Chapter 6: Modulation of Stress Protein Synthesis by *Echinacea* spp.

6.1 Introduction

The heat shock or stress proteins, which are among the most highly conserved proteins in nature, are found in all organisms from bacteria to humans and are expressed constitutively as well as induced in response to a mild, generally non-lethal stress. These proteins were originally observed in cells exposed to a mild heat shock, hence the name heat shock proteins (hsps). Later experiments reported that these proteins were also induced on exposure of cells to environmental stressors other than heat. These stress factors include inflammation, microbial infection (viral and bacterial), oxidative stress and cytotoxins. The more general term 'stress protein' has thus been applied to this set of proteins (Watson, 1990).

An important physiological function for the hsps is their role in the assembly and transport of newly synthesized proteins within cells as well as in the removal of denatured proteins. The hsps are therefore important in both preventing damage and in cellular repair processes after injury. There is well documented evidence that increased production of hsps protects cells against subsequent lethal stress induced by a number of conditions including oxidative stress, cytotoxins, heat stress and cellular damage after ischaemia or sepsis-induced injury (Jolly & Morimoto, 2000). Additionally, there is increasing evidence that the hsps play key roles as prominent antigens in the humoral and cellular immune responses mediated by antibodies and T cells respectively (Zugel & Kaufmann, 1999; Basu & Srivastava, 2000). The involvement of altered hsp expression in a number of disease states has emphasized the important role of these highly conserved proteins in the modulation of the immune response (Srivastava, 2002).

Many compounds have been postulated to modulate the immune response including the constituents found in Echinacea preparations. Echinacea, also known as purple coneflower and *Rudbeckia,* is a flowering plant member of the *Compositae* family (Figure 6.1) that is mainly used for the treatment of upper respiratory tract infections. It is, however, also used prophylactically as an immunomodulator and antioxidant and for the treatment of urinary tract infections, eczema and psoriasis as well as to

aid wound healing (Echinacea Monograph, 2001). Three species of Echinacea, *Echinacea purpurea*, *E. angustifolia* and *E. pallida*, are used in herbal preparations. Commonly, a combination of dried root, flowers or leaves from two or more species may be used in a variety of preparations including capsules or tablets, dried root or herb, liquid extract, tea, tincture or topical applications. Although Echinacea and its effects on the immune system have been studied since the late 1930's, its mode of action is still unclear. It has been concluded that there is a need for improved scientific evidence and quality control of Echinacea preparations with respect to acceptance by the medical community as to its efficacy as an immune stimulant (Melchart *et al.,* 2000; Barrett, 2003).

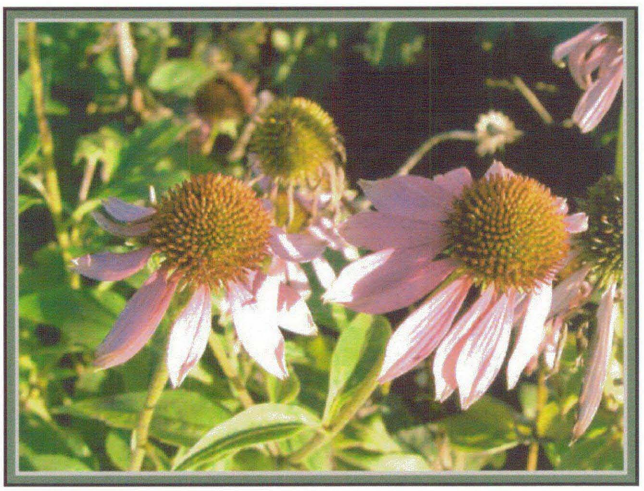


Figure 6.1: *Echinacea purpurea* flowers
(SUNY, 2005)

E. angustifolia and *E. pallida* roots and *E. purpurea* and *E. pallida* flower heads are known to contain a mixture of physiologically active constituents (Figure 6.2) including caffeic acid glycosides (echinacosides), alkyl amides (isobutyl amides), cichoric acid, polyenes, flavanoids and essential oils, a number of which have demonstrated immunomodulatory and antioxidant effects *in vitro* (reviewed in Matthias *et al.,* 2005).

The present study was a pilot scale clinical trial involving eleven subjects (five male, six female), orally dosed with the tablet form of an ethanolic extract of two species of *Echinacea – purpurea* and *angustifolia*. The aim of the study was to investigate the potential immunomodulatory effects of Echinacea in healthy subjects by measuring leukocyte hsp70 expression as a biomarker of the immune response. In addition, the

kinetics of erythrocyte haemolysis induced by the hydrophilic free radical generator AAPH [2,2-azobis(2-amidinopropane) dihydrochloride] and plasma total antioxidant status were measured as indicators of antioxidant effects of Echinacea.

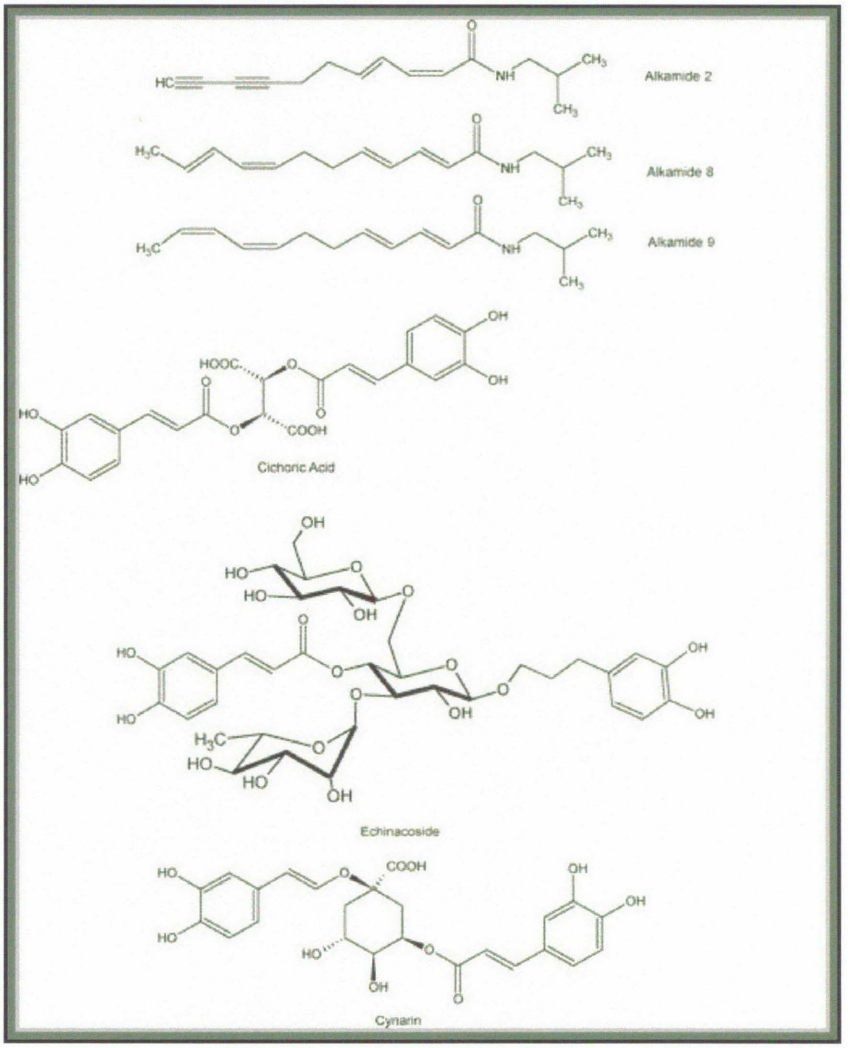


Figure 6.2: Structures of some alkylamides and caffeic acid derivatives present in *Echinacea* spp. (Adapted from Mølgaard *et al.*, 2003)

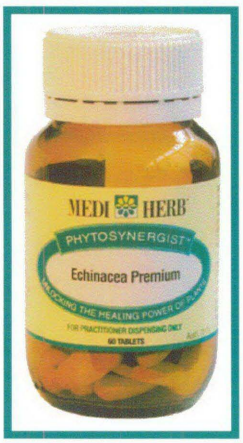
6.2 Study Design

6.2.1 Echinacea Premium tablets

Echinacea Premium tablets containing 675 mg of *E. purpurea* root extract and 600 mg of *E. angustifolia* root extract, prepared by ethanol extraction, were obtained from MediHerb, Warwick, Australia (Figure 6.3).

6.2.2 Study participants

Eleven individuals (five male and six female) aged between 26 and 61 years with body mass indexes (BMI) ranging from 18.8 to 30.0, participated in the study. All participants were in good health and not taking any other medications. The University of New England Human Research Ethics Committee (HE03/075) approved all procedures and all participants gave written consent to the study. On day one, a fasting, baseline, heparinised blood sample was collected. Participants then consumed two MediHerb Echinacea Premium tablets per day for fourteen days, a total of twenty-eight tablets per subject. A further fasting, heparinised blood sample was collected on the morning of day fifteen.



Extract equivalent (dry)	Amount
Echinacea purpurea root Containing alkylamides 2.65 mg	675 mg
Echinacea angustifolia root Containing alkylamides 2.50 mg	600 mg
Extracted by ethanol percolation	

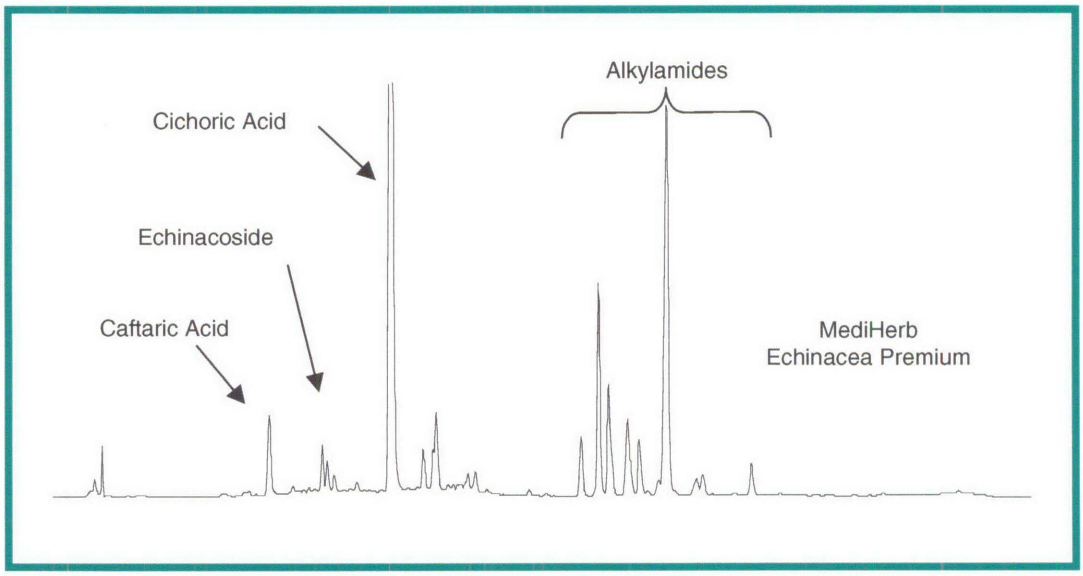


Figure 6.3: Echinacea Premium tablets and component ingredients. HPLC trace of MediHerb Echinacea Premium showing the major phytochemical groups (Matthias *et al.*, 2005)

6.3 Results

6.3.1 Erythrocyte Haemolysis

Figure 6.4 illustrates the kinetics of erythrocyte haemolysis as determined by progressive haemoglobin release after challenge with the free radical generator 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH). As a result of the differences observed in percent haemolysis at a number of the examined time points, the 50% haemolysis time increased from 135 ± 6 to 146 ± 5 min after Echinacea supplementation ($p = 0.006$).

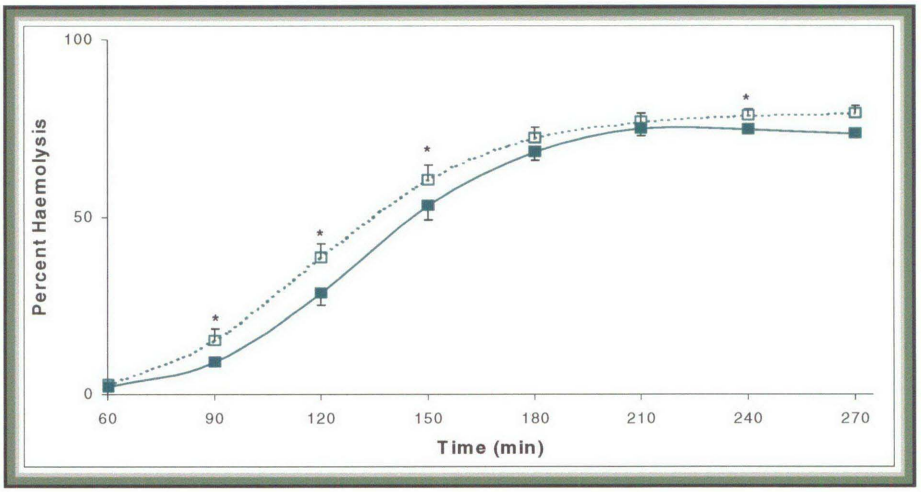


Figure 6.4: The kinetics of erythrocyte haemolysis as induced by the free radical generator AAPH. Each point represents the mean ± SEM (n = 11). Pre-Echinacea (□), after Echinacea (■). * $p < 0.05$ for pre compared to post for the given time points.

6.3.2 Total Antioxidant Status

Mean plasma Total Antioxidant Status (TAS) was not significantly altered by Echinacea supplementation with values of 0.832 ± 0.039 mM found under baseline conditions and 0.804 ± 0.029 mM measured after supplementation (Figure 6.5).

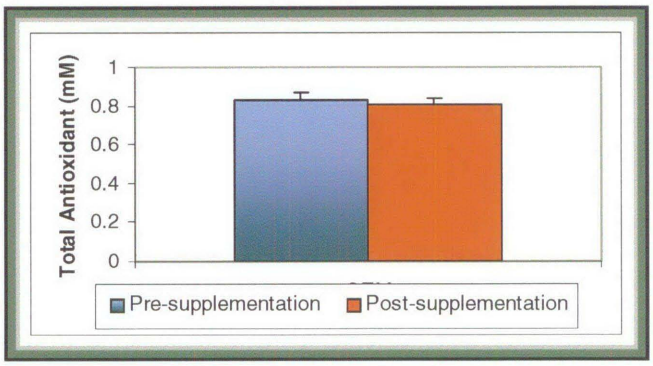


Figure 6.5: The plasma Total Antioxidant Status (TAS) as determined by the ABTS method. Values are means ± SEM (n = 11).

6.3.3 Expression of hsp70 as measured by Western immunoblot

Expression of hsp70 in leukocytes was measured by western immunoblot. In all samples, hsp70 expression increased after a mild heat shock (Figure 6.9). The expression of hsp70 (relative to β-actin, the internal control) measured at 37°C at baseline was 0.39 ± 0.10 and this did not differ significantly after Echinacea supplementation (0.29 ± 0.08). The expression of hsp70 (relative to β-actin) after heat shock at 42.5°C was also not significantly different with 0.66 ± 0.12 at baseline and 0.68 ± 0.13 after Echinacea intake. On the other hand, the ratio of control (37°C) versus heat shock (42.5°C) hsp70 expression, examined as a fold-increase was different. Mean hsp70 fold-increase at baseline was 2.21 ± 0.35 and this was significantly different after Echinacea intake at 3.25 ± 0.67 ($p = 0.029$).

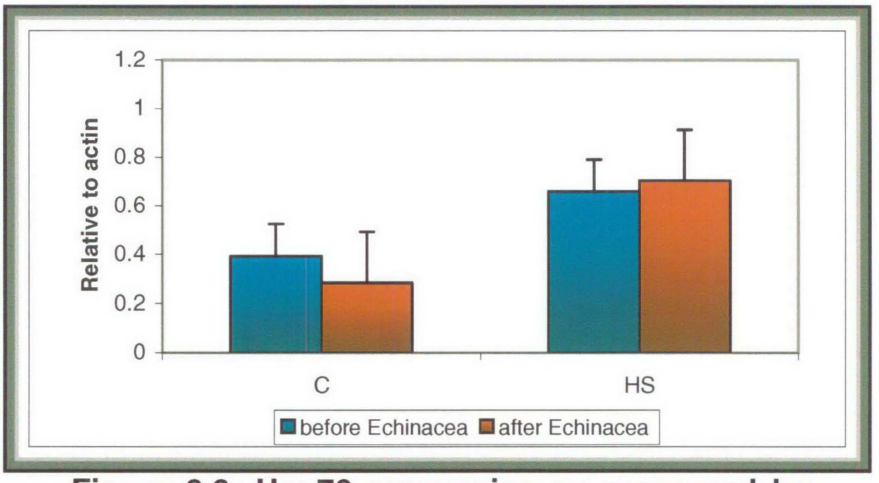


Figure 6.6: Hsp70 expression as measured by Western immunoblot. C = hsp70 expression measured under control conditions at 37°C. HS = hsp70 expression measured under heat shock conditions at 42.5°C. Values are means ± SEM.

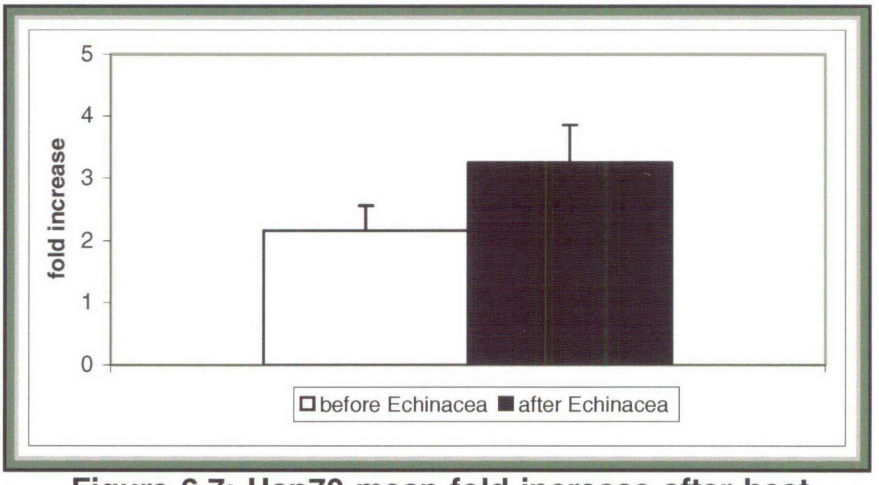


Figure 6.7: Hsp70 mean fold increase after heat shock. Values are means ± SEM.

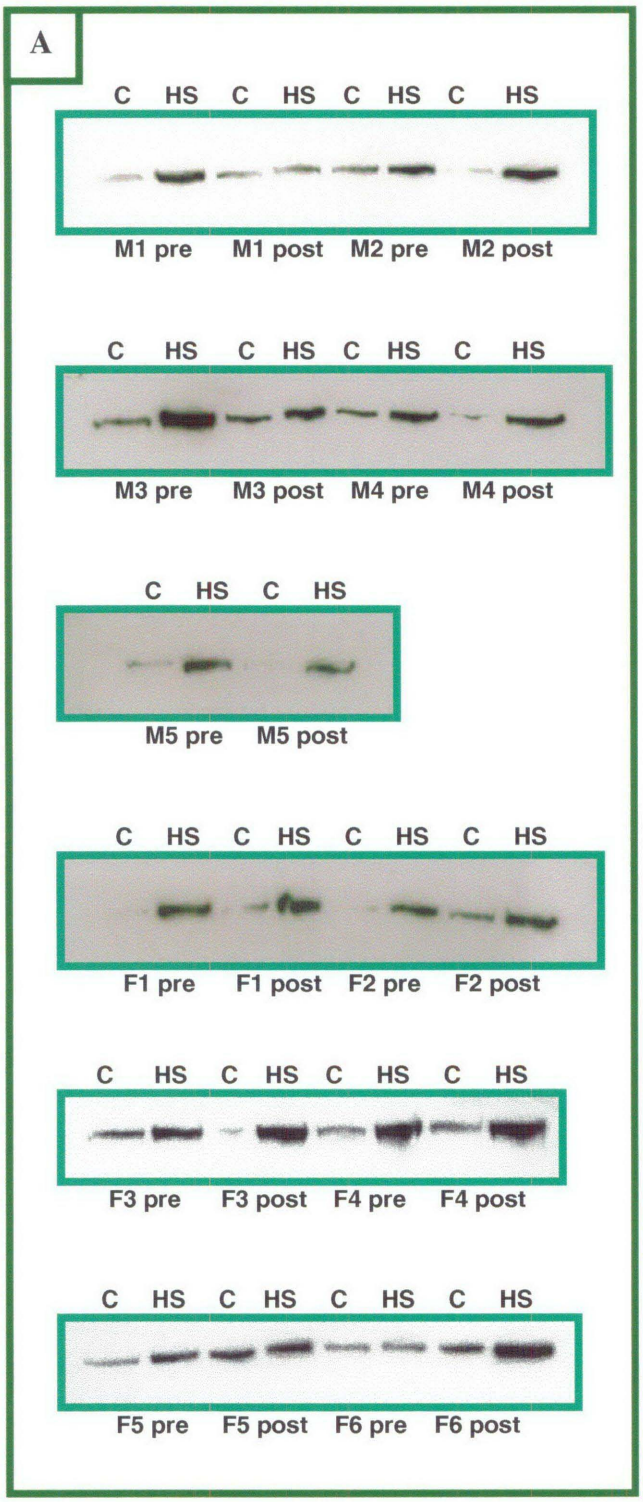


Figure 6.8A: Western immunoblots measuring Hsp70 expression. C = expression measured under control conditions at 37°C. HS = expression measured under heat shock conditions at 42.5°C. M = male. F = female.

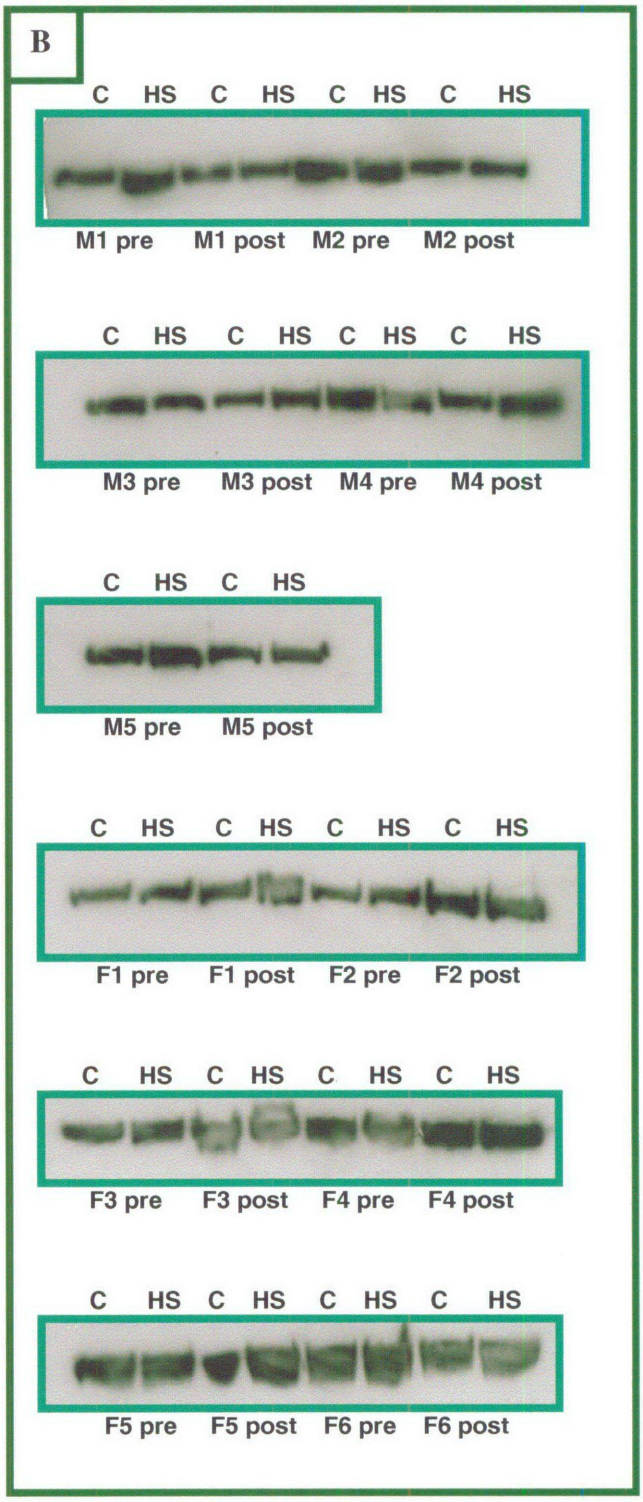


Figure 6.8B: Corresponding Western immunoblots measuring β-actin expression. C = expression measured under control conditions at 37°C. HS = expression measured under heat shock conditions at 42.5°C. M = male. F = female.

6.3.4 *De novo* synthesis of stress proteins

Proteins (10 µg) extracted from leukocytes, were run on 1D-SDS-PAGE to determine the effect of Echinacea supplementation on induction of hsps in leukocytes. Control (37°C) and heat shock (42.5°C) samples, collected from each subject before and after supplementation with Echinacea, were run side by side in each gel (Figures 6.12 – 6.15). Expression of hsps was calculated by densitometric analyses of peak intensity of bands relative to β-actin (43 kDa). The position of bands was determined by using a molecular weight marker for each gel. The expression of hsp70 (relative to β-actin, the internal control) measured at 37°C at baseline was 0.48 ± 0.22 and this did not differ significantly after Echinacea supplementation (0.28 ± 0.07). The expression of hsp70 (relative to β-actin) after heat shock at 42.5°C was also not significantly different with 1.07 ± 0.08 at baseline and 1.44 ± 0.27 after Echinacea intake. The *de novo* expression of hsp70 relative to β-actin before and after heat shock is illustrated in Figure 6.10. The ratio of control (37°C) versus heat shock (42.5°C) hsp70 expression was subsequently examined as a fold-increase. Mean hsp70 fold-increase at baseline was 2.21 ± 0.11 and this was not significantly different after Echinacea intake at 5.15 ± 1.25 ($p > 0.05$). The expression of hsp90 (relative to β-actin, the internal control) measured at 37°C at baseline was 0.14 ± 0.02 and this did not differ significantly after Echinacea supplementation (0.15 ± 0.02). The expression of hsp90 (relative to β-actin) after heat shock at 42.5°C was also not significantly different with 1.41 ± 0.41 at baseline and 1.05 ± 0.15 after Echinacea intake. The *de novo* expression of hsp90 relative to β-actin before and after heat shock is illustrated in Figure 6.11. The ratio of control (37°C) versus heat shock (42.5°C) hsp70 expression was subsequently examined as a fold-increase. Mean hsp90 fold-increase at baseline was 10.17 ± 5.1 and this was not significantly different after Echinacea intake at 6.79 ± 2.05 ($p > 0.05$).

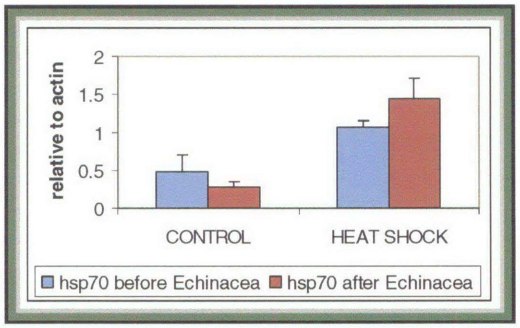


Figure 6.9: *De novo* expression of hsp70 before and after Echinacea intake. Values are means ± SEM.

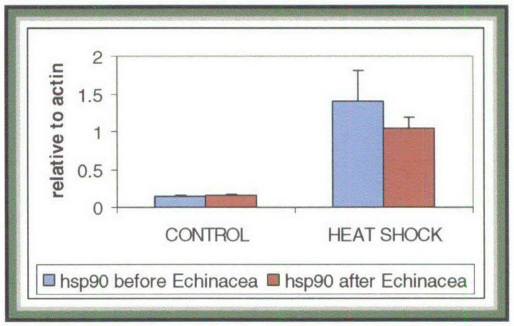


Figure 6.10: *De novo* expression of hsp90 before and after Echinacea intake. Values are means ± SEM.

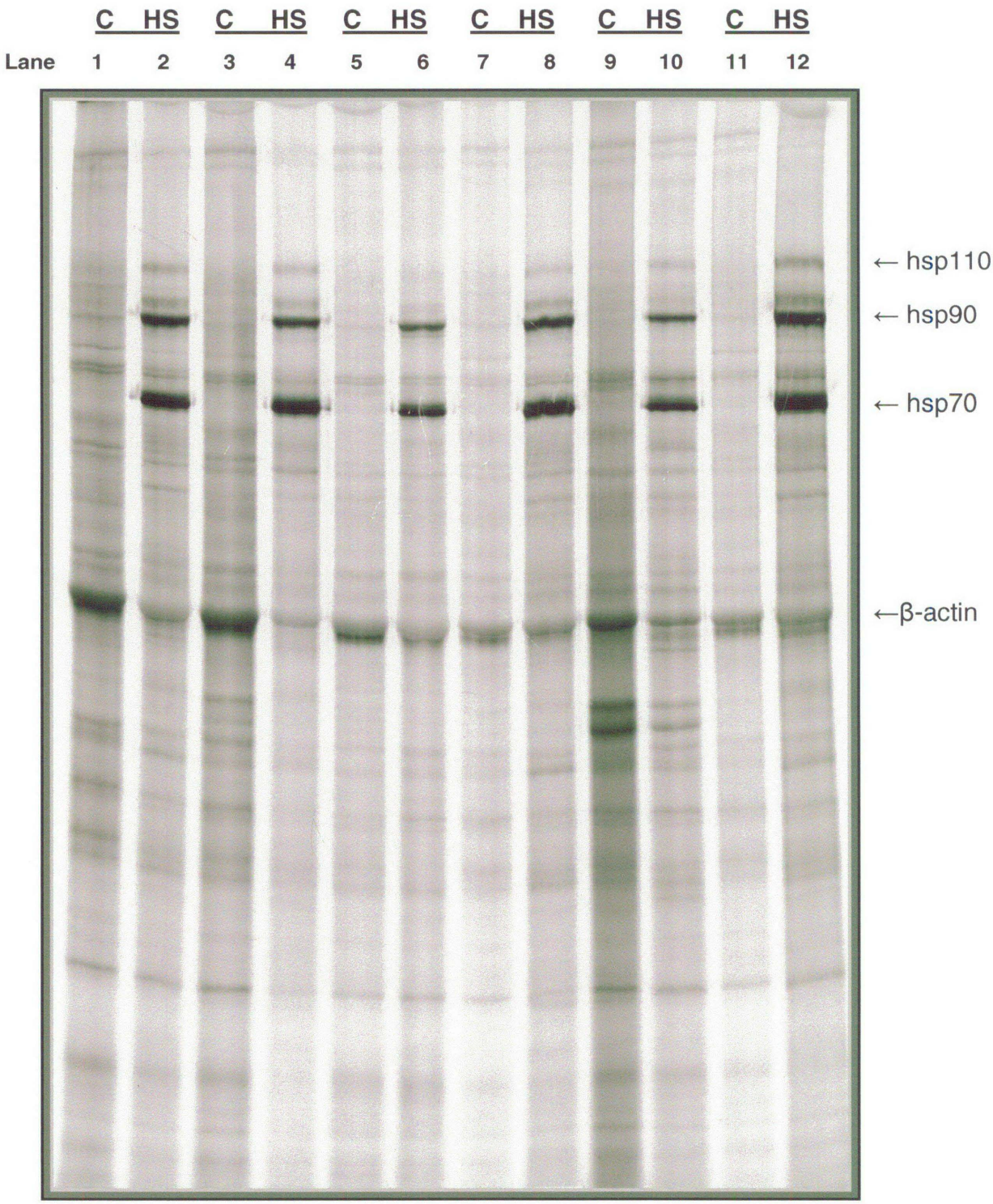


Figure 6.11: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from heat shocked lymphocytes from male study participants. **Lanes 1, 2, 5, 6, 9 & 10:** Before Echinacea supplementation. **Lanes 3, 4, 7, 8, 11 & 12:** After Echinacea supplementation. C = control samples incubated at 37°C for 4 h. HS = Heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

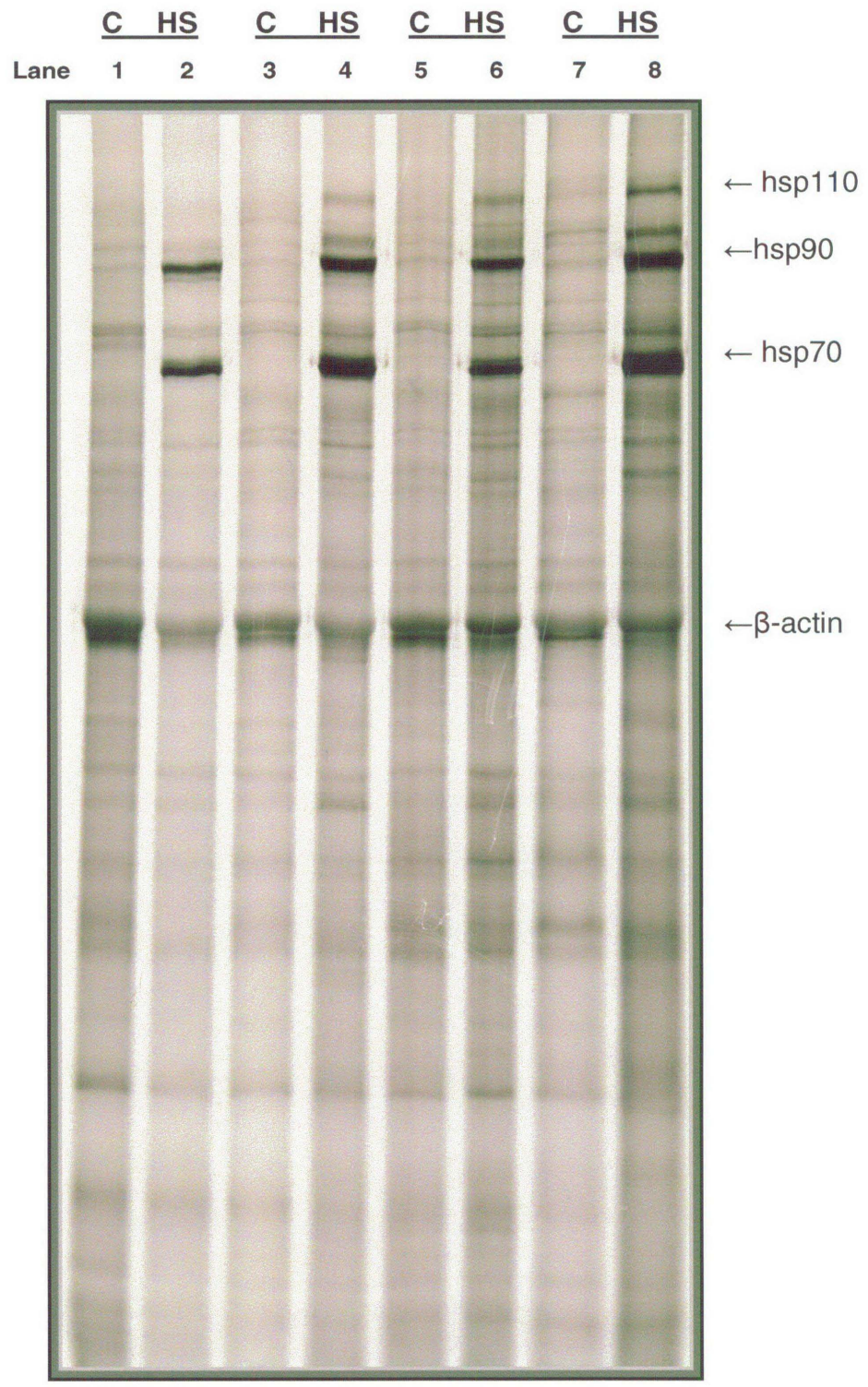


Figure 6.12: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from heat shocked lymphocytes from male study participants. **Lanes 1, 2, 5 & 6:** Before Echinacea supplementation. **Lanes 3, 4, 7 & 8:** After Echinacea supplementation. C = control samples incubated at 37°C for 4 h. HS = Heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

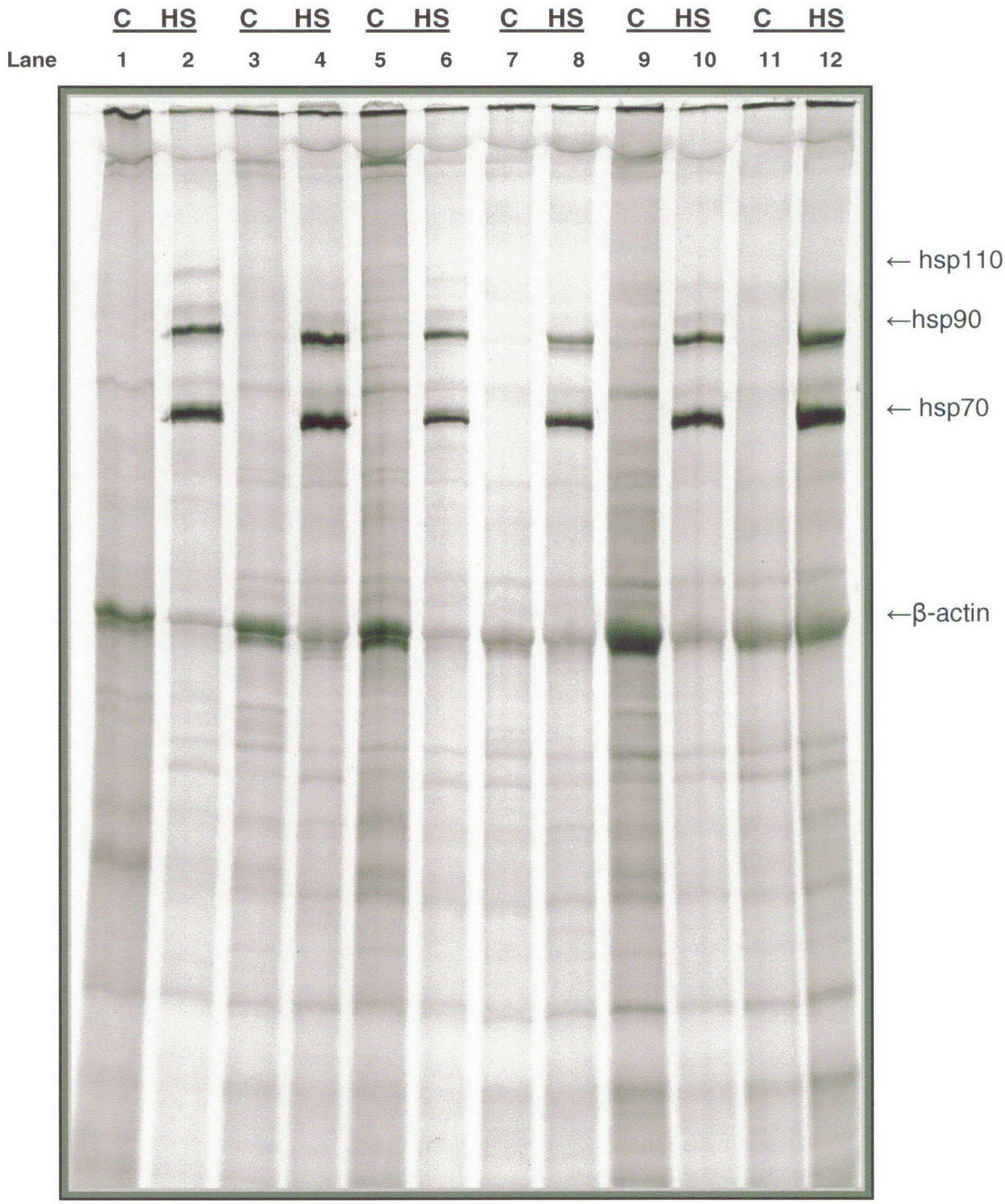


Figure 6.13: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from heat shocked lymphocytes from female study participants. **Lanes 1, 2, 5, 6, 9 & 10:** Before Echinacea supplementation. **Lanes 3, 4, 7, 8, 11 & 12:** After Echinacea supplementation. C = control samples incubated at 37°C for 4 h. HS = Heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

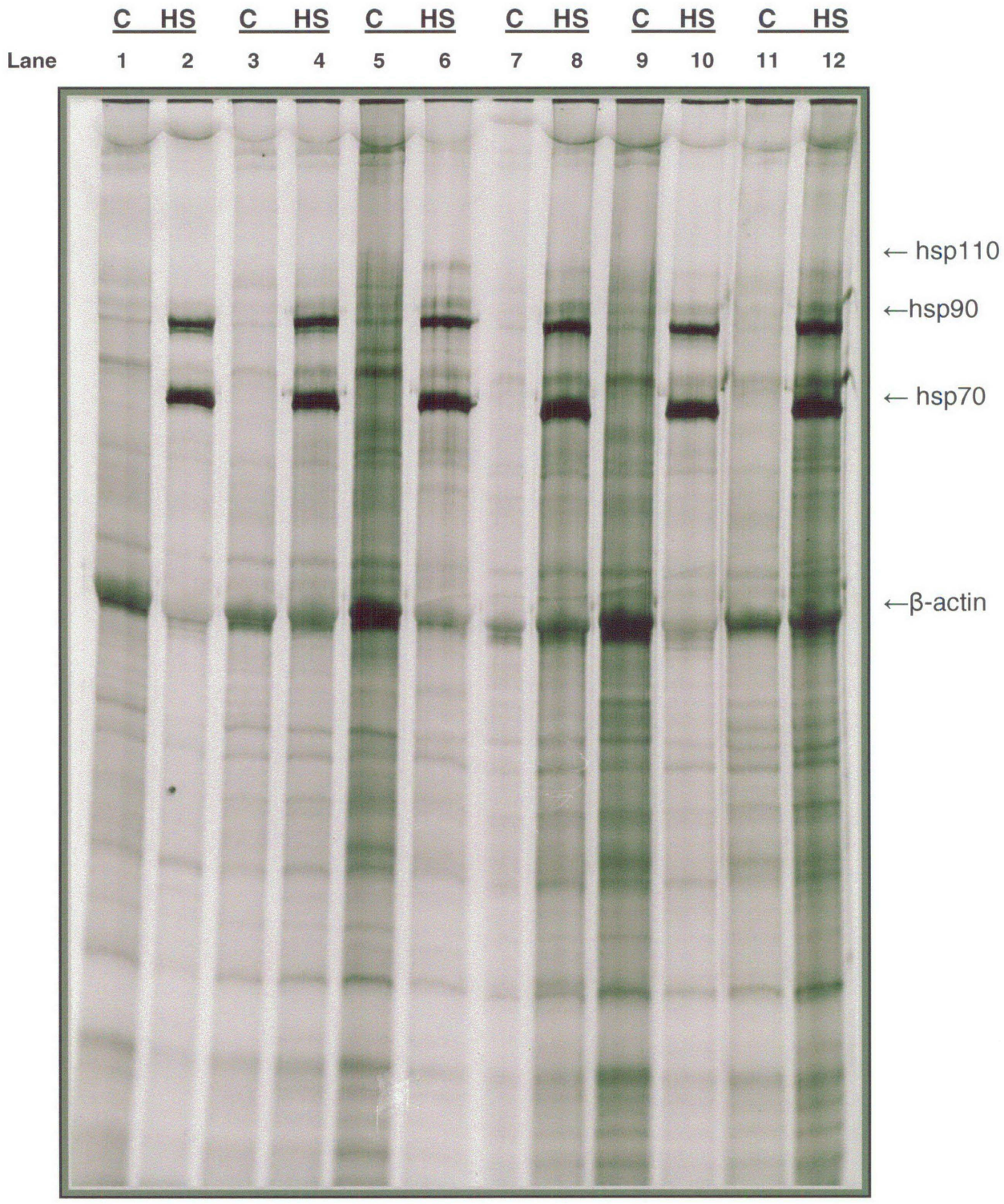


Figure 6.14: Effect of heat shock on induction of hsps in lymphocytes. Autoradiogram of SDS-PAGE gel obtained from heat shocked lymphocytes from female study participants. **Lanes 1, 2, 5, 6, 9 & 10:** Before Echinacea supplementation. **Lanes 3, 4, 7, 8, 11 & 12:** After Echinacea supplementation. C = control samples incubated at 37°C for 4 h. HS = Heat shock samples incubated at 42.5°C for 1 h then at 37°C for 3 h.

6.3.5 Serum Chemistry and Haematological values

The serum chemistry and haematological values for subjects after Echinacea intake, did not vary significantly from baseline levels (Table 6.1) with the exception of white cell counts (WCC). The mean baseline WCC was 6.6 ± 0.4 x 10^9/L whereas after supplementation it was 7.2 ± 0.3 x 10^9/L ($p = 0.043$) (Figure 6.16). Differential white cell counts were measured to determine whether any particular cell types showed significant increases or whether the trend was consistent across all cell types. Most cell types displayed modest increases after Echinacea intake, although only the lymphocyte sub-population approached significance ($p = 0.056$) (Figure 6.17 & Figure 6.18).

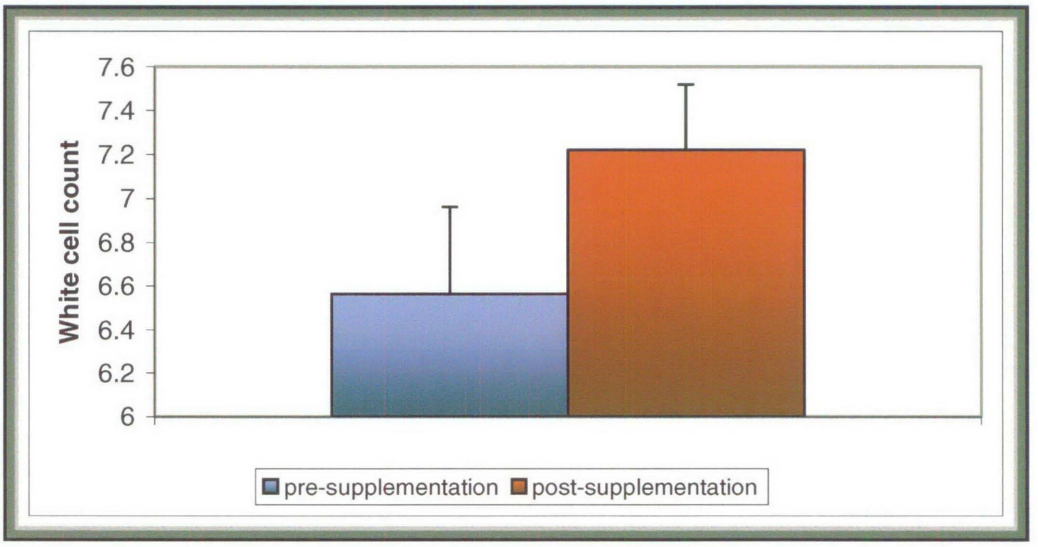


Figure 6.15: Mean white cell count pre- & post-Echinacea. Values are means ± sem and are expressed as cells x 10^9/L.

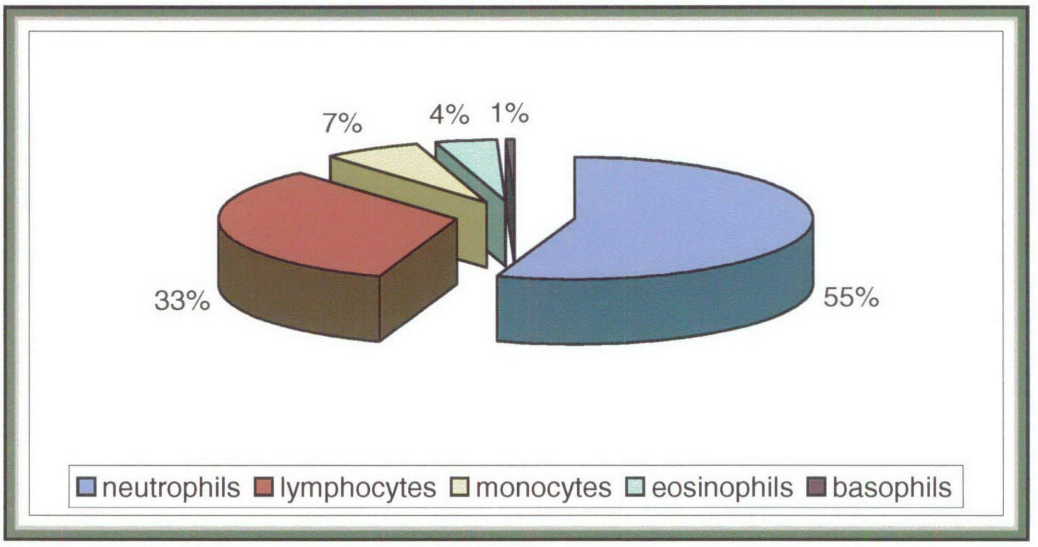


Figure 6.16: Mean differential white cell counts before Echinacea supplementation. Values are percentages of total white cell counts.

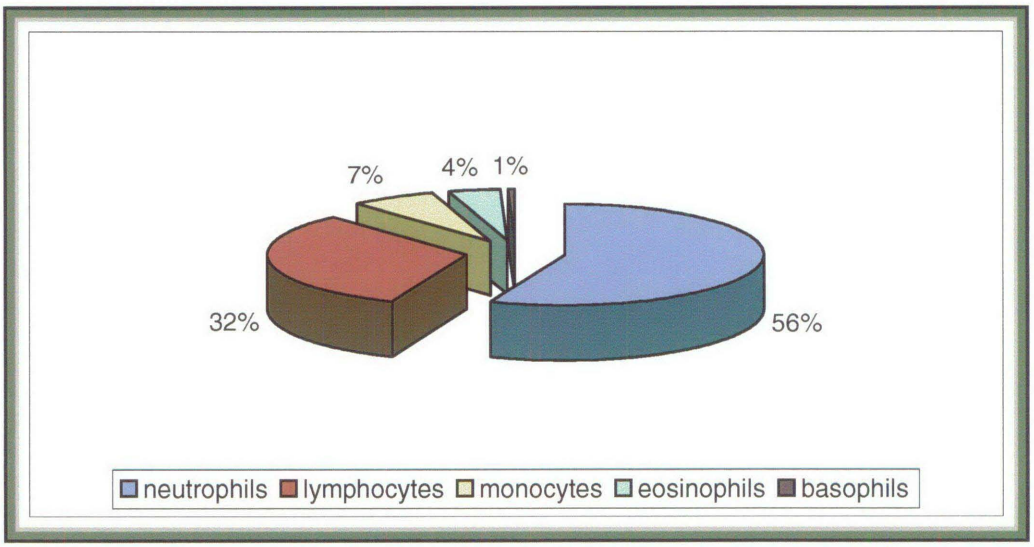


Figure 6.17: Mean differential white cell counts after Echinacea supplementation. Values are percentages of total white cell counts.

6.3.6 Alkylamide

Plasma alkylamide concentrations were determined following ingestion of one Echinacea Premium tablet. Only the major alkylamide, consisting of the mixed isomer (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide and (2E,4E,8Z,10E)-N-isobutyldodeca-2,4,8,10-tetraenamide, the main alkylamide found in both *Echinacea angustifolia* and *E. purpurea*, was clearly identified in all individuals (Figure 6.18). Alkylamide concentrations were found to be 11.5 ± 2.0 ng equiv/mL plasma (n = 7).

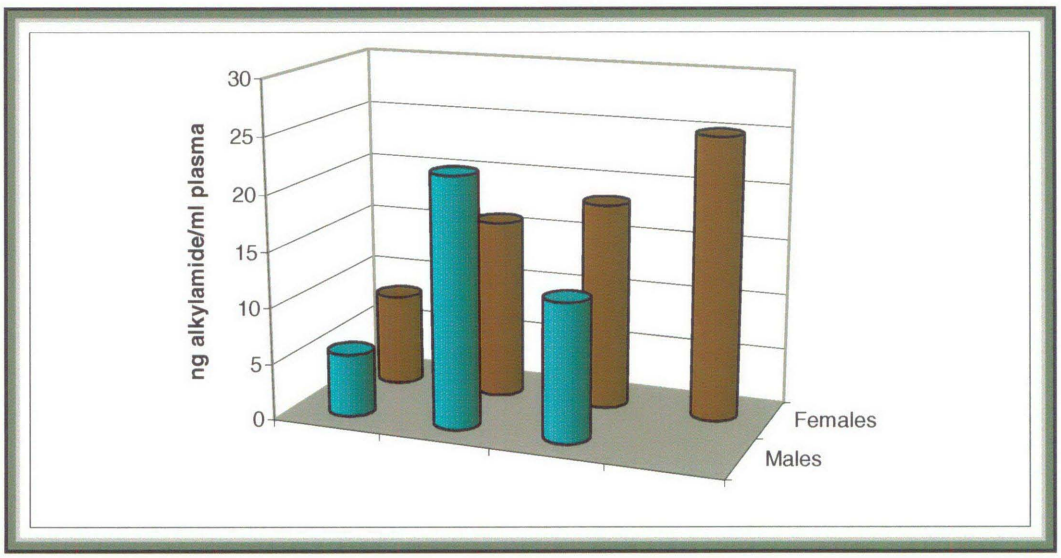


Figure 6.18: Alkylamide levels in plasma 1 h after ingestion off one Echinacea Premium tablet. Values are expressed as ng alkylamide/ml of plasma.

	Before Echinacea	After Echinacea
Liver Function		
Protein (g/L)	74 ± 2	74 ± 2
Albumin (g/L)	41 ± 1	42 ± 1
Calc. Glob. (g/L)	33 ± 1	32 ± 1
Bilirubin (μmol/L)	7 ± 1	7 ± 1
GGT (U/L)	17 ± 1	23 ± 5
Alk. Phos. (U/L)	71 ± 5	77 ± 10
ALT (U/L)	30 ± 2	31 ± 5
AST (U/L)	20 ± 1	23 ± 4
Haematology		
Haemoglobin (g/L)	146 ± 4	146 ± 4
WCC (10^9/L)	6.6 ± 0.4	7.2 ± 0.3 *
Platelets (10^9/L)	276 ± 20	272 ± 15
RCC (10^{12}/L)	4.84 ± 0.15	4.81 ± 0.16
Haematocrit (L/L)	0.45 ± 0.01	0.45 ± 0.01
MCV (fl)	92.9 ± 0.8	92.9 ± 0.8
MCH (pg)	30.2 ± 0.3	30.3 ± 0.3
MCHC (g/L)	325 ± 1	326 ± 1
RDW (%)	11.4 ± 0.1	11.3 ± 0.2
Neutrophils (10^9/L)	3.6 ± 0.3	4.0 ± 0.3
Lymphocytes (10^9/L)	2.2 ± 0.1	2.3 ± 0.1
Monocytes (10^9/L)	0.4 ± 0.0	0.5 ± 0.0
Eosinophils (10^9/L)	0.3 ± 0.1	0.3 ± 0.0
Basophils (10^9/L)	0.0 ± 0.0	0.0 ± 0.0

Table 6.1: Blood chemistry and haematological values. All values are mean ± sem (n = 11). Key: calcium globulin (calc. glob.), γ-glutamyltransferase (GGT), alkaline phosphatase (alk. phos.), alanine aminotransferase (ALT), aspartate aminotransferase (AST), white cell count (WCC), red cell count (RCC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW). * $p = 0.043$

6.4 Discussion

Following a two week dosing regimen, Echinacea was found to affect the immune system by increasing white cell counts and the response to heat shock of hsp70 in leukocytes as well as decreasing erythrocyte haemolysis.

The immunomodulatory effects of Echinacea are considered to be via non-specific activation of the immune system. Several possible modes of action have been implicated, including macrophage stimulation, phagocytic activity and NK cell activity. It has been reported that *Echinacea purpurea* is able to stimulate the production of cytokines by peripheral blood mononuclear cells (PBMC) *in vitro.* In particular, significantly higher levels of IL-1, IL-6, IL-10 and TNF-α were produced by macrophages cultured in low concentrations of Echinacea compared with unstimulated cells (Burger *et al.,* 1997). A number of studies have also demonstrated that *E. purpurea* has significant immunomodulatory effects, including the activation of polymorphonuclear leukocytes and natural killer (NK) cells and increased phagocytotic indices of macrophages (reviewed by Barrett, 2003). Furthermore, recent *in vitro* studies on exposure of NK cells in PBMC populations to *Echinace pupurea* demonstrated that Echinacea extracts are potent activators of NK cytotoxicity by augmenting the frequency of NK target conjugates and activating the programming for lysis of NK cells (Gan *et al.,* 2003).

The data presented in this Chapter showed that although neither basal nor heat shock (mild stress) hsp70 levels in leukocytes were different there was a greater fold-increase in hsp70 expression (the ratio of expression at 42.5°C compared with expression at 37°C) after Echinacea supplementation as measured by Western immunoblots. This was indicative of an improved leukocyte stress response. The stress response is a highly conserved, adaptive response that is present in both unicellular and multicellular organisms and it confers tolerance and stress resistance at both cellular and whole organism levels (Lindquist & Craig, 1988). This enhanced stress response is also indicative of an improved immune response given that increases in hsp expression following cellular stress may play critical roles in antigen presentation and lymphocyte effector function (reviewed in Prohaszka & Fust, 2004), cytokine (Asea, 2003) and β-chemokine induction (Lehner *et al.,* 2000), dendritic cell maturation (Kuppner *et al.,* 2001) and danger signalling (Matzinger, 1998).

There is also increasing evidence to suggest that non-specific activation of the immune system by Echinacea may be mediated by increased monocyte secretion of cytokines (Rinninger *et al.,* 2000; Goel *et al.,* 2002). Moreover, it has been established that monocytes express more hsp70 than any other leukocyte subtype (Bachelet *et al.,* 1998; Agnew & Watson, results unpublished) and, whilst the leukocyte populations used in the current studies were not exclusively monocytes, it is likely that the observed enhanced stress response is primarily related to monocyte expression of hsp70. This increased stress response was not apparently due to an increase in monocyte numbers *per se*, as demonstrated by no differences in monocyte counts before and after Echinacea intake (Table 6.1). It is therefore hypothesised that non-specific activation of the immune system by Echinacea may be mediated by monocytes synthesising increased amounts of hsp70 following cellular stress.

After a mild heat shock (from 37°C to 42.5°C for 1 h) lymphocytes displayed an augmented *de novo* synthesis of hsp90 and hsp70, relative to actin, in all individuals both before and after Echinacea supplementation. This finding is consistent with the literature, which holds that human leukocytes subjected to a non-lethal, thermotolerance-promoting increase in body temperature synthesize new and/or enhanced amounts of polypeptides (Rodenhiser *et al.,* 1985). This adaptive response is thought to complement the constitutive antioxidant defense system found in mammalian cells. It is noteworthy, however, that the presence of high levels of hsps does not necessarily imply stress tolerance (Gross & Watson, 1998). Despite the observation of an improved leukocyte hsp70 stress response, there was no difference in the *de novo* expression of either hsp70 or hsp90 after Echinacea supplementation. These results demonstrated that a two week dosing regimen of Echinacea did not alter the *de novo* synthesis of hsp70 or hsp90 in leukocytes. Therefore, the effects of Echinacea are likely to be an improvement in the ability of hsps to respond to stress rather than an increase in hsp synthesis *per se*.

The absence of significant changes in serum chemistry and haematological values for subjects' post-supplementation is consistent with a recent human Echinacea clinical trial result (Randolph *et al.,* 2003). Despite this lack of significant change, however, the observed increase in circulating white cell numbers after Echinacea supplementation indicated an enhanced immune response. Very recently, Jurksteine

et al. (2005) observed an increase in leukocyte numbers in their *in vivo* studies on the immune response of rabbits supplemented with Echinacea root extracts. Similar results were also reported by Cundell *et al.* (2003), who noted an increase in circulating white cells in Sprague-Dawley rats after supplementation with aerial parts of Echinacea.

There was no difference in the levels of plasma antioxidants as measured by the ABTS method. It should be noted, however, that this assay only measures the water-soluble antioxidants in the plasma and not the lipid-soluble antioxidant levels. The latter are more likely to be incorporated into cellular membranes. Nevertheless, we found that Echinacea intake provided some protection against free radical induced oxidative damage to erythrocytes. As the erythrocyte membrane contains high concentrations of polyunsaturated fatty acids that are susceptible to free radical induced peroxidation (Miki *et al.,* 1987), this experimental system was a useful tool to measure the ability of Echinacea to protect membrane lipids against free radical damage. A number of other recent *in vitro* studies have also demonstrated that Echinacea root extracts are a good source of antioxidants that possess free radical scavenging activities (Hu & Kitts, 2000; Sloley *et al.,* 2001; Pellati *et al.,* 2004). The antioxidant effects appear to be similar for each of the three species of Echinacea. Given that the combinations of constituents vary considerably between species, it has been postulated that these effects are cumulative, rather than being attributable to individual active constituents (Sloley *et al.,* 2001). As free radical damage is a well-established, causative factor in many disease states (Rice-Evans & Diplock, 1993; Halliwell & Gutteridge, 1999) and dietary antioxidant supplementation has been reported to improve cell-mediated immunity in humans (Meydani, 1999), the benefits of therapeutic agents such as Echiancea that improve the antioxidant status of an individual should be more closely evaluated.

The active phytochemical compounds believed to be responsible for the perceived immunomodulatory effects of Echinacea are the alkylamides and caffeic acid conjugates. It has been established, however, that alkylamides but not caffeic acid conjugates are able to cross the intestinal barrier thus being able to elicit pharmacological effects (Matthias *et al.,* 2004). The alkylamides are present in ethanolic extracts from *E purpurea* and *E angustifolia* roots and aerial parts (Bauer & Remiger, 1989). The detection of the major alkylamide isomer mix, (2E,4E,8Z,10Z)-N isobutyldodeca-2,4,8,10-tetraenamide and (2E,4E,8Z,10E)-N-

isobutyldodeca-2,4,8,10-tetraenamide, in the plasma of participants 1h after ingestion of one Echinacea tablet is consistent with the observations of Matthias *et al,* (2005) who very recently reported that alkylamides were detectable in plasma as soon as 20 min after ingestion of one Echinacea tablet and were detectable for up to 12 h. The range of alkylamide levels (5.56 – 25.08 ng alkylamide/ml plasma) measured in this cohort also highlights the individual nature of metabolism of active constituents of pharmaceuticals and may lend support to the notion of 'tailor made' patient-centred pharmacotherapeutic drug development.

A variety of herbs are commonly used in presumptive immune modulating formulations and Echinacea, in particular, is perhaps the most widely used of all medicinal herbs. The importance of the research presented in this Chapter and future research into the effects of Echinacea and other herbal products should be measured by the large and increasing proportion of the Australian adult population using such products, recently estimated as high as 52% (Expert Committee, 2003). The importance of this type of research to industry cannot be underestimated given that the complementary medicines industry in Australia is estimated to be worth $800 million. Moreover, the Therapeutic Goods Administration (TGA, 2005) has announced a requirement for the neutraceutical and complementary medicine industries to provide evidence-based support to substantiate claims of efficacy for their products. . The mechanisms of actions of most complementary therapies are poorly investigated and a scientific understanding of these mechanisms are thus critical both with respect to current efficacy and quality control issues as much as for the future development and marketing of these products.

Some major difficulties arise when reviewing the current literature on the efficacy of Echinacea products due to the wide variety of combinations of not only Echinacea species (*E purpirea, E angustifolia, E pallida*), but also plant parts (root, herb, flower) used, coupled with variability in extraction methods (hydrophilic or lipophilic), hence differences in active constituents present. These factors may explain the conflicting nature of results from different Echinacea studies, given the likelihood of significant differences in products being tested. It is also imperative to consider that many individuals participating in such studies may experience 'the placebo effect'. This effect has been widely discussed in the medical literature for many years and has contributed to the establishment of the 'gold standard' for clinical trials that include a placebo group in randomized, controlled, double-blind trials. The variability in content

and labeling is also of particular importance to consumers who self-medicate. In a review of frequently purchase herbs, Garrard *et al.* (2003), noted that of the 92 Echinacea products tested, 67% were not consistent in ingredients or dosage as compared with standards for ingredients and recommended daily dosage (RDD) as set by the regulatory arm of the US Federal Government.

The potential for herb-drug interactions has resulted in a number of warnings to consumers regarding the dangers of self-medicating and stressing the need for disclosure of the extent of complementary therapeutic use to health care providers. In particular, Echinacea is contraindicated for patients receiving immune suppressants such as those prescribed to prevent tissue rejection as the immune stimulating effects of Echinacea may interfere with the action of these drugs (Hardy, 2001).

The use of Echinacea by HIV-positive individuals is also quite controversial. Some patients with HIV/AIDS take Echinacea to treat the symptoms of colds and/or flu, however, many use Echinacea to stimulate the immune system. Potentially, stimulating the immune system by increasing the number of T lymphocytes may provide more 'target cells' for the virus to infect. It is also plausible that if the cells of the immune system are already activated in response to the HIV-infection, further activation by Echinacea may result in cell and tissue damage (AIDS Infonet, 2002). It is believed that stimulation of particular cytokines, such as TNF-α, may accelerate the pathology of HIV/AIDS and thus be detrimental for these patients (See *et al.,* 1997; Barak *et al.*, 2002).

Echinacea use is also contraindicated for patients receiving treatment for cancer (Block & Mead, 2003). In particular, individuals with lymphoma (Hodgkins, Non-Hodgkins, B-cell and unspecified types) are advised to avoid Echinacea products as the reported stimulation of B lymphocytes, phagocytosis, cytokine production by macrophages and increased activity and mobility of leukocytes are perceived to be detrimental to these patients (reviewed by Werneke *et al.,* 2004).

The results of this pilot study demonstrated the immunomodulatory properties of Echinacea and represented one of the very few studies involving a widely used, commercial preparation of Echinacea in tablet form. Importantly this Echinacea preparation consisted of chemically defined concentrations of alkylamides from the

roots of two distinct species of Echinacea. We suggest that supplementation with Echinacea may induce an immune response through altered expression of leukocyte hsp70 and increased white cell counts. There is also some support for the protective effects of Echinacea against free radical induced damage to erythrocytes. These immunomodulatory effects may be attributable to the alkylamide constituents of Echinacea and, as such, further research investigating the effects of alkylamide extracts on hsp expression is warranted. This research should also seek to investigate the mechanisms of altered hsp70 expression by examining the response to Echinacea and its constituents by individual leukocyte subsets. A greater understanding of the mechanisms of action of Echinacea and other herbal medicines is essential given the large and increasing percentage of the population using these products as passive immunomodulators.