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Research Article

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Immunomodulatory activity of *Withania somnifera* (L.)

Satish K. Verma*[1], Asima Shaban[1], Reena Purohit[3], Madhvi L. Chimata[2], Geeta Rai[4] and Om Prakash Verma[5]

[1]K.L.P.G College Naini Allahabad ,Uttar pradesh India.
[2]Dept. of Biotechnology, DPMC, Dehradun (U.K), India
[3]Dept. of Biotechnology SIPAS Dehradun, India
[4]Department of Pharmacy, United College Allahabad, Uttar Pradesh India
[5]Department of Molecular and Cellular Engineering SHIATS, Allahabad

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ABSTRACT

The present study was undertaken to evaluate the effect of some selected medicinal plants on immunomodulatory activity that comprises of screening to identify the activity of ethanolic extract of medicinal plants on humoral and cell mediated immunity (specific immune response). Experiments were conducted in vivo in Swiss albino mice. Withania somnifera (WS) ethanolic extract was found to enhance humoral immune response on 7th day by 12 % as compared to the standard control cyclophosphamide that exhibited 54% humoral immune response, where as cell mediated immune response was observed with an enhancement in the values (19.27%) in comparison with control cyclosporine (37.63%).

Keywords: *Withania somnifera* (L.) Dunal, Immunomodulation, haemagglutination titre (HT), delayed type of hypersensitivity (DTH) response.

INTRODUCTION

A large number of plants and their isolated constituents have been shown to have potential immunity. Some medicinal plants have been shown to exert anti-inflammatory, anti-stress and anti-cancer effects by modulating the immune functions[1,2].The genus *Withania* is a member of family Solanaceae. *Withania somnifera* (L.) Dunal commonly known as Ashwagandha is a highly valued medicinal plant with diverse therapeutic uses in the traditional Indian systems of medicines such as Ayurveda, Unani and Siddha. In English literature it is known as "winter cherry**"**. The plant is an erect branching under shrub reaching about 150cm in height, leaves ovate up to 10cm long; flowers greenish or lurid yellow in axillary fasicles, fruits globose berries which are orange coloured when mature enclosed in persistent calyx. In India, the species grows wild in North Western regions extending to hill regions of Punjab, Himachal Pradesh and Jammu up to an elevation of 1500m [3]. It prefers a sunny situation. The seeds are sown during June or July. The seedlings are transplanted at a distance of 60 cm x 30 cm. In the present communication, authors have set forth the objective of screening the immunomodulatory potential of this valuable plant in mice.

EXPERIMENTAL SECTION

Plant material and preparation of extracts

Withania somnifera (L.) Dunal plants were collected from February to March 2009, from Vikasnagar, Dehradun. The whole plants ethanolic extract was used for studying the immunomodulatory properties. Plant materials were dried at 37^{0}C, powdered and extracted in alcohol. Extract was fine-filtered and freeze dried. For the preparation of the extracts, dried ground plant material was percolated with 95% alcohol and concentrated to dryness under

reduced pressure. The samples were prepared in double distilled water along with 0.1% acacia gum for immunomodulatory test. Swiss mice were obtained from the Central Drug Research Institute (CDRI), Lucknow (average weigh 25±3 g). The animals were housed in standard environmental conditions.

Preparation of sheep red blood cells (SRBC) antigen

SRBC were collected aseptically from Jugular vein of sheep, stored in cold sterile Alsever's solution for immunization and challenge, at required time schedule. Stored sheep blood cells were centrifuged and washed three times with pyogen free sterile normal saline (0.85% NaCl w/v) and adjusted to a required concentration for immunization. Humoral antibody response (Hab) was analyzed using standard method. The mice were immunized by injecting 0.2 ml of 5×10^9 SRBC / ml i.p. and plant extracts were administered orally (100mg/Kg body. wt.) for 5 consequent days after immunization. Two parallel controls were run simultaneously. One of them received only normal saline water, named 'Normal Control', while the other received Levamisole (2.5 mg / Kg body wt.) and Cyclophosphamide (250 mg/Kg body wt. post oral). The mean titre values of the drug treated groups were compared with the normal control.

Delayed type hypersensitivity (DTH-CMI) method was employed to access SRBC induced DTH response in mice. Mice were immunized by injecting 20µl of 5×10^9 SRBC/ subcutaneously into the right hind footpad. The day of sensitization was designated as day 0. Seven days later the thickness of the left hind footpad was measured using a spheromicrometer (0.01mm pitch) and considered as control. Then the sensitized mice were challenged with the same amount of SRBC i/m into the left hind footpad. The test materials (doses= 100 mg/Kg body weight) were administered orally with a metal feeding cannula for 7 days from the day of immunization. The control animals were given an equal volume of 1% Gum acacia as vehicle. The challenging dose of 20µl of 5×10^9 SRBC/ml in mice were injected to assess the standard control response for DTH[4,5]. Swiss mice (n=6) bearing cancer were treated daily with *Withania somnifera* (L.) Dunal extract (100mg/kg) ip for 5 days. Blood samples were collected by puncturing the retro-orbital plexus. Total WBC and RBC count was determined using a hemocytometer. A normal control group received normal saline (5mg/kg/ip) and positive control group treated with 5-Fluorouracil (5-FU), an anticancer drug.

RESULTS AND DISCUSSION

Withania somnifera commonly known as Ashwagandha is a highly valued medicinal plant with diverse therapeutic uses in the traditional Indian systems of medicines such as Ayurveda, Unani and Siddha.There are several medicinal plants that are considered to possess immunomodulatory properties[6,7]. *Withania somnifera* (L.) Dunal ethanolic extract was found to enhance humoral immune response on 7th day by 12% as compared to the control cyclophosphamide (54%), where as cell mediated immune response was enhanced to 19.27% in comparison with control cyclosporine (37.63%). The effect of methanolic extract of the plant on the hematological parameters of the tumour bearing mice showed an increase in number of RBCs but a decrease in WBCs compared to the control mice. These data were based on the differential leucocyte count by Leishman staining. A number of plants used in traditional medicines have been shown to stimulate or inhibit immune responses, and several active principles have been isolated and characterized from plants[8]. The authors identified clinically useful immunomodulatory potential of *Withania somnifera* (L.) Dunal the study supported by earlier authors[9,10].

In clinical studies [11] WS root powder has been found useful in case of acute Rheumatoid Arthritis. Withaferin A, a major chemical constituent of Withania somnifera, has been reported for its tumor cell growth inhibitory activity, antitumor effects, and impairing metastasis and angiogenesis.A methanolic extract of aerial parts of WS had anti-inflammatory activities comparable to those of hydrocortisone sodium succinate .An 80% ethanolic extract of *Withania somnifera* (WS) displayed significant anti –inflamatory activity on carrageenan induced paw oedema.The locally induced graft (lymphocytes)-vs-host reaction in chicks was strongly inhabited by Withaferin. Extracts of WS, consisting of equimolar concentrations of sitoindosides VII-X and withaferin A, induced an increase in the levels of superoxide dismutase, catalase and glutathione peroxidase in rat brain, consistent with other research that reports antioxidant and immunomodulant activities[12]. WS has a sedative rather than stimulant action on the CNS, making it a superior medicine for exhaustion with nervous irritability.WS alters the concentration of neurotransmitters, chemical substances that are known to play an important role in the brain processes such as memory. The herbal drug is found to decrease the degree of anxity and depression and can be used as antidepressant. WS strengths immunity against colds, flu and other infections. An extract of root of WS enhance the levels of interferon gamma, interlukin-2 and granulocyte macrophage colony stimulating factor in normal and cyclophosphoamide treated mice, suggesting an immunopotentiating and myeloprotective effect WS is used as sexual tonic in the treatment of spermatopathia, impotence and seminal depletion.Men who used the herb enjoyed higher sexual performance.The roots contain Fe, K, Mg and Ni along with other elements, which are reported to play a significant role in the diuretic and aphrodisiac activity of the drug [13]. WS acts as an adaptogen to increase

the body's ability to withstand stresses of all types.WS improves physical energy, exercise capacity and overall health.WS is used for the treatment of bacterial infections, its roots and leaves exhibit marked activity against *S. aurens*[14]. Alkoids are the chief constituents that seem to be most likely candidates eliciting immunostimulating effects. However, the available evidence is not adequate to allow their use in clinical practice. There is a need for comprehensive, systematic, multi-disciplinary evaluation of various claims to make effective use of these products.

REFERENCES

[1] SK. Verma; SK. Singh, S Singh and A Mathur, *Journal of Chemical and Pharmaceutical Research* **2010**, 2 (4): 861-865.
[2] A Mathur, S K. Verma, R. Bhat, S K. Singh, A Prakash, GBKS Prasad and VK Dua *Journal of Chemical and Pharmaceutical Research* **2010,** 2 (4): 264-370
[3] S Singh; S Kumar. *Central Institute of Medicinal and Aromatic Plants: Lucknow, India*. **1998,** 65:179–204.
[4] NS Doherty. *Agents and Actions*, **1981,** 11 237–242.
[5] DS Nelson And P Mildenhall. *Australian Journal of Experimental Biology and Medical Sciences*, **1967** 45: 113–130.
[6] A Mathur; SK Verma; SK Singh; D Mathur; GBKS Prasad; VK Dua. *Recent Research in Science & Technology*. **2011**, 3(3): 40-43
[7] A Mathur; SK Verma**;** R Purohit; SK Singh; D Mathur; GBKS Prasad and V.K. Dua. *J. Chemical Pharm. Res* **. 2010**, 2(6):191-198
[8] SS Agrawal And VK Singh. *Proc Indian Natl Sci Acad, Part B,* **1999,** 65:179–204.
[9] AL Hindawi; MK Khafaji; S H Abdul; M H Nabi. *J Ethnopharmacol* .**1992,** 37:113-116.
[10] L Davis; G Kuttan. *Immunopharmacol Immunotoxicol.* **1999,** 21:695-703.
[11] NP Bector; AS Puri; D Sharma. *Indian J. Med. Res.* **1968,** 56:1581-1583.
[12] SK Bhattacharya; RK Goel; R Kaur; S Ghosal. *Phytotherap Res.* **1987,** 9: 10-13.
[13] DR Lohar; D Chaturvedi; PN Varma. *Indian Drugs.* **1992,** 29:271-273.
[14] PA Kurup. *Curr. Sci.* **1965,** 25:57-60.