UNIVERSITI PUTRA MALAYSIA

SCREENING OF MALAYSIAN MEDICINAL PLANTS FOR IMMUNOMODULATORY AND HAEMOLYTIC ACTIVITIES

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is specially dedicated to my beloved husband, *Mohd. Zaki*, parents *Samat* and *Zamzimar*, and daughters, *Nur Nadia Najiha* and *Nur Nadia Nazreen*.

Thank you for all the understanding, support, advice and opinion during the course of completing of this thesis.

For *Najiha* and *Nazreen*……..

Thanks for bringing me such joy and laughter

Thanks for everything.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman : Associate Professor Daud Ahmad Israf Ali, Ph.D.

Faculty : Medicine and Health Science

Twenty-six water-soluble extracts from eighteen local medicinal plant specirs and fourteen methanol extracts from ten medicinal plants were screened in order to find new immunomodulating substances from those medicinal plants. Effects on lymphocyte proliferation and macrophage function in host defense mechanism including the nitric oxide (NO) production, phagocytic activity and intracellular killing ability were tested. Lymphocyte or macrophage was treated with those extracts at the concentration of 2.5 - 50μg/ml. Four of the water-soluble extracts shows mitogenicity where it causes highest mitogenicity at different concentrations. Both methanol and water-soluble extracts failed to alter or improve macrophage function neither of its phagocytic activity nor its intracellular killing ability. The water extracts of *S. torvum* (stem bark), *C. asiatica, S. grandiflora, U. lobata* (stem bark) and *G. vulgaris* (leaves) produce NO significantly. Apart from that, haemolytic activity was also determined. Both

significantly. Apart from that, haemolytic activity was also determined. Both methanol and water-soluble extracts causes less than 32% haemolysis of SRBC as compared to haemolysis caused by saponin except for methanol extracts of E. guainensis leaf which causes 68.2% haemolysis. Overall results indicate that the extracts effect on all parameter were influenced by a few factor such as dose, cells involved, solvent used for extraction and constituents of each extracts.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENYARINGAN EKSTRAK TUMBUHAN UBATAN MALAYSIA BAGI AKTIVITI IMMUNOMODULATORI DAN HEMOLISIS

Oleh

NOR AZURA BT. SAMAT

September 2003

Pengerusi : Profesor Madya Daud Ahmad Israf Ali, Ph.D.

Fakulti : Perubatan dan Sains Kesihatan

Dua puluh enam ekstrak larutan air daripada lapan belas spesis tumbuhan ubatan tempatan dan empat belas ekstrak methanol daripada sepuluh tumbuhan ubatan tempatan telah disaring bagi mengesan ekstrak yang berpotensi dalam meningkatkan sistem imun. Kesan ekstrak terhadap proliferasi limfosit, fungsi-fungsi makrofaj dalam penghasilan nitrik oksida (NO), aktiviti fagositosis dan keupayaannya membunuh secara intraselular merupakan parameter yang dilihat dalam ujikaji ini. Ekstrak-ekstrak tersebut telah di inkubasi Limfosit dan makrofaj telah dieram bersama ekstrak-ekstrak tersebut di mana kepekatan ekstrak yang digunakan adalah di antara 2.5 - 50μg/ml. Empat daripada ekstrak larutan air telah menunjukkan peningkatan di dalam aktiviti proliferasi sel limfosit pada kepekatan yang berbeza. Walau bagaimanapun, kesemua ekstrak yang diuji tidak berupaya meningkatkan kadar fagositosis dan keupayaan makrofaj untuk membunuh secara

intraselular. Ekstrak larutan air bagi *S. torvum* (kulit pokok), *C. asiatica*, *S. grandiflora*, *U. lobata* (kulit pokok) dan *G. vulgaris* (daun) menunjukkan peningkatan yang signifikan dalam penghasilan nitrik oksida (NO). Selain itu, kadar hemolisis SRBC juga dikaji bagi memastikan ekstrak tidak toksik. Kesemua ekstrak menyebabkan hemolisis kurang daripada 32% jika dibandingkan dengan hemolisis yang disebabkan oleh saponin. Hanya ekstrak methanol bagi daun *E. guainensis* sahaja yang mengakibatkan kadar hemolisis yang agak tinggi iaitu 68.2%. Kesimpulannya, kesan ekstrak terhadap semua parameter yang diuji bergantung kepada beberapa faktor seperti kadar kepekatan ekstrak yang digunakan, sel-sel yang terlibat, jenis pelarut yang digunakan semasa proses ekstraksi dan juga kandungan setiap ekstrak yang diuji.

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CHAPTER 1

INTRODUCTION

Plants play an important role both as providers of foods in the form of carbohydrates, fibres, proteins and oils, and a source of natural products that can be used as traditional medicines. Preparations from leaves, seeds, stem bark and roots of plants have been widely used in traditional medicines. Although the use of plants for medicinal purposes has been practiced over thousands of years, it is only relatively recent time that scientific evaluation of plant extracts and decoctions have been assessed. Most studies described antiviral, antihelminthic, antitumoral, anti-inflammatory and antirheumatic activities (Nores, 1997). However, studies on effects upon the immune system are generally lacking.

Manipulation of the immune system is very important where it involves immunomodulation process. Immunomodulation can either suppress or enhance the immune system. It is also essential to improve protective immunity and reduce immune-mediated disease such as allergy and autoimmunity.

Previous studies showed that plants may consist of several compounds, which are immunomodulatory. Plant are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have

been found in vitro to have anti-microbial properties (Cowan, 1999). Plant constituents such as saikosaponin-d extracted from roots of *Bupleurum radix,* modifies immune response of T and B cells, and activates immunological function of the macrophage (Ushio, 1991). Apart from that, plants also may contain substances that cause haemolysis such as saponin from *Quillaja saponaria* (bark).

Traditional healers have long used plants to prevent or cure infectious conditions. Herbal medicines play an important and integral part in different communities and ethnic groups that make up the Malaysian society. Malaysian herbal medicine has its grass roots in the beliefs, religion, philosophical and practical aspects of health and diseases as defined by the Malays, Chinese, Indians and indigenous groups in Malaysia. However, scientific studies on these medicinal plants are very limited.

In Malaysia, the use of traditional medicine has been a part of life of multi-ethnic society for a long time (Khalilur, 1981). Traditional medication is common especially in rural areas. In urban areas, though modern medicines are widely used, traditional medicine is not discarded altogether. Very often, we find urbanities seek traditional medicine as an alternative when modern medicines does not bring desired results. Plant materials as herbal remedies are commonly practiced among different communities in Malaysia. Two common

local medicinal plants are *Eurycoma longifolia* (tongkat Ali) and *Centella asiatica* (pegaga). *E. longifolia* is consumed as an aphrodisiac and tonic whilst *C. asiatica* is widely used in health food and cosmetic industries. Research is actively pursued in order to characterize their bioactive compounds. Standardization of toxicity and efficacy evaluation is important to obtained herbal medicines, which are of high quality, safe and efficacious. Other popular herbs used are *Morinda citrofolia* (mengkudu), *Labisia pumila* (kacip Fatimah) *and Andrographis paniculata* (hempedu bumi). These products are being manufactured and sold regardless of the fact that concrete scientific data on the efficacy and even toxicity of the herbs used are inadequate or lacking.

Therefore, the objective of this study is to screen crude aqueous and organic extracts of selected indigenous plants for immunomodulatory and haemolytic activity.

CHAPTER 2

LITERATURE REVIEW

2.0 The Mammalian Immune System

The immune system provides the defense mechanisms to protect from hostile forces in the environment, among which are toxic substances and microorganisms. Its primary function is to discriminate between self and nonself. The mammalian immune system involves the action of cellular and soluble components of the blood. The cellular components are white blood cell or leukocytes, which consist of neutrophil, basophil, eosinophil, lymphocytes and monocyte-macrophages but it depends primarily on 3 major cell types: macrophage, thymus-derived lymphocyte (T cells) and bone-marrow derived lymphocyte (B cells). The mammalian immune system provides a mechanism for specific response to the invasion of particular pathogenic microorganisms and other substances. It is largely this specific physiological response that protects us against disease.

There are two types of immunity: innate and adaptive (refer Table 2.1). The important difference between these is that adaptive immune response is highly specific for a particular pathogen. Innate immunity is a non-specific response (not antigen specific), where it does not distinguish between self and non-self. It

is sometimes called natural immunity where it is present from birth and includes numerous nonspecific elements. Body surfaces, especially skin, form the first line defense against penetration by microorganisms. One of the important group of leukocytes is the phagocytic cells such as monocytes, macrophage and polymorphonuclear neutrophils. These cells bind to microorganisms, internalize them and then kill them (Riott et. al., 1996). In non-specific immunity, macrophage plays an essential role by ingesting and killing invading microorganism and by releasing many soluble factors that contribute to host defense and to inflammation.

The second type of immunity is called adaptive or acquired immunity. This system retains a memory of all the invaders it has faced. It is a specific response. The adaptive immune response is triggered by the presence of a foreign agents that escape early elimination by the innate immune system. It involves memory cell formation and antibody production by B cells. Adaptive immunity is classified into two responses: humoral response and cell-mediated response. Humoral response acts principally against extracellular phases of infection, that is, through the secretion of proteins called antibodies or immunoglobulins.

Table 2.1 : Comparison of innate and adaptive immunity

Property	Innate Immunity	Adaptive Immunity
Physical barriers	Skin and mucous membranes	None
Soluble factors	Enzymes (eg. lysozyme and complement) Acute-phase proteins (eg. CRP) α and β interferons	Antibodies Lymphokines
Cells	Macrophages, Polymorphonuclear neutrophils (PMN), NK cells, eosinophils	T and B lymphocytes
Self-nonself discrimination	Yes	Yes
Specificity	No	Yes
Memory	No	Yes

In contrast, the cell-mediated response is effective in eliminating infections by pathogenic microorganism that develop within host cells, which involves direct interaction between lymphocytes and the pathogens. It depends on the actions of T lymphocytes that divide and differentiate into cytotoxic T cells (killer T cells). Both lymphocytes are responsible for the defense mechanisms of the body.

T-lymphocytes are involved in cell-mediated immunity where it does not involve antibody production. It only recognizes antigen when following association with major histocompatibilty (MHC) molecules, which are on the cell surface. Two classes of MHC namely MHC Class I and Class II are involved in the elimination of virus infected cells and non-viral infected cells respectively. The recognition of MHC Class I-antigen will activate cytotoxic T cells to diminish the infected cells directly whereas MHC Class II-antigen activates effector T helper cells (T_h). T_h cells induce cytokine production that will bind to pathogens and activate cells in secreting proteolytic enzymes, which causes destruction of the pathogen.

On the other hand, B cells play an important role in the humoral response where they recognize non-self antigen directly. The development of B cells involves 4 stages; stem cell, pre-B cell, B cell and plasma cells. It enters the circulation and migrates to spleen and other peripheral lymphoid tissue. When antigen that is

complementary to surface immunoglobulin receptors and with the help of appropriate T cells, the B cell will differentiate into antibody secreting plasma and memory cells. There are five types of imunoglobulin: IgA, IgG, IgM, IgE and IgD. Each immunoglobulin has specific functions in the immune systems. Antibodies can also neutralize bacterial toxin and viruses. Apart from antibody production, the B cell also facilitates the engulfment of opsonized or agglutinated bacteria by phagocytes and initiates their destruction through activation of the complement system.

Involvement of soluble mediators in immune response is important. A wide variety of molecules are involved in the development of immune responses. These includes antibody as mentioned before and cytokines. During infections, these proteins concentration in serum increases rapidly. Cytokines are proteins or peptides. A number of cytokines produces by the immune cells are interferons, interleukins and tumor necrosis factors. Each cytokines plays a different role for example, interferons is important in limiting the spread of certain viral infections where else interleukins which consist of IL-1 to IL15 produced mainly by T cells involved in directing other cells to divide and differentiate.

2.1 Immunomodulation

Immunomodulation is a modification process of immune response, which is generally used to describe the pharmacological manipulation of immune system. It can either increase the magnitude of immune response; immunostimulation or decrease it; immunosuppression. This immunomodulation process can either be a specific immunomodulation which depends on a particular antigenic stimulus (e.g vaccination) or a non-specific where the immune system is altered by a wide range of antigenic stimuli or immunocompromised. The primary targets for this process include T cells, B cells and monocytes-macrophage.

The mechanisms involve in modifying the immune response are alteration of responsiveness either at cellular level or intracellular nucleotide level. The cellular level involves changes in membrane surface and metabolism of phospholipids, fatty acids and prostaglandins. In the intracellular level, the alteration of cyclic AMP (cAMP) and cyclic GMP (cGMP) in lymphocyte occur. High level of cAMP will inhibit the effector function of lymphocyte where as the increase in cGMP level will promote lymphocyte activity and thus have immunopotentiating properties. Substances that can increase cAMP are glucocorticoids. Plant lectins and lipopolysaccharides act in cGMP agonists. The effect of cyclic nucleotides on lymphocyte indicate that cGMP stimulates