-deficient ovariectomized rats (Sisodia and Bhatnagar, 2004; Bhatnagar *et al*., 2005; Nagareddy and Lakshmana, 2006; Sumantran *et al*., 2007a).

Withania somnifera has powerful antioxidants. It increases the levels of three natural antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidise in the brain of rats (Dhuley, 2000). Active principles of *Withania somnifera* root have antioxidant effects like anti-stress, cognition-facilitating, anti-inflammatory and anti-aging effects (Bone, 1996).

Anti-inflammatory effects: Ashwagandha acts as an effective anti-inflammatory agent and relives the symptoms of arthritis and variety of rheumatologic conditions. Naturally, it has much higher steroidal content than that of hydrocortisone (Anbalagan and Sadique, 1981). Begum and Sadique (1988) demonstrated that rats treated with powder of *Withania somnifera* root orally for 3 days, 1 h before injection of inflammatory agent produced anti-inflammatory responses comparable to that of hydrocortisone sodium succinate.

Effect on cardiovascular system: In humans, assessment of the hypoglycemic as well as diuretic and hypocholesterolemic effects of Ashwagandha root revealed that the treatment of subjects suffering from type 2 diabetes and mildly hypercholesterolemic can be initiated for a period of 30 days with a powder extract which results in a decrease in the glucose level in blood, comparable to that of an oral hypoglycemic drug. Significant increase in volume and sodium content in urine and decrease in cholesterol as well as triglycerides and low density lipoproteins in serum have also been observed (Bhattacharya and Muruganandam, 2003).

Hepatoprotective activity: Withaferin A has significant hepatoprotective effect in Ccl_4-induced hepatotoxicity in rats at a dose of 10 mg kg^{-1} b.wt. (Rastogi and Mehrotra, 1998; Khare, 2007).

Hypothyroid activity: An aqueous extract of dried Ashwaganda root was administered to mice daily for 20 days to test thyroid activity. Significant increase in serum T4 levels indicates the stimulating effect at the glandular level via its effect on cellular antioxidant systems. These results indicate ashwaganda is a useful treating agent for hypothyroidism (Panda and Kar, 1998).

Anti-hyperglycaemic effect: Transina is a commercial preparation, which contains active ingredient of Ashwaganda and other components. It decreases streptozocin (STZ) induced hyperglycaemia in rats due to its pancreatic islet free radical scavenging activity (Bhattacharya *et al*., 2001).

Musculotropic activity: Alkaloids of Ashwaganda have muscle relaxant and antispasmodic effects against several spasmogens on bronchial, blood vascular, intestinal, uterine and tracheal muscles. This smooth muscle relaxant activity of the alkaloids was similar to that of papaverine which is a direct musculotropic agent (Anonymous, 1982).

Effect on sexual behaviour: Impairment in libido and sexual performance, sexual vigour as well as dysfunction in penile erection can be corrected by root extract of *W. somnifera*. The roots contain Fe, K, Mg and Ni which plays significant role in the diuretic, aphrodisiac activity and in the treatment of spermatopathia and seminal depletion. On cessation of treatment, these effects are partially reversible and are attributed to hyperprolactinemic, Gamma Amino Butyric Acid (GABA), serotonergic or sedative activities of the extract instead of changes in levels of testosterone. Male sexual competence is detrimentally affected by roots of *W. somnifera* and thereby is contradictory. GABA mimetic activities of *W. somnifera* roots as well as serotonergic systems strongly depresses the libido (Ilayperuma *et al*., 2002).

Anti-cancer effects: The anti-cancer importance of the Ashwagandha plant has been well documented in a number of experimental studies and its extracts have potential use in cancer chemotherapy (Nath *et al*., 2005; Winters, 2006; Yang *et al*., 2007; Mathew *et al*., 2010; Dhama *et al*., 2013a). Literature reveals that Ashwagandha can be used as synergizer to support conventional chemo or radiation therapy due to its long term tumor growth inhibition property. Roots have been found to hamper the cellular growth and attachment of Chinese Hamster Ovary (CHO) carcinoma cells and thereby exerting the anti-tumor effect. This plant has also been found effective against uterine fibroids, dermatosarcoma, prostate cancer in humans, urethane induced lung-adenoma in mice, neuroblastomas in humans, ascitic lymphoma, benzopyrene induced lung cancer in male Swiss Albino rat and leukaemia in humans (Singh *et al*., 1986; Devi, 1996; Christina *et al*., 2004; Senthilnathan *et al*., 2006a, b; Winters, 2006; Senthil *et al*., 2007; Sumantran *et al*., 2007b; Srinivasan *et al*., 2007; Widodo *et al*., 2010; Kataria *et al*., 2013) (www.fibroids-and-endometriosis-help.com). Anti-carcinogenic effects are mainly on account of decreased expression of nuclear factor-kappa-B, suppression of intercellular Tumor Necrosis Factor (TNF) and potentiation of apoptotic signaling in cancerous cells of animals or cell lines (Singh *et al*., 2010; Dhama *et al*., 2013e). Withaferin A is shown to inhibit umbilical vein endothelial cell (HUVEC) that sprouts in three-dimensional collagen-I matrix at doses relevant to the inhibitory activity of NF-kappa B. In HUVECS, Withaferin A inhibits proliferation of cell at significant doses that are lower than those that are required for cell line of tumor origin via cyclin D1 expression inhibition. On the basis of these findings, it is proposed that in HUVECS NF-kappa B inhibition by Withaferin A occurs by interference of proteosome pathway mediated by ubiquitin. This is evident from the increase in the level of poly-ubiquinated proteins. Moreover, the finding that a potent anti-angiogenic activity is exerted by Withaferin A *in vivo* at lower doses than that required to induce anti-tumor activity *in vivo* highlights the use of this natural product obtained from *W. somnifera* to treat or prevent cancer (Mohan *et al*., 2004; Bargagna-Mohan *et al*., 2005; Ichikawa *et al*., 2006; Rao and Naresh, 2010; Dhama *et al*., 2013a).

A study regarding revealing the effect of *W. somnifera* root extracts on cell cycle and angiogenesis, as an anti-angiogenic compound showed Withaferin A and Withanolide D to inhibit growth of cancer (Maitra *et al*., 2003; Mathur *et al*., 2006). Ashwagandha plant extract inhibited benzo (a) pyrene-induced forestomach papillomagenesis, carrageenin induced air pouch granuloma and DMBA-induced skin papillomagenesis with up to 60 and 92% and 45 and 71% inhibition in tumor incidence and multiplicity, respectively in mice (Padmavathi *et al*., 2005). Genesis of papilloma of skin induced by 7, 12-dimethylbennzanthracene is inhibited by *W. somnifera*. In mice, however during the study this plant does not show any toxic effect apparently (Padmavathi *et al*., 2005). Sometimes, Ashwagandha (Indian ginseng) may produce interference in the immunoassay of serum digoxin level measurement (Dasgupta *et al*., 2007).

W. somnifera reduces tumor cell proliferation and mitigate undesirable side effects, hence increases overall animal survival time. It potentially supports radiation therapy and reduces the side effects produced by chemotherapeutic agents such as cyclophosphamide and paclitaxel without interfering with the tumor-reducing actions of the drugs. *W. somnifera* has been suggested to act as a novel complementary therapy in the field of oncology (Visavadiya and Narasimhacharya, 2007).

Recent *in vitro* studies in India has shown that the extract of the plant disrupt the ability of cancer cells to reproduce and is a significant step in fighting cancer. In addition it has been indicated by laboratory analysis that anti-angiogenic activity of ashwagandha extract against new blood vessels supporting unbridled growth. Oral administration ashwagandha extract effectively Inhibits the experimentally induced stomach cancers in laboratory animals. It reduces the incidence of tumor by 60% and multiplicity of tumor by 92%. Incidence and multiplicity of tumor are inhibited by 45 and 71%, respectively when study has been carried out in rodent model of skin cancer (Christina *et al*., 2004; Mathur *et al*., 2004; Padmavathi *et al*., 2005).

Anti-cancer activity of Ashwagandha has been attributed to Hypothalamic Pituitary Adrenal (HPA) axis and the neuroendocrine system. It modulates the activity of cytotoxic lymphocytes (CTL) for reducing the tumour and cancerous growth. Augmentation of the Natural Killer (NK) cell activity reduces the tumor growth and incidences and increases serum T3 and T4 in mice (Panda and Kar, 1998) Compared to doxorubicin, Ashwagandha showed better efficacy in arresting growth of breast and colon cancer cell line (Jayaprakasam *et al*., 2003). Extracts of Ashwagandha also possess potent antioxidant and detoxifying properties (Rasool and Varalakshmi, 2007). Withaferin A stoutly exerts I kappa B kinase beta hyperphosphorylation for inhibition of its kinase action thereby causing death of cancerous cells (Kaileh *et al*., 2007; Wang *et al*., 2012).

Radiosensitization action and activities: Studies have revealed that a good natural source of a potent and relatively safe radio sensitizer/chemotherapeutic agent is Ashwagandha. The radiosensitizing effect of *W. somnifera* has been studied *in vivo* on the B16F1 mouse melanoma. Volume doubling time (the time required for a quantity to double in size) and growth delay in dose dependent manner has been observed by treating 100 mm^3 tumors with intraperitonial injection of 10-60 mg kg^{-1} of Withaferin A. On the other hand, there has been a significant enhancement in tumor response due to gamma irradiation locally followed by injection of Withaferin A at the rate of 30-50 mg kg^{-1}. Such study has shown that the plant extract (Withaferin A) is effective mostly when injected intraperitonially 1 h prior to irradiation and thereby Withaferin A significantly enhance the radiation response of melanoma (Uma *et al*., 2000; Diwanay *et al*., 2004; Rao and Naresh, 2010).

Reducer of poisoning due to toxins and chemicals/drugs: Cancer is likely induced by chemotherapeutic agents like cyclophosphamide and cadmium and the side effects produced by chemotherapy as well as radiotherapy are sometimes more hazardous than the disease proper. Significant reduction in cyclophosphamide induced leucopenia has been observed due to administration of *W. somnifera*. When *W. somnifera* and cyclophosphamide together are used for treatment purposes, cellulairty of bone marrow increases significantly compared to cyclophophamide treatment alone. Alpha-esterase positive cells increase in number in the bone marrow of animals treated with cyclophosphamide due to administration of Ashwagandha because of stem cell proliferation (Kumar *et al*., 2011; Rahal *et al*., 2013). As per studies so far, toxicity induced by cadmium has not been reported to be regulated by any plant. But lipid peroxidation based results indicate that cadmium induced toxicity can be reduced by Ashwagandha, thereby indicating the potential of this plant to regulate metal induced toxicity (Panda *et al*., 1997). Ashwagandha is also known to significantly reduce the ochratoxin A induced suppression of chemotactic activity as well as interleukin IL-1 and TNF–alpha along with *Asparagus racemosus* (Satavari), *Tinospora cordofolia* and *Picrorhiza kurroa* (Katuki) (Mahima *et al*., 2012; Chakraborty and Pal, 2012; Dhama *et al*., 2013e). Tissue venom like hyaluronidase destroys the integrity of extracellular matrix thereby helping to spread toxin. It is an interesting finding that *W. somnifera* is a source of a hyaluronidase inhibitor, glycowithanolide (WSG) glycoprotein inhibits the hyaluronidase activity completely at a concentration of 1:1 w/w of snake venom to WSG, which could be help provide protection in case of Cobra (*Naja naja*) and Viper (*Daboia russelii*) bites. It is a scientific approach to use the Ashwagandha plant extract externally as an antidote to victims of snake bite in rural India. The *Naja naja* venom has got phospholipase A2 (PL-A2) activity which can be neutralized by glycoprotein isolated from *W. somnifera* known as antitoxin-PLA2. This has got implications in novel therapeutic reagent development as well as for treating snake evenomations along with implication in snake biology (Machiah *et al*., 2006).

Anti-aging activities: Double-blind clinical trial was conducted to test the anti-aging properties of Ashwagandha in a group of 101 healthy males, ageing about 50-59 years were given at a dosage of 3 g daily for one year. The volunteers showed significant improvement in hemoglobin, red blood cell count, hair melanin, seated stature, improvement in sexual performance, decrease in serum cholesterol and nail calcium was preserved (Bone, 1996; Ilayperuma *et al*., 2002).

Role against neurodegenerative disorders: Ashwagandha can be used as neuro-regenerative agent to treat Alzheimer's, Parkinson's, Huntington's and other neurodegenerative diseases at any stage of the disease as it can significantly reverse the neuritic atrophy and synaptic loss, along with GABA mediated anticonvulsant effect, GABA mimetic effect and promoting formation of dendrites due to therapeutic activity of glycowithanolides withaferin-A and sitoindosides VII-X present in the roots of Ashwagandha (Schliebs *et al*., 1997; Abbas *et al*., 2004, 2005; Ahmad *et al*., 2005). Ashwagandha enhances regeneration of the neurons along with reconstruction of synapse thereby acting as memory enhancer. The expression of Brain Derived Neurotropic Factor (BDNF) as well as Glial Fibrillary Acidic Protein (GFAP) is reversed by treatment with extract of Ashwagandha (Konar *et al*., 2011).

Alkaloids from the roots of Ashwagandha showed prolonged hypotensive, bradycardiac and respiratory stimulant activities in dogs. Hypotensive effect was mainly due to autonomic ganglion blocking action and was augmented by the depressant action on higher cerebral centres. In experimental animals, total alkaloids produced a taming and a mild depressant effect like tranquillizer-sedative type on the CNS (Rastogi and Mehrotra, 1998). Systemic administration of Ashwagandha root extract led to slightly enhanced acetylcholinesterase (ACHE) activity in the lateral septum and globus pallidus. Ashwagandha root extract affects mainly in the cortical and basal forebrain cholinergic signal transduction cascade. It increases cortical muscarinic acetylcholine receptor capacity leads to cognition-enhancing and memory-improving effects in animals and humans (Schliebs *et al*., 1997).

Anticonvulsant activity: Ashwagandha root extract significantly reduces the jerks in 70% animals and clonus in 10% animals caused by pentylene tetrazole (PTZ) induced convulsions when administered with dose of 100 mg kg^{-1} and it was evident from EEG wave pattern (Kulkarni and George, 1996). It also showed reduction in severity of motor seizures induced by electrical stimulation in right basilateral amygdaloid nuclear complex through bipolar electrodes. The protective effect was due to GABAergic mediation of Ashwagandha (Kulkarni *et al*., 1993).

Role of Ashwagandha in development of drug tolerance and dependence: Drug addiction has become a major health problem worldwide and is a very costly affair to be managed. In drug addict individuals, tolerance as well as withdrawal signs are observed due to chronic treatment with benzodiazepine as well as ethanol or opioids that can be blocked by BR-16A (Mentat) which contains *W. somnifera* as one of its important ingredient. The morphine induced analgesic effect is significantly attenuated when the Ashwagandha plant extract is administered repeatedly for a period of 9 days. As per assessment by naloxone precipitation, withdrawal signs of development of dependence to opiate viz., morphine withdrawal jumps can be suppressed by *W. somnifera*. Studies have revealed that there is no dependence liability of the plant even upon cessation abruptly which have got clinical implications without production of long-term tolerance and withdrawal effect (Kulkarni and Sharma, 1994; Kulkarni and Ninan, 1997; Kest *et al*., 2002).

Biotechnological techniques as a stand-pillar in upliftment of herbal therapy: Medicinal plants are broadly used to deal with an array of health problems. The major impediments in the study of therapeutic herbal plants consist of erroneous identification and less yield of active principles such as medicinally important withanolides in case of Ashwagandha. Biotechnological advance techniques such as deoxyribonucleotide (DNA) based techniques like Polymerase Chain Reaction (PCR), stochastic algebraic modelling language (SAMPL), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (Rapd), High Performance Liquid Chromatography (HPLC), molecular cloning and sequencing of any target gene can help to resolve uncertainties in identification of appropriate plants and their constituents (Sharma *et al*., 2007). The novel disciplines of bioinformatics and genomics involving recombinant DNA techniques, *in vitro* organ and tissue/cell culture methods can be employed to produce bioactive alkaloids such as withanolides under definite conditions (Patra *et al*., 2004; Jha *et al*., 2005; Negi *et al*., 2006; Supe *et al*., 2006; Wadegaonkar *et al*., 2006; Titanji *et al*., 2007).

Commercial preparations: Ashwagandha is the principal component of many polyherbal preparations viz., Immu-21, Amrit®, Su-Ruksh®, Ashwgandha® and

ImmuPlus® etc (Sangwan *et al*., 2004). Immu-Plus® has been reported to stimulate blastogenic capacity of T and B cells and increases antibody titer in dogs. Polyherbal preparations like Ashwagandharist act as a nervine tonic and Himalaya ashwagandha is a monoherbal extract used for management of stress. Stresswin is used for reduction of anxiety, strain as well as stress. Stresscom is a monoherbal extract that relieves anxiety. Himalaya massage oil is a polyherbal preparation used for relaxation of body along with relief from stress. It is a good immunopotentiating agent when used along with vaccines, like in pups it improves immune stimulation when used in conjunction with parvovirus and rabies virus vaccines (Chauhan, 1999). Similar beneficial immunomodulatory effects have been observed in poultry birds while using with infectious bursal disease and Newcastle disease vaccines (Dhote *et al*., 2005). Not only this but in few fishes also such as *Labeo rohita*, famous as Indian major carp, 'ImmuPlus' has helped in increasing immunity and providing enhanced resistance against the diseases at different growth stages of fish life (Kumari *et al*., 2007). Immunomodulatory effects of Immu-21® have been reflected in modest improvements in conditions of HIV patients (Singh *et al*., 2001).

CONCLUSION

The uses of botanical medicines continue to grow with the expansion of modern medicine. The revered herb (Ashwagandha) potentiates the immune functions, enhances the longevity and facilitates the restoration of homeostasis by reducing the stress. Along with these, the role of *Withania* to exert beneficial effect against anxiety as well as cognitive and neurological disorders, inflammation and Parkinson's disease are quiet noteworthy. Ashwagandha is a potent stress reliever and antidepressant with the property to strengthen immunity against cold, flu and other common infections. Root powder is useful in treatment of acute rheumatoid arthritis. The roots are used as potent diuretic and aphrodisiac, increases sexual performance and help to maintain vigour and vitality. The plant is also a good anti-inflammatory agent and is useful in graft-vs-host reaction. Ashwagandha extract supports antioxidant and immunomodulant activities. Withaferin A has powerful antitumor effects. It possesses metastatic and angiogenetic properties of decresing order. *Withania* increases the body's ability to withstand stress of all types signifying anti-stressor adaptogenic property. Roots and leaves of *Withania* exhibit marked antibacterial activity against *S. aureus*, *Neisseria gonorrhoea* and anti-fungal activity against *Candida albicans*. These facts indicate that *W. somnifera* can be regarded as a fine natural source of a potent and relatively safe radiosensitizer/chemotherapeutic agent. However the aforesaid benefits are documented in the literature but still multi-disciplinary evaluation is required with systematic approach before large scale commercialization of this miraculous herb. Proper caution should be taken while using this plant along with drugs that have anectodal effect (importantly barbiturates). The dose regimen should be given equal importance as in large dosage the plant extract causes gastrointestinal upset as well as diarrhea and vomition and may also have abortifacient effect (so better to avoid during pregnancy). Working hand-in-hand with oncologists as experienced natural medicine practitioners can effectively increase the therapeutic efficiency of Ashwagandha as well as decrease the side effects of *W. somnifera* when used for conventional treatments. In order to determine whether *W. somnifera* can duplicate the immunomodulatory and haematopietic activities in humans, optimal dosage for achieving these effects must be determined for which more research works are mandatory. Because of its wide pharmacological activities, Ashwagandha is considered as an important component of various polyherbal preparations. Thus the plant has got immense practical applicability in biomedicine as well as veterinary medicine focusing its potent role in the maintenance of sound health.

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