CHAPTER-I

INTRODUCTION
1.1 GENERAL

Ayurveda, the Indian system of medicine (ayur means life, Veda means knowledge) is the oldest system of medicine in the world, which explicitly reveals the potential of various herbs generally in polyherbal formulations as drugs (Dash et al., 1980). Ayurveda is a concept of strengthening or giving power to the host defenses against different diseases (Thatte et al., 1986). It is the chief source of Indian medicine originating from Brahma. Ayurveda essentially comprise of the knowledge of medicine and the healing art. The immune system is involved in the etiology as well as in pathophysiologic mechanism of many diseases. Modulation of the immune responses to alleviate the disease has been of interest for many years and the concept of ‘Rasayana’ in Ayurveda is based on related principles (Sharma, 1983).

Immunology was introduced as a branch of microbiology concerned with study of immunity to infection and deals with various aspects of immune system. It can be reckoned to have started in 1798, with Jenner’s study of vaccination against smallpox. Pasteur (1822-1895) developed attenuated vaccines for anthrax and rabies. Later on other scientists developed similar vaccines for typhus fever, yellow fever, poliomyelitis etc. The investigations were mainly concerned with chemistry of antigens and antibodies. During the last four decades there is increasing awareness of the role of immunological mechanism in the pathogenesis of a much wider range of pathological condition (Joshi et al., 1998; Ananthanarayan et al., 1996).
Infections remain the most common cause of disease and bacteria, viruses, parasites, fungi etc. produce them. To defend from these agents, the body devised astonishingly intricate systems. Microbes, attempting to enter the body must first find a space in the body’s external protection. The skin and the mucous membranes that line the body’s portals not only pose a physical barrier but also rich in scavenger cells and various antibodies.

The immune system uses various endogenous molecules in controlling invaders. The important characteristic is that these molecules can distinguish ‘self’ from ‘non self’ and mount defense reactions in a very selective and specific manner (Steven et al., 2002).

1.2. IMMUNE SYSTEM (Guyton, 1991; Roit, 1984)

Immune system comes from the word ‘immunitas’ means ‘freedom from’. All multicellular animals have such defense system that, prevent the entry of microorganism and eliminate the microbes, which infect our body system. Immunity is the resistance exhibited by the host towards microbe’s injury caused by microorganisms and their products. Protection against infection is one of the consequences of the immune response; immunity against infectious diseases is of two types: Innate immunity and Acquired immunity.

1.2.1. Innate immunity

Much of the immunity is caused by a special immune system that forms antibodies and activates lymphocytes that attack and destroy the specific organism or toxin. This type of immunity is called acquired
immunity. However, an additional portion of immunity result from general processes rather than from processes directed at specific disease organism. This is called innate immunity. It includes the following:

A. White blood cells and the cells of the macrophage system do phagocytosis of bacteria and invaders. Phagocytosis generally depends upon three selective procedures. First, if the surface of a particle is rough, the likelihood of phagocytosis is increased. Second, most natural substances of the body have protective proteins coat that repel the phagocytes; third, the body has a specific means of recognizing certain foreign material. The immune system develops antibodies against infectious agents like bacteria. There are two portions of antibody, constant and

![Diagram of phagocytosis]

**Fig. 1.1: Mechanism of phagocytosis**
variable. Constant portion of the antibody molecule that repel the phagocytes; third, the body has a specific means of recognizing certain foreign material. The immune system develops antibodies against infectious agents like bacteria. There are two portions of antibody, constant and variable. Constant portion of the antibody molecule combines with the C₃ product of the complement cascade. The C₃ molecule then attaches it to receptors on the phagocyte membrane, thus initiating phagocytosis. When foreign particle coated with antibody bind to the receptors on the phagocytic cell, the cell responds by engulfing the particle and forming the phagosome. (Fig. 1.1)

B. Destruction by the acid secretion of the stomach and the digestive enzymes of organism swallowed to the stomach.

C. Resistance of the skin for invasion by organism.

D. Presence in the blood of certain chemical compounds that attach to foreign organism or toxin to destroy them.

E. Natural killer (NK) cells and soluble factors.

Natural killer cells are leucocytes capable of recognizing cell surface changes on virus-infected cells. The N.K. cells bind to the target cells and can kill them. N.K. cells are activated by interferon that are themselves components of innate immune system.

The innate immunity might be species specific, race specific or individual specific.
Species-specific immunity:

Certain infections occur only in particular species *e.g.* syphilis, gonorrhea, leprosy, measles, and cholera etc. occurs in man only but not in lower animals.

Race specific immunity:

All races of same species are not found to exhibit the same degree of susceptibility to infection *e.g.* Algerian race of sheep is immune to anthrax, which is a common disease of other races of sheep.

Individual specific immunity:

Some differences in individuals of same species in susceptibility to certain infections do occurs *e.g.* individual of heterozygous traits of sickle cell anemia, thalassaemia, haemoglobin ‘C’ disease and deficiency of glucose-6-phosphate dehydrogenase are resistant to some types of malaria.

1.2.2. Acquired Immunity

The resistance that an individual acquires during life is known as acquired immunity. An immunoglobulin of the appropriate class *e.g.* human IgG, activates complement cascade by a separate pathway through binding C1q to its C1H2 domain thereby generating a C3 convertase; the C1H3 domain binds to specific Fc receptor on the phagocyte to activate it to initiate ingestion. The whole molecule is attached to the foreign invader through the antigen-binding region of the variable domain and
manufactures antibody molecule with a wide range of combining specificities.

The B lymphocyte system developed in order to produce antibodies and allowing each lymphocyte to synthesize only one type of antibody. The system of clonal triggering and formation of memory cells ensures that the body only concentrates its main energies on antigens, which it actually meets while retaining the potential to react against some obscure microbes, which might infect the body at any time in the future. The ability to respond specifically to a particular infection and to generate memory cell is the basis of acquired as distinct from innate immunity but it should be perfectly plain that antibody as one agent of acquired immunity acts to enhance the mechanism of innate immunity. The acquired immune response is of two types: Humoral immune response and cell mediated immune response. *(Fig.1. 3)*
1.2.2.1. **Humoral immunity**

In humoral immune response antibodies play a central role. The production of antibodies consists of three steps:

1. The entry or the invasion of the antigen, its distribution and fate in the tissue followed by its contact with appropriate immunocompetent cells.

2. The processing of antigen by cells and the control of the antibody forming process.

3. The secretion of antibody, its distribution in tissue and body fluids and the manifestation of its effects.

**Mechanism of action of antibodies:**

I. **By direct attack on invader:** The primary interaction between an antigenic determinant and the combining site of an antibody, governed by the affinity give rise to a number of secondary phenomenon such as precipitation, agglutination, phagocytosis, cytolysis, neutralization and so on. (Fig.1. 3)

II. **By the complement system:** The complement system is made up of a series of about 25 protein that work to "complement" the activity of antibodies in destroying bacteria either by facilitating phagocytosis or by puncturing the bacterial cell membrane.
Humoral Immunity

\textbf{Fig.1. 3: Different ways of Acquired immunity}

Complement proteins circulate in the blood in an inactive form. When the first of the complement substances is triggered usually by antibody interlocked with an antigen it sets in motion ripple effect. Each activated complement acts upon the next in a precise sequence of regulated steps known as the 'complement cascade' (\textbf{Fig.1. 4}).
There are two pathways 'classical' and 'alternative'. A series of proteins gives rise to a complex enzyme capable of cleaving a key protein, C₃. In alternative pathway, suitable targets trigger it in the absence of antibody C₃. But both pathways end in creation of a unit known, as the membrane attack complex, which constitutes a channel that allows fluids and molecules to flow in and out. The target cell rapidly swells to burst. During the course of cascade various products flung off which can produce other consequences. One by-product causes mast cells and basophills to release their contents, producing the redness, warmth and swelling of the inflammatory response. Another stimulates and attracts neutrophills.
<table>
<thead>
<tr>
<th>S.</th>
<th>Major characteristics</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgD</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Major characteristics</td>
<td>Most abundant Ig of internal body fluids particularly extra vascular where it combats microorganism and their toxins</td>
<td>Major Ig in sero-mucous secretions where it defends external body surfaces.</td>
<td>Very effective agglutinator; produced early in immuneresponse effective first line defence vs. bacteraemia.</td>
<td>Most, if not all, present on lymphocyte surface.</td>
<td>Protection external body surfaces recruit antimicrobial agents Raised in parasitic infections responsible for symptoms of atopic allergy.</td>
</tr>
<tr>
<td>2.</td>
<td>Complement fixation</td>
<td>Classical</td>
<td>++</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Alternative</td>
<td>–</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3.</td>
<td>Cross placenta</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.</td>
<td>Fix to homologous most cell and basophills</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>S.</td>
<td>WHO designation</td>
<td>IgG</td>
<td>IgA</td>
<td>IgM</td>
<td>IgD</td>
<td>IgE</td>
</tr>
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<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>1.</td>
<td>Sedimentation coefficient</td>
<td>75</td>
<td>75, 95, 115</td>
<td>195</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>2.</td>
<td>Molecular weight</td>
<td>150,000</td>
<td>160,000 and dimmer</td>
<td>900,000</td>
<td>185,000</td>
<td>200,000</td>
</tr>
<tr>
<td>3.</td>
<td>Number of basic four peptide units</td>
<td>1</td>
<td>1, 2</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>Heavy chains</td>
<td>γ</td>
<td>α</td>
<td>μ</td>
<td>δ</td>
<td>ε</td>
</tr>
<tr>
<td>5.</td>
<td>Light chains</td>
<td>k+λ</td>
<td>k+λ</td>
<td>k+λ</td>
<td>k+λ</td>
<td>k+λ</td>
</tr>
<tr>
<td>6.</td>
<td>Valency for antigen binding</td>
<td>2</td>
<td>2, 4</td>
<td>5 (10)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7.</td>
<td>Concentration range in normal serum</td>
<td>8-16mg/ml</td>
<td>1.4-4mg/ml</td>
<td>0.5-2mg/ml</td>
<td>0-0.4mg/ml</td>
<td>17-450mg/ml</td>
</tr>
<tr>
<td>8.</td>
<td>Total % immunoglobulin</td>
<td>80</td>
<td>13</td>
<td>6</td>
<td>0-1</td>
<td>0.002</td>
</tr>
<tr>
<td>9.</td>
<td>% Carbohydrate content</td>
<td>3</td>
<td>8</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>
Fig. 1. 4: Activation of Complement System
1.2.2.2. Cell-mediated immunity:

The cell-mediated immunity refers to the specific immune responses that do not involve antibodies. Induction of cell-mediated immunity consists of specifically sensitization of T-lymphocytes against the antigen. When a sensitized cell comes in to contact with the antigenic determinant, it undergoes blast transformation and clonal proliferation. The activated lymphocytes release biologically active products (lymphokines), those are responsible for the manifestation of cell-mediated immunity. Macrophage and other mononuclear cells under the effect of lymphokines affect the destruction of microorganism and other processes involved in cell-mediated immunity (Fig.1.5).

Types of T-cells and their role in immune system:

I. Helper T-cells  
II. Cytotoxic T-cells  
III. Suppressor T-cells

I. Helper T-cells: They serve as the major regulator of virtually all-immune functions. They form a series of protein mediators, called lymphokines that act on other cells of the immune system as well as on bone marrow cells (Fig.1.6).
T-cells are mobilized

When they encounter a cell such as a macrophage or a B-cell that has a digested an antigen

And is displaying antigen fragment bound to its marker molecule

Lymphokines help T-cell to mature

The T-cell, altered and activated secret lymphokines

Lymphokines

Some lymphokines spur the growth of T-cell

Infected Cells

Some T-cells become killer cells and attack down body cells infected by viruses

Fig. 1.5 Fate of T-cell
II. **Cytotoxic T-cells**: It directly attacks the cell and capable of killing microorganisms and at that time even kills the body's own cells. For this reason, these cells are frequently called killer cells. The receptor protein on the surfaces of the cytotoxic cells causes them to bind tightly to those organisms or cells that contain their binding specific antigen.

III. **Suppressor T-cells**: They are capable of suppressing the function of both cytotoxic and helper T-cells. It is believed that these suppressor functions serve the purpose of regulation the activities of the other cells, keeping them from causing excessive immune reactions that might be severely damaging to the body.
Fig. 1.6: Mechanism of Acquired Immunity
1.3 MODULATION OF IMMUNE RESPONSE

The immune response may be affected at various levels, either specifically or nonspecifically. Modulation of the immune response involves induction, expression or amplification or by alteration of immune products by specific immunization. Immunomodulation is the process by which we can alter the immune system of an organism by interfering with its functions. If any agent enhances the immune reaction and primarily implies stimulation of the non-specific system, *i.e.* stimulation of the function and efficiency of granulocytes, macrophages, complement, certain T-lymphocytes and related substances, is known as immunostimulant. In contrast with it, some chemical or natural substance reduces the resistance against infections, is known as immunosuppressant. The functions and efficiency of the immune system may be influenced by many exogenous and endogenous factors like various type of chemical or herbal drugs, physical and physiological stress, hormone etc. *(Fig. 1. 7).*

Compounds, which are capable to modulate the immune response, are called immunomodulators those which activate or enhance the immune response are called immunostimulators, these compounds activate macrophage by binding with it and activated macrophage displays increased phagocytosis, while some compounds suppress the immune reaction are called immunosuppressors, they interfere with cell growth, only after their activation in tissue, and are capable of inhibiting both the humoral and cell-mediated immune response.
Immunomodulators are chemicals from herbal origin as well as from plants, herbal immunomodulators are categorized in two classes. In the first class compounds, which are of light molecular weight like alkaloids, terpenoids, steroids, quinines, phenolic compounds etc. In the second-class relatively larger molecules like polysaccharides, peptides, glycoprotein, nucleotides are involved. (Upadhyay, 1997)

Fig.1. 7: Regulation of the Immune Response
1.3.1 Immunostimulation

Immunostimulation or immunopotentiation can be defined as a process, which directly enhances one or more specific immune function, or modifies one or more components of the complex immuno-regulatory network to achieve its effects through indirect mechanism. The idea of stimulating the immune system to top the balance of the struggle between an invading pathogen or malignant cell and host is almost as old as ideas that have evolved this century about the components and function of the system itself. An enormous array of crude biological extracts, plant and animal substances of varying degrees of purity and cloned cytokines and highly purified immunoglobulins have been used as immunopotentiating agents. The most effective way to increase the specific response to a given antigen is to administer the antigen along with an agent that will enhance the reactivity of the lymphoid system. Such type of agents is known as adjuvant.

Immunomodulating agents have been reported to act primarily on cellular immune system rather than humoral and to restore the immuno-competency of impaired host without hyper stimulating the normal. It augments macrophage chemotaxis, phagocytosis and promotes interaction with other immunoregulatory lymphoid cells.
1.3.2 Mechanism of Immunostimulation

Any immunostimulator activates the cell-mediated and humoral antibody response. When a specific antigen comes in contact with T-cells they become activated then it activates B-cells and helps in the formation of antibodies. There are million of different types of preformed T-cells and B-cells found in the system and these cells are capable of forming specific antibodies. After activation they form many duplicates lymphocytes by clonal selection method. Most antigens activate both T and B-lymphocytes at the same time. Complement cells can also cause Immunomodulation. There are two pathways by which antigen activates immune system i.e. alternate and classical pathway. Naturin, a bio-immunomodifier is made by many traditional Chinese herbs and, used as immunomodulator, may enhance the activity of human natural killer (NK) cells against tumor cells in vitro and significantly increase antibody dependent cell cytotoxicity mechanism (Shen et al., 1996).

Immunomodulator or stimulant may also enhance the activity of macrophage. Stimulation of macrophages causes the release of a mixture of cytokines. Cytokines do not act alone but normally functions together with a mixture of other cytokines (Fig. 1. 8).

To activate the immune response it is essential to activate T and B-cells and in order to response to the immunomodulator, both B-cells and T-cells carry special receptor molecules on their surface. For B-cell this receptor is a prototype of the antibody, which is manufacture by B-cell.
When B-cell encounters a matching antigen in the blood or to any other body fluid, this antibody like receptor allows the B-cell to interact with it very efficiently. T-cell cannot recognize antigen in its natural state. The antigen must first be broken down and the fragments bound to an MHC molecule, by an antigen-presenting cell. This complicated arrangement assured that T-cell affects other cells through either direct contact or by burst of secretion. It acts only on precise targets at close range.

![Diagram of immune system interaction](image)

**Fig.1. 8: Activation of Macrophages by immunomodulator**
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Diseases</th>
<th>Defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Primary Immunodeficiency Diseases</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Severe combined immunodeficiency diseases</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Reticular Dysgenesis</td>
<td>Failure to develop primitive marrow reticular cells.</td>
</tr>
<tr>
<td>(ii)</td>
<td>Thymic almphoplasia</td>
<td>No lymphoid stem cells.</td>
</tr>
<tr>
<td>(iii)</td>
<td>Agammaglobulinaemia</td>
<td>No lymphoid stem cells.</td>
</tr>
<tr>
<td>(iv)</td>
<td>Wiscott-Aldrich syndrome</td>
<td>Cell membrane defect of Laemopoietic stem cells, associated features are thrombocytopenia and eczema.</td>
</tr>
<tr>
<td>(v)</td>
<td>Ataxia telangisctasia</td>
<td>Defective T-Cell maturation.</td>
</tr>
<tr>
<td>2.</td>
<td>T-cell defect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Di George's syndrom</td>
<td>Epithelial component of thymus fails to develop.</td>
</tr>
<tr>
<td>3.</td>
<td>B cell defects</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Bruton's Xlinked agammaglobulinaemia</td>
<td>Defective, differentiation from pre B-to-B cell.</td>
</tr>
<tr>
<td>(ii)</td>
<td>Autosomal recessive agammaglobulinaemia</td>
<td>Defective differentiation from pre B-to-B cell.</td>
</tr>
<tr>
<td>(iii)</td>
<td>IgA deficiency.</td>
<td>Defective maturation of IgA synthesizing B-cells.</td>
</tr>
<tr>
<td>(iv)</td>
<td>Selective deficiency of other Ig types</td>
<td>Defective differentiation from B cells to specific Ig. Synthesizing plasma cells.</td>
</tr>
<tr>
<td>(v)</td>
<td>Immunodeficiency with thymoma</td>
<td>Defective pre B cell maturation.</td>
</tr>
<tr>
<td>S.No.</td>
<td>Diseases</td>
<td>Defects</td>
</tr>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>4.</td>
<td>Common predominant B-cell defect</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>With predominant B-cell defect</td>
<td>Defective differentiation from thymocytes to T-helper cells.</td>
</tr>
<tr>
<td>(ii)</td>
<td>With predominant B cell defect</td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Deficient T-helper cells</td>
<td>Defective differentiation from pre B to mature B cells.</td>
</tr>
<tr>
<td>(b)</td>
<td>Presence of activated T-suppressor cells</td>
<td>T-cell disorder of unknown origin.</td>
</tr>
<tr>
<td>(iii)</td>
<td>With auto antibodies to B and T-cells</td>
<td>Unknown differentiation defect.</td>
</tr>
<tr>
<td>B.</td>
<td>Secondary immunodeficiency diseases</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Infections</td>
<td>AIDS, other viral, bacterial and protozoan infections.</td>
</tr>
<tr>
<td>(ii)</td>
<td>Cancer</td>
<td>Chemotherapy by antimetabolites, irradiation.</td>
</tr>
<tr>
<td>(iii)</td>
<td>Lymphoid neoplasia</td>
<td>Deficient T and B cell function.</td>
</tr>
<tr>
<td>(iv)</td>
<td>Malnutrition</td>
<td>Protein deficiency</td>
</tr>
<tr>
<td>(v)</td>
<td>Sarcoidosis</td>
<td>Impaired T-cell function.</td>
</tr>
<tr>
<td>(vi)</td>
<td>Autoimmune diseases</td>
<td>Administration of high dose of steroids toxic to lymphocytes.</td>
</tr>
<tr>
<td>(vii)</td>
<td>Transplant cases.</td>
<td>Immunosuppressive therapy.</td>
</tr>
</tbody>
</table>
1.3.3 Immunosuppression

Some agents nonspecifically interfere with the induction or expression of the immune response and in this way suppress the activity of immune system. Inhibition of the immune response is more likely to occur, if the immunosuppressive therapy is began before rather than after, expose to the immunogen.

Immunosuppression is required for transplantation to prevent the rejection of transplanted organ, in autoimmune disease and in deadly disease of newborn foetus erythroblastosis.

1.3.4 Mechanism of Immunosuppression

Immunosuppressive drugs have relatively specific effect, such as CD₃ antibodies, anti thymocyte globulin and CD₄, which deplete and inhibit the T-cell population while some cytotoxic drugs have more generalized effect that inhibits proliferation of T and B-cells. If any, foreign material like virus, bacteria, fungi etc. make their way into the body, a complex immune response for destroying is set in to motion. Immunosuppressive agents like corticosteroids cause depletion of lymphocyte from the blood and lymphoid organs. They also stabilize the membranes of the cells and lysosomes, inhibiting the histamine release and inflammatory response. Antimetabolites are substances that interfere with the synthesis of DNA, RNA and thus suppress cell division and differentiation necessary for humoral and cellular immune responses e.g. alkylating agents and
analogue of purine. Some immunosuppressive agents like antilymphocytes serum (ALS) acts primarily against T-lymphocytes and therefore specifically on cell-mediated immunity. ALS acts only against lymphocytes in circulation and not the cells in lymphoid organs. In immunosuppression, there is not only a significant reduction in the number of the cells, which had incorporated opsonized erythrocytes ingested per single cell.

1.3.5 Chemical Agents as Immunomodulators

Ehrlich 1956 observed that certain chemical agents are able to alkylate biological material and later on it was found that aliphatic nitrogen mustards have a specific effect on lymphoid tissue like spleen, bone marrow, thymus and lymph nodes. Once it was appreciated that similar compounds might be useful in the treatment of lymph nodes lymphomas; many variants of the nitrogen mustard were prepared.

Alkylating agents were among the first classes of chemical substances to be evaluated for their immunosuppressive properties. Hektoen and Corper (1921) showed that the formation of agglutinin and hemolysin to sheep red blood cells (SRBC) could be inhibited in rabbits and dogs after the administration of Sulphur mustard. Later it was found that alkylating agents effectively suppress the immune response and interfere with antibody formation in several animal species when given at the very beginning or 1-2 days before antigen stimulus.

Soon it became very clear, however, as more experimental details become available that the efficiency of alkylating agents varies considerably,
qualitatively and quantitatively, depending on the time relationship between drug treatment and immunization. The potent alkylating agents are active against the primary and secondary immune responses; both the cellular and humoral antibody responses can be affected in animals and man. The most widely used alkylating agent is Cyclophosphamide, which has a broad spectrum of activity in animal and human malignancies.

![Chemical Structure of Cyclophosphamide](image)

**Fig. 1.9: Chemical Structure of Cyclophosphamide.**

Arnold and Bourseaux (1958) first synthesized Cyclophosphamide, which interferes with cell growth only after activation in tissue. It is capable of inhibiting both humoral and cell-mediated immune response (Shand et al., 1979, Doherty, 1981). *(Fig. 1.9)*

In some cases the purine antagonists are the classical immunosuppressant drugs. In 1960 Schwartz and Domeshek demonstrated that, the antileukemic agent 6-marcaptopurine suppressed the immune response to human serum albumin in rabbits *(Fig 1.10).*

![Chemical structure of 6-marcaptopurine](image)

**Fig. 1.10: Chemical structure of 6-marcaptopurine**
Azathioprine is the drug of choice in clinical immunosuppression, particularly for kidney transplant. β, β- Diethylcysteine or penicillamine, procarbazine etc. are the other chemical agents, which are used for immunosuppression (Hitchings, 1969). Cinaserin is a potent serotonin antagonist and it suppresses the formation of circulating antibodies, prolongs mouse skin homograft and exhibits activity in the EAE test as well as in adjuvant induced arthritis (Millonig et al., 1974).

In contrast to the well-recognized field of immunosuppression, stimulation of the immune response received less attention in the past. Now the restoration of an impaired immune response and the enhancement of exiting immune mechanism should be potentially useful in a variety of diseases. During recent years a number of unrelated substances with immunopotentiating properties have come into focus, and more attention is given to the use of adjuvant, their nature and the concept underlying immunostimulation (Regamey et al., 1967 and Walstenholme et al., 1973).

Adjuvants (Latin, adjuvare- to assist) are substances that aid in the development and manifestation of the immune system, when injected with an antigen; they enhance the antigenic properties of a weak antigen or convert a non-antigenic substance to an effective antigen. They lead to increased antibody production and to induce delayed hypersensitivity. For example incorporation of an antigen into water in mineral oil emulsion leads to enhance and prolong the period of antibody production (Freund et al. 1937). Certain salts of aluminium are useful adjuvant for
immunization against diphtheria and tetanus. Aluminium salts preferentially enhance the humoral antibody response.

Complementary copolymers such as polyuridylic acid, polyadenylic acid, polyinosinic acid and polycytidylic acid stimulate the formation of antibodies in mice immunized with SRBC; they appear to act directly on host cells rather than in a special complex with antigen. A number of small and chemically well-defined molecules e.g. tilorone, levamisole vitamin A and acid etc. devoid of antigenicity and free of the undesirable attributes of some adjuvant, have been shown to have a modulatory effect on the immune response in experimental models and in clinical trials.

Tilorone is an orally and parenterally active broad-spectrum antiviral substance and interferon inducer.

\[
\begin{align*}
(C_2H_5)_2NCH_2CH_2O & \quad OCH_2CH_2N(C_2H_5)_2 \\
\end{align*}
\]

**Fig. 1.11: Chemical structure of Tilorone**

SRBC in mice. Another synthetic agent that shows great promise to restore an impaired immune response is levamisole, which has been used successfully as a potent antihelminthic in humans and in animals against a wide range of nematodal infections. (Janssen, 1976) (Fig. 1.11)
Fig. 1.12 Chemical Structure of Levamisole.

The capacity of levamisole to stimulate aspects of cell-mediated immunity was first demonstrate by Renoux and Renoux, 1971. It affects primarily cellular immunity rather than humoral response. Lymphocytes, granulocytes and macrophages are all stimulated by it. Levamisole has been tried in a wide array of disease states ranging from cancer and autoimmune disease. (Renoux et al., 1972) (Fig. 1.12)

Isoprinosine is also use to modulate immune responses and it stimulates the action of thymic hormones. It acts through a receptor for an inosine like compound on T-cell precursors. It has been demonstrated to ameliorate a number of viral infections, particularly acute and recurrent herpes simplex virus, rhinovirus and appears to be able to arrest the progress of measles related subacute sclerosing panencephalitis (Kint et al., 1974; Ippen et al., 1975).

1.3.6. Limitations of Chemical Agents

There are several adverse effects of using these drugs. The major drawback of drug like cyclosporine is renal failure/toxicity. Nephrotoxicity
can occur in as many as 75% of patients. Other toxicities include hypertension, hepato-toxicity, hirsutism, gingival hyperplasia and gastrointestinal toxicity (nausea, vomiting, diarrhea, anorexia and abdominal pain) neurotoxicity like headache, tremor, insomnia, pain, cardiovascular toxicity, metabolic toxicity like hyperkalemia, hypomagnesaemia, hyperglycemia etc were the contra-indication of chemical drugs like Azathioprine which also affects rapidly growing cells, including bone marrow and gastrointestinal cells resulting in leucopenia, thrombocytopenia and gastrointestinal toxicity. (Diasio and LoBuglio, 1996)

Some other drugs cause hemorrhagic cystitis, severe pancytopenia. The major toxicities result from anti thymocyte globulin, being recognized as a protein, leads to serum sickness and nephritis. Other toxic symptoms include chills and fever, leucopenia, thrombocytopenia and skin rashes. In some cases it leads to infertility due to the destruction of testicular and ovarian cells.

1.4 NATURAL PRODUCTS AS IMMUNOMODULATORS

In nature, there are many plants, which exert their antitumour activities exclusively by an immune induced cytotoxicity when applied in the usual manner of traditional medicine. In recent years there has been an upsurge in the clinical use of indigenous drugs. Herbal plants, originally used in the traditional system of medicine are now effectively tried in a variety of pathophysiological states. (Darshan and Dorswamy, 1998) Attention has been gathering for natural products that have few side effects
along with an active ingredient, especially antitumour effect because the chemotherapeutic drugs or synthetic compounds exhibit potent antitumour activity with much side effect (Capelli et al., 2000). The concept of modulation of the immune response to alleviate the diseases has existed in ancient system of medicine like Ayurveda and Unani. Plants have been extensively used as a source of medicine in these systems to promote health and to maintain body's resistance against infections (Datta et al., 1999).

An increasingly important role is being attributed to various herbal products. Many proteins purified from seeds for example Concanavalin A phytohaemagglutinin (PHA), wheat germ agglutinin, pokeweed mitogen and some fungal immunomodulatory proteins isolated from Volvariella volvacea, Gonoderma lucidium and Flammulina velutipes have been shown to induce a cascade of events leading to cell activation, proliferation and thereby production of lymphokines (Hsu et al., 1997). Recent investigation has shown that nontoxic doses of galactoside specific mistletoe lectin have immunomodulatory potencies (Beuth, 1993). Studies on Azadirachta indica have been evaluated on some nonspecific and specific aspects of immunity in mice (Sen et al., 1990, Ray et al., 1996).

The plant may be considered a biosynthetic laboratory, not only for chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man but also for a multiple compounds like glycosides, alkaloids, volatile oils, tannin, simple polypeptides etc. that exert
physiological effects. The compounds, responsible for therapeutic effects are usually the secondary metabolites. Immunomodulatory activity of some medicinal plants was found to be more than of glucan and lithiumcarbonate (Thatte et al., 1988). *Asparagus racemosus* and *Tinospora cordifolia* have been claimed to possess potent immunomodulatory activity and have shown to produce significant leucocytosis and predominant neutrophilia in animal models (Dahanukar et al., 1986; Thatte et al., 1988). Pericorp oil of *Semicarpus anacardium* used in arthritic condition by Ayurvedic physicians has potent immunosuppressant activity in experimental animals (Saraf et al., 1989). Most of the plants described above have low molecular weight substances, which are responsible for alerting immunological sequenses. Number of other plants like *Echinacea purpurea* (Wagner et al., 1988), *Astragalus peregrinus* (Verotta et al., 2001) and *Viscum album* (Pelletier et al., 2001) have high molecular compounds mainly polysaccharides as immunomodulators.

Plants belong to family Meliaceae for example *Azadirachta indica*, *Mumronia pumila*, *Melia azedarch*, *Cedrela lilloi* and *Trichilia elegans* etc. display anti-inflammatory and antirheumatic properties. *Cedrela lilloi* and *Trichilia elegans* inhibit the phagocytic capability and the oxidative metabolism using opsonized zymosan as stimulus in peritoneal macrophages (Nores et al., 1997). Lima et al., 1999 revealed that *Pisum sativum* agglutinin (PSA) induces immunomodulatory effects, activating spleen lymphocytes in vivo. Recently many more plants are used to prepare
immunomodulatory drugs, therefore, it is important or in other words it is necessary to search out plant having immunomodulatory properties to combat many dreaded diseases.

Enormous diversity in plants, varieties of synthesis and difference in physiology to cope up, the stress of variable climatic condition is the few reasons to obtain different types of immunomodulator. Some important drugs from plant origin used in Ayurveda for long time to enhance the quality of health, vigour, vitality, freedom from disease, strength of body and mind, and they are obtained by vitaligers, thus it can be concluded that these drugs acts as immunomodulators and they may be inducers of enzymes, hormone etc. As it is known that herbal drugs have less toxic effects as compare to synthetic drugs, a few of among the many listed in the given table 1.4.
### Table 1.4 Plants with immunomodulatory activity.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Plant</th>
<th>Family</th>
<th>Part used</th>
<th>Extract</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td><em>Argyreia speciosa</em></td>
<td>Convolvulaceae</td>
<td>Roots</td>
<td>Ethanolic</td>
<td>Immunostimulant</td>
<td>Gokhale <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>3.</td>
<td><em>Angelica gigas Nakai</em></td>
<td>Umbelliferae</td>
<td>Roots</td>
<td>Aqueous</td>
<td>Immunostimulant</td>
<td>Han <em>et al.</em>, 1998</td>
</tr>
<tr>
<td>S. No.</td>
<td>Name of the Plant</td>
<td>Family</td>
<td>Part used</td>
<td>Extract</td>
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<tr>
<td>8.</td>
<td><em>Centrosema pubescens</em></td>
<td>Fabaceae</td>
<td>Seeds</td>
<td>Chloroform</td>
<td>Immunostimulant</td>
<td>Da Silva et al., 2000</td>
</tr>
<tr>
<td>10.</td>
<td><em>Hippophae rhamnoides</em></td>
<td>Elaeagnaceae</td>
<td>Leaves, Fruits</td>
<td>Ethanolic</td>
<td>Cytoprotective, Antioxidant, Immunomodulatory activity</td>
<td>Geetha et al., 2002</td>
</tr>
<tr>
<td>11.</td>
<td><em>Isatis cappadoica</em></td>
<td>Brassicaceae</td>
<td>Whole Plant</td>
<td>Ethanolic</td>
<td>Immunostimulant</td>
<td>Rezaeipoor et al., 2000</td>
</tr>
<tr>
<td>12.</td>
<td><em>Jatropha multifida</em></td>
<td>Euphorbiaceae</td>
<td>Latex</td>
<td>Aqueous</td>
<td>Increase the production of IL-2.</td>
<td>Kosasi et al., 1989</td>
</tr>
<tr>
<td>14.</td>
<td><em>Leucas aspera</em></td>
<td>Lamiaceae</td>
<td>Whole Plant</td>
<td>Ethanol/Aqueous</td>
<td>Anti-inflammatory</td>
<td>Singh et al., 2002</td>
</tr>
<tr>
<td>15.</td>
<td><em>Mahonia aquifolium</em></td>
<td>Berberidaceae</td>
<td>Stem Bark</td>
<td>Ethanolic</td>
<td>Enhance the production of IL-8</td>
<td>Kost alova et al., 2001</td>
</tr>
<tr>
<td>S. No.</td>
<td>Name of the Plant</td>
<td>Family</td>
<td>Part used</td>
<td>Extract</td>
<td>Activity</td>
<td>Reference</td>
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</tr>
<tr>
<td>22.</td>
<td><em>Panax ginseng</em></td>
<td>Araliaceae</td>
<td>Root</td>
<td>Hot Water</td>
<td>Stimulation of IL-8 production</td>
<td>Berechman and Dardymor 1969</td>
</tr>
<tr>
<td>S. No.</td>
<td>Name of the Plant</td>
<td>Family</td>
<td>Part used</td>
<td>Extract</td>
<td>Activity</td>
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<tr>
<td>27.</td>
<td><em>Platycodon grandiflorum</em></td>
<td>Campanulaceae</td>
<td>Roots</td>
<td>Aqueous</td>
<td>Immunostimulatory</td>
<td>Choi <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>33.</td>
<td><em>Trigonella foenum graecum</em></td>
<td>Leguminosae</td>
<td>Whole Plant</td>
<td>Aqueous</td>
<td>Immunostimulant</td>
<td>Hafeez <em>et al.</em>, 2002</td>
</tr>
<tr>
<td>34.</td>
<td><em>Tripterygium wilfordii</em></td>
<td>Celastraceae</td>
<td>Roots</td>
<td>Ethanolic</td>
<td>Immunosuppressive</td>
<td>Duan <em>et al.</em>, 1999</td>
</tr>
</tbody>
</table>
1.4.1 Important Plant Products

The plants are in fact, miniature factories of nature for the production of alkaloids, carbohydrates, proteins, essential oils, vegetable oils, gum and resins, glycosides, dyes, carotenes, flavonoids, tannin etc. Medicines, dyes, perfumes, cosmetics are the important fields of industrial applications of plant products. The medicinal value of the plants have ascribed to the presence of certain active chemical principles contained there in. These active principles may be put under the following groups of compounds:

- **Alkaloids:**

  Alkaloids are actually naturally occurring amines, the functions of alkaloids in the biochemical process of the living plant is not yet well understood, however a number of plant alkaloid exert a marked physiological action when they are administered to animals, some of these substances are toxic to the human organism while some other such as quinine have important medicinal value. The chemistry of alkaloids has been exhaustively dealt with in a number of standard works. Aristolochic acid, a 3,4-methylendioxy-8-methoxylnitrophenanthrenearconic (As) is an alkaloid isolated from *Aristolochia clemalites* have shown potent immunostimulatory activity. Experiments revealed a pronounced enhancement of phagocytosis of leucocytes and peritoneal macrophages and enhancement of granulocytic phagocytosis. (Manske *et al.*, 1950, Holubete *et al.*, 1965, Glasby *et al.*, 1977, Mose *et al.*, 1961, Kim *et al.*, 1996,
Vieregge et al., 1999, Rahman et al., 1992.) Mose et al., 1961, described a marked reduction in methylcholantherece induced transplantable tumors in animals treated with Aristolochic acid. Kang et al., 2000 explained antiproliferative effects of alkaloids isolated from Sedum sarmentosum on murine and human hepatoma cell lines.

- **Colouring Matters:**

These are phenolic compounds containing a pyran ring linked with benzene nucleus. The majority of the colouring matter occurs as glycosides from which the true colouring matter is obtained on hydrolysis. The variety of colours exhibited by flower is due to the presence of different anthocynins (Bently et al., 1960, Godwin et al., 1965).

- **Carbohydrates:**

The ratio of hydrogen to oxygen in these compounds is 2:1 as indicated by the general formula C\(_n\)(H\(_2\)O)\(_n\). Carbohydrates provides basic needs of life. They form important structural materials for plants and occur in all living cells. The main function of carbohydrate is to supply energy (Roy et al., 1965; Gathrie, 1968). The number of polysaccharides isolated from fungi and higher plants has been studied pharmacologically for immunomodulatory activity, Lentinan, a yeast polysaccharide, isolated from Lentinus edodes reported to have the antitumour activity. The antitumour activity of this compound is mainly the result of T-lymphocyte activation. It has been suggested that transmitter such as serotonin or 5
hydroxytryptophan are involved in the activation process (Chihara et al., 1969, Whistler, 1976, Haba, 1976.).

- **Enzymes and Protein:**

  Enzymes are chemically unstable nitrogenous compounds of colloidal nature. These are widely distributed in nature and are classified according to their action and are found associated with coenzymes (Paech et al., 1955, West et al., 1974) Protein is a necessary part of the diet of all animals, indeed human being can become seriously ill if they do not get enough protein hence, no form of life can exist without protein (Schulz et al., 1979, Bodansky et al., 1976, Bohinski et al., 1987, Lehninger et al., 1982). It is reported that α-Amanitin (A cylopeptide) isolated from *Amanita muscaria* induces interferon activity (Atherton, 1978).

- **Glycosides:**

  Glycosides are cyclic acetals formed by reactions of aldose with alcohol and may exist in α and β forms anameric. Glycosides derivatives of glucose called glucosides. Terpenoides and steroids are the other types of glycosides (Armstrong et al., 1931, Rodd et al., 1959). Several terpenoids possess antiarthritic or anti phagocytic activity similar to tylophorine. This is true for sesquiterpene-lactones with a α- methylene-γ lactone structure in its position to the cycloheptane ring. Their biological activities appear to be mediated by immunological process. The best antiarthritic activity among 20 sesquiterpen lactones studied in animal experiment, was observed with helenalin, tenulin and euaphyssopin. The effect of these
compounds on the immune system appears to be twofold, first the
production of antibodies is increased and second, the production of
T-lymphocytes decreased (Hall et al, 1979).

- **Tanin:**

  Several tannin exhibits strong inhibition of tumour produced
experimentally and this activity is due to enhancement of host mediated
antitumour activity of the tannin compound (Okuda et al., 1985).

- **Saponins:**

  The term saponin is applied to the group of plant products, which
produce soapy leather when they come in contact with aqueous solutions
and heamolyses the blood. On hydrolysis saponins yield one or more sugar
units and an aglycon called sapogenin (Price et al., 1987, Shibata, 1977,
Estrada et al., 2000). A steroid glycoside is also a type of saponin. A steroid
glycoside (Cynachoside) has been isolated from the roots of *Cynanchum
caudatum*. It increases macrophagocytosis and cellular immunity in mice

- **Flavonoids:**

  Flavonoids are a heterogeneous group of ubiquitous plant
polyphenols that abound in the human diet and are endowed with several
biological activities, including immunomodulatory and antioxidant
activities. Flavonoids are also important factors for plant growth,
development and immunity. Findings in vitro do not always agree with
observation in vivo. Moreover, the effects of different flavonoids may be antagonistic, in some cases they are immunosuppressive and in others, immunostimulating (Di Carlo et al., 1999) Numerous flavonoids have been seen to influence the function of enzyme system that are critically involved in the immune response and in the generations of inflammatory process, especially in the transudation of cellular activation signals (Lelpo et al., 2000). Flavonoids are among the natural biological response modifiers (Middleton, 1998).
1.5 OVERVIEW OF THE PREVIOUS WORK

Traditional medicine is an important cultural heritage, which is originated through man's continual need and search for protection and relief from various disorders. In the developed countries, chemical drugs have largely replaced this heritage. However, in third world countries, this heritage has largely been remaining the major remedy. The healing capabilities of many traditional medicines are real and reproducible to users. More and more scientific reports have confirmed the claimed values of herbal drugs, some of which even offered new leads and discoveries to enrich and fill the gap in western pharmaceutical profiles. Even the WHO recognizes and endorse the valuable contribution of traditional medicines in order to reach the WHO's goal of providing adequate health care to all the people. In spite of a rapidly expanding literature on photochemistry, only a small percentage of the total species have been examined chemically and there is a large field for future research.

However, man did not require the modern methods of investigation to collect for him a materia medica of plant, which he often used in conjunction with magical and other ritual practices. Such folk medicines naturally varied according to the plants available in a particular climatic area and can be studied today in those more or less undisturbed primitive society, which still exist. It is interesting to reflect that such collection of herbal medicines compiled over centuries by trial and error and presumably using the potent on the experimental animal, must surely
contain some material worthy by further investigation and should not be too readily discarded. Material produces adverse reaction because of chemical drugs was eliminated by using herbal drugs (Trease et al., 1985). Ancient Indian literature, abounds information on a large number of plants reported to have Immunomodulatory activity some are as follows:

Gao et al. (1989) found that water soluble and alkaline soluble polysaccharide fractions, prepared from roots and leaves of Panax ginseng contained pectins and glucans in roots and pectins and hetroglycans in leaves. The water-soluble polysaccharides fraction from the leaves of Panax ginseng showed higher anti-complementary activity than those from the roots. Hajto et al., (1986) reported that proprietary extract of mistletoe (Iscador) has federal approval for clinical application, can exhibit immunomodulatory capacity. Injections of nontoxic doses of the pacified lectin or even only its carbohydrate- binding subunit (0.25-1.0 ng/kg) in to rabbits yielded significant increase in natural killer cytotoxicity, frequency of large granular lymphocytes and phagocytic activity of granulocytes. Kosasi et al. (1989) isolated and characterized the anti- complement constituent(s) present in the latex of Jatropha mutifida called proanthocyanadin. The polymer inhibits CP activation of the complement cascade, while alternative pathway (A.P.) activation is relatively insensitive to the polymer for example, Panax ginseng, Astragalus membranaceus etc. Scaglione et al. (1990) showed the effect of Panax ginseng C.A. Meyer on immune system and found that intracellular killing was significantly increased.
Thabrew et al. (1991) studied the effect of aqueous extracts of Osbeckia octandra, Melothoia maderaspatana, Phyllanthus debelis on the human immune system, extract showed strong anti-complement effect on both the classical and alternate pathways of the human complement system. Effects were dose dependent and most pronounced in classical pathways. Wang et al. (1991) reported the activity of polysaccharide from Acanthopanax obovatus roots. It is found that it increased the spleen weight, number of spleen cells, and augmented the phagocytosis of peritoneal macrophages. Shen et al. (1991) observed that the polysaccharides from Acanthopanax senticosus inhibited transplanted tumor growth and ameliorated toxicities of the toxic substances in experimental animal.

Kuttan et al. (1992) isolated a peptide from Viscum album, which increases the natural killer cell activity and the number of antibody forming cells in the spleen.

Stienmuller et al. (1993) separated a polysaccharide from Echinacea purpurea, which enhances the resistance of immunosuppressed mice. Echinacea purpurea was effective in activating peritoneal macrophages isolated from animals after administration of Cyclophosphamide as cyclosporine.

Ingolfsdottir et al. (1994) purified a polysaccharide from Cetrariais landica the polysaccharide showed pronounced immunostimulating activity in an in vitro phagocytosis assay and in the in vivo carbon clearance assay.
Puri et al. (1994) reported immunostimulant activity in *Nyctanthes arbor-tristis* as evidenced by the increase in humoral and delayed type hypersensitivity. Wong et al. (1994) isolated and purified the bioactive polysaccharides from naturally occurring medicinal plants. The different fractions separated from Chinese medicinal plant show a range of immunomodulatory and antitumour activities.

Karaca et al. (1995) extracted a polysaccharide, Acemannan, from *Aloe Vera*, cultures of normal chicken spleen cells and HDII line cells produce nitric oxide (NO) in response to Acemannan, results suggest that Acemannan induced NO synthesis which may be mediated through macrophage mannose receptors. Kimura et al. (1995) studied the effect of stilbenes derivatives; resveratrol isolated from the roots of *Reynoutria japonica* and observed the effect on asachidonate metabolism and degranulation in human polymorph nuclear leucocytes. This derivative inhibited the release of lysosomal enzymes such as lysozyme and β-glucuronidase.

Gao et al. (1996) isolated a polysaccharide from *Panax notoginseng* and found that fraction PBGA12 has the most anti-complementary activity, which is mediated through both alternative and classical pathways. They also induced the production of significant amount of TNF-α in cell-culture. Wong et al. (1996) Demonstrated that hot water extract of *R. korthasic* was able to stimulate the proliferation of mouse lymphocytes. On the other hand ether fraction appeared to be cytotoxic against the tumor cells. Gao
et al. (1996) isolated three heteroglycans that heteroglycans induce human monocytes to produce interleukin-1, interleukin-6 and tumor necrosis factor in vitro.

Polysaccharide celosian, from Celosia argenta induces tumor necrosis factor-α and production of interleukin-β and nitric oxide in macrophage cell line. Celosian enhanced gamma-interferon production in mice spleen cells all these indicate that celosian is an immunostimulating agent in addition to antihepatotoxic effects (Hase et al., 1997). The compounds from Tinospora cordifolia possess anti-complementary and immunomodulatory properties that give rise to significant increase in IgG antibodies in serum. Kapil et al. (1997) observed that Humoral and Cell-mediated immunity were also dose dependent. Liang et al. (1997) found that Epimedium launarse significantly enhanced the response of spleen antibody forming cells, extract also significantly enhanced lymphocyte proliferation and caused a significant recovery of IL-2 production. Zhang et al. (1997) prepared polysaccharide fraction (DAP-1) which showed anti-complementary activity but also a stimulating effect on the mitogenic activity of lymphocytes. Nores et al. (1997) proved that aqueous leaf extracts of Cedrela lilloi and Trichelia elegans are anti-complementary and inhibited phagocytosis of opsonized sheep erythrocytes. The extract also activated oxidative metabolism of opsonized zymosan on peritoneal macrophages.

The acidic polysaccharide, ginsen from ginseng inhibited pulmonary metastasis of Blb-F10 melanoma cells and enhanced the inhibition of
malignant lung cell colonies by IL-2. Ginsen generates LAK-cells from both
NK and T cells through endogenously produced multiple cytokines
(Kim et al., 1998). Sonoda et al. (1998) found that acidic polysaccharide
from Ginseng induces the production of IL-8 by unstimulated and LPS-
Stimulated THP-1 cells. The results indicate that ginseng polysaccharides
act mainly on monocytic cell lines, but not on T-cells. Kulkarni and
Karande (1998) noticed that napthoquinone extract of leaves of Lawsonia
alba linn showed in vitro stimulation of human neutrophils at a low dose
range of 10g-70g/ml and in vivo, stimulation of macrophage phagocytic
activity in mice at the dose of 5mg/kg, intraperitoneally. Han et al. (1998)
isolated a polysaccharide, angelan, from Angelica gigas Nakai. This
polysaccharide increased the expression of IL-2, IL-4, IL-6 and IFN-γ. It also
activated B-cells, which in turn increased antibody production. Nose et al.
(1998) showed the immunomodulatory effect of crude polysaccharide
fraction obtained from the shoot of Glycyrrhiza glabra and from hairy roots
of Glycyrrhiza uralensis. The fractions induced nitric oxide production by
murin peritoneal macrophages in vitro.

Garbacki et al. (1999) identified that flower head of Centaurea
Cyanus, which is used in European phototherapy for the treatment of
minor ocular inflammation, contains a polysaccharide, which had anti-
inflammatory properties. Polysaccharides were found to be mainly
composed of galacturonic acid, arabinose, glucose, shampuses and galctose.
Some polysaccharides isolated from Imperata cylindrical enhance the
proliferation of murine splenocytes (Pinilla and Luu, 1999)
Chintalwar et al. (1999) isolated an arabinogalactan from the dried stem of *Tinospora cordifolia* with polyclonal mitogenic activity against B-cells. Shinde et al. (1999) studied the immunomodulatory activity of *Cedrus deodara* wood oil and found an inhibitory effect on humoral and cell-mediated immune responses. The volatile oil of *Cedrus deodara* wood administered orally and it significantly inhibited Type III and Type IV hypersensitivity. Subramoniam et al. (1999) reported that the butanol fraction of methanol extract of *Trichopus zyloanicus* did not inhibit antigen-induced degranulation of the mast cells in vitro, however, treatment of mice with *Trichopus zyloanicus* leaf resulted in inhibition of antigen-induced degranulation of sensitized peritoneal mast cells and it also reduced the ratio of mast cells in the exudate cells. Anesini et al. (1999) observed the stimulatory effect of the aqueous extract of *Tilia corelata* flower on lymphocyte proliferation; they suggested that extract could exert stimulatory effect by acting as an antagonist of the peripheral benzodiazepine receptor.

Saundane et al. (2000) investigated anti-inflammatory and analgesic activity of various extracts of *Leucas aspera Spreng*. The ethanol and distilled water extract exhibited significant anti-inflammatory activity, whereas significant analgesic effect was shown by petroleum ether and ethanol extract when compared with respective controls and with those of standard drugs. The diethyl ether extract of rhizomes of *Picorhiza scrophulariiflora* showed potent inhibitory activity towards the classical pathway of the complement system, and exhibited anti-inflammatory
activity towards carrageenan- induced paw edema Smit et al. (2000). Rezaeipoor et al. (2000) studied the effects of 20% ethanol extract of Isatis cappadocia on humoral immune responses in rabbits and mice. It was found that the 0.25g/kg intraperitoneally injected drug suppressed the primary immune responses while the dose of 0.5g/kg stimulated the secondary immune responses. Benencia et al. (2000) observed immunomodulatory activities of Trichilia glabra in aqueous leaf extract, the extract significantly diminished both antibody and delayed hypersensitivity response in mice.

Park et al. (2001) isolated an acidic polysaccharide from Panax ginseng, which enhanced the production of nitric oxide (NO). The number of peritoneal exudate cells and purified adherent macrophages were increased in BALB/c mice. Kulkarni and Desai (2001) isolated polyfructoscan, inulin, from the dried roots of Saussarea lappa. The insoluble form of inulin caused activation of macrophages and lymphocytes. Kost alova et al. (2001) investigated the effect of Mahonia aquifolium, the crude extract of its stem bark partly inhibited the IL-8 production after 48 hrs treatment of the cell whereas the purified polysaccharide when introduced or injected, it showed increase in the level of IL-8. Nicholl et al. (2001) reported the effect of extract of Osbeckia aspera as immunosuppressant. Results show that the inhibitory agent(s) in the aqueous extract of Osbeckia may have an effect on antigen presenting cell function. Da Silva et al. (2001) isolated a polysaccharide, glucan, from the mesocarp of fruits of Orbignya pharlerata. The polysaccharide enhanced phagocytosis in vivo and exhibited anti-
inflammatory activity. The aqueous suspension of fruit rind powder (100mg/kg) stimulated the cell mediated and humoral immunity (Ross et al., 2001). Iganacio et al. (2001) investigated the immunomodulatory role, both in vitro and in vivo, of aqueous extract of fresh and dried leaves of *Phyllanthus tenellus* and the production of NO by mouse peritoneal macrophages was detected in culture supernatants. NO is an important cytotoxic effectors molecule in the defense against tumor cells.

Geetha et al. (2002) investigated the antioxidant and immunomodulatory properties of Seabuckthorn (*Hippophae rhamnoides*) using lymphocytes as a model system. Alcoholic extracts of leaves and fruits of seabuckthorn at a concentration of 500mg/ml were found to inhibit chromium induced free radical production, apoptosis, and DNA fragmentation and restored the anti-oxidant status more effectively then that of control cells. Hafeez et al. (2002) found appreciable immunomodulatory activity in the aqueous extract of *Trigonella foenum graecum* L; it also facilitated haemopoietic stimulation in bone marrow. Goel et al. (2002) conducted a study to examine the immunomodulatory effects of the preparation of Echinacea. Phagocytic activity of alveolar macrophages was increased with increasing concentration of the Echinacea drug. An enhanced release of cytokines (such as TNF-α, IFN-γ) in response to higher concentration, Echinacea components was also detected in rat's spleen macrophages. Aguilar et al. (2002) showed the anti-inflammatory activity of two different extracts of *Uncaria tomentosa* and noticed that anti-inflammatory activity was significantly higher in the hydroalcoholic than
compared with aqueous extract. The extract also exerted little inhibitory activity on cyclooxygenase -1 and 2.

Freier et al. (2003) administered a volumetric dose of glycerin extract of *Echinacea purpurea* that enhanced humoral immunity as well as innate immune responses of female Swiss mice. Drozd and Anus Zewska (2003) studied the effect of aqueous extract of fine raw materials of tannin plant on the survival of thymocytes from the thymus of Balb-C mice, and found that only the *Fructus mystilli* extract has significant influence on the survival of thymocytes. Rivera et al. (2003) prepared dry extract of *Panax ginseng* C.A. Mayer roots, the extract have adjuvant properties. Immunization using PPV vaccines adjuvanted with ginseng fraction Rb1 which, induces higher antibody titres than the vaccine adjuvanted with Al (OH)3. Hu et al. (2003) also showed adjuvant effects of ginseng extracts on the immune responses to immunization against *Streptococcus aureae* in dairy cattle, addition of R (b1) resulted both in significantly higher antibody production and lymphocyte proliferation in response to PWM, Con A and *Streptococcus aureae* antigens than in control group. Orsolic and Basic (2003) studied the antimetastatic efficacy of water soluble derivatives of propolis, it is found that antimetastatic activity is mainly mediated by immunomodulatory activity, mainly toward augmentation of non-specific antitumour resistance in mice via macrophage activation.

Kumar et al. (2004) reported the anticancer and immunostimulatory compounds from *Andrographis paniculata*, their results indicate that the
dichloromethane fraction of the methanolic extract retains the active compounds for both anticancer and immunomodulatory activity. Sunila and Kuttan (2004) reported that the alcoholic extract of the fruits of *Piper longum* Linn. was 100% toxic at a concentration of 250 microg/ml to Delton’s lymphoma ascites (DLA) cells and 250 microg/ml to Elrich ascites carcinoma (EAC) cells. Piperine, one of the isolates from *Piper longum* Linn was also found to be cytotoxic toward DLA and EAC at a concentration of 250 mg/ml. Administration of *Piper longum* extract and piperine increased the total WBC count and number of plaque forming cells. Jayathirtha and Mishra (2004) reported Immunomodulatory activities of *Eclipta alba* and *Centella asiatica*. Thuy et al. (2004) isolated an immunosuppressive auronol glycoside maesopin 4-O glucoside from *Artocarpus tonkinensis*.

Klopp et al. (2005) showed the influence of *Viscum album* on microcirculation and on immune system of ear, nose and throat carcinoma patients treated with radiation and chemotherapy, the adverse effects of radiotherapy and chemotherapy on the microcirculation and immune system were decreased. Cottiglia et al. (2005) isolated two new pterocarpan and the new isoflavone from the fractionation of the petroleum ether and ethyl acetate extracts of *Bituminaria marisiana* leaves and did cytotoxicity test against cell related to the immune system and found that it showed a moderate activity and induce necrosis in leukaemia jurkat T-cells. Kodama et al., (2005) concluded that D-fraction, a polysaccharide, extracted from the maitake mushroom (*Grifola frondosa*) activates macrophages, dendritic cells, T-cells and inhibits the growth of tumour cells. D-fraction
significantly enhanced the cytotoxicity against NK sensitive YAC-1 cells and the expression of CD 223 on NK cells it also increased the level of IL-12 and expression of CD 86 on macrophages. Heitzman et al. (2005) explained in his review on Uncaria (Rubiaceae) genus is an important source of medicinal natural products particularly alkaloids and triterpenes. Review described all the processes related to immunomodulation affected by Uncaria, which established it as a potent immunomodulator. Silva et al., (2005) isolated a triterpenoidal saponin called pulcherrimasaponin from the leaves of Calliandra pulcherrima Benth. This saponin shows remarkable similarities of a potent adjuvant QS 21 saponin. The delayed type of hypersensitivity against leishmanial antigen was impressively increased. The safety analysis and the effect on humoral and cellular immune responses demonstrate this saponin as a potential candidate for a vaccine adjuvant.
1.6 RESEARCH ENVISAGED:

Rigveda and Atherveda describe medicinal plants and about 250 drugs have been subsequently identified from such descriptions. The vast knowledge about the properties of plant must have accumulated through trial and error. In this work three plants namely, *Cleome gynandra*, *Cocculus hirsutus* and *Lantana camara* are selected. The research work has been carried out under following headings.

1. Preliminary exploratory studies.
2. Qualitative chemical test.
3. Fractionation
4. Chromatographic studies with a view to characterize the extract.
5. Immunomodulatory screening to find out the effect of Ethanolic and Aqueous extract on phagocytic action, cell mediated and humoral immune response by following methods.
   A. Carbon clearance test to determine the effect over the innate immunity.
   B. Delayed type of hypersensitivity test (Paw edema) to measure the effect on cytotoxic T-cells.
   C. SRBC agglutination test to determine the effect over humoral immunity.
   D. Drug induced myelosuppression test to study the effect on various hematological parameters and cellular immunity.