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Journal of Ethnopharmacology 50 (1996) 69–76

Journal of ETHNO-PHARMACOLOGY

Studies on the immunomodulatory effects of Ashwagandha

Mohammed Ziauddin[a], Neeta Phansalkar[b], Pralhad Patki[c], Sham Diwanay[d], Bhushan Patwardhan*[e]

[a]*Indian Drugs Research Association, Medinova Diagnostics Center, Pune, India*
[b]*Department of Microbiology, Medinova Diagnostics Center, Pune, India*
[c]*Department of Pharmacology, Byramjee Jeejeebhoy Medical College, Pune, India*
[d]*Department of Microbiology, Abasaheb Garware College, Pune, India*
[e]*Interdisciplinary School of Health Sciences, University of Pune, Pune 411 007, India*

Received 1 June 1995; revision received 25 September 1995; accepted 6 October 1995

Abstract

The immunomodulatory activity of an Indian Ayurvedic medicinal preparation, Ashwagandna (*Withania somnifera* (L. Dunal)) was studied in mice with myelosuppression induced by one or more of the following three compounds: cyclophosphamide, azathioprin, or prednisolone. The assessment of immunomodulatory activity was carried out by hematological and serological tests. A significant modulation of immune reactivity was observed in all the three animal models used. Ashwagandha prevented myelosuppression in mice treated with all three immunosuppressive drugs tested. A significant increase in hemoglobin concentration ($P < 0.01$), red blood cell count ($P < 0.01$), white blood cell count ($P < 0.05$), platelet count ($P < 0.01$), and body weight ($P < 0.05$) was observed in Ashwagandha-treated mice as compared with untreated (control) mice. We also report an immunostimulatory activity: treatment with Ashwagandha was accompanied by significant increases in hemolytic antibody responses towards human erythrocytes.

Keywords: Ashwagandha; Immunomodulation; Immunostimulation; Myelosuppression; *Withania somnifera*

1. Introduction

Plant and animal products have been the basis of treatment of human diseases since time immemorial. Every country in the world has lists of herbal remedies for the treatment of diseases and different other unwanted conditions in humans (Satoskar and Bhandarkar, 1983). Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka and can be traced back to 6000 B.C. (Charak Samhita, 1949). Ayurveda, like other medical systems practised in the world, originated from folk medicine and now holds a commanding position in the countries mentioned. Ayurvedic medicines are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles (Patwardhan and Hooper, 1992).

The immune system is known to be involved in the etiology as well as pathophysiologic

* Corresponding author.

SSDI 0378-8741(95)01318-8

mechanisms of many diseases. Immunology is thus probably one of the most rapidly developing areas of biomedical research and has great promises with regard to the prevention and treatment of a wide range of disorders. Inflammatory diseases of the skin, gut, respiratory tract, joints and central organs, as well as infectious diseases, are now primarily considered immunological disorders, while neoplastic diseases may involve an immunosuppressive state (Samter, 1971). Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of 'Rasayana' in Ayurved is based on related principles (Charak Samhita, 1949). The function and efficiency of the immune system may be influenced by many exogenous and endogenous factors like food, phamaceuticals, physical and psychological stress, hormones etc., resulting in either immunosuppression or immunostimulation. The healthy state is believed to be based on a sophisticated fine-tuning of immunoregulatory mechanisms (Patwardhan et al., 1991).

Apart from being specifically stimulatory or suppressive, certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and are hence called immunomodulatory agents (Wagner, 1983).

Suppressive and cytotoxic activity affecting the function of the immune system has been reported for a great many of synthetic and natural therapeutic agents (Muftuogy, 1984). Among the synthetic substances, azathioprin and cyclophosphamide have been extensively studied (Shand and Howard, 1979). Azathioprin inhibits DNA synthesis and has an anti-inflammatory function by virtue of its myelosuppressive activity. Cyclophosphamide, an alkylating agent, is relatively selective for lymphoid tissue. Prednisolone, a glucocorticoid, also has selective immunosuppressive activity, acting at various levels in the immune system. Thus, these drugs have general myelosuppressive and specific immunosuppressive activities (Gilman, 1991).

Ashwagandha, *Withania somnifera* (L. Dunal) (Solanaceae), is an Ayurvedic medicinal plant which is popular as a home remedy for several diseases and human requirements. It is mentioned in Vedas as a herbal tonic and health food. It is an official drug and is mentioned in the Indian Pharmacopoeia. It is in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects. The chemical composition, and pharmacological and therapeutic efficacy have been established. Different investigators reported antiserotogenic, adaptogenic, anticancer and anabolic activity, and beneficial effects in the treatment of arthritis, geriatric problems (Asthana and Raina, 1989), and stress (Grandhi et al., 1994).

The effect of Ashwagandha on the immune system at various levels, i.e., hematopoiesis, nonspecific immune mechanisms and cellular responses, has not been extensively studied. Therefore, using the model of myelo- and immuno-suppression drugs in mice, the normalizing effects of Ashwagandha extracts were investigated.

2. Materials and methods

2.1. Test materials

Roots of Ashwagandha and their extract were supplied by Litaka Pharmaceuticals Ltd., Pune, India. The voucher sample was retained and has been deposited at the Indian Drug Research Association, Pune. Routine pharmacognostic investigations were carried out to confirm authenticity of these materials. These identifications were carried out at the Indian Drugs Research Association's Laboratory which has an approval for analytical testing from the Food and Drug Administration of Maharashtra State Government, and were confirmed as roots of *Withania somnifera* L. Dunal (Solanaceae). The root extract was prepared according to the procedure described in U.S. Patent Application No. 08/273, 189, 11,7,1994.

2.2. Chemical characterization

Thin-layer chromatography (TLC) was used to identify the steroidal lactones (withanolides) present in Ashwagandha. The solvent system used was chloroform:methanol:water (64:50:10, v/v) and spots were finally identified with vanillin phosphoric acid (Stahl, 1964). The percentage of steroidal lactones was estimated spec-

trophotometrically in the drug, according to the Indian Pharmacopoeia (1985). Estimations were carried out at 525 nm using the reagents, tetrazolium blue and tetramethyl ammonium hydroxide. The root extracts were converted to aqueous suspension by using 2% gum acacia as the suspending agent and 100 mg/ml (w/v) stocks were prepared.

2.3. Animals

Albino mice (Haffkine Institute, Kasauli strain) weighing between 30–40 g were used to evaluate the immunomodulatory activity of Ashwagandha. The animals were obtained from Hindustan Antibiotics Ltd. Pimpri, India. All the animals were given a balanced diet and maintained in the same environmental conditions.

2.4. Statistical analysis

Data were expressed as the means ± standard error of the means (S.E.M.) and statistical analysis was carried out employing Student's *t*-test.

2.5. Immunomodulatory activity

This activity was studied using drug-induced myelosuppression in animal models (Charles, 1990). Eighty albino mice were marked with picric acid and divided into eight groups designated as I to VIII, each group containing ten mice. The weight of individual mice were recorded. Before starting the treatment, on day zero all mice were sensitized with a thymus-dependent antigen, that is human 'O' group red blood cells 20% suspension in phosphate-buffered saline (PBS), 0.5 ml/mouse, by injecting intraperitonially.

Finely powdered drugs (cyclophosphamide, prednisolone, azathioprin) and Ashwagandha extract were suspended into 2% gum acacia solution as suspending agent and were administered orally daily.

The control group (I) received Ashwagandha extract at a dose of 100 mg/kg body weight. Groups III, V, and VII mice received cyclophosphamide at a dose of 3 mg/kg body weight, prednisolone at 5 mg/kg body weight or azathioprin at 3 mg/kg body weight, respectively. Groups IV, VI, and VIII mice received Ashwagandha extract at doses of 100 mg/kg body weight in combination with cyclophosphamide, prednisolone and azathioprin, respectively.

On the 16th day, five mice from all groups were sacrificed and blood was collected in a vial containing an anticoagulant for evaluating hematological parameters and to obtain serum for serological parameters.

The remaining mice received a secondary immunization with the same antigen and were then kept for recovery observations for the next seven days. No drug or extract was administered during this period. On the 22nd day, all remaining mice were sacrificed and blood was collected for the evaluation of hematological and serological parameters.

Blood and serum samples of animals for each group after treatment and the recovery period were subjected to hematological studies including hemoglobin content, total red blood cell count, total and differential white blood cell count, platelet count and serological studies, namely, quantitative estimation of agglutinin and hemolytic antibodies by hemagglutination and complement fixation, respectively (Talwar, 1983).

3. Results

The pharmacognostic investigations of Ashwagandha roots showed the presence of longitudinal striations, even fracture, dark yellowish brown colour, strong odor, and bitter and mucilaginous taste. The section of root observed under microscope showed the presence of cork, phellogen, phelloderm, phloem, xylem, medullary rays, pith, and also starch grains.

Upon physicochemical analysis, TLC showed the presence of four bluish spots (Rf 0.8–0.9). The spectrophotometric assay showed 2.8% of total mass to be steroidal lactones (withanolides).

Immunomodulatory studies were performed using the drug-induced myelosuppression animal model. The suppression of bone marrow activity reflecting myelosuppression by cyclophosphamide (group III), prednisolone (group V) or azathioprin (group VII) was considerable (Table 1) and was accompanied by a lowering of the hemoglobin concentration, red blood cell counts, platelet counts, and total white blood cell counts. In differ-

Table 1
Effect of Ashwagandha extract on blood cells of mice treated with cyclophosphamide, prednisolone and azathioprin for 15 days

Group	Haemoglobin concentration in g%	RBC count in million/cmm	Platelet count in thousand/cmm	WBC count in thousand/cmm
I	14.35 ± 0.23	4.80 ± 0.16	457.3 ± 0.31	10.0 ± 0.28
II	16.40 ± 0.23^{f}	6.74 ± 0.38^{f}	697.3 ± 0.23^{f}	11.2 ± 0.37 NS
III	10.86 ± 0.23^{a}	3.27 ± 0.25^{b}	280.6 ± 0.09^{b}	6.40 ± 0.26^{a}
IV	15.00 ± 0.11^{d}	6.06 ± 0.08^{d}	485.0 ± 0.14^{e}	10.76 ± 0.06^{d}
V	11.81 ± 0.11^{a}	3.48 ± 0.19^{b}	324.0 ± 0.32^{c}	6.76 ± 0.14^{a}
VI	14.60 ± 0.30^{e}	6.18 ± 0.17^{d}	513.3 ± 0.16^{e}	10.73 ± 0.64^{e}
VII	11.75 ± 0.40^{a}	2.88 ± 0.17^{b}	297.3 ± 0.17^{c}	9.26 ± 0.43 NS
VIII	14.80 ± 0.42^{e}	6.28 ± 0.25^{d}	508.6 ± 0.21^{e}	10.76 ± 0.39 NS

$n = 5$ per group; tabular values represent mean ± S.E.M.
(A) $^{a}P < 0.001$, $^{b}P < 0.01$, $^{c}P < 0.05$: Comparison between control and myelosuppressive drug treatment.
(B) $^{d}P < 0.001$, $^{e}P < 0.01$, NS, not significant: Comparison between drug-induced myelosuppression and combination of drug and Ashwagandha treatment.
(C) $^{f}P < 0.01$, NS, not significant: Comparison between control and Ashwagandha treatment.
Group I = control (without any drug treatment); Group II = Ashwagandha-treated mice group; Group III = treatment with cyclophosphamide; Group IV = treatment with cyclophosphamide and Ashwagandha; Group V = treatment with prednisolone; Group VI = treatment with prednisolone and Ashwagandha; Group VII = treatment with azathioprin; Group VIII = treatment with azathioprin and Ashwagandha.

ential white blood cell counts, a relative lowering of lymphocyte percentages and an increase in neutrophils was observed. No significant suppression of humoral antibody response was seen (Fig. 1).

Combined treatment of myelosuppressive drug and Ashwagandha extract resulted in a restoration of bone marrow activity as compared with myelosuppressive treatment only (Table 1). The

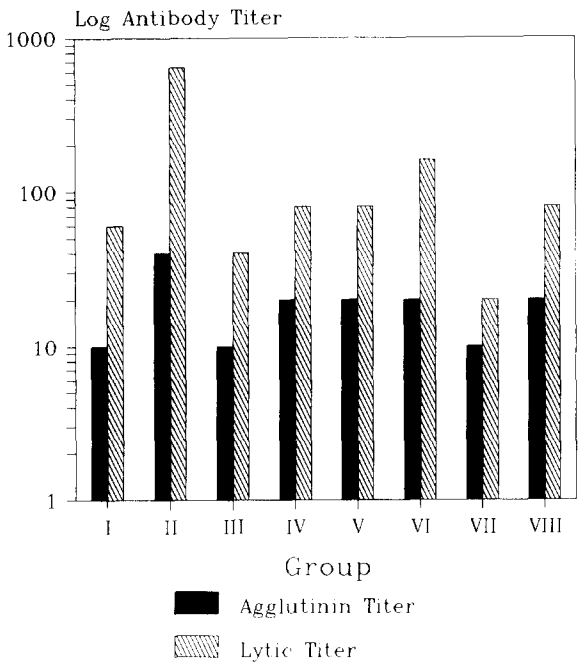

Fig. 1. Antibody titer after treatment period.

Table 2
Recovery period observations after 7 days with withdrawal of all drugs from all groups

Group	Haemoglobin concentration in g%	RBC count in million/cmm	Platelet count in thousand/cmm	WBC count in thousand/cmm
I	14.50 ± 0.18	5.33 ± 0.23	435.0 ± 0.16	11.26 ± 0.37
II	16.61 ± 0.11[f]	7.47 ± 0.03[f]	663.0 ± 0.14[f]	11.4 ± 0.19 NS
III	12.62 ± 0.12[a]	4.10 ± 0.06[b]	324.0 ± 0.25[b]	8.70 ± 0.36[a]
IV	14.61 ± 0.11[d]	5.56 ± 0.18[e]	456.0 ± 0.16[e]	11.70 ± 0.40[d]
V	12.16 ± 0.35[b]	3.68 ± 0.15[b]	305.0 ± 0.36[b]	9.33 ± 0.18[a]
VI	14.37 ± 0.13[e]	5.31 ± 0.12[e]	477.6 ± 0.39[e]	12.13 ± 0.39[d]
VII	12.07 ± 0.90[a]	3.45 ± 0.21[c]	357.6 ± 0.30[c]	9.60 ± 0.36[b]
VIII	15.15 ± 0.13[d]	6.76 ± 0.13[d]	560.0 ± 0.21[d]	11.67 ± 0.60[e]

$n = 5$ per group; tabular values represent mean ± S.E.M.
(A) [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$: Comparison between control and myelosuppressive drug treatment.
(B) [d]$P < 0.001$, [e]$P < 0.01$, NS, not significant: Comparison between drug-induced myelosuppression and combination of drug and Ashwagandha treatment.
(C) [f]$P < 0.01$; NS, not significant: Comparison between control and Ashwagandha treatment.
Group I = control (without any drug treatment); Group II = Ashwagandha-treated mice group; Group III = treatment with cyclophosphamide; Group IV = treatment with cyclophosphamide and Ashwagandha; Group V = treatment with prednisolone; Group VI = treatment with prednisolone and Ashwagandha; Group VII = treatment with azathioprin; Group VIII = treatment with azathioprin and Ashwagandha.

antibody response was not significantly altered, except in group VI, where a significant increase in hemolytic titer was observed (Fig. 1).

Ashwagandha-treated mice (group II) showed a significant improvement of bone marrow activity as compared with controls (group I), as indicated by the hemoglobin concentration ($P < 0.01$), red blood cell counts ($P < 0.01$), platelet counts ($P <$

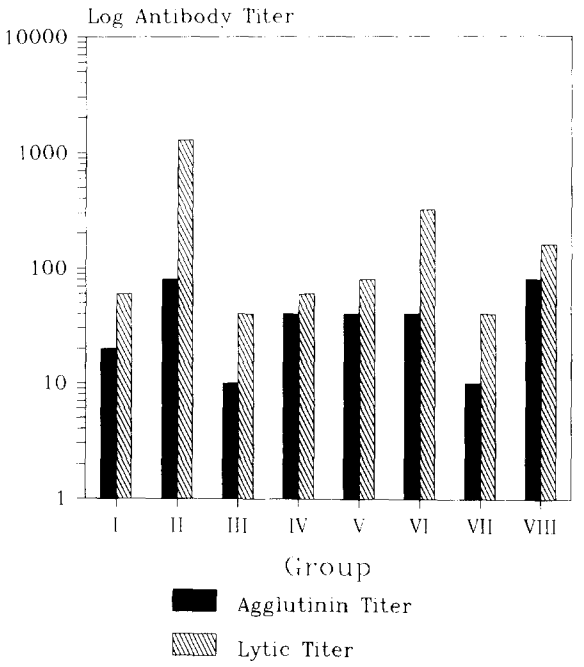

Fig. 2. Antibody titer after recovery period.

0.01), but did not significantly affect total white blood cell counts ($P < 0.1$). An immunostimulatory response was observed in group II mice, as indicated by a rise in antibody titer (control group (I) hemagglutinin titer 1:10 and hemolytic titer 1:60; group (II) hemagglutinin titer 1:40 and hemolytic titer 1:640). Body weight was significantly increased in group II mice as compared with control animals ($P < 0.05$).

Recovery period observations (Table 2) indicated that drug-induced myelosuppression was not restored to normal, even after discontinuous use of these drugs. It was observed that the animals in groups IV, VI, and VIII showed a more normal marrow activity, as compared with groups III, V, and VII. No significant changes in body weights were seen in any of the groups. With secondary immunization, Ashwagandha-treated mice (groups II, IV, VI, and VIII) showed even more enhanced hemoytic titers (Fig. 2).

4. Discussion

The main objective of this study was to focus on the immunomodulatory activity of Ashwagandha with special reference to it's putative protective and immunostimulatory activity in animal models.

Control of disease by immunological means has two aspects, namely the development and improvement of protective immunity and the avoidance of undesired immunological side reactions. Modulation of the immune system by cytostatic agents is emerging as a major area in pharmacology, especially in cases where undesired immunosuppression is the result of therapy. Cytotoxic drugs like cyclophosphamide and azathioprin act at various levels on cells involved in defence against foreign invaders. Bone marrow suppression resulting in cytopenia and subsequent suppression of humoral and or cellular, as well as nonspecific and specific cellular, responses is a major draw back of these drugs. Prednisolone is particularly responsible for a suppression of specific and nonspecific cellular defenses. Cyclophosphamide and prednisolone treatment resulted in significant lowering of hemoglobin concentrations, red blood cell counts, platelet counts and total white blood cell counts, as well as lymphocyte percentages. While azathioprin treatment had no significant effect on total white blood cell counts, albeit a lowering of hemoglobin concentration, red blood cell count and platelet count was observed. Thus, the cyclophosphamide and prednisolone-induced myelosuppressed animal model is a reliable, convenient, and reproducible experimental system.

Human doses of Ashwagandha are normally in the range of 4–6 g a day; therefore, a corresponding dose of 100 mg/kg was used in this study. The results of Ashwagandha-treated animals (group II) are supportive of its traditional use as a health tonic, improving the marrow activity without unwanted leucocytosis as side effect.

Animals in all groups were maintained at normal nutritional status and they were given the same antigenic stimulus. The response of the control group can be considered as normal. Animals treated with Ashwagandha extract caused increased agglutinin antibody titers and, even more significant, complement-fixing antibodies. This suggests that even for a normal healthy individual, treatment with Ashwagandha will serve as an immunostimulatory agent for immune system. The nonsignificant rise in agglutinating antibodies that in a hypersensitive condition may lead to immune-complex mediated reactions, with a significant rise in complement fixing antibodies definitely points out the development of protective immune responses and the counteraction of undesired immune reactions.

Supportive evidence for confirmation of the above-mentioned results was obtained when animals from all groups were subjected to an evaluation of hematologial and serological tests after the recovery period. It was clearly seen that animals treated with myelosuppressive drugs recovered at a slower rate as compared with animals treated with Ashwagandha extract. It was observed that myelosuppressive drug withdrawal can be supplemented with Ashwagandha therapy, resulting in a complete restoration of the immune response or even improved immunological parameters.

It is true that our present study does not clearly rule out the possibility that Ashwagandha would interfere with the activity of cyclophosphamide;

however, there is indirect evidence suggesting a direct effect of Ashwagandha on the immune system (Buddhiraja and Sudhir, 1987). Further, we have not actually studied the effect of cyclophosphamide on the individual cells or tissues. However, we examined the structural integrity of the gastrointestinal tract of all the animals during the course of the experiment. Animals which were treated with cyclophosphamide showed spot discontinuity in the mucous membrane, with bleeding spots near to the lower end of the esophagus and the greater curvature of the stomach. This finding was consistent even with the animals which are treated with Ashwagandha. This demonstrates the inability of Ashwagandha to produce any protective effect against cyclophosphamide-induced cytotoxicity.

In our study, we selected other drugs like azathioprin and prednisolone which could produce myelosuppression; however, the animals receiving these drugs show a different pharmacodynamic response to those fed with cyclophosphamide. This is similar to the response of the immune system to Ashwagandha, rather than an indirect modulation or interference with the action of the cytotoxic drugs cyclophosphamide or azathioprin.

In the course of this project, we also found that Ashwagandha exhibited nonspecific immunostimulatory activity in various models including oxazolone-induced erythema, plaque technique, carbon clearance test, and *Escherichia coli*-induced sepsis. For direct evidence, however, one has to screen the compounds in carcinoma-induced animal models and bone-marrow protection induced by chemotherapy (cyclophosphamide, prednisolone).

Many facets of Ayurvedic drugs, especially Ashwagandha, have been reported (Patwardhan et al., 1991). Use of Ashwagandha in various immunological and other disorders and also for normal individuals is recommended in Ayurveda. Recently, a possible role of Ashwagandha in the prevention of cataracts in animal models was demonstrated. This treatment was found to be very effective, probably via its effects on lipid peroxidation (Deshpande, 1995). Multidimensional investigations of Ashwagandha have revealed that the preparation is an emerging novel drug in therapeutics and general improvement of health.

5. Conclusions

The conclusions dealing with our experimental data can be extrapolated to a human situation or backchecked with Ayurvedic formulations and treatment schedules, reconfirming the immunomodulatory activity which is not directly mentioned in Ayurveda. The use of Ashwagandha as an immunomodulator to counteract the undesirable effects of myelosuppressive drugs and also for developing and improving protective immunity even in normal individuals may be possible in the future.

Acknowledgements

The authors are grateful to the Director of the Indian Drug Research Association, Dr. S.B. David for providing the necessary facilities, Dr. S.R. Naik (Manager, R & D, Hindustan Antibiotics), Dr. S.M. Mujumdar, Dr. Mrs. Desai, and Ms. Anuradha Venugopalan for their valuable guidance and help, Litaka Pharmaceuticals Ltd. for supplying us with the test preparations, and the Medinova Diagnostics Center for the facilities they offered us.

References

Asthana, R. and Raina, M.K. (1989) Pharmacology of *Withania somnifera* (Linn Dunal): A Review. *Indian Drugs* 26 (5), 199–204.

Buddhiraja, R.D. and Sudhir, S. (1987) Review of biological activity of withanolides. *Journal of Scientific and Industrial Research* 46, 488–499.

Charak Samhita (1949) *Translator: Shree Gulabkunverba Ayurvedic Society*. Jamnagar, India.

Charles, R., Craig, R.E. and Stitzel, A. (1990) *Modern Pharmacology*, 3rd edition. Little Brown and Co., London, pp. 821–831.

Dale, M.M., Foreman, J.C. and Fan, T.D. (1994) *Text book of Immunopharmacology*, 3rd ed. Blackwell Scientific Publication, London.

Deshpande, A. (1995) Effect of *Withania somnifera* Linn Dunal on lipid peroxidation in experimental cataract. *M. Pharm. Dissertation* submitted to University of Pune.

Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor, P. Ed. (1991)

The Pharmacological Basis of Therapeutics, 3rd ed. Pergamon Press, N.Y.

Grandhi, A., Mujumdar, A.M. and Patwardhan, B. (1994) A comparative pharmacological investigation of Ashwagandha and Ginseng. *Journal of Ethnopharmacology* 44, 131–135.

Indian Pharmacopoeia (1985) Appendix 3:3:10 pp. 69.

Muftuogy, A.U. and Barlas, N. (1984) *Recent Advances in Immunology*. Plenum Press, N.Y.

Patwardhan, B., Kalbag, D., Patki, P.S. and Nagsampagi, B.A. (1991) Search of Immunomodulatory Agents — A Review. *Indian Drugs* 28 (6), 249–254.

Patwardhan, B. and Hooper, M. (1992) Ayurveda and future drug development. *International Journal of Alternative and Complementary Medicine* 10 (12), 9–10.

Samter, M. (1971) *Immunological Diseases*, 2nd edn. Little Brown and Company, Boston.

Satoskar, R.S. and Bhandarkar, S.D. (1983) *Pharmacology and Pharmacotherapeutics*, Part I, 8th ed. Popular Prakashan, Bombay, India.

Shand, F.L. and Howard, J.G. (1979) *European Journal of Immunology* 9, 17.

Stahl, E. (1964) *Thin Layer Chromatography — A Laboratory Handbook*, 2nd ed. George Allen and Unwin, London, pp. 311–357.

Talwar, G.P. (1983) *A Handbook of Practical Immunology*. Vikas Publishing House Pvt. Ltd., Gaziabad, U.P., India, pp. 155–161.

Wagner, H. (1983) *Proceedings of Alfred Benzon Symposium*, 20. pp. 559.