Phytochemical and Pharmacological Activity of *Withania somnifera* (L.) Dunal

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Abstract

Withania somnifera (L.) Dunal is commonly called as Ashwagandha and it belongs to the family Solanaceae. It is an eminent medicinal plant widely used in the treatment of many clinical conditions in Indian subcontinent. It has natural source of withanolides (steroidal lactones) which are used as ingredients in many formulations prescribed for a variety of diseases. It has important medicinal capacity which has been used either single or in combination with other drugs in Unani as well as Ayurvedic system of medicine for centuries. Several therapeutic actions such as anti-inflammatory, neurostimulatory, hepatoprotective, anti-cancerous, immune-modulator and antioxidant activity. Observance in view the medicinal properties of Ashwagandha an effort has been made to explore various dimensions of the drug including phytochemical and pharmacological studies carried out on this herb. Pharmacological activity of W. somnifera needs number of scientific justification which would be helpful for the further future research.

Keywords: Ashwagandha, pharmacology, *Withania somnifera*, withanolides

1. Introduction

Withania somnifera (L.) Dunal is one of the most important medicinal plants in the Indian Ayurvedic system of medicine because of its valuable pharmaceutical and nutraceutical properties (Jain et al., 2012). Among the twenty three known species of Withania, only two (*Withania somnifera* and *Withania coagulans*) are economically significant (Negi et al., 2006). *W. somnifera*, commonly known as 'Ashwagandha', is the most exploited species under the family of Solanaceae. The roots are reputed to promote health and longevity by augmenting defense against disease, arresting aging, revitalizing the body in debility, increasing resistance to adverse environmental factors, and creating a sense of wellbeing (Tiwari et al., 2014). Several recent reports have demonstrated immunomodulator and antitumor effect of ashwagandha as well (Harikrishnan et al., 2012; Srivastava et al., 2013; Wadhwa et al., 2013).

Ashwagandha is commonly available as a churna, a fine sieved powder that can be mixedwith water, ghee (clarified butter) or honey. It improves the function of the brain, improves the memory and nervoussystem. It improves the function of the reproductive systempromoting a healthy sexual and reproductive balance. Being a powerful adaptogen, itenhances the body's resilience to stress. Ashwagandha improves the body's defense against disease by improving the cell mediated immunity. It also retains antioxidant properties that helps to protect against cellular damage caused by free radicals.

2. Anti-inflammatory Properties

The usefulness of *W. somnifera* in a variety of rheumatologic conditions may be due in part to its antiinflammatory properties, which have been studied by several authors. Anbalagan and Sadique (1981) reportedthat *W. somnifera* possesses efficient anti-inflammatory activity as compared with hydrocortisone, a common anti-inflammatory drug. The effect of *W. somnifera* on glycosaminoglycan synthesis in the granulation tissue of carrageenin induced airpouch granuloma was studied by Begum and Sadique (1987). Oral administration of *W. somnifera* root powder decreased the glycosaminoglycan content, which was much higher than that of the hydrocortisone and phenylbutazone (Jain et al., 2012). The methanolic fractions of the extract showed high anti-inflammatory activity as compared tothat of hydrocortisone sodiumto the high content of biologically active steroids in the plant, of which withaferin A is known to be a major component. Withaferin A is potent inhibitor of the proinflammatory transcription factors and a promising agent for the treatment of the inflammatory cascade of cardiovascular diseases (Kaileh et al., 2007). *W. somnifera* was found to cause considerable reduction in inflammation. Acute phase reactants of the blood monitored by crossed immunoelectrophoresis showed changes in the concentration

of many serum proteins like α2-glycoprotein, major acute phase α1-protein, and prealbumin in the Withania groups. The α2-glycoprotein found onlyin inflamed rat serum was decreased to undetectable levels in the group. On the other hand Phenylbutazone caused to considerable increase in the α2-glycoprotein in both arthritic and healthyrats. Another acute phase protein like peak2, α-1 major acute phase which increased by inflammation was brought back to normal levels by *W. somnifera* treatment. Several modulator proteins in Ashwagandha influencedin normal rats, suggesting that numerous plant chemicals possibly interact with the liver proteinsynthesis process (Anbalagan and Sadique, 1984).

3. Immunomodulatory Activity

Ashwagandha is a general tonic to increase energy and prevent disease may be partially related to its effect on immuno-activating and immunosuppressive properties. Ashwagandha enhancing the immune system is observed in innate immune response and modulating the activity of cell-mediated immune system (Harikrishnan et al., 2012). The withanolides inhibition of NFkB, a protein involved in many central pathways of immune regulation and proliferation it has received extensive appreciation throughout the last two decades (Maitra et al., 2009; Ozawa et al., 2013) Specifically active compound Withaferin A has specific immunosuppressive effects on human B and T lymphocytes viz. antigen recognition and proliferative capacity of B and Tlymphocytes (Bahr and Hansel, 1982). Glycowithanolides or withanolides and a mixture of sitoindosides IX and X isolated from *W. somnifera* were evaluated for their immunomodulatory and central nervous system effects in Swiss mice and Wistar strain albino rats (Bone, 1996). In another study, the aqueous suspension of the*W. somnifera* root powder inhibited the mitogen induced lymphocyte proliferation and DTH reaction in rats (Rasool and Varalakshmi, 2006). All the active compounds produced significant mobilization and activation of phagocytosis, peritoneal macrophages and increased activity of the lysosomal enzymes. Both compounds also created significant antistress activity in albino mice and rats, and augmented learning acquisitionand memory retention in both young and old rats. The root extract of *W. somnifera* also enhanced total white blood cell count, inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages (Davis and Kuttan, 2002). It is also tested on immunomodulatory effects in three myelosuppression models in mice cyclophosphamide, azathioprine, or prednisolone (Ghosal et al., 1989). Significant increases in hemoglobin concentration, red blood cellcount, white blood cell count, platelet count, and body weight were observed in *W. somnifera* treated mice compared to untreated control mice. Several authors also reported to increases in hemolytic antibody responses toward human erythrocytes which indicated immunostimulatory activity (Harikrishnan et al., 2012; Srivastava et al., 2013). The effect of Ashwagandha was alsostudied on the functions of macrophages obtained from mice treated with the carcinogen ochratoxin A (OTA) (Ziauddin et al., 1996). Treatment of OTA on mice for 17 weeks expressively decreased the chemotactic activity ofthe macrophages.

4. Antitumor Activity

In one study, *W. somnifera* was evaluated for its antitumor effect in urethane induced lung adenomas inadult male albino mice (Begum and Sadique, 1988). The alcoholic extract of the dried roots of theplant as well as the active componentWithaferin A isolated from the extract from leaves showed significant antitumor and radiosensitizing effects in experimental tumors *in vivo*, without any noticeable systemictoxicity. Simultaneous administration of *W. somnifera* (ethanol extract of whole plant, 200 mg kg^{-1} daily orally for seven months) and urethane (125 mg kg^{-1} without food biweekly forseven months) compact tumor incidence considerably (tumor incidence: untreated control, 0/25; urethane treated,19/19; *W. somnifera* treated, 0/26, and Withania somnifera plus urethane treated, 6/24, $p<0.05$) (Devi, 1996). Lungs histological appearance of animals protected by *W. somnifera* was similar to those observedin the lungs of control animals. No pathological evidence of any neoplastic alteration was observed in the brain, kidneys, stomach, heart, spleen, or testes of any treated or control animals. In addition to providing protectionfrom carcinogenic effects. Ashwagandha treatment also reversed the adverse effects of urethane on totalleukocyte count, lymphocyte count, body weight, and mortality. The growth inhibitory effect of *W. somnifera* was also observed in Sarcoma 180 (S-180), a transplantable mouse tumor (Singh et al., 1986). Ethanolextract of root (400 mg kg^{-1} and up, daily for 15 days) after intradermal inoculation of 5x105 cells of S-180 in BALB/c mice produced whole regression of tumor after the primary growth. In some cases, *W. somnifera* was also found to act as a radio and heat sensitizer in mouse S-180 and in Ehrlich ascites carcinoma (Devi et al., 1992). Antitumor and radio sensitizing effects of withaferin (a steroidal lactone) were also seen in mouse Ehrlich ascites carcinoma *in vivo*. Withaferin A from *W. somnifera*gave radio sensitizer ratio of 1:5 for *in vitro* cell killing of V79 Chinese hamster cell at a non-toxic concentration of about 2 mM l^{-1} (Devi, 1996). These studies are suggestive of antitumor activity as well as enhancement of the effects ofradiation by *W. somnifera*.

5. Neuropharmacological Activity

Progressive loss of structure or function of neurons by Neurodegeneration is the causing of death of neurons. Parkinson's, Alzheimer's and Huntington's diseases occur as a result of neuro-degenerative processes.Researchers found that ashwagandha can support the growth of nerve cell dendrites, which allow these cells to receive communications from other cells. Thus *W. somnifera* can restore the brain tissue changes that accompany promote the growth of both

normal and damaged nerve cells, suggesting that the herb may boost up healthy brain cell function as well as benefit diseased nerve cells. The bioactive metabolites isolated form Withania havebeen found to be effective in alleviating many centralnervous system disorders such as epilepsy, anxiety,depression, catalepsy, and sleep (Bhattacharya et al.,1997; Dhuley, 1998; Jain et al., 2001; Naidu et al., 2006). The extracts for the different parts of both the plants havethe capacity to modulate various neurotransmitters also. Bhatnagar et al. (2009) observed that the extract work asa suppressor of corticosterone release and activatingcholine acetyltransferase, which in turn increaseserotonin level in hippocampus. Withanolide A andwithanoside IV from *W. somnifera* roots promote neuriteoutgrowth in cultured neurons and in rodents injectedwith Aβ 25-35 and after oral administration of withanosideIV, sominone, an aglycone of withanoside IV, wasidentified as the main metabolite (Kuboyama et al., 2002). Recently, Sehgal et al. (2012) revealed that thesemipurified extract of the roots of *W. somnifera*reversed behavioural deficits, plaque pathology, accumulationof β-amyloid peptides (Aβ) and oligomers in thebrains of middleaged Alzheimer's disease transgenicmice by enhancing lowdensity lipoprotein receptor relatedprotein in brain micro vessels and liver.

6. Anticancer and Chemo Protective Activities

Cancer is one of the major causes of death and there is an increase in cancer mortality in all ages. In the last century, great advances were made by modern medical system in cure and prevention of this disease, but none of the attempts were completely successful. Thus search for novel safe and effective therapies are still continuing and exploration of traditional medicine for their anticancerous effects are found to be promising.The phytoconstituents of Ashwagandha are proved to have anticarcinogenic, radiosensitizing and chemopreventive properties in both *in vitro* and *in vivo* experimental models. WS also helps patients to recover from the adverse effects of chemotherapy.The anticancer effect of Withania has been studiedextensively (Devi et al., 1992; Devi, 1996; Davis and Kuttan, 2000; Prakash et al., 2002; Senthilnathan et al., 2006; Winters, 2006; Widodo et al., 2007; Wadhwa et al., 2013), and it wasfound that it is the most effective agent in preventingcancer through its ability to reduce the tumor size.Treatment of root extract of *W. somnifera* on induced skincancer in mice exhibited significant decrease in the incidenceand average number of skin lesions compared tocontrol group (Prakash et al., 2002). Withaferin A showedtumorinhibitory activity against cells derived from humancarcinoma of the nasopharynx (Jayaprakasam et al., 2003) and it also inhibited the growth of roots of *Allium cepa* by arresting the cell division at metaphase (Palyi et al., 1969). In another study, *W. somnifera* was evaluatedfor its antitumor effect in urethaneinduced lungadenomas in adult male albino mice. Simultaneousadministration of *W. somnifera* extract (200 mg kg^{-1} bodyweight daily orally for seven months) and urethane (125 mg kg^{-1} biweekly for seven months) reduced tumor incidencesignificantly (Singh et al., 1986). Additionally, in a different study the aqueous extract of WS was used for anti-cytotoxic effect in chicken lymphocytes and remarkable inhibitory activity of dimethyl sulfoxide (DMSO) induced cytotoxicity with a decrease in TNF-Gproduction was reported (Chattopadhyay et al., 2007).

7. Antibiotic Activity

The nervous system and brain are relatively more susceptible to free radical damagethan other tissues because they are rich in lipids and iron, both known to be important ingenerating reactive oxygen species (Halliwell and Gutteridge, 1989). Antibiotic activity of Withaferin A is due to the presence of the unsaturated lactonering. Thelactone showed strong therapeutic activity in experimentally induced abscesses in rabbits, the being somewhat stronger than that of Penicillin. It substantiates the reputation of the leaves as a cure for ulcers and carbuncles in the indigenous system of medicine. The brain also uses nearly 20% of the total oxygen supply (Ames et al., 1993). Free radical damage of nervoustissue may contribute to neuronal loss in cerebral ischemia and may be involved in normalaging and neurodegenerative diseases, e.g.,epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases (Scarfiotti et al., 1997). In traditional Ayurvedic system it has includedmany diseases associated with free radicaloxidative damage, it has been considered likelythe effects may be due to a certain degree ofantioxidant activity. The active principles ofWS, sitoindosides VII-X and withaferin A (glycowithanolides), have been tested for antioxidant activity using the major free radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontalcortex and striatum. Decreased activity of theseenzymes leads to accumulation of toxic oxidative free radicals and resulting degenerativeeffects. An increase in these enzymes wouldrepresent increased antioxidant activity and aprotective effect on neuronal tissue. Activeglycowithanolides of WS (10 or 20 mg kg^{-1} intraperitoneally) were given once daily for 21days to groups of six rats. Doserelated increases in all enzymes were observed; the increases comparable to those seen with deprenyl (a known antioxidant) administration (2 g kg^{-1} day^{-1} intraperitoneally). This implies that WSdoes have an antioxidant effect in the brainwhich may be responsible for its diverse pharmacological properties (Bhattacharya et al., 1997). Further studies onother parts of the brain (e.g., cerebellum, medulla, and hypothalamus) may provide information with respect to the effects of WS oncognitive behavior and other functions of thebrain, in both healthy and diseased individuals.

8. Hepatoprotective Activity

The extract of Ashwagandha roots exhibited hepatoprotective activity against carbon tetrachloride (CCl_4)induced hepatotoxicity in adult albino rats of either sexdue to the

presence of 3-β-hydroxy-2, 3-dihydrowithanolideF. The hepatoprotective effect of *W. somnifera* root powder was studied by Mohanty et al. (2008). The extract influenced the levels of lipidperoxidation and thereby provided the hepatoprotection.Verma et al. (2009) also examined the effect of *W. somnifera* aqueous root extract on the hepatic cell of*Clarias batrachus* and reported that the root extractcontains different flavonoids and neurotransmitters thatstimulated the neuroendocrine system, leading to hyperactivityof the endomembrane and the exit of moleculesthrough the surface via exocytosis.

9. Conclusion

For the use of ashwagandha as a multipurpose medicinal agent although the results from this revieware quite promising, severallimitations currently happen in the currentliterature. While ashwagandha has been usedeffectively in Ayurvedic system of medicine forcenturies, more medicaltribunals should beconducted to support its therapeutic use. It isalso important to recognize that *W. somnifera* may beeffective not only in isolation, but may actuallyhave a potentiating effect when given incombination with other herbs or drugs.

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