Immunomodulatory Effects of Traditional Chinese Herbal Formulation, Ginseng and Dang Gui Ten Combination (PS10)

Thesis submitted for the
Degree of
Master of Science

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Graduate School of Integrative Medicine
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Acknowledgements

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### Abbreviations and definitions

#### Abbreviations

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<td>BRM</td>
<td>Biological response modifiers</td>
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<tr>
<td>CFU assay</td>
<td>Colony-forming unit assays.</td>
</tr>
<tr>
<td>CSF</td>
<td>Colony-stimulating factors (also called haematopoietic growth factors)</td>
</tr>
<tr>
<td>E:T cell ratio</td>
<td>Effector to target cell ratio</td>
</tr>
<tr>
<td>GALT</td>
<td>Gut-associated lymphoreticular tissues</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatogram</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>HSC</td>
<td>Haemopoietic stem cell</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IL-2</td>
<td>Interleukin 2</td>
</tr>
<tr>
<td>K562</td>
<td>Human erythroleukemia cell line K562</td>
</tr>
<tr>
<td>LAK cells</td>
<td>Lymphokine-activated killer cells</td>
</tr>
<tr>
<td>LFT</td>
<td>Liver Function Test</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>NK cells</td>
<td>Natural Killer cells</td>
</tr>
<tr>
<td>NO</td>
<td>Nitrous oxide</td>
</tr>
<tr>
<td>PBL</td>
<td>Peripheral blood lymphocytes (PBL)</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PS10</td>
<td>The herbal test medication.</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>T-cells</td>
<td>T-lymphocytes</td>
</tr>
<tr>
<td>TCM</td>
<td>Traditional Chinese Medicine</td>
</tr>
<tr>
<td>Th1 and Th2 subset</td>
<td>T-lymphocyte helper type 1 and 2 subset</td>
</tr>
<tr>
<td>TJ-48</td>
<td>Product name for Japanese variation of the test medication.</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TLR-4</td>
<td>Human Toll-like receptor 4</td>
</tr>
<tr>
<td>TMP</td>
<td>Tetramethylpyrazine, activity constituent in Ligusticum wallichii</td>
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<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor</td>
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## Definitions

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<th><strong>Term</strong></th>
<th><strong>Definition</strong></th>
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<tr>
<td>Active ingredient or constituents</td>
<td>Active ingredients refer to ingredients of herbal medicines with therapeutic activity. In herbal medicines where the active ingredients have been identified, the preparation of these medicines should be standardized to contain a defined amount of the active ingredients, if adequate analytical methods are available. In cases where it is not possible to identify the active ingredients, the whole herbal medicine may be considered as one active ingredient.</td>
</tr>
<tr>
<td>Adaptogen</td>
<td>Increases the resistance to physical, environmental, emotional or biological stressors, restores normal physiological function to the body, also known as whole body tonic.</td>
</tr>
<tr>
<td>Alterative, depurative</td>
<td>Improves detoxification of the body (by improving digestion, and the function of liver/gallbladder, kidney/bladder and/or the immune system); also known as alterative or ‘blood purifiers’.</td>
</tr>
<tr>
<td>Biological Response Modifiers</td>
<td>Substances used in biological therapies may be referred to as <em>biological response modifiers</em> (BRMs). BRMs alter the interaction between the body's immune defences and cancer, thus improving the body's ability to fight the disease.</td>
</tr>
<tr>
<td>Biological therapy</td>
<td>Biological therapies use the body's immune system, either directly or indirectly, to fight cancer or to lessen side effects that may be caused by some cancer treatments.</td>
</tr>
<tr>
<td>Bitter, bitter tonic</td>
<td>Bitter herbs stimulate the digestive system via reflexes from taste buds, gastrin and vagus; improves absorption.</td>
</tr>
<tr>
<td>Blood tonic</td>
<td>Prescribed for the syndrome known as 'Blood deficiency' in TCM. There is no similar concept in Western medicine or Western herbalism: 'Blood deficiency' is not always due to anaemia and the 'Blood tonic' herbs do not always stimulate the</td>
</tr>
</tbody>
</table>
production of red blood cells. The 'Blood tonics' have some characteristics in common with nutritive tonics, adrenal tonics, adaptogens, and circulatory stimulants and are sometimes prescribed for longevity and fertility.

'Blood deficiency' often accompanies chronic disease, including heart failure, chronic hepatitis, prolonged emotional disturbance, menstrual irregularity, anorexia and low body weight. It is common after childbirth and breast feeding, and can be both the cause and the result of menstrual irregularity. It is commonly used for women, but can be given to men who have 'Blood deficiency' symptoms.

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<th>Carminative</th>
<th>Relieves flatulence, usually by relaxing intestinal sphincter muscles.</th>
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<td>Choleretic, cholagogue</td>
<td>Increases the production and flow of bile.</td>
</tr>
<tr>
<td>Complementary and Alternative Medicine (CAM)</td>
<td>The terms &quot;complementary medicine&quot; or &quot;alternative medicine&quot; are used inter-changeably with traditional medicine in some countries. They refer to a broad set of health care practices that may or may not be part of that country's own tradition and are not integrated into the dominant health care system.</td>
</tr>
<tr>
<td>Decoction</td>
<td>Hot water extract. Usually means to boil the herbs for one hour.</td>
</tr>
<tr>
<td>Depurative, alterative</td>
<td>Improves detoxification of the body (by improving digestion, and the function of liver/gallbladder, kidney/bladder and/or the immune system); also known as alterative or 'blood purifiers'.</td>
</tr>
<tr>
<td>Diaphoretic</td>
<td>Promotes sweating during a fever.</td>
</tr>
<tr>
<td>Diuretic</td>
<td>Increases urinary output and/or increases the excretion of metabolic waste products.</td>
</tr>
<tr>
<td>Ginseng and Dang Gui Ten Combination</td>
<td>English name for the traditional Chinese formulation that is the subject of the present study.</td>
</tr>
<tr>
<td>Hepatic</td>
<td>A remedy that improves the function of the liver and normalises the flow of bile.</td>
</tr>
<tr>
<td>Hepatoprotective</td>
<td>Protects the liver against damage from toxins.</td>
</tr>
<tr>
<td><strong>Term</strong></td>
<td><strong>Definition</strong></td>
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<tr>
<td>-----------------------</td>
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<tr>
<td>Juzen-taiho-to</td>
<td>Japanese name for the traditional formulation which is the subject of the present study.</td>
</tr>
<tr>
<td>Kampo</td>
<td>Traditional Japanese Medicine</td>
</tr>
<tr>
<td>Liquid extract, herbal</td>
<td>A hydro-ethanolic extract of crude herbal material with a drug solvent ratio of 1:1 or 1:2 (1 part herb to 1 or 2 parts solvent).</td>
</tr>
<tr>
<td>Liver tonic</td>
<td>A remedy that improves the function of the liver and normalises the flow of bile.</td>
</tr>
<tr>
<td>Lytic unit</td>
<td>The lytic unit is calculated as the sum of the absolute number of monocytes and absolute number of lymphocytes multiplied by the NK cell cytotoxicity gradient. It is a measurement of the combined effect of monocytes, lymphocytes and NK cells.</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td>Phytotherapy</td>
<td>Plant-therapy, modern term for scientifically validated herbal medicine.</td>
</tr>
<tr>
<td>Qi tonic</td>
<td>Energy tonic. Indicated for fatigue, tiredness of the extremities, sweating (including night sweating) and weakness of the digestive processes</td>
</tr>
<tr>
<td>Restorative</td>
<td>Restores normal function of the body, organ or system.</td>
</tr>
<tr>
<td>Saponins</td>
<td>Soap-like constituents found in herbs.</td>
</tr>
<tr>
<td>Shi Quan Da Bu Tang</td>
<td>Chinese name for the traditional formulation which is the subject of the present study.</td>
</tr>
<tr>
<td>Shimotsu-to (Dang Qui Four)</td>
<td>One of the sub-formulations containing four of the ten herbs in the test formulation PS10.</td>
</tr>
<tr>
<td>Synergy</td>
<td>When the combined action of two or more substances is greater than the action of the substances in isolation.</td>
</tr>
<tr>
<td>Vital force</td>
<td>A life giving energy, which permeates all living things, the energy, which heals, and repairs damaged tissue.</td>
</tr>
</tbody>
</table>
Abstract

Herbal Preparation – Ginseng & Dang Gui Ten Combination (PS10) and Immune Response in Humans

The severe debility and immune dysfunction associated with serious disease may respond well to treatment with the tonic formulas from Traditional Chinese Medicine (TCM). One of these, Ginseng and Dang Gui Ten Combination has gained prominence as the formula most suitable to assist convalescence after chemotherapy and radiotherapy. A literature review of the herbal combination suggests that it synergistically provides a broad range of pharmacological activity with a very low level of toxicity. The herbs may have haemopoietic, antimitogenic, antitumour, immunomodulatory and anticomplement activities and they seem to promote lymphocyte activation, interleukin production, protect various organs against toxicity, inflammation and ulceration, and promote drug delivery and radiation sensitising while protecting healthy tissue.

The specific immunomodulatory effects of PS10 combination were investigated in 10 healthy volunteers (7 males and 3 females aged 43 to 58 years). The study was a longitudinal study (28 days), using a repeated measures design to investigate the pre and then post intervention changes in Natural Killer (NK) cell activity as well as total and differentiated lymphocyte counts. Furthermore, liver function tests (LFT) were included to assess any adverse effects on the liver. It was envisaged that NK cells or other white blood cell subset variation could indicate an immunomodulatory effect of the herbal formulation, PS10.

Investigative methodologies included NK cell function assessment via the ability of peripheral blood lymphocytes (PBL) to lyse the human erythroleukemia cell line K562. (Lozzio and Lozzio, 1975) Target cells are labelled by incubation with radioactive chromium, washed, then added to a dilution series of PBMC’s and incubated for 4 hours. Supernatants are harvested and the amount of intracellular chromium-51 released into the supernatant is measured with a gamma counter.
The amount of chromium-51 released is proportional to the lytic activity of the NK cells. The gradient of the line of best fit through the plotted points was recorded as the measure of cytotoxicity or killing. (Brooks and Flannery, 1980) The steeper the gradient, the greater the cytotoxicity.

This pilot study demonstrated that the herbal preparation Ginseng and Dang Gui Ten Combination (PS10) increased the total number of lymphocytes cells in healthy human volunteers (n=10) (p<0.007). Although the test formulation increased NK cell activity in some participants, the change in mean NK cell cytotoxicity was not significant (p<0.07)
Statement of Originality

I declare that this thesis does not contain any material which has previously been submitted for the award of any other Degree or Diploma in any University or other institution and to the best of my knowledge and belief, the Thesis contains no material previously published or written by another person, except where due reference is made in the text.

I further declare that the ethical principles and procedures specified by the Swinburne University Human Research Ethics Committee have been adhered to in preparation of this report.

Michael Thomsen
Graduate School of Integrative Medicine
Swinburne University of Technology
1. Introduction

1.1 Objective of present thesis

The severe debility and immune dysfunction associated with serious disease may respond well to treatment with the tonic formulas from Traditional Chinese medicine. One of these, Ginseng and Dang Gui Ten Combination has gained prominence as the formula most suitable to assist convalescence after chemotherapy and radiotherapy.

The Department of Pharmacology, Toxicology and Therapeutics at the University of Kansas Medical Centre screened 116 Chinese herbal formulations for their potential ability to restore immunity in cancer patients, potentiate the therapeutic effect and ameliorate adverse toxicity of anticancer agents. ‘Ginseng and Dang Gui Ten Combination’ was selected as ‘the most potent biological response modifier’ after the evaluation of 116 different Chinese herbal formulas.

Ginseng & Dang Gui Combination has been shown to:
Modulate and potentiate the immune system by stimulating haematopoietic factors and production of interleukins in association with natural killer cells.
Potentiate therapeutic activity in chemotherapy (mitomycin, cisplatin, cyclophosphamide and fluorouracil) and radiotherapy
Inhibit the recurrence of malignancies, prolongs survival, as well as ameliorating and/or preventing adverse toxicities (GIT disturbances such as anorexia, nausea, vomiting, hematotoxicity, immunosuppression, leucopenia, thrombocytopenia, anaemia and nephropathy etc) of many cancer drugs.

The herbs in Ginseng and Dang Gui Ten Combination seem to work synergistically to provide a broad range of pharmacological activity with very low level of toxicity. The herbs possess haemopoietic, antimutagenic, antitumour, immunomodulatory and anticomplement activities. They promote lymphocyte activation, interleukin production, protect various organs against toxicity, inflammation and ulceration, and promote drug delivery and radiation sensitising while protecting healthy tissue.(Zee-Cheng, 1992)
The objective of this Graduate School of Integrative Medicine MSc Candidature is to investigate the specific immunomodulatory effects of ‘Ginseng and Dang Gui Ten Combination’ as well as to investigate the chemical profiles of the ten herbs in the formulation by thin layer and high performance liquid chromatography (TLC and HPLC). These tests are used to identify the correct herbal material and quantify the level of active constituents or marker compounds for quality control purposes.

1.2 Perspective on Traditional Medicine

The World Health Organisation defines traditional medicine as: “The sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.”

Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities.

Herbal Medicines include:

Plants including crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts.

Plant material including fresh juices, gums, fixed oils, essential oils and resins. In some countries, these materials may be processed by various local procedures, such as steaming, roasting, or stir-baking with honey, alcoholic beverages or other materials.

Finished herbal products may include comminuted or powdered plant materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.
Finished herbal products consists of herbal preparations made from one or more herbs. If more than one herb is used, the term herbal mixture is also used. Finished herbal products and herbal mixtures may contain excipients in addition to the active ingredients. However, finished products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal. (WHO, 2000)

1.2.1 Herbal medicine used worldwide

About 25% of modern medicines are descended from plants first used traditionally. The World Health Organisation estimates that up to 80% of people in the non-Western countries use traditional or complementary and alternative medicine as part of primary health care. Traditional medicine has been fully integrated into the health systems of China, Japan and other Asian countries. Traditional Chinese medicine is fully integrated into China’s health system where 95% of Chinese hospitals have units for traditional medicine. Ayurveda, siddha and unani systems of medicine have coexisted with yoga, naturopathy and homeopathy for centuries. Traditional medicine is widely used in India, particularly in rural areas, where 70% of the population lives. 2860 Indian hospitals provide traditional Indian medicine. A recent survey showed that 72% of registered western-style doctors in Japan use kampo medicine (the Japanese adaptation of Chinese medicine) in their clinical services. In Western countries, growing numbers of patients rely on alternative medicine for preventive or palliative care. In many Western countries including France, Germany and Australia it is estimated that over 60% of the population has used complementary medicine at least once. The worldwide importance of herbal medicine as a significant contributor in the health of the people is recognised by the inclusion of herbal medicines in the Essential Drugs and Medicines Policy of the World Health Organisation. (WHO, 2003)
1.3 Formula development in Traditional Chinese Medicine

The formulations in Traditional Chinese Medicine are not mere collections of medicinal substances in which the actions of one herb are simply added to those of another in a cumulative fashion. They are complex recipes of interrelated substances, each of which affects the actions of the others in the formula. It is this complex interaction that makes the formulas so effective, but also makes them more difficult. Every medicinal substance has its strength and weaknesses and a well-designed formulation is carefully balanced to accentuate the strengths and reduce the side effects. This concept is also known as synergy where the effect of the formulation is larger than the sum of the individual parts.

One has to become familiar with not only the actions and characteristics of individual herbs, but also with particular formulations that have over time become the building blocks of numerous formations.

Chinese herbal medicine is obviously very complex and impossible to fully comprehend unless one is properly trained in Traditional Chinese Medicine; there are certain concepts, which can relatively be incorporated into Western herbal medicine.

Apart from attention to the qualities as outlined in the section on the Humoral theory, there is another concept, which can easily be incorporated into the system of Western herbal medicine. This is the concept of the Hierarchy of Ingredients.

1.3.1 Hierarchy of Ingredients

In traditional Chinese society, rank was very important and this is reflected in the terminology used to grade the importance of the various herbs in a formulation.

Chief

The most important herb in a formulation is the Chief (also known as the monarch, ruler, king, emperor or principal). This herb is chosen primarily to treat the disease. The chief ingredient is usually given in the highest dosage.
Deputy
The deputy (also known as minister, adjutant, or associate) has two functions:
Aiding the chief ingredient in the treating the disease
Being the main herb to treat a coexisting disease

Assistant
The assistant (also known as the adjutant) has three functions:
Reinforcing the effects of the chief or deputy ingredients or directly treating a less important aspect of the disease (in such a case it is known as the helpful assistant)
Moderating or eliminating the toxicity or harshness of the chief or deputy ingredients (the corrective assistant)
Having an opposite effect to that of the chief ingredient (the opposing assistant). This is used in very serious or complex disorders.

Envoy
The envoy (also known as the messenger, guide or conductant) has two functions:
To focus the formulation on a certain channel or area of the body
To harmonise and integrate the actions of the other ingredients
Note that not all formulations, however, contain the full hierarchy of ingredients.

1.3.2 Formula Modification
Adjustments are often made to a formulation based on the strength of the patient, the season, climate and other environmental factors. This may involve altering the selection of herbs or their relative dosage, the method of preparation, or the means of administration.(Bensky and Gamble 1986)

1.4 Ginseng and Dang Gui Ten Combination
Ginseng and Dang Gui Ten Combination is a combination of the Blood and Qi tonic with the addition of Astragalus and Cinnamon. This formulation is used for general debility, anaemia, poor appetite and loss of weight, weak muscles, poor stamina, weak knees, and for
recovery after childbirth, surgery or chronic illness and cancer. It is a modified combination of Dang Gui Four and Four Major Herb Combinations. The formulation has been modified by the addition of *Cinnamomum cassia* and *Astragalus membranaceus*.

The combination was originally formulated by Tai-Ping Hui-Min Ju (Public Welfare Pharmacy Bureau) of the Chinese Song Dynasty in AD 1200. (Zee-Cheng, 1992)

**Dang Gui Four Combination** (Si Wu Tang) is essentially a ‘blood tonic’:

Blood tonics are prescribed for the syndrome known as 'Blood deficiency' in TCM. There is no similar concept in Western medicine or Western herbalism: 'Blood deficiency’ is not always due to anaemia and the 'Blood tonic' herbs do not always stimulate the production of red blood cells. The ‘Blood tonics’ have some characteristics in common with nutritive tonics, adrenal tonics, adaptogens, and circulatory stimulants and are sometimes prescribed for longevity and fertility.

‘Blood deficiency’ often accompanies chronic disease, including heart failure, chronic hepatitis, prolonged emotional disturbance, menstrual irregularity, anorexia and low body weight. It is common after childbirth and breast feeding, and can be both the cause and the result of menstrual irregularity. It is commonly used for women, but can be given to men who have 'Blood deficiency' symptoms.

**Four Major Herb Combination** (Si Tun Zi Tang) is essentially a Qi tonic indicated for fatigue, tiredness of the extremities, sweating (including night sweating) and weakness of the digestive processes.

**Ten Precious Tonic Combination** is a combination of the Blood and Qi tonic with the addition of Astragalus and Cinnamon. This formulation is used for general debility, anaemia, poor appetite and loss of weight, weak muscles, poor stamina, weak knees, and for recovery after childbirth, surgery or illness. (Trickey R, 1996)
1.4.1 Analysis of the Formulation

The Chief Ingredients
The chief herbs are warming, Panax ginseng (ren shen) augments the qi and Rehmannia glutinosa (shu di huyang) nourishes the blood.

The Deputy Ingredients
Two of the deputies, Atractylodes macrocephala (bai zhu) and Poria cocos (hoelen, fu ling), strengthen the Spleen and dry dampness, thereby assisting Panax ginseng in strengthening the qi of the Spleen and Lungs.

The other deputy ingredients, Paeonia lactiflora (bai shao) and Angelica sinensis (dang gui), nourish the blood and thereby reinforce the action of Rehmannia glutinosa.

The Assistant Ingredients
One of the assistant ingredients, Ligusticum wallichii (chuan xiong), invigorates the blood and promotes the movement of qi. The other, honey-fried Glycyrrhiza glabra (zhi gan cao), augments the qi and harmonises the Middle Burner (the Middle Burner is basically associated with digestion and transformation of energy). Ligusticum wallichii (also known as Cnidium radix, Chuan Xiong or Cnidium officinale) is a different herb to Cnidium monnieri, which is used for infections of the skin and mucous membranes and for infertility.

Formulation Modification
Astragalus membranaceus (Huang qi) has been added to the formula to further tonify the Spleen qi. Astragalus is indicated for anorexia, fatigue, diarrhoea and deficiency conditions with spontaneous sweating. Cinnamon cassia (rou gui) is warming and is used in Yang deficient formulations (complaints characterised by a sense of coldness, weakness and hypofunction associated with a deficiency in vital energy). The final formulation is listed in table 1.
<table>
<thead>
<tr>
<th>Name of Sub-formulation</th>
<th>Botanical Name</th>
<th>Chinese Name</th>
<th>English Common Name</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dang Gui Four (Si Wu Tang, Shimotsu)</td>
<td>Angelica sinensis</td>
<td>Dang Gui</td>
<td>Dang gui</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>Rehmannia glutinosa</td>
<td>Shu Di Huang</td>
<td>Prepared Chinese Foxglove</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>Paeonia lactiflora</td>
<td>Bai Shao</td>
<td>Peony</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>Ligusticum wallichii</td>
<td>Chuan Xiong</td>
<td>Szechuan Lovage Root</td>
<td>6%</td>
</tr>
<tr>
<td>Four Major Herb Combination (Si Jun Zi Tang, Shikunshi To)</td>
<td>Panax ginseng</td>
<td>Ren Shen</td>
<td>Korean Ginseng</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>Atractylodes macrocephala</td>
<td>Bai Zhu</td>
<td>White atractylodes</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>Poria cocos</td>
<td>Fu Ling</td>
<td>Hoelen</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>Glycyrrhiza uralensis</td>
<td>Zhi Gan Cao</td>
<td>Honey fried Chinese liquorice</td>
<td>5%</td>
</tr>
<tr>
<td>Formula modification</td>
<td>Astragalus membranaceus</td>
<td>Huang Qi</td>
<td>Milk-vetch</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>Cinnamomum cassia</td>
<td>Rou Gui</td>
<td>Cinnamon quills</td>
<td>6%</td>
</tr>
</tbody>
</table>

**Figure 1: Dried Herbs Used in the Test Preparation**

### 1.4.2 Traditional Preparation

The dried herbs (see figure 1) are grinded to a coarse powder and decocted (boiled) for one hour before being strained. Alternatively it may be prepared by steeping for several weeks in rice wine. The recommended dosage in TCM (traditional Chinese medicine) and Kampo (Traditional Japanese medicine) is anywhere from 9 to 35 grams by decoction or pills made from spray-dried decoction.

### 1.4.3 Traditional Indications

Ginseng & Dang Gui Combination has traditionally been used to treat patients with anaemia, anorexia or fatigue. Today, the main uses are convalescence, cancer support and disorders associated with immune suppression.
In Traditional Chinese Medicine it is indicated for persons with reduced vitality and general debility who have difficulty maintaining their own body heat and often complain of feeling cold. They have a pale cold face and extremities, poor appetite, weak digestion, loose stools, and abundant pale urine. They may suffer from weight loss, poor muscle strength, lack of stamina, and weak knees. Ginseng and Dang Gui Ten Combination is often used during winter as a general warming tonic.

1.5 Biological Cancer Therapy

Biological therapy (sometimes called immunotherapy, biotherapy, or biological response modifier therapy in the scientific literature) is, according to the National Cancer Institute (USA), a promising new addition to the family of cancer treatments that includes surgery, chemotherapy and radiation therapy.(National Cancer Institute, 2001) Biological therapies use the body’s immune system, either directly or indirectly, to fight cancer or to lessen side effects that may be caused by some cancer treatments. The body has a natural ability to protect itself against diseases, including cancer. The immune system may recognise the difference between healthy cells and cancer cells in the body and eliminate those that become cancerous. Cancer may develop when the immune system breaks down or is overwhelmed. Biological therapies are designed to repair, stimulate, or enhance the immune system’s natural anticancer function.

1.5.1 Biological Response Modifiers

Substances used in biological therapies may be referred to as biological response modifiers (BRMs). BRMs alter the interaction between the body’s immune defences and cancer, thus improving the body’s ability to fight the disease. BRMs (such as cytokines and antibodies) are substances that occur naturally in the body, however in recent decades a variety of BRMs have been produced as medical agents. BRMs may be used to enhance a cancer patient’s immune system, eliminate, regulate, or suppress body responses that permit cancer growth, make cancer cells more susceptible to destruction by the immune response, block or reverse the process that changes a
normal cell or a precancerous cell into a cancerous cell, reduce the side effects of other forms of cancer treatment, such as chemotherapy or radiation and reduce the risk of metastasises. BRMs under investigation include interferons, interleukins, tumour necrosis factor, colony-stimulating factors, monoclonal antibodies and cancer vaccines. Interferons were the first laboratory-produced cytokines. There are three major families of interferons, interferon alpha, beta and gamma. Interferon alpha currently is the most widely used in cancer treatment. Interferons may improve a cancer patient's immune response against cancer cells. In addition, interferons may act directly on cancer cells by inhibiting their growth or promoting their development into cells with more normal behaviour. Researchers believe that some interferons also may stimulate B cells and T cells, strengthening the immune system's anticancer function.

Like interferons, interleukins are cytokines that occur naturally in the body and can be made in the laboratory. Although many interleukins have been identified, interleukin-2 (IL-2) has been the most widely studied in cancer treatment. IL-2 stimulates the growth and activities of many immune cells including lymphocytes. Lymphocytes stimulated by IL-2, called lymphokine-activated killer (LAK) cells, have proven to be effective in destroying tumours.

Like the interferons and interleukins, TNF, another type of cytokine stimulates the body's immune cells to fight cancer. TNF is cytotoxic and may reduce angiogenesis. Although TNF has shown promising antitumour activity in laboratory studies, the dose needed for this level of activity is extremely toxic.

Unlike TNF, colony-stimulating factors (CSFs) (also called haematopoietic growth factors) usually do not directly affect tumour cells. Researchers have identified several CSFs (such as G-CSF and GM-CSF) that encourage bone marrow cells to divide and develop into various specialised white blood cells, platelets, and red blood cells. (National Cancer Institute, 2001)
Side Effects of Medical Biological Response Modifiers

Because BRMs are often administered by injection, rashes or swelling may develop at the site where the shots are given. Several BRMs, including interferons and interleukins, may cause flu-like symptoms including fever, chills, tiredness, and digestive tract problems. Blood pressure may also be affected. Side effects with IL-2 and TNF can often be severe, and patients need to be closely monitored during treatment. Side effects with antibody therapy vary, and allergic reactions may occur. Cancer vaccines may cause minor side effects including fever and muscle aches. (National Cancer Institute, 2001)

1.5.2 Herbal Biological Response Modifiers (BRM)

As evident by the serious side effects caused by chemotherapeutic agents, it is clear that herbal medicines should not be employed because of any direct cancer killing properties. In fact, perhaps we should not even refer to our cancer herbs as anti-cancer herbs, as the term anticancer is used to describe a drug, which has proven to be toxic to tumour cells in human clinical trials. Herbal cancer therapy should not aim at killing cancer cells, but should aim at supporting the body’s innate ability to fight cancer.

A new term has been coined to describe this process: Herbal Biological Response Modifiers (Herbal BRMs). Herbal BRMs include herbs with the following actions: adaptogen, immunomodulator, immunostimulant, nervine tonic and sedative, digestive, alterative (depurative), laxative, liver tonic, hepatoprotective, lymphatic, circulatory stimulant, antiplatelet and others. Some of these herbs may be used in fairly high doses to actively support the body to fight the cancer; others are more employed to support the body cope with the symptoms of cancer or the side effects of conventional cancer treatments.

1.5.3 Ginseng and Dang Gui Ten Combination – a herbal BRM

The severe debility and immune dysfunction associated with serious disease may be treated with tonic formulas in Traditional Chinese medicine. One of these, Shi Quan Da Bu Tang, has gained prominence as the formula most suitable to assist convalescence after
chemotherapy and radiotherapy. It is also known as Ginseng and Dang Gui Ten Combination, Ten Precious Substances Tonic, Ten Flavour Tea (Chinese patent medicine), Shih Chuan Ta Pu Tang, Juzen-taiho-to in Japanese. Ginseng and Dang Gui Ten Combination is made up of Dang Gui and Ginseng Eight Combination with two additional herbs, cinnamon and astragalus (see table 2).

**Table 2: Ginseng and Dang Ten Combination**

<table>
<thead>
<tr>
<th>Chinese Name</th>
<th>Shi Quan Da Bu Tang</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese Name</td>
<td>Juzen-taiho-to</td>
</tr>
<tr>
<td>Western Names</td>
<td>Ginseng and Dang Gui Ten Combination</td>
</tr>
<tr>
<td></td>
<td>Ten Precious Substances Tonic</td>
</tr>
<tr>
<td></td>
<td>Ten Significant Tonic Decoction</td>
</tr>
<tr>
<td></td>
<td>All inclusive Great Tonifying Combination</td>
</tr>
<tr>
<td>Kampo formulation number</td>
<td>TJ48 (Japanese numbering system for Kampo formulations)</td>
</tr>
<tr>
<td>Contents</td>
<td>Angelica sinensis (Dang gui)</td>
</tr>
<tr>
<td></td>
<td><em>Rehmannia glutinosa</em> (Prepared Rehmannia)</td>
</tr>
<tr>
<td></td>
<td>Paeonia lactiflora (Peony)</td>
</tr>
<tr>
<td></td>
<td><em>Ligusticum wallichii</em> (Szechuan Lovage Root, also known as Cnidium)</td>
</tr>
<tr>
<td></td>
<td>Glycyrrhiza uralensis (Liquorice)</td>
</tr>
<tr>
<td></td>
<td>Poria cocos (Hoelen)</td>
</tr>
<tr>
<td></td>
<td>Atractylodes macrocephala (Atractylodes)</td>
</tr>
<tr>
<td></td>
<td><em>Panax ginseng</em> (Korean Ginseng)</td>
</tr>
<tr>
<td></td>
<td>Astragalus membranaceus (Astragalus)</td>
</tr>
<tr>
<td></td>
<td><em>Cinnamomum cassia</em> (Cinnamon quills)</td>
</tr>
</tbody>
</table>
1.6 Scientific Investigation

1.6.1 Introduction
Since the early 1980s there have been many studies examining the activities of Ginseng & Dang Gui Ten Combination. This chapter reviews the clinical and pharmacological studies. The literature review was undertaken with the objective of examining the published research on the effects of the formulation Ginseng & Dang Gui Ten Combination and its individual herbs on the immune system as well as its general pharmacological effects especially in relation to cancer and debility. In the following chapters the term Ginseng and Dang Gui Ten Combination is used when describing the herbal formulation in general terms or when research refers specifically to the Chinese extract named Shi Quan Da Bu Tang. The terms Juzen-taiho-to and TJ-48 are used when the research is based on Japanese extracts.

1.6.2 Immunomodulatory activities

_Cytokine and Lymphocyte Activation_

T helper cells are classified into Th1 and Th2 subset according to the cytokines they secrete. The ratio of Th1 to Th2 is important for the balance of cellular and humoral immunity. An imbalance may be associated with excessive inflammation, allergies and autoimmune disorders. Cytokines IFN-gamma and IL-2 are known to stimulate cellular immunity while cytokines like IL-4 and IL-5 stimulate humoral immunity. Ginseng and Dang Gui Ten Combination has been shown in an early study to increase interferon-gamma and interleukin 2 (IL-2). The herbal formulation administered orally to mice after transplantation of Ehrlich ascites tumours increased survival rate compared to the control group. It was furthermore shown to increase INF-gamma and interleukin-2 in phyto-haemagglutinin-stimulated peripheral blood mononuclear cells. (Sakagami et al. 1988) Further studies have been performed as researchers attempt to identify the tissue most responsive to stimulation by the Ginseng and Dang Gui Ten Combination and uncover the nature of the cytokine stimulation. The gut-associated lymphoreticular tissues (GALT) in the intestinal
mucosa play an important role in host defence including IgA response. As the herbal formulation is administered orally this gastric mucosal immune system may be a target tissue. To examine this theory, TJ-48 was administered to mice for two weeks and the effects on cytokine production investigated. Lymphocytes from the spleen, mesenteric lymph nodes and Peyer’s patches were harvested and stimulated with concanavalin A. Administration of the herbal formulation caused enhancement of INF-gamma and IL-4 but decreased IL-5 from the spleen, while IFN-gamma production and IL-5 secretions were markedly increased from both mesenteric lymph nodes and Peyer’s patches. Secretion of IL-2 was decreased from both types of lymphocytes. These results seem to indicate the Ginseng and Dang Gui Ten Combination is better at stimulating cytokine secretion from GALT than from the spleen. Examination of various CD markers found that the cytokine modulation was due to functional changes rather than a change in lymphocyte population. (Matsumoto and Yamada, 2000)

Another possible mechanism for stimulating the cytokine network is via the liver. The liver is an important immune organ due to an abundance of lymphocytes in addition to the Kupffer cells. As the herbal formulation is taken orally and the portal vein drains into the liver, it is likely that active compounds in the herbs are delivered directly to the liver where they can exert an immunological effect. The effect of oral administration of Juzen-taiho-to on hepatic lymphocytes was therefore investigated. A study found a ten-fold increase in interferon gamma compared to controls. IL-4, IL-5, IL-6 and IL-12 secretions were also elevated. The stimulation of Th-1 associated cytokines (IFN-gamma) and Th-2 cytokines (IL-4, IL-5 and IL-6) suggests that Juzen-taiho-to induces a balanced production of the T helper cell subsets. IL-2 is one of the key cytokines stimulating proliferation of activated T lymphocytes. An excessive production of IL-2, however, may produce an imbalance of Th-1 to Th-2. The study found that the herbs reduced IL-2 (at least in the liver), which also points to an overall balancing effect of Juzen-taiho-to on the liver cytokine network. The herbal formulation was also shown to stimulate the induction of NK cells
possibly mediated through IL-12 and activation of macrophages (Kupffer cells) in the liver. (Matsumoto et al. 2000)

Tumour necrosis factor (TNF) is thought to cause tumour necrosis by inducing haemorrhagic infarction following circulatory disturbance characterised by hyperaemia, increased permeability and the formation of multiple fibrin thrombi in the tumour vessels. TNF activates the arachidonic acid cascade and necrosis is accompanied by infiltration by polymorphonuclear leukocytes. It is suggested that Juzen-taiho-to stimulates the potency of TNF while reducing its toxicity by reducing free radical damage and by inhibiting the release of inflammatory leukotrienes from the arachidonic acid pathway. (Satomi et al. 1989)

Juzen-taiho-to has also been shown to decrease TNF activity in postoperative cancer patients with advanced tumours and increase it in those with early tumours. Subjectively, patients reported feeling better with less malaise when taking Juzen-taiho-to. (Kurokawa T. and Tamakuma S., 1995)

TJ-48 has recently been shown to induce cytotoxic T lymphocytes specific for allogeneic tumour cells from peripheral blood mononuclear cells in vitro. TJ-48 was furthermore shown to decrease the monocyte to T cell ratio (M:T ratio) ratio in patients with gynaecologic cancer. (Maeda, 2004)

Increased Phagocytic Activity

Oral administration of Ginseng and Dang Gui Ten Combination and other Chinese formulations has been shown to enhance the function of the reticuloendothelial system in mice. The relative organ weights of thymus, spleen and liver have been shown to be increased as compared to control. One of the anti-tumour activities is thought to be due to stimulation of phagocytic activity. (Ito and Shimura, 1985b)

The protective efficacy of TJ-48 in Candida-infected mice varied between the two strains of the mice, which share the same H-2 genes but are different in their responsiveness of macrophages (C3He J and
C3H/He N mice strains). It is therefore strongly suggested that the enhanced protection against Candida toxicity is associated with macrophage function as TJ-48 only augmented the immunocompromised mice. The root extract of *Panax ginseng* was shown to be as active as Tj-48 in protecting against candida toxicity. Oral administration of Ginseng and Dang Gui Ten Combination as well as *Panax ginseng* exert strong anti-Candida activity by activating macrophage function. Ginseng and Dang Gui Ten Combination given orally once daily for 5 consecutive days in a dose of 2 g/kg after intravenous infection of Candida albicans prolonged survival of infected mice strain C3H/He J strain which is characteristic of functional deficiency of macrophages, but not a mice strain with normal macrophage function (C3H/He N). The two mice strains are known to have the same genetic backgrounds apart from certain immunological differences. Macrophages from C3H/He N and J mice have been proven to have high and low responsiveness to bacterial lipopolysaccharide (LPS) respectively. Both control groups (N and J strains which didn’t receive the herbal formulation) died within 11 days post-infection. Ginseng and Dang Gui Ten Combination prolonged the lifespan of infected J-strain animals of up to 30 days. However, no such protection of the herbal formulation was observed in mice with normal macrophage function.

To identify the herbal component of Ginseng and Dang Gui Ten Combination responsible for augmenting macrophage function, individual herbal components were tested. *Atractylodes lanceae, Panax ginseng, Ligusticum wallichii* and *Angelica sinensis* clearly augmented the anti-candida activity of macrophages inhibiting the fungal growth by 50% or more as compare with the value for unsupplemented controls. *Panax ginseng* was found to have the most potent activity. When *Panax ginseng* was tested in vivo it showed a macrophage stimulating activity comparable to the whole formulation. Ginseng and Dang Gui Ten Combination must be given a few hours following the Candida inoculation to exert a protective effect. This supports the theory that herbs augment the non-specific rather than the antigen-specific immune response, probably through the production of
cytokines. This is further supported by the fact that the differences between C3H/He J and N mice is that the former are not only hyposensitive to LPS but also deficient in production of cytokines in response to several kinds of stimulation. (Akagawa et al. 1996)

*Natural Killer cells*

Tj-48 has been shown to significantly increase the number of T cells with only a slight increase in NK cells in aged but not in young mice. NK cell activity, however, has been shown to increase both in young and old mice with treatment with TJ-48. A significant decrease was observed in metastatic pulmonary colonies of B16 melanoma cells both in young and old mice treated with Tj-48 for 16 weeks. (Utsuyama et al. 2001)

*Antibody Production*

Pectic polysaccharides fraction stimulates antibody production in mice. (Kiyohara et al. 1995b)

*Infections*

Human Toll-like receptor 4 (TLR4) has recently been identified and has been shown to be the main protein involved in recognizing Gram-negative bacteria. We examined the regulation of TLR4 surface expression in a human monocytic cell line (THP-1 cells) by two traditional Chinese herbal medicines. Bu-Zhong-Yi-Qi-Tang (TJ-41) and Shi-Quan-Da-Bu-Tang (TJ-48). TJ-41 and TJ-48 up regulated TLR4 surface expression in THP-1 cells, as well as enhanced TLR4 surface expression in these cells both dose- and time-dependently. These findings suggest that TJ-41 and TJ-48 increase the receptor involved in the response to Gram-negative bacteria and may enhance defences against these pathogens. (Mita et al. 2002)

*Intestinal immune stimulation*

TJ-48 has been found to show an enhancing activity on proliferation of bone marrow cells mediated by Peyer's patch cells (intestinal immune system modulating activity). (Kiyohara et al. 2000) When TJ-48 was fractionated by methanol-water extractions, ethanol precipitation and dialysis, the water-soluble dialyzable (F-3) and polysaccharide
fractions (F-5) both showed a significant intestinal immune system modulating activity in vitro while the other fractions had no activity. Oral administration of F-3 (150 mg/kg) also showed the intestinal immune system modulating activity in mice. Two active substances were purified by gel filtration from F-3. Chemical analysis indicated that the active substances comprised lignin as well as a carbohydrate consisting mainly of arabinose, galactose, glucose and galacturonic acid. These results suggest that lignin-carbohydrate complexes are involved in intestinal immune system modulation by TJ-48.

**Immunosuppression**

TJ-48 and its herbal components have been found to be protective against experimentally candidiasis in cyclophosphamide-induced immunosuppressive mice. ICR mice were immunosuppressed by intraperitoneal treatment with cyclophosphamide (day 4) and were given oral TJ-48 for four consecutive days. They were then challenged intravenously with a lethal dose of Candida albicans. TJ-48 significantly prolonged the life span of the mice. A similar protective effect was observed by treatment with the component herbs *Panax ginseng*, *Atractylodes macrocephala* and *Ligusticum wallichii*. (Abe et al. 1998)

**Increased Mitogenic and Complement Activity**

Mitogenic pectin polysaccharides have been identified in Ginseng and Dang Gui Ten Combination. (Kiyohara et al. 1991) Extracts of *Glycyrrhiza uralensis*, *Astragalus membranaceus* and *Atractylodes macrocephala* showed significant mitogenic activity whereas Cinnamon showed potent complement-activating activity. Exclusion of any single component herb whether active or not on its own did not result in a loss or an increase of the overall activity of TJ-48. (Yamada et al. 1992) A novel type of B-cell mitogen has also been isolated from Juzen-taiho-to. (Takemoto et al. 1994)

**Antiplatelet activity**

Suppression of platelet activation by anti-platelet glycoprotein antibodies without inhibiting antibody binding. (Kawakatsu et al. 1994)
Anti-inflammatory activity
TJ-48 has been shown to enhance the expression of inducible nitric oxide synthase in and nitric oxide (NO) production in RAW264.7 cells, a murine macrophage cell line. iNOS-mediated activation of biodefense mechanism.(Kawamata et al. 2000)
TJ-48 significantly reduced delayed-type hypersensitivity induced by a variety of stimulants (picryl chloride, sheep red blood cells) in a dose dependent manner in mice.(Nose et al. 1999)

Protection against Radiation Damage
Ginseng and Dang Gui Ten Combination has lately come into prominence as treatment for the physical debilitation of chronic disease, cancer or surgery. Traditionally, however, it was used for patients with anaemia, anorexia and fatigue. This let researchers to consider if the formulation may have restorative effects on the haemopoietic system. Juzen-taiho-to has been shown to be protective against radiation damage in mice. TJ-48 was given via the drinking water after whole-body radiation of 5.5 Gy. The 30-day survival rate was 77.5% compared to 32.5% in the control group. The radioprotective effect was also observed in a later study. Mice were irradiated with doses from 1 to 7 Gy, and then given TJ-48 for 7 days after which peripheral blood and bone marrow cells were examined. The viable cells of bone marrow, thymus, spleen, peripheral white blood cells and platelets decreased in a radiation dose-dependant manner in both experimental and control group. The bone marrow cells were used for various colony-forming unit assays. CFU-S assay on day 14 showed that the number of colonies in recipients of bone marrow cells from TJ-48 treated groups showed evidence of good general condition including body weight and had heavier spleens. Stem cells in the bone marrow have the capacity to self-replicate and differentiate. It seems that Juzen-taiho-to has the ability to stimulate recovery from radiation injury by acting on or stimulating the haemopoietic stem cells as detectable on day 14 CFU-S assay. It is possible that *Panax ginseng* is one of the herbs responsible for this action as evidence by other studies on protection against radiation injury. *Panax ginseng* has also
been shown to increase the 30-day survival rate after lethal radiation. (Ohnishi et al. 1990)

**Haemopoietic Recovery**

It has been reported that Juzen-taiho-to has a profound stimulatory effect on lymphohaemopoiesis in humans and mice. The unsaturated fatty acids oleic and linolenic acids, which are present at a concentration of 0.1% in TJ-48, have been identified to have haemopoietic stem cell (HSC)-stimulating activity. In vitro studies using mouse HSCs as well as studies using normal human bone marrow cells and umbilical cord blood cells have found that the fatty acids actively promote the proliferation of haemopoietic stem cells, and that the effect is mediated by stromal cells, rather than by any direct action on the HSCs. (Hisha et al. 2002)

Patients with Shwachman syndrome suffer diarrhoea due to exocrine pancreatic insufficiency as well as neutropenia and anaemia due to bone marrow hypoplasia. The syndrome may also be characterised clinically by short stature, skeletal abnormalities and various other less common findings.

The link between the pancreatic dysfunction and bone marrow hypoplasia is unknown but several genes control the functions of both the pancreas and haemopoiesis and chromosomal damage may elicit both dysfunctions. It is also possible that a copper deficiency in the newborn may elicit the syndrome. The general treatment for Shwachman syndrome is the administration of pancreatic enzymes such as pancreatin. G-CSF may also be administered for severe neutropenia and infections, although the effects are short-lived.

Juzen-taiho-to is reported to have induced a marked haemopoietic recovery in one patient with Shwachman syndrome. The patient had twice previously suffered severe pancytopenia but her condition was stabilised with the administration of TJ-48. Production of colony-stimulating activity from peripheral blood leukocytes appeared normal, thus it seems that the blood marrow dysfunction is caused by disorders at the haemopoietic stem and/or progenitor cell levels, and not at the humoral factor levels. Shwachman syndrome may progress gradually
to acute myelogenous leukaemia and myelodysplastic syndrome (MDS). However, no HSC stimulatory effect was observed in a patient with aplastic anaemia or in a patient with MDS. The fatty acids cannot ameliorate haemopoietic other than Shwachman syndrome, suggesting that it is indeed these fatty acids that are responsible for the haemopoietic dysfunction in Shwachman syndrome. (Hisha et al. 2002)

Cancer Prevention

Ten Precious Substances Tonic, with or without anticancer drug 5-flourouracil, prevented the body weight loss and the induction of the colonic cancer in rats treated with a chemical carcinogen. (Sakamoto et al. 1991)

TJ-48 protected against induced liver cancer in rats. The formulation significantly reduced the size, volume and number of hepatic lesions. The treatment also caused a significant increase in the proportion of interleukin-2 receptor-positive lymphocytes among the lymphocytes infiltrating the tumours. The effect is thought to be through activation of the immune system. (Tatsuta et al. 1994)

Inhibition of Tumour Growth

In an early study it was found that oral administration of Ginseng and Dang Gui Ten Combination was found to suppress the growth of carcinoma in mice and prolong survival time (oral administration). (Ito and Shimura, 1985a) Around the same time researchers found that certain Chinese herbs had the capacity to stimulate tumour necrosis factor, with activation of the reticuloendothelial system (RES), including macrophages. (Haranaka et al. 1985b) (Haranaka et al. 1985a) (Haranaka, 1989) Some of these herbs are also found in Ginseng and Dang Gui Ten Combination. Antitumour effects, suppression of thymidine kinase involved in salvage pathway for pyrimidine nucleotide synthesis. Juzen-taiho-to suppressed DNA-synthesising enzyme activity in mammary tumours in mice. (Sakamoto et al. 1994) Juzen-taiho-to was shown to inhibit tumour growth in mice. Test results suggest that the herbs inhibit excessive growth mediated by endogenous factors including antioxidant substances in
addition to improvement in host-mediated antitumour activity. (Ohnishi et al. 1996)

TJ-48 has been shown to inhibit the progressive growth of malignant clone cells derived from murine fibrosarcoma. Oral administration of TJ-48 at a dose of 40 mg/day for 7 days after tumour inoculation resulted in significant inhibition of tumour growth as compared to the control (P<0.01). In a second experiment it was shown that TJ-48 significantly (P<0.01) prolonged survival time compared to controls. The timing of administration with TJ-48 is important. Oral administration 7 days just before or after tumour inoculation significantly suppressed tumour growth on day 25. However, treatment given from day 8 to day 14 after tumour inoculation did not significantly affect the tumour growth. These results indicated that TJ-48 might be effective for preventing weakly malignant tumours from growing progressively. The effect may be due to increased host-mediated immune surveillance and antioxidant activity, but reduced PGE2 production during the pro-inflammatory process. (Ohnishi et al. 1996)

Oral administration of Juzen-taiho-to has been shown to suppress melanocytic tumours through potentiation of T-cell-mediated antitumour cytotoxic immunity in vivo (RET-transgenic mice). There was virtually no difference between the lengths of tumour-free stages in the juzen-taiho-to-treated mice and the untreated littermate control mice. The rate of tumour growth in the juzen-taiho-to-treated mice, however, was greatly suppressed during the entire period after the initial tumour development. Correspondingly, the life span of juzen-taiho-to-treated transgenic mice was longer (over 6 mo in mean value) than that of control mice. The addition of Juzen-taiho-to to in vitro cultures of Mel-Ret cells, a malignant melanoma cell line derived from a RET-transgenic mouse, caused neither cell death nor cell cycle arrest directly. The addition of Juzen-taiho-to to cultures of murine spleen cells, however, promoted their DNA synthesis. Furthermore, peritoneal exudate cells from the Juzen-taiho-to- treated transgenic mice, in which the ratio and number of T cells were increased, displayed an antitumour immunity against Mel-Ret cells in vitro. This immunity,
which was primarily conveyed by Thy-1+ T cells, was antigen (RET/melanoma) specific and cytotoxic. Amongst various chemical ingredients of Juzen-taiho-to examined in this study, glycyrrhizin displayed an action, partially replacing that of Juzen-taiho-to, in promoting anti-Mel-Ret immunity when supplementarily added in vitro. These results suggest that Juzen-taiho-to suppresses once-developed primary melanocytic tumours through potentiation of T-cell-mediated antitumour cytotoxic immunity in vivo. (Dai et al. 2001)

Reduction of Metastases
Two saponin-rich preparation from Red Ginseng inhibited lung metastasis from melanoma and cancer of the colon in mice. The effect was related to inhibition of the adhesion and invasion of tumour cells, and also to anti-angiogenesis activity. (Sato et al. 1994) Oral administration of TJ-48 for 7 days before tumour inoculation significantly reduced the number of liver metastatic colonies of colon carcinoma cells and attenuated the increase of liver weight. The herbal formulation also inhibited lung metastases from melanoma cells. (Kiyohara et al. 1995b) Oral administration of TJ-48 inhibited liver metastasis of colon cells, possibly through a mechanism mediated by the activation of macrophages and/or T-cells in the host immune system. (Hisha et al. 1997)

The combination of interferons with Juzen-taiho-to has been shown to increase the therapeutic potential of interferons and to decrease their toxicity in an experimental lung metastasis of murine renal cell carcinoma (Renca) cells. Five consecutive administrations of IFN-alpha to Renca-bearing mice resulted in dose-dependent inhibition of lung metastasis. IFN-alpha at the dose of 100,000 IU/mouse significantly inhibited the metastasis, but a marked loss of body weight was observed during and after the administration. In contrast, oral administration of Juzen-taiho-to (50 mg/mouse) alone tended to inhibit the metastasis, but the effect was not statistically significant. The combination treatment of suboptimal doses of IFN-alpha and Juzen-taiho-to markedly augmented the antimetastatic effect without causing any loss of body weight, as compared with either treatment
alone. Similar results were also obtained by treatment with IFN-gamma in combination with Juzen-taiho-to. The authors suggest that Juzen-taiho-to should be considered for the treatment of metastatic renal cell carcinoma. (Muraishi et al. 2000) Oral administration of a Kampo (Japanese herbal) medicine Juzen-taiho-to inhibits liver metastasis of colon 26-L5 carcinoma cells We investigated the inhibitory effect of Juzen-taiho-to in an experimental liver metastasis model, by means of inoculation of a highly liver-metastatic variant (L5) of murine colon 26 carcinoma cells into the portal vein. Oral administration of Juzen-taiho-to before tumour inoculation resulted in the dose dependent inhibition of liver tumour colonies. Oral administration of Juzen-taiho-to for 7 days before tumour inoculation led to a significant reduction in the number of metastatic colonies and liver weight even in NK-depleted mice as well as normal mice, but not in macrophage-deficient mice. This finding shows that the inhibition of metastasis by Juzen-taiho-to is associated with macrophage function. (Ohnishi et al. 1998)

Potentiation and Prevention of Side Effects of Chemotherapy or Interferon Therapy.

Juzen-taiho-to has been shown in animal studies to potentiate the effects of a variety of chemotherapeutic agents. ICR mice inoculated with sarcoma cells were given mitomycin, cytoxan or adriamycin with our without Juzen-taiho-to. In combination with Juzen-taiho-to, mitomycin resulted in a significantly greater tumour growth inhibition than could be obtained with mitomycin alone. The same results were obtained in mice inoculated with fibrosarcoma. The direct anti-tumour effects of Juzen-taiho-to were also tested and although there was a 38.7% increase in life span of mice inoculated with IMC carcinoma, no anti-tumour effect could be detected. (Komiyama et al. 1988)

Similar results were found in a later study where Juzen-taiho-to was found to potentiate a combination of mitomycin and hyperthermia in the treatment of mice inoculated with mouse sarcoma 180 tumours or B16 melanoma cells. Re-inoculation with the mice’ own tumours resulted in a stimulated growth in group receiving mitomycin alone
while the addition of Juzen-taiho-to not only potentiated the anti-tumour effects of mitomycin but also decreased its toxic and cancer-promoting side effects. (Komiyama et al. 1989)

In order to further test this findings, the direct interaction of Juzen-taiho-to (TJ-48) with mitomycin and cisplatin was examined. Administration of Juzen-taiho-to both once and for seven days both shifted the LD$_{50}$ and the dose response curve of mitomycin and cisplatin to the right. Administration for seven days markedly increased the survival curves and delayed death due to a lethal dose of the two chemotherapeutic agents. The herbal formulation was also found to reduce mitomycin-induced atrophy of the testes, thymus and spleen. It also had beneficial effects on mitomycin-induced leukopenia, anaemia and weight loss. (Iijima et al. 1989)

Juzen-taiho-to has also been tested with the 5-fluorouracil derivative, UFT. The body weights of rats were reduced to 80% compared to controls due to experimentally induced adenocarcinoma of the colon. Juzen-taiho-to or UFT reduce the loss of body weight but combining UFT with the herbal formulation completely prevented the body weight loss. Although Juzen-taiho-to did not increase the anticancer effect of UFT it did prevent body weight loss due to the cytotoxicity and/or carcinogenicity of the induced colon cancer. This indicates that Juzen-taiho-to may protect against carcinogens and that combination therapy with UFT may increase long-term survival of cancer patients. (Sakamoto et al. 1991)

Protective effect of sodium L-Malate, an active constituent isolated from Angelica sinensis on cis-Diamminedichloroplatium (II)-induced toxic side effect. (Sugiyama et al. 1994)

Ginseng and Dang Gui Ten Combination prevented decreases in white blood cell count, platelet counts, bone marrow cell count, relative spleen and thymus weight, food intake and body weight without reducing the antitumour activity of two chemotherapeutic agents. (Sugiyama et al. 1995a)

Ginseng and Dang Gui Ten Combination reduced nephrotoxicity, immunosuppression, hepatic toxicity and gastrointestinal toxicity
caused by cisplatin. Even when the dose of cisplatin was increased 20-fold, was the herbal formulation able to keep various parameters near normal. (Sugiyama et al. 1995b)

A pectic polysaccharide isolated from TJ-48 reduced the renal and lethal toxicities of cis-Diamminedichloroplatium (CDDP) in mice. The mice received TJ-48 fractions for 15 days, CDDP was administered i.p. on days 6-11. Survival rates of the mice were increased significantly by oral administration of fraction F-5. Removal of the methanol-soluble constituents did not reduce the protective effect. These results suggest that the pectic polysaccharides are protecting of damage to organs such as the kidneys by CDDP. All fractions were shown to reduce the renal toxicity of CDDP, F-1 , F-3 and F-4 seemed to be more effective than F-5, and F-2 was shown to have the weakest effect. These results suggest that the protective effect of TJ-48 is due to a combination effect of high and low molecular weight compounds as well as pectic polysaccharides. (Kiyohara et al. 1995b)

Ginseng and Dang Gui Ten Combination prevented immunodeficiency caused by chemotherapeutic agents in mice. After administration of oral doses of JTT, the body weight which had fallen to 75% recovered to 90% of the pre-treated weight. The number of white blood cells returned to 80% of their normal value. The phagocytic activity recovered to 131% compared to 11% in the control group. (Li et al. 1996) Juzen-taiho-to has also been found to have a preventative effect on endometrial carcinogenesis in mice. Shimotsu-to was found to b responsible for the inhibitory effects of Juzen-taiho-to on the oestrogen-related endometrial carcinogenesis in mice. (Lian et al. 2002)

TJ-48 accelerate recovery from hematopoietic injury induced by radiation and the anti-cancer drug mitomycin by stimulation of spleen colony-forming unit counts after 14 days. This effect may be due to the fatty acids, oleic and linolenic acids, found in the herbal formulation. The authors speculate that at least one of the signals is mediated by stromal cells, rather than any direct interaction with the hematopoietic stem cell. (Hisha et al. 1997)
1.6.3 Clinical Studies

There are not many published clinical studies on Ginseng and Dang Gui Ten Combination. One early study involved surgical departments of 40 university hospitals throughout Japan. The effects of Ginseng & Dang Gui (or Juzen-taiho-to as it is know as in Japanese) were tested in a controlled study involving 161 patients recently operated for gastric cancer. 100 patients received the test medication, 61 acted as controls. The herbal formulation was found to increase appetite and reduce the severity of leukopenia induced by treatment. Overall the improvement was rated at 60% for the test medication and 45% for the control group (see table 3). (Nabeya K, 1983)

Table 3: Clinical Trial – postoperative condition improvement (Adachi, 1990)

<table>
<thead>
<tr>
<th>Degree of improvement</th>
<th>Response Treatment Group</th>
<th>Response Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked improvement</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Improvement</td>
<td>18 (18%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Slight improvement</td>
<td>40 (41%)</td>
<td>18 (34%)</td>
</tr>
<tr>
<td>No change</td>
<td>37 (38%)</td>
<td>26 (49%)</td>
</tr>
<tr>
<td>Aggravation</td>
<td>2 (2%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Total</td>
<td>98 (100%)</td>
<td>53 (100%)</td>
</tr>
</tbody>
</table>

A randomised, controlled clinical study investigated the usefulness of Juzen-taiho-to as an adjunctive treatment to endocrine- and chemotherapy in patients with advanced breast cancer. 119 of 130 patients participating in the study were evaluated for subjective symptoms, laboratory tests and length of survival. The subjects were randomly divided into two groups. 58 patients in Group A received
endocrine- and chemotherapy plus Juzen-taiho-to while 61 patients in Group B received endocrine- and chemotherapy alone. The study found no significant differences in the patient characteristics between the two groups nor was there any differences in the rate of response to anticancer agents. No significant differences were observed in the length of survival between the two groups. Subjectively, the drug was useful for improvement of coldness of the limbs and anorexia. On further analysis, however, a significant improvement was found in a particular subgroup of patients. In Kampo as well as in traditional Chinese medicine, herbal medicine prescriptions are always individualised. The patients in the subgroup were identified as having signs and symptoms that matched the criteria for the prescription of Juzen-taiho-to according to traditional Chinese medicine diagnosis. These patients also reported better quality of life and the herbal formulation had a clear protective effect on the bone marrow as evidenced by a reduction of leukopenia due to chemotherapy. (Adachi, 1990)

These findings are supported by an earlier study by the same author which found that adding Juzen-taiho-to to endocrine- and chemotherapy increased the response rate from 54% to 70% in a randomised clinical trial involving 74 patients with a variety of different primary cancers and metastases. (Adachi, 1988)

During this same period in the mid-1980s, another Japanese researcher examined the effects of Juzen-taiho-to in 50 patients with invasive cancer of the bladder. The patients were treated with total cystectomy followed by adjuvant combination chemotherapy including cisplatin. In addition a natural product extracts known as OK-432 and TJ-48 (Juzen-taiho-to) were randomly prescribed in order to evaluate effects on the patients’ prognosis. Histologic grading of primary tumours and number of cycles of adjuvant chemotherapy administered had a significant correlation with patients’ survival: patients with grade 2 anaplasia had a better 3- and 5-year survival rates than those with grade 3 anaplasia, and patients receiving 3 or more cycles of chemotherapy had a better 3-year survival rate than those with 2 or less cycles. Administration of OK-432 or TJ-48 and pre-operative
treatment such as irradiation and intra-arterial chemotherapy had no favourable effects on the survival. Side effects of the adjuvant chemotherapy were minimal to moderate and more than 70% of the patients tolerated at least 3 cycles of chemotherapy.(Fukui et al. 1992) The study did not elucidate if the herbal extracts improved the patients’ tolerance to chemotherapy. Although this study did not show a statistically significant beneficial effect of combining TJ-48 with cystectomy combined with chemotherapy, it is possible that better results could be obtained when combining the herbal extract with the less invasive transurethral resection of a bladder tumour (TURBT) and MCV (methotrexate, cisplatin and vinblastine) chemotherapy for invasive bladder cancer. A recent study has found that (TURBT) (in suitable patients) combined with two cycles of MCV not only enabled patients to maintain normal bladder function also had a similar survival rate to patient treated with cystectomy.(Kachnic LA, 1997)

Juzen-taiho-to has been shown to decrease adverse effects of chemotherapy on the gastrointestinal system with an improvement in appetite in over 80% of patients.(Kurokawa T et al. 1994)

TJ-48 administered to postoperative gastric cancer patients has been shown to improve immunosuppression as measured by interleukin-2 receptor and reactivity studies.(Kato K et al. 19??)

A combination therapy of TJ-48 and a antineoplastic (Oral Uracil) agent was found to be a useful postoperative adjuvant chemotherapy for gastric cancer. Thirty-three patients who underwent surgery for gastric cancer were divided into two groups (UFT group and UFT and TJ-48 group). There was no difference in clinical or pathological variables, including sex, age, stage and lymph node metastases between the two groups. The administration of TJ-48 decreased the rate of suppressor T cell at 1 month (116.0+/-24.0 vs. 72.1+/-26.1; p<0.01), 3 months (101.6+/-38.9 vs. 95.6+/-37.5) and 6 months (129.6+/-53.2 vs. 86.7+/-32.4) after the initial treatment, and increased the rate of cytotoxic T cells at 1 month after treatment, compared with the values in UFT group. TJ-48 also reduced subjective symptoms of the patients. (Konno et al. 1997)
The effect of TJ-48 (Shi-Quan-Da-Bu-Tang, Juzen-taiho-to, Tsumura) on the host-immunity of gastric cancer patients was investigated. Thirty-three gastric cancer patients who underwent curative surgery were divided into 2 groups (UFT group and UFT and TJ-48 group). There was no difference of clinical or pathological variables, including sex, age, stage and lymph node metastases between the two groups. The administration of TJ-48 decreased the rate of suppressor T cell at 1 month (116.0+/-24.0 vs. 72.1+/-26.1; p<0.01), 3 months (101.6+/-38.9 vs. 95.6+-/-37.5) and 6 months (129.6+/-53.2 vs. 86.7+/-32.4) after the initial treatment, and increased the rate of cytotoxic T cells at 1 month after treatment, compared with the values in UFT group. TJ-48 also reduced subjective symptoms of the patients. The present results suggest that the combination therapy of TJ-48 and antineoplastic agent is useful as a postoperative adjuvant chemotherapy for gastric cancer.

The value of antioxidant therapy during chemotherapy is controversial. Conklin in a review article describes how cancer produces oxidative stress (free radicals) on the host organism. The highly effective antioxidant systems of individual tumour cells, however, are generally able to overcome the level of oxidative stress that is induced by cancer and maintain their rapid rate of proliferation. Administration of antineoplastic agents during chemotherapy results in a much greater degree of oxidative stress than is induced by cancer itself. Excessive oxidative stress, however, may decrease the cell proliferation and thereby interfere with the chemotherapy aimed at destroying rapidly dividing cells. Several studies have demonstrated that antioxidants do not inhibit, but actually enhance, the cytotoxic effect of antineoplastic drugs on cancer cells. Antioxidant therapy may enhance the effects of chemotherapy by combating free radicals while reducing certain side effects common to many anticancer drugs, such as gastrointestinal toxicity and mutagenesis. (Conklin KA, 2000)

Treatment with various combinations of mega-doses of beta-carotene, vitamin C, niacin, selenium, coenzyme Q10 and zinc compared to standard treatment has been tested in women with unilateral non-metastatic breast cancer. The addition of vitamins and minerals did not
improve breast cancer-specific survival and disease-free survival times. (Lesperance et al. 2002)

The antioxidant effect of Ginseng and Dang Gui Ten Combination was examined in a clinical trial with colo-rectal cancer. Patients. 60 randomly selected patients were divided into two groups. Group A (30 cases) received radiotherapy and Ginseng and Dang Gui Ten Combination, group B (30 patients) received radiotherapy alone. Another 40 healthy subjects were selected for a control group. Levels of superoxide dismutase (SOD) in serum, glutathione peroxidase (GSHPx) in blood, catalase (CAT) activity in blood and lipid peroxidase (LPO) in serum was measured before, during and after radiotherapy. The authors concluded that radiotherapy generates free radicals and that Ginseng and Dang Gui Ten Combination may both reduce the generation of free radicals and increase antioxidant enzyme activity. (Chiou JF and Tsai J, 2003)

TJ-48 has been found in two studies to be a beneficial adjunct in the treatment of brain tumour. In the first study the T cell subset and NK cell activity of lymphocytes in blood were investigated before and after administration of TJ-48 in 11 patients with brain tumours (7 cases of glioma and 4 cases of non-glial tumour). After initial treatment, Group A received TJ-48 (7.5 g/day, p.o.) with interferon-beta 300 million IU for 1-2 weeks. Group B was treated with only TJ-48 as an adjuvant therapy. Percent values of helper T cell (CD4+, CD45RA-), cytotoxic T cell (CD8+, CD11b-) and suppressor T cell (CD8+, CD11b-) were calculated with flow cytometry before and at 1 month, 2 months and 3 months of treatment. The decrease in Suppressor T cells was significant after 2 months of administration of JT-48. Group A, which received TJ-48 with interferon-beta, showed a greater decrease n suppressor T cells compared to group B. Administration of TJ-48 tended to increase cytotoxic and helper T cells. (Miyagami and Katayama, 2000) In the second study, 29 patients with brain tumours (15 cases of gliomas and 14 non-glial tumours) were divided into two groups. The methodology and the treatment protocol was identical to the previous study. Suppressor T cells decreased significantly after 2 months following administration of JT-48 in 16 of 17 cases tested.
(selected from both groups). After administration of TJ-48, helper T cells tended to increase slightly, but cytotoxic T cells and NK activity did not change. TNF-alpha productivity was measured in 8 cases of gliomas and increased to levels higher than they were before administration of TJ-48 in all of the cases that were examined more than 2 months after treatment. Adjuvant therapy with TJ-48 in both A and B group improved the host-immunity of the patients with brain tumours. (Miyagami and Katayama, 2003)

Juzen-taiho-to appears to contain bone marrow-stimulating compounds. TJ-48 was found in a recent study to ameliorate the reduction in haemoglobin levels in 67 chronic hepatitis C patients receiving interferon and ribavirin therapy. Interferon plus ribavirin therapy is currently the standard treatment for chronic hepatitis C. Haemolytic anaemia, however, is a serious side effect of this treatment, requiring reductions in or complete withdrawal of ribavirin. The reduction in haemoglobin levels was significantly ameliorated in TJ-48-treated patients (P<0.05). Consequently, only 13% (4/32) of TJ-48-treated patients received altered doses of ribavirin, while the ribavirin dose had to be reduced or withdrawn in 43% (15/35) of patients in the absence of TJ-48 administration (P<0.001). (Sho et al. 2004)

1.6.4 Active Constituents in Ginseng & Dang Gui Ten Combination

There have been several attempts to elucidate the active constituents in Ginseng and Dang Gui Ten Combination. The spray-dried decoction, TJ48, has been fractionised and extensively analysed. TJ48 fractionisation is produced by refluxing the extract with methanol for 1 hr thereby obtaining a methanol-insoluble and soluble fraction (F-a). Then, the MeOH-insoluble fraction is extracted with water at room temperature, and the MeOH-water insoluble precipitate (F-2) obtained. The water-soluble supernatant is then dialysed against water, and the dialyzable and the non-dialyzable fraction obtained. The dialyzable fraction is then recovered (F-3) by lyophilization. The additional of 4 volumes of ethanol to non-dialyzable fraction produces the precipitate (F-5) and the ethanol-sol supernatant (F-4). Yields of F-1, F-2, F-3, F-
4 and F-5 were 62.5%, 7.2%, 14.1%, 3.5% and 13% of TJ-48 respectively. Only the high molecular mass fractions showed potent anti-complementary and mitogenic activity. F-5 was shown to exert the most potent anti-complement activity. The methanol and water-insoluble fraction (F-2) also demonstrated significant activity. F-2 consisted mainly of a large quantity (87.9%) of carbohydrate, 3.5% of uronic acid and 3.9% of protein. The crude polysaccharide fraction F-5 was further fractionised and the subfraction, F-5-2, consisting of mainly pectin polysaccharides, was found to exert the most potent mitogenic activity. Both fractions contained an amylopectin-type alpha glucan. F-2 fraction may activate complement via the classical pathway whereas F-5 may activate via the alternative pathway. (Yamada et al. 1990) Antimetastatic effect of TJ48 may be mainly due to Shimotsu-to (Dang Qui Four). Oral administration of TJ48 for 7 days before tumour inoculation significantly reduced the number of liver metastatic colonies from colon carcinoma cells as well as lung metastasis from melanoma cells in vivo. Oral administration of Dang Gui Four for 7 days before tumour inoculation resulted in a significant reduction in the number of metastatic colonies, however, this effect was not observed when testing Shikunshi To.

Another formulation, Unsei In containing the four herbs in Dang Gui Four was also active in inhibiting liver metastasis. However, Toki Shakuyaku San and Ninjin Yoei To, which include all Dang Gui Four ingredients except Rehmannia glutinosa and Ligusticum wallichii respectively, did not show a significant anti-metastatic effect. Other Japanese formulations containing the four herbs found in Shikunshi To also failed to affect liver metastasis. The results suggest that the anti-metastatic effect of Juzen-taiho-to is partly associated with its Dang Gui Four-derived constituents (especially Rehmannia and Cnidium radix (Ligusticum wallichii)). (Onishi et al. 1998) Polysaccharides and other isolated constituents in various Chinese formulations show considerable immune enhancing and anticancer activity in vitro, but whether this always has relevance to oral use of the herbs in humans, is open to question. Nevertheless, the following in vitro and in vivo studies give us some understanding of how herbal BRMs may exert
their influence. Pectin polysaccharides in Ten Precious Substances Tonic have been shown to reduce the adverse effects of an antineoplastic agent and stimulate antibody production. (Kiyohara et al. 1995a) When investigating the protective effect against nephrotoxicity and bone marrow toxicity caused by a chemotherapeutic agent in the treatment of mice inoculated with sarcoma cells, it was found that Angelica sinensis (Dong Quai or Dang Gui) had the strongest effect against toxicity of the compounds in the formulation. The active compound was found to be sodium L-malate. (Sugiyama et al. 1994) (Ueda et al. 1998; Ohnishi et al. 1996). Ten Precious Substances Tonic prolonged the lifespan of immunocompromised mice receiving a lethal dose of Candida albicans. A similar protective effect was obtained by treatment with its component drugs, Ginseng, Liquorice, Atractylodes or Cnidium (Ligusticum wallichii). These herbal compounds were suggested to have a main role in the protective effect of TJ-48 against Candida infection.

Dang Gui Four, and especially Rehmannia, has been shown to inhibit ultraviolet radiation-induced cell damage by inhibition of PGE2 release from cutaneous target cells. (Sakuma et al. 1998) UV radiation of the skin produces both local, non-specific immune suppression that inhibits immune effector functions within irradiated skin, as well as systemic, specific immune suppression against antigens introduced at a critical (Abe et al. 1998)al time after exposure to UV radiation. This suppression is likely to be due to production of cytokines by epidermal cells in response to UV-induced DNA damage. O’Connor 1996

Dang Gui Four reduced angiogenesis, granuloma formation, inflammatory cell migration and fluid exudation in adjuvant-induced inflammatory model. Ligusticum wallichii represented the main ingredient for producing the anti-chronic inflammatory effects of Dang Gui Four. Ligusticum and Paeonia exhibited synergistic anti-inflammatory effects. (Kojimahara et al. 1995)

Antiproliferative effects of Dang Gui Four seems to be due to Cnidium (Ligusticum wallichii)-derived phthalides. (Kobayashi S et al. 1992)
Poria cocos (Hoelen or Fu-Ling) augmented the secretion of interleukins IL-1 beta and IL-6 six hours after in vitro cultivation of human peripheral blood monocytes. Tumour necrosis factor-alpha secretion was also increased. Hoelen also suppressed the secretion of an immune suppressor (TGF-beta) (Yu and Tseng, 1996) Poria cocos has been included in many combinations with other CM herbs for its traditionally claimed activities of inducing diuresis, excreting dampness, invigorating the spleen and tranquillising the mind and its modern pharmacological use of modulating the immune system of the body. Dehydrotumulosic acid, one of the effective constituents of Fu ling, was isolated from the chloroform-soluble material of ethanol extract of the fungus. After further purification by a high-performance liquid chromatographic method on a C18 column, the purified constituent was identified using modern analytical techniques, such as UV, 13C-NMR and EI-MS. A reversed-phase high-performance liquid chromatographic method has been developed for the determination of dehydrotumulosic acid in Poria cocos. The determination can be accomplished in less than 50 min using methanol-acetonitrile-2% glacial acetic acid as the mobile phase at a flow rate of 1.0 ml/min, with a UV detector setting at 242 nm and testosterone propionate used as an internal standard. This assay for dehydrotumulosic acid is simple, rapid and with good reproducibility. (Song et al. 2002b)

1.6.5 Toxicology Studies
Acute toxicity of ‘Ginseng and Dang Gui Ten Combination’ in mice and rats is very low. LD_{50} is >15g/kg in ICR mice and SR rats by oral administration. (Aburada et al. 1983)
No deaths or abnormal symptoms were observed during 13 weeks plus 4 weeks recovery period after oral administration at doses of 300, 1000 and 3000 mg/kg. (Minematsu et al. 1989)
1.7 Monographs of individual herbs

1.7.1 Angelica sinensis

Angelica sinensis of the Apiaceae family (formerly known as Umbelliferae) is a perennial fragrant plant. It is known as dong quai or gui (English), dang gui (Mandarin), toki (Japanese) and tanggwi (Korean). Its botanical synonym is *Angelica polymorpha*. The root is the medicinal part used. It is, however, important that the terminology used in Traditional Chinese Medicine (TCM) is not taken literally. The TCM term ‘spleen’ does not mean spleen in the modern sense; instead it refers to the entire gastrointestinal system. Similarly, the TCM term for ‘kidney’ does not mean kidney in the modern sense, and can mean the entire endocrine system. It is important to analyse these terms within the framework of TCM for the integration of traditional medicine into modern medicine. (Han J, 1988)

Active Constituents

*Angelica sinensis* contains an essential oil containing ligustilide, angelicide, n- butylidene phthalide, butylphthalide, 2,4-dihydrophthalic anhydride. The essential oil consists mainly of ligustilide and n- butylidene phthalide. (Zhu DP, 1987)

N-butyldiene phthalide is responsible for the characteristic fragrance of the oil. The high quality boxed root heads are extremely high in active constituents, containing as much as 5% ligustilide which is more than 10 times the level in normal commercial roots. The root also contains coumarins including angelol and angelicone (Zhu DP, 1987), phenylpropanoids including ferulic acid (Ji SG et al. 1999) and angelica polysaccharides (Choy YM et al. 1994).

Pharmacological activities

Cardiotonic Activity

Angelica sinensis has been shown to have a quinidine-like action on the heart. It can prolong the refractory period and correct experimental atrial fibrillation induced by atropine, pituitrin, strophanthin, acetylcholine or electrical stimulation. (Ikegami et al. 2003)
Antiplatelet Activity
An early study has demonstrated antiplatelet activity. (Yin ZZ et al. 1980) Ferulic acid, as well as the aqueous extract of Angelica sinensis, was shown to have an antiplatelet effect. (Ikegami et al. 2003)

Protection Against Reperfusion Injury
The injection of aqueous extract of Angelica sinensis exerted significant protective effects on myocardial dysfunction and myocardial injury in rabbits induced by ischemia/reperfusion. (Chen SG et al. 1995) Pre-treatment with a traditional formulation, ‘Dang gui decoction for Enriching the Blood’ which contains Angelica sinensis and Astragalus membranaceus as the main ingredients, was shown to give protection against ischaemia-reperfusion injury in isolated rat myocardiums. The effect was enhanced by the addition of another herb, Polygonum multiflorum. (Yim TK et al. 2000)

Prevention of Atherogenesis
Human umbilical vein endothelial cells were used to investigate the role of angelica in human vascular damage. Angelica sinensis protected against oxidation of low-density lipoproteins and inhibits changes to the function and structure of vascular endothelial cells thereby reducing the development of atherogenesis. (Xiaohong Y et al. 2000) Animal study found that Angelica sinensis can reduce atherogenesis through decreasing the serum triglyceride concentration, increasing whole blood viscosity and by regulating haematocrit and fibrinogen levels. (Zhui Y et al. 2000)

Antioxidant Activity
Angelica sinensis root extract demonstrated antioxidant activity and inhibition of lipid peroxidation of supernatant hepatic homogenate in mice. It was further demonstrated that different extract manufacturing processes affected the level of activity. (Wu H et al. 1996)

Effects on the Blood
Angelica polysaccharides were found to enhance haematopoiesis by directly or indirectly stimulating macrophages, fibroblasts and lymphocytes to secrete haematopoietic growth factor in healthy and
anaemic mice. (Wang Y and Zhu B, 1996) In TCM, Angelica sinensis is indicated for ‘stagnated blood’. A model of ‘blood stagnation’ was established in rats using adrenaline and a cold environment. The condition produced an increase in the viscosity of the blood and in the liability to coagulate. Astragalus membranaceus and Angelica sinensis both decreased whole blood specific viscosity while at the same time increase the plasma specific viscosity. The combination of the herbs had a more favourable effect. (Xue JX et al. 1994)

Angelica sinensis has been shown to increase red blood cell counts. (Miyagami and Katayama, 2003) Dang-Gui-Shao-Yao-San (DGSYS), which contains Angelica sinensis, is known to elevate haematopoietic functions during single X-irradiation. DGSYS given at doses of 10 and 20 mg/20 g body weight, once a day, for 7 consecutive days before irradiation protected mice from the sublethal effects of radiation in a dose-dependent manner. Prior administration of 20 mg/20 g DGSYS increased the number of femoral spleen colony-forming units (CFU-S) that survived irradiation, and significantly ameliorated leukopenia, thrombocytopenia and the depression of haematocrits after irradiation. These results suggest that DGSYS may be effective in the prevention of haematopoietic injury caused by sublethal dose irradiation. (Hsu HY and Lin CC, 1996)

**Uterine Effects**

Contrary to popular belief, Angelica sinensis has been found to be without any direct oestrogenic activity. (Ikegami et al. 2003) Angelica sinensis has been shown to increase sexual activity in female animals and a reduction in the signs of vitamin E deficiency has been noted in male mice. (Zhu DP, 1987) Butylidenephthalalde has been shown to inhibit rat uterine contractions. (Ko, 1980) Angelica sinensis decoction has been found to have a stimulating action on the uterus of mouse in vitro. (Shi M et al. 1995) Ferulic acid showed an inhibitory effect on uterine movement when given perorally and intravenously, respectively. (Ozaki Y and Ma JP, 1990)
**Immunity**

Ferulic acid has been shown to increase phagocytosis. (Xu LN et al. 1981)

Angelica sinensis can somewhat counter the immunosuppressive effects of hydrocortisone, although Astragalus membranaceus was found to be more effective. (Luo B et al. 1987) A low molecular weight polysaccharide consisting of protein (4.73%) and carbohydrate (85.85%) of which 5.2% is uronic acid, was shown to exert a strong anti-tumour activity on Ehrlich Ascites tumour bearing mice. It also exhibited immunostimulating activities, both in vitro and in vivo. (Choy YM et al. 1994) A TCM preparation (Dang gui Buxue Decoction) significantly (P < 0.001) promoted the production of interleukin-2 (IL-2) in the spleen of blood-deficient mice. Individual analysis of the herbal formula showed that IL-2 production could be promoted in splenic lymphocytes of blood-deficient mice given Angelica sinensis or Astragalus membranaceus (P < 0.001). (Chen YC., 1994)

**Anti-inflammatory Action**

Early experimental models have indicated that Angelica sinensis may have anti-asthmatic activity (Tao JY et al. 1984) and be of benefit in nephritis. (Zhang YK et al. 1986) Angelica sinensis injection increased the production of interleukin-2 in mouse spleen mononuclear cells. The stimulatory effect was totally abrogated by the addition of prostaglandin E2. (Weng XC et al. 1987)

Ferulic acid (FA) significantly inhibited the oedema induced by carrageenan, the increase of the dye leakage induced by acetic acid and the granuloma formation induced by cotton pellet. And also inhibited the number of writhes induced by acetic acid. From these results, it is suggested that ferulic acid has both anti-inflammatory effect and analgesic effects. The author furthermore suggested that ferulic acid exert an anti-inflammatory effect both at the early and the late stages of processes in the inflammatory pathology. (Ozaki Y., 1992)
Angelica sinensis markedly inhibited the formation of thromboxane A2 and mildly affected the formation of prostaglandin I2. (Wang SR et al. 1993)

Angelica polysaccharides were shown to have anti-inflammatory activity, perhaps through the inhibitory action on neutrophil infiltration in the gastrointestinal mucosa. Angelica polysaccharides could potentially be useful to prevent any neutrophil-dependent mucosal injury in the gastrointestinal tract. (Cho H et al. 2000) The crude extract of Angelica sinensis was shown to have a direct mucosal healing effect on gastric epithelial cells, promoting healing of gastric ulcer. (Ye YN et al. 2001b) A crude extract from Angelica sinensis (ASCE), which mainly consisted of polysaccharides, prevented ethanol- or indomethacin-induced gastric mucosal damage and promoted ulcer healing. It was found that ASCE significantly promoted the migration of epithelial cells over an artificial wound on the surface of an RGM-1 monolayer. The extract also stimulated DNA synthesis in a dose-dependent manner and concomitantly increased EGF RNA expression. Co-incubation of ASCE with anti-EGF antibody reduced the speed of migration and the DNA synthesis, which however were still higher than the control without ASCE. These results strongly suggest that ASCE has a direct wound healing effect on gastric mucosa, and this is acting partially through an EGF-mediated pathway. (Ye YN et al. 2001a)

**Antitumour Activity**

In vitro study found that ligustilide demonstrated an anti-proliferative effect on smooth muscle cells. (Kobayashi S et al. 1992)

**Hepatoprotective Activity**

Pre-treatment with sodium ferulate inhibited the activity of serum alanine aminotransferase, prevented the depletion of liver glycogen and glutathione, increased the liver homogenate and microsomal glutathione S-transferase activities, and reduced the malondialdehyde content, the membrane fluidity of liver microsome and the mitochondria in paracetamol-induced liver toxicity in mice. These results demonstrated the hepato-protective action of sodium ferulate in mice. (Wang H and Peng RX, 1994)
Cognitive Function

A methanolic extract and its hexane fractions were shown to improve drug-induced amnesia in rats and it was shown that this effect was related to the memory processes. The hexane fraction was more effective indicating that highly non-polar constituents may be responsible for this effect. (Hsieh MT et al. 2000)

Clinical Studies

Cardiovascular Disorders

Uncontrolled clinical studies have shown Angelica sinensis to be beneficial in the treatment of Buerger's disease and constrictive aortitis. (Ikegami et al. 2003)

40 patients with pulmonary hypertension in remission stage were equally divided into four groups, 10 cases in each. Group 1 was treated with an Angelica sinensis injection, Group 2 with nifedipine, Group 3 with both Angelica sinensis and nifedipine and Group 4 acted as the control group. The study was designed to investigate the changes of hemodynamics, pulmonary function and blood gas before and after the treatments by impedance rheopneumogram, lung function examination and blood gas analysis. The mean pulmonary arterial pressure was decreased and cardiac output, PaO2 were increased significantly (P < 0.05 or P < 0.01) in group 3. The effects of group 3 appeared to be better than in other groups. The side effect of PaO2 lowering in group 2 was overcome in adding Angelica sinensis. (Xu JY et al. 1992)

Endocrine Disorders

Long-term treatment (9 months) of infertility due to tubal occlusion resulted in successful pregnancies in over 50% of cases. (Fu YF et al. 1988)

A decoction of Angelica sinensis, Corydalis ambigua, Paeonia lactiflora and Ligusticum wallichii showed a 93% improvement rate for the treatment of dysmenorrhea. The decoction was given daily, starting 5 days before and until cessation of menstruation. (Liu MA et al. 1988)
**Hepatic Disorders**

Angelica sinensis reduced thymol turbidity in an uncontrolled study of 88 cases of chronic hepatitis or liver cirrhosis. (Ikegami et al. 2003)

**Contraindications**

Angelica sinensis is contraindicated during the first trimester of pregnancy and in menorrhagia.

**Drug Interaction**

Angelica sinensis affects the pharmacodynamics but not the pharmacokinetics of warfarin in rabbits. The root extract was found to not increase prothrombin time by itself, but may lower prothrombin time when combined with warfarin. (Lo AC et al. 1995) A 46-year-old African-American woman with atrial fibrillation stabilized on warfarin experienced a greater than 2-fold elevation in prothrombin time and international normalized ratio after taking dong gui concurrently for 4 weeks. No identifiable cause was ascertained for the increase except dong gui. The patient's coagulation values returned to acceptable levels 1 month after discontinuing the herb. (Page RL and Lawrence JD, 1999)

**Toxicology**

The oral LD$_{50}$ of a concentrated extract in rats was measured at 100g/kg body weight. (Mills and Bone 2000)

**Side Effects**

Can occasionally cause mild gastrointestinal upsets that are often alleviated with ginger, cinnamon or bitters, as indicated.

**Dosage**

The daily dose is 4.5 to 9.0 grams of the root, taken as a tea in divided doses

1.7.2 **Astragalus membranaceus**

Astragalus membranaceus, of the Leguminosae family, is known as Astragali, Beg Kei, Bei Qi, Hwanggi, Huang-Qi and Milk vetch. It is the root, which is used medicinally. Astragalus is traditionally used to invigorate and tonify qi and the blood, as an adaptogen, for severe
blood loss, fatigue, anorexia, organ prolapse, chronic diarrhoea, shortness of breath, sweating and to enhance recuperation.

Active Constituents
Astragalus contains saponins, including cycloastragenol, astragaloside, and cyclocanthoside which are suspected to be the main active group; flavonoids, polysaccharides, phytosterols (including beta-sitosterol), essential oil and amino acids (including gamma-aminobutyric acid).

Immunomodulatory activities
A number of in vitro and in vivo studies confirm the immune enhancing activity of astragalus. Astragalus has been found to stimulate macrophage activity and enhance antibody responses both in vitro and vivo(Chu et al. 1988; Jin et al. 1994; Sugiura et al. 1993) as well as enhance lymphocyte blastogenesis in vitro (Sun et al. 1983) The results of several in vivo and in vitro studies have found some negative effects on the enterovirus coxsackie B and a reduction in myocardial injury(Peng et al. 1995; Yang et al. 1990)

Anticarcinogenic effects
Both in vitro and animal studies indicate that Astragalus may have a role as adjunct therapy in the treatment of some cancers. In vivo studies suggest Astragalus extract exerts an anticarcinogenic effects in carcinogen-treated mice through activation of cytotoxic activity and the production of cytokines.(Kurashige et al. 1999) One constituent within the herb (Astragalan) increased the secretion of tumour necrosis factor-alpha and TNF-beta respectively.(Zhao and Kong, 1993) An animal study has suggested that a combination containing Astragalus membranaceus exerts antitumour effects by augmenting phagocyte and lymphokine-activated killer cell activities.(Lau et al. 1994)

Although no human studies could be located, encouraging results were obtained from an in vitro study investigating the effects of a proprietary product known as Equiguard on prostate cancer cells. It is prepared according to TCM principles and contains standardized extracts of nine herbs, respectively, Herba epimedium brevicornum Maxim (stem and leaves), Radix morindae officinalis (root), Fructus
rosa laevigatae michx (fruit), Rubus chingii Hu (fruit), Schisandra chinensis (Turz.) Baill (fruit), Ligusticum lucidum Ait (fruit), Cuscuta chinensis Lam (seed), Psoralea corylifolia L. (fruit), and Astragalus membranaceus (root). It is used in TCM to restore Qi in the urogenital region. The product was shown to significantly reduce cancer cell growth, induce apoptosis, suppress expression of the androgen receptor and lower intracellular and secreted prostate specific antigen (PSA).(Hsieh and Wu, 2002)

**Cardiovascular Activities**

The effect of Astragalus on heart function has been the subject of several investigations. Several constituents from the astragalus species have demonstrated effects on heart contractility, heart rate and blood pressure. 3-Nitropropionic acid (NPA), a compound obtained from the Astragalus species has been shown to decrease blood pressure and induce bradycardia when administered as an I.V. preparation in normotensive rats or renal hypertensive dogs.(Castillo et al. 1993) Another compound, astragaloside IV demonstrated positive inotropic activity in patients with congestive heart failure.(Luo et al. 1995)

**Digestive effects.**

Astragalus strengthens the movement and muscle tone of the small intestine (especially the jejunum) in vivo, which may account for its clinical application in a variety of common digestive symptoms. (Yang, 1993)

**Hepatoprotective actions.**

Astragalus has hepatoprotective qualities against stilbenemide, paracetamol, carbon tetrachloride and D-galactosamine.(Zhang et al. 1990) Increases in liver glutathione levels observed as a result of treatment may be partly responsible. Studies have identified the constituent betaine as an important contributor to this activity.
Clinical studies

Angina pectoris
Two clinical studies have suggested Astragalus may be an effective treatment for angina pectoris. One study used Doppler Echocardiogram to study the action of Astragalus on left ventricular function in 20 patients with angina pectoris. Treatment resulted in increased cardiac output after 2 weeks but no improvement in left ventricular diastolic function. (Lei et al. 1994) Another study reports that 92 patients with ischaemic heart disease were successfully treated with Astragalus as measured by electrocardiogram readings with the results obtained being superior to the use of nifedipine. (Li et al. 1995)

Hypercholesterolemia
A randomised double blind clinical trial compared the effects of a traditional Chinese herbal medicine combination known as Jian Yan Ling (which includes Astragalus as a main ingredient) to placebo in 128 hyperlipemic patients. After three months of treatment, it was found that total cholesterol and triglyceride levels, apoproteins and lipoprotein-a were significantly reduced in the treatment group compared to placebo. (Lu et al. 1994)

Asthma
A herbal combination consisting of Astragalus membranaceus, Codonopsis pilosula and Glycyrrhiza uralensis was found to improve lung function measures in a 6 week open study of 28 asthmatic patients. (Wang et al. 1998)

Dosage range
2-6 grams of dried root daily from a decoction. Often used as long-term treatment except in cases of acute infection.

Adverse reactions
None known

Toxicity
Doses as high as 100 g/kg given orally have not shown adverse effects in rats
Drug interactions
Due to astragalus immunostimulant activity antagonistic interaction with immunosuppressive therapy is theoretically possible it should be used with caution.

Contraindications and precautions
None known. According to the principles of TCM, Astragalus should not be used during the acute stages of an infection.

1.7.3 Atractylodes macrocephala
Atractylodes macrocephala of the Asteraceae family, is known in Chinese as Baizhu. The herb is sweet and bitter and has a warming property according to traditional Chinese medicine where it is used as an energy tonic and diuretic. It is principally used in the treatment of poor appetite, dyspepsia, chronic diarrhoea, oedema and abnormal foetal movements. The rhizome is used medicinally. (Bensky and Gamble 1986) (Chang and But 1986)

Atractylodes lancea or its less-desirable substitutes, such as A. chinensis, A. japonicum, and A. ovata, known in Chinese as cangzhu, are used interchangeably with A. macrocephala without always making it clear which species is used, making any scientific evaluation very difficult.

Active constituents
The rhizome contains about 1.5% volatile oil comprising mainly of atracylone and two structurally related lactones atracylenolide and atracylol and atracyllenolide. The lipophilic fraction contains juniper camphor, atracylone and atracylenolide. (Tang and Eisenbrand 1992)

The rhizome also contains polysaccharides (Chi et al. 2001) and sesquiterpene lactones (Zhang et al. 2000)

Hepatoprotective activity
Atractylon has been shown to have antihepatotoxic activity as it was shown to inhibit carbon tetrachloride-induced cytotoxicity in primary cultured rat hepatocytes and carbon tetrachloride-induced lipid peroxidation in rat liver microsomes. (Kiso et al. 1983) Atractylon has
been shown to inhibit hydroperoxide-induced cytotoxicity and lipid peroxidation in primary culture of rat hepatocytes. (Hwang et al. 1996)

**Tonic properties**

Intragastric administration of a decoction of the rhizome for one months has been shown to increase the body weight and swimming endurance of mice. (Bensky and Gamble 1986)

The atracylodes rhizome has been shown to inhibit stress ulceration in the stomach. (Yu et al. 1994)

**Immunomodulatory activities**

The extract has been reported to increase the phagocytic function of the reticuloendothelial system and increase leukocytes in patients with leukopenia. It has also been reported to increase the lymphocyte transformation, promoting cellular immunity and markedly increase serum IgG suggesting that atracylodes has immune enhancing properties. (Chang and But 1986) The polysaccharides from rhizomes of Atractylodes lancea have been shown to have intestinal immune system modulating activities. The polysaccharides were shown to stimulate the proliferation of bone marrow cells mediated by stimulating of Peyer's patch cells. (Yu et al. 1998)

**Anticancer activities**

A methanol extract of *A. japonica* as well as its component, atracylton, has been shown to inhibit tumour promotion in an experimental animal study of carcinogenesis. (Yu et al. 1994)

**Spasmolytic activity**

The sesquiterpene lactones have been shown to inhibit the movement of uterus smooth muscle, and the mechanism was shown to relate to the inhibition of cholinergic system as well as Ca2+ movement. (Huang et al. 2000)

**Anti-inflammatory activities**

The lipophilic extract of Atractylodes lancea rhizomes has been shown to potently inhibit the activities of 5-lipoxygenase and cyclooxygenase-1 in vitro. (Resch et al. 1998)
Toxicity
The LD<sub>50</sub> of the rhizome decoction in mice by intraperitoneal injection has been reported to be 13.3 g/kg. (Chang and But 1986)

Cautions and contraindications
Atractylodes is contraindicated in cases with excessive sweating and should be used with caution in patients with loose, watery stools according to traditional Chinese medicine.

Dosage
3-4.5 g daily, usually by decoction.

1.7.4 Cinnamomum cassia
Cinnamomum cassia of the Lauraceae family, is also known as Rou Gui (Mandarin), Cinnamon bark, Chinese cinnamon, false cinnamon, Cassia bark and Nikkei (Japanese). The inner bark is the part used medicinally. In Traditional Chinese Medicine, both cinnamon bark (Rou Gui) and cinnamon twigs (Gui Zhi) are used. The cinnamon bark is said to have superior inner warming qualities and is used as a component of many Yang deficient formulas (complaints which are analogous to a sense of coldness, weakness and hypo function associated with a deficiency in vital energy). Traditionally cinnamon bark is used in formulas lack of stamina, weakness, aversion to food, cold, weak legs, backache, impotence and frequency, abdominal pain, reduced appetite, diarrhoea, cold lower body, warm upper body, flushing, wheezing, to alleviate pain such as dysmenorrhoea, also used for chronic suppurative skin complaints and lack of vital energy.

There are two major species of cinnamon used. Cinnamomum cassia is used in TCM and is an evergreen tree, up to 10 meter high. It has alternate, petiolate, oblong-lanceolate leaves, small yellowish-white flowers and greyish brown, slightly coarse bark with irregular fine wrinkles. The plant is indigenous to South-East China, Laos and Vietnam but is now cultivated in many tropical countries.

Cinnamomum zeylanicum from Sri Lanka, sometimes called True Cinnamon, is the species commonly used in Western Herbal Medicine and as the culinary spice. It is carminative, depurative, aromatic
digestive and warming (circulatory stimulant), and is also used in
toothpastes, liniments and other external preparations.

**Active Constituents**

Cinnamon contains 1-2% essential oil containing mainly
cinnamaldehyde and to a lesser degree, cinnamic acid and methyl-
eugenol. It also contains diterpenes known as cinnassials (include
cinnassial, cinnassials, cincassiol D1 and their glucosides) Other
constituents: mucilage, tannins, coumarin, beta-sitosterol, choline,
procyanidins and other flavon-3-ol derivatives, cassioside and
cinnamoside. (Tang and Eisenbrand 1992)

Cinnamaldehyde has been shown to inhibit the allergic reaction caused
by the IgE antibody and antigen complex in basophils and mast
cells. (Tanaka et al. 1991) It has also been shown to suppress the
release of arachidonic acid from platelet membrane phospholipids and
reduced the formation of thromboxane resulting in reduced platelet
aggregation. (Takenaga et al. 1987) More recently is has been shown
that the anticancer and immunomodulating activities are related to
blockade of early steps in signalling pathway leading to cell growth
(inhibits lymphoproliferation and induces of T-cell
differentiation). (Kwon et al. 1998) *Cinnamomum cassia* bark in a
0.1% solution completely suppressed the growth of *E. coli*,
*Staphylococcus aureus and Candida albicans* as well as been shown to
be effective against periodontal bacteria. (Osawa et al. 1991)

**Clinical Trials**

None found in the literature.

**Contraindications and Cautions**

Cinnamon should be used with caution in pregnancy according to
traditional Chinese medicine.

**Toxicity**

The LD<sub>50</sub> in mice for oral dosages of cinnamaldehyde is 2225 mg/kg, at
normal dosages, Cinnamon extract has a very low risk of causing
hypersensitivity reactions or any other adverse reactions. (Tang
Eisenbrand 1992)
Dosage
Dried herb: 1-3 g daily

1.7.5 Glycyrrhiza glabra

Glycyrrhiza glabra of the Leguminosae family is known as liquorice, liquorice, and sweet root in English and as Gan cao (China) and Kanzo (Japan). Dioscorides named the plant Glycyrrhiza after the Greek glukos (sweet) and riza (root). Liquorice is one of the most widely-used herbs in both western Chinese, Japanese and Ayurvedic traditions. It also has a long history and was used by the ancient Chinese, Egyptians and Greeks. The roots are used medicinally. The Chinese species, Glycyrrhiza uralensis is used in many TCM formulas; in small amounts to harmonise the formula (to rove the palatability) and to port the spleen (improve the tolerance of the formula); and in large quantities to harness its therapeutic effects. It is a perennial plant, up to more than 1m in height. The root is cylindrical, fibrous, flexible, 20-22cm long and 15mm in diameter, with or without cork, cork reddish, furrowed, light yellow inside.

Active Constituents
The dried roots of Glycyrrhiza glabra contain about 2 to 9% of an intensely sweet saponin known as glycyrrhizin (Takino et al. 1979) It is 50-100 times sweeter than sugar and has many properties ranging including anti-inflammatory, antibacterial, antitussive, antiarthritic, antiviral and ant-allergic. Glycyrrhetinic acid (GA), the aglycone of glycyrrhizin (GL) is present in the root at about 0.5 to 0.9%. (Killacky et al. 1976) Intestinal flora is believed to hydrolyse GL, yielding GA and a sugar moiety resulting in absorption of both. Other triterpenes included liquiritic acid, glycyrrhetol, glabrolide, isoglabroii acid, and phytosterols. The root also contains a large quantity of flavonoids which impart the characteristic yellow colour to the root. Liquiritigenin, liquiritin (main flavonoid), rhamnoliquiritin, neoliquiritin, licoflavanol, licoisoflavones, licoisoflavanone, glabrol, glabrone, liquiritegol, glucoliquiritin apioside. (Kitagawa et al. 1994) Liquorice also contains coumarins including liqcoumarin, umbelliferone, herniarin, glycyrin;
polysaccharides, volatile oils and glycymarin, a bitter compound. *G. uralensis* is considered to be almost chemically identical to *G. glabra*. A recent review of the pharmacological activities of these constituents concluded that they had antioxidant, antibacterial, antitumour and inhibiting HIV activities. (Xing et al. 2003)

**Anti-inflammatory Effects**

Anti-inflammatory effects were demonstrated on a number of experimental models. *Glycyrrhiza glabra* extract has been shown to efficiently suppress both eicosanoids and leukotrienes formation in cell-free systems, implying that this extract directly acts as a dual inhibitor of 5-LO and COX-2 activities. With regard to the properties of dual COX-2/5-LO inhibitors, Glycyrrhiza glabra extract might be a potential drug possessing anti-inflammatory activity devoid of the most troublesome (gastric) side effects seen for drugs used as COX-2 and 5-LO inhibitors. (Herold et al. 2003) Isoliquiritigenin has been shown to suppress azoxymethane-induced colon carcinogenesis in mice and to decrease both prostaglandin E2 (PGE2) and nitric oxide (NO) production in mouse macrophage cells. The decrease of PGE2 was dependent on cyclooxygenase-2 (COX-2) expression and the decrease of NO appeared to be due to a decrease in inducible nitric oxide synthase (iNOS) protein expression. In mouse and human colon carcinoma cells, isoliquiritigenin treatment suppressed cell growth and caused apoptosis. Furthermore, in vivo administration of isoliquiritigenin inhibited the induction of preneoplastic aberrant crypt foci in the male rat colon. These results suggest that isoliquiritigenin has chemopreventive actions against colon carcinogenesis. (Takahashi et al. 2004) Licochalcone A, an oxygenated chalcone from liquorice has been shown to inhibit proliferation of T cells and production of cytokines. (Barfod et al. 2002) Rats with induced duodenal ulcers treated with liquorice has been shown to recover faster than controls. The mechanism was thought to be an increase in beta-glucuronidase activity in the Brunner’s glands rather than by an ‘antacid’ action. (Nadar and Pillai, 1989) The gastric anti-secretory action of
liquorice may be due to the inhibition of endogenous gastrin release. (Ishii and Fujii, 1982)

**Influence on Steroid Metabolism**

There is no doubt that GL and GA exert a powerful influence on human steroid hormone function. However their intrinsic glucocorticoid, mineralocorticoid and oestrogenic activities are extremely low. (Tamaya et al. 1986) There is strong evidence to suggest that GA and GL act by altering the way that certain steroid hormones are metabolised. Liquorice can produce pseudoaldosteronism, by inactivating 11beta-hydroxysteriod-dehydrogenase and by binding to mineralocorticoid receptors. Liquorice also has the ability to potentiate the action of cortisol, to reduce testosterone synthesis, especially in women, to exert an oestrogen-like activity and to reduce body fat mass. (Armanini et al. 2002) The effect of oral administration of doses of 100, 250 and 500 mg/kg of a water freeze-dried extract of Glycyrrhiza glabra has been shown to induced dose-dependent and mostly significant decreases in the concentration of cortisol, ACTH, aldosterone and potassium. There were concomitant dose-dependent increases in the concentrations of renin and sodium. These results suggest a strong and dose-dependent suppression of the adrenal-pituitary axis, accompanied by stimulation of renin production from the kidney. (Al-Qarawi et al. 2002) The aldosterone-like effects of liquorice and carbenoxolone are well documented, and these effects can also be produced by GA and GL. (Takeda et al. 1979) GA inhibits the enzyme which inactivates cortisol (to cortisone), leading to a high level of cortisol in the kidney, which in turn activates type 1 mineralocorticoid receptors to cause sodium retention. (Stewart et al. 1987) (Edwards et al. 1988) Glycyrrhizin was found to be the most active inhibitor of the enzyme 11-beta hydroxysteroid dehydrogenase involved in the degradation of prednisolone. (Homma et al. 1994) In various animal studies a derivative of glycyrrhizic acid, glyderabadine, was found to exert a pronounced antiinflammatory action (exceeding that of hydrocortisone and amidopyrine) via the adrenal cortex, and by suppressing vascular permeability and antagonising...
inflammation. (Azimov et al. 1988) Two compounds derived from liquorice root; glabridin, the major isoflavon, and glabrene, an isoflavene, both demonstrated oestrogen-like activities in vascular tissues in vitro and in vivo. Glabrene behaved differently to estradiol-17beta or glabridin, but similarly to raloxifene, being a partial agonist/antagonist of estradiol-17beta. Glabridin, on the other hand, demonstrated only estrogenic activity. (Somjen et al. 2004)

Anticancer activities
Liquorice and its derivatives may protect against carcinogen-induced DNA damage, inhibit lipoxygenase and cyclooxygenase, inhibit protein kinase C and downregulate epidermal growth factor receptors and induce apoptosis in cancer cells. (Wang and Nixon, 2001) Mice fed oral doses of glycyrrhizin have been shown to protect against skin tumour genesis (Agarwal et al. 1991) and isoliquiritigenin has been shown to inhibit the growth of prostate cancer. Isoliquiritigenin, flavonoids, is known to have an anti-tumour activity in vitro and in vivo. Isoliquiritigenin significantly inhibited the proliferation of prostate cancer cell lines in a dose-dependent and time-dependent manner. (Kanazawa et al. 2003) Licocoumarone has been identified as one of the apoptosis-inducing component in liquorice. A 70% methanol soluble fraction from a liquorice acetone extract was found to inhibit cell proliferation in human monoblastic leukaemia U937 cells by inducing apoptosis. Separation by the methods including preparative HPLC provided us with an active compound, which was identified as licocoumarone. Several lines of evidence indicated that licocoumarone induced apoptosis in U937 cells. (Watanabe et al. 2002) Liquorice extract and its active component liquiritigenin have also been shown to be cytoprotective against cadmium-induced toxicity. (Kim et al. 2004) Liquorice has been demonstrated to induce apoptosis via the mitochondrial route and involving caspase-3 in HL-60 cancer cells. (Nishida et al. 2003) Isoliquiritigenin was shown to induce apoptosis in human gastric cancer MGC-803 cells. (Ma et al. 2001) Isoliquiritigenin has also been found to suppress pulmonary metastasis of mouse renal cell carcinoma. Isoliquiritigenin significantly reduced
pulmonary metastasis, without any weight loss or leukocytopenia. Isoliquiritigenin suppressed the in vitro proliferation of carcinoma cells, potentiated nitric oxide production by lipopolysaccharide-stimulated macrophages and facilitated cytotoxicity of splenic lymphocytes in vitro. These findings suggest activation of macrophages, activation of cytotoxicity of lymphocytes, and direct cytotoxicity as possible mechanisms of metastasis suppression by isoliquiritigenin. In addition, isoliquiritigenin prevented severe leukocytopenia caused by administration of 5-fluorouracil. (Yamazaki et al. 2002)

**Antimicrobial Activity**

The methanol extracts of liquorice has been shown to have antibacterial activity against the cariogenic bacterium Streptococcus mutans. (Hwang et al. 2004) Liquorice flavonoids have been found to have antimicrobial activity against methicillin-resistant Staphylococcus aureus. (Fukai et al. 2002) Liquorice extract, glycyrrhizin and its metabolites exhibited has been shown to have rapid, concentration and strain-dependent bactericidal activity against Helicobacter pylori in vitro. (Krausse et al. 2004) GL but not GA inhibit virus growth and in some instances inactivate virus particles. It has been shown to be active against herpes simplex virus (Pompei et al. 1979) and human immunodeficiency virus. (Xing et al. 2003) With oral ingestion, GL is converted to GA, therefore oral doses of GL (or liquorice) will not have systemic anti-viral effects. GL exerts systemic anti-viral effects after injection, but this mode of application is not relevant to herbal medicine. Antimutagenic activity was shown by *Glycyrrhiza glabra*, with 18-beta glycyrrhetinic acid found to be the most active compound. (Zani et al. 1993)

**Hepatoprotective Effects**

GA has been shown to be hepatoprotective in carbon tetrachloride-induced liver injury. Pre-treatment with GA prior to the administration of carbon tetrachloride significantly prevented an increase in serum alanine, aspartate aminotransferase activity and hepatic lipid peroxidation in a dose-dependent manner. In addition, pre-treatment with GA also significantly prevented the depletion of glutathione
content in the livers of carbon tetrachloride-intoxicated mice. Carbon tetrachloride-induced hepatotoxicity was also prevented, as indicated by a liver histopathologic study. Cytochrome P450 2E1 is the major isozyme involved in bioactivation of carbon tetrachloride. GA decreased the P450 2E1 expression. GA also reduced lipid peroxidation in mice liver and promoted superoxide radical scavenging activity. These results show that protective effects of GA against the carbon tetrachloride-induced hepatotoxicity may be due to its ability to block the bioactivation of carbon tetrachloride, primarily by inhibiting the expression and activity of P450 2E1, and its free radical scavenging effects.(Jeong et al. 2002) Glycyrrhizin has furthermore been shown to inhibit the lytic pathway of complement possibly explaining its anti-inflammatory effect on liver cells in viral hepatitis. It is suggested that glycyrrhizin may prevent tissue injury not only in chronic hepatitis but in many autoimmune and inflammatory diseases.(Fujisawa et al. 2000)

GL has also been shown to have pro-apoptotic properties, whereas GA has been shown to be a potent inhibitor of bile acid-induced apoptosis and necrosis in a manner consistent with its antioxidative effect.(Gumpricht et al. 2005)

Antitussive activity
Liquorice contains a potent antitussive compound, liquilitin apioside, whose antitussive effect may depend on both peripheral and central mechanisms.(Kamei et al. 2003)

Contraindications and Cautions
Excessive consumption may cause hypertension (aldosterone-like effect) and oedema(Schambelan, 1994) Liquorice should not be used by those on diuretics (can exacerbate potassium loss). Liquorice should not be used with the drugs spironalactone or amiloride. Patients who are prescribed liquorice preparations high in GL for prolonged periods should also be placed on a high potassium and low sodium diet. Hypokalemic paralysis due to liquorice consumption is extremely rare, with only 40 cases in the English literature describing paralysis secondary to exposure to liquorice in candies, medications, chewing
tobacco, and herbal preparations. Aggressive fluid and potassium replenishment will usually insure complete and lasting recovery. (Elinav and Chajek-Shaul, 2003; Brouwers and van der Meulen, 2001)

A sample of 1,049 Finnish women and their healthy singleton infants was studied in 1998. Glycyrrhizin intake was calculated from detailed questionnaires on liquorice consumption. Heavy glycyrrhizin exposure during pregnancy has been shown not to significantly affect birth weight or maternal blood pressure, but it was significantly associated with lower gestational age. Babies with heavy exposure to glycyrrhizin were not significantly lighter at birth, but they were significantly more likely to be born earlier.(Strandberg et al. 2001)

1.7.6 Ligusticum wallichii

Ligusticum wallichii, of the Umbelliferae family, is also known as Cnidium officinale and its common names Szechuan Lovage Root, Chuan Xiong (Mandarin) and Cnidium radix. The root is used medicinally. In TCM, the herb is used to invigorate the ‘Blood’ and promotes the circulation of Qi. It is also used to treat headache and rheumatic arthralgia and angina (chest pain due to stagnant blood). In modern terms, the herb is indicated for suppressed and irregular menstruation, bleeding after childbirth, threatened miscarriage, prevention and treatment of cerebral ischaemia, hypertension, poor peripheral circulation, atherosclerosis, ischaemic retinal degeneration, angina pectoris, headache, convulsions, nerve damage, abdominal pain, diarrhoea and to maintain good immunity.

Active Constituents

*Ligusticum wallichii* contains phthalides including butyl-phthalide, butylidene-phthalide, cnidilide and senkyunolide, ligustilide, alkaloids including tetramethylpyrazine (TMP) and polysaccharides including cnidirhan

Cardiovascular Effects

TMP was shown to have strong effects of scavenging cytotoxic free radicals,(Huang et al. 1994) and when examined for its effects on retinal blood flow, choroidal blood flow, blood pressure and heart rate,
the researchers concluded that TMP could be used to prevent or alleviate certain ischaemic retinal degenerations without producing significant cardiovascular side-effects. (Chiou et al. 1991) Ligustilide, cnidilide and senkyunolide have centrally acting muscle-relaxing actions. (Ozaki et al. 1989) Senkyunolide H (a phthalide) reduces atherosclerotic proliferation. (Kobayashi et al. 1993)

The effects of purified tetramethylpyrazine (TMP) on blood pressure (in vivo) and vascular contractibility (in vitro) were examined. The data showed that TMP was hypotensive and had a direct vascular effect. It not only blocked the entry of extracellular calcium through calcium channels but also inhibited the release of intracellular stored calcium in the vascular smooth muscle cell. It was shown to be a true calcium antagonist. (Pang et al. 1996) In several studies on rats, extract from *Ligusticum wallichii* have been shown to be effective in reducing portal hypertension (TMP) (Chang et al. 1998), as a beta 1-adrenergic blocker with partial beta 2-agonist activity (ferulic acid) (Wu et al. 1998) and also as having selective anti-anginal effect (butylidene phthalide). (Ko et al. 1998) Ligusticum may reduce the severity of brain ischaemic damage. (Liu, 1990)

**Immune Modulating Effects**

In two studies on dogs, *Ligusticum wallichii* was given intravenously after muscle grafts were performed. In both studies, nerve regeneration and structural and functional recovery of the grafted site were improved. (Hua and En-tan, 1996) (Jiang et al. 1996) In a Japanese study, Shi-ka-ron containing *Lithospermum erythrorhizon*, *Astragalus membranaceus* and *Ligusticum wallichii* were given orally to mice also receiving the immunosuppressive antitumour agent, mitomycin C. The results suggest that the Shi-ka-ron and each of the herbs could resist immunosuppression and that its mechanisms might be correlated with stimulation of the reticuloendothelial system, activation of T cell blastogenesis and NK cell cytotoxicity. (Jin et al. 1995) *Rehmannia glutinosa* and *Ligusticum wallichii* in Japanese formulation Shimotsu have anti-metastatic activities. (Onishi et al. 1998) *Ligusticum wallichii* prolonged the lifespan of
immunocompromised mice receiving a lethal dose of *Candida albicans*. (Abe et al. 1998) *Ligusticum wallichii* reduced angiogenesis, granuloma formation, inflammatory cell migration and fluid exudation in adjuvant-induced inflammatory model. *Ligusticum* and *Paeonia lactiflora* exhibited synergistic anti-inflammatory effects. (Kojima et al. 1996) A beta-heteroglucan (polysaccharide), cnidirhan, was shown to have very pronounced stimulating effect on macrophages and anti-complement systems. (Tomoda et al. 1994)

**Antispasmodic and anti-inflammatory effects**

In rabbits with glycerol-induced acute renal failure, *Ligusticum* was shown to effectively inhibit platelet activation, correct prostaglandin PGF1 and thromboxane TXB2 imbalance and have a beneficial effect in the prevention and treatment of acute renal failure. (Hu and Ma, 1993) *Ligusticum* was also shown to have possible benefit in acute nephrotoxicity induced by cyclosporine A in rats. (Liu, 1992) A formulation containing *Astragalus membranaceus, Ligusticum wallichii, Paeonia lactiflora* and other herbs was shown to have significant protective effects on experimentally induced liver fibrosis. (Fu, 1992)

**Reproductive Effects**

Ferulic acid (intravenous) and tetramethylpyrazine (peroral) synergistically inhibited rat uterine contraction. (Ozaki Y and Ma JP, 1990) Butylidenephthalide (BdPh) inhibited rat uterine contractions induced by prostaglandin F2 alpha, oxytocin and acetylcholine. BdPh has a non-specific spasmolytic action. (Ko, 1980) *Angelica sinensis* and *Ligusticum wallichii* significantly increased progesterone secretion in female rats. (Usuki, 1991)

**Cognitive Effects**

Paeoniflorin (from *Paeonia lactiflora*) and tetramethylpyrazine has been shown to be cognitive enhancers. (Watanabe, 1997)

**Clinical Trials**

In Beijing general hospital, 49 cases of infantile pneumonia were studied during treatment with a traditional formulation containing *Ligusticum wallichii, Angelica sinensis, Paeonia lactiflora, Astragalus*
membranaceus and other herbs. The formulation reduced the activity of erythrocyte superoxide dismutase in the acute stage of pneumonia and returned it to normal during convalescence. This anti-oxidant effect was also shown in vitro tests. (Zong et al. 1993)

*Ligusticum wallichii* was shown to significantly improve a series of cerebrovascular haemodynamic parameters in patients with atherosclerosis compared to the control group. (Wang et al. 1993)

*Ligusticum wallichii* may effectively inhibit platelet activation and correct any thromboxane A2-prostacyclin F1 imbalance in patients with acute cerebral infarction. *Ligusticum* may be beneficial in the prevention and treatment of cerebral ischaemia. (Liu, 1991)

**Contraindications and Cautions**
Contraindicated in cases of excessive bleeding. Vomiting and dizziness may occur with overdose.

**Toxicity**
Oral doses of 5 or 10 mg/kg of tetramethylpyrazine (TMP) for 4 weeks caused no significant abnormalities in body weight, blood picture, liver and kidney functions and in pathohistological examination in mice. (Wang, 1983)

**Drug Interactions**
Due to its antiplatelet effect, *Ligusticum wallichii* should be used with caution in patients taking blood thinning or anticoagulant medication.

**Dosage**
Dried herb: 2 to 4 g per day

### 1.7.7 *Paeonia lactiflora*

*Paeonia lactiflora*, of the Ranunculaceae family, is commonly known as peony, white peony and in Chinese as, Bai Shao. Peony is a common and hardy flowering garden plant grown throughout Asia and Europe. Peony is cultivated for its medicinal root as well as the large, fragrant white flowers produced during spring. The root is used medicinally. Peony has a long history of use in Asia where it is used as a ‘blood tonic’, and for menstrual regulation. (Tsuda et al. 1998) *Dang Gui Four*
is a women’s tonic widely used in China which contains peony root as one of its components.

**Active constituents**

Paeonia lactiflora contains glycosides including the monoterpenoid paeoniflorin, albiflorin, oxypaeoniflorin, 8-debenzoylpaeoniflorin (Hsu et al. 1997), phenols, benzoic acid, polysaccharides, peonan SA, peonan SB (Takechi and Tanaka, 1981), pentagalloyl glucose, phytosterols and sitosterol (Wichtl, 1994) (Tsuda et al. 1998), (Shigetoshi et al. 1995)

**Antimicrobial activities**

Antiviral substances were isolated from *Paeonia* spp. and were found to be 1,2,3,4,6-pentagalloylglucose. (Takechi and Tanaka, 1981) A neutral and acidic polysaccharide called peonan SA and peonan SB were isolated from the root of *Paeonia lactiflora*.

**Immunostimulating activities**

Both polysaccharides, but especially peonan SB, showed remarkable reticuloendothelial system-potentiating activity in a carbon clearance test and considerable anti-complement activity. (Tomoda et al. 1993)

**Endocrine activities**

The direct action of herbal medicines on aromatase activity was investigated in cultured rat follicles. Follicles were incubated in the medium for 24 hour with three peony-containing formulas or their ingredients. The addition of an aromatase inhibitor reduced oestradiol secretion in a dose-response manner. Among the ingredients, *Paeonia lactiflora* stimulated oestradiol secretion in the medium in the presence or absence of the aromatase inhibitor. It is noteworthy that *Paeonia lactiflora* alone had a stimulatory action on aromatase activity and is the only one, which is equally contained in the three medicines. (Ota et al. 1989) Paeoniflorin reduces the production of the androgens in a dose-dependant manner. Paeoniflorin increases the rate of oestriadiol synthesis from testosterone via an effect on aromatase. (Takeuchi, 1988; Takeuchi et al. 1989)

Paeoniflorin and 8-debenzoylpaeoniflorin were isolated from the dried root of *Paeonia lactiflora*. The extracts produced a significant
hypoglycaemic action in streptozotocin-treated rats and had a maximum effect 25 minutes after treatment. Paeoniflorin appears to exhibit more hypoglycaemic activity than 8-debenzoylpaeoniflorin. The activity was observed in normoglycaemic rats at doses of 1 mg/kg, indicating an insulin-independent action. The glucoside also reduced the elevation of blood sugar in glucose challenged rats, thus suggesting paeoniflorin initiates an increase in glucose utilisation. (Hsu et al. 1997)

**Nervous system activities**
Paeoniflorin was found to exhibit the most potent antagonising effect on scopolamine-affected rats when compared with Japanese Angelica, Cnidium and Rehmannia. Extracts of the herbs were tested for their ability to improve performance of rats using an eight-arm radial maze task. Paeoniflorin, a constituent from *Paeonia lactiflora*, was found to significantly improve the working memory of the rats, a measure deemed analogous to recent memory in humans. (Hiroyuki et al. 1993)

**Anti-inflammatory activities**
In an *in vitro* serum was obtained from rats pre-treated with oral herbal medicines, *Paeonia lactiflora* and *Coptis japonica*. The serum sample inhibited AA biotransformation in a similar manner to indomethacin. (Umeda et al. 1988)

**Haematological activities**
The effects of an extract of *Paeonia lactiflora* on prothrombin time (PT) activated partial thromboplastin time (PTT); antithrombin effect and activity or plasminogen and urokinase *in vitro* were investigated. The extract prolonged PT and PTT, and was able to significantly inhibit thrombin and activate plasminogen. The inhibitory effect on thrombin and the efficacy of plasminogen activation were thought to be important mechanisms in explaining the action of *Paeonia lactiflora* in promoting blood circulation and removing blood stasis. (Wang and Ma, 1990)
Hepatic activities

Acute liver damage from D-galactosamine was initiated in rats that were then treated with *Paeonia lactiflora* and *Salvia miltiorrhiza*. Parameters measured in this study were changes in ALT, bilirubin, levels of plasma fibronectin and pathological histology. The results showed that both herbs increased plasma fibronectin and improved the reticuloendothelial system function. Aggregation of albumin, collagen fragments and immune complexes were markedly reduced. Liver immune damage and microcirculation disorders were avoided, justifying the important role of *Paeonia lactiflora* and *Salvia miltiorrhiza* in protecting hepatocytes. (Qi, 1991)

Cautions and Interactions

None reported.

Toxicology

None reported.

Uses in pregnancy and lactation

“Restless foetus” is given as an indication in TCM texts. This refers to overactivity of the uterus or excessive Braxton Hicks contractions that give the subjective sense of foetal overactivity.

Contraindications

Loose bowels (due to bitter component)

1.7.8 Panax ginseng

*Panax ginseng* C.A. Meyer of the Araliaceae family is commonly known as Korean ginseng, red ginseng, white ginseng and by its Chinese name, Ren shen. The medicinal plant part is the main and lateral roots. The smaller root hairs are considered an inferior source. There are two types of preparations produced from ginseng: white ginseng, which is prepared by drying the raw herb; and red ginseng, prepared by steaming before drying. Cultivated ginseng differs from wild ginseng and plants from different countries or regions may also differ greatly. Processing of crude herb to produce red ginseng appears to increase its potency. Steaming has been shown to alter the ginsenosides’
composition; for example, steaming produces the active 20(S)-ginsenoside-Rg(3) (Matsuda et al. 2003) and makes certain ginsenosides more cytotoxic. (Park et al. 2002a) The British Herbal Pharmacopoeia stipulates that ginseng should contain not less than 20% solids (70% ethanol). The German Pharmacopoeia requires not less than 1.5% total ginsenosides calculated as ginsenoside Rg1. *Gin* refers to man and *seng* to essence in Chinese, whereas Panax is derived from the Greek word *pan* (all) and *akos* (cure), referring to its use as a cure-all. Ginseng is a perennial herb native to Korea and China and has been used as a herbal remedy in eastern Asia for thousands of years. It is considered to be the most potent *qi* or energy tonic in traditional Chinese medicine. Modern indications include low vitality, poor immunity, cancer, cardiovascular disease and enhancement of physical performance and sexual function. However, a recent systematic review of randomised controlled trials found that the efficacy of ginseng root extract could not be established beyond doubt for any of these indications. (Coon and Ernst, 2002)

**Active constituents**

The most characteristic compounds in the ginseng roots are the ginsenosides, and most biological effects have been ascribed to these compounds. The ginsenosides are dammarane saponins and can be divided into two classes: the protopanaxatriol class consisting primarily of Rg1, Rg2, Rf and Re and the protopanaxdiol consisting of primarily of Rc, Rd, Rb1 and Rb2. Ginseng also contains other saponins, polysaccharides, essential oils and other compounds. Ginsenoside Rh-1, Rh-2, and Rg-3 are obtained from red ginseng as artefacts produced during steaming. The ginsenosides are transformed by human intestinal bacteria after oral administration (Shibata, 2001) and extrapolation from in vitro studies or studies where ginseng or isolated constituents were given by injection must be made very cautiously. Commercial ginseng preparations are variable. An analysis of 50 commercial ginseng products sold in 11 countries show that there is a large variation in commercial products with the concentration of ginsenosides varying from 1.9% to 9.0%. Some products were even
found to be void of any specific ginsenosides. Some ginseng products have also been discovered to be contaminated with ephedrine. Therefore, it is essential that only quality ginseng products are used. (Cui et al. 1994) Although the root hairs have a higher level of total ginsenosides than the main root, the main and lateral roots are the preferred medicinal parts. In all probability, it is the ratio of ginsenosides that is important and that other important compounds are also active.

Adaptogen activity
Adaptogens are innocuous agents, non-specifically increasing resistance against physical, chemical or biological factors (stressors), having a normalising effect independent of the nature of pathologic state according to the original definition of adaptogen by Brekhman, 1968. (Brekhman et al. 1966). Adaptogens are natural bioregulators which increase the ability of the organism to adapt to environmental factors and to avoid damage from such factors as described in a revised definition by Panossian et al, 1999(Panossian et al. 1999) The pharmacological effects of ginseng are many and varied, contributing to its reputation as a potent adaptogen. The adrenal gland and the pituitary gland are both known to have an effect on the body’s ability to respond to stress and alter work capacity(Filaretov et al. 1986) and ginseng is thought profoundly to influence the hypothalamic–pituitary–adrenal axis. Intraperitoneal injections of total ginsenosides, with Rc being the most potent single ginsenoside, have been shown to inhibit stress-induced increases in plasma corticosterone levels significantly by blocking ACTH action in the adrenal gland. (Kim et al. 2003) Ginseng has been shown in numerous animal experiments to increase resistance to a wide variety of chemical, physical and biological stressors. Ginseng extract or its isolated constituents have been shown to prevent immunosuppression induced by cold water swim stress, (Luo et al. 1993) to counter stress-induced changes from heat stress, (Yuan et al. 1989) food deprivation, (Lee et al. 1990) electroshock, and radiation exposure. (Takeda et al. 1981 Sep) As there are more than 1500 studies on ginseng and its constituents, it is outside the
scope of this monograph to include all studies so we have attempted to include those studies most relevant to the oral use of ginseng.

**Effects on Blood pressure**

Red ginseng has been used as an antihypertensive agent in Korea, but its clinical effect is unclear despite several in vivo and in vitro experimental studies. A study of isolated muscle preparations of animal left auricle, heart and aorta with an alcohol-based extract of ginseng suggest that the hypotensive effect of ginseng is associated with a direct inhibition of myocardial contractility due to a reduction of calcium ion influx into cardiac cells as well as the inhibition of catecholamine-induced contractility of vascular smooth muscles. (Hah et al. 1978)

**Antiplatelet effect**

A number of studies have found that several ginsenosides inhibit platelet aggregation in vitro and in live animals. Panaxynol has been shown to inhibit platelet aggregation induced by adenosine disphosphate (ADP), collagen and arachidonic acid. Panaxydol and ginsenosides Ro, Rg, and Rg2 inhibit rabbit platelets while panaxydol prevented platelet aggregation and thromboxane formation. (Kuo et al. 1990)

**Antihyperlipidaemia**

Oral administration of red ginseng powder reduced plasma total cholesterol and triglycerides, while elevating plasma HDL cholesterol in rats on a high cholesterol diet and in patients with hyperlipidaemia. Ginseng also decreases hepatic cholesterol and triglyceride levels in rats, indicating a potential use of ginseng in the treatment of fatty liver. (Yamamoto et al. 1983)

**Hepatorestorative activities**

Oral administration of Korean red ginseng (250 and 500 mg/kg) on liver regeneration has been investigated in 15 dogs with partial hepatectomy. All haematological values were within normal ranges except leukocyte counts for 3 days postoperatively. The levels of AST and ALT in the ginseng groups were significantly decreased compared
to those in the control group (p<0.05). The numbers of degenerative cells and area of connective tissue were significantly decreased in the liver of the dog treated with ginseng (p<0.01). (Kwon et al. 2003)

**Digestion**

Intraperitoneal administration of ginseng has been shown to reduce bile flow and secretion of total lipids and cholesterol into the bile, while increasing the secretion of proteins in a dose-dependent manner in rats. (Salam et al. 2002)

**Gastric ulceration**

Ginseng has been shown in several studies to protect against ulceration. Among the hexane, chloroform, butanol and water fractions, the butanol fraction of a ginseng extract has been shown to be the most potent inhibitor of HCl-induced gastric lesions and ulcers induced by aspirin, acetic acid and Shay (ulcer induced by pylorus ligation). The butanol fraction showed significant increase in mucin secretion, and inhibited malondialdehyde and H+/K+ATPasee activity in the stomach. These results indicate that the effectiveness of ginseng on gastric damage might be related to inhibition of acid secretion, increased mucin secretion and antioxidant properties. (Jeong, 2002)

**Peristalsis**

Ginseng root extract, and its components, ginsenoside Rb1 (4) and ginsenoside Rd (7), have been shown to significantly ameliorate chemically induced acceleration of small intestinal transit in vivo. The test results suggest that the protective mechanism involves both an inhibitory effect on the cholinergic nervous system and a direct suppressive effect on intestinal muscles. (Hashimoto et al. 2003)

**Anti-inflammatory activities**

Both a crude, and a standardised extract (G115) of ginseng varying in saponin concentrations, have been found to protect against muscle fibre injury and inflammation after eccentric muscle contractions in rats on a treadmill. The oral ginseng extracts significantly reduced plasma creatine kinase levels by about 25% and lipid peroxidation by 15%. Certain markers of inflammation were also significantly
reduced. (Cabral de Oliveira et al. 2001) The many and varied effects of ginseng may be partly associated with the inhibition of transcription factor nuclear factor kappa B (NF kappa B). NF kappa B is a transcription factor pivotal in the regulation of inflammatory genes. Inhibition of NF kappa B may reduce inflammation and protect cells against damage. Topical application of several ginsenosides (Rb-1, Rc, Re, Rg-1, Rg-3) significantly attenuated chemically induced ear oedema in mice. The ginsenosides also suppressed expression of cyclooxygenase-2 (COX-2) and activation of NF-kappa B in the skin. Of the ginsenosides tested, Rg-3 was found to be most effective. (Surh et al. 2002)

**Immunomodulation**

The immunomodulatory effect of ginseng is based on the production of cytokines, activation of macrophages, stimulation of bone marrow cells and stimulation of inducible nitric oxide synthesis. Inducible nitric oxide synthase (iNOS) produces high levels of nitric oxide in response to activating signals from Th1-associated cytokines and plays an important role in cytotoxicity and cytostasis (growth inhibition) against many pathogenic micro-organisms. In addition to its direct effector function, nitric oxide serves as a potent immunoregulatory factor. Ginseng polysaccharides have been shown to increase the cytotoxic activity of macrophages against melanoma cells, increase phagocytosis and to induce the levels of cytokines including tumour necrosis factor-alpha, interleukin-1 beta, interleukin 6 and interferon-gamma in vitro. (Shin et al. 2002) Ginseng has been shown to be an immunomodulator and to enhance antitumour activity of macrophages in vitro. (Song et al. 2002a) Ginseng has also been shown significantly to enhance natural killer cell function in an antibody-dependent cellular cytotoxicity of peripheral blood mononuclear cells in vitro. (See et al. 1997) Incubation of macrophages with increasing amounts of an aqueous extracts of ginseng showed a dose-dependent stimulation of inducible nitric oxide synthesis. Polysaccharides isolated from ginseng showed strong stimulation of inducible nitric oxide synthesis, whereas a triterpene-enriched fraction from an aqueous extract did not show
any stimulation. As nitric oxide plays an important role in immune function, ginseng could modulate several aspects of host defence mechanisms due to stimulation of the inducible nitric oxide synthase. (Friedl et al. 2001)

Ginseng promotes the production of granulocytes in the bone marrow (granulocytopoiesis). The ginseng saponins have been shown to directly and/or indirectly promote the stromal cells and lymphocytes to produce human granulocyte-macrophage colony stimulating factor (GM-CSF) and other cytokines and induce bone marrow haemopoietic cells to express GM-CSF receptors, leading to a proliferation of human colony-forming units for granulocytes and macrophages in vitro. (Wang et al. 2003)

Ginseng polysaccharides have been shown to have potent anti-septicaemic activity by stimulating macrophages and helping modulate the reaction against sepsis induced by Staphylococcus aureus. Ginseng polysaccharides have been shown to reduce the intracellular concentration of S. aureus in macrophages in infected animals by 50% compared to controls. Combination of the ginseng polysaccharides with vancomycin resulted in 100% survival of the animals whereas only 67% or 50% of the animals survived, respectively, when treated with the ginseng polysaccharides or vancomycin alone. (Lim et al. 2002)

Ginseng polysaccharides have anticomplement activity in human serum (in vitro). (Jia et al. 1989) The addition of 2 mg ginseng dry extract per vaccine dose has been shown to potentiate the antibody response of commercial vaccines without altering their safety. The enhancing effect of ginseng was demonstrated during the vaccination of pigs against porcine parvovirus and Erysipelothrix rhusiopathiae infections using commercially available vaccines. (Rivera et al. 2003)

**Analgesia**

Intraperitoneal administration of ginsenoside Rf has been shown to potentiate opioid-induced analgesia in mice. Furthermore, ginsenosides prevented tolerance to the opiate that was not associated with opioid or GABA receptors. (Nemmani and Ramarao, 2003)
Neuroprotection

Ginseng ginsenosides and especially Rg3 have been shown to be neuroprotective. The saponins were shown to inhibit chemically induced injuries in hippocampal neurons in vitro (Kim et al. 2002) and pre-treatment of ginsenosides (50 or 100 mg/kg for 7 days) by the intraperitoneal (i.p.) route have been shown to be neuroprotective in vivo. (Lee et al. 2002b) An in vitro survival assay, demonstrated that ginsenosides Rb1 and Rg1 protect spinal cord neurons against damage. The ginsenosides protect spinal neurons from excitotoxicity induced by glutamate and kainic acid, as well as oxidative stress induced by H$_2$O$_2$. The neuroprotective effects are dose-dependent. The optimal doses are 20–40 µM for ginsenosides Rb1 and Rg1. (Liao et al. 2002) The lipophilic fraction of ginseng has been shown to induce differentiation of neurons and promote neuronal survival in the rat cortex. The effect is thought to be mediated via protein kinase C dependent pathways. (Mizumaki et al. 2002)

Diabetic neuropathy

Aqueous extract of ginseng was shown to exert no significant effect on weight in normal rats, while it prevented weight loss in rats with streptozotocin-induced diabetes. Cell proliferation in the dentate gyrus of diabetic rats was increased by ginseng treatment, but it had no effect on cell proliferation in normal rats. These results suggest that ginseng may help reduce the long term central nervous system complications of diabetes mellitus. (Lim et al. 2002)

Epilepsy

Pre-treatment (30 minutes) with 100 mg/kg ginseng, significantly protected rats against pentylenetetrazole-induced seizures. (Gupta et al. 2001)

Steroid receptor activity

Ginseng has been shown to increase the mounting behaviour of male rats and increase sperm counts in rabbit testes. The effect is not by a direct sex-hormone-like function but probably via a gonadotropin-like action. Ginsenoside-Rb1 has been shown to increase LH secretion by
acting directly on the anterior pituitary gland in male rats. (Tsai et al. 2003) Ginsenoside-Rh1 failed to activate the glucocorticoid and androgen receptors, but did demonstrate an interaction with oestrogen receptors in vitro. The effect was much weaker than 17beta-oestradiol. Ginseng is therefore considered to contain phyto-oestrogens. (Lee et al. 2003)

Chemoprotection
There have been numerous studies on the cancer preventing activities of ginseng saponins and related triterpenoid compounds. Ginsenoside Rg-3 has recently been produced as an anti-angiogenic anti-cancer drug in China (Shibata, 2001)

Oral administration of red ginseng extracts (1% in diet for 40 weeks) significantly (P<0.05) suppressed spontaneous liver tumour formation in male mice. Oral white ginseng was also shown to suppress tumour promotion in vitro and in vivo. (Nishino et al. 2001)

Dietary administration of red ginseng in combination with 1,2-dimethylhydrazine suppresses colon carcinogenesis in rats (rats were fed 1% ginseng for 5 weeks). It is thought that the inhibition may be partly associated with inhibition of cell proliferation in the colonic mucosa. (Fukushima et al. 2001)

Oral administration of 50 mg/kg daily for 4 weeks of a ginseng intestinal metabolite has been shown to partially protect against doxorubicin-induced testicular toxicity in mice. The metabolite significantly (P<0.01) prevented decreases in body weight, spermatogenic activities, serum levels of lactate dehydrogenase and creatine phosphokinase induced by doxorubicin. The ginseng metabolite significantly attenuated germ cell injuries. (Kang et al. 2002)

The methanol extract of red ginseng has been shown to attenuate the lipid peroxidation in rat brain and scavenge superoxides in differentiated human promyelocytic leukaemia (HL-60) cells. Topical application of the same extract, as well as purified ginsenosides Rg3 have been demonstrated to suppress skin tumour promotion in mice. Rg3 also suppress cyclo-oxygenase, NF kappaB and extracellular-
regulated protein kinase which are all involved in tumour promotion. (Surh et al. 2001)

Pre-treatment with oral red ginseng extract significantly reduced the development of cancer from diethylnitrosamine-induced liver cancer nodules in rats (the developmental rate of liver cancer in the experimental group was 14.3% compared to 100% in the control group). When ginseng was given concomitantly with diethylnitrosamine, the hepatoma nodules were smaller than those of the control group, the structure of hepatic tissue was well preserved and the structure of hepatocytes was normal. Ginseng also prolonged the average life span. These findings suggest benefits of ginseng in the prevention and treatment of liver cancer. (Wu et al. 2001)

Irradiation protection

Ginsenosides and specifically panaxadiol have been shown to have radioprotective effects in mice irradiated with high-dose and low-dose gamma radiation. Jejunal crypts were protected by pre-treatment with extract of whole ginseng (i.p.: 50 mg/kg of body weight at 12 and 36 hours before irradiation, P<0.005). Extract of whole ginseng (P<0.005), total saponin (P<0.01) or panaxadiol (P<0.05) administration before irradiation (i.p.: 50 mg/kg of body weight at 12 and 36 hours before irradiation) resulted in an increase in the formation of the endogenous spleen colony. The frequency of radiation-induced apoptosis in the intestinal crypt cells was also reduced by pre-treatment with extract of whole ginseng, total saponin and panaxadiol. (Kim et al. 2003)

These radioprotective effects are partly associated with the immunomodulatory effect of ginseng. Ginsan, a purified polysaccharide isolated from ginseng, has been shown to have a mitogenic activity, induce LAK cells and increase levels of several cytokines. Ginsan has also been found to increase the number of bone marrow cells, spleen cells, granulocyte-macrophage colony-forming cells and circulating neutrophils, lymphocytes and platelets significantly in irradiated mice. (Song et al. 2003)
One of the causes of radiation damage is lipid peroxidation. Lipid peroxidation alters lysosomal membrane permeability leading to the release of hydrolytic enzymes. Ginseng has been shown to markedly inhibit lipid peroxidation and protect against radiation damages in testes of mice. (Kumar et al. 2003)

**Prevention of drug resistance**

Over expression of P-glycoprotein or multidrug resistance-associated protein may lead to multidrug resistance of cancer cells. Protopanaxatriol ginsenosides have been shown to sensitise cancer cells to chemotherapeutic agents in vitro by increasing the intracellular accumulation of the drugs through direct interaction with P-glycoprotein. (Choi et al. 2003)

**Antitumour, antimetastatic and apoptosis inducing**

Ginsenosides Rg3, Rg5, Rk1, Rs5 and Rs4 have been shown to be cytotoxic to Hep-1 hepatoma cancer cells in vitro. Their 50% growth inhibition concentration (GI50) values were 41, 11, 13, 37, and 13 µM, respectively. Cisplatin had a GI50 of 84 µM in the same assay conditions. (Park et al. 2002a)

Ginsenosides, especially 20(R)-ginsenoside Rg-3, has been shown to specifically inhibit cancer cell invasion and metastasis. (Shibata, 2001) and ginsenoside Rh-2 has been shown to inhibit human ovarian cancer growth in mice. (Nakata et al. 1998) It is likely that the anti-tumour promoting activity of Rg-3 is mediated through down-regulation of NF-kappaB and other transcription factors. (Keum et al. 2003)

Oral administration of 20(S)-Protopanaxatriol (M4), the main bacterial metabolite of protopanaxatriol-type ginsenosides, has been shown to inhibit melanoma growth in mice and pre-treatment was shown to reduce metastases to the lungs. This effect is thought to be due to stimulation of Natural Killer cell-mediated tumour lysis. (Hasegawa et al. 2002)

Constituents in ginseng have also been shown to inhibit proliferation of cancer cells. Panaxytriol isolated from red ginseng was shown to have significant dose-dependent cytotoxicity activity and inhibit DNA syntheses in various tumour cells tested. (Kim et al. 2002)
The antimetastatic effects of ginseng are related to inhibition of the adhesion and invasion of tumour cells and also to anti-angiogenesis activity. Ginsenosides Rg3 and Rb2 have been shown to significantly inhibit adhesion of melanoma cells to fibronectin and laminin as well as preventing invasion into the basement membrane in vitro. Other experiments have demonstrated that the saponins significantly decreased the number of blood vessels oriented toward the tumour mass. (Sato et al. 1994; Mochizuki et al. 1995)

Ginseng saponins have also been found to promote apoptosis (programmed cell death) in cancer cells in vitro. (Hwang et al. 2002)

**Lipidaemia and fibrinogen**

Ginsenoside Rb2 has been shown to enhance the fibrinolytic activity of bovine aortic endothelial cells (Liu et al. 2003) while ginsenoside Rb1 has been shown to lower triglyceride and cholesterol levels via cAMP production in the rat liver. (Park et al. 2002b)

**Prevention of damage from toxins**

Ginseng extract has been shown to be beneficial in the prevention and treatment of testicular damage induced by environmental pollutants. Dioxin is one of the most potent toxic environmental pollutants. Exposure to dioxin either in adulthood or during late foetal and early postnatal development causes a variety of adverse effects on the male reproductive system. The chemical decreases spermatogenesis and the ability to conceive and carry a pregnancy to full term. Pre-treatment with 100 or 200 mg/kg ginseng water extract intraperitoneal for 28 days prevented toxic effects of dioxin in guinea pigs. There was no loss in body weight, testicular weight or damage to spermatogenesis. (Kim et al. 1999)

**Blood pressure**

A prospective, randomised, double-blind, placebo-controlled study of 30 healthy adults, 200 mg ginseng extract for 28 days was found to increase the QTc interval and decrease diastolic blood pressure 2 hours after ingestion on the first day of therapy. These changes, however, were not thought to be clinically significant. (Caron et al. 2002)
**Anaemia**
Ginseng is traditionally used to treat anaemia. The total saponin fraction, and specifically Rg1 and Rb1, have been shown to promote haemopoiesis by stimulating proliferation of human granulocyte-macrophage progenitors. (Niu et al. 2001)

**Antioxidant**
In vitro studies did not find various extracts of ginseng to be particularly potent antioxidants against several different free radicals. (Kim et al. 2002)

**Hair growth**
Red ginseng extract (more so than white ginseng), and especially ginsenoside-Rb1 and 20(S)-ginsenoside-Rg3, has been shown to promote hair growth in mouse hair follicles in vitro. (Matsuda et al. 2003)

**Antiallergic activity**
Ginsenosides have been demonstrated to have antiallergic activity in vitro. One of the metabolites, 20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol, was found to inhibit beta-hexosaminidase release from rat basophil leukaemia cells and potently reduce passive cutaneous anaphylaxis reaction. The inhibitory activity of protopanaxadiol was more potent than that of disodium cromoglycate, an anti-allergic drug. The compound stabilised membranes but had no effect on hyaluronidase and did not scavenge free radicals. These results suggest that the antiallergic action of protopanaxadiol originates from its cell membrane stabilising activity and that the ginsenosides are prodrugs with antiallergic properties. (Choo et al. 2003)

**Anxiolytic**
Ginsenosides, and especially ginsenoside Rc, regulate GABA(A) receptors in vitro. (Choi et al. 2003)

**Hypoglycaemic effects**
The studies on Korean ginseng have used a variety of purified compounds mainly by injection. Intraperitoneal administration of
glycans (polysaccharides known as panaxan) and other unidentified compounds has been found to the have hypoglycaemic activity in both normal and alloxan-induced hyperglycaemic mice. (Kimura et al. 1981)

**Wound healing**
Ginsenoside Rb2 has been reported to improve wound healing. It is believed that ginsenoside Rb2 enhances epidermal cell proliferation by enhancing the expressions of protein factors related to cell proliferation, such as epidermal growth factor and fibronectin (and their receptors) keratin and collagenase. (Choi, 2002)

**Antiacne**
In an animal model of acne, ginseng extracts reduced the size of comedones by altering keratinisation of the skin and desquamating horny cells in comedones. In a study of experimentally induced hyperkeratosis, ginseng reduced the accumulation of lipids in the epidermis by regulating enzymes associated with epidermal metabolism. (Kim et al. 1990)

**Clinical use**
In practice, panax and the various ginsenosides are used in many forms and administered via various routes. This review will focus mostly on those methods commonly in use by the public and preparations, which are available over the counter such as oral dose forms, topical applications and inhalations.

**Cancer Prevention**
A 5-year prospective study of 4634 patients over 40 years of age found that ginseng reduced the relative risk of cancer by nearly 50%. (Yun et al. 1996)
A retrospective study of 905 case-controlled pairs taking ginseng showed that ginseng intake reduced the risk of cancer by 44% (odds ratio equal to 0.56). The powdered and extract forms of ginseng were more effective than fresh sliced ginseng, juice or tea. The preventative effect was highly significant (P<0.001). There was a significant decline
in cancer occurrence with increasing ginseng intake (P<0.05). (Yun et al. 1996)

Epidemiological studies in Korea strongly suggest that cultivated Korean ginseng is a non-organ-specific human cancer preventative agent. In case-control studies, odds ratios of cancer of lip, oral cavity and pharynx, larynx, lung, oesophagus, stomach, liver, pancreas, ovary and colorectum were significantly reduced by ginseng use. The most active compounds are thought to be ginsenosides Rg-3, Rg-5 and Rh-2. (Yun, 2003)

**Immune modulation**

Ginseng has been shown significantly to enhance natural killer cell function in healthy subjects and those suffering from chronic fatigue syndrome or AIDS (P<0.01). (See et al. 1997)

Ginseng extract (100 mg Ginsana G115 daily) improved the response to an influenza vaccine in a multicentre, randomised, double blind, placebo-controlled, two-arm study of 227 subjects. Compared to vaccine without the ginseng, the addition of ginseng resulted in fewer cases of influenza and common cold. Ginseng increase natural killer cell activity and increased antibody production. (Scaglione et al. 1990)

Ginseng polysaccharide injection has been shown in a randomised study to improve immunity in 130 patients with nasopharyngeal carcinoma and to reduce adverse reactions to radiotherapy compared with controls. (Xie et al. 2001)

Red ginseng powder has been shown to restore immunity after chemotherapy and reduce the recurrence of stage III gastric cancer. The five-year disease free survival and overall survival rate were significantly higher in patients taking the red ginseng powder during postoperative chemotherapy versus control (68.2% versus 33.3%, 76.4% versus 38.5%, respectively, P<0.05). In spite of the limitation of a small number of patients (n=42), these findings suggest that red ginseng powder may help to improve postoperative survival in these patients. Additionally, red ginseng powder may have some immunomodulatory properties associated with CD3 and CD4 activity in
patients with advanced gastric cancer during postoperative chemotherapy. (Suh et al. 2002)

**Menopausal symptoms**

Korean red ginseng is used to alleviate symptoms associated with menopause; 6 g ginseng for 30 days was shown in a small study of 20 women significantly (P<0.001) to improve menopausal symptoms, in particular fatigue, insomnia and depression. The women treated had a significant decrease in cortisol and cortisol-to-dehydroepiandrosterone ratio (P<0.05). No adverse effects were recorded. (Tode et al. 1999)

Formulations containing ginseng extract have been shown to be beneficial in the treatment of menopausal hot flushes and sleep disturbance. A morning capsule containing ginseng, black cohosh, soy and green tea extracts combined with an evening capsule containing black cohosh, soy, kava, hops and valerian extracts were shown to relieve menopausal symptoms of hot flushes and sleep disturbance. Healthy postmenopausal women, between 45 and 65 years of age, were asked to take the menopause formula orally, one capsule of the morning formula every morning and one capsule of the evening formula every evening for 2 months. The reduction in the number of hot flushes was observed as early as at the end of the second week. At the end of the second week, the number of hot flushes was reduced by 47%. The treatment also significantly reduced the Greene Climacteric Scale scores. At the end of the eighth week, the vasomotor, anxiety and depression scores were reduced by 50%, 56%, and 32%, respectively. Furthermore, the treatment significantly reduced global Pittsburgh Sleep Quality Index score and scores in five components (sleep quality, sleep latency, sleep duration, sleep disturbance and daytime dysfunction) by 18%–46%. (Sun, 2003)

**Erectile dysfunction**

There is good evidence that ginsenosides can facilitate penile erection by directly inducing the vasodilatation and relaxation of penile corpus cavernosum. Moreover, the effects of ginseng on the corpus cavernosum appear to be mediated by the release and/or modification of release of nitric oxide from endothelial cells and perivascular
nerves. (Murphy and Lee, 2002) Korean red ginseng has been shown to alleviate erectile dysfunction. Ginseng improves the ability to achieve and maintain erections even in patients with severe erectile dysfunctions. (Price and Gazewood, 2003)

In a double-blind, crossover study, 900 mg Korean red ginseng was found to significantly improve the Mean International Index of Erectile Function scores compared with placebo. Scores on Questions 3 (penetration) and 4 (maintenance) were significantly higher in the ginseng than in the placebo group (P<0.01). In response to the global efficacy question 60% of the patients answered that Korean red ginseng improved erection (P<0.01). Among other variables, penile tip rigidity on RigiScan showed significant improvement for ginseng versus placebo. (Hong et al. 2002)

Respiratory disease
Ginseng extract (G115) has been shown significantly (P<0.05) to improve pulmonary function test, maximum voluntary ventilation, maximum inspiratory pressure and maximal oxygen consumption (VO₂max) in a study of 92 patients suffering moderately severe chronic obstructive pulmonary disease (n=49, G115 100 mg bd for three months). (Gross et al. 2002)

Memory and concentration
Ginseng improves the quality of memory and the associated secondary memory. (Kennedy et al. 2001) In a randomised, placebo-controlled, double-blind, balanced, crossover study of healthy, young adult volunteers, 400 mg ginseng was shown to improve secondary memory performance on a Cognitive Drug Research computerised assessment battery and two serial subtraction mental arithmetic tasks. Ginseng also improved attention and the speed of performing the memory tasks. (Kennedy et al. 2001)

In a double-blind, placebo-controlled study of healthy young subjects, ginseng extract (G115) improved accuracy and slowed responses during one of the two computerised serial subtraction tests (Serial Sevens). Ginseng has also been shown to improve mood during these tasks. Combining ginseng with ginkgo dramatically improved the
memory, concentration and speed of completing mental tasks.(Kennedy et al. 2002; Scholey and Kennedy, 2002)

Standardised ginseng extract 400 mg was found in a double-blind, randomised, placebo-controlled, 8 to 9 week trial significantly to improve abstract thinking (P<0.005) and reaction time (not significant) in 112 healthy subjects over 40 years of age. Ginseng was found not to affect concentration nor memory.(Sorensen and Sonne, 1996)

Improvements were seen in psychomotor performance, but no difference from placebo was reported in pure motor function, recognition and visual reaction time in this double-blind, placebo-controlled study evaluating psychomotor variables such as attention, integrated sensory motor function, auditory reaction time and processing.

**Quality of Life**

An 8-week randomised, double-blind study found that 200 mg/day ginseng (n=15, placebo: n=15) improved aspects of mental health and social functioning after 4 weeks’ therapy but that these differences disappeared with continued use.(Ellis and Reddy, 2002) A review of 8 clinical studies with ginseng found some improvement in quality of life scores. However, the findings were equivocal. Despite some positive results, improvement in overall health-related quality of life cannot, given the current research, be attributed to *P. ginseng*. However, the possibility that various facets of quality of life may have improved and the potential of early transient effects cannot be discounted.(Coleman et al. 2003) A double-blind, placebo-controlled, randomised clinical trial of 83 subjects also did not find ginseng to enhance psychological wellbeing in healthy, young adults.(Cardinal and Engels, 2001)

A double-blind, placebo-controlled, crossover study found that 1200 mg Ginseng was only slightly more effective than placebo and not as effective as a good night's sleep in improving bodily feelings, mood and fatigue in 12 fatigued night nurses. Volunteers slept less and experienced less fatigue but rated sleep quality worse after ginseng administration.(Hallstrom et al. 1982)
Adaptogenic and tonic effects

A randomised, double-blind study involving 232 subjects between the ages of 25 and 60 years of age found that extract equivalent to about 400 mg ginseng root for 4 weeks significantly improved fatigue. Side effects were uncommon with only two subjects withdrawing from the study. (Le Gal and Cathebras, 1996)

A randomised, double-blind study of 83 subjects found that extract equivalent to 1 g ginseng root for 4 months improved the risk of contracting a common cold or bronchitis, improved appetite, sleep, wellbeing and physical performance. (Gianoli and Riebenfeld, 1984)

Ginseng is used by many athletes to improve stamina and to facilitate rapid recovery from injuries. To examine the effects of ginseng supplements on hormonal status following acute resistance exercise, eight male college students were randomly given water (control group) or 20 g of ginseng root extract treatment immediately after a standardised training exercise. Human growth hormone, testosterone, cortisol, and insulin-like growth factor 1 levels were determined by radioimmunoassay. The responses of plasma hormones following ginseng consumption were not significant between the control and the ginseng groups during the 2-hour recovery period. These results do not support the use of ginseng to promote an anabolic hormonal status following resistance exercise. (Youl Kang et al. 2002)

Although ginseng is commonly used to improve endurance, a double-blind study of 19 healthy, active women found that 400 mg of a ginseng extract (G115) did not improve supramaximal exercise performance and short-term recovery. Analysis of variance using pre-test to post-test change scores revealed no significant difference between the ginseng and placebo study groups for the following variables measured: peak anaerobic power output, mean anaerobic power output, rate of fatigue, and immediate post exercise recovery heart rates (P>0.05). (Engels et al. 2001) A recent study by the same authors also failed to find any benefit from ginseng (400 mg/day G115; equivalent to 2 g Panax ginseng C.A. Meyer root material for 8 weeks) on improving physical performance and heart rate recovery of
individuals undergoing repeated bouts of exhausting exercise. (Engels et al. 2003)

**Diabetes**
A double-blind, placebo-controlled study with 36 subjects found that 200 mg ginseng elevated mood, improved psychophysical performance and reduced fasting blood glucose and body weight in patients with newly diagnosed non-insulin-dependent diabetes mellitus. (Sotaniemi et al. 1995)

**Cardiovascular disease**
Although there are reports of ginseng causing hypertension, red ginseng is actually used as an antihypertensive agent in Korea. Its antihypertensive effect is not clear in spite of several in vivo and in vitro experimental studies. An open clinical study with 44 hypertensive patients found red ginseng to be useful as an adjuvant to antihypertensive medication. The dose of red ginseng was 4.5 g/day (1.5 g td). (Han et al. 1995) Korean red ginseng has also been shown to improve vascular endothelial function in patients with hypertension. The effect is thought to be mediated through increasing the synthesis of nitric oxide. (Sung et al. 2000)

A combination of red ginseng and digoxin was found to be more beneficial than either medicine alone in an open study of advanced congestive heart failure. There were no adverse reactions. (Ding et al. 1995) A combination of ginseng and ginkgo extracts has been found to improve circulation and lower blood pressure in a controlled single-dose study of 10 healthy young volunteers. (Kiesewetter et al. 1992)

**Hyperlipidaemia**
Red ginseng, 1.5 g td before meals, for 7 days, reduced liver cholesterol, decreased atherogenic index and elevated HDL cholesterol in 11 patients (5 normal subjects and 6 with hyperlipidaemia). Serum cholesterol was not significantly altered, but serum triglycerides were significantly decreased. (Yamamoto and Kumagai, 1982)
**HIV infection**

Clinicians have observed that CD4+ T cell counts in human immunodeficiency virus (HIV)-1-infected patients treated with only Korean red ginseng are maintained or even increased for a prolonged period. Long-term intake (60 +/- 15 months) of Korean red ginseng in HIV-1-infected patients has also been shown to delay the development of resistance mutation to zidovudine (AZT, Retrovir). (Cho et al. 2001)

**Dosage**

Extract equivalent to 0.9–3 g crude ginseng root. Many of the clinical studies published in the scientific literature have been conducted on a proprietary extract of ginseng standardised to 4% total ginsengsensenosides. Ginseng is usually given in the earlier part of the day. It should not be given in the evening unless it is used to promote wakefulness. Ginseng is usually not given to children.

**Adverse reactions**

Ginseng abuse syndrome (hypertension, nervousness, insomnia, morning diarrhoea, inability to concentrate and skin reactions) has been reported and there has been a report of a 28-year-old woman who had a severe headache after ingesting a large quantity of ethanol-extracted ginseng. Cerebral angiograms showed ‘beading’ appearance in the anterior and posterior cerebral and superior cerebellar arteries, consistent with cerebral arteritis. (Ryu and Chien, 1995)

However, the majority of the scientific data suggest that ginseng is rarely associated with adverse events or drug interactions. A systematic review found that the most commonly experienced adverse events are headache, sleep and gastrointestinal disorders. Data from clinical trials suggest that the incidence of adverse events with ginseng mono-preparations is similar to that of placebo. Any documented effects are usually mild and transient. Combined preparations are more often associated with adverse events, but causal attribution is usually not possible. (Coon and Ernst, 2002)

In a two-generation rat study, a ginseng extract fed at doses as high as 15 mg/kg/day did not produce adverse effects on reproductive
performance, including embryo development and lactation. (Hess et al. 1982)

Ginseng has very low toxicity. Subacute doses of 1.5–15 mg/kg of a 5:1 ginseng extract did not produce negative effect on body weight, food consumption, haematological or, biochemical parameters, or histological findings in dogs (Hess et al. 1983) and no effects have been observed from the administration of similar doses in two generations of rat offspring. (Hess et al. 1982)

Drug interactions
There have been two case reports of ginseng reducing the antithrombotic effects of warfarin. (Rosado, 2003; Janetzky and Morreale, 1997)

Ginseng contains glycosides with structural similarities to digoxin. Different interaction assays have yielded various results and there are no case reports of actual interaction. (Chow et al. 2003; Dasgupta et al. 2003)

Ginseng may act as an inhibitor of cytochrome CYP-1A; however, the clinical significance of this is unknown. (Lee et al. 2002a)

Contraindications and precautions
Korean ginseng is generally contraindicated in acute infections with fever, and hypertension and in persons who are very hot, tense and overly stimulated. Overuse may result in headache, insomnia and palpitation. Ginseng use should be discontinued two weeks before any surgery.

Pregnancy use
Ginseng is traditionally used in Korea as a tonic during pregnancy. The Commission E does not list any restrictions. Ginseng is not teratogenic, but long-term safety studies have not been conducted. Use with caution. (Blumenthal, 2001)

Indications
Ginseng is indicated for chronic immune deficiency, menopausal symptoms, erectile dysfunction, chronic respiratory disease, enhancement of psychomotor activity, memory and concentration,
tonic and adaptogenic effects in any chronic condition and for the elderly or infirm, non-insulin-dependent diabetes, cardiovascular disease (the effects on hypertension remain to be fully investigated) and to improve or maintain quality of life (equivocal scientific support). Commission E recommends ginseng as a tonic for invigoration and fortification in times of fatigue, debility and convalescence, or declining capacity for work and concentration. The World Health Organisation suggests that ginseng can be used as a prophylactive and restorative agent for enhancement of mental and physical capacities, in cases of weakness, exhaustion, tiredness and loss of concentration and during convalescence.

1.7.9 Poria cocos

Poria cocos of the Polyporaceae family is commonly known as hoelen, China root, Sclerotium of Tuckahoe, Fu Ling (Mandarin) and Bukuryo (Japanese). The medicinally part is the fungal body known as the sclerotium. Traditionally used as a diuretic and sedative in the treatment of oligouresis, insomnia, tachycardia and gastrointestinal atony. Hoelen is a fungus of the puff ball family. The fungus grows around pine roots, and consist of a large, ponderous tuberiform body, with reddish brown covering. They are white when young and are attached to the ground by a cord-like structure, a rhizomorph. The interior of the puffball, known as the gleba, is cottony when young and consists of the spores and capillitium. The interior consists of a compact mass of considerable hardness, varying in colour from cinnamon brown to pure white. The interior portion of this fungous body is odourless and slightly sweet; it is barely soluble in water. Fu Ling is the dried sclerotium of the fungus. The herb is mainly produced in the provinces of Anhui, Hubei, Henan and Yunnan. It is collected from July to September and dried in the shade.

Poria is traditionally used for disorder of the female reproductive tract including hypermenorrhea, dysmenorrhoea, fibroids, leucorrhoea and infertility. Poria is also used for non-specific fluid retention, renal and cardiac oedema, nephritis, neurasthenia and insomnia, nervous palpitations, nausea, diarrhoea and gastrointestinal bleeding as well as
for burn injury, premature ageing, inflammatory conditions, cancer prevention, cardiac disease, impaired memory, swelling, brownish urine, sputum, diarrhoea, difficult urination, anxiety, insomnia.

**Active Constituents**
The active constituents in poria include pachyman polysaccharides, triterpene acids including pachymic acid, tumulosic acid, eburicoic acid, pnicolic acid, poricodic acid A and B, hydroxylanostatrienoidioic acid, trienoic acid, hydroxytrametenoic acid, dehydroahcymic acid, epidehydrotumulosic acid, dehydroeburiconic acid, polyporenic acid C, poricoric acid D and AM. Poria also contain ergosterin, choline, histidine, adenine, lecithin and potassium.

**Pharmacological activities**

**Progesterone secretion**
Hoelen and Japanese Angelica root increase progesterone secretion from the corpus luteum.(Usuki, 1991)

**Immune modulation**
Hoelen hot ethanol extraction dose dependently increased the secretions of immune stimulators interleukins IL-1 beta, IL-6 and tumour necrosis factor (TNF) after in vitro cultivation with human peripheral blood monocytes while suppressing the secretion of immune suppressing transforming growth factor beta (TGF beta).(Yu and Tseng, 1996) Pachyman polysaccharides was shown to inhibit proliferation of mice carcinoma cell lines by a direct killing effect. The results suggested that the antitumour effect of the polysaccharides is not only related to the enhancement of immune function, but also related to changes in the cell membranes.(Tong et al. 1994)

**Antimutagenicity**
Poria cocos was shown to have antimutagenic properties in the Ames bioassay system.(Sakai et al. 1986)

**Scleroderma**
Cinnamon and Hoelen formulation containing *Cinnamomum cassia*, *Paeonia lactiflora*, *Paeonia suffruticosa*, *Prunus persica* and *Poria cocos*,

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significantly and selectively inhibited collagen synthesis in a dose-dependant manner which may help explain the clinical usefulness in the treatment of scleroderma. (Sheng et al. 1994)

**Hormonal Effects**
Cinnamon and Hoelen formulation used in the treatment of gynaecological disorders such as hypermenorrhea, dysmenorrhoea and infertility, decreases LH, FSH and oestradiol. The formulation is thought to act as an LH-RH antagonist and/or as a weak anti-oestrogen. (Sakamoto et al. 1988)

**Inflammation**
Hydroxybenzoyldehydrotumulosic acid showed anti-inflammatory effects by inhibiting tetradecanoylphorbol acetate (TPA) and arachidonic acid-induced ear inflammation in mice. (Yasukawa et al. 1998) The lanostane-type triterpenes, dehyrotumulosic and pachymic acids also proved effective against carrageenan, arachidonic acid and TPA-induced oedemas, chronic inflammation caused by TPA, and oxazolone delayed hypersensitivity. The inflammatory action may be due to competitive inhibition of phospholipase A2. (Cuellar et al. 1997) Triterpene carboxylic acids (dihydroxylanosta, trienoic acid, hydroxydehydropachymic acid, hydroxytrametenolic acid and dehydrotumulosic acid) also inhibited TPA-induced mouse ear oedema. (Nukaya et al. 1996) Pachymic acid, hydroxytrametnoic acid and proicoic acid B showed a strong anti-inflammatory and anti-tumour activity in mice. (Kaminaga et al. 1996)

**Nausea**
Triterpenes isolated from *P. cocos* inhibited emetic action induced by oral administration of copper sulfate pentahydrate to leopard frogs. (Tai et al. 1995)

**Nephritis**
Pachyman was shown to have anti-nephrotic activity. Pachyman prevented urinary protein excretion and elevation of serum cholesterol in original-type anti-GBM nephritic rats. The anti-nephrotic mechanism is thought to be due to inhibition of C3 deposition in the
glomeruli (Hattori et al. 1992) and enhancement of free radical scavenging in the renal cortex and glomeruli. (Akao et al. 1991)

**Burn injury**

Decoction of *Porcia cocos*, *Atractylodes macrocephala*, *Codonopsis pilosula* and *Glycyrrhiza uralensis* improved metabolism, nutrition and visceral organ function after burn injuries. (Ruan et al. 1994)

**Ageing**

Shou Xing Bu Zhi, containing thirteen herbs including *Porcia cocos* at 10 g/kg daily for 3 months effectively slowed ageing, as measured by reduction in lipofuscin of liver and brain tissues, in adult mice; in vitro inhibition of lipid peroxidation of rat liver homogeneous and a diminution of hydroxyproline of skin in both young and adult mice. (Chen, 1989) DX-9386, a formulation containing *Panax ginseng*, *Polygala tenuifolia*, *Acorus* and *Porcia cocos*, slowed the ageing process in terms of learning behaviour and lipid peroxidation, life span, prevention of body weight decrease and development of senility. Alcohol-induced impairment of learning and memory was ameliorated by the formulation which also exhibited sedative effects. The improvement in memory of the formulation was not due to direct activation of cholinergic transmission in the brain, but may, at least partly, be due to a direct action on the hippocampus. (Smriga et al. 1995)

**Digestion**

Both the fraction containing polysaccharide and the triterpenoid-rich fraction increased the activity of the vagus nerve.

**Post-ischaemic Brain Damage**

Cinnamon and Hoelen combination was shown, not only to have free-radical scavenging activity, but also to have a suppressive effect on the generation of lipid peroxidation in mice with post-ischaemic brain damage (after a stroke the brain is susceptible to lipid peroxidation and free radical damage). (Fushitani et al. 1995)
Clinical Trials

Oedema
Hoelen had a marked effect in 23 cases and improvement in 7 cases of 30 patients suffering non-specific, renal or cardiac oedema. (Yin and Guo 1993)

Schizophrenia
60 g Hoelen by decoction given daily to 53 schizophrenic patients for 1 to 3 months cured 3 patients, markedly improved 11 and improved 16 patients. (Yin and Guo 1993)

Diarrhoea
A formulation containing *Poria cocos* effectively treated infantile rotavirus diarrhoea in 83% of cases. (Wang et al. 1995) Another formulation containing *Poria cocos* cured 90% of 419 children with diarrhoea due to its antimicrobial and immune stimulating activities and by its ability to restoring proper digestion and absorption while inhibiting intestinal movement. (Li, 1991)

Toxicity
Hoelen has very low toxicity. The LD_{50} values of the warm water macerate of the herb were > 10 g/kg by oral administration. (Chang and But 1986)

Dosage
Dried herb: 5-10 g daily

1.7.10 Rehmannia glutinosa
Rehmannia glutinosa or Chinese Foxglove, from the Scrophulariaceae family, is known in mandarin as Di Huang. The uncured form is called Sheng Di Huang. If cooked with wine (cured) is called Shu Di Huang. The root the part used medicinally. Rehmannia is used to prevent the suppressive effects of corticosteroid drugs on endogenous levels of corticosteroids. For inflammatory disorders involving the immune system, eg allergies, especially skin rashes and auto-immune disorders. Also indicated for haemorrhages, eg haematuria, metrorrhagia.
**Active Constituents**

Active constituents include iridoid compounds including aucubin, catapol, ajugol, rehmanniosides, jioglutosides and rehmaglutin. Rehmannia also contains other glycosides known as jionosides A₁, B₁, C and D. (Sasaki et al. 1989) (Kitagawa et al. 1971)

**Adrenal Cortex Function**

Uncured Rehmannia antagonised the inhibitory action of corticosteroids on plasma corticosterone and inhibited the catabolism of cortisol. (Chang and But 1986)

**Immune Function**

Rehmannia has been shown to inhibit tumour necrosis factor-alpha and interleukin-1 secretion from mouse astrocytes. (Kim et al. 1999)

Constituents in Cured Rehmannia suppressed antibody formation. The immunosuppressive principles of Rehmannia were found to be jionoside A₁, jionoside B₁, acetoside, isoacetoside, purpureaside C, cistanoside A and cistanoside F. Cured Rehmannia abolished the suppressive effects of cyclophosphamide and dexamethasone on immunity.

Oral administration of a herbal preparation containing Rehmannia demonstrated protective effects on haematopoiesis, immunity, heart, liver and kidney functions during chemotherapy in tumour-bearing mice. (Xu, 1992)

Uncured rehmannia has been shown to inhibit the metabolism of cortisol by hepatocytes in vitro. (Yin ZZ et al. 1980) Oral administration of rehmannia has been shown to offset the suppressive effects of cyclophosphamide and dexamethasone on a variety of immunological factors. (Mills and Bone 2000)

**Anti-inflammatory effects**

Orally administered Uncured Rehmannia demonstrated improvement in haemorheology in arthritic and thrombotic rats. (Kubo et al. 1994)

Rehmannia glutinosas steamed root may be beneficial in the regulation of immediate type allergic reaction, as it has been shown to inhibit systemic allergic reaction both in vitro and in vivo. (Kim et al. 1998)
Uncured rehmannia has been shown to improve the haemorrheology in arthritic and thrombotic rats. The orally administered extract was shown to the peripheral microcirculation of various chronic diseases through the improvement of haemorheology. (Kubo et al. 1994)

**Chemoprotective**

Oral liquid rehmannia extracts has been shown to reduce side-effects of chemotherapy in tumour-bearing mice. The survival rate in the treated group was significantly higher than that in control (P < 0.01); the hemopoietic functions (HB, WBC, PL) in the treated group were also significantly better (P < 0.05 and P < 0.01); rehmannia also protected action the heart, liver and kidney tissues from damage (P < 0.01) and prevented drug-induced suppression of NK and T- and B-lymphocytes. The study suggests that rehmannia may improve hematopoiesis, immunity, while protecting heart, liver and kidney during chemotherapy. (Xu, 1992)

A decoction of rehmannia has been shown to protect mice against experimental toxic hepatitis induced by carbon tetrachloride and preventing a decrease in liver glycogen. (Chang and But 1986)

**Safety**

The mutagenic potential of Rehmannia was tested with the Ames test and in vivo. Uncured Rehmannia showed no mutagenic activity, whereas cured rehmannia was mutagenic in the in vivo mammalian (mice) assay when given by i.p injection. (Yin et al. 1991)

**Clinical Studies**

A decoction of rehmannia reduced blood pressure, serum cholesterol and triglycerides in 62 hypertensive patients. Cerebral flow and ECG also improved. (Chang and But 1986) Oral administration of a herbal preparation containing Rehmannia and Astragalus demonstrated therapeutic effects on chronic nephritis. Improvement was observed in 91% of the treatment group compared to 67% in the control group. The preparation also demonstrated anti-allergy effects and promotion and modulation of immunity. (Su et al. 1993)
**Dosage**

Up to 30 g/day of the dried (uncured) root. (Mills and Bone 2000)

Higher doses has also been recommended in the literature. (Chang and But 1986)

The actions and indications are summarised in table 4.

**Table 4: Summary of individual herb activities**

<table>
<thead>
<tr>
<th>Herb</th>
<th>Actions</th>
<th>Traditional and modern uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panax ginseng</td>
<td>Adaptogen, cardioprotective, hepatotonic, hepato-protective, anti-inflammatory, immunomodulator, anticancer, hypoglycaemic, tonic, male tonic.</td>
<td>Weakness, loss of physical stamina, exhaustion, tiredness, diminished concentration, memory or psychomotor activity, chronic immune deficiency, depression, any chronic condition including aging, infirmity or convalescence, cancer (prevention and supportive treatment), cardiovascular disease, chronic respiratory disease including asthma, emphysema, non-insulin-dependent diabetes, erectile dysfunction, menopausal symptoms.</td>
</tr>
<tr>
<td>Atractylodes macrocephala</td>
<td>Adaptogen, bitter tonic, digestive, diuretic.</td>
<td>Stress, exhaustion, anorexia, indigestion, diarrhoea, intestinal bleeding, cancer, oedema of face and extremities, night blindness</td>
</tr>
<tr>
<td>Poria cocos</td>
<td>Immune modulation, hormone modulation, anti-inflammatory, antioxidant.</td>
<td>Disorder of the female reproductive tract including hypermenorrhrea, dysmenorrhoea, fibroids, leucorrhoea and infertility. Non-specific fluid retention, renal and cardiac oedema, nephritis, neurasthenia and insomnia, nervous palpitations, nausea,</td>
</tr>
<tr>
<td><strong>Glycyrrhiza uralensis</strong></td>
<td>Adaptogen, adrenal restorative, aldose reductase inhibitor, anti-inflammatory, antiseptic (urinary), antispasmodic (muscles), antispasmodic (respiratory tract), antispasmodic (uterus), antitussive, anti-ulcerogenic (GIT), antiviral (topically), blood sugar regulating, demulcent, expectorant (relaxing), hypertensive, laxative, oestrogenic</td>
<td>Endocrine disorders including endometriosis, ovarian cyst, polycystic ovarian syndrome, androgen excess, Addison's disease, Respiratory conditions including asthma, bronchitis, cough, sore throat (gargle). Skin disorders including eczema, psoriasis and acne (topically), gastrointestinal disorders including gastritis, ulcer, irritable bowel syndrome, constipation, steroids therapy (to augment treatment), stress.</td>
</tr>
<tr>
<td><strong>Rehmannia glutinosa</strong></td>
<td>Antipyretic, antihaemorrhagic (systemic), anti-inflammatory, adaptogen, adrenal restorative</td>
<td>Inflammatory disorders involving immune system eg allergies, skin rashes and autoimmune disorders, Haemorrhages eg haematuria, metrorrhagia</td>
</tr>
<tr>
<td><strong>Paeonia lactiflora</strong></td>
<td>Antispasmodic (uterus), muscle relaxant, anticonvulsant, anti-inflammatory, cognition enhancer, anti-allergic, immune enhancer, anti-</td>
<td>Menstrual disorders – irregularity, erratic ovulation, spasmodic dysmenorrhoea, metrorrhagia, menorrhagia, leucorrhoea, menopausal symptoms, benign breast</td>
</tr>
<tr>
<td>Angelica sinensis</td>
<td>Anti-arrhythmic, anti-inflammatory, anti-platelet, antispasmodic (uterus), blood tonic, cardiotonic, circulatory stimulant, hepatoprotective, immunomodulator, uterine tonic.</td>
<td>Dysmenorrhoea, amenorrhoea, metrorrhagia, endometriosis, menstrual irregularity including erratic ovulation, premenstrual syndrome, infertility, liver disease, Hypertension, angina pectoris, arrhythmia, palpitations, anaemia. Cerebral ischaemia, constipation associated with aging or debility.</td>
</tr>
</tbody>
</table>
| Ligusticum wallichii | Antioxidant, hypotensive, anti-ischaemic, immunomodulating, anti-spasmodic, anti-inflammatory, hormone modulating. | Headache and rheumatic arthralgia and angina (chest pain due to stagnant blood). Suppressed and irregular menstruation, bleeding after childbirth, threatened miscarriage, prevention and treatment of cerebral ischaemia, hypertension, poor peripheral circulation, atherosclerosis, ischaemic retinal degeneration, angina pectoris, headache, convulsions, nerve damage, abdominal pain, diarrhoea and to
<table>
<thead>
<tr>
<th>Herb</th>
<th>Properties</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamomum cassia</td>
<td>Carminative, antidiarrhoeal, antiemetic, antimicrobial</td>
<td>Dyspepsia, intestinal colic, flatulence, diarrhoea, common cold, influenza, nausea, diabetes.</td>
</tr>
<tr>
<td>Astragalus membranaceus</td>
<td>Immunomodulator, tonic, adaptogen, cardiotonic, diuretic, hypotensive, antioxidant, renal tonic</td>
<td>Debility, immune deficiency, chronic infection and autoimmune diseases, wounds, gastrointestinal ulcers, chronic bacterial or viral infections, especially if combined with debility and spontaneous sweating (eg hepatitis, AIDS), cancer, hypertension (prevention of cardiovascular disease), congestive heart failure, menopausal night sweats, palpitations</td>
</tr>
</tbody>
</table>
2 Materials and methods

2.1 Test medication

Traditionally Ginseng and Dang Gui Ten Combination is prepared by decoction. Sometimes it is also made by maceration (soaking) in rice wine.

The test medication (table 5), given the code name PS10, contains the following herbs Angelica sinensis, Rehmannia glutinosa, Paeonia lactiflora, Panax ginseng, Ligusticum wallichii, Atractylodes macrocephala, Poria cocos, Glycyrrhiza uralensis, Astragalus membranaceus and Cinnamomum cassia. PS10 was made by packing 10 kg of the milled dried herbs into the percolator allowing it to steep for two days before commencing the percolation. A total of 30 litres of 50% ethanol-water v/v was used during the percolation. The soaking and percolation took place at 50°C. The initial extraction was then transferred to a Büchi Rotavapor R-220 (see figure 2), a rotary evaporator used to concentrate the extract at 50°C under vacuum (-100 kilopascal) to a final volume of 10 litres. By definition, the extract has a strength of 1:1 as 10 kg dried herbs were used to produce 10 litres of final extract.

Figure 2: Büchi Rotavapor R-220
<table>
<thead>
<tr>
<th>ID</th>
<th>Botanical Name</th>
<th>Chinese Name</th>
<th>English Name</th>
<th>Part Used</th>
<th>Amount per 10 ml dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Panax ginseng</td>
<td>Ren Shen</td>
<td>Korean ginseng</td>
<td>root</td>
<td>700 mg</td>
</tr>
<tr>
<td>2</td>
<td>Atractylodes macrocephala</td>
<td>Bai Zhu</td>
<td>atractylodes</td>
<td>root</td>
<td>1000 mg</td>
</tr>
<tr>
<td>3</td>
<td>Poria cocos</td>
<td>Fu Ling</td>
<td>hoelen</td>
<td>fungal body</td>
<td>1200 mg</td>
</tr>
<tr>
<td>4</td>
<td>Glycyrrhiza uralensis</td>
<td>Zhi Gan Cao</td>
<td>liquorice</td>
<td>root</td>
<td>500 mg</td>
</tr>
<tr>
<td>5</td>
<td>Rehmannia glutinosa</td>
<td>Shu Di Huang</td>
<td>prepared rehmannia</td>
<td>prepared root</td>
<td>1500 mg</td>
</tr>
<tr>
<td>6</td>
<td>Paeonia lactiflora</td>
<td>Bai Shao</td>
<td>peony</td>
<td>root</td>
<td>1200 mg</td>
</tr>
<tr>
<td>7</td>
<td>Angelica sinensis</td>
<td>Dang Gui</td>
<td>dong gui</td>
<td>root</td>
<td>1200 mg</td>
</tr>
<tr>
<td>8</td>
<td>Ligusticum wallichii</td>
<td>Chuan Xiong</td>
<td>Szechuan lovage root</td>
<td>root</td>
<td>600 mg</td>
</tr>
<tr>
<td>9</td>
<td>Cinnamomum cassia</td>
<td>Rou Gui</td>
<td>Cinnamon</td>
<td>quills</td>
<td>600 mg</td>
</tr>
<tr>
<td>10</td>
<td>Astragalus membranaceus</td>
<td>Huang Qi</td>
<td>Astragalus</td>
<td>root</td>
<td>1500 mg</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>10,000 mg</td>
</tr>
</tbody>
</table>

**ID Notes**

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Combination of the ten single herb extracts in the ratio as PS10 in water (H₂O) and Ethanol (EtOH)</td>
</tr>
<tr>
<td>2</td>
<td>Combination of the ten single herb extracts in the same ratio as TJ-48 in water (H₂O) and Ethanol (EtOH)</td>
</tr>
<tr>
<td>TT PE</td>
<td>Reconstituted TJ-48 in water (H₂O) and Ethanol (EtOH)</td>
</tr>
</tbody>
</table>
2.2 Chemical Analysis

Each of the ten herbs in the formulation was milled and extracted in 50% ethanol-water or hot water. The final extracts had a ratio of 1:10 (whereby 2 g dried herbs is used to produce 20 ml extract). The single extracts were then mixed according to the ratio defined in Table 5. In addition a sample of TJ-48 was obtained from a pharmacy in Japan. TJ-48 is spray-dried hot water decoction with a final drug-extract ratio of 5.7:1. It should be noted, however, that the percentages of individual herbs vary in TJ-48 and PS10. TJ-48 is based on the Japanese formulation that contains equal amounts of the herbs except *Glycyrrhiza spp.* which is added at half the level of the other herbs. 0.351mg of the TJ-48 powder was diluted in 20 ml water and 50% ethanol-water respectively with a final extract ratio of 1:10 (code: TT PE) This produced in total 24 extract samples, which were analysed by TLC and HPLC by Analytica Laboratory, Sydney.
2.2.1 HPTLC (High Performance Thin Layer Chromatography)
The extracts were analysed by reverse phase high performance thin layer chromatography on a Camag Linomat 4. Milled herbs were extracted in ethanol or water and subjected to sonication at 50°C for 30 minutes in stoppered test tubes. The extracts were filtered using 0.45 micron Teflon filters and spotted on silicagel 60F254 HPTLC plates. Chromatography paper was used to equilibrate (mix the gas and liquid phases) inside lidded twin trough glass tanks.

2.2.2 HPLC (High Performance Liquid Chromatography)
Approximately 1 ml of the extracts were filtrated through an HPLC filter (0.2 µm). From the filtrate, 10 µl was used for analysis with a high performance liquid chromatograph (HPLC).

2.2.3 Microbiology
PS10 was tested for microbial contamination according to the specifications listed in the European Pharmacopoeia 2nd Edition. The test medication was analysed for yeasts, moulds, Enterobacteria, E. coli, Staphylococcus aureus and salmonella.

2.3 Overall Study Design and Objectives
The overall aim of was to investigate the immunomodulatory effects of the Traditional Chinese Herbal formulation PS10 in healthy human subjects.

2.3.1 Study Design
The study was a longitudinal study (28 days), using a repeated measure design to examine pre-post intervention changes in Natural Killer cell activity as well as total and differentiated lymphocyte count, indicative of an immunomodulatory effect of the herbal formulation, PS10 (see figure 3).

A pre and post liver function tests were also performed to assess any adverse hepatic reaction to the herbal formulation.

Participants were interviewed before entering the research program to explain the purpose of the study and the requirements of the
participants, with written instructions given and consent form obtained. See Appendix B.

Participants were asked not to change their diet or exercise pattern for the duration of the study.

![Figure 3: Study Design](image)

2.3.2 Study Population and Size

Ten healthy volunteers, seven males and three females aged 43 to 58 were recruited to participate in the study.

2.3.3 Sample Selection

Participants were obtained by word of mouth and by a general notice posted at student notice boards at University of Swinburne, Hawthorn campus and Melbourne College of Natural Medicine, City campus.

2.3.4 Selection Bias

No selection bias was recorded.

2.3.5 Inclusions and Exclusions

This study was open to healthy subjects only. No participant on any prescription medication for serious conditions was included. Two participants, DXH and JXY, took Gemfibrozil and Zoccor for hypercholesterolemia.

2.3.6 Compliance

Weekly phone contact ascertained that all participants had consumed the required dosage of the test medication. At the end of the consumption of the test medication, the subjects were asked if any test medication remained. It was ascertained that the subjects had complied very well with taking the prescribed dosage and the majority
of subjects had consumed the correct amount with only one subject missing a single dosage.

2.3.7 Health Status
All participants were estimated to be in good general health.

2.3.8 Recent Medication Use
None of the participants required any new medication during the period of the study other than the test medication. One subject (JXY) continued treatment with Gemfibrozil for hypercholesterolaemia while subject DXH took Zoccor, Co-enzyme Q10 and fish oil for hypercholesterolaemia and Macuvision (antioxidant formulation) for mild macular degeneration.

2.3.9 Test Medication
All subjects received two glass bottle containing 140 ml PS10. Subjects were instructed to take precisely 10 ml PI10 in a little water twice daily for 14 days. A daily dosage of 10 ml extract twice daily is equivalent to a daily dose of 20 g dried herbs.

2.3.10 Blood Collection
All participants were asked to attend sample collection centre at Melbourne Pathology, Clifton Hill. 20 mL blood was taken from an antecubital vein of the right or left arm under tourniquet conditions with subjects seated in EDTA-containing glass tube.

Blood collection took place between 8.30 and 10.00 am on the day of testing. Blood collection took place on day 0, 1, 2, 14 and 28 of the study period. Blood samples were analysed within 1 hour for haematological data. Due to a serious storm and flooding, one subject, CXD, was unable to attend the blood collection venue on the required final day. Instead, blood was collected on day 35, three weeks after cessation of the test medication.
2.4 Assays

2.4.1 Natural Killer Cell Cytolytic Assay

The blood samples were analysed by Dr Graham R. Flannery and his team of the Department of Genetics, Faculty of Science, Technology and Engineering, La Trobe University, Bundoora, Victoria (see figure 4-6).

Natural Killer (NK) cell function was assessed by the ability of peripheral blood lymphocytes (PBL) to lyse the human erythroleukemia cell line K562.(Lozzio and Lozzio, 1975)

Tumour target cells were labelled with $^{51}$Cr, which was released from cells following membrane damage (lysis) and measured in a gamma-counter. Assays were performed in micro-titre plates in total volumes of 300 µl (using a fixed number of targets per well and appropriate numbers of effectors as listed in Table 6 below).

The assay was carried out in triplicate and 100 µl (1/3 of the well volume) samples were taken for counting (see figures 4 to 6 for a detailed description). Release of $^{51}$Cr from any E:T ratio was calculated as the sum of the triplicate values. Total (maximum) release from any test was determined by independently counting target cell aliquots. Percent release was calculated from released $^{51}$Cr /total (see Table 7 below).

Several effector to target (E:T) cell ratios were used in the 4-hour micro-cytotoxicity assay and the results were plotted as %Cr release versus E:T ratio. The gradient of the line of best fit through the plotted points was recorded as the measure of cytotoxicity or killing.(Brooks and Flannery, 1980)

**Table 6: Volumes for NK assay test**

<table>
<thead>
<tr>
<th>Ratio</th>
<th>0:1</th>
<th>6:1</th>
<th>12:1</th>
<th>25:1</th>
<th>50:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>200 µl</td>
<td>175 µl</td>
<td>150 µl</td>
<td>100 µl</td>
<td>0 µl</td>
</tr>
<tr>
<td>PBL</td>
<td>0 µl</td>
<td>25 µl</td>
<td>50 µl</td>
<td>100 µl</td>
<td>200 µl</td>
</tr>
<tr>
<td>Target cells</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Total</td>
<td>300 µl</td>
<td>300 µl</td>
<td>300 µl</td>
<td>300 µl</td>
<td>300 µl</td>
</tr>
</tbody>
</table>
Table 7: NK cytotoxicity equations

\[
% \text{ release} = \frac{100 \times \text{ cpm (mean of triplicates)}}{\text{total cpm}} \times (\text{mean of tumour cell aliquots})
\]

\[
% \text{ cytotoxicity} = \frac{100 \times (\% \text{ release with effectors (test)} - \% \text{ release with medium alone (spontaneous)})}{100 - \% \text{ release with medium alone}}
\]

CPM = count per minute (radio activity as determined by the gamma counter)

Whole blood, diluted in equal volume phosphate buffered saline (PBS) was layered onto a Ficoll-Paque Plus gradient. The density is less than that of erythrocytes (which pass through the gradient and sediment) but greater than that of mononuclear cells (the large granular lymphocytes including lymphocytes, monocytes and NK cells).

Sedimentation of blood cells through the gradient was facilitated by centrifugation (IEC HW-SII centrifuge and allowed to spin for 45 min. at 1500 rpm).
After centrifugation, the red blood cells were completely separated from the white blood cells.

The white blood cells formed a ‘buffy coat’ on the gradient, which was harvested using a Pasteur pipette.

The harvested white blood cells were washed in 2% FCS/RPMI to remove any residual Ficoll solution. The washing was repeated three times and centrifuged each time. Cells were then counted and diluted to the appropriate concentration for the assay.

**Figure 4: Separation of Peripheral Blood Lymphocytes**

The target cells, human myeloid leukemic cell lines K562, were fed with 10% FCS/RPMI, 24 hours prior to the assay commencing.
Target cells K562 were removed from the flask under aseptic conditions and centrifuged for 5 min. at 1000 rpm. The supernatant was discarded and the target cell pellet resuspended.

Target cells were labelled with 100µCi of $^{51}$Cr and incubated for 2 hours at 37°C with gentle mixing at 10-minute intervals.

Once the incubation was complete the target cells were washed with 2% FCS/RPMI. This process was repeated three times. After the final wash, the supernatant was then discarded and the pellet resuspended in RPMI supplemented with 10% foetal calf serum (FCS). The cells were counted and resuspended to a concentration of $5 \times 10^4$ cells/ml in RPMI supplemented with 10% FCS.

Finally the vials were tested obtain ‘spontaneous’ release of $^{51}$Cr (from targets dying independently of NK-mediated lysis) and this ‘background’ is subtracted from test values before plotting data.

Radioactivity is measured on a LKB mini gamma counter over 300 seconds.

**Figure 5: Preparation of the Target Cells**
The assay was performed in a flat bottom, sterile Cellstar (Greiner Bio-one) 96 well tissue culture plate. The tissue culture plates were filled with varying volumes of 10% FCS/RPMI media, lymphocytes (effectors) and K562 (target cells).

The effector cells (peripheral blood lymphocytes, PBL) concentration was $1.25 \times 10^6$/ml, whilst target cell concentration was at $5 \times 10^4$/ml. Various ratios of effector : target were plated out, and incubated for 4 hours in humidified conditions at 37°C with 5% CO₂.

After incubation the Cr51 is released from the target cells following membrane damage (lysis) and measured in a gamma-counter.

**Figure 6: Assay Preparation**

### 2.4.2 Lymphocyte Assay

The blood samples were analysed by Dr Graham R. Flannery and his team of the Department of Genetics, Faculty of Science, Technology and Engineering, La Trobe University, Bundoora, Victoria. Total as well as differentiated white blood cell counts were performed as described in figures 7 and 8. Staining with dyes highlights cell membranes (and hence their shapes) and intracellular structures such as the nuclei. This allows for determination of the relative proportions of each of the major cell types: granular cells (neutrophils and eosinophils) and agranular cells (lymphocytes and monocytes).
25 µl whole heparinized blood was diluted by adding 475 µl l buffer (3 ml acetic acid, 1 ml 0.1% gentian violet in dH2O). After the acetic acid had lysed the red blood cells, the sample was transferred to the haemocytometer for total white blood cell counting. Cells were counted using a haemocytometer.

Figure 7: Total White Blood Cell Count
Preparation of blood films
A small drop of whole blood was placed on a slide. The sample was then evenly spread across the slide leaving a single layer of cells. Once dry, the blood film was fixed by soaking the slide in 100% methanol for 10 min. It was then stained with May-Grunwald:H₂O (1:2) for 5 min and later with Giemsa:Gurr’s buffer (1:9) for another 15 min. The blood film was then rinsed in distilled water twice and soaked in distilled water for 2 min before being allowed to dry. The slide was then covered with Depex mounting medium and viewed under the microscope at 40x magnification.

Figure 8: Differentiated White Cell Count

2.4.3 Liver function test
Blood collected on day 0 and day 28 were analysed by Melbourne Pathology. Liver function test measures gamma-glutamyl transferase
(GGT); alanine aminotransferase (ALT or SGPT); aspartate aminotransferase (AST or SGOT); and alkaline phosphatase (ALP).

2.5 Lytic Unit

To present a more accurate picture of a person’s immunity, both the effects of NK cell cytotoxicity and number of mononuclear cells can be combined and expressed as ‘lytic units’.

**Lytic Unit Conversion**

The lytic unit is calculated as the sum of the absolute number of monocytes and absolute number of lymphocytes multiplied by the cytotoxicity gradient.

\[
\text{Lytic unit} = (\text{monocytes} + \text{lymphocytes}) \times \text{cytotoxicity gradient}
\]

2.6 Analyses of Data

The means for the variables, monocyte count, lymphocyte count, NK cell activity and lytic units were compared with a two-tailed T test from two time points namely at 0 days compared to 28 days as well as 14 days. The paired T Test is a two-sample test used to determine if two populations means are equal. In the paired T test, the data is dependent, i.e. there is a one-to-one correspondence between the values in the two samples. In the present study the same participant was measured before and after taking the test medication. The statistical packages SPSS and STATA software was used to statistical analyse the data. These statistical packages were utilised for spreadsheet data presentation and analysis. Means, standard deviations, medians and 25-75% inter-quartile ranges were calculated for continuous variables where necessary and box plot graphs produced.
3 Results

3.1 Analysis of data

Continuous data of blood parameters including:

- Liver function tests
- Monocyte cell counts
- Total lymphocyte cell counts
- Natural killer cell cytotoxicity
- Lytic units – representing combined NK cell cytotoxicity and number of mononuclear cells

were compared in a bivariate analysis from baseline versus the 28 days time point.

3.2 HPTLC Analysis

The HPTLC plates (figures 9-12) compare water versus ethanol-water extracts for the ten individual herbs as well as the TJ-48 hot water extract versus the ethanol-water extract produced by mixing the individual herbs according to the ratio specified in PS10 (ratio 1).

It is quite clear that there is a very good match between the water and the ethanol-water extracts of the single herbs (numbered 1-10) in both systems. The methanol-water extracts, however, appears to be stronger (the coloured bands are brighter or more pronounced).

There is also a close correlation between TJ-48 and the combined preparation PS10 although it appears that both the water and methanol-water extract of PS10 is stronger than TJ-48 with the methanol-water extract being the strongest. This is especially noticeable in the top end of the spectrum.
Figure 9: HPTLC Plate 1

Lane 1: water extract of Panax ginseng
Lane 2: ethanol extract of Panax ginseng
Lane 3: hot water extract of Atractylodes macrocephala
Lane 4: ethanol extract of Atractylodes macrocephala
Lane 5: hot water extract of Poria cocos
Lane 6: ethanol extract of Poria cocos
Lane 7: hot water extract of Glycyrrhiza uralensis
Lane 8: ethanol extract of Glycyrrhiza uralensis
Lane 9: hot water extract of Rehmannia glutinosa
Lane 10: ethanol extract of Rehmannia glutinosa
Lane 11: hot water extract of combined herbs according to PS10 formulation
Lane 12: ethanol extract of combined herbs according to PS10 formulation
Lane 13: hot water extract of combined herbs according to Tj-48 formulation
Lane 14: ethanol extract of combined herbs according to Tj-48 formulation.
Lane 15: Tj-48 formulation reconstitutet in water

To evaluate the possible differences between hot water versus ethanol extraction, the single ingredients as well as the combinations of them, were compared by HPTLC. Figure 9 shows a HPTLC plate for Saponin System: 60:32:12:8 (choloform:acetic acid:methanol:water) comparing hot water with ethanol extracts of Panax ginseng, Atractylodes macrocephala, Poria cocos, Glycyrrhiza, Rehmannia glutinosa, combination of all ten herbs as per the ratio described in the PS10 and Tj-48 formulations and the powder formulation Tj-48.
reconstituted in water. It is quite clear that there is a very good match between the water and the ethanol-water extracts of the single herbs (lanes 1-10). The ethanol-water extracts, however, appears to be stronger (the coloured bands are brighter or more pronounced). There is also a close correlation between TJ-48 and the combined preparation PS10 although it appears that both the water and ethanol-water extract of PS10 is stronger than TJ-48 with the ethanol-water extract being the strongest.

**Figure 10: HPTLC Plate 2**

Lane 1: hot water extract of Paeonia lactiflora
Lane 2: ethanol extract of Paeonia lactiflora
Lane 3: hot water extract of Angelica sinensis
Lane 4: ethanol extract of Angelica sinensis
Lane 5: hot water extract of Ligusticum wallichii
Lane 6: ethanol extract of Ligusticum wallichii
Lane 7: hot water extract of Cinnamomum cassia
Lane 8: ethanol extract of Cinnamomum cassia
Lane 9: hot water extract of Astragalus membranaceous
Lane 10: ethanol extract of Astragalus membranaceous
Lane 11: hot water extract of combined herbs according to PS10 formulation
Lane 12: ethanol extract of combined herbs according to PS10 formulation
Lane 13: hot water extract of combined herbs according to Tj-48 formulation
Lane 14: ethanol extract of combined herbs according to Tj-48 formulation.
Lane 15: Tj-48 formulation reconstituted in water
To evaluate the possible differences between hot water versus ethanol extraction, the single ingredients as well as the combinations of them, were compared by HPTLC. Figure 10 shows a HPTLC plate for Saponin System: 60:32:12:8 (chloroform:acetic acid:methanol:water) comparing hot water with ethanol extracts of Paeonia lactiflora, Angelica sinensis, Ligusticum wallichii, Cinnamomum cassia, Astragalus membranaceus, combination of all ten herbs as per the ratio described in the PS10 and Tj-48 formulations and the powder formulation Tj-48 reconstituted in water. It is quite clear that there is a very good match between the water and the ethanol-water extracts of the single herbs (lanes 1-10). The ethanol-water extracts, however, appears to be stronger (the coloured bands are brighter or more pronounced). There is also a close correlation between TJ-48 and the combined preparation PS10 although it appears that both the water and ethanol-water extract of PS10 is stronger than TJ-48 with the ethanol-water extract being the strongest.

**Figure 11: HPTLC Plate 3**

Lane 1: water extract of Panax ginseng  
Lane 2: ethanol extract of Panax ginseng  
Lane 3: hot water extract of Atractylodes macrocephala  
Lane 4: ethanol extract of Atractylodes macrocephala  
Lane 5: hot water extract of Poria cocos  
Lane 6: ethanol extract of Poria cocos  
Lane 7: hot water extract of Glycyrrhiza uralensis
Lane 8: ethanol extract of Glycyrrhiza uralensis
Lane 9: hot water extract of Rehmannia glutinosa
Lane 10: ethanol extract of Rehmannia glutinosa
Lane 11: hot water extract of combined herbs according to PS10 formulation
Lane 12: ethanol extract of combined herbs according to PS10 formulation
Lane 13: hot water extract of combined herbs according to Tj-48 formulation
Lane 14: ethanol extract of combined herbs according to Tj-48 formulation.
Lane 15: Tj-48 formulation reconstituted in water

To evaluate the possible differences between hot water versus ethanol extraction, the single ingredients as well as the combinations of them, were compared by HPTLC. Figure 11 shows a HPTLC plate for Flavonoid system: 100:11:11:27 (ethylacetate:formic acid:acetic acid:water) comparing hot water with ethanol extracts of Panax ginseng, Atractylodes macrocephala, Poria cocos, Glycyrrhiza, Rehmannia glutinosa, combination of all ten herbs as per the ratio described in the PS10 and Tj-48 formulations and the powder formulation Tj-48 reconstituted in water. It is quite clear that there is a very good match between the water and the ethanol-water extracts of the single herbs (lanes 1-10). The ethanol-water extracts, however, appears to be stronger (the coloured bands are brighter or more pronounced). There is also a close correlation between TJ-48 and the combined preparation PS10 although it appears that both the water and ethanol-water extract of PS10 is stronger than TJ-48 with the ethanol-water extract being the strongest.
Figure 12: HPTLC Plate 4

Lane 1: water extract of Paeonia lactiflora
Lane 2: ethanol extract of Paeonia lactiflora
Lane 3: hot water extract of Angelica sinensis
Lane 4: ethanol extract of Angelica sinensis
Lane 5: hot water extract of Ligusticum wallichii
Lane 6: ethanol extract of Ligusticum wallichii
Lane 7: hot water extract of Cinnamomum cassia
Lane 8: ethanol extract of Cinnamomum cassia
Lane 9: hot water extract of Astragalus membranaceus
Lane 10: ethanol extract of Astragalus membranaceus
Lane 11: hot water extract of combined herbs according to PS10 formulation
Lane 12: ethanol extract of combined herbs according to PS10 formulation
Lane 13: hot water extract of combined herbs according to Tj-48 formulation
Lane 14: ethanol extract of combined herbs according to Tj-48 formulation.
Lane 15: Tj-48 formulation reconstitutet in water

To evaluate the possible differences between hot water versus ethanol extraction, the single ingredients as well as the combinations of them, were compared by HPTLC. Figure 12 shows a HPTLC plate for Flavonoid system: 100:11:11:27 (ethylacetate:formic acid:acetic acid:water) comparing hot water with ethanol extracts of Paeonia lactiflora, Angelica sinensis, Ligusticum wallichii, Cinnamomum cassia, Astragalus membranaceus, combination of all ten herbs as per the ratio described in the PS10 and Tj-48 formulations and the powder formulation Tj-48 reconstituted in water. It is quite clear that there is a very good match between the water and the ethanol-water extracts of the single herbs (lanes 1-10). The ethanol-water extracts, however, appears to be stronger (the coloured bands are brighter or more pronounced). There is also a close correlation between TJ-48 and the combined preparation PS10 although it appears that both the water and ethanol-water extract of PS10 is stronger than TJ-48 with the ethanol-water extract being the strongest.
3.3 HPLC Analysis

It is very difficult to compare the HPLC chromatogram of TJ-48 (figure 13) and the combined ethanol-water PS-10 extract (figure 14) without further detailed analysis. It is noticeable that the PS-10 ethanol extract has higher peaks than the PS-10 hot water extract and that the ethanol-extract has more pronounced peaks at the non-polar (right hand side of the chromatogram). This is not surprising as the ethanol content is generally more superior in extracting non-polar constituents compared to hot water.

Figure 13: HPLC TJ-48

HPLC fingerprint overlay report comparing TJ48 reconstituted in water and 50% ethanol.
Figure 14: HPLC Combined ethanol-water Extract (PS10)

HPLC fingerprint overlay comparing hot water and ethanol extracts of PS-10.

Table 8 lists the laboratory analysis of the PS-10 ethanol formulations in regards to appearance (internal reference), total solids, specific gravity and ethanol content.

Table 8: Chemical analysis of PS10 (performed 27.06.2003)

<table>
<thead>
<tr>
<th>Test</th>
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<th>Units</th>
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<tr>
<td>Appearance</td>
<td>Conforms</td>
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<tr>
<td>Total Solids</td>
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<td>%W/V</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.0763</td>
<td>g/mL</td>
</tr>
<tr>
<td>Ethanol content by GC</td>
<td>36.3</td>
<td>%v/v</td>
</tr>
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</table>

3.4 Microbiology

PS10 was tested for microbial contamination and found to comply with the European Pharmacopeial specifications (see table 9).
Table 9: Microbial analysis of PS10 (performed 27.06.2003)

<table>
<thead>
<tr>
<th>Test</th>
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<th>Upper Limit/Test</th>
<th>Results</th>
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</thead>
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<td>&lt;100</td>
</tr>
<tr>
<td>Yeast Count</td>
<td>cfu/g</td>
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<td>&lt;100</td>
</tr>
<tr>
<td>Mould Count</td>
<td>cfu/g</td>
<td>100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Enterobacteriacea Count</td>
<td>cfu/g</td>
<td>100</td>
<td>&lt;100</td>
</tr>
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<td><em>Escherichia coli</em> (Presence/Absence)</td>
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<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (Presence/Absence)</td>
<td>g</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Salmonella</em> (Presence/Absence)</td>
<td>/10g</td>
<td>Absent</td>
<td>Absent</td>
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3.5 Liver Function Test

A liver function test was performed on day 0 (the day before the commencement of the test medication) and again on day 28 (two weeks after the discontinuation of the medication). All initial liver function tests apart from that of subject 6 were within the normal ranges before as well as after the test medication. Subject 6 has hypercholesterolemia and receives medication. The levels for ALT and T protein were outside normal levels prior to the test medication. On day 28, subject 6's ALT and AST were outside normal levels. The level for T protein had fallen to within normal levels. The actual values for each test in each of the subjects are listed in table 10 below. Using a two tailed t test, no significant differences (p>0.05) were observed for any participants in any parameter of the liver function tests from baseline values.

Table 10: Results of liver function tests on day 0 and day 28.

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<th>Participant 1</th>
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<tr>
<td><strong>Enzyme</strong></td>
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<tr>
<td>S ALP</td>
</tr>
<tr>
<td>S GGT</td>
</tr>
<tr>
<td>S ALT</td>
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<tr>
<td>S AST</td>
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<tr>
<td>S T Protein</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>S Albumin</td>
</tr>
<tr>
<td>S Globulin</td>
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**Participant 2**

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<td>S T-bili</td>
<td>2-20</td>
<td>Umol/L</td>
<td>8</td>
<td>6</td>
</tr>
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<td>S ALP</td>
<td>30-120</td>
<td>U/L</td>
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<td>83</td>
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<tr>
<td>S GGT</td>
<td>5-65</td>
<td>U/L</td>
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<td>17</td>
</tr>
<tr>
<td>S ALT</td>
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<td>U/L</td>
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<td>15</td>
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<td>S AST</td>
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<td>U/L</td>
<td>11</td>
<td>15</td>
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<td>S T Protein</td>
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<td>g/L</td>
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<td>68</td>
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<td>S Albumin</td>
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<td>g/L</td>
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<td>S Globulin</td>
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**Participant 3**

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**Participant 4**

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### Participant 9

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<td>30-120</td>
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<td>5-40</td>
<td>U/L</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>S T Protein</td>
<td>60-80</td>
<td>g/L</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td>S Albumin</td>
<td>35-50</td>
<td>g/L</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>S Globulin</td>
<td>20-36</td>
<td>g/L</td>
<td>25</td>
<td>23</td>
</tr>
</tbody>
</table>

### Participant 10

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Range</th>
<th>Units</th>
<th>Day 0</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>S T-bili</td>
<td>2-20</td>
<td>Umol/L</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
3.6 Monocytes

The test medication did not have overall notable influence on the total monocyte count. During the test medication (day 1 to day 14), the total monocyte count reduced for five subjects, increased for five patients. Calculating the changes from day 0 to day 28 (two weeks after the discontinuation of the medication), the total monocytes count increased for seven subjects and decreased for the last three subjects. The individual results are listed in table 11 and in graphical form in figure 15. Normal monocyte count in adults is 0.2-0.8 x10^9/L. All participants except participant 5 are within normal ranges. Participant 5 had a monocyte count of 0.97 x10^9/L and 0.84 x10^9/L on day 14 and day 28 respectively. Statistical analysis (see figure 16) using a paired samples t-test confirms that there is no significant difference between the mean level of monocytes from day 0 to day 14 nor from day 0 to day 28 (Paired samples t-test, power of the test 80%, p<0.055).

<table>
<thead>
<tr>
<th></th>
<th>30-120</th>
<th>5-65</th>
<th>5-40</th>
<th>60-80</th>
<th>35-50</th>
<th>20-36</th>
</tr>
</thead>
<tbody>
<tr>
<td>S ALP</td>
<td>30-120</td>
<td>U/L</td>
<td>68</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S GGT</td>
<td>5-65</td>
<td>U/L</td>
<td>13</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S ALT</td>
<td>5-40</td>
<td>U/L</td>
<td>21</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S AST</td>
<td>5-40</td>
<td>U/L</td>
<td>20</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S T Protein</td>
<td>60-80</td>
<td>g/L</td>
<td>68</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S Albumin</td>
<td>35-50</td>
<td>g/L</td>
<td>42</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S Globulin</td>
<td>20-36</td>
<td>g/L</td>
<td>26</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 11: Total monocyte count (x10^9/L ) day 0-28.

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant 1</td>
<td>0.245</td>
<td>0.364</td>
<td>0.396</td>
<td>0.44</td>
<td>0.19</td>
</tr>
<tr>
<td>Participant 2</td>
<td>0.23</td>
<td>0.3582</td>
<td>0.221</td>
<td>0.24</td>
<td>0.32</td>
</tr>
<tr>
<td>Participant 3</td>
<td>0.515</td>
<td>0.56</td>
<td>0.3438</td>
<td>0.578</td>
<td>0.5088</td>
</tr>
<tr>
<td>Participant 4</td>
<td>0.378</td>
<td>0.25</td>
<td>0.286</td>
<td>0.2718</td>
<td>0.488</td>
</tr>
<tr>
<td>Participant 5</td>
<td>0.3675</td>
<td>0.5</td>
<td>0.274</td>
<td>0.9728</td>
<td>0.8364</td>
</tr>
<tr>
<td>Participant 6</td>
<td>0.499</td>
<td>0.3125</td>
<td>0.1521</td>
<td>0.2322</td>
<td>0.7384</td>
</tr>
<tr>
<td>Participant 7</td>
<td>0.1992</td>
<td>0.3325</td>
<td>0.344</td>
<td>0.4488</td>
<td>0.3885</td>
</tr>
<tr>
<td>Participant 8</td>
<td>0.3465</td>
<td>0.392</td>
<td>0.2142</td>
<td>0.1472</td>
<td>0.627</td>
</tr>
<tr>
<td>Participant 9</td>
<td>0.528</td>
<td>0.15</td>
<td>0.2555</td>
<td>0.2115</td>
<td>0.4188</td>
</tr>
<tr>
<td>Participant 10</td>
<td>0.508</td>
<td>0.605</td>
<td>0.5118</td>
<td>0.4347</td>
<td>0.5236</td>
</tr>
</tbody>
</table>

**Figure 15:** Changes in total monocyte count (x10⁹/L) day 0-28

**Table 12: T-Test analysis of total monocytes day 0-14 and day 14-28**

<table>
<thead>
<tr>
<th>Monocytes</th>
<th>Mean</th>
<th>N</th>
<th>St.d. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>.3816</td>
<td>10</td>
<td>.1271</td>
<td>4.019E-02</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.3977</td>
<td>10</td>
<td>.2437</td>
<td>7.707E-02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monocytes</th>
<th>Mean</th>
<th>N</th>
<th>St.d.</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>.3816</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>0.3977</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deviation</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>---------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>.3816</td>
<td>.1271</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>.5040</td>
<td>.1921</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Paired Samples Correlations

<table>
<thead>
<tr>
<th>Monocytes</th>
<th>N</th>
<th>Correlation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0-14</td>
<td>10</td>
<td>-.029</td>
<td>.937</td>
</tr>
<tr>
<td>Day 0-28</td>
<td>10</td>
<td>.459</td>
<td>.183</td>
</tr>
</tbody>
</table>

Paired Samples Test

<table>
<thead>
<tr>
<th>Monocytes</th>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>Df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>Lowerr</td>
<td>Upper</td>
</tr>
<tr>
<td>Day 0-14</td>
<td>-1.61E-02</td>
<td>.2781</td>
<td>8.793E-02</td>
<td>- .215</td>
<td>.1828</td>
</tr>
<tr>
<td>Day 0-28</td>
<td>-.1223</td>
<td>.1756</td>
<td>5.553E-02</td>
<td>- .247</td>
<td>3.278E-03</td>
</tr>
</tbody>
</table>

123
3.7 Lymphocytes

The test medication showed an increase in total lymphocyte count. From day 0 to day 14 (period of test medication), the total lymphocyte count reduced for three subjects while it increased for the other seven subjects. Comparing the figures from day 0 to day 28 (two weeks after discontinuing the medication), one subject had a reduced total lymphocyte count, while nine subjects had an increased total lymphocyte count. The individual results are listed in table 13. The reference range for total lymphocyte count in adults is according to The Royal College of Pathologists is 1.5-4.0 x10^9/L. (McPherson and Thomas 1990) The reference intervals approximate 95% confidence limits of values observed in healthy adults. Three participants had levels below the reference intervals on day 0. By day 14, two of these had levels within the reference intervals and by day 28 all three participants had levels within the reference intervals.

T-test analysis shows that the overall change from day 0 to day 14 is not significant (p<0.487) whereas the overall change from day 0 to day 28 is significant (p<0.007) (see table 14 and figure 17).
Table 13: Total lymphocyte count (x10⁹/L) day 0-28.

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant 1</td>
<td>1.225</td>
<td>1.456</td>
<td>1.672</td>
<td>2.145</td>
<td>1.634</td>
</tr>
<tr>
<td>Participant 2</td>
<td>1.702</td>
<td>1.194</td>
<td>1.768</td>
<td>1.824</td>
<td>2.73</td>
</tr>
<tr>
<td>Participant 3</td>
<td>1.8025</td>
<td>2.31</td>
<td>1.2224</td>
<td>2.0808</td>
<td>3.2224</td>
</tr>
<tr>
<td>Participant 4</td>
<td>2.1</td>
<td>2.05</td>
<td>2.1164</td>
<td>1.4496</td>
<td>1.6104</td>
</tr>
<tr>
<td>Participant 5</td>
<td>1.5225</td>
<td>1.9</td>
<td>2.466</td>
<td>1.7024</td>
<td>2.3698</td>
</tr>
<tr>
<td>Participant 6</td>
<td>1.8962</td>
<td>2.3125</td>
<td>0.6591</td>
<td>0.9804</td>
<td>2.4992</td>
</tr>
<tr>
<td>Participant 7</td>
<td>1.162</td>
<td>1.197</td>
<td>2.494</td>
<td>2.1216</td>
<td>2.1645</td>
</tr>
<tr>
<td>Participant 8</td>
<td>1.4245</td>
<td>1.68</td>
<td>1.4637</td>
<td>0.9936</td>
<td>1.881</td>
</tr>
<tr>
<td>Participant 9</td>
<td>1.632</td>
<td>0.45</td>
<td>1.241</td>
<td>2.0304</td>
<td>1.8846</td>
</tr>
<tr>
<td>Participant 10</td>
<td>1.5748</td>
<td>2.09</td>
<td>0.9383</td>
<td>2.1735</td>
<td>1.87</td>
</tr>
</tbody>
</table>

Table 14: T-Test analysis of total Lymphocytes at day 0-14 and at day 0-28

Paired Samples Statistics

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Mean</th>
<th>N</th>
<th>St.d. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.6042</td>
<td>10</td>
<td>.2906</td>
<td>9.189E-02</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.7501</td>
<td>10</td>
<td>.4624</td>
<td>.1462</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Mean</th>
<th>N</th>
<th>St.d. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.6042</td>
<td>10</td>
<td>.2906</td>
<td>9.189E-02</td>
</tr>
<tr>
<td>Day 28</td>
<td>2.18866</td>
<td>10</td>
<td>.5188</td>
<td>.1641</td>
</tr>
</tbody>
</table>
Paired Samples Correlations

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>N</th>
<th>Correlation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0-14</td>
<td>10</td>
<td>.397</td>
<td>.256</td>
</tr>
<tr>
<td>Day 0-28</td>
<td>10</td>
<td>.239</td>
<td>.506</td>
</tr>
</tbody>
</table>

Paired Samples Test

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>Df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Day 0-14</td>
<td>-.146</td>
<td>.6363</td>
<td>.201</td>
<td>-</td>
<td>.601</td>
</tr>
<tr>
<td>Day 0-28</td>
<td>-.582</td>
<td>.5306</td>
<td>.167</td>
<td>-</td>
<td>.962</td>
</tr>
</tbody>
</table>

Time Points Samples (Days)
3.8 Natural Killer cell cytotoxicity

The test medication did not show an overall increase in total NK cell cytotoxicity. From day 0 to day 14 (period of test medication), the NK cell cytotoxicity reduced for five subject while it increased for the other five subjects. Comparing the figures from day 0 to day 28 (two weeks after discontinuing the medication), five subjects had reduced NK cell cytotoxicity, one subject maintained the same low level while the other four subject had an increase in NK cell cytotoxicity (see Table 15 below). A normal range for NK cell cytotoxicity has not been established. The following graphs illustrate Natural killer cell cytotoxicity as a gradient calculated as the line of best fit through the plotted points of %Cr release versus effector to target (E:T) cell ratio at each of the five assays. The full analysis for subject 1 is included in Attachment G, the full analysis for the other subjects have not been included. The essential cytotoxicity graphs for all subjects are included in figure 16 below. The steeper the gradient, the greater the cytotoxicity. The NK cell cytotoxicity is graphed in figure 18.

T-test analysis (see figure 21) confirms that there is no significant difference between the levels of NK cell activity from day 0 to day 14 nor from day 0 to day 28 (p<0.868 and p<0.806 respectively).

Participant 1
Participant 2

Participant 3
Figure 18: Cytotoxicity graphs for all subjects.

The graphs illustrate Natural killer cell cytotoxicity as a gradient calculated as the line of best fit through the plotted points of %Cr release versus effector to target (E:T) cell ratio at each of the five assays. The steeper the gradient, the greater the cytotoxicity. The NK cell cytotoxicity is graphed in figures 19 and 20 below.

Table 15: Levels of NK cell cytotoxicity day 0-28 expressed as gradient.

The higher the number, the greater the cytotoxicity.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.99</td>
<td>0.36</td>
<td>0.19</td>
<td>0.40</td>
<td>0.34</td>
</tr>
<tr>
<td>2</td>
<td>0.58</td>
<td>0.30</td>
<td>0.11</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>0.69</td>
<td>0.94</td>
<td>0.61</td>
<td>0.70</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>0.12</td>
<td>0.14</td>
<td>0.08</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>5</td>
<td>0.38</td>
<td>0.49</td>
<td>0.14</td>
<td>0.36</td>
<td>0.53</td>
</tr>
<tr>
<td>6</td>
<td>0.34</td>
<td>0.52</td>
<td>0.33</td>
<td>0.65</td>
<td>0.62</td>
</tr>
</tbody>
</table>
Figure 19: Changes in NK cell cytotoxicity day 0-28.
Figure 20: Changes in NK cell cytotoxicity day 0-28 grouped by individual participant.

Figure 21: T-Test analysis of mean changes in NK cell activity

Table 16: T-Test analysis of NK cell activity day 0-14 and at day 0-28

Paired Samples Statistic
<table>
<thead>
<tr>
<th>NK Cell Activity</th>
<th>Mean</th>
<th>N</th>
<th>St.d. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>.4060</td>
<td>10</td>
<td>.2874</td>
<td>9.089E-02</td>
</tr>
<tr>
<td>Day 14</td>
<td>.4240</td>
<td>10</td>
<td>.2891</td>
<td>9.144E-02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NK Cell Activity</th>
<th>Mean</th>
<th>N</th>
<th>St.d. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>.4060</td>
<td>10</td>
<td>.2874</td>
<td>9.089E-02</td>
</tr>
<tr>
<td>Day 28</td>
<td>.4330</td>
<td>.</td>
<td>.2629</td>
<td>8.315E-02</td>
</tr>
</tbody>
</table>

Paired Samples Correlations

<table>
<thead>
<tr>
<th>NK Cell Activity</th>
<th>N</th>
<th>Correlation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0-14</td>
<td>10</td>
<td>.329</td>
<td>.353</td>
</tr>
<tr>
<td>Day 0-28</td>
<td>10</td>
<td>.246</td>
<td>.493</td>
</tr>
</tbody>
</table>

Paired Samples Test

<table>
<thead>
<tr>
<th>NK Cell Activity</th>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>Df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.8E-02</td>
<td>.3399</td>
<td>.1056</td>
<td>-</td>
<td>.2568</td>
</tr>
<tr>
<td></td>
<td>-2.70E-02</td>
<td>.3384</td>
<td>.1070</td>
<td>-</td>
<td>.2691</td>
</tr>
<tr>
<td>Day 0-14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 0-28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.9 Lytic Unit

The individual results are listed in table 17 and presented in a graphical form in figures 22-24 below. The test medication showed a small, but not significant trend towards an increase in lytic units from day 0 to day 28. From day 0 to day 14 (period of test medication), the lytic count reduced for five subjects, while it increased for the other five subjects. Comparing the figures from day 0 to day 28 (two weeks after discontinuing the medication), two subjects had a reduced lytic unit count, while the other eight subjects had an increased lytic unit count, although the increase was only marginal for one subject. T-test analysis (see figure 25 and table 18) shows that neither the change between the levels of lytic units from day 0 to day 14 nor from day 0 to day 28 are significant (p<0.499 and p<0.070 respectively).

**Table 17: Levels of Lytic Units for all participants day 0 - 28**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant 1</td>
<td>1.46</td>
<td>0.89</td>
<td>0.71</td>
<td>1.30</td>
<td>0.75</td>
</tr>
<tr>
<td>Participant 2</td>
<td>1.22</td>
<td>0.72</td>
<td>0.42</td>
<td>0.62</td>
<td>0.87</td>
</tr>
<tr>
<td>Participant 3</td>
<td>1.76</td>
<td>2.73</td>
<td>1.09</td>
<td>2.03</td>
<td>2.35</td>
</tr>
<tr>
<td>Participant 4</td>
<td>0.63</td>
<td>0.54</td>
<td>0.46</td>
<td>0.30</td>
<td>0.84</td>
</tr>
<tr>
<td>Participant 5</td>
<td>0.95</td>
<td>1.43</td>
<td>0.62</td>
<td>1.59</td>
<td>2.09</td>
</tr>
<tr>
<td>Participant 6</td>
<td>1.14</td>
<td>1.52</td>
<td>0.37</td>
<td>0.87</td>
<td>2.29</td>
</tr>
<tr>
<td>Participant 7</td>
<td>0.69</td>
<td>0.75</td>
<td>1.39</td>
<td>2.42</td>
<td>2.44</td>
</tr>
<tr>
<td>Participant 8</td>
<td>0.83</td>
<td>0.56</td>
<td>0.26</td>
<td>0.48</td>
<td>1.61</td>
</tr>
<tr>
<td>Participant 9</td>
<td>0.82</td>
<td>0.18</td>
<td>0.32</td>
<td>1.37</td>
<td>1.10</td>
</tr>
<tr>
<td>Participant 10</td>
<td>0.54</td>
<td>0.65</td>
<td>0.53</td>
<td>0.59</td>
<td>0.56</td>
</tr>
</tbody>
</table>
Figure 22: Changes in Lytic Units from day 0-28

Figure 23: Changes in Lytic Units day 0-28 grouped by individual participant.
Figure 24: Linear trendlines for lytic unit expression in each participant.

Table 18: T-Test analysis of total Lytic Units day 0-14 and day 0-28

<table>
<thead>
<tr>
<th>Lytic Unit</th>
<th>Mean</th>
<th>N</th>
<th>St.d. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.0040</td>
<td>10</td>
<td>.3895</td>
<td>.1232</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.1570</td>
<td>10</td>
<td>.7068</td>
<td>.2235</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lytic Unit</th>
<th>Mean</th>
<th>N</th>
<th>St.d. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.0040</td>
<td>10</td>
<td>.3895</td>
<td>.1232</td>
</tr>
<tr>
<td>Day 28</td>
<td>1.4900</td>
<td>10</td>
<td>.7474</td>
<td>.2364</td>
</tr>
</tbody>
</table>

Paired Samples Correlations

<table>
<thead>
<tr>
<th>Lytic Unit</th>
<th>N</th>
<th>Correlation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0-14</td>
<td>10</td>
<td>.328</td>
<td>.355</td>
</tr>
<tr>
<td>Day 0-28</td>
<td>10</td>
<td>.260</td>
<td>.467</td>
</tr>
</tbody>
</table>

Paired Samples Test
Paired Differences

<table>
<thead>
<tr>
<th>Lytic Unit</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>Df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0-14</td>
<td>- .1530</td>
<td>.6861</td>
<td>.2170</td>
<td>-.6438</td>
<td>.3378</td>
<td>-.705</td>
<td>9</td>
</tr>
<tr>
<td>Day 0-28</td>
<td>-.4860</td>
<td>.7475</td>
<td>.2364</td>
<td>1.0207</td>
<td>4.872E-02</td>
<td>-</td>
<td>9</td>
</tr>
</tbody>
</table>

**Figure 25: T-Test analysis of changes in mean levels of Lytic Unit day 0-28**

3.10 Adverse Reactions

No side effects or adverse reactions were reported during the trial period.
Discussion

3.11 Overview of the Immunomodulatory activity of PS10

The study of this thesis investigated a group of herbs shown in the literature to have immunomodulatory activity. The herbs have been shown individually and collectively to have a varied influence on a variety of cytokines – increasing INF-gamma, IL-4 and IL-5 while possibly reducing IL-2 thereby having a balancing effect on the Th1 to Th2 response. The Ginseng and Dang gui Ten Formulation (TJ48) has also been shown to influence phagocytosis, lymphocytes, TNF-alpha and NK cell activity. The herbs were shown in this study to influence lymphocytes and NK cells.

Monocytes enter the blood from the bone marrow and circulate for about 72 hours. They then enter the tissues and become tissue macrophages, where they may persist for up to three months. Lymphokines from T lymphocytes activate the macrophages, which then migrate in response to chemotactic stimuli and engulf and kill micro-organisms by the process known as phagocytosis. Macrophages provide the body with powerful defences against tumours and viral, bacterial and parasitic infections.(Ganong 1999) During the test medication (day 1 to day 14), the total monocyte count reduced in five subjects, and increased for the other five subjects. Calculating the changes from day 0 to day 28 (two weeks after the discontinuation of the medication), the total monocyte count decreased in three subjects and increased in the other seven subjects. The mean changes in total monocytes (n=10) were not significant (p<0.859 and p<0.55 for day 0-14 and day 0-28, respectively).

Some lymphocytes are formed in the bone marrow but the majority are formed in the lymph nodes, thymus and spleen from bone marrow-derived precursor cells. At any given time only about 2% of the body lymphocytes are in the peripheral blood, the rest are in the lymphoid organs. The total lymphocyte count measures many different types of lymphocytes and does not differentiate the subclasses of lymphocytes. Low total lymphocyte count is associated with risk of viral infections.
including HIV and cancer. (Ganong 1999) The test medication also showed a certain trend towards producing an increase in total lymphocyte count. From day 0 to day 14, the total lymphocyte count reduced in three subjects while it increased in the other seven subjects. Comparing the figures from day 0 to day 28, one subject had a reduced total lymphocyte cell count, while nine subjects had an increased total lymphocyte count. The mean increase in total lymphocytes (n=10) was not significant at day 14 (p<0.487) but it was significant at day 28 (p<0.007). This is interesting because the stimulatory effect does not appear to have decreased during the washout period (from day 14 to day 28). The effect from the 14 days of medication seems to have continued during the following two weeks of not taking the medication.

In some individuals, the test medication increased the total NK cell cytotoxicity however the changes in mean NK cell cytotoxicity were not significant. From day 0 to day 14 the NK cell cytotoxicity reduced for five subjects while it increased for the other five subjects. Comparing the figures from day 0 to day 28, three subjects had reduced NK cell cytotoxicity; one subject stayed the same while six subjects had an increase in NK cell cytotoxicity. However, the changes in mean NK cytotoxicity were not significant (p<0.868 and p<0.806 at day 0-14 and 0-28 respectively). Like monocytes, NK cells also provide a first line defence against tumour and microbial infections. Although the test medications increased the NK cells activity in some participants, the effect was not significant. The study demonstrated that PS10 in stimulates lymphocytes but failed to show any statistical effects on total monocyte count and NK cell cytotoxicity.

3.12 NK Cells

NK cells are bone marrow-derived lymphocytes cells which act as part of the first line defence against some tumour cells and viral infections in the absence of specific recognition of viral or tumour-associated antigens. That is, NK cells have the ability to lyse tumour and virally affected cells without prior sensitisation, hence the term *Natural Killer* cells. Natural Killer cells play vital roles in both the innate and the
adaptive immune systems. NK cells are able to respond early to microbial assault and interact with other cells of the innate immune response; however, they also recognise and react to infections in highly specific ways that are similar to T lymphocytes. NK cells have been described as being positioned as a cellular bridge between the innate and the adaptive immune response. (Heusel and Ballas, 2003)

Contrary to earlier perception of NK cells as primitive and non-specific cells, it is now known that NK cells are specialised cells, which play an important role that is complementary to the cytotoxic lymphocytes. NK cells not only rapidly detect and kill highly dangerous cells; they have also evolved to cooperate with the adaptive immunity such as producing cytokines that regulate T cell function. NK cells can to kill MHC class-1 deficient target cells that cannot be detected by cytotoxic lymphocytes. (Moretta et al. 2002a) Activated NK cells release cytokines and chemokines that induce inflammatory response, modulate haematopoiesis, control monocyte and granulocyte cell growth and function and influence the type of subsequent responses. NK cells originate in the bone marrow. They share a common lymphoid progenitor with lymphocytes and B cells. Interleukins 2, 4, 7, 9, 15 and 21 are important for the development of NK cells with IL-15 being critical for the development of mature NK cells from NK progenitors in the bone marrow. (Heusel and Ballas, 2003) IL-15 also seems to be essential for NK cell homeostasis. IL-15 has been shown to promote survival of peripheral NK cells by maintaining anti-apoptotic factors. (Yokoyama et al. 2004) NK cells are mostly found in peripheral blood, spleen, liver and uterus but may ingrate to inflamed tissues in response to different chemoattractants. (Moretta et al. 2002a; Yokoyama et al. 2004) NK cells constitute only a small percentage (2.5%) of splenic leucocytes. NK cells are however stimulated by pro-inflammatory cytokines produced early in the course of an immune response and coupled with their multiple activation receptor ligands, NK cells can respond quickly with enough of a critical mass to effect significant immune response. (Yokoyama et al. 2004)

NK activity rises to adult values shortly after birth and is stable throughout life with activity in males slightly higher than females.
Systemic NK activity is easily suppressed by a number of factors including surgery, cytotoxic drugs, oestrogens, progesterone, stress and smoking. The life span of mature NK cells is unknown but they can be tracked in the circulation for at least 5 weeks (based on mice studies). Their half-life is estimated to be between seven to ten days. (Colucci et al. 2003) NK cells have two major triggering mechanisms: target recognition and cytokine stimulation. Target recognition leads to target killing while cytokine stimulation leads to cytokine production. NK cells recognise major histocompatibility complex (MHC) class 1 molecules through surface receptors generating signals that inhibit, rather than activate, NK cells. The inhibitory receptors are known as killer immunoglobulin-like receptors (KIRs). In other words, NK cells lyse target cells that have lost the MHC class 1 molecules. Such loss frequently occurs in tumours and in cells infected by certain viruses. This means that the NK cell is always “switched on” to kill potential target cells. NK cells can, however, also lyse MHC class 1 negative target cells. This mechanism has been described as the “missing self” hypothesis. The lack of expression of the relevant MHC by target cells (missing self) consequently activates NK cells and results in target-cell elimination. (Colucci et al. 2003)

The surface receptors responsible for activating NK cells have only recently been identified. The highly specific NK cell receptors are collectively referred to as natural cytotoxicity receptors (NCRs). NCRs have been shown to play a major role in the lysis of tumour cell lines and cells infected with certain viruses. (Moretta et al. 2002b)

Interferon alpha and gamma, IL-12, IL15 and IL-18 stimulate NK cells to rapidly produce other cytokines including IFNgamma, TNFalpha and granulocyte macrophage colony-stimulating factors (GM-CSF). (Yokoyama et al. 2004; Moretta et al. 2002a)

NK cells connect with target cells by the locking of lectin-like carbohydrates and other receptors on the NK cell with glycoprotein receptors on the target cells. This process can take place within one to four hours without prior activation priming or assistance by cytokines. The ensuing activation of NK cells leads to the release of granules into the space between the two cells. NK cells kill by activating apoptosis
(programmed cell death) in infected cells. A cascade of proteolytic enzymes known as caspases mediates apoptosis. Activation of the caspase cascade sends a death signal to the target cell by causing fragmentation of the nuclear DNA. There are two separate mechanisms for activating the caspases: perforin-dependent and FasL-dependent triggers.

Perforin, one of the granules, inserts itself into the membrane of the target where it polymerises to form a transmembrane pore, which allows the other granules to travel inside the target cell. The granules released by the NK cells also contain TNF alpha, lymphotoxin-beta and a family of serine proteases known as granzymes. Granzyme B acts as a NK cytotoxic factor by travelling through the perforin membrane pore into the cytoplasm of the target cell where it activates the caspase cascade. TNF-alpha induces apoptosis by activating tumour-necrosis factor-related apoptosis-induced ligan (TRAIL)-dependent receptors on the surface of the target cell membrane, which in turn activate the caspase cascade. Engagement of NK Fas-ligand receptor with the Fas receptor on the target cell activates a parallel killing mechanism that is also mediated via activation of the caspase cascade. (Roitt and Delves, 2001) (Colucci et al. 2003) Initially NK cells were thought to mainly act against tumour cells but during the last decades their scope and activity have significantly enlarged as listed in Table 19.

**Table 19: NK cell activity and function.**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyse target cells</td>
<td>First line defence against microorganisms especially certain viruses and cancer cells</td>
</tr>
<tr>
<td>Respond to immune complexes formed by IgG antibodies</td>
<td>Allows NK cells to kill IgG antibody-coated pathogens.</td>
</tr>
<tr>
<td>Release various cytokines including IFN-gamma, TNF and GM-CSF and chemokines, which may promote inflammatory</td>
<td>Regulation of T cell function is important in regulating immunity, tissue inflammation and autoimmunity</td>
</tr>
</tbody>
</table>
NK cells provide early defence against a variety of micro-organisms during the initial response period while the adaptive or specific immune system is being activated. Although NK cells respond to a variety of micro-organisms, including bacteria and protozoa, they are particularly important in viral infections. There is strong evidence that IL-15 is involved in mediating viral-induced NK cell proliferation. Following viral infection, a significant, specific NK cell proliferation is seen after a few days, peaking at day four to six. (Yokoyama et al. 2004)

Previously NK cells were referred to primitive or non-specific cells. However, NK cells have evolved to fit into various specific or adaptive immune responses. NK cells have for example developed the ability to kill antibody-coated target cells. Antibody-dependent cell-mediated cytotoxicity (ADCC) reactions are effective against opsonized microbes, parasites or tumour cells. NK cells are recruited to sites of microbial infection by a variety of chemokines where they interact with resident dendritic cells (a diverse collection of cells that initiate an adaptive immune response). These reciprocal interactions lead to dendritic maturation and NK cell activation. This interaction plays an important role in the early phase of infection. The dendritic cells induct the adaptive immune response and the NK cells initiate the early control of microbial replication through direct cytolytic destruction of infected cells.

The initial innate immune response is triggered by IFN-alpha beta production from plasmacytoid dendritic cells and parenchymal cells via Toll-like receptors (a receptor system for detection of microbes). In combination with other inflammatory mediators, additional dendritic cells, monocytes and macrophages are recruited and activated.
Subsequent NK cell recruitment provides a source of IFN-gamma, tumour necrosis factor-alpha and granulocyte macrophage colony-stimulating factors that lead to activation of T-cell mediated immune responses. This major source of IFN-gamma provided by NK cells in the early response to infection is thought to directly stimulate naïve helper T cells down a TH1 developmental pathway resulting in expansion of CD8+ cytolytic T cells. (Heusel and Ballas, 2003)

NK cell deficiency may be associated with significant immunodeficiency especially against herpes viruses, human papilloma virus and mucosal candidiasis. NK deficient animals have increased incidence of malignant disease. NK cell deficiency may also contribute to autoimmunity as NK deficiency may contribute to uncontrolled expansions of activated lymphocytes because of the absence of NK cell-mediated immune modulation. When assessing NK deficiency, it is important to appreciate that a functional deficiency may exist in the presence of normal NK cell numbers. In severe illness it may be essential to not only assess peripheral blood NK cell numbers, as measured by flow cytometry (eg. CD56 and CD16), but also to perform a NK functional assessment as used in the present study. (Heusel and Ballas, 2003)

Our understanding of the mechanisms and functions of NK cells have increased dramatically in the last decade due to the remarkable increase in NK cell research. It has become clear that the NK cells, far from being simple immune cells circulating the body looking for dangerous foreign cells to destroy, are actually integral to a much deeper immune surveillance and act as a bridge between the innate and the adaptive immune systems. The positive outcome of the present study is encouraging both in terms of the trend towards an increase in absolute numbers of monocytes and lymphocytes but especially due to the increase in NK cell activity. More research is needed to clarify the clinical significance of the test medication in human patients suffering immune suppression due to natural illness or as a consequence of chemotherapy.
3.13 Natural variation in immunity

When assessing immune function, it is important to realise that there are significant natural variations in immunity including variations based on sex, circadian rhythms, dietary and lifestyle factors and age. Cortisol as well as a subset of lymphocytes have been shown to present at higher levels in the mornings in ten healthy volunteers. (Mezzadra, 1971) A significant decrease in the concentration of interleukin-6 after breakfast was also observed in another study of twenty-two healthy subjects. (Dugue and Leppanen, 1998) It is therefore essential that blood be collected at the same time each day of the study. Five years of observational studies at La Trobe University, Melbourne, of healthy medical students have produced a range of average NK cell cytotoxicity (calculated as %Cr release in relation to effector to target (E:T) cell ratio (See Chapter 2: Materials and methods for a full description of the assay). The higher the number, the greater the cytotoxicity. The values are listed in table 20.

Table 20: Range and mean cytotoxicity in healthy medical students.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>0.18-1.96</td>
<td>0.46</td>
</tr>
<tr>
<td>Males</td>
<td>0.27-1.66</td>
<td>0.86</td>
</tr>
</tbody>
</table>

The immune defence develops differently in different people and seems to have a great capacity to compensate should any part of the immune defence not function optimally. A healthy response by monocytes and lymphocytes may thus counteract a less than optimal NK cell response. The concept of the lytic unit was developed to take into account the various compensations individuals have developed over the years. To present a more accurate picture of an individual’s immunity, both the effects of NK cell cytotoxicity and number of mononuclear cells can be combined and expressed as 'lytic units’. The lytic unit is calculated as the sum of the absolute number of monocytes and absolute number of lymphocytes multiplied by the cytotoxicity gradient. In some participants the test medication produced an
increase in lytic units. From day 0 to day 14, the lytic count reduced for five subjects, while it increased for the other five subjects. Comparing the figures from day 0 to day 28 however, two subjects had a reduced lytic unit count, while eight subjects had an increased lytic unit count. However, the changes were not significant (p<0.499 and p<0.070 on from day 0-14 and 0-28 respectively).

Overall, PS10 increased the absolute monocyte count in seven out of ten subjects, increased the total lymphocyte count in nine out of ten subjects and increased the NK cell cytotoxicity in six out of ten subjects. PS10 was shown in the present study to increase the lytic unit in six people out of the total of ten subjects. However, only the change in mean total lymphocyte was significant. The results do nevertheless suggest that the oral administration of the herbal medicine formulation PS10 may have a broad immunostimulating activity.

3.14 Psychological factors and immunity

Psychoneuroimmunology attempts to integrate immunology, endocrinology, and neuroscience with psychiatry and psychology. There is empirical evidence that cognitive behavioural stress management intervention positively affects mood, neuroendocrine sympathetic nervous system hormones and immune system status (lymphocyte subsets, anti-viral immune function) at least in HIV-infected persons. (Antoni, 2003) Research has also found suggestive links between emotional distress and immune and neuroendocrine measures in cancer patients. Furthermore, several studies have reported that participation in psychological support groups is associated with better health outcomes for cancer patients. However, controversy exists surrounding these findings, and the mechanisms behind such effects are unclear. Although strong evidence suggests that coping and psychosocial intervention can improve psychological outcomes for breast cancer patients, potential effects on physiological outcomes remain speculative. (Luecken and Compas, 2002)

Linkage between the central nervous system and the immune system is obvious and is accomplished through the hypothalamus-pituitary-
adrenal and sympathetic-adrenal medullary axes. Generally, mild stress has been shown in animal studies to enhance the immune response. The effects of stress also depend on the animal's behavioural profile, genetic background and pre-exposure to stressful conditions. Prenatal stress modifies the immune response of the offspring. Stress also modifies autoimmune reactions in animals and humans. (Zimecki and Artym, 2004)

A recent review critically and systematically reviewed all identifiable publications about psychological therapies used by cancer patients. The authors identified 627 relevant papers that reported on 329 intervention trials by searching MEDLINE™, Healthplan™, Psychlit™, and Allied and Complementary Medicine™ databases and in the bibliographies of the papers identified. Despite increased use of randomised, controlled trial designs over time, the methodological quality of the intervention trials was generally suboptimal, with only one trial achieving a quality rating of "good" for its methodology. Only 10 trials featured survival or immune outcomes and based on these studies, no intervention strategy could be recommended for improving patients' immune outcomes. In spite of this, the authors conclude that specific therapies should be considered for further investigation. (Newell et al. 2002)

Depression is also known to influence immunity. Depressed people are likely to have an increase in total white blood cell counts, however the relative number of lymphocytes is likely to be reduced. Depression is also associated with a suppression of lymphocyte proliferation and with a reduction in NK cell number and activity. (Irwin, 1999) In terms of the current study, it is interesting to note that three subjects appear to have low NK cell activity. Subject 2 (female, age 53) had a NK cell cytotoxicity on day 0 of 0.58 finishing with a score of 0.20 on day 28, subject 4 (male, age 55) had a NK cell cytotoxicity on day 0 of 0.12 finishing with a score of 0.22 on day 28 and subject 10 (female, age 54) had a NK cell cytotoxicity of 0.02 on both day 0 and day 28. Subject 2 experienced significant stress during the study period when her father was diagnosed with cancer. Subject 4 suffers from chronic,
moderately severe depression. Subject 10 is married to subject 4 and has suffered chronic stress and unhappiness for many years. The test medication was not able to prevent a reduction in NK cell cytotoxicity in subject 2, the activity remained low and did not change in subject 10 while subject 4 experienced a rise in activity from day 0 to day 28, although the level remained outside the normal range for males.

It is difficult to make any firm conclusions from these results except to say that psychological factors seem to negatively impact NK cell cytotoxicity and that the test medication, at least in the short term, was able to somewhat improve the NK cell activity in one of the subjects with a very low NK cell activity, but not in the other subject. A significant deficiency of NK cell activity is associated with an increased risk of viral infections. In adults, a NK deficiency may not be as evident as in children as the immune defence in an adult may have learnt to compensate and successfully fight infections by other mechanisms. It is however possible that low NK cell activity in adults may increase the risk of cancer. Subject 4 and 10 who in this study exhibited low NK cell activity were informed of the test results and counselled about the possible implications of such low activity. Subject 10, a female, had a NK cell cytotoxicity measurement of 0.02. The average range for healthy university students at La Trobe University is 0.18 to 1.96 with a mean of 0.46. Subject 10’s NK cell cytotoxicity was thus measured to be twenty times less than the mean. Subject 4, a male, had a NK cell cytotoxicity of 0.22. The range for healthy, male university students is 0.27 to 1.66 with a mean of 0.86. Subject 4’s NK cell cytotoxicity is thus about four times lower than the average for a young person. Even accounting for the age related decline in NK cell activity, these levels are disturbing. During the twelve months following the present study, contact with subject 4 and 10 has been maintained and they both suffer from repeated respiratory infections and general malaise, fortunately they do not suffer from any major illness. While the short-term administration did not improve the NK cell activity in subject 10, it did seem to improve the overall immunity for subject 4.
3.15 Herbal Research

Herbal medicine research is complex due to the chemical complexity of the herbs and their formulations. Herbal medicine is also sometimes applied with a different therapeutic framework from modern medicine. Herbal medicine is currently based on a mixture of empirical knowledge and modern research. There are basically three sources of herbal information. Traditional information, which is based on empirical knowledge accumulated by people over generations, modern pharmacology based on the research on isolated constituents and pharmacological and clinical studies on whole herb extracts whether they are single herbs or formulations. (Bone 1992) Human experimental studies and clinical trials will obviously supply the most clinically reliable information. In vitro research is fraught with issues concerning its relevance to clinical practice. In vitro trials frequently lead to misinterpretation of the data because of the biological effect of the herb when taken orally. Active constituents must be stable in the pharmaceutical preparation of the plant, it must not be changed by the digestive tract, it must be absorbed in significant quantities, it must survive its first passage through the liver, it must cross the blood-brain barrier (if relevant), it must be transported to the site of activity in significant quantities (based on the normal therapeutic dose and the concentration used in the in vitro study), it must not be inactivated by body fluids and it must gain access to the cell or receptor (if relevant). It follows that interpretation of in vitro research on plant extracts or isolated plant compounds is fraught with difficulties and uncertainties. Enthusiastic extrapolations of in vitro data often result in misguided interpretations of how a herb might be used therapeutically or how it might act in the body. (Bone 1997) Furthermore, extraction methods will affect the therapeutic action of the herb. A herbal tea made from chamomile will clearly be different from a industrial ethanol extraction of chamomile.

Fundamental Differences between herbs and drugs

The concept of the Vital Force is recognised by all herbal traditions. This life force or life energy is sometimes described as heat in the
Western tradition, *prana* in the Ayurvedic system, and *qi* in Traditional Chinese Medicine. It follows from the belief in the Vital Force that living things have an innate healing energy. According to Bone,(Bone 1992) the Vital Force can be illustrated by the basic law of thermodynamics, which states that any spontaneous process increases entropy and the observation that Life seems to work against entropy. Natural processes in life seek to create chemical order and organisation. The chemical organisation of a plant is related to its therapeutic properties. As plants are living things, they are seen to be supportive of the vital force or a self-sustaining energy that works against entropy and disintegration. There are many differences between herbs and pharmaceutical drugs but the fundamental differences are that herbs are chemically much more complex, they have a subtle and gentle activity and they are applied with a difference therapeutic strategy. A single herb may have dozens of different active constituents. Therefore a multi-herb formula may contain hundreds of active compounds. This chemical complexity in turn leads to the concepts of synergy, multi-faceted actions and the possibility of reduced side effects. Synergy is defined as the effects of the whole being greater than the sum of the individual parts. The synergy may be between the constituents in individual plants as well as in the combination of herbs used. Multifaceted actions refer to the fact that most herbs are used to treat a spectrum of symptoms, not just a single disorder. Reduced side effects occur because of synergy and/or multifaceted actions. This means that a therapeutic result can generally be achieved with minimal risk of side effects. Synergy may be created by increased absorption or biotransformation, or activation of active constituents, which is often not achieved until they are transformed by digestive enzymes or the liver.(Bone 1992) The combined effects of the test medication, PS10, are so numerous and more are being discovered as the research continues. Research has established that Juzen-taiho-to (Ginseng and Dang gui combination) stimulates the immune function of Peyer’s patch cells, yet none of its single component herbs shows such activity. Three-dimensional HPLC analysis has revealed that alterations occur in their composition during the decoction of the
component herbs. (Kiyohara et al. 2004) As discussed in Chapter 1, the formulation was developed from two traditional Chinese herbal medicine formulations, known as Si-Jun-Zi-Tang and Si-Wu-Tang, with the addition of cinnamon and astragalus. In terms of the intestinal immune stimulation, the formulation Si-Jun-Zi-Tang (Four Major Herb combination containing atractylodes, ginseng, hoelen and liquorice) has been identified as being the most active, although astragalus is also an essential ingredient. (Kiyohara et al. 2004) The Four Major Herb combination is considered the qi (life force) and energy tonic, while the Si-Wu-Tang (Dang gui) combination is considered to be a Blood tonic. Si-Wu-Tang has furthermore been shown to be mainly responsible for the antimetastatic activity of Juzen-taiho-to (Ginseng and Dang gui combination).

One can therefore conclude that it is the combined decoction of all ten herbs that is the active principle. The process of boiling the herbs together (decoction) may change the extraction rates of the active ingredients and/or produce new artificial substances, which may then exhibit new pharmacological activities. Or the observed effects may simply be due to a complex synergistic effect of the active constituents of the individual herbs.

The combination of chemical complexity and multifaceted actions may lead to reduced side effects. Various components may modify the actions of one another, i.e. one component of a herb may correct the side effects created by another component. (Bone 1992) Combining herbs not only may ensure reduced side effects of the herbs themselves but research has shown that TJ-48 can be used to reduce the side effects of common chemotherapeutic agents.

The strength of the therapeutic action can vary according to the condition of the individual. An unwell patient has a greater susceptibility to a given therapeutic agent than a well person. For example, the heating effects of a diaphoretic tea containing peppermint, ginger, yarrow, when a person is feeling well is minimal, however if a sick feverish person is given a diaphoretic tea, the effect will often be quite profound. Statistical analysis attempts to eliminate
individual responses, however patient susceptibility is actually one of the cornerstones of herbal medicine practice. Herbal treatments tend not to follow static or fixed combinations. In most cases herbalists would actually adjust the formulations to suit the individual. This increases the complexity of conducting research, but it would provide a more accurate picture of the efficacy of herbal medicines and the practice of phytotherapy if individual prescriptions were included in the analysis.

Control of symptoms is often the therapeutic aim of modern drug therapy. Herbalists often apply medicines with a different therapeutic strategy. For example, using tonics to support specific organs affected by the illness, or immune support herbs to assist the body to fight an infection. PS10 is not merely immunostimulating, is has a wide application and is used in Japan in the treatment of patients recovering from surgery or suffering from chronic disease, to improve their general debility. (Kiyohara et al. 2004) Juzen-taiho-to may also reduce or prevent drug-induced anaemia. Tj-48 was found in a recent study to ameliorate the reduction in haemoglobin levels in 67 chronic hepatitis C patients receiving interferon and ribavirin therapy. (Sho et al. 2004)

The aim of herbal therapy in immunocompromised patients is not just to improve general immunity, but also to prevent drug side effects, improve digestion, energy, mood and general wellbeing.

3.16 Quality assurance of herbal medicine

3.16.1 Raw Materials

Quality, efficacy and safety are parameters that are required for all medicines. In addition there are some issues that are particular to herbal medicine as discussed below. These are particularly important to herbal preparations as they gain in popularity. The past decade has witnessed significant worldwide growth in the use of medicinal herbs as teas and dietary supplements. Due to the increasing knowledge of both the traditional and scientifically documented benefits of herbs,
consumers are now more likely to consider herbal remedies as a viable alternative to conventional medicines.

The tremendous growth of the herbal sector has revealed the need for improved quality control in cultivation and collection of herbal raw material. Consistent and reproducible quality of herbal raw materials used for medicinal purposes is paramount for clinical efficacy, and for the reproducibility of beneficial effects as observed in clinical studies. Furthermore, numerous safety issues are dependant on consistent composition of botanical ingredients.

Ideally, raw material for the production of herbal medicines will come from a traceable and reproducible source. In reality, the trading habits co-evolving with the growth of the market frequently obscure the origin of the plant material and facilitate adulterations, which, especially in case of adverse events, have already damaged the reputation of otherwise relatively safe plants. (Schmidt et al. 2005)

In February 2004 the WHO released “WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants,” which addresses quality issues in the production of herbal raw material. (World Health Organization 2003) It covers recommendations that range from the selection of appropriate seed material and cultivation sites to the avoidance of contaminations in post-harvesting handling, training and working conditions of personnel, and general rules for handling and construction of tools and facilities.

The selection of the correct species for cultivation is still of major concern. The replacement of species with seeds from species that are closely or less closely related plants is a common feature in modern plant trading, even though the required species is frequently defined in pharmacopoeias, monographs or other scientific literature. For example, large tracts of the world market of liquorice root (Glycyrrhiza glabra) do not consist of batches of the species as defined in European pharmacopoeia (Ph. Eur.) and United States Pharmacopeia (USP), but from G. uralensis, such as permitted by the Japanese pharmacopeia. The mixture of species of various origins, partly from uncontrolled and destructive wild harvesting, is frequently sold as G. glabra. Current
trading practices often do not allow for a distinction between the raw materials of various different origins, which very likely will have varying chemical profiles, and therefore, produce variations in the reproducibility of effects in pharmacological and clinical studies. Finally, commercial orders for raw material of a given plant are often conducted using the local plant name or the English common names. One of the ingredients in the test medication is often listed by its English common name Cnidium radix (Chuan Xiong in Chinese). There are however two herbs by the name Cnidium which are used medicinally, Cnidium officinale which is used in this formulation and Cnidium monnieri, which is used for infections of the skin and mucous membranes and for infertility. (Bensky and Gamble 1986) To add to the confusion, Cnidium officinale is now officially known as Ligusticum wallichii.

Sometimes close substitutes are acceptable. In the case of Atractylodes macrocephala, A. lancea is an acceptable substitute and much of the research has actually been performed using this species. Atractylodes refers mainly to Atractylodes macrocephala (macro = big; cephal = head; so, big-headed atractylodes) known in Chinese as baizhu. Less frequently used is Atractylodes lancea (lancea = lance-like, so lance-leaved atractylodes) or its less-desirable (somewhat weaker) substitutes, such as A. chinensis, A. japonicum, and A. ovata, known in Chinese as cangzhu. (Dharmananda 2005)

The WHO guideline addresses this problem by stating the need for a proper botanical identification not only of the plant material, but also of the seeds used for cultivation. In GAP conform cultivation; the Latin binominal name and the definition of the subspecies/cultivar/chemotype (where applicable) must be included in the farmer’s documentation. The same documentation applies to plants issued from wild-harvesting, where the botanical identification should be even stricter than for plants grown under the controlled conditions associated with commercial cultivation, in order to take the local phytochemical variability into account.
The ecological and climatic conditions found on the cultivation site must meet the needs of the cultivated plant. Factors such as local rainfall, irrigation, water and soil quality and local climate have an important impact on plant quality. Too often, a decision to cultivate medicinal plants on a given site is made, not based on the specific requirements of the plant, but on the availability of the agricultural surface. Poor choice of a cultivation site may affect not only the local harvest, but, in extreme cases, can have a global impact on biodiversity. The WHO guideline provides recommendations for the choice of an appropriate cultivation site. It also points to the exclusion of sites with possible industrial contaminations with heavy metals, pesticides or herbicides, and radioactive contaminations. In practice this means that soil samples must be collected and analysed. Wherever possible, suggests WHO, organic growing techniques should be employed, thus avoiding the use of herbicides or pesticides. In addition, the impact of the growing of herbs on local biodiversity must be respected. Harvesting time and methods are in close relation to phytochemical parameters. Conditions associated with processing raw materials, especially drying, frequently have a major impact on drug quality. Inadequate drying and storage leads to microbiological contaminations and changes in the phytochemical composition. A major part of the WHO guidelines cover the various aspects of harvesting, storage and shipping. This section of the guidelines is essentially identical for cultivation and collection (wild harvesting or wildcrafting) of medicinal plants. In regard to harvesting times and post-harvesting processing, the guidelines refer to the specifications laid down in pharmacopoeias, with respect of experience published in the scientific literature. Cross-contamination during storage must be avoided, and organically grown material must be stored separately from conventionally grown herbs (i.e., herbs not grown organically and not certified as organic by an appropriate third party organization, regardless of whether the conventionally grown herbs have been sprayed with pesticides or not, or grown in artificially fertilized soil or not). The documentation of the harvest must contain essential indications that allow the identification and assessment of the key
steps. With the measures outlined in the GAP guidelines, the best possible harvest-to-harvest reproducibility and a full traceability of the herbal raw material should be guaranteed. (Schmidt et al. 2005)

Many medicinal plants are as yet unavailable from controlled cultivation. TRAFFIC (a division of the World Wildlife Fund) estimates that almost 75 percent of all botanical species in trade continue to be sourced from the wild. (Laird and Pierce 2002) One of the major goals of the WHO GACP guideline is to outline efficient, non-destructive, environmentally sound and sustainable procedures not only for cultivation, but also for controlled collection. In the case of wildcrafting, the guideline aims for the avoidance of negative impacts on plant population density and the maintenance of biodiversity.

Almost hidden within the GACP guidelines is the issue of intellectual property rights for plants endemic to a certain region. The brevity of the statement that “All intellectual property rights with regard to source materials must be respected” may seem to understate the importance of this topic for which the practical implications are still under discussion. The guidelines call for a scientific botanical survey to outline the distribution and assess the abundance of the species to be cultivated. A practical solution for the question of intellectual property rights would be to organize GACP projects in the regions where the plant naturally occurs. With this access, several problems are solved simultaneously: The plant grows in its natural habitat under conditions it is well adapted to. There is no problem with intellectual property rights, as the region the plant originates from immediately profits from the activities. GACP projects have a stabilizing impact on a regional economy, which is an important factor for the long-term sustainability of the cultivation. (Schmidt et al. 2005)

3.16.2 Manufacturing

Good Manufacturing Practice

Herbal extracts are not currently required to be manufactured under GMP. Only the finished consumer products are required by the Therapeutic Goods Administration of Australia to be manufactured under pharmaceutical GMP. The GMP rules however stipulate that the
herbal material must be identified by analytical methods such as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) in order to establish that it is the correct plant that is used.

**Active principles**

In an attempt to produce herbal medicines that may give a consistent and reliable clinical result, endeavours are made to identify the active constituents and standardise the medicines to those compounds.

The HPTLC plates (figures 9-12) compare water versus ethanol-water extracts for the ten individual herbs as well as the TJ-48 hot water extract versus the ethanol-water extract produced by mixing the individual herbs according to the ratio specified in PS10 (ratio 1). It is quite clear that there is a very good match between the water and the ethanol-water extracts of the single herbs (numbered 1-10) in both systems. The methanol-water extracts, however, appear to be stronger (the coloured bands are brighter or more pronounced).

There is also a close correlation between TJ-48 and the combined preparation PS10 although it appears that both the water and methanol-water extract of PS10 is stronger than TJ-48 with the methanol-water extract being the strongest. This is especially noticeable in the top end of the spectrum.

It is very difficult to compare the HPLC chromatogram of TJ-48 (figure 13) and the combined ethanol-water PS-10 extract (figure 14) without further detailed analysis. It is noticeable that the PS-10 ethanol extract has higher peaks than the PS-10 hot water extract and that the ethanol-extract has more pronounced peaks at the non-polar (right hand side of the chromatogram). This is not surprising as the ethanol content is generally more superior in extracting non-polar constituents compared to hot water.

The ethanol-water extract contains a broader range of constituents. It is not known what differences these constituents make to the overall effect of the formulation. The majority of the studies reviewed were based on the hot water extract. However many of the studies on the
individual extracts were performed on pure ethanol or ethanol extracts and both water-soluble and water insoluble fractions.

3.17 Safety of herbal medicine

3.17.1 Overview
The safety concerns in regards to herbal medicine are similar to those of pharmaceuticals and includes concerns about acute or chronic toxicity, side effects, safety in pregnancy, lactation and for children as well as concerns about drug interactions. There are also concerns about quality issues during the manufacturing processes including microbial contamination and the presences of heavy metals and agricultural chemicals.

3.17.2 Microbial analysis, agricultural chemicals and heavy metals
Under pharmaceutical GMP, all herbal products must be tested for microbial contamination. The PS10 was tested for microbial contamination and found to comply with the European Pharmacopeial specifications. Ethanol at a concentration of 25% and above is considered a reliable preservative. The PS10 formulation has an ethanol content of about 50%, which is sufficient to keep the liquid extract free of microbial contamination. The herbs were not tested for contamination of agricultural chemicals or heavy metals.

3.17.3 Herbal Hepatotoxicity
There is a perception in the medical community that herbal medicines are a likely cause of hepatotoxicity and herbs are often strongly suspected in cases of unexplained hepatitis. It is rare however that a direct link can be established, in other cases hepatotoxicity may have resulted from contamination of a herbal product or deliberate or unintentional substitution with another, toxic herb. (McLeod et al. 1996)

Several herbal medicines have been reported to be hepatotoxic. Hepatotoxins may have intrinsic, idiosyncratic, or immune-mediated mechanisms of liver injury. Toxic hepatitis may also occur sporadically due to differences in individual susceptibility related to factors such as
age, sex, underlying disease, concomitant medications, and genetic influences. (Haller et al. 2002) Establishing a diagnosis of herbal hepatotoxicity can be difficult. Some herbs are thought to be intrinsic hepatotoxins and show dose-related liver toxicity, either through direct hepatocellular damage, such as with amanita (found in Amanita mushrooms), or through the generation of a reactive metabolite, as is the case with pennyroyal oil (*Mentha pulegium*). Use of the American herb chaparral has also been associated with hepatotoxicity, especially in patients with existing liver conditions. The predominant type of liver injury was cholestatic hepatitis. (Haller CA et al. 2002) The reporting of adverse liver effects of herbal medicines have increased in recent years. (Whiting et al. 2002) Unfortunately the quality of the investigations have often been very inadequate. (Vitetta et al. 2003; Vitetta et al. 2003)

The identity of herbal medicines must be established, especially where the origin of particular plants is unknown or difficult to control. On closer examination of undesired drug effects attributed to the intake of herbal remedies, discrepancies regarding the listing of the single compounds are frequently found. It is often found retrospectively that adverse effects attributed to a certain plant were not likely to have been caused by the herbal preparation, but as the result of an intentional or accidental substitution with other plants, or by a contamination of the listed components with; a more toxic plant, a toxin (e.g. mould toxins of heavy metals), or even with a chemically defined drug. (Corrigan D., 2001; Fugh-Berman A. 2000; Li et al. 2000) (MacGregor et al. 1989) Tragic examples of such substitutions include reports of fatal kidney failure caused by the substitution of *Stephania tetranda* with the kidney-toxic plant *Aristolochia fangchi*. (Nortier et al. 2000; Violon, 1997; Martinez et al. 2002) It should be noted that frequently there is no effort made to identify the putative causal herbal agent or their adulterants in the corresponding preparation. If there is no such identification, the unsubstantiated correlation of toxic effects with the wrong plant will, in the future, lead not only to erroneous scientific citations, but also to unsubstantiated warnings for patients and practitioners and official claims for the
labelling of side effects on packages and leaflets demanded by the health authorities. (Corrigan 2001)

One example is the warning concerning the alleged kidney toxicity of juniper oil (Juniperus communis) found frequently in herbal literature, but based neither on case reports nor on relevant pharmacological studies or animal experiments (Schilcher and Heil 1994) When case reports of undesired effects from the intake of herbal medicines are more closely examined, not only the author publishing the report but also those citing the case report in journals and reviews should be required to verify certain key issues.

When discussing the potential hepatotoxic effects allegedly caused by herbal medicines it is paramount to analyse the composition of the corresponding herbal medicines. With the components of the herbal medicines not verified, the causality of the hepatotoxic reaction cannot indisputably be attributed to the intake of herbal medicines. All too frequently information regarding the plant parts used, the extraction medium, the amount of drug taken and the method of preparation is lacking. External factors may have contributed to the reported liver reactions. Examples potentially include past medical history, the use of recreational drugs, dietary factors and viral infections. Hepatitis for which no cause can be identified is not uncommon. In 5-10% of patients with viral hepatitis no increase in HBsAG is observed. The aetiology remained unclear in 17 (31%) of 55 newly diagnosed cases of hepatitis diagnosed in a population of 71,000 people in a managed care organisation in one year. There were no concurrent prescription medications or blood products that appeared related to the liver injury. The authors comment that this is a "background-rate" of hepatitis. (Walker 1992) Full pathophysiological or biochemical investigation into the specific reaction, is essential before any conclusion can be made as to the exact mechanism of hepatotoxicity. The documentation and examination of adverse reactions to herbal medicines is absolutely essential. It is without question that intentional or accidental adulterations of herbal medicines are absolutely unacceptable, and that companies producing herbal medicines should be expected to take every possible measure to avoid such events. Conversely, there are
many over-the-counter pharmaceutical drugs, such as analgesics, which are directly hepatotoxic. It is beyond the scope of this thesis to perform a complete analysis of the potential hepatotoxicity of the individual herbs or the combination PS10. However, the herbs are considered non-toxic in normal clinical dosages. The acute toxicity of ‘Ginseng and Dang Gui Ten Combination’ in mice and rats is very low. LD$_{50}$ is $>15$g/kg in ICR mice and SR rats by oral administration. (Aburada et al. 1983) No deaths or abnormal symptoms were observed during 13 weeks plus 4 weeks recovery period after oral administration at doses of 300, 1000 and 3000 mg/kg. (Minematsu et al. 1989) To test for acute lever toxicity, the subject in this study were given a liver function test before and after consuming the herbal medication.

A liver function test was performed on day 0 (the day before the commencement of the test medication) and again on day 28 (two weeks after the discontinuation of the medication). All initial liver function tests apart from that of subject 6 were within the reference range before as well as after the test medication. Subject 6 has hypercholesterolemia and receives statin medication. The levels for ALT and T protein were outside the reference range prior to the test medication. On day 28, subject 6’s ALT and AST were outside the reference range but level for T protein had fallen to within normal levels. The actual values for each test in each of the subjects are listed in table 10. A review of the published literature of the herbs in the test medication revealed that all the herbs have a very high safety margin. In the present study, the test medication, PS10, did not produce any noticeable changes in the liver function tests.

3.17.4 Side-effects, adverse reactions and drug interactions

The concerns regarding the safety of herbal medicines are mostly the same as for modern pharmaceuticals and include concerns about drug interaction, side effects, adverse reactions and toxicity and safety during pregnancy and lactation. The clinical significance of many drug interactions is still largely unknown as controlled trials are lacking in most cases. Reported interactions are often based on pharmacological
activity and case reports and although they may have a sound theoretical basis, their clinical significance has to be tested. (Braun and Cohen 2004) Potential drug interactions were not examined in the present study. The subject all completed the medication and none reported any side any adverse reactions.
Conclusion

The three main issues in herbal medicine are efficacy, safety and product consistency and the present study sought to explore various aspects of these issues. Ginseng and Dang gui Ten combination has previously been demonstrated to protect against radiation damage and reduce anaemia, anorexia and fatigue. It has been shown to enhance haemopoietic recovery, stimulate a variety of cytokines, and increase phagocytic activity, T lymphocytes, NK cell activity and antibody production. The herbal formulation has furthermore been shown to increase mitogenic and complement activity while reducing the inflammatory response. The effect on the cytokines is varied. The formulation seems to have a modulatory effect on the cytokines – increasing INF-gamma, IL-4, IL-5 while possibly reducing IL-2 thereby having a balancing effect on the Th1 to Th2 response. In addition the herbs have been shown to reduce the risk of cancer development, inhibit tumour growth and reduce metastases. It has been shown to potentiate the efficacy while reducing the side effects of common chemotherapeutic agents.

The few clinical studies conducted on Ginseng and Dang gui Ten Combination/TJ-48 suggests that it may be beneficial for cancer patients receiving conventional anticancer therapy. The herbal formulation has been found to increase appetite and reduce the severity of leukopenia induced by conventional anticancer treatment and generally improve the response and/or reduce the side effects of chemotherapy.

The present study found that PS10, an aqueous-ethanolic extract of ten traditional Chinese herbs, increased the absolute monocyte count in seven out of ten subjects, increased the total lymphocyte count in nine out of ten subjects and increased the NK cell cytotoxicity in six out of ten subjects. PS10 was shown in the present study to increase the lytic unit in six people out of the total of ten subjects. The changes in mean monocyte, NK cell cytotoxicity and total number lytic units were all insignificant, however the increase in mean total lymphocytes (n=10) was significant (p<0.007).
The study suffers from low number of participants (n=10), not having a placebo group or being a blinded study. The results do however suggest that the oral administration of the herbal medicine formulation PS10 may have a broad immunostimulating activity. Most herbs given for chronic conditions are taken over a length of time, and their effects in this case will be cumulative. In this study, the expected decrease in immune-stimulation was not observed during the wash-out period suggesting that the effect will continue for some time after cessation of the medication. Therefore the improvement will often be gradual, but sustained. In an acute illness the patient is more susceptible to the medicines and will generally require more frequent and higher doses. Much drug research is undertaken with a single high dose preparation in animals, however a single dose of a herbal extract will have little effect. The subtle and gentle activity of herbs can build up an effect over time, but the subtle activity of the herb may be missed by conventional research methods. Herbal medicine is normally given for many months and can be taken for years if indicated. The effects often manifest after a long treatment period. The present study suffered not only from low subject numbers but also a less than optimal treatment period. Subsequent studies based on long-term administration may well find that PS10 can stimulate NK cell cytotoxicity against cancer cells.

Further clinical research could also focus on establishing the most effective dosages, frequency of dosing and even the optimal time of day to consume the medication. More research is furthermore needed to identify the optimal extraction method. Further research could also aim at establishing some of the broader effects of the Ginseng and Dang gui Ten Combination in reference to fundamental differences between herbs and drugs. For example, a study could, in addition the effects on immunity, examine the herbs effect on the patients perception of wellbeing, energy level and mood in chronic disease or during chemotherapy.

The finding that two subjects who were suffering psychological depression had a very low level of NK cell activity suggest that further studies in regards to the psychological effects on NK cell activity are
warranted. While the short-term administration did not improve the NK cell activity in subject 10, it did seem to improve the overall immunity for subject 4. It would also be of interest to test if long-term administration of PS10 could improve the NK cell activity of these two individuals.

As Juzen-taiho-to (TJ-48) has been shown to induced cytotoxic T lymphocytes in vitro and to decrease the monocyte to T cell ratio (M:T ratio) ratio in patients with gynaecological cancers, further cancer research could examine the effects of the test medication on the M:T ratio, which has been found to be related to cancer recurrence. This could be important if it could be confirmed that the M:T ratio is a reliable early detection method for the recurrence of cancer compared to conventional tumour markers. Early detection of recurrence could result in earlier treatment and help prolong survival in cancer patients.

One of the main concerns for cancer patients seeking alternative and complementary therapies as an adjunct to their conventional therapy is the issue of adverse herb-drug interaction. Potential drug interactions were not tested, however, the literature seem to suggest that positive (beneficial) interactions can be expected when combining the herbal formulation with antibiotic or interferon therapy and during chemotherapy. (Adachi and Watanabe, 1989; Kurokawa T et al. 1994) It has also been found to augment antibiotic and interferon therapy. The interaction with other drugs has not been extensively explored by researcher to date. None of the subjects in this study reported any side effects or adverse reactions from consuming the test medication for two weeks. The liver function tests conducted before and after the consumption of the test medication suggest that the herbs do not have any acute toxic effects in healthy subjects. Further long-term studies with greater subject numbers are needed to confirm the lack of toxic effects. It would also require much larger trials and much longer running trials to properly examine the occurrence of side effects or adverse reactions. Long-term pharmacovigilance programs would also be essential in the establishment of a reliable safety profile of Ginseng and Dang gui Ten/ Juzen-taiho-to.
Much research has focused on attempting to identify the active principle(s) in Ginseng and Dang gui Ten combination. However the activities of the formulations are so varied and complex that it has not been possible to identify a single active principle. Research has for example established that that it stimulates the immune function of Peyer’s patch cells, yet none of its single component herbs shows such activity. (Kiyohara et al. 2004) Six herbs in the formulation were found to be the most active in terms of intestinal immune stimulation, however the other herbs have been shown to be most active in terms of inhibition of metastases. While this investigation did not attempt to identify the compounds responsible for the observed activity of the formulation, the review of the published literature seems to suggest that none of the individual ingredients are solely responsible for the activity and that it is the combination of a unknown number of herbs or indeed a combination of all of the ten herbs that provide the active principle of PS-10. The process of boiling the herbs together (decoction) may change the extraction rates of the active ingredients and/or produce new artificial substances, which may then exhibit new pharmacological activities. Or, the observed effects may simply be due to a complex synergistic effect of the active constituents of the individual herbs. PS10 is not a decoction but a 50% ethanol-water extract. Although the present study cannot conclusively show that PS10 was associated with an increased number of lymphocytes in human serum exposed to cancer cell lines, the result suggested a positive trend that requires further clarification and hence evaluation with further studies. It has been beyond the scope of this study to identify the differences between a hot water extract (decoction) and an ethanol-water extract. To ensure batch-to-batch reproducibility and therefore product consistency, it would be essential to standardise the formulation. In order to standardise the formulation certain compounds have to be identified as suitable marker compounds. These marker compounds may or may not be active compounds but it would make most sense if they had some relevance to the therapeutic activity of the formulation. Ideally all the herbal extracts in the formulation should be standardised. This would entail making the extracts
separately and then combining the herbal extracts afterwards to make
the final preparation. However, this would be a departure from the
original method of making a decoction of the combined dried herbs and
the differences should be examined before a choice can be made as to
the preferred method of manufacturing. Ensuring high quality and
standardised raw materials would be the first step to ensure product
consistency. The dried herbs used in the manufacturing of the PS10
formulation used in the present study were purchased from a trader in
traditional Chinese herbs in Sydney. The trader mainly supplies
practitioners of traditionally Chinese medicine. As these herbs have
passed through many companies before reaching the trader made it
impossible to establish traceability. It is not known from which
geographical area the herbs came, in what conditions they were
grown, harvested, dried or stored. Neither was any information about
the chemotype or precise species supplied. Unfortunately very little
information is generally available both in the market place for dried
herbs or their extracts, but neither does the researchers in the
reviewed articles place much emphasis on these issues.
Although Ginseng & Dang gui Ten Combination is a traditional Chinese
formulation, it has become integrated into the Traditional Japanese
medicine known as Kampo medicine. In Japan the formulation is
prepared by decoction followed by spray drying to produce a powdered
extract. The majority of the studies have been based on a particular
extract, TJ-48, produced by Tsumura Corporation, Japan. While the
formulations are essentially identical, that is, containing the same
herbs, there are variations based on quality and origin of raw herbs,
inter-herb ratio, substitution of certain species and manufacturing
methods. Unless bioequivalence have been demonstrated, the effects
of one particular extract used in a study are not necessarily
transferable to another extract even though it may contain the same
herb or herbs.
Further studies are required to establish the differences between the
hot water extract TJ-48 and the ethanol-water extracts PS10. Further
studies could examine the choice of extraction method, solvents, drug
to solvent ratio and other manufacturing parameters need to be examined and optimised.

The issues discussed above clearly need attention in future studies if herbal medicine is to play a significant role in modern health care.
Appendicis

Appendix A: Information sheet
Graduate School of Integrative Medicine Swinburne University of Technology

*Investigation of the immunomodulatory effects of traditional Chinese herbal formulation ‘ginseng and dang gui ten combination’ in healthy human subjects*

*Principal Investigators*
MSc student: Michael Thomsen
Senior Investigator: Dr Luis Vitetta
Supervisors: Professor Avni Sali

*The purpose of the study*
This study aims to investigate the effects of a traditional Chinese herbal formulation on immune function. The specific aim is to identify the mechanism whereby the formulation may stimulate the non-specific immune system by activation of certain compounds (such as interleukins), which can be identified in a blood sample.

*Procedure*

*Test medications*
The formulation consists of Panax ginseng, Atractylodes macrocephala, Poria cocos, Glycyrrhiza uralensis, Rehmannia glutinosa, Paeonia lactiflora, Angelica sinensis, Ligusticum wallichii, Cinnamomum cassia and Astragalus membranaceus.

All ten herbs used in the test formulation are listed on the Australian Register of Therapeutic Goods. Listed herbs are considered to be low risk medicines suitable for self-medication. It is not expected that you will experience any side effects from participating in the study.

You will need to take the herbs daily for two weeks.
Participants
Ten healthy volunteers will be recruited to participate in the study. If you are suffering any serious medical condition, you should not participate in this study.

Interviews
You are required to attend an interview prior to commencement of the study. The interview will be 20 mins in duration. During the interview the aim of the study, the test medication and what is required of you, will be explained. You will also be asked to fill out a health questionnaire and sign a form consenting to participating in the study. You are encouraged to inform your doctor that you are participating in the clinical trial.

Blood tests
In order to investigate the effects, we will need to take a blood sample from you before, during and after you have taken the herbal medication.

Contacts
Weekly phone contact will occur to ensure that you are consuming the required dosage of the test medication and to answer any questions that may arise. You are of course free, at any time, to withdraw from the study. If you have any questions or complaints regarding any of the study, please contact any of the following people:

Potential benefits of this study
The potential benefits are that this study may prove that administering essential oils may help improve immune function in the altered immune state that occurs in association with smoking. This may indicate uses for this type of treatment in other conditions. If proven effective the use of this treatment could potentially be tested for applications in other immune-altered states as an adjuvant treatment. If you agree to participate, please note that at any time you will be free to withdraw your consent and discontinue your participation in this study.
Any questions regarding the project entitled “Aromatherapy essential oils and immune function in smokers” can be directed to the Senior Investigator, Dr Luis Vitetta of the Graduate School of Integrative medicine, phone 9214 5975.

Privacy protection
All information collected will remain totally confidential.
The data collected will be protected and only the investigators named above will have access to it.
The results of the research will be published as part of a dissertation and PhD thesis, and if appropriate will be submitted to a peer-reviewed journal.
All participants are to be identified by a coded reference and only the principal investigators will have access to the keys to the codes. This information will be destroyed at the completion of the study, and no individual participant will be able to be identified in any of the published data.

Complaint procedure
If you have a complaint about any way in which you have been treated during this study, or any query that the Senior Investigator has been unable to answer satisfactorily, then complaints may be directed to:

Michael Thomsen
66 May St
Fitzroy North
3068
Tel: 03 9443 0061
Fax: 03 9443 0039
Mobile: 0438 700 287

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Appendix B: Informed Consent Form
Graduate School of Integrative Medicine Swinburne University of Technology

Investigation of the immunomodulatory effects of traditional Chinese herbal formulation ‘ginseng and dang gui ten combination’ in healthy human subjects

Informed consent
I understand that I have been asked to participate in a trial that will investigate the effects of a traditional Chinese herbal formulation on immune function.

I understand that this study has been designed to test whether a traditional Chinese herbal formulation has an effect on immune function. If there is a benefit this may improve immune function. This may benefit individuals and the wider community in general. If the theorised results are not found to be true, the results will guide future research.

I understand that I will be required to:
- take supplements daily in addition to any existing medication regime
- receive a weekly phone call from the researcher
- five blood tests, one prior to the commencement of the trial, three during the trial and one at the completion of the trial at a La Trobe collection centre

I understand that the data collected from my physical and psychological responses will be kept confidential to the extent permitted by law and that I may request an interpretation of my results.

I understand that the data I provide may be useful in future research studies and I give my permission for the blood test results to be available to future studies, which have ethics approval as long as my name has been removed.

I understand that if I have any questions about the study or if I experience any discomfort or have any concerns that I would like to express, I may contact Mr Michael Thomsen on 9443 0061 or Dr Luis
Vitetta at the Graduate School of Integrative Medicine on telephone 03 - 9214 5296 or fax 03 - 9214 8009.

I understand that my participation in this study is entirely voluntary and that I may discontinue my participation at any time without penalty to myself.

I acknowledge that the contents of this form have been explained to me, understood by me, and that I have been given the opportunity to ask questions. I have been given a copy of this form.

Participant Signature:__________________ Print
Name:___________________ Date: _________

Witness Signature:____________________ Print
Name:___________________ Date: _________
### Appendix C: Health questionnaire

*Graduate School of Integrative Medicine Swinburne University*

<table>
<thead>
<tr>
<th>Name:</th>
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</thead>
<tbody>
<tr>
<td>Occupation</td>
<td>Date of birth</td>
</tr>
<tr>
<td>Height</td>
<td>Weight</td>
</tr>
<tr>
<td>Address:</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Home phone</td>
<td>Work phone</td>
</tr>
<tr>
<td>Family doctor</td>
<td>Phone</td>
</tr>
<tr>
<td>Emergency contact</td>
<td>Phone</td>
</tr>
<tr>
<td>Relationship to emergency contact</td>
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</table>

Are you currently experiencing or have you had any of the following?  
Please tick yes or no

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<tr>
<th></th>
<th>Yes</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td>Heart problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest Pain/high blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent shortness of breath</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent fainting High blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression or anxiety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Currently pregnant</td>
<td></td>
<td></td>
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</table>
Asthma or other respiratory problems
Diabetes/blood sugar problems
Recurrent/frequent headaches
Ulcer/stomach problems
Kidney or urinary tract problems
Musculoskeletal problems
Major hospitalisation/Surgery (within the last year)
Symptoms of any acute illness?
Are you addicted to any pharmaceutical or recreational drugs?

List any medications (including over the counter drugs) you are currently taking

Appendix D: Call for volunteers
Graduate School of Integrative Medicine Swinburne University of Technology
Clinical trial: Investigation of the immunomodulatory effects of traditional Chinese herbal formulation ‘ginseng and dang gui ten combination’ in healthy human subjects

Commencement date: September 2003
Volunteers are invited to contact the Graduate School of Integrative Medicine to participate in a trial that will investigate effects of a traditional Chinese herbal formulation on immune function.
To participate in this study, applicants need to be healthy and not suffering any serious medical conditions.
Participants will take herbs daily for four weeks, attend a 20 minute interview prior to commencement of the study. This interview will explain the aim of the study, the test medication and what is required. You will also be asked to fill out a health questionnaire. During the trial period we will contact you weekly by phone.

We will need to take a blood sample from you before, during and after you have taken the herbal medication.

If you agree to participate, please note that at any time you will be free to withdraw your consent and discontinue your participation in this study.

You are encouraged to inform your doctor that you are participating in the clinical trial.

Privacy protection

All information collected will remain totally confidential. The data collected will be protected and only the investigators named below will have access to it.

The results of the research will be published as part of a dissertation and Master thesis, and if appropriate will be submitted to a peer-reviewed journal.

All participants are to be identified by a coded reference and only the principal investigators will have access to the keys to the codes. This information will be destroyed at the completion of the study, and no individual participant will be able to be identified in any of the published data.

**Principal Investigators**

MSc student: Michael Thomsen

Supervisor: Dr Luis Vitetta

Co-supervisor: Professor Avni Sali

For further information contact

Michael Thomsen

Tel: 03 9443 0061

Email: michael.thomsen@optushome.com.au
Appendix E: Ethics Approval

Swinburne University of Technology

Human Research Ethics Committee Certificate of Approval

Project Title: Investigation of the immunomodulatory effects of traditional Chinese herbal formulation 'Ginseng and Dang Gui Ten Combination' in healthy human subjects

HREC Register No.: 0242

Chief Investigator: Vitetta, Dr Luis

Other Investigators: Mr Michael Thomsen
                     Professor Avni Sali
                     Dr Wojciech Kielczynski

For period from: 27-Aug-03 to: 31-Mar-04

Approved for (max): 5 male participants
                    and 5 female participants

Approval is granted subject to the following conditions:
Researchers are required to immediately report anything which might warrant review of ethical approval of the protocol, including: (a) serious or unexpected adverse effects on participants; (b) proposed changes in the protocol; and (c) unforeseen events that might affect continued ethical acceptability of the project. If the research project is discontinued before the expected date of completion researchers must inform the HREC

A progress report must be submitted annually.
A final report must be submitted at the conclusion of the project.

Special Conditions as indicated below.

S

Professor K. Pratt
Chair, Human Research Ethics Committee

Wednesday, 27 August 2003
Appendix F: Poster
Second Prize, Swinburne 2003 Research Week Poster Competition

Immunomodulatory effects of traditional Chinese herbal formulation, Ginseng and Dang Gui Ten Combination

Graduate School of Integrative Medicine
Masters of Science Research Project - Michael Thomsen

Approximately 25% of modern medicines are descended from plants which were initially used in traditional cultures. The World Health Organisation estimates that up to 80% of people in non-Western countries use traditional or complementary medicine as part of primary health care.

The worldwide importance of herbal medicine as a significant contributor in the health of the population is recognised by the inclusion of herbal medicines in the Essential Drugs and Medicines Policy of the World Health Organisation.

The severe debility and immune dysfunction which is associated with serious disease may respond well to treatment with the tonic formulae from Traditional Chinese medicine.

One of these, *Ginseng and Dang Gui Ten Combination*, has gained prominence as the formula most suitable to assist convalescence after chemotherapy and radiotherapy.

*Ginseng & Dang Gui Combination* has been shown to:

- Modulate and potentiate the immune system
- Potentiate the therapeutic activity of chemotherapy and radiotherapy
- Reduce adverse reactions including anorexia, nausea, vomiting and immunosuppression of many cancer drugs.

The objective of this Graduate School of Integrative Medicine MSc Candidature is to investigate the specific immunomodulatory effects of ‘Ginseng and Dang Gui Ten Combination’ in healthy volunteers.

Ultimately, the herbal formulation will be tested for its benefits to cancer patients.
Appendix G: Results Participant 1

Results for Participant 1. Detailed results for other subjects not included.

Graph

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Missing Value Handling

Definition of Missing User-defined missing values are treated as missing.

Cases Used Statistics are based on cases with no missing values for any variable used.

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/NOORIGIN
/DEPENDENT cyto
/METHOD=ENTER ratio
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/CASEWISE PLOT(ZRESID)
OUTLIERS(3)
SAVE MAHAL.

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- a All requested variables entered.
- b Dependent Variable: Cell Cytotoxicity

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**Predictors:** (Constant), Cell Ratio  
**Dependent Variable:** Cell Cytotoxicity

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**Coefficients(a)**

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**Residuals Statistics(a)**

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a Dependent Variable: Cell Cytotoxicity

Charts
Graph

Notes

Output Created 12-NOV-2003 15:36:19

Comments

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Syntax

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### Regression

**Notes**

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**Comments**

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| Missing Value Handling |  
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| Definition of Missing | User-defined missing values are treated as missing. |
| Cases Used | Statistics are based on cases with no missing values for any variable used. |

**Syntax**

```
REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT cyto
/METHOD=ENTER ratio
/SCATTERPLOT=(*ZRESID ,*ZPRED
```
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OUTLIERS(3)
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Variables Entered/Removed(b)

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<tr>
<td>1</td>
<td>Cell Ratio(a)</td>
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<td>Enter</td>
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a All requested variables entered.
b Dependent Variable: Cell Cytotoxicity

Model Summary(b)

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a Predictors: (Constant), Cell Ratio

b Dependent Variable: Cell Cytotoxicity

ANOVA(b)
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a Predictors: (Constant), Cell Ratio

b Dependent Variable: Cell Cytotoxicity

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</tr>
<tr>
<td>(Constant)</td>
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a Dependent Variable: Cell Cytotoxicity

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Std. Residual | -1.162 | .867 | .000 | .866 | 5


Deleted Residual | -3.0734 | 8.2603 | 1.4440 | 4.45814 | 5

Stud. Deleted Residual | -1.668 | 5.863 | 1.002 | 2.918 | 5

Mahal. Distance | .104 | 2.504 | .800 | 1.003 | 5

Cook’s Distance | .012 | 6.726 | 1.506 | 2.923 | 5

Centered Leverage Value | .026 | .626 | .200 | .251 | 5

a Dependent Variable: Cell Cytotoxicity

Charts

Normal P-P Plot of Regression Stan
Dependent Variable: Cell Cytotoxicity

Scatterplot
Dependent Variable: Cell Cytotoxicity
Graph

Notes

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Syntax

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![Graph of Cell Ratio vs. Cell Toxicity](image)
**Regression**

**Notes**

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**Comments**

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**Missing Value Handling**

| Definition of Missing | User-defined missing values are treated as missing. |
| Cases Used | Statistics are based on cases with no missing values for any variable used. |

**Syntax**

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/DEPENDENT cyto
/METHOD=ENTER ratio
/SCATTERPLOT=(*ZRESID,*ZPRED )
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**Resources**

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### Variables Entered/Removed

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- a All requested variables entered.
- b Dependent Variable: Cell Cytotoxicity

### Model Summary

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- a Predictors: (Constant), Cell Ratio
- b Dependent Variable: Cell Cytotoxicity

### ANOVA

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- a Predictors: (Constant), Cell Ratio
Dependent Variable: Cell Cytotoxicity

### Coefficients (a)

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### Residuals Statistics (a)

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*a Dependent Variable: Cell Cytotoxicity*

*Charts*

- **Normal P-P Plot of Regression Stanc**
  - Dependent Variable: Cell Cytotoxicity

- **Scatterplot**
  - Dependent Variable: Cell Cytotoxicity
**Graph**

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![Graph of Grp 1 Patient #1 (19.11.03) with axes labeled Cell/Cytotoxicity vs Cell Ratio and a regression line with an R^2 value of 0.0600.]
Regression

Notes

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Variables Entered/Removed(b)

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a All requested variables entered.
b Dependent Variable: Cell Cytotoxicity

Model Summary(b)

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a Predictors: (Constant), Cell Ratio

b Dependent Variable: Cell Cytotoxicity

ANOVA(b)

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a Predictors: (Constant), Cell Ratio

b Dependent Variable: Cell Cytotoxicity

### Coefficients(a)

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a Dependent Variable: Cell Cytotoxicity

### Residuals Statistics(a)

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Mahal. Distance | .104 | 2.504 | .800 | 1.003 | 5
Cook’s Distance | .015 | 7.080 | 1.560 | 3.090 | 5
Centered Leverage Value | .026 | .626 | .200 | .251 | 5

a Dependent Variable: Cell Cytotoxicity

Charts

Normal P-P Plot of Regression Stanc
Dependent Variable: Cell Cytotoxicity

Scatterplot
Dependent Variable: Cell Cytotoxicity
Graph

Grp 1 Patient #1 (3.12.03)

Regression

Notes

Output Created 10-DEC-2003 12:35:00

Comments

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Missing Value Handling

Definition of Missing | User-defined missing values are treated as missing.
### Cases Used

Statistics are based on cases with no missing values for any variable used.

### Syntax

```
REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT cyto
/METHOD=ENTER ratio
/SCATTERPLOT=(*ZRESID ,*ZPRED )
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OUTLIERS(3)
/SAVE MAHAL .
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### Resources

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### Variables Entered/Removed(b)

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a All requested variables entered.

b Dependent Variable: Cell Cytotoxicity
### Model Summary(b)

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* a Predictors: (Constant), Cell Ratio

* b Dependent Variable: Cell Cytotoxicity

### ANOVA(b)

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* a Predictors: (Constant), Cell Ratio

* b Dependent Variable: Cell Cytotoxicity

### Coefficients(a)

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a Dependent Variable: Cell Cytotoxicity
Charts

Normal P-P Plot of Regression Stan:

Dependent Variable: Cell Cytotoxicity

Scatterplot:

Dependent Variable: Cell Cytotoxicity
References


213


Lesperance, M.L., Olivotto, I.A., Forde, N., Zhao, Y., Speers, C., Foster, H.,
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