

Immunotropic activity of *Echinacea*.

Part II. Experimental and clinical data

BARBARA J. BAŁAN¹, FILIP RÓŻEWSKI², EWA SKOPIŃSKA-RÓŻEWSKA^{3,4}, ANDRZEJ WOJDAS⁵,
ROBERT ZDANOWSKI⁶, WANDA STANKIEWICZ³

¹Department of Immunology and Nutrition, Warsaw Medical University, Warsaw, Poland

²Student of Warsaw Medical University, Faculty of Pharmacy, Warsaw, Poland

³Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

⁴Department of Pathology, Biostructure Centre, Warsaw Medical University, Warsaw, Poland

⁵Department of Otolaryngology, Military Institute of Medicine, Warsaw, Poland

⁶Department of Regenerative Medicine, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

Abstract

Nowadays *Echinacea* is world widely used and one of the most important medicinal herb for the treatment of upper respiratory tract infections, owing its anti-viral, anti-inflammatory and immunomodulatory properties. However, various *Echinacea* preparations tested in clinical trials differ greatly. There is some evidence that preparations based on the aerial parts of *Echinacea purpurea* might be effective for the early treatment of colds in adults, but results are not fully consistent. Beneficial effects of long lasting administration of high *Echinacea* doses, for preventative purposes, have not been shown in rigorous randomized trials.

Key words: *Echinacea purpurea*, immune system, antibacterial, anti-inflammatory, wound healing, respiratory infections, tumor angiogenesis.

(Centr Eur J Immunol 2012; 37 (1): 51-56)

Experimental data

A number of experimental *in vivo* and *in vitro* studies have shown that *Echinacea*-containing remedies increase immunologic activity and may protect experimental animals against systemic viral, bacterial, parasitic and fungal infections. They also exert antioxidative and anti-inflammatory activity, enhance antibody production, phagocytosis, cellular respiratory activity, and lymphocyte activation through release of various cytokines [1-21]. The mechanism of immunostimulatory effects is not fully understood, but it is probably connected with synergistic action of polyphenols, polysaccharides, glycoproteins and alkaloids. There is some evidence, that alkaloids present in *Echinacea* are agonists of cannabinoid receptor type 2 (CB2), and by stimulation of this receptor alkaloids upregulate tumor necrosis factor α (TNF- α) production. Cannabinoid receptor type 2 is expressed in different types of immune-competent cells. It is well established that cannabinoids (and their agonists) mediate both inhibitory and stimulatory

effects on the immune system by modulating cytokine expression [22-24].

Janusz Bany in the Military Institute of Hygiene and Epidemiology conducted his research on infection models with *Pseudomonas aeruginosa* and *Trichinella spiralis* in mice. He observed a significant inhibition of bacterial and parasite infection in those mice, which were treated with *Echinacea purpurea* and *Echinacea angustifolia* extracts, both separately and in mixtures, both before the actual infection and during the proliferation. Both intestinal and muscular phases of *Trichinella spiralis* infections were greatly inhibited [20, 21]. With the use of experimental model it has been showed that both *Echinacea angustifolia* and *Echinacea purpurea* activate macrophages to produce cytokines, including TNF- α and IFN- γ which play the role of cell-type immune response inducers.

Recently, it was reported, that herbal remedies based on *Echinacea* increase immunoglobulin production and may regulate antibody production by augmenting both Th1 and Th2 cytokine production [25]. Synergetic effects of oral

Correspondence: Ewa Skopińska-Różewska, Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Kozielska 4, 01-163 Warsaw, Poland, e-mail: ewaskop@hotmail.com

administration of levamisole and *Echinacea purpurea* on immune response in rats was also observed [26]. No effectiveness of *Echinacea* extract on acute and chronic rheumatoid arthritis rat model was observed [27]. In mice, genomic and proteomic analyses revealed stimulatory effect of *Echinacea purpurea* extract on the trafficking activity of mouse dendritic cells [28]. In fish, dietary administration of *Echinacea purpurea* exerted positive effects on growth and biochemical and haematological indices in rainbow trout [29].

The activity or the lack of activity of *Echinacea* preparations depends on several aspects, including the cultivation type, cultivation time and part of the plant that was used to produce the preparation. In our own researches we have observed some differences between the immune-stimulant activity of the preparations sent to us from various producers. Most probably, it was a result of the specificity of cultivation areas, purity and composition of raw-material and technological differences.

In some cases the doses recommended by the producer suppressed the immune response, whereas the actual immune-stimulation was demonstrated while using much lesser doses [30-35].

In therapy one can use liquid extracts: alcohol extracts (in a form of drops), oil extracts (ointments) and aqueous extracts (sirups) from the roots or herbs of *Echinacea*, as well as dry extracts in a form of tablets.

In Germany and Switzerland, approximately 200 preparations containing such extracts are in sale. In Poland there are also many of *Echinacea* products, unfortunately some of them occurred as food additives and therefore can avoid the registration process which takes place in case of regular medicines. The possible result of their activity is completely unknown.

However, there are preparations already tested by us and registered. Among them *Echinapur* (ointment, tablets, Herbapol Poznań), *Echinerba* (tablets, Labofarm), *Lymphosil* and *Lymphosil Forte* (tablets, Cesra), *Immunal* (drops) and *Immunal Forte* (tablets, Lek SA), *Succus Echinacea* (drops, Phytofarm), *Echinacea forte* (dr Theiss) and many others.

We also tested some complex remedies: *Echinasal* (sirup with the herb of *Echinacea purpurea*, *Grindelia camporum* and *Plantago lanceolata*, Herbapol Wrocław), *Reumaherb* (Herbapol Poznań, tablets containing extracts from the herb of *Echinacea purpurea*, the flowers of *Filipendula ulmaria* and the roots of *Harpagophytum procumbens*), *Alchinal* (sirup, Gemi), apart from *Echinacea* containing extracts from allium and cacao, and *Esberitox* (tablets and drops, Zymella), containing additionally the extract from the herb of *Thuja occidentalis* and *Baptisia*. We performed experiments on mice for evaluation the *in vivo* influence of *Echinacea*-containing remedies on cellular and humoral response of animals immune system on antigens and mitogens [30-35]. We also performed experi-

ments in human volunteers. Experiments on mice revealed that despite some differences observed between immunomodulatory action of various *Echinacea*-containing remedies, and their liquid and dry formulations, all of them stimulated various parameters of cellular and humoral immunity, without big differences between aqueous and hydro-alcoholic extracts.

Although the therapeutic spectrum of *Echinacea* is quite broad, it is to be remembered that the extracts from roots and herb have immune-modulating abilities. It means that, depending on the dose and/or initial state of the immune system, *Echinacea* can either stimulate or inhibit the activity of immune cells [35]. Indeed, we have observed it also in patients with oral candidiasis, receiving Echinapur tablets (300 mg daily for 10 days). An augmented chemiluminescence activity (a result of the oxygen free radical production) was observed only in patients, who had it low initially. It is worth emphasising, that in a proper dose that is exactly how a good immune-modulator should work [31].

In healthy human volunteers another *Echinacea purpurea*-containing remedy (Immunal forte tablets) administered for seven days in 160 mg daily doses, have stimulated granulocyte metabolic activity, have increased the incidence of CD16⁺ and CD56⁺ NK lymphocytes and have stimulated production of angiogenic growth factors by blood mononuclear cells, in comparison to placebo group. Also liquid form of *Echinacea purpurea* (Immunal drops) administered for 7 days in 6 ml daily dose, have stimulated the following parameters of immunologic reactivity of healthy humans blood leukocytes: release of angiogenic growth factors by mononuclear cells and their response to mitogen PHA (phytohaemagglutinin). Increase of chemiluminescent activity of granulocytes, and lymphocytes CD4/CD8 ratio, were also observed [36-38].

Schwarz *et al.* (2005) in a double-blind, placebo controlled, cross-over study, observed modulatory effect of oral administration of freshly pressed juice of *Echinacea purpurea* on the number of various subpopulations of B- and T-lymphocytes in healthy volunteers [39]. In Ritchie *et al.* study (2011) treatment with ethanolic extract of fresh *Echinacea purpurea* (Echinaforce) healthy volunteers for 8 days, reduced blood pro-inflammatory mediators TNF- α and IL-1 β , and increased anti-inflammatory IL-10 levels. An analysis of a subgroup of volunteers who showed low pre-treatment levels of the cytokines MCP-1, IL-8, IL-10 or IFN- γ showed significant stimulation of these factors upon Echinaforce treatment, whereas the levels in subjects with higher pre-treatment levels remained unaffected. This is another example demonstrating immune-modulating ability of *Echinacea* [40].

Clinical data

The immune system relies on numerous feedback, strictly correlated between each other and therefore, in case of

its excessively long and intense stimulation, the suppression mechanism starts to counteract, thereby limiting the immune response.

The very same regulation applies to the preparations made from *Echinacea*. It is already known for quite a long time, since the classic publication of Wagner [1], and supported by Coeugnet and Elek in 1987 [2]. In their study extracts of *Viscum album* (Plenosol) and *Echinacea purpurea* (Echinacin) were used clinically for their non-specific action on cell-mediated immunity. *In vitro* they proved that these two extracts have a stimulating effect on the production of lymphokines by lymphocytes and in the transformation test. A toxic effect on cells was produced only with very high, clinically irrelevant concentrations. Clinical application of these extracts produced a stimulation of cell-mediated immunity (one therapeutic administration followed by a free interval of one week) or had a depressive action (daily administrations of high doses). These observations were confirmed by lymphokine production and 3H-thymidine incorporation and a skin test with recall antigens (Multitest Merieux).

Regrettably, this knowledge rarely reaches the clinicians, which during the trials administer excessive doses of *Echinacea*, exceeding the proper time period. It is exactly the reason why there are so many negative outcomes in the trials of *Echinacea*-based preparations [41-45].

There are however numerous information about the benefits stemming from the use of *Echinacea* in infections. In 2004 Goel *et al.* [46] observed the reduction of symptoms in patients suffering from upper respiratory tract infections (URTIs) after a 7-day-long administration of Echinilin, a preparation produced from the herb of *Echinacea purpurea*. In his review Awang [3] refers to the researches of Braunig and his colleagues from 1992 [4], where one can find descriptions of beneficial effects, in comparison to placebo group, of an administration of 450-900 mg of the *Echinacea purpurea* roots to patients with URTIs.

Similar outcomes were published in 1992 by Schoneberger, who conducted the double-blind trials of fresh *Echinacea purpurea* succus [47]. Earlier in 1988 Baetgen described positive effects of *Echinacea purpurea* in paediatric patients with bronchitis [48]. Recently, the herbal compound of *Echinacea angustifolia*, β -glucan, vitamin C, arabinogalactan and zinc was presented as improving the quality of life in paediatric patients affected by recurrent pharyngotonsillitis and otitis media without contralateral effects [49]. Yakoot and Salem confirmed in adults efficacy of multiherbal formula Immumax (*Echinacea*, garlic, *Nigella sativa*, *Panax ginseng*, vitamin C, zinc) in reducing the duration and severity of common cold symptoms [50].

In the fifties and sixties of the past century one can find out that *Echinacea* extracts were used as an additive medicine in psoriasis. With their use 90% of the patients experienced the remission of their disease. Extracts were also administered intramuscularly to children suffering from

whooping-cough and during a 3-day treatment the frequency and severity of cough attacks was diminished. Patients with tuberculosis who did not respond well to the conventional therapy were also given the *Echinacea* extracts.

Given that the immunity to tuberculosis is a result of the ability of immune system cells to produce TNF- α and noting that the *Echinacea* extracts stimulate its production – it seems quite logical to use them as an auxiliary agents in the treatment of tuberculosis, instead of regarding tuberculosis as a counter-indication to the use of *Echinacea* preparations.

In another review of Schimmel and Werner [51], the authors describe the results of Sprockhoff's researches (1964), who stated the beneficial effect of the *Echinacea* extracts treatment in children with tonsillitis, otitis media, whooping-cough and bronchitis. In some cases *Echinacea* even replaced the antibiotic [52]. *Echinacea* was also used in osteoarthritis of bones and joints.

In 1990 Mowrey described the positive results of the persistent vaginal candidiasis treatment using *Echinacea* in a form of vaginal ointment and parallel administration *per os*. In 1978 in Germany a multi-directional trial on 4598 patients with various skin afflictions were conducted [53]. 538 clinicians reported that the ointment containing 16% of a fresh *Echinacea purpurea* succus demonstrated very good results in the treatment of skin inflammation (85.4% of patients), chronic wound healings (91.5%), eczema (82.3%), burns (96.3%), *Herpes simplex* infections (91.4%) and leg ulcers with varicose veins (71.1%).

These results were confirmed on other group in 1979 [54] and in 1994 with regard to leg ulcers [55].

In the literature there were also descriptions of the antibacterial and antiviral activity of *Echinacea*, especially against the *Herpes* group and VSV, which cause herpes and viral stomatitis, as well as against influenza virus, that has been recently confirmed [10-14].

Professor Jerzy Lutomski (2002) informed that a German E commission tested 50 clinically verified herbal preparations for skin problems, out of which 26 were marked as positive, including *Echinacea purpurea*. In his paper, he stated that „There is much hope for the treatment of atopic dermatitis with *Echinacea purpurea*. ECHINAPUR, made of standardised *Echinacea purpurea* extract, seems to be a perfect example as it accelerates the healing of surface wounds” [56].

Beneficial effect of short therapeutic treatment was also reported by Brinkeborn [57], Scaglione and Lund [58], Heinen-Kammerer [59], Cohen (composite herbal product in children aged 1-5 years) [60], Lindenmuth [61] and others [62, 63]. Schoop *et al.* performed a meta-analysis of three studies of *Echinacea* use in the prevention of induced *Rhinovirus* colds. This meta-analysis suggests that standardized extracts of *Echinacea* were effective in the prevention of symptoms of the common cold after clinical inoculation, compared with placebo [64].

In conclusion, *Echinacea* administered in low doses, within short periods of time should be widely used in pre-

vention and treatment of numerous infections, as it is a powerful stimulator of antiviral, antibacterial and antifungal response [65].

There are also some papers, presenting other effects of *Echinacea* on health. Hou *et al.* (2011) suggest a new application of *Echinacea* as a hepatoprotective agent [66]. Isbaniyah *et al.* show (2011) that the combination of *Echinacea purpurea*, zinc, selenium and vitamin c may alleviate exacerbation symptoms caused by URTI in chronic obstructive pulmonary disease [67]. In other study Echinaforce readily killed *Propionibacterium acnes*, and in cell culture models inhibited *Propionibacterium acnes* – induced the secretion of several pro-inflammatory cytokines, what could provide a benefit to acne individuals [68].

There are some papers suggesting a role of *Echinacea* and its phenolic components in tumors treatment [69-75]. We reported inhibitory effect of various *Echinacea*-containing remedies on neovascular reaction induced in murine skin after intradermal grafting of murine L-1 sarcoma cells or human lung and kidney cancer cells and cells homogenates [37, 76-78].

Benefits aside, it is also necessary to list the counter-indications for the use of *Echinacea*.

Above all those are: hypersensitivity, autoimmune and auto-aggression disease, leukaemia, AIDS and HIV infection. Now we know, that tuberculosis doesn't seem to count among them.

As AIDS is concerned, opinions vary. Kligler, in 2003 estimated the risk of the use of *Echinacea* in these patients to be theoretical, as it is not validated by any scientific reports. He shared the same point of view as the autoimmune diseases were discussed. Due to the possible interactions, *Echinacea* should be carefully administered to patients taking Itraconazole, Fexofenadine and Lovastatin. Recently, it was shown that co-administration of *Echinacea purpurea* with darunavir-ritonavir was safe and well tolerated in HIV-infected patients [79-83].

The problem of *Echinacea* use during pregnancy remains open. There is some evidence from human studies that *Echinacea* is not teratogenic. However, there are no formal studies, which may exclude the possibility that the absence of malformations in the living births resulted from the fact that consuming *Echinacea* may promote spontaneous abortion. In pregnant mice *Echinacea* reduced the number of viable fetuses, interfered with embryonal angiogenesis, and negatively influenced maternal lympho- and hemopoiesis [84].

Summarily, herbal remedies with *Echinacea purpurea* extracts exhibit immune-stimulation of numerous immune system parameters, which leads to the assumption that it could be highly beneficial in various types of infections, wound healing, bed sores, ulcerations and many others. They can be also effective used as a prevention in all situations of epidemiological risk such as: airway travel, massive public transportation, crowded events or current infections in close surroundings. Supplementation with standardized *Echinacea*

tablets, if taken before and during travel, may have preventive effects against the development of respiratory symptoms during travel involving long-haul flights [85]. Yet, both the recommended dosage and intervals of administration (7-14 days) are to not be exceeded, in view of contradictory effects which may occur. That means immune-suppression, instead of immune-stimulation. It is essential to test all available preparations on the market, measuring their immunomodulatory properties and the influence on both immune and tumour angiogenesis process activity, in order to determine the proper dosage and therapeutic intervals.

References

1. Wagner H (1986): Examination of the immune-stimulation effect of some plant homeopathic drugs. *Biol Ther* 4: 21-27.
2. Coeugnet EG, Elek E (1987): Immunomodulation with *Viscum album* and *Echinacea purpurea* extracts. *Onkologie* 10: 27-33.
3. Awang DVC (1999): Immune stimulants and antiviral botanicals: *Echinacea* and *Ginseng*. In: Janick J (ed.). *Perspectives on new crops and new uses*. ASHS Press, Alexandria, VA; 450-456, also www.hort.purdue.edu/newcrop/proceedings1999/v4-450.html.
4. Brauning B, Dorn M, Knick E (1992): *Echinacea purpurea* root for strengthening the immune response to flu-like infections. *Zeitschr Phytother* 13: 7-13.
5. Goel V, Chang C, Slama J, et al. (2002): *Echinacea* stimulates macrophage function in the lung and spleen of normal rats. *J Nutr Biochem* 13: 487.
6. Goel V, Chang C, Slama JV, et al. (2002): Alkylamides of *Echinacea purpurea* stimulate alveolar macrophage function in normal rats. *Int Immunopharmacol* 2: 381-387.
7. Sharma M, Arnason JT, Burt A, Hudson JB (2006): *Echinacea* extracts modulate the pattern of chemokine and cytokine secretion in Rhinovirus – infected and uninfected epithelial cells. *Phytother Res* 20: 147-152.
8. Schumacher A, Friedberg KD (1991): The effect of *Echinacea angustifolia* on non-specific cellular immunity in the mouse. *Arzneimittelforschung* 41: 141-147 [Article in German].
9. Gan XH, Zhang L, Heber D, Bonavida B (2003): Mechanism of activation of human peripheral blood NK cells at the single cell level by *Echinacea* water soluble extracts: recruitment of lymphocyte-target conjugates and killer cells and activation of programming for lysis. *Int Immunopharmacol* 3: 811-824.
10. Roesler J, Steinmüller C, Kiderlen A, et al. (1991): Application of purified polysaccharides from cell cultures of the plant *Echinacea purpurea* to mice mediates protection against systemic infections with *Listeria monocytogenes* and *Candida albicans*. *Int J Immunopharmacol* 13: 27-37.
11. Kapadia GJ, Azuine MA, Tokuda H, et al. (2002): Inhibitory effect of herbal remedies on 12-O-tetradecanoylphorbol-13-acetate-promoted Epstein-Barr virus early antigen activation. *Pharmacol Res* 45: 213-220.
12. Wacker A, Hilbig W (1978): Virus-inhibition by *Echinacea purpurea*. *Planta Med* 33: 89-102 [Article in German].
13. Skwarek T, Tynecka Z, Glowinski K, et al. (1996): *Echinacea L* – inducer of interferons. *Herba Pol* 42: 110-117.
14. Binns SE, Hudson J, Merali S, Arnason JT (2002): Antiviral activity of characterized extracts from *Echinacea* spp. (Helianthaceae: Asteraceae) against herpes simplex virus (HSV-1). *Planta Med* 68: 780-783.

15. See DM, Broumand N, Sahl L, et al. (1997): In vitro effects of Echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. *Immunopharmacol* 35: 229-235.
16. Tragni E, Galli CL, Tubaro A, et al. (1987): Anti-inflammatory activity of Echinacea angustifolia fractions separated on the basis of molecular weight. *Pharmacol Res Commun* 20 (Suppl 5): 87-90.
17. Tubaro A, Tragni E, Del Negro P, et al. (1987): Anti-inflammatory activity of a polysaccharide fraction of Echinacea angustifolia. *J Pharm Pharmacol* 39: 567-569.
18. Binns SE, Purgina B, Bergeron C, et al. (2000): Light-mediated anti-fungal activity of Echinacea extracts. *Planta Med* 66: 241-244.
19. Hu C, Kitts DD (2000): Studies on the antioxidant activity of Echinacea root extract. *J Agric Food Chem* 48: 1466-1472.
20. Bany J, Siwicki AK, Zdanowska D, et al. (2003): Echinacea purpurea stimulates cellular immunity and anti-bacterial defence independently of the strain of mice. *Pol J Vet Sci* 6 (3 Suppl): 3-5.
21. Bany J, Zdanowska D, Zdanowski R, Skopińska-Różewska E (2003): The effect of herbal remedy on the development of Trichinella spiralis infection in mice. *Pol J Vet Sci* 6 (3 Suppl): 6-8.
22. Clifford LJ, Nair MG, Rana J, Dewitt DL (2002): Bioactivity of alkamides isolated from Echinacea purpurea (L.) Moench. *Phytomedicine* 9: 249-253.
23. Raduner S, Majewska A, Chen JZ, et al. (2006): Alkylamides from Echinacea are a new class of cannabinomimetics. Cannabinoid type 2 receptor-dependent and independent immunomodulatory effects. *J Biol Chem* 281: 14192-14206.
24. Woelkart K, Bauer R (2007): The role of alkamides as an active principle of Echinacea. *Planta Med* 73: 615-623.
25. Yamada K, Hung P, Park TK, et al. (2011): A comparison of the immunostimulatory effects of the medicinal herbs Echinacea, Ashwagandha and Brahmi. *J Ethnopharmacol* 137: 231-235.
26. Sadigh-Eteghad S, khayat-Nuri H, Abadi N, et al. (2011): Synergistic effects of oral administration of levamisole and Echinacea purpurea on immune response in Wistar rat. *Res Vet Sci* 91: 82-85.
27. Arafa NM, Hamuda HM, Melek ST, Darwish SK (2011): The effectiveness of Echinacea extract or composite glucosamine, chondroitin and methyl sulfonfyl methane supplements on acute and chronic rheumatoid arthritis rat model. *Toxicol Ind Health* (Epub ahead of print).
28. Yin SY, Wang WH, Wang BX, et al. (2011): Stimulatory effect of Echinacea purpurea extract on the trafficking activity of mouse dendritic cells: revealed by genomic and proteomic analyses. *BMC Genomics* 11: 612.
29. Oskoi SB, Kohyani AT, Parseh A, et al. (2011): Effects of dietary administration of Echinacea purpurea on growth indices and biochemical and hematological indices in rainbow trout (Oncorhynchus mykiss) fingerlings. *Fish Physiol Biochem* (Epub ahead of print).
30. Skopińska-Różewska E, Furmanowa M, Guzewska J, et al. (2002): The effect of Centella asiatica, Echinacea purpurea and Melaleuca alternifolia on cellular immunity in mice. *Centr Eur J Immunol* 27: 142-148.
31. Sokolnicka I, Skopińska-Różewska E, Strzelecka H, et al. (2001): Adaptation of biological tests for estimation of the activity of Echinacea purpurea extracts. I. In vivo studies. *Terapia* 9: 38-42.
32. Skopińska-Różewska E, Strzelecka H, Wasiutyński A, et al. (2008): Aqueous and hydro-alcoholic extracts of Echinacea purpurea (L.) Moench as traditional herbal remedies with immunotropic activity. *Centr Eur J Immunol* 33: 78-82.
33. Skopińska-Różewska E, Wasiutyński A, Sommer E, et al. (2011): Modulatory effect of Echinacea pallida on cellular immunity and angiogenesis in mice. *Centr Eur J Immunol* 36: 18-23.
34. Skopińska-Różewska E, Wasiutyński A, Skopiński P, et al. (2011): In vivo effect of two complex herbal remedies Echinaseal and Bioaron C on antibody production and immunological angiogenesis in mice. *Centr Eur J Immunol* 36: 139-144.
35. Skopińska-Różewska E, Sokolnicka I, Siwicki AK, et al. (2011): Dose-dependent in vivo effect of Rhodiola and Echinacea on the mitogen-induced lymphocyte proliferation in mice. *Pol J Vet Sci* 14: 265-272.
36. Skopińska-Różewska E, Sokolnicka I, Radomska-Lesniewska D, et al. (2003): The in vivo effect of Echinacea purpurea succus on various functions of human blood leukocytes. *Centr Eur J Immunol* 28: 126-130.
37. Rogala E, Skopińska-Różewska E, Wasiutyński A, et al. (2008): Echinacea purpurea diminishes neovascular reaction induced in mice skin by human cancer cells and stimulates non-specific cellular immunity in humans. *Centr Eur J Immunol* 33: 127-130.
38. Skopińska-Różewska E, Sommer E, Bałan BJ, et al. (2010): The in vivo effect of dry hydro-alcoholic extract of Echinacea purpurea on angiogenic activity of human blood mononuclear cells. *Centr Eur J Immunol* 35: 223-226.
39. Schwarz E, Parlesak A, Henneicke-von Zepelin HH, et al. (2005): Effect of oral administration of freshly pressed juice of Echinacea purpurea on the number of various subpopulations of B- and T- lymphocytes in healthy volunteers: results of a double-blind, placebo controlled cross-over study. *Phytomedicine* 12: 625-631.
40. Ritchie MR, Gertsch J, Klein P, Schoop R (2011): Effects of Echinaforce® treatment on ex vivo stimulated blood cells. *Phytomedicine* 18: 826-831.
41. Barrett BP, Brown RL, Locken K, et al. (2002): Treatment of the common cold with unrefined echinacea. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 137: 939-946.
42. Yale SH, Liu K (2004): Echinacea purpurea therapy for the treatment of the common cold. *Arch Intern Med* 164: 1237-1241.
43. Barrett B, Brown R, Rakel D, et al. (2010): Echinacea for treating the common cold: a randomized trial. *Ann Intern Med* 153: 769-777.
44. Turner RB, Riker DK, Gangemi JD (2000): Ineffectiveness of Echinacea for prevention of experimental rhinovirus colds. *Antimicrob Agents Chemother* 44: 1708-1709.
45. Grimm W, Müller HH (1999): A randomized controlled trial of the effect of fluid extract of Echinacea purpurea on the incidence and severity of colds and respiratory infections. *Am J Med* 106: 138-143.
46. Goel V, Lovlin R, Barton R, et al. (2004): Efficacy of a standardized Echinacea preparation (Echinilin) for the treatment of the common cold: a randomized, double-blind, placebo-controlled trial. *J Clin Pharm Ther* 29: 75-83.
47. Schoneberger D (1992): The influences of immune-stimulating effects of pressed juice from Echinacea purpurea on the course and severity of colds: results from a double-blind study. *Forum Immunol* 8: 2-12.

48. Baetgen D (1988): Behandlung der akuten bronchitis im kindesalter – praxisstudie mit einem immunstimulans aus Echinacea purpurea. *Pädiatrie* 1: 66-70.
49. Minetti AM, Forti S, Tassone G, et al. (2011): Efficacy of complex herbal compound of Echinacea angustifolia (Imoviral® junior) in recurrent upper respiratory tract infections during pediatric age: preliminary results. *Minerva Pediatr* 63: 177-182.
50. Yakoot M, Salem A (2011): Efficacy and safety of a multi-herbal formula with vitamin C and zinc (Immumax) in the management of the common cold. *Int J Gen Med* 4: 45-51.
51. Schimmel KC, Werner GT (1981): Non-specific increase of the body own immunity by Echinacin administration. *Ther Ggw* 120: 1065-1076 [Article in German].
52. Sprockhoff O (1964): Pediatric experiences with myo-echinacin. *Landarzt* 40: 1173-1174 [Article in German].
53. Viehmann P (1978): Results of treatment with an Echinacea-based ointment. *Erfahrungshellkunde* 27: 353-358.
54. McLaughlin G (1992): Echinacea. *Austr J Med Herbalism* 4: 104-111.
55. McKeon K (1994): Herbal management of diabetic leg ulcers. *Austr J Med Herbalism* 6: 99.
56. Lutomski J (2002): The appreciated herbal medicinals for dermatologic uses. *Postępy Fitoterapii* 3: 39-44.
57. Brinkeborn RM, Shah DV, Degenring FH (1999): Echinacea and other Echinacea fresh plant preparations in the treatment of the common cold. A randomized, placebo controlled, double-blind clinical trial. *Phytomed* 6: 1-5.
58. Scaglione F, Lund B (1995): Efficacy in the treatment of the common cold of a preparation containing an Echinacea extract. *Int J Immunother* 11: 163-166.
59. Heinen-Kammerer T, Holtmannspötter C, Schnabel S, et al. (2005): Effectiveness of echinacin in therapy of chronic recurrent respiratory disease. *Gesundheitswesen* 67: 296-301 [Article in German].
60. Cohen HA, Varsano I, Kahan E, et al. (2004): Effectiveness of an herbal preparation containing Echinacea, propolis, and vitamin C in preventing respiratory tract infections in children: a randomized, double blind, placebo-controlled, multicenter study. *Arch Pediatr Adolesc Med* 158: 217-221.
61. Lindenmuth GF, Lindenmuth EB (2000): The efficacy of Echinacea compound herbal tea preparation on the severity and duration of upper respiratory and flu symptoms: a randomized, double-blind w zapobieganiu i leczeniu chorób. Skopińska-Różewska E, Siwicki AK (eds.). *MEDYK*, Warsaw; 93-104.
62. European Medicines Agency (EMA). List of references for assessment of Echinaceae purpureae herba. London, 8 March 2007. Doc. Ref: EMEA/HMPC/111536/2007.
63. Schoop R, Klein P, Suter A, Johnston SL (2006): Echinacea in the prevention of induced Rhinovirus colds: a meta-analysis. *Clin Ther* 28: 174-183.
64. Hudson JB (2012): Applications of the phytomedicine Echinacea purpurea (Purple Coneflower) in infectious diseases. *J Biomed Biotechnol* 2012: 769896.
65. Hou CC, Huang CC, Shyr LF (2011): Echinacea alkalimides prevent lipopolysaccharide/D-galactosamine-induced acute hepatic injury through JNK pathway-mediated HO-1 expression. *J Agric Food Chem* 59: 11966-11974.
66. Isbaniah F, Wiyono WH, Yunus F, et al. (2011): Echinacea purpurea along with zinc, selenium and vitamin C to alleviate exacerbations of chronic obstructive pulmonary disease: results from a randomized controlled trial. *J Clin Pharm Ther* 36: 568-576.
67. Sharma M, Schoop R, Suter A, Hudson JB (2011): The potential use of Echinacea in acne: control of Propionibacterium acnes growth and inflammation. *Phytother Res* 25: 517-521.
68. Gomes CA, da Cruz TG, Andrade JL, et al. (2003): Anticancer activity of phenolic acids of natural or synthetic origin: a structure activity study. *J Med Chem* 46: 5395-5401.
69. Xu F, Song D, Zhen Y (2004): Inhibition of tumor metastasis by sodium caffeate and its effect on angiogenesis. *Oncology* 67: 88-92.
70. Lersch C, Zeuner M, Bauer A, et al. (1992): Nonspecific immunostimulation with low doses of cyclophosphamide (LDCY), thymostimulin, and Echinacea purpurea extracts (Echinacin®) in patients with far advanced colorectal cancers: preliminary results. *Cancer Invest* 10: 343-348.
71. Kampa M, Alexaki VI, Notas G, et al. (2004): Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. *Breast Cancer Res* 6: R63-74.
72. Miller SC (2005): Echinacea: a miracle herb against aging and cancer? Evidence in vivo in mice. *Evid Based Complement Alternat Med* 2: 309-314.
73. Chicca A, Adinolfi B, Martinotti E, et al. (2007): Cytotoxic effects of Echinacea root hexanic extracts on human cancer cell lines. *J Ethnopharmacol* 110: 148-153.
74. Currier NL, Miller SC (2002): The effect of immunization with killed tumor cells, with/without feeding of Echinacea purpurea in an erythroleukemic mouse model. *J Altern Complement Med* 8: 49-58.
75. Bany J, Skopińska-Różewska E, Chorostowska-Wynimko J, et al. (2004): The effect of complex herbal remedy on the angiogenic activity of L1 sarcoma cells, L1 sarcoma tumor growth and on the bacterial infection in mice. *Centr Eur J Immunol* 29: 29-34.
76. Wasutyński A, Bałan BJ, Skopińska-Różewska E, et al. (2009): The effect of Echinacea purpurea on the morphology, angiogenic activity and VEGF concentration of murine L1 sarcoma tumors. *Centr Eur J Immunol* 34: 38-41.
77. Skopińska-Różewska E, Pastewka K, Wasutyński A, et al. (2010): The influence of herbal remedies on cutaneous angiogenesis induced in mice after grafting of human kidney cancer tissue. *Centr Eur J Immunol* 35: 199-203.
78. Kligler B, Einstein A (2003): Echinacea. *Am Fam Physician* 67: 77-80.
79. Dugo M, Gatto R, Zagatti R, et al. (2010): Herbal remedies: nephrotoxicity and drug interactions. *G Ital Nefrol* 27 suppl 52: S5-9 [Article in Italian].
80. Miller LG (1998): Herbal medicinals: selected clinical considerations focusing on known or potential drug-herb interactions. *Arch Intern Med* 158: 2200-2211.
81. Mathes A, Bellanger R (2010): Herbs and other dietary supplements: current regulations and recommendations for use to maintain health in the management of the common cold or other related infectious respiratory illnesses. *J Pharm Pract* 23: 117-127.
82. Moltó J, Valle M, Miranda C, et al. (2011): Herb-drug interaction between Echinacea purpurea and darunavir-ritonavir in HIV-infected patients. *Antimicrob Agents Chemother* 55: 326-330.
83. Skopińska-Różewska E, Makuch K (2010): Use of diet supplements, synthetic drugs and herbal remedies with immunotropic activity during pregnancy. I. Echinacea. *Centr Eur J Immunol* 35: 183-185.
84. Tiralongo E, Lea RA, Wee SS, et al. (2012): Randomised, double blind, placebo-controlled trial of Echinacea supplementation in air travellers. *Evid Based Complement Alternat Med* Epub 2012: 417267.