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Immunomodulators: A Review of Studies on Indian Medicinal Plants and Synthetic Peptides

Part I: Medicinal Plants

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In this review we have attempted to highlight the work on immunomodulators carried out in Indian laboratories including both plant extracts and synthetic peptides. The results for plant extracts are reviewed in part I of this review and synthetic peptides in part II. Various Indian plants which have the potential of immunomodulating activity are identified from various sources in the literature. Among these 14 have undergone *in vitro* and *in vivo* evalution, mostly in animals and to some extent in humans as well. While the leads are extremely promising for some of them such as *Asparagus racemosus, Azadirachta indica, Curcuma longa, Ocimum sanctum, Panax ginseng, Picrorhiza kurroa, Tinospora cordifolia, Withania somnifera* etc., considerable work remains to be done for the remainder plants.

Key Words: Immunomodulators, Plant extracts

Introduction

There have been remarkable advances in the field of basic Immunology during last three to four decades (Roitt et al. 1998). Starting with the distinction between cellular and humoral arms of immunity (Weissman & Cooper 1993) and recognition of cell surface phenotypes on T and B cells (Lal et al. 1998), we have come a long way in understanding molecular mechanisms of immune response. These include, (i) immunoglobulin and T-cell receptor gene rearrangements creating diversity as well as uniqueness of the immune response (Davis 1990, Alt et al. 1992), (ii) complexity of the MHC system and its role in antigen presentation and restriction of effector cytotoxic cells (Germain 1994, Kersh & Allen 1996), (iii) signal transduction (O'Shea 1997) and selection of Th1 and Th2 types of cellular responses (Abbas et al. 1996), and (iv) elimination of offending agent by the effector arm of the immune response.This knowledge has helped in unraveling mysteries of various key elements of immune response viz., recognition of self and non-self (Karlsen & Dyrberg 1998), regulation of immune response (Jerne 1984), termination of immune response after effective control of the offending agent (Parjis & Abbas 1998) and establishment of repertoire of memory cells for future. More recently the instructive role of innate immune system on acquired immune response has been recognised (Fearon & Locksley 1996). Simultaneously, defects and derangements in these mechanisms have been identified which lead to immune deficiency, allergy, autoimmunity and immuno-malignancies. However, networking and interactions within the immune system are so complex that modulation of the immune response at will 'to achieve designed therapeutic success is still 'in

the realm of philosophical editorials rather than of definitive efficacy studies' (Benson 1993).

Definition and Scope of Immunomodulators

An immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune response. One of the starting points in the field of immunomodulation has been the search for agents that could be used for treatment of residual cancer (Yamamoto 1996). The results with intralesional application of BCG (Patard et al. 1998) and systemic use of levamisole (Kurman 1993) have been quite promising. Discovery of cytokines has led to evaluation of efficacy of various interleukins (ILs) such as IL-1, IL-2 and interferon (IFN) in immunotherapy of cancer (Tartour & Fridman 1998). Interferon, both α and γ, have already entered in clinical practice in a big way. More recently cytokines have been used as immunoadjuvants along with vaccines (Linn et al. 1995). But this is in experimental stages. One immunostimulant, Isoprinosine, has been recently approved by FDA (Food and Drug Administration, USA) (Hadden 1994). It is recommended for controlling severity and duration of viral infections. The use of immunostimulants, particularly as adjunct to chemotherapy for control and prevention of infections (Chatterjee et al. 1988) holds great promise but has not yet received the attention that it deserves.

Most significant progress in the field of immunomodulators is represented by the discovery of cyclosporin (Walsh et al. 1992). It is a potent immunosuppressant that has proved to be a boon for prevention of graft rejection. The drug is also gaining ground for treatment of several autoimmune diseases, particularly in failures of prednisolone, azathioprin, cyclophosphamide and methotrexate. However, due to its very low therapeutic index and significant nephrotoxicity, search for an alternative to cyclosporine is being actively pursued (Morris 1991). Although several leads in this direction have been described in literature (FK506, Rampamycin, Mofetil etc), none has come to the market yet.

Classification of Immunomodulators

In clinical perspective immunomodulators can be classified into following three categories:

1. Immunoadjuvants

These agents are used for enhancing efficacy of vaccines and, therefore, could be considered as specific immune stimulants. One of the best known examples is Freund's adjuvant. However, since it contains Bacillus Calmette Guiren (BCG), it is not appropriate for human use (Claassen et al. 1992). Muramyl dipeptide (MDP) has been identified to be the active ingredient of BCG cell wall (Adam & Lederer 1984) but it has not been able to replace the BCG from Freund's adjuvant. Lack of availability of a suitable adjuvant for human use has been one of the important stumbling blocks in our ability to develop various vaccines e.g. malarial vaccine (Allison 1997). In recent years liposomes and other agents are being developed as a suitable alternative (Lasic 1998).

In addition to the above use, the immunoadjuvants hold the promise of being the true modulators of the immune response. It has been proposed to exploit them for selecting between cellular and humoral, Th1 and Th2, immunoprotective and immunodestructive, and reagenic (IgE) versus immunoglobulin G (IgG) type of immune responses, which poses to be a real challenge to vaccine designers.

2. Immunostimulants

These agents are envisaged to enhance body's resistance against infections (and may be against allergy, autoimmunity, and cancer as well). By this definition these agents are inherently non-specific in nature, but they can act through both

the innate and adaptive arms of the immune response. In healthy individuals the immunostimulants are expected to serve as prophylactic or promotive agent i.e. as immune potentiators by enhancing the basal levels of immune response, and in individuals with impairment of immune response as immunotherapeutic agent. The immunocompromised conditions include patients with primary (humoral, cellular or combined immune deficiency syndromes) as well as secondary immune deficiencies (AIDS, malignancy, cancer chemotherapy, patients receiving steroids etc).

Considering that these agents may not be effective by themselves, they may be used as adjunct to chemotherapy to remove residual cancer cells, as well as in treatment of chronic/ persistent/latent infections (viral, parasitic etc) with or without available chemotherapeutic agents.

3. *Immunosuppressants*

These agents could be used for control of pathological immune response in autoimmune diseases, graft rejection, graft versus host disease, hypersensitivity immune reaction (immediate or delayed type), and immune pathology associated with infections. Out of the list the maximum use of these agents has been for prevention of graft rejection and treatment of autoimmune diseases. A recent review by Kundu and Khare (1999) provides useful information about various immunosuppresants that are either already available or are in various stages of development.

Scope of Present Review

The readers are referred to 3 excellent reviews in this field, viz., (1) 'Immunostimulants' by Hadden (1993), (2) 'Adjuvants: current status, clinical perspectives and future prospects' by Audibert and Lise (1993) and (3) 'Selective immunosuppression' by Adorini et al. (1993) which appeared in Immunology Today. Although relatively old, these reviews have laid down conceptual framework that still holds true. Interested readers may cross-reference these articles. It should not be surprising that the 3 reviews cited above do not mention any of the Indian contributions in this field, what to say of the concept of 'Rasaynas', and indigenous plants with immunomodulatory activity which are unique to the Indian System of Medicine. In a bid to put together the Indian scene, this review will largely restrict to studies from Indian laboratories on immunomodulators, which include studies on crude and purified plant extracts. However, for sake of completeness of information on Indian medicinal plants, data from international laboratories is also included. For collecting information, we have carried out a Medline search (1964-98), as well as personally communicated with known active groups of scientists working on immunomodulators in India. Two monographs, viz. (i) Immunomodulation (Upadhyay 1997a), and (ii) Immunopharmacology (Upadhyay 1999) which have been brought out of the proceedings of national symposia on the subject have also been consulted.

Concept of 'Vyadhirodhak Chamatav' in Ayurvedic Medicine

There is a difference in the concept of body's resistance to disease in traditional Indian System of medicine, i..e., Ayurveda and the Modern System of medicine which is of Western origin (Nityanand 1990). According to Ayurvedic theory a harmonious balance between three humors of the body viz,. 'Vayu', 'Pitta' and 'Kaf' is needed for positive health; imbalance of these may cause disease(s). A significant part of Ayurvedic therapeutics is preventive in nature. It aims to promote positive health so that individuals do not suffer from disease. This is the concept of "Vyadhirodhak chamatav", i.e. capacity of the body to resist disease (Katiyar et al. 1997). Obviously, the immune system, as recognised in modern biology, which provides protection against microbes, should be a part of

it. An entire section of the Materia Medica of Ayurveda termed 'Rasaynas' is devoted to enhancement of body's resistance (Thatte & Dahanukar 1997). Interestingly, the prescribed procedures under this section include not only drugs ('Aushadhi') but also "Aachar" (daily routine including exercise), "Aahar" (diet and nutrition) and "Vyavhar" (mental attitude and discipline) which are equally important in achieving the desired goal (Sharma 1981). Interestingly somewhat similar role is ascribed to 'tonics' and various herbals in the Chinese and Eastern European systems of medicine. In comparison to this concept of 'Rasaynas' in Ayurveda, the only prophylactic intervention in the Modern System of medicine is the use of vaccines. Dietary considerations in Modern medicine are largely limited to the use of recommended daily allowances.

Candidate Plants of Indigenous Origin with Immunomodulatory Properties

A list of 34 plants identified as Rasaynas (Sharma 1981) in the Ayurvedic system of medicine is given in table 1. These substances have been described to possess various pharmacological properties such as immunostimulant, tonic, neurostimulant, antiaging, antibacterial, antiviral, antiseptic, anti-rheumatic, anticancer, antieczema, antiasthamatic, anti-inflammatory, adaptogenic, antistress, antisyphilitic, antileprotic, antipsoriatic activities Besides these, on the basis of various pharmacological actions attributed to plants classified under the category of Rasaynas (see above), a search has been made in the authoritative document of Dr R N Chopra (Chopra et al. 1996) for other indigenous medicinal plants for similar properties (Dev 1997). A total of 68 plants were identified which are listed in table 2. Out of these two groups, seven plants from each group have been investigated experimentally for immuno-modulating properties; viz., *Allium sativum, Aloe vera, Asparagus racemosus, Azadirachta indica, Curcuma longa, Tinospora cordifolia* and *Withania somnifera* from the first group and *Andrographis paniculata, Nyctanthus arbor-tristis, Ocimum sanctum, Panax ginseng, Panax pseudoginseng, Phyllanthus emblica* and *Picrorhiza kurroa* from the second group. The summary of the results of these investigations is given in table 3 and detailed finding for each plant is described separately.

***Allium sativum* ('Lasun' - Liliaceae):** Organosulfur compounds of garlic have been shown to inhibit growth of tumors in animals and to modulate activity of diverse chemical carcinogens (Chopra et al. 1958, Jain 1994, Fujiwara & Natata 1967, Aboul-Enein 1986, Corsi et al. 1998). This effect may be related to activation of natural killer (NK) cells, stimulation of T-lymphocytes and enhanced production of IL-2 (Tang et al. 1997).

Garlic extract has been shown to enhance cytotoxicity of human peripheral blood lymphocytes against both NK cell sensitive (K562) and resistant (M14) cell lines (Morioka et al. 1993). Lau et al. (1991) have demonstrated that garlic may augment macrophage (oxidative burst) and T lymphocyte (blastogenesis) functions. Reeve et al. (1993) have shown that garlic extract protect from UV induced suppression of contact hypersensitivity.

***Aloe vera* ('Ghrita kumari' - Liliaceae):** *A. vera* is known to contain several pharmacologically active ingredients including carboxypeptidase and salicylate. In addition, mucilaginous leaf-gel of *A. vera* possesses multiple therapeutic activities, the most reputed of which is its anti-inflammatory property (Davis et al. 1994). Vazquez et al. (1996) have demonstrated its inhibitory action on arachidonic acid pathway via cyclooxygenase. The polysaccharide isolated from gel inhibits opsonization of zymosan and shows adjuvant activity both for specific antibody production

and induction of delayed type hypersensitivity (DTH) in mice (t'Hart et al. 1989).

A. vera improves wound healing (Chithra et al. 1998a, 1998b). This effect is proposed to be mediated through mannose-6-phosphate (Davis et al. 1994, Visuthikosol et al. 1995). It appears that *A. vera* prevents dermal ischemia by reversing the effects of thromboxane synthetase. It may act synergistically with nitric oxide (NO) or could serve as an oxygen radical scavenger (Heggers et al. 1997). A recent study has shown that short-term exposure of macrophage to acemannan up-regulates the respiratory burst, phagocytosis and candidicidal activity (Stuart et al. 1997).

Acemannan is the major carbohydrate fraction obtained from the gel of *A. vera*. It has been shown to enhance production of IL-1 and TNF-α from peripheral macrophages (Peng et al. 1991, Zhang Tizard 1996). It also induces NO release, expression of surface molecules and morphologic changes in mouse macrophage cell line RAW 264.7 (Karaca et al. 1995, Zhang et al. 1996). Macrophage activation may be accountable for immunostimulating effects of acemannan (t'Hart et al. 1990). It may be responsible for regression of tumors in experimental animals (Peng et al. 1991). It also increases lymphocyte response to alloantigen via IL-1 production, which may explain its capacity to abrogate viral infections in animals and man (Womble & Helderman 1988).

The oligosaccharides from *Aloe* may prevent ultraviolet induced suppression of DTH by reducing keratinocyte derived immunosuppressive cytokines (Lee et al. 1997, Bycon et al. 1998).

***Andrographis paniculata* ('kirayat'-Acanthaceae):** Ethanol extract and purified diterpene andrographolides of *A. paniculata* have been shown to induce significant stimulation of antibody and DTH response to sheep red blood cells (SRBC) in mice (Puri et al. 1993). These preparations also stimulate macrophage migration, phagocytosis of ^{14}C-leucine labeled *E. coli*, and *in-vitro* proliferation of splenic lymphocytes (Puri et al. 1993). The stimulation was found to be both antigen specific and non-specific. It was lower with purified andrographolides than with the ethanolic extract indicating presence of substance(s) other than andrographolides that may be contributing towards immunostimulation. Recently Chiou et al. (1998) have reported that andrographolide from *A. paniculata* inhibits the induction of NO synthase by lipopolysaccharide (LPS) in RAW 264.7 cells. It is accompanied with reduced levels of nitrite in the cells. Since NO is involved in killing of cells, the plant extract may inhibit the effector arm of the immune response.

***Asparagus racemosus* ('satawar'-Asparagaceae):** Extract of *A. racemosus* has been tested for anticancer activity (Seena et al. 1993). Though the extract did not completely inhibit the development of solid tumors, it induced lag in tumor development.

The protective effect of *A. racemosus* against myelosuppression induced by single dose of cyclophosphamide has been reported by Thatte and Dahanukar (1988). Cyclophosphamide was administered as a single dose of 200 mg/kg subcutaneously to one group of mice, while the second group received 3 doses of 30 mg/kg intraperitoneally. Both groups received *A. racemosus* orally for 15 days prior to cyclophosphamide therapy. *A. racemosus* by itself produced leucocytosis with neutrophilia. When compared to untreated (control) groups, *A. racemosus* prevented leucopenia produced by cyclophosphamide to varying degrees. These authors have concluded that the plant is a potent immunostimulant, with effects comparable to lithium and glucan. It may be a good candidate for evaluation in patients receiving cytotoxic drugs.

It has been hypothesised that macrophages play an important role in development of intraperitoneal adhesions. As a corollary

modulation of macrophage activity is expected to prevent occurrence of adhesions. Effect of *A. racemosus* was evaluated in an animal model of intraperitoneal adhesions induced by caecal rubbing (Rege et al. 1989b). Animals were sacrificed 15 days following surgery. The peritoneal macrophages were collected to assess their activity. At the same time, peritoneal cavity was examined for presence of adhesions, which were graded. A significant decrease was observed in adhesion scores attained by animals receiving *A. racemosus*. These findings provide a novel approach for prevention and management of post-operative adhesions.

Treatment with *A. racemosus* significantly inhibited carcinogen ochratoxin A induced suppression of chemotactic activity and production of IL-1 and TNF-α by mouse macrophages (Dhuley 1997). *A. racemosus* induced excess production of TNF when compared with control.

***Azadirachta indica* ('neem'-Meliaceae):** *A. indica* is one of the most common wild growing trees in India. Recent studies have shown that it possesses significant non-specific immunostimulatory properties (Upadhyay et al. 1992). Intraperitoneal injection of neem oil in mice mobilises leukocytic cells into the peritoneal cavity which reach the peak on the third day following treatment. Peritoneal macrophages exhibited enhanced phagocytic activity and MHC class-II expression indicating enhancement of their antigen-presenting ability. Spleen cells of neem oil-treated animals showed significantly higher lymphocyte proliferative response to concanavalin A (Con A) and tetanus toxoid compared to controls. Pre-treatment with neem oil, however, did not augment anti-tetanus toxoid antibody response. It has also been shown that *in vitro* treatment of mice splenocytes with *A. indica* stimulates production of IL-1, IFNγ and tumor necrosis factor-α (TNF-α) reflecting activation of Th1 type of response (Upadhyay 1997b). Since Th1 type of response has been implicated to be protective in nature, the therapeutic effects of neem, as reported in the traditional medicine, may be mediated by activation of cellular immune responses.

Ray et al. (1996) evaluated the effects of *A. indica* leaf extract administration on humoral and cell mediated responses in ovalbumin immunized mice. Treated mice (100 mg/Kg) had higher IgM, IgG and anti-ovalbumin antibody titers compared with controls. There was also enhancement of macrophage migration inhibition and foot pad thickness.

In a clinical study on patients with psoriasis *A. indica* leaf extract was used orally at a dose of 300 mg per day in three divided doses supplemented with application of antipsoriatic coal tar ointment over skin lesions (Katiyar etal. 1997). Significant reduction in erythema, desquamation and infiltration of psoriatic lesions was reported in the treated group. It substantiates the immunostimulatory potential of *A. indica* in a wider sense.

Recently, Sai Ram et al. (1997) have described the immunomodulatory properties of NIM-76, a volatile fraction from *A. indica* oil. Pretreatment of rats with a single intraperitoneal injection of NIM-76 resulted in increase in polymorphonuclear (PMN) leukocytes with concomitant decrease in lymphocyte counts in blood. The immunomodulatory activity of NIM-76 was found to be dose-dependent. At 120 mg/kg body weight, there was enhanced macrophage activity and lymphocyte proliferation response, while humoral component was unaffected. At higher concentrations of NIM-76 (300 mg/kg body weight) there was stimulation of mitogen-induced lymphocyte proliferation, while macrophage activity remained unaffected. However, a fall in primary and secondary antibody titters was observed at this dose. The study indicates that NIM-76 primarily acts through cell-mediated mechanisms by activating macrophages and lymphocytes. The reason for decrease in antibody titers at higher doses will need to be investigated.

***Curcuma longa* ('Haldi' - Zingiberaceae):** *C. longa* rhizome (turmeric), commonly used as spice, is well known for its medicinal value in the Indian traditional system of medicine. Both the crude extract and active principle of this plant have been investigated for anti-inflammatory, anti-tumor and immunomodulatory activities (Nadkarni & Nadkarni 1976, Raghunath & Mitra 1982, Srimal 1997).

Curcumin (diferuloylmethane), which gives yellow color to turmeric rhizome, is one of the active ingredients responsible for the biological activity. It has been shown to possess anti-inflammatory effect in acute, subacute as well as chronic models of inflammation, in mice and rats. However, the effect is weaker than phenylbutazone (Srimal 1997). Sodium curcuminate, and volatile oil of Curcuma have also been shown to possess anti-inflammatory activity (Ghatak & Basu 1972) but the analogs of curcumin have not been found to be as effective as the parent molecule (Mukhopadhyay et al. 1982). Curcumin did not produce any side effect up to 1600 mg/kg/day for 4 weeks in phase I trials in male volunteers. Phase II clinical trials have been conducted in patients with rheumatoid arthritis and osteoarthritis. The first trial in 18 patients of rheumatoid arthritis showed significant improvement in morning stiffness, walking time and reduction in joint swelling score following 2 weeks of therapy (Deodhar et al. 1980). Satoskar et al. (1986) have carried out double blind trial with curcumin in acute inflammation and have found it to be effective.

Curcumin has also been investigated as chemopreventive agent against cancer (Kuttan et al. 1985, Rao et al. 1995). Both turmeric and curcumin inhibit cell growth of Chinese hamster ovary (CHO) cells and are cytotoxic to Dalton's lymphoma cells (Kuttan et al. 1985). Liposome encapsulated curcumin has been shown to inhibit tumor formation and increase survival of mice injected with Dalton's lymphoma cells. Oral administration of curcumin inhibited development of lung metastasis induced by B16F10 melanoma cells in mice (Menon et al. 1995) and colon tumorogenesis (Rao et al. 1995, Samaha et al. 1997).

As immunomodulator, turmeric has been reported to increase mitogenic response of splenic lymphocytes (Yasni et al. 1993). Japanese investigators have isolated a polysaccharide, named Ukonan A-D which stimulates carbon clearance (Gonda et al. 1992, Tomoda et al. 1993). They have also isolated a lipopolysaccharide from the root of Curcuma which is similar to bacterial lipopolysaccharide and is immunostimulant (Inagawa et al. 1992). Dietary curcumin (40 mg/kg) in rats for 5 weeks enhanced IgG levels but did not affect DTH and NK cell activity (South et al. 1997). Curcumin is a potent scavenger of reactive oxygen species like superoxide anions and hydroxyl radicals. It inhibits NO production in activated macrophages (Brouet & Ohshima 1995). The anti-cancer properties of curcumin may be mediated, at least in part by inhibition of inducible form of NO synthase.

***Nyctanthes arbor-tristis* ('harsinghar'- Oleaceae):** *N. arbor-tristis* L, a plant widely used in traditional medicinal system in India, has recently been reported to possess hepatoprotective, antileishmanial, antiviral and antifungal activities. In one study strong stimulation of antigen specific and non-specific immunity, as evidenced by increase in humoral and DTH response to SRBC and macrophage migration has been demonstrated in mice fed with 50% ethanolic extract of seeds, flowers and leaves (Puri et al. 1994). Maximum activity was found in seeds, in which the active principal(s) appear to be mainly associated with lipids. In flowers and leaves, however, major activity was found in the aqueous fraction. Khan et al. (1995) have shown protection of Swiss mice against *Candida albicans* as a result of stimulated humoral

response, DTH and macrophage activity on oral administration of iridoid glycosides of *N. arbortristis*.

***Ocimum sanctum* ('tulsi'-Labiatae):** Extract of *O. sanctum* was tested for anticancer activity (Seena et al. 1993). Though this plant extract did not completely inhibit the development of solid tumor, it induced significant lag in tumor development.

The radioprotective effect of the leaf extract of *O. sanctum* in combination with WR-2721 has been investigated by Ganasoundari et al. (1997, 1998). Mice were injected intraperitoneally with *O. sanctum*, 10 mg/Kg for 5 consecutive days or 100 to 400 mg/Kg WR-2721 or combination of the two, and whole body was exposed to 4.5 or 2 Gy gamma irradiation. The protective effect of water extract of *O. sanctum* (10 mg/Kg/day for 5 days) was greater than the aqueous ethanol extract in protecting mice against 11 Gy (LD100/30) of Co-60 gamma irradiation (Devi & Ganasoundari 1995).

The efficacy of its crude extract was compared with steroids (dexamethasone) in the treatment of patients with acute viral encephalitis (Das et al. 1983). The survival in the *O. sanctum* treated group was significantly higher ($p < 0.05$).

***Panax ginseng* and *P. pseudoginseng* ('ginseng'-Araliaceae):** *P. ginseng* is a Korean plant which is widely used as general health tonic. A number of steroidal saponins and glycosides from this plant have been shown to possess adaptogenic activity (Bhargava & Singh 1985). The crude extract and saponins of Indian *P. pseudoginseng* also possess similar activity (Dua et al. 1989). Saponins from Indian pseudoginseng were also found to be potent immunostimulant (macrophage migration, antibody plaque forming cells and haemagglutinating antibody titer against sheep red blood cells).

We have evaluated the extract of *P. ginseng* root obtained from Pharmaton, Switzerland. It was found to significantly potentiate the protection afforded by an IFN inducer (6-MFA) when ginseng extract was administered orally for 7 days prior to challenge with 100 X LD50 of the Semliki forest encephalitis virus (Singh et al. 1983a). The production of IFN was also enhanced, with change in the ratio of acid labile to acid stable varieties of IFNs (Singh et al. 1984). In another experiment circulating antibody titers and antibody plaque forming cells in response to SRBC, as well as macrophage migration inhibition factor against Semliki forest virus antigen were also enhanced by this extract (Singh et al. 1984). However, these results could not be reproduced with another batch of *P ginseng* root extract obtained from Ginseng Research Institute, Daejeon, South Korea (unpublished observation). The reason for this variation is not known. Further supplies of the ginseng preparation from Pharmaton, Switzerland could not be obtained.

In addition to above investigations in Indian laboratories, fractions of ginseng extract have been reported to possess anti-tumor activity (Xiaoguang et al. 1998, Zee-Cheng 1992) and stimulatory activity on reticuloendothelial system (Tomoda et al. 1993), T- cell proliferation by Con A *in-vitro* (Mizuno et al. 1994), phagocytosis (Solo'veva et al. 1989, Scaglione et al. 1990), chemotaxis (Scaglione et al. 1990), augmentation of NK cell activity (Kim et al. 1990, Yun et al. 1993, See et al. 1997, Lee et al. 1997, Kim et al. 1998), enhancement of antibody forming plaques and haemagglutinating antibody titers against SRBC (Nikitina et al. 1995, Kenarova et al. 1990), production of IL-1 (Kim et al. 1998), IL-2 (Ma et al. 1995, Lee et al. 1997, Kim et al. 1998), TNF-α (Gao et al. 1996), GM-CSF (Kim et al. 1998), increase in population of CD3, CD4, CD8 cells (Kenarova et al. 1990, Mizuno et al. 1994, Scaglion et al. 1990) and immunosuppression in virus infected mice (Yeung et al. 1982) by various workers internationally. It appears to be an extremely promising agent to be evaluated for immunostimulatory activity in humans.

***Phyllanthus emblica* (also known as *Emblica officinalis*) ('amla'-Euphorbiaceae):** *P. emblica* is one of the major constituents of many Ayurvedic tonics prescribed for rejuvenation, recuperation and vitality (Jain 1994, Hartwell 1969). It is also an excellent source of vitamin C. Suresh and Vasudevan (1994) have elucidated the immunomodulatory effect of *P. emblica* on immune profile of tumor bearing mice. When administered orally, *P. emblica* fruit powder was found to enhance NK cell activity and antibody dependent cellular cytotoxicity (ADCC) in syngeneic Balb/c mice bearing Dalton's lymphoma ascites tumor. It elicited 2-fold increase in splenic NK cell activity on the third day following tumor inoculation. Enhanced activity was significant on days 3, 5, 7 and 9 ($p < 0.05$) with respect to untreated tumor bearing control. A significant enhancement of ADCC in drug treated mice was demonstrated on days 3, 7, 9, 11 and 13. There was 35% increase in life span in tumor bearing mice treated with *P. emblica* in comparison to controls. This increased survival was abrogated when NK cell and killer cell activities were depleted either by cyclophosphamide or anti-asialo-GM1 antibody treatment. These results indicate: (a) requirement for functional NK cell or killer cell population for antitumor activity of *P. emblica* and (b) that the antitumor activity of *P. emblica* is mediated through cytotoxicity.

In *in vitro* studies, organic extracts of *P. emblica* leaves have been found to inhibit leukotriene B4-induced migration of human PMNs by 90% and FMLP (N-formyl-L-methionyl-L-leucyl-L-phenylalanine) induced degranulation by 25-35% (Ihantola-Vormisto et al. 1997). This might be the basis for anti-inflammatory and antipyretic properties ascribed to this plant in traditional medicine.

Picrorhiza kurroa ('kutaki'-Scrophulariaceae): *P. kurroa* has been reported by Atal et al. (1986) to be a promising immunomodulatory agent. Its ethanol extract has been observed to enhance DTH response by 80%, and also antibody production and phagocytic activity. The plant extract has been shown to hasten skin graft rejection.

The effect of *P. kurroa* on macrophages obtained from mice treated with carcinogen ochratoxin A has been investigated by Dhuley (1997). It significantly inhibited ochratoxin-induced suppression of chemotactic activity and production of IL-1 and TNF-α

A 50% ethanolic extract of *P. kurroa* leaves was found to elicit dose dependent increase in SRBC induced early (4 hr) and delayed (24-hr) hypersensitivity reaction in mice and rats. It also enhanced humoral immune responses in mice and rats and phagocytic function of reticuloendothelial cells in mice. It augmented responsiveness of murine splenocytes to T cell mitogens, viz., phytohaemagglutinin and Con A, and B cell mitogen viz., LPS (Sharma et al. 1994).

Picroliv is an iridoid glycoside derived from plant *P. kurroa*. It was found to significantly protect golden hamsters against challenge with *Leishmania donovani* promastigotes (Puri et al. 1992). Although no effect of picroliv could be detected on adult parasites of Litomosoides carinii in cotton rats, its administration along with ivermectin (which alone does not have any adulticidal action) exerted significant lethal effect (77%) on microfilariids ($p < 0.001$) (Fatma et al. 1994).

Picroliv was further studied for immunostimulant activity. Oral administration of Picroliv in mice prior to immunization with SRBC resulted in significant increase in haemagglutinating antibody titer, plaque-forming cells, and DTH response to SRBC (Puri et al. 1992). It also increased macrophage migration, ^{14}C-glucosamine uptake, phagocytosis of ^{14}C-leucine labeled *E. coli*, chemiluminescence of peritoneal macrophages and higher uptake of ^{3}H-thymidine in lymphocytes of picroliv treated mice.

***Tinospora cordifolia, T. sagittata,* and *T. malabarica* ('giloe'-Menispermaceae):** *T. cordifolia* is a traditional Indian medicinal plant that is ascribed to posses multiple medicinal properties (anti-bacterial, anti-allergic, anti-diabetic, analgesic and diuretic). It has also been used as tonic and vitaliser to enhance body's natural resistance. However, the mechanisms by which it acts remains to be elucidated. Extract of *T. cordifolia* was tested for anticancer activity by Seena et al. (1993). It was found to be cytotoxic to D11 and Ehrlich ascites carcinoma (EAC), but did not affect the growth of L929 cells.

Sainis et al. (1997) have examined the effect of crude extract (water extract) of dry stem of *T. cordifolia* and *T. malabarica* on lymphocyte proliferation. Maximum *in-vitro* mitogenic response was seen in splenic lymphocytes of mice. Lymph node cells also showed significant proliferation while thymus and bone marrow cells did not proliferate. It was poorly mitogenic to human lymphocytes. Among subpopulation of mouse spleen cells, B-lymphocytes showed strong proliferative response in presence as well as absence of macrophages. Nylon wool non-adherent cells (T-cells) did not proliferate. Purification of crude extract by removal of TCA precipitable constituents and subsequent acetone precipitation (fraction TCA-1) brought about significant improvement in mitogenic ability. TCA-1 was more mitogenic than crude extract even at 1/100 dose. Further purification of TCA-1 on Sephadex G 200 (TC-1a) and Sephacryl S 400 (TC-1a-scaq) resulted in further enrichment of mitogenic activity. TC-1a-scaq was more potent than TC-1a.

Recently Sainis et al. (1999) have further demonstrated that oral administration of *T. cordifolia* extract to mice for 15 days significantly enhanced the humoral immune response to SRBC but the T-cell response to Con A was suppressed. The levels of Th2 cytokines IL-4 and IL-10 in the supernatant of mouse spleen cells cultured with *T. cordifolia* extract were significantly reduced.

Dahanukar et al. (1988) have demonstrated immunotherapeutic potential of *T. cordifolia* against abdominal sepsis induced by caecal ligation in rats. Pretreatment with *T. cordifolia* reduced the mortality significantly. This was comparable to the group treated with metronidazole and gentamycin. It also helped in localization of infection and better sac formation. There was an increase in peripheral neutrophil count and peritoneal macrophages which was associated with increased phagocytic activity. Thatte and Dahanukar (1988, 1989) have demonstrated protective effect of *T. cordifolia* against myelosuppression induced by cyclophosphamide, and compared it with glucan and lithium carbonate. They have found that *T. cordifolia* was comparable to lithium and glucan. This plant like *A. racemosus* can also be useful to overcome cytotoxic drug's toxicity.

Immunotherapeutic modification of *E. coli* peritonitis and bacterimia by *T. cordifolia* has been investigated by Thatte et al. (1992). Pretreatment with *T. cordifolia* or gentamycin reduced mortality in mice injected with 1×10^8 *E. coli* intraperitoneally; from 100% in controls to 17.8% and 11.1%, respectively, in the treated mice. This was associated with significantly improved bacterial clearance as well as improved phagocytic and intracellular bactericidal capacities of neutrophils in *T. cordifolia* treated group. In gentamycin treated mice, although bacterial clearance was rapid, polymorphonuclear cell phagocytosis was depressed. *T. cordifolia* did not possess *in vitro* bactericidal activity. The results suggest that a prohost approach may be beneficial in the therapy of peritonitis.

To elucidate the mechanism of action of *T cordifolia,* Thatte et al. (1994) have measured the GM-CSF activity in the serum of mice treated with *T. cordifolia* (100 mg/kg/d for 10 days). It was found that serum from treated mice produced 255 ± 49.32 GM colonies compared to 38.51 ± 9.98 in controls ($p < 0.01$). It has been suggested that *T. cordifolia* activates macrophages to release GM-CSF activity.

Studies on patients with obstructive jaundice have shown that phagocytic and microbicidal activity of PMNs is significantly depressed (21.2 ± 3.7% phagocytosis and 20.85 ± 4.5% intracellular killing) compared to normal controls (30.37 ± 5.1% and 26.41 ± 4.3%, respectively) (Rege et al. 1989a). In rats also, cholestasis was found to cause significant depression in PMN and peritoneal macrophage activity. These cellular abnormalities were found to precede and predispose to infection. The rats also showed increased susceptibility to *E. coli* infection which was associated with high mortality (77.78%). A defect was detected in their serum responsible for depressing the function of phagocytic cells. An attempt was made to improve this immunosuppression by treating rats with water extract of *T. cordifolia* 100 mg/kg/day for 7 days, following development of cholestasis. The extract improved the cellular immune functions. Mortality rate following *E. coli* infection in treated animals was significantly reduced to 16.7%. This study has demonstrated that cholestasis (obstructive jaundice) results in immunosuppression which can be overcome by the use of immunomodulators. The plant *T. cordifolia* holds promise for such an application.

Kupffer cells are major determinants of outcome of liver injury. Their activity was therefore studied in a model of chronic liver disease. The effect of *T. cordifolia* was evaluated on Kupffer cell function using carbon clearance test as a parameter (Nagarkatti et al. 1994). Rats were divided into two groups. In Gp I which served as normal control, t 1/2 of carbon was 9.48 ± 4.14 min. Gp II received horse serum in a dose of 0.5 ml/100 gm body weight intraperitoneally for a period of 12 weeks and was divided into three sub-groups. In Gp IIA at the end of 12 weeks half-life of carbon was found to be significantly increased to 19.86 ± 7.95 min ($p < 0.01$) indicating suppressed Kupffer cell function in chronic liver damage. In Gp IIB treated with vehicle for 4 more weeks there was significant prolongation of half-life to 38.32 ± 10.61 min ($p < 0.01$), indicating perpetuation of damage in absence of damaging agent. Whereas in Gp IIC, treated with *T. cordifolia* t 1/2 was decreased to 14.24 ± 7.74 min ($p < 0.01$), as compared to vehicle control indicating significant improvement in Kupffer cell function and a trend towards normalization.

Recently Rege et al. (1999) have evaluated the effect of aqueous extract of *T. cordifolia* (powdered stem) administered through oral route in patients with hepatic disorders with evidence of fibrosis and immunosuppression (obstructive jaundice, asymptomatic carriers of Hepatitis B antigen and cirrhosis). In all the three conditions, having different etiologies and pathogenesis, the drug has proven its potential. Oral efficacy of this immunostimulant, coupled with wide therapeutic range, has provided a new weapon to fight uncontrolled fibrosis in chronic liver disease and other connective tissue disorders.

Treatment with *T. cordifolia* also significantly inhibited ochratoxin A induced suppression of chemotactic activity and production of IL-1 and TNF-α by mouse macrophages (Dhuley 1997).

The active principles, Syringin (TC-4) and Cordiol (TC-7) obtained from *T. cordifolia* have been found to possess anti-complement and immunomodulatory activity (Kapil & Sharma 1997). These compounds inhibited *in-vitro* immunohaemolysis of antibody coated SRBC by guinea pig serum which was found to be due to inhibition of C3-convertase of the classical complement pathway. The compounds also gave rise to significant increase in IgG antibodies in serum. Humoral and cell mediated immunity were also found to be enhanced in a dose dependent manner. Macrophage activation has been reported for cordioside (TC-2), cordiofolioside A (TC-5) and cordiol (TC-7) and this activation became more pronounced with increasing incubation times (Kapil & Sharma 1997).

***Withania somnifera* ('Ashwagandha'-Solanaceae):** Another plant, which has attracted the minds of immunologists, is *W. somnifera*. It is used in several Ayurvedic drug preparations. A number of withanolides isolated from this plant have been reported in literature to possess both immunosuppressive and immunostimulatory properties. Extract of *W. somnifera* was tested for anticancer activity (Seena et al. 1993). Though the plant extract did not completely inhibit development of solid tumor, it induced significant lag in tumor development. Administration of a 75% methanolic extract of the plant was found to significantly increase total WBC count in normal Balb/c mice and in mice with leucopenia induced by sublethal dose of gamma irradiation (Kuttan 1996).

A significant modulation of immune reactivity by Ashwagandha was observed in an animal model of myelosuppression induced by cyclophosphamide, azathioprin and prednisolone. Ashwagandha prevented myelosuppression in mice treated with all three immunosuppressive drugs (Ziauddin et al. 1996). A significant increase in white blood cells (WBC) count ($p < 0.05$) and body weight ($p < 0.05$) was observed in Ashwagandha treated mice as compared with untreated (control) mice. Treatment with Ashwagandha was accompanied with significant increase in haemolytic antibody responses towards human erythrocytes (Ziauddin et al. 1996). The effect of Ashwagandha on the function of macrophages obtained from mice treated with ochratoxin A has been investigated. Treatment with Ashwagandha significantly inhibited ochratoxin A induced suppression of chemotactic activity and production of IL-1 and TNF-α by macrophages (Dhuley 1997).

Ashwagandha given orally once a day at a dose of 100 mg/kg, after intravenous infection with *Aspergillus fumigatus* prolonged the survival period of infected Balb/cr. mice. This protective activity was related to increase in phagocytosis and intracellular killing of peritoneal macrophages induced by Ashwagandha (Dhuley 1998).

Withaferin A, a steroidal lactone from *W somnifera* root, inhibited EAC tumor growth and increased tumor free survival in a dose dependent manner when administered intraperitoneally after tumor cell injection with or without acute abdominal exposure to 7.5 Gy gamma irradiation in adult Swiss albino mice. Increase in life span and tumor-free survival were studied up to 120 days. The drug inhibited tumor growth and increased survival, which was dependent on the Withaferin A dose per fraction rather than total dose (Devi et al. 1995, Sharad et al. 1996). A dose of 30 mg/kg was optimum for combination with radiation. Withaferin A showed a radiosensitizer enhancement ratio of 1.5 for *in vitro* cell killing of V97 Chinese hamster cells at a concentration of 2-mM (Devi 1996, Devi et al. 1996). The studies so far indicated that *W. somnifera* could prove to be a good natural source of a potent and relatively safe radiosensitizer/chemotherapeutic agent.

A dose of 30 mg/kg of Withaferin A significantly enhanced the spleen colony forming unit (CFU-S) in irradiated (2 Gy whole body gamma irradiation) animals. Radiation reduced the CFU-S to less than 50% of normal. Withaferin A significantly restored this inhibition (Ganasoundari et al. 1997). Further studies are needed to explore clinical potential of this plant in cancer therapy.

Immunomodulator from *Aspergillus ochraceus*

In addition to angiospermic plants, there are few reports of immunomodulators from other sources as well. An interferon inducer designated 6-MFA, having broad spectrum of antiviral activity and high margin of safety, was isolated from fungus *Aspergillus ochraceus* (ATCC 28706) (Maheshwari et al. 1977,1978). This product was found to be a mixture of polysaccharides and nucleoprotein (virus like particles), and capable of significantly protecting Swiss mice against Semliki forest virus and Encephalomyocarditis virus. The active substance in 6-MFA is double stranded RNA found within virus like particles. There is evidence that 6-MFA induces production

of two types of interferon in mice, *p*H2 stable and *p*H2 labile (Singh et al. 1984). We have demonstrated that 6-MFA also stimulates haemagglutinating antibody titer as well as antibody plaque forming cell response against SRBC in mice when administered along with or prior to antigen (Singh et al. 1983b). *P. ginseng* extract was able to significantly enhance the anti-viral effect of interferon inducer, 6-MFA, when orally administered to Swiss mice (Singh et al. 1984).

Recommendations for Future Work

From the above review it should be evident that there are several Indian medicinal plants which possess immunomodulatory properties. However, the available evidence is not yet adequate to allow their use in clinical practice. Not only there is a need for comprehensive, systematic, multi-disciplinary evaluation of standardized products of a large number of plants listed in table 1 and 2 which remain uninvestigated, but more importantly there is a need to evolve a mechanism for rapid evaluation of clinical applications of these products. It is suggested that as far as Indian medicinal plant products are concerned, the process of drug development shall be reversed. The Ayurvedic medicinal formulations prepared according to Ayurvedic Materia and Medica incorporating the Rasaynas and the other plants listed in table 2 should be put to careful clinical trials without waiting for preclinical data. Care should, however, be taken to ensure that such trials are ethically valid. While there may be reservations against use of unknown substances where proved medicines already exist, such trials may be acceptable if the Ayurvedic medicines are used as adjuncts. It is also important that batch to batch variation in concentration of active components in different preparation of extract is monitored. Once the clinical utility is established, one can worry about identifying the active ingredients and their mechanism of action. This recommendation is made because preclinical studies are not needed for products which are already in use. The main handicap in establishing the use of Ayurvedic preparations has been the lack of data from appropriately controlled trials. It needs to be reminded that clinical trials are appropriate only when they incorporate principles of double-blinding and randomization. The results of the efficacy and toxicity of putative drugs against controls can be compared only when the two groups are comparable in all other respects except for the intervention. Planning and conduct of such clinical trials is not an easy task, particularly when preventive interventions are required to be evaluated. These trials are required to be conducted in the field, enlisting a large number of individuals at risk, and following them up adequately to monitor the outcome. Monitoring of compliance, particularly the dosage taken is difficult in field studies. Needless to say that the endpoint of efficacy also has to be sensitive as well as specific, besides being reliably measured. The number of subjects to be included in the trial could be large depending on attack rate of the condition studied. Such studies require a robust statistical design and could be quite expensive. But there is no shortcut to these studies. This lacuna can be resolved only by bringing together interested professionals from the Ayurvedic and the modern system of medicine. The current epidemic of AIDS, and periodic epidemics of Hepatitis, Dengue, cerebral malaria, Japanese encephalitis etc. should be exploited to the advantage of such trials.

Simultaneously appropriate animal models of disease are needed to be developed to evaluate the efficacy of these products. These can include inbred strains of animals with known susceptibility to infection (Zak & O'Reilly 1993), allergy (Irvin 1992), autoimmune disease (Myers et al. 1997) and cancer (Pariza 1997). As far as *in-vitro* studies for understanding the mechanism of action are concerned, the testing materials should be authentic and well

Table 1 *Plants identified as Rasaynas and their pharmacological properties*

Sr.No	Plants identified as Rasaynas in Charak Samhita (Sharma 1981)	Common name	Properties described in the treatise by Chopra et al. (1996)
1	*Acorus calamus*	Bach	Neurostimulant, antiaging
2	*Allium sativum*	Lahsuna	Antibacterial, antiviral
3	*Aloe vera*	Ghrit-kumari	Antiseptic
4	*Argyreia speciosa*	Samandar ka pat	Anti-rheumatic, tonic
5	*Asparagus racemosus*	Satawar	Anti-stress, anti-cancer, anti-septic, immunostimulator, antiaging
6	*Azadirachta indica*	Nimba, Neem	Antiseptic, anti-eczema
7	*Bacopa monnieri*	Brahmi	Antiasthmatic, antiaging
8	*Boerhaavia diffusa*	Sant	Anti-inflammatoy, anti-stress, adaptogenic, antiaging
9	*Cissampelos pareira*	Akanadi	Antiviral, effective in urinary tract infections
10	*Commiphora mukul*	Guggul	Antiseptic
11	*Convolvulus pluricaulis*	Shankhapushp	Tonic
12	*Curculigo orchioides*	Krishna Musali	Antiasthmatic, tonic
13	*Curcuma longa*	Haldi	Antiseptic, anti-inflammatory, tonic
14	*Desmodium gangeticum*	Shalaparni	Antiviral, antiasthmatic, tonic
15	*Dioscorea bulbifera*	Ratalu	Antibacterial, anti-syphillic, anti-inflammatory
16	*Embelia ribes*	Vidanga	Antiviral, tonic
17	*Emblica officinalis*	Amla	Antibacterial
18	*Glycyrrhiza glabra*	Yashtimadhu	Anti-inflammatory, antibacterial, antiviral, tonic
19	*Gmelina arborea*	Gamari	Antiseptic
20	*Hemidesmus indicus*	Ananta mul	Anti-syphillic, anti-rheumatic, cures skin disease - eczema
21	*Ipomoea digitata*	Ajvayan	Antiasthmatic
22	*Leptadenia reticulata*	Dori	Tonic, antiaging
23	*Piper longum*	Piplamul	Antiviral, tonic
24	*Plumbago zeylanica*	Chita	Anti-leprotic
25	*Psoralea corylifolia*	Babchi	Anti-leprotic, cure for skin disease
26	*Pterocarpus marsupium*	Bijasar	Antibacterial, cure for skin disease
27	*Semecarpus anacardium*	Bhilawa	Anti-rheumatic, anti-leprotic, antiaging
28	*Sida spinosa*	Gulsakari	Tonic
29	*Solanum nigrum*	Makoi	Anti-psoriasis
30	*Sphaeranthus indicus*	Mundi	Tonic
31	*Terminalia belerica*	Bahera	Immunostimulant
32	*Terminalia chebula*	Haritaki/Panhara	Helps wound healing, anti-asthmatic
33	*Tinospora cordifolia*	Guduchi	Antibacterial, antiaging, anti-allergic, anti-rheumatic, immunostimulant
34	*Withania somnifera*	Ashwagandha	Immunostimulatory, anti-inflammatory, anti-stress, anti-rheumatic

Table 2. *Indigenous medicinal plants with pharmacological properties similar to Rasaynas*

Sr.No	Plants	Common name	Properties
1	*Abrus precatorius*	Gunja	Immunostimulant
2	*Aconitum heterophyllum*	Atis	Tonic
3	*Albizzia lebbeck*	Shirisha	Immunostimulant
4	*Amoora rohituka*	Harin-hara	Antitumor
5	*Andrographis paniculata*	Kalmegh	Immunostimulant
6	*Aristolochia indica*	Isharmul	Immunostimulant
7	*Artocarpus lakoocha*	Dahua	Antibacterial
8	*Astragalus multiceps*	Sarmul	Antileprotic
9	*Bauhinia variegata*	Kachnar	Antihelminthic
10	*Berberis aristata*	Dar-hald	Immunosuppressor, antibacterial
11	*Blechnum orientale*	Rajhans	Anti-helminthic
12	*Bombax malabaricum*	Simul	Tonic, antibacterial
13	*Bupleurum falcatum*	Sipil	Tonic
14	*Butea monosperma*	Dhak	Antihelminthic, tonic, antibacterial, antiasthmatic
15	*Butea superba*	Palas	Wound-healing, cures eczema
16	*Calotropis procera*	Madar	Antibacterial
17	*Carissa carandus*	Karaunda	Anti-helminthic
18	*Catharanthus roseus*	Sada Bahar	Immunostimulant
19	*Celastrus paniculatus*	Malkangni	Anti-rheumatic
20	*Centella asiatica*	Madukaparni	Tonic, cures skin disease-eczema, cures syphillis, antiaging
21	*Cicer arietinum*	Chana	Tonic
22	*Citrullus colocynthis*	Indrayan	Anti-rheumatic
23	*Clerodendrun infortunatum*	Bhant	Antitumor, tonic
24	*Clitoria ternatea*	Aparajita	Immunostimulant
25	*Costus speciosus*	Keu	Anti-helminthic, tonic, anti-heumatic
26	*Cucumis sativus*	Khira	Tonic
27	*Cuminum cyminum*	Jira	Antibacterial
28	*Cymbopogon martini*	Gandh	Immunostimulator
29	*Dryopteris cochleata*	Jatashankar	Tonic, anti-rheumatic, anti-leprotic
30	*Elephantopus scaber*	Gobhi	Antiseptic, cure for skin disease - eczema
31	*Elytraria acaulis*	Sahustra muli	Antibacterial, used in boils
32	*Eupatorium cannabinum*	Tongollati	Antiseptic, antiviral
33	*Flacourtia indica*	Bilangra	Antiviral
34	*Gymnema sylvestre*	Merasingi	Anti-stress

Sr.No	Plants	Common name	Properties
35	*Hibiscus esculentus*	Bhindi	Antibacterial
36	*Holarrhena antidysentrica*	Kurchi	Antibacterial
37	*Hoppea dichotoma*	Kuki	Anti-inflammatory
38	*Hyoscyamus niger*	Parsikaya	Immunostimulant
39	*Jasminum sambac*	Motia	Antiseptic, tonic
40	*Lawsonia inermis*	Mehndi	Antileprotic
41	*Luffa acutangula*	Torai	Antileprotic, antiviral
42	*Luffa cylindrica*	Ghiatarui	Anti-stress
43	*Mallotus philippinensis*	Kamala	Anti-helminthic
44	*Mangifera indica*	Am	Antiasthmtic
45	*Manilkara kauki*	Khirni	Tonic, anti-leprotic
46	*Melia azedarach*	Bakain	Anti-rheumatic
47	*Mentha spicata*	Pudina	Antiviral
48	*Mucuna pruriens*	Kaunch	Tonic
49	*Nardostachys jatamansi*	Jatamansi	Immunostimulant
50	*Nelsonia campestris*	Patta Kamraj	Anti-leprotic, tonic
51	*Ocimum canum*	Mamiri tulsi	Antiviral
52	*Ocimum sanctum*	Tulsi	Antitubercular, antiviral, antifungal, antiasthmatic, anti-inflammatory
53	*Ougeinia oojeinensis*	Sandan	Antibacterial, used in boils
54	*Picrorhiza kurroa*	Kutaki	Immunostimulant, anti-oxidant
55	*Piper aurantiacum*	Shambhatuka	Tonic
56	*Piper betle*	Paan	Antiseptic, antiviral
57	*Pluchea lanceolata*	Sorahi	Anti-inflammatory
58	*Prosopis spicigera*	Jhand	Anti-rheumatic
59	*Randia dumetorum*	Mainphal	Anti-rheumatic
60	*Saraca indica*	Ashoka	Cures urinary tract infection
61	*Selaginella bryopteris*	Amarbooti	Anti-inflammatory, cures veneral diseases
62	*Solanum trilobatum*	Agnidamini	Anti-bacterial, antiviral
63	*Syzygium cumini*	Jamun	Anti-bacterial
64	*Valeriana wallichii*	Muskbala	Antiseptic
65	*Viscum album*	Banda	Antitumor, tonic
66	*Vitex negundo*	Nirgandi	Anti-rheumatic, tonic
67	*Woodfordia fruticosa*	Dhal	Antibacterial, used in boils, anti-inflammatory
68	*Zingiber officinale*	Adrak	Antiviral, anti-oxidant

Table 3. *Indigenous plants investigated in laboratory/clinic for immunomodulatory effects*

Sr No	Plants	Immunomodulatory properties *In-vitro*	*In-vivo*	Clinical	Reference
1	*Allium sativum*	Augments NK cells and macrophage activity	Augments NK cells, stimulates T cells and IL-2 production, inhibits tumor development, protects from UV induced suppression of contact hypersensitivity		Chopra et al. 1956, Fujiwara and Natata 1967, Aboul-Emein 1986, Tang et al. 1997, Lau et al. 1991, Morioka et al. 1993, Jain 1994, Reeve et al. 1993
2	*Aloe vera*	Enhances antibody production and DTH response, stimulates IL-6, TNF-α and NO production	Improves wound healing, stimulates production of IL-1, TNF-α and regresses tumor		t'Hart et al. 1989, Davis et al. 1994, Karaca et al. 1995, Zhang and Tizard 1996, Stuart et al. 1997, Corsi et al. 1998, Chithra et al 1998a, 1998b
3	*Andrographis paniculata*	Stimulates macrophage migration, phagocytosis of ^{14}C leucine labeled *E. coli*, proliferation of splenic lymphocytes, inhibits NO production	Induces stimulation of antibody and DTH response to SRBC in mice		Puri et al. 1993, Chiou et al. 1998
4	*Asparagus racemosus*		Induces lag in tumor development, prevents leucopenia produced by cyclophosphamide, inhibits ochratoxin A induced suppression of IL-1, TNF-α and macrophage chemotaxis		Seena et al. 1993, Thatte and Dahanukar 1988, Dhuley 1997
5	*Azadirachta indica*	Stimulates IL-1, IFN-γ, TNF-α production, enhances proliferative response of spleen cells to Con A and tetanus toxoid	Activates immune system, enhances macrophage phagocytosis, expression of MHC II antigen, IgM, IgG, antiovalbumin antibody, macrophage migration inhibition and DTH in psoriasis patients	Reduces erythema, desquamation and infiltration of psoriatic lesions	Upadhyay et al. 1992, Ray et al. 1996, Katiyar et al. 1997, SaiRam et al. 1997
6	*Curcuma longa*	Increases mitogenic response of lymphocytes, inhibits NO	Shows anti-inflammatory and antitumor activities production	Helps in rheumatoid arthritis	Ghatak & Basu 1972, Mukhopadhyay et al. 1982, Deodhar et al. 1980, Srimal 1997, Kuttan et al. 1985, Brouet and Ohshima 1995, Rao et al 1995

Sr No	Plants	Immunomodulatory properties *In-vitro*	*In-vivo*	Clinical	Reference
7	*Nyctanthes arbor-tristis*	Stimulates macrophage migration	Stimulates humoral and DTH response to SRBC in mice, protects mice against *Candida albicans* as a result of enhanced humoral, DTH and macrophage activity		Puri et al. 1994, Khan et al. 1995
8	*Ocimum sanctum*		Inhibits tumor development in mice, increases colony-forming units in spleen, and protects mice after irradiation	Enhances survival of viral encephalitis patients	Seena et al. 1993, Das et al. 1983, Devi and Ganasoundari 1995, Ganasoundari et al. 1997, 1998
9	*Panax ginseng*	Stimulates T cell proliferation, augments NK cells, enhances phagocytosis, chemotaxis, production of IL-1, IL-2, TNF-α, GM-CSF	Protects mice against viral encephalitis and enhances protective effect of interferon inducer, enhances circulating antibody and antibody forming cells to SRBC and macrophages migration inhibition factor against viral antigen, antitumor	Augments NK cell activity.	Singh et al. 1983a, 1984, Scaglione et al. 1990, Mizuno et al. 1994, Lee et al 1997, Kim et al 1998 Xiaoguang et al. 1998
10	*P. pseudoginseng*	Stimulates macrophage migration	Enhances circulating antibody and antibody forming cells to SRBC in mice		Dua et al. 1989
11	*Phyllanthus emblica*	Inhibits PMN activity induced by leukotriene B4 and FMLP	Enhances NK cell and ADCC activity against Dalton's lymphoma ascites tumor		Suresh and Vasudevan 1994, Ihantola-Vormisto 1997
12	*Picrorhiza kurroa*	Enhances phagocytosis, stimulates PHA, Con A and LPS induces lymphocyte proliferation, macrophage migration, inhibits antibody response against SRBC	Enhances antibody and DTH response to SRBC in mice, inhibits ochratoxin A induced suppression of IL-1, TNF-α and macrophage chemotaxis, protects animals against Leishmania and filarial infections	Orally effective in chronic liver disease	Atal et al. 1986, Dhuley et al. 1997, Sharma et al. 1994, Puri et al. 1992, Fatma et al. 1994, Rege et al. 1999
13	*Tinospora cordifolia*	Cytotoxic to D11 and EAC tumor cells, mitogenic to splenocytes and lymph node cells, enhances MHC class II expression and antigen presenting ability of macrophages	Induces lag in tumor development, inhibits myelosuppression induced by cyclophosphamide, enhances peritoneal macrophage number and phagocytic activity, stimulates phagocytic activity of macrophages and PMN in cholestatic animals, induces resistance to infection, reduces mortality in mice injected with *E. coli*, increases granulocyte-macrophage colony forming unit in mice serum, improves Kupffer cell function in rat model of chronic liver disease, enhances antigen specific antibody, inhibits ochratoxin A induced suppression of IL-1, TNF-α and macrophage chemotaxis, enhances IgG antibody and macrophage activation		Seena et al. 1993, Sainis et al. 1997, Thatte and Dahanukar et a1 988,1989,Rege et al. 1989 Thatte et al. 1992, 1994, Nagarkatti et al. 1994, Dhuley 1997

Sr No	Plants	Immunomodulatory properties *In-vitro*	*In-vivo*	Clinical	Reference
14	*Withania somnifera*	Enhances radiosensitization for V97 Chinese hamster cells	Inhibits tumor development, increases WBC count in irradiated mice, prevents myelosuppression induced by immunosuppressive drugs in mice, increases haemolytic antibody response towards human erythrocytes, inhibits ochratoxin A induced suppression of IL-1, TNF-α and macrophage chemotaxis, prolongs mice survival against *Aspergillus fumigatus* infection, enhances spleen colony-forming units		Seena et al. 1993, Kuttan 1996, Ziauddin et al. 1996, Dhuley 1997, 1998, Devi et al. 1995, 1996, Devi 1996, Sharad et al. 1996, Ganasoundari et al. 1997

Table 4 *In-vitro and in-vivo tests for evaluation of immunomodulators*

The agent can be added to *in-vitro* cultures or administered to experimental animals (with or without specific antigen) and thereafter the following tests can be performed.

1. Immunosuppresant activity:

In-vitro:
a) Inhibition of lymphocyte proliferation and cytokine production of either Th1 (IL-2, IFN-γ and TNF β) or Th2 (IL-4, IL-5 and IL-10) type following stimulation with mitogen or specific antigen
b) Inhibition of antibody forming cells in Jerne's plaque assay and antibody production
c) Inhibition of IL-1 and TNF-α production by macrophages in response to LPS
d) Inhibition of NK cell activity in absence or presence of IFN-γ

In-vivo:
a) Suppression of antibody production to specific antigens
b) Suppression of DTH response to specific antigens
c) Suppression of clearance by reticuloendothelial cells
d) Suppression of induction/progression of autoimmune disease in experimental animal models
e) Prevention of allograft (or xenograft) rejection.

2. Immunostimulant activity:

In-vitro:
a) Stimulation of lymphocyte proliferation and cytokine production of either Th1 (IL-2, IFN-γ and TNF-β) or Th2 (IL-4, IL-5 and IL-10) type following stimulation with sub-optimal dose of mitogen or specific antigen
b) Quantitation of the expression of cell activation markers on cell surface viz. -CD25, CD69, CD70 and CD86 by fluorescent activated cell scan (FACS)
c) Quantitation of different lymphocyte sub-populations by FACS - CD3, CD4, CD8, CD16/CD56, CD19, CD20 and CD45
d) Augmentation of NK cell cytotoxicity
e) Stimulation of IL-1 and TNF-α production by macrophages in response to LPS
f) Stimulation of antibody plaque forming cells and antibody production *in-vitro*

In-vivo:
a) Stimulation of antibody titer to specific antigens
b) Stimulation of DTH to specific antigens
c) Evaluation in rodent malaria and Japanese encephalitis virus models etc. for animal protection against infections with or without chemotherapy
d) Spleen foci (colony formation) assay as a measure of radioprotection

3. Immunoadjuvant activity: The agent is administered in combination with potential vaccine and the effect is estimated by:
a) Specific antibody profile and titer in immunized animals (*in-vivo*)
b) DTH response to the specific antigens (*in-vivo*)
c) Jerne's plaque assay using splenocytes from immunized animals (*in-vitro*)
d) Lymphocyte proliferation test using splenocytes from immunized animals against T-cell epitopes of the immunizing antigen (*in-vitro*)
e) Th1/Th2 cytokine profile (*in-vivo*)

4. Effector arm of the immune response:
a) Chemokine levels in the treated animal and *in-vitro* assay by chemotaxis
b) Inhibition/stimulation of phagocytosis (*in-vitro/in-vivo*)
c) Free radical production (*in-vitro*)
d) Nitric oxide production (*in-vitro*)

characterised and screening parameters should be such which can be automated and reliably quantified. Various tests that can be used for this purpose are listed in table 4. The potential of innate defense mechanisms to conquer problems of intractable infections, elimination of residual cancer cells and control of autoimmune diseases is worth harnessing.

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