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Influence of *Echinacea purpurea* intake during pregnancy on fetal growth and tissue angiogenic activity

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Abstract: The process of angiogenesis and control of blood vessels sprouting are fundamental to human health, as they play key roles in many physiological and pathological conditions. Intake of different pharmaceuticals with antiangiogenic activity by pregnant women may lead to severe developmental disturbances as it was described in case of thalidomide. It may also cause immunomodulatory effects as it was shown for antibiotics, theobromine, caffeic acid or catechins on the pregnant mice model. At present, *Echinacea purpurea*-based phytoceuticals are among the most popular herbals in the marketplace. Many compounds of *Echinacea* extracts (polysaccharides, alkamides, polyphenols, glycoproteins) exert immunomodulatory, anti-oxidative and anti-inflammatory activity. *Echinacea* is one of the most powerful and effective remedies against many kinds of bacterial and viral infections. In previous studies we had shown significant inhibitory effect of the *Echinacea purpurea* based remedy on tumour angiogenic activity using cutaneous angiogenesis test, and an inhibitory effect on L-1 sarcoma growth was observed .The aim of the present study was to establish whether pharmaceuticals containing alcoholic extracts of *Echinacea purpurea* given to pregnant mice influence angiogenic activity and tissue VEGF and bFGF production of their fetuses. We showed that angiogenic activity of tissue homogenates was increased in Esberitox group and diminished in case of Immunal forte as compared to standard diet group. In case of Echinapur group we did not find significant differences in angiogenic activity. VEGF and bFGF concentration were lower in all groups compared to the control. In the case of Echinapur and Esberitox number of fetuses in one litter were slightly lower as compared to control group, but the difference is on the border of statistical significance. In conclusion, there is some possibility that pharmaceuticals containing *Echinacea purpurea* might influence fetal development in human also, because they may interfere with embrional angiogenesis , and should not be recommended for pregnant women.

Key words: *Echinacea* - Pregnancy - Fetus - Angiogenesis

Introduction

Formation of new blood vessels (angiogenesis) and control of blood vessels sprouting (anti-angiogenesis) are fundamental to human health, as they play key roles in many physiological and pathological conditions.

Angiogenesis is a basic process in embryo development - responsible for organogenesis as well as in function and development of placenta, endometrium and corpus luteum [1,2].

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It also plays a crucial role in wound healing as well as in diabetic retinopathy [3], rheumatoid arthritis [4] and tumor growth and metastasis formation [5].

The initial signals for blood vessel sprouting towards embryo tissues are unknown, but tissue hypoxia is believed to be critical for commencement of the angiogenic cascade.

The development of the embryo occurs in close association with and proximity to capillary ingrowth.

In recent years an increase has been observed in the interest in drugs of natural origin having immunotropic activity. They may be a valuable complementation to treatment of infection, increasing cellular and humoral immunity of the organism in various clinical situations. *Echinacea purpurea*-based phytoceuticals

Table 1. The effect of *Echinacea*- based preparations given to pregnant mice on the angiogenic activity and VEGF and bFGF concentration in 18-days embryos tissues (n=number of fetuses)

Group of pregnant mice	4 mothers fed standard diet (controls) n=40	2 mothers fed standard diet + Immunal n=19	3 mothers fed standard diet + Echinapur n=23	3 mothers fed standard diet +Esberitox n=23
Mean number of blood vessels± SD	22.5 ± 4.3	18.2±5.4 p<0.01	21.3 ± 4.0 ns	28.2 ± 6.0 p<0.0001
Mean VEGF concentration (pg/ml) ± SD	325 ± 101	131 ± 8 p<0.0001	155 ± 35 p<0.0001	170 ± 16 p<0.0001
Mean bFGF concentration (pg/ml) ± SD	1529 ± 313	1104 ± 351 p<0.001	933 ± 110 p<0.0001	1022 ± 94 p<0.0001

are among the most popular herbals in the marketplace. Many compounds of *Echinacea* extracts (polysaccharides, alkamides, polyphenols, glycoproteins) exert immunomodulatory, anti-oxidative and anti-inflammatory activity [6,7]. *Echinacea* is one of the most powerful and effective remedies against many kinds of bacterial and viral infections. Several investigators describe stimulation by *Echinacea* extracts of various parameters of cellular and humoral immunity in different experimental models [8-14]. Different species of *Echinacea*, the part of the plant processed and the processing procedure are variables influencing its effectiveness and mode of action. However, many experts consider the juice and hydro-alcoholic extracts of *Echinacea purpurea*, in liquid or desiccated form (tablets), the best preparation, having the greatest level of clinical value [15].

Echinacea sp. products are shown as safe and harmless pharmaceuticals even during pregnancy. However, there is insufficient knowledge concerning the effects of using *Echinacea* species herbals in above period. While some data indicates that fetal malformations do not occur during pregnancy in humans consuming this herb [16], there are no formal studies aimed at assessing the possibility that *Echinacea* herbals intake may promote congenital abnormalities or fetal growth retardation.

In the previous studies we have shown significant inhibitory effect of the *Echinacea purpurea* based remedy on tumour angiogenic activity using cutaneous angiogenesis test, and an inhibitory effect on L-1 sarcoma growth was observed [17].

The aim of the present study was to establish whether pharmaceuticals containing desiccated alcoholic extracts of *Echinacea purpurea* succus, given to pregnant mice, influence number, angiogenic activity and tissue VEGF and bFGF production of their18-day fetuses.

Materials and methods

Drugs. Immunal Forte tablets (LEK, Slovenia) batch 4676311F, Echinapur tablets (Herbapol Poznań) batch 010905 and Esberitox tablets (GmbH,Germany) batch 623401.

Experimental protocol. Male and female 10-12-weeks old inbred Balb/c mice, about 25 g of body mass, derived from the breeding colony of Polish Academy of Sciences were matched among themselves. Female mice from the 1st day of fertilization until the 18th day of pregnancy were daily fed 0.6 mg of *Echinacea purpurea* extract dissolved in water (3 mothers were fed Esberitox, 2 mothers were fed Immunal, 3 mothers were fed Echinapur) or water as the control (four mothers), orally by Eppendorff pipette.

On the 18th day of pregnancy mice were narcotized and sacrificed, embryos were extracted and weighted. Embryos from one litter were pooled, suspended in phosphate buffered saline (PBS) 1g per 2 ml, homogenized (VirSonic ultrasound sonificator) and frozen at -78°C for further evaluation.

Embryo-induced angiogenesis test (EIA). The test was performed as previously described [18]. Briefly: 0.05 ml of homogenate samples were injected intradermally to anaesthetized (3.6% chloralhydrate, 0.1 ml per 10 g of body mass) syngeneic Balb/c mice (material from one litter was injected to 2-3 mice). Both flanks of each mouse were finely shaved with a razor blade, each flank was injected 2-3 times. Homogenates were supplemented with 0.05 ml/ml of 0.01% trypan blue in order to facilitate recognition of injection sites. After 72 hours mice were sacrificed and newly-formed blood vessels on the inner skin surface were counted in the dissection microscope, at 6x magnification, in central 1/3 of microscopic field. Identification of newly-formed vessels was based on the fact that they are thin, directed to the point of injection, and differ from the background vasculature in their tortuosity and divarications.

Cytokines level estimation. Measurement of VEGF and bFGF concentration in homogenates was done using ELISA test (R&D, USA) according to producer's instructions. Homogenate of each litter was tested in triplicate or quadriplicate for bFGF and VEGF. Optical density was measured at 450 nm.VEGF and bFGF concentrations were expressed as pg/ml.

Ethical issues. For all experiments animals were handled according to the Polish law on the protection of animals and NIH standards. Experiments were accepted by the local Ethical Committee.

Statistics. Statistical evaluation of the data from experiments was done by Statsoft, Inc. (1997) Statistika PL (Software system data analysis), version 5. ANOVA was used to test whether there was statistically significant difference in angiogenic activity, VEGF and bFGF concentration between groups of means (standard, Esberitox, Echinapur and Immunal diet group). To confirm these differences, the post hoc Tukey test for unbalanced designs was used for multiple comparisons ($p<0.05$).

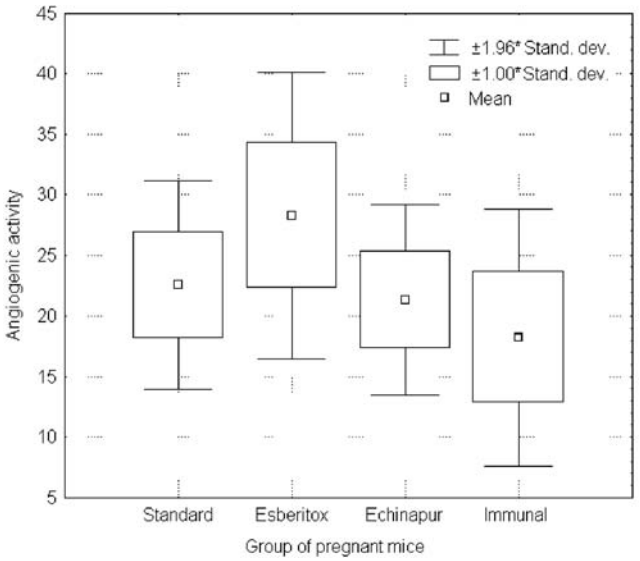

Fig. 1. The effect of *Echinacea* feeding of pregnant mice on the angiogenic activity of fetal tissue.

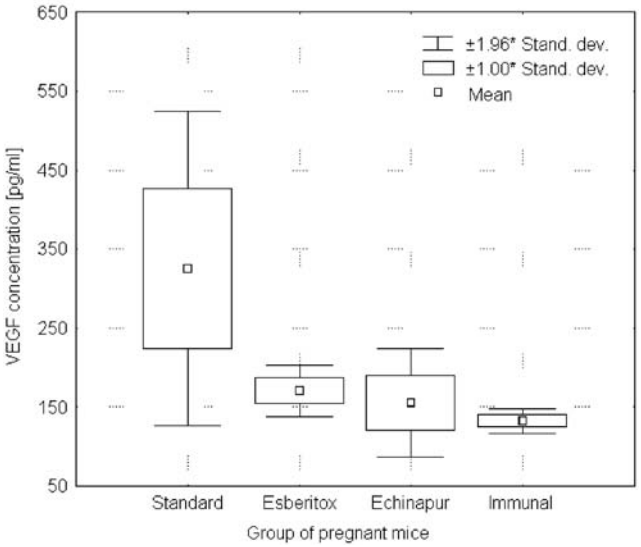

Fig. 2. The effect of *Echinacea* feeding of pregnant mice on the VEGF content activity of fetal tissue.

Results

We showed that angiogenic activity of tissue homogenates was highly significantly increased in Esberitox group and diminished in case of Immunal forte as compared to standard diet (Fig. 1 and Table 1). VEGF and bFGF concentration were lower in all examined than in control groups (Figs. 2 and 3 and Table 1).

In the case of Echinapur and Esberitox mean number of fetuses in one litter were slightly lower (7.6) as compared to Immunal-fed (9.5) and controls (10), but the differences are on the border of statistical significance ($0.05<p<0.1$).

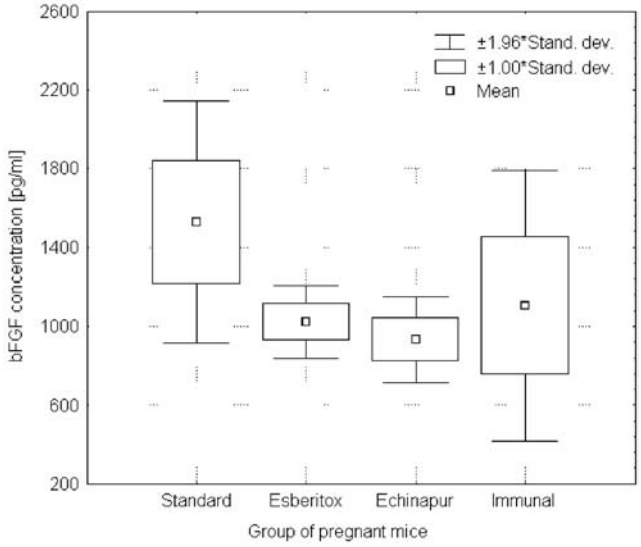

Fig. 3. The effect of *Echinacea* feeding of pregnant mice on the bFGF content of fetal tissue.

Discussion

Echinacea sp. came from North America and were traditionally used by the Indians for a variety of diseases, including mouth sores, colds, injuries, tooth pain and insect-bites[15]. According to clinical studies and spontaneous reporting programs adverse events with *Echinacea* are not commonly reported. Most frequently gastrointestinal upsets and rashes were observed. In rare cases, *Echinacea* can be connected with allergic reactions. Currently it is believed that short-term use of *Echinacea* is associated with a relatively good safety profile, with a slight risk of transient, reversible, adverse events [19].

It was shown that *Echinacea* influence different immunological features in animal models. Brousseau and Miller [20] showed that regular intake of *Echinacea* may be beneficial, because it maintains in an elevated state NK cells, prime elements in immunosurveillance against spontaneous-developing tumors, a phenomenon which increases in frequency with progressive aging.

In our previous studies we showed that phytoceuticals based on *Echinacea sp.* influence cell mediated immunity as well as antibody production and granulocytes activity [21].

Moreover there is a strong scientific evidence for the use of *Echinacea* in respiratory tract infection treatment. On the other hand there are no many publications concerning safety of *Echinacea* intake during pregnancy and lactation [22].

The evaluation of phytoceuticals safety during pregnancy and lactation seems to be of a great importance as according to some studies 36% women use herbal products while pregnant [22].

Maass and co-workers [23] investigated the inclusion of the dried herb *Echinacea purpurea* as feed additive in diets of sows, piglets, and grower/finisher pigs on growth performance, blood picture, plasma enzymes including proliferation of lymphocytes, antibody status, and protein and immune globulin content of colostrum. They did not find significant differences for growth performance, weight loss, blood picture, plasma enzymes, and colostrum composition. In our experiment in mice we found that two *Echinacea* drugs (Esberitox and Echinapur) lowered number of embryos in one litter but the results were on the border of statistical significance. On the other hand Chow and co-workers indicated in mice model that pregnancy-induced elevation in splenic lymphocytes and nucleated erythroid cells was all but eliminated in those females which consumed *E. purpurea* daily throughout their pregnancy. Moreover, they found that consuming *E. purpurea* during pregnancy reduced the number of viable fetuses [24].

Our recent studies suggest that the most important mechanism influencing fetal development in case of *Echinacea sp.* intake might be its influence on angiogenic activity and pro-angiogenic cytokines content of developing tissues.

In our previous studies we showed that Echinapur administration in mice (topically or subcutaneously) caused increase of LIA (lymphocytes induced angiogenesis). Moreover it caused increase of chemokinetic activity of granulocytes and mononuclear cells [25].

Additionally, we showed inhibitory effect of complex remedy containing *Echinacea purpurea* on tumor angiogenic activity and L-1 sarcoma growth [17].

The above results led to a suggestion that *Echinacea sp.* may influence embrionic angiogenesis and in the same time may cause developmental abnormalities.

In the present study we showed statistically significant decrease of fetal tissue angiogenic activity in Immunal Forte fed group. It may indicate possible adverse effects of above phytoceutical on embryo development. Echinapur did'nt influence this parameter, and Esberitox enhanced angiogenic ability of embryos tissues in EIA test, despite their lower than in the control VEGF and bFGF content. It may suggests the presence of some angio-stimulatory compounds in these drugs what is in agreement with our previous studies on lymphocyte-induced angiogenesis [25].

Angiogenesis cascade is promoted by hypoxia of tissues. As a result of hypoxia cells start to produce cytokines and growth factors that cause proliferation and migration of endothelial cells. VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) are known as the most potential angiogenesis promoters. VEGF plays a crucial role in organogenesis (liver and pancreas induction, kidney glomerulus, bone and nervous system development) [26].

Moreover VEGF and bFGF take part in placentation and cytotrophoblast proliferation during pregnancy [27,28]. As we showed the influence of examined phytoceuticals on angiogenic activity of embryo tissues we decided also to evaluate their influence on angiogenesis promoters (VEGF and bFGF). In all examined cases (all phytoceuticals used) we showed highly statistically significant decrease in VEGF and bFGF concentration in fetal tisuues. It may lead to decreased angiogenic activity and therefore to developmental abnormalities in offspring of *Echinacea sp.* fed mothers.

In conclusion it should be stressed that pharmaceuticals containing *Echinacea purpurea* may influence fetal angiogenesis in murine experimental model and without further studies should not be recommended for pregnant women.

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