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Echinacea root

(Echinacea angustifolia and/or purpurea)

Synonyms

Echinacea radix (Lat), Sonnenhut, Igelkopf (Ger), racine d'échinaeace (Fr), Echinacea (Ital), solhat (Dan).

What is it?

Three main species of Echinacea, commonly known as purple coneflower, are used medicinally: *Echinacea angustifolia* DC. (narrow-leaved purple coneflower), *E. purpurea* (L.) Moench. (common or broad-leaved purple coneflower) and *E. pallida* (Nutt.) Nutt. (pale purple coneflower). *Echinacea purpurea* has become the most cultivated and widely used of the various species because the whole plant (root, leaf, flower, seed) can be used and also because it is more easily cultivated. The root and rhizome of *E. angustifolia* and *E. pallida* are typically used medicinally, although *E. pallida* is sometimes considered to be less active, because it is low in alkylamides. In the past *E. pallida* preparations have been incorrectly labelled as *E. angustifolia*, particularly in Europe. *Parthenium integrifolium*, the Missouri snakeroot, is a documented adulterant of commercial Echinacea.

Echinacea has been the subject of a considerable amount of misinformation and misunderstanding concerning its active principles, mode of action, clinical efficacy and cautions and contraindications. One important reason behind such confusion is the many types of Echinacea products on the market. This has an historical background. The Native Americans, and the Eclectics physicians who adopted their use of Echinacea, preferred the root (see below). In fact, the Eclectics only used an aqueous-ethanolic (lipophilic) extract of dried *E. angustifolia* root high in alkylamides. After oral intake, these phytochemicals impart a persistent tingling sensation in the mouth and stimulate the flow of saliva, a sign of good quality according to *King's American Dispensatory*.[1] In Europe during the 1930's, the German herbalist Madaus promoted *E. purpurea* as it was easier to grow. Being somewhat influenced by the homeopathic approach of using fresh plant tinctures, his firm eventually developed a hydrophilic product prepared from the stabilised juice of fresh *E. purpurea* tops (aerial parts). This is still the most popular form of Echinacea in Germany. Another popular European product is a fresh plant tincture of the whole plant of *E. purpurea*.

As might be expected, these different products exhibit substantial variations in their phytochemical content (and hence by definition their pharmacological and clinical properties). Yet they are typically discussed in the literature under the generic term "Echinacea" as if they shared identical properties. Hence a rudimentary concept of phytotherapy, the overriding importance of the part of the plant being used, appears to have been overlooked or ignored. The mode of preparation also creates phytochemical differences. Specifically the hydrophilic type of product will be low in alkylamides and higher in water-soluble compounds such as polysaccharides, whereas a lipophilic product will be much higher in alkylamides (especially if prepared from the root).

As a counter to this poor academic rigour in the discussion of Echinacea, the decision has been made to limit this monograph to the roots of *E. angustifolia* and *E. purpurea*. These are, after all,

the species and plant part still preferred by herbal clinicians in the English-speaking world. Accordingly, scientific investigations of other plant parts or other Echinacea species have not been included, except by way of contrast. Furthermore, studies where the plant part used is not clear from the publication (as can sometimes be the case) and studies where mixed plant parts were used (for example root combined with aerial parts) have also been omitted from review (in most cases).

Effects

Immunostimulant, mainly acting on innate immunity, hence may modulate immune function in allergy and autoimmunity; enhances resistance to infections, particularly of the upper respiratory tract; assists in recovery from chemotherapy; anti-inflammatory, particularly after topical application.

Traditional view

As noted above, information about the use of Echinacea root first came from Native American tribes. Their use was adopted by the Eclectics, a group of practitioners who were prominent around the late 19th and early 20th centuries in the United States. By 1921 Echinacea (specifically the root of *E. angustifolia*) was by far the most popular treatment prescribed by Eclectic physicians. The Eclectics used Echinacea root for about 50 years. Given that their use was based on tribal knowledge and that they accumulated extensive clinical experience, their traditional use data is of a high quality. The best sources of these data are *King's American Dispensatory*[1] and Ellingwood.[2] The extensive range of conditions in these texts for which Echinacea root was prescribed included snakebite, syphilis, typhus, septic wounds, diphtheria, scarlet fever, dysentery and even cancer. It is clear from these writers that the limitations on Echinacea use suggested by some modern authors are not supported. The conditions treated were mainly infections and envenomations of various kinds, which probably attest to Echinacea's

influence on the immune system. However, the inclusion of tuberculosis and disorders related to autoimmunity such as diabetes, exophthalmic goitre, psoriasis and renal haemorrhage contrasts with contraindications proposed by some modern writers.

The Eclectics were also not averse to using Echinacea root long term. For example, according to Ellingwood, it was recommended for the following chronic conditions: cancer, chronic mastitis, chronic ulceration, tubercular abscesses, chronic glandular indurations and syphilis. With regard to syphilis, Ellingwood wrote: 'The longest time of all cases yet reported, needed to perfect the cure, was nine months.' He cites a dramatic case history of a vaccination reaction where Echinacea root was taken every 2 h for up to 6 weeks.

Summary actions

Immunomodulator, anti-inflammatory, vulnerary, lymphatic. Any significant clinical antibacterial and antiviral activity probably follows indirectly from immune enhancement.

Can be used for

Indications supported by clinical trials

Treatment of upper respiratory tract infections (clinical evidence is controversial); beneficial for the prophylaxis of upper respiratory tract infections and infections in general; to promote immune function.

Traditional therapeutic uses

Bacterial, viral and protozoal infections, including infections of the digestive, respiratory and urinary tracts; mild septicaemia; states of weakened, suppressed or imbalanced immunity, including allergies and autoimmune disease; inflammatory and purulent conditions, including

acne, abscess, furunculosis; envenomation. Topically for poorly healing wounds, inflamed skin conditions and bacterial infections.

May also be used for

Extrapolations from pharmacological

To increase phagocytosis; antiviral activity, probably indirect; for benign prostatic hyperplasia; to control anxiety. Topically: to improve wound healing, increase resistance to infection and to increase connective tissue regeneration.

Preparations

Echinacea purpurea or *E. angustifolia* root preparations include liquid extracts of fresh or dried root and rhizome and tablets and capsules based on these.

Dosage

Preventative doses or doses for chronic conditions are recommended as follows:

- 1 to 3 g/day of *E. angustifolia* dried root.
- 1.5 to 4.5 g/day of *E. purpurea* dried root.
- 2 to 6 mL/day of 1:2 liquid extract of *E. angustifolia* root.
- 3 to 9 mL/day of 1:2 liquid extract of *E. purpurea* dried root.
- 5 to 15 mL/day of 1:5 tincture of *E. angustifolia* root.
- 7.5 to 22.5 mL/day of 1:5 tincture of *E. purpurea* dried root.

Equivalent doses to these can also be used in tablet or capsule form as long as these have been carefully dried to preserve the alkylamide content.

These dosages may be substantially increased in the short term for acute conditions; for example, *E. angustifolia* root can be taken up to 10 to 15 g per day (or its equivalent in a liquid, capsule or tablet preparation).

Duration of use

Despite suggestions to the contrary, there is no evidence to suggest that long-term usage of Echinacea root will have an adverse effect on immune function. Echinacea root is most likely a benign agent acting mainly on innate immunity (although there is still much to be understood about its mode of action).

Summary assessment of safety

Echinacea root preparations may be safely prescribed for oral and topical use if the recommended dosage is not exceeded. Despite many reputed contraindications in the literature, the herb is unlikely to cause adverse effects in a wide range of applications including asthma, allergies and autoimmunity. However, care should be exercised when prescribing any preparation of Echinacea (including the root) to patients with known allergy to members of the Compositae (Asteraceae, daisy) family.

Technical data

Botany

Echinacea is a member of the Compositae (Asteraceae, daisy) family and grows to a maximum height of 50 to 180cm, depending on the species. The distinctive flower head consists of white, rose or purple drooping ray florets and a conical disc made up of numerous tubular florets. *E. angustifolia* is most easily identified by its low habit and the coarse hair and relatively straight ray florets; *E. purpurea* by the large, egg-shaped, serrated leaves and the bright purple ray florets; *E. pallida* by the white pollen and the longer length of the paler ray florets.[3,4]

In 1968 the botanist McGregor reported on a 15-year journey studying wild Echinacea plants from populations throughout its entire geographical range in North America.[5] As a result of this work McGregor recognised 9 species and 4 varieties of the genus Echinacea and the classification and names suggested are still currently used by both herbal clinicians and regulatory authorities (eg *Echinacea angustifolia, E. purpurea, E. pallida* and so on).

In 2001 and 2002, a group of US botanists undertook an extensive review of the Echinacea genus using morphometric analysis and the taxonomic results have recently been republished.[5] Their analysis found that the genus contained 2 subgenera, namely *Echinacea* subgenus *Echinacea* (containing a single species *E. purpurea*) and *Echinacea* subgenus *Pallida* (containing 3 species: *E. pallida, E. atrorubens* and *E. laevigata*). The botanists' classification also agreed with the earlier work of Cronquist.[5] Perhaps the most important outcome of this work from a herbal perspective is that under the new classification *Echinacea angustifolia* does not exist as a separate species, but becomes a subspecies of *E. pallida*, namely *E. pallida* var.*angustifolia*.[5] However, there is still controversy over this attribution.

Adulteration

Echinacea has been readily adulterated, particularly in the US, by species of Parthenium such as *P. integrifolium*.[6] The roots of *E. angustifolia* and *E. pallida* are very similar both macroscopically and microscopically[7] and are often confused. They can, however, be chemically differentiated.[8]

In the Unites States native species of Echinacea are dwindling in the wild due to loss of habitat and over-harvesting. *E. purpurea* is not as threatened as *E. angustifolia,* since the former is the most widely used species for cultivation.[9] Hence the use of *E. purpurea* root should be encouraged, either alone or preferably in combination with *E. angustifolia* root.

Key constituents

Roots

- Alkylamides (alkamides), mostly isobutylamides (which cause the characteristic tingling in the mouth).[10-13] Largely absent from *E. pallida*.[11]
- Caffeic acid esters: echinacoside (not present in *E. purpurea*), chicoric acid (significant quantities in *E. purpurea* only),[14] cynarin (in *E. angustifolia* only).[15]
- Essential oil;[16] polyacetylenes (including a distinctive series in *E. pallida*);[10,17] polysaccharides,[18] non-toxic pyrrolizidine alkaloids.[19]

Aerial parts

- Alkylamides as above.[20]
- Caffeic acid esters: including echinacoside (not present in *E. purpurea*), chicoric acid (abundant in *E. purpurea*), verbascoside (*E. angustifolia, E. pallida*), caftaric acid (*E. purpurea, E. pallida*), chlorogenic and isochlorogenic acids (*E. angustifolia, E. pallida*).[20]
- Flavonoids,[20] essential oil;[16] polysaccharides (notably in *E. purpurea*).[21]

The phytochemistry of Echinacea has been extensively reviewed, demonstrating considerable variation between species and plant part (as noted above).[22] One key difference is for the alkylamides, which are highest in *E. angustifolia* and absent from *E. pallida*. The roots of *E. angustifolia* and *E. purpurea* contain much higher concentration of alkylamides than their respective aerial parts.[23] Cynarin is found mainly in *E. angustifolia* roots.[23] Qualitative differences also exist for the alkylamides; those in *E. angustifolia* mainly exhibit 2-monoene and dodeca-tetraene structures, whereas the 2, 4-diene and dodeca-tetraene types predominate in *E. purpurea*.[24]

Studies have shown considerable phytochemical variation in Echinacea commercial products, with the concentrations of alkylamides especially exhibiting a particularly wide range.[25] One study found that alkylamides levels in stored *E. purpurea* roots dropped significantly over 64 weeks.[26] However, another found the major alkylamide in *E. purpurea* root hydro-alcoholic extract was quite stable, whereas the alkylamide content reduced over time in the dried powdered root.[27] Degradation of the major alkylamides in *E. purpurea* extracts was monitored under different conditions.[28] Alkylamides degraded faster in dry films than in solution and the Echinacea phenolic compounds acted as protective antioxidants. Predicted half-lives for alkylamides in extracts suggested very good stability.

Polysaccharides in *Echinacea spp* have received considerable research attention as possible immunologically active components. However, much of the research has been conducted on polysaccharides isolated from cell cultures rather than the naturally growing plant. The term "polysaccharide" is generic in nature, and encompasses starches and other potentially inert plant compounds. Indeed, some of the non-specific techniques used to analyse the polysaccharide content of plants also detect simple sugars. Hence, commercial Echinacea products claiming quantified levels of immunologically active polysaccharides should be viewed with caution, depending on the quantification methodology used.

While the polysaccharide research is more relevant to the aerial parts of Echinacea, the roots also contain these compounds. One study isolated arabinogalactan-proteins from the high molecular weight fraction of an aqueous extract of the root of *E. purpurea*.[29]

However, the true polysaccharides (as opposed to starches and sugars misidentified as polysaccharides) appear to be difficult to extract from Echinacea plant parts. An extensive research project isolated 2 polysaccharides from *E. purpurea* aerial parts that were structurally

characterised as a heteroxylan (molecular weight 35,000 Da), and an acidic arabinorhamnogalactan (molecular weight 450,000 Da). Polysaccharide I resembled other xylans found in plants and contained arabinose, xylose and 4-*O*-methylglucuronic acid in a molar ratio of 1:4.9:0.9. Polysaccharide II was composed of arabinose, galactose, glucuronic acid and galacturonic acid in a molar ratio of 0.8:0.6:1:0.6.[30] These polysaccharides were determined at around 15 mg/g in both the root and leaf of the freshly-harvested senescent plant, with less than half this level in root and leaf from the freshly-harvested mature plant. However, storage for just a few days resulted in substantial losses of polysaccharide (PS) II. Any solvent mixture containing more than 40% of ethanol was unable to extract the polysaccharides, while less than 4% of PS I and 17% of PS II was extracted by water at pH 5.5 from any dried plant part. Water extraction of the fresh plant was substantially more efficient for PS II, but PS I was absent from fresh plant root preparations. These results imply that polysaccharides are probably not present in sufficient quantities to influence the pharmacology of aqueous (hydrophilic) extracts of Echinacea dried root (even if the polysaccharides were bioavailable). They will certainly be absent from aqueous-ethanolic (lipophilic) extracts of Echinacea root prepared using more than 40% ethanol, unlike the alkylamides.

Pharmacodynamics

Immune-modulating activity

There is still much to understand about the way Echinacea root impacts the human immune system. Each in vitro study by its nature can provide just a narrow insight into a few specific aspects of immune function, with any clinical relevance potentially confounded by bioavailability, dosage issues and local tissue factors. The in vitro studies probably of most relevance are the ones investigating alkylamides, since these compounds have proven bioavailability (see later).

Indeed, one discussion paper has challenged the relevance of in vitro studies to the study of plant immunostimulants, suggesting that the whole concept may be a myth originating from such studies.[31] (This is of course notwithstanding the traditional uses of Echinacea, which certainly seem to point to enhanced immune function, as noted earlier.) The authors state: "From a historical prospective, these first reports on plant immunostimulants may have "seduced" a whole generation of researchers to adopt uncritically the view that certain plants strengthen the immune system by activating it."[31]

As this discussion paper points out, several in vitro studies of herbs on immune function (and possibly in vivo studies if the extract was administered by injection) have been confounded by endotoxin contamination from bacteria.[31] This is certainly the case for Echinacea, especially if aqueous extracts are used.[32] Based on their findings, one research group asserted that majority of in vitro macrophage activation exhibited by extracts of some immune enhancing plants is due to bacteria lipoproteins and lipopolysaccharides.[33] The majority of such activities for a range of samples of *E. angustifolia* root and aerial parts were abolished by treatment with agents that break down endotoxins.[34]

With these limitations in mind, the in vitro and in vivo data for Echinacea are reviewed below.

In early research, ethanolic extracts of the roots of all 3 main Echinacea species demonstrated an increase in phagocytic activity in vitro and after oral administration in vivo using the carbon clearance test. Of the three tested extracts, *E. purpurea* was the most active, both in vitro and in vivo.[35,36] Lipophilic (fat-soluble) and hydrophilic (water-soluble) fractions of these ethanol extracts also demonstrated activity, although this was weaker than the complete ethanolic extracts. The lipophilic fractions from *E. angustifolia* and *E. pallida* were considerably more active than their hydrophilic fractions, both in vitro and in vivo. In contrast, the hydrophilic fraction of the ethanol extract of *E. purpurea* significantly stimulated phagocytosis in vitro and

showed activity in vivo after oral doses, although not as great as the whole extract. Components of the lipophilic fraction included polyacetylenes, essential oil and alkylamides; the hydrophilic fraction contained caffeic acid derivatives. Polysaccharides were not present.[37] Immunostimulatory principles of Echinacea were therefore said to be present in both the lipophilic and the hydrophilic fractions of a pure ethanolic extract.

Some in vitro studies have compared lipophilic and hydrophilic extracts of Echinacea root. One study used a highly complex experimental design to conclude, on the basis of immunological responses in human whole blood samples, that the main immunostimulating activity of Echinacea plant parts (including root) resided in the water-soluble materials.[38] The authors assumed these water-soluble extracts contained polysaccharides, but did not confirm their presence. Also, no assessment was made of the confounding impact of bacterial endotoxins on their results. Ironically, the authors of this study suggest that their findings are consistent with the traditional use of Echinacea, but have at the same time completely discounted such traditional use by declaring the inactivity of the traditionally preferred lipophilic extracts.

Cytokine antibody arrays were used to investigate changes in pro-inflammatory cytokines released from human bronchial epithelial cells exposed to a rhinovirus.[39] Virus infection stimulated the release of at least 31 cytokine-related molecules and most of these were reversed by simultaneous exposure to the Echinacea extracts. The lipophilic extract of *E. purpurea* root was less active than the expressed juice of the aerial parts in this regard. However, in uninfected cells these cytokines were stimulated by Echinacea, with the lipophilic extract being more active. A follow-up study by the same investigators using gene array analysis suggested that production of various transcription factors involved in proliferative and differentiation signalling pathways were stimulated by the Echinacea extracts.[40]

A comparison of 5 Echinacea species using various in vitro models of immunological activity, such as monocyte cytokine secretion and mononuclear cell proliferation, suggested that the 50% ethanolic tincture of *E. angustifolia* was most active.[32] In another study, the alkylamide-rich Echinacea species were more active at stimulating cytokine production by peripheral blood mononuclear cells (PBMCs) taken from older volunteers 6 months after influenza vaccination.[41] This was confirmed in a second study for blood samples from unvaccinated volunteers.[42] However, cytokines from stimulated PBMCs taken from recently vaccinated individuals were not impacted by *E. purpurea* and *E. angustifolia* root extracts.[42]

Dendritic cells (DCs) are antigen-presenting cells that play a key role in mediating activities of various immune responses. Lipophilic extracts of the alkylamide rich plant parts of *E. purpurea* (root or flower) up-regulated expression of CD83 on DCs in vitro, whereas the leaf extract had the opposite effect.[43] CD83 is a key marker for dendritic cell maturation. Down-regulation of mRNA expression of specific chemokines and their receptors was observed in the leaf-treated DCs, whereas other chemokines and regulatory molecules involved in the c-Jun pathway were up-regulated in root-treated DCs.

Several in vitro studies have investigated the effects of alkylamide fractions or isolated alkylamides in immune models. Alkylamides inhibited lipopolysaccharide (LPS)-mediated activation of a murine macrophage line (anti-inflammatory activity).[44-46] They also countered the decrease in NF kappaB production by LPS-stimulated T cells, in vitro, as did chicoric acid,[47] and reduced IL-2 production in stimulated T cells.[48] Liver enzyme-mediated oxidation of *E. purpurea* alkylamides produced metabolites that were less active in terms of suppressing IL-2 secretion by stimulated T cells.[49]

Cynarin binds to CD28 in vitro, a receptor on T cells, and down-regulated CD-28 IL-2 expression in a T cell culture line.[50] Cynarin also effectively blocked the binding between CD80 on B cells and CD28 on T cells, thereby potentially exerting an immune modulatory effect.[51]

In early in vivo research, chicoric acid or an enriched alkylamide fraction from *E. angustifolia* and *E. purpurea* roots increased phagocytic activity after oral doses. Extracts of the aerial parts of the three species demonstrated lower activity than that of the roots.[36] In contrast, echinacoside from *E. angustifolia* and *E. pallida* roots (which is often used as a quality marker for these species) did not demonstrate immune-enhancing activity.[35]

A significant proportion of the in vivo investigations of Echinacea root (specifically *E. purpurea*) have been conducted by the research team of Sandra Miller in Canada. In an unusually entitled paper: *Echinacea: a Miracle Herb against Aging and Cancer*, Dr Miller reviewed her team's research on Echinacea, specifically *E. purpurea* root.[52] Their interest in Echinacea was triggered by research on the drug indomethacin, which is a cyclo-oxygenase inhibitor that reduces the endogenous suppressors of natural killer (NK) cells, namely the prostaglandins.[53,54] The drug resulted in statistically significant increases in NK cell numbers and functions in leukaemic mice. This led to the search for a safe agent without dangerous side effects that might function in the same way.

The observation that alkylamides in Echinacea can inhibit prostaglandin production in vitro, and the general reputation of Echinacea as an immune herb, led to the investigation of the potential of Echinacea in NK cell enhancement using in vivo laboratory models. In healthy young adult mice, oral doses of *E. purpurea* root (0.45 mg/ 25 g body weight, similar to human dose rates) stimulated NK cell production by bone marrow in the first 7 days, which resulted in significantly higher levels (around 25% more) of NK cells in the spleen by 2 weeks.[55] In addition, the accessory cells for NK cells, the monocytes, were also about 25% more numerous in both the

bone marrow and spleen of mice consuming Echinacea. The Echinacea treatment influenced no other white blood cell counts. Polysaccharides, even by injection, were not found to be responsible for this effect.[56]

NK cells decline in number and function with age and this is thought to be one factor behind the increase of various cancers with age. Experiments conducted in healthy, elderly mice found that 2 weeks of oral doses of Echinacea root returned NK cell numbers in bone marrow and spleen to the levels of young adults and also resurrected their functional capacity (target cell binding, lysis).[57] On this result Dr Miller writes:

"These observations appear to apply uniquely to this herb since we could never rejuvenate the NK cell-mediated component of the immune system in elderly mice by any of the other typical NK cell enhancers ..."

One of the persistent controversies about Echinacea is whether it is safe to be taken consistently for long periods of time. The answer, at least in mice, appears to be in the affirmative. Mice were fed *E. purpurea* root from 7 weeks of age to 13 months at the dose previously described.[58] Long-term use of Echinacea was in fact beneficial. By 13 months of age 46% of the control mice fed the standard chow were still alive, compared with 74% of those consuming Echinacea. As in previous experiments, the NK cell levels in the Echinacea-fed mice were considerably elevated compared with controls. On this Miller writes:

"Given that the key immune cells acting as the first line of defence against developing neoplasms in mice and humans are NK cells, it is not difficult to conclude that sustained enhancement of NK cells alone, throughout life, could readily account for the reduced frequency in deaths with advancing age. Spontaneous neoplasms, clinically undetectable, are well known to increase with advancing age in humans and mice. Thus, the logical corollary from this study indicates that

chronic daily intake of Echinacea, is clearly not detrimental to the immune system, but rather prophylactic."

Finally, the team investigated the question of whether Echinacea is effective once a cancer is in progress. Leukaemias and lymphomas are well known as targets for NK cell attack, and these cells are established as the first line of defence against these types of malignancies. Leukaemia-induced mice typically died after 3.5 weeks, whereas one-third of mice additionally fed Echinacea survived until 3 months after leukaemia onset and went on to live a normal lifespan.[59]

While these findings need to be regarded as work in progress, the implications are intriguing. In addition to helping to dispel some of the common myths about what is active in Echinacea and how it is best used (including the contraindication in leukaemia from the German Commission E), the research provides a possible insight into perhaps one of the key aspects of Echinacea's mode of action on the immune system. This is the boost in NK and monocyte number and function. NK cells and monocytes represent the innate immunity. Any agent acting largely on innate immunity will be beneficial in a range of areas including infection prevention and therapy, cancer prevention and therapy and even autoimmune disease (if stealth pathogens are involved as triggers via processes such as molecular mimicry). Above all, the consistent use of an agent that promotes innate immunity could well extend lifespan, since immunosenescence is a key aspect of poor health with ageing.

The NK cell connection for Echinacea root needs to be confirmed in humans.

The impact of *E. purpurea* root extract (2 mg/mouse/day) consumption was investigated in non-obese diabetic (NOD) mice, which are a model of human type 1 diabetes. NKT(natural killer T) cells are believed to be implicated in type 1 diabetes and their functional and/or numerical deficiency is thought to be largely responsible for the development of this disease in NOD

mice.[60] When NOD mice were fed Echinacea for varying times there was a substantial and significant increase in NK cell numbers. This was the only type of immune cell influenced by the Echinacea in these mice. The authors concluded:

"The observations of the present study have, at least in the animal model of human type 1 diabetes, led to 2 conclusions. First, daily consumption of Echinacea by animals afflicted with this particular autoimmune disease, leads to no negative repercussions, and indeed, may provide all the advantages, in vivo, that consuming this herb does for normal, unafflicted mice (humans). Second, the study may provide evidence for a possible new approach to the treatment of type 1 diabetes. That is, immuno-stimulation only of those cells (NK/NKT) involved in modulating the disease. Echinacea is one such uniquely tailored, immunostimulant, whose effect is on NK cells."

Other in vivo studies support the activity of Echinacea root on the macrophage/monocyte system. *E. purpurea* demonstrated a dose-dependent increase in the phagocytic activity of alveolar macrophages after oral administration to rats.[61] Alkylamides (12 mcg/kg/day, oral) significantly increased phagocytic activity and the phagocytic index of alveolar macrophages in rats.[62] These alveolar macrophages also produced significantly more TNF-alpha and nitric oxide after LPS stimulation in vitro. Chicoric acid and polysaccharides (oral doses) were not as active.

Rats given *E. angustifolia* root extract (equivalent to 3.3 g/L root in drinking water) for 6 weeks demonstrated a significantly higher primary and secondary IgG response to a novel antigen.[63] In contrast, 6 weeks of various Echinacea root extracts (tinctures and in glycerol) showed no activity in male rats in terms of NK cell activity, T cell-mediated delayed-type hypersensitivity, or specific antibody formation.[64] *E. purpurea* root at 2 and 4% of diet did not exhibit antiviral effects or show any evidence of immune enhancing properties in respiratory syndrome virus-infected nursery pigs.[65]

Alcohol extracts from roots of the 3 widely used Echinacea species, *E. angustifolia*, *E. pallida*, and *E. purpurea*, were investigated for immunomodulating properties.[66] Mice were gavaged once a day (for 7 days) with one of the Echinacea extracts (130 mg/kg) or vehicle and immunised with sheep red blood cells (sRBC) 4 days prior to collection of immune cells for multiple immunological assays. The 3 herb extracts induced similar, but different, changes in the percentage of immune cell populations and their biological functions, including increased percentage of CD49+ and CD19+ lymphocytes in spleen and NK cell cytotoxicity. The antibody response to sRBC was significantly increased equally by extracts of all 3 Echinacea species. Concanavalin A-stimulated splenocytes from *E. angustifolia*- and *E. pallida*-treated mice demonstrated significantly higher T cell proliferation. In addition, the Echinacea treatment significantly altered the cytokine production by mitogen-stimulated splenic cells. The 3 herbal extracts significantly increased interferon-gamma production, but inhibited the release of tumour necrosis factor- alpha and interleukin (IL)-1 beta. Only *E. angustifolia*- and *E. pallida*-treated mice demonstrated significantly higher production of IL-4 and increased IL-10 production. The authors suggested Echinacea is a wide-spectrum immunomodulatory agent that modulates both innate and adaptive immune responses. In particular, *E. angustifolia* or *E. pallida* may have more anti-inflammatory potential.

Following the observation that *E. angustifolia* and *E. purpurea* root extracts reduced cytokine production and intracellular killing activity by macrophages in vitro (although *E. angustifolia* enhanced bacterial phagocytosis), these effects were investigated in mice.[67] The Echinacea root extracts (130 mg/kg/day for 7 days, oral) did not substantially influence nitric oxide (NO) production and phagocytosis by LPS-stimulated peritoneal exudate cells (PECs) extracted from the mice. In addition, the anti-bacterial function of PECs was not affected, although NO production was somewhat decreased.

Twenty-four healthy men received 4.5 mL per day of standardised alcoholic extract of *E. purpurea* root (equivalent to 1 mg each of chicoric acid and alkylamides per day) or placebo for 5 days. A maximum stimulation of granulocyte phagocytosis was observed on the fifth day at 120% of the starting value. The rate of immune stimulation was much higher than that observed for administration by intramuscular injection.[68]

This study has been widely misinterpreted as demonstrating that Echinacea causes immune system tachyphylaxis if taken for more than a few days. A cursory examination of the figures published in this paper might lead to the conclusion that the use of Echinacea for more than a few days depletes the phagocytic response. However, this would be a misinterpretation of the results. The arrows at the bottom of the figures indicated the application of the test dose, which was administered for only the first 5 days. While the Echinacea was given, phagocytic activity remained high. Only when the Echinacea was stopped did the phagocytic activity decline to normal levels, a typical washout effect.

So the study in fact demonstrated the following:

- Phagocytic activity remained higher than normal while Echinacea was given.
- Oral doses of Echinacea stimulated phagocytic activity more than injected doses.
- When Echinacea was stopped, phagocytic activity remained well above normal for a few days, indicating that far from causing depletion, there was a residual stimulating effect when Echinacea was stopped.
- Phagocytic activity only returned to normal, that is, there was no depleting effect where activity drops to less than normal.

In terms of other probable misconceptions from the Echinacea research, immune-enhancing activity has been demonstrated for Echinacea polysaccharides in vitro.[69-72] However, these

results may not be translatable to effects in a living organism after oral administration because of the gastrointestinal breakdown, poor absorption and poor tissue mobility of the large polysaccharides. As noted previously, polysaccharides are probably not present in pharmacologically significant quantities in Echinacea preparations and are not absorbed in levels sufficient to achieve the concentrations used for in vitro studies.

In the only clinical trial to date on Echinacea polysaccharides, they were administered by injection because the uncertainties over their oral bioavailability. If the trial scientists had believed that the polysaccharides were orally active, then they would certainly have administered them by this simpler way. In this open, prospective study with matched historical controls, a polysaccharide fraction isolated from *Echinacea purpurea* cell cultures was tested to see if it could counter the undesired side effects of cancer chemotherapy.[73] Fifteen patients with advanced gastric cancer undergoing palliative chemotherapy with a range of cytotoxic drugs also received daily intravenous injections of 2 mg of a polysaccharide fraction from Echinacea. While the polysaccharide treatment did appear to increase white cell counts, there were no clinically relevant effects on phagocytic activity or lymphocyte subpopulations. The authors suggested that this form of treatment should be investigated in further studies.

Interestingly, there is a new voice in the Echinacea polysaccharide debate. An LPS-free preparation of a commercial aqueous extract of *E. angustifolia* has demonstrated immune activity in vitro and in vivo.[74-76] For example it reduced *Candida albicans*-induced mortality in both normal and in cyclosporine-A-treated mice.[76] This polysaccharide-targeted extract is being marketed as having superior immune activity to the traditional lipophilic extracts of *E. angustifolia* because it contains only low levels of alkylamides, which are being represented as inhibiting T cell function. Such assertions would be best supported by human data.

Excessive extrapolations of the early pharmacological research, especially the in vitro studies on Echinacea polysaccharides, have led to unsupported statements concerning the immunological activity of Echinacea. These include that Echinacea is mitogenic to T-lymphocytes, that ethanolic extracts of Echinacea are ineffective, that Echinacea will accelerate pathology in HIV/AIDS and that Echinacea will aggravate asthma.[77]

Cannabinoid receptor activity

A significant discovery, first presented at the International Congress on Natural Products 2004, was the observation by 2 separate research teams that the immune effects of Echinacea may be mediated by the interaction of Echinacea alkylamides with cannabinoid receptors. Two papers were presented on this topic. A Swiss research team found that an in vitro immune-modulating effect of a lipophilic *E. purpurea* extract (and individual alkylamides) on monocytes/ macrophages could be neutralised by the presence of agents that block CB2 cannabinoid receptors.[78] Bauer, in collaboration with US scientists, found that alkylamides from *E. angustifolia* bound to both CB1 and CB2 cannabinoid receptors.[79] In particular, certain alkylamides exhibited selectivity for CB2 receptors. The most potent binding to CB2 receptors (binding as strongly as THC from Cannabis) was exhibited by a monoene alkylamide found only in *E. angustifolia* (tetradeca-2E-ene-10,12-diynoic acid isobutylamide).[80]

CB1 receptors are highly localised in the central nervous system (CNS) and are believed to primarily modulate behaviour, while CB2 receptors predominate in immune tissues outside the CNS, especially in the spleen, and are believed to modulate immune function.[81] Cannabinoid receptors are remarkably preserved across the animal kingdom, which suggests they play an important developmental and physiological role.[82,83] Much of the immune activity of the cannabinoid system appears to be mediated by the cytokine network. Cytokines include the interleukins (IL-3, IL-6, etc), tumour necrosis factor alpha (TNF-alpha) and the interferons

(IFN). Both receptors mediate analgesic effects and CB2 effects can be mainly classed as anti-inflammatory.

The Swiss team mentioned above followed on from this ground-breaking research and confirmed that certain Echinacea alkylamides bind strongly to CB2 receptors.[84] In addition they have found that alkylamides exert additional effects on immune cells that are independent of CB2.[84] Their research has been particularly insightful into one aspect of the mode of action of Echinacea alkylamides.[85] A lipophilic extract of *E. purpurea* strongly stimulated TNF-alpha mRNA synthesis in peripheral monocytes, but not TNF-alpha protein production. In other words, the Echinacea-induced new TNF-alpha transcripts (mRNA) were not translated into TNF-alpha itself. When monocytes were treated with LPS (lipopolysaccharide or endotoxin, a powerful stimulator of the immune system) TNF-alpha protein production was substantially increased. However, co-incubation of monocytes with LPS and Echinacea extract resulted in a strong inhibition of this effect of LPS. Investigation over a longer time-span revealed that the lipophilic Echinacea extract, via interaction with CB2 receptors, modulated and prolonged TNF-alpha production following immune stimulation. The results of this study suggest that Echinacea acted more as a modulator or facilitator of the immune response, rather than as an immune stimulant. In resting monocytes it prepared them for a quicker immune response by inducing TNF-alpha mRNA. However, in overstimulated monocytes (as in the case of LPS) it first reduced and then extended their response in terms of TNF-alpha production. In particular, these key findings challenge the concept that traditional Echinacea extracts will "overstimulate and wear out" the immune system if taken continuously.

One point worth emphasis is the strong binding (and partial agonist effects) of certain Echinacea alkylamides at CB2 receptors is not shared by alkylamides from other plant species. As well as the monoene mentioned above, the tetra-ene alkylamides from both *E. purpurea* and *E. angustifolia*, especially the ZZ isomer, also bind strongly to the CB2 receptor.[86] The binding

of both synthetic and natural alkylamides to type 1 and 2 CB receptors was investigated in vitro.[87] Naturally occurring alkylamides from herbs such as maca *(Lepidium meyenii)* and *Spilanthes spp* showed a poor affinity for both CB1 and CB2 receptors. In contrast, the above alkylamides from *E. angustifolia* and *E. purpurea* bound more strongly to CB2 receptors than the endogenous cannabinoid anandamide.[84] Anandamide binds strongly to CB1 (as strongly as THC from Cannabis), whereas the Echinacea alkylamides only have a weak affinity for CB1 receptors (but perhaps sufficient to promote the sense of well-being that many patients report after taking Echinacea consistently).

An ethanolic root and herb extract of *E. purpurea* produced synergistic pharmacological effects at the CB2 receptor.[88] In particular, superadditive effects of alkylamide combinations were seen at the level of intracellular calcium release as a consequence of CB2 receptor activation.

A review of fatty acid amides and the human endocannabinoid system noted that alkylamides also partially inhibit the action of fatty acid amide hydrolase (FAAH), which controls the breakdown of endocannabinoids.[89] Hence they could exert also indirect agonistic effects at CB2 receptors. The CB2 receptor binding of alkylamides and their FAAH inhibition do not correlate.

An alkylamide from *E. angustifolia* that lacked affinity for the CB2 receptor inhibited IL-2 secretion in T cells through activation of PPARgamma, suggesting that cytokine modulation by alkylamides is not just due to CB2 effects.[90]

Antiviral activity

Given that the immune system attacks viruses, there is a degree of overlap between the antiviral and immune system activities of Echinacea root. In fact, it is most likely that any direct effects of Echinacea root on disabling viruses are relatively modest.

In early research, chicoric acid demonstrated antiviral activity against vesicular stomatitis viruses in vitro.[91] Later chicoric acid was found to inhibit HIV-1 integration into a host chromosome and was a non-competitive but reversible inhibitor of HIV-1 integrase in vitro.[92] Purified root extracts from the three *Echinacea species* demonstrated antiviral activity towards herpes simplex virus (HSV) and influenza virus in vitro. An indirect antiviral effect was also observed via stimulation of alpha- and beta-interferon production.[93]

Extracts of 8 species of Echinacea were found to have antiviral activity against HSV-1 in vitro when exposed to visible and UV light.[94] n-Hexane extracts of roots containing alkylamides and alkenes were more active than ethyl acetate extracts containing caffeic acids. Potent inhibitors included chicoric acid (MIC 45 mcg/mL) and *E. purpurea* root n-hexane extract (MIC 120 mcg/mL).

Interferons are cytokines that limit viral replication. The influence of Echinacea root extracts on virus-induced cell death and interferon secretion was investigated using HSV infection in murine macrophages.[95] Cells incubated with extracts prior to infection showed very modest enhancement of viability, and no increase in the secretion of interferons alpha or beta compared with control cells. Virus-infected macrophages treated with extracts from *E. purpurea* showed a small (<2-fold) induction of guanylate binding protein production, but no effect of extracts from other species was observed. In virus-infected cells, all the extracts increased the amount of inducible nitric oxide synthase (iNOS) protein, and this effect varied by type of extraction preparation. Together, these results suggested that any potential antiviral activities of Echinacea root extracts are likely not mediated through large inductions of interferon, but may involve iNOS.

Anti-inflammatory and wound-healing activity

In early research an anti-inflammatory effect was observed after the topical application of a crude polysaccharide fraction from *E. angustifolia* roots in the croton oil mouse ear test.[96] Topical application of an extract of *E. angustifolia* root inhibited oedema in the croton oil mouse ear test both at the maximum (6 h) and in the decreasing phase (18 h). Echinacea was more potent than the topical NSAID benzydamine.[97] Alkylamides from Echinacea demonstrated inhibitory activity against cyclo-oxygenase (COX) and 5-lipoxygenase (LOX) in vitro. The structure of the alkylamide determined the degree of activity, and most alkylamides were more active on COX-1 than COX-2.[98,99]

Alkylamides isolated from *E. angustifolia* root also inhibited COX-2-dependent prostaglandin E_2 (PGE_2) formation in vitro in human neuroglioma cells, but did not inhibit COX-2 expression at the transcriptional or translational level.[100] Extracts of the roots from 4 of 6 Echinacea species at 15 mcg/mL inhibited PGE_2 production by macrophages.[101] Synergy between alkylamides was suggested to be largely responsible. Ketones from Echinacea were also found to contribute to this activity.[102]

See also under Immune-modulating activity above for additional studies demonstrating anti-inflammatory effects for Echinacea root and its components.

Antimicrobial activity

In early research echinacoside demonstrated weak antimicrobial activity against *Staphylococcus aureus* in vitro.[103] Polyacetylenes from *E. angustifolia* and *E. purpurea* root also demonstrated bacteriostatic and fungistatic activity against *E. coli* and *Pseudomonas aeruginosa*.[17] *E. angustifolia* extract showed weak inhibitory activity in vitro against *Trichomonas vaginalis*[104] and *E. purpurea* extract inhibited the growth of *Epidermophyton interdigitale* in vitro.[105]

Results from an in vitro study suggest that any antifungal activity of *E. purpurea* root extract could be the result of disruption of the fungal cell wall.[106] Both lipophilic and hydrophilic extracts of Echinacea (including a lipophilic extract of *E. angustifolia* root) exhibited dose-dependent antileishmanial and trypanocidal activities in vitro.[107] Differences in antiadhesion activity against *Campylobacter jejuni* in vitro were found for the 2 main Echinacea species, with *E. purpurea* root displaying higher activity than *E. angustifolia* root.[108]

Other effects

Caffeic acid esters obtained from *E. angustifolia* root demonstrated antihyaluronidase activity in vitro.[109] The possible antihyaluronidase activity may help increase the resistance of tissue to the spread of certain infections and, in conjunction with the increased presence of fibroblasts, facilitate connective tissue regeneration. This effect would most likely be observed for topical application of Echinacea preparations.

Caffeic acid esters protected collagen from free radical damage in vitro. The protection occurred via a scavenging effect on reactive oxygen species. The authors concluded that topical Echinacea preparations may be useful in the prevention or treatment of photodamage of the skin by ultraviolet radiation.[110]

A synergistic antioxidant effect on human low-density lipoprotein was demonstrated in vitro for alkylamides, caffeic acid derivatives and polysaccharide fractions from *E. purpurea* root.[111]

Young rats were fed *E. purpurea* root extract (50 mg/kg) for 30 and 60 days.[112] Assessment of prostate glands indicated a decrease in prostrate weight and an increase in tissue lymphocytes. Results were more marked after 60 days of treatment. The same research group followed up these findings using an experiment rat model of benign prostatic hyperplasia (BHP).[113] Using the

same dose and treatment times as above, *E purpurea* root extract progressively reduced prostrate size and degenerative changes.

The anxiolytic activities of 5 different Echinacea preparations were investigated in mice.[114] Most consistently effective was a lipophilic extract of *E. purpurea* root, active at oral doses of 4 mg/kg. The authors described its anxiolytic potential as "considerable".

Splenic lymphocytes from mice treated with *E. purpurea* root extract and *Hypericum perforatum* herb extract (both at 30 and 100 mg/kg/day for 14 days, oral) were shown to be significantly more resistant to apoptosis.[115]

Hexane extracts of Echinacea root demonstrated cytotoxic activity against human pancreatic and colon cancer cells in vitro.[116] *E. pallida* was the most active, with the polyacetylenes exhibiting a significant portion of such activity.[117]

Pharmacokinetics

Using the Caco-2 monolayer as an in vitro model of intestinal permeability, the tetraene alkylamides demonstrated rapid passive diffusion across this artificial membrane.[118] A later study using the Caco-2 monolayer confirmed the ready permeability of both diene and monoene Echinacea alkylamides and also found that caffeic acid derivatives (caffeoylquinics) such as chicoric acid and echinacoside exhibited poor permeability.[119]

Alkylamides have also exhibited significant bioavailability in vivo. The tetraene alkylamides exhibited rapid absorption after a single oral dose in rats (2.5 mg/kg) and appeared in the brain within 8 minutes.[120] The Cmax in plasma was 26.4 ng/mL, while the Cmax in different brain regions varied between 33.8 and 46.0 ng/mL. This provides clear evidence that Echinacea

alkylamides cross the blood-brain barrier. A study in rats found that the absolute bioavailability of a tetraene alkylamide was 29.2%, which was increased to 47.1% by administration as part of a 60% ethanol *E. purpurea* root extract.[121] However, the administration of the Echinacea extract had no impact on the Cmax of the alkylamide, instead increasing blood exposure by prolonging half-life.

The majority of the pharmacokinetic studies on Echinacea, however, have been human trials. The first trial dates from Germany in 2001, where a tetraene alkylamide was detected in the blood of a single healthy volunteer after a 65 mL dose of a concentrated *E. purpurea* mother tincture.[122] Two more comprehensive studies were published close together in 2005. The first, a follow-up from the German study, used a single 2.5 mL dose of a 60% ethanol *E. angustifolia* root extract in 11 healthy volunteers.[123] The maximum concentration reached by the tetraene alkylamides was 10.9 ng/mL and occurred after 30 minutes. The second study was Australian and examined the pharmacokinetics in 9 healthy volunteers of a single dose of a tablet preparation containing lipophilic extracts of *E. purpurea* and *E. angustifolia*.[124] Caffeic acid conjugates could not be identified in any plasma sample at any time after tablet ingestion. Alkylamides were rapidly absorbed and remained detectable for up to 12 h. Tmax occurred at 2 to 3 h and Cmax was 336 ng/mL for the sum of alkylamides. There was no difference observed in alkylamide absorption between fasted volunteers and those who consumed a high fat breakfast prior to intake. Another study by the German team using a different preparation administered as a single dose to 8 healthy volunteers found considerably lower Cmax values for alkylamides, probably as a reflection of the lower doses given.[125]

Another pharmacokinetic study from the Australian team compared the relative bioavailabilities of single doses of a tablet and a liquid preparation of *E. purpurea* and *E. angustifolia* extracts delivering the same dose of alkylamides.[126] Alkylamides were rapidly absorbed from both preparations, with no quantitative difference between the two. Tmax increased from 20 minutes

for the liquid to 30 minutes for the tablet. The same team also investigated pharmacokinetic parameters following repeated doses of the Echinacea tablets (2 tablets twice a day for 14 days) in 6 healthy volunteers.[127] There was no evidence for either the induction or inhibition of alkylamide metabolism, as evidenced by a consistent elimination half-life of around 1.5 h and similar Cmax values (of around 100 ng/mL) at the beginning and the end of the trial.

The relative bioavailability of the major alkylamides, the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides, from *E. purpurea* root lozenges at 3 different dosage levels (0.07, 0.21 and 0.9 mg) was evaluated in a human pharmacokinetic study.[128] Alkylamides were found to be rapidly absorbed and measurable in plasma 10 minutes after administration of 0.21 and 0.9 mg via the lozenges and remained detectable for more than 3 h for the latter. Results of pharmacokinetic analysis revealed that a Cmax of 8.88 ng/mL was reached at 19 minutes with the 0.9 mg lozenges. Other results suggested that a fraction of the alkylamides was directly absorbed through the oral mucosa.

Following their initial pharmacokinetic study indicating a substantial first pass metabolism of alkylamides, the Australian group investigated the in vitro hepatic metabolism of these compounds using human liver microsomes.[129] No significant degradation of alkylamides was evident in cytosolic fractions. Time-and NADPH-dependent degradation of alkylamides was observed in microsomal fractions, suggesting they are metabolised by cytochrome P450 (P450) enzymes in human liver. There was a difference in the susceptibility of monoene and diene pure synthetic alkylamides to microsomal degradation, with (2E)-*N*-isobutylundeca-2-ene-8,10-diynamide (compound 1, a monoene) metabolised to only a tenth the extent of the diene (2E,4E,8Z,10Z)-*N*-isobutyldodeca-2,4,8,10-tetraenamide (compound 2) under identical incubation conditions. Markedly less degradation of the diene was evident in the mixture of alkylamides present in an ethanolic Echinacea extract, suggesting that metabolism by liver P450s was dependent both on their chemistry and the combination present in the incubation. Co-

incubation of compound 1 with 2 at equimolar concentrations resulted in a significant decrease in the metabolism of compound 2 by liver microsomes. This inhibition of metabolism by the monoene, which has a terminal alkyne moiety, was found to be time- and concentration-dependent, and due to a mechanism-based inactivation of the P450s involved. Alkylamide metabolites were detected and found to be the predicted epoxidation, hydroxylation and dealkylation products. The monoene alkylamides are predominant in *E. angustifolia* root.

Hence, put in simple language, the bioavailability of alkylamides in *E. angustifolia* root can be expected to be better than for *E. purpurea* root because of reduced first pass hepatic metabolism. Moreover, a combination of *E. angustifolia* with *E. purpurea* could be expected to improve the bioavailability of the *E. purpurea* alkylamides.

Further metabolic studies evaluated the human cytochrome P450 enzymes involved in the metabolism of an alkylamide mixture using recombinant P450s, human liver microsomes and a pure synthetic compound.[130] Epoxidation, N-dealkylation and hydroxylation products were detected, with different relative amounts produced by recombinant P450s and microsomes. The major isoforms showing activity toward the metabolism of N-isobutyldodeca-2E,4E,8Z,10Z-tetraenamide were CYP1A1, CYP1A2 (both producing the same epoxide and N-dealkylation product), CYP2A13 (producing 2 epoxides), and CYP2D6 (producing 2 epoxides and a hydroxylated metabolite). Several other forms showed less activity. In incubations with human liver microsomes and selective inhibitors, CYP2E1 was found to be principally responsible for producing the dominant, hydroxylation product, whereas CYP2C9 was the principal source of the epoxides and CYP1A2 was responsible for the dealkylation product.

Clinical trials

Treatment of upper respiratory tract infections

There are relatively few clinical trials of Echinacea root in the treatment of acute respiratory infections and the results of such trials are mixed. Acute viral respiratory infections were certainly not the mainstay of the traditional use of Echinacea root by the Eclectic physicians. This common conception (or perhaps misconception) of the role of Echinacea has developed in modern times, probably as an extrapolation of its immune system reputation and driven by companies wishing to exploit a ready over-the-counter sale. Such a popular use of Echinacea also fitted in well with the ill-advised notion that the herb could only be taken for a few days at a time. In fact, the clinical trial evidence is considerably more supportive of the value of Andrographis in the treatment of colds and mild influenza (see the Andrographis monograph).

However, it should be noted that several trials (both positive and negative) have been excluded from this monograph because they used the aerial parts, sometimes in combination with the root. One exception is a Canadian product from *E. purpurea* that may include aerial parts (see below). This was included because its phytochemical profile resembles one that could be achieved from the root alone.

The issue of the use of differing plant parts, with their substantially different phytochemical profiles, calls into question the value of most published systematic reviews and meta-analyses of Echinacea trials for the treatment of respiratory infections, including the Cochrane review.[131] In other words, the heterogeneity of the different treatments works against any meaningful analysis.

In an early randomised, double-blind placebo-controlled trial, 180 patients with upper respiratory tract infections received the equivalent of 1800 mg/day or 900 mg/day of *E. purpurea* root as a tincture, or a placebo. Patients receiving the high dose experienced significant relief of symptoms. Patients receiving the lower dose were not significantly different from the control.[132]

(Based on this trial result, the therapeutic dose for a 1:5 tincture of *E. purpurea* root would therefore begin at 9 mL/day during an infection.)

The Canadian product mentioned above has been assessed in 2 clinical trials. This is a liquid formulation prepared from "various parts" of freshly harvested *E. purpurea* and contains 0.25, 2.5 and 25 mg/mL respectively of alkylamides, chicoric acid and polysaccharides. (No information was provided on how the "polysaccharides" were measured. However, since the liquid was formulated in 40% ethanol the presence of true polysaccharides is questionable.) In the first trial, 282 people were randomly assigned to Echinacea or placebo using a double blind design.[133] At the onset of the first symptom related to a cold they took 10 doses on the first day (4 mL each) and 4 doses/day thereafter for 6 days. A total of 128 participants contracted a common cold (59 Echinacea, 69 placebo) and the total daily symptom scores were 23.1% lower in the Echinacea group ($p<0.01$). However, Echinacea did not impact the duration of symptoms. The herbal treatment was well tolerated.

In the second double blind, placebo-controlled trial, 150 volunteers were recruited and randomised. Of these, 62 participants (26 Echinacea, 36 placebo) contracted a common cold and completed the study.[134] The timing and doses were similar to above, with 8 doses on the first day (5 mL each) and 3 doses/day thereafter for 6 days. A modest decrease in daily symptoms was more evident in the Echinacea group ($p<0.05$) and Echinacea use was associated with a significant and sustained increase in circulating white blood cells, monocytes, neutrophils and NK cells.

In a well-designed trial involving 719 patients, a combination of *E. purpurea* and *E. angustifolia* roots standardised to 4.2 mg alkylamides/tablet did not substantially alter the course of the common cold.[135] Patients were assigned to 1 of 4 parallel groups: no tablets, placebo tablets (blinded), Echinacea tablets (blinded), or Echinacea tablets (unblinded, open-label). Echinacea

groups received the equivalent of 10.2 g of dried Echinacea root during the first 24 hours and 5.1 g during each of the next 4 days. The primary outcome was the area under the curve for global severity, with severity assessed twice daily by self-report using the Wisconsin Upper Respiratory Symptom Survey (WURSS), short version. Secondary outcomes included interleukin-8 levels and neutrophil counts from nasal wash, assessed at intake and 2 days later.

Of the 719 patients enrolled, 713 completed the protocol. Mean global severity was 236 and 258 for the blinded and unblinded Echinacea groups, respectively; 264 for the blinded placebo group; and 286 for the no-pill group. A comparison of the 2 blinded groups showed a 28-point trend (95% CI, -69 to 13 points) toward benefit for Echinacea (p=0.089). Mean illness duration in the blinded and unblinded Echinacea groups was 6.34 and 6.76 days, respectively, compared with 6.87 days in the blinded placebo group and 7.03 days in the no-pill group. A comparison of the blinded groups showed a nonsignificant 0.53-day (C1, -1.25 to 0.19 days) benefit (p=0.075). Median change in interleukin-8 levels and neutrophil counts were also not statistically significant.

In a randomised, double blind, controlled trial, a total of 154 patients (133 analysed in the per protocol collective) with acute sore throat present for not more than 72 hours were included in the study. They used either an Echinacea/sage spray or a chlorhexidine/lidocaine spray with two puffs every 2 hours, in a double-dummy blinded manner, up to 10 times daily until they were symptom-free, for a maximum of 5 days. The main outcome measure was the comparison of response rates during the first 3 days. A response was defined as a decrease of at least 50% of the total symptoms compared to baseline. The Echinacea/sage treatment exhibited similar efficacy to the chlorhexidine/lidocaine treatment in reducing sore throat symptoms during the first 3 days. Response rates after 3 days were 63.8% in the Echinacea/sage group and 57.8% in the chlorhexidine/lidocaine group. For all secondary parameters, such as time to becoming symptom

free, throat pain, and global assessments of efficacy by the physician and patient, no difference between the 2 treatments was seen. They were both very well tolerated.[136]

Infection prevention

Given the difficulties involved in undertaking infection prevention studies, the data on Echinacea root in this regard are reasonably good (especially if the experimental rhinovirus study is excluded). This is certainly consistent with the clinical experience of the authors (see also Chapter 8). In fact, the main value of Echinacea root probably lies in its capacity to prevent acute infections and resolve subacute or chronic infections, rather than to alter the course of short acute infections such as the common cold (where the immune system is probably functioning at its maximum anyway).

The safety and efficacy of two root extracts of Echinacea (*E. purpurea* or *E. angustifolia*) for preventing upper respiratory tract infections were assessed in a 3-armed, randomised, double blind, placebo-controlled trial involving 302 healthy volunteers over 12 weeks.[137] Although there was a numerical tendency towards a lower infection rate in both Echinacea groups, statistical significance was not achieved. Participants in the treatment groups believed they had more benefit from Echinacea than those in the placebo group (p=0.04). Adverse effects reports were 18 for *E. angustifolia*, 10 for *E. purpurea* and 11 for placebo. One problem with this trial was the relatively low doses of Echinacea used (about 200mg of dried root per day).

Three preparations with distinct phytochemical profiles were produced by extraction of *E. angustifolia* roots with supercritical carbon dioxide, 60% ethanol or 20% ethanol.[138] A total of 437 volunteers were randomly assigned to receive either prophylaxis (beginning 7 days before the virus challenge) or treatment (beginning at the time of the challenge) either with one of these preparations or with placebo. The results for 399 volunteers who were challenged with

rhinovirus type 39 and observed in a sequestered setting for 5 days were included in the data analysis.

There were no statistically significant effects of any of the 3 Echinacea extracts on rates of infection or severity of symptoms. Similarly, there were no significant effects of treatment on the volume of nasal secretions, on polymorphonuclear leukocyte or interleukin-8 concentrations in nasal-lavage specimens, or on quantitative-virus titre. The dose of *E. angustifolia* root used was 900 mg/day and the dose was not adjusted for the acute infection phase of the study. This is a relatively low dose for either prevention or treatment.

In an unpublished study presented by the late Dr Anna Macintosh at the 1999 Convention of the American Association of Naturopathic Physicians, an Echinacea root formulation was compared against a herbal adaptogenic formulation and a placebo in the prevention of winter colds over a 90-day period.[139] The trial recruited 260 medical students who were under stress from their studies. The placebo group averaged an infection rate of 10%, whereas this dropped to as low as 2% by day 70 (p=0.013) in the Echinacea group. The daily dose of Echinacea root was 1.7 or 3.5 g (two doses were consecutively trialled in the study).

A randomised, double blind placebo-controlled clinical trial was undertaken with 175 participants travelling return from Australia to America, Europe or Africa for a period of 1 to 5 weeks on commercial flights via economy class.[140] Participants were administered *E. purpurea* and *E. angustifolia* extract tablets (containing the equivalent of 1.275 g of root and standardised to 4.4 mg/tablet alkylamides) or placebo tablets, and trial dosing consisted of 3 protocols (priming, travel and sick), depending on the phase of travel of the participants and their health status. The priming dose was 2 tablets/day, travel dose 4 tablets/day and the dose when ill was 6 tablets/day.

Outcomes were assessed using questions about upper respiratory symptoms related to quality of life (based on WURSS-44). Each participant completed the survey before travel (baseline), less than 1 week after travel (return) and at 4 weeks after return from travel (follow-up). Compared with baseline, the average WURSS-44 scores for both groups increased immediately after travel (return) ($p<0.0005$). However, the placebo group had a significantly higher average WURSS-44 score (around double) compared with the Echinacea group ($p=0.05$). WURSS-44 scores tended to return to baseline levels for both groups at the 4-week follow-up. Hence, supplementation with Echinacea, if taken before and during travel, appears to have a protective effect against the development of respiratory symptoms during travel periods associated with long haul flights.

Other immunological effects

Two open-label pilot studies using 4 healthy volunteers found that *E. purpurea* root (0.93 g/day for 7 days) significantly increased CD25 and CD69 expression on T cells.[141,142] These results suggest a possible activation and regulation of T cell function.

In an open-label pilot trial, 11 healthy volunteers were evaluated at baseline (day 1) and on day 15 after consuming 2 Echinacea root tablets/day (containing 1.275 g of *E. purpurea* and *E. angustifolia* root, standardised to 4.4 mg alkylamides/tablet) for 14 days.[143] Echinacea root enhanced the increase in leucocyte heat shock protein (hsp70) expression after mild heat shock ($p=0.029$). White cell counts were mildly increased ($p=0.043$) and there was a preventative effect against free radical induced erythrocyte haemolysis ($p=0.006$), indicative of a clinical antioxidant effect.

A follow-up open-label trial in 24 healthy volunteers conducted by the same research team used the same design, except the Echinacea root dose was twice the above.[144] While Echinacea did not significantly change basal hsp70 expression in lymphocytes, it increased CD4, CD8 and NK cell stress-induced hsp70 expression. The effect was most marked in NK cells ($p<0.05$). The authors

suggested, since the differences were most evident when cells were exposed to a stressor (mild heat shock), this implies that Echinacea root may play a role in activating the immune system when the body encounters a challenge such as a virus.

Idiopathic autoimmune uveitis is usually treated by oral corticosteroids. It is an inflammation of part or all of the uvea, the middle (vascular) tunic of the eye, although it also commonly involves the sclera, cornea and the retina. On the basis of the known interaction of Echinacea alkylamides with cannabinoid CB2 receptors, which implies immune modulating and anti-inflammatory activities, a group of Italian clinicians investigated the safety and efficacy of *E. purpurea* (plant part not specified) in this autoimmune disease.[145] Fifty-one patients with low-grade autoimmune uveitis were treated with conventional therapy, including oral prednisone. In addition, 32 of these patients were given Echinacea as an add-on therapy. At the last follow-up, which was 9 months later, 87.5% of patients receiving Echinacea were in clinical remission compared with 82.3% of the control group. However, steroid-off time was significantly higher in the Echinacea group (indicating that patients receiving Echinacea needed less prednisone to induce remission). The authors concluded that the oral intake of Echinacea appeared safe and effective in the control of low-grade autoimmune uveitis. No patient showed any side effects or aggravation from the use of Echinacea for their autoimmune disease.

In the study of Echinacea lozenges in 6 healthy volunteers cited in the Pharmacokinetics section, inflammatory cytokine levels in collected plasma samples were down-regulated 24 h after lozenge administration.[128]

Toxicology and other safety data

Toxicology

The acute toxicity of an *E. purpurea* root extract has been determined at a level of more than 3000 mg/kg in mice.[146] Male rats given *E purpurea* root extract 50 mg/kg) for 4 or 8 weeks

exhibited a reduction in testicular mass after 8 weeks as well as changes in histological structure.[147]

Contraindications

Long-term use of Echinacea root is contraindicated in patients taking immunosuppressant medication (such as transplant patients). Short-term therapy only is suggested in this instance.

The German Commission E monograph states that in principle, Echinacea should not be used in 'progressive conditions' such as tuberculosis, leukaemia, collagen disorders, multiple sclerosis, AIDS, HIV infection and other autoimmune disease.[148] However, the key words here are 'in principle'. There are no clinical studies or case reports that credibly document any adverse effect resulting from Echinacea use in any of these conditions. Other authoritative sources do not support these restrictions.[149,150] In fact, their suggestion of use of Echinacea in infection prophylaxis implies long-term use.

A 1999 publication suggested that Echinacea is not beneficial for the immune systems of people living with HIV.[151] The basis for this recommendation appears to be extrapolated from the results of an in vitro study in which incubation with fresh expressed juice of *E. purpurea* stimulated the production of cytokines from human peripheral blood macrophages.[152] Seven other in vitro and two in vivo studies also reported stimulation of cytokines after application of Echinacea. However 6 of the 7 in vitro studies used either purified polysaccharides or extracts containing glycoproteins and polysaccharides; these same extracts were administered by intravenous injection to mice in the in vivo studies.[152,153,154] How the results of such studies relate to oral use of Echinacea is not known, particularly since the polysaccharides and glycoproteins may not be bioavailable. Echinacea root and alkylamides tend to reduce cytokine production in stimulated immune cells in vitro (see under Pharmacodynamics).

The above in vitro research may have triggered the concern expressed in a 1997 article published in the *Australian Medical Observer* which cautioned that Echinacea is a danger to asthmatic patients.[155] There is currently no sound evidence to suggest that Echinacea root cannot be used for the treatment of HIV, or that it should be used with caution in asthma.

The suggestion that Echinacea root is contraindicated in autoimmune disease assumes that any enhancement of any aspect of immune function is detrimental for these disorders. There is growing evidence that an inappropriate response to infectious micro-organisms, through phenomena such as molecular mimicry, may be a factor in the pathogenesis of autoimmune disorders. If so, Echinacea root may be beneficial in these disorders because it may decrease the chronic presence of micro-organisms. There are many herbal clinicians who routinely prescribe Echinacea root in autoimmune disease without apparent adverse effects in their patients.

Special warnings and precautions

Allergic reactions, mainly contact dermatitis, may occur rarely in susceptible patients sensitised to Echinacea aerial parts and to plants from the Compositae family. The likelihood of Echinacea root preparations causing allergy is low.

There is no sound evidence that it is detrimental to use Echinacea root for long periods. Indications listed in traditional sources (such as prophylaxis and treatment of chronic infections) suggest long-term usage is warranted[149,150] A randomised, double-blind, placebo-controlled trial investigating the efficacy of 2 herbal formulas in preventing the common cold in highly stressed medical students over a period of 15 weeks also found *E. angustifolia* root and *E. purpurea* root blend to be effective and well-tolerated (see under Clinical trials).

There is no evidence to suggest that Echinacea causes immune system tachyphylaxis. In a clinical study the oral administration of *E. purpurea* root tincture over a 5-day period increased

phagocytic activity compared with controls.[156] Only when the Echinacea was stopped did phagocytic activity decline to normal (pre-test) values, demonstrating a typical washout effect (see above).

Interactions

Echinacea should not be prescribed long-term with immunosuppressant medication as it may decrease the effectiveness of the drug. This is a theoretical concern based on the immune-enhancing activity of Echinacea. No case reports of this interaction have been published.

A 2008 review located 8 papers containing primary data relating to potential drug interactions for Echinacea (mainly *E. purpurea*).[157] Four of these papers included studies on Echinacea root. The authors of the review suggested that herbal products made from *E purpurea* appear to have a low potential to generate cytochrome P450 (CYP 450)-mediated herb-drug interactions, including effects on CYP1A2 and CYP3A4. Studies published subsequent to this review noted no significant effects in human volunteers of an *E purpurea* product high in alkylamides on CYP2D6[158] and P-glycoprotein.[159]

A 2009 in vitro study confirmed that Echinacea and selected alkylamides did not induce CPY3A4 mRNA expression in vitro.[160] However, an earlier study suggested that alkylamides can exert inhibitory effects on CYP3A4, 2D6 and 2C19 in vitro,[161] although the human studies cited above[157,158] suggest that these effects do not have clinical relevance.

Other human studies have found no impact of Echinacea root on warfarin pharmacodynamics[162] (although it did reduce plasma concentrations of S-warfarin) and the overall pharmacodynamics of the anti-HIV drugs darunavir and ritonavir in combination.[163]

Use in pregnancy and lactation

Category A: No proven increase in the frequency of malformation or other harmful effects on the foetus despite consumption by a large number of women.

Echinacea is commonly consumed by pregnant women, as has been demonstrated in several surveys.[164,165]

Pregnant mice were fed daily *E purpurea* (0.45 mg/mouse, plant part not specified, probably root) from pregnancy onset until gestational days 10, 11, 12, 13 and 14.[166] The pregnancy-induced elevations in splenic lymphocytes and nucleated erythroid cells were all but eliminated by Echinacea and the number of viable foetuses was reduced. The authors suggested caution with Echinacea during the early/mid stages of pregnancy.

However, a prospective, controlled study published in 2000 concluded that gestational use of Echinacea (typically for 5 to 7 days) during organogenesis was not associated with an increased risk of major malformations. There were no significant differences in pregnancy outcomes between the study group consisting of 206 women who had used Echinacea during pregnancy (112 during the first trimester, 17 for all three trimesters) and their matched controls (206).[167] It should be noted that no differentiation between Echinacea plant parts was undertaken in this study.

On available information Echinacea is compatible with breastfeeding. In contrast, a 2006 review recommended caution with Echinacea during breastfeeding.[168] However, a study in a breastfeeding woman found that only small quantities of alkylamides (about 0.5 % of the maternal dose) are passed to the infant during feeding[169] and these may in fact confer health benefits.

Effects on ability to drive and use machines

No adverse effects anticipated.

Side effects

In many published case reports the plant part(s) of the Echinacea product involved was not specified. Hence they need to be viewed in the context of this limitation.

Side effects are generally not expected for oral or topical administration of Echinacea root. As indicated below contact dermatitis may occur rarely in susceptible patients. Unsubstantiated reports of three deaths attributed to Echinacea products over a 6-year period occurred in the German media in 1996. However, no action was taken by the authorities as no causal link between the deaths and the taking of Echinacea could be established.[170]

A total of 1032 patients randomly chosen from 6 patch test clinics were patch tested with a series of 5 ointments and the components of the ointment bases. Two patients demonstrated a positive reaction to Echinacea. However, it is not certain that the reaction was to the plant material itself.[171] Anaphylaxis was attributed by an Australian immunologist to a woman with allergy after taking, among other dietary supplements, a commercial extract of *E. purpurea* and *E. angustifolia*.[172] However, it was suggested that the pharyngeal irritation experienced by the patient may have been due to the alkylamide content of the preparation (the patient took twice the recommended amount).[173]

A subsequent 2002 publication by the Australian immunologist evaluated 5 cases of adverse reactions to Echinacea.[174] Two patients experienced anaphylaxis, another 2 aggravation of asthma, and the fifth developed a rash. Three of the patients had a positive skin prick test (SPT) to Echinacea. Twenty per cent of 100 atopic subjects who had never taken Echinacea also exhibited a positive SPT to this herb when tested. However, it is unclear if any of the products

involved contained Echinacea root. Allergic reaction is far more likely to the aerial parts of Echinacea because of the likely presence of pollen proteins. Moreover, such a high risk of allergy has not been borne out by subsequent case reports.

Other published case reports attributed to Echinacea (species and plant part usually not specified) include erythema nodosum,[175] leukopenia,[176] aggravation of autoimmunity,[177,178] acute hepatitis[179] and hypereosinophilia.[180]

Misinformation exists that Echinacea is potentially hepatotoxic due to the presence of pyrrolizidine alkaloids (PAs). However the PAs found in Echinacea do not contain the 1,2-unsaturated necrine ring system which is essential for such reactions.

Overdosage

No incidents found in the published literature.

Safety in children

No specific information is available for Echinacea root, but adverse effects are not expected.

Regulatory status in selected countries

Monographs of *E. angustifolia* and *E. purpurea* roots appear in the *United States Pharmacopeia-National Formulary* USP31 NF26 2008 and the *British Pharmacopoeia* 2012 edition.

Echinacea angustifolia/pallida herb, *E. angustifolia* root and *E. purpurea* root are covered by null and negative Commission E monographs. *E. pallida* root and *E. purpurea* herb are covered by positive monographs. These herbs are listed with the following uses:

- to support the immune system with infections of the respiratory and lower urinary systems (*E. purpurea* herb);
- in supportive treatment of influenza-like infections (*E. pallida* root);
- externally for poorly healing wounds and chronic ulcerations (*E. purpurea* herb).

E. purpurea root is listed in the unapproved component characteristics section. The negative status of *Echinacea angustifolia/pallida* herb, *E. angustifolia* root and *E. purpurea* root is due to poor benefit:risk ratio and concerns about the risk from parenteral application (injection). The null status is due to a lack of substantiation of its activity for the listed conditions, leading to its therapeutic use not being recommended.

Echinacea is on the UK General Sale List. Echinacea root products have achieved Traditional Herbal Registration in the UK with the traditional indication of relief of symptoms of the common cold and influenza.

Echinacea does not have GRAS status. However, it is freely available as a 'dietary supplement' in the USA under DSHEA legislation (1994 Dietary Supplement Health and Education Act).

Echinacea root is not included in Part 4 of Schedule 4 of the Therapeutic Goods Act Regulations of Australia and is freely available for sale.

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