EVALUATION OF IMMUNOMODULATORY ACTIVITY OF

Jasminum sambac (L.) Aiton FLOWERS

BY *IN-VIVO* METHODS

A Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY,CHENNAI- 600 032

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Submitted By

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled, "**EVALUATION OF IMMUNOMODULATORY ACTIVITY OF *Jasminum sambac*** (L.) Aiton **FLOWERS BY *IN-VIVO* METHODS"** submitted to The Tamil Nadu Dr. M.G.R. Medical university,Chennai was carried out by **Reg.No:261825402** Department of Pharmacology, Nandha college of pharmacy, Erode -52 for the partial fulfillment for the award of degree of Master of Pharmacy in Pharmacology under my supervision.

This work is original and has not been submitted in part or full for any other degree or diploma of this or any other university.

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EVALUATION CERTIFICATE

This is certify that the work embodied in the thesis entitled "**EVALUATION OF IMMUNOMODULATORY ACTIVITY OF *Jasminum sambac (L.)* Aiton *F*LOWERS BY *IN-VIVO* METHODS** " to The Tamil Nadu Dr. M.G.R Medical University Chennai, was carried out by **Reg.No:261825402** in the department of Pharmacology, Nandha College of Pharmacy, Erode-52 for the partial fulfillment of the degree of "Master of Pharmacy" in Pharmacology under the supervision of **Mrs.V.Lalitha, Associate Professor,** Department of Pharmacology,Nandha College of Pharmacy, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university.

Internal Examiner **External Examiner**

DECLARATION

The work presented in this thesis entitled "**EVALUATION OF IMMUNOMODULATORY ACTIVITY OF *Jasminum sambac* (L.) Aiton FLOWERS BY *IN-VIVO* METHODS**" was carried out by me in the Department of Pharmacology, Nandha College of Pharmacy, Erode -52 under the direct supervision of **Mrs.V.Lalitha M.Pharm.,**Associate Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode-52.

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" Develop an attitude of gratitude, and give thanks for everything that happens to you, knowing that every step forward is a step toward achieving something bigger and better than your current situation. Success of any project depends solely on support, guidance and encouragement received from the guide and well wishers**"**

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ABBREVIATIONS

NK	Natural killer cells
MHC	Major histocompatability complex
Fc	Fragment crystallizable
TCTL	Cytotoxic T lymphocytes
CD4	Cluster of differentiation
PAMP	Pathogen associated molecular pattern
DAMP	Damage associated molecular pattern
DC	Dentritic cells
TNF	Tumour necrosis factor
IFN	Interferon neuron
NF*kB*	Nuclear factor kappa light-chain enhancer of activated B cells
TGF	Transforming growth factor
ROR	Retinoic acid receptor related orphan receptors gamma
EEJSF	Ethanolic extract of *jasminum sambac* flowers
LEV	Levamisole
ANOVA	Analysis of variance

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INTRODUCTION

Human beings are living in an open environment which is heavily contaminated with many pollutants. They are constantly exposed to varieties of physical, chemical, and environmental factors that causes adverse effects and damages on human. In nature our body has different strategy to control the above mentioned factors, and man is constantly trying to find and identify remedies for these problems. Plants are used since very ancient time to control different types of problem associated with human. There are reports that plants contain remedies for various diseases including AIDS, cancer, and diabetes. The use of plant-based medicines for healing is an ancient and universal as medicine itself. Until the dawn of this century, natural products have served as the mainstay of all medicines world-wide. Although herbalism has declined in the West, it continues to exist throughout the developing world. According to WHO, over 70% of the world population still relies on herbal remedies for their health care needs. Today there are a huge number of chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world.

Many of the plant products exert some effect on immune system. They either enhance immune response to help body clear undesirable agents from body or suppress immune response to control deterioration in the body.Compounds that alter immune response are considered Immunomodulatory agent.

The ubiquitous enemy:

Microbes are able to survive on animal and plant products by releasing digestive enzymes directly absorbing the food, or by growth on living tissues (Extra cellular), in which case they are simply bathed in nutrients. Other microbes infect (Invade) animal / human cells (intracellular), where they not only survive, but also replicate, utilizing host-cell energy sources.

Both extra cellular and intercellular microbes can grow, reproduce and infect other individuals. They are many different species of microbes and larger

organisms (such worms) which invade humans, some of which are relatively harmless and some even helpful (e.g. E.coli in our intestines). Many other causes diseases (human pathogens, and there is a constant battle invading microbes and immune- system. Some microbes can even cause the death of their hosts, although this should not be the property of the most successful microbes. The range of organisms that can infect humans. E.g. HIV.[1]

Range of infectious organisms

Bacteria : e.g. E.coli, Staphylococcus, Streptococcus, Mycobacteria

Viruses : e.g. Polio. Pox viruses, Influenza, Hepatitis-B, HIV

Worms (Helminthes) : e.g. Tape worms, Filaria

Protozoan : e.g. Trpanosomes, Leishmania, Malaria

Fungi : e.g. Candida, Aspergillus.

External defenses

1)Physical barriers to entry of microbes

Before a microbe or parasites can invade the host and causes infections, it must first attach to and penetrate the surface epithelial layers of the body. Organisms gain entrance into the body by an active or passive means. For eg.,they might burrow through the skin, or be ingested in food, inhaled into the respiratory tract or penetrate through an open wound. In practice, most microbes take advantage of the fact that we have to breathe and eat to live and therefore enter the body through the respiratory and gastrointestinal tract. Whatever their point of entry, they have to pass across physical barriers such as the dead layer of the skin or living epithelial cells layers which lines the cavities in contact with the exterior such as the respiratory, genitourinary tract or gastrointestinal tract.

ii) Secretions

Secretions at epithelial surfaces are important in defense. The aim is to provide a hostile environment for microbial habitation. Some substances are known to directly kill microbes e.g. lysozymes by digesting proteoglycans in bacterial cell walls: other competes for nutrients (e.g. transferring, Fe), and

others interfere with ion transport (e.g. NaCl). Mucus (containing mucin) secreted by the mucosal epithelial cells coat their surfaces and make it difficult for microbes to contact and bind to them-a prerequisite for entry in to the body. The washing action of tears, saliva and urine also help to prevent attachment of microbes to the epithelial surfaces. In addition, tears and saliva contain IgA antibodies which are secreted across epithelial cells and prevent the attachment of microbes. These antibodies are also present in genitourinary tract. Gastrointestinal, respiratory epithelia and Phagocytes throughout the body are also known to produce a number of small peptides which have potent anti-bacterial properties.

iii) Microbial products and competition

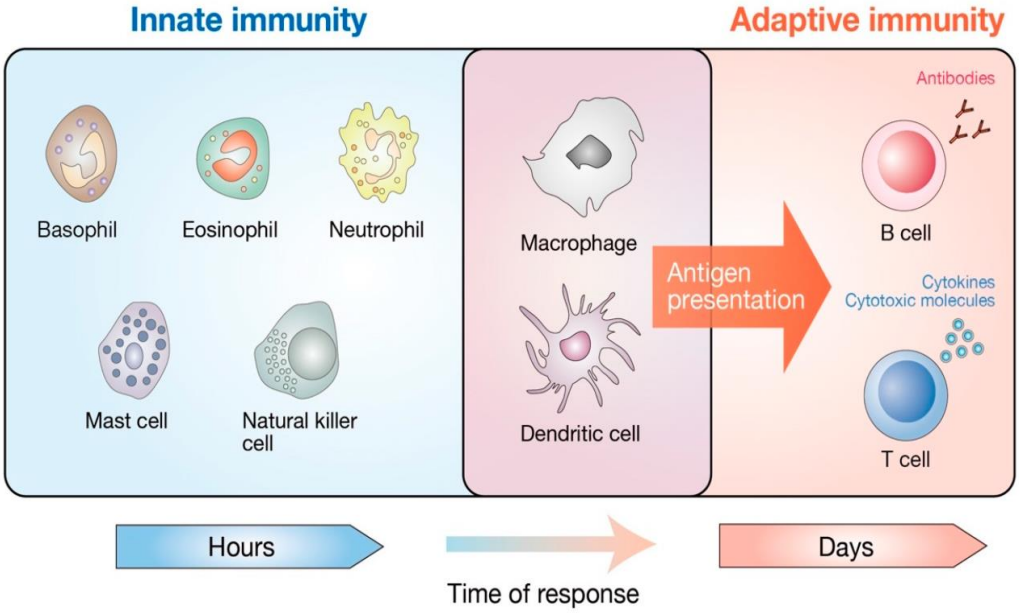
Non pathogenic bacteria also help to protect from infection. These non pathogenic microorganisms are found on the skin, in the mouth and in the reproductive and gastrointestinal tract. The gastrointestinal tract contains many billions of bacteria that have a symbiotic relationship with the host. These bacteria help to prevent pathogens from colonizing and releasing antibacterial substances such as colicins (anti-bacterial proteins) and short- chain fatty acid.[2]

1.1 The Immune System

Immune system is a adaptive defense system within vertebrates, to protect them from invading agents and cancer. It is able to generate varieties of cells and molecules capable of recognizing and eliminating limitless varieties of foreign invaders. Functionally the immune system is divided into two interrelated activities, recognition and response. Immune system is able to recognize foreign substances, and discriminate foreign molecules from own cells and proteins. Once foreign molecule or organism is recognized, immune system responds to eliminate or neutralize these by utilizing the initial recognition information. The same response could be repeated if the immune system encounters the same antigen (memory response).[3]

Immune system is a complex organization of white blood cells, antibodies, and blood factors that protects the body from foreign invaders,

while simultaneously maintaining self-tolerance. A series of specialized epithelial and stromal cells also provide the anatomic environment which regulate various functions of immune system by secreting several critical factors. The immune system is a network of cells, tissues, and organs that work together to defend the body against attacks by "foreign" invaders. These are primarily microbes (germs) -tiny, infection-causing organisms such as bacteria, viruses, parasites, and fungi. Because the human body provides an ideal environment for many microbes, they try to break in. It is the immune system's job to keep them out or, failing that, to seek out and destroy them.



The immune system is a system of biological structures and processes within an organism that protects against disease. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases, cancer and immunodeficiency. Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reaction, it is named as an immunostimulative drug which primarily implies stimulation of non-specific system. Immunosuppressant implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be considered in order to regulate the normal immunological functioning. Hence both immune stimulating agents and immune suppressing agents have their

own standing, so search for better agents exerting these activities is becoming the field of major interest all over the world.[4]

A number of Indian medicinal plants and various 'Rasayana' have been claimed to possess immunomodulatory activity .The use of plant products as immunomodulators is still in a developing stage. A variety of plant-derived materials such as polysaccharides, lectins, peptides flavonoids and tannins have been reported to modulate the immune system. Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine. Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system.

1.2 The Organs of the Immune System:

The immunological system is comprised of the lymphoid tissues and organs of the body. Immune cells have two main sites for their origin, and proliferation or activation (where they are located to target the foreign particles). These are the primary lymphoid organ and the secondary lymphoid organ.

a) Primary lymphoid Organs

Thymus gland and the bone marrows produce the specialized lymphocytes (T-cells and B-cells) and dispatch them through the lymph vessels to the secondary organs for their maturation, proliferation and storage. The bone marrow (soft tissue located in the cavities of the bones), produce and differentiate blood cells. It is the source of stem cells, which differentiate leukocytes. From the bone marrow lymphocytes (T Cell) are sent to the thymus gland to mature, and are then stored in the secondary organs of the lymph system and in the blood stream. B-cells get matured and differentiated in the bone marrow then send to the secondary organs.

b) Secondary Organs

Secondary Organs are the lymph nodes, spleen, tonsils, and Peyer's patches in the small intestines, liver, and appendix. They are the locations where the molecular parts of the immune system gather in readiness to do battle with germs, viruses, and allergens.[5]

Specialized Cells of the immune system

There are several types of cells responsible for specialized function in the immune system, they are mainly classified as two major cell type: leucocytes and lymphocytes.

Leucocytes

Leucocytes (White blood cells) are the main cells of the immune system provide either innate or specific adaptive immunity. They are motile with specialized functions. The number of leucocytes in normal blood ranges between 4,500 and 11,000 per mm^3 . Most of the leucocytes are outside the circulation, and the few in the bloodstream are in transit from one site to another. They are further classified into granulocytes, monocytes, and lymphocytes.

The most numerous of the leucocytes are important mediators of the inflammatory response. There are three types of granulocytes: neutrophils (50-70 %), eosinophils (1-4 %), and basophils (0.5 %).

Neutrophils

One of the major types of cells that recruit to ingest, kill and digest pathogens. They live only a few hours in the blood, some migrate to the tissues to areas of infection or injury. Neutrophils engulf and digest bacteria and other microorganisms and microscopic particles.

Eosinophils

Eosinophils are also motile and phagocytic , and migrate into the tissues. They are particularly important in the defence against parasites, and they participate in hypersensitivity and inflammatory (allergic) reactions. Their cytotoxicity is mediated by cytoplasmic granules.

Basophils

They have many large cytoplasmic granules, which contain heparin and histamine. When aggravated, they release histamine and other mediators that involve in allergic reactions. They also produce cytolcines. Basophils display high affinity surface membrane receptors for IgE antibodies.

Monocytes

Monocytes are also produced in the bone marrow. They constitute up to 10 % of the blood leucocytes. However, the majorities leave; the blood after a few hours and migrate into almost all tissues, where they develop into macrophages. Both monocytes and macrophages are highly adherent, motile and phagocytic. They marshal and regulate other cells of the immune system. They serve as antigen processing-presenting cells and act as cytotoxic cells when armed with specific IgG antibodies.[6]

Natural killer (NK) cells

. Natural killer cells, often referred to as NK cells, are similar to the killer T cell subset (CD8+ T cells). They function as effector cells that directly kill certain tumors such as melanomas, lymphomas and viral infected cells, most notably herpes and cytomegalovirusinfected cells. NK cells, unlike the CD8+ (killer) T cells, kill their targets without a prior "conference" in the lymphoid organs. However, NK cells that have been activated by secretions from CD4+ T cells will kill their tumor or viral infected targets more effectively.

Lymphocytes

Lymphocytes are small white cells found in lymphoid organs and in the blood. They get to the blood stream from the lymph nodes, which function to trap antigens and filter them out of the lymph fluid. Lymphocytes constitute about 25-50 % of the blood leucocytes. They are non-motile and enter the circulation through lymphatic channels. Some lymphocytes leave and renter the circulation, surviving for many years. They are found in large numbers in the secondary lymphoid organs. When stimulated by antigen for example or a

microbe, lymphocyte divides several times into daughter cells and eventually generates a clone of identical lymphocytes.

Some of these cells remain in the circulation; others patrol the tissues of the body. The larger number of lymphocytes capable of reacting to the same microbe is responsible for the immunological memory that is manifested, if the body encounters the same microbe later in life. Lymphocytes are divided into two main classes according to their origin and differentiation. T-Lymphocytes which are produced in the bone marrow pass through the thymus to get mature. The other class is the B- lymphocytes; they do not pass through thymus but get mature in gut associated lymphoid tissues (GALT)

T – Lymphocytes: There are two major classes of T-lymphocytes: T helper (Th) and T- cytotoxic lymphocytes (Tc).

T helper (Th) lymphocytes regulate the antibody-forming function of B- lymphocytes and participate in rejection of transplants. They posses CD4 surface molecules (also called CD4 cells). T helper cells are functionally further subdivided into at least two types, Th-1 and Th-2. The other T lymphocytes those involved in the defenses against virus infection, is called T-cytotoxic lymphocytes (Tc) or T-suppressor (Ts).

Cytotoxic T (Tc) cells are capable of destroying a target cell, that is infected with virus or that expresses some form of foreign antigen. These cells are the major immune effecter of the cellular immune response. They also express CDs molecules.

T-suppressor (Ts) cells act to diminish helper T-cell activity; they directly kill virus infected or cancer cells when the battle is over. They express CD8 molecules. In contrast to helper T cells, Ts cells down-modulate immune responses. Thus, the combination of helper and suppressor cells determines the level of the immune response to any specific antigen.

B- lymphocytes

They have a relatively short life span compared to T-cells. As B-cells mature, they turn into antibody-producing plasma cells found in lymph nodes and in

the spleen.Once the B-cells have created a specific antibody to attack a specific pathogen.[7]

1.3 Immunity

The system consists of innate componen`ts that act rapidly (within hours) but non-specifically (Innate Immunity - involves granulocytes, macrophages and natural killer (NK) cells for examples) and adaptive or acquired components that act specifically, but need time to respond (adaptive immunity - mediated by lymphocytes, initiate in 4-7 days).

Innate immunity

Innate immunity is considered to be antigen independent that occurs without prior exposure to antigens. It can be triggered upon the initial encounter with a foreign substance; its components are often called the first line of defence. Skin is considered as a component of innate immunity. Similarly, the following bodily functions contribute to host defence and are considered parts of innate immunity: the lysosomal enzymes in salivary, lacrimal and vaginal secretions, which have bacteriostatic properties, the cough reflex, which is an important mechanism to clear the bronchial passages of irritants and potential infectious microbes, and the fever response, which is an important reaction to an infection. Important components of the innate immune defence system include phagocytic cells such as Neutrophils, macrophages, NK cells, and the soluble products, type I interferon and complement.[8]

Receptors of the Innate Immune Response

In order to detect PAMPs or DAMPs, cells need tools to recognize them. These tools are protein receptors that can be found on the cell surface as well as internally. In general, they are called pattern recognition receptors or PRRs. These receptors come in families consisting of multiple members. Receptors that recognize PAMPs include the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), the NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and invariant T cell receptors. DAMP receptors are not so

clear-cut. TLRs have been implicated as well as the receptor for advanced glycation end products (RAGE). Also the purinergic receptors that recognize ATP would also fall into this category.[9]

Toll-like Receptors (TLR)

These receptors are found on most cells of the body. They recognize a variety patterns associated with a number of pathogens including virus-associated nucleic acids; bacterial-associated cell wall components, protein, ribosomal RNA and DNA; and protozoan-associated proteins. The majority is found extracellular, but a number are also found intracellular. When stimulated they activate the transcription factor NFκB, which is essential for activating a cell‘s immune functions and set off a signal cascade via MAP kinase (a phosphorylating enzyme).

C-type Lectin Receptors (CLR)

These receptors are specialized in recognizing carbohydrate structures, such as the sugar mannose, which is a common component of fungal cell walls. Thus, these receptors are found on the cell surface.. On the phagocytic cells, it is known that they can participate in endocytosis, the engulfment of particles or pathogens and respiratory burst. Somealso appear to initiate signal cascades similar to TLRs leading to NFκB and MAP kinase activation, but it also appears that they can work in concert with TLRs, enhancing or inhibiting their function.

NOD-like Receptors (NLR)

These receptors are found in the cytoplasm of cells. Traces of their expression is found in most organs of the body and it is probably safe to say that most immune cells express at least some members of the NLR family. These receptors are designed to detect intracellular bacteria and, possibly, endogenous stress molecules and allow the cell to produce one of the most potent inflammatory mediators, Interleukin (IL).

RIG-I-like Receptors (RLR)

Like NLRs, RLRs are also found in the cytoplasm of a cell. Instead of detecting bacterial products, these receptors help detect viral infection. They do this by binding to RNA produced during viral replication. Working together with nucleic-acid detecting TLRs, they lead to NFκB, MAP kinase activation and activation of Interferon regulatory factor (IRF) transcription factors.The IRF transcription factors are necessary to produce cytokines specialized for the control of viral infections. Cytokines are small, secreted proteins used as messengers between cells, which alert surrounding immune cells about danger.

Immune Cells of the Innate Response

Under epithelial layers are resident macrophages, neutrophils, dendritic cells, NK cells, mast cells and a number of T cell-related cells.

Macrophages

The name macrophage is derived from Greek, meaning - large eaters. Their main function is to phagocytize (engulf) pathogens and particles. It does this by wrapping its plasma membrane around particles until they are enveloped and pinched off to form an endosome inside the cell. Once inside the cell, the endosome merges with a lysosome that contains enzymes and acids that can digest the contents. Macrophages also have the ability to generate a respiratory burst, which is a release of oxygen radicals that damage surrounding pathogens and cells. They also can alert and attract other immune cells through inflammatory cytokine release.[10]

Neutrophils

Neutrophils are the main foot soldiers of the innate immune response and are certainly the most abundant. They also have a wide tools to deal with invaders. Like macrophages, neutrophils can phagocytize particles, release a respiratory burst and produce inflammatory cytokines. Unlike macrophages, neutrophils have the internal caches of anti-microbial substances called granules.[11]

Dendritic Cells (DCs)

Dendritic cells are also phagocytic cells, but they have the special ability of initiating an adaptive immune response. Unlike neutrophils and macrophages, Dendritic cells or DCs are not simple foot soldiers. Instead, they function more as spies and provide intelligence about invaders to T cells through a phenomenon called antigen presentation and through cytokine production.

NK Cells

The NK stands for Natural Killer and the name implies their function. These cells, however, do not kill pathogens directly. Instead, these cells have the ability to recognize when other cells are harboring internal pathogens using special receptors and then kill them. Situations where this might occur is during viral and mycobacterial infections. These pathogens easily reside in host cells, finding ways to block lysosome fusion and their own destruction.

Mast Cells

Mast cells are the cells that are responsible for the classic signs of inflammation, which include redness, swelling and heat. Though well known for their association with allergy, they also can detect PAMPs and DAMPs through receptors and become immunologically active. Mast cells exert their functions mainly through cytokine and granule release. Unlike neutrophils, which release antimicrobial substances, mast cells release histamine and heparin. Histamine is well known for its vasodilator function and ability to allow fluid to leak between cells, causing redness and swelling. It also causes inflammatory itching by triggering neurons (unmyelinated C-fibers) responsible for the itch feeling. Heparin prevents blood coagulation.

T cell-like Cells

Most T cells are part of the adaptive immune response as they have adaptive T cell receptors (receptors that learn to recognize pathogens).

NK T cells and UI T cells, however, use invariant T cell receptors (receptors that do not rearrange) or semi-invariant T cell receptors and participate in the innate immune response. The T cells are important for innate immune reactions and the adaptive immune response as they have invariant and variant T cell receptors. Their precise function remains unclear, but they can secrete cytokines and, like the NK T cells above, participate in alerting and strengthening local immune responses.[12]

Non-cellular Systems of the Innate Immune Response

Besides cells, there are also defenses in your body that are ready to react to pathogens as soon as they are encountered, much like booby traps. These systems rely on small proteins that are found within the bodily fluids.

Complement System

The liver synthesizes the proteins of the complement system and they work in concert to aid in phagocytosis, bacteria lysing and immune cell attraction. One can visualize it as a self-assembling machine that starts to assemble as soon as the first proteins are bound and in place. The complement machine is known to be initiated by three different pathways: the classical pathway, the alternative pathway and the lectin pathway. The classical pathway is triggered when antibodies are bound to a pathogen. The alternative pathway is triggered when the victim is unable to block the cascade (normal cells can, while pathogens cannot). The lectin pathway uses free lectin proteins (lectins are proteins that bind sugars) to bind sugars associated with bacterial cell walls).

Acute Phase Proteins

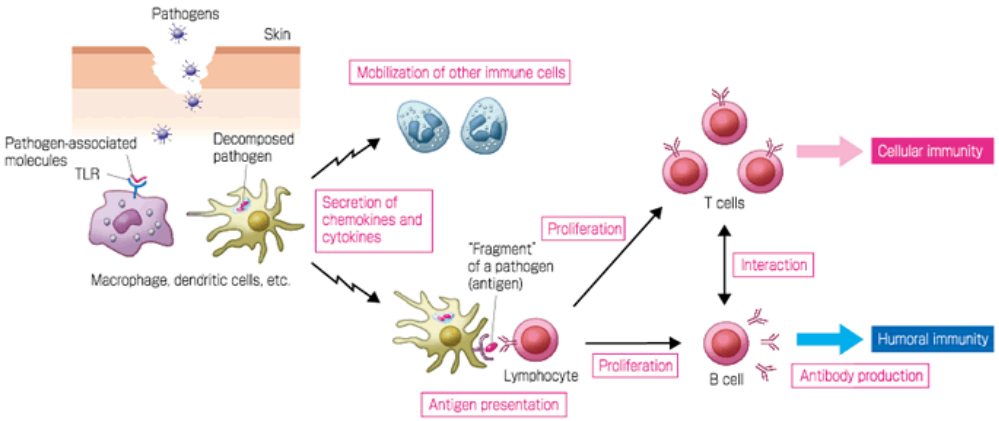
These proteins are also produced by the liver and especially during inflammation when pro-inflammatory cytokines are produced. Many are designed to coat pathogens and have chemotactic properties (have the ability to attract cells). Some inhibit microbial growth by sequestering iron from the environment. The lectins from the lectin pathway of complement activation are considered acute phase proteins.

Anti-microbial Peptides

Often called defensins, these peptides function as natural antibiotics and our produced by cells that guard the external surfaces and internal surfaces such as the skin and the gastrointestinal system. In the skin, the main sources are keratinocytes, mast cell neutrophils, sebocytes and eccine epithelial cells. In the intestines, one of the main producers are the Paneth cells of intestinal crypts.[13]

Acquired immunity

Acquired immunity is antigen dependent and comprises of all the specific immunological reactions associated with lymphocytes. Acquired immunity is subdivided into two effector arms,a) humoral immunity and b) cell mediated immunity. Humoral immunity is mediated by soluble protein molecules known as antibodies that are produced by B lymphocytes. Antibodies are specifically recognized microbial antigens, neutralize the infectivity of the microbes and target microbes for elimination by various effector mechanisms.[14]



Humoral immunity

The defence mechanism against extracellular microbes and their toxins, because secreted antibodies can bind to these microbes and toxins and

assist in their elimination. Antibodies themselves are specialized and different types of antibodies may activate different effector mechanisms.

Cell mediated immunity

It is the result of the activity of many leukocyte actions, reactions, interactions that range from simple to complex. This type of immunity is dependent on the actions of the T (Thymus) lymphocytes, which are responsible for a delayed type of immune response. The T lymphocyte becomes sensitized by its first contact with a specific antigen. It is also called cellular immunity.

1.4 Innate immune system

Innate immune system The term "innate" refers to that part of the immune system with which we are born; that is, it does not change or adapt to specific pathogens(unlike the adaptive immune system). The innate immune system provides a rapid first line of defence, to keep early infection in check, giving the adaptive immune system time to build up a more specific response. Innate immunity consists primarily of a chemical response system called complement, and the endocytic and phagocytic systems, which involve roaming "scavenger" cells, such as macrophages, that detect and engulf extracellular molecules and materials, clearing the system of both debris and pathogens.[15]

1.5 Adaptive immune system

The adaptive immune system is so-called because it adapts or "learns" to recognize specific kinds of pathogens, and retains a "memory" of them for speeding up future responses. The learning occurs during a primary response to a kind of pathogen not encountered before by the immune system. The primary response is slow, often first only becoming apparent several days after the initial infection, and taking up to three weeks to clear an infection. After the primary response clears an infection, the immune system retains a memory of the kind of pathogen that caused the infection.[16]

Should the body be infected again by the same kind of pathogen, the immune system does not have to re-learn to recognize the pathogens, because it "remembers" their specific appearance, and will mount much more rapid and efficient secondary response. The secondary response is often quick enough so that there are no clinical indications of a re-infection. Immune memory can confer protection up to the life-time of the organism (measles is a good example in this regard). The adaptive immune system primarily consists of certain types of white blood cells, called lymphocytes, which circulate around the body via the blood and lymph systems.

T helper Cells (Th cells)

T helper cells or Th cells are crucial cells in the adaptive immune response and they are characterized by a surface protein called, CD4. They hold the key to initiating the functions of cytotoxic T cells and B cells. Furthermore, they can also increase the efficacy of macrophages. Th cells interact with the MHC class II/peptide complexes presented by antigen presenting cells through its receptor, called the T cell receptor (TCR). If a T cell has never before seen antigen, it is called a naïve T cell. In this situation, the T cell will need instruction from a professional antigen presenting cells, usually a DC, about how to perform its function. DCs do this through cell surface proteins call costimulatory molecules and through cytokine expression. This process is consists of three main signals. The first signal is the antigen recognition; the second signal is co-stimulation and the third cytokine exposure. This whole process is referred to as priming of the naïve T cell.

Once primed, the T cells begin to divide; a process that is referred to as expansion or proliferation. The most important set of co-stimulatory molecules is CD80 or CD86 on the DC and CD28 on the T cells. This second signal is necessary to tell the Th cell that there is a problem. If signal one is given without this second signal, the T cell will assume that the antigen is actually harmless and become non-responsive in a process called anergy. Only a DC that has encountered a PAMP or another danger signal will express CD80 or CD86 on its surface reassuring the Th cell that there is, indeed, a problem. Signal three is the secretion of cytokines of the DC. There are several

cytokines important for Th cell education. They most important ones are IL-4, IL-12, IL-6, TGF and IL-10. Th cells will differentiate into different types of Th cells depending on which cytokines prevail. The main types of Th cells are T helper 1 (Th1) cells, T helper 2 (Th2) cells, T helper 17 (Th17) cells, and induced regulatory T cells.

Adaptive Humoral Immune Responses

The word - humor means fluid in Latin and, therefore, humoral immune responses relate to non-cellular systems found in the bodily fluids. Non-cellular components of the innate immunity, however, in immunology most people are not referring to these non - cellular systems when they use the term - humoral immune response. Instead, they are referring to the immune response mediated by antibodies and this is part of the adaptive immune response. The cell behind antibody responses is the B cell. Naïve B cells of the immune system produce rudimentary antibodies until other cells activate them. B cells, unlike the T cells, are not required to interact with DCs; instead B cells reside in lymphoid tissues and fish for antigens that they recognize using their B cell receptors or BCR.

The BCR looks like a surface bound antibody and once it binds a molecule, the B cell engulfs it and much like the phagocytes, digests it. Just like the DC, the B cell will then present pieces of the antigen to Th cells using MHC class II molecules. Primed and activated Th cells, which recognize the presented peptides, are then able to help the B cell through a CD40-CD40 ligand interaction. The Th cell also provides cytokine signals to tell the B cell which kinds of antibodies it should make. This process is reminiscent of the priming process of Th cells. Signal one is the MHC class II/peptide and TCR interaction between the B cell and the T cell. Signal two is the costimulatory help provided by the T cell in the form of CD40- CD40 ligand interactions. And, signal three is the cytokine message provided by the T cell. Helped B cells will then further differentiates into plasma cells, which can produce massive quantities of antibodies.

Antibodies

Antibodies, by themselves, cause very little harm. However, their strength lies in their ability to tag a molecule as harmful and block molecular functions. Antibodies enhance the functions of the innate immune system. They can bind to pathogens and particles to initiate the complement system and induce phagocytosis. They can also block/neutralize molecular interactions. Examples of this function would be an antibody that blocks the toxic effects of diphtheria toxin or antibodies the block viral binding sites to cells. Antibodies also interact directly with cells and can change their function by binding to specific antibody receptors found on the surfaces of immune cells.

Rudimentary Antibodies: IgM and IgD

The first types of antibodies that a B cell can produce are IgM and IgD . The - M and - D refers to different classes of the Fc region. IgM is found as a pentamer, with five individual IgM antibodies bound by their Fc regions in the center forming a star. They are effective at complement activation. IgD is found as a monomer and its function is undefined. However, it has the ability to bind mast cells via an Fcį receptor (į for D) and induce anti-microbial peptide secretion.

IgG

IgG antibodies are found as monomers and they are very potent at stimulating immune responses. They are capable of neutralization, inducing phagocytosis in macrophages and neutrophils via FcU receptors, activation of complement, and also the activation of NK cells.

IgE

IgE antibodies are monomers. They are known to cause mast cell degranulation via binding of Fcİ receptors (İ for E). They are induced during parasite infection and, unfortunately, also during allergy.

IgA

IgA is found as a dimer of two antibodies attached via their Fc regions. It is involved with mucosal defense: found in gastrointestinal system, the respiratory systems. They are particularly effective at neutralization of microbes and toxins. Once the adaptive immune system has formed a response, the body has a long-term record of the invading pathogen in the form of long-lived plasma cells, memory T cells (not covered here) and antibodies. This is why vaccination is so important. It allows your body to create an adaptive immune response against an invader without having to truly become infected. When a body encounters a pathogen for the second time, it's a completely different situation than the first encounter. During a second infection, T cells drawn to the inflammation site will have knowledge to help macrophages, recruit more neutrophils, and kill infected cells. Antibodies will be now present to assist complement activation, the phagocytosis of particles, and even kill microbes. The response will be quicker and more effective.[17]

1.6 Cytokines

Cytokines are a group of low molecular weight regulatory protein secreted by leukocytes and a variety of other cells, in response to a number of inducing stimuli. Cytokines as general act as immune "messenger molecules" that modulate, educate, stimulate, and regulate various aspects of the immune response by acting on cells. Cytokines bind to specific receptors on the surface of target cells. Cytokines that are secreted from lymphocytes are termed lymphokines, whereas those secreted by monocytes or macrophages ate termed monokines. Many of the lymphokines are also known as interleukins (ILs), since they are not only secreted by leukocytes but also affect on leukocytes.

Cytokines include interferons, stimulating factors, or necrosis factor. Some properties of cytokines are given below. Cytokines have been reported to be involved in the immunopathology of several autoimmune diseases including Type 1 diabetes. There is evidence that cytokines could have a direct role in N-cell death. The macrophage released cytokines, TNF-α and IL-1, are

cytotoxic to islet N-cells in vitro. Interleukin-6, also produced by macrophages, is a key mediator of multiple inflammatory and immune responses and regulates insulin secretion in vitro in concert with IL-1. IFN-y, produced by activated T lymphocytes, activates macrophages,enhances class I Major Histocompatibility Complex (MHC) antigen expression and induces class II expression in combination with TNF on normal cultured human islet cells. IFN-y also enhances TNF induced human islet cells cytotoxicity.

Cytokine antagonists

There are several proteins that can inhibit the activity of cytokines. These proteins act by two ways. Either the antagonist can bind to the receptor without activating it, or to the cytokines directly preventing their further binding to the receptors. The best characterized inhibitor is the IL-1 receptor antagonist (IL-1Ra) which binds to the IL-1 receptor (lL-1R) without activation, and blocks it from binding IL-l a or ß . The second group of the inhibitors is the soluble cytokine receptors that binds to cytokine and neutralize their activity. IL-2, IL-4, IL-6, IL-7, INF-y, TNF-a, TNF-13, and (Leukemia inhibitory factor) are among the soluble cytokine receptors that have been detected**.**[18]

Interleukin-2 (IL-2)

Interleukin 2 (IL-2) was one of the first well-characterized interleukin. Initially it was called T cell growth factor (TCGF), and its activity was detected in the supernatant of mitogen-stimulated peripheral blood lymphocytes.It is produced and secreted by activated T helper cells (CD4+), as the major interleukin responsible for proliferation and differentiation of T-cell and B-cell.IL-2 alone induced the proliferation of T-helper 2 (Th-2) cells, which produce predominantly IL-4 and IL-5 and generate IgGI- and IgE-secreting cells as well as eosinophilia. IL-2 as one of the first cytokines produced in draining lymph nodes several days after immunization which supports the important role of IL-2 in initiating T-cell activation in lymphoid tissues.

Interleukin-6 (IL-6)

Interleukin-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. In humans, it is encoded by the IL6 gene. IL-6 is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. IL-6 also plays a role in fighting infection, as IL-6 has been shown in mice to be required for resistance against bacterium Streptococcus pneumonia.

Clinical use:

IL-6 is one of the most important mediators of fever and of the acute phase response. It is capable of crossing the blood and brain barrier and initiating synthesis of PGE2 in the hypothalamus, thereby changing the body's temperature set point. In muscle and fatty tissue, IL-6 stimulates energy mobilization which leads to increased body temperature. IL-6 can be secreted by macrophages in response to specific microbial molecules, referred to as pathogen associated molecular patterns (PAMPs). These PAMPs bind to highly important group of detection molecules of the innate immune system, called pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). These are present on the cell surface and intracellular compartments and induce intracellular signaling cascades that give rise to inflammatory cytokine production. IL-6 is relevant to many diseases such as diabetes atherosclerosis, depression , Alzheimer's Disease, systemic lupus erythematosus , prostate cancer and rheumatoid arthritis.

1.8 Immunomodulation

Modulate and potentiate the weapons of your immune system keeping them in a highly prepared state for any threat it may encounter. With this balancing effect, all subsequent immune responses improve. When your immune system is in this highly prepared state, the invading organisms do not have the time to build up force and strength before the immune system attacks destroys and/or weakens the invader. Immunomodulation is the process of modifying an immune response in a positive or negative manner by administration of a drug

or compound. Many proteins, amino acids, and natural compounds have shown a significant ability to regulate immune responses, including interferon-γ (IFN-γ), steroids, DMG. These are biological or synthetic substances, which can stimulate, suppress or modulate any of the immune system including both adaptive and innate arms of the immune response. Clinically immunomodulators can be classified into following three categories**.**[19]

Immunoadjuvants

These agents are used for enhancing vaccines efficacy and therefore, could be considered specific immune stimulants 31 example in this regard is of Freund‟s adjuvant. The immunoadjuvants hold the promise of being the true modulators of immune response. It has proposed to exploit them for selecting between cellular and humoral, Th1 (helper T1 cells) and Th2, (helper T2 cells) immunoprotective and immunodestructive, and reagenic (IgE) versus immunoglobin G (IgG) type of immune responses, which poses to be a real challenge to vaccine designers**.**

Immunostimulation

These agents are inherently nonspecific in nature as they envisaged enhancing body's resistance against infection. They can act through innate immune response and through adaptive immune response. In healthy individuals the Immunostimulant are expected to serve as prophylactic and promoter agents i.e. as immunopotentiators by enhancing basic level of immune response, and in the individual with impairment of immune response as immunotherapeutic agents

Immunomodulators have the ability to mount an immune response or defend against pathogens or tumors and can safely be used to alleviate hyper- or hypoimmune responses or against various diseases that accompany immune suppression viz. Acquired Immune Deficiency Syndrome (AIDS), Leishmaniasis, Filariasis, Tuberculosis and Malaria. Immunomodulators may also serve as immunological adjuvants to presently available standard drugs or vaccines to boost their efficacy. The capacity of adjuvants to activate antigen presenting cells (APCs) during induction of

primary immune response is of critical importance for development of protective immunity against a number of pathogens which can be cured by these immune modifiers when used prophylactically.

In spite of the availability of drugs against several diseases newer agents are required to fulfill drawbacks of the currently used drug and to combat drug resistance commonly encountered or fast emerging against some pathogens. A number of plants have been identified to possess immunomodulatory or therapeutic efficacy and even active chemical constituents have also been identified.

Aims of Immunostimulation

Immunostimulation constitutes an attractive alternative to conventional chemotherapy and prophylaxis of infections, especially when the host defense mechanisms have to be activated under conditions of impaired immune responsiveness. Immunostimulants or immunopotentiators are compounds leading predominantly to a nonspecific stimulation of the immunological defense system. They do not affect immunological memory cells. Therefore the terms immunomodulation or immunoregulation, denoting any effect on or change of immune responsiveness, very often seem to be more appropriate. Immunoadjuvants are substances that enhance the production of antibodies without acting as antigens themselves. Their effects are often thymus-dependent.

Mechanism of Immunostimulation

Immunological defence is a complicated interplay between nonspecific and specific, cellular and humoral immune responses, stimulation and suppression of immune competent cells, and the influence of endocrine and other mechanisms upon the immune system. Primary targets of the immunostimulant are T or B lymphocytes or the complement system, an increase in phagocytosis by macrophages and granulocytes plays a central role in immunostimulation. Activation of macrophages is probably important for the stimulating agents to remain in contact with the reactive cell. The second

most important role is the stimulation of T lymphocytes, which can be achieved either directly or indirectly, via macrophages.

IMMUNODEFICIENCY DISEASES

Immunodeficiency diseases are condition where the defence mechanism of the body are impaired, leading to repeated microbial infections of varying severity and sometimes enhance susceptibity to malignancies.Deficiency of defence mechanism may involve specific immune functions,humoral immunity cell mediated immunity or both or nonspecific mechanism such as phagocytosis and complement which act in conjunction with specific immune process.immunodeficiency may be classified as primary and secondary.

Primary immunodeficiencies

a) Disorders of specific immunity

1) humoral immunodeficiencies(B cell defects)

- common variable immunodefeciencies
- Trancecobalamine II deficiency
- X –linked agammaglobulemia
- Immunodeficiencies with hyper-IgM
- Selective immunoglobulin immunodeficiencies

2) cellular immunodeficiencies(T cell defects)

- Purine nucleosidephophorylase
- Thymic hypoplasia

3) combined immunodeficiencies(B and T cell defects)

- Ataxia telangiectasia
- Immunodeficiency with short limbed dwarfism
- Wiskott-aldrich syndrome

b) Disorders of complement

1) inhibitor deficiencies

2) Complement component deficiencies

C) Disorders of phagocytosis

1) Jobs syndrome
2) Hyper IgE syndrome
3) Myeloperoxidase[20]

Secondary immunodeficiencies

A variety of factors such as malnutrition malignancy infection metabolic disorders and cytotoxic drugs may lead to deficit in specific and non-specific immunity.Deficiencies of humoral and cellular immune responses may occur secondariliy during the course of many disease process.

PHYSICAL IMMUNOSUPPRESSION METHODS

Surgical manipulation can have a major impact on immune responsiveness. Surgical removal of the bursa, or the thymus, or both in the neonatal period block the development of immunologic competence in the correspondingly dependent lymphoid system. Surgical removal of these tissues after immunologic development, however, has very little effect on immune competence.

Radiation: acts on the lymphoid cells and bone marrow. Radiation damages DNA; thus, cells that are in the process of division or that need to divide to express their immunologic role will be most affected by this exposure. It is used in the treatment of lymphoid malignancies (i.e. Hodgkins disease) and has been attempted in other T-suppressor cell autoimmune disorders. The most common adverse effects of TLI are moderate constitutional symptoms including fatigue, anorexia, diarrhea, abdominal pain, nausea, weight loss, xerostomia, and herpes zoster .

Pheresis: Pheresis is the discriminating removal of a specific blood component (e.g., platelets, plasma, red blood cells, leukocytes, and lymphocytes). Within hours to days, plasmapheresis removes antibodies, immune complexes, hormones, drugs, and other plasma-soluble substances. Plasmapheresis is used in the following immunologically mediated disorders: hyperviscosity syndromes associated with myelomas, mushroom poisoning,

theophylline toxicity, Goodpastures syndrome, autoimmune hemolytic anemia, myasthenia gravis, and systemic lupus erythematosus. Plasma exchange is the process of separating and removing plasma, then replacing it with either fresh frozen plasma or plasma protein fractions. Selective forms of cellular pheresis are used to treat a variety of disorders. For example, plateletpheresis is used for essential thrombocytosis, hemapheresis for sickle cell anemia, and lymphocytopheresis for leukemias.

Blood transfusion:

Transfusions have also altered immune function by either improving renal allograft survival or decreasing bone marrow transplant engraftment survivals

Immunosuppressive Drug Cyclophosphamide: Cyclophosphamide also known as cytophosphane, is a nitrogen mustard alkylating agent from the oxazophorines group. Cyclophosphamide has been shown to supress primary and secondary humoral immune response, delayed type of hypersenstivity, skin graft rejection and animal diseases of autoimmunity. It is used to treat various types of cancer and some autoimmune disorders. A prodrug, it is converted in the liver to active forms that have chemotherapeutic activity. Cyclophosphamide also decreases the immune system's response to various diseases and conditions. Therefore, it has been used in various non-neoplastic autoimmune diseases where disease-modifying antirheumatic drugs have been ineffective it is also used to treat minimal change disease, severe rheumatoid arthritis, Wegener's granulomatosis and multiple sclerosis. Recent clinical studies have shown cyclophosphamide induces beneficial immunomodulatory effects in the context of adaptive immunotherapy. Although the mechanisms underlying these effects are not fully understood, several mechanisms have been suggested based on potential modulation of the host environment, including

1. Elimination of T regulatory cells (CD4+CD25+T cells) in naive and tumor bearing hosts

2. Induction of T cell growth factors, such as type I IFNs, and

3. Enhanced grafting of adoptively transferred tumor-reactive effectors T cells by the creation of an immunologic space.

Azathioprine: Azathioprine (IMURAN) is a purine antimetabolite which is used as a firstline therapy or as an alternative to nitrogen mustard, alkylating agents, for treatment of immune-mediated disease. Azathioprine, an imidazole derivative of 6- mercaptopurine, that acts as an Antimetabolite to inhibit nucleic acid synthesis both de novo and salvage pathways. Rapidly dividing cells, including not only T and B-cell lymphocytes but also, for eg, gut endothelium and bone marrow elements, are most susceptible to the drug's antiproliferative effects. In addition, it has been suggested that the imidazole residue of Azathioprine alkylates thiol groups on T-cell surface membranes block antigen recognition. Myelosuppression, particularly leukopenia, is the most common and serious side effect of Azathioprine therapy. It induced hepatotoxicity due to endothelial damage and may cause veno-occlusive disease. Other toxicities relate antimitotic effects, including alopecia, oral ulcers, nausea, vomiting, diarrhea, anorexia and esophagitis. The increased incidence of malignancies with Azathioprine tends to be less than that observed with other immunosuppressants, probably owing to its modest relative potency.

Methods for Testing Immunological Factors

The routine process for screening is to extract single ingredient or single distilled fraction from herbal drugs, determine its bioactivity by the classic pharmacological means. The whole animal model is the most classic pharmacological screening model, which is very important at the aspect of medicine evaluation because it can apparently respond to the efficacy, side effect and toxicity of medicines in whole.

In vitro methods:

1) Mitogen induced lymphocyte proliferation
2) Inhibition of T cell proliferation
3) Inhibition of histamine release from mast cells

4) Chemiluminescence in macrophages

5) PFC (plaque forming colony) test in vitro

6) Inhibition of dihydro-orotate dehydrogenase

In vivo methods:

1) Spontaneous autoimmune diseases in animals

2) Acute systemic anaphylaxis in rats

3) Passive cutaneous anaphylaxis

4) Anti-anaphylactic activity (Schultz-Dale reaction)

5) Arthus type immediate hypersensitivity

6) Delayed type hypersensitivity

7) Reversed passive arthus reaction

8) Adjuvant arthritis in rats

9) Collagen type II induced arthritis in rats

10) Proteoglycan-induced progressive polyarthritis in mice

11) Experimental autoimmune thyroiditis

12) Coxsackievirus B3-induced myocarditis

13) Porcine cardiac myosin-induced autoimmune myocarditis in rats

14) Experimental allergic encephalomyelitis

15) Acute graft versus host disease (GVHD) in rats.

LITERATURE REVIEW

Dash et al., 2006, *Heracleum nepalense* The immunostimulatory potential of this plant was investigated by in vitro phagocytic index and lymphocyte viability tests, using IFN-a-2b, a known immunostimulant drug, as the standard. Other tests such as carbon clearance, antibody titer and DTH were studied in mice by using levimasole as the standard. The dried root extract and isolated quercetin glycoside significantly increased the in vitro phagocytic index and lymphocyte viability in all assays. They also showed a significant increase in antibody titer, carbon clearance and DTH in mice[42].

Lu Yin et al., 2007, *Actinidia macrosperma* (AM) is a medicinal plant in China and has been well known for its activities against cancers, especially of lung, liver and digestive system. The immunomodulatory effects of AM aqueous extract were examined using S180-bearing mice. The immunomodulatory effect was dosedependent in a nonlinear fashion with the optimal dose of 100 mg/kg. The AMinduced antitumor effects were at least partially indirect and were associated with the modulation of immune functions.[43]

Huang et al.,2009, *Withania coagulans* (Family Solanaceae) contain six new withanolides, withacoagulins A-F (1-6, resp.), together with ten known withanolides, 7-16, were isolated from the aerial parts of Withania coagulans. These compounds, including the crude extracts of this herb, exhibited strong inhibitory activities on the T- and B cell proliferation. From, that the results showed Withania coagulans has a immunosuppressive activity.[44]

Ankur et al., 2010, *Aegle marmelos,* belongs to family Rutaceae is generally known as bael fruit. They were evaluated for potential immunomodulatory activity using the in vitro Polymorphonuclear leukocyte function test. Both Methanolic and Ethanolic extract of Aegle marmelos leaves were evaluated for their immunomodulatory activity. The extract was tested for hypersensitivity and hemagglutination reactions; using sheep red blood cells (SRBC) antigen methanol extract exhibited a significant increase in the

percentage phagocytosis versus the control. The Methanolic extract was found to stimulate cell mediated and antibody mediated immune responses in rats.[45]

Atul et al., 2011, To study the immunomodulatory activity of saline extracts of leaves of *Aloe vera Linn*. (Family: Liliaceae) on the albino mice. The saline extract of leaves of Aloe vera was administered orally according to their body weight in mice. The assessment of immunomodulatory activity on specific and nonspecific immunity was studied by administration of test extract. The study demonstrates that A. vera triggers both specific and non-specific responses to a greater extent.[46]

Jagdap et al., 2011, The immunomodulatory activity of an Indian medicinal plant i.e. ethanolic extract from *Dodonaea viscosa* L.F. namely DV was studied for there phagocytic activity, cell mediated and humoral immune system on rat/mouse. Immunomodulatory effect was assessed in carbon clearance test, delayed type of hypersensitivity (DTH), T-cell population test, and sheep erythrocyte agglutination test (SEAT) in animal treated with DV at doses of 200 and 400 mg/kg.[47]

Jasminum sambac

Upaganlawar A B et al., 2009, Different extracts viz. ethyl acetate and water extract of leaves of *Jasminum sambac* at a dose of 300mg/kg, p.o on blood glucose level for 21 days were evaluated in alloxan induced diabetic rats. Aqueous extract showed significant ($p<0.01$) reduction of elevated blood glucose level. Ethyl acetate extract was found to be less active as compared to aqueous extract at 300mg/kg dose level .[31]

Radu S et al., 2002 The screening of antitumour activity and antimicrobial activity against Gram +ve bacteria, Gram -ve bacteria, yeast and fungi was carried out on isopropanol extracts prepared from isolates of endophytic fungi isolated from *Jasminum sambac* (L.) Ait. in Malaysia. Sixteen endophytic fungal isolates tested were also found to exhibit antitumor activity in the yeast cell-based assay test around the disk of 6 mm or more were defined as positive for biological activity.[48]

Hussaini A L et al., 2011 Antimicrobial activity using ethanol extract of *Jasminum sambac* Ait. (Oleaceae) leaves, flowers, fruits and stem bark was evaluated against nine bacteria and four fungi using Agar diffusion assay and Minimum Inhibitory Concentration (MIC) determinations. Study shows that flowers and leaves extracts of Jasminum sambac exhibited almost good activity (10-15mm inhibition zone) against Gram +ve bacteria including the Methicillin resistant Staphylococcus aureus (MRSA) and Bacillus subtilis while a moderate activity was recorded against Gram -ve bacteria including Escherichia coli and Klebsiella pneumonia.[49]

Abdoul-Latif et al., 2010 Antioxidant activity using essential oil and methanol extract of *Jasminum sambac* (L.) Ait. leaves was evaluated by two complementary test systems, namely DPPH free radical scavenging and -carotenelinoleic acid assays. Butylated hydroxytoluene (BHT) was used as positive control in both test systems. In the βcarotene-linoleic acid system, oxidation was effectively inhibited by *Jasminum sambac.*[32]

Rahman et al., 2011 Analgesic activity using dried leaves extract of *Jasminum sambac* (L.) Aiton (Family - Oleaceae) was evaluated. The extract produced significant ($P<0.001$) writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg of body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.[33]

Harisaranraj R., 2010, Ethanol flower extract of *Jasminum sambac* (L.) Ait. was evaluated against the etiologic agents of acne vulgaris using agar well diffusion and broth dilution methods. The results from the disc diffusion method showed that ethanol extract of Jasminum grandiflorum could inhibit the growth of Propionibacterium.[34]

Chiang L C et al., 2003 Anti-herpes simplex viruses (HSV; including HSV-1 and HSV-2) and antiadenoviruses (ADV; including ADV-3, ADV-8 and ADV-11) activities of hot water (HW) extract of Jasminum sambac flowers was evaluated using XTT-based colorimetric assay. Results showed that the tested hot water extracts exhibited anti-HSV and antiADV activities at different magnitudes of potency.[35]

Shrivastav P et al., 1988 Efficacy of jasmine flowers (*Jasminum Sambac* (L.) Ait.) applied to the breasts to suppress puerperal lactation was compared with bromocriptine. Both bromocriptine and jasmine flowers brought about a significant reduction in serum prolactin, the decrease was significantly greater with bromocriptine. Jasmine flowers seem to be an effective and inexpensive method of suppressing puerperal lactation and can be used as an alternative in situations where cost and nonavailability restrict the use of bromocriptine.[36]

Hongratanaworakit T et al,2010, The effect of aromatherapy massage with jasmine oil (*Jasminum sambac* L., Oleaceae) on humans was investigated. Human autonomic parameters, i.e. blood pressure, pulse rate, blood oxygen saturation, breathing rate, and skin temperature, were recorded as indicators of the arousal level of the autonomic nervous system. In conclusion, our results demonstrated the stimulating/activating effect of jasmine oil and provide evidence for its use in aromatherapy for the relief of depression and uplifting mood in humans.[37]

Kalaiselvi M et al .,2011, Anti-lipid peroxidation effect of methanol extract of J. sambac (L.) Ait. using the standard antioxidants Butylated hydroxytoluene (BHT), Vitamin C, Vitamin E and Rutin was evaluated. The methanol extract of the J. sambac (L.) Ait. flowers showed anti-lipid peroxidative effect which is similar to that of all standards. Results of this study suggests that the methanol extract of J. sambac (L.) Ait. can be used as therapeutic agents to treat against various diseases caused by free radicals and other chemical agents.[38]

Kuroda K et al., 2005 The effects of the odor of jasmine tea on autonomic nerve activity and mood states in a total of 24 healthy volunteers. R–R intervals and the POMS test were measured before and after inhalation of the odors for 5 min. Both jasmine tea and lavender odors at perceived similar intensity caused significant decreases in heart rate and significant increases in spectral integrated values at high-frequency component in comparison with the control ($P < 0.05$). In the POMS tests, these odors produced calm and vigorous mood states. Thus, the low intensity of jasmine tea odor has sedative effects on both autonomic nerve activity and mood states, and (R)-(–)-linalool, one of its components, can mimic these effects.[39]

Baby et al., 2010 ,The antistress activity of the methanol extract of *Jasminum sambac* (L.) Ait. leaves was studied against swimming stress induced gastric ulceration in rats and swimming endurance test in mice. Antistress activity of Jasminum sambac Linn. leaves was compared with that of Geriforte (43mg/kg), which was used as standard. Methanol extract of *Jasminum sambac* at a dose of 100 mg/kg and 200 mg/kg p.o., exhibited good antistress effect in both the tested models.[40]

Lis-Balchin M et al,2002, Spasmolytic activity of Jasmine on guinea pig ileum (post synaptic and not atropine like) and rat uterus was evaluated. The mechanism of action of the spasmolytic activity, studied in vitro using a guinea-pig ileum smooth muscle preparation, was postsynaptic and not atropine-like. The spasmolytic effect of Jasmine absolute was most likely to be mediated through cAMP, and not through cGMP. The contradictory effect in vitro and in vivo has been suggested probably due to the solely physiological effects of jasmine absolute in vitro (producing a relaxation) compared with that in vivo, where it has a strong psychological input, producing a stimulant effect in man and enhanced movement in animals.[41]

Talib et al., 2010 Ethanol extract of Jasminum sambac (L.) Ait. used traditionally as anticancer agents was evaluated in vitro for their antiproliferative activity against Hep-2, MCF-7, and Vero cell lines. *Jasminum sambac (L.) Ait.* extract demonstrated significant antiproliferative activity against one or more of the cell lines[50]

Rath et al., 2008 Antibacterial activity of jasmine (Jasminum sambac L.) flower hydro distilled essential oil, synthetic blends and six major individual components was evaluated against Escherichia coli (MTCC 433) strain. Study proved the antibacterial activity and mechanism of action of jasminum sambac natural oil and its synthetic blends against E. coli strain[51]

AIM AND OBJECTIVE

General objective is to evaluate *in- vivo* immunomodulatory activity of ethanolic extract of *Jasminum sambac* flowers.

The literature revealed that chemical constituents namely phenols, flavonoids,saponins, steroids,cardiac glycosides, coumarins, essential oils are present in the flower of Jasminum sambac. Leaves contain major phytoconstituents as alkaloids, glycosides, saponins, flavonoids and terpenoids. Mainly the Iridoid glycosides are present. These include sambacin, Jasminin, Sambacoside A, Sambacolingoside. Flavonoids include quercetin , isoquercetin , rutin, kempferol and luteolin , Secoiridoid glucoside- sambacolignoside along with oleoside 11-methyl ester.Oligomeric irridoids like molihuasides A is a dimeric irridoid glycoside and Molihuasides C-E is a trimeric irridoid glycoside.[18,19]

And previous studies revealed that it shows antidiabetic activity. From the literature, *Jasminum sambac* have been screened for some pharmacological activities and found to possess spasmolytic, anti-inflammatory, analgesic, antimicrobial, antiulcer, antioxidant, cytoprotective, chemopreventive, breast cancer, wound healing and anti-acne activities but number of other pharmacological activities are yet to be explored.[18,19]

The immunomodulatory activity of *Jasminum sambac* in flowers was not proven still now. So I have selected crude extract of this flowers to evaluate immunomodulatory activity.

PLAN OF WORK

1) Collection and authentification of plant and plant materials.
2) Clean and perform size reduction of plant materials.
3) Plant material dried in shade and pulverized dried materials.
4) To perform the extraction and characterisation of ethanolic extract.
5) To perform preliminary phytochemical studies.
6) To evaluate the acute toxicity study of ethanolic extract.
7) To evaluate in vivo immunomodulatory activity of ethanolic extracts of *jasminum sambac* flowers by following methods.

    a) Complete blood count
    b) Neutrophil adhesion test
    c) Heamagglutinating antibody titre
    d) Delayed type hypersentivity reaction

8) Statistical analysis using ANOVA followed by Dunnett's test

MATERIALS AND METHODS

Collection of Materials, Chemicals and Drugs

- 96% ethanol,carboxy methyl cellulose.
- The Sheep Red Blood Cells (SRBCs) were collected from Veterinary College IRT, Perundurai.
- The cyclophaspamide vial and Levamisole (Cipla Limited- India) was purchased from local pharmacy, perundurai.
- The Humoral antibody tests and complete blood count were performed in origin diagnostic laboratories, Coimbatore.

Selection of animals

Wistar albino rats weighing around 120-330gms is selected for the experiment. The animals were checked to confirm that they were free from any disease. The rats were collected from the animal house of Nandha College of Pharmacy and Research Institute, Erode-52.

Maintenance of animals

The rats that were selected were brought into the laboratory 2 days before the commencement of experiment for acclimatization. They were provided with standard laboratory rodents chow diet obtained from (Pranav agro industries Ltd, Bangalore) and free access to water. A 12 hour day and dark cycle and room temperatures at 27^{o}C were maintained.

Plant collection and Authentification

The plant was collected from Coimbatore and authenticated by the botanist, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. The voucher specimen no. 659 was deposited in the herbarium for future reference

Plant material

After identification of the plant, flowers are dried in a shade. It was then grind into powder for extraction.

Animal approval

The study was conducted after obtaining approval from Institutional Animal Ethics Committee (IAEC) and the experimental procedures were in accordance to the guidelines of proposal No: NCP/IAEC/2019-20/23.

EXTRACTION OF FLOWER MATERIAL

Ethanolic extraction

Jasmine flowers were collected and cutting the flower to small. Moreover, jasmine flowers were extracted to ethanol (96%) through soxhlet at room temperature for three dsys. Furthermore, filtrate and residue were separated by filter paper. Filtrate evaporated using heating process and were stored at the room temperature protected from sunlight. The extract were used for the in vivo study by dissolving in 1.0% w/v carboxymethylcellulose sodium (CMC-Na).[53-55]

PHYTOCHEMICAL SCREENING

The qualitative phytochemical studies were performed for testing different chemical groups present in the extract.

REAGENTS

Dragendroff's Reagent

Solution (A): Dissolve 0.85 g basic bismuth nitrate in glacial acid and 40 ml of distilled water under heating.If necessary filter**.**

Solution(B): Dissolve 8 gm of potassium iodide in 30ml of distilled water.

Stock solution: solution (A) +(B) are mixed 1:1.

Libermann- Burchard Reagent

50 ml of acetic anhydride and 5ml of conc.sulphuric acid were added carefully to 50ml absolute ethanol**.**

Wagner's Reagent

Dissolve 1.27gm iodine and 2 gm of potassium iodide in 5 ml of water and make up to volume100ml with distilled water.

Lead Acetate Reagent

25 mg of lead acetate dissolved in 100ml of sodium hydroxide solution.

Hager's Reagent

3ml of saturated aqueous solution of picric acid.

TEST FOR ALKALOIDS

Wagner's test:

2 mg of aqueous extract was acidified with 1.5 per cent v/v of hydrochloric acid and a few drops of Wagner's reagent were added.

Formation of yellow or brown precipitate indicates the presence of alkaloids.

Hager's test:

To 2 mg of the aqueous extract taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow precipitate confirms the presence of alkaloids.

Dragendorff's test:

To 2 mg of the aqueous extract 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To above solution 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

TEST FOR FLAVONOIDS

Shinoda's test :

To 0.5 ml of the aqueous extract in test tube magnesium turnings were added followed by dil. HCl. Formation of pink, reddish or reddish-brown colour indicated the presence of flavonoids.

Lead acetate test:

5ml of extract solution was added with 1ml of lead acetate solution.white precipitate indicated the presence of flavonoids

Zinc hydrochloride test:

To the test solution, add a mixture of zinc dust and conc.HCL.It gives red colour after few minutes.

TEST FOR TANNINS

Ferric chloride test : To 1-2 ml of the aqueous extract, few drops of 5 % w/v ferric chloride solution were added. Appearance of green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudotannins.

Gelatin test: To 2 ml of extract, 1% gelatin solution containing 10 % sodium chloride was added. White Precipitate formation indicated the presence of tannins.

TEST FOR STEROIDS

Liebermann-Burchard's test: 2 mg of the extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.

Salkowski reaction Test : The extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Red colour indicated the presence of steroids

TEST FOR SAPONINS

Froth formation test: 2 ml of the aqueous extract was shaken vigorously with water in a test tube and left for 3 min. Formation of constant froth indicated the presence of saponins

Heamolysis test

0.2ml of solution of saponin(prepared in 1% normal saline) to 0.2 ml of blood in normal saline and mix well. Centrifuge and note the red supernatant compare with control tube containing 0.2 ml of 10%blood in normal saline diluted with 0.2ml of normal saline.

TEST FOR PHENOLS

Ferric chloride test: To 2ml of aqueous extract 1ml of ferric chloride is added. Blue,purple, violet or red brown colour indicates the presence of phenols.

Bromine water test:

To 1ml of extract add bromine water.if color of bromine disappear indicates the presence of phenol

Acute Oral Toxicity Test

Acute toxicity for ethanolic extract of flower will be done according to the office of pollution prevention and toxics (OPPT.) The overnight fasted rat is weighed and selected. The extracts will be dosed in a stepwise procedure, by using up and down or stair case method. The two animals selected with a dose of 50 mg/kg. Orally and examined for 24h for mortality. Subsequent dose are then increased to attain maximum non lethal and minimum lethal dose. The extract was found to be safe at the dose of 5g/kg per oral. Maximum safe dose (5g/kg) corresponding to 400 mg/kg and 200 mg/kg were selected as high and low doses respectively.[56]

Immunomodulatory activity

Group treatments and dosing of animals

Wistar albino rats were divided into 5 groups .each group consist of five animals.

Rats in the normal control group (Group I,VI) received 1%carboxy methyl cellulose throughout the 2 weeks of study period. Rats in the test groups (Group II–V and VII-X)were treated with an immunosuppressant (cyclophosphamide) at doses of 100 mg/kg bwt on day 0 of the study by subcutaneous injection

FOR 1-14 DAYS

Group I,VI : Normal control receiving 1% carboxy methyl cellulose orally.

Group II, VII: Negative control .

Group III ,VIII : Positive control receiving lavamisole50mg/kg orally.

Group IV,IX : EEJSF dose of 200mg/kg orally.

Group V,X : EEJSF dose of 400mg/kg orally.

INNATE IMMUNE RESPONSE

DETERMINATION OF COMPLETE BLOOD COUNT (CBC)

Two milliliters of fresh blood was drawn by Retro orbotal puncture for each of the animals in Group. Group I, II , III, IV, V on the 14th day into ethylenediaminetetraacetic acid (EDTA)-containing vacutainers. It was then analyzed at the laboratory using an automated Beckman coulter A-T Pierce hematology analyzer for the complete and differential blood cell counts.[57]

NEUTROPHIL ADHESION TEST

On the 14th day of the treatment, blood samples from all the groups were collected by puncturing retro-orbital plexus under mild ether anesthesia. Blood was collected in vials pre-treated by disodium EDTA and analysed for total leukocyte count (TLC) and differential leukocyte count (DLC) by fixing blood smears and staining with Leishman's stain. After initial counts, blood samples were incubated with nylon fiber (80 mg/mL of blood sample) for 15 min at 37 °C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and percent neutrophil gives neutrophil index of blood sample. Percent neutrophil adhesion was calculated as follows: Neutrophil adhesion=NIu-NIt ×100÷NIu Where NIu is neutrophil index before incubation with nylon fiber; NIt is neutrophil index after incubation with nylon fiber.[58]

ADAPTIVE IMMUNE RESPONSE AGAINST SHEEP RED BLOOD CELLS (SRBCS)

ANTIGEN PREPARATION

Fresh blood was collected from healthy sheep at IRT veterinary college perundurai and mixed with sterile Alsever's solution (1:1). The blood was then centrifuged at 1609.92×g for 5 min to enable red blood cells to settle at the bottom of the test tube. The supernatant was discarded, leaving sheep red blood cells (SRBC) pellets that were washed three times with pyrogen-free phosphate buffered saline (pH 7.2). They were then kept under refrigeration for use in the immunization and challenge study.

HAEMAGGLUTINATING ANTIBODY (HA) TITRE

On 14th day blood samples from group VI - X were collected in centrifuge tubes from all animals separately by retro orbital puncture and subjected to centrifugation at 5000 rpm for 15 minutes. Sera were obtained and antibody levels were determined using haemagglutinating technique . Individual 96 well plates were employed for evaluation of each

sample. 25µl of individual test serum was poured in the microtitration plates separately. Two-fold serial dilutions were prepared in 25µl of phosphate buffer saline (PBS). Afterwards, 25µl of 10% (v/v) SRBCs in PBS was placed in all wells of each plate and mixed gently. The microtitration plates were incubated at 37ºC for 2 h and observed visually for agglutination. The reciprocal of the highest dilution of the test serum giving 50% agglutination was expressed as the HA titre. The percentage of agglutination was calculated by the method of estimating fifty percent end-points in serological titrimetry.[59]

DELAYED TYPE HYPERSENSITIVITY (DTH) RESPONSE

Delayed type hypersensitivity response was determined by inducing immunogenic response using SRBCs in rats with little modifications.. On day 7 of the study, all the Group VI- X were primed by subcutaneously injecting 0.1 mL of suspension containing 1×108 SRBC into the right hind footpad. The contralateral paw also received an equal volume of 0.1% phosphate buffered saline (PBS). The administration of EEJSF was continued until the 14th day. On the 14th day, the animals were challenged by subcutaneously injecting 0.1 mL of 1×108 SRBCs into the left hind footpad of the rats. The extent of delayed-type hypersensitivity (DTH) response in the rats was determined by measuring the footpad thickness after 0 4, 8, and 24 h of challenge using vernier callipers. The difference in the thickness of the right hind paw and the left hind paw was then used as a measure of DTH reaction and was expressed as a mean percent increment in thickness/edema.[60]

STATISTICAL ANALYSIS

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.P values < 0.05 were considered significant.[61]

PLANT PROFILE

Jasminum sambac (L.) Aiton.

SCIENTIFIC CLASSIFICATION:

Taxon : Jasminum sambac (L.)

Family : Oleaceae

Tribe : Jasmineae

Group : Dicot

Class : Magnoliopsida – Dicotyledons

Subclass : Asteridae

Order : Scrophulariales

Genus : Jasminum

Species : Jasminum sambac[21]

Synonym: Arabian jasmine or Sambac jasmine

VERNACULAR NAMES

Tamil- Mallikaipu, Anangam, Iruvachi, Iruvadi, Karmugai, Malli, Maladi, Peramalli

Kannada- Dundu Mallige

Malayalam -Cherupichakam, Chirakamulla

Bengali- Bel/Beli

Hindi and Marathi-Mogra, Chamba, Bel, Motia, Mugra

Telugu- Mallepuvvu, Boddumalle, Bondmalle, Gundemalle.[22]

BOTANICAL DESCRIPTION

Jasminum sambac is an evergreen vine or shrub reaching up to 0.5 to 3 m (1.6 to 9.8 ft) tall. The species is highly variable, possibly a result of spontaneous mutation, natural hybridization, and autopolyploidy. Cultivated *Jasminum sambac* generally do not bear seeds and the plant is reproduced solely by cuttings, layering, marcotting, and other methods of asexual propagation.

The leaves are ovate, 4 to 12.5 cm (1.6 to 4.9 in) long and 2 to 7.5 cm (0.79 to 2.95 in) wide. The phyllotaxy is opposite or in whorls of three, simple (not pinnate, like most other jasmines). They are smooth (glabrous) except for a few hairs at the venation on the base of the leaf.

The flowers bloom all throughout the year and are produced in clusters of 3 to 12 together at the ends of branches. They are strongly scented, with a white corolla 2 to 3 cm (0.79 to 1.18 in) in diameter with 5 to 9 lobes. The flowers open at night (usually around 6 to 8 in the evening), and close in the morning, a span of 12 to 20 hours.[23]

DISTRIBUTION

Jasminum sambac is native to southern Asia, in India, Myanmar and Sri Lanka. Jasmine plant is found in almost all the parts of India[24].

PHYTOCHEMICAL CONSTITUENTS

Flowers: contains 3-hexenol, 2- vinylpyridine, indole , myrcene, linalool , geranyl linalool), alpha terpenol, beta terpenol, linalyl acetate , nerolidol, phytol , isophytol , farnesol, eugenol, benzyl alcohol, methyl benzoate, benzyl cyanide, benzyl acetate, methyl dihydrojasmonate, methyl anilate, cisjasmone, methyl N-methylanthranilate, vanillin , cis-3-hexenylbenzoate, benzoate, methylpalmitate and methyl linoleate, 8,9-dihydrojasminin, 9- deoxyjasminigenin.

Glycosidic aroma precursor like benzyl 6-Oβ-D xylopyranosyl-β-D-glucopyranoside (βprimeveroside), 3(2-phenylethyl 6-O-α-Lrhamnopyranosiyl-β-D-glucopyranoside (βrutinoside) are identified from the flowers.

Benzyl acetate, benzyl alcohol and cisjasmone give the fruity aromatic jasmine qualities. Linalyl- β-D-glucopyranoside and its 6-O-malonate, the aroma precursor of linalool and farnesol give the flowery character and are the main compounds involved in the production of scented tea. Yellow-flower of some Jasminum spp. contains verbascoside but are absent from the white-flowered J. sambac.[25]

TRADITIONAL AND MODERN USES

Jasminum sambac has many medicinal properties like anti-depressant, antiseptic, cicatrisant, aphrodisiac, expectorant, anti-spasmodic, galactogogue, sedative, parturient, uterine etc. The *Jasminum sambac* flower is used for removing intestinal worms and is also used for jaundice and venereal diseases.

The flower buds are useful in treating ulcers, vesicles, boils, skin diseases and eye disorders. The leaves extracts against breast tumours. The leaves are antiseptic and are useful for wounds or acne when used as a poultice. Drinking Jasmine tea regularly helps in curing cancer.

The dried flowers of *Jasminum sambac* are used by the Chinese to flavor jasmine tea. Jasmine tea is most commonly consumed with or after meals as a digestive aid.

Jasminum sambac oil is used for making perfumes, creams, shampoos, soaps. Its flowers are used to flavor Jasmine tea and other herbal or black tea. In India Jasmine flowers are stringed together to make garlands.[26,27]

PHARMACOLOGICAL STUDIES:

FLOWER :

1) Anti bacterial and antimicrobial activity
2) Antiacne activity
3) Anti-herpes Simplex Viruses and Antiadenoviruses Activity
4) Suppression of puerperal lactation
5) Antilipidemic activity
6) Anticancer activity

LEAVES :

1) Antidiabetic activity
2) Antitumour activity
3) Antimicrobial activity
4) Antioxidant activity
5) Analgesic activity
6) Antistress activity
7) Autonomic Nervous Sedative Effect

JASMINE OIL:

1) Antibacterial activity
2) Autonomic Nervous Stimulating Effect[28-30]

RESULTS

PERCENTAGE YIELD OF ETHANOLIC EXTRACT OF *Jasminum sambac* FLOWERS

Percentage yield of ethanolic extract of *jasminum sambac* flowers that was used in the study was calculated as follows. Percentage yield of extract = [weight of final extract/ sample material weight] x 100

=[18 grams/300 g] x 100

=6.00 %

TABLE 1: QUALITATIVE PYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF *Jasminum sambac* FLOWERS

Compounds	Results
Alkaloids	-
Flavonoids	+
Phenols	+
Saponins	+
Steroids	+
Tannins	+

+ : moderate, - : negative

TABLE 2: EFFECT OF VARYING DOSES OF ETHANOLIC FLOWER EXTRACT OF *Jasminum sambac* ON HEMATOLOGICAL PARAMETERS OF WISTAR ALBINO RATS TAKEN ON DAY 14 OF THE STUDY

Parameter	**Group I (1% cmc)**	**Group II Negative control**	**Group III (LEV 50mg/kg)**	**group IV EEJSF (200mg/kg)**	**group V EEJSF (400mg/kg)**
WBC (10^3/µL)	12.24±0.89	9.81 ^{a} ±0.78	15.29 ^{b} ±0.89	11.40^{b}±0.56	13.85^{b}±0.67
NEUT (10^3/Ml	7.14±0.24	5.95^{a}±0.15	8.50^{b}±0.27	6.96^{b}±0.12	8.09^{b}±0.15
LYMP (10^3/Ml)	3.75±0.10	2.96^{a}±0.21	4.73^{b}±0.11	3.11^{ns}±0.20	3.96^{b}±0.12
MON (10^3/µL)	0.9±0.03	0.5^{a}±0.01	1.42^{b}±0.14	0.82^{b}±0.04	1.24^{b}±0.15
EO (10^3/µL)	0.44±0.02	0.39^{a}±0.02	0.59^{b}±0.02	0.49^{ns}±0.01	0.52^{b}±0.01
BAS (10^3/µL)	0.02±0.001	0.01^{ns}±0.002	0.04^{b}±0.003	0.02^{ns}±0.002	0.04^{b}±0.003
RBC (10^3/µL)	6.92±0.14	5.45^{a}±0.15	7.78^{b}±0.25	6.32^{b}±0.67	6.98^{b}±0.23
HGB (g/dL)	12.24±0.71	10.06^{a}±0.1	13.12^{b}±01	11.34^{b}±0.62	13.05^{b}±0.31
PLT (103/µL)	674±15.02	589^{a}±11.6	714^{b}±14.6	601^{ns}±12.1	708^{b}±10.8

Values expressed as mean±SEM,

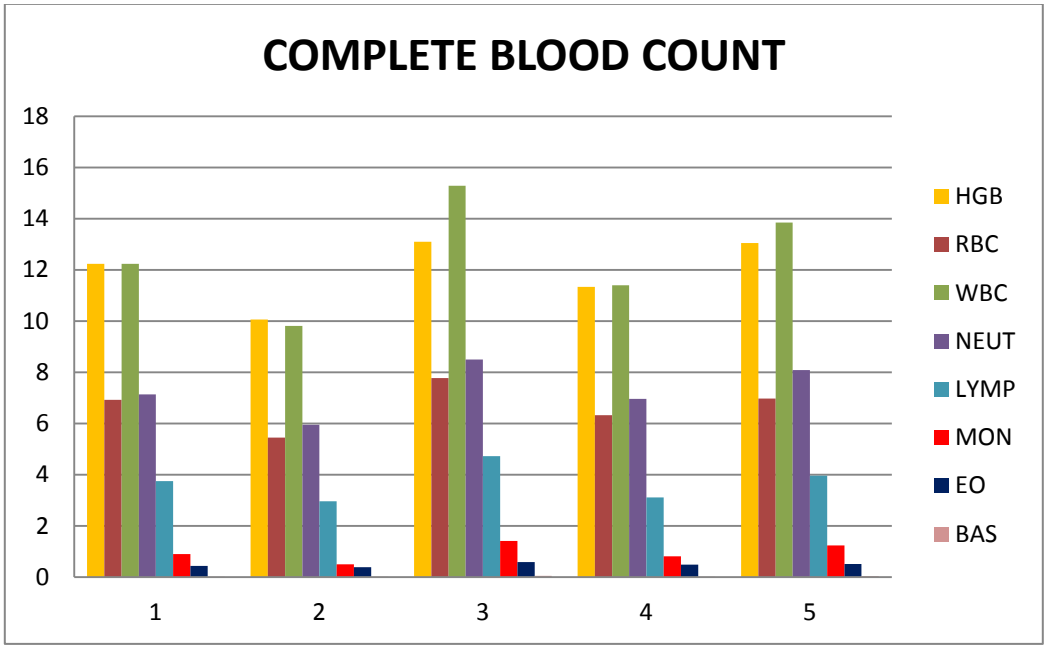
$^{a}P < 0.05$ when compared to control to control

$^{ns}P > 0.05$, $^{b}P < 0.05$ when compared to negative control.

CYP-Cyclophosphamide; Lev, levamisole; **WBC**- white blood cell count; **NEUT**-neutrophils; **LYM-**lymphocytes; **MON-** monocytes; **EO**-eosinophil; **BAS**- basophils; **RBC**-red blood cell count; **PLT**-platelet count

In Table 2 Effect of varying doses of extract on complete blood count in cyclophospamide treated animals during the study period showed a net reduction in heamatological parameters such as RBC, WBC,HGB, PLT When compared($P < 0.01$) to normal control. Group of animals which received levamisole showed significant increment in WBC,RBC,PLT as compared to control. Group which received EEJSF (200mg/kg and 400mg/kg) both doses showed significant increase in count when compared to normal control.

Figure1: Effect of varying doses of ethanolic flower extract *of jasminum sambac* on hematological parameters of Wistar albino rats taken on day 14 of the study



Group 1: 1%cmc,

Group 2: CYP 100mg/kg or negative control

Group 3: LEV 50mg/kg

Group 4: 200mg/kg of EEJSF

Group 5: 400mg/kg of EEJSF

TABLE 3 EFFECT OF VARYING CONCENTRATIONS OF *Jasminum sambac* FLOWER EXTRACTS ON THE NEUTROPHIL ADHESION

OF WISTAR ALBINO RATS

Group	Dose mg/kg	Neutrophil adhesion (%)
I	1%cmc	10.24±1.26
II	Negative control CYP 100mg/kg	8.76^{a} ±0.82
III	LEV 50mg/kg	24.18^{c} ±1.68
IV	EEJSF(200mg/kg)	16.42^{b} ±1.24
V	EEJSF(400mg/kg)	22.84^{c} ±1.96

Values are presented as mean ± SEM (standard error of the mean); $^{a}p < 0.01$ when compared to control .$^{b}P< 0.05$, $^{c}P< 0.01$ when compared to negative control.One way ANOVA followed by Dunnet's test.

CYP, cyclophosphamide; LEV, levamisole;CMC: Carboxy methyl cellulose.EEJSF: Ethanolic extract of *jasminum sambac* flowers.

In Table 3 ,The mean % neutrophil adhesion from the rats who were dozed with 50 mg/kg bwt of levamisole, 400 mg/kg bwt EEJSF were higher than normal control. From the treatment groups, statistically significant results were observed in the groups that received 400mg/kg extract.

Figure 2 :Effect of varying concentrations of *Jasminum sambac* flower extracts on the neutrophil adhesion of Wistar albino rats

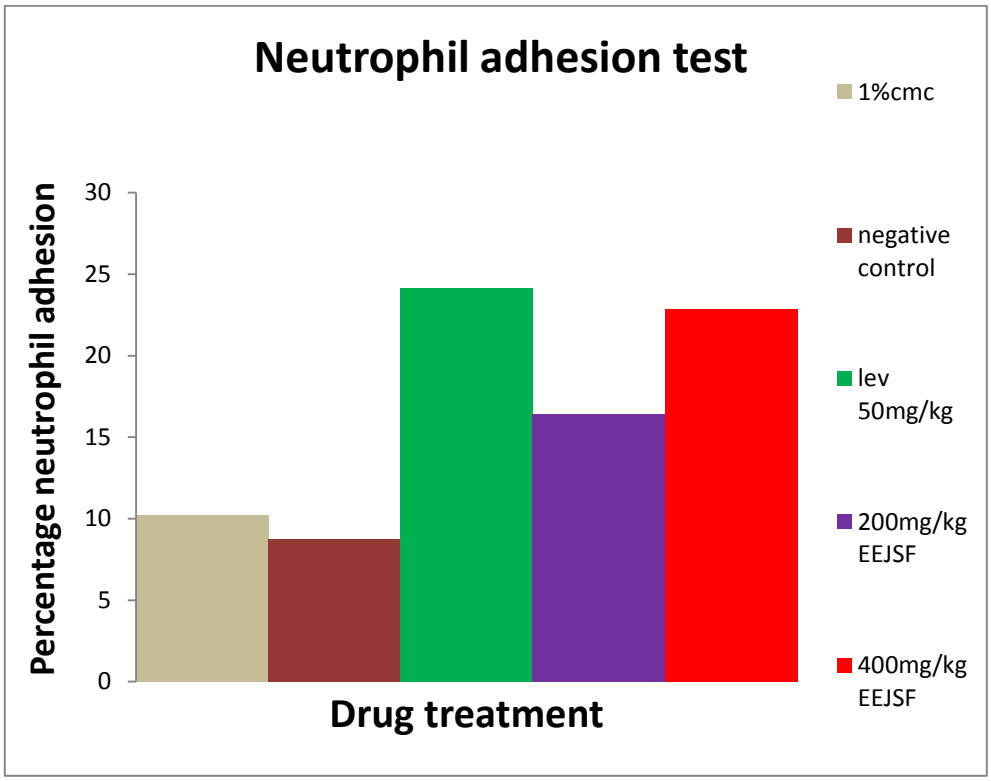


TABLE4: EFFECT OF DIFFERENT DOSES OF *Jasminum sambac* FLOWER EXTRACTS ON THE OF HUMORAL ANTIBODY RESPONSE TO SRBC AS DETERMINED BY HEMAGGLUTINATION ANTIBODY TITERS IN WISTAR ALBINO RATS.

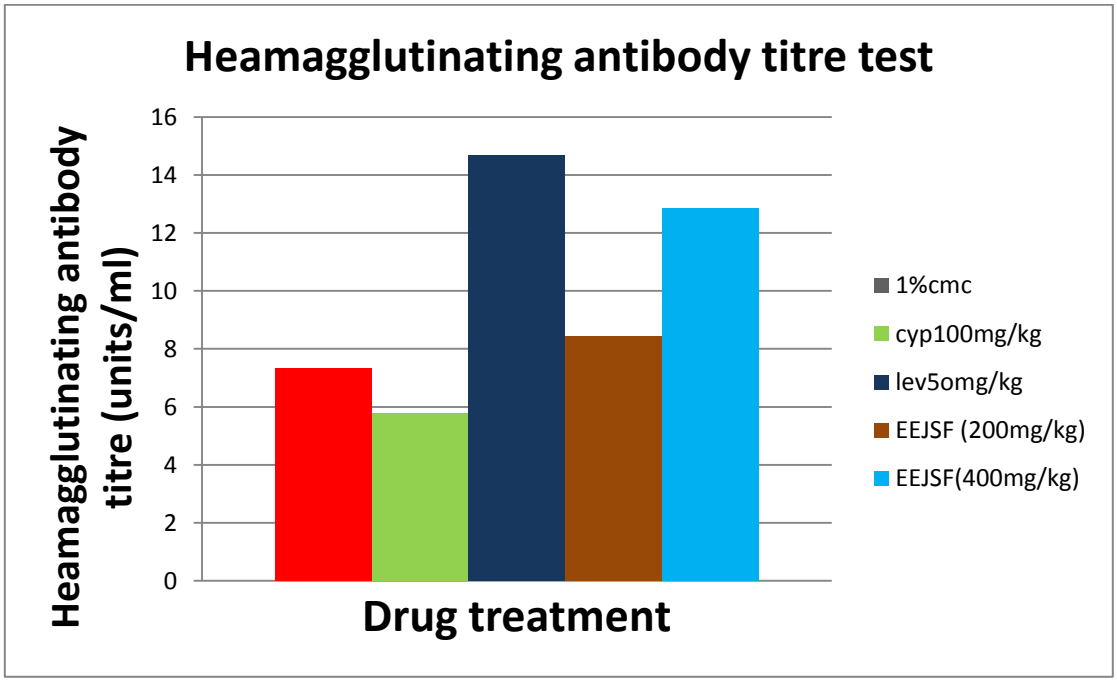
Group	Treatment	Dose	Mean hemagglutination antibody titer (±SEM) HA units/Ml
VI	1%cmc	0.1ml/kg	7.33±0.64
VII	Negative control	CYP 100mg/kg	5.78 ±0.98[a]
VIII	Levamisole	50mg/kg	14.67±1.24[b]
IX	EEJSF	200mg/kg	8.42 ±0.88[b]
X	EEJSF	400mg/kg	12.84±1.72[b]

Values expressed as mean± SEM; [a] P< 0.05 when compared to control.

[b]P < 0.01, when compared to negative control.one way ANOVA followed by Dunnet's test

In Table 4, The mean hemagglutination antibody titer to SRBC showed a dose-dependent increment for the rats dosed with 400mg/kg EEJSF as compared to normal. The group that receives only CYP shows net reduction in titre value when compared to normal.

Figure 3: Effect of different doses of *Jasminum sambac* flower extracts on the of humoral antibody response to SRBC as determined by hemagglutination antibody titers in Wistar albino rats



Group VI: 1%cmc **GroupVII : CYP 100mg/kg(negative control)**

Group VIII: LEV 50mg/kg **Group IX: 200mg/kg of EEJSF**

Group X :400mg/kg of EEJSF

TABLE 5: EFFECT OF DIFFERENT CONCENTRATIONS OF Jasminum sambac ETHANOLIC FLOWER EXTRACT ON THE DELAYED HYPERSENSITIVITY REACTIONS IN WISTAR ALBINO RATS BY MEASURING PAW SIZE.

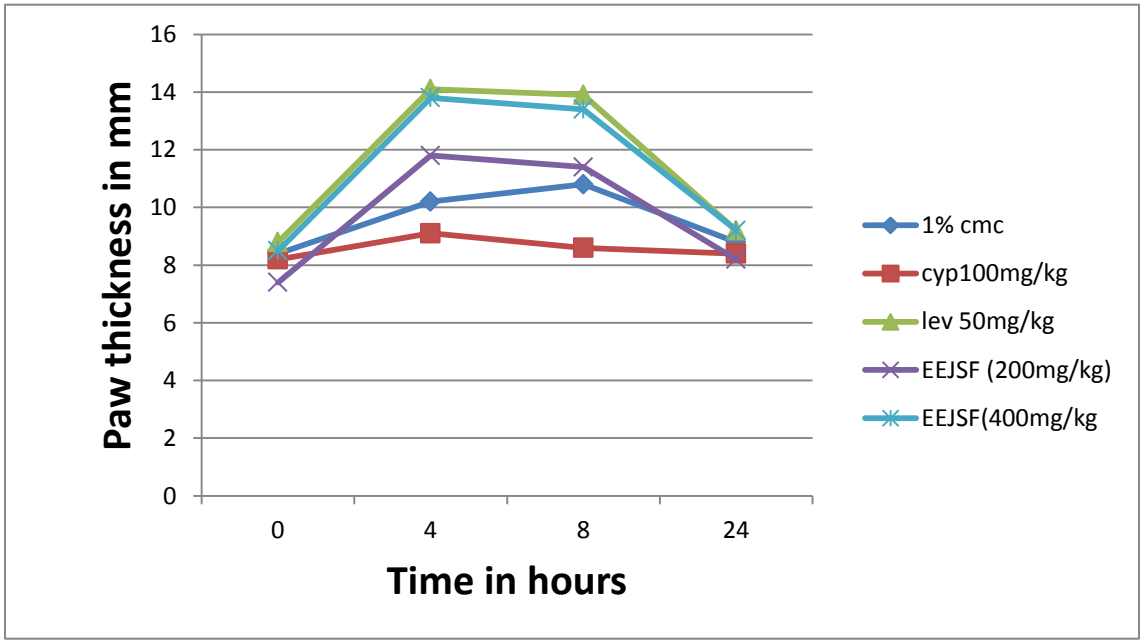
Group	Dose	Paw thickness in mm (Time in hrs)				% Inhibition on 8hr
		0hr	4hrs	8hrs	24hrs	
VI	1%cmc + SRBC	**8.4**±0.4	**10.2**±0.2	**10.8**±0.4	**8.8**±0.8	-
VII	CYP 100mg/kg + SRBC	**8.2**±0.2^{ns}	**9.1**±0.6^{a}	**8.6**±0.6^{a}	**8.4**±0.8	21%
VIII	LEV 50mg/kg + SRBC	**8.8**±0.6^{ns}	**14.1**±0.6^{b}	**13.9**±0.8^{b}	**9.2**±0.4^{ns}	28%
IX	EEJSF (200mg/kg) + SRBC	**7.4**±0.4^{ns}	**11.8**±0.8^{b}	**11.4**±0.4^{b}	**8.2**±0.6^{ns}	5%
X	EEJSF (400mg/kg) + SRBC	**8.5**±0.8^{ns}	**13.8**±0.2^{b}	**13.4**±0.7^{b}	**9.2**±0.8^{ns}	24%

Values expressed as mean± SEM; ^{ns} P> 0.05 , ^{a}P< 0.05, ^{b}P<0.01 when compared to control.

one way ANOVA followed by Dunnet's test

Mean percentage inhibition of paw edema thickness showed increase in EEJSf of 400mg/kg when compared to normal.There is increase in % inhibition of LEV treated group when compared to normal.

Figure 4 : Effect of different concentrations of *Jasminum sambac* ethanolic flower extract on the delayed hypersensitivity reactions in wistar albino rats by measuring paw size.



DISCUSSION

The immune system is the defense mechanism of the body and it helps to protect it from foreign bodies and infection thus playing a part in homeostasis of the body. Modulation of the immune system by way of stimulation or suppression helps in maintaining a disease-free state within an individual. Immunomodulators have been therefore used globally to control disease conditions. The study explore the immunomodulatory activity of the EEJSF by evaluating its effect on neutrophil adhesion, DTH reactions, hemagglutination antibody titers and on the complete blood count.[62]

Based on these results, In complete blood countEEJSF that was administered to the initially immunosuppressed animals had a stimulatory effect on the WBC, neutrophil, and lymphocyte counts .The observed increment in WBC counts could have been due to the presence of different nutritional elements in the EEJSF.

Previous study and review revealed that Phytochemical analysis of Jasminum sambac revealed the presence of carbohydrates, proteins, amino acids, coumarins, glycosides, tannins, phenolic compounds, flavonoids, phenolics, saponins, steroids, fats, essential oils, fixed oils, terpines, resin, and salicylic acid.[63]

It is possible that the ethanol extract almost all these compounds which could contribute to the observed effects on the hematological parameters. These compounds are essential for the development and maturation of the body's immune systems especially the cellular components of hemopoiesis. The micronutrients in EEJSF are essential in the growth, differentiation, and proliferation of immune system cells

Elements especially vitamins B12, B6, C and E, folic acid, and riboflavin are essential for the synthesis of DNA and in the final maturation of the red blood cell .

The amino acids found in EEJSF are also important in the formation of globin which is essential for hemoglobin synthesis, Iron, also a trace element found in

EEEJSF is one of the single most important elements in the formation of hemoglobin found in the RBC. The micronutrients also play a key role in balancing the redox state of leukocytes thus protecting them from oxidative stress.

These processes are vital in cell proliferation and survival and hence the observed increment of the WBC counts in animals dosed with the EEJSF especially at high doses. The dose-dependent increment in the WBC, lymphocyte, and neutrophil counts could be attributed to the dilution effect of the micronutrients present in EEJSF.

Increase in WBC count suggests that there is a high probability that the extracts of the flower contain agents that have the ability to stimulate the production of leucocytes Degenerative body systems are strengthened and harmonize with immune boosters which provide assistance to the immune system to combat foreign invading agents like bacteria and viruses.

It was concluded from this study that the herb contains various compounds that increase hemopoiesis and the observed increased production of WBC and RBC, thereby justifying its use widely for the management of anemias and immunodeficiency syndrome.

In neutrophil adhesion test, Neutrophils are the main components of immune protection system from infections by migration towards the challenge. The neutrophil adhesion to nylon fibres describes the margination of polymorphonuclear lymphocyte in the blood vessels and the number of macrophages reaching to the site of inflammation.

Both the doses (200 mg/kg and 400 mg/kg) of EEJSF showed a substantial rise in the neutrophil adhesion to nylon fibres. This might be due to the upregulation of the β2 integrins, present on the surface of the neutrophils through which they adhere to the nylon fibres. Hence it was inferred that extract causes stimulation of neutrophils towards the site of inflammation.

In heamagglutination antibody titre test was performed to determine the effect of EEJSF on the humoral immune response. Humoral immunity involves interaction of B- cells with the antigen and their subsequent proliferation and differentiation into plasma cells that secretes antibodies.

Antibodies thus function as the effectors of the humoral response by binding to the antigens and neutralizing them or facilitating their elimination by cross linking to form clusters that are then ingested by phagocytic cells .

The study results demonstrated that EEJSF had a stimulatory effect on the humoral immune response. This was evidenced by the mean hemagglutination antibody titer to SRBC that showed a dose-dependent increment for the rats dosed with EEJSF as compared to the rats that received only cyclophosphamide.[64]

Immunoglobulins and antigen-binding fragments are essential in the humoral immune responses that are products of amino acid chains and glycoproteins most of which are present in EEJSF.

Other compounds such as fatty acids, vitamin C, vitamin B6, vitamin B12, manganese, and selenium are also essential for the maturation of the B-lymphocytes in the bone marrow.[65]

Results from this study therefore demonstrated that the EEJSF contains compounds that can stimulate the production of antibodies in an immunocompromised animal. This may justify the common usage of the herb as an immune stimulant..

Delayed type hypersensitivity reaction is a type IV cell-mediated immune response. The test provides a functional *in -vivo* assessment of the cell-mediated immunity. It is often used as a skin test which capitalizes on intradermal inoculation of an antigen.

It is therefore used to assess the skin response following intradermal inoculation of the antigen which is dependent on antigen specific memory T-cells and the observed results were due to the recruitment of mononuclear cells and neutrophils.

Activation of the T cells leads to the release of lymphokines which causes the activation and accumulation of macrophages, increases vascular permeability, induces vasodilatation and produces inflammation.

It also produces a boost in phagocytic activity and increases the concentration of lytic enzymes for more effective killing of microorganisms . This results in the net increase in the thickness of the foot pad in previously immunized animals.

This increment in footpad thickness of the Wistar albino rats that were subjected to EEJSF in this study could be attributed to the ability of the extract to activate lympohcytes and their accessory cell types leading to enhancement in the production of antibodies in the previously immunosuppressed animals thereby increasing cell-mediated immunity[66]

Amino acids also present in EEJSF are also important in the formation of immunoglobulins and major histocompatibility complexes which are essential in the mediation of the DTH reaction. Trace elements are also essential for the proliferation of the T-cells and Langerhan cells and the activity of the lytic enzymes which are important components of the DTH reaction to antigen.[67]

The results of the study therefore showed that the EEJSF can be used to boost the immune system as there was a dose-dependent increment in paw size in response to antigen.

CONCLUSION

Based on the findings from the study that EEJSF (200 AND 400 mg/kg) increases both the cell-mediated and humoral immune responses in rats. This could be due to the different macronutrients, micronutrients and phytochemicals present in the plant.The preliminary phytochemical analysis determined the presence of steroids,flovonoids,phenols saponins. So the study revealed that the ethanolic flower extract of *Jasminum sambac* therefore has a potential therapeutic alternative value in several immunosuppressing clinical conditions and hence the reason for its use in local communities to alleviate various disease conditions.

Further studies can be undertaken at the cellular and molecular level, which may further elucidate its mechanism in detail. The present investigation has also opened an avenue for further research especially with reference to the development of potent formulation for enhancement of immunity from *Jasminum sambac* flowers.

REFERENCES

1) BIOS Instant Notes in Immunology, By Peter Lydyard, Alex Whelan, Michael Fanger Third edition.
2) Yasmine Belkaid, Timothi Hand ,Role of the Microbiota in Immunity and inflammation *Pubmed central* 2015 Mar 27.
3) Jean S Marshall,Richard Warrington, An introduction to immunology and immunopathology. *Allergy Asthma and clinical Immunology* 2018; 14(Suppl 2): 49.
4) Karim M.Yatim,Faddi G.Lakkis. Brief journey through immune system. *Clinical Journal of American Society of Nephrology*. July 2015, 10 (7) 1274-1281
5) Makare N., Bodhankar S., Rangari V., Immunomodulatory activity of alcoholic extract of Mangifera indica L. in mice, *Journal of Ethnopharmacology*,,2001,133-137. 26.
6) Naik Suresh R, Hule Amolkumar, Evaluation of Immunomodulatory Activity of an Extract of Andrographolides from Andographis paniculata, *Planta Med*, 75,2009,785-791.
7) Doherty NS. Selective effect of immunosuppressive agents against the delayed hypersensitive response and humoral response to sheep red blood cells in mice. Agents Actions 1981;11:237-242
8) Gaikward SB, Mohan GK, Reddy KJ. Moringa oleifera leaves: immunomodulation in Wistar albino rats. Indian journal of Pharmacyand Pharmceutical Sciences 2011; 3(Suppl 5):1–5.
9) Riadh Ben Massour,Toll like receptors and Innate Immunity, Journal of Immunology Research 2019.
10) Immunology ;Introductory text book by Nandini shetti.

11) Introduction to Immunology by Professor Charles Bangham.

12) Lecture Notes: Immunology by Ian Todd, Gavin Spickett, Lucy Fairclough

13) Immunobiology 6th Edition by Charles Janeway, Paul Travers, Mark Walport, Mark Shlomchik.

14) Cellular and molecular immunology by Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai.

15) Doherty NS. Selective effect of immunosuppressive agents against the delayed hypersensitive response and humoral response to sheep red blood cells in mice. Agents Actions 1981;11:237-242.

16) Limbasiya KK, Kachchhi NR, Vekaria RH, Desai TR, Tirgar PR. Immunomodulatory effect of hydroalcoholic extract of Triticum aestivum on laboratory animals. Journal of Pharmacology and Toxicology 2011; 1(4): 15-19

17) Chandua S, Kailash H, Studies on immunomodulatory activity of Aloe vera (Linn), International Journal of Applied Biology and Pharmaceutical Technology; 2,2011,19-22.

18) Gupta A, Gautam MK, Singh RK, Kumar MV, Rao CV, Anupurba RK. Immunomodulatory effect of Moringa oleifera Lam on cyclophosphamide induced toxicity in mice. Indian Journal of Experimental biology Biol. 2010; 48:1157–60. [PubMed: 21117458]

19) Satpute KL, Jadhav MM, Karodi RS, Katare YS, Patil MJ, Rub R, Bafna AR. Immunomodulatory activity of fruits of Randia dumetorum Lamk. Journal of pharmacognosy and phytotherapy 2009; 1(3): 36-40

20) Clement F, Pramod SN., Venkatesh YP., Identity of the immunomodulatory proteins from garlic (Allium sativum) with the major garlic lectins or agglutinins, Int Immunopharmacol; 10,2010,316-24

21) Tropical Plants Database, Ken Fern. tropical.theferns.info. 2020-03-18. <tropical.theferns.info/viewtropical.php?id=Jasminum+sambac>

22) T. Gowdhami, A. K. Rajalakshmi ,Ethnobotany and Pharmacognostical studies of Jasminum sambac Linn. *International Letters of Natural Sciences* ISSN: 2300-9675, Vol. 37, pp 39-45

23) Stone BC. The flora of Guam. Micronesica 1970;6:1-659.

24) Grohmann F. Oleaceae. In: Nasir, E. and Ali SI [eds.]. Flora of West Pakistan 1974: 20-22

25) Anonymous, The wealth of India, A Dictionary of Raw Materials and Industrial Products, Raw Materials, first supplement series, Vol-4: j-q. Publications and Information Directorate, New Delhi: 3-4.

26) Rastogi R P & Mehrotra B N: Compendium of Indian medicinal plants. Vol 2. C D R I, Lucknow & N I S C, New Delhi. 1970-1979: 395-396.

27) Rastogi R P & Mehrotra B N. Compendium of Indian medicinal plants. Vol. 4. C D R I, Lucknow & N I S C, New Delhi. 1985-1989: 407-408

28) Neeraj Mourya, Devendra Bhopte, Rakesh Sagar Sri Sathya Sai. A review on Jasminum sambac: A potential medicinal plant.*International journal of indigenous herbs and drugs* 2017; 2(5): 13-16.

29) Swati sabharwal, Swati sudan, Vadi ranja. Jasminum sambac linn (motia): a review *International Journal of Pharmaceutical Research and Bio-science*2013; Volume 2(5):108-130.

30) Liu, Haiyang, Ni, Wei, Yuan, Minhui, Chen & changxiang: Chemical constituents of Jasminum sambac. Yunnan Zhiwu Yanjiu 2004; 26/6: 687-690.

31) Upaganlawar A B, Bhagat A, Tenpe C R & Yeole P G: Effect of Jasminum Sambac leaves extracts on serum glucose and lipid profile rats treated with alloxan. Pharmacologyonline 2009: 1-6.

32) Abdoul-Latif F, Edou P, Eba F, Mohamed N, Ali A & Djama A: Antimicrobial and antioxidant activities of essential oil and methanol extract of Jasminum sambac from Djibouti. *African Journal of Plant Science* 2010; 4/3: 38-43.

33) Rahman A, Hasan S, Hossain A & Biswas N N: Analgesic and cytotoxic activities of Jasminum sambac(L) Aiton. Pharmacologyonline 2011; 1: 124-131.

34) Harisaranraj R, Babu S S & Suresh K: Antimicrobial properties of selected Indian medicinal plants against acne-inducing bacteria. Ethno botanical Leaflets 2010; 14: 84-94

35) Chiang L C, Cheng H Y, Liu M C, Chiang W & Lin C: In vitro anti-herpes simplex viruses and anti-adenoviruses activity of twelve traditionally used medicinal plants in Taiwan. *Biological & Pharmceutical Bulletein* 2003; 26/11: 1600-1604

36) Shrivastav P, George K, Balasubramaniam N, Jasper M P, Thomas M, & Kanagasabhapathy A S: Suppression of puerperal lactation using Jasmine flowers (Jasminum Sambac). *Australian & Newzeland Journal Obstetrics & Gynaecology* 1988; 28/1: 68-71.

37) Hongratanaworakit T: Stimulating effect of aromatherapy massage with Jasmine Oil. Natural Product Communications 2010; 5/1: 157-62.

38) Kalaiselvi M & Kalaivani K P L: Phytochemical analysis and antilipid peroxidative effect of *Jasminum sambac* (L.) Ait. Oleaceae. Pharmacologyonline 2011; 1: 38-43.

39) Kuroda K, Inoue N, Ito Y, Kubota K & Sugimoto A: Sedative effects of the jasmine tea odor and (R)-(−)-linalool, one of its major odor components, on autonomic nerve activity and mood states. Europeon Journal of Applied Physiology 2005; 95: 107–14.

40) Baby & Aimy A, Pharmacological investigations of antistress Activity of *Jasminum sambac (*linn) leaves, [Online]. [cited 2010 Sep 15] Available from: URL: http://hdl.handle.net/123456789/928.

41) Lis-Balchin M, Hart S & Lo WH: Jasmine absolute and its mode of action on guinea pig ileum in vitro. *Phytotheraphy Research* 2002; 16: 437–39.

42) Dash S,Nath L K,Bhise S,Kar P,Bhattacharya S. Stimulation of immune function activity by the alcoholic root extract of heracleum *nepalense* D.Don.*Indian Journal of Pharmacology* 2006;38:336-40.

43) Lu Y, Fan J, Zhao Y, Chen S, Zheng X, Yin Y, Fu C Immunomodulatory activity of aqueous extract of *Actinidia macrosperma, Asian Pacific Journal Clinical Nutrition.* 2007;16 Suppl 1:261-5.

44) Huang C F, Immunosuppressive withanolides from withania coagulans,*chemistry and biodiversity*,2009,6;9 ,1415-1426.

45) Ankur Choubey, Aadarsh Choubey,Ashish Mishra, Shilpi Mishra, U.K.Patil ,evaluation of the immunomodulatory activity of methanolic and ethanolic extract of leaves of *Aegle*

marmelos in rats. *International Journal of Drug Development & Research* July-Sept 2010 Vol. 2 Issue 3 ISSN 0975-9344.

46) Atul N Chandua , Santhosh kumar. C studies on immunomodulatory activity of *Aloe vera (Linn*), *International Journal of Applied Biology and Pharmaceutical Technology*, Volume: 2: Issue-1: Jan-Mar -2011.

47) A.Jagtap, Immunomodulatory activity of ethanolic extract of *Dodonaea viscosa* L.F Pharmacologyonline1:685-701 · January 2011

48) Radu S & Kqueen C Y: Priliminary screening of endophytic fungi from medicinal plants in Malaysia for antimicrobial and antitumour activity. *Malaysian Journal of Medical Science* 2002; 9/2: 23-33

49) Hussaini A L & Mahasneh A M: Antibacterial and antifungal activity of ethanol extract of different parts of medicinal plants in Jordan. *Journal of Pharmaceutical Sciences* 2011; 4/1: 57-68.

50) Talib W H & Mahasneh A M: Antiproliferative activity of plant extracts used against cancer in traditional medicines. *Journal of Scientia Pharmceutical* 2010; 78: 33-45.

51) Rath C C, Devi S, Dash S K & Mishra R K: Antibacterial potential assessment of Jasmine essential oil against E Coli. *Indian Journal of Pharmaceutical science 2008*; 70/2: 238-241.

52) Ari Yuniarto, Ika Kurnia and Muhammad Ramadhan. Anti-obesity effect of ethanolic extract of jasmine flowers *(Jasminumsambac*(l)Ait) in high-fat dietinduced mice: potent inhibitor of pancreatic lipase enzyme, *International journal of advances in pharmacy, biology and chemistry*, Vol. 4(1), Jan - Mar, 2015 ISSN: 2277 – 4688.

53) Kunhachan P, Banchonglikitkul C, Kajsongkram T, Khayungrannawee A, Leelamanit W. Chemical Composition, Toxicity, and Vasodilatation Effect of the Flowers Extract of *Jasminum sambac* (L) ait. "G.Duke F Tuscanny". Evidence-Based Complementary and Alternative Medicine, 2012;1-7

54) Rahman MA, Hasan MS, Hossain MA, Biswas NN. Analgesic and Cytotoxic Activity of *Jasminum sambac* (L) Aiton ,Pharmacologyonline, 2011; (1): 124-131

55) Bhangale J, Patel R, Acharya S, Chaudhari K. Preliminary Study on Anti-Inflammatory and Analgesic Activities of *Jasminum sambac* (L) Aiton in Experimental Animal Models. *American journal of pharmatech research* 2012; 2(4): 804-813

56) Joshua Nfambi, Godfrey S. Bbosa, Lawrence Fred Sembajwe, James Gakunga, Josephine N. Kasolo, Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats, *Journal of Basic Clinical Physiology and Pharmacology*. 2015 November 1; 26(6): 603–611. doi:10.1515/jbcpp-2014-0104.

57) Endang Kumolosasi, Siti Nur Aisyah Ibrahim, Siti Munirah Ahmad Shukri, Waqas Ahmad. Immunostimulant activity of standardised extracts of Mangifera indica leaf and Curcuma domestica rhizome in mice. *Tropical Journal of Pharmaceutical Research* January 2018; 17 (1): 77-84

58) Sunil Kumar, Shalini Sharma, Dinesh Kumar, Karunesh Kumar, Renu Arya. Immunostimulant activity of Phyllanthus reticulatus Poir: a useful plant for infectious tropical diseases. *Asian Pacific Journal of Tropical Disease*. 2014; 4(Suppl 1): S491-S495

59) Chandua S, Kailash H, Studies on immunomodulatory activity of Aloe vera (Linn), *International Journal of Applied Biology and Pharmaceutical Technology*; 2,2011,19-22.

60) Clement F, Pramod SN., Venkatesh YP., Identity of the immunomodulatory proteins from garlic (Allium sativum) with the major garlic lectins or agglutinins, *Immunopharmacology;* 10,2010,316-24.

61) Naik Suresh R, Hule Amolkumar, Evaluation of Immunomodulatory Activity of an Extract of Andrographolides from Andographis paniculata, Planta Med, 75,2009,785-791.

62) Makare N., Bodhankar S., Rangari V., Immunomodulatory activity of alcoholic extract of Mangifera indica L. in mice, *Journal of Ethnopharmacology*,78,2001,133-137. 26.

63) Gabhe SY, Tatke PA, Khan TA, Evaluation of the immunomodulatory activity of the methanolic extract of Ficus benghalensis roots in rats. *Indian Journal of Pharmacology*, 38, 2006, 271-275.

64) Karthikumar S, Jegatheesan K, Thangaraja A, Banupriya K, Dhivya T, and Malarvizhi JM, Immunomodulatory activity of Eclipta prostrata in SRBC immunized mice. *Journal of Pharmacognosy and Phytotherapy*, 3, 2010, 52-55.

65) Agrawal SS, Khadase SC, Talele GS, Studies on immunomodulatory activity of Capparis zeylanica leaf extracts. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 3, 2010, 887-892.

66) Pradhan D. Panda K.P.Thripathi G. Evaluation of Immunomodulatory activity of the methanolic extract of *Couroupita guinensis* Flowers in Rats.Natural Products Radiance, 8, 2009, 37-42.

67) Gaikward SB, Mohan GK, Reddy KJ. Moringa oleifera leaves: immunomodulation in Wistar albino rats. *Indian journal of Pharmacy and Pharmceutical Sciences* 2011; 3(Suppl 5):1–5.

68) Gupta A, Gautam MK, Singh RK, Kumar MV, Rao CV, Anupurba RK. Immunomodulatory effect of Moringa oleifera Lam on cyclophosphamide induced toxicity in mice. *Indian Journal of Experimental biology* 2010; 48:1157–60. [PubMed: 21117458]

69) Gaikward SB, Mohan GK, Reddy KJ. Moringa oleifera leaves: Immunomodulation in Wistar albino rats. *Indian journal of Pharmacy and Pharmaceutical Sciences* 2011; 3(Suppl 5):1–5.

70) Wilkinson, PC. Neutrophil adhesion test. In: Vane, JK.; Ferreria, SH., editors. *Handbook of Experimental Pharmacology* I. 1. Berlin: Springer Verlag; 1978. p. 109

71) Banji OJ, Banji D, Kavitha R. Immunomodulatory effects of alcoholic and hydroalcoholic extracts of Moringa oleifera Lam leaves. *Indian Journal of Experimental Biology 2012; 50:270–6.* [PubMed: 22611915]

72) Shah AS, Juvekar AR. Immunostimulatory Activity of Aqueous Extract of Murraya Koenigii (Linn.) Spreng Leaves. *Indian Journal of Natural Products and Resources* 2010; 1(4): 450-455.

73) . Patel S, Banji D, Banji OJF, Patel MM, Shah KK. Scrutinizing The Role of Aqueous Extract of Trapa bispinosa as an Immunomodulator in Experimental Animals. *Indian Journal of Research in Pharmaceutical Sciences* 2010; 1(1): 13-19.

74) Kannan M, Singh AJAR. An Immuno-Pharmacological Investigation of Indian Medicinal Plant *Nyctanthes arbortristis Linn*. *World Applied Sciences Journal* 2010; 11(5): 495-503.

75) Sudha P, Asaq SB, Dhamingi SS, Chandrakala GK. Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in animals. *Indian Journal of Pharmacology 2010; 54:133–40.*

76) Limbasiya KK, Kachchhi NR, Vekaria RH, Desai TR, Tirgar PR. Immunomodulatory effect of hydroalcoholic extract of Triticum aestivum on laboratory animals. *Journal of Pharmacology and Toxicology* 2011; 1(4): 15-19

77) Hajra S, Mehta A, Pandey P. Immunostimulating activity of methanolic extract of Swietenia mahagoni seeds. *International Journal of Pharmacy and Pharmaceutical sciences* 2012; 4(1): 442-445.

78) Satpute KL, Jadhav MM, Karodi RS, Katare YS, Patil MJ, Rub R, Bafna AR. Immunomodulatory activity of fruits of Randia dumetorum Lamk. *Journal of Pharmacognosy and Phytotherapy* 2009; 1(3): 36-40

79) Dashputre NL, Naikwade NS. Immunomodulatory activity of Abutilon Indicum Linn. on albino mice. *International Journal of Pharmaceutical Sciences* Res 2010; 1(3): 178-184

80) Ankur Choubey1, Aadarsh Choubey, Ashish Mishra, Shilpi Mishra, U.K.Patil Evaluation of the immunomodulatory activity of methanolic and ethanolic extract of leaves of Aegle marmelos in rats. *International Journal of Drug Development & Research* | July-Sept 2010 | Vol. 2 | Issue 3 | ISSN 0975-9344 |.

81) Doherty NS. Selective effect of immunosuppressive agents against the delayed hypersensitive response and humoral response to sheep red blood cells in mice. Agents Actions 1981;11:237-242.

82) Ali Esmail Al-Snafi, Pharmacological and therapeutic effects of *Jasminum sambac*- A REVIEW. *Indo American Journal of Pharmaceutical Sciences*. 2018, 05 (03), 176.

SUMMARY

The use of potential natural medicines as immunomodulators is a new concept in medicine used as traditional medicine. An immunomodulator is a compound that can improve the body's defense mechanism. The importance of the body's defense system to health is to prevent or protect the body from infection and also the immune system into a physiological system to regulate body homeostasis. Herbs have been used extensively in ethnopharmacology as a potential immunomodulatory effect[68-73]

The flowers of *Jasminum sambac* was collected on Dec 2019 from market in perundurai. It was then dried for one month and later powdered.This powder was then extracted with ethanol for 24 hrs using soxhlet apparatus.

After extraction Phytochemical analysis was carried out to determine the presence of flavonoids,phenols, saponins, steroids, tannins etc.,

Ethanolic extract of *Jasminum sambac* flowers was dissolved in 1%cmc. Acute toxicity study was carried out and then selected different doses namely 200mg/kg and400mg/kg body weight. Immunostimulant activity is studied by using suppressing drug namely cyclophospamide and then by grouping animals into different groups.

On day 0 all the animals was suppressed by cyclophospamide and then after day 1 to 14, all groups were treated with different doses. At the end of day 14 blood from all the groups were collected in EDTA tubes for estimation of complete blood count.

In complete blood count following parameters are counted. White blood cells, Neutrophil , lymphocytes,monocytes, basophil, eosinophil, Red blood cell, platelet count , heamoglobin.

For neutrophil adhesion test, On day 14 after administration of the extract, blood samples from rats in Group 1-5 were obtained by ventricular

puncture and analyzed for total leucocyte counts (TLC) and differential leukocyte counts (DLC). After the initial counts, the blood samples were incubated with 80 mg/mL of nylon fibers for 15 min at 37 °C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil were given as the neutrophil index (NI) of blood sample Percent neutrophil adhesion was calculated.These two test are done for innate immune response.[74-78]

For adaptive immune response antigen was prepared using sheep red blood cells. Heamagglutinating antibody titre test is carried out. On day 7 blood from second set of group of animals were collected and centrifuged to get serum. Antibody titre method was carried ou to obtain hemagglutination antibody titer (HA units/Ml).

Delayed type hypersensitivity response was determined by inducing immunogenic response using SRBCs in rats with little modifications. After 7 days of immunization with SRBCs, the animals were administered 50 µl of SRBCs (0.5 x 109 /ml) into the right hind foot pad; while, the left hind foot pad was challenged with 50 µl of PBS.

The thickness of right and left hind foot pads was measured by micrometer screw gauge or vernier calliper after 4 h, 8 h and 24 h of challenge. The difference in millimeter (mm) between right and left hind foot pad and percentage of footpad swelling was taken as DTH response to specific antigen.[79-83]

From all the study there was significant increase and this effect was due to phytochemical constituents such as tannins, steroids, saponins, phenols,flavonoids and aminoacids present in flowers.